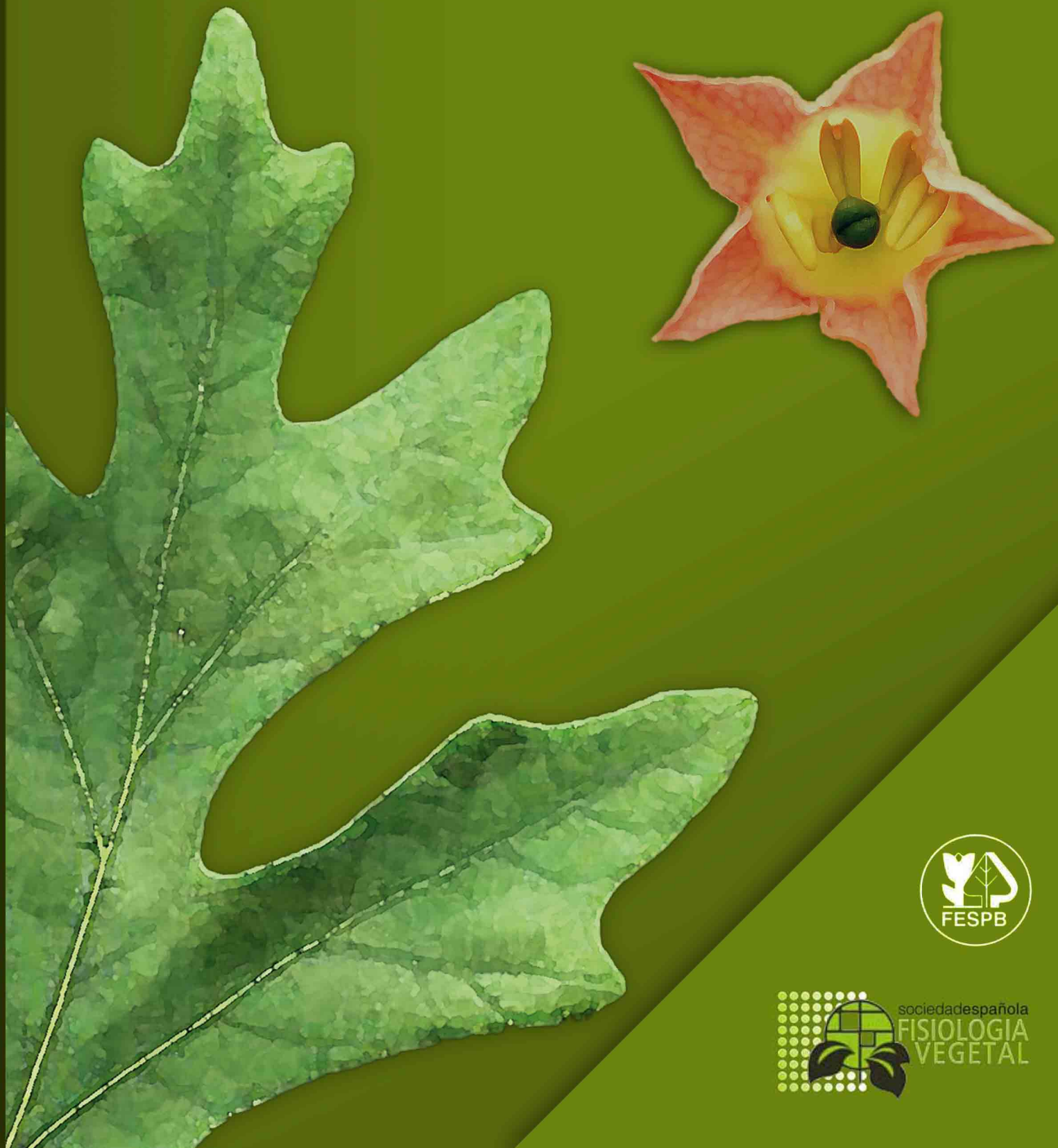
FESPB 2010

XVIII Congress of the Federation of European Societies of Plant Biology (FESPB)

Book of Abstracts

4-9 July 2010
Valencia, Spain



FESPB

sociedadespañola FISIOLOGIA VEGETAL

Wel

Welcome

On behalf of the FESPB 2010 Organising Committees we welcome all plant scientists attending the XVII Congress of the Federation of European Societies of Plant Biology in Valencia.

The Congress is organised by SEFV, the *Sociedad Española de Fisiología Vegetal*. SEFV was founded in 1974 and since then it has been very active in promoting knowledge in plant physiology and plant adaptation. SEFV organised in 1980 the II Congress of FESPB in Santiago de Compostela. Currently this Society has over 600 members and is one of the major contributors to FESPB.

The program of FESPB 2010 includes all aspects of plant physiology covering plant biology of organisms and systems and is intended to merge classical plant physiology with plant molecular biology and biotechnology. Moreover, we have included two special sessions, one dedicated to emerging techniques: modelling meristem development, and the other one to *hot topics*: a mechanistic analysis of endodermis as a selective and protective root-soil interface.

Welcome to Valencia, a city giving special relevance to research.

Recently it has been undertaken a joint venture among the University of Valencia, the Polytechnical University of Valencia and the Spanish Research Council (CSIC) for the establishment of a Campus of International Excellence, VLC/CAMPUS, focused on sustainable development, health, and technologies of information and communication, in which plant biologists will play a relevant role.

The Valencia Congress is officially under the patronage of the Spanish Ministry of Science and Innovation, the Spanish Research Council, The University of Valencia and the Polytechnical University of Valencia.

The Conference will take place at the “Palacio de Congresos” a building designed by Norman Foster equipped with the most advanced technologies, in which more than 900 attending scientists from 54 different countries will present their latest progress in research, bringing a unique opportunity to establish new links and collaborations with colleagues from all European countries.

Last but not least, we wish you a pleasant stay in Valencia, a melting pot of 2000 years of diverse cultural influences in which you will find a friendly atmosphere.

José Pío Beltrán María
President of FESPB

Dolores Rodríguez
President of SEFV

Abs

Abstracts

PL PLENARY SESSIONS LECTURES
PS PARALLELL SESSIONS LECTURES
S SELECTED ABSTRACTS FOR ORAL PRESENTATIONS
P POSTERS

TOPICS

PLENÀ
SESSIO
LECTUI

PLENARY SESSIONS LECTURES

PL01: SUBMERGENCE STRESS AND ACCLIMATION: AN ECOGENOMICS APPROACH

Voesenek, R.
Plant Ecophysiology, Institute of Environmental Biology, Utrecht University.
**Corresponding author, e-mail: L.A.C.J.Voesenek@uu.nl*

The semi-aquatic dicot *Rumex palustris* responds to complete submergence by upward movement of leaves (hyponastic growth) and elongation of young petioles. These two escape responses together can bring leaves above the water surface, thus restoring gas exchange with the atmosphere and increasing survival in flood-prone environments. So far our work suggests that these two responses are regulated via an ethylene-driven signaling network in which apoplastic acidification, expansin action and the activity of the hormones abscisic acid (ABA), gibberellin (GA) and auxin (IAA) are important.
Recently, several genes are identified in R. palustris that code for ethylene response factor (ERF) DNA binding proteins. These genes are regulated by submergence and by some of the hormones that are important for the submergence-induced elongation response. The results will be presented and discussed.

PL02: LEAF MORPHOGENESIS: THE MARGINAL POINT OF VIEW

Adroher, B.[1] - Blein, T.[1] - Boudaoud, A.[2] - Hasson, A.[1] - Hay, A.[3] - Johansen, E.[4] - Morin, H.[1] - Nikovics, K.[1] - Plessis, A.[1]
*Pulido, A.[1] - Tsiantis, M.[3] - Laufs, P.[1]**
[1]Institut Jean Pierre Bourgin, INRA Versailles
[2]RDP, ENS Lyon
[3]University of Oxford
[4]Danish Institute of Agricultural Sciences
**Corresponding author, e-mail:* Patrick.Laufs@versailles.inra.fr

Leaf margins show various levels of dissection/outgrowth such as lobes or serrations in simple leaves, or leaflets in compound leaves, and contribute to a large extent to inter- and intra-species variation of leaf shape. Here we report the contribution of the *NAM/CUC3* genes to leaf dissection in Arabidopsis and other Eudicots. These genes, which code for plant specific transcription factors of the NAC family, were initially identified for their role in organ separation and meristem promotion. We show that they have a conserved expression pattern at the leaf margins and that they are essential for all levels of marginal dissection/outgrowth. We further analysed the role of the *CUC1, CUC2* and *CUC3* genes in the serration of Arabidopsis leaves. We show that in addition to *CUC2, CUC3* contributes to leaf dissection, though via a different mechanism. In contrast, *CUC1* is not involved in Arabidopsis leaf serration, though *CUC1* can efficiently replace CUC2 when expressed at the leaf margin. We provide evidences that CUC2 orchestrates a regulatory network involving *CUC* genes and *MIR164a* to fine-tune the level of Arabidopsis leaf serration. Specific and overlapping roles of the *CUC* genes during leaf development will be interpreted in the light of the evolutive history of the CUC genes within the Brassicales.

PL03: MOLECULAR NETWORKS REGULATING LEAF ORGAN SIZE

Inzé, D.*
VIB Department of Plant Systems Biology, UGent
**Corresponding author, e-mail:* diinz@psb.ugent.be

Understanding the mechanisms that govern tissue, organ and organism size are amongst the most mysterious and fascinating open questions in biology. Our long term goal is to unravel the molecular pathways that govern leaf size and biomass production in *Arabidopsis*. One of our approaches is based on studying the action mechanisms of genes which, when mutated or overexpressed, enlarge leaf size (hereafter called “intrinsic yield genes” (IYG)). Currently, we have confirmed the positive effect of numerous IYGs operating in seemingly unrelated pathways on *Arabidopsis* leaf size. In all cases examined so far enlarged leaf size and increased biomass production results from an increased cell number, mostly without any significant effect on cell size. Our results indicate that, by a yet unknown mechanism, the instructor network must affect the developmental timing of cell division. Cell cycle control genes are likely targets for the instructor genes. Various approaches are now being used to decipher how leaf size is determined. Combining IYGs by crossing lines overexpressing single genes yielded unexpected synergistic effects and different ‘omics’ technologies are also being applied to determine the molecular networks governing enhanced organ growth.

PL04: OF ION CHOREOGRAPHY AND THE REGULATION OF APICAL CELL GROWTH: MOLECULAR PARTNERS AND INTEGRATIVE THEORETICAL MODELS

*Feijó, J. A.**
Universidade de Lisboa, Faculdade de Ciências
**Corresponding author, e-mail: jfeijo@fc.ul.pt*

Despite copious amounts of detailed physiological and molecular data, the mechanistic regulation of growth in pollen tubes still lacks a consensual integrative model. Transcriptomics reveals the presence of about 7.000 genes, but theoretical modeling shows that cooperation of all of these into two processes- wall surface and cytoplasmic volume production- is condition enough to all the morphogenic events that characterize these cells. Spatial and temporal integration of extended biochemical and biophysical processes is mandatory, and in the past we have propose and demonstrated that, at some levels, ion dynamics is common denominator of these regulatory mechanisms. To test the hypothesis that membrane transport activity is sufficient condition for the formation of the intracellular patterns of cytosolic ion concentration, we developed stringent 3-D theoretical modeling of ion fluxes and cytosolic diffusion based on the current knowledge of the system. These models showed that while the current knowledge about membrane fluxes is sufficient to explain cytosolic pH patterning, it is not for Ca2+, where intracellular sequestering must play a role. We will further discuss the minimal needs for channels to explain all the available evidence, and will present physiological, molecular and genetic data showing the presence of novel chloride and calcium influx mechanisms.

PL05: ENGINEERING PHENYLPROPANOID PRODUCTION FOR HEALTHY FOODS

*Martin C.**
John Innes Centre, Norwich, UK
**Corresponding author, e-mail: cathie.martin@bbsrc.ac.uk*

The past 20 years has seen an enormous rise in publicity about super foods that promote health and reduce the risk of cardiovascular disease, cancer and age-related degenerative diseases, related specifically to the metabolic syndrome. These claims are supported by robust evidence from cell studies, animal feeding trials, human intervention studies and epidemiological studies. However, despite all the positive messages about the value of eating fruit and vegetables (the 5-aday program has been running for 25 years) the numbers of people meeting these dietary recommendations in the US remains below 25% of the population, numbers are falling, and chronic diseases, especially those

associated with obesity and the metabolic syndrome, are reaching epidemic proportions in Western societies. There is a need to engineer high levels of protective bioactives in the foods that people actually do consume, to help combat this rise in chronic diseases. Most attempts at engineering the levels of bioactives have focused on increasing the activity of key, rate-limiting steps, but such strategies usually result in only modest improvements in flux to bioactive end-products. Use of transcription factors to up-regulate entire pathways of plant secondary metabolism is a far more effective strategy and results in food material with very significantly elevated levels of health-promoting bioactives. While such improvements may, in part, be achievable for some crops through selective breeding, genetic modification offers bigger improvements because it can overcome limits in the natural variation available in transcription factor specificity and activity. Use of genetically improved foods in animal feeding studies with models of tumorigenesis have revealed that protection is afforded by diets enriched in high bioactive foods. Such health-promoting foods will offer consumers tangible improvements in the products available to them, and have the potential for public approval of genetically improved plant varieties and foods derived from them, in Europe.

PL06: HORMONAL CONTROL OF ROOT MERISTEM DEVELOPMENT

*Sabatini, S.**
Università di Roma Sapienza
**Corresponding author, e-mail: sabrina.sabatini@uniroma1.it*

Upon seed germination, meristems rapidly grow due to a prevalence of cell division over cell differentiation and eventually reach their final size and a constant number of cells. At this stage, meristem maintenance and organ growth are ensured by the balance between cell division and cell differentiation. We have shown that in the *Arabidopsis* root meristem this balance is the result of the interaction between cytokinin (promoting differentiation) and auxin (promoting division) through a regulatory circuit where the ARR1 cytokinin-responsive transcription factor activates the gene *SHY2* that negatively regulates the *PIN* genes encoding auxin transport facilitators. We have thus clarified how the size of the root meristem is maintained, but it is still unknown how a defined final meristem size is set, i.e. how a change in the relative rates of cell division and cell differentiation is brought about for meristem growth to stop. Here, we show that in allowing growth of the root meristem after seed germination and for the meristem to reach its final size, the ARR1/SHY2/PIN circuit necessary to maintain final root meristem size is integrated by two additional components: the cytokinin-responsive ARR12 transcription factor, and gibberellins.

PL07: MOLECULAR MECHANISMS IN INTRACELLULAR PH HOMEOSTASIS

*Serrano, R.**
Instituto de Biología Molecular y Celular de Plantas (IBMCP)
**Corresponding author, e-mail: rserrano@ibmcp.upv.es*

The homeostasis of intracellular pH is a fundamental activity of living cells. In fungi and plants the plasma membrane H+ -ATPase and K+ transport have previously been identified as crucial factors in pH homeostasis. To identify novel components we have utilized the yeast Saccharomyces cerevisiae and the plant Arabidopsis thaliana as model systems and a functional genomic approach based both on transcriptomics studies and on random over-expression of genes and selection for acid tolerance. In yeast we have identified leucine transport and the leucine-tRNA-leu synthetase as targets of intracellular acid pH toxicity. Inhibition of these systems triggers activation of the protein kinase Gcn2, which is required for activity of leucine transporters. In Arabidopsis Weak Acid Tolerance 1 (WAT1) encodes the beta subunit of an AP-3 adaptin complex and loss of function results in acid tolerance.

PL08: IMPACTS OF THERMAL HISTORY ON PLANT RESPIRATION: AN ORGANELLE, ORGAN AND GLOBAL PERSPECTIVE

*Atkin, O.**
The Australian National University
**Corresponding author, e-mail: Owen.Atkin@anu.edu.au*

Climate-mediated changes in plant respiration (*R*) are now accepted as an important component of the biosphere's response to global climate change. Because *R* is temperature-sensitive, several studies have predicted that *R* will increase in a future, warmer world, with important implications for terrestrial C storage and atmospheric CO_2. The extent to which warming increases *R* will depend, however, on whether respiratory metabolism acclimates to sustained increases in growth temperature. There is growing evidence that acclimation does occur, with acclimation being associated with a change in the shape of the temperature response curve of *R*. Acclimation occurs in response to cold as well as warmth, and can eventually result in complete metabolic homeostasis (i.e. identical rates of *R* in plants growing at contrasting temperatures). It can also result in the balance between *R* and photosynthesis remaining constant in plants experiencing contrasting growth-temperatures. In this talk, I will discuss our current understanding of the mechanistic basis of thermal acclimation of *R* at the organelle and whole tissue level, the impacts of acclimation on the C budgets of individual plants and whole ecosystems, and the importance of accounting for acclimation of *R* into a coupled global climate-vegetation models

PL09: IMMUNE SYSTEM DIVERGENCE AND ITS ROLE IN GENETIC INCOMPATIBILITY

*Bomblies, K.**
Harvard University
**Corresponding author, e-mail: kbomblies@oeb.harvard.edu*

Plants boast an elaborate arsenal of defenses to minimize exploitation. Mirroring the diversity of pathogens and herbivores, genes encoding components of the plant immune system are numerous, often highly diverse and frequently found in complex clusters. But an immune system, though critical, is a dangerous weapon – aberrantly activated it can unleash a cascade of unwanted deleterious effects, culminating in growth suppression, widespread tissue necrosis, or even death of the plant. There is growing evidence that errant pathogen response activation is involved in hybrid necrosis, a common type of hybrid failure in plants, and that this is triggered by interactions among diverged immune system components. Thus rapid evolutionary diversification of the defense portfolio must occur in the context of compatibility with co-evolving partners. In other words, pathogen pressure, by promoting divergence of resistance genes, may indirectly promote genetic incompatibility. This points to an important role for intragenomic co-evolution in preventing deleterious immune hyperactivation. We are examining the pattern of resistance gene diversification and its implications in *Arabidopsis thaliana*, and are beginning to examine a related outcrossing species, *A. arenosa* to ask how mating system differences and ploidy differences may affect patterns of resistance gene evolution.

PL10: REPRESSION OF JASMONATE RESPONSES: BEYOND THE JA-SIGNALLING CORE MODULE

*Solano, R.**
Laboratorio de Genómica., Centro Nacional de Biotecnología, CSIC
**Corresponding author, e-mail: rsolano@cnb.csic.es*

Jasmonates (JAs) are essential phytohormones structurally similar to metazoan prostaglandins. In spite of their importance for plant development and survival in nature the molecular details of their signalling pathway are not fully understood.
The identification of COI1 as an F-box protein almost a decade

ago suggested the existence of a repressor of JA responses targeted by SCF^{COI1} for degradation by the proteasome in response to JA. Another important step in the pathway is represented by the transcription factor MYC2, which regulates several responses to JA. However, several key questions such as the link between these two steps in the pathway (MYC2 and SCF^{COI1}), the nature of the bioactive hormone and the identity of its receptor remained unknown. We have recently identified a novel family of JA-regulated nuclear targets of SCF^{COI1}, named JAZ (Jasmonate ZIM-domain proteins). JAZ proteins are repressors of AtMYC2, linking the previous known steps in the pathway. Moreover, the identification of JAZ repressors has also paved the way to identify the jasmonate receptor (the F-box COI1) and the bioactive form of the hormone [(+)-7-*iso*-JA-Ile]. Recently, the identification of a Novel-Interactor-of-JAZ proteins (NINJA) has uncovered the mechanism by which JAZ proteins repress MYC2 activity. How these discoveries help to understand the molecular mechanisms underlying JA-signalling will be discussed during the seminar.

PL11: FROM PLANT-PATHOGEN INTERACTIONS TO ECOGENETICS OF PLANT-MICROBE COMMUNITIES

*Schulze-Lefert, P.**
Max Plankc Institute for Plant Breeding Research, Köln, Germany
**Corresponding author, e-mail: schlef@mpiz-koeln.mpg.de*

Parasitic and symbiotic associations between plants and microbes are merely the two extreme outcomes of a continuum of inter-organismal interactions affecting plant productivity. Little is understood about plant-microbe interactions that are, at first glance, symptomless. Complex communities of poorly studied plant-associated microbes are an untapped reservoir that can promote plant health and productivity. We have begun to examine the microbiome of the Arabidopsis rhizosphere using T-RFLP and 454 pyrosequencing profiling methods. I will describe the structure of root-associated bacterial communities found in natural soil and our attempts to examine the genetic basis of their formation.

PL12: MECHANISMS AND PHENOTYPIC CONSEQUENCES OF DNA METHYLATION IN ARABIDOPSIS

*Colot, V.**
Institut de Biologie de l'Ecole Normale Supérieure (IBENS), CNRS, Paris, France
**Corresponding author, e-mail: colot@biologie.ens.fr*

DNA methylation plays key roles in the control of genome activity in plants and mammals. It is critical for the stable silencing of repeat elements and is also involved in the epigenetic regulation of some genes. Despite similarities in the controlling functions of DNA methylation, its dynamics and deposition patterns differ in several respects between plants and mammals. One of the most striking differences is that plants tend to propagate pre-existing DNA methylation states across generations, whereas mammals re-establish them genome wide at every generation. Our recent findings on the transgenerational stability of DNA methylation patterns in Arabidopsis will be presented. The role of RNAi in the incremental methylation and silencing of repeat elements over successive generations and in the preservation of normal expression of neighboring genes will be highlighted

PL13: MAKING OIL IN BIOMASS BY REGULATING FATTY ACID BREAKDOWN AND LIPID SYNTHESIS PATHWAYS.

Graham, I; Slocombe, S; He, Z; Dyer, J; Hernandez, L; Larson, TR.
University of York
**Corresponding author, e-mail: iag1@york.ac.uk*

Plant oils in the form of triacylglycerol (TAG) are used for food, industrial feedstock and biofuel manufacture. Although TAG is typically harvested from the fruit or seeds of oil crop species, plants can also accumulate small amounts of TAG in the leaves and other vegetative tissues. Partitioning of fatty acids into TAG involves several endoplasmic reticulum associated acyltransferases. We have found that when fatty acid breakdown is blocked in seedlings of the model plant Arabidopsis, recycling of fatty acids back into oil body TAG occurs. A soluble cytosolic acyltransferase appears to be involved in this process. In older vegetative leaves, we have found that TAG levels can be increased significantly (10–20 fold) by blocking fatty acid breakdown, particularly during extended dark treatments or leaf senescence. Generation of a double mutant in fatty acid breakdown and diacylglycerol acyltransferase 1 (DGAT1) results in a severe vegetative growth phenotype suggesting that partitioning of fatty acids to TAG in leaves is carried out predominantly by this acyltransferase. Ectopic expression of LEC2, a seed development transcription factor involved in storage product accumulation results in accumulation of seed oil type species of TAG in senescing tissue that are blocked in fatty acid breakdown. Our data suggests that recycled membrane fatty acids can be re-directed to TAG by expressing the seed-programme in senescing tissue or by a block in fatty acid breakdown. This work raises the possibility of producing significant amounts of oil in vegetative tissues of biomass crops such as Miscanthus.

PL14: PLANTS RESPONSES TO GLOBAL CHANGE

*Valladares, F.**
Instituto Recursos Naturales CSIC
**Corresponding author, e-mail: valladares@ccma.csic.es*

Current environmental change is having important impacts on plant performance. Global change involves not only a general trend of increasing air temperatures but also an increased frequency of extreme climatic events, habitat fragmentation and degradation, and high rates of biotic exchange leading to assemblages of novel communities of plants, animals and microorganisms. Plant responses to these changes involve plastic phenotypic changes (e.g. acclimation), passive tolerance of the increased stress, rapid microevolutionary changes, and, if all this fails, local extinctions. There are two main novelties of global change pressures for plants: the speed of the environmental change and the fact that it involves changes in several biotic and abiotic factors simultaneously. While plant stress physiology has promoted active research, plant physiology under multiple stresses still requires extensive attention. I illustrate what we know and what we should know about plant physiology under global change conditions with examples of Mediterranean ecosystems where water limitations are exacerbated by climate change. Impacts of changing water availabilities coupled to changes in the light environment are mediated by other co-occurring factors such as soil fertility and air temperature, but also by the presence and performance of other competing or symbiotic organisms. Co-existing plants can either facilitate or outcompete the study species and the stress level imposed by water is profoundly affected by organisms in the soil.

PL15: MECHANISTIC ANALYSIS OF THE ENDODERMIS AS A SELECTIVE AND PROTECTIVE ROOT-SOIL INTERFACE

*Geldner, N.**
University of Lausanne
**Corresponding author, e-mail: Niko.Geldner@unil.ch*

We analyse the molecular mechanisms that underlie the development and function of the root endodermis in Arabidopsis. The endodermis is a cell layer in the root of all higher plants and thought to be of central importance for plant nutrition and stress resistance. We have established molecular markers which demonstrate that the endodermal plasma membrane has two separate polar domains of distinct function. Our description of endodermal

development reveals that the plasma membrane domain at the Casparian Strips (CSD) separates these two complementary polar domains and has features analogous to animal tight and adherens junction, establishing the endodermis as a new cellular system that displays many of the features of polarised epithelia in animals. We have identified a family of conserved, plant-specific transmembrane proteins of unknown function that predict and mark the site of CSD formation. We show that their lack-of-function leads to disorganised formation of Casparian Strips. Molecular analysis of these proteins suggests that they are major constituents of this plant tight junction equivalent. In addition we have undertaken forward genetic screens and have identified a number of mutant with strong, but apparently specific defects in the formation of the endodermal barrier. Our developmental and cell biological analysis of endodermal differentiation now allows us to manipulate endodermal differentiation. We investigate the role of the endodermis in lateral root formation, pathogen infection and plant nutrition. In addition, we use the endodermis as a model to address fundamental questions of plant cell polarisation and the biosynthesis, degradation and localised deposition of cell wall material.

PL16: HOW GROWING PLANTS TRANSFORM GENE EXPRESSION INTO SHAPE CHANGES: MAKING EXPLICIT THE ROLE OF MECHANICS DURING MERISTEM GROWTH AT CELL RESOLUTION

*Godin, C.**
INRIA - UMR DAP - Développement et Amélioration des Plantes , Montpellier, France
**Corresponding author, e-mail: christophe.godin@inria.fr*

The generation of new organs at the apex of meristems is controlled by physiological processes that have been extensively studied in the past decade. Auxin transport for instance makes it possible to accumulate auxin at key locations in the meristem which in turn triggers primordia outgrowth, while growth itself was shown to be decisively affected in each cell by the orientation of cortical microtubules. We are now at a point where growth can be interpreted quantitatively as a result of these processes and their control by genes. At the core of this system, the physical forces between cells make it possible to transform physiological information at cell level into shape changes at tissue level. In this talk I will describe the recent advances made to model this mechanical interaction from cell to cell during plant growth and show preliminary applications of these models to interpret tissue deformation corresponding to organ outgrowth at the shoot apical meristem.

PL17: RETROMER RELOADED: A REAPPRAISAL OF POST-GOLGI TRAFFICKING

Robinson, D.G - Niemes, S - Pimpl, P - Scheuring, D - Viotti, C.*
HIP-Cell Biology, Univ. Heidelberg
**Corresponding author, e-mail: david.robinson@urz.uni-heidelberg.de*

Contrary to previous reports, both the large (VPS35, VPS29, VPS26) and small (sorting nexins 1 and 2) subunits of retromer locate to the trans Golgi network (TGN) and not the multivesicular, prevacuolar compartment (PVC). This fact, together with the knowledge that in plants the TGN functions as an early endosome has consequences for our understanding of biosynthetic and endocytic trafficking to the vacuole. Retromer recycles vacuolar sorting receptors (VSRs) and our data suggest that recycling occurs from the TGN to the ER. We also have data which indicate that VSRs already interact with their cargo ligands in the ER, and exit the ER in a COPII-independent manner. Endocytosed proteins which are destined for degradation are selectively internalized into the luminally located vesicles of the PVC with the help of the ESCRT complex. Immunolocalization studies indicate that the different ESCRT complexes are arranged sequentially downstream of the Golgi apparatus. Recent observations highlight the dynamic nature of the TGN. This organelle appears to be continually formed and released from the Golgi stack suggesting that the separation of secretory and vacuolar traffic must occur through a division of the TGN. The “vacuolar“ part of the TGN presumably then matures into the PVC.

PL18: MAPPING CONNECTIONS BETWEEN THE GENOME, IONOME AND THE PHYSICAL LANDSCAPE

*Salt, D. E.**
Purdue University
**Corresponding author, e-mail: dsalt@purdue.edu*

Understanding how organisms control their ionome or mineral nutrient and trace element composition, could have a significant impact on both plant and human health. Furthermore, associating the genetic determinants that underlie natural ionomics variation, with the landscape of the individuals that carry these genotypes, will provide insight into the genetic basis of adaptation and speciation. Using *Arabidopsis thaliana* we have employed high-throughput mineral nutrient and trace element profiling to determine the biological significance of connections between an organisms genome and its ionome. We have used PCR-based positional cloning, DNA microarray based approaches, QTL and association mapping to identify genes that control the ionome. Association of polymorphic loci with the landscape is starting to reveal the genetic architecture underlying specific adaptations to the environment. We are also finding specific ionomic “fingerprints” associated with functionally related sets of genes, and with the physiological status of the organism. Further, we have developed a publicly searchable online database containing over 3.7 million ionomic data elements from over 1900 different experiments (www.ionomicshub.org), and the database is being updated regularly.

FESPB AWARDS LECTURES

PL19: LIGHT AND TEMPERATURE SIGNAL CROSSTALK IN PLANT DEVELOPMENT

*Franklin, K. A.**
University of Bristol
**Corresponding author, e-mail: kerry.franklin@bristol.ac.uk*

Light and temperature are two of the most important environmental signals regulating plant development. It is perhaps therefore not surprising that complex crosstalk exists between these signalling pathways to optimise plant environmental adaptation. Plants monitor their ambient light environment using specialised information-transducing photoreceptors which include the red and far-red light-absorbing phytochromes. A major role of the phytochromes in natural environments is the detection of competing vegetation and initiation of architectural responses to avoid shading. Different light foraging strategies are, however, observed at different ambient growth temperatures. We are currently exploiting natural genetic variation in temperature-mediated light foraging strategy to identify the regulatory mechanisms involved. Light quality-mediated elongation growth responses are regulated, in part, through the bHLH transcription factors PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and PHYTOCHROME INTERACTING FACTOR 5 (PIF5). We have recently shown that PIF4 functions as a master regulator of auxin-mediated elongation growth at high temperatures, thereby identifying a key molecular mechanism through which plants integrate multiple environmental stimuli.

PL20: REGULATION OF AQUAPORINS BY THE ARBUSCULAR MYCORRHIZAL (AM) SYMBIOSIS. SOIL RESOURCES USE OPTIMIZATION

*Aroca, R.**
Estación Experimental del Zaidín (CSIC)
**Corresponding author, e-mail: raroca@eez.csic.es*

Almost all plants in nature establish a symbiosis with AM fungi. By this symbiosis the fungus can complete their vital cycle and it receives from the host plant carbon resources. At the same time, the host plant receives from the fungus mineral nutrients (especially P) and water located in places non accessible to plant roots. On the other hand, it is known that aquaporins (membrane intrinsic proteins able to transport water and other small solutes following an osmotic gradient) determine water uptake capacity of plat roots. In the past few years how AM symbiosis regulates aquaporin expression under different abiotic stress conditions has been studied. Thus, it was observed that each aquaporin gene responded in a different way to a specific environmental factor, and also its response depends if the root was inoculated or not with an AM fungus. In other studies was found that the response of aquaporins to the AM symbiosis was related to the endogenous levels of ABA in host plant tissues. At the same time, AM plants responded stronger to potassium soil addition than non AM plants in terms of dry mass production and enhancement of root hydraulic conductance. Also, it has been found a kind of communication between intra- and extra- radical AM mycelium regarding the expression of an aqauporin of the AM fungus and those of the host plant. Most recently, when AM symbiosis was combined with application of biological treated agro-waste residues to the soil, this particular combination improved water use efficiency of the host plants and also regulates differentially

PARALL
SESSIO
LECTU

PARALLELL SESSION SECTURES

PS01: ENVIRONMENTAL STRESSES & ACCLIMATION
Session lead lectures

PS01-001: COMMON ELEMENTS OF ARABIDOPSIS RESPONSES TO ANAEROBIOSIS AND HEAT

*Banti, V.[1] - Mafessoni, F.[1] - Loreti, E.[2] - Novi, G.[1] - Pucciariello, C.[1]- Alpi, A.[3] - Perata, P.[1]**
[1]Scuola Superiore Sant'Anna
[2]IBBA, National Research Council
[3]University of Pisa
**Corresponding author, e-mail: p.perata@sssup.it*

Genomic and transcriptomic studies suggest the existence of a "core stress response" gene cluster, confirming the overlapping of physiological responses to abiotic stress, often observed in plants. Heat Shock Proteins (HSPs) have been proposed to be involved in different kinds of environmental conditions, well beyond heat shock: they resulted also induced by anoxia in rice and Arabidopsis seedlings and a mild heat pre-treatment can enhance anoxia-tolerance in Arabidopsis. Transcript profiling revealed up-regulation of HS genes following oxygen deprivation and a significant overlapping between the anoxic and heat response. The heat shock transcription factor HsfA2, notably involved in heat-acclimation, is strongly up-regulated under anoxia and its induction appears to be mediated by an H_2O_2 burst following the first minutes of anoxia. We demonstrate an important role of *HsfA2* in Arabidopsis response to anoxia: an *HsfA2* knock-out, differently from the wild type, cannot cross-acclimate to anoxia following a mild heat pre-treatment, whereas p35S:HsfA2 seedlings show enhanced tolerance to anoxia and a more lasting and strong immuno-signal for target of HsfA2 (HSP17.6-CI) during anoxia. The role of the putative targets of HsfA2 will be discussed.

PS01-002: STRUGGLING FOR LIGHT: HORMONE INTERACTIONS REGULATE SHADE AVOIDANCE RESPONSES

Pierik, R. - Sasidharan, R. - Keuskamp, D. H. - de Wit, M. - Voesenek, L. A. C. J.*
Utrecht University
**Corresponding author, e-mail: r.pierik@uu.nl*

Plants growing in dense vegetations compete with proximate neighbors for light. They can ensure growth and survival through an escape syndrome known as shade avoidance. Upon perception of neighbors plants elongate their shoots and move their leaves upwards. Neighbor detection occurs through spectral changes in the light reflected from or transmitted through neighboring vegetation. Red light (R) is absorbed for photosynthesis whereas far-red light (FR) is reflected, thus lowering the R:FR ratio which can be sensed by the phytochrome photoreceptors. We showed more recently that plant neighbor detection also involves chemical cues, including the volatile plant hormone ethylene. Both light quality signals and ethylene regulate a variety of hormones to control adaptive growth responses. We show here that ethylene emissions are enhanced by low R:FR, as are endogenous levels of auxin and gibberellins. These three hormones interact at the level of DELLA proteins, which are transcriptional regulators that inhibit growth, but in addition have DELLA-independent functions as well to control shade avoidance. We further show that downstream targets for these light-hormone interactions include cell wall modifying proteins such as expansins and XTH's. The functional implications of this network of interactions for plant growth under natural competitive conditions will be discussed.

PS02: VEGETATIVE DEVELOPMENT
Session lead lectures

PS02-001: DUPLICATION AND DIVERGENCE OF BRANCHED1-LIKE GENES AND THE EVOLUTION OF THE CONTROL OF SHOOT BRANCHING IN TOMATO

*Martín Trillo, M.[1] - González Grandío[2], E. - Serra, F.[3] - Rodriguez Buey, M. L.[2] - Dopazo, H.[3] - Cubas, P.[2]**
[1]Universidad de Castilla la Mancha, Facultad de Ciencias del Medio Ambiente.
[2]Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología/CSIC.
[3]Evolutionary Genomics Unit. Bioinformatics and Genomics Department. Centro de Investigación Príncipe Felipe. Valencia.
**Corresponding author, e-mail: pcubas@cnb.csic.es*

Duplication and divergence of genes and pathways controlling developmental programmes are thought to have played a fundamental role in the evolution of morphological diversity. However the molecular mechanisms underlying functional divergence following duplication and the relationship between gene evolution and the emergence of new traits are still not well understood. In angiosperms, branching patterns greatly determine overall plant architecture and affect key aspects of plant life. Recent studies suggest that branch development is controlled by a conserved genetic pathway evolved before the radiation of flowering plants. However, despite the general conservation of genes and pathways, a wide diversity of branching patterns is found in angiosperms. One of the central genes controlling branching in Arabidopsis, *BRANCHED1 (BRC1)*, encodes a transcription factor of the TCP family which is a putative target gene for selection during the evolution of new branching patterns is *BRC1*. To investigate the relevance of the molecular evolution of *BRC1* genes during the evolution of branching patterns, we have isolated and analyzed the function of *BRC1*-like genes in *Solanum lycopersicum* (Solanaceae, Asteridae) a dicot species distantly related from Arabidopsis (Brassicaceae, Rosidae) and with divergent branching patterns. We have found that a duplication of the BRC1 gene has taken place in this species. Our view of the molecular evolution and divergence of these two gene copies will be presented.

PS02-002: FEEDBACK CONTROL OF CELL FATES IN PLANT MERISTEMS

*Rüdiger, S.**
Heinrich-Heine University
**Corresponding author, e-mail: Ruediger.Simon@uni-duesseldorf.de*

Primary plant meristems are the shoot and root meristems that are initiated at opposite poles of the plant embryo. They contain stem cells, which remain undifferentiated, and supply new cells for growth and the formation of tissues. The maintenance of a long-lasting stem cell population in meristems is achieved by signal exchange between organizing regions and the stem cells, and also by feedback signals emanating from differentiating cells. I will discuss the role of peptide signals that make use of different receptor classes to control the stem cell populations in both meristem types by regulating evolutionarily conserved homeodomain transcription factors.

PS03: SYSTEMS BIOLOGY & Omics
Session lead lectures

PS03-001: SYSTEMS ANALYSIS OF THE CONTRIBUTION OF METABOLISM TO GROWTH IN ARABIDOPSIS

*Stitt, M.**
Max Planck Institute of Molecular Plant Physiology, Golm, Germany
**Corresponding author, e-mail: stitt@mpimp-golm.mpg.de*

Plants are exposed to a continually changing environment, including the diurnal light-dark cycle, and changes that are superimposed on this cycle. Starch provides the major carbon store for growth at night. We have used 'omics analysis of responses during the diurnal cycle to investigate how growth is coordinated with the momentary and longer-term changes in the carbon. Thousands of transcripts show large diurnal changes of their levels. These can be predicted using a simple linear model, in which the clock, sugar and light as the major inputs. Some of the most striking and best-predicted changes are for genes involved in protein synthesis. Polysome analyses reveal that the global translation rate is tightly tied to the momentary carbon availability. To explore the relation between translation and plant growth more closely, we have carried out quantitative analyses of rRNA, transcripts, polysome loading and protein abundance. This allows us to model the relationship between basic molecular parameters like ribosome and transcript concentrations and the whole plant carbon budget and growth. The results show that protein synthesis represents a significant component of the total plant energy budget, and is regulated to optimise energy costs on a diurnal basis, and probably also during long term adaptations to environmental conditions. These results receive independent support from a complementary approach, in which we have identified integrative molecular and metabolic parameters that determine biomass production in a panel of 100 Arabidopsis accessions. These reveal that starch and protein are correlated with each other, act as integrators of the metabolic response, and are negatively correlated with rosette biomass, and also reveal a strong impact of ribosome abundance and the efficiency of ribosome use on biomass formation.

PS03-002: CONSTRAINT BASED MODELLING – A NEW APPROACH TO SYSTEMS-LEVEL STUDY OF PLANT METABOLIC NETWORK

*Zhu, X.**
CAS-MPG Partner Institute for Computational Biology
**Corresponding author, e-mail: zhuxinguang@picb.ac.cn*

Identifying new ways to improve photosynthesis is one feasible approach to increase crop potential yields. Building dynamic systems models of photosynthesis and plant primary metabolism is one option to identify targets to increase photosynthesis. This method however is often limited by the shortage of kinetic parameters, which demands development of high throughput methods to characterize the compartment-specific information about the metabolomics, proteomics and enzyme activities related to photosynthesis and plant primary metabolism. Constraint based modelling on the contrary requires less detailed kinetic information. It has been used widely in microbiology community to study metabolism and regulation at a genomic scale. A few constraint based models have been developed in the last five years to study plant metabolic network properties. In this lecture, I will briefly discuss the concept, methodology, major applications and challenges of using this approach in plant biology research. The potential of linking this method to high throughput data to sudy the response of plants to climate change and identifying new options to increase photosynthesis will be discussed.

PS04: REPRODUCTIVE DEVELOPMENT
Session lead lectures

PS04-001: SELF-INCOMPATIBILITY SIGNALLING NETWORKS: CONVERSATIONS THAT TELL "SELF" POLLEN TO COMMIT SUICIDE

*Wheeler, M.J. - de Graaf, B.H.J. - Wu, J. - Poulter N.S. - Wilkins, K.A. - Vatovec, S. - Franklin-Tong, V.**
University of Birmingham
**Corresponding author, e-mail: v.e.franklin-tong@bham.ac.uk*

Self-incompatibility (SI) is controlled by a multi-allelic *S* locus that allows discrimination between "self" pollen from "non-self" pollen. In *Papaver rhoeas*, the pistil *S* determinant (recently renamed as *PrsS*, *Papaver rhoeas stigma S*) encodes a small novel protein that interacts with incompatible pollen, triggering a Ca^{2+}-dependent signalling network. We recently identified the *Papaver* pollen *S*-determinant *(Papaver rhoeas pollen S), PrpS*, which encodes a novel ~20 kDa transmembrane protein with no homology to sequences in existing databases. I will present our data showing that PrpS has the attributes expected of a pollen *S* locus determinant, including functional data5. I will also present recent data suggesting that PrsS acts as a ligand, stimulating non-specific cation conductance permeable to Ca^{2+} and K^{+}. Downstream of interaction of PrsS with PrpS, we have identified several events that are triggered specifically in an incompatible situation. These include rapid alterations to the actin cytoskeleton[1,2] and programmed cell death, involving caspase-like activities[3,4]. I will present unpublished data identifying further SI-specific events triggered in incompatible pollen. We have recently begun studies of PrpS in *Arabidopsis* and I will present preliminary data showing that poppy SI appears to function in *Arabidopsis.*

References
1. Snowman BN, et al. (2002) Plant Cell 14, 2613-2626.
2. Poulter NS, et al (2010) Plant Physiol 10.1104/pp.109.152066
3. Bosch, M. & Franklin-Tong, V. E. (2007) PNAS 104, 18327-18332.
4. Thomas, S. G. & Franklin-Tong, V. E. (2006) Nature 429, 305-309.
5. Wheeler, M. J. et al. (2009) Nature 459, 992-995.

PS04-002: CELL-CELL COMMUNICATION DURING FERTILIZATION IN ARABIDOPSIS: A SURPRISING LINK TO DISEASE RESISTANCE

*Kessler, S.[1] - Escobar-Restrepo, J. M.[1] - Huck, N.[1] - Asano, H.[1] - Kienath, N. F.[2] - Panstruga, R.[2] - Grossniklaus, U.[1]**
1Institute of Plant Biology & Zürich-Basel Plant Science Center, University of Zürich
2Max-Planck Institute for Plant Breeding Research, Cologne
**Corresponding author, e-mail: grossnik@botinst.uzh.ch*

Research in our laboratory focuses on the developmental genetics of plant reproduction. Our studies have shown that both genetic and epigenetic mechanisms play a key role in plant reproduction. We have isolated a female gametophytic mutant, *feronia*, which disrupts double fertilization: in *feronia* mutant embryo sacs the pollen tubes, even if wild-type, are unable to release the sperm cells to effect fertilization (Huck et al., 2003, Development 130: 2149). This phenotype suggests that the female gametophyte plays a crucial role in pollen tube reception and, thus, controls the behaviour of the male gametophyte. The *feronia* mutant defines novel signalling processes between the male and female gametophytes in the process of double fertilization FERONIA was shown to encode a receptor-like kinase of a plant-specific subfamily (Escobar-Restrepo et al., 2007, Science 317: 656). Interestingly, some interspecific crosses result in phenotypes that are very similar to those observed in the feronia mutant. I will report on the molecular and biochemical characterization

of *FERONIA* and on our search for additional components of this signal transduction process using genetic and biochemical approaches. Our recent attempts to identify novel components of the *FERONIA* signal transduction pathway have identified surprising links to disease resistance in plants. The evolutionary implications of these findings will be discussed.

PS05: BIOTECNOLOGY
Session lead lectures

PS05-001: METABOLIC AND TRANSPORT ENGINEERING OF GLUCOSINOLATES

*Halkier, B. A.**
Department of Plant Biology, Faculty of Life Sciences, University of Copenhagen
**Corresponding author, e-mail: bah@life.ku.dk*

Epidemiological studies have demonstrated reduced risk of developing cancer upon consumption of diets rich in cruciferous vegetables. Key players in this chemoprevention are the natural products glucosinolates, in particular the methionine-derived glucoraphanin which is highly abundant in broccoli. Improved nutrition by functional foods or health-promoting dietary supplements is an attractive means for prevention of lifestyle-based diseases. Towards this goal, we have transferred the entire glucoraphanin biosynthetic pathway consisting of thirteen genes from Arabidopsis into the non-cruciferous tobacco by transient expression. The engineering involves the chloroplast-localized chain elongation machinery (5 genes) that converts methionine to dihomomethionine, and the cytosolic, ER-anchored core structure pathway (8 genes) that converts dihomomethionine to the glucoraphanin. Transport engineering is important to ensure efficient channeling of intermediates between compartments and proper storage of end product to prevent feedback inhibition, but not much attention has so far been given to this aspect of engineering. Our progress in development of a technology platform for transport engineering will be discussed, as will our technology platform for engineering plant pathways into yeast.
1) Mikkelsen et al. (2010) Reconstitution of the glucoraphanin biosynthetic pathway. Molecular Plant (in press)Ç

PS05-002: TRANSGENIC NUTRITIONAL ENHANCEMENT: THE PRODUCTION OF OMEGA-3 LONG CHAIN POLYUNSATURATED FATTY ACIDS IN PLANTS

Napier, J. - Sayanova, O. - Ruiz-Lopez, N. - Haslam, R. - Venegas-Caleron, M.*
Rothamsted Research, Harpenden, Herts AL5 2JQ, UK
**Corresponding author, e-mail: johnathan.napier@bbsrc.ac.uk*

There is now considerable evidence as to the importance of omega-3 long chain polyunsaturated fatty acids (LC-PUFAs) in human health and nutrition. Unfortunately, current sources are either in severe decline (fish oils) or expensive (via microbial fermentation), leading to the search for an alternative source. We have been evaluating the possibility of producing omega-3 LC-PUFAs in transgenic plants, to provide a sustainable source of these important nutrients, since no native higher plant species synthesise these fatty acids. We have transgenically assembled the primary biosynthetic pathway for LC-PUFAs in both model plants and crop species. Our data indicate that whilst the transgenic synthesis of C20 LC-PUFAs such as arachidonic acid and eicosapentaenoic acid is clearly feasible, a number of factors may limit the efficient heterologous reconstitution of this pathway. We have attempted to address this problem in a systematic manner by firstly identifying different metabolic "bottlenecks" and then seeking genetic interventions to overcome them. It seems likely that a generic bottleneck resides within the primary LC-PUFA biosynthetic pathway as a result of the "substrate dichotomy" between the lipid-dependent desaturases and the acyl-CoA-dependent elongases which catalyze the reactions. Attempts to overcome this bottleneck, through the use of acyl-CoA dependent desaturase or acyltransferases will be presented. In addition, the impact (in terms of substrate-channelling) of endogenous plant lipid metabolism on the heterologous LC-PUFA pathway will be considered. The outcomes from our recent iterations of this transgenic metabolic engineering will be presented, and the future prospects for GM-derived LC-PUFAs will be discussed.

PS06: ROOT BIOLOGY
Session lead lectures

PS06-001 AUXIN RESPONSE AND CELL COMMUNICATION IN EMBRYONIC ROOT FORMATION

*Weijers, D.**
Laboratory of Biochemistry, Wageningen University
**Corresponding author, e-mail: dolf.weijers@wur.nl*

Plant growth and development is controlled through the activity of stem cells within specialized niches, the meristems. These meristems are first established in the early embryo, when the organism consists of few cells. The work in my group aims at understanding the mechanisms underlying the initiation of the root meristem in the early embryo.
Root initiation is first manifested by the specification of an extra-embryonic suspensor cell as the hypophysis, the future quiescent center. This cell specification event is triggered by signals from the adjacent embryonic cells. The auxin-dependent transcription factor AUXIN RESPONSE FACTOR5 / MONOPTEROS (ARF5/MP) is a critical regulator of hypophysis specification, and acts in the embryonic lineage to promote cell-cell communication. We have recently identified a number of direct MP target genes, and among these found a small mobile bHLH transcription factor that acts as a novel intracellular signal that mediates MP-dependent root formation.
MP also promotes transport of auxin to the hypophysis. However, the response machinery in this cell is not known. In a systematic effort to define auxin-dependent patterning steps and ARF gene expression patterns, we have found a novel auxin response module that operates in the extra-embryonic suspensor to promote hypophysis specification, but also to prevent transformation to embryonic cell fate. Hence, auxin triggers several different responses in the early embryo, depending on the cellular context. Our current focus is to 1) dissect MP-dependent cell-cell communication, 2) understand the basis for context-dependent auxin responses in the embryo and 3) identify the gene networks that are controlled by auxin in the different cell types of the embryo.

PS06-002 TOWARD NEW REGULATORY NETWORKS IN THE ROOT MERISTEM

*Blilou, I.**
Department of Molecular Genetics, Utrecht University, The Netherlands
**Corresponding author, e-mail: i.blilou@uu.nl*

In the Arabidopsis root meristem, a dynamic transcription factor networks regulate stem cell specification and meristem maintenance. One of the best studied subnetworks is that involving the SHR/SCR GRAS family transcription factors. It involves transmission of positional information between cell layers in the meristem. We have previously shown that JKD (a C2H2 type transcription factor) regulates SHR movement and nuclear localization. Here we provide evidence that new members of the JKD clade act redundantly in this process, we show that their expression overlaps with JKD in the ground tissue and that they form protein complexes with SHR to control asymmetric cell division in the ground tissue.
We also show that JKD controls epidermal cell fate and thus root hair patterning.

PS07: MOLECULAR MECHANISM OF ABIOTIC STRESS
Session lead lectures

PS07-001 TARGETING PROTEIN KINASE SIGNALING CASCADES TO IMPROVE STRESS TOLERANCE IN PLANTS

*Hirt, H.**
URGV Plant Genomics, Evry, France
**Corresponding author, e-mail:Hirt@evry.inra.fr*

Signal transduction pathways relay information of the extracellular environment to the cellular interior, most often resulting in changes in gene expression programmes.
Signalling pathways are highly conserved modules that are most commonly composed of a number of protein kinases that phosphorylate and thereby change the activity of their respective target proteins. Because the activation of a signaling pathway generally changes expression of a large number of genes, failure or modification of the activity of signalling pathways are often related to pathologic conditions in man, animals and plants.
However, careful modification of protein kinases can also have beneficial effects for the organisms as evidenced by the enhanced tolerance against environmental conditions or pathogen attack. Therefore protein kinases are ideal targets for genetic modification as well as biochemical agonists and antagonists. The usefulness and potential of targeted protein kinase approaches will be discussed with respect to the potential to improve plant performance.

PS07-002 NEW ROLES OF THE POLYAMINE CATABOLIC PATHWAY IN STRESS RESPONSES

Roubelakis-Angelakis, K. - Moschou, P.N. - Angelini, R. - Fincato, P. - Federico, R. - Tavladoraki, P.*
Department of Biology, University 'Roma Tre',Rome, Italy
**Corresponding author, e-mail: poproube@biology.uoc.gr*

The stress-induced Polyamine exodus into the apoplast reveals a novel signalling pathway leading either to tolerance-effector responses or to execution of cell death, depending on the level of apoplastic H_2O_2. Engineering the PA catabolic pathway leads to increased tolerance to biotic and sensitivity to abiotic stress.
The pathway is controlled partially by abscissic acid (ABA). ABA induces expression of *AtPAO3*, a peroxisomal *Arabidopsis* PAO and GUS activity post-treatment with ABA is localized to guard cells, implying a direct role of PAO-derived H_2O_2 in stomatal closure. Moreover, the identification and analysis of AtPAOs in *Arabidopsis* reveals that all four AtPAO1-4 backconvert Spm to Spd and additionally AtPAO2 and AtPAO3 backconvert Spd to Put.
Thus, *Arabidopsis* seems to lack PAOs involved in terminal catabolism of PAs in contrast to maize, in which the until now characterized PAOs produce 1,3-diaminopropane and 4-aminobutanal or *N*-(3-aminopropyl)-4-aminobutanal from Spd or Spm oxidation, respectively. Additionally, the organ/tissue specific expression of AtPAOs implies functional diversity inside the AtPAOs family. Surprisingly, H^1-NMR studies reveal that AtPAOs produce 3-aminopropanal from their substrates, which can be further converted to the osmoprotectant molecule β-alanine and pantothenate in a pairwise reaction.
All these results along with the involvement of AtPAOs in catabolism of thermospermine, a Spm isomer involved in vascular differentiation and stress adaptation, reveal novel roles of the PA catabolic pathway.

PS08: PHOTOSYNTHESIS & RESPIRATION
Session lead lectures

PS08-001 PHOTOSYNTHETIC LIMITATIONS IN RESPIRATORY MUTANT PLANTS

Flexas, J. - Florez-Sarasa, I.D. - Galle, A. - Medrano, H. - Ribas-Carbo, M.*
Grup de Recerca en Biologia de Plantes en Condicions Mediterranies, Departament de Biologia, Universitat de les Illes Balears
**Corresponding author, e-mail: jaume.flexas@uib.es*

Under stress conditions, plant growth and survival is often limited due to reductions of plant carbon balance, which is dependent on the balance between photosynthesis and respiration. Although both processes are intimately linked, photosynthesis responses to mitochondrial alterations remain relatively poorly evaluated. Here we review the current knowledge on photosynthesis responses of respiratory mutants. In general, any knockout or anti-sense reduction in a protein involved in respiration results in altered photosynthesis rates, although by different mechanisms. For instance, when protein impairments lead to potentially decreased availability of ATP, such as depleted mitochondrial Complex I or decreased fumarase, it results in impaired photosynthesis due to restricted CO2 diffusion due to reduced stomatal and mesophyll conductances to CO2. In contrast, impairments resulting in increased availability of NADH in mitochondria (e.g., cytochrome oxidase and/or alternative oxidase in mitochondrial electron transport chain) decrease photosynthesis by limiting chloroplast electron transport rate, presumably operated by a mechanism involving the malate valve. Similarly, impairments likely resulting in reduced Gly to Ser interconversion for photorespiration induce a metabolic limitation to photosynthesis. Surprisingly, impairing some proteins such as MDH or aconitase results in increased rather than decreased photosynthesis. The implications of these findings are discussed.

PS08-002 REACTIVE OXYGEN SPECIES AND RETROGRADE SIGNALLING FROM MITOCHONDRIA AND CHLOROPLASTS

*Møller, I. M.**
Department of Genetics and Biotechnology, Aarhus University
**Corresponding author, e-mail: ian.max.moller@agrsci.dk*

Reactive oxygen species (ROS) production increases in plants under stress. ROS can damage cellular components, but they can also act in signal transduction to help the cell counteract the oxidative damage in the stressed compartment. H_2O_2 might induce a general stress response, but it does not have the required specificity to selectively regulate nuclear genes required for dealing with localized stress, e.g., in chloroplasts or mitochondria. I will here argue that peptides deriving from proteolytic breakdown of oxidatively damaged proteins have the requisite specificity to act as secondary ROS messengers and regulate source-specific genes and in this way contribute to retrograde ROS signalling during oxidative stress. Likewise, unmodified peptides deriving from the breakdown of redundant proteins could help coordinate organellar and nuclear gene expression.

PS09: NATURAL VARIATION & ADAPTATION
Session lead lecture

PS09-001 GENETICS OF ADAPTATION AND SPECIATION IN MIMULUS

*Willis, J.**
Duke University
**Corresponding author, e-mail: jwillis@duke.edu*

How do new species arise? What is the genetic basis of adaptations and reproductive isolating barriers, and what does this tell us about how they evolved? Here we take advantage of the on-

going development of genomic tools for the closely related wildflower taxa in the *Mimulus guttatus* species complex to analyze the genetic basis of reproductive isolation between coastal perennial and inland annual ecogeographic races of *M. guttatus.* We first show that these races are locally adapted to their coastal or inland environments, and that this local adaptation of life history and physiology results in habitat-based reproductive isolation and temporal pre-zygotic isolation. We show that this isolation appears to result in differentiation at molecular markers throughout the genome. Replicated QTL mapping reveals that the adaptive morphological and life history differentiation between the races is primarily due to two loci of large effect. Finally, we use additional mapping analysis to investigate the chromosomal and molecular basis of these QTLs, and field experiments to elucidate their ecological function and contribution to reproductive isolation in the wild. Together these results provide insights into how local habitat adaptation can drive speciation in plants.

PS09-002 KEY INNOVATIONS IN THE EVOLUTION OF THE GENUS CAPSELLA (BRASSICACEAE)

*Neuffer, B.**
Botany Department, Biology, University of Osnabrück
**Corresponding author, e-mail: barbara.neuffer@biologie.uni-osnabrueck.de*

Mainly due to almost universal parallel evolution in nearly all morphological characters which have been used for classification, intergeneric relationships within the Brassicaceae are to a large extent still unresolved. Recent studies demonstrate that e.g. hybridisation and polyploidisation, changing of the mating system followed by consequences for the flower architecture and ecotypic differentiation are substantial driving forces or "key innovations" in the evolution of the Brassicaceae. Information about genomic sequences and gene function gained with the model plants *Arabidopsis* provides a new foundation of organismal biology. Moving out from this basis, the study of processes of phenotypic evolution occurring in present-day natural populations will substantially contribute towards understanding evolution in the field. In the Brassicaceae, the transition from a self-incompatible (SI) to a self-compatible (SC) mating system has happened often and independently and often goes hand-in-hand with colonising success. *Capsella* comprises diploid taxa (C. *grandiflora*, SI, endemic to Western Balkan peninsula and Northern Italy and C. *rubella*, SC, occurring in Mediterranean climates worldwide) and tetraploid taxa (C. *bursa-pastoris*, SC, one of the most common species worldwide).
The overall phenotypic variation revealed macrogeographic patterns superimposed by patchiness depending partly on microhabitat conditions (e.g., field versus trampling habitats). In 2009 and 2010 a reciprocal transplantation experiment with provenances from various regions of Eurasia has been performed at six stations.

PS10: SIGNALLING AND GENE EXPRESSION
Session lead lectures

PS10-001 MOLECULAR ASPECTS OF THE ETHYLENE RESPONSE PLASTICITY IN ARABIDOPSIS

*Alonso, J.**
North Carolina State University
**Corresponding author, e-mail: jmalonso@unity.ncsu.edu*

In order to survive, sessile organisms need to tune their developmental programs to the ever-changing environment where they get to spend their entire lives. Central to this integration process are plant hormones that act as executers of both internally and externally generated signals. Since the number of possible signal combinations is much larger than the number of hormones, plant utilize a combinatorial approach where interactions between hormones and spatiotemporal signals are utilized to achieve this wide diversity of plant responses. Our recent work indicates that regulation of auxin biosynthesis plays a critical role in modulating several ethylene responses in Arabidopsis. Due to our very limited knowledge of auxin biosynthesis and its regulation, and to better understand how this essential hormone is produced in plants, we are taking a combination of genetic, cellular, and molecular approaches. Specifically, we are currently focusing on the following three key questions: (1) which genes are responsible for auxin production in plants, (2) what the relationship is between the YUC and the TAA1 routes of auxin production (the two most characterized auxin biosynthetic pathways in plants), and (3) what the role of local auxin production is in the generation of auxin gradients required for the essential developmental processes such as embryogenesis or the maintenance of root stem cells. Our recent progress in addressing these critical questions of auxin biology will be presented.

PS10-002 REGULATION OF ABA SIGNALLING THROUGH THE PYR/PYL/RCAR ABA RECEPTORS, PP2CS AND SNRK2S

Rodriguez Egea, P. - Gonzalez-Guzman, M. - Rodríguez, L. - Santiago, J. - Antoni, R.*
CSIC.IBMCP
**Corresponding author, e-mail: prodrig1@upvnet.upv.es*

Control of ABA signalling by PYR/PYL/RCAR ABA-receptors involves direct inhibition of clade A PP2Cs, which are key negative regulators of the pathway. On the other hand, the ABA-activated SnRK2s, i.e. SnRK2.2, 2.3 and 2.6/OST1, are well known positive regulators of ABA signalling Indeed, a triple mutant deficient in the three ABA-activated SnRK2s, displays a dramatic ABA-insensitive phenotype and global changes in the induction or repression of ABA-responsive genes. We have performed a comprehensive analysis of the interactions among clade A PP2Cs and ABA-activated SnRK2s as well as the corresponding biochemical characterization, and we have examined the effect of some ABA-receptors on this interaction. As a result, we have found that dephosphorylation of SnRK2s by clade A PP2Cs can be blocked by ABA-receptors in an ABA-dependent manner. Finally, although genetic redundancy has been observed for PYR/PYL/RCAR proteins, generation of different combinations of loss-of-function mutations *(pyr1, pyl1, pyl2, pyl3, pyl4, pyl5, pyl6* and *pyl8)* reveals specific roles for some ABA-receptors depending on the ABA-response analysed, organ and developmental stage.

PS11: CELL BIOLOGY
Session lead lectures

PS11-001 WHY PLANT CELLS NEED MUSCLES: THE ACTIN-AUXIN OSCILLATOR

*Nick, P.**
Karlsruhe Institute of Technology
**Corresponding author, e-mail: peter.nick@bio.uni-karlsruhe.de*

Actin filaments are essential for tip growth and cytoplasmic streaming, but they are also found in cells that are not growing in a polar fashion and do not exhibit prominent streaming. This actin function seems to be related to the directional transport of the plant hormone auxin. This transport depends on transcellular gradients of auxin-efflux carriers that continuously cycle between plasma membrane and intracellular compartments depending on actin filaments. However, the role of actin for the polarity of auxin transport has been disputed. To get insight into this question, actin bundling was induced by overexpression of the actin-binding domain of talin in tobacco BY-2 cells and in rice plants. This bundling can be reverted by addition of auxins, which allows to address the role of actin organization on the flux

of auxin. In both systems, the reversion of a normal actin configuration can be restored by addition of exogenous auxins and this fully restores the respective auxin-dependent functions. These findings lead to a model of a self-referring regulatory circuit between polar auxin transport and actin organization. To further dissect the actin-auxin oscillator, we used photoactivated release of caged auxin in tobacco cells to demonstrate that auxin gradients can be manipulated at a subcellular level. Our findings support the model of an actin-auxin oscillator that might represent a central element of a morphogenetic Turing-system.

PS11-002 THE ROLE OF THE SECRETORY PATHWAY IN THE REGULATION OF CELL WALL METABOLISM IN PLANTS.

*Vernhettes, S. - Crowell, E. - Bischoff, V. - Desprez, T. - Gasciolli, V. - Gonneau, M. - Höfte, H.**
IJPB, INRA, Versailles
**Corresponding author, e-mail: herman.hofte@versailles.inra.fr*

Cellulose microfibrils are remarkable structures consisting of crystalline arrays of a large number of parallel ß1,4-linked glucan chains. This structure is thermodynamically unstable and its synthesis requires a specialized cellular machinery. The cellulose synthase is the largest known membrane-bound complex, it forms hexameric structures of 25nm diameter in the plasma membrane and most likely has a cytosolic domain that is twice this diameter. Genetic and co-immunoprecipitation studies show that each complex contains 3 types of cellulose synthase catalytic subunits (CESA). In this presentation, I'll first discuss our recent findings on the stoichiometry of the CESAs in the complex and the presence of other components. Next I'll discuss how cellulose synthesis is regulated through the selective insertion and retrieval of the complexes from the membrane and the role of the cortical microtubules and specialized intracellular compartments in this process.

PS12: EPIGENETICS
Session lead lectures

PS12-001 MECHANISMS OF SPECIATION BY POLYPLOIDY

Erilova, A. - Brownfield, L. - Exner, V. - Köhler, C. - ETH Zurich*
**Corresponding author, e-mail: koehlerc@ethz.ch*

Polyploidization is a widespread phenomenon among plants and is considered a major speciation mechanism. Polyploid plants have a high degree of immediate post-zygotic reproductive isolation from their progenitors, as backcrossing to either parent will produce mainly nonviable progeny. This reproductive barrier is called triploid block and it is caused by malfunction of the endosperm. Nevertheless, the main route to polyploid formation is via unreduced gametes and unstable triploid progeny, suggesting that there are ways to overcome the triploid block. Until recently, the mechanistic basis for unreduced gamete formation and the triploid block were completely unknown. Recent findings from our group have revealed a genetic pathway leading to unreduced gamete formation as well as the underlying genetic basis for the triploid block in Arabidopsis. These novel findings provide the basis for a genetic understanding of polyploid formation and subsequent speciation in plants.

PS12-002 TESTING THE ROLE OF DNA CYTOSINE METHYLATION ON MEIOTIC RECOMBINATION FREQUENCIES

*Henderson, I.**
University of Cambridge
**Corresponding author, e-mail: irh25@cam.ac.uk*

Meiosis is the reductive cell division that generates haploid gametes necessary for sexual reproduction. During meiosis homologous chromosomes pair and undergo exchange of genetic material, or recombination. Recombination, or crossover (CO), frequency is known to vary dramatically between chromosomes, individuals and species. Despite the fundamental importance of differences in recombination rate for breeding and evolution, the causes of this variation remain largely unknown. Analysis of marker segregation following controlled crosses is a robust method to identify CO locations. Advances in sequencing technology have greatly increased the number of markers that it is feasible to score and therefore have also increased our CO mapping ability. We are combining the powerful genetics of *A.thaliana* with high-throughput sequencing to describe genome-wide patterns of recombination frequency. Our recombination map will be correlated with the abundant genomic data available in this species to better understand CO hotspot distributions. As a first step to dissecting this control we are generating maps in mutant plants lacking DNA cytosine methylation, a key epigenetic silencing mark, to test its role in determining crossover patterns. An increased understanding of recombination control will find important applications in plant breeding and crop development.

PS13: METABOLISM
Session lead lectures

PS13-001 METABOLOMICS FROM FUNDAMENTAL UNDERSTANDING TO MARKER-ASSISTED BREEDING

*Fernie, A.**
MPI of Molecular Plant Physiology
**Corresponding author, e-mail: fernie@mpimp-golm.mpg.de*

Metabolic profiling has recently gained great exposure in all fields of biological science as a novel method to find biomarkers of cellular circumstance it is also widely being adopted in functional genomics approaches aimed at gene identification or systems descriptions of conditional responses. Here I will discuss its application to the understanding of crop chemical composition. To evaluate components of fruit metabolic composition, we have previously metabolically phenotyped tomato introgression lines (ILs) containing segmental substitutions of wild species chromosome in the genetic background of a cultivated variety. These studies facilitated the identification of a vast number of quantitative trait loci (QTL) for a large number of primary metabolites. To place these results in a relevant content we carried out morphological phenotyping in parallel. Cartographic network analyses revealed that fruit metabolite composition was broadly conserved within compound classes but perhaps more significantly negatively correlated with harvest index. These data prompted us to expand our work in two directions. Firstly, to analyse the metabolite content in other tissues of the ILs in order to gain a better physiological understanding of this finding and secondly to adopt a range of approaches including candidate gene mapping and reverse genetic strategies and transcriptomics in order to achieve higher genetic resolution. The current status of this work and a perspective of the possibilities of metabolomics assisted selection will be presented

PS13-002 ENZYMES AND ENZYME-LIKE PROTEINS THAT FUNCTION IN METABOLISM, METABOLIC REGULATION AND CELLULAR SIGNALING

*Zeeman, C.S.**
Institute of Plant Sciences, ETH Zürich
**Corresponding author, e-mail: szeeman@ethz.ch*

Plant genomes frequently contain multiple genes coding for a particular enzyme. The duplicated genes may be differentially regulated or encode enzymes isoforms with different properties and/or sub-cellular locations. Alternatively, the duplicated genes may become specialised and adopt new, non-enzymatic functions.

The nine-member β-amylase (BAM) gene family in Arabidopsis is a good example of this. β-Amylase is known as a key enzyme involved in starch degradation. In Arabidopsis, starch is accumulated as a primary product of photosynthesis in leaves during the day, serving as a transitory store of carbohydrate for use during the night. β-Amylases liberate maltose molecules from the ends of the glucan chains that comprise starch. Mutations in Arabidopsis that cause a deficiency in chloroplastic β-amylase (BAM1 and BAM3) result in a block in starch breakdown and an accumulation of leaf starch. However, the chloroplast contains another type of β-amylase-like protein which appears to be non-catalytic (e.g. BAM4). Mutational studies show that these proteins are also important in starch breakdown, although the mechanism by which they act is as-yet unclear. Surprisingly, two Arabidopsis β-amylase-like proteins, BAM7 and BAM8, are nuclear localised and share an amino-terminal DNA-binding domain with a family of plant-specific transcriptional regulators involved in plant steroid hormone signalling. Deregulation of BAM7 and BAM8 expression results in altered plant growth and development, to altered brassinosteroid sensitivity, but not to altered starch metabolism. We have identified the DNA motif to which these two proteins bind and obtained in-vivo evidence that they are transcriptional activators. Homologous genes have been identified in other plants including gymnosperms, and angiosperms (both monocot and dicot species), implying that their functional specialisation occurred early in higher plant evolution. Our hypothesis is that the duplication of the genes encoding β-amylases has given rise to metabolic sensors that control metabolism and provide a regulatory link between carbon availability and growth control.

PS14: PLANTS & GLOBAL CHANGE
Session lead lectures

PS14-001 GLOBAL CHANGE AND THE EFFECTS OF SOLAR UV RADIATION ON TERRESTRIAL ECOSYSTEMS

*Ballaré, C.**
Universidad de Buenos Aires and CONICET
**Corresponding author, e-mail: ballare@ifeva.edu.ar*

UV radiation (280-400 nm) is a minor component of the solar spectrum reaching the ground surface; yet, it has important effects on organisms and biogeochemical cycles. Many research efforts during the past two decades have thoroughly characterized the effects of the UV-B (280-315 nm) component. In this talk, I will summarize the lessons from this previous work, and highlight some of the important knowledge gaps in connection with the effects of climate change. I will address the following points. A) The effects of UV-B radiation on the growth (biomass accumulation) of terrestrial plants are relatively small. B) On the other hand, UV radiation affects plant secondary chemistry and the activities of canopy arthropods and phyllosphere microorganisms. Therefore, trophic interactions in terrestrial ecosystems are likely to be significantly affected by future variations in UV irradiance. C) Changes in UV resulting from climate change (e.g., variations in cloud cover) may have more important consequences on terrestrial ecosystems than those derived from ozone depletion. This is because the resulting variations in UV may affect a greater range of ecosystems, and will not be restricted solely to the UV-B component. D) Several processes that are not particularly sensitive to UV-B can be strongly affected by UV-A radiation (315-400 nm). One example is the physical degradation of plant litter. Recent work suggests that increased photodegradation (in response to reduced cloudiness or reduced canopy cover) may have important direct and indirect effects on carbon sequestration in terrestrial ecosystems.

PS14-002 BEYOND 2050: CAN WE EXPECT CO2 SATURATION OF LEAVES AND ECOSYSTEMS?

*Ellsworth, D.**
University of Western Sydney
**Corresponding author, e-mail: D.Ellsworth@uws.edu.au*

As atmospheric CO_2 concentration is rising, one important suite of responses of ecosystems to the atmosphere are those involving the carbon cycle, mediated by photosynthesis. However, leaf photosynthesis saturates at $[CO_2]_{air}$ of 500-700 ppm, and hence further increases in $[CO_2]$ may suggest no additional impact on ecosystem C cycles beyond these concentrations. Others have suggested that ecosystems are already saturated at current atmospheric $[CO_2]$ levels, in part due to nutrient limitations and severe water stress. Here, I will consider the question: are further increases in ecosystem CO_2 flux or productivity in native ecosystems possible beyond the photosynthetic CO_2 saturation threshold? And which systems are closest to this threshold? Results from a series of elevated CO_2 experiments, ecosystem CO_2 flux measurements, and from natural phenomena such as the European drought of 2003 are used to provide evidence of plant and ecosystem CO_2 saturation. Whilst the short-term mechanism of photosynthetic response would suggest a large CO_2 stimulation effect under drought, the longer-term response is very different. The findings have relevance to the Mediterranean region as well as to the significant fraction of global ecosystems that are nutrient- and water-limited.

PS17: PLANT-MICROBE INTERACTIONS
Session lead lectures

PS17-001 THE TOMATO – FUSARIUM OXYSPORUM PATHOSYSTEM

Rep, M. - Houterman, P. - Gawehns, F. - Ma, L. - de Sain, M. - Lukasik, E. - van der Does, C. - Cornelissen, B. - Takken, F.*
University of Amsterdam
**Corresponding author, e-mail: M.Rep@uva.nl*

The tomato xylem-colonizing fungus *Fusarium oxysporum f.sp. lycopersici* (Fol) secretes small proteins into xylem sap of its host. Three of these 'effectors' trigger effector-mediated immunity: Avr1, Avr2 and Avr3 (or their activities) are recognized by the resistance proteins I, I-2 and I-3, respectively. Interestingly, Avr1 suppresses *I-2* and *I-3*-mediated resistance. Several Fol effectors were shown through gene knock-out to contribute to virulence towards susceptible plants. The genes for the effectors in Fol that we identified reside on a 'pathogenicity chromosome'. This chromosome can be transferred between genetically isolated strains of the asexual fungus, conferring host-specific pathogenicity to the recipient.
We aim to uncover the molecular mechanisms by which effectors of Fol trigger susceptibility (suppression of resistance) and immunity (activation of R proteins), beginning with localization of effectors in plant cells and identification of host proteins with which they interact.

PS17-002 PLANT TARGETS OF BACTERIAL TYPE III EFFECTOR PROTEINS

*Bonas, U.**
Department of Plant Genetics, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany
**Corresponding author, e-mail: bonas@genetik.uni-halle.de*

We study the interaction between pepper and tomato and the Gram-negative plant pathogenic bacterium *Xanthomonas campestris pv. vesicatoria (Xcv)*, which causes bacterial spot disease on its host plants. Successful interactions of Xcv with the plant depend on a functional type III secretion (T3S) system, a molecular syringe, which injects more than 20 effector proteins (termed Avr or Xop = *Xanthomonas* outer protein) into the plant cell cytoplasm. Among the Xops we find suppressors of the plant innate immunity, putative enzymes and transcription factors. One of the

best-studied type III effector proteins in our laboratory is the 122 kDa-protein AvrBs3, which acts as transcription factor and induces phenotypic changes in both susceptible and resistant plants. *Xcv* strains expressing AvrBs3 induce the hypersensitive reaction (HR) in pepper plants carrying the resistance gene *Bs3*. The HR is a rapid local programmed cell death that halts bacterial multiplication. In pepper plants lacking the *Bs3* gene and other solanaceous plants AvrBs3 induces a hypertrophy of mesophyll cells. AvrBs3 activity depends on a central region of 17.5 tandem 34-aa repeats, its localization to the plant cell nucleus and the presence of an acidic activation domain. One of the direct targets of AvrBs3 is *UPA20* (*UPA*, upregulated by AvrBs3) which encodes a transcription factor and is a key regulator of hypertrophy. Recent insights into the mechanism of AvrBs3 action will be discussed.

PS18: WATER & MINERALS
Session lead lectures

PS18-001 PHYSIOLOGICAL AND GENETIC DISSECTION OF AQUAPORIN FUNCTIONS IN ROOTS AND LEAVES

Maurel, C. - Postaire, O. - Tournaire-Roux, C - Boursiac, Y. - Sutka, M. - Li, G. - Prado, K. - Santoni, V. - Wudick, M. - Doan-Trung, L.*
Biochemistry and Plant Molecular Physiology, Integrative Biology Institute for Plants, CNRS/INRA UMR5004, 2 place Viala, F-34060 Montpellier, France
Corresponding author: maurel@supagro.inra.fr

Uptake of soil water by roots and its delivery from xylem vessels to inner leaf tissues are crucial for maintaining the plant water status. Knock-out mutants for plasma membrane aquaporins (PIPs) were used to dissect the osmotic and hydrostatic modes of water transport in the *Arabidopsis* root. The variability of root hydraulic architecture and of aquaporin expression in a set of 13 natural accessions of *Arabidopsis* provided complementary insights into root water uptake and its regulation by salt stress. The latter process involves a Reactive Oxygen Species (ROS)-dependent signalling path that triggers an internalisation of PIPs. The mechanisms and routes of ROS-dependent trafficking of PIPs were dissected in detail using a combination of biochemical and cell biological approaches. Pharmacological and reverse genetic approaches also showed that PIPs contribute to water transport in the inner tissues of leaves and can account for light-dependent changes in their hydraulic conductivity. The contribution of specific PIP isoforms to light-dependent water transport in the veins and/or the mesophyll is being elucidated.

PS18-002 PIDENTIFICATION OF ARABIDOPSIS GENES INVOLVED IN NUTRIENT ACQUISITION OR HOMEOSTASIS

*Giehl[1] - Takahashi, H.[2] - Von Wiren, N.[3]**
[1]Molecular Plant Nutrition, University of Hohenheim
[2]RIKEN PLant Science Center Yokohama
[3]Leibniz-Instuitute for Plant Genetics & Crop Plant Research
**Corresponding author, e-mail: vonwiren@ipk-gatersleben.de*

Plant growth and propagation depend on the acquisition of mineral nutrients by the root, their root-to-shoot translocation and their re-translocation to sink tissues when plants undergo senescence. For most nutrients, molecular mechanisms involved in their acquisition from soils have been described. However, much less is known about the regulatory pathways underlying the uptake and translocation of nutrients in plants. The possibility to perform large-scale elemental analysis and data mining offer the opportunity to screen large populations of mutant lines in the search for genes affecting a plant's nutriome. In order to search for transcription factors involved in the regulation of nutrient accumulation in plants, we have screened transposon-tagged lines with altered expression of transcription factors for their nutrient profiles. For that purpose, 313 Ds-transposon-tagged lines were grown in nutrient solution and leaves and roots were analyzed for 13 mineral elements by ICP-MS and ICP-OES. For each mineral element approximately 1-4% of the lines showed nutrient concentrations in roots and/or shoots that differed from the wildtype. Among these lines, line no. 117 accumulated significantly more K and P in the shoot. Further phenotypical analysis of this line and of an independent mutant allele as well as expression analysis support a role of the transcription factor 117 in root responses to low P availability.

SELECTED ABSTRACTS FOR ORAL PRESENTATIONS

S01-001: REGULATION OF THE MECHANO-SENSITIVE GENE *PTAZFP2*

Gourcilleau, D. - Martin, L. - Leblanc-Fournier, N. - Julien, J.L.*
Université Blaise Pascal
**Corresponding author e-mail: delphine.gourcilleau@univ-bpclermont.fr*

In their natural environment, plants are continuously exposed to wind loads, characterized by a high variability of frequencies and intensities. In response to mechanical loadings, plants exhibit generally a decrease in longitudinal growth, an increase in diameter growth and in rooting. This syndrome of growth responses has been called thigmomorphogenesis. In order to grow in continuously changing windy conditions, plants may have to develop acclimation processes. However, molecular mechanisms involved in plant acclimation to recurring and successive mechanical loadings are not well characterized.
Recently, through the analysis of the short-time effects of quantified stem bending on young poplars, we demonstrated the rapid induction of *PtaZFP2* expression, a gene encoding a putative Cys2/His2 zinc finger transcription factor. The *PtaZFP2* transcripts accumulate 10 min after a single bending and only in strained tissues. The relative abundance of *PtaZFP2* transcripts was linearly correlated with the amount of applied mechanical solicitation. By comparing the effect of successive bending on this early mechano-sensitive gene, our results indicate that *PtaZFP2* mRNA accumulate to a lesser extent after two bendings than after a single one. These results clearly show a partial desensitization of plants to recurrent successive bendings.
Our objectives are now to identify molecular actors upstream PtaZFP2 in order to understand its regulation in the mechanosensing pathway. Furthermore, to characterize the kinetics of accommodation processes and the tissues involved in mechanosensing, the effect of single and repeated bending are studied, at the protein level, on PtaZFP2 accumulation and localization.

S01-002: ARABIDOPSIS RESPONSE TO HIGH TEMPERATURE IS MEDIATED BY LIGHT INTENSITY

François, V. - Pantin, F. - Dauzat, M. - Rolland, G. - Bédié, A. - Muller, B. - Vile, D.*
INRA-SupAgro – UMR 759 Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux (LEPSE), Montpellier, France
**Corresponding author e-mail: fvasseur95@hotmail.fr*

Environmental constraints, such as temperature, water and light, often act simultaneously in the field limiting plant growth and development. Although the effects of separate stresses on plants have been described at the molecular level, little is known about the mechanisms involved in their integrated response to interacting environmental factors, particularly to long-term exposure to moderate stresses. Moreover, only a few studies have shown relationships between gene expression, physiological processes and growth in different environmental conditions.
Arabidopsis thaliana ecotypes and mutants were cultivated in controlled conditions to study the influence of light and water availability on plant responses at high temperature. Precisely, we investigated the links between rosette growth, leaf morphology, photosynthesis, and molecular markers of carbon status. Furthermore, the analysis of particular biological functions was complemented using specific mutants. Plants showed a multilevel response to high temperature. Growth was reduced by high temperature while specific leaf area increased. Furthermore, drastic hyponastic movements of leaves occurred, suggesting that responses could be modulated by light intensity. While low light (70 µmol m^{-2} s^{-1}) amplified the deleterious effects of high temperature, high light (330 µmol m^{-2} s^{-1}) restored the growth achieved at control temperature and abolished hyponastic movements. Taken together, results showed that responses to high temperature depend on light availability. Physiological and gene expression data, together with interacting effects of water deficit, confirm the major role of energy balance in plant response to high temperature.

S01-003: AQUATIC ADVENTITIOUS ROOTS OF THE WETLAND PLANT HALORAGIS BROWNII CAN PHOTOSYNTHESIZE: IMPLICATIONS FOR FLOODING TOLERANCE

Rich, S. - Ludwig, M. - Pedersen, O. - Colmer, T.*
The University of Western Australia
**Corresponding author e-mail: sarah.rich@grs.uwa.edu*

Whan flooded, many plants produce adventitious aquatic roots from submerged stems. These roots supplement, or replace, the primary root system that often dies during soil flooding. Adventitious aquatic roots can form chloroplasts and,therefore, potentially contribute to plant survivial during flooding through photosynthate and O_2 production. The wetland plant *Haloragis brownii* (Hook.f.) Schindl.) grows an extensive stem-borne aquatic roots system (up to 29 % of total plant dry mass) during flooding. Chlorophyll is detected in these roots within days of emergence, even when plants are grown at low light levels. The aquatic roots have a complete photosynthetic pathway, producing O_2 and fixing carbon at rates similar to submerged stems, but four-fold lower than leaves.
Microelectrode and $^{14}CO_2$-uptake experiments showed that these roots are able to produce significant amounts of carbon and O_2, lowering inputs needed from the shoot for growth and development. The contribution of *H. brownii* aquatic adventitious roots to their own metabolic needs is beneficial to whole plant survival during flooding as it mediates the negative effects of energetic stress and explains the capacity for prolific growth of these new roots, which accompanies a flooding event.

S01-004: NEW INSIGHTS INTO AN OLD STORY: ZMASR1 EXPRESSIONENHANCED DROUGHT STRESS TOLERANCE IN MAIZE

Virlouvet, L.[1] - Jacquemot, M.P.[1] - Corti, H.[1] - Gilard, F.[2] - Bouton, S.[1] - Remoue, C.[2] - Valot, B.[1] - Riviere, N.[3] - Tcherkez, G.[4] - Trouverie, J.[1] - Damerval, C.[2] - Gerentes, D.[3] - Falque, M.[1] - Rogowsky, P.[1] - Noctor, G.[4] - Zivy, M.[2] - Coursol, S.[1]*
[1] Institut national de la recherche agronomique (INRA), France
[2]Centre National de la Recherche Scientifique (CNRS), France
[3]Biogemma Auvergne, Ferrand Cedex, France
[4]Univ Paris-Sud, Institut de Biotechnologie des Plantes, Orsay, France
**Corresponding author: rennesson@moulon.inra.fr*

Maize is particularly sensitive to drought stress at reproductive stages with a strong impairment of gynoecium development. As such, identification of factors that confer drought stress tolerance would pave the way for increasing agricultural productivity. Here, we assessed the role of the candidate gene *Zea mays abscisic acid-, stress- and ripening-induced 1 (ZmASR1)* for drought stress tolerance in maize. Quantitative RT-PCR studies with

gene-specific primers for six of the nine *ZmASRs* identified in the maize genome showed that *ZmASR1* was the major expressed isoform in leaves and kernels. We found that up-regulation by drought in leaves was a common feature to all *ZmASRs*, except ZmASR3, in contrast to kernels where only *ZmASR2* transcript levels increased. Transgenic maize plants over-expressing *ZmASR1 (ZmASR1-OE)* displayed increased shoot biomass yield under fully irrigated condition and increased ear leaf area, kernel yield weight and kernel number under both fully irrigated and water-limited conditions in the field. Comparative transcriptomic and proteomic analyses of *ZmASR1-OE* and wild-type sister leaves led to the conclusion that *ZmASR1-OE* triggers small-scale changes on the transcriptional and protein levels that concern mainly genes involved in the raffinose family oligosaccharides or branched-chain amino acid metabolic pathways.
Metabolomic analysis confirmed the impact of *ZmASR1-OE* on these pathways and revealed that *ZmASR1-OE* decreased the levels of metabolites displaying a negative correlation to biomass in Arabidopsis. Collectively, these data demonstrate the feasibility of engineering drought stress tolerance and yield new insights into the function of the *ZmASR1* protein.

S02-001: DISSECTING THE MOLECULAR REGULATION OF CORK CAMBIUM

Miguel, A.[1] - Milhinhos, A.[1] - Pinto Ricardo, C.[2] - Jones, B.[3] - Miguel, C.[1]*
[1]IBET/ITQB-UNL
[2]ITQB-UNL
[3]University of Sydney-Australia/UPSC-Sweden
**Corresponding author e-mail: andreiamiguel@itqb.unl.pt*

In plant stems, the cork cambium usually initiates in the subepidermis. Cork cambium cells divide periclinally, giving rise to phelloderm cells on the inside and to phellem (cork) cells on the outside. Together, the cork cambium, cork and phelloderm form the periderm which protects the live tissues from damaging factors. Cork extracted from the cork oak is also a renewable material of high economic value due to its unique properties. Our major goal is to identify molecular regulators of this lateral meristem and we are presently focusing on the role of a few transcription factors, namely SHORT-ROOT *(SHR)* from the GRAS family. This gene has been extensively studied in the Arabidopsis root where it is described as a key component in the developmental pathway regulating the specification of the root stem cell niche and radial patterning (1, 2, 3). Through an integrated approach combining cell biology and gene expression characterization tools we aim to provide evidence of the putative involvement of *SHR* in the regulation of cork cambium. *SHR* sequences have been cloned from poplar and cork oak transcriptome and the effects of down-regulation and ectopic expression of *SHR* in transgenic poplar lines are being investigated. A comparative analysis of *SHR* expression patterns in poplar and cork oak will be conducted in order to validate the use of poplar as a model for these studies. References: 1. Benfey PN, et al. (1993) Development 119: 57-70; 2. Helariutta Y, et al. (2000) Cell 101: 555-567; 3. Nakajima K, et al. (2001) Nature 413: 307-311.
Acknowledgments: Financial support provided by FCT through project PTDC/AGRGPL/ 098369/2008 and grant SFRH/ BD/44474/2008.

S02-002: INSIGHTS IN THE DISCOVERY OF NOVEL REGULATORS THAT CONTROL PLANT ARCHITECTURE

Alos Ros, E. - Wigge, P. - Kumar, V. - Lucyshyn, D.*
John Innes Cente
**Corresponding author e-mail: enriqueta.alos@bbsrc.ac.uk*

In *Arabidopsis*, a key role in maintaining indeterminate vegetative meristems is carried out by a molecule closely related to FLOWERING LOCUS T (FT), TERMINAL FLOWER 1 (TFL1), that acts in combination ith FD to repress expression of flowering genes. While mutants that lack *FT* are very late flowering, *tfl1* mutations have the opposite phenotype, causing the plants to flower early and terminate their growth with a profusion of flowers, resembling gain-of-function *35S::FT* plants (1, 2, 3). To address both plant architecture and the limiting role of FT in plant development, we have performed a genetic screen for flowering time and architecture mutants in the tfl1- *1* background. 3400 M2 families have been screened and 20 mutants selected as modifiers of the *tfl1-1* phenotype. Amongst the isolated mutants, mutations in already described genes have been identified such as *APETALA 1* and *LEAFY.* Interestingly, novel modifiers of the *tfl1-1* phenotype have been obtained and mapped. The functional characterization and the roles of these genes in plant architecture are discussed in the present work.
1. Bradley, D., Ratcliffe, O., Vincent, C., Carpenter, R., and Coen, E. (1997). Science 275, 80-83.
2. Kardailsky, I., Shukla, V.K., Ahn, J.H., Dagenais, N., Christensen, S.K., Nguyen, J.T., Chory, J., Harrison, M.J., and Weigel, D. (1999). Science 286, 1962-1965.
3. Prusinkiewicz, P., Erasmus, Y., Lane, B., Harder, L.D., and Coen, E. (2007). Science 316, 1452-1456.

S02-003: CLOCK-MEDIATED CONTROL OF GIBBERELLIN RESPONSES IN ARABIDOPSIS

Arana, M. V. [1] - Marín, Nora A.[1] - Maloof, J. N.[2] - Alabadi, D.[1] - Blázquez, M.A.*
[1]IBMCP - Universidad Politécnica de Valencia. Valencia. España
[2]Section of Plant Biology, College of Biological Sciences, University of California, Davis
**Corresponding author e-mail: arana@agro.uba.ar*

The circadian clock acts as central coordinator of plant activity, and it regulates key traits for plant fitness such as seed germination, gas exchange, flowering and growth.
Particularly, growth of germinating seedlings is restricted to certain times of the day, showing a maximum rate near dawn. This pattern can be explained by the lightmediated degradation of PIF proteins during the day combined with the clock-mediated repression of PIF transcript accumulation early in the night. This combined action yields high levels of PIF proteins at the end of the night, which are responsible for growth. In addition to this mechanism, growth is controlled by several plant hormones such as gibberellins (GA), auxins and brassinosteroids, and GA action has been shown to involve the relief of the DELLA-mediated inhibition of PIF transcriptional activity. Although GA signaling has been thoroughly studied in constant environments its contribution to plant growth under predictable daily environmental changes such as day/night cycles is still unknown. Here we show that the circadian clock gates hypocotyl GA sensitivity, resulting in the promotion of GA signaling late in the night, the moment when maximum growth occurs. This effect involves the transcriptional control of the GA receptors and influences both the daily patterns of DELLA accumulation and the rate of hypocotyl elongation. Taken together, our results show that anticipation of biological events to external day and night cycles requires a functional GA signaling, and that the GA pathway is a relevant clock output for the control early plant developmental traits, such as daily growth rhythms.

S02-004: DEVELOPMENT AND VALIDATION OF A SENSITIVE AND RAPID METHOD FOR THE DETERMINATION OF UNLABELED AND DEUTERIUM LABELED PLANT HORMONES IN DIFFERENT PLANT TISSUES USING UPLC-MS/MS

*Müller, M. * - Munné-Bosch, S.*
University of Barcelona
**Corresponding author e-mail: maren.muller@ub.edu*

Plant hormones play a pivotal role in several physiological processes during a plant's life cycle, from germination to senes-

cence, and the determination of endogenous concentrations of phytohormones is essential to elucidate the role of a particular hormone in any physiological process. Availability of a sensitive and rapid method to quantify multiple classes of plant hormones simultaneously will greatly facilitate the investigation of hormone-induced signalling networks in controlling specific developmental pathways and physiological responses. Due to the presence of plant hormones at very low concentrations in plant tissues (10-9 M to 10-6 M) and their different chemistries, the development of a high-throughput and comprehensive method for the determination of phytohormones is challenging. The present work reports a rapid, specific and sensitive method using UPLC-MS/MS for the quantitative and simultaneous analysis of the major phytohormones found in plant tissues, including auxins, cytokinins, gibberellins, abscisic acid, 1-amino-cyclopropane-1-carboxyic acid (the ethylene precursor), jasmonic acid and salicylic acid. Sample preparation, extraction procedures and UPLC-MS/MS conditions were optimized for the determination of all plant hormones in a single run. This new method is applicable to the analysis of dynamic changes in endogenous concentrations of phytohormones in different plant tissues to study plant developmental processes or plant responses to biotic and abiotic stresses. An example is shown in which simultaneous analyses of phytohormones is performed in leaves of plants exposed to salt stress, both in the model plant, Arabidopsis thaliana and in an aromatic plant, Salvia officinalis.

S03-001: OXYLIPIN-INDUCED TYROSINE PHOSPHORYLATION OF PLANT PROTEINS

Yakusheva, O. - Karimova, F.*
Kazan Institute of Biochemistry and Biophysics, RAS
**Corresponding author: vov1985@mail.ru*

Oxylipins are products of oxygenated polyunsaturated fatty acids, biologically active signaling molecules. Nowadays molecular mechanisms of oxylipin effects are the object of close attention. In plant cells the signaling pathway of jasmonates is the most studied. Earlier the researchers of out Institute showed that one of the main products of legume lipoxygenase metabolism is (9Z)-12-hydroxy-9-dodecenoic acid (HDA). It was shown that HDA is a growth stimulator causing an increase in soybean callus biomass up to 400%. Previously we showed HDA-induced Ca^{2+}- and cAMP-dependent plant protein phosphorylation for 2h of exposure. Protein tyrosine phosphorylation is known to be critical for cell proliferation and differentiation. In this context plant protein tyrosine phosphorylation is of great interest. We investigated dynamics of HDA effect *in vivo* on the tyrosine phosphorylation level (TPL) of leaf soluble proteins in pea plants. Our results indicate that TPL quickly changes in control and HDA-treated plants during different time periods. To detect factors critical for HDA effect on TPL plants grown on the nutrient solution without Ca^{2+} were used. We showed that Ca^{2+}-deficiency in the growth medium caused a decrease in TPL of all polypeptides in comparison with control plants grown on the optimal solution. These data suggest that there is Ca^{2+}- dependence of protein phosphorylation/dephosphorylation enzymes activity. The HDA effect on TPL was also Ca^{2+}-dependent. As known in vertebrate cells, protein tyrosine phosphatase (PTP) activity is 10-100 times higher than protein tyrosine kinase activity. Using PTP inhibitor phenylarsinoxide we showed contribution of PTP activity to Ca^{2+}- dependence of HDA-induced TPL in pea plants.

S03-002: ANALYSIS OF THE ARABIDOPSIS GLYCOPROTEOME

Van Der Krol, S.[1] - Song, W.[1] - Mentink, R.[2] - Henquet, M.[3] - Cordewener, J.[3] - Bosch, D.[3] - America, T.[3]*
[1]Lab. of Plant Physiology, Wageningen University
[2]Hubrecht Inst. Utrecht Netherlands
[3]Plant Research International, Wageningen, Netherlands
**Corresponding author: sander.vanderkrol@wur.nl*

Arabidopsis contains ~4500 secreted proteins with one or more of the N-glycosylation consensus site N-x-S/T. However, for only few glycoproteins has the presence of an Nglycan actually been confirmed and mapped experimentally. Here we present the characterization of the glycoproteome from Arabidopsis, as extracted from leaves, seedlings and developing seeds. Extracted proteins were first digested with trypsin, after which (activated) glycopeptides were coupled to Hydrazide resin. After extensive washing, bound peptides were released by the enzyme PNGaseF and were measured by LCMS DDA and MS^E. Because PNGaseF converts the N to D, it leaves a 'glycansignature' in the peptide sequence to be analyzed. Moreover, PNGaseF cleaves mannose glycans (on glycopeptides in ER), but not complex glycans (on glycopeptides in and from Golgi). This allowed distinguishing between ER- and Golgi-derived glycoproteomes, by comparing glycopeptide profiles from WT (only ER-derived glycopeptides) and glycopeptide profiles from mutant plants without complex glycans *(cgl)* (full glycoproteome).
Using this method we confirmed glycan occupancy of over 800 consensus sites on more than 300 proteins. We show that some glycoproteins (e.g. LRR receptors) have heterogeneous glycosylation (mannose and complex glycans within the same protein). N-glycan site occupancy mapping was also used to correct THMM-predicted membrane protein topology of eight membrane proteins. The results show that our method allows for High Through Put proteomics of this important subset of the plant proteome. This is now used to map changes in the glycoproteome in response to protein secretion stress in seeds and in pathogen-effector/plant interactions.

S03-003: A SYSTEM BIOLOGY APPROACH TO UNDERSTAND FUNCTIONS OF RAPTOR1 IN ARABIDOPSIS THALIANA

*Li, Y. * - Caldana, C. - Giavalisco, P. - Leisse, A. - Willmitzer, L.*
Max-Planck-Institute of Molecular Plant Physiology
**Corresponding author: yli@mpimp-golm.mpg.de*

RAPTOR/KOG1 proteins, conserved WD-40 repeat proteins, are binding partners of the target of rapamycin (TOR) kinase that plays a central role in metabolism, such as cellular growth in response to nutrients, mitogens and growth factors in eukaryotes. In Arabidopsis, *RAPTOR1* interacts with TOR and S6K1 in vivo, and overexpression of *RAPTOR1* rendered the S6K1 osmotic stress insensitive. We developed computational and experimental methods to identify *RAPTOR1* by using artificial microRNA lines. It is shown that, by quantitative RT-PCR, *RAPTOR1* expression level decreased after estradiol induction. Interestingly, amiRaptor1 plants were much smaller after transfer to MS medium containing estradiol. Further, I will focus on the function of *RAPTOR1* in TOR signaling pathway by tanscriptomics, proteomics and metabolomics data analysis at system-level.

S03-004: STEADY-STATE 13C METABOLIC FLUX ANALYSIS: FOCUS ON DEVELOPING BARLEY SEEDS

*Krach, C. **
Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung
**Corresponding author e-mail:christian.krach@gmx.de*

Metabolic Flux Analysis has become a well-established tool in microbial metabolic engineering. It has been successfully adopted to rational redirections of carbon metabolism and so increasing the yield of desired fermentation products. In contrast affords to manipulate a plant's metabolism beyond the scope of secondary metabolites were less successful, resulting in data hard to interpret. Accordingly a system-wide analysis and a more general understanding of metabolic processes are necessary.
A cell's set of metabolic fluxes represents a very comprehensive phenotype of its metabolic activity and therefore includes extremely important information for the targeted improvement of crop metabolism. Intracellular fluxes themselves cannot be measured

directly but have to be deduced from ^{13}C steady-state labelling experiments. The additional use of labelled substrate turns it possible to generate detailed flux maps including bidirectional or cyclic fluxes, exceeding the possibilities of the well established flux balancing. Furthermore the application of special software makes it possible to calculate the fluxes from GC-MS fractioning patterns of metabolites and positional isotopomer enrichment.
To achieve these goals we set up barley spike- and single grain cultures. After feeding labelled substrates metabolites are extracted and subjected to GC-MS analysis. The data are corrected for natural isotope abundance and then can be used to calculate the fluxes using 13CFlux software.

S04-001: ODDSOC2, A MADS BOX FLORAL REPRESSOR THAT IS DOWN-REGULATED BY VERNALIZATION IN TEMPERATE CEREALS

Greenup, A.[1] - Sasani, S.[2] - Oliver, S.[1] - Talbot, M.[1] - Dennis, E.[1] - Hemming, M.[1] - Trevaskis, B.[1]*
[1]CSIRO
[2]University of Tehran
**Corresponding author e-mail: u4378396@anu.edu.au*

In temperate cereals the transition from vegetative to reproductive development can be accelerated by exposure to extended periods of cold (vernalization). We investigated the function of a grass-specific MADS box gene *ODDSOC2* in the vernalization response of barley *(Hordeum vulgare)*. In barley *HvOS2* is expressed in the shoot apex and leaves but is repressed by vernalization. Repression of *OS2* can occur independently of the central regulator of vernalization, *VERNALIZATION1 (VRN1)*. In addition to regulating *OS2* as part of the vernalization pathway, barleys that carry active alleles of *HvVRN1* have reduced expression of *HvOS2*, suggesting that *HvVRN1* down-regulates *HvOS2* during development. Ectopic expression of *HvOS2* in a 'spring' barley delayed flowering and reduced spike, stem and leaf length. Microarray analysis of plants overexpressing *HvOS2* revealed that expression of barley homologues of the *Arabidopsis thaliana* gene *Floral Promoting Factor 1* were reduced *(FPF1)* and expression of RNase-S-like genes was increased. *FPF1* promotes floral development and enhances cell elongation in Arabidopsis, rice and tobacco, so down-regulation of *FPF1-like* genes might explain the phenotypes observed in barley plants over-expressing *HvOS2*. Based on our findings an extended model of the genetic pathways controlling vernalizationinduced flowering in cereals has been developed. The model describes the regulatory relationships between *VRN1*, *OS2* and *FPF1-like* genes. These findings further highlight the differences between the vernalization responses of temperate cereals and the model plant Arabidopsis.

S04-002: AN ANCIENT PHOTOPERIODIC MECHANISM CONTROLLING DEVELOPMENT AND CARBON METABOLISM IN PLANTS AND ALGAE

Valverde, F. - Ruiz, M.T. - Albi, T. - Cano, B. - Girón, B. - Laureano, A. - Ortiz, M.I. - Ribeiro, M.A. - Said, F.E. - Romero, J.M.*
Instituto de Bioquímica Vegetal y Fotosíntesis. CSIC-USE
**Corresponding author e-mail: federico@ibvf.csic.es*

The decision to flower is a crucial developmental process in a plant because it determines the reproductive success of the individual. It demands essential energetic resources and failing to flower at the correct time of the year seriously impinges plant vitality. Carbon metabolism in plants is connected to their developmental stage; i.e. Mutants in the photoperiod pathway, that present a longer vegetative growth phase, such as *CONSTANS (CO)* accumulate more starch than wild type plants. Our group has recently published the role of a *CO* ortholog *(CrCO)* in the control of photoperiod in the green alga *Chlamydomonas reinhardtii* and its influence over several key metabolic and cell cycle processes. To better understand the process, we propose to make a comparative study of the role of photoperiod in the control of carbon metabolism in green algae and plants.Several observations indicate that the effect of CO on leave starch accumulation may be related to its capacity to increase the expression of a starch synthese *(GBSSI)* and that it could involve a new regulatory mechanism. For this reason we have isolated *gbssi* mutants, characterized their flowering time phenotype and capacity to synthesize starch. gbssI mutants have been crossed to plants overexpressing CO and their effect on flowering time checked. We will also show *GBSSI expression* in 35S::*CO* plants and co mutants in LD and SD conditions to asses the effect of *CO* on *GBSSI* expression in a circadian manner.

S04-003: IDENTIFICATION AND FUNCTIONAL ANALYSES OF GENES INVOLVED IN FRUIT SET IN TOMATO

Gómez-Mena, C. - Medina, M. - Cañas, L. - Beltrán, J.P.*
Instituto de Biología Molecular y Celular de Plantas (IBMCP-CSIC)
**Corresponding author e-mail: cgomezm@ibmcp.upv.es*

Tomato has become a plant model system for fleshy fruit to study fruit set and development. The shift from the static flower ovary to fast-growing young fruit is a phenomenon known as fruit set, and is an important step in the development of all sexually reproducing higher plants. In general, fruit set is induced after pollination and successful fertilization of the egg cells in the ovules. However fruit development can be uncoupled from fertilization and seed development to generate seedless (parthenocarpic) fruits. Male-sterile tomato plants have been obtained by anther ablation at early stages of development (Roué et al 2007). The ovaries of these transgenic plants quickly develop and fruit set is established in the absence of fertilization. Using these lines, we have carried out a genomic approach in order to identify the genes involved in the process. A set of 173 unigenes coding transcription factors are differentially expressed at early stages of ovary development in the male-sterile plants pEND1::*barnase*. The function of these genes is being investigated using VIGS technology. The identification and characterization of these genes will make possible to develop biotechnological tools to gain control over fruit set in tomato. Roque, E., Gómez, M.D., Ellul, P., Wallbraun, M., Madueño, F., Beltrán, J.P., Cañas, L.A. (2007). Plant Cell Rep. 26: 313-325.

S04-004: THE ROLE OF MIRNAS DURING GERM CELL SPECIFICATION IN ARABIDOPSIS POLLEN

Borges, F.[1] - Slotkin, R. K.[2] - Gardner, R.[3] - Martienssen, R. A.4 - Becker, J.D.[1]*
[1]Plant Genomics, Instituto Gulbenkian de Ciência, Oeiras, Portugal
[2]Plant Cellular and Molecular Biology, The Ohio State University, Columbus, OH, USA
[3]Cell Imaging Unit, Instituto Gulbenkian de Ciência, Oeiras, Portugal
[4]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA
**Corresponding author e-mail: jbecker@igc.gulbenkian.pt*

Plant meiocytes undergo subsequent mitotic divisions to form the gametes, which must rapidly reprogramme their epigenome before fertilization. In Arabidopsis, the male germline differentiates by asymmetric division of haploid uninucleated microspores, giving rise to a vegetative cell enclosing a smaller generative cell that divides before anthesis to originate two sperm cells. The vegetative nucleus (VN) retains a somatic nature, orchestrates pollen tube growth and does not contribute with genetic material to the next generation. However, recent observations indicated that DNA demethylation and expression of particular transposable element (TE) loci occurs in the VN, producing siRNAs that might reinforce epigenetic silencing of TE activity in the gametes.
Transcriptional profiling of FACS-purified mature pollen and sperm cells has shown that transcripts involved in small RNA biogenesis and RNA-directed DNA methylation are enriched in

sperm cells, suggesting active epigenetic reprogramming as well as posttranscriptional regulation of gene expression. Our deep sequencing analysis of small RNA libraries from pollen and sperm cells revealed that 49 known miRNA families are enriched in the male gametes. We could predict 31 potentially novel miRNAs in sperm cells and can show cleavage of some of their predicted target transcripts in pollen samples. Thus our comparative sRNA sequencing coupled with the transcriptome data and pollen 5'RACE analysis suggest that miRNA pathways are active during sperm cell specification. Moreover, we are testing the hypothesis that some miRNAs accumulate in the male gametes to be delivered to the female gametes upon fertilization and only play a role during early embryonic development.

S05-001: MOLECULAR APPROACHES TO IMPROVING SORGHUM QUALITY: A CEREAL CROP FOR FOOD, FEED AND BIOMATERIALS

Godwin, I. D.[1] - Gilding, E.[1] - Prentis, P.[1] - Frere, C.[1] - Mudge, A.[1] - Liu, G.[1] - Lambrides, C.[1] - William, S. B.[1] - Campbell, B.C.[1] - Mace, E.S.[2] - Cruickshank, A.[2] - Jordan, D.R.[2]*
[1]The University of Queensland
[2]AgriScience Queensland
**Corresponding author e-mail: i.godwin@uq.edu.au*

The sorghum genome sequence will facilitate the development of more highly saturated genome maps with database mining for Single Nucleotide Polymorphisms (SNP). This will also enable rapid *in silico* identification of candidate genes in mapped regions known to be involved in the expression of Quantitative Trait Loci (QTL) and allelic diversity for important grain quality, cell wall quality, abiotic stress tolerance and developmental genes. Internationally, great interest in the sorghum genome derives from its importance as a grain and forage crop, as a model for the maize and sugarcane genomes, and most recently because of its emerging potential as a biomass crop. The combination of new tools such as whole genome mapping and selection, developments in association mapping, robotics and computational biology for high throughput sequencing and sorghum transgenics make for a powerful package for sorghum genetic improvement. We have produced sorghums with altered protein:starch matrix by targeting the S-S cross-linking of the beta- and gamma-kafirins. We have characterised a range of cultivated and wild sorghum germplasm for grain quality parameters, and are in the process of manipulating genes involved in seed storage protein and starch biosynthesis to alter the nutritional and bio-industrial quality of the endosperm. Additionally, forward and reverse genetics approaches are underway to improve stover quality for nutritional and bio-industrial applications.

S05-002: NOVEL INSIGHTS INTO THE PHYSIOLOGICAL FUNCTION OF LATEX IN TARAXACUM SPP

Wahler, D.[1] - Colby, T.[2] - Schulze Gronover, C.[3] - Prüfer, D.[1]*
[1]University of Münster - Institut für Biochemie und Biotechnologie der Pflanzen (WWU-IBBP)
[2]MPIZ Cologne
[3]Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany
**Corresponding author e-mail: daniela.wahler@uni-muenster.de*

Ten percent of all angiosperms contain specialized cells with a remarkable milky cytoplasm called latex. The genera *Taraxacum* is probably most familiar among latexcontaining plants. So far, no physiological function of latex could be determined but a participation in plant defense is discussed. Additionally, some latex-containing plants such as *T. koksaghyz* synthesize natural rubber and the question rose whether this biopolymer also contributes to defense responses. Here, we present a proteomic approach on *Taraxacum spp*. to get a better insight into the biology of latex and particularly into rubber biosynthesis. Through establishment of reliable methods for protein purification, visualization on two-dimensional gels and subsequent crossspecies identification 39% of all visible spots were identified. A high proportion could be related to plant defense giving yet another hint that a prime role of latex is defense. By comparative analyses of the latex proteomes of the closely related rubber producer *T.koksaghyz* and non-rubber producer *T. officinale*, differentially expressed proteins were detected that are thus likely to be factors of rubber biosynthesis. Among them three members of the rubber elongation factor superfamily that are known to be essential factors in rubber biosynthesis exhibited a deviating expression pattern in the two *Taraxacum* species.

S05-003: IMPROVEMENT OF PLANT DEFENSES THROUGH METABOLIC ENGINEERING OF LIMONENE IN TRANSGENIC ORANGE FRUITS

Rodriguez, A.[1] - San Andres, V.[1] - Redondo, A.[1] - Alquezar, B.[1] - Cervera, M.[1] - Shimada, T.[2] - Gadea, J.[3] . Rodrigo, M.J.4 - Zacarias, L.4 - Castañera, P.5 - Palou, L.[1] - Lopez, M.M.[1] - Peña, L.[1]*
[1]IVIA - Instituto Valenciano de Investigaciones agrarias
[2]National Institute of Fruit Tree Science (NIFTS)
[3]Instituto de Biología Molecular y Celular de Plantas (IBMCP)
[4]Instituto Agroquímica y Tecnología de Alimentos (IATA – CSIC)
[5]Centro de Investigaciones Biológicas (CIB – CSIC)
**Corresponding author e-mail: rodriguez_anabai@gva.es*

Plants produce a wide diversity of secondary metabolites, many of which are volatile compounds. Terpenes are major components of fruits, which suggests that serve for seed protection against pests and pathogens and as attractants of frugivorous animals. However, proof that a specific fruit terpene attracts or repels a specific animal remains to be obtained. The extraordinarily high amount of limonene that accumulates in orange oil glands suggests an important biological role for this terpene compound in interactions with the environment. To test this, we manipulated oil gland chemistry by antisense downregulation of limonene expression in orange fruits. Transgenic plants had reduced limonene accumulation in fruit peel. A gene expression analysis was performed with a 20K citrus microarray and results indicated that monoterpene downregulation was activating the immune response in fruit peel. To test whether antisense suppression resulted in an improvement in the response of the fruit against pest and pathogens, antisense (AS) and control (EV) fruits were challenged with the Medfly *Ceratitis capitata*, the fungus *Penicillium digitatum*, and the bacterium *Xanthomonas axonopodis*. Medfly males were more attracted to EV fruits, indicating that limonene emission was attracting insects to the fruit. Moreover, AS fruits showed a marked resistance against both pathogens characterised for their inability to establish infection in peel tissues.
These results provide a more comprehensive view of the role of terpene volatiles as attractors of insects and microorganisms, which break the external peel layer, thus promoting pulp and seed feeding by frugivores. The strategy represents a very promising alternative for increasing resistance to pests and pathogens in plants.

S05-004: PENETRATION AND TRANSPORT OF NANOPARTICLES IN LIVING PLANTS AS A TOOL FOR DIRECTED DELIVERY OF DRUGS IN CROP PROTECTION

Risueño, M.[1] - Corredor, E.[1] - Marquina, C.[2] - Ibarra, M.R.[2] - De La Fuente, J.M.[3] - Rubiales, D.4 - Pérez-de-Luque, A.5 - Testillano, P.S.[1]*
[1]Centro de Investigaciones Biológicas, CSIC
[2]Inst. Ciencia de Materiales, ICMA, CSIC-Univ. Zaragoza
[3]Inst. Nanociencia de Aragón, INA, Univ. Zaragoza
[4]Inst. Agricultura Sostenible, IAS, CSIC
[5]ÍFAPA
**Corresponding author e-mail: risueno@cib.csic.es*

The use of nanotechnology for the targeted delivery of substances has been subject of special attention in biomedicine, being this

technology of interest also in the treatment of phytopathologies, even though reports on plant bionanotechnology are very scarce. .In this work the penetration and movement of iron-carbon nanoparticles in plant cells has been analyzed in living plants of *Cucurbita pepo*. A nanoparticle suspension was applied *in planta* by injection and spraying, and magnets were used to retain the particles in movement in specific areas of the plant. Correlative light and electron microscopy was used for the analysis, results providing evidence of intracellular localization of nanoparticles and their displacement from the application point. Long range movement of the nanoparticles through the plant body was also detected, with their presence in the proximity of the magnets used to immobilize and concentrate them. Results support the applicability of carbon coated magnetic particles for the directed delivery of substances into plant cells and open new possibilities for the treatment of phytopathologies by specific drugs conjugated to nanoparticles, as well as for the design of early diagnostic methods. CORREDOR E, TESTILLANO PS, CORONADO MJ, GONZÁLEZ-MELENDI P, FERNÁNDEZ-PACHECO R, MARQUINA C, IBARRA MR, DE LA FUENTE JM, RUBIALES D, PÉREZ-DE-LUQUE A, RISUEÑO MC. (2009) Penetration and transport of nanoparticles in living plants as a tool for directed delivery: in situ detection into plant cells. BMC *Plant Biology*, 9: 45. Work supported by MICINN International Project EUI2008-00157 in the Iberian Laboratory of Nanotechnology and CSIC Project 200520F0043, PIF-NANOAGRO2005.

S06-001: A MOLECULAR CLOCK SETS COMPETENCE FOR PERIODIC BRANCHING IN THE ARABIDOPSIS ROOT

Moreno Risueno, M. - Van Norman, J. - Benfey, P.*
Duke University
**Corresponding author e-mail: mm141@duke.edu*

Formation of periodic modular structures is a common developmental mechanism in both animals and plants. However in plants, the developmental mechanisms by which newly formed organs are positioned in time and space along the primary axis remain largely uncharacterized. In Arabidopsis, lateral roots (LR) are formed from pericycle cells that are re-specified into LR founder cells. Competence to specify new LR can be tracked by expression of the auxin signaling reporter DR5, which rhythmically pulses in the oscillation zone (OZ) at the root tip independently of changes in auxin content; and reports future branching at the prebranch sites. Interestingly, prebranch site initiation as well as root bending follow a periodic temporal pattern and compensate for changes in temperature and in different environmental conditions, which is characteristic of endogenous mechanism that track time. To further understand this molecular oscillatory mechanism we performed genomic transcriptomic analyses of the OZ. Some of our results include the identification of novel transcription factors oscillating in the OZ and impaired in root periodic responses. We provide evidence that branching in the Arabidopsis root depends on two sets of genes oscillating in opposite phases that establish the temporal and spatial distribution of lateral roots along the primary root axis.

S06-002: ABI4 MEDIATES ABSCISIC ACID AND CYTOKININ INHIBITION OF LATERAL ROOT FORMATION BY REDUCING POLAR AUXIN TRANSPORT

Bar-Zvi, D. - Shkolnik-Inbar, D.*
Dept of Life Sciences, Ben-Gurion University
**Corresponding author e-mail: barzvi@bgu.ac.il*

Lateral roots (LRs) formation is an essential process in plant's development and adaptation to the environment. LR development is controlled by a balance between three plant hormones: auxin is the key hormone promoting LR formation, whereas cytokinin and ABA inhibits this developmental process. We present here direct evidences for *ABSCISIC ACID INSENSITIVE 4 (ABI4)* encoding an ABA-regulated AP2-domain transcription factor role in root branching. *ABI4* is intensively studied in ABA and glucose signaling in seed germination. Mutation in *ABI4*, results in an increased number of LRs and its overexpression impairs LRs development. Root expression of *ABI4* is enhanced by ABA and cytokinin and repressed by auxin. *ABI4* also affects the profiles of the auxin and cytokinin hormones in the root, as determined by the activities of the respective hormone-response promoters *DR5* and *ARR5*. LRs are initiated in xylem-pole pericycle cells accumulating threshold level of auxin, leading to a serious of divisions, resulting in the LR primordia formation. *ABI4* is expressed in phloem companion cells, and its expression reduces the level of the auxin-efflux carrier PIN1, abrogating auxin accumulation, and thus, LR initiation. We therefore suggest that *ABI4* plays a key inhibitory role in LR development by affecting auxin polar transport, in a mechanism regulated by ABA and cytokinin.

S06-003: CHARACTERIZATION OF ROOT ARCHITECTURE MUTANTS IN MEDICAGO TRUNCATULA

Laffont, C.[1] - Blanchet, S.[2] - Lapierre, C.[3] - Cosson, V.[2] - Ratet, P.[2] - Crespi, M.[2] - Mathesius, U. - Frugier, F.[2]*
[1]Centre National de la Recherche Scientifique (National Center for Scientific Research)
[2]Institut des Sciences du Végétal ((ISV - CNRS)
[3]UCB, AgroParisTech–INRA
[4]ARC-ANU Canberra
**Corresponding author e-mail: laffont@isv.cnrs-gif.fr*

Root system architecture is crucial to adapt plant growth to changing soil environmental conditions and consequently to maintain crop yield. In addition to root branching through lateral roots, legumes can develop another lateral organ, the nitrogen-fixing nodule, upon a symbiotic bacterial interaction. We identified several *M. truncatula* mutants, referred to as *cra* for *compact root architecture*, showing root developmental defects but able to form nodules.
Transcriptomic characterization of the *cra1* mutant revealed only few significant changes, mainly related to cell wall metabolism. The most down-regulated gene in *cra1* mutant encoded a Caffeic Acid O-Methyl Transferase, an enzyme involved in lignin biosynthesis, and accordingly whole lignin content was decreased in *cra1* roots. This correlated with differential accumulation of specific flavonoids and decreased polar auxin transport. The *CRA1* gene may therefore control legume root architecture through regulation of lignin and flavonoid profiles leading to polar auxin transport changes. We also characterized *Tnt1 insertional mutants* identified in a screen done with Drs. Kyran Mysore and Tadege Million at the Noble Fundation (USA). The *cra2* mutant notably shows a strong increase in lateral root density but no major defect in the development of their aerial parts. Cloning of the mutated gene through systematic sequencing of *Tnt1* borders and segregation analyses is in progress.

S06-004: THE ADVENTITIOUS ROOTS FORMATION IN POPLAR IS MEDIATED BY THE AINTEGUMENTA–LIKE TRANSCRIPTIONAL FACTORS

Rigal, A.[1] - Tisserant, E. - Yordanov, Y.[2] - Busov, V.[2] - Martin, F.[1] - Legue, V.[1] - Kohler, A.[1]*
[1]INRA and Nancy University
[2]Michigan Technological University
**Corresponding author e-mail: adeline.rigal@nancy.inra.fr*

Adventitious rooting (AR), i.e. regeneration and development of roots on any organ but not roots, is an essential step in the vegetative propagation of economically important horticultural and woody species. The formation of AR is a complex process that involves successive developmental phases including cell cycle re-activation, primordium formation and finally root emergence. These cellular events are regulated by unknown signalling mole-

cules and transcriptional factors (TFs). In order to identify TFs and gene networks involved in AR development in *Populus trichocarpa*, we have carried out a series of genome-wide transcript profilings during the development of AR.
Aintegumenta–like TFs *(PtANTs)* are dramatically upregulated during primordium formation and root emergence, suggesting a role for this TFs in AR formation. The differential expression of *PtANTs* has been validated by qPCR. Poplar transgenic lines over-expressing *PtANT1* showed an increased capacity in AR formation compared to the wild-type confirming the importance of *ANT* TFs in AR development. The function of this gene in the root formation will be discussed.
This research project is funded by the European project ENERGYPOPLAR, the Région Lorraine Research Council (Project FORBOIS), and the French National Space Agency (CNES).

S07-001: COLD SHOCK DOMAIN PROTEIN GENES IN THE EXTREMOPHYTE THELLUNGIELLA SALSUGINEA: IDENTIFICATION AND DIFFERENTIAL EXPRESSION

*Taranov, V. * - Berdnikova, M. - Nosov, A. - Galkin, A. - Babakov, A.*
All-Russia Research Institute of Agricultural Biotechnology, Russian Academy of Agricultural Sciences
**Corresponding author e-mail: v.taranov1@gmail.com*

Four genes encoding cold shock domain (CSD) proteins have been identified in salt cress [*Thellungiella salsuginea (halophila)*, an extremophyte currently recognized as a promising model for studying stress tolerance]. The deduced proteins prove highly homologous to those of *Arabidopsis thaliana* (up to 95% identity) and are accordingly enumerated TsCSDP1--TsCSDP4; after the N-proximal conserved CSD, they have respectively 6, 2, 7, and 2 zinc finger motifs evenly spaced by Gly-rich stretches. Much lower similarity (~45%) is observed in the regions upstream of TATA-box promoters of *TsCSDP1 vs. AtCSP1*, with numerous distinctions in the sets of identifiable *cis*-regulatory elements. Plasmid expression of *TsCSDP1* (like AtCSP1/3) rescues a coldsensitive *csp*-lacking mutant of *E. coli*, confirming that the protein is functional. In leaves of salt cress plants under normal conditions, the mRNA levels for the four TsCSDPs relate as 10:27:1:31. Chilling to 4°C markedly alters the gene expression; the 4-day dynamics are different for all four genes and quite dissimilar from those reported for their Arabidopsis homologues under comparable conditions. Thus, the much greater cold hardiness of *Thellungiella* vs. *Arabidopsis* cannot be explained by structural distinctions of its CSDPs, but rather may be due to expedient regulation of their expression at low temperature.

S07-002: TRANSCRIPTOME ANALYSIS OF A M. TRUNCATULA SALT-ADAPTED GENOTYPE REVEALED AN APETALA2- DEPENDENT PATHWAY ASSOCIATED TO ROOT GROWTH UNDER SALT STRESS

Zahaf, O.[1] - Blanchet S.[2] - de Zélicourt, A.[1] - Alunni, B. - de Lorenzo, L.4 - Imbeaud, S.5 - Ichanté, J.L.5 - Diet, A.[1] - Plet, J.[1] - Badri, M.6 - Delacroix, H.4 - Frugier, F.[1] - Crespi, M.[1]*
[1]ISV CNRS
[2]CEA
[3]IBP
[4]Departamento de Microbiología y Parasitología, Universidad de Sevilla
[5]DNA MicroArray Platform and Centre de Génétique Moléculaire, CNRS
[6]Laboratoire des Interactions Légumineuses-Microorganismes
**Corresponding author e-mail: ons.zahaf@isv.cnrs-gif.fr*

Evolutionary diversity can be driven by the interaction of plants with different environments. Global molecular bases involved in these ecological adaptations can be explored using genomic tools. Legumes due to their capacity to establish symbiotic associations are able to grow in nitrogen poor soils and are major crops worldwide. As soil salinity is a major stress in legumes, we compared the root transcriptomes of two *M. truncatula* genotypes having contrasting responses to salt stress. The genotype TN1.11, isolated from salty Tunisian soils, shows increased root growth and symbiotic nodulation under salt stress when compared to the reference model legume *M. truncatula* Jemalong A17. Genomic analysis revealed specific gene clusters differentially regulated by salt in the TN1.11 genotype. Among those, functional clustering of regulatory pathways pointed to a link with auxin and, accordingly, TN1.11 and A17 roots show a differential response to this phytohormone. In addition, several transcription factors (TFs) were differentially regulated between the two genotypes and 6 TF genes were over-expressed in roots of the Jemalong A17 genotype. Overexpression of an *APETALA2*-type transcription factor, regulated by auxin and ABA, conferred a significant increase in root growth under salt stress conditions. Hence, an *APETALA-2* pathway may play a critical role in the adaptation of *M. truncatula* to saline soil environments.

S07-003: THE ARABIDOPSIS VACUOLAR ANION TRANSPORTER, ATCLCC, IS INVOLVED IN THE REGULATION OF STOMATAL MOVEMENTS AND SALT TOLERANCE

Leonhardt, N.[1] - Kroniewicz, L.[1] - Jossier, M. - Dalmas, F.[1] - Le thiec, D. - Barbier-Brygoo, H.[2] - Ephritikhine, G.[2] - Filleur, S. [2]*
[1]CEA Cadarache-IBEB-LEMS
[2]CNRS
[3]INRA
**Corresponding author e-mail: nathalie.leonhardt@cea.fr*

In plants, the main function of chloride transport is the net salt accumulation responsible for the high cell turgor, involving the creation and maintenance of a large vacuolar volume. In recent years, various plant chloride channels and transporters have been identified to be involved in specific function such as plant nutrition, stomatal movement, and metal tolerance. In addition, plant chloride channels play a predominant role in signal perception and transduction since a large number of signals such as pathogen-derived elicitor or hormones induce membrane depolarization by stimulating anion efflux. In this study, we report for the first time evidence that a member of the CLC family in *Arabidopsis thaliana*, *AtCLCc*, plays an important role in stomatal movements and salt tolerance. The *AtCLCc* protein is localized to the tonoplast and *AtCLCc* is highly expressed in guard cell and up-regulated by ABA and salt treatment in the whole plant. Four T-DNA mutants in *AtCLCc* of two ecotypes (WS and Col-0) are impaired in light-induced stomatal opening and ABA-induced stomatal closing. These alterations are associated to modifications in chloride content in guard cells. Concomitantly, the *clcc* mutants exhibit a hypersensitive phenotype to salt stress compared to wild-type. Our recent data on the role of AtCLCc in salt tolerance and stomatal movement will be presented and the importance of the chloride in these processes will be discussed.

S07-004: ROLE OF SOS1 IN POTASSIUM NUTRITION

Tello, C. - de Luca, A. - Leidi, E.O. - Pardo, J.M.- Quintero, F.J.*
Instituto de Recursos Naturales y Agrobiología de Sevilla
**Corresponding author e-mail: ctello@irnase.csic.es*

Potassium nutrition is vital for plants, since this cation plays a major role in plant growth, stomatal movements, enzyme activation and osmoregulation. *SOS1*, a plasma membrane Na^+/H^+ antiporter which determines sodium homeostasis in saline conditions, was first described as an essential locus for potassium acquisition, as *sos1* plants are unable to grow under low potassium conditions. However, biochemical and transport assays in *SOS1* showed this protein is highly specific for Na^+ and doesn't transport K^+ or other monovalent cations. The role of *SOS1* in potassium uptake has been thus thought to be indirect, by preventing inhibition of potassium channels, such as *AKT1*, by sodium. This hypothesis was tested in our study by growing *sos1* and *akt1*

mutants under controlled sodium and potassium conditions. When grown under low potassium levels, sos1 growth was severely affected but only if the medium contained sodium. Removal of sodium abrogated the potassium phenotype.
Growth of *akt1* mutant was reduced at low potassium levels but not affected by sodium at the low concentration of this cation used in the experiment. Moreover, an *akt1 sos1* double mutant behaved like *akt1* single mutant in normal conditions but, when sodium was added to the medium, growth was reduced compared to that of sos1 and akt1 single mutants in all the potassium concentrations tested. Our results shed light on the interaction of both *SOS1* and *AKT1* in potassium nutrition.

S08-001: EFFECTS OF SALICYLIC ACID ON PHOTOSYNTHESIS

Janda, K.[1] - Hideg, É.[2] - Janda, T.[3] - Szalai, G.[3] - Kovács, L.[2]*
[1]University of Szeged
[2]Biological Research Center of the Hungarian Academy of Sciences
[3]Agricultural Research Institute of the Hungarian Academy of Sciences
**Corresponding author e-mail: gumimacihu@yahoo.co.uk*

Salicylic acid (SA) is a phenolic phytohormone with important roles in plant development, transpiration, endogenous signalling and defence against pathogens.
One of the pathways of SA biosynthesis is located in the chloroplasts. The aim of the present work was to investigate the possible regulatory effects of SA on the photosynthetic electron transport processes. Here we show that SA also affects leaf photosynthesis, via inducing stomatal closure and also by slowing down Photosystem (PS) II electron transport. Photosynthetic CO_2 incorporation, and stomatal conductivity (measured with an infrared gas analyser) were much lower in SA-infiltrated tobacco leaves than in untreated or water-infiltrated controls.
Data of tobacco and pea leaves show that PS II electron transport (calculated from PAM chlorophyll fluorescence data) was more sensitive to SA than PS I (measured with far red absorption). Direct probing of PS II charge separation and stabilization (measured with thermoluminescence), however, showed that these events were less affected in isolated thylakoid membranes than in leaves, suggesting that the effect of SA on PS II is indirect and different from that of phenolic herbicides.
Our data also suggest that the effects of SA may differ in different plant species.

S08-002: MONITORING CHLOROPHYLL A FLUORESCENCE IN ARABIDOPSIS PLANTS AFTER CITRAL TREATMENT

*Sotelo Pérez, T. * - Graña, E. - Reigosa, M.J. - Sánchez-Moreiras, A.M.*
University of Vigo
**Corresponding author e-mail: tamaras@uvigo.es*

Imaging of chlorophyll a fluorescence has been recognized as a valuable, non-invasive technique for the investigation of photosynthesis and the detection of stress in plants1.
Fluorescence emission allows to estimate effective PSII quantum yield Y(II); quantum yield of regulated energy dissipation Y(NPQ), quantum yield of non-regulated energy dissipation Y(NO), coefficient of photochemical quenching (qL), coefficient of nonphotochemical quenching (qN), maximal PSII quantum yield (Fv/Fm) and electron transport rate (ETR).
The knowledge of these parameters allows estimate how plant metabolism copes with environmental constrains.
Citral is an essential oil known to show anti-microbial, anti-insecticide and anti-tumoral activity. The previously demonstrated toxicity on other organisms suggests the potential of this compound on weed control. However, this plant secondary metabolite has been never assayed on plants. Therefore we decided to investigate the phytotoxic activity and the mode of action of Citral on plant metabolism.
Arabidopsis plants were sprayed or watered for 21 days with different citral concentrations. Our results showed a decrease for the two treatments in effective PSII quantum yield confirmed by an increase in Y(NO), while no significant change occurred in Y(NPQ). However, spraying affected Fv/Fm and ETR in a very effectiveway.
The results of photosynthetic activity, growth rate, pigment content and total proteins suggest a general reduction of the metabolism in citral-watered plants, while the results obtained for spraying could be suggesting more direct by-contact damage. In conclusion, citral appears as a plant growth regulator with potential as bioherbicide.
1 Oxborough K (2004) J. Exp. Bot., 55:1195-1205.

S08-003: IN VIVO CYTOCHROME AND ALTERNATIVE PATHWAY RESPIRATION IN ARABIDOPSIS THALIANA PLANTS WITH ALTERED ALTERNATIVE PATHWAY CAPACITY UNDER LOW AND HIGH LIGHT CONDITIONS

Florez-Sarasa, I.[1] - Flexas, J.[1] - Umbach, A.L.[2] - Siedow, J.N.[2] - Gallé, A.[1] - Ribas-Carbo, M.[1]*
[1]Universitat de les Illes Balears
[2]Duke University
**Corresponding author e-mail : igor.florez@uib.es*

Plant respiration is characterised by the existence of a cyanide-insensitive respiratory pathway, alternative to the cytochrome respiratory pathway. Alternative oxidase (AOX) activity is thought to be regulated by several factors: a) inter-disulfide bond, b) α-ketoacids interaction c) AOX protein expression.
Arabidopsis thaliana plants with altered levels of AOX protein (*AtAOX1*a antisense, AS-12 and overexpressor, XX-2) as well as wild type Columbia 0 plants (Col-0) were grown under low light conditions (80 µmol quanta m-2 s-1) and moved to high light (800 µmol quanta m-2 s-1) for 2 hours. The alternative pathway capacity was different between lines while no differences were observed on total respiration neither on electron partitioning through alternative pathway (τa) under low light. However, when plants were exposed to high light for 2 hours, τa in AS-12 plants decreased while no changes were observed in Col-0 and XX-2 plants. Despite changes on τa in AS-12 under high light conditions, effects on photosynthesis and chlorophyll fluorescence parameters were similar in all 3 lines.
In vivo regulation of the mitochondrial electron partitioning in relation to AOX expression levels under photoinhibitory conditions will be discussed.

S08-004: STOMATAL DENSITY LINKED TO LEAF INTERNAL CO_2 CONCENTRATION

Santrucek, J.[1] - Vaskova, M.[1] - Tomsickova, J.[1] - Simkova, M. - Hronkova, M.[2] - Kveton, J.[1] - Vrabl, D.[1] - Schreiber, L.[3]
[1]Univ. South Bohemia
[2]BC, Inst. Plant Mol. Biol.
[3]Univ. Bonn, ICMB

Stomata are hydraulically controlled pores between two guard cells in epidermis allowing gas, primarily water vapor and CO_2, exchange between plants and atmosphere. It was recently shown that number of stomata per unit of leaf area, stomatal density SD, changed with changing atmospheric CO_2 concentration, c_a, over geologic time. SD is used as a proxy for paleo-CO_2 reconstructions. However the SD/c_a proportionality factor, estimated from glasshouse cultivation data or leaf remains, is affected also by light, air humidity, drought and other environmental factors. Moreover, stomata evolve in an early stage when the folded leaf is sheathed or covered by other leaves primordia evolving local massive flux of respiratory CO_2.
This doubts the role of ambient CO_2 as an exclusive environmental factor controlling SD. Here, we show results of cultivation

experiments with garden cress *(Lepidium sativum)* where carbon isotope ratio ($\delta^{13}C$) and SD were measured in cotyledon leaves. Relative enrichment in ^{13}C by 1 ‰, caused by various environmental factors, increased stomatal density by 9 %. Similar proportionality (12-17 %) was obtained from analyses of published and our own data for number of herbaceous and tree species. Based on leaf internal CO_2 concentration (ci) manipulative experiments, we show that c_i rather than ca or the c_i/c_a ratio is the factor controlling SD and integrating multitude of environmental stimuli effecting SD including atmospheric O_2 concentration and (photo)respiration.

S09-001: FUNCTIONAL ANALYSES OF THE NATURAL VARIATION OF ONE MICRORNA IN ARABIDOPSIS THALIANA

Jinyong, H.U. - de Meaux, J.
Max-Planck-Institute for Plant Breeding Research

MicroRNAs (miRNAs) play pivotal roles in post-transcriptional regulation. These small RNAs are normally produced by manufacturing of hairpin featuring precursors. Only very recently the base region of the stem-loop structure was shown to be important for the processing of miRNAs, however, rare case concerning the natural variation of the miRNA structure has been found. Here we report the natural variation of a miRNA precursor, which harboring thermo-sensitive secondary structure polymorphisms, from *Arabidopsis thaliana.* We first show that the natural variation of the precursors of this miRNA could affect the processing efficiency to mature microRNA, leading to the variation of its target gene transcripts level. We continue to show that this variation could influence various life cycle traits of *Arabidopsis*, hence suggest that this variation may play important micro-tuning roles in the adaptation to various environments. By setting up a multiple-generations field assay we could reveal that fluctuating selection could act in maintaining this variation at this microRNA locus. Finally we propose a novel mechanism of modulating important and complex gene networks, which play essential roles in the development and adaptation of *Arabidopsis*.

S09-002: COMPARISON OF NEUTRAL AND ADAPTIVE MICROSATELLITE DATA IN THE RED ALGA FURCELLARIA LUMBRICALIS

Olsson, S.[1] - Kostamo, K.[2] - Korpelainen, H.[2]*
[1]Department of Agricultural Sciences
[2]University of Helsinki
**Corresponding author e-mail: sanna.olsson@helsinki.fi*

The target plant of the study, the red alga Furcellaria lumbricalis, occupies a range of aquatic habitats varying in salinity, some habitats being clearly marginal for the species.
We investigated the genomic structure and genetic variability of F. lumbricalis populations originating from different habitats using microsatellite data. First we used microsatellites that are assumed to be neutral, but since neutral genetic variation does not present a valid picture of the adaptive capabilities of algae in a changing environment, we also studied the genetic variation present in expressed genes. This may link genetic variation patterns found within populations with environmental factors. To develop presumably neutral, species-specific microsatellite markers for F. lumbricalis, we utilized an alternative protocol based on genomic screening with ISSR primers.
For the discovery of adaptive microsatellite and other possible markers, EST libraries were created and sequenced. The level, structure and distribution of genetic variation based on both neutral and adaptive markers were investigated and compared. F-statistics was applied to test whether the divergence pattern among populations differs between the neutral and adaptive marker sets. Selection is considered to act on adaptive loci only, whereas genetic drift, gene flow and reproductive patterns affect genetic variation at all loci to the same extent.

S09-003: NATURAL VARIATION OF QLTG3-1, A MAJOR QTL CONTROLLING LOW-TEMPERATURE GERMINABILITY IN RICE

*Fujino, K.**
HOKUREN
**Corresponding author e-mail: fujino-kenji@hokuren.jp*

Tolerance to abiotic stress is an important agronomic trait in crops and is controlled by many genes, which are called quantitative trait loci (QTLs). Identification of these QTLs will contribute not only to the understanding of plant biology but also for plant breeding to achieve stable crop production around the world. Previously, we mapped three QTLs controlling low temperature tolerance at the germination stage (called low temperature germinability). To understand the molecular basis of one of these QTLs, qLTG3-1 (quantitative trait locus for low temperature germinability on chromosome 3), map-based cloning was performed, and this QTL was shown to be encoded by a protein of unknown function. qLTG3-1 is strongly expressed in the embryo during seed germination. Prior to and during seed germination, specific localization of GUS staining in the tissues covering the embryo was observed. Expression of qLTG3-1 was tightly associated with vacuolation of the tissues covering the embryo. This may cause tissue weakening resulting in reduction of the mechanical resistance to the growth potential of the coleoptile. Although rice originated in a tropical region, now rice is grown under various climatic conditions. To elucidate the relationships between wide adaptability of rice and low-temperature germinability, we surveyed natural variation of qLTG3-1 using the rice core collection, which represents the diverse genetic diversity among cultivated rice.

S09-004: NATURAL GENETIC VARIABILITY IN FREEZING TOLERANCE OF ARABIDOPSIS THALIANA USING CONTROLLED CHAMBER EXPERIMENTS AND DATA FROM OVERWINTERING FIELD TRIALS

Zuther, E. - Fürstenberg-Hägg, J. - Schulz, E. - Tohge, T. - Fernie, A. - Köhl, K. - Hincha, D. K.*
MPI of Molecular Plant Physiology
**Corresponding author e-mail: zuther@mpimp-golm.mpg.de*

Most plants from temperate regions increase their freezing tolerance in response to low, non-freezing temperatures in a process termed cold acclimation. The natural variability in freezing tolerance of *Arabidopsis thaliana* was phenotyped by electrolyte leakage analysis in 54 accessions including the Versailles Arabidopsis Core Collection under non-acclimated and acclimated conditions in controlled chamber experiments. Additionally a comprehensive analysis of primary and secondary metabolites was performed and data correlated with freezing tolerance. Furthermore, 80 accessions were investigated in three field studies in Potsdam from 2007 to 2009 to monitor overwintering capacity. Developmental parameters, content of selected primary and secondary metabolites and seed weight were determined. From these data, characteristic metabolite patterns for different developmental stages were identified. Significant differences were found for anthocyanins, sugars and organic acids between flowering and non-flowering accessions. Furthermore, LT_{50}–values for acclimated and non-acclimated plants under controlled conditions were correlated with the anthocyanin levels of the field grown plants. Using a multiple linear regression model with eight variables measured under field conditions, we were able to predict LT_{50}-values for accessions that had not been previously investigated under controlled conditions. The results of our ongoing tests of these predictions will be presented.

S10-001: BR-GA CROSS-TALK IN REPRESSION OF PHOTOMORPHOGENESIS

De Lucas, M. - Bernardo, S. - Davierè, J. M. - Prat, S.*
Centro Nacional de Biotecnología-CSIC

**Corresponding author e-mail: mdelucas@cnb.csic.es*

Wild type seedlings germinated in darkness show an etiolated growth characterized by an elongated hypocotyl, closed and undifferentiated cotyledons and the presence of an apical hook.
Different bHLH transcription factors (PIFs) appear to mediate this response and to be rapidly destabilized by the PhyA and B photoreceptors that mediate responses to red (R) and far red (FR) light (Reed et al., 1994).
In the light, the photoactivated (Pfr) conformer of these photoreceptors translocates into the nucleus and induces PIF destabilization, mutations in this transcription factors exhibiting a deetiolated phenotype in the dark (Leivar et al., 2008). Work aimed to characterize gibberellin (GA) function in seedling etiolation, uncovered as well an additional mechanism of regulation of PIFs transcriptional activity involving formation of an inactive complex with the DELLA repressors (de Lucas et al., 2008).
These results evidenced a key role of PIFs in the control of cell elongation, these transcription factors being involved in the diurnal rhythm of hypocotyl growht (Nozue et al., 2007) and in temperature-mediated control of seedling growth (Koini et al., 2009). In the dark, the brassinosteroid (BR) mutants *det2* and *cpd* have a de-etiolated phenotype that resembles that of seedlings grown in the light.
Whereas BR-application is able to rescue the phenotype of GA-deficient seedlings, the de-etiolation traits of BRdeficient mutant are not recued by GA treatment.
The lack of response to GAs in the BR-deficient mutants, suggest that BR are neccesary to complete the effect of GA signalling.
In this work, we analyze the regulation of PIFs activity by brassinosteroids, and build a more complete model on how the plant integrates light and hormones signalling to control growth promotion.

S10-002: THE TRANSCRIPTIONAL ACTIVATORS ZML1 AND ZML2 MEDIATE THE CRYPTOCHROME1-DEPENDENT RESPONSE TO EXCESS LIGHT IN ARABIDOPSIS THALIANA

Shaikhali, J. - Eriksson, M.J. - Barajas-Lopez, J.D. - Srivastava, V. - Wingsle, G. - Strand, Å.*
Umea Plant Science Center
**Corresponding author: jehad.shaikhali@plantphys.umu.se*

When photon fluence exceeds the photon utilization capacity of the chloroplast the production of reactive oxygen species increases, which causes photo-oxidative damage and inhibition of photosynthesis, as well as dramatic changes in nuclear gene expression.
In Arabidopsis, the cryptochrome1 photoreceptor is essential for the induction of genes encoding photoprotective components, which counteract the photooxidative damage. Using bioinformatic analysis the putative *cis*-element CryR1 (GnTCKAG) was found to be enriched in the CRY1-regulon. We demonstrate an interaction between CryR1 and the zinc finger GATA type transcription factor ZINC FINGER PROTEIN EXPRESSED IN INFLORESCENCE MERISTEM LIKE2 (ZML2). The *ZML2* protein specifically binds to the CryR1 *cis*-element but not to its mutagenized variants *in vitro* and TCTAG was shown to be the core sequence required for binding.
In addition, *ZML2* activated transcription of the YFP reporter gene driven by the CryR1 cis- element in Arabidopsis leaf protoplasts.
T-DNA insertion lines for *ZML2* and its homologue *ZML1* demonstrated not only mis-regulation of several CRY1- dependent genes in response to excess light, but also a high irradiance-sensitive phenotype with significant photoinactivation of PSII, indicated by reduced Fv/Fm and severe photobleaching.
Here we report the identification of the ZML2 and ZML1 GATA transcription factors as two essential components of the CRY1-mediated photoprotective response.

S10-003: NUCLEAR SHUTTLE OR STRESS GENE ACTIVATOR - VIP1 TIPS THE SCALES

Pitzschke, A.[1] - Djamei, A.[2] - Hirt, H.[3]*
[1]University of Natural Resources and Applied Life Sciences (BOKU)
[2]Max-Planck-Institut für terrestrische Mikrobiologie, Marburg, Germany
[3]URGV Plant Genomics, Evry, France
**Corresponding author e-mail: andrea.pitzschke@boku.ac.at*

Stress survival requires a rapid, efficient and specific signal transduction. Mitogenactivated protein kinase (MAPK) cascades play a prominent role in the early stress response. Through phosphorylation, they modify target proteins which then initiate transcriptional reprogramming and, finally, stress adaptation. The soil-borne pathogen *Agrobacterium tumefaciens* genetically manipulates numerous plant species by transferring (vir) factors and transfer DNA (T-DNA) into the plant nucleus. Transformation efficiency depends on the initial host stress status. Arabidopsis thaliana bZIP protein VIP1 (virE2-interacting protein 1) undergoes cytonuclear translocation upon phosphorylation by stress-activated MAPK3 and has been hijacked by *A. tumefaciens* as nuclear shuttle of the virE2/T-DNA complex (1). We now found that VIP1 controls target gene expression through binding to a novel stressassociated DNA motif (VRE – VIP1 response element), thus filling the gap between MAPK activation and transcriptional reprogramming in the stress response (2, 3). Due to its dual function VIP1 may be one of the factors that "tips the scales", i.e. that decides between successful transformation and failure of transformation because of elevated basal stress levels. The battle between Agrobacterium and plant therefore is to "compete" for one of the two VIP1 functions.
1) Djamei A, Pitzschke A, Nakagami H, Rajh I. and Hirt H. (2007) Trojan horse strategy in Agrobacterium transformation. Science 318
2) Pitzschke A, Djamei A, Teige M. and Hirt H. (2009) VIP1 response elements MAPK3-induced stress gene expression. PNAS 106
3) Pitzschke A and Hirt H, (2010) Mechanism of MAPK-targeted gene expression unraveled in plants. Cell Cycle 9

S10-004: TRANSCRIPTIONAL REGULATION OF GIGANTEA, A CIRCADIAN-CLOCK REGULATED FLOWERING TIME GENE IN ARABIDOPSIS THALIANA

*Berns, M. C. * - Cremer, F. - Coupland, G.*
Max-Planck-Institute for Plant Breeding Research, Cologne, Germany
**Corresponding author e-mail: berns@mpiz-koeln.mpg.de*

The initiation of flowering is a key step in the life cycle of all higher plants, marking the transition from the vegetative to the reproductive state. A key player in regulating flowering time in Arabidopsis thaliana is *GIGANTEA (GI)*, a circadian-clock regulated protein that is most abundant in the evening.
The precise timing of *GI*-expression in the evening is crucial for it to fulfil its functions during flower initiation and in the circadian clock, raising the question of how *GIGANTEA* itself is transcriptionally regulated. Using a phylogenetic shadowing approach, we identified three highly conserved Evening Elements (EEs) within the GI promoter. Mutagenesis of these EEs and subsequent characterization of stably transformed *Arabidopsis* lines revealed an altered timing of *GI* expression under various conditions, which clearly shows that EEs are functional within the *GI* promoter. Moreover, we discovered an unexpected function of EEs in regulating the transcriptional response of *GI* to cold temperatures, suggesting that the EE is a shared motif both for circadian and for temperature regulation in plants.
Currently we are investigating transcription factors that control the EE-mediated regulation of *GI* as well as the impact of EE mutations on flowering time and the plant's response to cold stress.

S11-001: A NOVEL CLATRHIN-DEPENDENT TRAFFICKING MEDIATES TYROSIN MOTIF-BASED PIN EXPORT FROM THE ENDOPLASMATIC RETICULUM

Kleine-Vehn, J. - Barbez, E.[1] - Zhang, J.[1] - Petrasek, J.[2] - Zazimalova, E.[2] - Friml, J.[1]
[1]VIB-Plant systems biology
[2]Institute of Experimental Botany, ASCR

Clathrin-coated vesicles and COP-coated vesicles are known to be the two main classes of transport vesicles in eukaryotic cells. Current models assume protein export from the endoplasmatic reticulum (ER) to be mediated solely by COPII coated vesicles, while clathrin facilitates endocytosis and post golgi trafficking.
Here, we present an unexpected clathrin-dependent trafficking mechanism in plant cells that mediates ER export of the plasma membrane (PM) proteins, PIN auxin efflux carriers. Tyrosin motifs in the cargo mediate recruitment into clathrin coated pits via interaction with clathrin adaptor protein (AP) complex. Site directed mutagenesis of the tyrosin residue in an evolutionary conserved tyrosin motif in PIN1 led to disrupted ER export. To address the requirement of the clathrin AP complex for PIN export from the ER, we investigated the μ-adaptin subunits of the clathrin APcomplex which are the main binding partners for the tyrosin motif in cargo proteins (Happel et al., 2004).
To gain further insight into the pathway, we engineered dominant negative (DN) version of all μ-adaptin homologs in *Arabidopsis*. Gain and loss of adaptin μ1-μ4 function resulted in distinct phenotypes. In particular, 35::μ1 gain-offunction lines displayed severely affected, dwarf plants with short internodes. In contrast, DN-μ1 based loss-of-function lines led to seedling lethality which precluded cell-biological investigation.
To circumvent this limitation, we transiently expressed DN-μ1 in tobacco BY-2 cell suspensions, which led to ER retention of PIN1-RFP. This effect was highly specific, because induction of other DN-μ-adaptins did not affect PIN sorting at the ER. Moreover, ER export of another plasma membrane cargo appeared to be μ1-adaptin-independent. Notably, also the interference with clathrin activity led to the specific ER retention of PIN1-RFP, indicating clathrin dependency for PIN1 export from the ER. Our data strongly suggest that PIN sorting at the ER requires adaptin μ1-dependent tyrosin motif recognition and subsequent recruitment of the clathrin coat.
These findings are in contrast with present models and illustrate that plant cells evolved, beside COPII-dependent bulk flow, a clathrin-dependent pathway for differential, cargo specific ER export.

S11-002: CHARACTERIZATION OF THE ENIGMATIC P24 PROTEIN FAMILY

Aniento, F.[1] - Montesinos, J.C.[1] - Niemes, S.[2] - Marcote, M.J.[1] - Robinson, D.G.[2]*
[1]Universidad de Valencia
[2]University of Heildelberg
**Corresponding author e-mail: Fernando.Aniento@uv.es*

In animals and yeast, p24 proteins have been proposed to act as cargo receptors in the early secretory pathway (ER-Golgi) and in the organization and/or biogenesis of the Golgi complex. To perform their functions, p24 proteins cycle between ER and Golgi via signals determining their COPII-dependent anterograde (ER-Golgi) or COPIdependent retrograde (Golgi-ER) transport. The p24 family can be subdivided into four subfamilies (α, β, δ, γ) by sequence homology. Intriguingly, plants contain only representatives of the δ and β subfamilies. We have previously shown that an Arabidopsis p24 protein of the δ subfamily (Atp24δ5) localizes exclusively to the ER (in contrast with their mammalian counterparts, which localize mainly to the ERGIC and the Golgi), via a dilysine motif in the C-terminal cytosolic tail which interacts with the COPI coat.
We therefore proposed that plant p24 proteins could perform plantspecific functions, since plants lack ERGIC –an intermediate compartment between ER and Golgi-. We have now analyzed the relative contribution of the luminal (cargobinding and coiled-coil) domains as well as the cytosolic tail to the in vivo trafficking and localization of Atp24δ5. We have also investigated the in vivo trafficking and localization of members of the beta subfamily, as well as the interaction between
members of both subfamilies.

S11-003: ARABIDOPSIS EXOCYST COMPLEX IS INVOLVED IN CYTOKINESIS

Fendrych, M. - Synek, L. - Pecenkova, T. - Žárský, V.*
Institute of Experimental Botany, ASCR
**Corresponding author e-mail: fendryc1@natur.cuni.cz*

During its division, the plant cell must distribute genomes, organelles and endomembranes between daughter cells and later reestablish information flow across the cells. Secretory pathways are crucial for cytokinesis, the final stage of cell division.
From the cell plate initiation to the maturation of the new cell wall, delivery of secretory vesicles is necessary to sustain successful daughter cell separation. In eukaryots, secretory vesicles are tethered to plasma membrane by the exocyst, a heterooligomeric protein complex.
Using GFP fusions we observed localization maxima of exocyst subunit EXO84b in stages of cytokinesis with possibly highest demand for vesicle fusion – at the cell plate initiation and at the moment of cell plate contact with the maternal plasma membrane and cell plate maturation.
In seveely dwarfed mutants of *Arabidopsis* EXO84b, leaf epidermis cell division and morphogenesis are compromised. Our results indicate that *Arabidopsis* exocyst is involved in cytokinesis and further support concept of evolutionary relatedness between plant and animal cytokinesis.

S11-004: A NEW PROTEIN TRANSPORT PATHWAY BETWEEN THE ENDOPLASMIC RETICULUM AND THE CHLOROPLAST IS LINKED TO LIPID TRAFFICKING IN ARABIDOPSIS

Martinez, A. - Ortega-Villasante, C. - Martínez, F. - Villarejo, A.*
Universidad Autónoma de Madrid
**Corresponding autor e-mail: andrea.martinez@estudiante.uam.es*

Recently, firm evidence for a chloroplast protein transport pathway involving the endoplasmic reticulum (ER) and Golgi in *Arabidopsis thaliana* was presented. Proteins following this route are N-glycosylated, harbouring complex type N-glycans. Preliminary results also showed the occurrence of chloroplast N-glycoproteins containing high-mannose type N-glycans (HMGP) in *A. thaliana.*
This type of N-glycans is exclusively acquired in the ER and would imply a direct transport of proteins from this compartment to the chloroplast, avoiding passage through Golgi.
A direct trafficking pathway between ER and chloroplast has been only described for the transport of lipid precursors. This pathway has been genetically dissected and most of its components have been identified. In addition, direct contact sites between ER and chloroplast (PLAMs) have been previously described.
Mutants defective in different steps of the lipid transport pathway have been used to study whether transport of HMGP is linked to transport of lipid precursors. Our results show that lack of TGD4, -an ER protein involved in lipid transport and potentially in PLAM formation-, also disrupts HMGP trafficking. This suggests that functional PLAMs would be required not only for lipids but also for protein transport. Our data indicate that HMGP would be translocated across the chloroplast envelope through a translocon system, which differs from that used by lipid precursors. In order to find a model protein for characterization of this pathway, around 20 stroma HMGPs have been purified, using a method based on their intrinsic characteristics, such as the affi-

nity of their N-glycans to the lectin Concanavalin A. Data from mass spectrometric and biochemical analysis of these proteins will be presented.

S12-001: CAN DNA METHYLATION EXPLAIN THE ROLE OF DOSAGE-SENSITIVE GENES IN POLYPLOID PHENOTYPES IN ARABIDOPSIS?

Zielinski, M.[1] - Donoghue, M.[1] - McKeown, P.[1] - Clifton, R.[2] - Spillane, C.[2]*
[1]Genetics and Biotechnology Lab, Botany and Plant Science, National University of Ireland Galway
[2]Genetics & Biotechnology Lab, Dept of Biochemistry & Biosciences Institute, University College Cork, Cork, Ireland
**Corresponding author e-mail: marielui@yahoo.de*

Although more than 70% of flowering plants have undergone polyploidy events during their evolutionary history many aspects of how polyploidy affects regulation of genes and genomes still require elucidation. To address this, we investigated the relative importance of genome dosage and epigenetic control using a series of autopolyploid Arabidopsis plants with ploidy levels of 2n, 3n and 4n. The dosage series included triploids generated with excess of both maternal and paternal genomes, additionally allowing us to study phenotypes associated with cross direction. High levels of phenotypic variation were observed among the F1 offspring generated from interploidy crosses including effects on seed size, germination rate, cell size, and heterochromatin structure. To link these observations to transcrioptom changes, we used the Arabidopsis AtSNPtile1 platform. This suggested that the most Arabidopsis genes were unaffected by altered ploidy level. However, a small subset were shown to display wide ranging genome dosage effects. Analysis of the expression levels of these genes in reciprocal triploids shows that these changes may be either dependent or independent of cross direction. Finally, to identify expression changes with epigenetic control via DNA methylation pathways, we analysed our polyploids and consider the extent of CG methylation and methylation polymorphism to relate these data to current theories of inheritance of methylation in patterns in autopolyploid plants.

S12-002: HISTONE MODIFICATIONS IN RNA-DIRECTED TRANSCRIPTIONAL GENE SILENCING

Kuhlmann, M. - Schmidt, R. - Mette, M.F.*
Leibniz Institut für Pflanzengenetik und Kulturpflanzenforschung
**Corresponding author e-mail: kuhlmann@ipk-gatersleben.de*

RNA-directed transcriptional gene silencing (RdTGS) of transgenes provides a versatile experimental system for the study of epigenetic gene regulation in plants. In our experimental setup in *Arabidopsis thaliana*, transcription of a promoter-inverted repeat construct provides an RNA signal that can trigger transcriptional gene silencing (TGS) and *de novo* methylation of a homologous nopaline synthase promoter (pNOS) *in trans*. Target transgenes integrated at different positions of the genome show different silencing efficiency. Generally, the degree of silencing is correlated with proportional levels of DNA methylation in the pNOS region. 3 target transgenes with different properties (strong silencing with strong DNA methylation, weak silencing with weak DNA methylation, weak silencing with substantial DNA methylation) were selected for quantitative analysis of histone modification changes in reaction to the silencing signal. They showed a positive correlation between levels of DNA methylation and H3K9me2 and H3K27me2 at the target pNOS. In a reverse-genetic screen for factors involved in RNA-directed *de novo* DNA methylation at endogenous AtSN1 sequences, we could identify putative histone methyltransferases SUVH2 and SUVH9. Analysis of histone modifications of several regions of an AtSN1 locus in wild type and mutant plants revealed a correlation of increased H3K9me2 and H4K20me1 with the presence of DNA methylation.

We found that these modifications can be correlated with accumulation of RNA homologous to the analysed region. From this we can conclude that SUVH2 and SUVH9 are factors involved in TGS.

S12-003: CIS-REGULATORY ELEMENTS AND CHROMATIN STATE CO-ORDINATELY CONTROL TEMPORAL AND SPATIAL EXPRESSION OF FLOWERING LOCUS T

Farrona, S. - Adrian, J. - Reimer, J.J. - Albani, M.C. - Coupland, G. - Turck, F.*
Max Planck Institute for Plant Breeding Research
**Corresponding author e-mail: farrona@mpiz-koeln.mpg.de*

FLOWERING LOCUS T (FT) gene is one of the main regulators of flowering in Arabidopsis. It has been shown that *FT* encodes the systemic florigen signal and integrates different environmental response pathways, such as those involved in temperature and photoperiod perception.
FT transcription has to be tightly regulated in the leaf veins to ensure that flowering occurs at the right moment. Different transcription factors and chromtin related proteins have been involved in *FT* regulation, indicating the complexity of *FT* control. However, the cis-regulatory sequences required for *FT* regulation and how these elements are integrated in a chromatin context is still not well understood. We have found a proximal and a ≈5 kb upstream *FT* promoter region containing evolutionary conserved blocks.
These sequences that are depleted for the chromatin mark H3K27me3, are essential for *FT* activation by CONSTANS (CO). In plants constitutively overexpressing CO, changes in chromatin status, such a decrease in binding of LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) and increased acetylation of H3K9 and K14, were observed throughout the *FT* locus, although these changes appear to be the consequence and not the cause of *FT* activation. In addition, binding of LHP1 was required to repress enchancer elements located between the COcontrolled regions, which show a more "open" chromatin configuration that could facilitated the accesibility of transcription factors to *FT*. In summary, our data show that far located regulatory regions are essential for *FT* transcription and that chromatin mediated *FT* repression is involved in fine-tunning rather that cementing the expression state of *FT*.

S12-004: DNA METHYLATION AND EXPRESSION OF MET1 ARE REGULATED DURING POLLEN DEVELOPMENT AND POLLEN REPROGRAMMING TO EMBRYOGENESIS

Testillano, P. - Solís, M.T. - Rodríguez-Serrano, M. - Risueño, M.C.*
Consejo Superior de Investigaciones Científicas, CSIC. Centro de Investigaciones Biológicas, CIB
**Corresponding author e-mail: testillano@cib.csic.es*

In this work we have analyzed the dynamics of DNA methylation patterns, as defined epigenetic mark of the chromatin functional state, in relation to nuclear architecture during pollen development and the stress-induced pollen reprogramming to embryogenesis, in *Brassica napus* L and *Nicotiana tabacum* L, as well as during the development and programmed cell death (PCD) of tapetum, the nursing tissue of pollen. Quantification of global DNA methylation by high performance capillary electrophoresis (HPCE) and immunofluorescence of 5-methyl-citidine (5mdC) and confocal analysis were performed at different developmental stages. Expression of *MET-1* DNA methyl-transferase, one of the enzymes responsible of DNA methylation, was analyzed by semiquantitative RT-PCR and fluorescence in situ hybridization (FISH) on semithin cryosections.
Results showed that DNA methylation increased during the process of pollen maturation and tapetum PCD whereas methylation decreased after pollen reprogramming to embryogenesis.

An increase in DNA methylation was found at later stages of pollen embryogenesis, accompanying differentiation. *BnMET-1* expression was developmentally regulated in both pollen pathways, being upregulated during pollen maturation and tapetum PCD, and downregulated with the switch to embryogenesis. This report showed new evidences of changes in DNA methylation that accompany the reorganization of the nuclear architecture during plant cell differentiation, proliferation and PCD, giving new insights in the knowledge of the epigenetic control of plant development.

Work supported by MICINN projects BFU2008-00203 and AGL2008-04255.

.

S13-001: TREHALOSE METABOLISM AND SUGAR SIGNALLING IN PLANTS

Lunn, J. - Feil, R. - Yadav, U. P. - Martins, M. - Ivakov, A. - Krause, U. - Wahl, V. - Stitt, M.*
Max-Planck-Institute of Molecular Plant Physiology
**Corresponding author e-mail: lunn@mpimp-golm.mpg.de*

Trehalose is a disaccharide sugar that is commonly found in bacteria, fungi and insects, where it can function as a compatible solute, storage reserve, transport sugar or stress protectant. Trehalose was once thought to be rare in higher plants, but genomic and other sequence data revealed that the capacity to synthesise trehalose is present throughout the plant kingdom.

Mutants and transgenic plants with altered trehalose metabolism show pronounced morphological phenotypes, which are linked to changes in the level of trehalose 6-phosphate (Tre6P), the intermediate of trehalose synthesis, rather than to trehalose itself. Using a new mass spectrometry-based assay, we found that the amount of Tre6P in plant tissues reflects changes in the level of sugars, particularly sucrose, leading us to propose that Tre6P acts as a signal of sucrose status [Lunn *et al*. (2006) *Biochem.* J. 397; 139–148]. We are now investigating both the upstream and downstream pathways of Tre6P signalling in plants.

Inhibitor studies showed that protein synthesis is required for the response of Tre6P to changes in sucrose content, and that phosphorylation and turnover of proteins could also be involved. Inducible over-expression of trehalose-phosphate synthase in *Arabidopsis thaliana* showed that night-time degradation of starch in leaves is sensitive to increased levels of Tre6P.

This indicates that Tre6P could form part of a feedback loop linking starch turnover in the leaves to demand for sucrose from sink organs. We are also testing the hypothesis that Tre6P acts as a signal of sucrose status in meristems and organ primordia, thus tying the growth and development of the plant to the availability of sucrose from the leaves.

S13-002: DOWNREGULATING SUBERIN BIOSYNTHETIC ENZYMES TO BETTER UNDERSTAND SUBERIN STRUCTURE AND PERIDERM PHYSIOLOGY.

Serra, O. - Soler, M. - Molinas, M. - Figueras, M.*
University of Girona
**Corresponding author e-mail: olga.serra@udg.edu*

Periderm develops to protect mature stems, roots and tubers from dehydration and pathogens. It is composed of phellem or cork layer at the external side, phellogen or mother layer and phelloderm. The phellem cell walls are impermeable to water due to the deposition of suberin, a complex polymer made of an aliphatic domain linked to an aromatic domain. The aliphatic suberin is a polyester of fatty acids and derivatives (C16-C30), glycerol and ferulic acid. Although the chemical composition of suberin is widely known, the synthesis and polymerization of its aliphatic monomers and their contribution to the barrier function is poorly understood.

To shed light in this subject, we constructed a phellem SSH library that yielded a list of suberin candidate genes (1) and developed a strategy to analyze their role using a reverse genetic approach. Three genes encoding key enzymes for aliphatic suberin biosynthesis have been characterized: a fatty ω-hydroxylase (P450) (2), a fatty acid elongase (3) and a BAHD acyl transferase (4). For each gene, the composition, ultrastructure and water permeability of tuber periderm has been analyzed in downregulated potato plants. Altogether, the results reveal the importance of suberin composition in the ultrastucture and physiological function of periderm.

(1) P Phys 2007, 144:419;
(2) P. Phys.2009, 149:1050;
(3) J Exp Bot 2009) 60:697;
(4) Plant J 2010, doi: 10.1111/j.1365-313X.2010.04144.x.

S13-003: THE KEY ROLE OF FATTY ACID DESATURATION IN THE RESPONSE TO ENVIRONMENTAL FACTORS AND IN THE AROMA BIOGENESIS OF OLIVE AND TOMATO FRUITS

Hernandez, L.[1] - Padilla, M.[1] - Domínguez, T.[2] - Sanmartin, M.[2] - Sanchez Serrano, J. J. [2] - Sanz, C.[1] - Martinez-Rivas, J.M .[1]*
[1]Instituto de la Grasa (CSIC)
[2]Centro Nacional de Biotecnologia (CSIC)
**Corresponding author e-mail: mlhdez@cica.es*

The lipoxygenase (LOX) pathway is responsible for the biosynthesis of oxylipins and its involvement in different plant physiological processes such as stress resistance and aroma biogenesis has been long demonstrated.

The first step of this pathway is catalyzed by LOXs enzymes that produce a fatty acid hydroperoxide, derived from the corresponding fatty acid. These hydroperoxides are subsequently metabolised through a number of potentially competing pathways to generate a wide array of oxylipins.

Linoleic and linolenic acid, the main precursors of the LOX pathway, are synthesized from oleic acid by the consecutive action of ω-6 and ω-3 desaturases. In plants, two sets of these enzymes have been reported; one is located in the endoplasmic reticulum (FAD2 and FAD3) and the other in chloroplastic membranes (FAD6 and FAD7/8). In this work, we have studied in olive fruit the effect of different environmental stress situations such as low and high temperature, dark exposure and wounding, on the fatty acid composition and on the expression levels of *FAD2, FAD3, FAD6* and *FAD7/8*, and *LOX* genes. The analytical and expression data indicate that some of these genes are coregulated at the transcriptional level.

On the other hand, we have over-expressed the ω-3 desaturases FAD3 and FAD7 in tomato. Fruits and leaves of transgenic tomato plants exhibit a modification not only in the fatty acid composition by increasing the linolenic/linoleic acid ratio, but also in the aroma profile with a significant alteration of the (*Z*)-hex-3-enal/hexanal ratio. The results obtained from both approaches point out the involvement of fatty acid desaturation in the response to environmental factors and in the aroma biogenesis of fruits.

S13-004: DIGITALIS PURPUREA p5βr2 IS A NEW STEROID 5β- REDUCTASE THAT BELONGS TO THE NOVEL SDR75R FAMILY

Tuñón, I.[1] - Ferrer, S.[2] - Moya García, A.[3] - Pérez-Bermúdez, P.[4]- Tarrío, R.[5] - Rodríguez-Trelles, F.[6] - Gavidia, I.[4] -
[1] Dept. Química Física, Univ. Valencia, Burjassot, Spain
[2]Dept. Química Física I Analítica, Univ. Jaume I, Castellon, Spain
[3]CIBER de Enfermedades Raras, Dept. Biología Molecular y Bioquímica, Univ. Málaga. Spain
[4]Dept. Biología Vegetal, Univ. Valencia, Burjassot, Spain
[5]Grupo de Medicina Xenómica–CIBERER, Univ. Santiago de Compostela, Spain
[6]Dept Genètica i Microbiologia, Univ. Autonòma de Barcelona, Bellaterra, Spain

Progesterone 5β-reductase (P5βR) catalyzes the 5β-reduction of progesterone to 5β-pregnan-3,20-dione, and is considered the first committed step in the branch pathway leading to cardenolides (1), plant metabolites widely used in patients with compromised cardiac function. ere we describe the characterization of a new clone, designated P5βR2.
A database search revealed no significant homology to other proteins than those corresponding to P5βR. P5βR is conserved throughout the plant kingdom (1) whereas P5βR2 is restricted to some species. Like P5βR, the recombinant form of P5βR2 can catalyze the reduction of several steroids with a 3-oxo,$\Delta^{4,5}$ structure; the highest substrate specificity was obtained with progesterone (3).
A primary structural analysis of the P5βR2 protein revealed the presence of several conserved sequences of the short chain dehydrogenases/reductases (SDR) as well as the novel motifs specific for a new family represented by P5βR as a prototype (1). A structural model of P5βR2 and a feasible reaction mechanism of this protein are depicted. A functional subdivision of the SDR superfamily has been recently established (2). P5βR family belongs to a different type of SDRs distinguished by sequence patterns at the active site region; we propose to be named “Restricted SDR type”, and according to (2) the family designation is SDR75R (3). Finally, a comparative structural analysis has been carried out by means of Molecular Dynamics simulations on the holo form (including the substrate progesterone and the cofactor NADPH) of P5βR and P5βR2.
1. Gavidia I et al. *Phytochemistry* 2007, 68: 853-864.
2. Persson B et al. *Chem Biol Interact* 2009, 178: 94-98.
3. Pérez-Bermúdez P et al. *New Phytol* 2010, 185: 687-700

S14-001: PRINCIPAL FACTORS CONTROLLING GREENHOUSE FLUXES IN EVERGREEN OAK FOREST OF SOUTHERN PORTUGAL

Shvaleva, A. - Lobo do Vale, R. - Cruz, C. - Castaldi, S. - Rosa, A.P. - Chaves, M.M. - Pereira, J.S.*
Instituto de Tecnologia Quimica e Biologica, Universidade Nova de Lisboa, Oeiras, Portugal
**Corresponding author e-mail: shvaleva@itqb.unl.pt*

Soil water content, soil temperature, pH, ammonium and nitrate concentrations were studied over one year in an evergreen oak forest to examine the principal factors controlling greenhouse gases (GHGs) emission, namely CO_2, CH_4 and N_2O fluxes in Mediterranean-type ecosystems of southern Portugal.
To characterize the seasonal variations in gas fluxes and to examine the effect of treatments, i. e. simulated rainfall (water-addition) and rain-fall exclusion on GHG fluxes, a static chamber technique was used.
Although we did not detect statistically significant effect of treatments, our results showed that soil moisture and soil temperature are important variables controlling soil CO_2 fluxes in Mediterranean forest ecosystems.
Soil respiration (CO_2 fluxes) showed a strong increase from summer to autumn. This must be the “Birch effect”, which describes increases in soil heterotrophic respiration as a result of stimulation of microbial activity and of structural alterations in soil micro- and macro-aggregates following autumn rains. Our results also showed that the soil was a consistent CH_4 sink independently of the soil water content in the range between 6-20%, and supported the concept that seasonally dry ecosystems (Mediterranean) are a significant sink of atmospheric CH_4.
We hypothesized that in evergreen-forest ecosystems of southern Portugal the biological oxidation of atmospheric CH_4 takes place by methanotrophic microorganisms in presence of low soil ammonium and nitrate contents.

S14-002: WHERE HAS ALL THE CARBON GONE? SEASONAL AND NUTRIENT EFFECTS ON CARBON ALLOCATION IN SCOTS PINE

Campbell, C. [1] - Keel, S.[2] - Metcalfe, D.[3] - Högberg, M. N.[3] - Linder, S.[3] - Högberg, P.[3] - Nasholm, T.[3] - Hurry, V.[4]*
[1]SLU
[2]Princeton University
[3]Swedish University of Agricultural Research
[4]Umeå University
**Corresponding author e-mail: catherine.campbell@plantphys.umu.se*

It is becoming increasingly essential that we understand the carbon balance of whole ecosystems. Nutrient uptake and mycorrhizal symbionts are a significant carbon sink in the field, and carbon flux is highly seasonal, making field trials an essential component in understanding the global carbon balance.
Elevated CO_2 may increase overall carbon uptake, but environmental and developmental factors will determine the allocation and thus ultimate fate of that carbon. Using a ^{13}C pulse-chase labelling technique, we determined the flow of carbon through intact stands of fertilized and unfertilized *Pinus sylvestris*. We compared carbon allocation early (June) and late (August) in the growing season, and made a comparison of allocation in fertilised and unfertilised plots.
Carbon allocation belowground was very low early in the growing season, when most carbon was allocated to growing shoots, and much higher near the end of the season. This late season belowground allocation was greatly reduced by one year of nitrogen fertilization.
Overall carbon uptake was increased by the fertilization treatment, so reduced allocation to roots resulted in a very large fraction of carbon remaining aboveground, in wood and storage to support growth in early spring. This reduction in belowground allocation may mean a reduction in carbon sequestration in belowground biomass and soils under nitrogen deposition, while forest growth and wood production are increased.

S14-003: THE ROLE OF C “MANAGEMENT” ON RESPONSIVENESS OF SHRUBS AT THE NEVADA DESERT FACE FACILITY

Aranjuelo, I.[1] - Clark, NM.[2] - Ebbets, A.L.[3] - Evans, R.D. - Smith, S.D.[3] - Nogués, S.[1] - Nowak, RS.[2]*
[1]University od Barcelona
[2]Department of Natural Resources and Environmental Science, University of Nevada Reno, USA
[3] School of Life Sciences, University of Nevada Las Vegas, Las Vegas Nevada, USA
[4]School of Biological Sciences, Washington State University, USA
**Corresponding author e-mail: iaranjuelo@yahoo.es*

The effect of environmental growth conditions on C source/sink balance of two desert shrubs (*Larrea tridentata* and *Ambrosia dumosa*) exposed to elevated [CO_2] (average 521 µmol mol-1 versus average ambient [CO_2] of 376 µmol mol- 1) was examined at the undisturbed Nevada Desert FACE Facility (NDFF).
We took advantage of differences in isotopic 13C/12C composition ($\delta^{13}C$) of air above elevated CO_2 plots ($\delta^{13}C$ ca -18.2‰) *versus* that above ambient plots (ca. - 8.0‰) to investigate C allocation and partitioning. C labeling analyses confirmed that during the summer dry season, decreases in leaf photoassimilate accumulation could have been caused by the translocation (especially in *Ambrosia*) of C compounds from leaves to roots (and probably main stems).
Total soluble protein and N concentration data suggest that the lack of elevated [CO_2] stimulation of photosynthetic activity during the primary spring growing season was explained by the depletion of protein content in elevated [CO_2] treatments, which was a result of carbohydrate build-up and a reallocation of nitrogen away from leaves to other processes more limiting for growth.
The fact that environmental conditions (drought and elevated temperature) during the summer decreased photosynthetic activity and induced the senescence of leaves in the deciduous *Ambro-*

sia implies that photoassimilate concentration diminished and, consequently, no CO_2-associated differences on photosynthetic rates were observed.
Consequently, this study revealed that the responsiveness of desert shrubs to elevated [CO_2] is conditioned by environmental effect on the capacity of these desert shrubs to export C away from leaves with the consequent balance in leaf C sink/source and the overcoming photosynthetic acclimation.

S14-004: THE RESPONSE OF PLANTS IN AN OLIGTOTROPHIC SEMI-NATURAL GRASSLAND TO LONG-TERM CO2 ENRICHMENT

*Winkler, J.B.**
Helmholtz Zentrum München, Institute of Biochemical Plant Pathology, Dept. of Environmental Engineering
**Corresponding author e-mail: bwinkler@helmholtz-muenchen.de*

Since 1999, the long-term responses of a semi-natural grassland community to elevated CO_2 concentration and different cutting regimes were investigated.
The grassland was exposed to either ambient or elevated CO_2 concentration (600 ppm) using Free-Air CO_2 Enrichment (FACE) technique. There was no artificial irrigation and no fertilisation. The above-ground biomass was harvested once or twice per season. The grassland is composed of a high number of different species representing different functional plant groups regarding morphological and physiological characteristics.
Under elevated CO_2 concentration, annual community biomass production was stimulated significantly for the first time after four years of investigation and in 2003 when precipitation was only about 60 % of average precipitation the effect of CO_2 enrichment was highly significant. Functional plant groups responded differently to CO_2 enrichment causing a clear shift in botanical composition since 1999 towards a higher proportion of legumes under elevated CO_2 concentration. In this nutrient-poor grassland community, the higher competitiveness of the legumes under CO_2 enrichment might be due to their ability of symbiotic nitrogen fixation. However, varying climatic conditions may superimpose the response of the plants to the CO_2 enrichment.

S17-001: THE ROLE OF ARGONAUTE PROTEINS IN CONSTITUTIVE AND INDUCED ANTI-VIRAL RESPONSES

*Moffett, P.**
Université de Sherbrooke
**Corresponding author e-mail: peter.moffett@usherbrooke.ca*

To successfully infect a plant, a virus must usurp the cell host machinery and overcome plant defense mechanisms. A major mechanism of constitutive antiviral immunity is ensured by RNA silencing which relies on the recognition and degradation of viral double-stranded RNA into virus-derived small RNA (vsRNA) by DICER-like enzymes.
Once incorporated into complexes containing members of the Argonaute (Ago) family of endonucleases, these vsRNA act as guides to target viral RNA for degradation or inhibition of translation. Induced resistance to viruses is also afforded by the products of plant disease resistance (*R*) genes encoding NB-LRR proteins. Using several viruses, including potato virus X (PVX), we have investigated the role of different Ago family members in both constitutive and induced anti-viral defenses. We find that induced during induced anti-viral responses PVX RNAs are generated but virus-encoded proteins do not accumulate and viral RNAs do not associate with ribosomes. Furthermore, this response was dependant on Ago4-like proteins, but not Ago1-like proteins.
These results suggest that NB-LRR proteins induce Ago-dependant anti-viral mechanisms that specifically inhibit the translation of viral transcripts. At the same time, we find that constitutive defenses against PVX are directed by members of the Ago7/Ago2 family, which represents a different clade from Ago4-like proteins. Our results indicate a specialization in Ago function during different types of anti-viral defenses and provide new insights into the mechanisms of induced defense responses.

S17-002: TYPE I METACASPASES CONTROL CELL DEATH IN ARABIDOPSIS

Sanchez Coll, N.[1] - Vercammen, D.[2] - Smidler, A.[1] - Van Breusegem, F.[2] - Epple, P.[1] - Dangl, J.L.[1]*
[1]University of North Carolina at Chapel Hill
[2]Ghent University
**Corresponding author e-mail: nuria@email.unc.edu*

Programmed cell death is essential for plant survival and development. Ten years ago metacaspases were proposed by homology modeling to be distant homologues of animal caspases. The genome of *Arabidopsis thaliana* encodes nine putative metacaspases (AtMCs), which can be classified into two groups: type I metacaspases (AtMC1, AtMC2 and AtMC3) contain an LSD1-like zinc finger prodomain, whereas type II metacaspases (AtMC4-9) lack any prodomain.
Metacaspases are thought to be positive regulators of cell death, while the zinc finger protein LSD1 has been shown to negatively regulate cell death and disease resistance.
Our data demonstrate that two type I metacaspases, AtMC1 and AtMC2, antagonistically control cell death in Arabidopsis. AtMC1 acts as a positive regulator of cell death requiring caspase-like catalytic residues for its function. Activation of AtMC1 is complex and developmentally regulated. AtMC1 is required for the cell death that accompanies successful innate immune responses mediated by intracellular NB-LRR receptor proteins. The regulatory function of both AtMC1 and AtMC2 is enhanced by removal of the putative prodomain similar to the activation mechanism of some animal caspases.The inhibitory function of AtMC2 does not require classic cysteine catalytic residues. This is reminiscent of the mode of action of animal caspase-12, which negatively regulates caspase-1, dampening the inflammatory response to bacterial infection. Caspase-12 also inhibits NOD-like receptor-mediated innate immunity, in line with our observation that AtMC2 inhibits the analogous plant innate immune receptors. Our data suggest an ancient link between cell death control and innate immune receptor function.

S17-003: REGULATION OF IMMUNITY AND PLANT DEVELOPMENT BY OXYLIPINS

Castresana Fernández, C.[1] - Vellosillo, T.[1] - Martinez, M.[1] - Bennemberg, G.[1] - Vicente, J.[1] - Lopez, M. A.[1] - Kulasekaran, S.[1] - Cascon, T.[1] - Hamberg, M.[2]*
[1]Centro Nacional de Biotecnología-CSIC, Madrid, Spain
[2]Karolinska Institutet, Sweden
**Corresponding author e-mail: ccastresana@cnb.csic.es*

Oxylipins are a big family of lipid derivatives synthesized from fatty acids through complex enzymatic pathways initiated by the action of lipoxygenases (9-LOX and 13-LOX) and alpha-dioxygenases (alpha-DOX).
Production of oxylipins can also occur non-enzymatically by singlet oxygen or free radicals oxidation of fatty acids. The importance of the 13-LOX pathway in the defense against necrotrophic pathogens and plant fertility, have been demonstrated. However, the biological function of most oxylipins, especially those from the 9-LOX and alpha-DOX branches, remains poorly understood. Functional analyses of these pathways indicated their participation in plant defence and developmental responses through the activation of specific signalling pathways. Results in Arabidopsis thaliana with the 9-lipoxygenase genes, LOX1 and LOX5, indicated that 9- lipoxygenase-derived oxylipins are both modulators of root architecture and part of the defence mechanisms against pathogen attack. In addition, studies with alpha dioxygenases, revealed the implication of alpha-DOX1 in controlling the cell

death response associated with the activation of a hypersensitive defence reaction. In contrast, the alpha DOX2 protein is not involved in defence but it plays a role in plant development. Mutation of the tomato alpha DOX2 gene caused severe developmental alterations, whereas deletion of alpha-DOX2 in Arabidopsis did not provoke any visible alteration. The phenotype of the tomato mutant was complemented by transgenic expression of the α-DOX2 genes from both tomato and Arabidopsis indicating the functional exchangeability of both proteins and that the relative importance of α-DOX2 in plant physiology is species-specific.

S17-004: THE BENEFICIAL INTERACTION BETWEEN THE ROOTCOLONIZING ENDOPHYTE PIRIFORMOSPORA INDICA AND ARABIDOPSIS

Oelmüller, R.
Institute of General Botany and Plant Physiology, Friedrich-Schiller-University of Jena, Germany

Piriformospora indica, a wide-host root-colonizing fungus, promotes growth, biomass and seed production and confers resistance against various biotic and abiotic stresses. We used a genetic approach to identify mutants which do not respond to the fungus. Activation of several defence mechanisms is required to restrict root colonisation. Early recognition of the two symbionts requires an extracellular MATH protein and an atypical receptor kinase located in the plasma membrane. Downstream signalling is entirely dependent on cytoplasmic calcium elevation. Furthermore, a central target of the fungus in Arabidopsis is the sulphur metabolism. I will discuss novel data on these topics.

S18-001: REGULATION OF FE ACQUISITION AND HOMEOSTASIS

*Bauer, P.**
Saarland University
**Corresponding author e-mail: p.bauer@mx.uni-saarland.de*

Progress has been made in identifying the players of Fe homeostasis in plants. In Arabidopsis, Fe acquisition is controlled by FIT, a bHLH transcription factor. FIT positively regulates the expression of *FRO2*, a gene coding for an iron reductase as well as *IRT1*, coding for the major transporter responsible for iron uptake into roots. FIT is regulated not only on transcriptional level but also on post-translational level.
To study post-translational control we constructed targeted FIT lines which allowed us to study the involvement of signal molecules on FIT regulation. Since differential protein interactions may also account for regulation at protein level we have aimed at identifying putative FIT interaction partners. Two FIT protein interactors were identified that are central regulators in hormone signalling responses. Knockout mutant lines, overexpression lines and crosses were analyzed to elucidate the role of these protein interactions in the iron deficiency response process.
Finally, we present new results on long-distance transport of Fe such as mediated by nicotianamine, a chelator of metals.

S18-002: SELECTIVE (DIS)ADVANTAGES TO MAINTAIN A MUTANT TRANSPOSON-INSERTION ALLELE OF THE METAL HOMEOSTASIS NICOTIANAMINE SYNTHASE 1 GENE IN A NATURAL THLASPI CAERULESCENS POPULATION

Ya-Fen, L.[1] - Nguyen, D.[1] - Cheng, X.[1] - Schat, H.[2] - Aarts, M.G.[1]*
[1]Laboratory of Genetics, Wageningen University, the Netherlands
[2]Institute of Molecular and Cellular Biology, Vrije Universiteit Amsterdam, the Netherlands
**Corresponding author e-mail: ya-fen.lin@wur.nl*

Thlapi caerulescens, a Zn/Ni/Cd hyperaccumulator, is a good model to study the evolution of heavy-metal hyperaccumulation and tolerance in plants. Compared to related metal non-accumulator species, several metal homeostasis genes show enhanced expression, including the *Nicotianamine Synthase (NAS)* genes. NAS activity is needed for the production of nicotianamine (NA), a metal chelator essential for proper distribution of Fe and Cu through the plant. Altered NAS function will also affect Zn, Mn and Ni homeostasis. Out of the four *NAS* genes found in *T. caerulescens*, *NAS1* shows the highest expression, predominantly in shoots. Despite the apparent importance of this gene, we identified a transposon insertion mutant *nas1* allele in a natural population of *T. caerulescens* growing at zinc mine smelter deposits around Plombières (Belgium). The transposon disrupts the normal reading frame and although the gene can be transcribed, the protein will be terminated prematurely at a.a. position 216 instead of 323.
The insertion appears to be stable, and both transposon and wild-type alleles are found in the population, suggesting either a neutral effect on fitness or balancing selection. When tested for metal tolerance, isogenic *nas1/nas1* transposon mutants are clearly more sensitive to Fe deficiency and Ni or Cd exposure than *NAS1/NAS1* wild type plants. In addition, they accumulate more Fe, Ni and Cd in their aboveground parts. The implications which this mutation will have on fitness will be discussed.

S18-003: O-CARBOXYL- AND N-METHYLTRANSFERASES ACTIVE ON PLANT AQUAPORINS

Sahr, T. - Adam, T. - Fizames, C. - Maurel, C. - Santoni, V.
SupAgro/INRA/CNRS/UM2

A mass spectrometry approach revealed that the N-terminal tail of *At*PIP2;1, a plasma membrane aquaporin of plants, is methylated. Using bioinformatical and biochemical screens, we identified two methyltransferases of *A. thaliana*, SDG7 (At2g44150) and OMTF3 (At3g61990), as acting on AtPIP2;1 Lys3 and Glu6, respectively. The two enzymes are associated to the endoplasmic reticulum. An *in vitro* assay using various AtPIP2;1 N-terminal peptides as a bait allowed to characterize the enzymatic properties of recombinant enzymes that showed apparent KM values for their substrates, S-adenosyl-methionine and peptide, in the range of 5-8 μM and 2-9 μM, respectively. SDG7 almost exclusively monoor di-methylated Lys3. OMTF3 specifically methylated Glu6, this methylation being dependent on the methylation profile of the neighboring Lys3 residue. This study allows to characterize the first methyltransferases acting on plant transmembrane proteins. It may be critical to identify new regulatory mechanisms of membrane transport.

S18-004: COMPARTMENTATION OF ALUMINIUM IN LEAVES OF TEA PLANTS (CAMELLIA SINENSIS L.) GROWN IN HYDROPONICS: A LEXRF SPECTRO-MICROSCOPY STUDY

Poschenrieder, C. [1] - Vogel-Mikus K.[2] - Tolra, R.[1] - Hajiboland, R.[3] - Kump, P.4 - Pongrac, P.[2] - Kaulich, B.5 - Gianoncelli, A.5 - Babin, V.5 - Barceló, J.[1] - Regvar, M.[2]*
[1]Universidad Autónoma de Barcelona
[2]University of Ljubljana
[3]University of Tabriz, Iran
[4]Jozef Stefan Inst. Ljubljana, Slovenia
[5]Elettra–Sincrotrone Trieste, Italy
**Corresponding author e-mail: charlotte.poschenrieder@uab.es*

Characterization of Al localization and speciation in tea leaves is of fundamental interest for a better understanding of both the high Al tolerance in this Alaccumulating plant and the bioavailability of the high Al concentrations in teabased beverages. Here low-energy X-ray fluorescence (LEXRF) spectromicroscopy was used for localizing Al and other elements (C, O, Mg, Si and P), in fully developed leaves of tea plants. (*Camellia sinensis* L.). Plants were grown from seeds and pre-cultured for 3 months in nutrient solution without Al. After this, plants were exposed to 200 μM $AlCl_3$ in nutrient solution for 2 weeks. After fast freezing

in propane cooled with liquid N2 the leaf pieces were sectioned with a cryo-microtome (20 μm) and freeze dried. Two regions of tealeaf cross-section (epidermis–mesophyll, xylem–phloem; 80 x 80 μm^2) were raster scanned with 1.7 keV excitation energy to reach the Al-K absorption edge, and then again with 2.2 keV excitation energy to reach the P-K absorption edge. Al was localized mainly in the cell walls of the leaf epidermal cells where Al was not associated with P or Si. Mesophyll cell walls emitted a weak Al signal, while no Al signal was obtained from the symplast. Contrastingly, Al was more-or-less evenly distributed in the xylem–phloem region, with a slightly higher signal obtained from the phloem region. The Al signal was significantly lower than that recorded from the epidermis–mesophyll region. However, the localization of Al in the phloem region suggests that tea plants are able to retrieve Al from the transpiration stream and to redistribute it via phloem transport. LEXRF spectro-microscopy proofed to be a reliable tool for Al localization in plant tissues.

Acknowledgements: Supported by BFU2007-60332/BFI and EU TWIN MIC 20095369

POST

04 POSTERS

P01 Environmental Stresses and Acclimation

P01-001: EFFECT OF DROUGHT STRESS AND DIFFERENT FERTILIZERS ON SOME PHYSIOLOGICAL ASPECTS IN CHAMOMILE

Heidari, M.[1] - Arazmjo, A.[2]*
[1]*University of Zabol*
[2]*MSC student of agronomy, University of Zabol*
**Corresponding author, e-mail: Haydari2005@gmail.com*

In order to study the effects of water stress and three kind of fertilizers on physiological parameters (Ch a, Ch b, proline and carbohydrates concentration) and sodium and potassium concentration in Chamomile (*Matricaria chamomoilla* L.) a field experiment was laid out in randomized complete block design in split plot arrangement with three replications in 2008 at university of Zabol. Drought treatments included 90% FC or non stress (W_1), 70% FC (W_2) and 50% FC (W_3) as main plot and fertilizer treatments included non fertilizer (F_1), chemical fertilizer (F_2), manure (F_3) and compost (F_4) as sub plot. Results showed that water stress at W_3 treatment, reduced dry flower yield about 18.1%. In this study, however the highest flower yield was obtained from W_1 and use of chemical fertilizer treatments but at W_3 treatment, among the fertilizer, manure had the best effect on flower yield in Chamomile.
Water stress increased percentage and yield of Essential oil but the highest of that was obtained in W_2 and use of chemical fertilizer. In this experiment, Chlorophyll a, b and K^+ contents in leaves decreased by impact of water stress but free proline, total soluble carbohydrate concentration and Na^+ were increased under water stress. Use of manure fertilizer had the highest positive effects on physiological parameters and potassium uptake under water stress condition in chamomile.

P01-002: NOVEL STRESS EFFECTS OF NANOSIZED COLLOIDS IN THE EXTERNAL AND INTERNAL ENVIRONMENT OF PLANTS

Neumann, P. - Asli, S.*
Technion
**Corresponding author, e-mail: agpetern@tx.technion.ac.il*

Nanosized colloid particles are ubiquitous in the aqueous phase of the rhizosphere (clays, humic acids) and plant internal environment (xylem proteins, polysaccharides). We report on time and concentration dependent inhibition of root and xylem hydraulic conductivity by colloids.
The inhibition is shown to cause reductions in whole plant resistance to water deficits, leaf growth and transpiration. The mechanisms of inhibition, possible plant adaptations and practical consequences of these novel phenomena are discussed. Asli S, Neumann PM 2009 Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. Plant Cell & Environment 32: 577-584 Neumann PM. 2008 Coping mechanisms for crop plants in drought prone environments. Annals of Botany 101:901-907

P01-003: PHOTOSYNTHETIC PERFORMANCE OF A NON-ENDEMIC CROP SPECIES UNDER DROUGHT AND HIGH-IRRADIANCE IN MEDITERRANEAN AREAS

Montanaro, G. - Dichio, B. - Xiloyannis, C.*
Dipartimento di scienze dei Sistemi Colturali, Forestali e dell'Ambiente / Università degli Studi della Basilicata
**Corresponding author, e-mail: giuseppe.montanaro@unibas.it*

Distribution at a global scale of some cultivated (high profitable) crops may be affected by economic pressures, leading these crops to grow often under sub-optimal climatic conditions. This is the case with kiwifruit (*Actinidia deliciosa*) which has been introduced also in semi-arid Mediterranean areas where summer are dry and irradiation typically high being in contrast with the habitat of origin. Therefore we investigate the physiological and structural leaf response of field-grown kiwifruit to Mediterranean climate conditions coupled with water shortage
At mid-summer, groups of plants were progressively droughted. Daily variations in leaf gas exchange and midday chl-a fluorescence were determined once plants had -0.6 MPa (moderate stress) and -1.0 MPa pre-down leaf water potential (severe stress). Results suggest that high-light *per se* does not greatly affect the efficiency of photosystem (PS)2, but instead predisposes it to be synergistically reduced by drought co-occurrence. Fluorescence indices showed transiently photodamage of PS2 with a complete recovery in the afternoon in both droughted and irrigated plants. Also we documented that a 50 % shade application maintained efficiency of PS2 (Fv/Fm) close to 0.8 even under severe drought so that to prevent its large decline (0.65) recorded in sunlit leaves. Under moderate stress level stomata behaviour dominated upon metabolic impairments of PS2. Reduction of irradiance increased WUE (15-20 %) in droughted vines. Efficiency of PS2, photosynthesis and stomatal conductance upon rewatering is discussed.We conclude that kiwifruit photosynthetic apparatus is prepared to cope with temporary water shortage under adverse Mediterranean-type-climates

P01-005: DYNAMICS OF CYTOGENETIC DISTURBANCES AND STRUCTURE OF ECOLOGICAL-GENETIC VARIABILITY IN SCOTS PINE POPULATIONS EXPERIENCING TECHNOGENIC IMPACT

Oudalova, A. , Geraskin, S.*
Russian Institute of Agricultural Radiology & Agroecology
**Corresponding author, e-mail: oudalova@mail.ru*

Environment contamination is one of the most important ecological factors essential for biota existence and development. Studies after radiation accidents in the South Urals and Chernobyl showed that mutation and recombination processes accelerate in plant and animal populations experiencing chronic exposure. This result in a rearrangement of population genetic structure and increases genetic load. Surveying temporal trends in populations under stress and studying changes in their genetic variability give a basis for better understanding adaptation in populations. To test the pine tree population stability against additional acute impact, a portion of seeds was exposed to 15 Gy of γ-rays and a higher resistance in the impacted populations was found. A family-related analysis of variability components revealed alterations in intrapopulation variability structure. A degree of these alterations related to the impact severity. Scots pine populations in vicinity of a nuclear waste storage plant and under an urban pressure were monitored during six years (Leningrad region, Russia.) Cytogenetical damage in the impacted pine tree populations was significantly over reference level. Temporal dynamics of aberrant cell occurrence was studied, and cyclic fluctuations with time were found in the reference population, whereas in the impacted populations stress appeared strong enough to destroy the natural regularities. In cytogenetic variability, ecological and genotypic components were distinguished, and their proportion was studied

in dependence on time and technogenic impact. A tendency for dynamics destabilization in the variability structure was revealed under chronic stress.

P01-006: PRIMARY AND SECONDARY STRATEGIES OF EDAPHIC PLANT FUNCTIONAL TYPES OF THE CERRADO

Meira Neto, J. - Viana Neri, A. - Gomes Ferreira Junior, W. - Lopes de Souza, A. - Schaefer, C.E.*
Universidade Federal de Vicosa - UFV
**Corresponding author, e-mail: j_meiraneto@hotmail.com*

It is hard to believe that a large and megadiverse tropical ecosystem has only one important gradient for woody species: soils with higher calcium contents to acidic soils with high contents of Al3+. In addition to the calcium-aluminum gradient, we hypothesize that there are other important environmental variables and a greater diversity of plant functional types than the two that have previously been described. The study site is a small patch that has a broad range of soil variation and fire protection. We employed 25 20x20m plots positioned in transects of five plots per soil unit. Soil samples were a mix of ten sub-samples from 0 to 20 cm depth in each 20x20 m vegetation plot.
A Canonical Correspondence Analysis showed four types of species. Mechanistic models showed that plant functional types were correlated with calcium-aluminum and organic matter/drainage-aluminum gradients.
Primary and secondary plant strategy nomenclature follows Grime's primary and secondary plant strategies theory: Competitors (CC), Aluminum-Semi-Tolerant-Competitors (ASC), Aluminum-Tolerant-Competitors (ATC) and Aluminum Tolerant (AT). Regressions for 38 species (from 44 analyzed) were significant ($p<0.05$). These models permit raw predictions about shifts in savanna-forest boundary floristics on such soils variations under influence of climate change, and allow interpretations applying neutral and niche based theories. Besides calcium-aluminum gradient, organic matter/drainage causes another important soil gradient of Cerrado vegetation. Sponsored by FAPEMIG and CNPq - Brazil

P01-007: CHANGES IN THE PROTEIN PROFILE OF POPLAR GROWN ON TANNERY WASTE

Zemleduch A - Lorenc-Plucinska, G.*
Institute of Dendrology PAS
**Corresponding author, e-mail: agata.zemleduch@wp.pl*

Worldwide chromium emissions, mainly from metallurgy, chemical pigments production, electroplating, wood preservation, and tanneries, exceed 898 000 tonnes. In the last decades, phytoremediation technology has emerged to restore lands contaminated in this way. The use of trees is especially advantageous, considering their natural abilities to detoxify and store heavy metals in their high biomass, or to stabilize them in the ground in a non-bioavailable form. Pollution often requires serious changes in tree metabolism, because trees cannot move and find better growth conditions, but have to deal with their environment by some molecular adaptations. The proteomic approach allows visualization of these various changes at the same time, giving a general view of the metabolic state of plants. In our study, for about 17 weeks, we observed similar growth of hybrid poplar(*Populus tremula* × *alba*) saplings in uncontaminated soil and in solid Cr-rich tannery waste, regardless of Cr concentration exceeding the critical level both in the substrate and in plants. Next, with 2-D electrophoresis, we evaluated changes in the proteome of roots and leaves after the treatment. We found differences in expression of about 10% of protein spots between the control and waste-grown plants. Using mass spectrometry we identified 10 selected proteins characterizing each treatment variant in leaves and 10 in roots, revealing some aspects of metabolic alterations in poplar due to growth in Cr-rich tannery waste.

P01-008: MINERAL COMPOSITION AND ROOT MORPHOLOGY OF WHITE LUPIN (LUPINUS ALBUS L.) UNDER PHOSPHORUS DEFICIENCY IN NUTRIENT SOLUTION

Lazarevic B [1] - Vodnik, D. [2] - Poljak, M. [3] - Horvat, T. [1]*
[1]Faculty of Agriculture, University of Zagreb
[2]Department of Agronomy, Biotehnical Faculty, University of Ljubljana
**Corresponding author, e-mail: blazarevic@agr.hr*

Cluster roots are an adaptation for nutrient acquisition from nutrient-poor soils. It is known that phosphorus (P) deficiency in white lupin (*Lupinus albus* L.) enhances cluster roots formation. Their morphology is variable but tipically, large number of determinate branch roots develop over short distances of main root axes. It has been shown that changes in the architecture of the root system, especially in root length, surface area and branching patterns, can profoundly affect the capacity of plants to uptake nutrients and water.
The aim of this study was to explore root morphology and mineral composition of white lupin grown under different phosphorus concentrations in nutrient solution.
White lupin plants were planted in 1 L pots containing continuously aerated nutrient solution with 0, 0,05 and 0,25 mM L -1 P. After 40 days root morphology and mineral composition of root and shoot were analysed. Plants grown in 0 P nutrient solution had significantly higher total root length, surface area, volume, total number of root tips and branches. No differences in number of primary lateral roots, depending on various P treatments, were found.
Dry matter content both in shoots and roots and shoot to root dray matter ratio of plants grown in 0 P treatment was significantly lower in comparation to plants grown in 0,05 and 0,25 mM L-1 P treatments. Differences in nutrient uptake depending on analysed plant part and phosphorus treatment, were found.

P01-009: THE CORRELATION BETWEEN OXIDATIVE STRESS AND SENESCENCE IN TOBACCO (NICOTIANA TABACUM L.) LEAVES

Glavas, K. [1] - Radic, S. [1] - Vujcic, V. [1] - Lepedus, H. [2] - Cvjetko, P.[1] - Pavlica, M.[1] - Pevalek+-Kozlina, B.[1]*
[1] Faculty of Science, University of Zagreb
[2] Agricultural Institute Osijek
**Corresponding author, e-mail: kglavas@zg.biol.pmf.hr*

The process of senescence is characterized by decline in photosynthesis and induction of oxidative stress. We investigated the process of natural senescence in tobacco leaves old 14, 30, 120 and 270 days and the process of premature senescence induced by hydrogen peroxide in 14d old leaves. Both natural and H_2O_2-induced senescence was accompanied by a decrease of chlorophylls content and by an increase of oxygen consumption. Effective quantum yield of PSII, DF/F'm, declined in naturally senescent leaves and was not affected by exogenous H_2O_2. Both natural and H_2O_2-promoted senescence caused oxidative damage to lipids and proteins while DNA damage was noticed only in the oldest leaves (270 d).
The process of natural senescence induced *superoxide dismutase activity* and inhibited catalase and peroxidase activities (H_2O_2-destroying enzymes) Accordingly, endogenous H_2O_2 levels increased nearly linearly with *progression of natural senescence.* However, the promotion of senescence by the exogenous H_2O_2 was not associated with an increase in its endogenous level which is probably the result of increased ascorbate and pyrogallol peroxidase activities.
Presented results show that increased radical levels displayed during natural senescence are not only caused by the elevated production of radicals but also by a loss in antioxidant capacity. The results also show the importance of hydrogen peroxide as a signal molecule.

P01-010: THE EFFECT OF TEMPERATURE STRESSES ON PROTEIN EXPRESSION IN DIFFERENT ORGANS OF ZEA MAYS L SEEDLINGS IN EARLY STAGES OF VEGETATIVE DEVELOPMENT

Kosakivska, I. - Rasevich, I.*
Institute of Botany National Academy of Sciences of Ukraine
**Corresponding author, e-mail: lgkos@ukrpost.ua*

Using disc polyacrylamide gel electrophoresis (PAGE), we demonstrate that moderate heat (2h. +400C) and cold (2h.+20C) stresses resulted in qualitative and quantitative changes in the composition of soluble proteins in different organs of 72-hour maize seedlings. Under stress conditions, we observed an elevated expression of a 75 kDa polypeptide from the Heat Shock Protein (HSP) 70 family, and the formation of new polypeptides from the HSP 60 family, namely: a 61 kDa protein in the leaves, a 62 kDa protein in the mesocotyl, and a 64 kDa protein in the roots. A comparison of changes in HSP protein expression profile in maize seedlings to those of *Phaseolis vulgaris* L, obtained previously under analogous conditions, revealed marked differences between monocotyledons (maize) and dicotyledons (common bean). Monocotyledons with C4 carbon fixation (maize) possess characteristic constitutional stress proteins, which maintain stable protein expression profiles. C3 dicotyledons (common bean), on the other hand, rely primarily on inducible polypeptides. We discuss the defensive role of stress proteins during early stages of vegetative development in plants.

P01-011: INOSITOL-PHOSPHOLIPID SIGNALING COORDINATES ENVIRONMENTAL STRESS ADAPTATION: FROM STRESS PERCEPTION IN PLASMA MEMBRANE TO GENE EXPRESSION IN NUCLEI

Levine, A. - Kaey, Y. - Golani, Y.*
The Hebrew University of Jerusalem
**Corresponding author, e-mail: AlexLevine@huji.ac.il*

Plants are able to adapt to adverse environmental conditions. The process requires removal of multiple proteins, lipids and other molecules and replacement with better suited ones. It involves many metabolic changes that require coordination of gene expression and membrane/protein trafficking. Plasma membrane is first barrier to outside conditions. It is ideally positioned for stress perception and inward signaling. Phosphatidylinositides (PtIns) were shown to regulate signaling by phosphorylation of specific sites in the inositol ring by specific kinases and phosphatases. We studied the role of PtdIns 5-phosphatases (5PTases) in salt and drought stress by reverse genetics. From 9 mutants tested, only *5ptase7* was sensitive. Surprisingly, it was more toleranto osmotic stress. Molecular analysis of stress responses showed reduced ROS production and Ca^{2+} influx in cytosol and nuclei of the *5ptase7* mutants. They also showed reduced endocytosis and salt-responsive gene expression. 5PTase7 localizes in plasma membrane and nucleus, in line with the locations of ROS production, endocytosis, gene expression.
The regulation of abiotic stress by 5PTases, described here, is in accord with PtdIns 3-kinase activity; described before. Taken together, our results show that PtdIns coordinate plant stress responses on several levels: by affecting ROS production, endocytosis and gene expression, through regulation of membrane and protein trafficking.

P01-012: USE OF CHLOROPHYLL FLUORESCENCE IMAGING IN AGRICULTURAL RESEARCH

Calatayud, A. - Gorbe, E.*
Instituto Valenciano de Investigaciones Agrarias
**Corresponding author, e-mail: calatayud_ang@gva.es*

The technique of chlorophyll fluorescence, which has been traditionally based on point measurements, has been successfully used in the evaluation of plant photosynthetic activity with the advantages of being rapid, non-destructive and inexpensive. However, it has the disadvantage of ignoring the typical heterogeneity of photosynthetic activity over the leaf surface. To overcome this source or errors, chlorophyll fluorescence imaging (CFI) has been developed to permit the study of the spatial-temporal heterogeneities in the fluorescence emission pattern within cells, leaves or plants. CFI has been used in agricultural research for several purposes, mainly for the diagnosis of biotic or abiotic stresses in both preharvest and postharvest conditions. For example, CFI has been used for the early identification of genotypes with high tolerance to stress, due to its high potential to detect stresses before visual symptoms appear and to its capacity of screening a large number of plants simultaneously. This work provides an overview of the contribution of CFI in agricultural research and, more specifically, in the detection of abiotic stresses (due to nutrient deficiency, water deficit, extreme temperatures, excessive light intensity, herbicides or air pollution) and biotic stress (caused by different pathogens) during preharvest conditions and during postharvest life of fruits and flowers.

P01-013: SCREENING OF TURKISH BREAD WHEAT VARIETIES FOR THE PRESENCE OF DURABLE DISEASE RESISTANT GENE, Lr34/Yr18/Pm38

Yildirim, K. - Atici, E. - Akkaya, M.S. - Boylu, B.*
METU
**Corresponding author, e-mail: ykubilay@metu.edu.tr*

Rust diseases of wheat are among the oldest and important diseases of wheat worldwide. Development of resistant wheat cultivars, which is the main objective for many breeding program, is the most economical and environmentally safe control measure. Wheat cultivars that carry durable or race-nonspecific resistance are identified. Inheritance of this resistance indicates that these cultivars often carry a few slow rusting gene locus that have small-to-intermediate effects on fungal pathogens. Oneof these gene loci, Lr34/Yr18/Pm38, is found to confer partial and durable resistance against the rust pathogens as well as powdery mildew. This important resistance was found to be controlled by a single gene, which encodes an adenosine triphosphate–binding cassette transporter (ABC-transporter) of the pleiotropic drug resistance subfamily. Alleles of resistant and susceptible cultivars differed by only three sequence polymorphisms, which enable researchers to develop five allele-specific markers based on a 3 bp deletion in exon 11 of the *Lr34*-gene, and another marker from a single nucleotide polymorphism in exon 12. In this study, 62 different Turkish bread wheat cultivars were screened by the gene specific molecular markers, developed from those Lr34 gene mutation sites. The 14 cultivars determined to posses the gene. This is the first screening of Turkish cultivars for the presence of these genes. The gene now can be affectively used for marker assisted selections in breeding improved varieties.

P01-014: EVALUATION MYCORRHIZAL ASSOCIATION FORMED BY FUNGI ISOLATED FROM POLISH ECOSYSTEMS WITH CRANBERRY CV. 'PILGRIM', USING CHLOROPHYLL A FLUORESCENCE METHODT

Borkowska, B. - Krzewinska, D.*
Research Institute of Pomology and Floriculture
**Corresponding author, e-mail: Bozenna.Borkowska@insad.pl*

Cranberry cultivars are originated from *Vaccinium macrocarpon* (Ait), genus native to North America. Cranberry, requires a specific fungal partner for developing mycorrhizal association, desired for growth and fruiting. Thus, finding fungi from polish ecosystems which are able to develop mycorrhizal symbiosis with American cultivars was the purpose of presented experiments.
Chlorophyll a fluorescence (ChF) provides an opportunity for ecophysiological research through the analysis of the changes in activity of photosynthetic apparatus, under stress conditions. As mycorrhization is recognized as biotic stress, measurements of

ChF could help to determine response of host plants to inoculated fungi. Rooted microshoots were inoculated in 2006 year. Spring 2007 they were transplanted into the field. Each year (until 2009), ChF was measured using MINI-PAM fluorometer. Morphological response and later on, yielding were also measured.
On the base of ChF measurements and morphological evaluation we concluded:

- Fungi isolated from *Vaccinium spp.* growing in polish ecosystems were able to form mycorrhizal symbiosis with *V. macrocarpon*, genus native to North America.
- Among multiple ChF parameters, ETR and qP were the most suitable to assess host plant response to symbiotic fungi.
- At beginning, fungi played a role of partial parasites, decreasing photosynthetic activity of host plants.
- Mycorrhizal symbiosis established only in the third year of plants growth in the field. Values ETR and qP were higher for mycorrhized plants than non-mycorrhized. Acknowledgement

The experiments were conducted within the framework of a project No DPN/N83/COST/2009

P01-015: EFFECTS OF AQUEOUS EXTRACT FROM DRY OLIVE RESIDUE (ADOR) ON THE PHYSIOLOGY OF TOMATO PLANTS: OXIDATIVE STRESS AND CHANGE IN THE ANTIOXIDANTS SYSTEMS

García Sánchez M - Aranda, E. - Reina, R. - Ocampo, J.A. - Garcia-Romera, I.*
Consejo Superior de Investigaciones Científicas
**Corresponding author, e-mail: mercedes.garcia@eez.csic.es*

The aim of this work was to analyse the effect of aqueous extracts of dry olive mill residue (ADOR), non treated and treated with saprobe fungi, in the physiology of *Solanum lycopersicum* plants in order to recycle dry olive residue for fertilizers. In addition, we tried to determine if the ADOR use enhanced lipid peroxidation (MDA) and H_2O_2 levels and induced changes on antioxidant systems: Superoxido Dismutase (SOD), Ascorbate Peroxidase (APX) and Glutatione Reductase (GR). ADOR, obtained by orbital-shaking of dry olive residue, was used as the growth medium of the following fungi: *Coriolopsis rigida* and *Penicillium chrysogenum*. ADOR treated and not treated with saprobe fungi at 5% dose were applied to tomato plants during 4, 10 and 30 days.
We observed that treated and non treated ADOR with saprobe fungi induces higher MDA and H_2O_2 levels on roots and leaves of tomato plants after 4 and 10 days of exposure. However, after 30 days of exposure, MDA and H_2O_2 levels only increased in response to non treated ADOR. On roots, after 4 and 10 days of exposure, we observed an increase in APX and GR activities whereas, on leaves, the increase was found on SOD, APX and GR activities. After 30 days of exposure we observed an increase in APX and SOD on roots and leaves of tomato plants.
We concluded that ADOR induces an oxidative stress through the generation of ROS and activates the antioxidant system of tomato plants. However, we observed that the treatment of ADOR with saprobe fungi palliated this effect mainly in a long-term exposition due to the bioremediation effect by these fungi.

P01-016: USE OF REDOX-SENSING ROGFP1 FOR MONITORING REDOX CHANGES IN THE CYTOSOL AND MITOCHONDRIA IN WATER STRESSED ARABIDOPSIS THALIANA PLANTS

Brossa Gonzalez, R.[1] - Jubany-Marí, T.[1] - Alegre Batlle, L.[1] - Jiang, K.[2] - Feldman, L. J. [2]*
[1]Universitat de Barcelona
[2]University of California, Berkeley
**Corresponding author, e-mail: rbrossa@ub.edu*

Water deficit can induce redox changes in plant cell compartments and therefore maintenance of redox homeostasis is one of the plant’s mechanisms to cope with stress. Knowledge of where redox changes occur, and their kinetics and magnitude, is crucial to understanding the responses of plants to environmental stress. The expression of Reduction-Oxidation Green Fluorescent Proteins (roGFP1) in plants has become a useful tool as it provides in vivo direct measurement of redox state, as well as dynamic measurements over the time and in different intracellular locations.Real-time measurements of redox state in A.thaliana plants under a drought stress treatment were reported. For this effort seeds of A.thaliana ecotype Col-0 were transformed with roGFP1 which was expressed either in the cytosol (c-roGFP1) or in the mitochondria (m-roGFP1). Five-week-old plants of the two transformed lines were subjected to two irrigation treatments: Well watered (WW), and Water stressed (WS). The time course of leaf water relations, content of reduced and oxidized forms of ascorbate and glutathione, and cytosolic and mitochondrial leaf redox state (measured using c-roGFP1 and m-roGFP1) were monitored.In this work changes in the redox state were reported in both the cytosol and mitochondria of leaf cells during the time course of drought stress. As the plant became more dehydrated the redox state of the leaf cytosol became more oxidized compared to its initial redox state. However, the mitochondria of WS plants decreased their redox potential (becoming less oxidized) with increasing periods of WS. This suggests that under WS the cellular compartments respond differently (presumably adaptively) to WS, exhibiting an enhanced protection against oxidative stress in mitochondria.

P01-017: REDOX CHANGES DURING COLD ACCLIMATION IN WHEAT GENOTYPES WITH DIFFERENT FREEZING TOLERANCE

Kocsy, G - Soltész, A. - Tímár, I. - Kellos, T. - Vashegyi, I. - Szalai, G. - Galiba, G.*
Agricultural Research Institute of the Hungarian Academy of Sciences
**Corresponding author, e-mail: kocsyg@mail.mgki.hu*

The aim of the present study was to find out whether redox changes induced by cold hardening are related to freezing tolerance and vernalization requirement in a specific genetic system containing chromosome 5A substitution lines. The amounts of H2O2 and ascorbate, the ratio of ascorbate to dehydroascorbate exhibited a rapid transient increase in the crown, followed by a gradual increase during the subsequent two weeks. The amount of glutathione (GSH) and its ratio to glutathione disulphide (GSSG) first decreased, while later increased. The H2O2 (measured in crown extract and visualized by fluorescence staining in the shoot apex), ascorbate and GSH concentrations, the ascorbate/dehydroascorbate and GSH/GSSG ratios and the half-cell reduction potential of the GSH/GSSG couple were higher in the freezing-tolerant genotype and the corresponding substitution line than in the sensitive genotypes during the second half of the hardening period. In contrast to H2O2 and the non-enzymatic antioxidants, the lipid peroxide concentration and the activity of the four antioxidant enzymes studied, exhibited a transient increase during the first week, with no significant difference between the genotypes. While the observed redox changes correlated with freezing tolerance in the crowns, they were independent from the vegetative/generative transition state monitored by the apex morphology and the vernalization gene expression. This work was supported by the Hungarian Scientific Research Fund and the National Office for Research and Technology (NKTH-OTKA K67906 and K68894).

P01-018: OSBADH1 IS POSSIBLY INVOLVED IN THE OXIDATION OF ACETALDEHYDE IN RICE PEROXISOMES

Mitsuya, S. - Yokota, Y. - Fujiwara, T. - Takabe, T.*
Graduate School of Bioagricultural Sciences, Nagoya University
**Corresponding author, e-mail: mitsuya@agr.nagoya-u.ac.jp*

Betaine aldehyde dehydrogenase catalyzes the last step in the synthesis of glycine betaine, a compatible solute accumulated by many plants under various abiotic stresses. Although rice (*Oryza sativa* L.) produces little glycine betaine, it was reported that rice has two BADH genes (*OsBADH1* and *OsBADH2*). To characterize BADH enzyme of rice, we investigated the enzymatic properties of recombinant OsBADH1 and OsBADH2 proteins. The affinity of OsBADH2 for betaine aldehyde (Km of 231 μM) was similar to that found in the BADH of other glycine betaine-accumulating plants BADHs. However OsBADH1 showed an extremely low affinity for betaine aldehyde with apparent Km of 2590 μM. OsBADH1 and OsBADH2 catalyzed the oxidation of ω-aminoaldehydes such as 4-aminobutyraldehyde, 3-aminopropionaldehyde, 4-*N*-trimethylaminobutyraldehyde, and 3-*N*-trimethylaminopropionaldehyde. We also found that both OsBADH proteins catalyzed the oxidation of acetaldehyde, but OsBADH1 showed a higher V_{max} value for acetaldehyde than OsBADH2 did. The accumulation of *OsBADH1* and *OsBADH2* mRNA was decreased by submergence treatment and recovered by re-aeration. Analysis of the subcellular localization of OsBADH1 protein using green fluorescent protein indicated that OsBADH1 was localized in peroxisomes. These results suggest that OsBADHs show dehydrogenase activity for a range of aldehydes, and that OsBADH1 has a possible physiological function in the oxidation of acetaldehyde in rice plant peroxisomes.

P01-019: CHANGES IN THE SALICYLIC ACID PATHWAY IN WHEAT DURING LEAF RUST INFECTION

Szalai, G. - Vida, G. - Janda, T.*
Agricultural Research Institute of the Hungarian Academy of Sciences
**Corresponding author, e-mail: szalaig@mail.mgki.hu*

Salicylic acid (SA) has been known as a signal molecule in the induction of defence mechanisms in plants for a long time. Exogenous SA treatment may also induce the expression of pathogenesis related proteins and has been shown to establish systemic acquired resistance (SAR). Although SA is not considered to be the signal translocated from the infection site, its accumulation in distant tissues is essential for the induction of SAR. The aim of the present work was to investigate the SA pathway and the antioxidant response during leaf rust (*Puccinia triticina*) infection in wheat plants. Near isogenic lines containing various leaf rust resictance genes were used in the experiments. Plants were grown under field and greenhouse conditions. The activity of the antioxidant enzymes (glutathione-S-transferase, ascorbate peroxidase, guaiacol peroxidase and catalase) increased after infection. The level of ortho-hydroxy-cinnamic acid decreased while the SA level increased in infected plants during the filed experiments. Further experiments were made in greenhouse to check the SA contents of various leaf levels after infection.

P01-020: LIGHT-DEPENDENT REGULATORY MECHANISMS DURING COLD HARDENING IN WHEAT

Janda, T.[1] - Majláth, I.[1] - Szalai, G.[1] - Soós, V.[1] - Sebestyén, E.[1] - Balázs, E.[1] - Vanková, R.[2] - Dobrev, P.[2] - Tari, I.[3] - Tandori, J.[1]*
[1]*Agricultural Research Institute of the Hungarian Academy of Sciences*
[2]*Institute of Experimental Botany of the Czech Academy of Sciences*
[3]*University of Szeged*
**Corresponding author, e-mail: jandat@mail.mgki.hu*

Frost tolerance is the result of a wide range of physical and biochemical processes that allow functioning at low temperatures. Earlier results showed that frost hardening at low temperature under low light conditions is much less effective than under normal light conditions, and several processes, including the lipid, polyamine, or salicylic acid metabolism and antioxidant activity, may contribute to the light-induced freezing tolerance. The aim of the present work was to discover what other changes in the regulatory processes were responsible for the light-enhanced freezing tolerance of wheat plants. Young winter and spring wheat varieties were cold hardened under medium or low light conditions. Microarray and RT-PCR analyses show that the light intensity during the hardening period significantly affected the expression of several genes, which may have role in the development of frost hardiness of wheat plants. In order to get more information about the regulatory processes during cold hardening period, the changes in the levels of plant hormones were also detected. While the abscisic acid level was lower in the 12-d cold hardened plants than in the controls in both genotypes, independently on the light intensity, changes in the plant growth regulator cytokinins, signal transducer NO, ethylene precursor ACC, and proline levels showed a strong light and variety dependencies. Results suggest that temperature and light regulate the development of frost hardiness in a complex way.

P01-021: A TRUNCATED FORM OF A B-ADAPTIN CONFERS WEAK ACID RESISTANCE IN ARABIDOPSIS

Niñoles, R.[1] - Alejandro, S. [2] - Fernández, J.A[3] - García-Sánchez, M.J.[3] - Serrano, R.[1]*
[1]*IBMCP*
[2]*Institute of Plant Biology University of Zurich*
[3]*Universidad de Málaga*
**Corresponding author, e-mail: renioro@ibmcp.upv.es*

The homeostasis of intracellular pH is a fundamental activity of living cells. In order to identify new components of the Arabidopsis pH regulation system, an activation-tagged mutant seed collection was screened using acetic acid as a selection agent. The dominant mutant wat1-1D (Weak Acid Tolerant) is more resistant to the acid stress generated by weak acids such as acetic, propionic and sorbic acid, and this tolerance correlates with its T-DNA insertion. Wat1-1D also shows lithium sensitivity and ABA resistance during the germination stage. Measurements of intracellular pH show that during acid stress mutant plants maintain a higher cytosolic pH than the wild type, although their acetate uptake is normal. The plasma membrane (PM) H+ATPase activity and the PM potential are similar in the wild type and the mutant in normal conditions. However, under acid stress the wild type undergoes a higher PM hiperpolarization. The T-DNA insertion of wat1-1D interrupts the At3g55480 gene, an adaptin family protein, and causes an induction of the adjacent gene At3g55470. Surprisingly it is the expression of a truncated form of the adaptin and not the overexpression of At3g55470 what causes the observed phenotypes, as transgenic plants expressing the truncated adaptin or the antisense gene have similar phenotypes to those of the wat1-1D mutant. In adition, a SAIL T-DNA insertion mutant in At3g55480 also shows the same phenotypes. Our working hypothesis is that under acid stress this mutant introduces the excess of protons into the vacuole.

P01-022: THE ROLE OF PROLINE AND ITS METABOLISM ENZYMES IN CUCUMBER CELL CULTURES DURING ACCLIMATION TO SALINITY

Naliwajski, M. - Sklodowska, M.*
Department of Plant Physiology and Biochemistry, University of Lodz, Poland,
**Corresponding author, e-mail: nalmar@biol.uni.lodz.pl*

Proline is one of the most widely distributed osmolytes and acts as a reservoir of nitrogen and carbon source for post stress growth, a stabilizer of the membranes and as a sink of energy to regulate redox potential. The aim of this study was to investigate the role of the activities of pyrroline-5-carboxylate: synthetase (P5CS), reductase (P5CR), proline dehydrogenase (PDH) and proline level in salinity adaptation in cell cultures. All biochemical analyses were carried out in two cucumber cell cultures:

non acclimated (NAC) and acclimated to this stress by treatment with 20 mM (AC20) NaCl. Finally all groups were stressed 150 and 200 mM NaCl and measurements were performed in: 24th, 48th and 72nd hour after stress. Our result showed that 150 and 200 mM NaCl stress caused significant increase in P5CS activity in AC20 throughout the experiment whereas in NAC no changes were noticed. Both NaCl concentration caused significant increase in P5CR activity in AC20, between 24th to 48th h whereas in NAC, 200 mM NaCl caused decrease in P5CR activity between 48th and 72nd h. In both examined cell cultures salt stress inhibited PDH activity. In contrast to NAC where proline concentration significantly increase in 48th and 72nd h, in AC20 salt stress caused earlier growth of proline level, starting from 24th h. The above results indicate that acclimation process to salinity is indirectly connected with high P5CS and P5CR activity as well a earlier proline accumulation in response to stress. It can suggest that in AC20 changes in proline level and its metabolism enzyme activities play a role in coping with salt stress. Ministry of Science and Higher Education Grant No NN 302117735.

P01-023: STRESS REACTION OF PRIMARY CARBOXYLASES OF C3 AND C4 PLANTS AFTER SHORT-TERM UV-B IRRADIATION OF WHOLE LEAVES

Lyubimov, V.
Institute of basic biological problems RAS
**Corresponding author, e-mail: valyu@online.stack.net*

Investigations were carried out using maize and wheat . The attached leaves of three-week-old plants was irradiated with different doses of UV-B . Then plants were exposed under white light continuously up to 4 days. During this period PEP-carboxylase and RuBP-carboxylase activities of total protein extract (TP) and purified enzyme preparation (EP) were measured in irradiated and untreated leaves. At low UV-B dose (1.2 kJ) and “0” time activities of both enzymes and preparations not changed. Later (3, 6, 12, 24, 48 and 96 hours) examinations exhibited three-phase dynamics of enzymes activity. In 1st, the diminishing of carboxylases activities was occurred with minimum at 3-4 h. Under higher UV-dose (3.0 and 6.0 kJ) this inhibition gradually increased in both preparations of Rubisco and in the TP of PEP-C, and sharp inhibition was observed in the EP of PEP-C starting at “0“ time. In 2nd, activities of both carboxylases in TP and EP raised up to control level or higher depending on dose of UV-irradiation. In 3d, activity of PEP-C returned gradually to the control level in the course of 12-18 hours, and Rubisco activity returned to the control level with oscillations after 96 h. So, primary carboxylases of C3 and C4 plants have the same three-phase reaction under UV-B stress. Quantitative and temporal data indicate that Rubisco activity is changed under control of the high-molecular mechanism (Rubisco activase). PEP-C activity is modulated by the high-molecular processing too (phosphorylation), and by the low-molecular reversible inhibition additionally.

P01-024: INFLUENCE OF PLANT MATURITY, SEX AND STRESS MEMORY ON THE TOLERANCE OF THE DIOECIOUS PLANT, URTICA DIOICA TO WATER DEFICIT

Oñate Gutiérrez, M. - Blanc, J. - Munné-Bosch, S.*
Universidad de Barcelona
**Corresponding author, e-mail: martaonate@ub.edu,*

It is generally accepted that reaching maturity has drastic effects on the physiology of plants, especially in stress conditions, but are there differences between sexes in dioecious plants? And, how stress history affect plant stress tolerance of mature plants? This study was aimed at examining the effects of plant maturity and sex on the water stress response of the herbaceous dioecious perennial, *Urtica dioica*. Furthermore, we evaluated the effects of a stress treatment during the juvenile phase on the stress tolerance of mature plants, and also the differences in the physiology of leaves of reproductive and non-reproductive shoots to unravel the effects caused by reproduction at local level. Plant maturity drastically reduced plant tolerance to water deficit (mature plants showing severe reductions in the F_v/F_m ratio compared to juveniles). Differences between sexes were apparent, females showing smaller leaf water contents and chlorophyll levels, with smaller reductions in these parameters under stress. These effects were evident in the mature phase, but not in the juvenile phase (when plant sex was still not expressed). Stress treatment in juveniles led to constitutive higher lipid peroxidation levels and smaller leaf water contents during the mature phase, and a similar performance to water deficit, with smaller chlorophyll a/b ratios. In conclusion, (i) plant maturity reduces plant stress tolerance in *U. dioica*, (ii) effects of plant maturity on stress sensitivity are evident both in males and females, and (iii) stress history during the juvenile phase determine the performance of mature plants to water deficit, leaves of non-reproductive shoots being more stress tolerant, allowing plant survival to repeated periods of water deficit.

P01-025: INVOLVEMENT OF NO AND REACTIVE OXYGEN SPECIES IN SALICYLIC ACID-INDUCED STOMATAL CLOSURE IN ABAXIAL EPIDERMAL PEELS OF TOMATO

Tari, I. - Poór, P. - Szepesi - Gémes, K.
Department of Plant Biology, University of Szeged, Hungary
**Corresponding author, e-mail:*

Salicylic acid (SA), a signalling component in the acclimation to biotic and abiotic stressors may lead to cell death or to hardening of tissues. SA induces the accumulation of reactive oxygen species (ROS) and NO, the compounds that can turn on defence reactions or at higher concentrations they can trigger the cell death program. The addition of SA at 10^{-7}-10^{-2} M to the hydroponic culture of tomato plants resulted in stomatal closure on intact leaves. At 10^{-3}-10^{-2} M, but not at lower concentrations, SA decreased the maximal CO_2 fixation rate (A_{max}), and the initial slopes of the CO_2 (A/C_i) and light response (A/PPFD) curves, increased the H_2O_2 and NO content of leaf tissues, and resulted in the death of plants. Since the guard cells are generally more resistant to stressors, than the cells of the mesophyll, the question is, whether the SA-induced H_2O_2 or NO participate in SA-induced signal transduction leading to stomatal closure or their accumulation is a part of the cell death program. The stomatal aperture in the abaxial epidermal peels incubated in increasing SA concentrations, exhibited different pattern, than in the intact leaves. The apertures on epidermal peels were closed in the buffer containing 10^{-3}-10^{-2} M and 10^{-7} M SA but remained open at 10^{-4} M. In short-term experiments the guard cells exposed to 10^{-3}-10^{-2} M remained viable. As a function of SA concentrations the chlorophyll a fluorescence induction parameters exhibited similar tendencies in single guard cell of epidermal peels, than in intact leaves. It is suggested that the burst of H_2O_2 and NO at 10^{-3}-10^{-2} M SA initiates the cell death program and at 10^{-7} M SA the increase in H_2O_2 may mediate the SA-induced stomatal closure.

P01-026: FACTORS INVOLVED IN THE RISE OF PHOSPHOENOLPYRUVATE CARBOXYLASE -KINASE ACTIVITY CAUSED BY SALINITY IN SORGHUM LEAVES

García-Mauriño, S.[1] - Monreal, J. A.[1] - Arias, C.[1] - Vidal, J.[2] - Echevarría, C.[1]*
[1]University of Seville
[2]Université Paris-Sud
**Corresponding author, e-mail: sgarma@us.es*

Salinity has been shown to increase phosphoenolpyruvate carboxylase-kinase (PEPC-k) activity in sorghum leaves[1,2]. At least two different processes are involved in this phenomenon:
a) Salinity triggers *PPCK1* expression at dark. This gene is res-

ponsible for the synthesis of the mesophyll isoenzyme, and its expression is up-regulated by light.
b) ABA decreases the rate of PEPC-k degradation[3]. The rise in ABA level, as a consequence of salinity, might account for a lower rate of PEPC-k degradation.
We have used LiCl, which decreases the degradation of PEPC-k[4], as a tool to investigate the molecular mechanisms controlling this process. PEPC-k has been has been proposed to be ubiquitinated and catabolised by the proteasome[5]. In this respect, the ubiquitination of 1-aminocyclopropane-1-carboxylate synthase (ACS) is regulated by phosphorylation by a calcium-dependent protein kinase (CDPK)[6]. The treatment with 10 mM LiCl of sorghum leaves causes a marked rise of a CDPK activity while decreasing the rate of PEPC-k disappearance. This late effect was lost in the presence of W7, an inhibitor of CDPK activity. These preliminary results suggest that the phosphorylation of PEPC-k by a CDPK could be regulating the degradation of the first.

1.- Echevarría et al (2001) Planta, 214, 283-287.
2.- García-Mauriño et al (2003). Planta, 216, 648-655.
3.- Monreal et al (2007a). FEBS Lett., 581, 3468-3472.
4.- Monreal et al (2007b). Planta, 225, 801-812.
5.- Agetsuma et al (2005). Plant Cell Physiol, 46, 389-398
6.- Tatsuki and Mori (2001). J Biol Chem, 276, 28051-28057.

P01-027: INFLUENCES OF HIGH TEMPERATURE DURING GRAIN FILLING STAGE ON ACCUMULATION OF STORAGE PROTEINS AND GRAIN QUALITY IN RICE (ORYZA SATIVA L.)

Lur, H.[1] - Lin, SK. [2] - Liu, YH. [1] - Lin, CJ.[1]*
[1]Department of Agronomy, National Taiwan University
[2]Sustainable Environment Research Center, Natl. Cheng-Kung University
**Corresponding author, e-mail: lurhs@ntu.edu.tw*

High temperature (HT) can reduce the grain yield and quality of rice. Storage proteins are important for both the development and quality of rice grains, but effect of the HT on the accumulation of storage proteins is unclear. Our study was to understand the effects of HT during filling stage on the expression of storage proteins and the quality of rice grains. Storage proteins were analyzed by 1D SDS-PAGE, and differential expressed gel bands were further identified by LC\MS\MS. Transcriptions of the genes for key proteins were also determined. Results showed that HT reduced the wight, amylose content, and flour gel consistency of grains. HT increased accumulation of storage proteins at early filling stage, but decreased the accumulation of prolamines and globulins at maturation. Among storage proteins prolamins and globulins were most sensitive to HT. Proteins of cyclophilin 2, peroxireducxin, glyoxalase I, RAB24 and HSP16.9 were differentially enhanced by HT. At transcription level, HT enhanced the expressions of genes for glutelin, prolamins, globulins, and protein disulfide isomerase at early filling stage; but decreased that of these genes at later stage. HT also decreased the expressions of starch biosynthesis related genes *GBSS* and *SSIIa*, and HT increased the expression of stress responsive genes *PRDX*, *RAB24*, *HSP16.9c*, and *GloI*. A schematic model is proposed to depict the influence of HT on grain quality formation in rice.

P01-028: THE IMPACT OF PROLINE ON POLYAMINE METABOLISM DURING HEAT STRESS RESPONSE IN TOBACCO PLANTS

*Martincova, O.**
Institute of Experimental Botany v.v.i., AS CR, Rozvojová
**Corresponding author, e-mail: martincovao@ueb.cas.cz*

Heat stress represents very fast, acute stress associated (at least at the initial phase) with the decrease of leaf water potential. Defense pathways include synthesis of osmolytes and production of other key protective compounds (polyamines, PAs). PA involvement in abiotic stress adaptation could be due to their roles in osmotic adjustment, membrane stability and free-radical scavenging. Responses to heat were compared in tobacco plants constitutively over-expressing a modified gene for the proline biosynthetic enzyme Δ1-pyrroline-5-carboxylate synthetase (TR) and in the corresponding wild-type (WT).
Initial phase of heat stress (2 h at 40 °C) was associated with an increase in free PAs (more expressive in TR), especially of spermidine and spermine in WT plants and in addition putrescine (Put) in TR. The rise in PA contents correlated with a stimulation of the activity of arginine, ornithine and S-adenosylmethionine decarboxylases. Decrease in free PAs, especially of Put, coincided with down-regulation of the activity of PA biosynthetic enzymes after 6 h of heat stress. The use of plants with elevated proline content enabled us to evaluate the effect of enhanced stress tolerance on the dynamics of PA levels during the heat stress.
This work was supported by Ministry of Education CR project No. OC 08013.

P01-029: PHYTOTOXICITY OF NANOSIZED MOLYBDENUM OCTAHEDRAL CLUSTERS

Cabello-Hurtado, F. - Aubert, T. - Esnault, MA. - Cordier, S.- Grasset, F.*
University of Rennes
**Corresponding author, e-mail: francisco.cabello-hurtado@univ-rennes1.fr*

Manufactured nanoparticles (<100nm), are being increasingly produced for a wide range of applications within industry. We have recently shown that $Cs_2Mo_6Br_{14}@SiO_2$ nanoparticles, containing $[Mo_6Br_{14}]^{2-}$ cluster units, exhibit photonic properties with potential applications in bio-imaging [Grasset *et al*. 2008, *Adv. Mater*. 20: 143]. If these cluster-based nanoparticles are meant to be used for biological applications in living organisms, their toxicity in all its forms should be perfectly known. Here the study aimed to provide information about phytotoxicology of $Cs_2Mo_6Br_{14}$ (CMB) clusters compared to $CMB@SiO_2$ nanoparticles. Moreover, CMB clusters proved to be an interesting system for fundamental aspects of nano-toxicity studies since, as we showed here by SEM, they aggregated or remained nanosized depending on the dispersing medium.
The effects of CMB clusters, dispersed either with water-sorbing or ethanol-sorbing solutions, and $CMB@SiO_2$ nanoparticles on plant growth were investigated using rape. Seed germination was not affected. Clusters greatly inhibited plant growth, roots being more affected than shoots. These effects were much more important for nanosized-CMB (ethanol-sorbing solutions) than for aggregated-CMB (water-sorbing solutions). In addition to higher growth inhibition, nanosized-CMB affected root morphology in a different manner as shown by SEM. While roots treated with aggregated-CMB showed high proliferation of root hairs, nanosized-CMB treatments affected root cap, external tissues and root gravitropism. So far $CMB@SiO_2$ nanoparticles did not show any toxicological potential. In the light of these results, we will propose and discuss different hypothesis for explaining cluster impacts.

P01-030: INCREASED SALT TOLERANCE, K ACCUMULATION AND HAK5 EXPRESSION IN TRANSGENIC TOMATO OVEREXPRESSING LENHX2

Huertas, R. - Cagnac, O. - Venema, K. - Rodríguez Rosales, M.
Consejo Superior de Investigaciones Científicas

LeNHX2 is an endosomal ion transporter which exchanges H^+ with K^+ and, to a lesser extent, Na^+ (Venema et al 2003, Rodríguez-Rosales et al 2008). We have investigated the impact of LeNHX2 overexpression on the response to salt stress and K^+ deprivation in tomato plants. Transformed tomato plants grew better in NaCl supplemented media than untransformed controls. Analysis of mineral composition showed a higher K content in roots and shoots of transgenic plants and no differences in Na

content between transgenic and untransformed plants grown either in the presence or the absence of 120 mM NaCl. Growth in 120 mM NaCl provoked an increase in Na and a decrease in K content in roots and shoots of all plants analyzed as well as a decrease in water content in roots, shoots and leaves, although the decrease was lower in transgenic tomato than in untransformed plants. An increased expression of the high affinity K uptake system HAK5 in roots of transgenic plants grown under K limitation was also detected. Together, the increased expression of LeNHX2 and HAK5 in transgenic plants is consistent with their higher K content and indicates the fundamental role of K homeostasis in the better performance of LeNHX2 overexpressing plants grown under NaCl stress.

Venema et al 2003. J Biol Chem 278: 22453-9
Rodríguez-Rosales et al 2008. New Phytol 179: 366-377

Work financed by grant BIO2008-01691 from MICINN, Spain

P01-031: MAPMAN VISUALIZATION OF GENE EXPRESSION IN DIFFERENT ORGANS OF WINTER BARLEY DURING COLD ACCLIMATION

Janská, A.[1] - Aprile, A.[2] - Ovesná, J.[1] - Zámecník, J.[1] - Attivelli, L.[3]*
[1]Crop Research Institute
[2]University of Salento
[3]Agricultural Research Council of Italy
**Corresponding author, e-mail: janska@vurv.cz*

MapMan software is a very useful tool for visualizing a big amount of transcriptional data in metabolic pathways which help to organize the whole data set to the functional groups. We have compared the transcriptional profiles in the progress (0, 1, 3, 7, 21d in 3 °C, 1d in -3 °C) of cold acclimation in two distinct organs (crown and leaf) of winter barley. Freezing tests of both leaves and crowns were also provided. Using Affymetrix chips and GeneSpring software 6197 differentially expressed genes were identified in leaves comparisons and 2370 were significantly modulated in crowns. The comparisons were visualized in MapMan software and analysed the up/down regulated genes involved in the metabolic pathways, especially that involved in sugar responses. Moreover, the three cluster analyses were carried out in the GeneSpring software on the list of common differentially modulated genes (1371 probe sets), on the crown specific modulated genes (999 probe sets) and on the leaf specific modulated genes (4827 probe sets). Each cluster was characterized by a typical course and functional categories determined by MIPS database. Interesting clusters, functional cathegories and examples of differentially expressed genes will be presented.

The work was supported by the Grant Agency of Charles University (project no. 84309), by the National Agency for Agricultural Research (QH 81287) and by the Czech Ministry of Agriculture (MZe0002700604).

P01-032: FEATURES OF REACTION OF SEEDLINGS OF SOFT WHEAT AND ITS WILD RELATIVES ON ACTION OF ABIOTIC STRESSES

Terletskaya, N. - Rysbekova, A. - Iskakova, A. -*
Khailenko, N. - Polimbetova, F.
Institute of Plant Biology and Biotechnology National, Center of Biotechnology Ministry of Education and Science Republic of Kazakhstan
**Corresponding author, e-mail: teni02@mail.ru*

Lack of water of soil and air, salt are the most abiotic stresses in Kazakhstan which create threat of a life of plants, brake their growth and reduce productivity. Modern selection uses various approaches for improvement of cultivars of wheat, including a genofund of world collections and leaning as on methods of the remote hybridization, and direct carrying over of genes. But the basic mechanisms of salt tolerance, wheat inherent in wild relatives are not known practically and their chromosomes are not conjugation in meiosys with genome of *Triticum aestivum* L., technologies transgenesys are almost unsuitable now. Other approaches of increase of wheat tolerance to stresses are focused on studying of physiological mechanisms for detection of new genes of stress-stability of grain cereals. Wild representatives *Triticeae Dum*. are growing in a wide series of conditions all over the world and have the big genetic variations. It is revealed that among representatives of its, containing even halophytes, there is a considerable variability in stability to osmotic and salt stress. Revealing of features of reaction of wheat – T. aestivum L. – and its wild relatives on abiotic stresses action are caused by doubtless interest.

As material for researches kinds of wheat served: T. *monococum* L. (AuAu), T. pseudomonococcum L. (AuAu), T. *sinskaya* Filat. et Kurk. (AbAb), T. *polonicum* L. (AuAuBB), T. *aethiopicum* Jakubz. (AuAuBB), T. *dicoccum* Shuebl. (AuAuBB), T. *turgidum* L. (AuAuBB), T. *macha* L. (AuAuBB), T. *compactum* L. (AuAuBBDD), T. V. rufulum (AuAuBBDD), T. *spelta* L. (AuAuBBDD), T. *kiharae* Dorof. et Migusch. (AtAtGGDD), and also T. *aestivum* L. (AuAuBBDD) (grades Caratovskaja-29, Mironovskaja-808, Leningradka) and *Secale cereale* L.

Features of reaction on action of abiotic stresses of wheat T. *aestivum* L. and its wild relatives were studied. Various reaction of wheat species to stresses was established, that testifies to necessity of variety of approaches at selection of genetic material for improvement of modern wheat cultivars.

P01-033: SHORT-TERM SALT AND OSMOTIC STRESS IN MAMILLARIA GRACILLIS PFEIFF. TISSUE CULTURE

Balen, B.[1] - Rogic, T.[1] - Simac, M.[1] - Peharec, P.[1] - Bar-Zvi, D.[2] - Krsnik-Rasol, M.[1]*
[1]University of Zagreb, Faculty of Science, Department of Molecular Biology
[2] Department of Life Sciences, Ben-Gurion University of the Negev
**Corresponding author, e-mail: bbalen@zg.biol.pmf.hr*

In vitro propagated *M. gracilis* plants develop calli without any exogenous growth regulators. This habituated calli spontaneously regenerate morphologically normal and hyperhydric shoots. Since the habituation and hyperhydricity are both part of a neoplastic progression, cactus cells were transformed with *A. tumefaciens* strain B6S3. Tumor line, which was established, never expressed any morphogenic capacity.

The aim of this study was to investigate the effect of short-term salt and osmotic stress on the *M. gracilis* callus and tumor culture. Tissue explants were grown in a liquid nutrient and exposed for 15 min and 3 h to growth medium supplemented with 250 mM NaCl or 500 mM mannitol. Following treatments, tissue was collected and protein extracts were prepared.

They were examined with regard to abundance and phosphorylation of ASR1 protein by western blotting and Pro Q Diamond fluorescent dye, respectively. Glycosylation pattern was examined by Pro Q Emerald fluorescent dye and lectin assay. ASR1 was present in both cactus tissues with three isoforms of approximately 42, 26 and 17 kDa.

No difference in abundance or phosphorylation of ASR1 in callus and tumor was observed after either 15 min or 3 h treatment with salt. More intensive phosphorylation signal of ASR1 was noticed in callus grown on medium with mannitol for 3 h. Analysis of glycosylation pattern with lectins Con A and GNA indicated stronger protein glycosylation in callus exposed to mannitol after 15 min and 3 h.

P01-034: VARIABILITY OF THE ARABIDOPSIS THALIANA PHENOTYPE – GENOME AND ENVIRONMENT

Jansen, M. - Gilmer, F. - Braun, S. - Schurr, U. - Walter, A.*
Forschungszentrum Jülich
**Corresponding author, e-mail: m.jansen@fz-juelich.de*

Plants are growing in heterogeneous and dynamically changing environments and are faced to stress by adverse biotic and abiotic factors. Human activities that pollute the environment and negatively affect the climate enforce stress. Plant phenotypes result from interplay of environmental factors and the genetic features of the plant. Plant phenotyping needs sensitive methods to detect phenotypic developments and modifications resulting from both genetic and environmental factors. GROWSCREEN FLUORO analyses revealed that *Arabidopsis thaliana* reacts very sensitively to changes in environmental conditions. When one ecotype of A. thaliana (Col-0) was subjected to different environments in terms of PAR, water supply, and cultivation substrates, pronounced modulations of growth, morphology and photosynthesis were observed. Stress generated by sub-optimal light, water or nutrient supply strongly diminished plant performance. Growing different *A. thaliana* ecotypes in one substrate and one set of environmental conditions, modulations of the phenotype caused by genetic properties could be stated in a similar intensity as those caused by the environment. These observations in the *A. thaliana* model system have impact on both laboratory experiments and breeding of crop plants. For the lab they suggest that it is crucial to precisely control and monitor the environment to which the plants are exposed. For breeding, the observations underline the importance of stress tolerance as a prerequisite for good plant performance in agriculture.

P01-035: THE ROLE OF PHOTORESPIRATION DURING WATER STRESS IN TWO TOMATO CULTIVARS WITH CONTRASTING DROUGHT TOLERANCE

Sánchez-Rodríguez, E - Rubio-Wilhelmi, M.M. - Blasco, B. - Leyva, R. - Melgarejo, R. - Romero, L. - Ruiz Juan, M.*
University of Granada
**Corresponding author, e-mail: evasr@ugr.es*

Drought is a major limitation to the productivity of many crops. Stomatal closure in response to drought stress restricts CO_2 entry into leaves while decreasing water loss from the leaves. Inhibited CO_2 assimilation reduces electron consumption by photosynthesis. Consequently mechanisms protecting the photosynthetic apparatus become increasingly important. It has been suggested that photorespiration is key for energy dissipation in order to prevent photoinhibition. In addition, photorespiration can generate metabolites, such as serine and glycine, which can be exported out the leaf or used in other metabolic pathways. Thus, photorespiration may also be a useful process in plants. The present work analyses the variations in activity of some photorespiration enzymes in two cherry tomato cultivars under water stress, with the aim of establishing whether the photorespiration of the plants is related to the degree of sensitivity or tolerance to this type of stress. To study photorespiration cycle, we analysed the enzymes ribulose-1,5-biphosphate carboxylase/oxygenase (rubisco), glycollate oxidase (GO), glutamate:glyoxylate aminotransferase (GGAT) and hydroxypyruvate reductase (HPR). The results show that the cv. Zarina enhanced enzymatic activities of cycle with the greatest RGR and LRWC. However, the activity of these enzymes in cv. Josefina either did not augment or maintained with respect to well-watered conditions. These results could indicate that photorespiration in cv. Zarina played a protective role by consuming photoinhibition-induced excess electrons under moderate drought stress.
ACKNOWLEDGEMENT This work was financed by the PAI programme and by a grant from the FPU of the Ministerio de Educación y Ciencia awarded to ESR.

P01-036: INVOLVEMENT OF CYTOKININS DURING NITROGEN DEFICIENCY IN TOBACCO PLANTS: OXIDATIVE STRESS

Rubio Wilhelmi, M.[1] - Sanchez-Rodriguez, E.[1] - Blasco, B.[1] - Leyva R.[1] - Romero L.[1]. - Blumwald, E.[2] -*
Ruiz Juan M.[1]
[1]Universidad de Granada
[2]Uc Davis
**Corresponding author, e-mail: mmrubio@ugr.es*

Nitrogen (N) is often defined as the limiting factor for plant growth. For this reason, the inefficient use of nitrogenous fertilizers in agriculture has increased in recent decades. The reduction of environmental pollution and the economic cost of applying these fertilizers requires the use of plants with high efficiency in utilizing this nutrient. N deficiency induces senescence and intensifies the production of reactive oxygen species, a situation that can be counteracted by augmenting the synthesis of cytokinins. Therefore, the aim of the present work was to determine the effect of abiotic stress, such as N deficiency on oxidative metabolism in two tobacco plants lines, Wild Type (WT) and PSARK::$IPT_{4\text{-}24}$ (IPT). These transgenic plants express isopentenyltransferase, an enzyme that catalyses the rate-limiting steps in cytokinin synthesis. The N was applied to the nutrient solution in the form of $NaNO_3$ at 10mM (Control), 7mM, 4mM and 1mM. The results indicated that the application of 1mM depressed the relative leaf growth rate of the WT, increased the enzymes SOD and CAT activities, whereas the foliar accumulation of MDA, ascorbate total, superoxide radical and H_2O_2. IPT showed a rise in the relative leaf growth rate under all the N treatments with regard to WT. In fact, this rise was highest in the case of plants under 4mM treatment, showing greater SOD activity but diminished CAT activities, and lower foliar accumulation of total ascorbate and superoxide radical with regard to control treatment. The results suggest the possibility of defining the increased cytokinin synthesis as an effective mechanism to improve N-use efficiency.
Acknowledgement This work was financed by the PAI programme and by a grant from the FPU of the Ministerio de Educación y Ciencia awarded to MMRW.

P01-037: PROTEOMIC ANALYSIS OF XYLEM SAP UNDER SALT STRESS IN BRASSICA OLERACEA.

Fernandez Garcia N[1] - Hernandez, M.[1]* - Casado Vela, J.[2] - Bru, R.[3] - Elortza, F.[2] - Hedden, P.[4] - Garcia de la Garma, Jesus[1] - Olmos, E.[1]*
[1]CEBAS-CSIC
[2]CIC-BIOGUNE, Vizcaya
[3]Universidad de Alicante, Dept. Agroquimica y Bioquimica
[4]Dept. Plant Science, Rothamsted Research, UK
**Corresponding author, e-mail: nieves@cebas.csic.es*

The movement ofsolutes from root to the aerial part of the plant is accomplished by the tracheary elements of the xylem. Its main function was traditionally considered as the main conduit for water and minerals from root to shoots. Probably for these reasons, xylem sap analysis has been mainly focused in the mineral contents. However, xylem sap contains also organic solutes including carbohydrates, amino acids, organic acids, hormones and proteins. It is considered that root-to-shoot signalling could be an important physiological process that can supply signal molecules different to water and nutrient from the root system. Therefore, modifications in these signal molecules under abiotic stress can play important roles in plant adaptation to stress.
Xylem sap proteome of maize, *Brassica napus, Glycine max, Vitis vinifera* and poplar have been recently studied using separation in 2-D gel and mass spectrometry identification. In this work, the xylem sap of *Brassica oleracea* was analyzed following the 2-D DIGE technique. More than 200 protein spots were observed in the gel. Gel images analysis identified 76 proteins that were differently expressed in xylem sap of control and salt treated plants. Most of these proteins, 41 spots, matched to proteins or genomic sequences. The identified proteins whose abundance changed fell into four major biological categories: cell wall metabolism, programmed cell death, plant defence metabolism and plant metabolism. Our results indicate that salt stress is inducing xylem differentiation and lignification.

P01-038: EFFECT OF MELAMPSORA RUST ON THE PHOTOCHEMISTRY AND WATER RELATIONS OF DIFFERENT POPULUS SP. CLONES

Elena, G.[1] - Fernàndez, J.[1] - Zacchini, M.[2] - Moret, A.[1] - Leck, I.[1]*
[1]Departament de Biologia Vegetal. Unitat Fisiologia Vegetal. Facultat, Biologia. Universitat de Barcelona
[2]Istituto di Biologia Agroambientale e Forestale IBAF, Roma.
**Corresponding author, e-mail: gelenajimenez@gmail.com*

Rust caused by *Melampsora* spp. is the most widespread and frequent disease of poplars (*Populus* spp.), affecting their development and productivity. The effect of *Melampsora* sp. on the photochemistry of photosynthesis was studied in leaves of three clones of poplar: Luisa Avanzo - (*P. x canadensis* Mönch.), Lux - (*P. deltoides* Bartr.) and Adige - (*P. x canadensis* Mönch.). In these clones, different tolerances to *Marsonnina brunnea* and to the poplar mosaic virus (PMV) have been reported. The study was conducted in September 2009 and the poplar clones were taken from a collection of fifty clones established since 2001 at the CNR-IBAF–Institute experimental fields in the Tevere valley, near Rome.
L. Avanzo was the most susceptible clone to the infection, showing high occurrence and severity. Moreover, the chlorophyll fluorescence images and results obtained with the fluorometer Imaging-Pam Walz showed lower maximum quantum yield (Fv/Fm) in infected leaves. These results were supported by the analyses of total chlorophyll content (evaluated by SPAD-502 Minolta) and by the radiometric parameter PRI (Photochemical reflectance index). However, no differences between clones were found in relative water content (RWC) or water index (WI). We conclude that Lux and Adige showed a notable tolerance to infection by *Melampsora* sp. and that their photochemical process in the leaves was unaltered.

P01-039: OLIGALACTURONIDES INVOLVEMENT IN ROOT GROWTH, ROS ACCUMULATION AND EXTRACELLULAR ALKALINIZATION IN ALFALFA SEEDLINGS.

Jiménez, A.[1] - Camejo, D.[1] - Martí, M.C.[1] - Cabrera, J.C.[2] - Olmos, E.1 - Sevilla, F.[1]*
**Corresponding author, e-mail: ajimenez@cebas.csic.es*
[1]CEBAS-CSIC
[2]FUNDP

Oligogalacturonic acids (OGA) are biologically active oligosaccharides with diverse biological effects in plants, being the earliest detectable response the transient formation of ROS. The mechanisms involved and the relationship between ROS and the physiological effects inducing plant growth brought about by OGAs are still not well established. In this work, the effects of an OGA pool on root growth of intact alfalfa seedlings (*Medicago sativa* L.) as well as on extracellular pH and both, extracellular and intracellular O_2.- dynamics together with H_2O_2 are examined to provide an overall view of the mechanisms that may influence root growth under these treatments. Alfalfa seedlings were soaked in OGA solutions at 25, 50, 75 and 100 µg mL^{-1} for 120 minutes. Spectrophotometry measurements, histochemical detection and confocal laser scanning microscopy were used for ROS detection in control and OGAs-treated seedlings. The lower OGA concentrations were found to promote root growth, but 50 µg mL^{-1} had higher promoting effect, while the higher OGA concentration had not significant effect. The extracellular pH dynamics of the treated seedlings provided evidence that the OGA-induced root growth cannot be explained by the "acid growth theory", but is mediated mainly by O_2.- accumulation. The 50 µg mL^{-1} OGA concentration induced the generation of O_2.- in the root, mainly in the elongation zone which seems to be significant at 60 and 120 min of treatment. We suggest that "increasing and maintaining" but not toxic level of O_2.- generation could drive several processes associated with root elongation in the seedling treated with 50 µg mL^{-1} OGA.
Work supported by DGI-FEDER (BFU 2008-00745 project) and SENECA Foundation (04553/GERM/06 project)

P01-040: MONITORING EARLY STRESS CAUSED BY THE ALLELOCHEMICAL BOA IN ARABIDOPSIS THALIANA PLANTS

Sánchez-Moreiras, A. - Martínez-Peñalver, A. - Reigosa, M.J.
University of Vigo
**Corresponding author, e-mail: adela@uvigo.es*

Experiments conducted so far on the mode of action of BOA (secondary metabolite belonging to the benzoxazinone group) resulted in the establishment of a general model for the response induced by BOA in plant metabolism. Many physiological and biochemical mechanisms related to plant oxidative metabolism were altered after BOA treatment, which led to postulate oxidative stress as a possible way for the phytotoxic action of BOA (Sánchez-Moreiras and Reigosa, 2005, Batish et al., 2006).
However, in the most of the work carried out with adult plants the damage was measured more than 7 days after treatment (Batish et al., 2005, Sanchez-Moreiras and Reigosa, 2005, Sanchez-Moreiras et al., 2009), so that the early response to BOA, and therefore the primary effect thereof, has not been properly established. That is why in this study we sought to detect early signs of stress in Arabidopsis plants exposed to BOA by monitoring the physiology of the plant (by means of chlorophyll a fluorescence-image analysis) and establishing valid biomarkers for the detection of stress (pigments, proteins, LIP POD, etc).
This study managed to differentiate the primary effects from the side effects that occur after several days of treatment. Our results showed that oxidative stress is not part of the primary effect of BOA although is critical to its long-term phytotoxic action. The results suggest the presence of an induced senescence mechanism as a possible cause of the damage observed by BOA in the metabolism of treated plants.
Sánchez-Moreiras AM, Reigosa MJ (2005) J. Chem. Ecol. 31: 2689-2703. Batish DR, Singh HP, Setia N, Kaur S, Kohli RK (2006) Plant Physiol. Biochem. 44: 819-27.

P01-041: THE TWO TREBOUXIA ALGAE, EVER-PRESENT IN LICHEN THALLI OF RAMALINA FARINACEA, DISPLAY DIFFERENT PHYSIOLOGICAL RESPONSES TO OXIDATIVE STRESS. COEXISTENCE VS. COMPETENCE?

Casano Mazza, L.[1] - Del Campo, E.[2] - del Hoyo, A.[2] - Álvarez, R.[2] - Gasulla, F.[3] - Guéra, A.[2] - Esteban-Carrasco, A.[2] - Barreno, E.[3]*
[1]Universidad de Alcalá
[2]Department of Plant Biology, University of Alcalá, Madrid
[3]ICBIBE, Department of Botany, University of Valencia, Faculty of Biology,
**Corresponding author, e-mail: leonardo.casano@uah.es*

Ramalina farinacea (L.) Ach. is an epiphytic fruticose lichen relatively abundant in areas with mediterranean, subtropical or temperate climates, suggesting a wide ecophysiological plasticity of this symbiotic association to cope with changing and often stressful environmental conditions. We have previously demonstrated that *R. farinacea* constantly contains two distinct photobionts belonging to *Trebouxia* genus (provisionally named TR1 and TR9) in each thallus. An initial physiological characterization indicated a better performance of TR9 under relatively high temperatures and irradiances while TR1 seems to prefer more temperate and shadow conditions.
In the present study we studied the response of TR1 and TR9 to stress. Since different environmental stresses result in enhanced production of reactive oxygen species (ROS), we analyzed the effects of oxidative stress, generated through treatments with Cumene Hydroperoxide (CuHP), an intracellular ROS propagator. Compared to TR1, TR9 showed a higher capability to preserve

key chloroplast components, such as chlorophyll a, carotenoids and D1 protein, which in turn helped to maintain a higher PSII photochemical efficiency under strong oxidative treatments. In addition, CuHP caused in TR1 a higher decrease of non-photochemical dissipation of energy (NPQ) and also diminished the activity of key antioxidant enzymes, like SOD, GR and APx, while in TR9 a lesser NPQ decrease and a significant increase of antioxidant enzymes were observed. We propose that the constant presence of TR1 and TR9 in every *R. farinacea* thalli could be favoured by the different and probably complementary physiological behaviour of each photobiont.
(Grants: CGL2009-13429-C02-00 and PROMETEO 174/2008 GVA)

P01-042: RESPONSES OF TRANSGENIC TOBACCO PLANTS WITH INCREASED PROLINE CONTENT TO WATER AND/OR HEAT STRESS

Pospisilova, J. - Haisel, D. - Vankova, R.*
Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague
**Corresponding author, e-mail: pospisilova@ueb.cas.cz*

Net photosynthetic rate, transpiration rate, stomatal conductance and pigment contents in transgenic tobacco plants (M51-1) constitutively over-expressing a modified gene for the proline biosynthetic enzyme Δ2-pyrroline-5-carboxylate synthetase (*P5CSF129A*) and the corresponding wild-type plants (WT) were compared during water stress and heat stress alone or in combination. The proline content was several times higher in M51-1 than in WT which coincided with abscisic acid content higher in transformant. In non-stressed plants, transpiration rate and stomatal conductance of M51-1 were lower than those of WT, while differences in net photosynthetic rate were not significant and water use efficiency and contents of chlorophyll and xanthophyll cycle pigments were higher in M51-1 than WT. Cessation of watering for 7 d decreased all gas exchange parameters and pigments contents, the response being similar in M51-1 and WT plants. After heat stress (40 °C/60 min) applied to control or water-stressed plants the gas exchange parameters decreased considerably in both M51-1 and WT plants. Short-term heat stress alone, however, did not affect pigment contents.

P01-043: ROOT SPECIFIC EXPRESSION OF THE TRANSCRIPTION FACTOR OSNAC10 IMPROVES DROUGHT TOLERANCE AND GRAIN YIELD IN RICE UNDER FIELD DROUGHT CONDITIONS

Jeong, JS.[1] - Kim, YS.[1] - Baek, WH.[1] - Park, HK.[1] - Ha, SH. [2] - Choi, YD.[3] - Kim, M.[3] - Reuzeau, C.[4] - Kim, JK. [1]*
[1]School of Biotechnology and Environmental Engineering, Myongji University
[2](National Academy of Agricultural Science, RDA),
[3](School of Agricultural Biotechnology, Seoul National University)
[4](CropDesign N.V., Technologiepark),
**Corresponding author, e-mail: jsjeong74@gmail.com*

Drought poses a serious threat to the sustainability of rice yields in rainfed agriculture. Here we report the results of a functional genomics approach that identified a rice NAC-domain gene, *OsNAC10*, which improved performance of transgenic rice plants under field drought conditions. Of the 140 *OsNAC* genes predicted in rice, 18 were identified to be induced by stress conditions. Phylogenic analysis of the 18 *OsNAC* genes revealed the presence of 3 subgroups with distinct signature motifs. The *OsNAC10* is expressed predominantly in roots and panicles, and induced by drought, high salinity and ABA. Overexpression of *OsNAC10* in rice under the control of the constitutive promoter *GOS2* and the root-specific promoter *RCc3* increased the plant tolerance to drought, high salinity and cold at the vegetative stage. More importantly, the *RCc3:OsNAC10* plants showed significantly enhanced drought tolerance at the reproductive stage, increasing grain yield by 25-42% and 5-14% over controls in the field under drought and normal conditions, respectively. Grain yield of *GOS2:OsNAC10* plants in the field, in contrast, remained similar to that of controls under both normal and drought conditions. These differences in performance under field drought conditions reflect the difference in expression of *OsNAC10*-dependent target genes in roots as well as in leaves of the two transgenic plants, as revealed by microarray. Root diameter of the *RCc3:OsNAC10* plants was thicker by 1.25-fold than that of the *GOS2:OsNAC10* and NT plants due to the enlarged stele, cortex and epidermis. Overall, our results demonstrated that root specific overexpression of *OsNAC10* enlarges roots, enhancing drought tolerance of transgenic plants, which increases grain yield significantly under field drought conditions.

P01-044: SALT STRESS-INDUCED CHANGES IN MITOCHONDRIA FROM LUPINE EMBRYO AXES

Garnczarska M[1] - Wojtyla, L.[1] - Grabsztunowicz, M.[1] - Rog, M.[1] - osmala, A.[2]*
[1]Department of Plant Physiology, A. Mickiewicz University, Poland
[2] Insitiute of Plant Genetics, Polish Academy of Sciences, Poland
**Corresponding author, e-mail: garnczar@amu.edu.pl*

In this work, an attempt was made to investigate the response of mitochondria from lupine (*Lupinus luteus*) embryo axes to salinity stress. Changes in mitochondria from isolated lupine embryo axes grown on modified Heller medium with or without addition of 0,1M NaCl for 24, 48 and 72 h were analysed. 2D-IEF-PAGE electrophoresis of mitochondria proteins revealed that after 24 hours 38% of proteins had lower and 27% higher level of expression in salt treated axes as compared to control. After 48 and 72 hours approximately 33% of proteins were both less and more abundant in salt stressed organs than in non-stressed. The profile of antioxidant enzymes was also analysed. Native electrophoresis of mitochondrial proteins revealed higher activity of catalase and superoxide dismutase in mitochondria from salt treated axes in comparison with control axes. Salt stress caused ultrastructural changes of ER but no deformation of mitochondria was observed. We have also established a salt-induced PCD model in lupine embryo axes. Our results indicated that NaCl treatment lead to specific characteristic of PCD in lupine embryo axes, such as DNA laddering and cytochrome c release from mitochondria into the cytoplasm. Changes in mitochondrial proteome during salt-induced PCD were also studied.
This work was partially supported by Polish Ministry of Science and Higher Education research grant N N303 471038.

P01-045: ROLE OF SILICON IN MITIGATION OF CADMIUM TOXICITY IN MAIZE

Vaculik, M.[1] - Lux, A.[1] - Luxova, M.[2] - Tanimoto, E.[3] - Lichtscheidl, I.[4]*
[1]Department of Plant Physiology, Faculty of Natural Sciences, Comenius University in Bratislava
[2]Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovakia
[3]Department of Information and Biological Sciences, Graduate School of Natural Sciences, Nagoya City University, Mizuho, Japan
[4]Cell Imaging and Ultrastructure Research, University of Vienna, Austria
**Corresponding author, e-mail: vaculik@fns.uniba.sk*

Silicon, the second most abundant element in the earth crust, is not considered as an essential element in general, but its beneficial influence in alleviation of various kinds of abiotic and biotic stresses in plants is known. Recently, several studies described Si-induced alleviation of negative effects of dangerous toxic metal cadmium on plants, but the role of Si in this process is poorly understood. This contribution brings novel insight into

the function of Si in mitigation of toxic effects of Cd in widely used crop – maize. Seedlings of *Zea mays* L. cv. Jozefina were cultivated in hydroponics in Hoagland solution in standard control conditions and in excess of Cd, Si and both Cd+Si. Various treatments have been compared: control (C), Cd (5μM $Cd(NO_3).4\ H_2O$), Si (5 mM Si in the form of sodium silicate solution) and Cd+Si. Cadmium caused decrease of growth parameters (root length, root and shoot fresh and dry weight, leaf area). Extensibility of root cell walls was decreased by Cd, this was significantly alleviated when Si was added to Cd treatment. When Cd+Si was applied, the content of Cd was higher both in the below-and above-ground plant parts when compared with the control. This corresponded with the changes in apoplastic barrier development – endodermal suberin lamellae formed more distant from the root apex when Si was applied together with Cd. Activity of four antioxidative enzymes and non-enzymatic antioxidant ascorbate, as well as content of chlorophyll and carotenoids in the first two fully developed leaves were also significantly influenced by Si when compared with non-Si treated plants. We suppose that beneficial role of Si in mitigation of Cd toxicity is based on “in planta mechanism” and Si is probably actively involved in several metabolic pathways running in plants.

P01-046: ENHANCED SALT STRESS TOLERANCE OF CYANOBACTERIUM SYNECHOCYSTIS EXPRESSING PLANT AND MICROBIAL MEMBRANE PYROPHOSPHATASES

Serrano, A - Drake, R. - Perez C.*
IBVF, CSIC-Univ. de Sevilla
**Corresponding author, e-mail: aurelio@ibvf.csic.es*

Cyanobacteria are unique prokaryotes performing oxygenic photosynthesis, and possessing plasma- and thylakoid membranes. However, in contrast to other major groups of photosynthetic prokaryotes they lack of proton-translocating pyrophosphatases (H-PPases), the simplest primary proton pumps known to date. Genes encoding H-PPases of the embriophyte *Arabidopsis thaliana* (AVP1 isoform), the euglenozoan protist *Trypanosoma cruzi* and the green non-sulfur photobacterium *Chloroflexus aurantiacus* have been functionally expressed in the freshwater (moderately salt tolerant) cyanobacterium *Synechocystis* PCC6803. In contrast to control cells, the transformed clones exhibited high levels of membrane-bound PPase activity and the 70 kDa H-PPase subunit was immunodetected in both thylakoid and plasma membranes. Noteworthy, the transformed PCC6803 clones show enhanced tolerance to severe salt stress, being able to growth in the presence of 1.0-1.5, M NaCl, which are lethal conditions for control cells. The comparatively higher PSII activity measured in the transformed clones indicates a better preserved photosynthetic apparatus. These results strongly suggest that pyrophosphate (PPi) can be used as alternative energy source by the transformed clones, and are in agreement with our proposal of PPi and H-PPases being involved in a PPi-based sustainable bioenergetics that should be an adaptative advantage under environmental conditions that severely constrain the cellular energy status. Supported by grant BFU2007-61887/BMC (MICINN, Spain) and PAIDI group BIO-261 (Junta de Andalucía)

P01-047: EFFECT OF AQUAPORIN EXPRESSION ON LOW ROOT TEMPERATURE RESPONSES IN ARABIDOPSIS

Zwiazek, J. - Lee, S-H. - Chung, G-C. - Oh, M-N - Hong, S-W. - Kang, H.*
University of Alberta
**Corresponding author, e-mail: jzwiazek@ualberta.ca*

Effects of low root temperature were studied in the wild-type and transgenic Arabidopsis that constitutively overexpress PIP1;4, PIP2;5 aquaporins (AQPs) and in double knockout (PIP1;1×PIP2;6, PIP1;2×PIP2;6, TIP3;1×TIP3;2) lines. Plants were grown in solution culture in a growth room at 25oC with their roots exposed to temperatures ranging from 5ºC to 25ºC. No differences in growth were observed in the wild-type, overexpressing PIPs and double knockout lines at higher root temperatures. However, plants overexpressing PIP2;5 had higher growth rates under the low root temperature (10ºC). When measured at 20 and 25ºC, hydraulic conductivity of root cortical cells (Lp) was similar in the wild-type plants and plants overexpressing PIPs. However, in the double knockout plants, Lp was lower compared with the wild-type plants. Descending temperatures series (from 25 to 10ºC in 5ºC steps) caused a strong reduction of L_p in the wild-type, double knockout PIP and TIP plants, but not in the plants overexpressing PIP2;5 and PIP1;4. Whenthe temperature was increased from 10 to 25ºC, irreversible changes of half-times of water exchange ($T_{1/2}$) were obtained in the wild-type, double knockout PIP and TIP plants suggesting longer-term conformation changes of AQPs. Application of HgCl2 (AQP blocker) reversibly inhibited L_p by about 3-4-fold in the wild-type and double knockout PIP plants, and caused smaller reductions of L_p in plants overexpressing PIPs and double TIP knockout plants. The results suggest that increased abundance of PIPs was helpful in maintaining water uptake under low root temperature and that the overexpression of AQPs may be a useful trait in plants growing in cold soils.

P01-048: ARE THERE CULTIVAR DIFFERENCES ALONG EARLY RICE DEVELOPMENT THAT REFLECT THE FURTHER EFFECT OF SALINITY ON PRODUCTIVITY?

*Wankhade, S. - Cornejo, M.J. - Mateu, I. - Sanz, A.**
Universitat de València
**Corresponding author, e-mail:amparo.Sanz@uv.es*

The traditional rice cultivar Bomba is highly appreciated due to the grain organoleptic properties. Its productivity, however, is affected not only by high plant size but also by salt sensitivity. We compared the effects of saline stress on development and leaf anatomical features of this cultivar with other *japonica* cultivars less affected by salinity (Taipei 309, Bahia). Our results show that salt sensitivity is mainly associated with the ionic component of salinity. Thus, the inhibition of growth in cv Bomba seedlings was stronger under saline than under osmotic stress and some anatomical features, particularly those related to the conducting tissue (i.e. size of xylem vessels) were altered to a higher extent by NaCl than by sorbitol. Along the vegetative growth stage the pattern of anatomical variations caused by salinity in leaves persisted. Concomitantly, there was a stronger reduction in chlorophyll content and in maximum potential efficiency of PSII (Fv/Fm), together with a decrease of net CO_2 assimilation rate (P_N). Since transpiration rates (E) were scarcely affected, it results in lowered water use efficiency (WUE) values. Only plants subjected to low NaCl concentrations (10 and 20 mM) reached the reproductive stage. The anatomical variations observed at this stage showed that even 20 mM NaCl appeared to be an excessive dosage for this cv, which reduced four-fold the number of panicles formed in comparison to cv Bahia. Thus, differences in sensitivity to osmotic and saline stress between developing seedlings of these cultivars reflect further differences in salt sensitivity during flowering. The genetic relations among these cultivars has been studied by SSR markers and will also be presented.

P01-049: CELL LINES WITH DIFFERENT LEVELS OF STRESS TOLERANCE ASA MODEL SYSTEM TO STUDY RESPONSES TO DEHYDRATION STRESSES IN RICE.

*Sanz, A. - Pons, R. - Cornejo, M.J.**
Universitat de València
**Corresponding author, e-mail: Maria.J.Cornejo@uv.es*

As compared to plants, cell lines are a relatively homogeneous and simplified experimental system which avoids tissue speci-

fic responses and allows the establishment of relations between some cell responses and environmental stress tolerance, i.e. osmotic adjustment, proline accumulation or membrane transport (*Luttset al. JPP 149: 186-95, 1996; Kerkeb et al. PPlant 116: 37-41, 2002; Yang et al. PC Rep 26: 229-35, 2007*). We have further developed this experimental system by the use of cell lines subjected to dehydration stresses of different magnitude and selecting those with high or low tolerance to compare their responses to stress. Thus, we studied putative physiological and genomic variations related to cryopreservation procedures (*Moukadiri et al. PPlant 105: 442-9, 1999*), the pattern of endocytosis under osmotic and saline stress (*Bahaji et al. PCP 44: 1100-11, 2003*), and demonstrated the relationship between tolerance and ability of ABA synthesis under stress (*Perales et al. PPB 43: 786-92, 2005*). More recently, we used transformed rice cell lines to study the induction of a poliubiquitin promoter by dehydration stresses (*Perales et al JPP 165: 159-71, 2008*) and, at present, we are investigating the involvement of (plasmalemma and tonoplast) H^+ pumps in NaCl tolerance mechanisms (*Pons et al, MS in prep.*). Results from this investigation will be presented and discussed in relation to previous research performed with this experimental system.

P01-050: CHANGES IN FREE AND CONJUGATED ABSCISIC ACID (ABA AND ABAGE) CONCENTRATIONS IN FIVE CULTIVARS OF HORDEUM VULGARE PLANTS UNDER WATER STRESS CONDITIONS

Thameur, A.[1] - Ferchichi, A.[1] - López-Carbonell, M.[2]
[1]Institue Arid Zones, Medenine, Tunisie
[2]University of Barcelona- Department of Plant Biology

Changes in endogenous abscisic acid (ABA) and its glucose ester conjugate (ABAGE) as well as in proline content, water relations and growth parameters in five barley genotypes (Ardhaoui, Manel, Pakistan, Roho, Rihane) with different drought resistance characteristics have been studied. The aim of this work was to study the balance between free and conjugate ABA and how it can affect the development of these plants grown under water deficit conditions. Differences among the five genotypes lead to changes in the pattern of growth and development. Water stress led to a reduction in relative water content, as well as an increase in proline and endogenous ABA and ABAGE concentrations in all tested genotypes. The increase of proline ranged between 2-fold for var. Rihane and 1.3 fold for var. Manel. The lack of water lead to increase in endogenous ABA concentrations between 5-fold for cv. Ardhaoui and 1.4-fold for var. Roho. Also small increases in endogenous conjugated ABA in all genotypes, except for cv. Ardhaoui, were observed. Nevertheless, the increases in free ABA were more pronounced in tolerant cultivars than in the less tolerant ones. Our results show that changes in growth parameters were correlated with variations of endogenous concentrations of ABA and ABAGE, and will contribute to the knowledge of the involved mechanisms in drought adaptation of *Hordeum vulgare* plants.

P01-051: LONG-TERM RESPONSES TO WATER STRESS IN SELECTED CLONES OF EUCALYPTUS GLOBULUS L.

Granda, V.[1] - de la Torre, C.[1] - Feito, I.[2] - Cuesta, C.[1] - Hernández, B.[1] - Kidelman, A.[2] - Majada, J. [2] - Ordás, R.J.[1]- Rodriguez, A.[1]*
[1]Departamento de Biología de Organismos y Sistemas, Área de Fisiología Vegetal. Universidad de Oviedo
[2]Área de Cultivos Hortofrutícolas y Forestales, Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA) , Asturias.
**Corresponding author, e-mail: biovgg@gmail.com*

The response of juvenile forest crop plants to water stress is key to the survival of forest populations. In this stage of development plants are very sensitive to water stress; this in fact being the major source of plant loss in commercial plantations. Therefore early detection of tolerant genotypes/varieties using physiological markers would be useful to avoid major loss during field establishment of plantations.In order to understand the physiological changes and adaptation occurring in juvenile plants of *Eucalyptus globulus* L., selected clones were subjected to long-term stress in controlled conditions. Half of the plants of every clone were maintained at 90-100% of field water capacity, whereas the water supply to the remainder was reduced; firstly to 40% of field capacity (first sampling), and finally, until the death of the plant (final sampling). Plants were monitored throughout the experiment to detect changes in foliar area and growth. Hydrical, photosynthetic, metabolic and other physiological measures were also taken to compare the behavior of the different clones and the establishment of physiological markers for water stress tolerant clones.Acknowledgements. This work was supported by the projects of Ministerio de Educación (AGL2006-13912-C02-01 and GEN2006-27791-C2-1E/VEG). V. Granda is funded by a predoctoral grant by Ministerio de Educación y Ciencia (FPI BES-2007-15663). The eucalyptus clones were provided by ENCE group.

P01-052: TOMATO NHX ANTIPORTERS

Galvez, F.J. - Cagnac, O. - Abushamsiya, K. - Rodríguez Rosales, M.P. - Venema, K.
EEZ-CSIC

Plant NHX antiporters can be subdivided in tonoplast Class I proteins, with a suggested role in salt tolerance by Na^+ accumulation in vacuoles, and Class II proteins. We previously determined an endosomal localization of the Class II LeNHX2 protein in plants and confirmed this expression pattern in yeast. We also determined a predominantly K^+/H^+ exchange reaction of the protein in vitro. These data suggest that the salt tolerance phenotype in over-expressing yeast does not rely strictly on vacuolar Na+ accumulation, which we confirmed in transgenic tomato.
We have identified several more tomato NHX isoforms. The expression of the isoforms was induced more by salt stress in salt tolerant tomato species as compared to salt-sensitive species, confirming that NHX genes are determinants of salt tolerance. Of these isoforms, Class I LeNHX4 shows a high expression level in Fruit and Flower tissue, and could thus play an important role in K^+ accumulation in these tissues, essential during flowering and fruit development. Like LeNHX2, LeNHX4 confers salt, KCl and Hygromycin resistance to yeast with a disruption in the yeast ScNHX1 gene. The LeNHX4 protein has a tonoplast localization in tomato plants, as is to be expected for a Class I NHX protein. In yeast the majority of the fluorescence signal from GFP tagged protein is found inside the vacuoles, and not in the membrane, pointing to a degradation of the protein. This is also indicated by SDS-PAGE electrophoresis, were the protein appeared as a small band of the expected molecular weight and a large amount of smaller molecular weight. As a consequence, we could so far not determine the antiporter activity in vitro. New results regarding function and functioning of the LeNHX4 protein will be presented

P01-053: AGEING AND IRRADIANCE ENHANCE VITAMIN E CONTENT IN GREEN EDIBLE TISSUES FROM CROP PLANTS

Garcia Plazaola, J. - Lizarazo, K. - Fernández-Marín, B. - Becerril, J.M.*
Universidad del País Vasco
**Corresponding author, e-mail: joseignacio.garcia@ehu.es*

Tocopherol (vitamin E) is an antioxidant essential in human nutrition. Several approaches have aimed to enhance tocopherol content in crops by the genetic modification of plants, a practice that generates some social concern. As tocopherol accumulates with leaf age in some wild plants and the antioxidant mechanisms respond plastically to stress conditions, we hypothesize

in this study that tocopherol content can be increased in edible plants by the manipulation of harvesting time and growth conditions, in particular irradiance. We have studied ontogenic changes in tocopherol concentration in photosynthetic tissues of edible leaves (lettuce, spinach, corn salad and dandelion) and green fruits (cucumber and pepper). In all species tocopherol content increased with tissue age. Spinach showed the fastest rate of tocopherol accumulation, and the growth at higher irradiance had a synergistic effect over the rates of accumulation. The same irradiance dependency of this accumulation was observed in fruits, but a final decrease with senescence occurred in cucumber. This study demonstrates that the content of tocopherol in vegetables can be notably enhanced (or reduced) by simply selecting the adequate harvesting time and/or by manipulating the environmental conditions during the growth period

P01-054: PHYTOREMEDIATION CAPABILITY OF BRASSICA NAPUS GROWN ON SOILS, CONTAMINATED WITH HEAVY METALS

Koshkin, E. - Vagun, I.*
Russian State Agrarian University-Moscow Timiryazev Agricultural Academy

Summer oilseed (*Brassica napus* L.) variety Podmoskovniy was grawn in a greenhouse in a pot culture with sod-podzol soil contaminated (in mg/kg soil) with Pb (from 50 up to 400 in every 50 mg/kg dosage interval), Cd (from 2 up to 14 in every 2 mg/kg dosage interval) and Zn (from 100 up to 800 in every 100 mg/kg dosage interval). Linear relationship was established between Zn and Pb accumulation in shoots at seed maturity stage and their concentrations in aboveground biomass. As for Cd, linear relations between above parameters exists only up to its concentration of about 15 mg/kg DW and reached a plateau there after. Harvest index (Hi) decreased with increasing Zn and Pb concentration in the seeds and remained almost constant with Cd.With increasing rates of heavy metals (HM) contamination the ratio of their accumulation in the seeds to that in shoots decreased for Pb and Zn and remained almost stable for Cd. Accumulation of Pb and Cd in shoots increased with increasing HM concentration in seeds. No regular pattern was established for Zn due to reverse relationship between its concentration in seeds and shoot DM accumulation.

P01-055: NA^+-ATPASES IN MARINE GREEN MICROALGAE.

Popova, L. - Balnokin, Y.*
K.A.Timiryazev Institute of Plant physiology, Russian Academy of Sciences
**Corresponding author, e-mail: lora_gp@mail.ru*

All organisms surviving high substrate salinity prevent excessive Na^+ accumulation in cytoplasm due to operation of Na^+-translocating proteins localized to the plasma membrane/tonoplast and exported Na^+ from the cytoplasm to the external medium/vacuole. A wide diversity of the enzymes executing primarily active sodium export from cells operates in cell membrane of prokaryotes. In eukaryotes, only P-type ATPases resided in plasma membranes mediate primarily active sodium export from cells. Mammalian Na^+,K^+-ATPase is the first and most extensively studied representative of this ATPase family. More recently, Na+-translocating ATPases of P-type were also discovered in marine golden-brown microalga *Heterosigma akashiwo* (the kingdom Chromista) (Wada et al., 1992) and some yeast species (the kingdom Fungi) (Benito et al., 2002). In halotolerant plants the existence of a primary Na+-transporter was debated for a long time. Nevertheless, primary Na+-pumps have been found in some representatives of the kingdom Plantae. Na^+-ATPases of P-type were found in two marine green microalga species, *Tetraselmis viridis* (Balnokin and Popova, 1995) and *Dunaliella maritima* (Popova et al., 2005). Both species belong to the class Prasinophyceae which may be a paraphyletic basal group to all green plants. The Na^+-ATPases from the algae demonstrate near similarity. Both ATPases are electrogenic enzymes and operate in the weakly alkaline pH range with maximal activity at pH 7.5 – 8.0. They are highly specific to Na^+ and could not transfer K^+ thus differing from both animal-type Na^+,K^+-ATPase that exchanges Na^+ for K^+ and fungal-type Na^+-ATPase that does not discriminate between Na^+ or K^+ (Benito et al., 2002).

P01-056: TEMPERATURE DROP APPLIED AT EARLY STAGES OF ONTOGENESIS CAN ENHANCE PLANT DEVELOPMENT

Sysoeva, M. - Markovskaya, E. - Sherudilo, E. - Shibaeva, T.*
Institute of Biology, Karelian Research Centre RAS
**Corresponding author, e-mail: sysoeva@krc.karelia.ru*

It is well known that a short duration temperature drop may affect plant morphogenesis. However, limited data are available on its effect on plant development.
The experiments were conducted with different plant species: cucumber, cabbage, marigold, pansy, petunia. Seeds or plants at early stages of ontogenesis were treated with temperature drops for 6-7 days.
The intensity and duration of the temperature drop varied with plant species. Temperature drop increased the number of leaves in cucumber young plants and cabbage, accelerated flowering and improved plant quality in marigold but not in petunia.
Pre-sowing seed treatment with temperature drop has also hastened flowering in marigold and pansy.
Possible underlying mechanisms which contribute to these effects will be discussed.
The study was supported financially by the Russian Foundation for Basic Research (N 07-04-00063).

P01-057: CHANGES IN CHLOROPLAST LIPOXYGENASE 6 LEVEL AND LOCALIZATION UNDER DARK-CHILLING CONDITIONS IN COMMON BEAN (PHASEOLUS VULGARIS L.)

Rudowska, L.[1] - Mazur, R.[1] - Rumak, I.[1] - Kozlowski, P.[1] - Trzcinska-Danielewicz, J.[1] - Hapka, M.[1] - Michalski, W.[2] - Mostowska, A.[1] - Garstka M.[1]*
[1]University of Warsaw
[2]CSIRO Australian Animal Health Laboratory
**Corresponding author, e-mail: lucja.rudo@gmail.com*

It was demonstrated that low temperature induced changes in the chloroplast structure and function of chilling sensitive (CS) plant species. These changes were due to the rearrangement of chlorophyll-protein complexes inside the thylakoid membranes. We found out that in CS Common bean (*Phaseolus vulgaris* L.) the dark-chilling stress induces association of the lipoxygenase 6 (LOX6) with the thylakoid membranes. LOX6 is probably involved in the oxylipin synthesis against wounding and non-host pathogen infection. For detailed analysis of LOX6 we used both molecular (immunodetection, mass spectrometry and northern-blot) and microscopy (electron microscopy with immunogold labeling) techniques. Our analysis has shown increased *PvLOX6* mRNA and LOX6 protein levels in thylakoids during dark-chilling. Furthermore we have observed reverse changes in LOX6 molecular weight. Microscope images have conformed the chloroplast localization of bean lipoxygenases. More than half of the gold particles for LOX proteins were localized in the thylakoid membranes in all experimental variants. We have observed that there are differences in specific localization of the LOX proteins in the thylakoid and granum compartments.

P01-058: ECO-PHYSIOLOGICAL TRAITS AND POLY(ADP-RIBOSYL)ATION ACTIVITY IN WINTER AND SUMMER LEAVES OF THE MEDITERRANEAN SPECIES CISTUS INCANUS L.

Arena, C.[1] - Vitale, L.[2] - Mistretta, C.[1] - De Maio, A.[1]*
[1]Università di Napoli Federico II
[2]ISAFOM-CNR, Ercolano, Napoli
**Corresponding author, e-mail: c.arena@unina.it*

Mediterranean-type ecosystems are characterised by a peculiar regime of temperature and precipitations that limits plant growth in both summer and winter seasons. The environmental constraints promote in Mediterranean woody species several mechanisms at eco-physiological, structural and biochemical level that allow them to survive the stress periods. In this work the modification of eco-physiological traits as well as the modulation of poly(ADP-ribosyl) ationactivity have been investigated in winter and summer leaves of the semi-deciduous species C. *incanus* L. Winter and summer leaves, collected in the field, were compared on the basis of photosynthetic rate, photochemistry, functional leaf characteristic and chlorophyll content. The poly(ADP-ribosyl)ation of proteins, a post-translational reversible modification operated by poly(ADP-ribose)polymerases, was utilized as a marker of cell energy metabolism. The results indicate that compared to winter leaves, summer leaves showed a lower specific leaf area and a higher leaf dry matter content, a strong reduction of photosynthesis, quantum yield of PSII electron transport and poly(ADP-ribosyl)ation activity, as well as higher levels of thermal dissipation. Nevertheless no difference in the maximal PSII photochemical efficiency and total chlorophyll content were detected. The increase of protection at the photochemical level together with the modifications of leaf functional traits may protect C. *incanus leaves* from potentially photoinhibitory conditions in summer. The decline of poly(ADP-ribosyl)ation activity in summer leaves is interpreted as a strategy to maintain cell energy homeostasis and improve stress tolerance under drought.

P01-059: POLY(ADP-RIBOSYL)ATION AND PHOTOCHEMICAL BEHAVIOUR DURING LEAF ONTOGENESIS IN THE EVERGREEN SPECIES CISTUS INCANUS L.

De Maio, A. - Mistretta, C. - Porzio, E. - Faraone - Arena, C.*
Dipartimento di Biologia Strutturale e Funzionale, Università di Napoli, Federico II
**Corresponding author, e-mail: andemaio@unina.it*

Poly(ADP-ribosyl)ation is a posttranslational protein modification in which ADPR units derived from NAD^+ are attached to proteins by poly(ADP-Ribose) polymerase (PARP) enzymes. ADPR groups are removed from these polymer chains by the enzyme poly(ADP-Ribose) glycohydrolase (PARG). PARP homologs have been also identified in plants, where, as in mammalians, they impact a variety of biological processes including DNA repair, genome stability, and cell death.
It has been suggested a link between DNA-damage induced poly(ADP-ribosyl)ation and mammalian aging, but little is known about plants. In this work, the poly(ADP-ribosyl)ation has been assessed in C. *incanus* L. leaves of different age in relation with their photochemical behaviour. Young (14 days old), mature (28 days old) and old (45 days old) leaves have been analyzed for PARP activity and protein expression. As indicators of leafphysiological performance, photochemical activity and photosynthetic pigment content have been determined.
The results showed that the PARP enzyme is always expressed in leaves, but in dipendence of age, different levels of enzymatic activity were measured . The highest activity was found in young leaves. As regards photochemistry, compared to young and mature leaves, old leaves showed lower values of maximal PSII photochemical efficiency, quantum yield of PSII linear electron transport and chlorophyll content. The highest photochemical activity was found in young leaves.
We have hypothesized that poly(ADPribosyl)ation plays an important role in the three leaf growth stages. Both the highest PARP activity and photochemical efficiency suggest in young leaves a best healthy state.

P01-060: PHOTOPERIODIC AND VERNALIZATION TIMING IN CANOLA PLANTS AND LOW TEMPERATURE ADAPTATION

*Tarakanov, I. * - Rajabian, M. - Panichkin, L.*
Russian State Agrarian University - Moscow Timiryazev Agricultural Academy
**Corresponding author, e-mail: ivatar@yandex.ru*

To properly time flowering and cope with low temperature stress, winter plants, *e.g.*, cereals, Brassicas, regulate their development through adaptive mechanisms that are responsive to day-length and temperature. We studied plant responses to photoperiodic conditions and thermo-induction in spring and winter canola, *Brassica napus* L., varieties of diverse geographical origin in the field (step-wise sowing) and in the controlled environment. A high level of genetic variability in photoperiodic sensitivity and vernalization requirements was observed within varietal populations. Thus, low latitude populations from Iran consisted of spring and alternative (double response) biotypes.
Although both cold acclimation and vernalization are responses to sensing low temperature, the duration of cold exposure that is required to initiate these responses is distinct. Screening for inherent and acclimation specific freezing tolerance was conducted under natural conditions and in phytotron using a series of plant chilling pre-treatments. It was assessed by rates of electrolyte leakage from leaf discs of treated plants after the exposure to a range of freezing regimes. A linkage between freezing tolerance and vernalization requirement was observed in the winter and alternative biotypes in our experiments. Vernalization and photoperiod responses could regulate the expression of low temperature tolerance genes through their influence on the rate
of plant development. This linkage could be controlled at the level of transcriptional factors like CBF1.

P01-061: PHYSIOLOGICAL ADAPTATIONS OF PLANT SPECIES FROM CALAMINE COMMUNITIES TO HEAVY METAL STRESS

Przedpelska-Wasowicz, E. - Wasilewska, W.- Parys, E. - Wróblewska, B. - Zienkiewicz, M. - Wierzbicka, M. - Romanowska, E.*
Department of Molecular Plant Physiology, Warsaw University
**Corresponding author, e-mail: przedpelska@biol.uw.edu.pl*

Plant species from calamine communities are the best model to study physiological adaptations to heavy metal stress. We carried out research on Armeria maritima, Arabidopsis arenosa and Arabidopsis halleri from metallicolous (M) and non-metallicolous (NM) populations. The aim of our study was to compare plant responses to heavy metal stress (Pb, Zn) in aspect of their origin: from M and NM sites. Influence of Pb on photosyntesis parameters was tested on A. maritima, while Zn toxicity on water relation on the cell level were investigated using Arabidopsis plants. In A. maritima the chlorophyll a fluorescence parameters and PSII activity were insensitive to Pb. In both populations, lead, even at highest concentration, did not inhibit CO2 exchange. In contrast to CO2 exchange, O2 uptake and ATP production was stimulated by Pb, but only in leaves from M plants. Leaves treated with Pb showed increased transpiration and it was accompanied with permanent stomata opening indicating that closure of stomata was inhibited. We tested also aquaporins (AQPs) activity in leaf epidermal cells. In A. halleri the AQPs activity did not differ significantly between both populations, whereas in A. arenosa tested plants differed in water permeability. The results suggest that both population of A. maritima and A. halleri developed similar strategies to metal toxicity, while A. arenosa from M sites showed different physiological strategy in response to Zn stress. It seems that the structure of leaves and stomatal movements affecting the rate of CO2 exchange as well as water relations are a part of complex mechanism of metal tolerance.
AcknowledgementThe work was supported by the grant from the Polish Ministry of Science and Higher Education NN303393636.

P01-062: HEAVY METALS-INDUCED OXIDATIVE STRESS IN SELECTED CULTIVATED CROPS

Azevedo, R. - Gratão, P.L. - Capaldi, F.R - Monteiro, C.C. - Cia, M.C. - Rolão, M.B. -*
Universidade de São Paulo
**Corresponding author, e-mail: raazeved@esalq.usp.br*

A major environmental problem is the soil and water contamination with toxic metals, leading to considerable losses in plant productivity. Exposure to toxic metals can intensify the production of reactive oxygen species (ROS). Heavy metals and normally are present in low concentrations in the environment, however, human activities have considerably altered such a scenario. We have been studying the biochemical and physiological aspects related to the antioxidative responses by plants (tomato, coffee, sugarcane) to heavy metal (Cd, Ni, Al and Se). The analyses carried have shown that glutathione reductase (GR) normally respond more effectively to the metal-induced stress. Other enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST) and other peroxidases vary considerably in their responses, which appear to be dependent on plant tissue, metal concentration and developmental stage. We have also designed a new strategy to investigate stress signaling in plants using the grafting technique and initiated a metallomic approach. Analysis of grafted tomato plants between control and cadmium or aluminum grown plants revealed specific changes and responses to the oxidative stress induced in distinct plant organs. Moreover, depending on the growth condition control tomato plants acquired tolerance to high cadmium concentrations. Financial support from FAPESP and CNPq.

P01-063: ORGANIC ACIDS AND SUB-CELLULAR SEQUESTRATION STUDIES IN ZINC ACCUMULATOR SOLANUM NIGRUM L.

Samardjieva, K. - Ferreira, D. - Freire, A. - Tavares, F. - Pereira , S. - Pissara, J.*
Instituto de Biologia Molecular e Celular
**Corresponding author, e-mail: kas@ibmc.up.pt*

Reclamation of soils contaminated by heavy metals has been the focus of intense research. The use of plants to scavenge these pollutants is a promising bioremediation approach, and it is acknowledged that chelation by organic acids and cellular compartmentalization are important mechanisms for plants' tolerance and accumulation. Previous studies showed that *Solanum nigrum* is tolerant to zinc and cadmium, although not much is known about the molecular, cellular and histological basis for *S. nigrum* Zn remediation potential.

By exposing *S. nigrum* plantlets to stressful zinc concentrations in hydroponics for 35 days, and using biometric parameters, it was possible to determine the highest concentration at which no toxicity symptoms were evident. The results showed that Zn accumulated in leaves, stems and roots, although the highest concentration was obtained in the roots with 220 mg Zn/g.f.w. Ultrastructural studies by autometallography of root tissues, showed Zn accumulation in the cell walls of root epidermal and sub-epidermal cell layers, intercellular spaces and vascular tissues. In stems, Zn accumulation seemed to occur mainly in the external phloem parenchyma, the starch sheath and the collenchyma of the cortex. Regarding the role of organic acids as Zn chelators, contrary to what has been shown for other species, malic and citric acids do not vary. Recently, using a HPLC approach to screen a wider assortment of organic acids, it was observed that oxalic acid levels rise with Zn accumulation. Further experiments are in progress to understand the role of oxalate in *S. nigrum* Zn remediation capacity.

P01-064: CADMIUM RETENTION CAPACITY IN RICE ROOTS IS INFLUENCED BY CADMIUM AVAILABILITY, CHELATION AND TRANSLOCATION.

Lancilli, C. - Dendena, B. - Sacchi, G.A. - Nocito, F.F.*
Università Degli Studi Di Milano - Dip. Produzione Vegetale
**Corresponding author, e-mail: Clarissa.Lancilli@Unimi.It*

Cadmium (Cd) presence in soils is an increasing concern with respect to human food-chain accumulation as well as crop production. This non-essential metal can be easily taken up by roots and accumulated in vegetative and reproductive plant organs. Among cereals, rice presents the highest risk of Cd accumulation in grains due to agricultural and genetic reasons; such a trait may be influenced by different processes, related to root retention of Cd taken up by roots and its translocation to shoots and grains. Our data show that phytochelatins (PCs), mediating Cd chelation and compartmentalization into the vacuole, play a pivotal role in defining the total Cd amount retained in the roots of rice plants exposed to different Cd concentrations (0,01, 0,1 and 1 μM). However, it seems clear that other processes different from those based on the Cd-PC complex formation, such as the control of Cd translocation, may influence Cd root retention capacity and thus the total Cd translocated to shoots and grains. Since Cd translocation, as well as its uptake, may depend on essential cation transporters belonging to various families, we focused our attention on some proteins belonging to the P1B-type ATPase family of rice. Here we also present a first characterization of OsHMA4 and OsHMA2; interestingly, their heterologous expressions in *Saccharomyces cerevisiae* confer some Cd resistance to yeast, suggesting a role of these transporters in moving non-essential elements, such as Cd, in rice.

P01-065: EFFECT OF DROUGHT STRESS ON GROWTH, PHOTOSYNTHESIS AND OXIDATIVE STRESS STATUS IN DIFFERENT BARLEY CULTIVARS

Alsamrah, A - Gilbert, M. - Wilhelm, C.*
Institute of Biology I, Plant Physiology
**Corresponding author, e-mail: afraa81@hotmail.com*

Drought as one of the main abiotic stress factors induces different physiological responses resulting in reduced growth, lower photosynthetic performance and oxidative damage. Drought stress experiments were carried out with 8 cultivars of barley. The effect of drought stress on photosynthetic performance was studied by measuring thermoluminescence (TL), variable fluorescence (FL) and delayed luminescence (DL). About 60-75% of cultivars showed significant effects in most TL, FL and DL parameters. The influence on FL was relatively small with reduction in the Fo and Fm level and very small changes in the optimal quantum yield of PS II (Fv/Fm). Drought stress lowered the intensity of the TL B band ($S_2Q_B^-$) in many cultivars and decreased the intensity of delayed luminescence resulting primarily from S2QA-. In most cultivars the peak temperature of the TL B band was shifted to higher temperatures. The intensity of the C band ($Y_D^+Q_A^-$) also decreased in most cultivars. An increase in the high temperature band (120-180°C) was detected in only 3 cultivars indicating oxidative stress in the respective leaf samples. Therefore, drought stress induced a stabilization of the radical pair $S_2Q_B^-$, i.e. an increase in the activation energy between $S_2Q_B^-$ and the excited state of the primary donor (P680*). Furthermore, this effect changed the equilibrium between QB^- and QA^-, lowering the concentration of QA^-, and consequently decreasing the TL C band and the DL intensity. The measurements of photosynthetic parameters were supplemented by estimates of biomass and oxidative stress markers (TBARS, protein carbonyl).

P01-066: REVERSIBLE SALT CRYSTAL DEPOSITION INCREASES PHOT OPROTECTION IN AVICENIA GERMINANS

Esteban, R.[1] - Fernández-Marín, B.[1] - Becerril, J.M.[1] - Jiménez, E.T.[2] - León, A.[2] - Alvarez, R.[2] - García-Mauriño, S.[2] - Echevarría, C.[2] - Silva, C.D.[3] - Dávila, M.[3] - Pastora, R.M.[3] - Dolmus, J.R.[3] - Dolmus, C.M.[3] - Molina, M.J.[3] - Gutierrez, N.N.[3] - Jimé-*

nez, M.S.[4] - Morales, D.[4] - Olano, J.M.[5] - García-Plazaola, J.I.[1]
[1]University Of Basque Country
[2]Universidad de Sevilla
[3]UNAM-León
[4]Universidad de La Laguna
[5]Universidad de Valladolid
**Corresponding author, e-mail: raquel.esteban@ehu.es*

Mangrove forests are worldwide distributed in tropical and subtropical shores. Several tree species co-exist in this environment displaying different strategies to cope with salinity. One of them, the black mangrove (*Avicennia germinans* L), excretes salt taken up by roots through specialised glands located in leaves. As a consequence, at noon when relative humidity is low, leaves become whitish because of the deposition of salt crystals that liquate again at the end of the day. As mangrove ecosystems are exposed to strong light the reversible formation of crystals during periods of strong photoprotective demand could have a photoprotective role for leaves, as light intensity intercepted by photosynthetic tissues is attenuated.
It was therefore the objective of this study to verify this hypothesis. Experiments were performed in Juan Venado Island Nature Reserve (Nicaragua) in both dry (November) and rainy seasons (August). Salt was removed from one half of the leaves and both parts were compared. In August no differences were observed in any of the physiological parameters analysed, but in the dry season, when crystal deposition is more conspicuous, leaves with salt showed higher photochemical efficiency during the afternoon, indicating higher photodamage in exposed leaves. Furthermore, salty leaves showed a tendency to have higher values of Photochemical Reflectance Index, which indicates that more photons are used photosynthetically. Thus, the ability of *A. germinans* leaves to excrete the absorbed salt, apart from contributing to osmoregulation, may represent a photoprotective mechanism that generates dynamic changes in leaf reflectance, making it more tolerant to extreme conditions of the mangrove ecosystem.

P01-067: EFFECT OF PHENOLIC COMPOUNDS IN PISUM SATIVUM AND LUPINUS LUTEUS PLANTS UNDER SOIL DROUGHT

Skrzypek, E.[1] - Czyczylo-Mysza, I.[1] - Marcinska, I.[1] - Waligorski, P.[1] - Dziurka, K.[1] - Dziurka, M.[1] - Stawicka, A.[1] - Dubert, F.[1] - Plazek, A.[2]- Koscielniak, J.[2]*
[1]The F. Gorski Institute of Plant Physiology, Polish Academy of Sciences
[2]Department of Plant Physiology, University of Agriculture
**Corresponding author, e-mail: skrzypek@ifr-pan.krakow.pl*

Increase in phenolic compounds biosynthesis have been observed in a variety of biotic and abiotic stresses. Legume species showed high ability to endure intense dehydratation and return to normal turgor after soil rehydratation.
The aim of the investigation was to determine the drought resistance diversity in chosen genotypes of yellow lupine and pea cultivated in Poland.
Influence of soil drought on changes of plants growth, leaf water content, endogenous level of phenolics in pea and lupine genotypes were compared. Plants were grown in 4.5 l pots with soil (70% of soil field water capacity, FWC) during the late spring and early summer time in the open-air shelter. Drought stress (25% FWC) was subjected to the plants for 14 days, when the plants were after flowering phase. After stress treatment plants were well watered and recovery of their vitality was observed. Drought differentiated, dependently on the genotype, seedlings growth and leaves injury.
Leaf water content (lower under drought stress than in control) and endogenous level of phenolics (higher under stressed plants) allowed to select tolerant and susceptible pea and lupine genotypes. Research funded by grant 621/N-COST/09/2010/0

P01-068: COLD-INDUCED CHANGES OF CELL WALL STRUCTURE AND COMPOSITION IN TRITICALE LINES THAT ARE SENSITIVE OR RESISTANT TO FUNGAL PATHOGEN MICRODOCHIUM NIVALE

Szechynska-Hebda, M.[1] - Hebda, M.[2] - Wedzony, M.[1]*
[1] Institute of Plant Physiology PAS
[2] Institute of Material Engineering CUT
**Corresponding author, e-mail: szechynska@wp.pl*

The work was based on the observation that exposure of triticale seedlings to cold promotes their resistance to infection with the fungal pathogen *Microdochium nivale*. Since, the expression of resistance is dependent on the plant genotype, two lines, namely Hewo (pathogen-tolerant) and Magnat (pathogen-sensitive) were used in the study of the cell wall properties during cold harvesting. Two types of plant resistance are suggested: resistance to the establishment of the initial infection and resistance to hyphal invasion through the plant tissue. The physiological and chemical state of the cell wall, brought by their exposure to cold determined the resistance efficiency of both types.
The first type of resistance we studied using coupling techniques: TG, DSC and QMS. We showed that the expression of plant resistance strongly depend on the cell wall structure and composition. The signal was assigned using model substances. In this way we showed that pectin was degraded first, then hemicellulose, cellulose and finally lignin. Different thermal behavior was found between the cell wall components of Hewo and Magnat treated with cold. The peaks, assigned to the cellulose and lignin thermal decomposition, occurred at a different temperature and had remarkably differences in the shape of the curves. It can be explained by the different amount of cellulose and lignin and its different thermal stability.
The second type of resistance was confirmed by physiological tests. Resistant plant responded defensively to hyphae invasion with the events at the place of the first contact: callose deposition for surrounding the necks of invasion hyphae and generation of H_2O_2 by cell wall peroxidases.
The research was supported by a projects: 595/N-COST/2009/0 and MERG-CT-2007-207350.

P01-069: TOLERANCE OF ARABIDOPSIS THALIANA PLANTS TO THE ALLELOCHEMICAL PROTOCATECHUALDEHYDE (PCA)

Reigosa, M. - Martínez-Peñalver, A. - Sánchez-Moreiras, A.M.*
University of Vigo
**Corresponding author, e-mail: mreigosa@uvigo.es*

Protocatechualdehyde (PCA), is a phenolic compound found in many plant organs of different species (stems of *Ilex litseaefolia*, roots of *Salvia miltiorrhiza*, leaves of *Vitis vinifera*, etc). This plant secondary metabolite has many beneficial effects for human health as anticarcinogenic, anticoagulant, etc (Zhou et al., 2005). However, there are very few studies evaluating its role as allelochemical. Reigosa and Pazos-Malvido (2007) showed the phytotoxic capacity of PCA during root growth and germination, but we have found no experiments in the literature, either short or long term, with information about PCA phytotoxicity on adult plants. Therefore we studied the phytotoxicity of PCA on *Arabidopsis* plants, monitoring the effect by imaging chlorophyll a fluorescence, pigment content, concentration of free radicals (O_2- and H_2O_2), lipid peroxidation, total protein and glutathione transferase at various times during 8 days treatment. Photosynthetic efficiency and fluorescence emission values of PCA treated plants remained broadly in values close to control suggesting a non-phytotoxic effect of PCA. However, just minutes after the addition of the allelochemical oxidative burst was observed with increased values of O_2- and H_2O_2. This burst was followed by a very significant increase of lipid peroxidation in the early hours of measurement. But plants were able to cope with PCA toxicity showing very low values of MDA content after 48 h treatment, as

a symptom of recovery. The values of antioxidant enzymes and the morphological change of the leaves with edges rolled inwards suggest stress tolerance mechanisms in the treated plants.
Reigosa MJ, Pazos-Malvido E (2007) J Chem Ecol 33: 1456-1466. Zhou Z, Liu Y, Miao AD, Wang SQ (2005) Eur J Pharmacol 513: 1-8.

P01-070: SUBCELLULAR STUDY OF THE ENDOMEMBRANE SYSTEM IN A TOBACCO BY-2 CELL LINE ADAPTED TO SALT STRESS.

Olmos Aranda, E.* - Garcia de la Garma, J. - Fernandez-Garcia, N.
CEBAS-CSIC
**Corresponding author, e-mail:* eolmos@cebas.csic.es

Cell adaptation to high salinity levels implicates the modification of different cellular, physiological and molecular mechanisms. Recently, we have obtained a stable tobacco cell line adapted to grow at 250 mM of NaCl. Salt-adapted cells showed a lower relative growth than control cells. The morphology of salt-adapted cells was altered, cellular size was highly reduced and the cytoplasm showed abundant microvacuoles. To follow the endocytic pathway and its contribution to the formation of the microvacuoles in tobacco salt-adapted cell line, the FM4-64 dye and laser confocal microscopy has been used.The FM dyes have been widely used in tracking processes of membrane trafficking in eukaryotic organism. The amphiphilic character of FM4-64, the most commonly used FM dyes, allows us to analysis the dynamic of the endomembrane system. Cell labelling with FM4-64 during 2-3 hours, followed by 12-16 hours of culture, showed the main labelling in the tonoplast. When cells were labelled at short pulse of few minutes with FM4-64, the plasma membrane and endocytic vesicles were observed. Finally, the ultrastructure of control and adapted cell was also studied by transmission electron microscopy.

P01-071: HSP70 EXPRESSION IN LYCOPERSICON SPP. IN RESPONSE TO ABIOTIC AND BIOTIC STRESSES AND THEIR COMBINATION

Piterková, J. - Luhová, L. - Petrivalský, M. - Matulková, Z. - Mieslerová, B.*
Palacky university, Faculty of science
**Corresponding author, e-mail: jana.piterkova@seznam.cz*

Plants are daily exposed to biotic and abiotic stress factors, such as low and high temperature, heavy metals, UV radiation or pathogen attack. Exposure to stressful conditions leads to increased formation of reactive oxygen (ROS) and nitrogen (RNS) species, which include namely nitric oxide (NO) and hydrogen peroxide (H_2O_2). Temperature stress and other stresses cause protein denaturation or aggregation and cell death. Heat shock proteins are synthesized to regulate protein homeostasis and membrane fluidity and to prevent the cell death.
The present work was conducted to examine the expression of Hsp70 in two *Lycopersicon* spp. caused by temperature stress, biotic stress (*Oidium neolycopersici*) and treatment with modulators of ROS and RNS concentration. Substances that modulate ROS and RNS concentration, like NO donor (GSNO), NO scavenger (PTIO) and inhibitor of NADPH oxidase (DPI) were tested. An increased production of Hsp70 protein was observed when *Lycopersicon* spp. were exposed to heat stress, whereas cold stress had no significant influence on the production of studied proteins.
Two proteins of Hsp70 family with different molecular masses were detected: heat-inducible 72 kDa protein and constitutive 75 kDa protein. The pathogenesis or the application of modulators of ROS and RNS levels influenced the increased expression of Hsp75 protein. Correlation between ROS and RNS and Hsp70 expression was found.
This research was supported by grants MSM 6198959215 and 522/08/H003 from Czech Grant Agency.

P01-072: KESTOSES IN TABLE GRAPES DURING POSTHARVEST STORAGE

Sanchez-Ballesta, M. - Blanch, M. - Escribano, M.I. - Merodio, C.*
Instituto del Frio-CSIC
**Corresponding author, e-mail: mballesta@if.csic.es*

Low temperature storage is one of the most effective technologies for extending the postharvest life of fruit and vegetables. Although *Vitis vinifera* is tolerant to chilling, activation of defence responses associated with storage at 0ºC has been reported in cv. Cardinal table grapes. Among the soluble carbohydrates, high relative sucrose levels are most often associated with increased cold hardiness in a wide range of plant species. Nevertheless, the effect of low temperature on fructan metabolism and accumulation in fruits are largely unknown. Fructans consist of a series of homologous oligo and polysaccharides of fructose which can be considered as derivatives of sucrose. The determination of kestose was carried out using anion-exchange chromatography with pulsed amperometric detection. In the present study, the levels of 1-kestose and neokestose were analyzed in grapes during low temperature storage at 0ºC with and without CO_2 treatment (20% CO_2 for 3 days) and further shelf-life at 20ºC. Our results indicate that low-temperature storage drastically increased levels of 1-kestose after 3 days, and a decrease in the abundance was observed after 12 days at the time of the increases in accumulation of neokestose. In CO_2-treated grapes the accumulation of 1-kestose was smaller than in non-treated grapes. On the contrary, the highest level of neokestose was detected in CO_2-treated grapes. The transfer to 20ºC after cold storage drastically decreased the levels of neokestose of both non-treated and CO_2-treated grapes meanwhile an increase in the content of 1-kestose was recorded. These results provide new evidence that 1-kestose and neokestose appear to be related to the response of table grape to low temperature.

P01-073: SALT STRESS RESPONSE IN ABSCISIC ACID AND SALICYLIC ACID MUTANTS OF ARABIDOPSIS THALIANA

Asensi-Fabado, A. - Munné-Bosch, S.*
Departament de Biologia Vegetal, Universitat de Barcelona
**Corresponding author, e-mail: masensif@ub.edu*

Abscisic acid (ABA) and salicylic acid (SA) modulate a variety of developmental processes in plants, from seed germination to senescence, as well as plant responses to abiotic stress. In the present study, we aimed at better understanding plant responses to salt stress in the model plant *A. thaliana*, with an emphasis on the interplay between SA and ABA. Different stress indicators (relative water contents, plant growth, Fv/Fm ratios and chlorophyll levels), as well as endogenous concentrations of phytohormones (cytokinins, auxins, gibberellins, ABA, SA and jasmonic acid) were measured in SA- and ABA-deficient and insensitive (*sid2*, *eds5*, *aba3* and *abi4*) *A. thaliana* mutants exposed to salt stress (100 mM NaCl) for 19 days. A deficiency in ABA (*aba3* mutants) reduced the relative water contents in well-watered conditions, but showed smaller reductions in this parameter under salt stress compared to the wild type. This was associated with changes in plant growth. *aba3* mutants grew less in well-watered conditions but showed smaller reductions in growth when exposed to salt stress compared to the wild type. Furthermore, these mutants maintained the F_v/F_m ratio above 0.80 throughout the study, while this parameter was reduced below 0.75 after 19 days of stress in the wild type. These effects were not observed neither in the abi4 mutant, which is insensitive to ABA nor in the *sid2* and *eds*5 mutants, which are deficient and insensitive to SA, respectively. It is discussed here to what extent an altered hormonal balance explains the differences observed between mutants. However, it is noteworthy that maintenance of a constant F_v/F_m ratio under salt stress in the *aba3* mutants is made at the expense of reducing plant growth under well-watered conditions.

P01-074: BIOCHEMICAL CHARACTERIZATION OF PEA ORNITHINE DELTA-AMINOTRANSFERASE: SUBSTRATE SPECIFICITY AND INHIBITION BY DI- AND POLYAMINES

Stranska, J.[1] - Tylichova, M.[1] - Kopecny, D.[1] - Snegaroff, J.[2] - Sebela, M.[1]*

[1] Palacky University, Faculty of Science, Department of Biochemistry

[2] Laboratoire de Chimie Biologique, INRA-AgroParisTech

**Corresponding author, e-mail: jana.stranska@volny.cz*

Ornithine δ-aminotransferase (OAT, EC 2.6.1.13) catalyzes the transamination of L-ornithine to L-glutamate γ-semialdehyde. The physiological role of OAT in plants is related to proline biosynthesis and extends to the processes of drought and salinity stress adaptation. We investigated the enzyme from pea (PsOAT) to assess whether diamines and polyamines may serve as substrates or they show inhibitory properties.

First, a cDNA coding PsOAT was cloned and expressed in *Escherichia coli* to obtain a recombinant protein with a C-terminal 6xHis tag. Recombinant PsOAT was purified under native conditions by immobilized metal affinity chromatography and its molecular and kinetic properties were characterized. Protein identity was confirmed by peptide mass fingerprinting after proteolytic digestion. The purified PsOAT exists as a monomer of 50 kDa and shows typical spectral properties of enzymes containing pyridoxal-5'-phosphate as a prosthetic group.

The cofactor content of PsOAT was estimated to be 0.9 mol per mol of the monomer by a spectrophotometric analysis with phenylhydrazine. L-Ornithine is the only good substrate (K_m = 15 mM) but PsOAT also slowly converts N_α-acetyl-L-ornithine. In these reactions, 2-oxoglutarate is the exclusive amino group acceptor (K_m = 2 mM). The enzyme has a basic optimal pH of 8.8 and displays relatively high thermal stability.

Diamines and polyamines are not accepted as substrates. On the other hand, putrescine, spermidine and others represent weak non-competitive inhibitors. This might have a biological consequence in mitochondria. A model of the molecular structure of PsOAT was obtained using the crystal structure of human OAT as a template.

P01-075: EFFECT OF OSMOTIC STRESS ADJUSTED WITH PEG IN HYDROPONIC CULTURE ON BIOCHEMICAL CHANGES IN WHEAT SEEDLINGS

Marcinska, I.[1] - Skrzypek, E.[1] - Czyczylo-Mysza, I.[1] - Waligorski, P.[1] - Dziurka, K.[1] - Dziurka, M.[1] - Filek, M.[1] - Stawicka, A.[1] - Quarrie, S.A.[2]*

[1] The F. Gorski Institute of Plant Physiology, Polish Academy of Sciences

[2] Visiting Professor, Institute for Research on Environment and Sustainability, Newcastle University

**Corresponding author, e-mail: i.marcinska@ifr-pan.krakow.pl*

Drought tolerant or sensitive wheat cultivars exposed to low water potential can be characterized by growth response and by the changes in accumulation of compatible solutes. Wheat seedlings were hydroponically grown in half strength Hoagland nutrient solution. After three weeks of culture seedlings were exposed for 7 d to three osmotic stress levels: D1, D2 and D3, which was adjusted with PEG 6 000T. he aim of the experiment was to reveal the differences in sensitivity to osmotic stress in two differed in drought tolerance wheat genotypes. After 7 d of stress treatment, the leaves were excised and lyophilized. Polyamines were measured by HPLC procedure. Phenolics, proline, reducing and nonreducing sugars, chlorophyll a, b and carotenoids content were estimated spectrophotometrically on microtiter plate reader. Only small differences in the case of amount of soluble carbohydrates and phenolics compounds between two tested genotypes were observed.

For the other measured traits, more significant changes in its amount were noticed in sensitive genotype CS, compared to tolerant SQ1. The concentration of proline and polyamines, especially putrescine, were higher in CS genotype than in SQ1. On the contrary, concentration of chlorophyll a and b was lower in CS than in SQ1 genotype. On the basis of obtained results we supposed that these biochemical parameters could be useful for further drought stress studies.

Research funded by grant 480/N-COST/2009/0

P01-076: PHYSIOLOGICAL AND MOLECULAR BASICS OF ADAPTATION TO PERIODICAL FLOODING IN DESCHAMPSIA WIBELIANA (SOND.) PARL. AND D. CESPITOSA (L.)

Ambroselli, V - Müller, D. - Rink, F. - Heydel, F. - Reisdorff, C. - Jensen, K. - Hanelt, D. - Lorbiecke, R.*

Universität Hamburg

**Corresponding author, e-mail: volker.ambroselli@gmx.de*

This project focusses on the ecological differentiation of the two Poaceae species *Deschampsia cespitosa* and *D. wibeliana* and the underlying physiological and molecular mechanisms. *D. cespitosa* is a widespread distributed species adapted to stagnant moisture, whereas the endemic *D. wibeliana* inhabits periodically flooded marshes in the Elbe river estuary. Individuals of both species are cultivated in the greenhouse and an experimental model system simulating periodical flooding under controlled conditions is set up.

Thus a homogenous basis for the following studies is achieved. Based on this system physiological and molecular experiments will be conducted. In the physiological approach abiotic constraints on photosynthesis appearing during submergence are in the center of interest. The molecular approach focuses on the comparative investigation of genes that are differentially regulated during hypoxia and anoxia. For this purpose experimental techniques such as PAM analysis, gas exchange measurements, enzyme assays, (QRT-)PCR analysis, Northern Blots, and subtractive hybridizations will be applied. Concluding, combination of the collected data should help revealing the molecular and physiological principles of ecological differentiation of both species. First data will be presented.

P01-077: GENETIC DIVERSITY OF SESAME IN DROUGHT STRESS CONDITION USING ROOT SYSTEM STUDY IN CASPIAN BORDER REGION OF IRAN

Gharib Eshghi, A.[1] - Azizova, I.[2] - Mozzaffari, J.[2]*

[1]Seed and Plant Improvement Institute

[2]Institute of Botany, Baku, Azerbaijan ,Seed & plant improvement institute of Iran

**Corresponding author, e-mail: dara_eshghi@yahoo.com*

In order to determine the relation between root system characters and drought stress condition this study designed and conducted through 3 years. During first 2 years, 20 sesame genotypes planted inNorth West region of Iran during 2007 and 2008 for two years with 3 Replication and experimental design was RCBD. Two separate experiments were done.

First in Normal irrigation condition and second with dry condition with only one time irrigation after planting. To study of response of genotypes to drought resistance, 5 different indices were Used. Including: SSI , STI, TOL , MP and GMP. In third year(2009), 10 genotypes through genotypes mentioned above, selected for more study using root system characters. During root study these genotypes planted in a split plot experiment design with 3 replication, in three irrigation levels, A)Normal irrigation ,B)75% irrigation and C)50% irrigation .seeds planted in special polythene sheets that were dug into soil .finally end of season , plants extract slowly and completley , and roots prepared and soils washed away with a thin water spray. Root length, root length density and root diameter determined. Results showed that: 1) sesamegenotypes showed significant differences in root

length, and root length density at 1% and root diameter in 5%. 2) More tolerant genotypes had more root length, root length density and less root diameter. 3) the rooting density of sesame in surface layers is higher 4)sesame rooting depth differs with water condition 5) non branching genotypes has a better root distribution in the soil profile in comparison to branching types.

P01-078: BIOCHEMICAL MECHANISMS RELATED TO ENHANCED CHILLING TOLERANCE IN CHERIMOYA FRUIT BY POSTHARVEST HIGH-CARBON DIOXIDE TREATMENT

Goñi, O. - Sanchez-Ballesta, M.T. - Merodio, C. - Escribano, M.I.*
Instituto del Frio (CSIC)
**Corresponding author, e-mail: oscargr@if.csic.es*

The tolerance of plants species to chilling can be achieved by accumulation of substances as osmoprotectants or cryoprotective proteins. In cherimoya fruit (*Annona cherimola* Mill.), a treatment with high concentrations of CO2 enhances its chilling tolerance during storage at 6ºC. LT-SEM studies in the mesocarp of treated fruits showed a consolidated structure and maintenance of membrane integrity.
Consistent with these observations, an accumulation of betaine was noted in the fruit throughout the storage period. Moreover, the gaseous treatment induced the synthesis of a low molecular mass basic chitinase, BChi14, and acidic 1,3-β-glucanase, AGlu19, which were associated with a raise in the cryoprotective activity *in vitro* of protein extracts. Functional study of purified PR proteins revealed that BChi14 and AGlu19 are very effective in protecting the cold-labile enzyme LDH from freeze-induced inactivation. On a molar basis, both enzymes are about 3-times more effective than the cryoprotective protein BSA.
These results indicate that BChi14, AGlu19 and betaine may be concerned in the cherimoya active cold defence mechanism induced by postharvest CO_2 treatment. This protection against chilling injury could be explained in the context of cross-resistance between storage at chilling temperatures and a gaseous treatment.

P01-079: PHYTOCHELATINS GOVERN ZINC/COPPER HOMEOSTASIS AND CADMIUM DETOXIFICATION IN THE PARASITIC INTERACTION BETWEEN CUSCUTA CAMPESTRIS AND DAUCUS CAROTA

Sanita' di Toppi, L. - Vurro, E. - Ruotolo, R. - Ottonello, S. - Elviri, L. - Maffini, M. - Falasca, G. - Zanella, L. - Altamura, M.M.*
Universitá Degli Studi di Parma
**Corresponding author, e-mail: luigi.sanitaditoppi@unipr.it*

Cuscuta sp., a member of the Convolvulaceae family, known with the common name of "dodder", is an obligate parasite capable of invading stems and leaves of a wide range of host plants. Dodder stem usually coils counterclockwise around the host and, within a few days, develops haustorial structures at each point of contact. As soon as dodder haustoria reach the host vascular bundles, they start tapping water, photosynthates and minerals from the host. Metal ions such as zinc (Zn) and copper (Cu) are essential for normal dodder growth and metabolism, although an exceedingly high (over-homeostatic) supply of these micronutrients can result in growth inhibition and cellular toxicity. Even more so, non-essential metals such as cadmium (Cd), if transferred from the host to the parasite, need to be neutralized by timely detoxification mechanisms.
The first goal of this work was thus to demonstrate that *Cuscuta campestris* can indeed parasitize a model plant such as carrot (*Daucus carota*) and establish effective haustorial connections, capable of transferring Cd and essential metal ions such as Zn and Cu from the host vascular bundles to the parasite. Having proven the above point, we subsequently addressed the hypothesis that the presence of glutathione (GSH) as well as GSH derivatives such as phytochelatins (PCs) might be particularly important in dodder for Zn and Cu homeostasis and Cd detoxification. In fact, throughout its life-cycle, dodder is exposed to simultaneous fluxes of essential and non-essential metal ions coming from the host. Last but not least, we wished to verify whether PCs could be synthesized by C. campestris on its own, rather than being massively imported from the host.
Results on the above issues will be presented and discussed.

P01-080: ISOLATION AND QUANTITATIVE ANALYSIS OF HEMOGLOBIN 1 INTO SPINACH TISSUES IN RESPONSE TO ANAEROBIOSIS

Antonacci, S. - Ferrarese, M. - Faoro, F. - Ferrante, A.*
DiProVe - Università degli Studi di Milano
**Corresponding author, e-mail: simona.antonacci@unimi.it*

Class I Hemoglobins (Hb1s) have an extremely high affinity for oxygen and are induced in plants during hypoxic and anoxic stresses or by the oversupply of nitrogen (N) compounds. It has been proposed that in case of hypoxia Hb1 acts as a nitric oxide scavenger within a NO_3-/NO_2-/NO cycle in which most intracellular nitrate is utilized. In this case Hb1 activity would have a dual effect of detoxifying excessive NO production and tuning NO in its action as a stress signal. Inorder to study Hb1 expression and activity in response to different external stimuli a full-length coding sequence showing high similarity to known anaerobiosis-induced non-symbiotic Class1 Hemoglobins was isolated from anoxic spinach roots. The full length sequence encoded for a putative protein of 167 aa. Blast analysis showed conserved protein domains that confirmed the similarity with Hb1-like protein class. Microarray experiments on 3 h anoxic roots and qPCR analysis of *SoHb1* expression after a prolonged anaerobic stress showed a strong upregulation in roots and in leaves collected in the dark from waterlogged spinach plants. *In vivo* NO levels were analyzed on anaerobic root samples through DAF staining and confocal microscopy. The experiments showed an augmented fluorescence into stressed samples. To further investigate on Hb1-NO interactions an RNAi construct was designed with an anti-LyHb1 and tomato plants were transformed. Transformed lines will be assayed for nitrate metabolism and NO content under anaerobiosis.

P01-081: PHOTOSYSTEM II EFFICIENCY EXPLAIN A SIGNIFICANT PART BUT NOT ALL THE REDUCTION OF RADIATION USE EFFICIENCY OF MAIZE CULTIVATED UNDER CHILLING CONDITIONS.

Postaire, O. - Louarn, G. - Fournier, C. - Andrieu - Giauffret C.
INRA

The higher light interception efficiency of the maize is reached at flowering, which usually occurs at the end of July when solar radiation is already decreasing. Moreover, water availability after flowering is often limiting grain filling. Because earlier sowing would allow a better fit between plants crop cycle and overall resource availability, breeders are seeking for strategies enabling plants to grow more efficiently under cool temperature. Biomass production depends on the amount of photosynthetically active radiation (PAR) intercepted by the crop and on radiative use efficiency (RUE). Suboptimal temperatures have negative impact on PAR interception and on RUE. The aim of this work was to evaluate the targets for improving maize tolerance to chilling stress under the field conditions. Field experiments were carried out from 2005 to 2009 with a flint inbred line from temperate climate. Each year, two sowing dates were used to generate contrasted temperatures regimes during seedling establishment. The relative contribution of the intercepted PAR and the RUE on the biomass reduction at silking in the early sowings were similar for four years. Low temperatures reduced the surface of laminas, even for leaves that were growing after the cold period. Lamina size reduction is strongly depended on the climatic scenario. RUE was higly correlated with mean air temperature ($r^2 = 0.91$). The RUE variation during the sowing-silking period can be very high

(from 0.5 to 4 g/MJ). Maximum quantum efficiency of PSII (Fv/Fm) and photochemical quenching of PSII (phiPS2) of the last ligulae leaf were measured during the cycle. Variations of Fv/Fm and PhiPS2 can in some extent explain the RUE variations observed.

P01-082: UPTAKE AND TRANSLOCATION OF HEAVY METALS IN RAPHANUS SATIVUS : A COMPARISON OF TWO GROWING SYSTEMS

Di Salvatore, M. - Carafa, A.M. - Aprile, G.G. - Carratù, G.*
DIARBOPAVE - Università degli Studi di Napoli Federico II
**Corresponding author, e-mail: mdisalva@unina.it*

In this study we compare the heavy metals effect on radish plants grown in soil and in hydroponic culture to check whether the plant response to heavy metals (Cd, Cu, Ni and Zn) is affected by the cultural system. In hydroponics all nutrient elements are fully available to the plant, while in soil only a fraction of the total nutrients are available. In order to obtain comparable nutrient availability in both cultural systems, we prepared artificial soils where the available fraction of each element was the same as in the hydroponic nutrient solution. In both growing substrates the heavy metals were given together. The following parameters were monitored: 1) germination percentage 2) biomass production 3) heavy metals quantity in the shoot. In both growth substrates, soil and hydroponic culture, the seed germination percentage (> 90%) was not affected by heavy metals. With no heavy metals added, biomass production of plants grown in soil or in hydroponics was not statistically different. In hydroponics, the addition of heavy metals resulted in a reduction of radish growth while in soil, the differences between the biomass of the controls and the respective treatments was in no case statistically significant. At the same available concentration of heavy metals in both substrates, radish plants grown in hydroponics absorb higher concentrations of heavy metals in comparison with plants grown in soil. These results suggest that when studying the absorption and translocation of metals in vegetables, it is not possible to compare the concentration of heavy metals in a hydroponic solution with the heavy metals concentrations chemically extracted from a soil.

P01-083: POTENTIAL REDOX IN POTATO (SOLANUM TUBEROSUM) UPON WATER DEFICIENCY

D.Boguszewska - M.Grudkowska - B.Zagdanska*
Plant Breeding and Acclimatization Institute
**Corresponding author, e-mail: dominika@boguszewska.net*

Drought is one of the major abiotic stresses affecting plant growth, development and productivity. Plants exposed to water deficiency undergo changes in their metabolism in order to cope with the unfavourable environmental conditions. One of the biochemical consequences occurring under dehydration of plants is the production of reactive oxygen species (ROS). Mechanisms that minimize oxidant concentrations and maintain the internal reducing environment ranged from reducing molecules such as glutathione and ascorbate to many enzymes such as superoxide dismutase, catalase and other peroxidases, that further reduce ROS to water. Mechanisms that initiate the production of ROS are central to understanding not only the stress physiology but the pattern of plant growth as well.
Therefore, the question arises whether ROS-scavenging mechanisms of plants including activity and pattern of superoxide dismutase, peroxidase and catalase (EC 1.11.1.6) are changed under soil drought applying in tuberisation phase of potato development. Experiments were carried out on 2 potato cultivars: Cekin and Tajfun differing in dehydratation tolerance. Analytic electrophoresis of tuber extracts under non-denaturating conditions (native PAGE) was performed according to Laemmli (1972).
The proteins subjected to SDS-PAGE were transferred electrophoretically to a nylon membrane. The membrane blot was incubated with anti-cAPX antibodies or anti-Gluthatione reductase.
The obtained results clearly indicate that soil drought induced the new bands of peroxidase and superoxide dismutase activities in leaves and tubers. However, the observed induction of antioxidant responses seems to be genotype dependent.
The proteome analysis of potato tubers during drought has been initiated. The knowledge of potato stress-related proteins could help to understand the molecular basis of potato drought tolerance

P01-084: CHANGES IN HYDROGEN PEROXIDE PRODUCTION AND THE CONTENTS OF ASCORBATE AND GLUTATHIONE IN ROOTS OF TWO WHEAT CULTIVARS DURING EXPOSURE TO WATER STRESS

Koenigshofer, H. - Loeppert, H.*
University of Natural Resources and Applied Life Sciences BOKU
**Corresponding author, e-mail: helga.koenigshofer@boku.ac.at*

Drought like other stress conditions frequently enhances the level of reactive oxygen species (ROS) in plants. In green leaves an excess of ROS during water deficit is mainly the result of increased photorespiratory activity upon drought-related stomata closure. However, less is known about the role of ROS in roots under drought stress. Therefore, in the present study we have monitored the contents of hydrogen peroxide (H_2O_2) and the major water-soluble antioxidants ascorbate and glutathione during water-stress conditions in roots of two wheat cultivars differing in drought tolerance. The level of H_2O_2 in the unstressed roots of both cultivars did not change significantly during the daily light-dark cycle. Deprivation of water did not affect the level of H_2O_2 in the roots of the drought-sensitive cultivar (*Triticum aestivum* L. cv. Manhattan). In contrast, the drought-tolerant cultivar (*Triticum aestivum* L. cv. Josef) showed a transient increase in H2O2 in response to water deficit. Since the relative water content (RWC) of the roots decreased in “Manhattan” but not in “Josef” after prolonged exposure to drought, the H_2O_2 increase in the roots of “Josef” might act as a signal triggering adaptive processes which allow to maintain a high water status. In the course of exposure to water stress the concentration of ascorbate increased markedly in the roots of both cultivars indicating that this antioxidant substantially contributes to the control of the H_2O_2 level in the roots.

P01-085: PLANT CELL WALLS, TOXIC METALS AND THE ENVIRONMENT

Vatehová, Z.[1] - Malovíková, A.[2] - Kollárová, K.[2] - Lišková, D.[2]*
[1]Department of Plant Physiology, Faculty of Natural Sciences, Comenius University
[2]Institute of Chemistry, Slovak Academy of Sciences
**Corresponding author, e-mail: zuzana.vatehova@savba.sk*

The plant cell wall is a dynamic extracellular structure with characteristics depending on the species, developmental stage of the plant/cell cycle, type of the tissue, and growth conditions. It provides cells with structural support and protection, and also acts as a filtering/immobilization mechanism limiting the entry of molecules that may be toxic to the cell. The aim of our work is to identify cell wall structure/components and mechanism or mechanisms coupled with the cell wall which could be responsible for plant tolerance and/or sensitivity to toxic metals. For cell wall isolation we selected two clones of *Zea mays*, tolerant and sensitive. The seeds were germinated for 72 hours at 25 oC, 70% humidity in the dark. Uniform seedlings were selected and cultivated 10 days in solutions containing various concentrations of $Cd(NO_3)_2$ (10^{-5}, $5x10^{-5}$ and 10^{-4} M) at 25 oC, 70% humidity, in light conditions (130-140 μmol m^{-2} s^{-1}, 16^{-h} photoperiod). Single cell wall components were extracted by chemical procedures from aboveground plant parts and roots. Growth parameters (elongation and fresh/dry mass of aboveground plant parts and

roots) were determined. The effect of cadmium on cell wall composition and its content in various plant organs has been assessed by the AAS analysis. Results of this study can contribute not only to plants protection against abiotic stress, but they can resolve some remediation problems of the environment.
Acknowledgement: This work was supported by grants - VEGA 1/0472/10, 2/0046/10 and
APVV-SK-ZA-0007-07, APVT 51-013304, APVV-COST 0004-06, COST Action FA0905.

P01-086: ECOPHYSIOLOGICAL CHARACTERIZATION OF ARTEMISIA LERCHIANA WEB. INHABITING STEPPE ZONE OF THE LOWER VOLGA REGION

*Orlova J., Balnokin Y.**
K.A.Timiryazev Plant Physiology Institute RAS
**Corresponding author, e-mail: orl-jul@mail.ru*

In the context of possible climate aridization, investigation of mechanisms underlying plant adaptation to stresses of steppe zone is of a great importance. Growth, morphology, anatomy, cell ultrastructure, photosynthesis, water and ionic relations of warmwood, *Artemisia lerchiana* Web., inhabiting steppe of Lower Volga Region have been studied. Anatomical, ecophysiological, and biochemical features that provide warmwood viability under drought, soil salinity, high temperature, and excessive insolation were revealed. These features are: (i) entering rest at the time of maximal strength of stress factors in the middle of the vegetation, (ii) water reservation by paraveinal parenchyma in vascular bundles, (iii) apoplast loading of phloem with assimilates low sensitive to high temperatures, (iv) stability of photosynthetic apparatus under excessive insolation and water defict, (v) ability of the plant under drought and soil salinity to decrease cell water potential below the level lower than that in the environment, (vi) ability to sustain water potential gradient in the soil-root-shoot system. It was shown that maintenance of the intracellular water potential at low levels is achieved by accumulation of inorganic ions (Na^+, Cl^-, K^+) and organic osmolytes in the cells. Among organic osmolytes the main role belongs to mono-, di-, and trisaccharides. The gradient distribution of K^+ as well as mono- and disaccharides along the plant establishes water potential gradient allowing ascending water transportation in even in the absence of transpiration.

P01-087: DIFFERENCES OF PHOTOCHEMICAL RESPONSE TO PB IONS OF MAIZE CHLOROPLASTS

Wasilewska, W.* - Krzemianowski, L. - Parys, E. - Romanowska, E.
Faculty of Biology, University of Warsaw
**Corresponding author, e-mail: wiolaw@biol.uw.edu.pl*

Maize is a C4 plant in which two distinct cell types, mesophyll (M) and bundle sheath (BS), cooperate during photosynthesis. The environmental factors as light intensity and/or heavy metals cause changes in the efficiency of photosystems and in relative levels of thylakoid components in chloroplasts. Up to date little information is available about acclimation strategies of maize chloroplasts in plants treated Pb ions and growing under different light intensities. Maize plants were grown under low (LL) and high (HL) light intensity. Lead was introduced into detached leaves with transpiration stream. We observed that accumulation of Pb ions in the leaves was higher in plants grown under LL than HL. It results probably from the differences in structure of bundle sheath cell wall in this light condition. Effects of Pb ions were independent on light intensity during growth. Amount of LHCI proteins and PSI activity decreased in response to Pb2+ and it was more evident in BS chloroplasts. It seems that in agranal chloroplasts Pb disturb cyclic electron transport and ATP production. Simultaneously, the higher respiration rate in Pb2+ treated leaves accompanied with ATP synthesis can contribute substantially to maintain the high adenylate level in M cells. Fluorescence parameters and PSII activity were not affected by Pb ions. Interestingly, we observed the difference in chloroplast proteins phosphorylation what would imply protection mechanism. We therefore propose that in maize during Pb treatment cellular homeostasis (in M and BS) is maintained as long as polls of ATP/ADP and redox potential remain at balanced ratios.
AcknowledgementThese studies were financed by the grant NN303 393636 from the Ministry of Science and High Education of Poland.

P01-088: INTERACTION BETWEEN POLYAMINES AND PROLINE IN COMMON SAGE (SALVIA OFFICINALIS L.) PLANT IN NORMAL GROWTH CONDITION AND UNDER UV-B LIGHT IRRADIATION

Mapelli, S. - Radyukina, N.L. - Shashukova, A.V. - Shevyakova, N.I. - Kuznetsov, Vl. V.
Timiryazev Institute of Plant Physiology, RAS, Moscow, Russia

Common sage plants, grown in water culture to the stage of 4–5 true leaves, were treated with 5 mM proline (12 - 48 h) added to the medium, irradiated with UV-B (12.3 kJ/m^2 for 10 min), or to the combined action. In control plants, the content of endogenous proline was close to zero. In the presence of proline in medium, its content in the roots was 9 μmol/g fr wt in 12 h of exposure, whereas in the leaves the proline increased in 24 h to 1 & μmol/g fr wt. The content of PUT increased in the leaves and especially in the roots after 10 min UV-B irradiation. The UV-B affected not only the synthesis of PUT but also that of SPD and SPM; it also induced accumulation of their soluble conjugates. The presence of proline in medium enhanced PUT but not the formation of soluble conjugate. At combined treatment of the two factors, the content of free PUT in the leaves displayed a tendency to the rise and in the roots to the decrease. At the same time, the content of polyamine free and conjugates increased in both tissues. All these facts are an indirect indication of relationship between proline and polyamine. It can also state that artificially created high proline concentration in common sage tissues, resulted in homeostasis disturbance of low-molecular metabolites and induced a requirement in its restoration by diverse ways. Activation of PDH, a key enzyme of proline degradation, changes in the polyamines content and of their soluble conjugates might be the ways for such restoration.

P01-089: CELL WALL PLASTICITY OF MAIZE CELL CULTURES HABITUATED TO DICHLOBENIL

Melida, H. - García-Angulo, P. - Alonso-Simón, A. - Encina, A. - Acebes, J.L. - Alvarez, J.M.
Universidad de León

This work addresses the characterisation of a maize cell line able to grow in the presence of high concentration of dichlobenil, a specific inhibitor of cellulose biosynthesis in plants.
A dichlobenil-habituated cell line was obtained by a stepwise increase in the concentration of the inhibitor in the culture media. Habituation to dichlobenil implied slower growing rates and irregularly shaped cells among other changes. Dichlobenil-habituated cells presented a modified cell wall architecture characterized by: i) reduced (75%) cellulose content, ii) increased amount of phenolics iii) increased amount of arabinoxylans. Proteomic analysis revealed that habituation is linked to modifications in several metabolic pathways: carbohydrate, nitrogen and ethylene metabolism and stress-related pathways. The results of RT-PCR analyses of genes involved in synthesis of cellulose (*ZmCesA1-12*) and phenolics (*PAL, C4H, 4CL, HCT, C3H, COMT, CCoAOMT*) show that: 1) *ZmCesA5* and *ZmCesA7* have an outstanding role in the habituation, ii) the expression of the majority of the genes involved in phenolic synthesis is induced during exponential cell growing phase and repressed during the stationary phase. Based on the increased levels of cell wall phenolics and expression levels of genes of phenolic synthesis, we deeper analysed these compounds. In summary cell wall pheno-

lics have a central role in the habituation of maize cell cultures to dichlobenil.

P01-090: ADAPTIVE PECULIARITIES OF DECIDUOUS MAGNOLIAS DURING THE INTRODUCTION IN UKRAINE

Vovk, M. - Palagecha, R.
T. Shevchenko Kyiv National University, Fomin Botanical Garden
**Corresponding author, e-mail:* slawawowk@yahoo.com

For the first time are established reasons of different hardiness of annual shoots in 11 species of *Magnoli a* L. introduced in Ukraine. The anatomy-morphological peculiarities of shoot structure connected with different winter resistance are presence of thick cuticle (10-28 mkm), multilayer periderm, subepidermal sclerenchymatous elements; formation of secretory containers which accumulates secondary metabolism matters in bark; intensive lignification of cell membranes; different density of lenticels arrangement. In experiments with artificial freezing of shoots (under -25, -30 and -35°C) was established decreasing of tissue hardiness in sequence from bark to medulla. High water storage capacity and more contents of bound water in annual shoots are presupposition for formation of winter resistance. In the shoots of winter resistant species was the bound water twice as more then in species with low winter resistance. Increase of flavonoides quantity in bark during vegetation and its decrease in winter testifies about their participation in processes of wintering. Acclimatization in natural conditions (temp. from +3,2 to -10°C) and in laboratory (temp. -30 and -35°C) accompanied by changes of total flavonoides contents (decreasing in middle on 34%).
In annual shoots tissue established differences in localization of lipid compounds and dynamics of starch and lignin and has been determined their specific role in winter resistance of different species of magnolias. The adaptive changes of lipidic index of resistance proved in increasing of galactolipids content in bark during preparation of plants to the wintering and their decreasing in winter. Shown also decreasing of sulfolipids content in bark of magnolia shoots owing to decreasing of temperature.

P01-091: EFFECTS OF TREATMENT WITH ADOR ON OXIDATIVE ACTIVITY IN AXENIC SUNFLOWER ROOTS

*Garrido, I.[1] - García-Sánchez, M.[2] - Garrido-Romera, I.[2] - Ocampo, J.A.[2] - Espinosa, F.[1]**
[1] Universidad Extremadura
[2] Estación Experimental Zaidín, CSIC
**Corresponding author, e-mail: espinosa@unex.es*

The aim of this work was to analyze the effect of aqueous-extract of dry olive-mill residue (ADOR), uninoculated and inoculated with saprobic fungi, on sunflower growth. The O_2^- formation and exogenous NADH oxidation activities, apoplastic peroxidases (DMAB-MBTH POX and CA-POX), total antioxidant capacity, total phenols, flavonoids, and phenylpropanoids, and membrane lipid peroxidation were determined in intact sunflower roots germinated and grown for 36 h without (control) or with 50% ADOR, and with or without prior incubation with saprobic fungi (*Pychoporus cinnabarinus*, *Coriolopsis rigida*, *Trametes versicolor,* and *Penicillium crysogenum*). Treatment of the control roots with ADOR for 10 min induced an increase in both O_2^- formation and DMAB-MBTH EC-POX activity, possibly due to oxidative shock in response to the stress caused by the ADOR. The roots germinated in uninoculated ADOR, however, presented marked decreases in oxidative activity, total antioxidant capacity, and phenol and phenylpropanoid contents. These activities were partially recovered in the roots germinated in the inoculated ADOR, although without reaching the normal values. The most effective fungal were P. cinnabarinus and P. crysogenum. Treatment with ADOR was also observed to increase membrane lipid peroxidation.
The effects induced by ADOR may be caused by alterations in the plasmamembrane, which could then affect membrane-linked enzyme systems.

P01-092: ROLE OF HEAT DISSIPATION MECHANISMS IN PHYSCOMITRELLA PATENS ACCLIMATION TO DIFFERENT LIGHT CONDITIONS

Gerotto, C.[1] - Alboresi, A.[2] - Bassi, R.[2] - Giacometti, G.[1] - Morosinotto, T[1]*
[1]Dipartimento di Biologia, Università di Padova
[2]Dipartimento di Biotecnologie, Università di Verona
**Corresponding author, e-mail: caterina.gerotto@unipd.it*

Sun light provides energy supporting life of photosynthetic organisms but also leads to the formation of reactive oxygen species when in excess.Thus, plants and algae evolved several photoprotective processes to survive in a variable environment. The fastest one, called Non Photochemical Quenching (NPQ), consists in the dissipation of excess energy as heat, which is triggered by the generation of a pH gradient across thylakoid membranes. Green algae and plants are all able to induce NPQ, but its activation depends on two different proteins: LHCSR and PsbS, respectively. The moss *Physcomitrella patens* is the only known organism where both PsbS and LHCSR are present and active in NPQ.
Here we show that acclimation to different light conditions has a strong influence on NPQ in *P.patens*: when acclimated to high light WT mosses showed an enhanced NPQ which is correlated to the increased expression of both PsbS and LHCSR. Overexpression of PsbS and LHCSR in transgenic plants confirmed that the level of both proteins controls NPQ amplitude. Conversely, KO mutants depleted in PsbS and LHCSR showed reduced capacity of NPQ which, interestingly, was accompanied by an enhanced susceptibility to long-term light stress. Our results thus point to the relevance of NPQ in the long-term acclimation besides being a short-term response: despite it is a fast activated mechanism, NPQ modulation is also fundamental for acclimation to prolonged light stress.

P01-093: THE STUDY OF PHYSIOLOGICAL RESPONSES OF MUSA ACUMINATA VAR. MAS TO INTERACTION OF SALINITY AND CADMIUM

Farzami Sepehr, M.[1] - Harikrishna J.A.[2] - Norzulaani, K.[2]*
[1]Dep of Biology, Faculty of Agriculture, Islamic Azad University Saveh Branch
[2]Genetics and Molecular Biology, Institute of Biological Sciences ,Faculty of Science, University of Malaya ,Kuala Lumpur, Malaysia
**Corresponding author, e-mail: mfsepehr48@yahoo.com*

Soil salinity affects plant growth and development due to harmful ion effects and water stress caused by reduced osmotic potential in the soil solution. Furthermore, Cd is a pollutant that has been emitted into the environment for decades. Major anthropogenic sources are Cd-containing phosphate fertilizers, sewage sludge and industrial emissions. Plants undergo one or more stress during their life cycle. The effects of 0,25,50 µM Cd^{2+} ($Cd(NO_3)_2.4H_2O$) and 0,50,75,100,125,150 mM NaCl on growth , the content of some ions and proline contents in Banana (*Musa acuminata* var. Mas) were investigated in present study. With increasing concentrations of Cd^{2+} or NaCl alone in culture media, growth parameters, Chlorophylls and proline contents decreased. Combination treatment with salinity and cadmium decreased the negative effects observed following the two stress alone. Plants exhibiting growth retardation, none cadmium accumulation in response to one mild stress factor (75,100,125 mM NaCl).the exposure of plants to cadmium caused a partial reversal of effect of salinity. Root and shoot growth, ion accumulation, sensitivity index and other physiological responses were improved at moderate concentrations of two stress factors imposed jointly.

P01-094: RIN2, A NOVEL CHLOROPLAST PROTEIN INVOLVED IN RETROGRADE SIGNALING IN RESPONSE TO EXCESS LIGHT

Kindgren, P. - Kremnev, D. - Barajas-Lopez, J.D. Strand, Å.*
**Corresponding author, e-mail: peter.kindgren@plantphys.umu.se*

Plants have evolved numerous protective and scavenge systems to survive in a fluctuating light environment. Retrograde signals originate in the plastid and regulate nuclear expression in response to excess light. In order to elucidate these signals we performed a screen for redox insensitive mutants and isolated redox *insensitive 2 (rin2)*.
Following high light treatment *rin2* demonstrates impaired regulation of nuclear encoded photosynthesis genes. The transcription of plastid encoded photosynthesis genes are repressed while ribosomal genes are up regulated, suggesting an impaired activity of the plastid encoded RNA polymerase. Positional cloning of the mutant revealed a point mutation in a gene with unknown function. RIN2 is a plant specific protein with no sequence homology to any other protein of known function.
The rin2 mutation creates a premature stop codon in the C-terminus of the protein and leads to a striking phenotype where the cotyledons are albino and the newly developed leaves are pale green. A T-DNA insertin mutant has a similar but more severe phenotype. Both mutants demonstrates a lower electron transport rate and the T-DNA insertion mutant show retarded growth. Interestingly, the pale phenotype is partly rescued when seedlings are grown under low light intensities, suggesting a role for RIN2 in the photooxidative stress response. Using a RIN2:YFP fusion protein we could show that RIN2 is localized to the chloroplast. A working model for the RIN2 mediated retrograde signal will be presented.

P01-095: IN VIVO SUBSTRATE PREFERENCE OF ARABIDOPSIS PLD REVEALED BY A LIPIDOMICS STUDY.

Ruelland, E. - Rainteau, D. - Vergnolle, C. - Zachowski, A.
Université Pierre et Marie Curie/CNRS
**Corresponding author, e-mail: eric.ruelland@upmc.fr*

In plants, phospholipases D (PLDs) have been implicated in responses to abiotic stresses or to hormones, such as abscisic acid or salicylic acid (SA).
The product of PLD is phosphatidic acid (PtdOH). It has been suggested that, depending on their acyl chains, the different PtdOH might not necessarily have the same signalling roles. However, the analysis of the composition of PLD products was till now tedious.
The development of lipidomics techniques gives us the possibility to easily analyze the product of PLD activity. Because other enzymes than PLD can produce PtdOH, we chose to analyse phosphatidylbutanol (PtdBut), the product of the transphosphatidylation, specifically catalyzed by PLDs in presence of primary butanol.
In Arabidopsis suspension cells, the composition of PtdBut was analyzed by mass spectrometric multiple reaction monitoring and compared to that of the major phospholipids. We show that the profiles of PtdBut in cells challenged or not by SA do not correctly match those of any of the major phospholipids. When compared to that of phosphatidylcholine (PtdCho) or phosphatidylethanolamine (PtdEtn), an over representation of 16:0/18:2 and 16:018:3 species is observed. However, when microsomes extracted from Arabidopsis cells are used as a PLD source, and Soybean PtdCho or PtdEtn used as substrates for *in vitro* PLD assays, the resulting PtdBut exactly match the profiles of the substrates. Therefore, the apparent mismatch in the *in vivo* experiments is unlikely to be due to a selection of acyl chains by the PLDs. Our results hint at the non-homogeneous repartition of the different species of phospholipids in the different cellular membranes, or in the so-called membrane microdomains, combined to a non-homogeneous localization of PLDs.

P01-096: EFFECTS OF EXTERNALLY ADDED RIBITOL ON PRIMARY PHOTOSYNTHETIC PROCESSES AT LOW AND FREEZING TEMPERATURE IN LICHEN THALLI

Hájek, J.[1] - Smejkal, L.[1] - Barták, M. [1] - Vaczi, P.[1] - Lipavská, H.[2]*
[1]Masaryk University, Faculty of Science, Department of Experimental Biology
[2]Charles University Prague, Faculty of Science
**Corresponding author, e-mail: hajek@sci.muni.cz*

In lichens, polyols (sugar alcohols, *e.g.* ribitol, arabitol, mannitol) have several physiological roles.
They are effective carbon storage and anti-freezing substances. Natural levels of polyols in lichens are species-specific and vary within 1.4-8.8 $mg.g^{-1}$ DW (ribitol), 0.4-29.0 $mg.g^{-1}$ (mannitol). In our study, we tested positive effects of extrenally added ribitol on primary photosynthetic processes at low/freezing temperature and evaluated interspecific differences between 3 fruticose species (*Cetraria islandica, Cetraria nivalis, Cetrariella delisei* collected in Southern Norway and single foliose lichen collected in Svalbard. Lichen thalli were exposed to ribitol concentrations of 0 (control), 16, 32, 40, 50 mM for 168 h at 0 °C and two subzero temperatures. The chlorophyll fluorescence parameters (potential yield of photochemical processes in photosystem II (FV/FM), effective quantum yield in PS II (ΦPSII), and non-photochemical quenching (NPQ) were monitored in 24-h intervals using a chlorophyll fluorescence imaging (Handy Fluor Cam, HFC-010, *PSI*, CZ). Contents of polyols and other non-structural saccharides (NSS) were evaluated before and after the treatment by a HPLC. Contents of chlorophyll *a*, *b*, and total carotenoids were measured by a spectrophotometer (*Spekord*, Germany). The results indicated importance of polyols in antifreezing tolerance of lichens since positive affect of ribitol addition was apparent at freezing temperature. Funded by the GAAV KJB601630808.

P01-097: EFFECT OF SALINITY AND ITS INTERACTION WITH ASCORBATE ON SOME PHYSIOLOGICAL AND BIOCHEMICAL ACTIVITY IN CUMINUM CYMINUM L.

*Ghorbanli, M.**
Azad University Gorgan Branch
**Corresponding author, e-mail: ghorbanli@yahoo.com*

In this investigation growth factors, the amount of photosynthetic pigments including chlorophyll a, chlorophyll b, carotenoid, the amount of soluble and insoluble carbohydrate in aerial organ and root ,activity of catalaseand ascorbate peroxidase, the amount of proline, phenol and MDA in medicinal plant *Cuminum cyminum* L. seedlings were investigated .The plants were cultivated in green house conditions and were treated randomly with three replications. Plants were treated by different concentrations of NaCl (02, 5, 50, 75, 100, 125 mmolar) and ascorbate (0.9mmolar). In salt treated plants with increasing NaCl concentrations, germination, growth parameters, the amount of photosynthetic pigments, insoluble carbohydrate and the amount of phenol were decreased but the amount of soluble carbohydrate, enzymes activity and MDA were increased. The plants that were treated by NaCl and ascorbate at the same time in same NaCl concentrations, their germination, growth factor, the amount of photosynthesis pigments and soluble and insoluble carbohydrate and the amount of proline and phenol were increased.The results indicated that the ascorbate is the one of the antioxidant that caused increase in *Cuminum cyminum* L. plant resistance in salt stress conditions.Key words: *Cuminum cyminum* L.,salt stress, ascorbate antioxidant, photosynthesis pigments.

P01-098: IMPACT OF LIGHT SPECTRAL COMPOSITION AND OZONE FUMIGATION ON CHLOROPHYLL CONTENT CHANGES AND OPTICAL PROPERTIES OF BROCCOLI LEAVES

*Skoczowski, A.**
Polish Academy of Sciences, The Franciszek Gorski Institute of Plant Physiology
**Corresponding author, e-mail: skoczowski@ifr-pan.krakow.pl*

Broccoli leaves were injured by the ozone dose applied (70 ppb, 6 hours daily) mainly under WBL (WBL - white supplemented by blue radiation). Studies were carried out on leaf fragments from the outer (OP) and the inner parts (IP). The spectrophotometric analysis did not reveal differences in the Chl content. The variations in reflectance (R) in the visible region (400-700 nm) were not significant with respect to tissue age, ozone fumigation and growth conditions (WBL or WL - white radiation). Only in plants grown under WBL an increase in green R in ozone-treated leaves was observed, indicting a decrease in the chlorophyll content. Measurements of the optical properties of leaves provide more precise information about changes in the content of chlorophyll than spectrophotometry. Leaves under WBL showed significant differences in R within 800-1100 nm, which depended on the physiological age of the tissue. For physiologically older tissues, values of R were lower than those for physiologically younger tissues. However, under WL, a decrease in R within the 800-1100 nm range depended only on ozone fumigation. Response of broccoli leaves to spectral composition of irradiation and ozone stress was much higher for irradiation transmission (T) than for R. Ozone fumigation leaves grown in WBL increased T in OP and IP leaf fragments, both in the 500-700 and 750-1100 nm ranges. For plants growing in WL no influence of ozone fumigation on the T of irradiation within the visible range was observed. However, fumigation with ozone resulted in a significant decrease in the T within the infrared range (750-1100 nm). The results indicate the major role of irradiation spectral composition in plant response to ozone stress.

P01-099: POST-TRANSCRIPTIONAL REGULATION OF STRESS-INDUCED MRNA DECAY BY 3'UTR OF GENES IN RICE

Kim, Y.[1] - Park, S.H.[1] - Chung P.J.[2] - Felipe Redillas, M.C.[1] - Kim, J.K.[1]*
[1]Myongji University
[2]Rockefeller University, NY, USA
**Corresponding author, e-mail: younshic@gmail.com*

Many environmental stimuli including water potential, temperature extremes, and high salinity, regulate gene expression at transcriptional and post-transcriptional levels. We are taking a genomics-based approach to unravel the regulation in response to such environmental stresses in rice. Expression profiling with the 135K Rice Whole Genome Microarray revealed that transcripts of a group of genes involved in light and dark reactions are decayed much earlier than the others under stress conditions. Changes in polysomal mRNA are similar with those of total RNA. The stress-induced mRNA decay was discovered a post-transcriptional event by using RNA pol II chromatin immunoprecipitation assay. To delineate functional determinant(s), we chose two representative genes, *RbcS1* and *Cab1*, and dissected them into several components. Transgenic rice plants expressing different combinations of the components were analyzed under stress conditions using the real-time qPCR method, demonstrating that 3'UTR is the major mRNA sequence determinant that mediates such stress-induced mRNA decay.

P01-100: EPR INVESTIGATION OF LONG LIVED RADICALS IN WHEAT SEEDS

Filek, M.[1] - Labanowska, M.[2] - Bidzinska, E.[2] - Biesaga-Koscielniak, J.[1]*
[1]The F. Gorski Institute of Plant Physiology, Polish Academy of Sciences
[2]Faculty of Chemistry, Jagiellonian University
**Corresponding author, e-mail: mariafilek@gmail.com*

Electron Paramagnetic Resonance (EPR) is one of the very useful method to study of various types of radicals generated in biological materials during life processes. Among these radicals long lived species seems to be responsible for protection of cells against different stresses. The aim of this work was investigation of long lived radicals in seeds of two wheat genotypes, Polish and Finnish, which exhibit various stress response. EPR spectra of both genotypes reveal the similar signals characteristic for sugar radicals. In the Finnish wheat, the intensity of these signals was about two times lower than in Polish one. Cutting of seeds stimulates the appearing of the additional signal but only in the EPR spectrum of the Polish seeds. This phenomenon was accompanied with the disappearance of the intensive signal of iron species visible in not cutting seeds. The latter was not observed in the seed of Finnish genotype.
This work was supported by COST FA0605, Nr: DPN/N110/COST/2009

P01-101: DROUGHT STRESS TOLERANCE AND THE ANTIOXIDANT ENZYME SYSTEM IN SPRING WHEAT

Biesaga-Koscielniak, J. - Filek, M. - Dziurka, M. - Koscielniak, J. - Pitera, R. -
The F. Gorski Institute of Plant Physiology, Polish Academy of Sciences
**Corresponding author, e-mail: j.koscielniak@ifr-pan.krakow.pl*

Plants experience drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high. Although the general influence of drought on plant growth are fairly well known, the primary effects of water deficit at the biochemical and the molecular levels are not well understood. Drought induces oxidative stress in plants by producing reactive oxygen species (ROS). Oxidative damage in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant metabolisms. The relationship between the antioxidant enzyme system and drought stress tolerance during leaf rolling in the leaf of sprig wheat was studied. 20 cultivars of spring wheat were used in the study. Plants were cultivated in growth room in pots filled at a 16-h photoperiod, at irradiance of 450 $\mu mol \times s^{-1} \times m^{-2}$. Some plants were well watered (control) whereas the other plants were subjected to drought stress. Analyses were carried out after a 3-week period of drought. Changes in non-enzymatic and enzymatic antioxidant responses were used to assess the effects of drought stress. The reaction of all investigated genotypes to drought stress was typical i.e. an increase of the antioxidative enzymes level was observed. At the same time the levels of tocopherols, carotenoids and phenols increase also when comparing to the non-stressed plants.
This work was supported by COST FA0604 Tritigen Nr: 192/C0ST/2008/0 and COST FA0605, Nr: DPN/N110/COST/2009

P01-102: QUANTITATIVE DETERMINATION OF REDOX-ACTIVE POLYPHENOLICS FROM NITROGEN STRESSED RED BEETROOT (BETA VULGARIS L.) PLANTS BY ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY.

Papasavvas, A[1] - Stathi, P.[1] - Salahas, G[1] - Hela, D.[2] - Deligiannakis, Y.[1]
[1]Lab. of Physical Chemistry, Dept. of Environmental and Natural Resources Management, University of Ioannina
[2] School of Natural Resources and Enterprise Management, University of Ioannina

Plant polyphenolics are the most widely distributed secondary plant products (30-45%) of plant organic matter, and have been shown to accumulate frequently as a reaction to environmental stress, including nitrogen starvation. The total Stress-Induced Polyphenolics (SIP) can be determined by liquid chromatography (HPLC), or spectrophotometric assays. However the antoxidant activity of the SIPs is the redox-active fraction, which is

able to scavenge free radicals. In the present work, the redox-active fraction of SIPs has been determined in plant extracts obtained from red beetroot (*Beta vulgaris* L.) leaves from plants hydroponically grown. We used nutrient solutions containing different concentrations (gradient) of nitrate ions supplied by nitrogen source i.e. 86, 173, 260, 560, 876 ppm. Plant leaves extracts in 50 % methanol/ 48,5% H_2O/ 1,5 % formic acid solution where studied by Electro Paramagnetic Resonance spectroscopy EPR as a function of the pH and redox potential. The EPR data show that in the plant exctracts polyphenolic radicals can be stabilized under ambient redox potentialsi.e. under O2 oxidation, at alkaline pH. At least two types of phenolic radicals characterized by g-values in the range 2.0035-2.0045 and ΔH=12-16 Gauss have been identified. These represent redox-ative polyphenolics which account for 95% of the total SIPs. The concentration of the redox-active polyphenolics, determined using Gallic Acid as spin standard, is quantitatively correlated with the growth-stress conditions applied during the plants' cultivation protocol. Moreover the concentration of the redox-ative polyphenolics parallels the accumulation of the total SIPs.

P01-103: BIOCHEMICAL RESPONSES OF SELECTED SPECIES OF THE BRASSICACEAE FAMILY TO POLLUTION WITH METALS IN DIFFERENT VEGETATION TYPES

Boštjan, G.[1] - Lasnik, C.R.[1] - Romih, N.[1] - Feifhofer Hartwig, W.[1] - Batic, F.[2]*
[1]Institute of the Environment and Spatial Planning
[2]Biotehnical faculty, University of Ljubljana
**Corresponding author, e-mail: bostjan.grabner@iop.si*

To determine biochemical response to heavy metal pollution in the soil, total ascorbic acid, phytochelatins, total glutation, glucosinolates, chlorophylls, carotenoids and tocopherols were measured in five species of the *Brassicaceae* family, characteristic of the vegetation types. *A.petiolata* at the forest edge, *C. bursa-pastoris* on arable land, *D. tenuifolia* on road margins, *B. laevigata* on closed, permanent grasslands and *C. enneaphyllos* as forest ground layer vegetation were collected at three locations in Slovenia, differing in soil pollution. Additionally, two hybrids Brassica *napus* L. var. napu) ere also analysed.
The results show the correlation between metal pollution of the soil, the concentrations of metals in the upper parts of the plants and the expression of defence mechanisms in the plants to the metal pollution. The determination of response markers is giving us the possibility to use selective plants as a biomonitorsin environmental monitoring of metals in different habitat types of natural and semi natural vegetation, separately for short-term changes in heavy metal pollution in cultivated arable land and urban areas and also for long term metal pollution in forests.

P01-104: IMPACT OF OZONE AND SALT STRESS SINGLY AND IN COMBINATION ON PHYSIOLOGY AND GROWTH OF TOMATO PLANTS (LYCOPERSICON ESCULENTUM MILL) GROWN UNDER FIELD CONDITIONS

Al Husini, M.
King Khalid University

.
The main route for ozone entry in plants is through the stomata. Consequently, environmental factors that may expose plants to any stress that will eventually lead to stomatal closure, will also reduce the rate of ozone entry into the plant and will possibly counteract ozone damages. In addition, several abiotic stresses activate the synthesis of antioxidant compounds which will further contribute to neutralize toxic ozone derivatives. Due to this complex response, it is critical to assess how abiotic stresses and ozone toxicity will interactively affect plant growth and yield, especially in those areas, such as the coastal Mediterranean regions, where these types of stress and ozone exposure typically coexist. In this research we exposed tomato plants to salt stress in presence and absence of ozone to assess how salinity and ozone may interfere in terms of physiological responses and final yield. Plants grown in absence of ozone had a greater total biomass and higher yield compared to those grown in presence of ozone. Nevertheless these differences disappeared upon salinization. The reduced ozone damage in saline environment must be interpreted on relative terms, however, since salinity by itself caused a general inhibition of plant growth and yield. Based on these results, it was concluded that defining environment-specific ozone toxicity thresholds is necessary for developing reliable prediction models and/or assessing environmental safeguard levels.
Additional index words: stomatal conductance, Photosynthetic rates, antioxidant activity, open top chambers, plant growth, tomato.

P01-105: REGULATION OF WATER TRANSPORT IN ARABIDOPSIS ROOTS BY MULTIPLE ABIOTIC AND NUTRITIONAL STIMULI.

di Pietro, M. - Li, G. - Maurel, C. - Santoni, V. -
Université Montpellier
**Corresponding author, e-mail: dipietro@supagro.inra.fr*

The uptake of soil water by roots is a key function for maintaining the plant water status. The root water permeability (root hydraulic conductivity, Lp_r) is mediated in large part by water channel proteins named aquaporins and is highly dependent on environmental conditions. Our group previously showed that NaCl and H_2O_2 treatments decrease the Lp_r of *Arabidopsis* and alters the phosphorylation status of some root aquaporins. With a final aim of extending these studies, the present work thoroughly characterizes in *Arabidopsis* the kinetics of Lp_r inhibition by a large array of osmotic, oxidative and nutritional stresses. Measurements by means of the pressure chamber technique, showed that a treatment of roots by 200 mM mannitol inhibits Lp_r by up to 63% with a half time of 1 h. We also showed that increasing concentrations of nitric oxide (NO), from 50 µM to 500 µM inhibit Lpr by 13% to 57%. Nitric oxide specificity was assessed by the counteracting effects of a NO scavenger. After 6 days of nitrate or phosphate starvation, the Lp_r was decreased by 45% and 77% respectively. Nitrate resupply did not allow Lp_r to recover, while phosphate resupply induced a 95% recovery of Lp_r from the starved conditions.
The present work provides the time frame for future studies on the multiple molecular and cellular mechanisms involved in Lp_r regulation.

P01-106: TWO PLEIOTROPIC DRUG RESISTANCE TRANSPORTERS OF NICOTIANA TABACUM ARE INVOLVED IN PATHOGEN-RESISTANCE

Drozak, A.[1] - Bultreys, A.[2] - Vos, C.[1] - Crouzet, J.[1] - Baijot, A.[1] - Fourmeau, J.[1] - Trombik, T.[1] - De Waele, D.[1] - Boutry, M.[1]*
[1]Catholic University of Leuven, Belgium
[2]Centre wallon de Recherches Agronomiques, Gembloux, Belgium
**Corresponding author, e-mail: anna.drozak@uclouvain.be*

The ATP-binding cassette transporter family contains several subfamilies among which the pleiotropic drug resistance (PDR) subfamily is specific to fungi and plants. Five PDR transporter genes have been identified and studied in *Nicotiana tabacum*. There are two PDR1 homolog genes - *NpPDR1* (originally cloned from *N. plumbaginifolia*) and *NtPDR1* (cloned from *N. tabacum*)- which share 84% amino acid identity. They also have a similar but not identical expression profile in roots, leaf trichomes and flowers of *N. tabacum*. These transporters are involved in the plant response to biotic stress. Indeed, their expression was strongly induced in the whole leaf upon treatment with jasmonates, signaling molecules involved in plant defence. Moreover, transgenic plants silenced for both *PDR1* genes were frequently

spontaneously infected by fungal pathogens, resulting in death before the flowering stage. Controlled infection showed increased susceptibility of *PDR1*-silenced plants to *Botrytis cinerea*, *Fusarium oxysporium*, *Rhizoctonia solani* and *Phytophthora nicotianae*. Susceptibility of the silenced plants to the root-knot nematode Meloidogyne incognita is being tested. Transgenic plants specifically silenced for either *NpPDR1* or *NtPDR1* have been obtained and are being characterized to decipher their respective roles. To identify putative substrates, *NpPDR*1 was expressed in yeast. Susceptibility of transgenic yeast to the anti-microbial diterpenes sclareol and cembrene was clearly modified, indicating that these N. tabacum antimicrobial molecules are substrates of NpPDR1.

P01-107: IMPACT OF OZONE ON CHEMICAL CHANGES IN PLANT TISSUE DETECTABLE BY FT-RAMAN SPECTROSCOPY

Miszalski, Z.
Polish Acamy of Sciences
**Corresponding author, e-mail: miszalski@excite.com*

To study the influence of ozone on plant tissue, two species of Brassica vegetables were treated with an elevated concentration of ozone (70 ppb). By using classical spectrophotometric methods, the impact of ozone on assimilation pigments in leaves was determined. In white cabbage and, to a lesser extent, in broccoli leaves ozone fumigation reduced the content of chlorophyll a and b as compared with control units. On the other hand, carotenoids content was not affected by ozone stress. However, when FT-Raman spectroscopy was applied to the analysis of plant tissue, it was shown that ozone strongly influenced carotenoid content in leaves. Cluster analyses used to the obtained spectra allow for clear separation of ozone fumigated samples and non-processed control samples into distinct groups. The discrimination was achieved mainly on the basis of the wavenumber range. This is the first report on the use of FT-Raman spectroscopy for a non-destructive analysis of the impact of air pollutant on plants.

P01-108: IMPROVEMENT OF NITROGEN REMOBILISATION DURING OILSEED RAPE LEAF SENESCENCE

Orsel, M.[1] - Laperche, A.[1] - Bissuel, C.[1] - Bregeon, M.[1] - Bloquel, E.[1] - Clouet, V.[1] - Canoy, A.S.[2] - Leflon, M.[3] - Nesi, N. [1] - Pinochet, X.[1]*
[1] Univ. Renne, Le Rheu, France
[2]Biogemma, France
[3]CETIOM, Campus Grignon, France
**Corresponding author, e-mail: mathilde.orsel@rennes.inra.fr*

Winter oilseed rape (WOSR) is characterized by high N-uptake efficiency (*Laine et al.*, 1993) but only half the N originating from fertiliser application is recovered in the seeds (Schjoerring *et al.*, 1995). WOSR (*Brassica napus*) is also characterised by early leaf shedding and unusual high N loss through falling leaves: up to 15% of its entire N content (Rossato *et al*., 2001). Leaf senescence generally corresponds to the mobilisation of N reserves from source leaves to sink organs such as seeds (Masclaux-Daubresse et al., 2008). Improvement of N remobilisation efficiency during leaf senescence is likely to improve significantly the overall plant NUE, particularly in WOSR in which N remobilisation is relatively inefficient (Rathke et al., 2006).
In order to identify the key elements (such as genomic regions, genes, enzymes and metabolites) involved in the control of N remobilisation during WOSR leaf senescence we are developing a dual approach based on genetic and functional genomic strategies. We are currently assessing field grown genotypes for traits related to leaf senescence and N remobilisation in order to identify a genetic population and pertinent traits to perform a QTL analysis. We are also taking advantage of the knowledge already accumulated on model species such as *Arabidopsis thaliana* to identify *B. napus* genes involved in N remobilisation during leaf senescence. Using Arabidopsis candidate gene sequences, potentially orthologous genes have been identified in *B. napus*. Their expression patterns are under investigation and genetic mapping is in process. This approach will be reinforced with transcriptomic experiments based on the new Affymetrix *B. napus* array and original material from our large collection of *Brassica napus* genotypes.

P01-109: DIFFERENTIAL RESPONSES OF ANTIOXIDATIVE SYSTEMS AND PHOTOSYNTHETIC PIGMENTS IN THE LEAVES OF QUERCUS AUCHERI (BOZ PıRNAL OAK) UNDER DROUGHT STRESS CONDITION

Hakan, Ö. - Gulriz, B.
Istanbul University

Drought is the most important stress factor determining plant growth and productivity world-wide. The aim of the present study was to investigate disturbances of antioxidative systems and photosynthetic pigments in the leaves of *Quercus aucheri* which are endemic for Turkey under drought stress condition. 6 months old plants were treated with drought stress for 4 weeks. At the end of the treatment period, contents of photosynthetic pigments, levels of lipid peroxidation, levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) and activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), guaicol peroxidase (GPX) and catalase (CAT) were determined in the leaves of *Quercus aucheri*. 4 weeks drought stress significantly decreased contents of photosynthetic pigments while increased the levels of lipid peroxidation in the leaves of *Quercus aucheri*. Levels of GSH were significantly decreased while levels of GSSG were significantly increased in the leaves of *Quercus aucheri* at the 4 weeks drought stress treatment. The activities of CAT, AP and GR were significantly decreased while activities of SOD and GPX were significantly increased in the leaves of *Quercus aucheri* at the 4 weeks drought stress treatment. The results indicated that 4 weeks drought stress may induce oxidative stress and cause differential responses on the antioxidant enzymes in the leaves of *Quercus aucheri*. The present study would contribute to future studies on Quercus species which are endemic for Turkey under drought stress conditions.

P01-110: STRESS-INDUCED ETHYLENE AND ROS BIOSYNTHESIS ARE SYNERGISTICALLY INTERACTED FOR DETERMINING THE EXTENT OF CELL DAMAGE

Jang, S. - Ri, J.N. - Young, P.K.
Sunchon National University
**Corresponding author, e-mail: sj1208@sunchon.ac.kr*

ROS, which are inevitable by-products of many redox reactions in eukaryotic cells, play a crucial role in many cellular responses and signaling pathways. The interactions between ROS and ethylene biosynthesis in biphasic manner are studied in tobacco plants under the environmental stresses. The ROS production was peaked twice at 30 min and 3 h after treatment of abiotic stress such as oxidative stress and high salinity. Ethylene production was also peaked twice at 1 h and 30 h after abiotic stress. These results implied that ROS could act as a signal upstream of ethylene biosynthesis. Not only the treatment of ACC, a precursor of ethylene biosynthesis, was increased ROS accumulation in phase I, but also the treatments of AVG and NBD, which are inhibitors of ethylene biosynthesis and ethylene signaling, respectively, were significantly reduced ROS accumulation during phase I. We already reported that stress-induced ethylene production was significantly inhibited in *rbohD-AS* and *rbohF-AS*, in which antisense expression of *NADPH oxidase* genes was performed. These results suggested that ethylene and ROS act in a positive feedback cycle, which results in mutual enhancement of ethylene and ROS production. This means that after onset of

stress treatment the first peak of endogenous ROS accumulation was followed by ethylene production during phase I, and then ROS and ethylene biosynthesis were occurred for a larger and more prolonged response with necrosis during phase II.

P01-111: PLANT HORMONE-INDUCED BIPHASIC ACCUMULATION OF ETHYLENE AND REACTIVE OXYGEN SPECIES (ROS)

Jung, M. - Young, P.K.*
Sunchon National University
**Corresponding author, e-mail: jmh223@sunchon.ac.kr*

In this study, we investigated the interrelation of plant hormones and well-known signaling molecules such as ethylene and ROS. In usually, biphasic productions of ethylene and ROS in response to abiotic and biotic stress are reported. A biphasic ethylene production was appeared in treatment with stress-related plant hormones such as 50 μM ABA, 300 μM SA, and 50 μM JA. Also those hormones induced a biphasic ROS accumulation, which was determined by confocal image with DCFH-DA. Biphasic peaks of ethylene production occurred at 1 h and 30 h after hormone treatments, which were resulted from gene-specific expression of *NtACS4* at 1 h and *NtACS1* at 30 h. ROS accumulation was peaked twice at 30 min and 3 h after treatment with ACC, ABA, JA and SA. However, we detected a biphasic production of ethylene and ROS accumulation after treatment of growing-promoting plant hormones such as gibberellin, auxin, and cytokinin. The treatment of auxin, 10 μM IAA and 50 μM NAA, also induced a biphasic ROS accumulation at 30 min and 3 h. Also, treatments with cytokinin, 50 μM BA (benzyladenine) and 50 μM Kinetin, and gibberellin, 25 μM GA3, were induced a biphasic ROS accumulation at 30 min and 3-4 h. Hormone-induced ROS was mainly produced in most cell components such as cytosol, nucleus, plasma membrane and chloroplast. Therefore, it was suggested that ethylene and ROS, which were reacted in biphasic manner, were signaling molecules during plant hormone-induced physiological cellular response.

P01-112: PROTEIN PHOSPHATASE 2A IN CROSS-TALK BETWEEN LIGHT ACCLIMATION AND DEFENSE PATHWAYS IN ARABIDOPSIS

Kangasjärvi, S.[1] - Wrzaczek, M.[2] - Scharte, J.[3] - Rahikainen, M.[1]- Konert, G.[1] - Tikkanen, M.[1]- Aro E-M[1]
[1] University of Turku
[2]University of Helsinki
[3]University of Munster
**Corresponding author, e-mail: saijaliisa.kangasjarvi@utu.fi*

Serine/threonine protein phosphatase 2A (PP2A) family members carry out crucial functions in the regulation of signalling through phosphorelay cascades in animals and plants. The predominant form of PP2A is heterotrimer, consisting of a catalytic subunit C, a scaffold subunit A, and a highly variable regulatory subunit B, which is thought to determine the target specificity of subunit C in the PP2A holoenzyme. We found that a specific PP2A-B subunit is required for accurate light acclimation and jasmonic acid (JA) and salicylic acid (SA) dependent disease resistance in *Arabidopsis thaliana*. Knock-down *pp2a-b* mutants show age-dependent formation of yellowing lesions when grown under moderate light intensity. Promoter::GUS analysis indicates activity of *PP2A-B* promoter in patches that highly resemble the yellowing lesions on pp2a-b mutant leaves. On ultra-structural level, symptoms of cell death appear particularly in the spongy mesophyll tissue of visually pre-symptomatic *pp2a-b* leaves. The cell death phenotype of *pp2a-b* is accompanied by accumulation of reactive oxygen species through a pathway that requires the activity of CONSTITUTIVE EXPRESSION OF PR GENES 5 (CPR5). Moreover, similarly to *cpr5*, *pp2a-b* shows constitutive activation of JA- and SA-dependent defense pathways. In both *cpr5* and *pp2a-b*, these characteristics become alleviated upon acclimation to high irradiation levels. Currently, PP2A-B-dependent signalling interactions are being studied by biochemical tools in attempts to reveal the mechanism by which PP2A-B prevents unnecessary defense reactions in *Arabidopsis thaliana*.

P01-113: THE KEY REQUIREMENT FOR SUCCESSFUL PLANT CRYOPRESERVATION IS INDUCTION OF TOLERANCE TOWARDS DEHYDRATION STRESS

Panis B[1], Carpentier, S.C.[1] - Zhu, G-HV [1] - Dussert S [2] - Geuns J.M.C.[1] - Swennen R [1]*
[1] K.U.Leuven, Belgium
[2] IRD, GeneTrop, UMR DGPC, Montpellier, France),
**Corresponding author, e-mail: bart.panis@biw.kuleuven.be*

Plant germplasm stored in liquid nitrogen (-196°C) does not undergo cellular divisions. In addition, metabolic and most physical processes are stopped at this temperature. The most damaging event during cryopreservation is the irreversible injury caused by the formation of intracellular (or more precisely intraprotoplasmatic) ice crystals. The physical damage to the membrane is especially lethal because this results in the loss of its semi-permeability. The only way to prevent ice crystal formation at ultra-low temperatures without an extreme reduction in cellular water is through vitrification, i.e. non-crystalline solidification of water. The main requirement for a solution to vitrify is that it needs to be concentrated enough. Cryogenic strategies rely on air-drying, freeze dehydration, osmotic dehydration, addition of penetrating cryoprotective substances and adaptive metabolism (hardening) or combinations of these processes. They result in more concentrated intracellular solutes, most of them associated with cell volume reduction. Most hydrated tissues, however, do not withstand dehydration to moisture contents needed for vitrification (20-30%) due to solution and mechanical effects. The key for successful cryopreservation thus lies in the induction of tolerance to dehydration and not to the freezing itself.
In this study we examined physiological changes associated with an increase of cryopreservation ability in different banana cultivars. For this we analysed sugars, membrane composition (membrane lipids as well as sterols), water thermal behaviour, polyamines and the proteome of meristem cultures of banana cvs. with a differential response.

P01-114: DIFFERENTIAL GENE EXPRESSION ANALYSIS PROVIDES NEW INSIGHTS INTO THE MOLECULAR BASIS OF IRON DEFICIENCY STRESS RESPONSE IN CITRUS.

Forner-Giner, M.A.[1] - Llosa, M.J. [1] - Carrasco, J.L.[2] - Perez-Amador, M.A. [2] - Navarro, L [1] - Ancillo, G [1]
[1] Instituto Valenciano de Investigaciones Agrarias
[2] Instituto de Biologia Molecular y Celular de Plantas

Iron chlorosis is one of the major abiotic stresses affecting fruit trees and other crops in calcareous soils, which results in a decrease in growth and yield. Usual remediation strategies consist of iron amendments to soil, which is an expensive practice, or the use of tolerant cultivars, which are difficult to develop when not available. However these practices are expensive and sometimes difficult to apply.
To better understand the mechanisms underlying the associated physiopathy, and thus develop new strategies to overcome the problems resulting from iron deficiency, we have examined the differential gene expression induced by iron deficiency in the susceptible citrus rootstock *Poncirus trifolita* (L.) Raf. Identified genes are putatively involved in cell wall modification, in determining photosynthesis rate and chlorophyll content, and reducing oxidative stress. Additional studies on cell wall morphology, photosynthesis and chlorophyll content and peroxidase and catalase activities support their possible functions in the response to iron deficiency in a susceptible genotype, and the results are discussed.

P01-115: QUANTITATIVE ANALYSIS OF ROOT RESPONSE TO MULTIPLE PHYSICAL CONSTRAINTS: EFFECTS OF SOIL PORE STRUCTURE.

Valentine, T.A. - Binnie, K. - Squire, G.R. - Hawes, C. - Hallett, P.D. - Bengough, A.G.*
SCRI
**Corresponding author, e-mail: tvalen@scri.ac.uk,*

The impact of soil physical conditions on barley root elongation and gene expression was assessed by repacking soil cores at 20 combinations of water content and dry bulk densities, and by sampling intact soil (59 fields from across the east of Scotland). Field cores were equilibrated to a matric potential of -20kPa. Repacked core matric potential was measured using tensiometers and a psycrometer. Root elongation was measured after destructive harvest at 48h with maximum rates of 0.7mmh^{-1} and 0.63 mmh^{-1} in repacked and field cores respectively, compared with 1 mmh^{-1} in loosely packed sieved soil at optimum water content. In 50% of field cores root elongation was less than 50% of the maximum growth rate achieved suggesting soil physical conditions were limiting root elongation. 32% of variation in root growth in repacked cores was accounted for by dry bulk density variation (p=0.005), compared with 15% in field cores (p=0.001). In contrast, large air-filled pore volume accounted for 61% of the root growth variation in field cores (repacked ns.). This suggests different root growth behaviour in soil cores with structural pores verses uniform discontinuous pores. A complementary *invitro* model system based on *Arabidopsis*, confocal microscopy and the new image analysis techniques (PlantVis) has also shown differences in meristem behaviour depending on pore spatial structure. A high throughput method to assess gene expression of barley roots in this system will also be presented.

P01-116: SALINITY EFFECTS ON PHOTOSYNTHESIS PARAMETERS AND SECONDARY METABOLITE OF TWO LETTUCE VARIETIES

Mahmoudi, H.[1] - Kaddour, R.[1] - Huang, J.[2] - Hannoufa, A.[2] - Lachaâl, M.[1] - Ouerghi, Z.[1]
[1] Faculté des Sciences de Tunis
[1] Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre

Our study is interested to evaluate the effect of NaCl and Na_2SO_4 treatments on photosynthesis parameters and secondary metabolite of two lettuce varieties, Verte and Romaine. The experiments were carried out under greenhouse conditions. Plants of lettuce were acclimated for 7 days in hydroponic solution and supplemented with or without 100 mM NaCl or 77 mM Na_2SO_4, for 12 days. Greater concentrations of chlorophyll were found in Verte compared to Romaine, under both control and salinity treatment. Net photosynthetic rate was similar in both varieties with and without NaCl. This capacity was equally reduced for both varieties with Na_2SO_4 treatment. The stomatal conductance was strongly reduced by salts, especially under Na_2SO_4 treatment. In the absence of salt treatment, leaves of both varieties had similar total carotenoid levels. In presence of salts, ß-carotene, lutein, and total carotenoids were significantly higher in Verte. The carotenoid levels did not significantly change under either type of salt stress, in Romaine. For polyphenol, both lettuce varieties contained mainly phenolic acids with only a minor fraction of flavonoids, in the absence of salt treatment. Under salt treatments, an increase of total flavonoids was detected in Romaine compared to Verte.
The enhanced salt tolerance of Verte relative to Romaine is partly reflected in the differential accumulation of carotenoid and phenolic antioxidants. Verte accumulated higher levels of total carotenoids and mainly individual carotenoids. These changes in carotenoid contents of the two lettuce varieties cultivated under salinity treatments did not correlate with the expression profiles of *PSY, PDS* and *ε-CYC* genes that encode key enzymes in the carotenoid pathway.

P01-117: ROLE OF CD AND CU ON THE ANTIOXIDANT BEHAVIOUR OF BRACHYCHITON POPULNEUS (SCHOTT & ENDL.) R. BR. (STERCULIACEAE)

Baycu, G.[1] - Bakacak, S.A.[1] - Rognes, S.E.[2]*
[1] Istanbul University
[2] Oslo University
**Corresponding author, e-mail: gbaycu@gmail.com*

Brachychiton populneus (Bottle tree) occurs naturally in southern Australia. Young seedlings distribute resources to form a large tap-rooted tuber that has considerable starch and water reserves, allowing the tree to survive in the dry and hot periods of Mediterranean environment. *Brachychiton* is commonly cultivated and is hardy in a range of climates and soils. Oxidative stress is induced by a wide range of environmental factors including drought, heat and trace metal stresses. The aim of this study was to examine the antioxidant behaviour of this drought tolerant plant to trace metal toxicity. Plant seeds were collected from Cyprus Island, which has a typical Mediterranean climate. We had some difficulties during the germination and plant growth in hydroponic cultures was impossible. For this reason sand & perlite cultures were set up and plants were grown for several months in the phytotrone conditions. The seedlings were then treated with 50-150µM Cd and 500-1500µM Cu for 2-week to investigate the role of Cd and Cu on the activity of catalase (CAT), glutathione reductase (GR) and guaiacol peroxidase (GPOX) in the leaves and roots. The activity of CAT was diminished both by Cd and Cu toxicity in the plant organs. GR activities were reduced by both metals in the leaves except a slight increase in the low Cd treatment. No changes in GR activity were observed in the roots. Conversely, both Cd and Cu produced a raise in GPOX activity in the leaves. Whereas an increase was observed with low Cd and low Cu treatments in the roots, high Cd and high Cu reduced the activity. GPOX could be exerting a stronger antioxidant function. These results suggest that antioxidant behaviours may change with various trace metal concentrations in different plant organs.

P01-118: THE ROLE OF SALT (NACL) STRESS AND CALCIUM ON ION ACCUMULATION (MN, MO, ZN, CU, FE) IN SOYBEAN [GLYCINE MAX (L.) MERR.]

Cetin E[1] - Caglayan A[2] - Kiziler A R[3]*
[1] Istanbul University
[2] Bahcesehir Ataturk Highschool
[3] Namik Kemal University
**Corresponding author, e-mail: ergulc@istanbul.edu.tr*

In this study, the role of NaCl stress and Ca on the accumulation of Mn, Mo, Zn, Cu, Fe ions in the stem and leaves of *Glycine max* A-3935 was investigated. In the first part of the experiment carried out in hydroponic cultures, six different concentrations of NaCl (0, 20, 40, 60, 80, 100 mM) were applied. In the second part, two diffferent Ca concentrations (60+6, 80+6, 100+6, 60+9, 80+9,100+9 mM NaCl+Ca) were added.
The accumulation amounts of Mn, Mo, Zn, Cu and Fe ions (µg/g DW) in stem and leaves of the plants were measured by Atomic Absorption Spectrophotometer (AAS).
Along with the increased NaCl concentration in the hydroponic medium Mo, Zn and Cu ion accumulations were increased while Mn ion concentration was decreased in the stems. We have not found any significant decrease or increase in Fe ion accumulation. After the addition to Ca concentrations, Mo, Zn, Cu and Fe ion accumulations were decreased. However Mn ion accmulation was increased in the stems.
Along with the increased NaCl concentration in the hydroponic medium Mo, Cu and Fe ion accumulations decreased in the leaves. 8Whereas Mn and Zn ions were not significantly increased or decreased.
The effect of additional Ca concentrations caused a decrease in Zn and Cu ion accumulations. According to 100 mM NaCl concentration Fe ion accumulation was increased.

P01-119: COMBINED EFFECT OF MANGANESE AND UV-B RADIATION ON PHYSIOLOGICAL FEATURES IN HIGHBUSH BLUEBERRY CULTIVARS WITH CONTRASTING MN TOLERANCE

Rojas-Lillo, Y. - Reyes-Díaz, M, - Mora, M.L. - Alberdi, M.
Universidad de La Frontera

A Mn-tolerant blueberry cultivar (Brigitta) and a Mn-sensitive ones (Bluegold) were subjected in Hoagland solution under greenhouse conditions, to following treatments: 500 μM Mn as MnCl2 (Mn), 500 μM Mn + UV-B (Mn+UVB), +UV-B and control (-UV-B and 2 μM Mn). A typical daily summer cycle of UV-B radiation for 38º SL was simulated during 30 days. Maximum quantum yield (Fv/Fm), CO_2 assimilation, antioxidant capacity (AC) and total phenol (TP) contents were analyzed. Fv/Fm decreased in the treatments +UV-B up to 16 days of treatment in both cultivars ($P<0.01$), but no differences between cultivars were found. In Bluegold, CO_2 assimilation decreased (~30%) in +UV-B treatments, while in Brigitta a lower decrease (~15%) was found. AC of Brigitta augmented 68% ($P<0.01$) in the treatment with Mn and twice in the Mn+UV-B combined treatment with respect to the control, whereas in Bluegold a lower increase in both treatments was found. TF were strongly increased with UV-B in both cultivars in relation to the controls, being this increase higher (4-fold) in Bluegold than in Brigitta. It is concluded that the combined effect of Mn and UV-B affected the investigated parameters in both cultivars, being the UV-B the preponderant factor in the differences between treatments and cultivars.

Acknowledgments: Fondecyt 1080372 and PSD- 26 projects.

P01-120: DESICCATION TOLERANCE IN RUSTYBACK FERN (ASPLENIUM CETERACH L.): CHARACTERIZATION OF ANTIOXIDANT SYSTEMS

Zivkovic, S. - Momcilovic, I. - Ghalawenji, N. - Grubisic, D.*
Institute for Biological Research "Sinisa Stankovic"
**Corresponding author, e-mail: suzy@ibiss.bg.ac.rs*

Certain plant species, termed desiccation tolerant or resurrection plants have evolved the remarkable ability to withstand extreme dehydration and rapid rehydration of vegetative tissues without cell damage. These plants have well developed enzymatic and non-enzymatic defense systems and contain number of secondary products with antioxidant properties. *Asplenium ceterach* is a resurrection fern widespread in Western and Central Europe, including the Mediterranean region. Fronds of fern sporophyte contain unusually large amount of chlorogenic acid (CGA), phenolic compound known for its *antioxidant activity.* Results of our studies indicate that CGA as well as phenol oxidizing enzymes, peroxidases (PODs) and polyphenol oxidases (PPOs), may participate in the response of *A. ceterach* to dehydration and desiccation. Furthermore, we have investigated the contribution of superoxide dismutases (SODs) to antioxidant protection in rustyback fern. The expression and activity of antioxidant enzymes were analyzed at the protein level in the fronds of *in vitro* grown sporophytes as well as greenhouse cultivated ferns. Since the mechanisms underlying desiccation tolerance in resurrection ferns are not well known, the study on *A. ceterach* as a model-system may contribute to better understanding of this phenomenon.

P01-121: CHANGES IN THE TRANSCRIPTIONAL PROFILE OF THE GENES CODING FOR THE LIGHT HARVESTING COMPLEX PROTEINS UNDER CD STRESS IN POPLAR

Basa, B. - Solti, A. - Sárvári, E. - Tamás, L.
Eötvös Loránd University

Lhc proteins composing the peripheral antennae of photosystems in the thylakoids of higher plants are deeply involved in the stress responses. During the process of stress acclimation reorganization of the antenna complexes occurs. Therefore, we aimed to study the changes in the gene expression pattern of the whole *Lhc* gene family under Cd stress in poplar plants. *Populus jaquemontiana* var. glauca cv. Kopeczkii plants were grown in hydroponics up to four-leaf stage (mature leaves), and treated with 10 μM Cd through the roots for two weeks. To monitor the changes in the mRNA levels of the *Lhc* genes Real-Time PCR was chosen as the most sensitive and precise approach. RNA samples were collected from Cd-treated and control plants at different stages of development. To be able to quantify the responses different strategies for normalization of qPCR data were examined. The tested six reference genes showed alterations, especially in young leaves. An alternative strategy of normalization, the RiboGreen method proved to be more reliable than using internal reference genes.
Cd seemed to have a strong impact on the transcription level of *Lhc* family genes. Genes coding for the major LHC-s (*Lhca*1-4, *Lhcb*1-6) and also the less abundant *Lhca*5, showed similar expression pattern: slight decrease in mature leaves and strong reduction in leaves developed during the treatment. However, the transcription level of *Lhca*6 increased slightly in mature leaf samples and *Lhcb*7 showed higher expression level in each Cd treated samples compared to the corresponding controls. Preliminary proteomic studies showed similar changes in the amounts of apoproteins of the main Lhca-s referring to transcriptional control.

This work was supported by grant NN-74045 (OTKA).

P01-122: OPTIMIZING SOMATIC EMBRYOGENESIS IN PINE FOR CHARACTERIZATION OF CANDIDATE GENES INVOLVED IN PINEWOOD NEMATODE RESPONSE

Miguel, C.[1] - Oliveira, A.M.[2] - Marum, L.[2] - Simões, M.[2] - Milhinhos, A.[2]*
[1] ITQB
[2] IBET/ITQB-UNL
**Corresponding author, e-mail: cmiguel@itqb.unl.pt*

The pinewood nematode, *Bursaphelenchus xylophilus*, has a highly destructive capability towards conifers and represents an extremely serious threat to the Portuguese pine forests. The identification and characterization of genes which may be involved in the maritime pine response to this pathogen is therefore of utmost importance. Somatic embryogenesis (SE) is an essential step in the recovery of transgenic pine plants, and the availability of an efficient SE system is critical for the functional characterization of candidate genes involved in the response to biotic factors. The objective of the present work is to improve SE in pine for use in genetic transformation and validation of the role of candidate genes in the response to the pinewood nematode disease. The most widely used SE protocols in conifers require 2,4-D and BAP for initiation of embryogenic cultures. However, the overall efficiency of the SE process is still low. In this work we have studied the effect of brassinolide (BL) on the induction of SE in maritime pine in comparison to other plant growth regulators including 2,4-D. The percentage of embryogenic cell lines initiated from zygotic embryos was higher on initiation media containing BL. These embryogenic cell lines were afterwards used in maturation experiments under the same conditions as the lines initiated on 2,4-D containing media. However, after somatic embryo conversion and transfer to ex vitro conditions, the early growth of somatic embryo plants derived from embryogenic lines initiated on BL-containing medium was enhanced. Results will be presented and discussed. Acknowledgements: This work was supported by Autoridade Florestal Nacional through the project "Resposta Biotecnológica ao Problema do Nemátode da Madeira do Pinheiro".

P01-123: SUGAR MEDIATED ACCLIMATION: THE IMPORTANCE OF SUCROSE METABOLISM

Carpentier, S.[1] - Swennen R. [1] - Vertommen, A.[1] - Souza, M. [2] - Witters, E.[3] - Panis, B.[1]*

[1] *KULEUVEN*
[2] *WUR*
[3] *UA*
**Corresponding author, e-mail: sebastien.carpentier@biw.kuleuven.be*

Mulitiple shoot meristems are an exceptional model to study meristematic cells (Carpentier et al 2007) and proteomics is an efficeint way to characterize plants (Carpentier et al 2008a, 2008b). We have designed an *in vitro* experimental setup to study the role of sucrose in sugar mediated acclimation of meristems. It is a first step towards the systems biology of a meristem and the understanding of how it can survive severe abiotic stress. Using the 2D-DIGE proteomic approach and a meristem specific EST library, we describe the long term acclimation response of banana meristems and analyze the role of sucrose in acclimation. Sucrose synthase is the dominant enzyme for sucrose breakdown in meristem tissue which is most likely related to its lower energy consumption. For an efficient acclimation, metabolizing sucrose and respiration needs to be carefully balanced. Metabolizing sucrose is of paramount importance to survive but the uptake of sugar and its metabolism drives respiration which may result in limited oxygen levels. Our data point towards a reduced breakdown of sucrose and an induction of fermentation likely by a lack of oxygen.

P01-124: OXIDATIVE STRESS PRODUCED BY ALUMINIUM TOXICITY DECREASED BY CALCIUM IN HIGHBUSH BLUEBERRY

Reyes-Díaz, M. - Meriño, C. - Deppe, M. - Alberdi, M.
Universidad de La Frontera

Brigitta, Legacy and Bluegold highbush blueberry cultivars growing in hydroponic solution (Hoagland) were subjected to following treatments: control (Hoagland solution); 2.5 mM $CaSO_4$; 5 mM $CaSO_4$; 100 µM $AlCl_3$; 100 µM $AlCl_3$ + 2.5 mM $CaSO_4$; 100 µM AlCl3 + 5 mM $CaSO_4$ during 15 days. Thereafter, relative growth rate (RGR), lipid peroxidation (LP), antioxidant activity (AA) and superoxide dismutase (SOD) activity were performed. RGR in Brigitta and Bluegold decreased ~50% under aluminium (Al) treatment and with 5mM $CaSO_4$ with respect to the controls, whereas RGR of Legacy practically did not change. The addition of 2.5 and 5 mM $CaSO_4$ in presence of Al-stress ameliorated the negative effect on RGR in Bluegold, while in Brigitta it occurred at the highest $CaSO_4$ concentration. In roots, LP increased 3.5-fold with Al-stress in Brigitta and Bluegold with respect to the control, decreasing with $CaSO_4$ application. In roots and leaves, AA increased with Al and $CaSO_4$ treatments, especially in Brigitta. SOD activity also increased with $CaSO_4$ treatments, mainly in leaves. In conclusion, CaSO4 treatment could be a promissory tool in the amelioration of oxidative stress produced by Al toxicity in blueberry cultivars.
Acknowledgments: Fondecyt Nº 11080231 project, technical assistance and Berries San Luis, Lautaro, Chile.

P01-125: BIOHERBICIDE POTENTIAL OF MENTHA ROTUNDIFOLIA AND EUCALIPTUS GLOBULUS

Puig, C.G - Fernández, N. - Forján, R. - Garabatos, A. - Iglesias, L. - Álvarez-Iglesias, L. - Sánchez-Moreiras, A.M. - Reigosa, M.J. - Pedrol, N.*
University of Vigo
**Corresponding author, e-mail: cgpuig@uvigo.es*

In the search of new eco-friendly bioherbicides, the phytotoxic activity of water extracts of *Mentha rotundifolia* L. and *Eucaliptus globulus* Labill were tested on the model target species *Lactuca sativa* L. and *Agrostis stolonifera* L. *In vitro* phytotoxicity tests of 1:15 (dw/v) water extracts of mint and eucalyptus fresh biomass were carried out on the target species at 10, 25, 50 and 100 % (v/v) dilutions of the original extracts, using water and the pre-emergence herbicide metholachlor as negative and positive controls, respectively. Water extracts of both species at IC 80 obtained from dose-response curves were tested on pre-grown *L. sativa* young plants as post-emergence bioherbicides. Three weeks old lettuce plants were treated by spraying vs. watering during 6 days. Effects of plant extracts on the functioning of photosystem II were monitored by means of chlorophyll a fluorescence-image analysis, obtaining the parameters Y(II), Y(NPQ), Y(NO), qN, qL, ETR and Fv/Fm every 24 h. Both 50 and 100 % dilutions of *M. rotundifolia* and *E. globulus* water extracts resulted highly inhibitory of seed germination and radicle growth of lettuce and creeping bentgrass, being 100 % 1:15 water extracts even more phytotoxic than metholachlor at the recommended dose. Used in post-emergence, we obtained significant phytotoxic effects of mint and eucalyptus water extracts measured on chlorophyll fluorescence, mainly when applied by watering. From our results, water extracts of *M. rotundifolia* and *E. globulus* biomass are effective for being used as bioherbicides in organic agriculture, as well as potential sources of active compounds in the search of new agrochemicals for sustainable agriculture.

P01-126: TRANSCRIPTION FACTORS INVOLVED IN THE REGULATION OF THE SALT STRESS RESPONSIVE GENE OSRMC

Serra, T. [1] - Bogre, L.[2] - Oliveira, M.M. [1] - Saibo, N. [1]
[1] ITQB/IBET, Estação Agronómica Nacional
[2] RoyaHl olloway, University of London

Soil salinity imposes constrains on plant ability to grow and efficiently produce seeds. Rice is extremely sensitive to high salt concentrations, showing harmful effects such as leaf necrosis and photosynthesis impairment. To cope with environmental changes, plants need to coordinate the activation and/or repression of stress-responsive genes, mainly through transcription factors (TFs).The rice *Root Meander Curling* (*OsRMC*) gene was described as an apoplast protein involved in the repression of salt stress tolerance mechanisms, even though its expression is induced by salt stress. The main goal of this work was to identify and characterize novel TFs involved in the regulation of *OsRMC* gene expression. A yeast-one-hybrid system was used to screen a cDNA expression library enriched in salt stress responsive genes, allowing the identification of two ERF binding to the *OsRMC* promoter. These TFs were initially characterized regarding their gene expression pattern under several abiotic stress conditions. Our results showed that *EREBP1* was not significantly regulated at transcriptional level; therefore we are now conducting studies to investigate if the EREBP1 protein is regulated by post-translational modifications, such as phosphorylation, ubiquitination and/or SUMOylation. In contrast, the *EREBP2* gene expression was induced by salt, drought and cold stress conditions. The identified TFs are also being characterized regarding their *in vivo* localization, transcriptional activity and biological function.
We acknowledge funding from FCT through the PhD scholarship (SFRH/BD/31011/2006) and the projects POCI/BIA-BCM/56063/2004 and PTDC/BIA-BCM/099836/2008.

P01-127: ENJOYING PLANT ECOPHYSIOLOGY: WATER STRESS X COMPETITION, KEY OF SPECIES DISTRIBUTION ALONG AN ENVIRONMENTAL GRADIENT

Pedrol, N. - Reigosa, M.J.*
University of Vigo
**Corresponding author, e-mail: pedrol@uvigo.es*

Holcus lanatus L., *Koeleria glauca* (Schrader) DC. and *Dactylis glomerata* L. from the margins of the Ría of Vigo and Cíes Islands (NW Spain) were ecophysiologically characterized. This area offers very contrasted environmental conditions for plant life, caused by habitat diversity, a pronounced bioclimatic gradient of summer drought, and the more or less proximity to the

sea. We observed a differential distribution of species in the field. As initial hypotheses, we suggested the probable influence of water stress (caused by drought and/or salinity) and competition in such distribution. In order to test the effects of each environmental variable, we combined field observations with several greenhouse experiments of water stress and competition, with the analysis of different morphological, physiological and biochemical parameters, including stress markers (free proline and polyamines). Overall discussion revealed that the differential distribution of grass species is due to complex interactions among biotic and abiotic factors: *K. glauca* is not able to compete with *H. lanatus* and *D. glomerata* under its physiological optimum. But the increase of stress along bioclimatic gradient gradually buffers the effects of competition, so that all species can coexist in the driest coastal locations. Water deficit balances competitive abilities of the species. Finally, the presence of *K. glauca* seems to improve the adequation of the other two species to salinity through niche divergence and/or the establishment of positive interactions. Biotic and abiotic stress interactions are shown as sources of biodiversity and stability.

P01-128: EFFECT OF SHORT-TERM SALINIZATION AND DROUGHT ON THE RATE OF PLANT GROWTH AND WATER TRANSPORT IN SHOOTS AND ROOTS

*Budagovskaya, N.**
Institute of Plant Physiology
**Corresponding author, e-mail: postnabu@mail.ru*

Rapid (min) and slower (h) growth response reactions of leaves and stems of oat, barley, wheat, rice and buckwheat plants on increase and decrease in NaCl concentration and drought at the root zone have been studied using a highly sensitive method - laser interference auxanometry. Addition of NaCl in increased concentration to the root zone of plants caused a two phase response reaction of leaves: decrease and the following increase in their growth rate in each phase. Duration of the 1st phase was shorter than that of the 2nd. Growth rate of leaves was restored by the end of the 2nd phase (in few h after addition of NaCl). The 1st phase may be related to rapid adaptive reactions and changes in leaf turgor, the 2nd – to slower adaptive processes –*de novo* synthesis of protectors. Introduction of NaCl in high concentration caused stoppage in leaf and stem growth and shrinking of their tissues as result of dehydration. Reversal of water transport in roots under salinization has been demonstrated. Washing the roots of NaCl rapidly restored the turgor of leaves and increased their growth rate. Under drought conditions the growth rate of shoots decreased rapidly. Shrinking of leaf and stem tissues was observed after the stoppage of shoot growth under drought as well as at high level of salinization. The data obtained provide information on dynamics of response reactions of shoots and roots on increase and decrease of NaCl concentration, drought and watering.

P01-129: DEPTH-DEPENDENT RESPONSE TO LIGHT OF THE SEAGRASS POSIDONIA OCEANICA (L.) DELILE

Ortolà, A.G. - Moliner, M. - Rodilla, M. - Ballester, I.
Universitat Politècnica de València

Posidonia oceanica is a seagrass endemic to the Mediterranean Sea which is considered highly sensitive to reductions in light availability, where small decreases can cause significant declines in growth and depth distribution. The objetive of this study was to characterize in *Posidonia oceanica* plants the effect of light attenuation depth- dependent on whole plant carbon distribution, using the activity of sucrose synthase (EC 2.4.1.13) as a general indicator of the degree of metabolic activity. Posidonia plants were collected at the Denia coast (Alacant, Spain) in depths of 3, 6 and 9 metres. The plants were divided into leaves, rhizomes and roots for analysis. The increment in light attenuation within the water column induces an increase in leaf length, a reduction in shoot density and chlorophyll content, and a change in the distribution pattern of carbohydrates. Soluble carbohydrates and sucrose accumulate in below-ground tissues and their levels decreases with the light attenuation. Starch is accumulated in the leaves of plants growing in the deeper meadow. Sucrose synthase (SS) activity decreases in leaves under reduced light conditions but does not change in below-ground tissues. The results presented show that under sub-optimal light conditions *Posidonia oceanica* adjusts their carbon-budget to maintain growth and metabolic processes.

P01-130: SULPHUR METABOLITES OF DURUM WHEAT SEEDLINGS GROWN UNDER SALT STRESS

Nacca, F. - Parisi, D. - Massaro, G. - Annunziata, M.G. - Carillo, P. Fuggi, A.*
Dipartimento di Scienze della Vita, Seconda Università di Napoli, Caserta
**Corresponding author, e-mail: amodio.fuggi@unina2.it*

Keywords: Glutathione, sulphur nutrition, salt stress, Triticum durum Desf. cv. Ofanto.
Salt stress can strongly limit growth and productivity of crop plants, mainly because of the oxidative stress caused by the increased release of Reactive Oxygen Species (ROS). To prevent or repair the oxidative damages, plant cells activate a complex defence system involving enzymes and low molecular compounds like glutathione, the major thiol metabolite occurring in plants. Glutathione has key roles in sulphur metabolism, being involved in the maintenance of the cellular redox state, detoxification of xenobiotics, synthesis of phytochelatins, as well as in regulation of sulphur allocation. Even if its role in the control of sulphate assimilation has been demonstrated, minor information are available in relation with salt stress. Here the effect of sulphur nutrition on the levels of glutathione and its precursors has been determined in leaves and roots of durum wheat (Triticum durum Desf. cv ofanto) grown in hydroponics in Hoagland medium containing different sulphate concentrations and kept in a growth chamber under controlled conditions. Sulphur compounds were determined as bimane-derivatives, separated in reverse phase HPLC and quantified fluorimetrically. The findings evidenced that, salt treatment decreased the tissue levels of glutathione and cysteine, in the leaves and increased their content in the roots.
Financial support was obtained by “Seconda Università degli Studi di Napoli”, “Ministero dell’Università e della Ricerca” (PRIN 2006077008_005; 2008S9T3KK_003), “Regione Campania”.

P01-131: BIOCHEMICAL MECHANISM OF METHYURE EFFECT IN CORN SEEDLING ROOTS UNDER SALT STRESS CONDITIONS

Palladina, T. – Ribchenko, I. – Konturska, O.
M.G. Kholodny Institute of Botany National Academy of Science of Ukraine

Salinity is the hard negative factor for plant organisms prevented agriculture in many regions. This actual problem can be resolved by plant salt tolerance increasing. Its main stream is consisted in salt tolerant transgenic forms creation. However there is another way with adaptogenic preparations using. Early we have demonstrated preference of nontoxic synthetic preparation Methyure permitted to breed corn on salinized fields. In experiments on corn seedlings exposed at 0.1M NaCl presence is was find that seed soaking in 10-7M Methyure prevented salt stress display. This treatment normalized peroxidation process and homeostasis. Besides Methyure influenced transport processes in root cell membranes by lipid content stabilization which restricted their permeability and by activation of H+-pumps and Na+-H+-antiporters in plasmalema and tonoplast. Thus Methyure adaptation effect under salt stress conditions can be explained mainly by Na+ removing from cytoplasm.

P02 VEGETATIVE DEVELOPMENT

P02-001: EVALUATION OF SOME SUBSTRATES AND FERTILIZATION IN CRANBERRY (VACCINIUM MACROCARPON AIT.) GROWING.

Krzewinska, D. - Borkowska, B. - Treder, W. - Tryngiel-Gac, A.*
Research Institute of Pomology and Floriculture, Skierniewice, Poland
Corresponding author e-mail: Danuta.Krzewinska@insad.pl

The aim of the study, performed in 2008-2009, was to determine the effect of some substrates, fertilization and mycorrhizal fungi, on growth of cranberry cv. 'Pilgrim.' There were five soil treatments (mineral soil, and mineral soil amendment by: peat, milled pine bark, compost, brown coal); and three ways of fertilization (none fertilizer, low realized fertilizer, liquid fertilizer). Before planting, one-half of plants were inoculated by mycorrhizal fungi (ERM). Vegetative growth of plants, photochemical activity and chlorophyll status (index SPAD) in leaves, also pH and EC value in substrates were evaluated.
It was found that amendment of organic matter effected more than mycorrhization and fertilization on evaluated features. Vegetative growth and photochemical activity (ETR, Yield, Fv/Fm) were significantly reduced in compost substrate and were increased in peat substrate. Index SPAD value was higher when brown coal and peat were applied. Different fertilizer treatments effected vegetative growth and photochemical activity. Low realized fertilizer stimulated number of runners, and in some cases EC, index SPAD value and photochemical activity, while liquid fertilizer induced mean length of one runner.
Mycorrhizal fungi decreased photochemical activity, increased index SPAD value (in 2009), and not induced on other features.
The research was financed by the Polish Ministry of Science & Higher Education within project PBW-0364/B/P01/2007/33

P02-002: ECTOPIC EXPRESSION OF A RICE TCP TRANSCRIPTION FACTOR MODIFIED LEAF GROWTH AND DEVELOPMENT IN TRANSGENIC ARABIDOPSIS

Yoon, I. - Lee, IS. - Lee, YN. - Kim, DY. - Kim, DH. - Byun, MO.*
National Academy of Agricultural Sciences
**Corresponding author e-mail: yooninsun@hotmail.com*

Tillering is an important agronomic trait for grain production of rice. Microarray analysis revealed that many TCP genes are highly up-regulated in the meristemic region of rice high tillering mutant d10. TCP family transcription factors are known as important regulators of plant cell division and organ development. OsTCP11 is a nuclear localized protein. In situ hybridization analysis revealed that OsTCP11 gene is highly expressed in shoot apical meristem, leaf axile and crown root primordia, implicating that its function is related to meristemic and/or cell division activity. Ectopic expression of TCP11 in transgenic Arabidopsis under the control of 35S promoter displayed markedly narrow leaves and elongated hypocotyls compared to wild type, whereas root or leaf length was not significantly changed. We found that cell size was increased and irregular cell arrangement pattern was observed in the leaves of transgenic Arabidopsis. Gene expression analysis with microarray and RT-PCR revealed that genes related to cell expansion such as xylogucan endotransglucosylase and expansin-like genes are up-regulated in the transgenic Arabidopsis, whereas cell cycle-related genes was not significantly affected. This implicates that TCP11 may regulate centrolateral axis development of leaf by control of cell expansion. Thiswork was supported by the grants from RDA, Republic of Korea.

P02-003: ACCELERATING BREEDING FOR BIOMASS YIELD IN SHORT ROTATION COPPICE WILLOW BY EXPLOITING KNOWLEDGE OF SHOOT DEVELOPMENT IN ARABIDOPSIS.

Ward, S.[1] - Salmon, J.[2] - Hanley, S.[2] - Karp, A.[2] - Leyser, O.[1]*
[1] University of York
[2] Rothamsted Research
**Corresponding author e-mail: spw6@york.ac.uk*

Willows are amongst the most advanced biomass crops in temperate regions because of their potential for high yields in short time periods, ease of vegetative production, broad genetic base and ability to resprout after multiple harvests. Exploitation of these inherent characteristics in a production system is integral to the way in which willows have been developed as biomass crops. In contrast to willow, *Arabidopsis* is the model plant in which a substantial body of knowledge exists on the regulation of axillary bud formation and activity, along with clear evidence of conservation of these regulatory mechanisms across higher plants. Limited studies have shown that resprouting, and thus coppicing response in SRC willow is due entirely to release of pre-existing buds through repression of apical dominance. Robust yield QTL have already been identified at Rothamsted Research as part of a Defra-funded BEGIN project (A. Karp and S. Hanley). Our research has shown that *MAX* genes, which affect shoot branching in *Arabidopsis*, map to willow yield QTL which co-associate with stem diameter and height. Key allelic differences in these *Salix MAX* genes are being tested through transformation of their corresponding *Arabidopsis max* mutant and analysis of degree of rescue of the highly-branched phenotype. We will present an update on our research into whether members of the *MAX* family are regulating bud behaviour in coppicing response of SRC willows; whether bud behaviour and bud number are under separate control in coppicing response; and physiological studies of regulation of axillary bud outgrowth in SRC willow.

P02-004: TOLERANCE OF POPULUS SP. TO HERBICIDES. A PHYSIOLOGICAL AND AGRONOMICAL VIEW OF THE CHEMICAL WEED CONTROL MANAGEMENT

Iván, J. - Lasa, B. - Lecumberri, J. - Irigoyen, I. - Muro, J. - Moran, JF. - Aparicio-Tejo, P.*
Public University of Navarra (UPNa)
**Corresponding author e-mail: ivan.jauregui@unavarra.es*

Weed control in short rotation forestry of Populus sp. plantations is an essential practice to assure the profitability of the crop. The allowed herbicides for populous cultivation in Spain are oxyfluorfen, ammonium gluphosinate and glyphosate. The objective of the presente study is to evaluate the response to these three herbicides by poplar clone I-214. We have grown poplar clone I-214 in greenhouse under drip irrigation for 4 months in 35 L pots. There were three different group tests. One with oxyfluorfen sprayed after planting. A second with gluphosinate under three dosages: recommended dose (5 L ha-1), half, and double of recommended dose), which were sprayed on three developmental periods (1, 2 and 3 months after planting). A third experimental group included plants sprayed with glyphosate at 2.5 L ha-1 (recommended dose) for the three mentioned developmental stages. Thereafter, we analysed death and growth parameters, stem fresh weight, and leaf area. In the gluphosinate assay, we also measured leaf GS activity and ammonium contents. For every treatment and parameter 4 replicates were obtained.

The clone did not show toxicity to the oxyfluorfen treatment. However, the clone exhibited low tolerance to gluphosinate and glyphosate applications, as indicated by differences in growth parameters which were dependent on both dose and application stage. After the second gluphosinate application, GS activity was less affected and the leaf ammonium content showed a positive correlation to the plant death. Therefore, we conclude that without the suitable protection of poplar, post-emergency herbicides application is not recommended.

P02-005: AERENCHYMA, A REMARKABLE TISSUE IN BROMELIADS

Croonenborghs, S. - De Proft, M.P.*
KULeuven
**Corresponding author e-mail: sarah.croonenborghs@biw.kuleuven.be*

The bromeliad family comprises more than 2900 species in 56 genera which inhabit various neotropical sites. These genera are divided into 3 subfamilies: Pitcairnioideae, Bromelioideae and Tillandsioideae. They all exhibit charachteristic morphological features which allow them to cope with diverse types of environmental stress which translates into the colonisation of diverse habitats. A C3 *Guzmania* hybrid (Tillandsioidea) forms the subject of this study. The ancestors of this commercial hybrid, loved for its striking inflorescence, reside in Central and South America and are shade loving epiphytes. In this study the macromorphological growth is monitored in a controlled greenhouse environment for a period of one year. As plants get older, a display of higher biomass and plant water tank volume is linked with clear changes in micromorphological leaf traits. More specifically, an extended network of stellate mesophyll cells arises, forming air channels throughout the length of the leaf. This aerenchyma, which is characteristic for bromeliads, shows a well-organised structure that is clearly distinctive of spongy parenchyma and of aerenchyma found in water plants. Our hypothesis is that the aerenchyma plays a crucial role in the leaf gas exchange and is formed as an answer to a need for better gas diffusion of the larger leaves formed by the fully matured plants.

P02-006: A NEW CLASS OF PROTEIN WITH TANDEM REPEATED SEQUENCES, AND THEIR FUNCTION AS STORAGE PROTEIN

Labrador, E.*- Albornos, L. - Martin, I. - Dopico, B.
Universidad de Salamanca, CIALE, Spain
*Corresponding author e-mail: labrador@usal.es

We study two chickpea ST proteins encoded by CanST-1 and CanST-2 clones, which were isolated from a cDNA library constructed from the mRNA of Cicer arietinum epicotyls. The ST-1 and ST-2 proteins have molecular mass of 36 and 28.5 kDa, respectively, and a peptide signal in their N-terminal end. A feature of these proteins is the presence of a sequence of 25 or 26 amino acids repeated several times, including the hexapeptide EFEPRP or DFEPRP, for ST-1 or ST-2 respectively. These proteins are limited to a group of taxonomic families in the plant kingdom, appearing only in six families of dicotyledoneous plants, mainly in the Fabaceae and Esteraceae families, 50% of the genus where these sequences were founded belonging to the Fabaceae. The absence of ST proteins in plants such as Arabidopsis thaliana, made this specie a valuable tool to study their function. After the construction of transgenic plants of A. thaliana expressing the chickpea st-1 and st-2 ORFs, we established that ST proteins have a double sub-cellular location, both in cell wall and in the cytoplasm.Our studies of immunolocalization of the protein ST-1 and the pattern of transcription of the corresponding gene throughout the development of the seed, as well as in the cotyledons during seed imbibition and germination, led us to postulate a function as a storage protein, preformed in the seed. The immunolocalization in seedling also point out a role as vegetative storage protein. Other functions for the ST proteins could not be excluded.

P02-007: GENETIC AND MOLECULAR CHARACTERIZATION OF ARABIDOPSIS GENES ENCODING MITOCHONDRIAL TRANSCRIPTION TERMINATION FACTORS (MTERFS)

Robles, P. - Micol, JL. - Quesada Pérez, V.*
Universidad Miguel Hernández, Elche, Alicante, Spain
**Corresponding author e-mail: probles@umh.es*

In Metazoa and plants it has been recently identified a wide and complex protein family named the mitochondrial transcription termination factor (mTERF) family which, in vertebrates, is constituted by 4 subfamilies (MTERF1-MTERF4). The mTERF proteins are encoded by nuclear genes and those hitherto characterized are involved in the regulation of transcription initiation and/or termination of the mitochondrial genome. Recent experimental results obtained in mice demonstrate that some *mTERF* genes are essential for life since loss of their function proves lethal very early in development. The information currently available about the function of these genes in plant development is scarce, although new findings demonstrate that some of them are important for chloroplast and mitochondria homeostasis. We have performed a genome-wide *in silico* analysis of the mTERF-transcription factor gene family in plants, focusing on *Arabidopsis thaliana* and rice. We are conducting an extensive functional analysis of the mTERF proteins in Arabidopsis by characterizing T-DNA tagged mutant alleles of different *mTERF* genes at the genetic, phenotypic and molecular levels and already identified several loss-of-function mutants displaying developmental phenotypes. We have intercrossed and crossed the *mTERF* mutants to others affected in organelle transcription in order to establish genetic interactions. The identification and characterization of the double mutants is in progress. We will present further advances on the study of the *mTERF* mutants that will contribute to elucidate the function that the *mTERF* gene family plays in plant development.

P02-008: A SCREENING OF T-DNA MUTANT ALLELES OF GENES CODING PROTEINS INVOLVED IN THE FLUX OF GENETIC INFORMATION IN THE ARABIDOPSIS CHLOROPLASTS

Quesada Pérez, V. – Micol, JL. – Robles, P.*
Universidad Miguel Hernández, Elche, Alicante, Spain
**Corresponding author e-mail: vquesada@umh.es*

The chloroplast genome of *Arabidopsis thaliana* was completely sequenced more than a decade ago, its size is 154.5 kb and comprises 133 genes, 88 and 45 encoding for proteins and RNAs, respectively. The expression of the chloroplast and nuclear genomes has to be carefully coordinated in order to fulfill the metabolic requirements of the plant cells and establish the correct developmental program of this organelle. Although bioinformatics predictions have estimated that the number of different proteins contained in the chloroplasts ranges from 2000 to 3000, there are not so many putatively involved in the control of transcription and/or translation. Using a reverse genetics strategy, we are trying to identify new genetic functions, that are nucleus-encoded and chloroplast-localized, involved in the flux of genetic information inside this organelle. For this purpose, we are screening insertional T-DNA alleles for 75 nuclear genes coding chloroplast proteins, from several publicly available knock-out collections aimed to find loss-of-function mutations causing visible and abnormal developmental phenotypes. To date we have identified more than twenty mutants, most of them not previously isolated or described, whose genetic and phenotypic characterization is in progress. We will report in the meeting the preliminary results obtained from our screening which, when finished, will shed new light on chloroplast function.

P02-009: NITRIC OXIDE (NO) INTERACTION WITH THE GAS PATHWAY IN THE REGULATION OF HYPOCOTYL ELONGATION IN ARABIDOPSIS SEEDLINGS

Albertos Arranz, P. - Fernández-Marcos, M. - Curto-Prieto, M. - Sanz, L. - Lorenzo, O.*
Universidad de Salamanca. Departamento de Fisiología Vegetal. Centro Hispano Luso de Investigaciones Agrarias.
**Corresponding author e-mail: paa@usal.es*

Nitric oxide (NO) is a gaseous free radical playing a role in different plant developmental and stress responsessuch as seed germination, root organogenesis, etiolation, plant defense and programmed cell death, among others. It has also previously been reported that NO is able to prevent hypocotyl elongation during dark growth in Arabidopsis seedlings and lettuce. It remains, however, to be determined at which level NO regulates this process and which target genes are essential for the individual NO response. To uncover the role of NO during the inhibition of hypocotyl elongation in etiolation responses, a genetic screening using an Arabidopsis EMS-mutant collection has been performed. Rougthly, 67 putative mutants have been isolated from 35.000 EMS-mutagenized M2 Arabidopsis plants belonging to 32 M1 families. Plants were grown in the presence of NO donors under dark conditions and scored for their hypocotyl length. Preliminary data of the phenotypical analysis and molecular screening for point mutations of interest will be presented. In addition and using a different approach, we will describe how NO could be interacting with the gibberellins (GAs) pathway in the regulation of hypocotyl elongation in etiolated seedlings. NO-treated seedlings induce the expression of DELLA repressor genes and interestingly, quadruple *della* mutant (impaired in GA signalling) shows higher levels of insensitivily to the NO-mediated inhibition of hypocotyl elongation in dark grown plants. Taken together, these findings suggest that NO could be playing a main role in GAs regulation of hypocotyl growth.

P02-010: OVER-EXPRESSION OF GID1, ENCODING A GIBBERELLIN RECEPTOR, MODIFIES THE DETERMINATE PHENOTYPE IN TOMATO

Carrera Bergua, E. - Ahuir, A. - Ruiz-Rivero, O. - Chamarro, J. - García-Martínez, JL.*
Instituto de Biología Molecular y Celular de Plantas (IBMCP), UPV-CSIC
**Corresponding author e-mail: ecarrera@ibmcp.upv.es*

Fruit-set and growth in tomato depend on gibberellins (GA) and over-expression of GA20ox, encoding an enzyme of GA biosynthesis, induces limited parthenocarpic capacity of unpollinated ovaries. We wanted to test whether over-expression of OsGID1, a gene from rice coding for a GA receptor, would also induce parthenocarpy. With that purpose, we isolated seven homozygous 35S:GID1 lines of tomato cv MicroTom, all of them expressing the transgene. Total fruit yield and number of seeds per fruit were higher in some transgenic plants. However, parthenocarpic capacity of unpollinated ovaries was not induced in any of the lines. Some of them also presented advanced germination compared to control plants, displayed flowering delay (they had more leaves before the first truss), and were taller because the internodes developed immediately before flowering were longer, as well as the shoot grown from the axillary bud in the upper leaf. MicroTom has a determinate phenotype due to the presence of the *self-pruning (sp)* mutation. Interestingly, *GID1* over-expression also affected the determinate phenotype because the upper axillary shoot produced more leaves and consequently was more longer and indeterminate than in control plants. Quantification of transcript levels of the *SELF-PRUNING* gene family (*Sp, Sp2I, Sp3D, Sp5G, Sp6A* and *Sp9D*) showed that while expression of *Sp, Sp2I* and *Sp6A* was not affected, that of *Sp5G* was upregulated and those of *Sp3D* and *Sp9D* was downregulated in *GID1* overexpressor lines. This indicates the existence of interaction between the GA-signal transduction pathway and some Sp affecting the expression of determinate phenotype in tomato.

P02-011: BRASSINOSTEROIDS ACT UPSTREAM OF TMM AND TTG/BHLH/MYB/GL2 TO PROMOTE STOMATA FORMATION IN THE ARABIDOPSIS HYPOCOTYL

Fuentes, S. - Serna, L.*
Universidad de Castilla-La Mancha
**Corresponding author e-mail: sonia.fuentes@uclm.es*

Stomata are an excellent model system for examining the mechanisms that regulate cell fate determination and pattern formation. Here, we show that brassinosteroids both positively regulate stomatal formation in the hypocotyl and accelerate their development. Hormone tests and reporter gene studies show that brassinosteroids act upstream of the membrane receptor *TOO MANY MOUTHS* (*TMM*) and of the transcriptional factors *CAPRICE* (*CPC*) and *GLABRA2 (GL2).* However, in spite of that the brassinosteroid receptor *BRASSINOSTEROID INSENSITIVE1 (BRI1)* controls stomatal production and pattern in the cotyledon, the steroid hormones seem to play no role in this plant organ. A model is proposed highlighting the differences between the genetic control of stomatal development in these two organs.
This work is supported by grants from both the Communities Council of Castilla-La Mancha (PCI08-0041-1136) and the Ministry of Education and Culture of Spain (BIO2008-02149).

P02-012: PHYTOHORMONES IN DIFFERENT ORGANS OF EQUISETUM ARVENSE L. DURING THEIR GROWTH.

Vedenicheva, N. - Vasjuk, V. - Voytenko, L. - Musatenko, L.
N.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine

Phytohormones studies in cryptogamous plant are necessary for more deep understanding of origin and evolution of plant hormonal regulation system. We paid attention to vascular cryptogamous plant because unlike fungi and algae their phytohormones system is investigated weakly and connection with growth and development is unknown. The purpose of this study was to clarify the changes of endogenous hormones content in organs of *Equisetum arvens*e L. at different developmental stages. Low content of cytokinins (zeatin (Z), zeatinriboside, isopentenyladenin (IP), isopentenyladenosine (IPA) and zeatinglucoside) was detected in risomes and leaf-like branches of young plants. Relatively high level of Z and IPA was present in their vegetative stems. Cytokinins levels decreased in adult plants excepting risome, where high levels of iPA and IP were detected. High activity of gibberellin-like substances was determined in the top internodes and leaf-like branches of adult plants. ABA and IAA content was the highest in risome. Plants development was accompanied by decrease in both ABA and IAA content in risome whereas free and bound IAA accumulation was shown in leaf-like branches and vegetative stems. Possible role of phytohormones in regulation of growth and development of *E. arvense* will be discussed.

P02-013: EFFECT OF NACL ON THE GERMINATION AND SEEDLING GROWTH OF MEDITERRANEAN HARTWORT

Liopa-Tsakalidi, A. – Barouchas, P. - Panagiotopoulos, L.
Department of Mechanical & Water Resources Engineering,TEI Mesolonghiou

Tordylium apulum L. (Apiaceae), also called Mediterranean Hartwort, is an annual herb and delicious wild green. This investigation was performed in order to study the effect of NaCl concentration (0, 40, 60 and 80 mM NaCl) on seed germination and on seedling growth of Mediterranean Hartwort, under greenhouse conditions. The seeds were sown using an automatic

seeder into cell-plug-trays filled with soil, and were covered with vermiculite. The monitoring of seed germination and seedling growth was performed in regular time intervals from the placement of the seeds in the cell-plug-trays up to the day where the seeds were not germinating anymore. The seed germination percentage of Mediterranean Hartwort in all NaCl concentrations and in control was low. The germination percentage of 40 mM NaCl (12%) and the germination velocity index (0,52) were higher throughout the experiment than those of control and of the 60, and 80mM NaCl concentrations, however the difference was not statistically significant. The root, the hypocotyls, the cotyledons, the petiole of leaf No1 and the seedling growth was inhibited by all NaCl concentrations. At the 47th day after the seeding, the 40 mM NaCl concentration solutions increased the stem of seedlings and did not affect the leaf blade of leaf No1.

P02-014: INVESTIGATING THE ROLES OF KRP1 AND KRP3 IN SHOOT APICAL MERISTEM FORMATION AND LEAF MORPHOGENESIS

*Jun, S.E.[1] - Ha, C.M.[2] - Cho, K.H.[1] - Yi,Y.B.[1] - Okushima, Y.[3] - Nam, J.[1] - Umeda, N.[3] - Kim, G.T.[1]**
[1] Department of Molecular Biotechnology, Dong-A University
[2] Plant Gene Expression Center, USDA/UC Berkeley
[3] Nara Institute of Science and Technology
**Corresponding author e-mail: kimgt@donga.ac.kr*

Cell division is strictly regulated for proper development and appropriate shape during plant development. Recent study of Kip-related proteins (KRPs), which are inhibitor of cyclin-dependent kinase, indicated that negative regulation of cell division plays an important role in plant morphogenesis. To investigate how cell division affects the architecture of shoot apical meristem (SAM) and leaf organ, we have characterized transgenic plants overexpressing *KRP1* and *KRP3* genes which are highly expressed in the vicinity of SAM and leaves. As a result, we have observed reduced sized leaves with serration and reduced sized SAM in KRP overexpressing transgenic plants. In addition, overexpression of KRP1 and KRP3 affected the structural of SAM by the reduction of cell number and the increase of cell size. Our results suggest that KRP1 and KRP3 might be key regulators of cell division in SAM and leaf development.

P02-015: RELATIONSHIPS BETWEEN XYLEM ANATOMY, ROOT HYDRAULIC CONDUCTIVITY, LEAF/ROOT RATIO AND TRANSPIRATION IN CITRUS TREES ON DIFFERENT ROOTSTOCKS

Rodríguez-Gamir, J. - Intrigliolo, DS. - Primo-Millo, E. - Forner-Giner, MA.*
Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain
**Corresponding author e-mail: juanrodriguezgamir@hotmail.com*

The aim of the study was to determine the extent in which leaf and whole plant transpiration (Tp) were influenced by root hydraulic conductance (K_r), leaf to root ratio and leaf mass. Also, the relationships between the anatomic characteristics of roots and K_r were investigated. To this end, 9-month-old seedlings of the citrus rootstocks Cleopatra mandarin (CM), *Poncirus trifoliata* (PT), and their hybrids Forner-Alcaide no 5 (FA-5) and Forner-Alcaide no 13 (FA-13) and 15-month-old trees of Valencia orange budded on these four rootstocks were tested. The hybrid FA-13 and PT had higher values of K_r and leaf transpiration rates (E) than FA-5 and CM. There was a positive curvilinear correlation between E and K_r. Furthermore, E levels in the different types of plants decreased with increased leaf/root (L/R) ratios. Pruning of the roots and defoliation confirmed that transpiration rates were strongly influenced by the L/R ratio. However, variations in E because of differences in L/R ratios were less pronounced in trees budded on FA-13 and PT than on the other two rootstocks. In addition, there was a positive correlation between Tp and leaf biomass, although differences between rootstocks may be attributed to differences in K_r. The average lumen diameter of xylem vessel was greater in rootstocks with high K_r. Size of epidermal and hypodermal cells of fibrous roots may also restrict K_r.

P02-017: DUAL REGULATION OF EXPRESSION OF ETTIN GENE BY ASYMMETRIC LEAVES2 FOR ESTABLISHMENT OF THE LEAF POLARITY

Machida, Y.[1] - Iwasaki, M.[2] - Iwakawa, H.[2] – Machida, C.[2]*
[1]Division of Biological Science, Grad. Sch. Sci., Nagoya University
[2]Plant Res. Center, Chubu University
**Corresponding author e-mail: yas@bio.nagoya-u.ac.jp*

ASYMMETRIC LEAVES2 (AS2) gene is one of key regulators for leaf morphogenesis along three axes of Arabidopsis leaves: the proximal-distal, the medio-lateral, and the adaxial-abaxial axes because mutations in this gene cause formation of leaves with averrant morphology in all axes (1). Our intesive analysis of gene expression have demonstrated that AS2, its interacting factor AS1 and other factors such as HDACs negatively control expression of small numbers of genes including class 1 KNOX genes and abaxial-identity genes such as *ETTIN* (*ETT*) (2, 3). Recently, we have also shown that ectopically expressed class 1 *KNOX* genes are involved in limited morphological abnormalities in as2 leaves but not all *as2* phenotypes (4). We here report that *ETT* and *ARF4* are responsible for the asymmetric formation of leaf lobes, the abaxialization of leaves and the increased ability of shoot regeneration of as2 leaves. In addition, AS2 and AS1 control expression of *ETT* by two distinct mechanisms. We will discuss relevance of the dual regulation of *ETT* expression to the leaf development.

1. Semiarti et al., Development 128: 1771 (2001)
2. Ueno et al., Plant Cell 19: 2855 (2007)
3. Iwakawa et al., Plant J. 51: 173 (2007)
4. Ikezaki et al., Plant J. 61: 70 (2010)

P02-018: UNCOVERING NOVEL GENE FUNCTIONS INVOLVED IN LEAF DEVELOPMENT

Candela Antón, H. - Casanova-Sáez, R. - Muñoz-Nortes, T. - Martínez-Asperilla, A. - Micol, J.L.*
División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche (Alicante), Spain
**Corresponding author e-mail: hcandela@umh.es*

The leaf of *Arabidopsis thaliana* comprises a limited number of differentiated cell types and hence is an ideal model for dissecting plant developmental processes, such as vasculature patterning or the establishment and maintenance of abaxial-adaxial polarity. We are following a two-pronged strategy to molecularly identify genes important for leaf development.
On the one hand, we are assigning a set of 28 viable leaf mutations to genomic intervals between 100 and 200 kb, which we plan to sequence using next-generation technologies to identify the causal mutations. On the other hand, we are systematically using the so-called cell autonomy (CAUT) lines to induce leaf sectors for embryo-lethal mutations, in an attempt to identify developmental functions that have eluded previous screens, which have mainly focused on viable leaf mutants.

P02-019: INCURVATA13 UNCOVERS A ROLE FOR THE REGULATION OF THE SCF COMPLEX DURING VEIN SPECIFICATION IN ARABIDOPSIS LEAVES

Esteve-Bruna, D. - Pérez-Pérez, J.M. - Micol, J.L.
División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche (Alicante), Spain

To build an almost flat wild-type organ, the growth of the different tissue layers must be coordinated within an expanding leaf. Mutations affecting the genes involved in such process should produce leaves deviating from flatness. We aimed to test this

hypothesis by studying a large collection of viable mutations that visibly disrupt the shape of *Arabidopsis thaliana* leaves. We focused on the *incurvata (icu)* mutants, which exhibit hyponasty (leaf incurvature). We positionally cloned the *incurvata13 (icu13)* mutation and found it to be an allele of *AUXIN RESISTANT6 (AXR6)*, which encodes a core subunit of the SCF complex of E3 ubiquitin ligases.
The *icu1*3 mutation causes mRNA missplicing and is predicted to truncate the *AXR6* protein. Both the *icu13* and *eta1* (*enhancer of tir1-1 auxin resistance*) alleles of AXR6 exhibit hyponastic leaves and a simple leaf venation pattern, and are defective in auxin signaling. To understand the role of AXR6 during leaf growth and vein patterning, we are analyzing the genetic interactions of *icu13* and *eta1* with available mutations affecting other components of the SCF pathway.

P02-020: GENETIC AND MOLECULAR ANALYSIS OF THE ARABIDOPSIS MAS GENES

Sánchez-García, A. - Jover-Gil, S. - Aguilera, V. - Qunito, P. - Micol-Ponce, R. - Micol, J.L. - Ponce, M.R.
División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche (Alicante), Spain

The Arabidopsis ARGONAUTE1 (AGO1) protein is the core component of the RNA-induced silencing complex (RISC) that mediates the regulation of gene expression by microRNAs (miRNAs). The *ago1* loss-of-function alleles of the *AGO1* gene alter many developmental processes and often cause lethality or sterility. With a view to identify novel genes involved in miRNA-guided gene silencing, we mutagenized with EMS M_1 seeds of the viable and fertile *ago1-52* mutant, which had been isolated in our laboratory.
We screened 36,810 M_2 seeds and identified 17 lines in which the phenotype caused by ago1-52 is from partially to almost completely suppressed. We have already mapped five of the suppressor mutations, which we named mas (*morphology of argonaute1-52 suppressed*), and have positionally cloned three of them. We will present our results on the genetic and molecular characterization of the *MAS* genes.

P02-021: ARABIDOPSIS TCU2 IS REQUIRED FOR LEAF BILATERAL SYMMETRY

Ferrández-Ayela, A. - Lapeña-Muñoz, A. - Ponce, M.R. - Micol, J.L.
División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche (Alicante), Spain

Leaves of the *transcurvata2-1* (*tcu2-1*) mutant are folded downwards in a slightly asymmetrical manner relative to the midvein, and exhibit a venation with reduced length, density and number of bifurcations.
Mesophyll cell size heterogeneity, stem length and flower size are increased compared to the wild type. *tcu2-1* also shows early flowering, delayed anther dehiscence, and its siliques are short and thick, and many are three-valved. The first two leaves are fused in 10% of the seedlings. We positionally cloned the *TCU2* gene, which encodes a protein of unknown function. We are making constructs for the phenotypic rescue of the mutant, constitutive expression of the *TCU2* gene, visualization of its spatial expression pattern, and the subcellular localization of the TCU2 protein. We have also designed an artificial microRNA targeting *TCU2*, and are conducting double mutant analyses in order to study the genetic interactions of *TCU2* and its role in leaf and whole-plant development.

P02-022: CARBAMOYL PHOSPHATE SYNTHETASE IS ESSENTIAL FOR MESOPHYLL CELL DEVELOPMENT IN ARABIDOPSIS

Sarmiento-Mañus, R.[1] - Mollá-Morales, A.[1] - Robles, P. [1] - Quesada, V. [1] - Pérez-Pérez, J.M. - González-Bayón[1] - Gónzalez-Torres, P.[1] - Hannah, M.[2] - Willmitzer, L. - Ponce, M.R.[1] - Micol, J.L. [1]
[1]División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche (Alicante), Spain
[2]Marx-Planck-Institute of Molecular Plant de Bioingeniería, Postdam-Golm, Germany

The leaf vascular network of the *venosa (ven)* mutants of Arabidopsis can be clearly distinguished as a green reticulation on a paler lamina. We have previously shown that leaf reticulation may reveal an altered internal leaf architecture. We isolated the *ven3* and *ven6* mutants, which carry semidominant mutations that had been induced by EMS and cause the primary and secondary veins to stand out as a colour difference on the lamina. A metabolomic analysis of *ven3* and *ven6* leaves revealed increased ornithine levels and decreased arginine and citrulline levels. Supplementation of the growth medium with citrulline, an intermediate of the arginine biosynthetic pathway, completely suppressed the mutant phenotype at the rosette and tissue levels. Consistent with this, the ven3 and ven6 mutants were more sensitive than the wild type to the inhibition of growth shown on media supplemented with ornithine. We positionally cloned the *VEN3* and *VEN6* genes, which respectively encode the large and small subunits of carbamoyl phosphate synthetase (CPS). In *Escherichia coli*, CPS is a heteromultimer consisting of four large and four small subunits, and catalyzes the conversion of glutamine into glutamate and carbamoyl phosphate. Carbamoyl phosphate condenses with ornithine to produce citrulline in the arginine biosynthetic pathway. In plants, most of these reactions occur in the chloroplast. Our genetic and molecular analyses of the *ven* mutants indicate that CPS function is essential for mesophyll cell development in the interveinal tissues of vegetative leaves.

P02-023: CHARACTERIZATION OF GENES REQUIRED FOR LEAF GROWTH

Rubío-Díaz, S. - Muñoz-Viana, R. - Pérez-Pérez, J.M. - Hernández-Romero, D. - Antón- Bolaños, N. - Ponce, M.R. - Micol, J.L.
División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche (Alicante), Spain

We have isolated several hundreds of EMS-induced, viable and fertile Arabidopsis mutants with abnormal leaf morphology. More than forty of them have already facilitated the molecular identification of genes required for leaf organogenesis. In our large-scale screen, we identified dozens of mutants with small leaves, and assumed that their reduced leaf size indicates loss of function of genes required for leaf growth. Several of these mutants were named *exigua (exi)* and display small, dark green vegetative leaves, with no obvious perturbations in lamina proportions or flatness. The *EXI1*, *EXI2* and *EXI5* genes were positionally cloned and found to respectively encode the CESA8/IRX1, CESA4/IRX5 and CESA7/IRX3 cellulose synthases, which are involved in secondary cell wall formation. For the positional cloning of additional growth regulatory genes, we are also studying mutants belonging to the Ondulata (Ond), Serrata (Sea), Orbiculata (Orb), Angusta (Anu) and Apiculata (Api) phenotypic classes, which also exhibit reduced leaf size. *OND2* and *OND3* have been positionally cloned and found to encode the AtMinE1 and ARC6 proteins, which are required for chloroplast division. *ANU7* and *API6* were found to respectively encode a chloroplastic protein of unknown function and a ribosomal protein.

P03 System Biology And Omics

P03-001: ASSESSMENT OF CITRUS QUALITY BY GC-MS ANALYSIS AND AN AROMA SENSOR

González-Mas, M.¹ – Cortes, S.¹ - Bernet, G.¹ - Granell, A.² - Rambla, JL.² - López, S.¹ - Marqués, M.¹ - Gutierrez-Suanzes, A.¹*
¹Instituto Valenciano de Investigaciones Agrarias
²Instituto de Biologia Molecular y Celular de Plantas
**Corresponding author e-mail: gonzalez_mde@gva.es*

One of the main characteristics of citrus fruit quality is defined by the aroma of both fruit and fruit juice. This work describes the characterization of the aroma of citrus juices and the volatiles evolving from the whole fruit, using varieties of good organoleptic quality. The varieties studied for the analysis of juice aroma were Powell, Clemenules, Fortune, Chandler Pummelo and two hybrids of the latter two (FxCh 90 and FxCh 77), and included a diversity of fruits with different aroma. The variety used for the evolution of fruit aroma was Navel Late. Two different analytical techniques were used for analysis: SPME-GC-MS (solid phase microextraction coupled to gas chromatography-mass spectrometry), and an aroma sensor. In order to study the evolution of volatile composition of the citrus aroma during fruit ripening, whole fruits were enclosed in small climatic chambers and gas samples withdrawn and analysed at given times. More than eighty volatile organic compounds were detected by SPME-GC-MS in the juice from the varieties indicated above. Our six varieties showed characteristic volatile profiles with mainly quantitative differences in their juice aromatic profiles. Whole fruits during storage evolved very few volatile compounds in the chamber atmosphere. Despite of that, the volatile profile, with the most prominent compounds being limonene and valencene, allowed to follow the evolution of fruit ripening during fruit storage in the cold.
The correlation between the results obtained by GC-MS and aroma sensor should allow the development of simple and quick methods to monitor some aspects of quality in citrus fruits. This work has been supported by the research Projects GVPRE/2008/164 and INIA FEDER RTA2007- 00029-C02-01 (Sensogest).

P03-002: PROTEOMIC APPROACH TO NITROGEN EFFICIENCY IN CONTRASTING ZEA MAYS HYBRIDS.

Allue, J. - Chaves, L. - Barcelo, J.*
Fisiologia Vegetal, BABVE, Facultad de Biociencies, Universitat Autonoma de Barcelona
**Corresponding author e-mail: josep.allue@uab.cat*

Two Zea mays hybrids differing in nitrogen efficiency according to field data were used for a proteomic study under N deficiency conditions (NL) imposed in hydroponics. Changes in protein patterns in roots and shoots of the N-efficient HS20x724 and the non-efficient HS11x723 were assessed using Difference Gel Electrophoresis (DiGE), a highly sensitive method that enables accurate analysis of differences in protein abundance (125 pg of protein). After 4 days of germination, seedlings of both hybrids were transferred to a hydroponic system and grown for further 10 days under controlled conditions (RH: 50%-70% day, 45%-55% night; light intensity increasing from 150 to 1,000 μEs-1m-2; photoperiod 16 h light / 8 h dark; maximum temperatures, 27ºC day and 18ºC night). Control plants were grown in Hoagland nutrient solution and NL plants in the same nutrient solution deprived of nitrogen. The proteomic study was conducted by analyzing the soluble protein fractions extracted from roots and leaves. Image analysis and statistical quantification were evaluated. Adopted ANOVA criterion was a ratio ≥ 1.2 and ($p \leq 0.005$) for comparing HS11x723C vs HS11x723NL and HS20x724C vs HS20x724NL groups, and a ratio ≥ 1.2 y ($p \leq 0.001$) for comparing hybrids. The study detected 181 Spots with significant differences and their analysis allowed identifying 59 proteins related to nitrate and carbohydrate metabolism, cell rescue, and stress responses.
Acknowledgements: Supported by the Spanish Government BFU2007-60332/BFI

P03-003: PLASTID STROMAL PROTEOME OF IN VITRO GROWN HORSERADISH (ARMORACIA LAPATHIFOLIA GILIB.) PLANTLET, TUMOR AND TERATOMA TISSUES

Peharec, P. - Razdorov, G. – Balen, B. - Krsnik-Rasol, M.*
University of Zagreb
**Corresponding author e-mail: ppeharec@zg.biol.pmf.hr*

Crown-gall tumors were induced on horseradish leaf fragments with a wild strain B6S3 of *A. tumefacien*s. During subcultivation, two different transformed phenotypes were established: unorganized tumor-TN and teratoma-TM, composed of malformed shoots. The study has been focused on plastids. During tumor transformation, these organelles change from developed chloroplast to juvenile proplastide stage. Stromal proteome of tumor plastids and chloroplasts from leaf cells were compared. Intacpt lastids were lysed under non-denaturating conditions and stromal proteins were digested with trypsin. Triptic peptides were separated by C18 nano-chromatography and measured online with LTQ-Orbitrap Discovery MS. Peak lists (generated by MaxQuant) were used by X!Tandem to search *A. thaliana* proteome -TAIR database. 321 leaf proteins, 70 TM and 54 TN proteins were identified. They were classified according to MapMan Bin classification, reported in PPDB. Leaf proteins are involved in photosynthesis, major/minor CHO, amino acid, nucleotide and RNA metabolism, glycolysis, OPPP, organic transformation, lipids, hormone, isoprene, tetrapyrrole, protein synthesis and redox regulation. TM proteins were missing in minor CHO and RNA metabolism, isoprene synthesis. TN proteins were missing in minor CHO and nucleotide metabolism, lipid, hormone, isoprene and tetrapyrrole synthesis. Our results show that cell transformation and tissue developmental stage influence the composition of stromal proteome.

P03-004: COMBINING ENHANCED ROOT AND SHOOT GROWTH REVEALS CROSSTALK BETWEEN PATHWAYS THAT CONTROL PLANT ORGAN SIZE IN ARABIDOPSIS THALIANA

Vercruyssen, L.¹ - Werner, T.² - Gonzalez, N.¹ - Schmülling, T.² - Inzé, D.¹*
¹Department of Plant systems Biology, Flanders Institute for Biotechnology (VIB) - Ghent University
²Institute of Biology/Applied Genetics, Free University of Berlin
**Corresponding author e-mail: licru@psb.vib-ugent.be*

Improving plant yield will be of great importance to serve the increasing demand for food, feed and bio-energy of the growing world population. To this end functionally distinct transgenes were combined, that increase root or shoot growth when ectopically expressed. Enhanced root growth resulting from cytokinin deficiency was exploited by overexpressing *CKX3 (Cytokinin oxydase/dehydrogynase* 3) from a root specific *PYK10* promoter.

Plants harboring PYK10-CKX3 were crossed with four different transgenic lines with enhanced leaf growth. For all combinations both phenotypic traits could be combined resulting in overall yield increase. Both leaf and root growth were synergistically enhanced in plants ectopically expressing *CKX3* and *BRI1* (*Brassinosteroid Insensitive* 1), indicating crosstalk between cytokinins and brassinosteroids. Treatment of *PYK10-CKX3* plants with brassinolide showed a dramatic increase in lateral root growth that could not be observed in wild type plants. Co-expression of *CKX3* and *GRF5 (Growth Regulating Factor* 5) antagonized the effects of *GRF5* overexpression, revealing interplay between cytokinins and *GRF5* during leaf cell proliferation. Combined overexpression of *CKX3* and *GA20ox1 (Gibberellin 20-oxidase 1*) led to a synergistic increase in leaf growth, suggesting that cytokinins act together with gibberellins. On the other hand, only additive effects on root and shoot growth could be observed in plants ectopically expressing both *CKX3* and the vacuolar pyrophosphatase *AVP1*, indicating an independent mode of action.

P03-005: METABOLOMIC ANALYSIS OF VANILLA PLANTS FROM DIFFERENT SPECIES AND IMPACT OF CYMBIDIUM MOSAIC VIRUS (CYMMV) ON THE GROWTH AND METABOLOME OF VANILLA VINES

Palama, T.[1] - Grisoni, M.[1] - Fock, I.[1] - Jade, K.[1] - Bartet, L.[1] - Choi Y, H.[2] - Verpoorte, R.[2] - Kodja, H.[1]*
[1]UMR PVBMT, Université de La reunión
[2]Division of Pharmacognosy, Section Metabolomics, IBL, Leiden University
**Corresponding author e-mail: tony.palama@univ-reunion.fr*

More than 110 species are comprised in *Vanilla* genus which belongs to the Orchidaceae family. Nevertheless, only three species are commercially cultivated (*V. planifoliaV, . tahitensis* and *V. pompona*) with V. planifolia pods being the major source of natural vanilla flavour. In order to increase vanilla pods production, a more intensive cultivation system is now employed. However, this cultivation system facilitated the spread of viruses like the Cymbidium mosaic virus (CymMV). Investigation of the effect of CymMV on the growth and metabolome of vanilla plants was performed by growing four *Vanilla* accessions: CR01 (*V. planifolia*), CR17 (*V. tahitensis*),CR03 (*V. planifolia* × *V. tahitensis*) and CR18 (*V. pompona*). CymMV infected plants of CR01, CR03 and CR17 have a reduced growth (vines and internodes length, stem diameter and number of leaves). Nevertheless, there is no significant difference in the growth of CR18. Methanol-water extracts of *Vanilla* leaf were analyzed by ^{1}H NMR spectroscopy. Metabolomic analysis of the leaves showed a difference of profile according to the species. It appears that CR18 leaves had qualitatively more phenolic compounds than the others accessions. However, no discrimination based on the CymMV infection status was possible for samples collected from the field. Another metabolomic analysis was performed under *in vitro* conditions with *V. planifolia* plants infected by CymMV. An increase of amino acids and sugars levels of CymMV infected leaves was observed, whereas phenolic compounds and malic acid levels decreased. This study was the first metabolomic analysis performed on Vanilla plants from different species. Nevertheless, further studies are required on the earlier mechanism of CymMV infection.

P03-006: VANILLA REGENERATION THROUGH SHOOT FORMATION FROM PROTOCORM CALLUS: METABOLOMIC AND PROTEOMIC ANALYSIS AT EARLY STAGE.

Kodja, H.[1] - Palama, T.L.[1] - Fock, I.[1] - Choi Y, H.[2] - Bourdon, E.[1] - Verpoorte, R.[2]*
[1]Université de La Reunión
[2]University of Leiden
**Corresponding author e-mail: kodja@univ-reunion.fr*

Vanilla planifolia is an important Orchid commercially cultivated for the production of natural vanilla flavour. Because mass production of *V. planifolia* through indirect shoot differentiation from callus culture is rare and may be a successful use of *in vitro* techniques for producing somaclonal variants, we have established a novel protocol for the regeneration of vanilla plants and investigated the initial biochemical and molecular mechanisms that trigger shoot organogenesis from embryogenic/organogenic callus. By associating proteomics and metabolomics analyses, the biochemical and molecular markers responsible for shoot induction have been studied in 15-day-old calli at the stage where no differentiating part was visible on calli. The subculture of embryogenic/organogenic calli onto shoot differentiation medium triggers the stimulation of cell metabolism principally at three levels namely (i) initiation of photosynthesis, glycolysis and phenolic compounds synthesis; (ii) amino acid – protein synthesis, and protein stabilization; (iii) sugar degradation. These results might contribute to elucidate the complex mechanism that leads to vanilla callus differentiation and subsequent shoot formation into PLB organogenesis. Moreover, histological analysis showed that the nearby presence of starch could be an important factor in organogenesis of PLB. These observations confirm that Orchid seeds cannot reach the seedling stage without an external supply of carbohydrates which is provided in nature by fungi mycorrhizae.

P03-007: THE ART NOUVEAU OF SYSTEMS BIOLOGY (ON HOW TO MODEL NATURAL ORGANIC FORMS)

Sauret-Gueto, S. - Sablowski, R. – Coen, E.*
John Innes Centre
**Corresponding author e-mail: susana.sauret-gueto@bbsrc.ac.uk*

Developmental biology have long tried to understand how the organisms acquire their mature shape. A fascinating example is flower development, where undifferentiated primordia will shapeinto one of the four types of floral organs: sepals, petals, stamens and carpels. In the last two decades we have begun to understand how genes products control the identity, number and positioning of floral organs in each whorl but If we want to understand how gene activity is translated into a morphological form we need to address growth as a dynamic process and construct mechanistic models. In plants, shape is generated as a result of two local variables: growth rate and growth direction. Two basic types of data provide information on how growing shapes emerge: 1) growth studies such as tracking and clonal analysis provide access to growth patterns and 2) molecular genetic studies provide information on patterns of gene activity. A major challenge is to link these two types of data. We have generated a quantitative framework for the Arabidopsis petal and clonal analysis is being used to infer its growth patterns. We are following a computational approach to understand how gene activity influences the development of tissue shape. Tissue is treated as continuum and genetically controlled factors, that interact and propagate, are inputs that locally control growth rate and growth direction. As a result these factors specify local strain fields and elasticity theory is used to compute the resulting deformations at a higher level, the continuum tissue. In a crosstalk of approaches, computational generated models are contrasted with mutant phenotypes and gene activities to generate new hypothesis on how Arabidopsis petal shape is generated at the tissue level.

P03-009: XYLEM METABOLOMICS AND IRON DEFICIENCY

Rellán-Álvarez, R.[1] - El Jendoubi, H.[1] - Wohlgemuth, G.[2] - Abadía, A.[1] - Fiehn, O.[2] Abadia, J.[1] - Álvarez-Fernández, A.[1]
[1]Aula Dei Experimental Station-CSIC
[2]University of Calfornia-Davis

Xylem sap provides an interesting system to study plant metabolism. Xylem is the highway for metabolite traffic between roots

and shoots, and can provide a different picture of plant metabolome changes from that obtained from whole tissue extracts, where all cell compartments are mixed together.
It is known that Fe deficiency causes changes in the levels of metabolites related to the glycolytic and TCA cycles, reflecting an alteration of the carbohydrate metabolism.
This has been associated to an anaplerotic root carbon fixation *via* phospho*enol*pyruvate carboxylase, with fixed carbon being exported to leaves through the xylem sap [1].
In this work, the effects of Fe deficiency in the xylem sap metabolome of different plant species, two grown in controlled environments (white lupin and tomato) and one in the field (peach) were studied. Metabolites were extracted and analyzed following the recommendations of the Metabolomics Standards Initiative [1]. Approximately 70 metabolites were identified in the different experiments.
The main metabolite changes induced by Fe deficiency include relative increases in TCA cycle metabolites and decreases in amino acids, among others.
[1] Abadía et al. (2002) Plant Soil 241: 75
[2] Fiehn et al. (2007) Metabolomics 3: 195.
Acknowledgements: Supported by the Spanish Ministry of Science and Education (projects AGL2007-61948, AGL2009-09018 and PLANT-KBBE EUI2008-03618), and the Aragón Government (group A03).

P03-010: PROTEOMIC ANALYSIS OF VIROID-INFECTED TOMATO PLANTS

Lisón, P. - Tárraga, S. - Hernández-Buendía, A. - López-Gresa, M[a]P. – Rodrigo, I. – Bellés, JM[a] - Conejero, V.*
(IBMCP) Instituto de Biología Molecular y Celular de Plantas UPV-CSIC
**Corresponding author e-mail: plison@ibmcp.upv.es*

Viroids are single-stranded, circular, noncoding RNAs that infect plants. These small RNAs are able to overcome the defensive mechanisms of plants provoking devastating diseases.
Recently, advances in proteomics studies have been driven by the development of fluorescence 2-D difference in gel electrophoresis (2D-DIGE).
In the present work, we use this technology to analyze the response of tomato plants to the infection with Citrus Exocortis viroid (CEVd).
A total of 1481 spots were detected in the four gels analyzed. Quantitative comparisons of viroid-infected samples versus control-type counterparts resulted in many differences. A total of 409 spots were statistically significant; 224 out of them showed difference in volume and they were abundant enough to enable identification.
Among the proteins showing a high average ratio, 80 of them were up-regulated and 12 were down-regulated. These proteins were picked up and analyzed by mass spectrometry. A total of 43 proteins were successfully identified; some of them were present on the gel as two or more spots, suggesting the existence of different isoforms and/or post-translational modifications that shift the mobility in 2-D.
These roteins could be classified into functional groups: defense response, transcription and translation, metabolism and energy, and others. Results from proteomic analysis were validated by RT-PCR. Some of the differentially expressed proteins showed variations at RNA level while some others exhibited the difference only at protein level, thus suggesting a posttranscriptional regulation. These results indicate that the proteomic analysis can provide with a valuable information, complementing genomics.

P03-011: METABOLIC RESPONSE OF TOMATO LEAVES UPON DIFFERENT PLANT-PATHOGEN INTERACTIONS

López-Gresa, M.[1] - Bellés, JM[a1] - Lisón, P. [1] – Rodrigo, I. [1] – Conejero, V. [1] - Hae Choi, Y.[2] - Verpoorte, R.[2]*
[1]Instituto de Biología Molecular y Celular de Plantas UPV-CSIC
[2]Institute of Biology, Leiden University
**Corresponding author e-mail: mplopez@ceqa.upv.es*

Plants utilize various sophisticated defense mechanisms against their potential biotic stressing agents such as viroids, viruses, bacteria, fungi and abiotic environmental challenges. Among them, metabolic alteration is a common response in both compatible and incompatible plant-pathogen interactions.
However, the identification of metabolic changes associated with defense response is not an easy task due to the complexity of the metabolome and the plant response. To address the problem, a metabolomics approach using nuclear magnetic resonance (NMR) spectroscopy in combination with multivariate data analysis was performed. To identify a wide range of pathogen-induced metabolites, two different plant-pathogen systems were studied: *Solanum lycopersicum* leaves infected by citrus exocortis viroid (CEVd) or *Pseudomonas syringae* pv. *tomato*. NMR-based metabolomics of crude extracts from tomato-infected plants allowed the identification of different metabolites implicated in the systemic and hypersensitive response of tomato to CEVd and *P. syringae*, respectively. Xylosylated gentisic acid was the most prominent induced metabolite in tomato plants infected with viroid, while phenylpropanoids and the flavonoid rutin were found associated to bacterial infection. NMR metabolomics is a potent platform to analyze the compounds involved in different plant infections. A broad response to different pathogenic infections was revealed at metabolomic levels in the plant. Also, metabolic specificity against each pathogen was observed.

P03-012: IDENTIFICATION OF GENES AND GENE PROMOTERS RESTRICTED TO NEMATODE FEEDING CELLS BY TRANSCRIPTOMIC ANALYSES AND CANDIDATE GENE APPROACHES

Barcala, M. - García, A. - Portillo, M. - Cabrera, J. - Sánchez, M. - Escobar, C. - Fenoll, C.
Universidad de Castilla-la Mancha

Sedentary endoparasitic root-knot nematodes (*Meloidogyne* spp.) induce root galls that harbor specialized feeding cells called giant cells (GCs). Gene expression changes in galls have been reported, but the key molecular events that trigger the GCs differentiation remain unknown. Gall transcriptomic analyses provide limited information, since GC-specific transcripts are diluted by the contribution of other cell types. We provide detailed information on this dilution effect by microarray analysis of laser microdissected GCs in Arabidopsis and tomato GCs, as compared to whole galls. Only 120 differentially expressed genes out of 1161 in GCs were shared with galls in Arabidopsis. Fc values for most co-expressed genes were lower in galls than in GCs, suggesting that relative contribution of GC transcripts to galls was low. Transcription patterns were also different between galls and GCs for several functional categories. Several genes related to auxin metabolism were differentially regulated in GCs as compared to root vascular cells, perhaps reflecting an increase in auxin sensitivity or accumulation in early developing GCs. LBD16 functions downstream of ARFs-dependent auxin signaling in lateral root initiationa, nd its promoter is induced only 24h after infection by *Meloidogyne* spp. *LBD16* reacts to *Meloidogyne* spp. secretions microinjected in Arabidopsis roots and added to protoplasts. We are addressing *LBD16* roles in galls and GCs by monitoring the IAA-responsive promoter DR5 and by studying the effects of LBD16 fused to a dominant repressor domain on nematode infectivity. *LBD16* and geminiviral promoters induced in GCs are being used to design biotechnological tools for nematode control.

P03-013: REGULATION OF BIOLOGICAL SIGNALLING BY TEMPERATURE: ROBUST. CRY1, CRY2 AND PHYA REGULATE TEMPERATURE COMPENSATION OF THE CIRCADIAN CLOCK

Gould, P.[1] - Foreman, J.[2] - Domijan, M.[3] - Piñas Fernández, A.[2] - Costa, M.[3] - MacGregor, D.4 - Zielinski, T. [2] - Steward, K.[2] - Bird, S.4 - Steel, G.[2] - Williams, M.[2] - White, M.[1] - Graham, I.[4] - Penfield, S. 4 - Finkenstadt, B.[3] - Rand, D.[3] - Millar, A.[2] - Hall, A[1] - Halliday, K.[2]
[1]University of Liverpool
[2]University of Edinburgh
[3]University of Warwick
4University of York

Changes in ambient temperature can have dramatic consequences for plant development and physiology. Temperature changes alter the reaction rates of individual biochemical processes; therefore, a biological network must balance the effects of these alterations in each of its components to retain signalling integrity over a temperature range. ROBuST seeks to understand the principles that underlie SENSITIVITY and ROBUSTNESS of a biological network. Our study network comprises the interconnected network of light, cold acclimation and the circadian pathways. Our work has identified a new and prominent function for cry1, cry2, and phyA as regulators of temperature buffering of the circadian clock. Deficiencies in cry1 and cry2 resulted in failure of the clock transcriptional feedback loop under warm temperatures, and phyA deficiency under cold. This work has led to i) the development of temperature compensated plant clock model, and ii) evidence the co-evolution of light and temperature signal transduction.

P03-014: METABOLITE ANALYSIS OF AN ABA-DEFICIENT MUTANT OF SWEET ORANGE DURING RIPENING AND POSTHARVEST STORAGE

Romero, P.[1] - Lafuente, M.T.[1] - Reyes-de-Corcuera, J.I.[2] - Alférez, F. [1] - Zacarías, L.[1] - Rodrigo, M.J.[1] - Burns, J. K.[2]*
[1]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC)
[2]Citrus Research and Education Center, University of Florida
**Corresponding author e-mail: ciepro@iata.csic.es*

'Pinalate' is a yellow-colored spontaneous mutant fruit from the 'Navelate' orange (*Citrus sinensis* L. Osbeck). Carotenoid biosynthesis is blocked in this mutant, resulting in abscisic acid (ABA) deficiency and accumulation of non-coloured carotenoids. Fruit of this mutant display altered ripening and increased susceptibility to peel pitting during storage at non-chilling temperatures (12ºC). Flavedo (colored part of the peel) samples from both cultivars were collected at several ripening stages and stored at various durations at 12 ºC to characterize their metabolic profile by GC-MS.
Compounds were identified and grouped into amino acid, sugar, polyalcohol and organic acid families. All identified sugars increased during ripening in both cultivars; however, sucrose content fluctuated and reached higher values in mutant mature fruit. Other compounds such as serine and ribitol
decreased with ripening while indole-3-acetic and mannoonic acids sharply increased at the last ripening stage in both varieties. The results also showed that at 12 ºC most sugars similarly decreased in both cultivars. Among organic acids, only hexadecanoate showed a different pattern, reaching higher levels in 'Pinalate' after prolonged storage. Taken together, the results revealed that only sucrose and hexadecanoate of studied compounds showed differences between 'Navelate' and 'Pinalate' fruits, suggesting that these changes may be associated with the altered behavior of 'Pinalate' mutant.

P03-015: A REVERSE GENETICS APPROACH TO THE ANALYSIS OF LEAF DEVELOPMENT

Muñoz-Viana, R. - Rubio-Díaz, S. - Pérez-Pérez, J.M. - Wilson-Sánchez, D. - Ponce, M.R. - Micol, J.L.
División de Genética and Instituto de Bioingeniería, Universidad MiguelHernández, Campus de Elche, Alicante, Spain

Because of their photosynthetic activity, leaves are the ultimate source of most of the oxygen that we breathe and of the food that we eat. Yet the processes by which these organs grow are poorly understood. Previous forward genetics studies yielded a large number of mutations affecting Arabidopsis leaf development, shape or size. However, none of these earlier attempts reached genome saturation. The group of Prof. J.R. Ecker at the Salk Institute is obtaining a large collection of gene-indexed homozygous T-DNA insertion mutants that will cover 25,000 genes of the Arabidopsis genome. To identify novel genes required for leaf growth regulation, we have begun a reverse genetics screening using the 14,000 T-DNA insertion lines available in batches from the ABRC, which correspond to 10,800 different Arabidopsis genes. These lines are grown *in vitro* and those exhibiting aberrant leaf phenotypes are documented and kept for further studies. In order to saturate the Arabidopsis genome with viable and fertile leaf mutations, we plan to screen the entire Salk homozygous T-DNA insertion collection for visible leaf phenotypes.

P03-016: A COLLECTION OF AMIRNAS TARGETING GROUPS OF TRANSCRIPTION FACTOR-ENCODING PARALOGS

Ponce, M. - Jover-Gil, S. - Micol, J.L.
División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche (Alicante), Spain

Our understanding of the function of individual Arabidopsis genes is obscured by the existence of gene families that include redundant members. In fact, there is an expanding list of single null mutants not exhibiting a mutant phenotype. In addition, examples are known of double and even triple combinations of non-allelic, loss-of-function mutations affecting paralog genes that cause no visible phenotypes. The masking effects of redundancy in gene families can be overcome with new technologies based on gene silencing using artificial microRNAs (amiRNAs). We are obtaining transgenic Arabidopsis lines expressing amiRNAs designed to repress groups of paralog genes encoding transcription factors. These amiRNAs were designed to target groups of two or more transcription factor-encoding genes that are arranged in tandem in the Arabidopsis genome. Following the design principles that can be found at http://wmd.weigelworld.org, we already designed 197 amiRNAs, 164 of which have already been transferred to Arabidopsis plants. Three well known transcription factor-encoding genes with easily visible loss-of-function phenotypes were chosen as controls: *GL1*, *AG* and *PAP1*. In most, but not all cases the transgenic plants obtained exhibited the phenotype expected from downregulation of the target gene.

P03-017: METABOLIC PROFILES CAN ASSIST TO DISCRIMINATE CAULIFLOWER GROWN UNDER DIFFERENT FARMING PRACTICES

Annunziata, M. – Massaro, G. – Iannuzzi, F. – Nacca, F. – Carillo, P. - Fuggi A.
Dipartimento di Scienze della Vita, Seconda Università di Napoli, Caserta Keywords: cauliflower, Brassica oleracea L. Subsp botrytis, carbohydrates, aminoacids, glucosinolates The conventional cauliflower culture needs high inputs of fertilizers and pesticides, that can cause soil pollution and degradation. On the other hand, the demand of better foods and the use of more sustainable agricultural practices is increasing. In this view the aim of this work was to verify if metabolite profiling can discriminate among farming practices and assist in tracing the produce. Cauliflower (Brassica oleracea subsp. botrytis cv Atalaya) plants were grown under traditional farming system (TFS) and conservative (low tillage) farming system (CFS). At the harvest the corymb heads were divided in curd (immature flowers) and stem, frozen and homogenised in liquid nitrogen. Aliquots were used to determine the content of protein, carbohydrates, ascorbate and glutathione, inorganic and organic acids, free amino acids and glucosinolates. Five individual plants were used as replicates.

Multivariate analysis of the results related to such metabolites allowed to discriminate between curd and stem of the cauliflower heads along the first principal component accounting for about 60% and between the farming practices along the second principal component for about 25%. Financial support was obtained by “Seconda Università degli Studi di Napoli”, “Ministero dell’Università” and “Ricerca Scientifica e tecnologica” of Italy (PRIN 2006077008_005; 2008S9T3KK_003).

P04 Reproductive Development

P04-001: THE EFFECTS OF HEAVY METAL SALTS ON THE PHYTOHORMONAL STATUS AND SEX EXPRESSION IN CANNABIS SATIVA L.

*Khryanin, V.**
State Pedagogical University, Dep. of Plant Biology, Russian Federation
**Corresponding author e-mail: khryanin@spu-penza.ru*

We studied the effects of heavy metal salts ($Pb(NO_3)_2$, $CuSO_4$, and $ZnSO_4$) on phytohormonal status and sex expression in various cultivars of marijuana (*Cannabis sativa* L.), a dioecious plant, grown on Knop nutrient medium. $Pb(NO_3)_2$ and $ZnSO_4$ were added to the medium at the concentration of 10^{-9} M, and $CuSO_4$, at the concentration of 10^{-10} M. Plant were grown under controlled conditions at luminescent illumination, 22–24°C, and 80% humidity. The contents of GA and zeatin were determined by HPLC. Copper and zinc salts induced plant feminization, and this effect was coupled with zeatin accumulation. Lead salts favored plant masculinization coupled with GA accumulation. Thus, a shift in sex expression in marijuana plants was correlated with the heavy metal action on the balance of phytohormones, GA and zeatin. In general, we have shown that the addition of cop•per and zinc salts to the growing medium resulted in zeatin accumulation and a significant decrease in the GA content in treated plants. Application of $Pb(NO_3)_2$ reduced the level of zeatin and increased the level of GA. Copper and zinc salts favored plant feminization, whereas lead enhanced plant masculinization. Thus, HM salts affected sex expression in marijuana plants via changes in the hormonal balance, the ratio of GA and zeatin. Their effects displayed species-specificity.

P04-002: STUDY OF LIFE CYCLE OF PTERIS CRETICA L. AND THE IMPACTS OF ARSENIC ON THE THE GROWTH OF SPORE AND THE DISTINCTION OF SEXUAL ORGANS

Tajadod, G. - Majd, A.*
ISLAMIC azad university-north tehran branch
**Corresponding author e-mail: tajadodg@yahoo.com*

Pteris fern belongs to pteridaceae family and Pteris cretica is one of the existing types of this family in Iran. The fern's spore underwent the process of culture in the soil and the stages of germination (protonema, prothalli, sexual notch, appearance of antheridium and archegonium) were studied till the emergence of the young plant (sporophyt). Arsenic is a toxic pollutant of the environment. Identification of *Pteris* and its species and the accumulation of arsenic in its frond led to the creation of photoremediation technology for decontaminating arsenic –contaminated places. By adding 0.001 gr/lit of arsenic, its impacts on the growth of spores, protonema, prothalli and the sexual organs (gametophyte) till the emergence of sporophyte were studied. Spores treated with arsenic grow slowly and the growth of protonema and prothalli takes place with a delay. The prothallis are small and have few sexual notches with low depth. There are more lateral buds in prothallis and they emerge more quickly. The prothallis that have undergone culture have great number of rhizoids. The male and female sexual organs grow with little difference of time compared to the mature plant and their number has decreased in proportionate with the decreasing size of the prothalli. The young sporophyte in the treated plant grows similar to the mature plant.

P04-003: KANADI MODULATION OF AUXIN TRANSPORT DURING S. TUBEROSUM SSP. ANDIGENA TUBER DEVELOPMENT

Abelenda, J.A.[1] - Navarro, C.[1] - Cuellar, C.A.[1] - Bjorn, K.[2] - Bachem, C.WB.[2] - Prat, S.[1]*
[1]Centro Nacional de Biotecnología-CSIC
[2]Wageningen UR Plant Breeding, Wageningen University and Research Centre
**Corresponding author e-mail: jaabelenda@cnb.csic.es*

Tuber formation in potato plants is a complex process regulated by different environmental and endogenous factors. In wild potato subspecies such as *S. tuberosum ssp. andigena* there is a strict requirement of short-days for tuberization. Previous results from our group showed that the pathway mediating this photoperiodic control in potato has many elements in common with that of daylength flowering control in *Arabidopsis*. As in *Arabidopsis*, a potato FT-like protein functions as part of the mobile signal, moving from leaf to the stolon, where it promotes differentiation of the subapical region into a tuber. While in *Arabidopsis* floral transition relies on the interaction of FT with a bZIP transcription factor called FD, in potato, using 2H screen, we identified an additional interaction with the KANADI transcription factor, a Myb-Like protein that belongs to GARP subfamily. KANADI has been described as a master regulator of polarity and abaxial identity, and recently it was demonstrated to control expression of the auxin efflux-transporter protein PIN1 (Ilegems et al. 2010). KANADI affects auxin localization through changes in PIN1 expression and therefore organ polarization during development. We found that *andigena* potato plants over-expressing this gene can tuberize under non inductive long day conditions, probably through a redistribution of auxin maxima in the stolon. At the same time and using transgenic plants expressing the auxin DR5::GUS marker and StPIN1p::GUS we have observed substantial changes in the distribution of auxin along stolon-to-tuber transition. The interaction of FT with KANADI and its effect on PIN1 mediated auxin distribution will be discussed. Llegems et al. 2010. Development 137, 975-984

P04-004: TOCOPHEROL COMPOSITION IN FLOWER ORGANS OF LILIUM AND ITS VARIATIONS DURING NATURAL AND ARTIFICIAL SENESCENCE

Arrom Mascasas, L. - Munné-Bosch, S.*
Universitat de Barcelona
**Corresponding author e-mail: laia.arrom@ub.edu*

Although the biosynthesis and function of tocopherols (vitamin E) in leaves and seeds have been studied in detail, their occurrence within other plant organs/tissues is still poorly understood. In an attempt to better understand the occurrence and possible functions of tocopherols in flowers, we measured the concentrations of the four tocopherol homologues in floral organs of *Lilium* (including the gynoecium, androecium, and inner and outer tepals), and evaluated their variations in tepals of cut, senescing flowers (artificial senescence) compared to controls (natural senescence). Results showed that flowers accumulated α-tocopherol at significant amounts, while γ-tocopherol was present at much lower concentrations. The androecium was the organ showing the highest amounts of tocopherols, with a specific accumulation in the pollen, while tocopherols were not present in the gynoecium. Inner and outer tepals also contained significant amounts of α- and γ-tocopherol, whose levels increased during senescence. α- Tocopherol increased in both outer and inner tepals earlier and to a higher extent during senescence of cut flowers than in controls. The lowest concentrations of toco-

pherols were found at the beginning of tepal development (in green tepals), while the highest concentrations were found in chlorophyll-free, senescing tepals, especially in cut flowers. It is concluded that (i) tocopherols accumulate in outer and inner tepals, and in the androecium of *Lilium* flowers, particularly in the pollen, and (ii) tocopherols increase with the progression of tepal senescence, and most particularly in cut flowers, whih show advanced senescence (reduced longevity).

P04-005: CHARACTERIZATION OF OLIVE S-ADENOSYL METHIONINE DECARBOXYLASE AND SPERMIDINE SYNTHASE: INDUCTION AND SPATIAL ORGANIZATION OF POLYAMINE BIOSYNTHESIS DURING FLOWER OPENING AND EARLY FRUIT DEVELOPMENT

Gomez-Jimenez, M.[1] - Gallardo, M.[2] - Sanchez-Calle, IM.[3] - Paredes, MA.[1]*
[1]University of Extremadura
[2]University of Vigo
[3]University of Granada
**Corresponding author e-mail: mcgomez@unex.es*

Polyamines (PAs) are required for cell growth and cell division in eukaryotic and prokaryotic organisms. The present study is aimed at understanding the developmental regulation of PA biosynthesis and catabolism during flower opening and early fruit development in relation with fruit size and shape. Two full-length cDNA clones coding for S-adenosyl methionine decarboxylase (SAMDC) and spermidine synthase (SPDS) homologues, key steps in the PA biosynthesis pathway, in the stone-fruit of olive (*Olea europaea* L.) were identified and the spatial and temporal organization of these genes were described. In olive flowers, *OeSAMDC* gene transcripts were highly expressed in ovary wall, placenta, and ovules, while the *OeSPDS* transcript is confined to the ovules of ovary at anthesis stage. A correlation was detected between the SAMDC enzyme activity/accumulation transcript and spermidine (Spd) and spermine (Spm) levels during olive-fruit early development, implying that the synthesis of decarboxylated SAM might be a rate-limiting step in Spd and Spm biosynthesis. *OeSAMDC* and *OeSPDS* transcripts were co-expressed in fruit mesocarp and exocarp at all developmental stages analysed as well as in nucellus, integuments, and inner epidermis tissues of fertilized ovules. The results provide novel data
about localization of PA biosynthesis gene transcripts, indicating that transcript levels of PA biosynthesis genes are all highly regulated in a developmental and tissue-specific manner. Moreover, the results suggest that PA synthesis, conjugation, and oxidation are essential to the homoeostatic mechanism controlling PA levels and their involvement in processes such as flower opening and early cell division in stone-fruit development.

P04-006: THE REGULATION OF GERMINATION PROCESSES OF DECIDUOUS MAGNOLIA SEED BY USE HE-NE LASER

*Dmytriiev, O.**
Taras Shevchenko national university of Kyiv
**Corresponding author e-mail: m_sieboldii@ukr.net*

The receipt of plants from the seed of local reproduction is important part of introduction process. Introduction process by this way is promoted by stability of next generations to the unfavorable terms of environment. In Ukraine magnolia species bear fruit sporadically and not enough abundantly. The magnolia seeds are characterized by difficult and deep calm periods which complicate the process of reproduction. For improve introduction processes we can use treatment of seeds by red light (with use HENE LASER, λ=623,8 nm). The duration of seeds treatment was 20 seconds.The seeds treatment by red light promote to increase the hemolitic activity of lectins at the seedlings. In same time the citostatic activity of cells these seedlings has been decreased. The blood group specificity has been found for the *M. obovata Thunb, M. kobus DC, M. sieboldi K. Koch, M. x soulangiana Soul-Bod.* The highest titre of agglutination for lectins extracts from magnolias leaves was shown for plants after red light treatment. A laser irradiation can promote germination process of magnolia plants after calm periods of seeds. After laser irradiation biennial seeds were showed 85% germination. The germination level of seeds without laser irradiation has been not more than 15%. Consequently, He-Ne laser irradiation can be effective factor for activation physiology processes in the germinating seeds of magnolia.

P04-007: HERITABILITY OF FLOWERING INTENSITY IN HALF-SIB FAMILIES OF EUCALYPTUS CLADOCALYX GROWING UNDER ARID CONDITIONS: A BAYESIAN APPROACH

Mora, F.[1] - Vargas-Reeve, F.[1] - Cané-Retamales, C.[1] - Perret, S.[1] - Santos, A.I.[2]*
[1]Facultad de Ciencias Forestales, Universidad de Concepción
[2]Universidade Estadual de Maringá, Brazil
**Corresponding author e-mail: freddymora@udec.cl*

In the dry regions of Chile, prolific flowering from forest plantation is particularly advantageous for honey production, in order to supplement the erratic flowering in native plants. For this reason, the components of flowering are becoming an important selection criterion in *Eucalyptus cladocalyx*; a species suitable for areas with low water availability and their flowers provide a reliable source for the production of honey. The aim of this study was to examine the heritability of flowering intensity in 49 half-sib families of *E. cladocalyx* in southern Atacama Desert, Chile. Flowering intensity was estimated visually in 8-year-old trees. Bayesian methods were used in data analysis. Flowering intensity was found to be highly heritable. The estimate of posterior mean of the heritability (90% of credible interval) was 0.51 (0.36–0.71). The posterior mean of the genetic correlation between basal diameter and flowering intensity was positive (r=0.26) but, according to the credible interval (-0.05–0.55), it was not significantly different from zero, indicating that tree selection for only flowering intensity would have little impact on growth. Scientific information generated on the additive genetic control of flowering intensity could be used by plant breeders to improve the honey production in situations where native species are not available in sufficient quantities

P04-008: FUNCTIONAL CHARACTERIZATION OF BZIP TRANSCRIPTION FACTOR IN ARABIDOPSIS POLLEN

Gibalová-Bérešová, A.[1] - Renak, D.V.[2] - Šolcová, K.[2] - Duplakova, N.[2] - Honys, D.[2]*
[1]Charles Univerzity
[2]Institute of Experimental Botany ASCR
**Corresponding author e-mail: gibalova@ueb.cas.cz*

Haploid male gametophyte, plays a key role in plant fertility and crop production. Our ability to control and guide this process represents an effective tool for crop breeding and genetic optimalisation. We have very limited understanding of the regulatory mechanisms that have evolved to specify the gametophytic developmnetal programs. Therefore, it is necessary to identify transcription factors that are part of such haploid regulatory networks. Here we focus on bZIP transcription factors playing critical roles also in plants. We report the functional characterization of Arabidopsis thaliana AtbZIP34 that is expressed in both gametophytic and surrounding sporophytic tissues during flower development. T-DNA insertion mutants in AtbZIP34 show pollen morphological defects that result in reduced germination efficiency and slower pollen tube growth both in vitro and in vivo conditions. Light and fluorescent microscopy revealed misshapen and mispaced nuclei with large lipid inclusions in the cytoplasm of *atbzip34* pollen. Scanning and transmission electron microscopy

revealed defects in exine shape and micropatterning and a reduced endomembrane system. Several lines of evidence including the AtbZIP34 expression pattern and the phenotypic defects observed, suggest a complex role in male reproductive development that involves a sporophytic role in exine patterning, and a sporophytic and/or gametophytic mode of action of AtbZIP34 in several metabolic pathwatys, namely regulation of lipid ,etabolism and/or cellular transport. Acknowledgment: Authors gratefully acknowledge the financial support from the Grant Agency of the Czech Republic (grant 522/09/0858) and Ministry of Education of the Czech Republic (grant LC06004 and OC10054).

P04-009: IDENTIFICATION AND CHARACTERIZATION OF PECTINASE ENZYMES IN THE OLIVE POLLEN GRAIN

Rejón, J. - Rodríguez-García, M.I. - Castro, A.J.*
Consejo Superior de Investigaciones Científicas (CSIC)
**Corresponding author e-mail: jdavid.rejon@eez.csic.es*

Pectinases might play a key function in pollen tube entry through the papilla cells and pollen tube growth regulation [1, 2]. In the olive, the composition and distribution of pectins in the pollen tube wall have been studied [3]. With the aim of determining the existence of pectinolytic activity in this species, we carried out *in vitro* enzyme assays using esterified pectin as substrate. We confirmed the existence of active pectinases in the mature pollen grain. The pectinolytic activity remained unchanged during the hydration and the early steps of pollen tube growth, but abruptly decreased after 6 h of germination. We also detected the presence of pectinase activity in the germination medium, suggesting that pectinolytic enzymes might be released from pollen during the process. The pectinase enzymes pattern was also determined in polyacrylamide gels using β-naphtyl acetate as substrate. Thus, two pectinolytic bands of 24 and 27.5 kDa, respectively, were detected in the mature pollen grain and during germination. The intensity of these two bands correlated well with the pectinase activity levels reported *in vitro*. These proteins were excised from gels and analyzed by mass spectrometry. They showed homology with a partial sequence of a putative pectin methylesterase from olive (accession no. ABS72005). At ultrastructural level, the pectinase activity was visualized as electron-dense precipitates, and it was mainly located in the vicinity of the plasmalemma and the intine wall layer. [1] Bosch et al. (2005) Plant Physiol. 138: 1334 [2] Chen & Ye (2007) J. Integ. Plant Biol. 49: 94 [3] Majewska-Sawka et al. (2002) Sex. Plant Reprod. 15: 21 This work was supported by the MICINN (project AGL2008-00517/AGR) and the Junta de Andalucía (project P06-AGR-01791)

P04-010: DEVELOPMENTAL CHANGES IN THE PRESENCE OF ROS (REACTIVE OXYGEN SPECIES) IN THE STIGMA AND THEIR RELATIONSHIPS WITH STIGMA FUNCTIONALITY.

Alché, J. - Rejón, JD. - Jiménez-Quesada, M.J. - Rodríguez-García, M.I. – Zafra, A.*
Estación experimental del Zaidín.csic.
**Corresponding author e-mail: juandedios.alche@eez.csic.es*

The presence of reactive oxygen species (ROS) and NO in the reproductive tissues of several Angiosperms has been investigated. Early studies have shown that these molecules play putative roles as signaling molecules during the interaction pollen-pistil. However, their occurrence in other reproductive phases has not been investigated. We have analyzed the presence of ROS and NO in floral tissues over different developmental stages (unopened flower buds, recently opened flowers, dehiscent anthers and flowers after fertilization) by using different fluorophores and confocal laser scanning microscopy. The study was carried out in species with different types of stigmas and systems of compatibility (olive tree, orange, tomato, pepper, pea and *A. thaliana*). Although the presence of ROS in the stigmas was detected at higher levels during those developmental phases considered “receptive” to pollen interaction, these molecules were also present at early (unopened flower) or later (post-fertilization) stages. The biological significance of the presence of these products may differ between these stages, including defence functions, signaling and senescence. The study confirms the enhanced production of NO by pollen grains and tubes during the receptive phase, and the decrease in the presence of ROS when NO is actively produced [1].
[1] Zafra et al. BMC Plant Biology 2010, 10:36
Acknowledgments. This work was supported by MICINN (project BFU2008-00629/BFI), and by JA (project P06-AGR-01791). The authors want to thank S. Hiscock (U. Bristol U.K.) for helpful comments.

P04-011: CELLULAR STUDY OF TEOSINTE ZEA MAYS SUBSP. PARVIGLUMIS CARYOPSIS DEVELOPMENT SHOWING SEVERAL PROCESSES CONSERVED IN MAIZE

Kladnik, A.[1] - Dermastia, M.[2] - Dolenc Koce, J.[1] – Chourey, PS.[3]*
[1]University of Ljubljana, Biotechnical Faculty, Department of Biology
[2]National Institute of Biology, Slovenia
[3]U.S. Department of Agriculture–Agricultural Research Service, and University of Florida, USA
**Corresponding author e-mail: ales.kladnik@bf.uni-lj.si*

The evolutionary history of maize (*Zea mays* subsp. *mays*) is of general interest because of its economic and scientific importance. Here we show that many cellular traits described previously in developing caryopses of maize are also seen in its wild progenitor teosinte (*Zea mays* subsp. *parviglumis*). These features, each with a possible role in development, include: (1) an early programmed cell death in the maternal placento-chalazal (P-C) layer that may lead to increased hydrolytic conductance to the developing seed; (2) accumulation of phenolics and flavonoids in the P-C layer that may be related to antimicrobial activity; (3) formation of wall ingrowths in the basal endosperm transfer layer (BETL); (4) localization of cell wall invertase in the BETL, which is attributed to the increased transport capacity of photosynthates to the sink; and (5) endoreduplication in endosperm nuclei suggested to contribute to increased gene expression and greater sink capacity of the developing seed. In maize caryopsis, these cellular traits have been previously attributed to domestication and selection for larger seed size and vigor. Given the conservation of the entire cellular program in developing teosinte caryopses described here, we suggest that these traits evolved independently of domestication and before human selection pressure.

P04-012: HOW TO IDENTIFY NEW & IMPORTANT CIS-ELEMENTS? BIOINFORMATIC APPROACH AND FUNCTIONAL ANALYSIS OF REGULATORY ELEMENTS IN PROMOTERS OF POLLEN EXPRESSED GENES.

Šolcová, K.[1]* - Gibalová, A.[2] - Honys, D.[1]
[1]Institute of Experimental Botany of the AS CR
[2]Faculty of Science, Charles University in Prague
**Corresponding author e-mail: matczuk@ueb.cas.cz*

For understanding of regulatory mechanisms specifying and controlling male gametophytic developmental program, it is necessary to identify regulatory sequence motifs (cis-elements) in promoters of pollen-expressed genes and to isolate interacting protein partners responsible for their transcriptional activation or repression. Regulatory elements present in gene promoters include several classes of functional DNA sequence motifs recognized by appropriate proteins (trans-elements), components of the RNA polymerase transcription machinery and complementary transcription factors. Here we demonstrate identification and

analysis of gene promoters by bioinformatic tools and experimental methodology. Analysed promoters were selected from a substantial fraction of putative pollen-specific genes showing early or late expression profiles and candidate motifs were identified using bioinformatic tools. Putative regulatory motifs were functionally analysed. First, the activity of candidate promoters was tested using appropriate promoter::eGFP:GUS constructs. Selected motifs were excluded from corresponding genes using PCR-based mutagenesis and altered promoters were subcloned into eGFP::GUS-harbouring vectors. Both stable and transient transformation into Arabidopsis and tobacco pollen was performed. Authors gratefully acknowledge the financial support from Grant Agency of the Czech Republic 522/09/0858, from Ministry of Education of the Czech Republic OC10054 and from the European Fund for Regional Development, the Operational Programme Prague - Competitiveness, project no.: CZ.2.16/3.1.00/21159

P04-013: PROTEOMIC DISSECTION OF THE STIGMA EXUDATE

Castro, A.[1] - Rejón, JD. - Delalande, F.[2] - Schaeffer-Reiss, C.[2] - Van Dorsselaer, A.[2] - Rodríguez- García, MI.[1]*
[1]Consejo Superior de Investigaciones Científicas (CSIC)
[2]University of Strasbourg/CNRS
**Corresponding author e-mail: antoniojesus.castro@eez.csic.es*

At anthesis, the olive pistil is composed of a bilobed wet stigma covered with intact surface cells that protrude as papillae [1]. The stigma surface is coated by a viscous secretion containing proteins, lipids and polysaccharides. In the present work, we carried out for the first time a large scale analysis of a plant stigma secretome using a proteomic approach. Thus, after analytical separation by SDS-PAGE, the olive exudate yield up to 28 distinguishable protein bands in a molecular mass range from 14 to 120 kDa. All these bands were systematically excised and digested with trypsin followed by MS/MS and *de novo* sequencing analyses. This dual approach allowed us to identified 64 different proteins in a plant species that is not annotated in databases. Identified proteins were grouped in different functional categories, including protein metabolism (13), carbohydrate and energy metabolism (12), defence and stress response (6), signalling (6), amino acid biosynthesis (5), transport (5), cell wall remodelling and metabolism (4), cytoskeleton dynamics (3), lipid metabolism (1), miscellaneous (6) and some other proteins of unknown function (3). The functional meaning of these data in the context of the progamic phase is discussed.

[1] Serrano et al. (2008) Sex. Plant Reprod. 138: 1334

This work was supported by the MICINN (project AGL2008-00517/AGR) and the Junta de Andalucía (project P06-AGR-01791). JD Rejón thanks the MICINN for providing FPU grant funding.

P04-014: THE ESSENTIAL ROLE OF THE PLASTIDIAL GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IN VIABLE POLLEN DEVELOPMENT IN ARABIDOPSIS

Muñoz-Bertomeu, J.[1] - Cascales-Miñana, B.[1] - Anoman, AD.[1] - Tornero-Flores, M.[1] - Nunes-Nesi, A.[2] - Fernie, A.R.[2] - Segura, J.[1] - Ros, R.[1]
[1]Universidad de Valencia
[2]Max Planck Institut

In this work we show that the deficiency in the plastidial glycolytic glyceraldehyde-3-phosphate dehydrogenase (GAPCp) leads to male sterility in *Arabidopsis thaliana*. Pollen from homozygous *gapcp* double mutant plants (*gapcp1gapcp2*) have shrunk and collapsed shapes and are unable to germinate when cultured in vitro. The pollen alterations observed in *gapcp1gapcp2* were attributed to a disorganized tapetum layer. Accordingly, the expression of some of the genes involved in tapetum development was down-regulated in *gapcp1gapcp2*. The fertility of *gapcp1gapcp2* was rescued by transforming this mutant with a construct carrying the *GAPCp1* cDNA under the control of its native promoter (*pGAPCp1::GAPCp1c*). However, the *GAPCp1 or GAPCp2* cDNA under the control of the 35S promoter (*p35S::GAPCp*), which is poorly expressed in the tapetum, did not complement the mutant fertility. Mutant GAPCp isoforms deficient in the catalytic activity of the enzyme were unable to complement the sterile phenotype of *gapcp1gapcp2*, thus confirming that both the expression and catalytic activity of GAPCp in anthers are necessary for mature pollen development. A metabolomic study in flower buds indicated that the most important difference between the sterile (*gapcp1gapcp2, gapcp1gapcp2-p35S::GAPCp*) and the fertile(wild type plants, *gapcp1gapcp2-pGAPCp1::GAPCp1c*) lines was the increase in the signalling molecule trehalose. We provide evidence for the crucial role of plastidial GAPCps in pollen development and suggest for the first time that plastidial glycolysis may regulate plant development through the trehalose signalling pathway.

Funding: Spanish Government (BFU2009-07020; FPU fellowship to B. C-M and AECI to A.D. A.), Valencian Government (ACOMP/2009/328, PROMETEO/2009/075).

P04-015: THE ROLES OF DORNOESCHEN AND DORNROESCHEN-LIKE IN FLORAL ORGAN PATTERNING VIA AUXIN

Chandler, J. - Chandler, JW - Comelli, P. - Jakobs, B. - Werr, W.*
Departement of Developmental Biology, Cologne University
**Corresponding author e-mail: flori43@hotmail.com*

Auxin maxima are required for floral organ initiation, and therefore, floral organ merosity is affected by auxin synthesis, polar transport and signalling. Mutations in the ERF AP2 transcription factors *DORNROESCHEN (DRN)* and *DORNROESCHEN-LIKE (DRNL)* play a fundamental redundant role in embryonic patterning but also control stamen number. In the embryo, *DRN* has been placed both upstream and downstream of auxin function and under transcriptional control by MONOPTEROS. To further place *DRN* and *DRNL* genetically in embryogenesis and floral development with respect to genes in auxin biosynthesis, transport and signalling pathways, we have combined *drn* and *drnl* with *yucca, pin1*, and *pid* mutants. Mutations in *DRNL*, but not in *DRN* show genetic interactions with those in *YUC4, EBP* and *PID*, different to those interactions with the same genes during embryogenesis, indicating distinct functions for *DRN* and *DRNL* in floral development. Higher order mutants show that *DRN* and *DRNL* act redundantly with local auxin biosynthesis in floral organogenesis and also that *DRN*, *DRNL* and a further *ERF* gene, *ETHYLENE BINDING PROTEIN (EBP)*, redundantly control carpel development. Expression of the *DRNL* gene and protein is specific to all floral organ anlagen, but absent from the meristem, whereas that of *DRN* and its protein is more diffuse, and in the meristem. Evidence for discrete *DRN* and *DRNL* functions in floral development is also provided by promoter swap experiments and complementation of a strong *drnl* allele. EMS mutagenesis to isolate enhancers of *drnl* and suppressors of *drnD* has led to putative floral mutants which will be presented and discussed. Taken together, our data provide evidence for novel transcription factor functions which integrate auxin-regulated floral organogenesis.

P04-016: REGULATION OF FLOWERING IN TIMOTHY (PHLEUM PRATENSE)

Jokela, V. – Seppänen, M.*
University of Helsinki
**Corresponding author e-mail: venla.jokela@helsinki.fi*

Timothy (*Phleum pratense* L.) is the most important perennial forage grass in Scandinavia and also cultivated in other cool areas of Europe and North America. It has good winter hardiness and the nutritive value and palatability of harvested biomass is

good. Stems constitute major portion of the yield, both amount and quality. We have examined the role of different flowering pathways (vernalization, photoperiod, GA) on the regulation of canopy structure in different timothy genotypes. Timothy flowers when critical day length exceeds and it does not require double induction for flower induction. Our results show that vernalization enhances flowering and the number of flowering tillers. There exists difference between genotypes in the flowering rate and number of flowering tillers after vernalization. Transcript of two flowering genes, *VRN1* and *VRN2*, were examined after vernalization. The level of *VRN1* expression remained high during prolonged vernalization. The role of *VRN2* gene is unclear in timothy. In 12 h photoperiod all examined genotypes remained at a vegetative stage, but in 16 h these genotypes formed flowers. A single GA treatment didn´t affect flower formation in timothy similarly as it has been shown for example in *Lolium temulentum*. Our preliminary results show that the regulation of flowering induction in timothy is unique compared to other grasses, ad currently we are analyzing sequencing data on various flowering pathways.

P04-017: STUDYING THE MOLECULAR REGULATION OF VERNALIZATION IN LILY

Zaccai, M.- Lugassi-Ben Hamo, M. – Mazor, I.*
Ben Gurion University
**Corresponding author e-mail: mzaccai@bgu.ac.il*

In lily (*Lilium longiflorum*), bulb vernalization is not only an obligatory requirement for flowering, but also the most important factor affecting flowering time and quality. However, the molecular regulation of the response to vernalization and of floral transition remains largely unclear in lily. Evidence mainly obtained from Arabidopsis and wheat indicates that the general pattern of the vernalization response, namely a floral repressor inhibited by cold exposure, is conserved among different species. Yet, the type of genes involved in the process differ between species. To identify candidate genes involved in the vernalization response in lily we took a differential expression approach. Lily bulbs were vernalized at 4oC (V) or kept at 25oC (Non Vernalized, NV) for 9 weeks and subtractions libraries were generated to detect differentially expressed genes from V and NV meristems. Clone sequences were analyzed using annotations databases. Overall transcription (amount of mRNA from total RNA), as well as homology to known genes were much higher in V than in NV meristems. Clones from the subtraction libraries showed homology to genes involved in dormancy, chromatin modification and floral transition. This study represents a first step towards elucidation of the molecular regulation of vernalization in lily, and addresses fundamental questions regarding the conservation of the vernalization response among higher plants. Vernalization-related genes could be used in the future, to manipulate this trait in important crops.

P04-018: IMPROVEMENTS ON MICROSPORE EMBRYOGENESIS INDUCTION IN OLIVE: EFFECT OF VARIOUS ENDOGENOUS AND EXOGENOUS FACTORS

Prem, D.[1] - Bueno, MA.[2] – Testillano, PS.[1] - Risueño, MC.[1]*
[1]Centro de Investigaciones Biológicas, CSIC
[2]CIFOR-INIA
**Corresponding author e-mail: deepakandrewprem@gmail.com*

DP and MAB contributed equally to this work. The existing knowledge for the switchover from gametophytic to embryogenic pathway of microspores in trees is still scarce. Induction of microspore embryogenesis in olive has been reported by the development of isolated microspores *in vitro* system (1), and cellular markers of the reprogramming process have been characterized (2). In this work we have studied several critical endogenous (plant genotype, time of bud collection, microspore stage, thermal pre-treatment) and exogenous (media composition, carbon source, stress treatments after microspore isolation, culture temperature, cell density) factors that affect microspore embryogenesis induction. Some modifications on the pre-treatment, processing and microspore isolation methods have been developed to improve the protocol of the culture system. Monitoring of the microspore development and multicellular proembryo formation was accomplished by cytochemistry at light and fluorescence microscopy. Cold pre-treatment of buds and cold treatment of isolated microspores with or without starvation inducing agents such as polyols showed a marked increase in frequency of embryogenesis induction. A cell density of 20 – 50,000 cells was essential to keep a congenial osmotic environment and the choice of carbon source had a marked effect on the development of embryogenic cells. Maltose provided an appropriate osmotic balance leads to the development of multicellular structures. These new insights offer several clues to fine tune the microspore embryogenesis process in olive.
Bueno et al. 2005. Acta Physiol. Plantarum 27, 695-702. Solís et al. 2008. Plant Sci. 174, 597-605.
Work supported by MICINN projects AGL2008-04255 and BFU2008-00203.

P04-019: UNCOUPLING DELLAS FROM GA-SIGNALLING DURING ARABIDOPSIS FRUIT DEVELOPMENT

Fuentes, S.[1] - Alvey, L.[2] - Ljung, K.[3] - Sorefan, K.4 – Harberd, N.P. [2] - Østergaard, L.[1]*
[1]John Innes Centre
[2]University of Cambridge, Department of Plant Sciences
[3]Umeå Plant Science Centre
4University of East Anglia
**Corresponding author e-mail: sara.fuentes@bbsrc.ac.uk*

Fruit initiation and fruit development ensure the efficient survival and dispersal of flowering plants. These processes are energetically costly and, consequently, fruit development is tightly controlled by a complex regulatory network. The hormone gibberellin (GA) plays a pivotal role in this network and previous studies have shown that GA promotes growth by inducing degradation of the growth-repressing DELLA proteins. However, the extent to which DELLA proteins contribute to GA-mediated gynoecium and fruit development remains to be clarified.
To shed light on this area, we provide an in-depth characterisation of the role of DELLA proteins in fruit-set and growth. We show that DELLA proteins not only regulate reproductive organ size but are also involved in the control of wider aspects of plant sexual reproduction. By utilising the facultative parthenocarpy phenotype of *della* mutants we show that RGL1 and RGL2 are major repressors of pistil growth in the absence of fertilisation. And, for the first time, we demonstrate the existence of a DELLA-independent GA response which functions during fruit development. Our results show that DELLA proteins are key regulators of gynoecium and fruit development. Moreover, control of GA-signalling during fruit development is likely to rely on additional levels of complexity as suggested by the existence of a DELLA-independent GA response.

P04-020: GENETIC AND HORMONAL CONTROL BY INDEHISCENT FOR FRUIT PATTERNING IN ARABIDOPSIS

Girin, T.[1] - Sorefan, K.[1] - Arnaud, N.[1] - Paicu, T.[2] - Fuentes, S.[1] - O'Brien, M.[2] - Wood, T.[1] -*
Sablowski, R.[1] - Smyth, D.R.[2] - Østergaard, L.[1]
[1]John Innes Centre
[2]Monash University
**Corresponding author e-mail: girint@bbsrc.ac.uk*

The bHLH transcription factor INDEHISCENT (IND) is essential to pattern the Valve Margins (VMs), which are specialised tissues where dehiscence of the silique takes place to release the seeds at maturity. We have previously shown that IND regulates

the expression of *PID* and *WAG2* kinase genes, thus affecting the subcellular localisation of PIN auxin efflux carriers and creating an auxin minimum necessary for VM formation. Our recent work, primarily based on expression analysis and ChIP experiment, has led to the identification of two other *IND*-dependant pathways required for proper development of VM tissues. IND directly regulates Gibberellins biosynthesis, which appears to be crucial as a local depletion in bioactive Gibberellins affect the formation of VMs. IND also directly activates the expression of *SPATULA (SPT)*, a bHLH-encoding gene previously described in the patterning of other fruit tissues. IND and SPT proteins interact when expressed in epidermal onion cells and in yeast, and the analysis of over-expressing *Arabidopsis* lines suggests that this interaction is important for the function of the proteins in planta. Finally, phenotypic analysis of single and double mutants shows that *IND* and *SPT* are together required for the patterning of several fruits tissues ; this leads to the identification of previously unknown roles for *IND* in stigma and transmitting tract formation and for *SPT* in VM development. Cross talks between the three *IND*-regulated pathways involved in VM patterning will be discussed.

P04-021: B-FUNCTION MADS-BOX GENES IN LEGUMES: GENE DUPLICATION AND SUBFUNCTIONALIZATION IN MEDICAGO TRUNCATULA

Roque, E. - Rochina, MC. – Serwatowska, J. – Yenush, L. - Beltrán J.P. - Cañas L.A.*
Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV)
**Corresponding author e-mail: edroque@ibmcp.upv.es*

Using the MADS-box of the *AmDEFICIENS* gene as a probe to screen a floral cDNA library of *Medicago truncatula*, we have isolated four B-function genes involved in the specification of petal and stamen identity: two *APETALA 3* (*MtAP3* and *MtNMH7*) and two *PISTILLATA* (*MtPI* and *MtNGL*9) orthologs. The expression patterns of these genes have been studied by *in situ* hybridization analysis and we have also performed two-hybrid assays to identify possible protein-protein interactions. Both MtPI and MtNGL9 proteins lack the PI motif described as essential for the functionality of PISTILLATA in *Arabidopsis*. Using RNAi and *Tnt1* mutagenesis, we generated *MtPI* loss-of-function mutants. These plants showed flowers with sepaloid petals and carpelloid stamens. Our results suggest that the differences in the functional constrictions among the paralogs *MtPI* and *MtNGL9* could have generated a high degree of structural conservation in *MtPI*, which maintains a predominant role during floral development, whereas *MtNGL9* could have accumulated mutations in the expression activation elements of the regulatory regions, in addition to acquiring new *cis*-regulatory elements, thus providing new spatial expression patterns. Therefore, these genes could have followed a quantitative subfunctionalization process in parallel, followed by a possible neofunctionalization process. The *M. truncatula AP3* orthologs could have undergone a qualitative subfunctionalization with the subsequent partition of the ancestral functions. This hypothesis is reinforced by the non-overlapping spatial expression patterns in the 2nd and 3rd floral whorls and by the phenotypes of partial loss-of-B function in both mutants.

P04-022: GENOME-WIDE ANALYSIS OF FLORAL ORGAN FORMATION

Raganelli, A. - Wellmer, F.*
Smurfit Institute of Genetics Trinity College Dublin
**Corresponding author e-mail: andrea.raganelli@gmail.com*

How complex organs are formed by multicellular organisms is a key question in developmental biology. Flower formation is an excellent system for studying the molecular mechanisms underlying organogenesis in plants. Several genes that control flower development have been identified in the model plant *Arabidopsis thaliana* but only few of them are known to specifically control the formation of the different types of floral organs, i.e.: sepals, petals, stamens and carpels. Most of these genes encode transcription factors or other proteins involved in the regulation of transcription, indicating the existence of a complex gene regulatory network that underlies flower development. Nevertheless, the target genes of the known transcriptional regulators, the regulatory elements, and how these genes interact is still for the most part unknown. We focus on the dissection of the genes network that underlies floral organ formation by the identification and functional characterization of target genes of key regulatory transcription factors at a global level. To this end, we are generating and testing different induction systems to separately control the onset of flowering with a floral induction system that allows the isolation of a large number of synchronized floral buds and the expression of artificial microRNAs (amiRNAs) directed against the transcription factor analyzed. This will enable us to specifically knock down the expression of floral regulators at different stages of flower development and through the use of genome-wide expression profiling by DNA microarray analysis, to identify the downstream responsive genes.

P04-023: FUNCTIONAL STUDY OF TRANSCRIPTION FACTORS POTENTIALLY INVOLVED IN THE JUVENILE TO ADULT PHASE TRANSITION IN CITRUS

Castillo, MC.[1] - Gas, M.E.[2] - Navarro, L.[1] - Ancillo, G.[1]*
[1]Instituto Valenciano Investigaciones Agrarias
[2]Institut de Génétique et de Biologie Moléculaire et Cellulaire
**Corresponding author e-mail: mccastillo@ivia.es*

In higher plants, development has two different phases, juvenile and adult. Plants are not reproductively competent until they reach the adult phase. In citrus the juvenile phase can be as large as 5-20 years depending on the variety what is a serious constraint for molecular and conventional breeding of citrus genotypes. With the aim of identifying regulatory genes involved in the process of juvenile to adult phase transition we developed a citrus transcription factors (TF) microarray, and used it to screen for TF differentially expressed between juvenile and adult plants in four citrus species: Sweet Orange (*C. sinensis* (L.) Pineapple), Tangor Murcott (*C. reticulata x C. sinensis*), Grapefruit (*C. paradisi* Macf. Duncan) and Rough Lemon (*C. limon* (L.)). Several transcription factors were identified as differentially expressed. Some of these genes showed high homology with MADS-box genes which are a diverse class of TF that are involved in regulating developmental processes, particularly meristem and organ identity during floral development. On the other hand, some other genes showed no significant homology to genes of known function, indicating that they may be specific factors. Since the juvenility in citrus and *Arabidopsis* has differential aspects, those genes could be good candidates to study these particular aspects.
To deep into their functional study, transgenic *Arabidopsis* lines over expressing some of these genes have been generated. Number of leaves without abaxial trichomes, flowering time and morphology of leaves, siliques and hole plant have been analysed. Some lines showed shortening of the juvenile phase, indicating that those genes are most likely involved in determining the reproductive phase transition in citrus.

P04-024: STRUCTURE AND DNA BINDING SPECIFICITY OF THE LEAFY FLORAL TRANSCRIPTION FACTOR

Moyroud, E. – Blanchet, S. – Minguet, E.G. – Hames, C. – Thevenon, E. – Parcy, F.*
Physiologie Cellulaire Végétale. CNRS-INRA-CEA-UJF
**Corresponding author e-mail: eugeniogomez@ono.com*

The LEAFY gene is present in all land plant and plays a central role in flower development of angiosperms [1]. It encodes a plant

specific transcription factor with no resemblance to other proteins and unknown origin. In Arabidopsis, LEAFY participates to triggering the floral transition and subsequently patterns the floral meristem by inducing the expression of several floral organ identity genes. Characterizing LEAFY's molecular action and evolution is central to understand the evolution of plant reproductive structures. By combining biochemical and structural analyses, we have shown that LFY is a novel type of Helix-Turn-Helix transcription factor, which binds DNA as a cooperative dimer [2]. Combining SELEX (binding sites selection assay) experiments with quantitative affinity measurements, we have established a position specific weight matrix that allows accurate prediction of binding site affinity. The implications of these findings in Arabidopsis and other plants will be discussed.[1] Moyroud et al. (2009) J. Plant Biology 52:177.[2] Hamès et al. (2008) EMBOJ 27:2628.Acknowledgements. This work was supported by an ATIP+ from the CNRS, a PhD fellowship from the Rhône-Alpes Region Cluster 9 and the French ANR (ANR-07-BLAN-0211-01 'Plant TF-Code' and ANR-BSYS-002-03 'Flower Model').

P04-025: AUXIN POLAR TRANSPORT AND PIN LOCALIZATION PATTERN DURING CONIFER EMBRYO DEVELOPMENT

Hallberg, H. - Palovaara, J. – Hakman, I.*
School of Natural Sciences, Linnaeus University
**Corresponding author e-mail: henrik.hallberg@lnu.se*

Evidence implicates the plant hormone auxin and its polar transport, mainly established by the PIN family of auxin efflux transporters, in the patterning of plant embryos. We recently characterized the gymnosperm homologue *PaPIN1*, from the conifer Norway spruce (*Picea abies* [L.] Karst.), and followed its expression pattern during somatic embryo development, where it correlate with the auxin distribution pattern as shown by using an immunohistochemical method (Palovaara et al. 2010. Tree Physiol. 30:479-489). We have here used a polyclonal antibody raised against the PaPIN1 protein for immunolocalization studies, and show that the PIN distribution pattern in seed- and somatic embryos has many similarities to that seen in angiosperm embryos. In addition to common features seen during embryo development of gymnosperms and angiosperms, we also discuss certain differences in the embryogeny of the two taxa.

P04-026: ULTRASTRUCTURE OF THE MEGAGAMETOPHYTE DEVELOPMENT IN TILLANDSIA (BROMELIACEAE)

Papini, A. - Mosti, S. – Tani, G. - Di Falco, P. – Brighigna, L.*
University of Florence
**Corresponding author e-mail: alpapini@unifi.it*

Tillandsia is a genus of Bromeliaceae, lacking an absorbing root system, substituted by absorbing trichomes specialized for direct uptake from air (1). Many aspects of the reproductive biology have been investigated in this genus. Nevertheless few data are available about the ultrastructure of the megagametophyte development. We investigated this structure by Transmission Electron Microscope (TEM), Light/Fluorescence Microscope and TUNEL assay for Programmed Cell Death (PCD). The nucleus of the survived megaspore showed a huge nucleulus. Many cup-shaped chloroplasts were present. At 8-nuclei stage autophagy phenomena in the cytoplasm were evident. At the final stage the central binucleate cell had a vacuole filling about 50% of the cytoplasm. One-two layers of nucellar cells around the gametophyte showed signs of PCD such as cell and nucleus shrinkage, enlarging of the ER system, persisting mitochondria and TUNEL positivity. The Thiery staining showed that starch was scarce in the gametophyte and more abundant in the nucellus chloroplast. The megagametophyte development in Tillandsia was monosporic of the Polygonum type (2) with some specific ultrastructural features distinguishing it from other gametophytes, such as the presence of autophagic events in the 8 nuclei phase and the scarce presence of starch in the mature gametophyte. Literature cited
1- Papini, A., G. Tani, P. Di Falco, and L. Brighigna. 2010. The ultrastructure of the development of *Tillandsia* (Bromeliaceae) trichome. *Flora* 205(2): 94-100.
2- Willemse M. T. M. and van Went J. L. (1984) The female gametophyte. In B. M. Johri (Ed.) Embryology of the angiosperms. Springer Verlag, Berlin, Germany.

P04-027: PARENTAL EFFECTS AS DETERMINANTS OF POLYPLOID FERTILITY IN ARABIDOPSIS

Duszynska, D.[1] - McKeown, P.[1] - Vilhjalmsson, B.[2] - Comte, A.[1] - Donoghue, M.T.A.[1] - Pietraszewska, A.[3] - Nordborg, M.[2] - Juenger, T.E.[4] – Sharbel, T.F.[5] – Spillane, C.S.[1]
[1] Department of Botany and Plant Science, Aras de Brun, National University of Ireland, Galway
[2] Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria and Molecular and Computational Biology, University of Southern California, Los Angeles
[3] Molecular Cytology, Swammerdam Institute for Life Sciences (SILS), University of Amsterdam, The Netherlands
[4] Section of Integrative Biology & Institute for Cellular and Molecular Biology, University of Texas, Austin, USA
[5] Apomixis Research Group, Dept of Cytogenetics & Genome Analysis, Leibniz Institute of Plant Genetics & Crop Plant Research (IPK), Gatersleben
**Corresponding author e-mail: p.c.mckeown.99@cantab.net*

Hybridization and polyploidy events trigger genomic shock and lead to reduced fertility. However, many plants tolerant these events well, including many crops. To understand the basis for this tolerance, we generated a series of 169 Arabidopsis triploids, each consisting of a different accession into which an extra copy of the Landsberg-0 genome had been introduced. The resulting plants had very variable reproductive modes with a strong heritable component. More surprisingly, some accessions had significant fertility differences depending on whether the additional genome copy was maternally or paternally inherited. Differences in cross direction also affected the size of seed produced by these triploids, and altered features of the following generation, including their tendency to chromosome loss and aneuploidy. This suggests that parent-of-origin effects can have an epigenetic impact on the later plant generations. Finally, we performed linkage disequilibrium association mapping (LDAM) on SNPs defined between our accessions, and mapped the loci, genome features and putative protein-protein complexes which are likely to be responsible for the effects of genetic backgrounds and cross directions on polyploid fertility in Arabidopsis.

P04-028: NOVEL REGULATORS OF TERMINAL FLOWER 1 AFFECT PLANT ARCHITECTURE

Fernández-Nohales, P. - Zambrano Rodriguez, J.A. – Jiménez, C. – Madueño, F.*
Instituto Biología Molecular y Celular de Plantas (CSIC-UPV)
**Corresponding author e-mail: pedferno@ibmcp.upv.es*

During the floral transition, the shoot apical meristem (SAM) changes its identity from vegetative, when it produces leaves and shoots, to inflorescence, when it produces flowers. According with the identity of the SAM, the inflorescences can be classified as indeterminate, where the inflorescence SAM grows continuously, or determinate, where the SAM forms a terminal flower. In *Arabidopsis*, the expression of the *TERMINAL FLOWER 1 (TFL1)* gene in the centre of the inflorescence SAM prevents the expression of floral meristem identity genes in this meristem, which impedes its conversion into a flower and, therefore, the determination of the inflorescence. Thus, *TFL1* is an inflorescence meristem gene with a key role in the control of plant architecture, a function that is related to its particular expression pattern. In our lab we are interested in the identification of transcription

factors that regulate the expression of *TFL1*. A first step toward that aim has been the study of the *TFL1* promoter, where we have combined deletion analysis with phylogenetic footprinting to identify promoter regions that are critical for the expression of *TFL1*. One of these regions has been used in a screening with the yeast one-hybrid system. This has resulted in the isolation of two transcription factors, belonging to the *TCP* and *Zn-finger* families, respectively, for which no function has been reported so far. Currently, we are characterizing mutant and overexpression lines for these two genes. In both cases, we have observed phenotypes related to the architecture of the inflorescence, which supports the idea that these two transcription factors control plant architecture through the regulation of *TFL1*.

P04-029: HIGH NITRATE DECREASES SORGHUM BICOLOUR GROWTH UNDER ELEVATED CO2

Lacuesta, M.[1] - Saiz-Fernández, I.[1] - Aguirre Igartua, E.[1] - Wargent, J.[1] - Muñoz-Rueda, A.[1] - España-Diez, S.[1] - Paul, N.[2]*
[1]Department of Plant Biology and Ecology. University of Basque Country
[2]Lancaster Enviromnetal Center, Lancaster University
**Corresponding author e-mail: alcemorris@hotmail.com*

Atmospheric CO2 concentration has varied through geological times and is expected to double the current during the next 100 years. As a consequence, carbon, water and nitrogen relationships could be affected. Nitrogen is one of major factors limiting crop yield and its central role on leaf growth has long been recognized. An adequate supply of N is fundamental to optimize crop yields, but its mismanagement, such as an excessive application, can result in contamination of groundwater. In addition, although no negative effects of nitrate excess on plant development have been described, preliminary studies in our laboratory have showed a decreased growth in sorghum plants watered with high N solution. The aim of this work is to describe how different N supplies, in an enriched CO2 ambient, affect the plant development, especially at leaf level. Sorghum bicolor plants were grown in a Fitotron at 900 µL.L-1 of CO2 and watered three times a week using modified Hoagland solution containing 5, 15 and 30mM of N. Data about dry weight and leaf area were recorded every 15 days and leaf impressions were taken for further epidermic cell analysis. After 45 days, plants which were watered with higher N content showed less biomass in both shoots and roots and smaller leaves. However, leaf impressions showed higher epidermic cell area with higher N supply which suggests that the excess of nitrogen could have a negative effect on cell division.

P05 Biotechnology

P05-001: GENETIC DIVERSITY STUDIES OF COARSE AND FINE RICE USING RAPD MARKERS

Iqbal, M. - Iqbal, A. - Saeed, A. - Nisar, A.*
CABB, University of Agriculture, Faisalabad, Pakistan
**Corresponding author e-mail: ahsanuaf2003@yahoo.com*

The availability of genetically diverse gene pool is vitally important in the varietal development program. Molecular Markers are being extensively utilized to explore the genetic diversity among native and exotic germplasm.
This study was designed to reveal the genetic diversity and patterns of relationships among the 20 accessions/genotypes representative of Basmati and Non-Basmati Rice from the existing rice gene pool using RAPD (Random Amplified Polymorphic DNA) Markers.
Employing RAPD, Thirty (30) decamer oligonucleotide primers were used to estimate the genetic diversity. Out of these thirty primers, seventeen (17) primers give the polymorphic results and produced a total of 116 fragments, out of which 101 were polymorphic (87.06%) while 15 fragments were monomorphic (12.93%). Similarity coefficients had ranged from 0.47 to 0.90. The average genetic similarity was calculated 0.68 (68%). In this study, the Coarse rice genotypes showed more polymorphism (85.84%) than the Fine rice genotypes (61.76%).
Genotypes were clustered into 8 distinct groups: A, B, C, D, E, F, G and H but two genotypes i.e. Shadab and Kangni-27 showed divergence from all the genotypes of the groups. So, these diverse genotypes should be included in the breeding programme.

P05-002: VARIATIONS IN BARE-1 INSERTION PATTERNS IN BARLEY CALLUS CULTURES

Gozukirmizi, N. - Evrensel, C. – Yilmaz, S. – Temel, A.*
Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics
**Corresponding author e-mail: nermin@istanbul.edu.tr*

Stability of aging barley calli was investigated with *BARE-1* transposon specific Inter-Retrotransposon Amplified Polymorphism (IRAP) technique.
Mature embryos of barley (*Hordeum vulgare* cv. Zafer-160) were cultured on callus induction MS (Murashige and Skoog) medium supplemented with 3 mg/L 2,4-D and maintained on same medium for 30, 45 and 60 days. Ten IRAP primers were used in 25 different combinations and as a result, the variation level of DNA isolated from 30-, 45- and 60-days old calli, have been found to change 14-25%, depending on the mature embryo material and the age of callus.
While the similarity level between 30- days and 45-days old calli is 84%, it is 79% between 30-days and 60-days old calli and it is 76% between 45-days and 60-days old calli. As a result, culture conditions cause genetic variations and there are also evident *BARE-1* transposon alterations. The findings are expected to contribute to genetic engineering studies to get better results and also to understand, how transposons contribute to features like tissue culture – especially callus tissue – formation and the ability of regeneration thereof. To our knowledge, this is the first report of employment of IRAP technique in barley in terms of callus development.
Keywords *Hordeum vulgare* L.- Transposon - Tissue culture – *BARE-1* – Inter Retrotransposon Amplified Polymorphism

P05-003: METABOLIC ENGINEERING OF POTATO (SOLANUM TUBEROSUM) FOR ENHANCED ASCORBIC ACID (VIT C) ACCUMULATION

Upadhyaya, C.P. - Hemavathi – Nookaraju, A. – Mayank, A.G. – Park, S.W.*
Department of Molecular Biotechnology, College of Life & Environmental Sciences, Konkuk University, Seoul, Korea
**Corresponding author e-mail: cpupadhyay@gmail.com*

Potato (*Solanum tuberosum* L.) is an important vegetable crop which ranks fourth among the staple foods of mankind after wheat, rice and maize. Unlike most of the animals, humans lack the ability to synthesize ascorbic acid on their own due to a mutation in the gene encoding the last enzyme of ascorbate biosynthesis. As a result, vitamin C must be obtained from dietary sources like plants. The potato (*Solanum tuberosum* L. cv. Taedong Valley) was engineered for enhanced ascorbic acid (Vit C) ovr-accumulation which also leads to increase in tolerance for various abiotic stresses. A gene encoding D-galacturonic acid reductase (*GalUR*) isolated from strawberry and l-gulono-γ-lactone oxidase (*GLOase* gene), from rat cells under the control of CaMV 35S promoter was introduced in potato plants resulted in 1.6-3.0 folds increase in AsA in transgenic potato.
Integration of the *GalUR* and *GLOase* gene in the plant genome was confirmed by PCR, RT-PCR and Southern blotting. Ascorbic acid (AsA) levels in transgenic tubers were determined by high-performance liquid chromatography (HPLC). The increases in levels of AsA were positively correlated with increased GalUR and GLOase activity in both kinds of transgenics. The transgenic lines with enhanced vitamin C content showed enhanced tolerance to abiotic stresses induced by methyl viologen (MV), NaCl or mannitol as compared to control plants. The leaf disc senescence assay showed better tolerance in transgenic lines by retaining higher chlorophyll as compared to the untransformed control plants. Present study demonstrated that the over-expression of *GalUR* or *GLOase* gene enhanced the level of AsA in potato tubers and these transgenics performed better under different abiotic stresses as compared to untransformed control.

P05-004: ANIONIC SOYBEAN PEROXIDASE AND ITS APPLICATION IN ENZYME IMMUNOASSAY

*Sakharov, I.**
M.V.Lomonosov Moscow State University
**Corresponding author e-mail: sakharovivan@gmail*

Cationic horseradish peroxidase (HRP) is usually used in chemiluminescent enzyme immunoassay (CLEIA), one of highly sensitive analytical methods. However, HRP has drawbacks connecting with quick decay of CL signal and, in some cases, insufficiently high sensitivity. We demonstrated that anionic plant peroxidase isolated from soybeans (SbP) is more effectively biocatalysts than HRP-C and is able to oxidize luminol in the absence of enhancers. In addition, unlike HRP-C, SbP produces a long-term CL signal. Although SbP is a more potent biocatalyst in luminol oxidation than HRP-C itself, in the presence of enhancers HRP-C produces a higher CL intensity than SbP. On the other hand, HRP-C enhancers do not practically increase SbP-induced CL. At screening of some phenothiazines we showed that 3-(10'- phenothiazinyl)propane-1-sulfonate (SPTZ) is a first potent enhancer of CL induced by anionic SbP. Also, the simultaneous introduction of SPTZ and 4-morpholinopyridine (MORP) in the substrate mixture resulted in an additional increase of CL as well as a decrease of the lower detection limit (LDL) of SbP. The SbPcatalyzed chemiluminescent signals in presence of SPTZ and SPTZ/MORP are long-term. At comparison of the three fully optimized systems, SbP–SPTZ– MORP versus HRP-C–SPTZ–MORP versus HRP-C– PIP, it demonstrated that the SbP system possessed significantly higher sensitivity and lower LDL value.
The SbP-SPTZ-MORP system was successfully employed in CL-EIA for determination of human thyroglobulin, one of mar-

kers of thyroid gland cancer. These results open up very promising perspectives for using the SbP-SPTZ-MORP system in ultra-sensitive immunoassays.

P05-005: CALCIUM REGULATES TUBERIZATION IN POTATO THROUGH ENHANCED EXPRESSION OF CALMODULIN, CALCIUM DEPENDANT PROTEIN KINASE AND LIPOXYGENASE

Won, P. - Nookaraju, A. – Upadhyaya, C.P. - Chun, S.C. - Kim, D.H.*
Department of Molecular Biotechnology, Konkuk University, Seoul, Korea
**Corresponding author e-mail: sewpark@konkuk.ac.kr*

Calcium plays an important role in plant physiology and various plant cell signaling pathways, and alters many biochemical processes by activating specific enzymes. Cytosolic Ca^{2+} regulates the activity of different Ca^{2+} dependant protein kinases, which specifically phosphorylate various key metabolic enzymes. In the present study, the expression of two Ca^{2+} regulator proteins, calmodulin (CaM1) and Calcium Dependant Protein Kinase (StCDPK; EC 2.7.1.37), as well as the lipoxygenase (LOX; EC 1.13.11.12) were studied upon Ca^{2+} application to the single node segments of potato cv. Desiree inoculated for tuberization. Calcium at higher levels (6 mM) significantly improved the tuber number, tuber growth and tuber yield under *in vitro* conditions.
The mRNA transcript expression levels of the CaM1, StCDPK and LOX were found to be significantly higher in stolons showing positive correlation with supplemental Ca^{2+} and tuberization response.
Similar trends were observed with LOX enzyme activity, which has increased by 18% and 25% with the addition of Ca^{2+} at 3 and 6 mM, respectively to the tuber induction medium, when compared to control.
The present study reports that the increase in tuberization, tuber growth and tuber yield with the supplementation of Ca^{2+} could be attributed to the increased expression of the Ca^{2+} dependant proteins and enhanced lipoxygenase activity that played an important role in tuberization in potato.

P05-006: MOLECULAR INVESTIGATION OF TERPENE SYNTHASE GENES FROM SALVIA FRUTICOSA AND SALVIA OFFICINALIS

Bacu, A.[1] - Makris A.[1] – Owen, C.[2]*
[1]Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania
[2]Maich, CIHEAM, Greece
**Corresponding author e-mail: a_bacu@yahoo.com*

The aim of this study was to investigate the extent of variation in monoterpene synthase genes in the sage plants *Salvia fruticosa* and *Salvia officinalis*. Gene fragments from four different but closely related monoterpene synthase genes from *Salvia fruticosa* and three from *Salvia officinalis* were cloned. This was achieved by using a PCR amplification and cloning strategy based on similarities between previously reported monoterpene synthase genes from different plant species. The isolated gene fragments were sequenced and compared with each-other and with previously isolated genes. The results of sequence analysis showed that the isolated clones represented novel sequences derived from monoterpene synthase genes, which had not been previously described. Subsequently, efforts were made to isolate the full-length gene sequence of the most interesting *Salvia fruticosa* clone.
In order to produce monoterpe synthase-specific polyclonal antibodies fusion proteins were produced from two of the clones. To achieve this, subfragments of the two genes corresponding to open reading frames were PCR amplified and then cloned into the vector pBAD-TOPO. The His-tag produced fusion proteins have been used for the production of polyclonal antibodies by immunizing rabbits. It is anticipated that these antibodies will be of value to demonstrate the expression patterns of these genes.

P05-007: UPTAKE AND ACCUMULATION OF CD IN TWO WILLOW CLONES EXPOSED TO ELEVATED CONCENTRATIONS OF CD, NI AND PB-EDTA

Borisev, M.[1] – Pajevic, S.[2] - Nikolic, N.[2] - Krstic, B.[2] - Pilipovic, A.[3] - Orlovic, S.[3]
[1]Department for Biology and Ecology, Faculty of Sciences, University of Novi Sad
[2]Department for biology and ecology, Faculty of Sciences, Serbia
[3]Institute for Lowland Forestry and Environment, Serbia
**Corresponding author e-mail: milan.borisev@dbe.uns.ac.rs*

Two willow clones (*Salix alba* – clone 68/53/1 and *Salix nigra* – clone 0408) were exposed to elevated concentrations of Cd, Cd+Ni, Cd+Pb-EDTA and Cd+Ni+Pb-EDTA, each in two concentrations (10^{-4} M and 10^{-5} M) in water culture solutions. Cd content accumulated in the root, shoot and leaves was measured and analysed in order to determine phytoextraction potential of investigated genotypes. Results display supstantial amounts of Cd accumulated in aboveground tissues, proving considerable Cd phytoextraction possibilities.
Inhibition of uptake and translocation of Cd was detected in the presence of Ni in the hydroponic solution indicating to antagonism between these two metals. Similar inhibiton effect in the presence of Pb-EDTA was less expresed because of the heavy metal helation by EDTA. Competition of Cd, Ni and Pb to same metal transporters and carriers is the probable cause of the detected antagonism.

P05-008: A NEW STRATEGY TO ENHANCE THE PRODUCTION OF PHYTOSTEROLS IN DAUCUS CAROTA CELL CULTURES

Sabater, A. - Sabater-Jara, AB. – Almagro, L. - Belchí-Navarro, S. – Pedreño, M.A.*
Dpto Biología Vegetal. Facultad de Biología. Universidad de Murcia. Spain.
**Corresponding author e-mail: asj00482@um.es*

We have developed a new innovative procedure to produce phytosterols from *Daucus carota* cell cultures (1). The method is based on elicitation using cyclodextrins and methyljasmonate (MJ), separately or in combination. MJ is a key compound of the signal transduction pathway involved in the production of secondary metabolites (2) and cyclodextrins are cyclic oligosaccharides which induce a cascade of cellular events that gives rise to the accumulation of some metabolites (3), in this case, phytosterols. This new strategy to enhance phytosterol production combines the addition of these inducing factors at their optimal concentrations, optimal UV irradiation dose and time of elicitation at an optimal culture growth stage of *D. carota* cells. The results open up a new strategy for producing phytochemicals with nutraceutical and medical applications. References
1.S abater-Jara, A.B. Almagro, L. Bru, R and Pedreño, MA. 2008. P200803107
2.Gundlach H, Müller MJ, Kutchan TM and Zenk MH. 1992. PNAS 89: 2389–2393
3.Bru, R; Sellés, S; Casado, J; Belchí-Navarro, S; and Pedreño, MA. 2006. J. Agric.Food Chem.54: 65- 71
Acknowledgements
Sabater-JaraA, .B. and Almagro L. held a grant from the MICINN. This work has been partially supported by MICINN (BIO2008-02941), by Consejería de Educación, Ciencia e Investigación de la Región de Murcia (BIO BVA 07 01 0003) and, by Fundación Séneca (08799/PI/08).

P05-009: EARLY SIGNALLING EVENTS IN RESPONSE TO METHYLJASMONATE AND CYCLODEXTRINS IN TOBACCO CELLS

Pedreño, M.[1] - Almagro, L. – Pugin, A.[2]*
[1]Dpto de Biología Vegetal. Facultad Biología. Universidad de Murcia. Spain

[2]UMR Plante-Microbe-Environnement. INRA. Université de Bourgogne, Dijon. France
**Corresponding author e-mail: mpedreno@um.es*

The events occurring in elicitor-induced defence responses include reversible phosphorylation of plasma membrane and cytosolic proteins, cytosolic calcium flux, plasma membrane depolarization, defence gene expression, MAPK and NADPH oxidase activation, H_2O_2 and NO and secondary metabolite production. Methyljasmonate (MeJA) has been proposed as a key compound of the signal transduction pathway involved in the production of secondary metabolites which take part in plant defence reactions. Likewise, special attention has been paid to the use of cyclodextrins (CDs) as elicitors that induce defense responses, including phytoalexin synthesis. However, the intracellular signalling pathway induced by CDs alone or in combination with MeJA in cell cultures is completely unknown. The aim of this work is to study the early signal events in tobacco cells elicited with MeJA and CDs, particularly, the activation of cytosolic [Ca^{2+}] fluxes and ROS and NOS production. The results could generate a model of intracellular signalling pathways activated by the elicitation of plant cell cultures with CDs and MeJA.

Acknowledgements
Almagro L. held a grant from the MICINN. This work has been partially supported by MICINN (BIO2008-02941), Consejería de Educación, Ciencia e Investigación de Región de Murcia (BIO BVA 07 01 0003), and Fundación Séneca (08799/PI/08).

P05-010: BARLEY (HORDEUM VULGARE) AS A BIOREACTOR FOR PRODUCTION OF RECOMBINANT HUMAN LACTOFERRIN

*Tanasiienko, I.[1] * - Yemets, A.[1] - Radchuk, V.[2] - Blume Ya.[1]*
[1]Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, Osipovskogo, Ukraine
[2]Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany
**Corresponding author e-mail: iratanasienko@gmail.com*

Plants used as bioreactors may soon represent one of the most important developments in agriculture, pharmaceutical and chemical industries for production of therapeutic proteins, drugs and vaccines. Biotechnology plays a key role in the obtaining of high-molecular drugs and human proteins. Here we describe a system for transformation of commercial barley varieties by *hLF* gene. Two vector constructs carrying *hLF* gene driven under glutelin promoter and terminator were created. Binary vector pBiLF carrying gene of interest and selectable marker gene hpt was used for Agrobacterium–mediated transformation. Construction pHLFTubA, for biolistic transformation, included *hLF* gene and a mutant α-tubulin gene conferring resistance to dinitroaniline herbicide, trifluralin. Spring barley (11 commercial cultivars) were used for elaboration of successful transformation protocol. The regeneration potentials of these genotypes were established and four varieties revealing high level of somatic embryogenic capacity were used for further transformation. For this, mature embryos were treated with tungsten M17, then embryos were immersed into bacterial suspension and subjected additionally to vacuum infiltration. Barley callus culture was bombarded according to Abumhadi et al. (2001). Transformed cells were transferred then to respective media containing selective concentration of hygromycin or trifluralin. Seeds collected in vivo from all selected barley plants were analyzed for the stable integration of the foreign DNA by PCR analysis. The 542 length fragment of the hLF gene was amplified during molecular analysis from transgenic plants that confirm a successful integration and expression of recombinant hLF gene in transgenic barley seeds.

P05-011: COMPARATIVE STUDY OF SOMATIC EMBRYOGENESIS FROM ZYGOTIC EMBRYOS IN OLIVE TREE CULTIVARS (OLEA EUROPAEA L.)

Mazri, M.[1] - Belkoura, I.[1] - Pliego-Alfaro, F.[2]*
[1]Ecole Nationale d'Agriculture de Meknes
[2]University of Malaga, Faculty of Sciences
**Corresponding author e-mail: m.a.mazri@gmail.com*

The control of the biotechnologies relatives to the improvement and multiplication of olive trees represents an important factor in the plant improvement strategies, their conservation as well as the massive multiplication of plant free-pathogen with very short unproductive period. The *in vitro* manipulations constitute the main technique to reach these purposes. Besides, among the various possibilities of the micropropagation, the somatic embryogenesis offers several perspectives, like the massive multiplication of interesting genotypes / variety and natural hard-rooting varieties as well as massive propagation from manipulated cells. Indeed, the genetic improvement programmes are directed to solve several agronomic and commercial problems such as the regulation of fruit ripening, increase of oil content and quality, increase of cold, drought and salt tolerance, resistance to biotic stress. Radicles and cotyledons were taken from young seeds of two foreign cultivars and two Moroccan ones: Arbequine, Picual, Dahbia and Moroccan Picholine. The explants were cultivated according to a protocol performed by Pliego-Alfaro and al. (not published) which is constituted by four steps: somatic embryogenesis initiation, development, proliferation and expression. The somatic embryos obtained were then taken into germination medium.
Results have shown that Arbequine was the cultivar which gave the best average of somatic embryogenesis (7 %), followed by Dahbia (3 %) and Picual (2 %). Morrocan Picholine did not give any embryo. This comportment underlined the genotype difference described in olive trees micropropagation (Sghir and al., 2005).

P05-012: THE INDUCTION AND GROWTH OF POTATO'S MICROTUBERIZATION (SOLANUM TUBERSUM L.) VARIETY SANTA IN RESPONSE TO MISCELLANEOUS CONSISTENCIES OF BAP AND SUCROSE IN HISTOLOGICAL TISSUE CULTURE CONDITIONS

Iranbakhsh, A.[1] - Ebadi, M.[2]*
[1]Islamic Azad University, Aliabad Katoul Branch
[2]Islamic Azad University, Damghan Branch
**Corresponding author e-mail: iranbakhshar@yahoo.com*

In histological tissue culture conditions, the impact of different consistencies of BAP and Sucrose as inducing compounds on microtuberization and also on some parameters such as time, number, dry and fresh weights of microtubers was investigated in the present study. In order to induce the microtubers in MS liquid media, different consistencies of sucrose (30, 40, 60, 80 gl^{-1}) and BAP (1, 2, 5, 10 mgl^{-1}) and also perpetual darkness were applied. In induced media containing low densities of sucrose (30 g^{-1}), the increment of BAP consistency was of no inducing effect on microtuberization. Following the augmentation of sucrose consistency up to (40 gl^{-1}) and only in high consistency of BAP, the microtubers were induced at the end of 4th week with a short delay. Howsoever, the microtubers grew emanating from the alteration in <u>*Meristm*</u> growth pattern and biomass of <u>*Stolon*</u> sub-apical area. Moreover, the microtubers became larger and began to emerge as attached to rhizome following the enhancement in BAP consistency. Whilst the sucrose level was augmented to (60 gl^{-1}) and even in low levels of BAP, the induction of microtubers occurred with a short delay during the first two weeks until the sixth week. The aforementioned microtubers didn't survive in media culture in vitro. In high consistencies of Sucrose and BAP, the average numbers of microtubers were influenced along with the induction of microtubers until the second week. In iduction media comprising high consistencies of Sucrose (80 gl^{-1}) and BAP (10 mgl^{-1}), the microtubers dormancy and health were more likely. The topmost proportion of arid weight

of microtubers to arid weight of branches was attained in high consistencies of BAP (10 mgl^{-1}) and Sucrose (8%). The media having high consistencies of BAP (5 mgl^{-1}) and Sucrose (80 gl^{-1}), were of the utmost number of microtubers whereas the maximum fresh weight of microtubers appeared in media containing BAP (5 mgl^{-1}) and Sucrose (60 gl^{-1}). Howsoever, high consistencies of Sucrose along with high consistencies of BAP reduced the induction period and also decreased the microtubers formation to two weeks. Both Sucrose and BAP were of a paramount role on decrease and increase in microtubers' fresh weights. An escalation in Sucrose consistency had a significant impact on rise of dry weight and microtubers' biomass. To sum up, in order to select the most suitable induction media, not only the number and fresh weight of induced microtubers are to be considered but also other parameters such as health, dormancy period and the proportion of dry weight of microtubers to dry weight of branches should be fully taken into regard.

P05-013: THE EFFICIENCY OF OAT (AVENA SATIVA L.) HAPLOID PLANT PRODUCTION VIA POLLINATION BY MAIZE (ZEA MAYS L.)

Stawicka, A. - Skrzypek, E. - Czyczylo-Mysza, I. – Marcinska, I.*
The Franciszek Gorski Institute of Plant Physiology Polish Academy of Science
**Corresponding author e-mail: stawicka@gmail.com*

Oat (Avena sativa L.) is an important cereal cultivated both as animal fodder and valuable source of nutrients for people. Doubled haploids (DH) allow enhancing new cultivars breeding by improving quality of grains and shortening the time of production. Obtaining DH in cereals is possible mostly by anthers culture and wide crossing. Oat DH production still remains difficulties comparing with other cereals.

The aim of the study was to improve the oat haploid embryo production and ability to developing green plants. Forty four oat genotypes were used in the experiment. Florets were emasculated before anthesis and after that they were pollinated with maize pollen. Next picloram and dicamba were applied on ovaries. Embryos were isolated 3 to 4.5 weeks after pollination and placed on TL3 and 190-2 media with maltose. They were grown at 4°C for 0, 1 and 2 days in darkness and then at 21°C in 16h photoperiod. Ovaries treated with dicamba produced more embryos (4.1/100 florets) compared to picloram (3.5/100 florets). After 3 weeks of culture 66.7% of embryos treated with dicamba and 57.8% with picloram germinated. After 6 weeks 24.2% and 27.7% of them (respectively) developed into

plants. Embryo germination decreased with time of their isolation (from 92.3% after 3 weeks to 56.9% after 4.5 weeks). Kind of medium significantly influenced embryo development. After 3 weeks 40.4% embryos germinated on 190-2 and after 6 weeks 21.2% of them produced plants, whereas on TL3 23.6% embryos germinated and 5.6% produced plants. One day of treatment with cold increased embryo development into plants (30.6%) compared to 2 days of cold (15.3%) and 0 days of cold (24.6%). Chromosome doubling using colchicine and acclimatization until grain maturation were successful.

P05-014: LITHOSPERMUM CANESCENS (MICHX.) LEHM. HAIRY ROOTS CULTURE AS A SOURCE OF RED NAPHTHOQUINONES DEMONSTRATING CYTOTOXIC ACTIVITY

Pietrosiuk, A.[1] - Syklowska-Baranek, K.[1] – Kawiak, A.[2] - Jeziorek, M.[1] - Lojkowska, E.[2] - Chinou, I.[3]
[1]Department of Biology and Pharmaceutical Botany, Faculty of Pharmacy, Medical University of Warsaw
[2]Intercollegiate Faculty of Biotechnology, Medical University of Gdansk, Department of Biotechnology, Poland
[3]University of Athens, School of Pharmacy, Deptartment of Pharmacognosy, Greece
**Corresponding author e-mail:agnieszka.pietrosiuk@wum.edu.pl*

Lithospermum canescens (Boraginaceae) is native to Northern America. It contains shikonin type pigments:
acetylshikonin (ACS) and isobutyrylshikonin (IBS) [1]. Biological studies of shikonin derivatives showed a broad spectrum of their activities [2]. Hairy roots of *L.canescens* were maintained in liquid LS medium [3]. To enhance the red pigment production roots were cultivated for 3 weeks in liquid M9 medium [4]. In this conditions, the total ACS and IBS content (182.01 mg/l) increased almost 10 fold [5]. Apart ACS and IBS, the detailed phytochemical analysis of the red coloured fraction revealed the presence of four alkanines and shikanofurans C and D [6]. The extracts from transgenic roots were submitted to cytotoxicity assay against HL-60, HeLa and HaCaT cell lines. They proved to be the most potent against HL-60 cells (IC_{50}= 4 ± 0.3 μg/ml) after 24 hours while for HeLa and HaCaT cells the IC_{50} values were 20 ± 1.2 μg/ml and 45 ± 2.5 μg/ml, respectively.

1. Pietrosiuk A., Wiedenfeld H. (2005). Pharmaceutical Biology, 43: 189-191.
2. Papageogiou V.P. et al. (2006). Current Organic Chemistry,10: 2123-2142
3. Linsmaier E.F., Skoog F. (1965). Physiologia Plantarum, 18: 100-127.
4. Fujita Y. et al. (1981). Plant Cell Reports, 1: 61-63.
5. Sykłowska-Baranek K. et al. (in press).
6. Graikou K. et al. (in press).

P05-015: CELL SUSPENSION AND CALLUS CULTURES OF ARNEBIA EUCHROMA (ROYLE) JOHNST. FOR PRODUCTION

Syklowska-Baranek, K.[1] – Pietrosiuk, A.[1] – Kawiak, A.[2] - Jeziorek, M.[1] - Lojkowska, E.[2] - Chinou, I.[3]*
[1]Medical University of Warsaw, Department of Biology and Pharmaceutical Botany
[2]Intercollegiate Faculty of Biotechnologym – Medical University of Gdansk, Department of Biotechnology, Poland
[3]University of Athens, School of Pharmacy, Department of Pharmacognosy, Greece
**Corresponding author e-mail: k.syklowska@yahoo.com*

Arnebia euchroma (Boraginaceae) is a perennial herbaceous plant which grows widely on the mountains between 2100 and 3300 m altitude in Tianshan, Xinjiang [1]. The roots of *A.euchroma* are rich in naphthoquinone compounds, shikonin and its derivatives which shows several medicinal properties [2]. In comparison to *Lithospermum erythrorhizon*, *A.euchroma* contains much higher pigment contents and is regarded as a better source of shikonin-related compounds [3, 4]. The callus tissue of *A euchroma* were maintained on MSA solid medium [5]. The cell suspension culture was established by transferring callus tissue to liquid MSA medium. The chemical investigation of deep red pigment fraction revealed the presence of two major constituents: acetylshikonin, isobutyrylshikonin [4] and eight alkanins [6]. Combined extracts prepared from callus and cells suspension were submitted to cytotoxicity assay against HL-60, HeLa and HaCaT cell lines and proved to be the most potent against HL-60 cells (IC_{50}= 0.75 ± 0.01 μg/ml) after 24 hours while for HeLa and HaCaT cells there was no activity observed.

1. Jiang B. et al. (2005). In vitro Cellular and Developmental Biology – Plant, 41: 677-681.
2. Papageogiou V.P. et al. (2006). Current Organic Chemistry,10: 2123-2142
3. Ge F. et al. (2003). Chinese Traditional Herbal Drugs, 34: 7-10.
4. Pietrosiuk A. et al. (1999). Herba Polonica, 45:354-361.
5. Davydenkov V.N. et al. (1991). Him Farm Zh, 1: 53-55.
6. Damianakos H. et al. (in press).

P05-016: THE STUDY OF POTATO'S MICROTUBERIZATION RESPONSES (SOLANUM TUBERSUM L.) IN HISTOLOGICAL TISSUE CULTURE CONDITIONS TO THE VARIOUS LEVELS OF BENZYL AMINO POURINE

Ebadi M.[1] - Iranbakhsh, A.[2]*
[1]Islamic Azad University, Damghan Branch
[2]Islamic Azad University, Aliabad Katoul Branch
**Corresponding author e-mail: iranbakhshar@yahoo.com*

In this study, potatos cultivated through mono-nodule explanation, were moved from shoot-formation induction media to MS induction media or the purpose of microtuberization of which the consistence/density of BAP (1, 2, 5 & 10 mg^{-1}) and Sucrose (30, 40, 60 & 80 mg^{-1}) were shifted in darkness. Although no microtuberization was observed in the induction media containing 3% Sucrose, the number of white branches sprouted from peripheral buds were enhanced. It is observed that increase in BAP consistence on one hand led to deacrease in the number of branches. In media containing 4% sucrose and with low BAP consistency (1 mgl^{-1}, 2 mgl^{-1}) shoot-formation (branching) was not perceived. While BAP in the levels (5 mgl^{-1} to 10 mgl^{-1}) was augmented in the induction media containing 4% sucrose, the growth of lateral buds accompanied by delayed microtuberization was four weeks after induction. In such media, the microtubers were created from the alteration to the growth pattern in sub-apical area with positive geotropism. In BAP 10 mgl^{-1} consistency, 50% of tubers were attached to the branches. In induction media containing 6% sucrose and BAP 1 & 2 mgl^{-1}, the microtubers were grown on peripheral branches until the end of week2. In such groups, no tubers bigger than 7 mm, was seen. In sucrose with 8% consistency, the highest percentage of microtuberization was perceived in BAP different consistencies. In BAP 10 mgl^{-1} consistency joined by 8% Sucrose, the maximal number of normal tubers attached to the pedicle/rhizome with larger dimension was formed. In such induction media, the formation of microtubers in the 1st week was commenced and the 2nd week was completed. The growth of microtubers continued up to the 7th week. The latency of microtubers was protracted of which the duration was about 30 months.

P05-017: A GENETIC ENGINEERING APPROACHES TO INCREASE STARCH PRODUCTION IN PLANT

Halil, I. - Baris, I. – Tuncel, A. – Ozber, N. - Keskin, O.*
Koc University
**Corresponding author e-mail: hkavakli@ku.edu.tr*

ADP-glucose pyrophosphorylase (AGPase), a key allosteric enzyme involved in higher plant starch biosynthesis, is composed of pairs of large (LS) and small subunits (SS). Current evidence indicates that the two subunit types play distinct roles in enzyme function. Recently the heterotetrameric structure of potato AGPase has been modeled. In the current study, we have applied computational tools and identified critical amino acids of the potato AGPase LS and SS subunits that interact with each other during the native heterotetrameric structure formation. We have further shown the role of the LS amino acids in subunit-subunit interaction by yeast two-hybrid, bacterial complementation assay and native gel. During ouranalysis we have found that lateral interaction of the LS-SS is much stronger than the longitudinal one, and it is mainly mediated by hydrophobic interactions. Also, we have utilized a reversion genetic approach to obtain stable heterotetrmeric AGPase using one of the LS AGPase mutant. Currently, we are characterizing the mutants in *E.coli* system. Thistudy will not only enhance our understanding of the interaction between the SS and the LS of AGPase, but will also enable us to engineer proteins to obtain better assembled variants of AGPase which can be used for the improvement of plant yield.

P05-018: SOMATIC EMBRYOGENESIS IN THE GRAPEVINE: SOMACLONAL VARIANTS AND DEVELOPMENTAL PHYSIOLOGY

Rey, M.[1] - Domínguez, C[1] - Acanda, Y.[1] - Rey, L.[1] - González, M.V.[2] - Rodriguez, E.[3] - Santos, C.[3] - Centeno, M.L.[4] - Prado, M.J.[1]*
[1]Universidad de Vigo
[2]Universidad de Santiago de Compostela
[3]Universidad de Aveiro
4Universidad de León
**Corresponding author e-mail: mrey@uvigo.es*

We have developed a complete protocol for plant regeneration through somatic embryogenesis for six autochthonous grapevine cultivars from Galicia (north-western Spain). Somatic embryogenesis was induced in anthers and ovaries collected all along the binucleate pollen microsporogenesis stage, indicating a wide window of competence for induction.
The trueness-to-type of the somatic embryogenesis-regenerated plants was tested by flow cytometry and microsatellite analysis. Results showed that several somaclonal variants by ploidy level changes were obtained, including octoploid, tetraploid and mixoploid (diploid plus tetraploid cytotypes) plants.
A particular case was that of the cultivar 'Brancellao' from which both tetraploid and diploid plants were regenerated. As we found that 50% of the adult field-grown mother 'Brancellao' plants analysed were mixoploid, this suggests that regenerated plants originated either from somaclonal variation or by separation of genotypically different cell layers through somatic embryogenesis. All somatic embryogenesis-regenerated plants were true-to-type according to the microsatellite genotypes, with the exception of six 'Torrontés' plants showing a mutant allele (231) instead of the normal one (237) at the locus VVMD5.
Despite these results, deficiencies in the maturation of the somatic embryos reduce the efficiency of the somatic embryogenesis regeneration system.
We are studying the role phytohormones like ABA and IAA are playing during somatic embryo development, to understand the physiological mechanisms for such deficiencies. Preliminary results are presented, with the aim to further contribute to increase the efficiency of the somatic embryogenesis process in the grapevine.

P05-019: DEVELOPING DROUGHT AND BROOMRAPE RESISTANT SUNFLOWER GERMPLASM UTILIZING WILD HELIANTHUS SPECIES

Petcu, E. - Joita Pacureanu, M.*
National Agricultural Research and Development Institute, Fundulea, Romania
**Corresponding author e-mail: petcue@ricic.ro*

The production of sunflower, the most important oil plant in Romania, is quantitatively and qualitatively affected by the extreme meteorological events during last time. Among climatic factors, drought and heat are the most important. Broomrape (*Orobanche cumana*) is a very harmful root parasitic weed of sunflower under dry area. The project aim is the increasing of sunflower adaptability to stresses by improving biological material tolerance to drought, heat and broomrape.
For this, introgression of resistance genes from wild species was attempted, using embryo rescue techniques, combined with classical procedures to improve crossing, selfpollination and backcrossing.
Interspecific F_3 hybrids were produced between six cultivated Romanian inbred sunflower line and wild species *H. argophyllus* and F_2. hybrids between *H. argophyllus* and four cultivated inbred sunflower. Crosses between four cultivated inbred Romanian sunflower line and *H. maximiliani* were backcrossed with same cultivated inbred line advanced to BC_1F_3 and BC_2F_3 generation in 2008 and 2009 for seed increase. Replicated green house tests with several progeny were screened for drought and broomrape resistance. The results indicated good resistance, suggesting successful gene introgression. Identified resistant lines will retest in 2010 and the results will be used to release germplasm, providing new resistance genes to enhance drought and broomrape resistance in sunflower.

P05-020: IN VITRO STRESS INDUCED BY THE PHOMOPSIS HELIANTHI FILTRATE ON SOME PHYSIOLOGICAL INDICES AND OIL QUANTITY AND QUALITY ON SUNFLOWER

Florentina, S. - Sauca, F. - Petcu, E.*
National Agricultural Research and Development Institute, Fundulea, Romania
**Corresponding author e-mail: tina@ricic.ro*

Using *in vitro* screening, the goals of this study were to contribute to the knowledge regarding of the influence of stress induced by *Phomopsis helianthi filtrate* on some Romanian inbred lines and identification the inbred lines with high level of tolerance to the pathogen. For *in vitro* testing to *Phomopsis helianthi* pathogen a total of 14 Romanian inbreed lines were used. As follows of the treatment applied on MS culture medium supplemented with 150ml/l filtrate and on the basis of the results obtained regarding the leaf index, chlorophyll contend, TKW, seed oil percentage and its composition determination. Genotypes with increased resistance to this pathogen have been selected. The determination have been performed by the Minolta Chlorophyll meter (SPAD units) for chlorophyll contends, RMN methods for oil contend and gas-chromatography method (Shimadzu-GC-14B) for fatty acid contends from oil. The results obtained at gas chromatograph underline the fact that from the five fatty acids from sunflower oil, the oleic acid decreases after treatment in all genotypes, excepting the LC 4010 line. We positively notice the fact that the linolenic acid which reduces the oil stability, was detected only in three genotypes but in very small quantities. . Eight genotypes in which the leaf area was not diminished by the treatment as compared with control have been identified.
As regards the chlorophyll content, all tgenotypes, at the treatment variant, the average / variant was diminished with 5.2 SPAD units vs. the control. In variant treated with filtrate, TKW was diminished at seven out of the 14 genotypes. The oleic acid content was more decreased in all lines excepting the LC 4010 line.
Key words: *Phomopsis helianthi; in vitro* culture; chlorophyll contend; in vitro.

P05-021: LIPID CHANGES IN CELL CULTURES OF SILYBUM MARIANUM IN RESPONSE TO ELICITATION BY METHYL JASMONATE

Corchete, P. - Cacho, M.*
Dep. Fisiología Vegetal. Universidad de Salamanca
**Corresponding author e-mail: corchpu@usal.es*

The biosynthesis of silymarin, a medicinal compound of the fruits of *Silybum marianum*, is severely reduced in cell cultures. Methyl jasmonate (MeJA) elicits silymarin synthesis and its release to the culture medium. Recent work has shown that phospholipase D (PLD) and its product phosphatidic acid (PA) mediates silymarin secretion.
To comprehensively determine the overall effects of MeJA on cellular membranes, glycerolipid composition of cell cultures and changes during elicitation were analyzed by ESI-MS/MS (Kansas Lipìdomics Centre, USA).
With this methodology 152 molecular lipid species were identified. Phosphatidyl choline (PC) (42,46%), P ethanolamine (PE) (23,56%) and P inositol (PI) (22,52%), were the main glycerophospholipids; monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), in less proportion (2 and 3%), were also present in membranes. Acyl species were represented by insaturated C16 and C18 fatty acids. A quantitative decrease of PC and PE was observed after 24h of MeJA treatment; galactolipids or PI levels were not changed. Elicitation induced rapid and transient increases in LysoPC and LysoPE. PA also increased and its levels remained high up to 8h of elicitation. The results suggest that besides PLDs, PL As were also activated by MeJA. However, the low level of the PLA products indicates that PLD is more active than PLA in *Silybum* cultures.
These findings show that elicitation causes a selective hydrolysis of membrane lipids, indicate which are the affected molecular species and inform that phospholipases and not galactolipases are involved in the process. In addition, the decrease in both PC and PE suggests that the two lipids are potential substrates for PLDs.
Work was financed by MICINN (BFU-02876) Spain

P05-022: OVER-EXPRESSION OF THE ARABIDOPSIS HISTONE ANTI-SILENCING FACTOR A, SGA1, INCREASES STABLE TRANSFORMATION OF PLANT CELLS

Tenea, G.[1] - Spantzel, J. – Oltmanns, H.[2] - Gelvin, S.B.[2]*
[1]University of Bucharest, Institute of Genetics
[2]Department of Biological Sciences, Purdue University
**Corresponding author e-mail: tenea.gabriela@gmail.com*

Agrobacterium-mediated genetic transformation is the dominant technology used for the production of genetically modified transgenic plants. Extensive research has been conducted which is aimed at understanding the role of host proteins, especially chromatin proteins, in the transformation process. A long-term goal is to manipulate the expression of these proteins to improve transformation. RNAtiargeted against 109 *Arabidopsis* chromatin genes demonstrated a role for many chromatin proteins, such as the anti-silencing factor SGA1, in *Agrobacterium*-mediated transformation. We investigated the effects of over-expressing a *SGA1* cDNA on transgene expression and *Agrobacterium*-mediated transformation. Unlike the situation with many histone cDNAs, over-expression of a *SGA1* cDNA did not increase transient expression of a *gusA* gene co-transfected into tobacco BY-2 protoplasts. Moreover, over-expression of *SGA1* did not increase expression of a previously integrated *gusA* transgene. However, transgenic *Arabidopsis* plants containing additional copies of a *SGA1* cDNA displayed increased accumulation of *SGA1* mRNA and greater susceptibility to transformation. Moreover, DNA blot analysis showed an increase in T-DNA integration into the genome of *Arabidopsis* plants over-expressing *SGA1*. We speculate that SGA1 might lead to enhanced plant transformation by allowing T-DNA and complexed proteins greater access to plant target DNA, thus facilitating T-DNA integration.

P05-023: PRODUCTION OF SOYBEAN GRAINS AND TOBACCO SEEDS HAVING HIGHER METHIONINE CONTENT

Amir, R.[1] - Godo, I.[1] - Matityahu, I.[1] - Shikui, S.[2] - Wensheng, H.[2] - Tianfu, H.[2]*
[1]Migal, resreach institute
[2]Chinese Academy of Agricultural Sciences
**Corresponding author e-mail: rachel@migal.org.il*

This study describes the first modification of methionine biosynthesis in seeds of tobacco, the model plant, andof soybean, the crop legume plant (*Glycin max* L.). Unlike vegetative tissues, the seed-specific expression of *Arabidopsis* in its unregulated deleted form of cystathionine gamma synthase (At D-CGS) in tobacco seeds led only to a slight accumulation of methionine. It was previously found that the seed-specific expression of feedback-insensitive bacterial aspartate kinase (AK), which operates up-stream to CGS in the methionine biosynthesis pathway, led to two-fold higher methionine content in transgenic seeds. This finding suggests that in tobacco seeds, the availability of the carbon/amino skeleton of methionine limits its synthesis and less the activity rate of CGS. To further test this assumption, plants’ seeds-specific expression of AtD-CGS were crossed with those expressing AK. The methionine level increased up to four-fold higher in seeds of progenies expressing both foreign genes compared to wild-type seeds, suggesting that indeed the carbon/amino skeleton limits methionine content in seeds. These results are encouraging us to try to elevate methionine content in

legume grains whose proteins have a low level of methionine. Therefore, the At D-CGS was seed-specific expressed in soybean seeds. The level of soluble methionine increased up to seven-fold, while the methionine that was incorporated into the protein seeds increased up to 18-fold. The phenotype and germination rate did not alter in the transgenic seeds compared to the wild-type seeds. These results have shown new ways of elevating methionine content in seeds, thus enhancing their nutritional qualities.

P05-024: AN IMPROVED PROTOCOL FOR TRANSFORMATION OF ANTIRRHINUM MAJUS

Manchado Rojo, M. - Portero Martinez, M.A. - Weiss, J. - Egea-Cortines, M.*
Universidad Politécnica De Cartagena
**Corresponding author e-mail: maria.manchado@upct.es*

Genetic transformation is a cornerstone to obtain information of gene functions. We have developed an improved protocol for transformation and regeneration of *Antirrhinum majus*, obtaining a highly reproducible method that has yielded up to a high efficiency, close to 10% (the final results will be discussed in the congress). Several aspects affect transformation efficiency. We tested two lines, 165E and Vilmorin Nain and two different explants, leaf discs and hypocotyls from seedling of two and four weeks. As a proof of concept we transformed *A. majus* with a pHellsgate12 construct expressing RNAi of the homeotic gene *Deficiens*. Two week old hypocotyls explants from the line Vilmorin Nain had the highest transformed rate. Putative transformants were tested by PCR using NPTII primers and by their phenotypes. The resulting plants showed classic phenotypes corresponding to hypomorphic alleles of Def, which included sepaloid petals and sterile stamens and fell somewhere in strength between *deficiens chlorantha* and *deficiens nicotianoides*.

P05-025: MAKING A TRANSGENIC RICE WITHOUT SELECTION

Jung, H. - Bang, S.W. – Kim, Y.S. – Kim, J.K.*
School of Biotechnology and Environmental Engineering, Myongji University
**Corresponding author e-mail: harin0723@gmail.com*

Over the past several years, consumer and environmental groups have expressed concern about the use of antibiotic-and herbicide-resistance genes against ecological and food safety perspective. Although no scientific basis has been established for these concerns, generating marker-free plants would certainly contribute to the public acceptace of transgenic crops. Here we present a technology that allows us to make transgenic plants without using selectable markers. This technology relies on an efficient *Agrobacterium*-mediated transformation method and PCR-based selection of transformants. After co-cultivated, transformed cells were allowed to regenerate on MS medium without any antibiotics or herbicides. In about 2-3 weeks after regeneration, a leaf disc of regenerated plants from one callus and was punched and pooled in one tube. We performed PCR with the leaf discs for the presence of transgene in the pools. We then select the positive pools and punched a leaf disc of individual rice plants in the positive pools. Second PCR from individual plants of the PCR-positive pools led us to identify transgenic plants. This series of process was referred to as the Clean T-DNA technology. Such transgenic rice plants are marker-free and further analyzed after grown in a paddy field. Thus, our Clean T-DNA technology may provide marker-free transgenic rice plants that can be used directly for commercial purpose

P05-026: USSING OF TRANSGENOSIS FOR INDUCTION OF VIRUS RESISTANCE IN PE

Hanacek, P.[1] - Rohrer, M.[1] - Reinöhl, V.[1] - Prochazka, S.[1] - Safarova, D.[2] - Navratil, M.[2] - Horacek,*
J.[3] - Svabova, L.[3] - Smykal, P.[3] - Griga, M.[3]
[1]Mendel University in Brno
[2]Palacky University Olomouc
[3]Agritec, Research, Breeding & Services,Ltd.
**Corresponding author e-mail: hanacek@mendelu.cz*

Viral diseases are one of the serious problems that can cause great economical loss during pea cultivation. In the Czech Republic *Pea enation mosaic virus* (PEMV) and *Pea seed-borne mosaic virus* (PSbMV) were the most frequently detected viruses in samples collected from inspected pea fields. Integrated plant protection is a modern trend against pest and diseases, and includes also creation of resistant varieties. Breeding for resistance could be used, but the process is lengthy and sometimes the resistance sources are not available.
This goal can be achieved utilizing biotechnological tools, especially transgenosis. Here we present results of testing of this alternative approach – the use of genetic modification to achieve the induction of resistance to viral diseases by inverted repeats post-transcriptional gene silencing (IR-PTGS). For both PEMV and PSbMV we cloned sense and antisense *CP* cDNA between the 35S promoter and CAMV terminator that result in hairpin (hp)RNA. This dsRNA is processed by a dsRNase resulting in 21-23 nt siRNAs (small interfering RNA). Incorporation of the siRNAs into a nuclease complex that degrades ssRNA in a sequence specific manner induces viral resistance. Preparation and testing of plasmid vectors as well as results of testing of transgenic pea plants will be presented. This research was supported by a project of the Czech Ministry of Agriculture QI91A229.

P05-027: PHYTOREMEDIATION OF STABLE CESIUM AND LEAD FROM SOLUTIONS BY CHENOPODIUM ALBUM

Moogouei, R.[1]- Borghei, M.[2] - Arjmandi, R.[1] - Vosoughi, M.[2]*
[1]Department of Environmental Management, Faculty of Environment and Energy, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Sharif University of Technology
**Corresponding author e-mail: r_moogoui@iau-tnb.ac.ir*

Chenopodium album plants were tested for their potential to remediate stable cesium and lead from solutions in 15 d. Hydroponically grown Plants were exposed to CsCl and $Pb(C_2H_3O_2)_2$ solutions at three different concentrations (0.6, 2 and 5 mg l^{-1}). When plants were incubated in CsCl solutions 68.08 ± 2.12%, 39.66 ± 3.48% and 56.37 ± 1.90% cesium was found to be remediated after 15d, respectively. Moreover more than 99% lead was removed from the $Pb(C_2H_3O_2)_2$ solution in all three concentrations after 15d. When both CsCl and $Pb(C_2H_3O_2)_2$ were supplemented together to the solution, 14.53 ± 1.62%, 47.25 ± 0.96% and 48.01 ± 1.43% cesium and 71.22 ± 0.25%, 94.31 ± 0.24% and 98.40 ± 0.05% lead were removed after 15d. The present study suggests that hydroponically grown *Chenopodium album* could be used as a potential candidate plant for phytoremediation Cesium and lead from solutions, however plants were found to be more efficient for the remediation of lead than cesium.

P05-028: THE DEVELOPMENT OF TRANSGENIC FLAX WITH ENHANCED HEAVY METALS BINDING CAPABILITY

Vrbová, M.[1] - Horacek, J.[1] - Vetrovcova, M.[1] - Macek, T.[2] - Griga, M.[1]*
[1]AGRITEC Ltd., Plant Biotechnology Department, Šumperk, Czech Republic
[2]Joint Laboratory of IOCB and ICT Prague, Institute of Organic Chemistry and
Biochemistry CAS, Prague, and Department of Biochemi
**Corresponding author e-mail: vrbova@agritec.cz*

Flax is an industrial crop utilized mainly for technical purposes and for this reason is a good candidate for phytoextraction of

heavy metals (HM) from polluted soils. Because the HM accumulation by commercial flax varieties is not high enough, genetically modified flax with inserted heavy metal binding gene may improve heavy metal binding and detoxification capacity. *Agrobacterium tumefaciens* strain EHA 105 containing binary vector pBI-αMT with α-domain of metal-binding mammalian metallothionein *α-MT* (with potential for heavy metal detoxification) was used in the experiments. With the aim to obtain the genotype with high transformation ability, we screened 16 flax/linseed genotypes for sensitivity/recalcitrance to transformation. The genotype AGT-0917 was found as the most responsive one, and thus used for further experiments. The transgene integration in GUS-positive transformants was confirmed by PCR. The difference between stable (non-segregating) transgenic and non-transformed regenerants of T2 generation was observed in cadmium (Cd) effect on growth parameters *in vitro* as well as in Cd-accumulation by explants grown *in vitro*. The Cd-content in stems and roots from GM and non-GM flax plants grown in the field was determined. The results indicate enhanced heavy metals binding capability of transgenic flax with inserted *α-MT* gene. Acknowledgement: Authors thank for support of projects 1M06030, MSM 2678424601, MSM 6046137305 and Z 40550506 of the Czech Ministry of Education.

P05-029: PROPAGATION AND CALLUS INDUCTION IN DIANTHUS ANTICARIUS SUBSP. SAORINII

Agulló Antón, M. - Bañón-Arnao, M. – Acosta, M.*
Universidad de Murcia. Department of Plant Biology (Plant Physiology)
**Corresponding author e-mail: maagullo@um.es*

Dianthus anticarius subsp. *Saorinii* is a vulnerable species, whose few individuals are restricted to the Sierra de Almenara (SW Murcia, Spain). In this study, different biotechnology techniques are applied in an attempt to propagate and conservate this subspecie. *Saorinii* seeds were germinated *in vitro* on solid MS medium after being decontaminated. Root and stem node explants obtained from the *in vitro* seedlings were cultured on MS medium supplemented with different concentrations of 2,4-D and kinetin (KN). Callus induction, root and stem regeneration were assessed. The best response for callus induction was observed from stem node segments on MS medium containing 0.5 mgL^{-1} or more 2,4-D. No calli were observed in the absence of 2,4-D in either kind of explant and, in the case of root explants, no calli were observed at low concentrations (0.2 mgL^{-1}), either. For stem node explants, balanced or high auxin/cytokinin (2,4-D/KN) ratios in the culture media seemed to be beneficial for callus formation. Root explants generally resulted in a lower callus formation than stem explants, and required higher auxin/cytokinin ratios. Regarding plant propagation, only the node segments produced shoots or complete plants. Root and shoot growth was specially evident from stem node explants when no 2,4-D was applied and the KN concentration was below 1 mgL^{-1}. In the case of root explants, high root elongation was observed when no 2,4-D was applied. The results described provide useful information for developing tools for the large-scale multiplication and conservation of germplasm of *Dianthus anticarius* subsp. *Saorinii*.
ACKNOWLEDGEMENTS: Project CARMurcia/PEPLAN (S4 and S13) and CARMurcia-Dirección General de Patrimonio Natural y Biodiversidad (F.J. Sánchez Saorín).

P05-030: PROTEINACEOUS ELICITOR OF INDUCED RESISTANCE FROM LEPTOSPHAERIA MACULANS

Valentova, O- .Dinh Kim, P. – Sasek, V.[2] - Burketova, L.[2]
[1]Institute of Chemical Technology Prague
[2]Institute of Experimental Botany AS CR
*Corresponding author e-mail: olga.valentova@vscht.cz

The efficiency of plant defence mechanisms against pathogens depends on the ability of the plant to recognise its effector molecules followed by signal transduction leading to the expression of pathogenesis related (PR) proteins. These proteins represent a group of *de novo* synthesized proteins, first described in plant tissues infected with pathogens but later on also in plants treated with various elicitors and chemical compounds. They are suggested to be in a tight correlation with disease resistance and/or tolerance, as well as with the level of systemic acquired resistence (SAR). Elicitors are molecules secreted by pathogen during microbial entry or derived from their cell walls capable to activate plant defence mechanisms.
Cultivation media of *Leptosphaeria maculans*, fungus causing "blackleg" of oilseed rape (*Basic napus*) induce the expression of systemic acquired resistence marker genes (PR1 and WRKY 70) in cotyledons of *B. napus* plants. Analysis of extracellular proteins of cotyledons treated with cultivation media showed induction of at least two acidic chitinase isoenzymes. In biological assay we show that cultivation media induces resistance towards *L. maculans*. For further characterization the compounds with the elicitation activity were partially purified by dialysis, ultrafiltration, ion exchange and size exclusion chromatography. Digestion of the active fractions with trypsin, beta-glucosidase and alpha-amylase indicated the proteinaceous character of the elicitor supported by its heat instability.
Acknowledgement
This study was supported by grants of Ministry of Agriculture no. QH 81201 and Czech Science Foundation no. 522/08/1581.

P05-031: THE ROLE OF TS AND BAPT GENE EXPRESSION IN THE TAXOL BIOSYNTHETIC PATHWAY IN ELICITED CELL CULTURES OF TAXUS BACCATA

Moyano, E.[1] - Onrubia, M.[1] - Bonfill, M.[2] - Palazón, J.[2] - Cusidó, R.M.[2]*
[1]Departament de Ciències Experimentals i de la Salut (UPF)
[2]Facultad de Farmacia, Universidad de Barcelona
**Corresponding author e-mail: elisabeth.moyano@upf.edu*

Biotechnological production of valuable secondary metabolites in plant cell or organ cultures is an alternative to their extraction from whole plant material. However, the use of plant cell factories has had only limited commercial success. The biotechnological production of the anticancer compound taxol and related taxanes has become a commercial reality for various companies, but the low productivity of *Taxus* cell cultures requires a wide use of elicitors. Here, we describe the action of methyl jasmonate (MeJ 100 mM) and vanadyl sulphate (VS 50 mM), as well as the joint action of both elicitors on a two-stage *Taxus baccata* culture. The elicitors were added when cells had adapted to the production medium, 8 days after being transferred from the growth medium and the transcript levels of the genes encoding taxadiene synthase (TXS) and baccatin III 13-O-(3-amino-3-phenylpropanoyl) transferase (BAPT) were determined by qPCR. The results were related to taxol and related taxane production in the selected *T. baccata* cell line. Elicitation with MeJ, VS or both caused quite marked changes in the total taxane production, varying according to the elicitor. Cell cultures treated with MeJ achieved the highest levels of total taxanes almost throughout the experiment. Regarding individual taxane production, MeJ significantly increased both taxol and baccatin III accumulation, but VS only activated taxol production to the same extent. Regarding gene expression, MeJ clearly increased the transcription level of the *txs* and *bapt* genes but the presence of VS in the medium only increased the accumulation of the *bapt* gene mRNA. These results suggest that the elicitors have different and probably interfering mechanisms of action in taxane biosynthesis.

P05-032: A PLATFORM FOR HIGH LEVEL ISOPRENOID PRODUCTION IN NICOTIANA TABACUM

van Deenen. N.[1] - Post, J.[1] - Schulze Gronover, C.[2] - Prüfer, D.[1]*
[1]Department for Plant Biochemistry and Biotechnology, Westphalian Wilhelm´s University Muenster, Germany

[2]Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany
**Corresponding author e-mail: nickivd@web.de*

Isoprenoids are the largest group of natural products with more than 30,000 compounds identified. The prenyl diphosphate precursors (IPP and DMAPP) for all plant isoprenoids derived from two separate, compartmentalized, biosynthetic pathways, the cytoplasmic mevalonic acid (MVA) and the plastidial methylerythritol phosphate (MEP) pathway. Beyond their specific functions in plant primary and secondary metabolism, many isoprenoids have been shown to have industrial and medical importance. In the present work, we present the generation of a platform for high level production of isoprenoids in *N. tabacum* by engineering the MVA and the MEP pathway to increase the IPP pool both in the cytosol and the plastids. A higher production of IPP in the cytosol could be reached by the expression of the catalytic domain of an HMG-CoA reductase (HMGR) – a key enzyme of the MVA pathway. The coexpression of a farnesyl diphosphate synthase (FPS) which uses IPP as substrate for the synthesis of isoprenoid precursors let to a 50fold increase in squalene production. A further increase in isoprenoid production rate was achieved by the upregulation of the plastidial isoprenoid pathway. The expression of a synthetic operon which was transformed to the plastom via particle bombardment, carrying ten different genes amongst others encoding enzymes involved in the MEP pathway resulted in remarkable changes in isoprenoid levels.

P05-033: PROTECTION OF RARE WILD ORCHID SPECIES IN LATVIA EX SITU

Belogrudova, I. - Jakobsone, G. - Roze, D. - Megre, D.*
University of Daugavpils
**Corresponding author e-mail: visio@versija.lv*

Ex situ conservation of terrestrial orchid species of Latvia in National Botanic Garden (NBG) is attempted in two ways – in field conditions in expositions and in the Department of Plant Diversity In Vitro - Conservation.
There are three objectives for this research: elaboration of methodology for various wild terrestrial orchid species to ensure their prolonged cultivation *in vitro* to approach the natural development (i); optimization of acclimatization process *ex vitro* (ii); invention of models to create artificial plant communities in NBG, ranging into account ecology of each orchid species and similar to those in natural habitats (iii). Elaboration of methodology for *in vitro* cultivation involves several problems: supplementary addition of Ca^{2+} in culture medium without inorganic stuffs for calciferous species, suppression of browning of culture medium resulting in plant necroses; initiation of t of new shoots to keep the microculture in rejuvenescence under study by us. The cold storage (5ºC in the dark for 5 months) was necessary for subsequent acclimatization *ex vitro*. The colonization with symbionts was achieved with addition to substrates the soil from natural meadow when transplanting *ex vitro*.
Plant communities and ecology peculiarities of various orchids were documented *in situ* to make a cultivation strategy in expositions of NBG. Our future aim will be to establish plant communities in the field in artificial way for each *in vitro* acquired orchid taxon.

P05-034: IDENTIFICATION AND CHARACTERIZATION OF PROTEINS INVOLVED IN RUBBER BIOSYNTHESIS IN TARAXACUM KOKSAGHYZ

Hillebrand, A.[1] - Schmidt, T.[1] - Schulze Gronover, C.[2] - Prüfer, D.[1]*
[1]Department for Plant Biochemistry and Biotechnology, Westphalian, Wilhelm´s University Muenster, Germany
[2]Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany
**Corresponding author e-mail: a_hill05@uni-muenster.de*

Natural rubber (*cis*-1,4 polyisoprene) is one of the most important raw materials in the world and nowadays the para rubber tree *Hevea brasiliensis* is the sole crop whose latex is used for commercial rubber production. As the global demand for natural rubber increases it probably can no longer be satisfied by *Hevea* rubber. Since the special characteristics of natural rubber, like enormous elasticity and capacitance, can not be mimicked by artificially produced polymers, there is a need for alternative rubber crops. Amongst others *T. koksaghyz* has been considered as a potential alternative rubber source due to its ability to produce large amounts of high quality rubber. The elucidation of rubber biosynthesis and proteins involved in this process in *T. koksaghyz* is crucial to improve rubber harvest and production. In *H. brasiliensis* a cis-prenyltransferase is supposed to be responsible for polymerization of IPP subunits to form long chains of rubber, while small rubber particle protein and rubber elongation factor influence rubber biosynthesis in a supportive manner.
In *T. koksaghyz* we identified orthologous cDNAs encoding three *cis*-prenyltransferases (*TkCPT1-3*) and five small rubber particle proteins (*TkSRPP1-5*). The expression profile, localization studies as well as functional analyses implicate these proteins in rubber biosynthesis.

P05-035: CROPS2INDUSTRY

Rigas, S.[1] - Margaritopoulou, T.[1] – Hatzopoulos, P.[1] – Alexopoulou, E.[2] - Christou, M.[2] - Milioni, D.[1]*
[1]Agricultural University of Athens, Department of Agricultural Biotechnology, Athens, Greece
[2]Center for Renewable Energy Sources, Pikermi, Greece
**Corresponding author e-mail: srigas@aua.gr*

Agricultural and forestry resources can provide renewable raw materials for a broad range of non-food products such as chemicals, fibres, construction materials, lubricants and fuels. The cultivation of crops for non-food use is a long-existing concept. Despite the considerable investment in research and development, little progress has been made towards the commercial exploitation of such products. Crops2Industry overall objective is to examine the potential use of non-food crops for selected industrial applications namely oils, fibres, resins, pharmaceuticals or other specialized products within the EU27 context. Biotechnology will play a key role in producing biomaterials that conform to technical specifications. This project aims to outline and prioritise crops-to-products schemes suitable for individual Member States, which will support a sustainable, economically viable and competitive European bio-based industry and agriculture. The project consortium is composed of fourteen partners from nine European countries, involving four Universities, five research organizations/institutes and five companies. The partners will gather data on the potential establishment of non-food crops in combination with biotechnology to overcome breeding constraints, the assessment of market needs, product quality standards, prices and yield costs. Emphasis will be given to the dissemination of information and data to farmers and end-users in terms of the most promising non-food crops. Crops2Industry is expected to assess whether and under which terms Europe has the potential and technical competence to develop a competitive bio-industry fed by sustainable agriculture and become a world leader in the field of bio-based products.

P05-036: MICROPROPAGATION OF SCOTS PINE FROM SEEDLING EXPLANTS

Lebedev, V. - Schestibratov, K.*
Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry
**Corresponding author e-mail: vglebedev@mail.ru*

Conifer clonal forestry as a form of plantation forestry has great potential advantages. However conifers are most recalcitrant objects for cultivation in vitro. Scots pine is one of the most wides-

pread forest trees in Russia. We investigated influence of various factors, such as basal nutrient media, cytokinins, ethylene, activated charcoal, light intensity and photoperiod length on bud induction, shoot elongation and rooting of explants from young seedlings of Scots pine. Basal media used in our study include MS, PM1, SH, WPM, TE, MCM and DCR media. WPM and MS media induced vitrification of most explants. Use of TE medium resulted in explant necrosis. SH medium gave the best results. BA, kinetin, zeatin, thidiazuron and 2iP at various concentrations were tested for the induction of adventitious buds. Kinetin and 2iP had little bud-inducing effect compared to BA and zeatin. Optimal conditions for pulse treatment of explants with BA were defined. Addition of activated charcoal inhibited bud production but promoted shoot elongation. Root initiation up to 44% were obtained after exposition on NAA-containing medium. Rooted plantlets were transferred to the greenhouse and acclimatized with 90% survival. Our results may be applied for development of clonal micropropagation method of Scots pine using vegetative tissue explants from mature trees.

P05-037: OPTIMIZING DNA DELIVERY ON SOMATIC EMBRYOGENIC TISSUE OF MARITIME PINE

Blasco Carlos, M. - Humanez, A. - Mendoza-Poudereux, I. - Muñoz-Bertomeu, J. - Almazan, V. - Brisa, C. - Segura, J. - Arrillaga, I.*
Universitat de València
**Corresponding author e-mail: miblas@alumni.uv.es*

Maritime pine (*Pinus pinaster* Ait.) is a characteristic species in Mediterranean forests, with its main populations located in the Iberian Peninsula. The species is also the most advanced conifer model for genomic research in Europe. Genetic transformation is the best tool to allow gene functional analysis and for rapidly increasing yield and wood quality. The objective of this work is to develop strategies to efficiently deliver DNA into somatic embryogenic tissue by *Agrobacterium tumefaciens* coculture techniques. Embryogenic lines were initiated from immature megagametophytes and maintained by 2-week subcultures on Litvay medium (mLV). Embryogenic callus was resuspended in liquid mLV and mixed with the same volume of the bacterial suspension. After infection, cells were recovered on filter paper and transferred to the same medium for coculture. Transient Gus Assays were performed following standard protocols afterthree days of coculture. Factors tested were: *Agrobacterium* strain (AGL1, EHA105 and LBA4404); infection by sonication or vacuum infiltration; coculture time (10, 30 and 60 min); and method to recover embryogenic material after infection (by a low-pressure pulse on a Buchner funnel or poured on filter paper over a pile of paper towels). Among factors tested, AGL1 strain applied under vacuum infiltration (1 min) followed by 10 min infection and further recover of cells on a filter paper over paper towels produce the higher number of blue foci per plate (average of 130). Additiona l r esults on stable integration will be presented. This work is funded by the Spanish MICINN and FEDER funds (AGL2007-66345-CO2-02); Generalitat Valenciana (Prometeo 2009/075) and a Research Fellowship to M. B.

P05-038: STRATEGY FOR PRODUCTION OF RICE WITH INCREASED ISOFLAVONE GENISTEIN LEVEL BY METABOLIC ENGINEERING OF PHENYLPROPANOID PATHWAY

Park, H. - Choi, M.S. – Lee, Y.Y. – Shon, J.Y. – Choi, I.S. – Yun, H.T. – Lee, J.Y. – Kim, Y.J.*
National Institute of Crop Science, RDA
**Corresponding author e-mail: parkhm2002@korea.kr*

Isoflavonoids are a diverse group of plant natural products synthesized from phenylpropanoid pathway which play important roles in plant growth and development.
There have been considerable attentions as health-promoting nutraceuticals because of their antioxidant and estrogenic anticancer activity. In our study, we attempted to develope genistein-enriched rice for recommendable daily consumption through engineering the isoflavone pathway. Both overexpression and RNAi suppression strategies were used to manipulate the expression of several genes encoding key enzymes in the flavonoids/isoflavonoids pathway in transgenic rice. Rice plants were transformed with two soybean (*Glycine max* L) isoflavone synthase (*GmIFS1, 2*) genomic DNA under the control of the seed specific rice globulin promoter. HPLC-MS analysis demonstrated the presence of genistein as the major isoflavone metabolite in the transgenic plants. Substantial amounts of genistein (up to 87.0 μg/g FW) were found in seeds. However, the amounts of genistein showed annual variation in the field condition. For producing transgenic rice seeds with high level of genistein in a stable manner, we transformed maize C1 and R-S genes that together activate most structural genes in the flavonoid biosynthetic pathway. Furthermore, we constructed a RNAi vector to suppress the flavanone 3-hydroxylase (F3H) gene expression for blocking anthocyanin synthetic pathway. We obtained transgenic rice plants harboring RNAi-F3H vector or C1 and R-S overexpression vector and then we pyramided these three genes (IFS1/ / C1R-S/RNAiF3H) by crossing. These plants will be used for further analysis of isoflavone detection and molecular characterization. (Supported by RDA Biogreen 21 and NICS grant)

P05-039: MOLECULAR CHARACTERIZATION AND DEVELOPMENT OF MOLECULAR MARKER FOR BREEDING LOW ALLERGY SOYBEAN CULTIVARS BY NULLIFYING MAJOR ALLERGEN P34

Choi , M. - Jeong, K.H. – Lee, S.K. – Seo, M.J. – Yun, H.T. – Park, H.M. – Kim, Y.H.*
National Institute of Crop Science (RDA)
**Corresponding author e-mail: mschoi73@korea.kr*

Soybean *(Glycine max* (L.) Merr.) is an important source of vegetable oil and high protein. Use of soybean meal by the food industry is increasing, but severely limiting dietary choices and the quality of life of food-allergic individuals. Gly m Bd 30K (P34) is known as the main seed allergens in soybean-sensitive patients. The objective of this work was to determine the molecular basis of the low mutation of soybean P34 and to design molecular marker for the selection of the causative mutations for wild homozygous, heterozygous and mutant homozygous.
Using soybean genome assembly, we knew that soybean P34 genes are existing 2 copies in LG A1 and 1 copy in LG A2 in soybean genome. We confirmed three copies of P34 genes in soybean genome by Southern blot analysis and found that all of those genes are expressing during the seed filling stage through RT-PCR analysis. Especially, Glyma08g12270 of those was expressed at significantly higher level compared with Glyma08g12280 and Glyma05g29130. However this gene was not expressed in the low-P34 germplasm accessions. We developed a co-dominant marker based on the sequence of Glyma08g12270 containing a four-base pair insertion at the P34 start codon. Also, we made a polyclonal antibody for investigation of P34 protein levels. Using a co-dominant marker and a polyclonal antibody, polymorphism and amount of protein for Glyma08g12270 were analyzed in F2 and F3 generation crossing PI 567476 and Hwanggumkong, Korean cultivar. As results, the polymorphism analysis was accustomed to a difference of protein level of wild homozygous, heterozygous and mutant homozygous.

P05-040: INCREASED VITAMIN E CONTENT IN SOYBEAN PLANTS OVEREXPRESSING HGGT GENE FROM RICE ENHANCED TOLERANCE TO ABIOTIC STRESS AND ANTIOXIDANT ACTIVITY

Kim, Y. - Park, H.M. - Lee, Y.Y. – Kim, Y.H. – Choi, M.S. – Jeong, K.H. – Lee, S.K. – Seo, M.J. – Yun, H.T.*
National Institute of Crop Science, RDA
**Corresponding author e-mail: kimyuh77@korea.kr*

Vitamin E (tocopherols and tocotrienlos), with powerful antioxidant properties, is important for human and animal health and play essential roles to environmental stresses such as drought, low temperature condition. Many studies have been carried out to increase vitamin E content in plants through engineering the tocochromanol biosynthesis pathway for meeting human daily consumption and improving tolerance abiotic stresses. In this study, we used genetic approaches to develop soybean plants showing enhanced vitamin E levels in both plant leaves and seeds for human heath and cultivating benefits. Rice homogentisate geranylgeranyl transferase (*HGGT*) which catalyzes the committed step of tocotrienol synthesis, was over-expressed in soybean under the control of the seed specific rice globulin promoter. Two transgenic soybean plants were produced and their progenies were analyzed. Introduced rice *HGGT* gene was expressed at significantly higher levels in soybean leaves and seeds, and resulted in 2-fold increase in the tocopherol content, and yielded tocotrienols which not existing in soybean. Transgenic soybean plants exposed to drought and low temperature conditions, they showed decreased lipid peroxidation, electrolyte leakage. In addition, we found that those lines increased antioxidant activity in soybean oil. These soybean plants with increased vitamin E content could have a potential to increase the dietary intake of vitamin E as well as to enhance tolerance to abiotic stresses. (Supported by RDA Biogreen 21 and NICS grant)

P05-041: FUTURE PERSPECTIVES AND LIMITS TO PRODUCE BIOFUELS FROM LARGE SCALE ALGAL BIOTECHNOLOGY

*Wilhelm, C.**
University of Leipzig, Department of Plant Physiology
**Corresponding author e-mail: cwilhelm@rz.uni-leipzig.de*

Due to future limited use and availability of fuels from fossil sources biofuels are the most promising technology to produce carbon based fuels for private and industrial applications. Because of the up to ten times higher productivity per area algal biotechnology opens the perspective to replace fossil energy to a significant extent. However, in the context of climate burden algal based biofuels must not be as cheap as other sources but also has to fullfil the requirement that the energy and carbon balance must be a real win from photon to wheal. Based on complete energy balances from photon to biomass the most important losses can be quantified. To our surprise these balances show that the process of photosynthesis can be considered to be optimized in algae, whereas the major losses can be attributed to the metabolic processes which convert the primary metabolites into real cellular biomass. Complete energy balances show that an efficient biofuel technology with algae should not be based on the accumulation of lipids instead of carbohydrates. The conversion of the latter into biofuels by anaerobic microbial fermentation processes is the most promising approach.
References:
Jakob T. et al. Uncoupling of growth rates and biomass production under nitrate limitation in combination with dynamic light conditions in the diatom *Phaeodactylum tricornutum.* J. Exp Bot. 58: 2101-2113, 2007. Langer, U. et al. A complete energy balance for *Chlamydomonas reinhardtii* and *Chlamydomonas acidophila* under neutral and extremely acidic growth conditions. Plant Cell Environm 32: 250-258, 2009

P05-043: SUNFLOWER MUTANT LINES AND TRANSGENIC OILSEED RAPE WITH AN ENLARGED ROOT SYSTEM SHOW AN INCREASED TOLERANCE AND METAL ACCUMULATION ON A METAL-CONTAMINATED SOIL

Nehnevajova, E. - Herzig, R. - Gerdemann-Knörck, M. - Schmülling, T.*
Institute of Biology/Applied Genetics, Dahlem Centre of Plant Sciences, Freie Universität Berlin
**Corresponding author e-mail: nehnevaj@zedat.fu-berlin.de*

Oil crops, such as sunflower and oilseed rape have been proposed for the decontamination of soils polluted by heavy metals. However, the time needed for cleaning soil is still too long because of only a moderate metal accumulation in the above-ground parts of high yielding plants. Efforts in plant breeding and genetic engineering seek to generate a plant showing high shoot metal accumulation and high yield.
One possibility to improve plant yield and crop quality under stressful conditions is the development of plants with an enhanced root system and an improved potential for uptake of inorganic pollutants from the soil. Sunflower mutants with an increased metal tolerance and an enhanced capacity of metal extraction were generated by chemical mutagenesis and selected for four generations. M5 sunflower mutant lines were investigated for metal tolerance and metal accumulation on sewage sludge contaminated soil. Transgenic oilseed rape plants overexpressing a cytokinin-degrading CKX gene were generated and root development was characterized on the same metal-polluted soil in the greenhouse. Mutant lines with an enlarged root system showed a 20-30% increased Cd and Zn concentration in leaves and roots as compared to the original cultivar IBL 04.
Cadmium and zinc accumulation in shoot tissue of transgenic *Brassica* seedlings was also 30 % higher than in wild type seedlings. Young sunflower mutants grown on metal-contaminated soil showed a higher specific activity of gluthatione reductase, peroxidase, monodehydroascorbate reductase and dehydroascorbate reductase than IBL 04. Together, a tolerance index indicated an enhanced tolerance of sunflower and *Brassica* lines toward stress caused by toxic metals.

P05-044: PLANTS DEFICIENT IN FRUCTOSE-1,6-BISPHOSPHATASE (FBPASE) ISOFORMS INDUCE CHANGES IN CARBOHYDRATE BIOSYNTHESIS AND DISTRIBUTION

Sahrawy-Barragán, M. - Rojas, J.A. – Sandalio, L.M. – García, A. – Chueca, A. – Serrato, A.*
Estación Experimental Del Zaidin -Consejo Superior De Investigaciones Cientificas
**Corresponding author e-mail: sahrawy@eez.csic.es*

Sucrose and starch are the final products of the CO_2 fixation during the photosynthesis. The enzymes involved in carbon metabolism are responsible for preserving the best balance between sucrose and starch in plant development and fructose-1,6-bisphosphatase (FBPase) occupies key positions in this process. FBPase catalyses the breakdown of fructose-1,6-biphosphate to fructose-6-phosphate and Pi. Until now three FBPases have been described, two in the chloroplasts and one in the cytosol (Serrato et al, 2009, J.Exp.Bot 60: 2923-2931).
The cytosolic isoform (cyFBPase) is involved in sucrose synthesis and is regulated by FBP and AMP. In the chloroplast, one of the two present isoforms, known as cpFBPseI, is directly implicated in starch formation. Its tertiary structure displays a redox domain with three cysteines able to form disulphide bonds that can be reduced by plastidial thioredoxins. Finally, a recently discovered new chloroplastic isoform (cpFBPaseII) lacks the redox domain and is resistant to H_2O_2 inactivation. In this work we study the role of plant FBPases in the carbohydrate distribution by analysing three *Arabidopsis* knock out mutant lines affecting to each FBPase isoform.
We show that the lack of cpFBPaseI induces a lower photosynthesis rate, a higher content of soluble sugars and a diminution of starch accumulation.
On the contrary, repression of cyFBPase increases the number of starch granules in the chloroplasts. Interestingly, the phenotype of cpFBPaseII mutant plants has been compared to the other plant lines revealing significant differences between them CO_2 assimilation, pigment contents and leaves size. The results point out to new biotechnological approaches for generation of novel high-quality crops.

P05-045: DEVELOPMENT OF STS MARKERS FOR ANALYSIS OF GENETIC DIVERSITY USING EEG LIBRARY IN SOYBEAN

Seo, M. – Lee, S.K. – Kim, Y.H. – Jeong, K.H. – Hwang, T.Y. – Choi, M.S. – Yun, H.T.*
National Institute of Crop Science, RDA
**Corresponding author e-mail: mjseo77@korea.kr*

Several molecular markers are available for identifying germplasms and for analyzing their genetic diversity. Some molecular markers like RAPD and AFLP are undefined elements, whereas SSR and STS markers are derived from defined elements. Especially, sequence tagged site (STS) marker as a source of locus- specific codominant marker is distinctive in that it is combined advantages of PCR and RFLP. We performed this study to develop the gene based DNA library and STS marker derived from gene rich region for identifying of soybean cultivar. To develop the euchromatin enriched genomic DNA (EEG) library of soybean, we used DH5 alpha bacteria cell with the Mcr A and Mcr BC system. One thousand four hundred forty EEG colonies have been constructed in total. We analyzed blast search of NCBI and Phytozome for the genetic information of sequenced colonies. More than one hundred forty STS primer sets have been designed based on the sequencing data of selected colonies. The developed primer sets were applied for Hwanggeumkong to select promising primer sets and more than one hundred Korean cultivars were tested to analyze genetic diversity with selected primer sets. Twelve primer sets were polymorphic in Korean cultivars using five restriction enzymes. It is thought that these primers could be useful for specific allele tagging in diverse germplasms and for the functional study of soybean. (This work was supported by grants from the R&D project (#200901FHT020609503) of the National Institute of Crop Science of the Rural Development Administration)

P05-046: THREE ENDO-ß-MANNANASE GENES EXPRESSED IN THE RADICLE TIP AND MICROPYLAR ENDOSPERM PRIOR TO RADICLE EMERGENCE INFLUENCE GERMINATION OF ARABIDOPSIS THALIANA SEEDS

Iglesias-Fernández, R.[1] - Rodríguez-Gacio, M.C.[2] - Barrero-Sicilia, C.[1] - Carbonero, P.[1] - Matilla, A.[2]*
[1]Universidad Politécnica de Madrid
[2]Universidad de Santiago de Compostela
**Corresponding author e-mail: raquel.iglesias@upm.es*

Mannans are hemicellulosic polysaccharides in the plant primary cell wall (CW). Mature seeds, specially their endosperm cells, have CW rich in mannan-base polymers that confer a strong mechanical resistance for the embryo protrusion. The rupture of the seed coat and endosperm are two sequential events during the germination of *Arabidopsis thaliana.* Endo-β–mannanases (MAN; EC. 3.2.1.78) are hydrolytic enzymes that catalyze cleavage of β1→4 bonds in the mannan-polymer. In the genome of Arabidopsis, the endo-beta-mannanase (MAN) family is represented by eight members. We have systematically explored the expression of the eight MAN genes in different organs of this plant and found that only four of them (*AtMAN7*, *AtMAN6*, *AtMAN2* and *AtMAN5*) are induced upon seed germination. Moreover, in situ hybridization analysis shows that their transcript accumulation is restricted to the radicle and to the micropylar endosperm before protrusion, and this expression disappears soon after radicle emergence. T-DNA insertion mutants in these genes (K.O. MAN7, K.O. MAN6, K.O. MAN5), except that corresponding to *AtMAN2* (K.O. MAN2) that is weakly expressed in germinating seeds, show a germination rate slower than that of the wild type (Wt). K.O.MAN6 is the most affected in the germination time course with a t_{50}=48h, almost double than that of the Wt (t_{50}=25h). Our data demonstrate that *AtMAN6*, and at a lower degree *AtMAN7* and *AtMAN5*, are crucial players in the germination process of *A. thaliana* seeds, possibly by facilitating the endosperm rupture by the emerging radicle.

P05-047: ANALYSIS OF FIVE NOVEL CONSTITUTIVE GENE PROMOTERS IN TRANSGENIC RICE PLANTS

Kim, J.[1]* - Park, S.H.[1] - Yi, N.[1] - Kim, Y.S.[1] - Bang, S.W.[1] - Choi, Y.D.[2]
[1]Myongji University
[2]Seoul National University
**Corresponding author e-mail: jukon306@gmail.com*

Novel constitutive gene promoters are essential components of crop biotechnology. We report our analysis of five such promoters, *APX, SCP1, PGD1, R1G1B* and *EIF5*, in transgenic rice plants. The five promoter regions were linked to the gfp reporter gene and transformed into rice. Using fluorescent microscopy and qPCR, promoter activities were analyzed in comparison with *OsCc1, Act1* and *ZmUbi1*, previously characterized strong constitutive promoters. The *APX* and *PGD1* promoters direct high levels of gene expression in all tissues and stages, producing GFP at levels up to 1.3% of the total soluble protein. *PGD1* is particularly active in flowers and mature roots. The *R1G1B* is active in the whole grain including the embryo, endosperm and aleurone layer, and thus represents a constitutive promoter with activity in whole seeds that has not been described previously. The *ZmUbi1* and *R1G1B* promoters are markedly less active in young roots and mature leaves whilst the *APX, PGD1, OsCc1* and *Act1* promoters are highly active in both vegetative and reproductive tissues. Overall, our results demonstrate that *APX, PGD1* and *R1G1B* are novel constitutive gene promoters that are highly active at all stages of plant growth with distinct levels of activity.

P05-048: INHIBITION OF ENDOGENOUS PROTEASES IN NICOTIANA TABACUM CV BRIGHT YELLOW 2 (BY-2) SUSPENSION CELLS IMPROVES RECOMBINANT PROTEIN PRODUCTION

Mandal, M.[1] - Mandal, M.K.[2] - Fischer, R.[2] - Schillberg, S.[1] - Schiermeyer, A.[1]*
[1]Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Department Plant Biotechnology
[2]Rwth Aachen University Institute for Molecular Biotechnology
**Corresponding author e-mail: mkmiit2002@gmail.com*

Recombinant proteins secreted to the plant suspension culture medium are often degraded by endogenous plant proteases resulting in low yields. Recently we have cloned different protease cDNAs from BY-2 cells (NtAspP, NtCysP, NtMMP1 and NtSerP). We have exploited the sequence information to generate protease deficient tobacco BY-2 cell lines through the simultaneous expression of antisense-RNAs against these endogenous proteases. All established antisense cell lines showed reduced levels of endogenous protease expression and activity at late stages of the cultivation cycle. Strikingly, knockdown of the endogenous proteases led to an elongated cell shape indicating involvement of proteases in cell growth and development.
One of the antisense cell lines showing reduced proteolytic activity in the culture medium was selected for the expression of the recombinant full-length antibody 2F5 recognizing the gp41 surface protein of HIV-1. This cell line showed significantly reduced degradation of the 2F5 heavy chain resulting in four fold higher accumulation of the intact antibody heavy chain when compared to transformed wild type cells expressing the same antibody. These data provide a basis for further improvement of plant cells for the production of recombinant proteins.

P05-049: REGULATION OF XYLEM DEVELOPMENT IN BRACHYPODIUM DISTACHYON

Ruth Valdivia, E. – Gianzo, C. – Fidalgo, J. – Cortiñas, L. – Freijeiro, S. – Herrera, MT. – Revilla, G. – Zarra, I. – Sampedro, J.*
Universidad de Santiago de Compostela

**Corresponding author e-mail: ignacio.zarra@usc.es*

Lignocellulosic biomass, composed mainly of secondary walls, could be an abundant and economical source of biofuels. The manipulation of its composition could reduce processing costs significantly. Currently there is very little information on the regulation of secondary cell wall synthesis in monocots, one of the most promising sources of biofuels. In *Arabidopsis*, on the other hand, genes from several families of transcription factors (TFs) are known to be involved in the regulation of secondary cell wall synthesis.
In particular AtVND6 and AtVND7 overexpression constructs can induce ectopic transdifferentiation of different types of cell into tracheary elements (TEs). These genes are both direct activators of MYB46 and MYB83, which are also capable of inducing transdifferentiation. Several other TFs with effects on xylem secondary walls have been described. We have identified orthologs of several of these genes in the genome of the model grass *Brachypodium dystachion*, including six VND genes. We have made transient transformations of tobacco leaves with overexpression constructs of these genes and observed in several cases transdifferentiation of parenchyma and epidermal cells into tracheary elements. It appears that the function of these genes is conserved between monocots and eudicots. These results suggest
that the binding motifs in their target promoters are also conserved. We are currently trying to identify these motifs through cotransformation with promoter::reporter constructs. We will test in *Brachypodium* the function of several TFs that gave positive results in tobacco. We have achieved transient transformation of calli and seedlings and are currently in the process of obtaining stable transformation lines.

P05-050: SEEDS GERMINATION OF THE ENDANGERED SPECIES THYMUS LOTOCEPHALUS G. LÓPEZ & R. MORALES (LAMIACEAE)

Coelho, N.[1] - Gonçalves, S.[1] - Gonzalez-Benito, M.E.[2] - Romano, A.[1]*
[1]Laboratory of Plant Biotechnology, Faculty of Sciences, University of Algarve and Institute
for Biotechnology and Bioengineering (IBB/CGB-UTAD)
[2]Departamento de Biología Vegetal, Escuela Universitaria de Ingeniería Técnica Agrícola, Universidad Politécnica de Madrid
**Corresponding author e-mail: a22545@ualg.pt*

Thymus lotocephalus is a rare species endemic from the Algarve region, Portugal, and little information is known about this plant. The species is legally protected under the European Habitats Directive 92/43/CEE and by the Portuguese law (reference 140/99 from April 24).
Biotechnological approaches offer several conservation possibilities which have the potential to support *in situ* protection strategies and provide complementary conservation options. However, before the application of *ex situ* conservation approaches (e.g. cryopreservation, *ex situ* plant propagation and populations restoration) it is important to study the seed germination requirements of the species. Thus, the goal of this work was to investigate the germination requirements of *T. lotocephalus* seeds under controlled conditions. The presowing treatments studied were: dry heat, cold, mechanical scarification, acid scarification, soaking in distilled water and soaking in gibberellic acid.
Untreated (control) and pre-treated seeds were incubated under a 16-h light photoperiod at 25 ± 2 ºC. It was observed that untreated seeds presented a final germination percentage around 50% and dormancy of *T. lotocephalus* seeds was not broken by heat. From the treatments tested, only soaking in gibberellic acid significantly improved germination, with a germination percentage higher than the control (more than 80%). From these preliminary results, it can be concluded that the main cause of seed dormancy present in *T. lotocephalus* seems to be physiological and can be overcome by immersing the seeds in gibberellic acid. Acknowledgments: Coelho N and Gonçalves S acknowledge a grant from Portuguese Science and Technology Foundation (SFRH/BD/63501/2009 and SFRH/BPD/31534/2006, respectively).

P05-051: LARGE-SCALE INSERTION MUTAGENESIS USING TOBACCO RETROTRANSPOSO (TNT1) IN MEDICAGO TRUNCATULA

Hee-Kyung, L.[1] - Serrani Yarce, J.C.[1] - Tadege, M.[2] - Blaylock, L.[1] - Bishop, B.[1] - Gallaway, J.[1] - *Elles, C.[1] - Jiangqi, W.[1] - Cheng, X.[1] - Ratet, P.[3] - Kirankumar, M.[1]*
[1]The Samuel Roberts Noble Foundation
[2]Oklahoma State University
[3]Institut des Sciences du Vegetal, CNSR

Autonomous long-terminal repeat (LTR) retrotransposons are retrovirus like elements which encode functions required for their own replication and transposition and move in the genome via a so called 'copy and paste' mechanism.
Retrotransposons can be activated by tissue culture to transpose in multiple copies. The absence of excision during transposition makes retrotransposons ideal tools for saturation mutagenesis with stable tags.
We are using tobacco retrotransposon *Tnt1* to mutagenize and tag the whole genome of model legume *Medicago truncatula*. We have generated over 18,000 independent *Tnt1*-containing lines encompassing approximately 450,000 insertion events. Over 20,000 *Tnt1* flanking sequence tags (FSTs) have been recovered. We have pooled genomic DNA from 14,000 lines for customized reverse-genetic screening, and the frequency of insert identification in this pool for an average-sized-gene is approximately 90 percent. All FST sequences have been deposited in the publicly available database (http://bioinfo4.noble.org/mutant/database.php). Mutant screening workshops are open to the scientific community on an annual basis. The range and diversity of mutant phenotypes suggest that *M. truncatula* offers a great opportunity to dissect symbiotic and developmental pathways for a comprehensive understanding of legume biology.

P05-052: OPTIMIZATION OF BIODIESEL PRODUCTION FROM MICROALGAE: LIGHT INFLUENCE ON NANNOCHLOROPSIS GADITANA LIPID ACCUMULATION

Simionato, D. - Corteggiani Carpinelli, E. . Giacometti, G. – Morosinotto, T.*
University of Padova, Department of Biology, Padova, Italy
**Corresponding author e-mail: diana.simionato@unipd.it*

Exploiting microalgae for biodiesel production is an interesting perspective as renewable energy source. Several studies are still needed to improve the process productivity and make it economically competitive. In this work we focused on *Nannochloropsis gaditana*, a seawater microalga, which combines two essential qualities to be a good candidate for biodiesel production: a fast growth rate and a good lipid productivity. We paid attention to the relationship between photosynthesis and lipid accumulation of this alga to find the best conditions for maximal lipid productivity.
We compared growth rate, photosynthetic parameters and lipid production of *N. gaditana* cultures exposed both to continuous illumination and fluctuating light. In the first case we tested different illumination intensities (from 15 to 2000 μE m^{-2} s^{-1}) and results showed that in all cases *N. gaditana* maintained
good growth rate and lipid productivity thanks to the activation of acclimative responses in its photosynthetic apparatus. At high light, these responses included carotenoids accumulation and regulation of photoprotection mechanisms; at low light, an increase of chlorophyll content per cell was observed. In order to simulate outdoor photobioreactor conditions, cells of *N. gaditana* were exposed to light–dark cycles and the response of the photosynthetic apparatus to light fast changes was analyzed and compared to that of a model alga such as *Chlamydomonas reinhardtii*.

P05-053: BIOTECHNOLOGICAL MODIFICATION OF B-CAROTENE CONTENT IN ORANGE FRUITS

Pons, E. - Alquezar, B. - Rodriguez, A. - Rodrigo, M.J. - Zacarias, L. - Peña, L.
Instituto Valenciano de Investigaciones Agrarias (IVIA)

Color of citrus fruits, which greatly influences its visual quality and marketability, is the result of a complex and heterogeneous accumulation of carotenoids. Moreover, carotenoids have important nutraceutical properties, mainly derived from its antioxidant and/or provit-A activity.
The main carotenoid with provit-A activity is β-carotene, a compound hardly detectable in the pulp of most oranges. The most important provit-A compound in oranges is β-cryptoxanthin, which shows a 1:1 conversion (versus 1:2 conversion for β-carotene) to all-trans-etinol (provit-A). β-cryptoxanthin is a intermediate in the formation of zeaxanthin
from β-carotene, in the reaction catalysed by β-carotene hydroxylase (β-CHX). There is an increasing public interest in healthy and flavorful plant products. Because of the massive consumption of Citrus fruits worldwide as both fresh fruits and juice, they are an important source of carotenoids, besides other health-promoting compounds such as vitamin C, flavonoids and folic acid, which occur in few fruits and vegetables at the same combination and high levels. In order to increase orange nutritional value, we have attempted to increase its provit-A content by blocking the expression of the endogenous *β-CHX* using either antisense or RNA interference technology. Transgenic plants were obtained that showed important changes in carotenoid complement in both fruit peel and pulp, with β-carotene increases accompanied by a general decrease in the accumulation of downstream xanthophylls and enhanced production of flavor-related apocarotenoids and terpene volatiles.
The implications of these changes in the nutritional value and volatile composition of transgenic oranges will be discussed.

P05-054: LARVICIDAL ACTIVITIES AGAINST CERATITIS CAPITATA (DIPTERA; TEPHRITIDAE) OF ESSENTIAL OILS FROM MOROCCO

*Loubna, A.**
Faculty of Sciences Semlalia Marrakesh (FSSM)
**Corresponding author e-mail: loub163@hotmail.com*

The Mediterranean fruit fly, *Ceratitis capitata*, is a polyphagous and multivoltine species with a long history of invasion success. Female flies may lay their eggs in more than 250 varieties of fruits, the majority of which are economically important.
In morocco, argan forest covers about 700,000 ha in the Souss region (southern of Morocco) and act as an enormous sink of medflies for the 20,000 ha of Citrus trees cultivated in the Souss plain. The fruit is suitable for larval development which, when completed, is followed by pupation in the soil. The aim of this work was to evaluate the larvicidal activity of essential oils obtained from leaves of some plants against *C. capitata*.
The essential oil of *Salvia officinalis* and *Lavandula dentata*, collected from Central Morocco, were extracted respectively by Microwave-assisted hydrodistillation and hydrodistillation technics. Larvicidal activities of the oils were tested against 24 first-instar larvae of C. capitata using 3 concentrations: 25% 50% and75% of artificial
diet. The bioassays were conducted at constant ambient temperature (25 ± 1ºC), 60 ± 10% R.H in dark conditions.
Larvae mortality was recorded after 7 days. The *L. dentata* and *S. officinalis* oils were toxic, killing respectively 87.5% and 83.33% at concentration of 75%. The LC50 value estimated for these oils were 0.553 mg/g for *S. officinalis* and 1.710 mg/g for *L. dentata.*
In conclusion this study show that oils of *S. officinalis* and *L. dentata* have a significant toxic effect and could be useful in search for newer, safer, and more effective natural larvicidal agents against *C. capitata* and other insects pest.

P05-055: BARE-1 RETROTRANSPOSON ACTIVITY IN BARLEY TISSUE CULTURE AND RESPONSE TO ANTI-RETROVIRAL DRUG COMPOUNDS

Campbell, B.[1] - Piperidis, G.[2] - Godwin, I.D.[1]*
[1]The University of Queensland
[2]BSES, Central Sugar Experiment Station
**Corresponding author e-mail: b.campbell2@uq.edu.au*

Transgenic plants are widely used in agriculture and gene expression network analysis. However, the question arises as to whether the observed phenotype is entirely explained by the transgene, or there has been other genetic changes as a result of the transformation procedure. Application of GM techniques and the possibility of unintended mutations have therefore triggered systematic research on somaclonal variation (SCV). Most transformation protocols apply an *in vitro* selection and regeneration step. These procedures frequently induce SCV, which changes numerous plant characteristics. A direct mechanistic link between tissue culture and transposable element activation is becoming clearer, and accumulated evidence suggests that transposition, insertion, excision, chromosome breakage and ectopic recombination make a significant contribution to SCV. Transformation protocols utilise many components that potentially provide stress to cells grown in culture. The type, age of the culture and various factors present in the culture media such as hormones influence the extent of SCV. Further, osmotic treatment and selection with herbicides or antibiotics has been shown to trigger extensive cytological aberrations in transgenic barley plants. The present study was aimed at investigating the retrotransposon *BARE-1* of barley, in particular, quantifying *BARE-1* expression levels under different stress inducing treatments, as well as the effectiveness of anti-retroviral compounds on down-regulating *BARE-1* expression. Preliminary results indicate that *BARE-1* activity was elicited under most stress inducing treatments and specific contact with anti-retroviral compounds on reverse transcriptase expression had an effect on *BARE-1* down-regulation.

P05-056: GROWTH DEFECTS IN LIGNIN DOWN-REGULATED ALFALFA ARE ASSOCIATED WITH HORMONAL IMBALANCE AND CONSTITUTIVE EXPRESSION OF STRESS RESPONSE GENES

Gallego-Giraldo, L.[1] - Jikumaru, Y.[2] - Kamiya, Y.[2] - Dixon, R.A.[1]*
1The Samuel Roberts Noble Foundation
2Plant Science Center, RIKEN, Japan
**Corresponding author e-mail: imgiraldo@noble.org*

Genetic modification of lignin biosynthesis pathway in alfalfa (*Medicago sativa*) can greatly improve forage quality and saccharification efficiency for bioethanol production. However, transgenic lines down-regulated in *HCT* (shikimate hydroxycinnamoyl transferase) and *C3H* (coumaroyl shikimate 3-hydroxylase) genes, which leads to lower lignin levels and higher cell wall processing efficiency, exhibited the greatest reductions in plant
height, biomass, changes in plant architecture and a flowering delay. To determine whether we could avoid pleiotropic growth effects in low content lignin plants and maintaining their beneficial agronomic features, we performed a detailed phenotypic, physiological and molecular characterization on those transgenic lines. Auxin transport was not affected in *HCT* or *C3H* down-regulated plants despite an increase in flavonoid biosynthesis pathway. Levels of the bioactive gibberellins GA_1 and GA_4, as well as gibberellin sensitivity, were reduced in *HCT* lines, consistent with the reduced plant stature. Additionally, levels of the stress response hormones/signals abscisic acid, jasmonic acid, and salicylic acid were elevated in the transgenic lines, and transcriptome analysis of *HCT* and *C3H* down-regulated lines revealed massive up-regulation of pathogenesis and abiotic stress-related genes. These changes were associated with enhanced tolerance of the lignin-modified plants to *Collectotricum trifolli* infection and drought. We conclude that the growth phenotypes of severely

lignin down-regulated plants likely result from a combination of hormonal imbalance and constitutive activation of defense responses.

P05-057: ANALYSIS OF CIS-ACTING ELEMENT REGION FOR FRUIT SPECIFICITY IN SLHD-2 PROMOTER

*Kim, A.Y. – Park, S.H. – Kim, J.H. – Jeong, B.R. – Joung, Y.H.**
Chonnam National University
**Corresponding author e-mail: yhjoung@jnu.ac.kr*

Result of micro-array analysis, expression level of the histidine decarboxylase gene -2(SlHD-2) was high in tomato fruit and continuous increased during ripening. The SlHD-2 promoter was isolated from tomato (Solanum lycopersicum cv. Micro-Tom) by genome walking method. The isolated SlHD-2 promoter region was characterized here for their levels of expression and tissue-specific location of expression when transformed into tomato. GUS expression with the SlHD-2 promoter was 10 folds higher than 35S promoter in mature green and red stage fruit. On the contrary, GUS expression with the SlHD-2 was 14.4 folds lower than 35S in leaf. Histochemical staining showed that GUS was highly expressed in jelly and pericarp tissues in tomato fruit. These results show that SlHD-2 promoter was fruit specific, especially in mature stage. For analysis cis-acting elements which are related to fruit specific expression, serial deletion from 5' region of promoter was performed. The GUS activity for the deleted promoter (▲ SlHD610) was not detected in matured fruit. In this result, cis-acting elements which are related to fruit specificity was contained in -910~-610 region.
KEY WORDS: fruit specific promoter, transgenic tomato, histidine decarboxylase gene (SlHD-2)

P05-058: PRODUCTION OF HEMAGGLUTININ AS VACCINE AGAINST INFLUENZA VIRUS USING TRANSGENIC PLANT

*Jeong, B.R. - Kim, A.Y. - Park, S.H. - Kim, J.H. – Joung, Y.H.**
Chonnam National University
**Corresponding author e-mail: yhjoung@jnu.ac.kr*

Hemagglutinin(HA) is surface protein of influenza virus. For production of influenza virus vaccine, full and partial genes of HA(A/Puerto Rico/8/34(H1N1) strain) were cloned into plant expression vector, pCAMBIA2300-HE. These full and partial genes were controlled by 35S promoter and tagged by Histidine. The recombinant vectors were transformed into tobacco (*Nicotiana tabacum* cv. Xanthi NC) using Agrobacterium-mediated leaf disc transformation. The transgenic tobaccolines were verified by genomic DNA PCR. HA gene expressed transgenic lines were selected by real-time PCR. HA protein was purified by TALON affinity column from these transgenic tobacco and expression level of HA gene was evaluated by ELISA. This results suggest that plant can be used as an influenza virus vaccine production system.
Key worlds : Hemagglutinin, Agrobacterium mediated transformation, vaccine

P05-059: CHARACTERIZATION OF TOMATO HR7 GENE(SLHR7) PROMOTER IN ARABIDOPSIS

*Kim, J.H. - Kim, A.Y. - Park, S.H. - Jeong, B.R. – Joung, Y.H.**
Chonnam National University
**corresponding author e-mail: yhjoung@jnu.ac.kr*

According to Micro-array data, the *HR7* gene was specific expression in seed of hot pepper. For developing tomato seed specific promoter, pututive promoter region of tomato *HR7* gene(*SLHR7*) was isolated by genome waking method. 981bp promoter include 5'-UTR was isolated and cloned into pCAMBIA1391Z which was promoter analysis binary vector contained GUS gene. *HR7* promoter was characterized here for the levels of expression and tissue specific location of expression when transformed the pCAMBIA1391z-HR7 into abidopsis.
Levels of GUS expression were higher in flower then other tissues with the *SLHR7* promoter. Histochemical staining showed that GUS was highly expressed at stigma and anther in flower but GUS was not detected at petal.
KEY WORLDS: flower specific promoter, transgenic arabidopsis, GUS , 35S promoter

P05-060: COLORECTAL CARCINOMA (CRC)-ASSOCIATED ANTIGEN GA733-2 GENE EXPRESSION IN TOBACCO

*Park, S.H. - Kim, A.Y. - Kim, J.H. - Jeong, B.R. – Joung, Y.H.**
Chonnam National University
**Corresponding author e-mail: yhjoung@jnu.ac.kr*

Production of vaccine in plants has become an important issue in plant biotechnology field. Human colorectal carcinoma antigen GA733-2 gene and Fc region fused recombinant GA733-Fc gene were expressed into tobacco (*Nicotiana tabacum* cv. Xanthi-nc) plant using by Agrobacterium-mediated transformation. Plant expression vector used to this experimentation was pCAMBIA2300-HE. The GA733-2/GA733-Fc genes were controlled by CaMV 35S promoter and tagged by histidine. Several GA733-2/GA733-Fc transgenic tobaccos were selected. The GA733-2/GA733-Fc proteins were detected in these transgenic plants and protein expression was quantified by ELISA. Also GA733-2/GA733-Fc genes were expressed in tobacco using by Agroinfiltration method. Compare the expression level of GA733-2, transient expression method was more efficient then transformation.
Key worlds: Human colorectal carcinoma antigen GA733-2, Agrobacterium mediated transformation, Agroinfiltration, immuno-protein, transgenic tobacco

P05-061: TOOLS FOR REVERSE GENETICS AND SNP DISCOVERY

Schmidt , J. – Malvoisin, P.
AELRED

AELRED is a recently established biotechnology start-up company, and has a technology transfer agreement with INRA and Genoplante-Valor on the latest developments of the reverse genetics TILLING® technology. AELRED provides mutagenesis and TILLING or Eco-TILLING services to its customers, either for commercial seed companies or for academic laboratories wishing to obtain new alleles of a given gene, or to confirm the function of unknown genes.On a longer term, AELRED plans to become an integrated operator in the green chemistry sector, using its technology to develop ingredients extracted from improved plants for industrial, food, pharmaceutical or cosmetic uses.
AELRED is currently developing several TILLING platforms on various plant species for private and public customers, and is implementing an Arabidopsis thaliana (cv. Col 0) TILLING platform based on a collaboration with INRA-Versailles; this platform will be publicly available in 2010. AELRED hosts as well INRA's Brassica napus (cv. Tanto) TILLING platform which is publicly open for screening. Besides, AELRED is partner in several submitted scientific projects.
AELRED establishes itself as a putative partner in any project in the field of crop improvement or plant genomics, by providing the appropriate mutated plant collections and reverse genetics TILLING or Eco-TILLING screening.
TILLING® r:egistered trade mark of Anawah Inc (Arcadia Biosciences).

P05-062: EXPLORING ENDOGENOUS CEREAL PHYTASES FOR THE IMPROVEMENT OF PHOSPHORUS AND MINERAL BIOAVAILABILITY

Krogh Madsen, K. - Dionisio, G. - Bæksted Holme, I. - Bach Holm, P. - Brinch-Pedersen, H.*

University of Aarhus, Faculty of Agricultural Sciences, Dept. of Genetics and Biotechnology, Research Centre Flakkebjerg, Denmark
**Corresponding author e-mail: ClausKrogh.Madsen@agrsci.dk*

Increasing the endogenous phytase activity in cereal grains is of importance in order to improve the sustainability of animal intensive agriculture. Furthermore it may help counter some forms of human malnutrition
such as zinc deficiency which affects millions.
Understanding the molecular basis for the observed variation between species and cultivars would be valuable for the effort to increase endogenous phytase activity by breeding or cisgenic approaches. Two families of phytases are known in cereals, the Multiple Inositol Phosphatases (MINPP's) and the Purple Acid Phosphatase Phytases (PAPhy's). Both families are highly conserved at the mature protein level and variation in specific activity within each family appears to be small.
Hence we propose that the variation in phytase activity between species and cultivars are mainly attributable to gene regulation. To investigate this hypothesis we have used genomic library screening and PCR to isolate promoters of phytase genes from wheat, barley and rye. Promoters from cultivars representing the whole spectrum of grain phytase activity in wheat have been PCR amplified, cloned and sequenced. The promoters are analyzed for known regulatory elements and compared. Already, we have shown that PAPhy genes are regulated by two distinctly different types of promoters that are active primarily during grain filling and germination, respectively. In order to account for the variations in grain phytase activity we wish to correlate promoter polymorphisms with variations in expression level or pattern. These parameters are examined by proteomics, qRT-PCR, reporter gene constructs and immunolocalisation. Selected proteins are expressed in *Pichia pastoris* and characterized *in vitro*.

P05-063: BIOLOGICAL EFFECT EVALUATION OF A TRITERPENIC COMPLEX TOWARDS CELL LINE DU 145

Olariu, L. – Constantinovici, M. – Dumitriu, B. - Zglimbea, L. – Dociu, N.*
BIOTEHNOS S.A.
**Corresponding author e-mail: lolariu@biotehnos.com*

Objective: Determine the triterpenic acids complex actions on DU 145, human prostate carcinoma *cell line*, for the purpose of studying the biological effect of this new biological active product, obtained from *Salviae sp*., on this neoplastic cell type. Methods: There were applied several types of cell biology specific protocols for a complete screening of its biological effects: viability and citotoxicity evaluation, using ELISA technique; proliferation, apoptosis and cell cycle analysis, using flow cytometry. The viability and cytotoxic evaluation were made after 24 h of DU 145 cells exposure to increasing doses of this complex and its carrier DMSO, ranging from 8 to 16 μM; the positive control was methothrexate 10 μM. The DNA synthesis analysis using flow cytometry were done after 48 h exposure to specific doses of triterpenic complex, DMSO and methothrexate. The statistical analysis of raw data was made with FCS Express software. Results: The triterpenic complex effect on proliferation and viability status of DU 145 cells was negative, inhibiting them in a dose dependent manner; citotoxicity was moderate until 12 μM, after that raising drastically its value. There was a significant reduction of the proliferation index which had half the value measured for untreated cells. Also this complex induced early apoptosis (30% of treated cells compared to 9% for control) and had a blocking effect on DNA synthesis (compared to DMSO and methothrexate 10 μM), both being more evident at 10 μM concentration.
Conclusions: These results could be used as a starting point for more profound evaluation of biological implications of triterpenic derivates as therapeutical agents of neoplastic diseases.

P05-064: SCREEN FOR DEVELOPMENTAL PHENOTYPES IN TAXON RESTRICTED GENES

Chikkaputtaiah, C. – Donoghue, M. – Li, W. - Swamidatta, S. – Spillane, C.*
Genetics and Biotechnology lab, Botany and Plant science, National University of Ireland Galway, Ireland
**Corresponding author e-mail: chinnatal@gmail.com*

Taxon restricted genes are protein coding genes which have no recognizable homologs in other species or within existing protein sequence databases. Even though they constitute up to one third of the genes found in any particular species, very little is known about their function and evolutionary genesis. Our group has defined a set of taxon restricted genes in *Arabidopsis thaliana* and whole genome selection scans indicated that some of the taxon restricted genes are fast evolving with a dN/dS>1 suggesting that these genes could be under positive Darwinian selection. We are performing systematic genetic, molecular and biochemical characterization of taxon restricted genes to elucidate their role in different developmental processes including reproduction and seed development in plants.

P05-065: BIOCHEMICAL ANALYSIS OF DOMINANT POLLEN TYPES REPRESENTED BY LOCAL HONEY SAMPLES

Tidke, J. - Nagarkar, S.S.*
Department of Botany,Sant Gadge Baba Amravati University, Amravati (MS), India
**Corresponding author e-mail: jaikirantidke@rediffmail.com*

Qualitative and quantitative biochemical analysis of pollen grains of fifteen plant species i.e. *Brassica campestris, Gossypium hirsutum, Azadirachta indica, Moringa oleifera, Butea monosperma, Cajanus cajan, Syzygium cumini, Coriandrum sativum, Helianthus annuus, Parthenium hysterophorus, Tridax procumbens, Vernonia cineria, Ipomoea fistulosa, Vitex negundo* and *Ricinus communis* was undertaken during the year 2008-2009. The pollen grains of these plant species were found to be dominantly present in the local honey samples. The pollen samples were biochemically investigated for carbohydrates and sugars, free amino acids, protein, free lipids, moisture and ash contents. The maximum amount of reducing sugar and total carbohydrates was found to be 3.08% and 5.76% in *Vernonia cineria*. Maximum crude protein and soluble protein i.e. 48.4% and 32.1% was found to be present in pollen grains of *Parthenium hysterophorus.* Free amino acids and lipid contents were encountered maximum in *Helianthus annuus* pollen; i.e. 3.09% and 4.10%. The maximum amount of moisture and ash was 12.83% and 7.05% in *Tridax procumbens* and *Brassica campestris* respectively. The role of some biochemical was found to be responsible for the visits of flower visiting bees in general and honey bee (*A. dorsata*) in particular. The data obtained through pollen biochemical analysis is being interpreted with pollen frequency class and the honey bee visits. Some pollen types such as *Moringa oleifera, Helianthus annuus, Ricinus communis* and *Parthenium hysterophorus* having more amounts of protein, carbohydrates and amino acids were dominantly represented in studied honey samples.
Key Words: Biochemical, pollen, visitor relevance

P06
Root Biology

P06-001: THE SHR/SCR PATHWAY DIRECTLY ACTIVATES GENES INVOLVED IN ASYMMETRIC CELL DIVISION IN THE ARABIDOPSIS ROOT

Sozzani, R. [1] - Moreno Risueno, M. [1] - Wolfgang Busch [1] - Jaimie Van Norman [1] - Walter Dewitte [1] - Jim Murray [1] - Philip Benfe [1]*
1 (Duke University)
2 (Cardiff School Of Biosciences)
**Corresponding author e-mail:* rs93@duke.edu

Multicellular organisms rely on asymmetric cell division to generate diverse cell types. The molecular mechanisms responsible for this process are still poorly understood, in particular how developmental pathways trigger asymmetric divisions. Asymmetric divisions in the Arabidopsis root are controlled by a finely orchestrated interplay between the transcription factors SHORTROOT (SHR) and SCARECROW (SCR). To understand the dynamics of the SHR/SCR regulatory network, we performed time-course microarrays after SHR and SCR induction. To examine the transcriptional effects specifically to the ground tissue, we sorted the GFP positive cells from the J0571 enhancer trap present in both inducible lines. The proportion of transcription factors downstream of SHR/SCR network was significantly higher than expected by chance, as demonstrated by GO category enrichment. Thus, this suggests that SHR and SCR activate a regulatory cascade involved in several biological processes including asymmetric cell division. Moreover, we observed that that joint activity by both SHR and SCR is necessary to activate this group of genes and that their temporal regulation is important to determine the timing of asymmetric cell division in the ground tissue.

P06-002: LONGITUDINAL AND TRANSVERSAL EXPANSION IN RESPONSE TO INCREASED ETHYLENE IN MAIZE ROOTS

Alarcón, M.V.[1] - Lloret, P.G.[2] - Iglesias, D.J.[3] - Talón, M.[3] - Salguero, J. [4]
[1]Centro de Investigación Finca La Orden. Junta de Extremadura
[2]Biología Celular. Universidad de Extremadura
[3]Genómica y Poscosecha. IVIA
[4]Universidad de Extremadura

Ethylene is involved in the regulation of root growth, particularly in roots growing under mechanical impedance or other stresses. The increase of ethylene production has been previously related to reductions of root length and induction of radial growth (observed as swelling in root tips). In this work, we show the effect of ACC treatments upon ethylene production compared with those on root elongation and radial growth in maize roots.
The presented results demonstrate that ACC treatments increase ethylene production and vary with the concentration of ACC applied.
The ethylene antagonist silver thiosulphate (STS) does not modify the increased ethylene production in response to ACC treatment. Moreover, the raise in ethylene level inhibited root elongation and increased radial expansion (swelling) as ACC concentration was increased. STS completely reversed the effects of increased ethylene levels upon the elongation and swelling without affecting the ethylene production. However, the root elongation and the radial expansion showed different sensitivity to increase in ethylene levels. Low ACC concentrations (1 μM), which significantly augmented ethylene levels, inhibited elongation but did not affect radial growth. Taken together, the results showed that root elongation is more sensitive than transversal expansion in response to increased ethylene.
Financed: 2PRI01B009 Junta de Extremadura, Spain.

P06-003: MTNAC969, AN ENVIRONMENTAL RESPONSIVE TRANSCRIPTION FACTOR REGULATING MEDICAGO TRUNCATULA ROOT ARCHITECTURE

de Zélicourt, A.[1] - Zahaf, O.[1] - Plet, J.[1] - Diet, A.[1] - Marcel, F.[2] - Crespi, M[1] - Frugier, F.[1] - Gruber, V.[1]*
[1]ISV CNRS
[2]URGV
**Corresponding author e-mail: dezelicourt@isv.cnrs-gif.fr*

Legumes, due to their capacity to establish symbiotic associations, are able to grow in nitrogen poor soils and are major crops worldwide. Transcription factors (TF) are able to integrate environmental and endogenous cues to control root architecture. As soil salinity is a major abiotic stress affecting crop yield, we developed transcriptomic approaches in the *Medicago truncatula* model to search for regulatory genes linked to salt response in roots. Forty-six salt regulated TFs were identified, three of them belonging to the NAC transcription factor family: *MtNAC969*, *1081* and *1126*,. These TFs are specifically induced in response to salt stress in roots when compared to other abiotic stresses such as mannitol, cold or heat. The three NACs were overexpressed in *M. truncatula* roots and only de-regulation of *MtNAC96*9 yield a root phenotype. This TF is closely related to *AtNAP*, involved in *Arabidopsis* leaf senescence.
Overexpression of *MtNAC969* reduces root growth and lateral root formation whereas symbiotic nodules showed an accelerated senescence. In addition, RNAi against *MtNAC969* led to an increased root growth under salt stress, a higher number of lateral roots and a reduction of their nodulation capacity. *MtNAC969* could be a new TF involved in the regulation of legume root architecture in presence of symbiotic bacteria or an abiotic stress.

P06-004: POLAR IAA TRANSPORT DURING ARABIDOPSIS ROOT GRAVITROPISM: QUANTITATIVE ANALYSIS WITH CYTOCHEMISTRY AND GC/MS

Pozhvanov, G. - Medvedev, S.*
Saint-Petersburg State University, Faculty of Biology, dept. of Plant Biology and Physiology
**Corresponding author e-mail: pozhvanov@gmail.com*

Process of polar auxin transport and its level are of great interest in cell biology. IAA accumulation in root inhibits cell elongation and thus regulates growth direction in response to gravity. Our study extends the existing methods for measuring the content of IAA through the use of cytochemistry with GC/MS. Arabidopsis DR5::GUS transformants were used.
Plants were grown on a medium with different IAA concentrations for calibration and then stained with X-Gluc according to a standard protocol. Auxin content was estimated indirectly by computer analysis of stained roots digital microphotographs and matched to direct measurement of total IAA content in root 1cm sections with GC/MS, SIM version of metabolomic analysis. One 6 days old DR5::GUS seedling of Arabidopsis contained 175.6 pg IAA per g of dry mass.
We also examined the IAA content *in situ* not only in single plant but also in different organs and specifically in gravistimulated roots.
Auxin content in the bottom side of roots (30÷35 fMole/px) was 2-3 times larger than in the upper side (10÷15 fMole/px). We studied the interconnection between IAA lateral transport and cytokinin content in roots in both DR5::GUS and ARR5::GUS seedlings. Auxin content in seedlings grown on medium with BAP 10^{-8} to 10^{-5} M was not correlated with BAP treatment. Pos-

sible auxin and cytokinin interactions during root gravitropism are discussed.
Project was supported with RFBR Grant No. 08-04-00566a, St.-Petersburg Government Grant for Young Researchers No. 2.6/22-04/004.

P06-005: THE ROLE OF APL AS A TRANSCRIPTIONAL REGULATOR IN SPECIFYING VASCULAR TISSUE IDENTITY

Lichtenberger, R. - Satu Lehesranta - Ove Lindgren - Sari Tähtiharju - Ykä Helariutta*
University of Helsinki
**Corresponding author e-mail:raffael.lichtenberger@helsinki.fi*

The vascular system of higher plants confers efficient conduction and provides mechanical support. It consists of two kinds of conducting tissues, xylem and phloem. Phloem transports the products of photosynthesis and provides paths for translocation of proteins and mRNAs involved in plant growth and development. Although there are some reports of gene expression characteristic to phloem, the molecular basis of phloem development is still largely unknown.
The APL transcription factor (Altered Phloem Development) was identified as the first gene specifying vascular tissue identity. Based on cell sorting coupled with genome-wide microarray analysis, we have been able to uncover phloem abundant regulatory genes dependent on APL. The results indicate that APL is a key node for transcriptional activation of gene expression characteristic to phloem development and for transcriptional repression of gene expression characteristic to xylem development. We are currently studying the possible functions of the identified genes in phloem development.

P06-006: MIR390, TAS3 TA-SIRNAS AND THEIR ARF TARGETS DEFINE AN AUTO-REGULATORY NETWORK QUANTITATIVELY CONTROLLING LATERAL ROOT GROWTH

Marin, E.[1] - Jouannet, V.[2] - Herz, A.[3] - Lokerse, A.S.[4] - Weijers, D.[4] - Vaucheret, H.[5] - Nussaume, L.[3] - Crespi, M.[2] - Maizel, A.[2]*
[1](CEA)
[2]Institut des Sciences du Végétal
[3] Laboratoire de Biologie du Développement des Plantes, SBVME, IBEB, DSV, CEA, CNRS, Université Aix Marseille
[4]Laboratory of Biochemistry, Wageningen University
[5]Laboratoire de Biologie Cellulaire, Institut Jean-Pierre Bourgin
**Corresponding author e-mail: elena.marin@cea.fr*

Plants adapt to environmental conditions by forming new organs in response to morphogenetic signals. Lateral roots branch from the main root in response to local auxin maxima. How a local auxin maximum translates to a robust pattern of gene activation ensuring the proper growth of the newly formed lateral root is not known. Here we demonstrate that miR390, *TAS3*-derived ta-siRNAs (trans-acting siRNAs) and ARFs form an auxin-responsive regulatory network controlling lateral root growth. Spatial expression analysis using reporter gene fusions, ta-si/miRNA sensors and mutant analysis, showed that miR390 is specifically expressed at the sites of lateral root initiation where it triggers the biogenesis of trans-acting siRNAs. These ta-siRNAs inhibit ARF2, ARF3 and ARF4, thus releasing repression of lateral root growth. In addition, ARF2, ARF3 and ARF4 control auxin-induced miR390 accumulation.
Positive and negative feedback regulation of miR390 by ARF2, ARF3 and ARF4 thus ensures the proper definition of the miR390 expression pattern and maintains ARF expression in a concentration range optimal for controlling the timing of lateral root growth, a function similar to its activity during leaf development. These results also show how small regulatory RNAs integrate with auxin signalling to quantitatively control organ growth during development.

P06-007: THE INHIBITION OF PRIMARY ROOT ELONGATION UNDER LOW BORON SUPPLY COULD BE MEDIATED BY HORMONES IN ARABIDOPSIS SEEDLINGS

Martín-Rejano, E.M. - Camacho-Cristóbal, J.J. - Herrera-Rodríguez, M.B. - Rexach, J. - Navarro-Gochicoa, M.T. - González-Fontes, A.
Departamento de Fisiología, Anatomía y Biología Celular, Universidad Pablo de Olavide, E-41013, Sevilla, Spain
**Corresponding author e-mail: emmarrej@upo.es*

Soil nutrients are critical elements for plant growth and productivity. Thus, in response to the bioavailability of nutrients in soils, plants have evolved various adaptive mechanisms among which the plastic development of the root system is of crucial importance. In this work we study in detail the temporal responses of the Arabidopsis root architecture to low boron (B) supply. For this purpose Arabidopsis seedlings were grown in 10 μM B during 5 days and then transferred to a low B medium (0.4 mM) or control medium (10 mM) for a 4-day period. Neither the length nor the number of lateral roots were affected by B availability during the 4 days of experiment; however, plants grown in the low B conditions had a reduced primary root (PR) length when compared to control plants from day 1 onwards. This is a very interesting result since lateral root growth should also be inhibited by low B supply if the primary effect of B was only on cell elongation (i.e. a consequence of the structural role of B in the cell wall). B availability also affected the number and elongation of roots hairs in the PR; thus, low B supply induced root hair proliferation and elongation.
Furthermore, by using chemicals that alter hormone metabolism or signalling and several suitable mutants, we provide evidence showing that hormones could play a key role in the root architectural changes caused by low B availability.
Research supported by BFU2009-08397 and Junta de Andalucía CVI-4721 and BIO-266, Spain

P06-008: TOMATO MORPHOLOGICAL AND ANATOMICAL ROOT TRAITS IN RELATION WITH PLANT WATER UPTAKE

Romero Aranda, M.[1] - Jurado-Lavado, O.[2] - Fernández-Muñoz, R. [2]*
[1]Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Estación Expe
[2]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA)
**Corresponding author e-mail: rromero@eelm.csic.es*

Limited work has been performed to explore the potential for genetic improvement of tomato roots in relation to water uptake. The main goal of this study was to establish possible relationships between morphological or anatomical root characters and plant water uptake.
We examined root traits in two tomato species (wild *Solanum pimpinellifolium* acc. TO-937 and domesticated *Solanum lycopersicum* cv. Moneymaker) differing in plant water use.
The study was developed in a growth chamber in hydroponically cultivated plants which allowed assessing plant water uptake. Key root traits for water uptake, including root length and number of root tips were identify.
Root anatomical traits, including root diameter, thickness of the cortex, number of xylem poles, number of metaxylem vessels and root and shoot biomass were also determined. In a period of 21 days, the cumulative plant water consumptions of TO-937 and Moneymaker were 1368±419 and 3209±696 ml/ plant, respectively. Shoot dry mass determined on Moneymaker was threefold that of TO-937 but root to shoot ratio were 0.17±0.03 and 0.27±0.07 for Moneymakeer and TO-937, respectively. Plant water uptake and biomass production are discussed in relation with root development, root morphological traits and root internal anatomy.

P06-009: AUXIN AND SUGAR METABOLISM INTERACTIONS DURING ADVENTITIOUS ROOTING

Acosta Echeverría, M.[1]* - *Albacete, A.*[2] - *Pérez-Alfocea, F.*[2] - *Sánchez-Bravo, J.*[1] - *Agulló-Antón, M.A.*[1]
[1]*Universidad de Murcia. Department of Plant Biology*
[2]*CEBAS-CSIC. Department of Plant Nutrition*
**Corresponding author e-mail: macosta@um.es*

Auxins are essential phytohormones for the rooting of cuttings. Exogenous auxin treatment of carnation (*Dianthus caryophyllus* L.) cuttings before planting is a common procedure in the commercial production of carnation plants and flowers. On the other hand, since adventitious root formation implies high cell activity, the presence of carbon skeletons becomes essential. Freshly harvested carnation cuttings were planted after being treated or not (control) with an auxin solution.
Carbohydrate concentrations were measured in the basal stem before treatment and at different intervals up to 72 h after planting. The activity of different enzymes working in the carbohydrate metabolism was also determined for the same intervals. Glucose concentration gradually decreased until 48 h after planting in the basal stem of control cuttings, whereas in auxin-treated cuttings glucose continued diminishing at least until 72 h. Sucrose synthase (SS) activity increased between 24 and 72 hours as a result of the auxin treatment.
We conclude that auxin increases SS activity in a step that is subsequent to the beginning of root differentiation (first 24 hours following plantation).
From that moment on, high amounts of sugar are released, coinciding with a phase of high cell activity and carbohydrate consumption (as the carbohydrate measurement showed). These changes in SS activity might also contribute to endogenous auxin regulation, because UDP-glucose is a substrate for the conjugation and inactivation of indole-3-acetic acid.
Acknowledgements: Project MICINN/FEDER AGL-2008-02472, plant material supplied by Barberet&Blanc S.A.

P06-010: GROWTH, MORPHOLOGICAL AND ANATOMICAL CHARACTERISTICS OF ROOTS AND ROOT HAIRS IN ARABIDOPSIS SPECIES WITH DIFFERENT STRATEGY IN ZINC TOLERANCE

Stanova, A. - Valasekova, E. - Ciamporova, M. - Ovecka, M.*
Institute of Botany, Slovak academy of sciences
**Corresponding author e-mail: andrea_stanova@centrum.sk*

We focused on strategies in heavy metal tolerance, roots and root hairs characteristics of three *Arabidopisis* sp. originating from localities with non-contaminated (N) and contaminated (C) soils in Slovakia, and their responses to zinc excess.
Sensitive A. thaliana (Ratkovo, N) did not accumulate zinc. Based on the ratio of leaf and soil zinc concentrations (BCF) for the tolerant species the following strategies were suggested: *A. arenosa* (Richtarova -- luka, N) and *A. halleri* (Uhorna, N) as an accumulators, *A. arenosa* (Stiavnicke bane -- Terezia, C) as an excluder. Based on amount of zinc in leaves, we confirmed *A. halleri* (Krompachy, C) as a hyperaccumulator.
Only in the sensitive *A. thaliana* the experimentally-induced stress of zinc excess (1000 μM $ZnSO_4$) inhibited root growth, reduced the distance between root tip and the first root hair, reduced number and length of root hairs. Morphology of root hairs was deformed. Extremely high zin concentration caused disturbances in pattern and integrity of root tissues in *A. thaliana.* Atypical site of root hair outgrowth appeared in A. arenosa (Richtarova -- luka).
Otherwise, in tolerant species differences between control and zinc-treated seedlings were statistically insignificant. Responses of the three *Arabidopsis* sp. to experimentally induced zinc stress corresponded to their adaptation ability to survive in soils (C) of the natural habitats.
Supperted by VEGA 2/0149/08 and VEGA 2/0200/10.

P06-011: SUPPRESSION OF LATERAL ROOT INITIATION IN BARLEY IN RESPONSE TO TRANSIENT ROOT WATER SHORTAGE: A NOVEL ROLE FOR ABA IN LATERAL ROOT INITIATION?

*Babe, A.**
University of Louvain-la-neuve (UCL)
**Corresponding author e-mail: aurelie.babe@uclouvain.be*

The plasticity of branching patterns of plant roots plays a crucial role in the capture iof resources from unpredictable, heterogeneous and dynamic soil environement. This plasticity is largely achieved through the regulation of the site and time of initiation of lateral roots.
The seminal root of Hordeum v. seedlings grown in aeroponics lack branches in the segment which is formed during transient mild root water shortage episodes.This response is highly reproductible and is not due to changes of mineral concentrations in the root zone that might result from the treatment. Surprisingly in view of abscissic acid effects in Arabidopsis root development, transient application of 50μM ABA produces the same response as the water shortage.
A miscrocopy approach was perfromed to characterise the stage at which primordium development is blocked by this treatment. We shown that LR formation in Barley is restricted to the apical region of the seminal root and follows an acropetal sequence. The first asymmetric division, though to be the earliest visible stages of LR initiation, takes place between 10 and 15 mm from the tip. No primordia were found in the zone devoid in LR (under water stress or ABA treatment).
In addition, careful time lapse imaging of the seminal root reveals that the region devoid of LR under water stress or ABA treatment is offset by about 8 mm proximal to the SR segment formed during the treatment.
These results suggest a checkpoint-like regulation of lateral initiation operating at about 8 mm from the tip, i.e. in the very early phases of LR initiation (before or around the first asymmetric divisions). Experiments to validate and elucidate the role of ABA in this process are under way.

P06-012: EFFECTS OF CYTOKININ ON LATERAL ROOTS INITIATION AND DEVELOPMENT

Bielach A - Agnieszka Bielach, E.B.*
VIB Department of Plant Systems Biology, Ghent University, Ghent, Belgium
**Corresponding author e-mail: agbie@psb.vib-ugent.be*

Development of root architecture is influenced by environmental parameters and hormones. It has been shown that plant hormones auxin and cytokinin are key regulators of lateral root (LR) organogenesis.
Many reports describe the inhibitory effect of cytokinins on lateral root, thus acting antagonistically to auxin, but the mechanism of cytokinin regulation is not known.
To investigate the role of cytokinin in LR organogenesis a detailed expression analysis of cytokinin marker TCS (*Two Component Outputsensor*) was performed. TCS::GFP exhibits increased response in lateral root cap, columella cells, in a tip of emerging primordium and in pericycle cells. In general, TCS expression in root revealed complementary pattern of enhanced cytokinin response when compared to auxin distribution.
To monitor the cytokinin perception involved in lateral root organogenesis detailed expression and phenotype characterization of cytokinin receptor mutants was performed. Analysis of *ahk2-2*(*arabidopsis histidine kinase2*), *ahk3-3, ahk4/cre1-12* and their double mutant combinations revealed specific and partially overlapping function of cytokinin receptors in LR organogenesis.

P06-013: FUNCTIONAL ARCHITECTURE OF BLACK COTTONWOOD (POPULUS TRICHOCARPA TORR. & GRAY) FINE AND PIONEER ROOTS

Bagniewska-Zadworna, A.[1] - Byczyk, J.[1] - Zadworny, M.[2]*
[1]Department of General Botany, Institute of Experimental Biology, Adam Mickiewicz University
[2]Laboratory of Root System Pathology, Institute of Dendrology, Polish Academy of Science
**Corresponding author e-mail: agabag@amu.edu.pl*

The function of the roots is partially exhibited by their anatomical structure. The aim of the experiments was to investigate the diverse of the new developing fine and pioneer roots to determine their functional structural design. The daily root growth rate in the relation to the weather conditions was examined in the field conditions, using root boxes. Since the function of pioneer roots is not well known, it is important to verify their character and the nature of relationship between growth speed and histological arrangement. In general pioneer roots are attributed to play structural function whereas fibrous are responsible for water and nutrient absorption. The anatomical structure separated these two classes of roots. Pioneer and fibrous roots significantly varied for basic of the parameters analyzed. We found that the root and stele diameters, proportions between stele and cortex as well as archic structure ranged between fine and pioneer roots. The cytological analysis aimed also to examine the xylogenesis process in those two types of roots of the known age. Anatomical construction of apical first root order confirmed that these two classes of roots, varied in absorptive ability, even if produced by the same plant. It seems that black cottonwood might generate more pioneer roots to forage for nutrient rich areas at large soil distance and then specifically "install" fine roots. Since those results are only preliminary, it will be necessary to study how flexible pioneer roots of different plants could be in response nutrient rich zones or investigate why the same species produces functionally diverse lateral roots.
Acknowledgement This work was supported by grant no. NN309007437 from the Polish Ministry of Science and Higher Education.

P06-014: IDENTIFICATION OF THE MOLECULAR COMPONENTS OF THE AUXIN-CYTOKININ INTERACTION DURING LATERAL ROOT ORGANOGENESIS

Vanstraelen, M. - Duclercq, J. - Naouar, N. - Parizot, B. - Benková, E.*
VIB Dept. of Plant Systems Biology, UGent, Gent, Belgium
**Corresponding author e-mail: mastr@psb.ugent.be*

Lateral root organogenesis in *Arabidopsis* is governed by a complex network of hormonal regulations.
The plant hormones auxin and cytokinin have been demonstrated to act as key regulators of lateral root organogenesis and their mode of interaction is antagonistic. To identify novel molecular components regulating auxin-cytokinin interaction, we used transcript profiling on sorted pericycle cells after treatment with auxin, cytokinin or both. Of the 29666 genes analyzed, 3172, 1457 and 3660 were differentially regulated (FC>1.5 or <-1.5 and p<0.01) by auxin, cytokinin and simultaneous treatments, respectively.
These genes were classified into 27 clusters depending on their expression profile after the hormone treatments. Using ANOVA 2 way analysis, we identified 1117 genes showing an auxin-cytokinin interaction (p<0.01). Three major profiles were discerned representing an additive, antagonist or synergistic mode of interaction. Our expression analysis revealed that auxin treatment affected expression of the cytokinin metabolism and signaling pathway genes significantly more than the cytokinin expression of genes regulating auxin metabolism and perception.

P06-015: NITRIC OXIDE (NO) ROLE AS A MOLECULAR REGULATOR OF PRIMARY ROOT DEVELOPMENT.

Fernández Marcos, M. - Sanz, L. - Curto-Prieto, M. - Rodríguez, D. - Lorenzo, O.*
Universidad de Salamanca
**Corresponding author e-mail: maria@usal.es*

Nitric Oxide (NO) is a bioactive molecule and an essential regulator involved in several plant developmental processes, such as seed germination, root organogenesis and etiolation. Since the molecular bases of the NO action in early plant development are currently unknown, the identification of the elements involved in this response is essential to understand the NO perception and signalling by the plant. We get insight into NO action in early seedling growth by performing a phenotypical, genetic and molecular analysis of NO responses using NO donors (SNP and SNAP) and NO scavengers (cPTIO). It has been previously described that NO is able to diminish primary root (PR) growth in tomato. In this work, we demonstrate that wild type Arabidopsis seedlings show inhibition of PR growth after NO treatment compared to control plants. Comparisons of the number and size of cells in the root meristem reveal significant differences after the addition of NO donors or scavengers, supporting a pivotal role of NO in the modulation of cell division and cell elongation in root growth in Arabidopsis. To find out molecular targets of NO we are adopting three different approaches: (1) a differential transcriptome analysis from whole seedlings and from laser-capture-microdissected epidermis cells in presence or absence of NO donors and scavengers (2) a genetic screening using EMS-mutagenized Arabidopsis collection after addition of NO and score for their length of primary root and (3) a genetic screening of key hormonal impaired mutantas (i.e. auxins, GAs…) and score for NO root-related phenotypes.

P06-016: THE ROLES OF AUXIN AND OTHER HORMONES IN ARABIDOPSIS ROOT MERISTEM DEVELOPMENT

Zolla, G. - Brian, J. - Karin, L.*
Umeå Plant Science Centre, The Swedish University of Agricultural Sciences
**Corresponding author e-mail: gaston.zolla@genfys.slu.se*

Auxin (IAA, indole-3-acetic acid) and cytokinins are essential plant growth regulators that are known to influence cell division, elongation and differentiation in various developmental contexts. Localized IAA biosynthesis and polar IAA transport combine to form an IAA gradient and maximum in the root apex. This, in turn, mediates pattern formation(Blilou et al. & 2005; Grieneisen et al., 2007; Petersson et al., 2009). We have characterized different auxin biosynthesis mutants and show that both tryptophan and indole-3-pyruvic acid (IPA) biosynthesis pathways play critical roles in determining root meristem size in *Arabidopsis* seedlings. Moreover, we show that cytokinins regulate auxin biosynthesis and as a result, show that cytokinins can modulate the auxin gradient and maximum in the root apex. ABA and gibberellins also play roles in determining meristem size. We are currently investigating the potential role of these hormones and how they interact to modulate root meristem development. Blilou et al. (2005) Nature 433: 39-44.
Grieneisen et al. (2007) Nature 449: 1008-1013. Petersson et al. (2009) The Plant Cell 21: 1659-1668.

P06-017: ARABIDOPSIS TREHALOSE-6-PHOSPHATE PHOSPHATASES FUNCTION IN LATERAL ROOT DEVELOPMENT

López-Galvis, L. - Vandesteene, L. - Beeckman, T. - Van Dijck, P.*
VIB-Department of Molecular Microbiology-K.U.Leuven, Belgium
**Corresponding author e-mail: Lorena.Lopez@mmbio.vib-kuleuven.be*

Trehalose (T) is a disaccharide widely distributed in nature playing an important role in carbohydrate storage and stress protection. It was thought to be absent in plants, but genome sequencing and mutant analysis using ectopic expression of microbial T biosynthesis genes showed that its metabolism is essential for normal

growth and development as specific phenotypes related to sugar partitioning, carbon allocation and stress resistance were found. This is more linked to the change in the level of the intermediate Trehalose-6-Phosphate (T6P), which is strongly related to sugar status, than to T itself. Arabidopsis has 21 genes putatively involved in T biosynthesis, only one has showed T6P synthase activity and surprisingly 10 of them, known as T6P Phosphatases (TPPA-TPPJ), are active TPPs. Identification of TPP promoter cell-type activity revealed specific expression patterns of these genes in different organs of the plant. By analyzing loss and gain of function mutants we could find that some TPPs are involved in the development of lateral root primordia and their outgrowth. Phenotypes related to the formation of flowers and high/low biomass have been found also. These observations suggest that TPP genes could be tightly controlling T6P levels in particular cells and times of plant growth instead of producing more or less T. Nevertheless sugars, T6P and metabolite measurements are needed to confirm this hypothesis and to understand why T metabolism is so relevant for plant growth.

P06-018: BELOWGROUND VOLATILES FROM WHEAT (TRITICUM AESTIVUM L.) AND FROM PLANT GROWTH-PROMOTING BACTERIA OF THE WHEAT RHIZOSPHERE

du Jardin, P. - Delaplace, P. - Ormenõ, E. - d'Ans, S. - Fauconnier, M.L. - Wathelet, J.P.*
University of Liège - Gembloux Agro-Bio Tech
**Corresponding author e-mail: patrick.dujardin@ulg.ac.be*

The relevance of organic volatile compounds (VOC) released by leaves in plant–to–plant communication and in the interaction with herbivores, pathogens and their natural enemies is well acknowledged, but their roles in the root environment are much less known. In order to contribute to the unravelling of the nature, origins and functions of volatiles emitted by roots, wheat (*Triticum aestivum* L.) was chosen as a model with the aim to characterize VOC-mediated interactions between roots and rhizospheric bacteria defined as promoters of plant growth („Plant Growth Promoting Bacteria, or PGPRs). Regarding the plant partner, three questions were first raised: what is the profile of extractable volatiles of roots (in the absence of biotic or abiotic stress), how similar/different is this profile with that of leaf volatiles, and do the released volatiles match the extractable VOCs found within the root organs? Regarding the bacterial partners, 19strains from 8 genera (*Azospirillum, Azotobacter, Bacillus, Burkholderia, Paenibacillus, Pseudomonas, Raoultella, Serratia*) were selected. Their growth parameters were defined in order to standardize the physiological conditions used when analyzing their VOC emissions. Volatiles were analysed by gas chromatography – mass spectrometry (GC-MS) after both extraction from plant tissues and adsorption of the released volatiles on solid-phase micro-extraction fibers. The results indicate that wheat roots produce and release a blend of VOCs mainly derived from the enzymatic oxydation of unsaturated fatty acids. Preliminary results on the VOC profiling of the selected rhizobacteria will also be presented.

P06-019: RETINOBLASTOMA-RELATED PROTEIN CONTROLS ROOT EPIDERMAL CELL DIFFERENTIATION IN ARABIDOPSIS THALIANA.

Desvoyes, B. - Gutierrez, C.
Centro de Biología Molecular Severo Ochoa, CSIC-UAM

The role of the retinoblastoma related protein (RBR) in restricting cells to enter S-phase by modulating the activity of E2F transcription factors is now well established. New roles of RBR in cell differentiation processes are now emerging. To assess the relevance of the RBR pathway in cell fate specification we are using Arabidopsis root epidermal cells as a model. Root epidermal cells are generated at the root apical meristem and differentiate either in hair or hairless cells in a cell-position dependent manner. The genetic network that control root hair cell patterning is well known and relies on the expression of the homeodomain protein *GLABRA2 (GL2)*, an inhibitor of hair cell specification. Ectopic expression of RBR prevents cell proliferation of meristematic cells and alters cell fate specification provoking the appearance of ectopic hairs. Likewise, inactivation of RBR by expression of the RBR-binding RepA protein affects root hair patterning. We will present evidence that RBR is implicated in the control of epidermal cell fate in a dual manner: one that is cell cycle-dependent and, another by regulating specifically the expression of cell fate genes. Our study identifies a novel role of RBR in linking cell proliferation control and cell fate determination during root growth.

P06-020: LOCAL SUPPLY OF IRON DISTINCTLY DEFINES LATERAL ROOT NUMBER AND ELONGATION IN ARABIDOPSIS THALIANA

Ricardo F. H. Giehl - Joni E. Lima - Nicolaus von Wirén*
University of Hohenheim
**Corresponding author e-mail: hetgiehl@yahoo.com.br*

Morphological adaptations of the root system to a localized nutrient source are seen as an indication for nutrient sensing by plant roots. With regard to the low mobility of iron (Fe) in soils, we investigated changes in the root system architecture of Arabidopsis plants in response a localized supply of Fe. Increasing Fe concentrations in a homogenous or localized supply on separated agar plates enhanced lateral root number in a similar manner. Lateral root length, however, was twofold higher under localized relative to homogenous Fe supply. With further increasing Fe concentrations lateral root length was repressed even though shoot growth was unaffected. Observing early lateral root development by the use of CYCB1::GUS reporter lines indicated that in particular the emergence of lateral root initials was stimulated by local Fe. We then investigated lateral root growth in the Fe uptake-defective mutant irt1 and in the frd3-1 mutant, which is defective in root-to-shoot translocation of Fe. Based on these observations we propose a differential regulation of lateral root initiation and elongation in response to localized Fe supply that is subject to a local regulation by Fe and involves the high-affinity Fe transporter IRT1.

P06-021: ANATOMICAL CHARACTERIZATION OF ROOT SYSTEM OF MAIZE MUTANT LRT1

Husáková, E. - Soukup, A.
Charles University in Prague, Faculty of Science, Department of Experimental Plant Biology

Maize mutant *lrt1 (lateral rootless1)* is one of few mutants out of *Arabidopsis* with defect in lateral root formation. Since its description we present the first detailed anatomical-histochemical analyse along the developing main root.
Mutant *lrt1* was described as incapable of lateral root initiation during early post germination growth. Our data indicate that *lrt1* competency to initiate of the lateral root primordium is highly dependent on environmental factors. However, *lrt1* primordia didn't emerge from the main root, their cells are more vacuolized and the cellular organization of primordia is affected coparing to wildtype. Disturbances with oxidized polyphenolic substances were often found in pericycle where initiation normaly takes place. The outer layers of *lrt1* cortex were disorganized and continual ring of exodermal layers were also interrupted. The permeability test of root surface detected changes in exodermis function. Strong activity of peroxidase was detected in all tissues of *lrt1* root. The highest activity appeared in central cylinder, mainly in pericycle, and in the subepidermal layers of the root.
Our results indicate, that phenotypical traits described for the mutant might be related to cell wall modifications and subsequent higher rigidity of cell walls and/or differentiation of cells.

P06-022: THE ROLE OF TTL GENES IN ROOT INITIATION AND DEVELOPMENT

Šefrnová, Y.[1] - Hilgert, D.A. [1] - Fischer, L. [1] - Dubrovsky, J.G.[2] Vielle-Calzada, J.-P [3] - Soukup, A. [1]
[1]Department of Plant Experimental Biology, Faculty of Science, Charles University in Prague
[2]Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, Universidad Nacional Autónoma de México
[3]Laboratorio Nacional de Genómica para la Biodiversidad and Departamento de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados del I

Lateral root initiation and growth are crucial determinants of interaction of plant with rhizosphere, controlled via complex network of regulatory elements (Péret *et al.*, 2009; Fukaki & Tasaka, 2009). *TTL3* gene (AT2G42580, Tetratricopetide-repeat Thioredoxin-Like 3) was identified during the forward screening of a collection of gene-trap lines aimed to identify new genes involved in lateral root initiation and subsequent development. In *Arabidopsis TTL* genes comprise family of four members. TTL proteins contain repeating TRP motif, which is considered to be a protein-protein interaction domain shared among numerous proteins (Schapire *et al.*, 2006) in combination with thioredoxin fold. TTL1 was described as a novel protein taking part in salinity and abscisic acid response (Rosado *et al.*, 2006). TTL3 (VIT) was previously identified as an interaction partner of BRL2/VH1 brassinosteroid receptor and appears to play a role in brassiosteroid and auxin signaling (Ceserani *et al.*, 2009). Transcriptional fusions of *Arabidopsis TTL* promotors and *GUS* gene were constructed and their expression pattern is described under various conditions. The phenotypic effects of *TTL* mutations in selected publically available mutants are described with special emphasis on lateral root initiation and growth.

Ceserani,T., Trofka,A., Gandotra,N., and Nelson,T. (2009). Plant J 57, 1000. Fukaki,H. and Tasaka,M. (2009). Plant Mol Biol 69, 437-449.
Péret,B., De Rybel,B., Casimiro,I., Benkova,E., Swarup,R., Laplaze,L., Beeckman,T., and Bennett,M.J. (2009). Trends Plant Sci 14, 399-408.
Rosado,A., Schapire,A.L., Bressan,R.A., Harfouche,A.L., Hasegawa,P.M., Valpuesta,V., and Botella,M.A.
(2006). Plant Physiol. 142, 1113-1126.
Schapire,A.L., Valpuesta,V., and Botella,M.A. (2006). Plant Signal Behav 1, 229-230.

P06-023: MOBILE MIRNA165/6 TARGET HD-ZIP III IN THE ROOT STELE PERIFERY FOR PROPER XYLEM PATTERNING

Carlsbecker, Annelie[1] - Lee, Ji-Young[2] - Roberts, Christina J.[1] - Dettmer, J.[3] - Lehesranta, S.[3] - Zhou, Jing[2] - Lindgren, O.[4] - Moreno-Risueno, M.A.[5] - Vaten, A.[3] - Thitamadee, S.[3] - Campilho, A.[3] - Sebastian, J.[2] - Bowman, J.L.[6] - Helariutta, Ykä[3] - Benfey, P.[5]
[1]*Dept. of Physiological Botany, EBC, Uppsala University*
[2] *Boyce Thompson Institute for Plant Research and Graduate Field of Plant Biology, Cornell University*
[3] *Institute of Biotechnology/Department of Biosciences, University of Helsinki*
[4] *Institute of Technology, University of Tartu, Estonia*
[5] *Biology Department and IGSP Center for Systems Biology, Duke University*
[6] *School of Biological Sciences, Monash University*

A fundamental aspect of developmental biology is information exchange between cells resulting in proper cell identity. In Arabidopsis, the root xylem pattern is very consistent: radially, xylem forms in an axis with protoxylem at either end and metaxylem in the center. How is this pattern determined? We have identified a mutant, *phb-7d*, harboring a mutation in the microRNA165/6 (miR165/6) target site of the class III homeodomain leucine zipper (HD-ZIP III) gene *PHABULOSA (PHB)*, which leads to an expanded expression domain of *PHB* now encompassing not only the central, but also the peripheral stele. This mutant develops metaxylem in the place of protoxylem. In contrast, multiple mutants in *HD-ZIP III* genes form protoxylem in the place of metaxylem. Hence, the HD-ZIP III transcription factors act together to determine the xylem cell type. We show that their activity domain is determined by the movement of miR165/6 from the endodermal cell layer. Therefore, we describe a bi-directional signaling pathway where stele-produced SHORT-ROOT protein moves out to the endodermis to activate *miR165/6*, which then acts non-cell autonomously to restrict *HD-ZIP III* transcripts from the stele perifery, ultimately leading to proper xylem patterning in the stele.

P07 Molecular Mechanism Of Abiotic Stress

P07-001: EXPRESSION OF SMALL MICRORNA MIR398 UNDER ABIOTIC STRESSES IN THELLUNGIELLA HALOPHILA PLANTS

Pashkovskiy, P.[1] - Ryazansky, S.[2] - Kuznetsov, V.L. [1]*
[1]Institute of Plant Physiology
[2]Institute of Molecular Genetic
**Corresponding author, e-mail: biomaquinademuerte@gmail.com*

miRNA is a big class of small, 21-23 nt, RNAs capable to post-transcriptionaly regulate expression of plant genes by inducing the degradation of complementary mRNAs (target mRNAs). miRNA-dependent regulation of gene expression is important for many biological processes including growth, development and metabolism. In *Arabidopsis thaliana* MIR398 and its target Cu/Zn superoxiddismutese encoded by *CSD1* have a key role in formation of plant response to an abiotic oxidative stress. The oxidative stress induces the decrease of the miR398 abundance leading to accumulation of mRNA *CSD1*.We have found that salt-resisted *Thellungiella halophila* probably have a similar mechanism of response formation to oxidative stress. First, high concentration of NaCl or UV-B irradiation results in changing of MIR398 and mRNA *CSD1* abundances dose-dependent manner. Second, these alterations of expressions have oppositely directed character and occur both in leaves and roots. Third, irradiation of plant by high intensity light also lead to alterations of MIR398 expression. Thus, we suggest that MIR398-dependent regulation of mRNA *CSD1* can take place not only in glycophyte *A. thaliana* but also in halophytes *Th. halohila* and have stress- and organ-nonspecific character.

P07-002: LIPOXYGENASE ACTIVITY AND ROS FORMATION IN PLANT CELL COMPARTMENTS UNDER HYPOXIA AND CO2-MEDIA

Ershova A - Berdnikova, O.*
Voronezh State Pedagogical University
**Corresponding author, e-mail: aershova@vspu.ac.ru*

Under hypoxia in plants lipid peroxidation processes are activated. Activity of lipoxigenase (LOX) and reactive oxygen species (ROS) in cytoplasm, mitochondria and chloroplasts of pea seedlings exposed to 3-9h hypoxia and CO_2-media (100%) in dark were studied. It was discovered that superoxide anion content was increasing in mitochondria for 50% after 6h, in chloroplasts for 2.7 fold after 3h and then decreasing to aerated plants. In cytoplasm superoxide content was increasing only to experiment end to 250%. Hydrogen peroxide level, the most long-lived ROS form, was 40% higher after 3h of hypoxia in mitochondria and was 376% in chloroplasts, but it was decreasing for 20-30% in cytoplasm of aerated plants. It was determined that LOX activity in cytoplasm was 67%, in chloroplasts – 18% and 9% in mitochondria of total activity. LOX activity was rising in mitochondria till 170.1 after 3h of hypoxia, was 156.2 after 6h and than decreasing to 87.9 U/mg protein that was lower than aerated plants. LOX activity was consistent to control plants in chloroplasts and cytoplasm of seedlings. When studying dynamics of ROS accumulation in cytoplasm, mitochondria and chloroplast of pea it was shown that only in mitochondria LOX can reinforce ROS formation due to lipoperoxides accumulation in first hours of hypoxia. It was noted that high CO_2 cocentrations increased hypoxia effects on these processes.

P07-003: IDENTIFICATION OF GENES INVOLVED IN NON-TARGET-SITE-BASED RESISTANCE TO HERBICIDES IN THE ARABLE GRASS WEED ALOPECURUS MYOSUROIDES (BLACK-GRASS)

Délye, C. - Petit, C.*
INRA
**Corresponding author, e-mail:* delye@dijon.inra.fr

Arable weeds are essentially annual plants infesting crops. They are mostly non-model plant species dwelling in highly anthropised, highly disturbed environments (i.e., agricultural fields). To secure crop yield, weeds are destroyed using herbicide applications. The repeated use of herbicides selected for adapted ('resistant') weed individuals surviving herbicide applications. In *A. myosuroides*, non-target-site-based resistance (NTSR) plays the major role in resistance. NTSR is generally considered a quantitative trait endowed by many genes belonging to the general pathways of plant response to stresses. Such genes are differentially regulated in resistant and sensitive individuals. Despite the considerable economical consequences of herbicide resistance in weeds, hardly any data is available regarding the nature of the genes involved in NTSR. No genomic data being available for *A. myosuroides*, we used heterologous hybridisation on wheat DNA microarrays to identify genes with a moderate level of differential expression between resistant and sensitive plants, and suppression subtractive hybridisation to identify genes strongly up-regulated in resistant plants. Nine candidate genes were identified. Eight encoded proteins homologous to enzymes that may degrade herbicides or compensate for herbicide action. One potentially encoded an homologue of a protein involved in signal transduction during plant response to stress. Our data will aid gaining an insight into the processes driving the selection for adaptive life traits in weeds.

P07-004: ON MOLECULAR MECHANISMS OF TUNGSTATE TOXIC EFFECT ON PEA ROOT GROWTH

Gazizova, N. - Karimova, F.*
Kazan Institute of Biochemistry and Biophysics
**Corresponding author, e-mail: natgazizova@mail.ru*

The anthropogenic contamination of environment by heavy metal salts can exert stress actions on plant cells leading to cell metabolism disturbance, alteration of cell proliferation activity and growth inhibition. Tungsten is widely used in the industrial, civilian andmilitary activities. Its concentration in environment can be also increased by the application of phosphate fertilizers. Tungsten belongs to toxic heavy metals and is biologically significant for very limited range of organisms. Protein phosphorylation/dephosphorylation is known to be the main events in cell signaling and plant responses to abiotic stresses. Protein tyrosine phosphorylation is critical for cell division and growth. The aim of this work was to study the effect of sodium tungstate on cell proliferation activity, root growth, and root protein tyrosine phosphorylation. It was shown that tungstate caused the alteration of cell mitotic activity, which was accompanied by the increase of mitotic phase duration and the delay of root growth. Tungstate induced an increase of phosphotyrosine proteins amount and level of protein tyrosine phosphorylation. Regulatory, metabolic and defense proteins were among the identified ones. The increase of tyrosine phosphorylation of 14-3-3 proteins is of special interest. It is known that activity of some enzymes depends on their binding with 14-3-3 proteins and on their phosphorylation. The data obtained reveal the molecular mechanisms of tungstate toxic effect on pea root growth.

P07-005: CHARACTERIZATION OF KNOCK-OUT LINES OF TWO SALT- INDUCED GENES IN ARABIDOPSIS THALIANA

Abo-Ogiala, A. - Göbel, C. - Feussner, I. - Polle, A.*
Georg-August Universität Göttingen, Forstbotanik und Baumphysiologie
**Corresponding author e-mail: aabo@gwdg.de*

Soil salinity is a global problem leading to loss of arable land, especially, in arid areas. To improve salt tolerance of plants it is important to understand the adaptation mechanisms for life in a saline environment. Towards this goal, gene regulation was investigated in a salt tolerant poplar species (Fayyaz, 2008). Among the salt responsive candidate genes, one gene of the family of temperature induced lipocalin-like protein (TIL) and another yet uncharacterized gene denominated as SIS (salt-induced serine rich protein) were selected for further investigations using their closest homolog in *Arabidopsis thaliana.* The transcript abundances of *TIL* and *SIS* were strongly increased after 10 and 20 days of salt exposure in wild type (WT) plants of *A. thaliana.* To corroborate a function of TIL and SIS in salt tolerance, two homozygous T-DNA insertion lines of *A. thaliana (Attil1-1, Attil1-2, Atsis1-1, Atsis1-2)* were isolated for each gene and used for phenotypic and biochemical characterization.. Leaf growth, biomass of the rosette and photosynthesis of the knock-out (KO) lines were more strongly reduced after salt exposures than in the WT. Protein carbonyl and malondialdehyde contents revealed significant differences between WT and KO lines indicating oxidative stress. Elements analysis suggested changes in ion allocation in the KO plants under salinity stress. Increased electrolyte conductivity and changes in the fatty acid profiles suggest a role of TIL and SIS for stability of the plasma membrane.

P07-006: REGULATION OF PHOTOSYSTEM II RESPONSE UNDER HEAT STRESS

Pshybytko, N.[1] - Kabashnikova, L.[1] - Lysenko, E.[2]*
[1]Institute of Biophysis and Cell Engineering of NAS Belarus
[2]Institute of Plant Physiology of RAS
**Corresponding author e-mail: pshybytko@rambler.ru*

Photosystem II (PSII) is considered to be the most thermosensitive component of thylakoid membranes. In the present research, we have studied the mechanisms of PSII thermoinactivation and adaptation under high temperature impact and participation of hydrogen peroxide at these processes. The twice suppression of oxygen evolving activity of thylakoids with simultaneous decrease in D1 protein content and the release of extrinsic 33 kDa polypeptide from water oxidizing complex after 15-20 min heating of thylakoid membranes at 40ºC were registered. Using inhibitor analysis it was shown that thermoinduced degradation of D1 protein after 20 min heating occurred by proteases. The participation of FtsH protease in thermoinduced D1 protein degradation was observed. Level of transcription of *psbA* gene in chloroplast raised after 20 min heating and decreased through 1 h. The content of hydrogen peroxide increased three time after 20-30 min of heating and decreased to normal level through 1 h and raised after 2.5 h again. It is interesting that level of peroxidation lipids products increased after 2.5 h heating only.
Received data indicated that hydrogen peroxide is signal molecular at the photosynthetic apparatus under heat stress. During heating the inactivation of the most thermosensitive sites (WOC and D1 protein) is occurred. As result the ROS is generated. Hydrogen peroxide as signal molecule activates transcription of psbA. Turnover of PSII is occurred. More long heating induces degradation of proteins and lipids and hydrogen peroxide represents as the destructive agent.

P07-007: EFFECT OF HYDROGEN PEROXIDE ON CARBONYLATED PROTEIN LEVELS DURING PEA SEED GERMINATION

Barba Espín, G.[1] - Díaz-Vivancos, P.[2] - Clemente-Moreno, M.J.[2] - Job, D. [3] - Belghazi, M. [4] - Hernández, J.A. [2] - Job, C. [3]*
[1]Centro De Edafologia y Biologia Aplicada Del Segura (CEBAS-CSIC)
[2]Grupo de Biotecnología de Frutales-Dept. Mejora Vegetal, CEBAS-CSIC,
[3]Centre National de la Recherche Scientifique/Bayer CropScience Joint Laboratory,
[4](Proteomics Analysis Center, (CAPM), Faculty of Medicine, Marseille, F-13916 France)
**Corresponding author e-mail: gbespin@cebas.csic.es*

In a previous work, we showed that the imbibition of pea seeds whydrogen peroxide increased the percentage of germination as wellas the growth of the seedlings after 48 h of incubation. This increase in seedling growth correlated with the accumulation of proteins related to plant growth, cellular signaling and cell cycle control as well as with substantial decreases in ABA, SA and JA contents (Barba-Espín *et al.,* 2010). Protein carbonylation is an irreversible oxidative process leading to a loss of function of the modified proteins. Carbonylation in seeds is not necessarily a harmful phenomenon but at the opposite has been associated with the completion of germination (Job *et al.,* 2005). In this context we analyzed the effect of 20 mM H_2O_2 on the level of protein carbonylation in pea seedlings to correlate changes in this parameter with changes in plant growth. After 12 hours of H_2O_2-imbibition the amount of oxidized proteins, detected by specific Western blot analysis, increased notably. At the same time an increase in lipid peroxidation contents occurred. MALDI-TOFF analyses resulted in the identification of proteins related with several cellular events, including a polypeptide showing homology with the dormancy related protein from Arabidopsis thaliana. These results provide the first evidence that connect H_2O_2 treatment and increases in protein carbonylation levels with beneficial effects in the growth of pea seedlings. Barba-Espin et al. (2010) *Plant, Cell & Environment* (in press). Job et al. (2005) *Plant Physiology*. 138, 790–802.
This work was supported by the projects 05571/PI/07 and 11883/PI/09 (Fundación Séneca, Agencia de Ciencia
y Tecnología de la Región de Murcia).

P07-008: VESICLE TRAFFICKING AND NUCLEOLI ARE PRIMARY TARGETS OF ALUMINIUM TOXICITY IN MAIZE ROOT TIPS.

Barceló, J.[1] - Amenos, M.[1] - Corrales, I.[1] - Illéš ,P.[2] - Baluška, F.[3] - Poschenrieder, C.[1]*
[1]Universidad Autónoma de Barcelona)
[2]Institute of Botany Slovak Academy of Science, Bratislava, Slovakia
[3]Institut für Zelluläre u. Molekuläre Botanik, Univ. Bonn , Bonn Germany
**Corresponding author e-mail: juan.barcelo@uab.es*

Aluminium toxicity is an important stress factor limiting crop production on acid mineral soils. In order to clarify the primary toxicity mechanisms of Al-induced inhibition of root growth, this study analyzed the influence of 50 µM Al (16 µM Al^{3+} activity) on root elongation, Al-induced oxidative stress, boron-cross linked rhamnogalacturonan II (RGII)-containing brefeldin A compartments, F-actin cytoskeleton, and Al distribution in root tip cells of two contrasting maize varieties. In Al sensitive variety 16x36, but not in the tolerant Cateto, Al exposure caused fast inhibition of root elongation, oxidative stress, and alterations of the F-actin structure of cells of the central part of the root tip transition zone. In var. 16x36 Al caused a strong but transient inhibition of the formation of BFA compartments. The time sequence of Al effects on pectin recycling matched the growth effects in the sensitive variety. Confocal microscopy images of lumogallion stained root tips revealed a fast preferential accumulation of Al in the nucleoli of cells in the transition zone of

variety 16x36, but not in those of variety Cateto. Our results suggest that Al-induced inhibition of root elongation has two components. A reversible inhibition probably linked to Al binding to pectin fraction in the cell wall and an irreversible component that may operate at the level of BFA-induced inhibition of root elongation. The Al-induced amelioration of BFA-induced inhibition of root elongation suggests that Al could act as an activator of G-protein-mediated signaling.
Acknowledgement: Supported by Spanish GovernmenBt FU2007-60332/BFI

P07-009: FUNCTIONAL ANALYSIS OF METALLOTHIONEIN GENES IN GENUS SILENE

Hudzieczek, V. - Nevrtalova, E. - Hobza, R. - Vyskot, B.*
Institute of Biophysics, v.v.i., ASCR
**Corresponding author e-mail: hudzieczek@ibp.cz*

Metallothioneins are one of the most important genes in heavy metal resistance and homeostasis. These genes code low molecular, cysteine-rich proteins acting as metal binding chelators in heavy metal detoxification. It has passed more than seventy years since it was reported that closely related species *Silene vulgaris* and *S. dioica* can sustain high concentrations of copper in soil. On the other hand, still is little known about the molecular mechanisms of this phenomenon in genus *Silene*. We have cloned and characterized *metallothionein3* gene (*MT3)* from different ecotypes growing on copper-polluted soils (mainly from Špania Dolina copper mines, Slovakia). Here we manifest that *Silene MT3* gene is - based on complementation assays in copper sensitive mutant yeast strain - associated with heavy metal resistance.
The work was supported by the Grant Agency of AS CR grant nos. M200040905 and KJB600040901.

P07-010: THE CONTRIBUTION OF CAX TRANSPORTERS IN HEAVY METALS TOLERANCE OF CUCUMBER PLANTS

Papierniak, A. - Migocka, M. - Klobus, G.*
Wroclaw University, Faculty of Biological Science, Department of Plant Physiology
**Corresponding author e-mail: anna.papierniak@gmail.com*

Plant possess a wide range of heavy metal transporters engaged in the various ions homeostasis. They include the proteins of CAX (*CA*ation e*X*changers) family, which are likely to be involved in the active sequestration of metals in the plant vacuole. Indeed, our preliminary studies clearly indicate that the vacuole membranes isolated from cucumber roots accumulate Cd, Mn and Ni via H^+/Me antiport. We have assumed that proteins of CAX family could mediate that process. Therefore we screened the cucumber genome in search for the genes encoding CAX transporters and sequenced 6 orthologs of *Arabidopsis* typical *CAX1-6* genes. Following this, we performed a wide analysis of the expression pattern of each *CsCAX* gene in both, inflorescence and florescence organs and in response to heavy metals stress. All *CsCAX* transcripts were clearly detectable in all tissues of both 7-day-old seedlings and 2- and 6-week-old plants with the exception of *CAX3*, which was not expressed in the roots of seedlings. However, in the 2 week-old plants shoots and leaves the levels of *CAX3* RNA significantly increased after Mn^{2+}, Pb^{2+}, Cd^{2+} and Ni2+ treatment. According to this, the *CAX2* expression was also enhanced by metals in roots, shoots and leaves of seedlings and older plants. Our findings suggest that of all the CsCAX transporters in cucumber, CAX2 and CAX3 may increase the metal transport properties of tonoplast membrane in all plant tissues, and thus determine cucumber tolerance to heavy metals stress. Moreover, these studies provide a profound insight in the potential function of CAXs in the distribution of various metals among different cell tissues. The results also provide a basis for futher functional studies of CAX genes in cucumber.

P07-011: CHARACTERIZATION OF METALLOTHIONEIN OF TYPE 3 GENE AND ITS ROLE IN HEAVY-METAL TOLERANCE AND ACCUMULATION IN THE GENUS SILENE

Nevrtalova, E. [1] - Vojtech, H.[2] - Roman, H.[2]- Boris, V.[2]*
[1]Institute of Biophysics ASCR
[2]Department of Plant Developmental Genetics, Institute of Biophysics, ASCR, Brno Czech Republic
**Corresponding author e-mail: nevrtalova@ibp.cz*

Metallothioneins (MTs) are a small Cys – rich cytoplasmatic proteins with capacity to chelate different metal ions. Although it is known that MTs play a general role in metal homeostasis, recent data show that they participate mainly in copper tolerance and accumulation. We have isolated a *MT3* gene in heavy metal tolerant plants *Silene vulgaris* and *S. dioica*. Molecular analysis indicates different levels of expression induction of *MT3* gene in some populations of *S. dioica* and *S. vulgaris* species. To study the mode of regulation of *MT3* gene we also analyzed flanking regulatory regions of the gene and we found an insertion of retroelement into the promoter sequence. We conclude that the activation of gene expression via the retrotransposon activation in stress conditions (a high copper concentration in medium) could play a role in the copper homeostasis in different *Silene* species.
Key words: metallothioneins, heavy metal resistance and accumulation, copper, *Silene vulgaris*
Acknowledgements: The work was supported by AS CR grant M200040905 and GACR 20409H002.

P07-012: A MUTANT SCREEN FOR REVERTANTS OF H2O2-INDUCED CELL DEATH IN ARABIDOPSIS THALIANA

Hoeberichts, F. - Mühlenbock, P. - Vandorpe, M. - Van Breusegem, F.*
VIB Department of Plant Systems Biology, Ghent University
**Corresponding author e-mail: frhoe@psb.vib-ugent.be*

Photorespiration is the most important source of H_2O_2 production in leaves in the light. This proces is initiated from the Calvin cycle, when oxygenation of ribulose-1,5-biphosphate by Rubisco is favoured over carboxylation. Oxygenation occurs at high rates in the leaves of C3 plants and increases during adverse environmental conditions that limit CO_2 availability and/or increase light influx, such as drought or high light. H_2O_2 is released during the peroxisomal conversion of glycolate to glyoxylate, and catalases are the principal H_2O_2-scavenging enzymes present in the peroxisomes. Perturbation of catalase activity results in the accumulation of photorespiratory H_2O_2, and studies using catalase-deficient plants have revealed that photorespiratory H_2O_2triggers extensive transcriptional reprogramming, bleaching of the leaves, and cell death. In order to isolate mutants disturbed in their response to photorespiratory H_2O_2, we screened an EMS-mutagenised population of the Arabidopsis thaliana catalase-deficient T-DNA insertion line cat2-2. The obtained revertants all display a reduction in the cell death phenotype and are expected to carry mutations in genes involved in photorespiration and/or H_2O_2-dependent cell death pathways. Results relating to the phenotypic, biochemical and molecular characterisation of the obtained revertants will be presented.

P07-013: MODULATION OF PLASMA MEMBRANE H+-ATPASE UNDER HEAVY METALS.

Janicka-Russak, M. - Klobus, G.*
Wroclaw University, Institute of Plant Biology
**Corresponding author e-mail: russak@biol.uni.wroc.pl*

Plasma membrane functions are rapidly altered by heavy metals present in the environment at high concentrations. The first diagnostic symptom of membrane damage by heavy metals is an increase in its permeability with a subsequent disturbance in the

ionic balance of the cell. The ATP-dependent proton pump of the plasma membrane has a central function in the regulation of ion homeostasis in the cytosol. To explain the mechanism of metal action on the plasma membrane proton pump, the activity of H^+-ATPase were measured in plants treated short and long time with metals (Cd and Cu) simultaneously with the expression of genes encoding the enzyme. In the longer time the plants were grown in medium containing 10 μM Cd or Cu for 6 days. Part of the plants after 3 days exposure to metals were transferred to control conditions for next 3 days (post-stressed, PS). In the case of short time treatment six-day-old cucumber seedlings were transferred into 10 μ M Cd or Cu solutions and after 2 hours plant roots were used to the isolation of plasma membranes. In membranes the activity of proton pump were measured as ATP hydrolysis and H+ transport. Total RNA was also isolated from roots and expression of *Cucumis sativus* plasma membrane H^+- ATPase genes were measured with semi-quantitative RT-PCR. Treatment of the cucumber seedlings with heavy metals decreased the hydrolytic and transporting activities of H^+-ATPase in the plasma membranes. However, the activity of proton pump was stimulated in plants treated with heavy metals for longer time (6 days). In PS plants the activity of H^+-ATPase was the highest. The results have shown that the effect of metals on plasma membrane proton pump activity was dependent on time exposure plants to metals.

P07-014: INTERACTION BETWEEN NITRIC OXIDE- OR SALICYLIC ACID-MEDIATED PATHWAYS AND ASCORBATE LEVELS IN COLD-HARDENED ARABIDOPSIS PLANTS

Majláth, I.[1] - Szalai, G.[1] - Vanková, R.[2] - Papp, I.[3] - He, Z.[4] - Janda T[1]*

[1]Agricultural Research Institute of the Hungarian Academy of Sciences

[2]Institute of Experimental Botany AS CR, Czech Republic

[3]Department of Plant Physiology and Plant Biochemistry, Corvinus University in Budapest, Hungary

[4]National Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institute for Biological Sciences, Chinese Academ

**Corresponding author e-mail: majlathi@mail.mgki.hu*

Plants have evolved intricate mechanisms to respond to changes in their environment, such as low temperature stress, which is one of the most important limiting factors in the spread of plants species or cultivated plant varieties. Ascorbic acid, which is both a metabolite with strong antioxidant activity and a cofactor for enzymes catalysing numerous biochemical reactions, including those neutralizing the effects of reactive oxygen species, acts as one of the major defence components accumulated by plant cells in response to stress factors. Salicylic acid has long been known as a signal molecule in the induction of defence mechanisms in plants. Nitric oxide is
a signal molecule, and there is increasing evidence of its role not only in plant growth and development, but also
as a plant defence signal against various stressors. The aim of the present work was to find a connection between the nitric oxide- or ascorbate-dependent protective mechanisms, and the salicylic acid-mediated signal pathways under cold hardening conditions in Arabidopsis thaliana plants. Freezing survival tests show that Arabidopsis mutant plants with reduced ascorbate or nitric oxide levels exhibited reduced frost tolerance. Preliminary experiments show that altered levels of ascorbate or nitric oxide may also have an effect on the salicylic acid content. The possible interactions among these compounds in Arabidopsis will be discussed.

P07-015: CONSEQUENCES OF ECTOPIC EXPRESSION OF ATHMA4 FULL-LENGTH AND ATHMA4-TRUNCATED IN TOBACCO TO PLANT METAL HOMEOSTASIS

Siemianowski, O.[1] - Mills, R.F.[2] - Williams, L.E.[2] - Antosiewicz, D.M.[1]*

[1] University of Warsaw, Faculty of Biology, Institute of Plant Experimental Biology

[2]University of Southamp

**Corresponding author e-mail: osiemianowski@wp.pl*

AtHMA4, a P_{1B}-ATPase of the Zn/Cd/Pb/Co subclass, has a common P1B-ATPase structure with the longest C-terminal domain. *AtHMA4* play a key role in the control of root-to-shoot Zn translocation and xylem loading (Mills et al. 2005, Verret et al. 2004). Based on these results we tested whether *AtHMA4* could be a candidate gene for engineering modifications of metal root/shoot partitioning for biofortification and phytoremediation. In this study, we expressed (i) *AtHMA4* full-length (*AtHMA4*-F) and (ii) *AtHMA4*-trunc (lacking C-terminus), under the 35S CaMV promoter in tobacco. Transformed and wild type plants were subjected to a range of Zn (0.5, 10, 100, 200 μM) and Cd (0.25, 5 μM) concentrations. Expression of *AtHMA4*-FL enhanced Zn translocation to the shoots only at 10 μM Zn. With respect to the importance of the C-terminus for the activity
of *AtHMA4*, our study demonstrates a decrease or lack of the phenotype in *AtHMA4*-trunc plants compared to AtHMA4-FL expressing tobacco. For example, the moderate facilitation of Zn translocation to the shoots at 10 μM Zn was not seen in the *AtHMA4*-trunc plants. Although exposure of AtHMA4-F and AtHMA4-trunc transformed plants to 0.25 and 5 μM Cd resulted in the pattern of Cd accumulation different to that found in Zn-exposed plants, the activity of AtHMA4 protein also seemed to be reduced when the C-terminal part was deleted. Therefore these results are consistent with an important role of the C-terminus in Cd transport function as well as Zn. In general, it was shown that the response of transformants depended both on the metal used and its concentration. There appears to be a an interplay between the activity of the transgene and the tobacco metal-homeostasis-system that contributes to the observed modifications of metal root/shoot partitioning.

P07-016: GENES INVOLVED IN ETHYLENE SYNTHESIS AND ETHYLENE SIGNALLING ARE UP-REGULATED IN FE-DEFICIENT ARABIDOPSIS PLANTS

Alcantara, E. - García, M.J. - Lucena C. - Romera F.J. - Pérez-Vicente, R.*

Universidad de Córdoba

**Corresponding author e-mail: ag1alvae@uco.es*

In *Arabidopsis* (Strategy I plant), Fe deficiency up-regulates several genes involved in Fe acquisition, like the ferric reductase *FRO2*, the Fe(II) transporter IRT1 and the FIT transcription factor *FIT*, which is necessary for the activation of both *FRO2* and *IRT1*. Several years ago, Romera *et al.* (1999, Ann. Bot. 83: 51) found enhanced ethylene production by roots of Fe-deficienSt trategy I plants. More recently, Lucena et al. (2006, J. Exp. Bot. 57: 4145) showed an involvement of ethylene in the up-regulation of *FIT* and, consequently, of *FRO2* and *IRT1*. In this work we have studied whether or not Fe deficiency up-regulates genes involved in ethylene synthesis and signalling. For this study, *Arabidopsis thaliana* "Columbia" plants were grown in nutrient solution with or without Fe (1 to 2 days), and roots were collected to later analyse gene expression by RT-PCR. The results obtained show that Fe deficiency up-regulates the expression of genes involved in ethylene synthesis (*MTK, SAM1, SAM2, ACS4, ACS6, ACS9, ACO1* and *ACO2*) and signalling (*ETR1, CTR1, EIN2, EIN3, EIL1* and *EIL3*). These results give additional support to the hypothesis proposed by Lucena *et al*. (2006) suggesting an involvement of ethylene in the regulation of Fe acquisition genes. AcknowledgementsP: roject AGL2007-64372; Project AGR-3849; Research Group AGR115.

P07-017: IDENTIFYING NOVEL PLANT DEFENCE RESPONSES TO SIMULTANEOUS BIOTIC AND BIOTIC STRESSES

Atkinson, N. - Urwin, P.E-*
University of Leeds
**Corresponding author e-mail: bsnjsj@leeds.ac.uk*

Plants respond to different biotic and abiotic stresses using specific gene signalling pathways leading to stress tolerance or acclimation. Traditionally each stress and its molecular effect on a plant have been studied independently, although field environments are likely to present coincident stresses. Recent research suggests that the molecular and metabolomic reaction of plants to multiple stresses is different to that for individual stresses. Furthermore it has been found that stress signalling pathways may act antagonistically. To fully understand the nature of plant responses it is therefore essential to study stress factors in combination. In this study Affymetrix ATH1 microarray chips were used to study the transcriptome response of *Arabidopsis thaliana* to combined abiotic and biotic stress. Plants were exposed to infection by the plant-parasitic nematode *Heterodera schachtii* and then subjected to drought stress by dehydration under stringently controlled conditions. The response of plants to combined drought stress and nematode infection was found to be distinct from that of each stress individually. In roots 1067 gene transcripts were differentially regulated specifically in response to joint stress ($P < 0.05$) whilst in leaves 1282 such genes were identified. Transcription factors were highly represented amongst these gene subsets, as well as genes induced by stress signalling-related hormones abscisic acid and jasmonic acid. Thus a new pattern of multiple stress defence response has been identified. The identification of genes controlling the crossover between multiple stress signalling pathways may provide opportunities for the future development of broad-spectrum stress tolerant crops.

P07-018: CATION SELECTIVITY OF THE NA+/H+ EXCHANGER ATSOS1

Feki, k.[1] - Pardo, J. M.[1] - Masmoudi, K.[2] - Quintero, F.J [1]*
[1]Instituto de Recursos Naturales y Agrobiología de Sevilla (CSIC)
[2]Center of Biotechnology of Sfax
**Corresponding author e-mail: kaouther_feki@yahoo.fr*

The plasma membrane Na^+/H^+ antiporter AtSOS1 is a key determinant for salt tolerance. This protein mediates Na^+ extrusion from plant cells and shows a high specificity for Na^+ in biochemical and in vivo assays. Interestingly, the Arabidopsis transporter AtNHX8, phylogenetically related to AtSOS1, was characterized as a Li^+/H^+ antiporter with little affinity for Na^+. Although the substrate specifity is different in the two proteins, they show a high degree of similarity at the protein sequence level. Little is known about topological determinants involved in the cation specifity of antiporters. Critical residues of the pore domain and the regulatory cytosolic
C-terminal domains are both thought to be important. We have studied the effect of the C-terminal part of the Arabidopsis proteins in the process of cation selectivity. The C-terminal regions of AtSOS1 and AtNHX8 were swapped and the transport activity of the chimerical proteins was analyzed in a Na^+ and Li^+ sensitive yeast strain. Results supporting a role for the C-terminal region in determining the substrate specificity of the transporter will be presented.

P07-019: GENETIC AND PHYSIOLOGICAL ANALYSES OF THE ROLE OF VACUOLAR NHX EXCHANGERS IN POTASSIUM METABOLISM

Andrés González, Z. - Andrés, Z. - Barragán, V. - Leidi, E. O. - Pardo, J. M. - Cubero, B.*
Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNASE-CSIC)
**Corresponding author e-mail: zandres@irnase.csic.es*

The physiological role of tonoplast NHX-type cation/H+ antiporters is thought to relate mainly to the plant salt stress response by mediating compartmentation of Na+ in vacuoles. However, all isoforms characterized so far catalyze both Na+/H+ and K+/H+ exchange. Genetic evidence will be presented that the regulation of K+ homeostasis by NHX-type antiporters is essential for normal plant growth and development, and plays an important role in the response to osmotic stress by improving K+ accumulation. Thus, NHX-typeproteins are likely candidates for the H+-linked K+-transport that is thought to facilitate active K+ uptake at the tonoplast and the partitioning of K+ between vacuole and cytosol. This critical function should be taken into consideration for understanding the salt tolerance phenotype of plants overexpressing NHX-type exchangers.

P07-020: PIN-DRIVEN AUXIN REGULATION OF SHADE AVOIDANCE

Keuskamp, D. - Voesenek, L. A. C. J. - Pierik, R.*
Plant Ecophysiology, Institute of Environmental Biology , Utrecht University
**Corresponding author e-mail: d.h.keuskamp@uu.nl*

Plants usually grow in a dynamic environment with oftentimes severe competition for light with surrounding neighbours. As a result, not only light intensity is affected, but also the light quality and the latter is exploited by plants to sense neighbours. Light quality signals are a reduced red to far-red ratio (R:FR) and blue light depletion, which are perceived by the phytochrome and cryptochrome photoreceptors respectively. These signals can independently induce shade avoidance responses, which include shoot elongation to consolidate light capture. It has been shown for low R:FR-exposed plants that elevated auxin levels1 and proper auxin transport2 are essential to this response. We show here that PIN proteins, which control polar auxin transport (PAT), are essential regulators of light quality-mediated shoot elongation in Arabidopsis. We show that low R:FR conditions regulate PINs at both the transcriptional level and cellular location. Knockout mutants display inhibited shade avoidance responses to low R:FR and are consequently out-competed in dense stand mixtures with WT neighbours. These data show how auxin transport is regulated to control shade avoidance responses, and how this regulation determines plant competitive vigour.

1) Tao Y, et al. Cell 2008; 133:164-176.
2) PierikR, et al. Plant Physiol 2009; 149:1701-1712.

P07-021: OVEREXPRESSION OF ARABIDOPSIS COPPER TRANSPORTERS AFFECTS THE EXPRESSION OF CCA1 AND LHY CIRCADIAN CLOCK COMPONENTS

Andrés-Colás, N. - Perea-García, A. - Puig, S. - Peñarrubia, L.*
Universitat de València
nuria.andres@uv.es

Copper is an essential cofactor for key processes in plants, but it becomes harmful when in excess. The overexpression of either Arabidopsis COPT1 or COPT3 high affinity copper transporters in transgenic plants drives increased endogenous copper levels and sensitivity to the copper in the growth medium. Additional phenotypes include decreased hypocotyls growth in red light and differentially affected flowering times depending on the photoperiod, as well as compromised plant survival in the absence of environmental cycles such as light and temperature. Furthermore, the expression of the nuclear circadian clock genes CCA1 and LHY is substantially reduced in these transgenic plants. Copper induces the down-regulation of the expression of CCA1 and LHY in wild type plants and it also drives a reduction in the expression of circadian clock output genes. These results reveal that the spatial-temporal control of copper transport is a key aspect of metal homeostasis that is required for Arabidopsis fitness, especially in the absence of environmental clues. In this sense, Cu homeostasis could participate in the integral cellular circadian system, possibly through the effect of Cu on the expression of

circadian clock components maybe fulfilling the cell-specific and appropriate timing requirements of Cu daily intracellular traffic.

P07-022: METABOLOMIC CHANGES IN OAT (A. SATIVA) DURING PROGRESSIVE DROUGHT STRESS

Prats, E. [1] - Sanchez-Martín, J.[1] - Mur, L.A.J.[2] - Rubiales, D.[1] - Prats, E.[1]*
[1]Consejo Superior de Investigaciones Científicas (CSIC)
[2]Institute of Biological, Environmental and Rural Sciences, University of Aberystwyth, UK
**Corresponding author e-mail: elena.prats@ias.csic.es*

Drought is one of the major environmental factors determining plant yield. Tolerance to this abiotic stress is a complex phenomenon, comprising of a number of physio-biochemical processes and including elaborate orchestration of signaling pathways and reprogramming of metabolites. In this work we used a metabolomic approach to determine key metabolites and pathways involved in oat drought tolerance. Samples from leaves and roots were taken from resistant and susceptible oat plants during a drought time course. After an initial fingerprinting using Fourier Transform Infrared (FT-IR) spectroscopy, metabolite profiles were obtained from different genotypes, time points and tissues using Direct Injection Electrospray Ionisation-Mass Spectrometry. Key metabolite differences were elucidated using multivariate statistical approaches, particularly Discriminant Function Analysis (DFA).
Taken together the discriminatory metabolites suggested that biochemical pathways involved in alleviating photo-oxidative stress was a key factor for drought resistance. These included antioxidant pathways and those contributing to cell membrane stability. In addition metabolites from pathways related with osmotic adjustment and signaling molecules involved in the root-shoot communication such as ABA, malate and threalose were also found in a higher concentration and at early time points in the resistant plants. Independent targeted analyses are currently carried out in order to substantiate the role of the differentially expressed metabolites.

P07-023: ROOT GDH1 AND GDH2 GENES ARE UP-REGULATED IN TOBACCO PLANTS SUBJECTED TO SHORT-TERM BORON DEFICIENCY

Rexach, J. - Beato, V.M. -Navarro-Gochicoa, M.T. - Herrera Rodríguez, M.B. - Camacho-Cristóbal, J.J. - González-Fontes, A.*
Universidad Pablo de Olavide
**Corresponding author e-mail: jrexben@upo.es*

It is well known that nutrient deficiencies favour degradation of proteins leading to release of ammonium that is re-assimilated as asparagine (Asn) and glutamine (Gln)1.
Recently it has been reported that boron (B) deficiency increases the expression of tobacco asparagine synthetase (AS) gene in roots, and that AS might play a main role as a detoxifying mechanism to convert ammonium into Asn2. The aim of this work was to ascertain whether glutamate dehydrogenase (GDH) gene expression is also increased under short-term B deficiency given that this enzyme acts as an ammonium supplying via from proteolysis. Interestingly, roots of plants subjected to B deprivation for 24 h increased their transcript levels of GDH1 and GDH2, in addition to AS, when compared to controls. Consistently, Asn and Asp concentrations were higher under B deficiency. Furthermore, glucose and fructose contents decreased with B stress, while ammonium concentration was kept enough low and with similar values to that of control plants. In conclusion, we propose that overexpression of GDH1 and GDH2 genes under B deficiency might supply ammonium to be re-assimilated via GS/GOGAT and AS. In addition, root tobacco AS gene expression seems to be regulated by sugar levels rather ammonium concentration.
1Vierstra RD (1993) Annu Rev Plant Physiol Plant Mol Biol 44: 385-410 2Beato et al (2010) Plant Sci (doi:10.1016/j.plantsci.2009.12.008) Research supported by BFU2009-08397 and Junta de Andalucía CVI-4721 and BIO-266, Spain

P07-024: LOCALIZATION AND FUNCTION OF HIGH-AFFINITY COPPER TRANSPORT PROTEINS IN PLANTS

Garcia-Molina, A. - Andrés-Colás, N. - Perea-García, A. - Puig, S. - Peñarrubia, L.*
Universitat De València
**Corresponding author e-mail: Angarmo3@Uv.Es*

Copper (Cu) is an essential micronutrient for aerobic organisms, because it participates as a redox cofactor in multiple processes including respiration and protection against oxidative stress. In plants, Cu also participates in critical processes such as ethylene perception and photosynthesis. Little is know about the molecular mechanisms that mediate Cu acquisition, distribution and utilization in plants. Over the past years, we have used the budding yeast Saccharomyces cerevisiae to identify a family of five putative CTR1-type high-affinity Cu transport proteins (the COPT family) in the model plant Arabidopsis thaliana. To date, COPT1 is the only member of the family whose function in plant Cu homeostasis has been characterized. COPT1 is a plasma membrane protein that is highly expressed upon Cu-starvation mainly in root tips and pollen grains. Interestingly, copt1 knockout mutants exhibit a reduction of 50 % in Cu acquisition and accumulation, as well as morphological alterations in pollen grains. Here, we utilize transcriptional fusions of COPT promoters to GUS reporter gene to elucidate the expression pattern of the other COPT family members. Furthermore, we have constructed traductional fusions of COPT-open reading frames to the green fluorescent protein and determined the subcellular localization of COPT proteins in Arabidopsis protoplasts. Our studies suggest an overlapping but different function in Cu homeostasis for the COPT-family members.

P07-025: PP2CS AS A REGULATORY NODE IN THE SALINITY STRESS RESPONSE

Villalta, I. - Pardo, J.M. - Quintero, F.J.*
Instituto de Recursos Naturales y Agrobiología de Sevilla
**Corresponding author e-mail: ivillalta@irnase.csic.es*

The Na+/H+ antiporter SOS1 is the most important regulator of Na+ homeostasis in Arabidopsis thaliana by controlling net Na+ uptake and translocation from roots to shoots. SOS1 activity is regulated through phosphorylation by the protein kinase complex SOS2-SOS3. SOS2 is a Ser-Thr protein kinase belonging to the SNF1-related kinase (SnRK) family (also known as SnRK3.11 and CIPK24). SOS3 is a myristoylated Ca2+ sensor belonging to the family of calcineurin B-like (CBL) protein. Recently the PYR/PYL (Pyrabactin resistance/Pyrabactin resistance-like protein) family of abcisic acid receptors have been shown to interact with the type 2C protein phosphatases (PP2C) in an ABA dependent manner. ABA-bound PYR/PYLs are inhibitors of the PP2Cs activity. PP2Cs counteract ABA signalling by interacting, dephosphorylating and inhibiting the SnRK2 kinases, which are positive regulators of the pathway. Initial studies of interactions between SOS1, SOS2 and several PP2Cs (PP2CA, PP2CB, ABI1, ABI2, HAB1 and HAB2) indicate that SOS2 interacts with PP2CA and ABI1, suggesting a regulatory role of PP2Cs in the activity of SOS2 and, in turn, the Na+ transporter SOS1. Since PP2Cs also interact with the SnRK3 member CIPK23, which regulates K+ uptake through channel AKT1, the PP2C family constitute a common link for the coordinate regulation of ion transport processes mediated by SnRK3 and ABA signalling by the SnRK2 kinases in the salt stress response.

P07-026: PP2CS AS A REGULATORY NODE IN THE SALINITY STRESS RESPONSE

Villalta, I. - Pardo, J. M. - Quintero, F.J.*
Instituto de Recursos Naturales y Agrobiología de Sevilla
**Corresponding author e-mail: ivillalta@irnase.csic.es*

The Na+/H+ antiporter SOS1 is the most important regulator of Na+ homeostasis in Arabidopsis thaliana by controlling net Na+ uptake and translocation from roots to shoots. SOS1 activity is regulated through phosphorylation by the protein kinase complex SOS2-SOS3. SOS2 is a Ser-Thr protein kinase belonging to the SNF1-related kinase (SnRK) family (also known as SnRK3.11 and CIPK24). SOS3 is a myristoylated Ca2+ sensor belonging to the family of calcineurin B-like (CBL) protein. Recently the PYR/PYL (Pyrabactin resistance/Pyrabactin resistance-like protein) family of abcisic acid receptors have been shown to interact with the type 2C protein phosphatases (PP2C) in an ABA dependent manner. ABA-bound PYR/PYLs are inhibitors of the PP2Cs activity. PP2Cs counteract ABA signalling by interacting, dephosphorylating and inhibiting the SnRK2 kinases, which are positive regulators of the pathway. Initial studies of interactions between SOS1, SOS2 and several PP2Cs (PP2CA, PP2CB, ABI1, ABI2, HAB1 and HAB2) indicate that SOS2 interacts with PP2CA and ABI1, suggesting a regulatory role of PP2Cs in the activity of SOS2 and, in turn, the Na+ transporter SOS1. Since PP2Cs also interact with the SnRK3 member CIPK23, which regulates K+ uptake through channel AKT1, the PP2C family constitute a common link for the coordinate regulation of ion transport processes mediated by SnRK3 and ABA signalling by the SnRK2 kinases in the salt stress response.

P07-027: THELLUNGIELLA SALSUGINEA SHOWS HIGHER SALT TOLERANCE THAN ARABIDOPSIS THALIANA AT THE CELLULAR LEVEL

Nosov, A.[1] - Taranov, V.[1] - Baranova, E.[1] - Shugaev, A.[1] - deBoer, A.[2] - Babakov, A.[1]*
[1]Russian Academy of Agricultural Sciences
[2]Faculty of Earth and Life Science Vrije Universiteit, Amsterdam, The Netherlands
**Corresponding author e-mail: alex_n@ippras.ru*

Thellungiella salsuginea (Ts) is a close relative of Arabidopsis. thaliana (At) and displays high tolerance to harsh environments including extreme salinity. It is commonly accepted that restriction of Na+ influx into roots of Ts is an important mechanism of salt tolerance in this plant. Ts leaves accumulate proline under salinity, what means that leaf cells respond to elevated Na+ around the roots. This raises the question whether Ts tolerates salt stress at the cellular level? Stable cell-suspension cultures from calli generated from mature leaves of Ts ecotype Yakutsk and At ecotype Columbia plants were established. Both cell suspensions were grown in SH medium with equal inoculums and growth conditions. Growth indices of At and Ts cell cultures were reduced by 50% at 75 mM and 200 mM of NaCl, respectively. After two-week-long salinity (100 mM) the respiration (KCN sensitive) and TTC reduction were doubled in Ts cells, and the reverse situation was measured in At cells. Moreover, two-fold higher Na+ and proline concentrations were measured in Ts as compared to At cells, along with the detection of increased vacuolar volume and energized mitochondria structure in Ts cells. Thus, Ts can adapt to and tolerate salinity at the whole plant and the cellular level as well.

P07-028: METALLOTHIONEIN PARTICIPATION IN COPPER AND ZINC DETOXIFICATION IN PLANTS

Volkov, K. - VlV Kuznetsov - Ivanova, E.M. - Grinin, A.L. - Kholodova, V.P.*
IPP RAS
**Corresponding author e-mail: volkov_ks@mail.ru*

Metallothionein (MT) participation in Cu and Zn excess detoxification was investigated in Mesembryanthemum crystallinum, Brassica napus and B. juncea. Expression of genes McMT2, McMT2a, BnMT2, BjMT2 was evaluated with RT-PCR technique using 18S RNA as the control. The prolonged action of HM excess (7-10 days) increased MT mRNA levels compare to control in leaves of all plants studied. Maximal values - 3-fold higher than in the control variant - were obtained in leaves of M.crystallinum plants under 50 M CuSO4, ZnSO4 (500 M) increased gene McMT2 expression not more than 1,5 times. Detailed dynamic of HM effect to gene activation was followed for rape plants. Significant activation of BnMT2 was registered in 24 hours of HM action, later the effect was enhanced and maintained to the level exceeding 5-times the control variant during 5-7 days then mRNA content dropped almost to the control level. In distinction to BnMT2, activation of BjMT2B expression started later but retained longed in leaves of rape plants. It was determined that sum mRNA content of the two genes investigated correlated much stronger to the rates of Cu accumulation than to its total content in leaves. It may witness that the role of MT in HM detoxification is limited to HM active center blockage until they were in cytoplasm, in the zone of an active metabolism. After HM sequestration for prolonged storage in vacuole or apoplast, phytochelatins seems to serve as HM chelators. The conclusion was supported by increased activation by Cu and Zn of PCS, gene encoding phytochelatinsynthase during the later stage of B. napus adaptation to HM excess. The work was partially supported by the RFBR and by the Presidium of RAS program (Cell and Molecular Biology).

P07-029: SCREENING FOR DROUGHT RESISTANCE IN AN OAT GERMOPLASM COLLECTION.

Sanchez-Martín, J. - Rubiales,D. - Prats, E.*
Susteinable Agriculture Institute
**Corresponding author e-mail: javiersanchez84@ias.csic.es*

Drought is the main abiotic stress on cereal yield. To date cereal breeding has mainly based on empirical selection for yield per se. However this is far from being optimal, since yield is characterized by a low heritability and a high genotype x environment interaction. The sought of new sources of resistance, as well as dissecting the complex resistance traits into different components, highly heritable, easy to measure and repeatable is crucial to improve breeding for drought resistance To this aim, an oat germplasm collection consisting on 141 accessions of A. sativa and A. byzantina together with 32 oat cultivars (A. sativa) was screened for drought tolerance. A visual scale of symptoms able to discriminate among genotypes was set up in seedlings, under controlled conditions and along a treatment of progressive drought. According to this evaluation, 11 genotypes were classified as highly resistant. In these ones together with three susceptible controls several physiological traits associated with resistance were assessed to determine which parameters better reflected the resistance observed. Relative water content (RWC) and cell membrane stability (CMS) was measured at 0, 6, 9, 12, 15 and 18 days after withdrawing the water. CMS at a similar and low RWC reflected better the plant symptoms that RWC alone. In addition, we determined daily the stomatal response to drought stress. Unlike resistant genotypes, the susceptible ones were not able to maintain the circadian rhythm from the sixth day following drought and kept conductance at levels similar to the dark period. In several genotypes, this stomatal closure lead to photo inhibition and oxidative damage such as lipid peroxidation that later correlated with burned and rolled tip leaves

P07-030: CHARACTERIZATION OF P RESPONSIVE GENES IN RELATION TO ROOT HAIR GROWTH

Bremer, M.
Leibniz Universität Hannover
**Corresponding author e-mail: melanie-bremer@web.de*

web.de Enhanced P efficiency hab been attributed to the forma-

tion of longer root hairs in Brassica carinata cultivars. A transcriptome analysis using Suppression Subtractive Hybridization (SSH) and microarray was performed in order to identify differentially expressed genes involved in P induced root hair formation in two contrasting cultivars. The expression pattern of candidate genes was characterized in response to changes in P, N and K supply and along the root. Root hair length was enhanced with P and N starvation but not with K depletion. Additionally, changes in P and N supply resulted in longer root hairs 4h after removal of P and in shorter root hairs after P and N resupply to previously starved roots after 2h and 8h, respectively. Likewise, transcription of a leucine rich receptor like protein kinase (BcLRR) was induced by P and N starvation and suppressed by resupply while unaltered during K starvation. In contrast, expression of a hydroxyproline rich glycoprotein (BcHRGP) was reduced in response to P and N starvation and most pronounced in mature parts of the root. Thus, a function of these genes in root hair development is suggested. The expression pattern of BcLRR suggests a function in root hair growth induced by P and N stress whereas BcHRGP may be involved in negative regulation of root hair growth by strengthening the cell wall.

P07-031: THE SOS SYSTEM IS ESSENTIAL FOR THE SALT TOLERANCE OF RICE

Elmahi, H.[1] - Espartero, J.[1] - Aguilar, M.[2] - Quintero, F.J.[1] - Pardo, J.M.[1]*

[1]Instituto De Recursos Naturales Y Agrobiología (CSIC)

[2]Centro De Investigacion y Formacion Agraria Las Torres y Tomejil, Consejeria De Agricultura Y Pesca, Junta De Andalucía

**Corresponding author e-mail: Houda@Irnase.Csic.Es*

Sodium homeostasis in plants is controlled by Na/H antiporters in the plasma membrane and tonoplast. In Arabidopsis thaliana, the Na/H antiporter SOS1 regulates sodium efflux in roots and the long-distance transport of sodium from roots to shoots. SOS1 activity is regulated through phosphorylation by the protein kinase SOS2 together with the calcium-sensing regulatory subunit SOS3. Functional homologues of the SOS genes have been identified in rice. OsSOS1, OsCIPK24 and OsCBL4 are the rice orthologues of Arabidopsis SOS1, SOS2 and SOS3 genes, respectively. They suppressed the salt sensitivity of the corresponding mutants of Arabidopsis. We have used reverse genetics to demostrate the importance of the SOS system in the salt tolerance of rice plants. Mutant lines in public rice collections bearing gene disruptions in OsSOS1, OsCBL24 and OsCBL4 that had been created by insertion of T-DNA or retrotransposons, have been analyzed for salt tolerance. Our data indicates that mutants with reduced activity of OsSOS1 or OsCIPK24 are indeed salt sensitive. The gene expression pattern of OsSOS1 will be analyzed using promoter::GUS transcriptional fusions to corroborate a role in sodium efflux and long-distance transport in rice plants. SOS1 proteins contain self-inhibitory domains located at their carboxy termini. The truncation of this inhibitory domain in OsSOS1 resulted in a much greater transport activity and enhanced salt tolerance in yeast cells, that were both independent of CIPK24/CBL4.

P07-032: IDENTIFICATION OF QTLS FOR CHLOROPHYLL AND CAROTENOIDS CONTENT IN WHEAT UNDER THREE LEVELS OF WATER AVAILABILITY

Czyczylo-Mysza, I.[1] - Marcinska, I.[1] - Skrzypek, E.[1] - Chrupek, M.[1] - Stawicka, A.[1] - Grzesiak, S.[1] - Quarrie, S.A.[1]*

1The F. Górski Institute of Plant Physiology, Polish Academy of Sciences

2Visiting Professor, Institute for Research on Environment and Sustainability, Newcastle University

**Corresponding author e-mail: czyczylo-mysza@wp.pl*

Drought is one of the most common environmental stress worldwide that affects growth and development of plants through alterations in metabolism and gene expression. Set of 94 doubled haploid lines obtained from Chinese Spring x SQ1, mapped with 450 markers (Quarrie et al., 2005), was evaluated for chlorophyll a, chlorophyll b and carotenoids under moderate (MD) and severe drought (SD) stress, and compared with results for well-watered plants, as a control. Both drought conditions were imposed for 4 weeks during the late vegetative stage, to be relieved at around the time of flowering. QTLs were identified using Windows QTLCartographer version 2.5 software and results were analysed using single-marker analysis (SMA) and composite interval mapping (CIM). The genetic control of pigments varied considerably between drought-stressed and non-stressed plants. Two QTLs under MD on chromosomes: 4B, 7D and 1 QTL under SD on chromosome 3A for chlorophyll a, with R2 values 11.7, 13.31 and 17.72% respectively were identified. For chlorophyll b, 1 QTL on chromosome 3D under well-watered conditions and 2 QTLs on chromosomes 3D and 7D under MD were identified. Mapping QTL for carotenoids revealed 2 QTLs on chromosomes 1B (LOD=3.8) and 6D (LOD=5.2) under well-watered and 1 QTL on chromosome 6B (LOD=3.4) under moderate drought. Higher content of chlorophyll in crops may be an effective way to increase the biomass production and the grain yield. Therefore, the genetic analysis of these traits provided an excellent tool to understand better the mechanisms regulating responses of wheat to drought stresses.

Research funded by grant 479/N-COST/2009/0

P07-033: MOLECULAR RESPONSES TO IONISING RADIATION EXPOSURE IN ARABIDOPSIS THALIANA

Morgane, G.[1] - Esnault, M.A.[1] - Jorrín-Novo, J.V.[2] - Cabello-Hurtado, F. [1]*

[1]University of Rennes

[2]University of Cordoba

**Corresponding author e-mail:* morgane.gicquel@univ-rennes1.fr

Plants are exposed to ionising radiations (IR) in their environment by the cosmic and telluric radiances and can be subjected to higher doses from anthropogenic sources. The harmful effects of IR derive from the oxidative stress due to hydroxyl radicals generated during the water radiolysis phenomenon and other reactive species secondary produced. Their interactions with biomolecules lead to lipid peroxides in membranes, denaturation of proteins and DNA strand breaks. Plants are more radioresistants than animals and understanding their adaptive responses will allow to improve their selection and to discover radioprotective compounds

We used proteomics as an approach to study global metabolic changes occurring in Arabidopsis thaliana (Col-0) after exposure to 2 sub-lethal doses of X-Rays, 10 and 40 grays (Gy). Our model was 10-days-old plants irradiated at the 2-leaves-spread-out stage. Leaves and roots were harvested separately at 2, 24 and 72 hours after treatment and proteins were extracted with TCA-acetone / phenol method. The analysis of changes in the 2-DE protein profiles in both tissues revealed 138 spots differentially expressed as a function of the doses and the kinetic points, and the corresponding proteins were identified by MALDI-TOF-TOF mass spectrometry. Results showed both quantitative and qualitative differences between proteins regulated by 10 and 40 Gy doses. In both cases, numbers of up- and down-regulated spots tends to equilibrate, showing an active response. Hierarchical Clustering Analysis showed 6 different expression patterns. Hypothesis on activated mechanisms will be completed with the analysis of data from a twin transcriptomic study.

P07-034: PLANT ABA-INDUCED, TSPO-RELATED PROTEIN: A POTENTIAL FREE HEME SCAVENGER DURING STRESS?

Vanhee, C. - Gumy, C. - Masquelier, D. - Batoko, H.*

ISV, université catholique de louvain

**Corresponding author e-mail: celine.vanhee@uclouvain.be*

Arabidopsis AtTSPO is a yet-to-functionally-identify, TSPO/MBR domain containing membrane protein potentially involved in multiple stress regulation. It's expression is tightly regulated both at the transcriptional and post-transcriptional levels. AtTSPO is detectable in seeds and senescent leaves and we showed previously that AtTSPO can be induced in vegetative tissues by ABA and water-related stress.
We found that ABA-dependent induction of AtTSPO is transient and the protein is rapidly degraded in vegetative tissues. Degradation of AtTSPO is enhanced by boosting tetrapyrroles biosynthesis. Heterologously expressed and purified or endogenous AtTSPO binds heme. Free heme level is transiently increased by ABA treatment of plant cells. ABA-dependent increase of free heme was higher in AtTSPO knockout plant and lower in transgenic lines overexpressing AtTSPO. ABA-induced AtTSPO or overexpressed AtTSPO were more stable when we inhibited chloroplastic biosynthesis of heme in plant cell. In addition, we found that the stability of AtTSPO in plant cell can be enhanced by inhibiting phosphoinositide 3-kinases, key players in bulk protein degradation through autophagy.
Taken together, these results suggest that AtTSPO is a hemoprotein whose stability is regulated by tetrapyrroles metabolism. The potential degradation of the hemoprotein through autophagy may be a mean of getting rid of excess free heme transiently induced by ABA.

P07-035: EVIDENCE FOR LEAF SIGNAL AND REGULATION OF ANTIOXIDANT SYSTEM IN TOMATO FRUITS IN RESPONSE TO STRESS: INVOLVEMENT OF ABA, ASCORBATE REDOX STATE, H2O2 AND NO.

Lopez-Lauri, F. - Murshed, R. - Sallanon, H.*
Université d'avignon et pays de Vaucluse (UAPV)
**Corresponding author e-mail: felicie.lauri@univ-avignon.fr*

To begin, tomato plants were treated with mercury (5 ppm of HgCl2) for 24 hours to induce arrest of water flux in plants and evaluate oxidative parameters and antioxidant defence mechanisms. Mercury treatment induced oxidative damages in leaves and fruit peduncles; whereas the fruits were not affected. Nonetheless, the activities of antioxidant enzymes (SOD, CAT, APX, DHAR and MDHAR) as well as the transcript levels of some genes encoding antioxidant enzymes (SlAPXcyto, SlAPXt, SlDHAR1, SlDHAR2 and SlMDHAR) in fruits increased with mercury treatment. That led us to suppose that the oxidative stress in leaves induced a signal which in turn alerted the fruit antioxidant enzymes. To test this hypothesis, tomato fruits were treated with different molecules considered as putative stress signals: abscisic acid (ABA), ascorbate redox state, H2O2 and nitric oxide (NO). Changes in the oxidative parameters (H2O2 and MDA), the ascorbate levels and the activities and transcript levels of antioxidant enzymes were found in fruits treated with different concentrations of H2O2 (250, 500 and 1000 µM), 0.1 mM of ABA, 0.5 mM of SNP as NO generator, 0.5 mM of SNP and 1 mM of PTIO as NO scavenger or different levels of ascorbate redox state (0.5, 0.75 and 1) for 4, 8 and 24 hours, suggesting that ABA, AsA redox state, H2O2 and NO act as stress signals provided by leaves and play important roles in the regulation of the fruits’ antioxidant mechanisms.

P07-036: ACTIVATION OF PLASMA MEMBRANE H+-ATPASE IN WHEAT ROOTS BY WATER STRESS

Planes, M. D.[1] - Montesinos, C.[1] - Younis, H.M.[2] - Serrano, R.[1]*
[1]IBMCP,UPV-CSIC,Valencia (SPAIN)
[2]Dept.Pesticide Chemistry, Faculty Agriculture, El-Shatby, Alexandria(EGYPT)
**Corresponding author e-mail: dplanes@ibmcp.upv.es*

Drought is the most important stress limiting crop productivity and plant defenses based on osmolytes, LEA and HSP proteins and antioxidants are well known. Relatively little work, however, has been done on the response of plant ion transporters to water stress. We have found in the roots of three egyptian wheat (Triticum aestivum) cultivars that the plasma membrane H+-ATPase is activated 1.35-fold by water stress. This activation correlates with increased amount of the enzyme (1.4-fold) as determined by Western blot with two antibodies against Arabidopsis AHA3. The ATPase band was inmunodecorated with an anti-phosphothreonine antibody, pointing to at least partial activation of the enzyme.

P07-037: COMPARATIVE ANALYSIS OF THE TWO CONTRASTING FLAX CULTIVARS UPON CADMIUM EXPOSURE

Hradilová, J.[1] - Mazanec, K.[2] - Griga, M.[3] - Brzobohatý, B.[1]*
[1]Mendel University in Brno
[2]Institute of Analytical Chemistry of the ASCR
[3]AGRITEC Ltd.
**Corresponding author e-mail: hradilov@mendelu.cz*

Phytoremediation represents an effective low-cost approach for removing pollutants from contaminated soil and water. Cadmium (Cd) is a wide-spread and serious pollutant due to its high toxicity and carcinogenicity. Flax (Linum usitatissimum L.) is a crop with a high phytoremediation potential. However, significant differences in Cd tolerance were previously detected among commercial flax cultivars. Notably, cv. Jitka showed substantially higher tolerance to Cd in soil and in suspension cultures than cv. Tábor. In our study, significant differences were revealed in physiological and biochemical parameters between Cd-treated seedlings of cv. Jitka and cv. Tábor. Higher levels of glutathione were detected in cv. Jitka than in cv. Tábor. In contrast to cv. Tábor, no significant increase in malondialdehyde level was observed in roots of cv. Jitka exposed to the highest Cd concentration. Comparative proteomic analysis was carried out to detect changes in protein expression in these two contrasting flax cultivars. Differentially expressed proteins were identified by two-dimensional gel electrophoresis followed by mass spectrometry. Analysis of the root proteome revealed four proteins selectively up-regulated in cv. Jitka. In contrast, no significant differences in response to Cd were found in the shoot proteome. The identified changes could facilitate marker-assisted breeding for Cd tolerance and the development of transgenic flax lines with enhanced Cd tolerance and accumulation capacities necessary for phytoremediation

P07-038: LEAD ACCUMULATION AND DETOXIFICATION IN TWO CONTRASTING ECOTYPES OF DIANTHUS CARTHUSIANORUM

Wojcik, M. - Oniszczuk, M. - Wojcik, A. - Tukiendorf, A.*
Maria Curie-Sklodowska University
**Corresponding author e-mail: mwojcik@umcs.pl*

Dianthus carthusianorum plants were identified as facultative metallophytes showing ecotypical differentiation for heavy metal tolerance. In this study, two contrasting ecotypes of D. carthusianorum, one populating a Zn-Pb waste heap in an ore-mining and smelting region in southern Poland and the other from an unpolluted site were cultivated in hydroponics to investigate their response to various Pb concentrations. The two ecotypes showed significant morphological differences even when cultivated in the same and favourable conditions in a vegetative chamber. Although the waste heap ecotype accumulated almost twice as much Pb both in roots and in shoots in comparison with the reference ecotype, it exhibited less pronounced symptoms of Pb toxicity. Accumulation of thiol peptides and organic acids as potential ligands for Pb detoxification in plant cells was examined. The glutathione level was similar in both ecotypes; however, phytochelatins were accumulated in higher concentrations in the roots of the ecotype from the unpolluted site. The content of organic acids, especially of citrate, was higher in the waste heap ecotype; however, it seemed to be insufficient to explain the

higher Pb tolerance of these plants. Thus, it seems that it is not chelation by ligands but rather various Pb compartmentation in plant tissues or cells that may be responsible for the different Pb tolerance of these two ecotypes.

P07-039: CU EXCESS INFLUENCE ON THE PHOTOPROTECTIVE MECHANISMS IN SECALE CEREALE PLANTS

Janik, E. - Maksymiec, W. - Gruszecki, W. I.*
Department of Biophysics, Institute of Physics, Maria Curie-Sklodowska University
**Corresponding author e-mail: ewa.janik@poczta.umcs.lublin.pl*

Cu is an essential microelement for maintaining optimum photosynthesis but its excess may perturb this process. The influence of excess Cu ions and high light treatment on the function of photosystem II was investigated in order to examine how this heavy metal modifies the photoprotective mechanisms operating at the molecular level in Secale cereale plants. Thus, non-treated plants and those treated with 5 or 50 μM Cu, simultaneously illuminated with 150 μmol m–2 s-1 or 1200 μmol m–2 s-1 light intensity, were studied. The parameters of Chl a fluorescence induction kinetics indicated that the photosynthetic apparatus adapted to the high light condition.
This phenomenon was based on the effective utilization of excitation energy in the light and dark phases of photosynthesis. HPLC measurement of the xanthophyll pigment content showed that Cu excess under high light condition induces violaxanthin de-epoxidation and zeaxanthin accumulation.
The strong zeaxanthin accumulation was accompanied by an increase in non-radical dissipation of the absorbed energy within the antenna complexes. Cu treatment caused trans-cis violaxanthin isomerization in proportional correlation to concentration, which suggests a direct metal-pigment molecule interaction confirmed by in vitro study. It can be assumed that Cu excess enhances yield of the photoprotective mechanisms operating at the molecular level.

P07-040: PROTEOMIC AND EXPRESSION ANALYSIS OF TREBOUXIA ERICI. RESPONSE TO DEHYDRATION AND REHYDRATION

Gasulla, F.[1] - Barreno, E.[1] - Guéra, A.[2] - Oliver, M.[3]*
[1]Universitat de València
[2]Universidad de Alcalá de Henares
[3]University of Missouri
**Corresponding author e-mail:* francisco.gasulla@uv.es

The study of desiccation tolerance of lichens, and of their photobionts in particular, has frequently focused on the antioxidant system that protects the cell against photo-oxidative stress, produced by an increase in ROS during dehydration and rehydration cycles.
As far as we know, few studies have dealt with the regulation of the responses during dehydration and/or rehydration in lichen photobionts at a molecular level. Thus, we decided to carry out proteomic and genetic expression analyses of the changes associated with desiccation and rehydration in the isolated photobiont Trebouxia erici. Algae were dried slowly (5 - 6 h) and rapidly (< 60 min), and after 24 h of desiccation were rehydrated. To identify those proteins that accumulate during the drying and the rehydration process, we have employed a strategy of 2-D Difference Gel Electrophoresis (DIGE) coupled with individual protein identification using trypsin digestion and LC-MS/MS.Proteomic analysis showed that desiccation caused up-regulation of around 19 proteins and down-regulation of 43 proteins in T. erici. Some of the up-regulated proteins in the desiccated and rehydrated algae were identified as proteins involved in transport, protection, cytoskeleton, cell cycle and targeting and degradation.
Three and two of the most highly up-regulated proteins were Heat Shock Protein 90 (Hsp90) and β-tubulin proteins, respectively. The Hsps protect cells and help to return to equilibrium during recovery after stress. Microtubule skeleton seems to play a key role in the recovery of the ultrastructure of cells after desiccation. We observed that five Hsp90 and two β-tubulin genes were activated during dehydration and mRNA was accumulated until the cell was completely dried.

P07-041: NORSPERMIDINE INDUCES A CYTOSOLIC HEAT-SHOCK RESPONSE IN ARABIDOPSIS THALIANA.

Sayas, E. - Alejandro, S. - Niñoles, R. - Serrano, R.*
Instituto de Biología Molecular y Celular de Plantas (IBMCP)
**Corresponding author e-mail: ensamon@etsia.upv.es*

Polyamines are small polycationic molecules found ubiquitously in all organisms and function in a wide variety of biological processes. The most important polyamines in plants are putrescine, spermidine and spermine.
Recently, it has been reported that spermidine is involved in the post-translational hypusine modification of the eukaryotic initiation factor 5A (eIF5A), a process that is essential in all eukaryotic cells. Here we used the uncommon polyamine norspermidine (NE), which structurally resembles spermidine, to study ionic stress responses in Arabidopsis thaliana. An expression analysis of plants treated with NE showed an induction of genes related to the antioxidant response and also of genes related to the cytosolic heat-shock response (CHR).
We analyzed overexpression and knock-out mutant lines of two different polyamine oxidases, PAO1 and PAO3, which are enzymes that oxidize polyamines producing H2O2, and we found that, unexpectedly, the overexpression plants were tolerant to NE. This suggests that the H2O2 generated by the oxidation of NE is secondary, and that the main toxic effect of NE could be caused by cytosolic protein denaturation, as suggested by the induction of the CHR. Cytosolic protein denaturation could be caused by NE interfering with eIF5A hypusination, due to its homology with spermidine. Alternatively, NE could directly interact with the ribosomal RNA and perturb protein synthesis.

P07-042: CHANGES IN GENE EXPRESSION OF ETHYLENE BIOSYNTHESIS AND SIGNALLING IN TOCOPHEROL-DEFICIENT ARABIDOPSIS MUTANTS

Cela Udaondo, J.[1] - Chang, C.[2] - Munné-Bosch, S.[2]*
[1]Universidad de Barcelona
[2]University of Maryland
**Corresponding author e-mail: reciproca@hotmail.com*

Tocopherols are a group of lipid soluble antioxidants that are synthesized only by photosynthetic organisms. Plants accumulate α-tocopherol, and to a lesser extent its immediate precursor, γ-tocopherol, in plant responses to abiotic stress. Although it is well known that tocopherols protect photosynthetic membranes from lipid peroxidation, recent studies suggest that γ-tocopherol could play specific roles in abiotic stress tolerance, but mechanisms are still unknown.
In this study, we analysed changes in gene expression of ethylene biosynthesis, perception and signalling in vte1 and vte4 Arabidopsis mutants exposed to water deficit. vte1 and vte4 mutants lack α-tocopherol, but only the vte1 mutant is additionally deficient in γ-tocopherol. The expression of some genes of ethylene biosynthesis (ACO1, ACO4, ACS2 and ACS6) and signalling (ERF1) increased in wild type plants exposed to water stress. The expression of ACO4, which encodes for ACC oxidase, increased in vte4 mutants under water stress. Furthermore, the expression of ERF1, which encodes for a transcription factor that regulates ethylene response genes, increased in water-stressed plants of both vte1 and vte4 mutants, but to a smaller extent compared to the wild type. It is concluded that a deficiency in tocopherols may alter ethylene biosynthesis and signalling at the gene expression level in Arabidopsis plants exposed to water stress.

P07-043: ROLE OF PROTECTIVE REACTIONS IN DIFFERENT KINDS PLANTS AT STRESSFUL CONDITIONS

Gambarova, N. - Gambarova, N.G.*
Baku State University
**Corresponding author e-mail: nailya-gambarova@rambler.ru*

The purpose of the dissertation is investigate of mechanisms of protective reactions in different kinds C3 and C4 plants at action of the various nature of stresses. Obtained results are generalized on the basis of investigations of action of the high temperature on transport electron chloroplast of different sort of wheat, distinguished with thermal resistance, various concentration NaCl in the sort of barley, distinguished with salt-endurance, various speciation plants of the amarant, distinguished with contenting of pigment betacyanin nature of amarantin.
Despite of the different nature, effective stressful factor, changing functional activity of electronic transport in investigated kinds C3 and C4plants have a general orientation of the processes adducting to the change of mechanism of regulation of electronic transport in extreme conditions. In particular, generally is - at activity of above described factors – switching of the basic linear stream of electrons (acyclic transport of electrons) on alternative ways: cyclic around ΦCI and also pseudo-cyclic with participationspace separated photoreaction supervised redox-status of components – carriers of electrons in ETC (pools of plastokhinones, O2, oxidized НАДФ).
The basic purpose of the above-stated alternative ways at action of the different nature of stressful influences is, first of all, prevention of inflowing of surpluses of energy in ΦCII, bound with forming of singlet O2, elimination reduction ETC by carrying of electrons on O2 with forming H2O2which probably serves as the basic signal molecule in transcription chloroplast and nucleur genes, and also synthesis of adenosinethrephosphate (ATP), necessary for realization of the important processes in the cells.

P07-044: DISTINCT PROTECTIVE PATHWAYS OF 24 EPIBRASSINOLIDE AND 6-BENZYLAMINOPURINE IN WHEAT SEEDLINGS

Yuldashev, R. - Avalbaev, A. - Lastochkina, O. - Shakirova, F.
Institute of Biochemistry and Genetics

Earlier we found that 0.4 μM 24-epibrassinolide (EB) and 4.4 μM 6-benzylaminopurine (BAP) comparably decreased the damaging action of water deficit on growth of wheat seedlings. Further we have found that EB and BAP stimulated gene expression of TADHN dehydrin and accumulation of proline (Pro), osmoprotectants which content are regulated by ABA. Interestingly that ABA accumulation preceded the BAP-induced TADHN dehydrin gene expression and increase in Pro content while EB had no effect on these processes.
We have suggested that BAP induced defense reactions is ABA-mediated. Experiments with fluridon (Flu), an inhibitor of ABA biosynthesis, confirmed the key role of ABA in BAP-induced Pro accumulation.
There was no effect of BAP on Pro content in Flu-pretreated plants. Meanwhile there was the same EB effect both in Flu-pretreated and non-pretreated plants. Alongside with brassinosteroids and cytokinins protective effect in response to abiotic stresses there were data about their defense action in response to viral, bacterial and fungal infection. So it was interesting to investigate EB and BAP action on gene expression of pr-1 protein which is related to pathogenesis-related proteins that accumulate in plants following attack by different types of pathogens and analyzed the role of ABA in this process. It was revealed that both EB and BAP increased the pr-1 gene expression in plants. Using Flu it was found that like in case of proline BAP realized its effect in ABA-dependent manner. However action of EB was ABA-independent because Flu did not affect the EB-induced pr-1 gene expression. Thus these data indicate on distinction between protective pathways of EB and BAP in wheat plants.This work is supported by Grant RFFI 08-04-01563.

P07-045: MODIFICATIONS IN PLANT TRANSFER RNA AT NORMAL AND STRESS CONDITIONS

Atanasova, L.
Institute of Plant Physiology, Bulgarian Academy of Sciences

Transfer RNA (tRNA) is the most extensively modified nucleic acid in the cell. The four normal nucleosides adenosine, guanosine, uridine and cytidine can be modified into derivatives with different chemical structures and location along the tRNA chain. The modifications belong to different tRNA species, and affect their activity and stability. The appearance and the extent of a tRNA modification depend on various factors, especially developmental and environmental conditions can change the modifying of tRNA. The levels of most abundant modified nucleosides were examined in total tRNA from two plant species grown at normal and stress conditions. Total RNA was isolated from organs of maize (Z. mays L.) and pea (P.sativum L.) plants grown at normal and prolonged stress conditions provoked by salinity (NaCl), metal toxicity (Cd) and herbicide (atrazine). Total tRNA was fractioned and digested to nucleosides which were analyzed by HPLC (Umeå University, Sweden).
Numerous modified nucleosides were demonstrated in tRNA preparations. Variations in the levels of several modifications between plant organs and under stresses were found. Prominent changes were noticed in the levels of queuosine, N4-acetyl-cytidine and derivatives of N6-isopentenyl adenosine. The observed trends in the distribution of tRNA modifications along the plant as well as in the effects of stressors on their levels allow proposing that these modifications exert specific functions in plant growth and development as well as in surviving at the stresses.
Projects by the NSF (B1208/02, PISA/2005-08i)n Bulgaria; grant by the SI Sweden (2005).

P07-046: ABSCISIC ACID IS AN INTERMEDIATE IN PROTECTIVE ACTION OF SALICYLIC ACID ON WHEAT PLANTS UNDER SALINITY

Lastochkina, O. - Maslennikova, D. - Shakirova, F.
Institute of Biochemistry and Genetics, Ufa Scientific Centre, Russian Academy of Sciences
**Corresponding author e-mail: shakirova@anrb.ru*

Earlier we found that salicylic acid (SA) exerts protective effect to dehydration in wheat. It was revealed that treatment of wheat seedlings with 50 μM SA causes reversible abscisic acid (ABA) accumulation. Also it causes essential increase in osmoprotectant levels - TADHN dehydrin gene expression and proline - both of which are ABA-regulated. SA actively influence on pro- and antioxidant (AO) systems connected with parallel and reversible reactive oxygen species accumulation and increase in activities of peroxidase and phenylalanine-ammonia-lyase (PAL) which gene expression are ABA-controlled. To reveal the crucial role of endogenous ABA in development of SA-induced defense reactions in wheat plants, there was compared the effects of SA and SA in mix with fluridon (Flu) (inhibitor of ABA biosynthesis) on proline concentration, peroxidase and PAL activities, malonaldehyde (MDA) content and dynamics of lignin deposit in the cell wall of root basal part under salinity. Flu prevented SA-induced proline accumulation, increase in PAL and peroxidase activities that was reflected in delay of SA-induced lignification. Meanwhile Flu completely prevented the SA-induced protective effect which was visualized as decrease of salinity-induced MDA accumulation which probably evoked through SA-induced ABA-mediated activation of AO enzymes. Also we carried out comparative analysis of SA and ABA influence on PR-1 gene expression which is known marker of SA-induced systemic acquired resistance. We have revealed the high sensitivity of PR-1 gene not only to SA but also to ABA. Thus, the obtained

data indicate on ABA as an intermediate in SA protective action on wheat plants under salinity.This work is supported by Grant NS - 915.2008.4

P07-047: THE ROLE OF MIRNAS IN ENERGY SIGNALING

Martinho, C. - Rodrigues, A.*
Instituto Gulbenkian de Ciência, GIRM, ESTM/Int Politecnico de Leiria
**Corresponding author e-mail: cmartinho@igc.gulbenkian.pt*

Baena-González E (Instituto Gulbenkian de Ciência)
As sessile organisms, plants must be able to adapt to the local environmental conditions and face a range of biotic and abiotic stresses. Perception of stress cues and their proper integration with other physiological and developmental signals are key steps for the establishment of stress tolerance. Besides triggering stress-specific responses, recent findings have suggested that various types of stress induce also largely overlapping transcriptional changes via a common energy-deficiency signal. This convergent transcriptional response is orchestrated by the energy-sensing SnRK1 (Snf1-related kinase1) protein kinases, which thereby allow the re-establishment of homeostasis and the elaboration of a more targeted adaptive response. The mechanisms involved in the sensing and transduction of the energy-deficiency signal triggered by environmental stresses are still largely unknown. Recent studies have shown that microRNAs are key players in the response to various nutrients as well as to other abiotic and biotic stresses. Moreover, some miRNAs are similarly regulated by different stress conditions and are oppositely regulated by sugar. We thereby postulate that SnRK1 regulation of gene expression is partly exerted through miRNAs. In support of this, several of the genes known to be under SnRK1 control are known miRNA targets. We have now identified by deep sequencing miRNAs specifically associated with energy deprivation. Current efforts aim at validating the deep sequencing results and characterizing the role of specific miRNAs in the SnRK1 signaling chain.

P07-048: ROLE OF THE PLASMA MEMBRANE NA+/H+ ANTIPORTER SOS1 IN TOMATO PLANTS UNDER SALINE CONDITIONS

Olías, R.[1] - Eljakaoui, Z.[1] - Li, J.[2] - Alvarez de Morales, P.[1] - Aly, M.[1] - Pardo, J M [3] - Belver Cano, A.[1]
[1]Estación Experimental del Zaidín, CSIC, Granada
[2]Instituto de Agrobiotecnología y Recursos Naturales, CSIC, Pamplona
[3]Instituto de Recursos Naturales y Agrobiología, Sevilla

Maintaining a high K+/Na+ ratio in the cell cytosol, along with the processes of transport implicated in the xylem and phloem loading/unloading of Na+ in plants (long-distance transport) are key aspects in plant salt tolerance. The Ca2+-dependent regulatory SOS pathway involved in salinity tolerance has been reported in Arabidopsis, by regulating Na+ and K+ homeostasis as well as long-distance Na+ transport. We recently isolated the SlSOS1 gene, encoding a Na+/H+ antiporter from tomato. By silencing of SlSOS1 in tomato plants we have shown that, besides its main action in extruding Na+ out the root, SlSOS1 is critical for the partitioning of Na+ in plant organs and the ability of tomato plants to retain Na+ in the stems, having an effect on K+ nutrition. We hypothesized that the action of SOS1 to achieve Na+ (and K+) homeostasis should be coordinated with class I HKT1 transporters, as previously suggested in Arabidopsis. Thus, dysfunction of either system might alter long-distance transport and adequate partition of Na+, thereby resulting in salt-sensitive phenotypes. We are studying whether the depletion of SOS1 in suppressed tomato does indeed affect the function of the AtHKT1;1 orthologous protein. We believe that SlSOS1 could participate in the redistribution of Na+ from young to old leaves via xylem (although via phloem should not ruled out), with a significant part being held in the stem (probably in close association with SlHKT1).

P07-049: PLANT HORMONE RESPONSE TO WATER STRESS IN SIX PINUS RADIATA D. DON ORIGINS.

De Diego, N.[1]* - Moncalean, P.[1] - Perez-Alfocea, F.[2] - Lacuesta, M.[3]
[1]Neiker-Tecnalia
[2]CSIC Murcia
[3]Basque Country University
**Corresponding author e-mail:* ndiego@neiker.net

Radiata pine is a species widely distributed in Northern Spain due to its importance in the timber sector and fast growth. The global change and the climatology fluctuations (with year cycles with low pluviometry) make necessary to select adaptable species to stress conditions (biotic and abiotic stress) for their use either for restoration of the landscapes or commercialization. Thus, breeding programs need good quality planting stock for a successful establishment of plantations. Droughits considered the main factor limiting production, growth, and development of forest species and can affect the outcome of reforestation programmes. Physiological characterization of plants is a good tool to determine markers which permit select elite plants. In this study, water relations as well as growth regulator content (ABA, IAA, Z, RZ, salicylic acid, jasmonic acid and ACC) were analyzed on 2 year-old plants of Pinus radiata from 6 different origins as possible markers under water stress.
The plants were exposed to a short water stress cycle (4 week), followed by rehydration of stressed plants, and a second cycle of long water stress (finished when 50% of stressed plants from each origin showed apical curvature).
The results about the use of these parameters as water stress tolerance and the stress tolerance behaviour for each origin will be discussed. This work was funded by MEC (Science and education department) (AGL2005-08214-CO2-O2 and SUM-2006-00007-CO2). Nuria De Diego was supported by FT grant from the Basque Government.

P07-050: FKBP PROTEINS ARE IMPORTANT DETERMINANTS OF INTRACELLULAR ACID STRESS TOLERANCE IN YEAST AND IN ARABIDOPSIS

Bissoli, G. - Niñoles, R. - Fresquet, S. - Montesinos, C. - Hueso G. - Planes, D. - Serrano, R. - Mulet José, M.
IBMCP-CSIC-UPV

Our previous work in yeast has demonstrated that overexpression of FPR1, an FKBP immunophilins, conferred tolerance to weak organic acids such as acetic and sorbic acid. FK506 binding proteins (FKBP) where originally identified as the cellular targets of the immunosupressant drugs rapamycin and FK506. FKBPs are peptidyl-prolyl cis-trans isomerases (PPIase EC 5.1.2.8) that catalize the isomerization of peptidyl prolyl bonds between cis and trans configurations. FKBP are ubiquitous proteins that can be found either as a single catalytic domain proteins or being part of more complex proteins. To assess the implication of FKBP proteins in weak acid tolerance in plants we have generated lines of Arabidopsis thaliana overexpressing two different proteins: yeast FPR1 and Arabidopsis FKBP65 (ROF2). In presence of acetic acid transgenic lines overexpressing any of these genes grew better than wild type plants. On the other hand an AtFKBP65 loss-of-function mutant line showed weak acid sensitivity. In absence of stress we have observed a gain of apical dominance in 35S::AtFKBP65 mutants and its loss in FKBP65 knock-out line. We have also found that 35S::AtFKBP65 plants showed enhanced response to ABA and IAA. The roots of AtFKBP65 knock-out mutants have reduced number of lateral roots and exogenous application of IAA was able to revert this phenotype. Our hypo-

thesis is that ROF2 is a positive regulator of either auxin biosynthesis or perception.

P07-051: CRIO4, A PATL LIKE GENE FROM SUGAR BEET IS ABLE TO CONFER TOLERANCE TO COLD STRESS BY OVEREXPRESSION

Izquierdo-García A. C. - Serrano R. - Mulet J. M.*
IBMCP
**Corresponding author e-mail: anizgar@ibmcp.upv.es*

Abiotic stress is one the main limiting factors for agricultural yield. There are not extensive descriptions on which molecular processes are compromised by suboptimaltemperatures. Aiming at identifying plant genes related to cold tolerance weconstructed a cDNA library of sugar beet (Beta vulgaris) in a yeastexpression vector, isolating CRIO4 by its ability to formcolonies at 10ºC. This gene is conserved in plants and presentsseveral functional domains. Among them, a SEC14 domain. This domain issimilar to the SEC14 gene of yeast (Saccharomyces cerevisae). SEC14 encodesa Phosphatidylinositol/Phosphatidylcholine transfer protein involved incoordinate regulation of PtdIns and PtdCho metabolism. Our results indicatethat enzymatic activity should be conserved, given that our sequence datashows a conservation of the key aminoacids identified in other organisms.Aiming at confirm this observation we have purified the CRIO4 protein andperformed in vitro interaction assays with phospholipids. In addition ourstudy has identified a GOLD domain (related to protein-protein interactionin the Golgi), a Poliproline domain and several PXXP domains. CRIO4 ishomologue to the PATELLIN1-6 gene family of Arabidopsis thaliana. We haveidentified the members of this family which exhibit higher conservation toCRIO4, isolated homozygotic mutants, and we crossed them to obtain doublemutants. Phenotypic analysis of those double mutants, as well aslocalization data will be presented.

P07-052: THE ROLE OF ABA IN THE INDUCTION OF WGA ACCUMULATION BY 24-EPIBRASSINOLIDE AND 6-BENZILAMINOPURINE IN WHEAT SEEDLING ROOTS UNDER SALINITY

Bezrukova, M. - Àvalbaev, A. - Allagulova, Ch. - Murzabaev, A, - Shakirova, F.*
Institute of Biochemistry and Genetics
**Corresponding author e-mail: lectin@anrb.ru*

It is well known that gene expression and quantity of wheat germ agglutinin (WGA) are regulated by ABA which plays a key role in the regulation of plant protection to environmental stresses. Meanwhile, the essential WGA accumulation, preceded by the rapid transit increase in endogenous ABA level, is observed in wheat plants in response to biotic and abiotic stresses. Since WGA is Rab (responsive to ABA) protein it is possible to conclude that it is involved in ABA-controlled nonspecific antistress reactions of wheat plants. However we have shown that other phytohormones 24-epibrassinolide (EB) and cytokinin 6-benzylaminopurine (BAP), which possess antistress activity, induced gene expression and accumulation of WGA in wheat seedling roots. With the use of fluridone, an effective inhibitor of ABA biosynthesis, we have investigated the role of endogenous ABA in the regulation of quantitative level of WGA by EB and BAP in wheat seedlings in the normal and salt stress conditions. It was revealed that BAP-induced reversible accumulation of endogenous ABA is necessary link in the regulation by BAP of WGA level because fluridone prevented the BAP-induced WGA accumulation both in normal and stress conditions. At the same time fluridone did not affect the EB-stimulating effect on lectin accumulation in normal conditions and maintenance of WGA increased level in plants under salinity. These results serve as the proof of existence of ABA alternative pathways of hormonal regulation of concentration of WGA as component of nonspecific wheat resistance under both normal and adverse conditions and distinctions in the mechanisms of EB and BAP protective action on plants.

This work was supported by Grant RFFI 08-04-01563 and Grants MK-4081.2008.4 and NSh-915.2008.4.

P07-053: RNA&DNA CONTENT AFTER TEMPERATURE STRESSES IN PLANTS WITH DIFFERENT ECOLOGICAL STRATEGIES

Negretsky, V. - Kosakivska, I. - Demirevska, K.
Institute of Botany NAS Ukraine

We investigated the nature of nucleic acid content changes in response to heat (2h. +40oC) and cold (2h. +2oC) temperature stresses in six plants following different ecological strategies. RNA and DNA were extracted from frozen leaf material using TRIZOL LS (Sigma, USA).
The concentration of nucleic acids was measured using a Nanodrop spectrophotometer and integrity – by agarose gel electrophoresis (DNA) and capillary electrophoresis
(RNA). RNA quality, integrity and concentration were determined using electrophoresis on an Agilent 2100 bioanalyzer with Nanochips. DNA quantification was performed on a Nanodrop spectrophotometer, and with 1% agarose electrophoresis with the SYBR. The results revealed both specific and non-specific properties of DNA and RNA content changes following temperature stresses. The competitor Festuca pratensis L. possessed the most stable measured levels of nucleic acids. DNA and RNA reaction was sufficiently pronounced in the ruderal Brassica napus L., which grows in low-competitiveness environments and is sensitive to biotic and abiotic stresses.
Quantitative changes in ruderals were markedly stress-induced. RNA and DNA content following a temperature stresses were differentiated between C3 and C4 plants. We discuss the role of nucleic acids in response to abiotic stresses and how DNA and RNA content in plants with different strategies are affected by exposure to temperature stresses.

P07-054: NTE2F OVEREXPRESSION PREVENTS G2/M CHECKPOINT ARREST NECESSARY FOR GENOTOXIC-INDUCED PCD IN TOBACCO CELL LINE BY-2

Smetana, O.[1] - Houlné, G.[2] - Opatrny, Z.[1] - Chaboute, M. E.[2]
[1]Charles University
[2](IBMP Strasbourg)

Various endogenous and exogenous stresses such as genotoxics induce DNA damage leading to differential gene expression, cell cycle arrest, and DNA repair or programmed cell death (PCD). The main actor responsible for sensing DNA damage induced by double strand breaks inducers (γ- and x-rays, bleomycin, zeocin) is the kinase ATM. Recently, it was reported that ATM is necessary for autophagic-type PCD induction in stem cells of Arabidopsis root meristem upon x-rays irradiation and zeocin treatment (Fulcher and Sablowski, 2009).
E2F transcriptional factors play an important role during cell cycle regulation, DNA repair response or cell differentiation. In mammals and flies, E2F1 overexpression leads to increased genomic instability and subsequent cell death via transcriptional activation of several PCD genes in p53-dependent or p53-independent manner. We have investigated the effect of bleomycin (BLM) on the cell cycle progression, viability, autophagy rate, and expression of PCD-related genes in tobacco cell line BY-2. Caffeine, a specific inhibitor af ATM, is able to inhibit BLM-induced cell death. Next, to reveal the role of E2F transcriptional factor in BLM-response we generated BY-2 cell line overexpressing the only one known tobacco NtE2F transcriptional activator. Upon genotoxics, NtE2F increases genomic instability and inhibits G2/M checkpoint activation leading to decreased cell death. Thus, we suggest an important role of G2/M checkpoint during BLM-induced PCD.This work was supported by grants MSM 21-2061157 and GAUK43-259157.

P07-055: ANALYSIS OF ABIOTIC STRESS RESPONSES USING PROTEOMIC APPROACHES

Hedtmann, C.[1] - Kaspar, S.[1]- Lippmann, R. [1] - Doell, S. [1] - Matros, A.[1]- Mock HP[1] - Witzel K[2]
[1]*Leibniz Institute of Plant Genetics and Crop Plant Research*
[2]Instituts für Pflanzenernährung und Bodenkunde der Universität Kiel

The presentation will focus on the application of proteomics performed on model and crop plants to elucidate mechanisms and traits related to abiotic stress tolerance. Although proteomics still is limited in comprehensiveness when compared with transcriptomics, it can provide valuable information not obtained by other "omics"-techniques. We apply 2-D gel based as well as LC-MS-based approaches to study the responses of plants towards abiotic stresses on the protein level. A major focus of recent work relates to the function of co-chaperones which are induced by various stresses. These co-chaperones with homology to the human HOP protein are represented as dimers and within larger protein complexes and are located both in the cytosol and the nucleus. Central for our further work is the use of the wide genetic diversity represented e.g. in the plant genebank of our institute. As an example, barley was evaluated for contrasting salt tolerance using mapping populations.

P07-056: ARSENATE SIGNALLING IN ARABIDOPSIS THALIANA

Leyva Tejada, A. - Castrillo Molina, G. - Pablo Catarecha, Z. - Eduardo Sánchez, B. - Leo Del Puerto, Y.
Centro Nacional de Biotecnología-CSIC
**Corresponding author e-mail: aleyva@cnb.csic.es*

In soils arsenic is primarily in the form of arsenate [As(V)] (Tamaki and Frankenberger, 1992, Brown et al 1999). Due to its chemical similarity to phosphate (Pi), As(V) is highly toxic to plants since it is readily incorporated through the high affinity phosphate transporter (McNair et al., 1992). This transport system is induced by Pi starvation and repressed in its presence. We have demonstrated that the genes induced by Pi are properly down regulated by As (V) (Catarecha et al 2007). In fact, some of these genes (Pi transporter PHT1;1 included), are repressed by As (V) more efficiently than by Pi. Analyzing the transcriptomic profile of the As(V) response in Arabidopsis thaliana, we have found that this behavior occur at least in 10 % of the Pi-inducible-genes. These data indicates that in the case of As(V) and Pi their signaling mechanisms shares common elements. In order to identify these and others different elements, we start working in the characterization of mutants alters in the PHT1;1 repression by As(V). In this regard, we have analyzed the kinetics of PHT1;1 repression using a transgenic line expressing the gen of luciferase drive by PHT1;1 promoter. This tool provide us the possibility to concluded that this promoter are rapidly repressed by As(V) and in the case of Pi this repression is retarded (30 min and 36 h respectively). In addition, the screening of an EMS mutagenized population expressing PHT1;1:LUC led us to the identification of mutants alters in the repression
by As (V). The mapping of these genes will allow us the characterization of key elements in the As (V)
perception.

P07-057: MITOCHONDRIAL PROTEASE, ATFTSH4, IS REQUIRED FOR ARABIDOPSIS GROWTH AND DEVELOPMENT UNDER CONTINUAL MODERATE HEAT STRESS.

Smakowska, E. - Kicia, M. - Gibala, M, - Janska, H.*
University of Wroclaw
**Corresponding author e-mail: elwira@ibmb.uni.wroc.pl*

We found, that a loss of mitochondrial AtFtsH4 protease significantly affect Arabidopsis growth and development under continual moderate heat stress (long days at 30oC). The ftsh4 mutant plants have shorter roots and stems, beside an emergence of true leaves but not cotyledons is delayed compared with wild-type plants. The true leaves are smaller and an important characteristic of the adult leaves is the asymmetric shape and irregular serration of leaf blades. Moreover, the inflorescence development of the ftsh4 plants growing at 30oC is arrested and, as a consequence, ftsh4 plants are unable to produce seeds.
Although the growth and development of the ftsh4 mutants is highly susceptible to continual moderate heat stress, the mutants are not defective in acquired thermotolerance based on physiological experiments. In agreement with this result, the transcript level of AtHsp 101, which is a cytosolic heat shock protein required for acclimation to high temperature, is similar in the wild-type and ftsh4 when plants are exposed to both a short severe heat stress (1h, 38oC) or continual moderate heat stress (1-4 weeks, 30oC). We also established that the mitochondrial AtFtsH4 protease is not a typical heat shock protein like Hsp101, because we haven't found an increased accumulation of the AtFtsH4 transcripts upon a short time of high temperature stress. These results suggest that the thermosensitivity observed in the ftsh4 mutants is not caused by a defect in induction of cytosolic Hsp101 expression.

P07-058: POLY ADP-RIBOSE POLYMERASE INVOLVEMENT IN PROGRAMMED CELL DEATH OF TOBACCO BY-2 CELLS

Locato, V.[1] - Cimini, S.[2] - Novo Uzal, E.[2] - de Pinto, M.C.[3] - Foyer, C.H.[4] - De Gara, L.[1]
[1]*CIR-University Campus Bio-Medico di Roma*
[2]*Departamento de Biología Animal, Vegetal y Ecología, Universidad de A Coruña*
[3]*Dipartimento di Biologia e Patologia vegetale, Università di Bari*
[4]*Centre of Plant Science, Leeds University, UK*

The term "programmed cell death" (PCD) refers to an apoptotic-like cell death that occurs during plant development and/or as consequence of various injuries. Plant PCD has many common features with animal apoptosis, but it also has some unique features related to the morphological and functional differences between the plant and animal systems. Like apoptosis, plant PCD initiation generally requires an accumulation of reactive oxygen species (ROS). Enhanced ROS production or depletion of antioxidants leads to oxidative stress, which can cause damage to cell structure and DNA. Moreover, ROS can act as molecular signals that regulate the progression of PCD. Animal and plant cells have defence and repair systems that counteract cell injury and DNA breakage. Poly-ADP ribosylation is a major process involved in DNA repair during oxidative stress and apoptosis that has been intensively studied in animal systems. However, little information is available on this process in plant cells. Here we report a study in which tobacco BY-2 cells were subjected oxidative stress-triggered PCD. Our results showed that poly ADP-ribose polymerase (PARP) activity was increased rapidly as consequence of oxidative stress. However, within hours of PCD activation PARP activity was decreased. PARP uses NAD+ to drive poly-ADP ribosylation. Thus, we suggest that regulated activation and deactivation of PARP might avoid energy depletion during PCD in order to sustain the energy-demanding death process.

P07-059: LOOKING FOR CADMIUM TOLERANT MUTANTS

Agorio, A.[1] - Dardennes, J.S.[2] - Thomine, S.[1]
[1]*Institut des Sciences du Végétal (CNRS)*
[2]*Agroparistech*

Exposure to cadmium (Cd) has adverse health effects. Human uptake of Cd occurs mainly through the food-chain and tobacco smoke, as a consequence of heavy metal accumulation by crop

plants grown in contaminated soils. Phytoremediation strategies and control of Cd accumulation in crop may contribute to prevent Cd intoxication, although require a deep understanding of plant heavy metal tolerance and accumulation. The NRAMP was identified as one of the gene families highly expressed in metal hyperaccumulating plants. These genes play important roles in metal ion homeostasis. The tonoplast transporters AtNRAMP3 and AtNRAM4 function in the mobilization of iron and manganese from the vacuole. AtNRAM3 and AtNRAMP4 proteins are also able to transport Cd (Thomine et al., 2000, PNAS 97(9): 4991). The Arabidopsis nramp3nramp4 double mutant exhibit a strong hypersensitivity to Cd (Oomen et al., 2009, New Phytologist 181(3): 637).
To bring insights about the genetic determinants of Cd targets in plants, we have performed a screen for suppressors of nramp3nramp4 Cd-hypersensitive phenotype. As a result of this screen we have identified a number of nramp3nramp4 Cd-hypersensitive suppressors (nns). Here we present some characteristics of these nns mutants: (i) the nns mutants partially suppress nramp3nramp4 Cd-hypersensitivity (ii) not all the nns mutants suppress other nramp3nramp4 phenotypes (allowing us to classify the nns mutants).

P07-060: QUANTITATIVE PROTEOMICS OF NITROSYLATED ARABIDOPSIS PROTEINS UNDER SALT STRESS

Fares, A. - Falk, C. - Chay, S. - Rossignol, M. - Peltier, J.B.
INRA

Nitric oxide (NO) is a key player in signal transduction pathways leading to activation of plant defense against biotic or abiotic stress. Moreover, nitric oxide has been shown in many cases to be one of the earliest event occuring after a stress and preceding other post-translational modifications as protein phosphorylation. Here, we investigated the effect of a salt stress on protein nitrosylation in Arabidopsis culture cells. Thank to a modified Biotin Switch method we were able to identify and quantify nitrosothiol modified peptides using nanoLC-ESI-MS/MS mass spectrometry. During the time course of a 30 minutes salt stress barely hundred proteins went to hypo- or hyper-nitrosylated forms highlighting the dynamics of nitric oxide response in the early event. Direct link of different candidates with oxidative stress is also discussed.

P07-061: A COMPARATIVE STUDY OF ANTIOXIDANT ACTIVITIES BETWEEN DICHLOBENIL-HABITUATED CELL CALLUS OF DICOTILEDONEOUS (PHASEOLUS VULGARIS L.) AND MONOCOTILEDONEOUS (ZEA MAYS).

Largo Gosens, A. - Melida, H. - de Castro, M. - García-Angulo, P. - Alonso-Simón, A. - Acebes Jose, L. - Encina Antonio, E. - Álvarez Jesús, M.
Área de Fisiología Vegetal, Dpto de Ingienería y Ciencias Agrarias, 24071 Universidad de León

We have obtained bean [1] and maize [2] callus-cultured cells habituated to lethal concentrations of dichlobenil, a cellulose biosynthesis inhibitor. Together with changes on growth pattern and morphology, dichlobenil-habituated cells showed altered cell walls in which the lacking in cellulose was compensated by means of a modified network of matrix polysaccharides (i.e. esterified pectins in bean and feruloyl-arabinoxylans in maize) both at the compositional and structural level.
An alternative mechanism to tolerate dichlobenil would be the control of a putative oxidative damage caused by this compound. In order to know whether or not dichlobenil habituation relies on a high antioxidant capacity, several activities (i.e. ascorbate peroxidase, guaiacol peroxidase and glutathione reductase) have been tested in bean and maize dichlobenil-habituated cells.
In habituated bean cells, our results show that dichlobenil-habituation is linked to a constitutive increase in the antioxidant capacity where guaiacol peroxidase plays a major role. The enhanced guaiacol peroxidase activity is stable and this could explain why bean habituated cells cultured in a medium lacking dichlobenil for a long time (dehabituated cells) retain a high tolerance to this compound. However, in habituated maize cells our results show that there is not a significant increase in the antioxidant capacity. The mechanisms of habituation in maize cells seem to be due related to cell wall modifications.
We suggest that cells with type II (bean) and type I (maize) cell walls have different strategies to tolerate high concentrations of dichlobenil.
[1] Encina, AE. et al (2002). Physiol Plant. 114: 182-191.
[2] Mélida, H. et al (2008). Planta. 229: 617-631.

P08 Photosyntesis And Respiration

P08-001: THE EFFECT OF SOURCE AND SINK ACTIVITY ON CARBOHYDRATE METABOLISM IN CITRUS

Nebauer, S. - Renau-Morata, B. - Guardiola, J.L. - Molina, R.V.*
Universidad Politécnica de Valencia
**Corresponding author, e-mail: sergonne@bvg.upv.es*

The effect of the activity of source and sinks on carbon metabolism were studied in Salustiana sweet orange (Citrus sinensis L.). Changes in leaf carbohydrates and photosynthesis were provoked by source-sink imbalances after girdling. The experiments were performed in one year-old shoots bearing one fruit, by removing a ring of bark and remaining 10 to 50 leaves distal to the girdle. Fruit growth was positively correlated to leaf-to-fruit ratio, due to the increase in soluble sugar availability. The photosynthetic rate remained unchanged when the number of leaves per fruit increased in spite of the high levels of accumulated soluble sugars (21% of dry weight for 40 leaves per fruit). Between 25 and 40 leaves per fruit were enough to guarantee fruit growth. Only the lack of a strong sink activity leads to a decrease in photosynthetic rate. In these conditions, beside a high level of soluble sugars, starch strongly accumulated in leaves (17% of dry weight vs 9% in controls). However, a feedback inhibition can be discarded, since photosynthesis and stomatal conductance reduction occurred prior to any significant accumulation of carbohydrates. Gas exchange and fluorescence parameters suggested biochemical limitations to photosynthesis. In addition, the expression of carbon metabolism-related genes was altered within 24 hours when strong sinks were removed. Sucrose synthesis and export genes were inhibited, while the expression of ADP-glucose pyrophosphorilase was increased to cope with the excess of assimilates.

P08-002: CHLOROPLAST STRUCTURE AND THYLAKOID PROTEIN COMPOSITION IN PEA AND BEAN PLANTS GROWN UNDER DIFFERENT LIGHT INTENSITY

Mazur, R. - Rudowska, L. - Waleza, M. - Mostowska, A.*
University of Warsaw
**Corresponding author, e-mail: rmazur@biol.uw.edu.pl*

Garstka M (Department of Metabolic Regulation, Faculty of Biology, University of Warsaw) We demonstrated relationship between the entire chloroplast structure and thylakoid protein changes in pea (Pisum sativum) and bean (Phaseolus vulgaris) under low (LL) and high (HL) light conditions. We chose these two plant species because they have different chlorophyll-protein (CP) complexes organization within thylakoid membranes which determines distinct chloroplast structure. We found with the help of electron and confocal laser scanning microscopy that HL conditions induce starch grain accumulation which disturb thylakoid membrane network, both in pea and bean chloroplasts. Formation of large appressed regions and growth of grana diameter were observed in bean chloroplasts under LL conditions. On the contrary no such changes were noticed in pea. Western-blot analysis showed a decrease of PSII core protein D1 and an increase of major LHCII proteins levels in bean thylakoids. These observations might suggest different arrangement of bean CP complexes under low light intensity.

P08-003: PHOTOSYNTHESIS TOLERANCE TO DROUGHT, HEAT AND HIGH ILLUMINATION IN SUN AND SHADE PLANTS

Quiles, M. - Ibañez, H. - Muñoz, R.*
University of Murcia
**Corresponding author, e-mail: mjquiles@um.es*

Chrysanthemum morifolium (sun plant) and Spathiphyllum wallisii (shade plant) were used to study the effects of drought, heat and high illumination on the photosynthesis.
The stress conditions caused a greater accumulation of hydrogen peroxide in C. morifolium than in S. wallisii leaves, and resulted in down–regulation of linear electron transport in the leaves of both species and an increase in the non-photochemical quenching of fluorescence. Only a slight decrease in Fv/Fm was observed under stress conditions in either plant species, suggesting that the chloroplast is protected by mechanisms that dissipate excess excitation energy to prevent damage to photosynthetic apparatus. Changes were also observed in the plastidial NADH dehydrogenase complex and the PGR5 polypeptide.
The NADH dehydrogenase activity in the thylakoid membranes was similar in control plants of both species and increased in stressed plants, particularly in S. wallisii.
The level of PGR5 polypeptide was higher in C. morifolium than in S. wallisii control plants, while, after stress, the quantity of PGR5 increased significantly in C.morifolium and remained constant in S. wallisii.
The results indicate that the relative importance of chlororespiration and the cyclic electron pathways in the tolerance of photosynthesis to drought, heat and high illumination differs in sun and shade plants, indicating different adaptive mechanisms to the environment.
This work was supported by the Spanish MCyT (BFU2008-00331)

P08-004: THE SALICYLIC ACID AND NITRIC OXIDE INFLUENCE ON PHOTOSYNTHESIS AND RESPIRATION OF PLANTS EXPOSED TO ELEVATED CONCENTRATIONS OF HEAVY METALS

Rakhmankulova, Z. - Gilvanova, I.R. - Enikeev, A.R. - Stepanov S.Y.
Bashkir State University

The influence of salicylic acid (CK) and nitric oxide (NO) on photosynthesis (P) and respiration (R) of Triticum aestivum L., exposed to elevated concentrations of copper and zinc was investigated. Under the influence of toxic concentrations of heavy metals (HM) was seen a deterioration in the energy balance of the studied plants, that was expressed in the ratio of R to P (R/P) increasing, on copper in a 2 times and zinc in 1,9 times. CK or NO stabilized energy balance, by R/P reducing in average 1,2 times.
Identified that under high HM concentrations, less effective respiratory pathways, associated with alternative oxidize (AOX), were increased significantly. CK or NO treatment resulted in a reduction of alternative respiration in shoots and roots of the wheat. In the case of shoots of plants grown in excess zinc, the treatment of the CK or NO caused increasing of alternative respiration in 2 times.
In the presence of toxic concentrations of HM the level of malondialdehyde (MDA) increased, due to the occurrence of oxidative stress. CK or NO had reduced level of MDA, contributed improvement of antioxidant balance of plants and decreasing lipid peroxidation. Similarities in the protective reactions of the CK and NO in plants under influence of HM on the level of the main energy processes were revealed.

P08-005: HEAT DISSIPATION IN THE MOSS PHYSCOMITRELLA PATENS: EVOLUTION OF PROTECTION MECHANISMS UPON LAND COLONIZATION

Morosinotto, T. - Gerotto, C. - Alboresi, A. - Giacometti, G. - Bassi, R.
Università di Verona

Light is the source of energy for photosynthetic organisms but, when in excess, it also drives the formation of reactive oxygen species and consequently photoinhibition. Plants and algae, thus, have evolved mechanisms to regulate light harvesting efficiency in response to variable light intensities as to avoid oxidative damage.
Non Photochemical Quenching (NPQ) consists in the rapid dissipation of excess excitation energy as heat. Although widespread among oxygenic photosynthetic organisms, NPQ shows important differences in its machinery: in land plants, such as Arabidopsis thaliana, it depends on the presence of PsbS, while a different protein, called LHCSR (or Li818), is required in the green alga Chlamydomonas reinhardtii. The moss Physcomitrella patens is the only known organism where both proteins are present. By generating knock out mutants lacking PSBS and/or LHCSR we demonstrated that both gene products are active in P. patens NPQ. Plants lacking both proteins are also more susceptible to high light stress, implying that these gene products are indeed fundamental for photoprotection. Furthermore, generation of plants over-expressing either PsbS or LHCSR also showed that these proteins are active independently in triggering NPQ.
Taken together, these results suggest that NPQ is a fundamental mechanism for survival in excess light and that upon land colonization photosynthetic organisms evolved a new mechanism for excess energy dissipation before losing the ancestral one found in algae.

P08-006: FUNCTIONAL ANALYSIS OF ALB3 IN THE CPSRP-DEPENDENT LHCP TRANSPORT TO THE THYLAKOID MEMBRANE OF CHLOROPLAST

Schünemann D
Ruhr University Bochum, Molecular Biology of Plant Organells
**Corresponding author, e-mail: danja.schuenemann@rub.de*

Alb3 belongs to the recently identified YidC/Oxa1/Alb3 protein family that facilitates the insertion and assembly of membrane proteins in bacteria, mitochondria and chloroplasts. In chloroplasts, the signal recognition particle (cpSRP) and its receptor cpFtsY form a complex with the translocase Alb3 during the posttranslational insertion of members of the light-harvesting chlorophyll-binding proteins (LHCPs) into the thylakoid membrane. CpSRP consists of an evolutionarily conserved 54-kD subunit (cpSRP54) and a unique 43-kD subunit (cpSRP43) (1). In this study we showed an interaction between full-length Alb3 and cpSRP43 and analysed the binding interface. We used a combination of in vivo techniques as bimolecular fluorescence complementation using Arabidospsis protoplasts and the yeast split ubiquitin system and in vitro techniques using Alb3-proteoliposomes. In addition we present data to analyse the interplay of the SRP pathway components at the Alb3-translocase that lead to release of LHCP and recycling of the SRP components.(1) Schünemann D. (2007) Mechanisms of protein import into thylakoids of chloroplasts. Biol. Chem., 388: 907-915

P08-007: EFFECTS OF LIGHT INTENSITY ON PHOTOSYNTHESIS DURING ACCLIMATIZATION OF U. MINOR

Dias, M. - Pinto, G. - Correia, C. - Moutinho-Pereira, J. - Guerra, C.C. - Monteiro, C. - Santos, C.*
CESAM, University of Aveiro
**Corresponding author, e-mail: celeste.dias@ua.pt*

Ulmus spp. is among the Europe´s Noble Hardwoods. It is important as a source of high quality wood and is largely used for landscape and amenity purposes. However, elm population faces a drastic decline since the beginning of the 20th century due to the Dutch Elm Disease. Several efforts have been performed for preserving elm species and research dealing with in vitro propagation has been successfully conducted. Although acclimatization of micropropagated Ulmus minor was already achieved, no comprehensive study on the physiological responses has been done. The goal of this study is to optimize light acclimatization conditions for the maximization of plant performance. Hence, this contribution aims to reduce plant losses and to establish and optimize large scale breeding programmes. In our experiments, we transferred plants growing under in vitro conditions to ex vitro and acclimatized under low and high light condition. We analysed the effects of light intensity on plant performance through the measurement of photosynthesis, chlorophyll fluorescence, RuBisCO activity, pigments and carbohydrates in leaves under in vitro conditions and after 7, 14, 25 and 42 days of ex vitro transfer. Under in vitro conditions, U. minor leaves presented a positive photosynthetic response. The results obtained during the acclimatization indicate a different behavior of U. minor in response to different irradiance. Plants acclimatized under high light conditions have a more positive effect on plant performance than those under low light conditions.
Acknowledgements
This work was supported by Portuguese Foundation for Science and Technology (FCT) through a post-doctoral fellowship of M. C. Dias (SFRH/BPD/41700/2007) and a doctoral fellowship of C. Monteiro (SFRH/BD/48204/2008‏).

P08-008: DROUGHT ADAPTATION MECHANISMS: THE BENEFICIAL INTERACTION OF PHOTOSYNTHESIS AND RESPIRATION IN NICOTIANA SYLVESTRIS

Galle, A.[1] - Flexas, J.[1] - Florez-Sarasa, I.[1] - Thameur, A.[2] - De Paepe, R.[3]- Medrano, H.[1] - Ribas-Carbo, M.[1]*
[1]Universitat de les Illes Balears
[2]Laboratory of Dryland and Oasis Cropping, Institute of the Arid Regions of Medenine, 4119 Elfjé, Tunisia
[3]Laboratoire Mitochondries et Métabolisme, Institut de Biotechnologie des Plantes (IBP), UMR-CNRS 8618, Batiment 630, Université Paris Sud, F-91405 Orsay cedex, France
** Corressponding autor e-mail: alexander.galle@uib.es*

More frequent drought events will impair plant carbon balance and thus primarily affect photosynthesis and respiration. Due to their close interconnection, an improved plant adaptation to drought and survival may be derived from the beneficial interactions of photosynthetic and respiratory processes.
Nicotiana sylvestris plants were grown under different environmental conditions (indoors & outdoors), subjected to severe drought stress by withholding water and thereafter re-watered until photosynthesis recovered. In vivo photosynthetic and respiratory pathway activities, as well as related leaf compounds were determined. The response to drought and re-watering was similar under all growth conditions, while the rate of decline in net photosynthesis and respiration differed among them. Moreover, mitochondrial respiratory pathway activities were different in outdoors and indoors experiments, most likely to adjust ATP supply for maintained cell functioning. Additional data on mitochondrial complex I mutants (CMSII) support the essential role of respiratory ATP supply during drought-inhibited photosynthesis. Further implications on photosynthetic limitations and the rate of recovery are discussed.

P08-009: SALICYLIC ACID-INDUCED CHANGES IN PHOTOSYSTEM II REACTIONS IN BARLEY PLANTS

Losanka Popova Staevska
Institute of Plant Physiology, Bulgarian Academy of Sciences
**Corresponding author, e-mail: lpopoba@bio21.bas.bg*

In vivo effect of salicylic acid (SA) on PSII reaction activity

in barley (Hordeum vulgare L.) was assessed by monitoring of Hill reaction activity, kinetic behavior of oxygen evolving centers, thermoluminescence emission and polypeptide analysis of thylakoid membranes. Increasing concentrations of SA (0.1mM, 0.5 mM and 1mM), imposed through the root medium for a period of 7 days have a marked inhibitory effect on the number and the energetics of PSII alfa reaction centers that is consistent with some specific alterations in polypeptide composition of thylakoid membranes. On the other hand, when barley seedlings were supplied with SA through the transpiration stream for 24 h no marked changes in investigated parameters were observed. The results obtained are in support of the idea that SA, applied exogenously to the root medium, acts as moderate stressor having a direct effect on photosynthetic apparatus and on the PSII reactions in particular. A possible role of PSII β centers situated in stroma lamellae regions is discussed.

P08-010: PHYTOTOXICITY OF THE PLANT SECONDARY METABOLITE TRANS-CHALCONE CAN BE DETECTED BY IMAGING OF CHLOROPHYLL A FLUORESCENCE

Graña, E. - Sotelo, T. - Sánchez-Moreiras, A.M. - Reigosa, M.J.
University of Vigo
**Corresponding author, e-mail: eli-grana@uvigo.es*

Chalcone (1,3-diphenyl-2-propen-1-one) is an aromatic ketone, precursor of important molecules in plants, like flavonoids or anthocyanins. This compound has been found to show phytotoxic activity1, but no deeply studies were done to elucidate the mode of action of this compound on adult plants.
Therefore, we tested the phytotoxic effect of different chalcone concentrations by watering or spraying for 21 days on Arabidopsis thaliana plants. Plants were analyzed every day to obtain Y(II) (effective PS II quantum yield), Y(NO) (quantum yield of non-regulated energy dissipation), Y(NPQ) (quantum yield of regulated energy dissipation), Fv/Fm (maximal PS II quantum yield), ETR(electron transport rate), qN and qL coefficients (non-photochemical and photochemical quenching, respectively). We observed growth reduction and bolting rosettes in chalcone-watered plants. In addition to these morphological changes we found an increase in Y(NO), closely linked with a decrease in Y(II) and electron transport rate (ETR), while no effects were detected in Y(NPQ) and Fv/Fm. This could suggest a slowing down in Calvin cycle, induced by an energy diversion to reproduction in detriment of growth. The results previously found for chalcone-watered plants in Y(II), Y(NO), Y(NPQ) and ETR were also found in chalcone-sprayed plants. However, Fv/Fm showed a highly significant decrease after spraying, which could be suggesting a physical damage at the antenna complex and a concomitant effect on the photochemical phase of the photosynthetic process.
The previously discussed results confirm the phytotoxic activity of chalcone on adult plants and its potential use on weed management.
1: Chen WJ, Yun MS, Deng F, and Yogo Y. 2004. Weed Biol. Manage. Vol4, 235-238.

P08-011: DETECTION OF PHOTOSYNTHETICALLY-GENERATED REACTIVE OXYGEN SPECIES USING NOVEL SPIN-TRAPS: THE BENEFITS OF THE ENCAPSULATION OF SPIN-TRAP ADDUCTS BY CYCLODEXTRINS

Šnyrychová, I.[1] - Stolze, K.[2]*
[1]Palacky University Olomouc, Faculty of Science, Dept. of Experimental Physics, Laboratory of Biophysics
[2]Molecular Pharmacology and Toxicology Unit, Department of Biomedical Sciences, University of Veterinary Medicine Vienna
**Corresponding author, e-mail: snyrychi@prfnw.upol.cz*

Electron paramagnetic resonance (EPR) spin trapping spectroscopy is one of the best methods that can be used for the detection of oxygen radicals, however, its application in biological systems is limited by the instability of spin trap adducts. This instability is caused namely by the reduction of paramagnetic spin trap adducts to EPR silent species by the action of biological reductants. It has been suggested that this undesirable reduction can be partially avoided by the inclusion of the spin trap adducts into the cavity of cyclic oligosaccharides - cyclodextrins. A recent study with spin trap EMPO has already proven that the presence of cyclodextrins can considerably improve the sensitivity of superoxide detection in illuminated photosystem II particles [1]. In our current work we have tested several novel spin traps with respect to their ability to trap photosynthetically-generated oxygen radicals and we have evaluated the extent of the stabilization of their spin adducts by various cyclodextrins.
[1] Šnyrychová I. (2010) Free Radic Biol Med 48:264-274
Acknowledgement: I.Š. was supported by the grant MŠMT ČR MSM 6198959215.

P08-012: SPECIFIC SUPPRESSION OF THE CHLOROPLAST N-GLYCOSYLATED CARBONIC ANHYDRASE (CAH1) HAS MAJOR IMPACT ON THE PHOTOSYNTHETIC PERFORMANCE OF ARABIDOPSIS THALIANA

Ortega-Villasante, C.[1] - Burén, S.[2] - Blanco-Rivero, A.[1] - Samuelsson, G.[2] - Villarejo, A.[1]*
[1]Universidad Autónoma de Madrid
[2]Umeå Plant Science Centre
**Corresponding author, e-mail: cristina.ortega@uam.es*

A recently described alpha-type carbonic anhydrase (CAH1) has been localized in the Arabidopsis thaliana chloroplast after being N-glycosylated in the endomembrane system. Although its trafficking pathway to the chloroplast through the endoplasmic reticulum and the Golgi apparatus has been studied in some detail, the function of this protein remains unknown. Genomic analysis with bioinformatic tools annotated CAH1 to a cluster involved in chloroplast development and photosynthesis. To unravel eventual role of CAH1 in photosynthesis, we have used T-DNA knockout mutant lines of Arabidopsis in which the expression of the CAH1 gene was suppressed.
Mature plants of two mutant alleles exhibited reduced CO2 exchange rates as well as lower accumulation of starch, suggesting that CAH1 may play a crucial role in the photosynthetic performance of the plant. Plants from a mutant allele with stronger photosynthetic phenotype were also affected in growth and showed altered levels of soluble carbohydrates linked to chloroplast function. To complement the mutant phenotype, different epitope-tagged versions of CAH1 were expressed in the suppressed plants. Interestingly, N-terminally tagged CAH1 could fully restore wild-type levels of starch and photosynthesis, while expression of the C-terminally tagged protein had little or no complementary effect. Our results indicate that complementation was only achieved when the tagged CAH1 protein was correctly targeted to the chloroplast, and that tagging of the C-terminus inhibited this process. The data also suggest that CAH1 plays a pivotal role in the photosynthetic performance of the plant cell, despite the fact of being a very low abundant protein in the stroma.

P08-013: SUPRAMOLECULAR COMPLEXES AND CALVIN CYCLE REGULATION: THE INITIAL STEP OF GAPDH/CP12/PRK COMPLEX FORMATION COMES TO LIGHT

Sparla, F.[1] - Thumiger, A.[1] - Fermani, S.[1] - Trivelli, X.[2] - Marri, L.[1] - Pupillo, P.[1] - Falini, G.[1] - Trost, P.[1]
[1]University of Bologna
[2]University of Lille

In oxygenic photosynthetic organisms, the activities of two Cal-

vin cycle enzymes (glyceraldehyde-3-phosphate dehydrogenase, GAPDH, and phosphoribulokinase, PRK) are regulated by CP12-mediated complex formation. Free CP12 is an intrinsically disordered protein with limited propensity to fold into an ordered and stable structure, even if contains two internal disulfide bonds under oxidizing conditions typical of darkned chloroplasts. NMR analysis identified a stable α-helix (Pro59-Asp66) at the C-terminal end of free oxidized CP12. CP12 soaking on pre-formed GAPDH crystals and co-crystallization methods were adopted in order to study the structure of CP12 and the interaction between the two proteins. High resolution structure of crystallized binary complexes demonstrated that two CP12 molecules could bind one tetramer of GAPDH in two deep clefts. The C-terminal fragment of CP12 embedded in each cleft includes α-Helix-C (Pro59-Asp66) followed by loop-C (Asn67-Glu72) and a disulfide, plus an extended C-terminal tail (Tail-C; Arg74-Asn78). The last four amino acids of Tail-C are involved in a great number short-distance interactions with GAPDH and occupy the catalytic site of the enzyme. We propose that thanks to its high flexibility, oxidized CP12 could easily reach its partner GAPDH. Initial recognition events could be driven by the α-Helix-C, already present in free oxidized CP12. Subsequently, the flexible Tail-C would slip into the catalytic site of GAPDH to be fixed into an ordered, extended conformation. The rest of CP12 would remain disordered, possibly an essential requisite for the successive interaction with PRK.

P08-014: ANALYSIS OF PHOTOSYNTHESIS RELATED GENES UNDER SALINITY IN DIVERSE RICE CULTIVARS

Chander, S. - Saibo, N. - Oliveira, M. M.
ITQB, University of New Lisbon

The fundamental mechanism of salt tolerance in plants is a complex phenomenon and remains to be completely understood. High salinity alters plant osmotic and ionic homeostasis, thus leading to reduction of net photosynthesis, one of the major factors limiting plant growth and productivity. Two major genes, Rubisco Activase (RCA) and Sedoheptulose-Bisphosphatase (SBPase), which encode key enzymes in the Calvin cycle, play a vital role in photosynthesis and their expression is modulated by salinity. In addition, over-expression in rice showed enhanced photosynthesis performance in transgenic plants under stress conditions. However, little is known about how salinity affects the expression of these genes in diverse rice cultivars showing differences in salt stress tolerance, which could help to understand the molecular mechanisms underlying salinity tolerance in this species.
In the present investigation, two-weeks-old rice seedlings, from four different rice cultivars (LC-93-4, FL478, IR29 and Nipponbare), were grown under control conditions and then exposed or not to salt (150mM NaCl) stress.
To study the expression pattern of the RCA and SBPase genes under salinity stress, control and treated seedlings were sampled at specific time points after treatment.
The expression analysis of these genes is under progress and will be discussed. Additionally, we are sequencing the promoters of these genes in diverse rice cultivars. Altogether, our results will provide insight into the transcriptional regulation of the photosynthetic response under salt stress conditions.
Key words: Photosynthesis, rice, salinity stress, RCA, SBP
Acknowledgement: We acknowledge Foundation for Science and Technology (FCT) for PDF grant (SFRH/BPD/64193/20099) to Subhash Chander.

P08-015: INTERCEPTION AND USE OF PHOTOSYNTHETICALLY ACTIVE RADIATION UNDER MEDITERRANEAN CONDITIONS IN WHEATS CARRYING THE DWARFING GENE RHT-B1B

Garcia del Moral, L.F.[1] - Isidro, J.[1] - Royo, C.[2] - Alvaro, F. [2] - Nuñez, V.[1]*
[1]Dpt. Fisiologia Vegetal, Facultad de Ciencias, Univesridad de Granada
[2]IRTA, LLeida
**Corresponding author, e-mail: lfgm@ugr.es*
To study the effect of dwarfing genes on canopy architecture and crop-biomass production under Mediterranean conditions, 5 fields experiments were conducted for 5 years in southern Spain. Twenty-four durum wheat cultivars were selected to represent the germplasm grown during the 20th century before and after the introgression of Rht-B1b dwarfing gene developed during the "Green Revolution". Fractional absorbed radiation (FRa), was determined at anthesis and maturity by measuring the Photosynthetic Active Radiation (PAR) aboveground and at ground level below the canopy by using a 1-m-long linear ceptometer. The extinction coefficient for light transmission (k) was calculated as the value of the slope of the regression of ln (1-FRa) on Leaf Area Index and Green Area Index at anthesis. Radiation-use efficiency at anthesis and maturity (RUE) was calculated as the ratio between total crop biomass and the sum of the fraction of the daily global radiation absorbed until these stages, respectively. FRa and k did not significantly differ between cultivars carrying Rht-B1b allele or not, while the crop growth rate (CGR), net assimilation rate (NAR) and leaf:grain ratio (G) during the grain-filling period registered higher values in the cultivars carrying the Rht-B1b allele. Whereas RUE before anthesis was greater in cultivars without the Rht-B1b allele, after anthesis it was significantly greater in the cultivars with the Rht-B1b allele, possibly due to their greater sink capacity, suggesting the existence of a photosynthetic feedback mechanism in cultivars with higher sink capacity. The chlorophyll content at anthesis measured on flag leaves in SPAD units appears to be a reliable predictor of k under Mediterranean conditions.

P08-016: CHANGES AT THE ENTRANCE OF THE RUBISCO CENTRAL SOLVENT CHANNEL INDUCE ALTERATIONS IN ENZYME STABILITY

Esquivel, M.G.1 - Nogueira, S.A.
Spreitzer, R J,[2]
[1]Centro de Botânica Aplicada a Agricultura CBAA, Technical University of Lisbon
[2]Department of Biochemistry, University of Nebraska

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is one of the most important photosynthetic enzymes because it is responsible for the fixation of CO2. The holoenzyme is composed of eight large subunits (55 kDa) and eight small subunits (15 kDa). The X-ray crystal structure has revealed that large subunits are arranged as a tetramer of dimers around a central solvent channel, thereby defining a four-fold symmetry axis. Small subunits are arranged as two tetramers at polar positions of the axis. Four isoleucine-58 (I58) residues, one from each of the four small subunits, define the narrowest point of the central solvent channel entrance in Chlamydomonas reinhardtti Rubisco.
To examine the role of the central solvent channel, a mutant was created by directed mutagenesis in which I58 was replaced by three tryptophan residues (I58WWW) to close the entrance of the central solvent channel.
We have been looking for structural alterations that are detected by changes in thermal inactivation in vitro and degradation of the enzyme in vivo under oxidative stress.
The results show that the purified mutant enzyme is inactivated at a lower temperature than the wild type control enzyme. However, Rubisco degradation under oxidative stress induced by hydrogen peroxide is delayed in the mutant cells compared with the control. These results show that the closure of the central solvent channel by the presence of three tryptophans has an influence on the thermostability of the purified enzyme and gives stability against oxidative stress in vivo.

P08-017: THE THYLAKOID FORMATION 1 PLASTID PROTEIN IN PARASITIC DODDER PLANTS

Ludmila Slovakova - Viktor Demko - Andrej Pavlovic
Department of Plant Physiology, Faculty of Natural Sciences, Comenius University in Bratislava

The Thylakod formation 1 (THF1) protein has been identified in various plant species. Based on a sequence similarity and different functions that were attributed to the protein initially, various names of the same protein exist: THF1, Psb29, chloroplast inositol phosphatase-like protein, ToxA-binding protein, SlALC1, etc. It was suggested previously that THF1 protein is involved in thylakoid membrane differentiation, sugar signaling and responses to fungal and bacterial infections. Even though the THF1 is highly conserved in many oxygenic phototrophs, its precise cell and tissue distribution as well as its biochemical functions in photosynthetic and non-photosynthetic plastids have not yet been elucidated.

In our experiments, we use parasitic plants belonging to the genus Cuscuta. Our aim is to elucidate possible role
of the THF1-mediated signaling in parasitic plant development. Here we show and discuss the THF1 experssion and localization in dodder cells in respect to the seedling development and plastid differentiation.

This research was supported by the grant VEGA 1/0040/09

P09 Natural Variation And Adaptation

P09-001: CADMIUM RESISTANCE IN CONTRASTING THLASPI ECOTYPES

Llugany, M. - Martín, S.R. - Poschenrieder, C. - Barcelo, J.*
Universitat Autónoma de Barcelona
**Corresponding author, e-mail: merce.llugany@uab.es*

Growth, total proteins, cadmium and nutrient accumulation, as well as the activity of several antioxidative enzymes, i.e. superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) were investigated under Cd stress in hydroponically growing plants of two contrasting Thlaspi arvense accessions: ecotype Aigues Vives (South France) from a commercial source (B&T seeds) and ecotype Jena (Centre-East Germany) from a nearby industrial area. Ecotype Agues Vives exhibited higher root growth and biomass production than ecotype Jena in control conditions but was severely affected by 50 µM Cd treatments. On the other Jena ecotype showed considerable Cd resistance expressed by an enhanced root elongation under Cd exposure. Ecotype Jena had lower tissue Cd concentration than Aigues Vives (the highest concentration of both was in roots) but accumulated more Zn, Fe, Cu, Mn, Ca, Mg and P in the shoots. Cadmium stimulated the synthesis of proteins in shoots of Aigues vives ecotype but strongly decreased the activities of antioxidant enzymes in comparison to Jena ecotype where the level of proteins and the SOD and APX activities remained unaffected or in the case of CAT was increased by Cd. The results suggest that Thlaspi arvense ecotype Jena is characterized by an efficient Cd exclusion mechanism that together with an undisturbed mineral nutrition and an increase in CAT activity confers Cd tolerance to the population. This is the first time that differences in Cd resistance between T. arvense populations are reported. This resistant accession could be a new tool to compare the contrasting mechanisms of heavy metal tolerance with its hyperaccumulating congener Thlaspi caerulescens.
Supported by: BFU200760332

P09-002: EFFECTS OF MOIST CHILLING AND GIBBERELLIC ACID ON DORMANCY AND GERMINATION IN TWO ENDEMIC THLASPI (BRASSICACEAE) SPECIES OCCURRING IN THE ULUDAğ MOUNTAIN, TURKEY

Kirmizi, S. - Arslan, H. - Güleryüz, G. - Sakar F. S.
Uludag University, Faculty Of Science And Arts, Department Of Biology
**Corresponding Author, E-Mail: Skirmizi@Uludag.Edu.Tr*

We investigated the germination requirements of two endemic plant species Thlaspi papillosum Boiss. & Huet and Thlaspi lilacinum Boiss. (Brassicaceae) from alpine site of Uludağ Mountain, Turkey. We studied the effects of moist chilling (+4°C) for 30 days, different doses of gibberellic acid (GA3; 250, 500 and 1000 mg/l) and combined hormone and moist chilling treatments under dark (20°C) and photoperiod (20/10ºC; 12/12 h, respectively) conditions. T. papillosum seeds were germinated more than 70 % per cent under both dark and photoperiod conditions with distilled water. T. lilacinum seeds were failed to germinate in moist chilling treatments with distilled water and non chilled controls under dark and photoperiod conditions. Maximum 23 % of the T. lilacinum seeds were germinated by GA3 application in dark, but it enhanced with moist chilling combined by GA3 to 63 %. The promotive effect of the moist chilling was not observed for photoperiod conditions. The lowest mean germination times (MGT) were also found for moist chilling and 1000 mg/lt GA3 combination under photoperiod conditions. T. lilacinum seeds probably exhibit non-deep physiological dormancy (PD) that can be broken by moist chilling and GA3 treatments.

Our results show that these two endemic Brassicaceae species have different germination requirements.

Key words: seed germination, Thlaspi papillosum, Thlaspi lilacinum, endemics, moist chilling, gibberellic acid

P09-003: USE OF DNA BARCODING GENES IN GENETIC ANALYSIS OF THE CISTUS HETEROPHYLLUS SUBSP.CARTHAGINENSIS UNIQUE POPULATION

Pawluczyk, M. - Vicente Colomer, M.J. - Weiss, J. - Egea-Cortines, M.
Universidad Politécnica de Cartagena
**Corresponding author, e-mail: marta.pawluczyk@upct.es*

Cistus heterophyllus subsp. carthaginensis is an endangered species from the Cistaceae family. This unique natural population is situated in Peña del Águila (Murcia). Within the population of pure Cistus heterophyllus there were found also individuals of putative hybrids C. heterophyllus x C. albidus also described as Cistus x clausonis.We used DNA barcoding as an initial approach to identify the genetic structure of the plant population compared to bona fide C. heterophyllus and C.albidus. We analyzed four genes: rbcL, matK, rpoB, rpoC1 and
an intergenic spacer: trn L-F in individuals of C. heterophyllus, C. albidus and C. x clausonis from Peña del Águila. The genes rbcL, matK and the intergenic spacer trnL-F did not show any polymorphisms in the analyzed individuals. In contrast we found two different alleles of the genes rpoB and rpoC1 in individuals of C. heterophyllus and C. x clausonis suggesting heteroplasmy in the Cistaceae. Our results show that rpoB and rpoC1 could be useful as markers to study the Cistaceae family evolution.

P09-004: THE INHIBITORS ACTIVITY OF HYDROLYTIC ENZYMES L. DECEMLINEATA IN THE POTATO LEAVES

Ibragimov, R.[1] - Mardanshin, I.[2] - Shevchenko, N.[1] - Shpirnaya, I.[1]*
[1]Bashkir State University
[2]Birsk Research Institute of Russian Agricultural Academy
**Corresponding author, e-mail: ibragimov_rinat@yahoo.com*

Digestive enzymes (hydrolases) are a major factor in aggressiveness and pathogenesis of insect herbivores. There are compounds in the tissues of plants which are able to inhibit the activity of hydrolases and to ensure, thereby, their resistance to parasite attacks.
We have measured the inhibitory effect of potato leaves' extracts of the varieties differ in the stability to the digestive enzymes activity of the gastrointestinal tract of Colorado potato beetle: cellulases, pectinases, proteases and lipases.
The dependence was detected between the varieties resistance to bacteriosis and the activity of cellulase inhibitors in the plant leaves. A direct dependence between the varieties stability to the attacks of the potato beetle and the level of activity of the lipase and proteinase inhibitors in potato leaves was determined. So,extracts of leaves of unstable varieties(Nevsky, Lugovskoy)

exhibit a low level of antilipase activity (not more 1 IE/g). Varieties with high resistance are characterized by high activity of lipase inhibitirs (up to 7.4 IE/g).
In our opinion, the inhibition of lipolytic activity leads to disturbance of lipid metabolism, and, accordingly, to maldevelopment of insects fat body. The supression of insects proteolytic enzyme activity by inhibitors from the tissues of resistant genotypes leads to a slowdown in intensity of protein metabolism that causes a decrease in functioning of the gastrointestinal tract and worsen the violations of fat assimilation.

P09-005: UNIVERSAL PRIMERS FOR AMPLIFYING AND SEQUENCING A WIDE RANGE OF BACTERIA AND PLASTID-BEARING ORGANISMS: APPLICABILITY IN SPECIES INVENTORYING AND PHYLOGENETIC ANALYSES

Del Campo, E. - Hoyo, A. - Royo, C. - Casano Mazza, L. - Barreno, E.*
University of Valencia
**Corresponding author, e-mail: Eva.Campo@Uah.Es Del*

Progress in biodiversity studies and phylogenetic investigations has been facilitated by the development of methods for identifying species based on short standardized DNA sequences, known as “DNA barcodes”. Currently, a number of sets of “universal primers” are available in the literature, specifically for animals and plants. However, the scarcity of universally applied molecular markers for both micro- and macro-algae has resulted in the development of multiple, independent and not easily comparable systems. The goal of this work is to increase the number of available molecular markers and to generate easily comparable systems for their future application in the two main fields of construction of sequence libraries for a broad range of plastid bearing organisms (especially for algae) and the study of the evolution of photosynthetic eukaryotes. This study will compensate for the limited sequences available for certain algae in public databases. To reach this goal we have designed a primer pair capable of amplifying a broad range of organisms: Bacteria, Chlorophytes, Chlorarachniophytes, Euglenoids, Heterokonts, Rhodophytes, Cryptophyta, Glaucocystophyceae and Streptophyta including plants. This primer pair can amplify a DNA fragment of the 23S rDNA with sufficient variability to identify species across a broad range of taxa and perform phylogenetic studies alongside other available markers.

P09-006: EFFECT OF NITROGEN DEFICIENCY ON GROWTH AND PRIMARY METABOLISM IN A LARGE SET OF ARABIDOPSIS ACCESSIONS

Antonio, C.[1] - Tshoep, H.[1] - Yves, G.[2] - Usadel, B.[1] - Ishihara, H.[1] - Fernie, A.[1] - Sulpice, R.[1] - Stitt, M[1]*
[1]Max-Planck-Institute of Molecular Plant Physiology
[2]INRA Bordeaux, University of Bordeaux
**Corresponding author, e-mail: antonio@mpimp-golm.mpg.de*

Nitrogen (N) is essential to plant growth. It is a basic nutrient found in healthy soils, and plants draw in nitrogen through their roots. In this study, a set of 98 genotypically-diverse Arabidopsis thaliana accessions was used to investigate the effect of N deficiency on growth and primary metabolism covering three different levels of function: structural components (protein and chlorophyll), metabolite levels (GC-MS), and rosette fresh weight as an indicator for biomass production and growth. Accessions were grown on low N soil in a 12h light/ 12h dark cycle. Control accessions were grown on standard soil conditions for Arabidopsis in a 12h light/ 12h dark cycle. Spearman rank correlation analysis was performed on the LSM values for all pairs of measured traits across the whole population. In the control set of accessions, biomass highly correlated with starch, whereas this correlation was lost in low N condition and new metabolites then correlated significantly with biomass. This result reveal a shift in metabolism in response to N deficiency which was further analysed by network analyses and predictive tools such as CCA or PLS analyses. In a next step, the best predictors were then used either individually or together for the detection of master genes potentially regulating growth and metabolism by the use of genome-wide association mapping using the 250K SNP data available via the HapMap project *(http://walnut.usc.edu/)*.

P09-007: NATURAL MODIFIERS OF HYBRID NECROSIS IN ARABIDOPSIS THALIANA

Rowan, B.[1] - Bomblies, K.[2] - Weigel, D.[1]*
[1]Max Planck Institute for Developmental Biology
[2]Harvard University
**Corresponding author, e-mail: beth.rowan@tuebingen.mpg.de*

The maintenance of allelic diversity at loci encoding components of the pathogen recognition system is expected to be evolutionarily favorable because it allows individuals within a species to recognize and defend against various pathogens. However, such diversity can also inadvertently lead to the formation of reproductive barriers, as was previously observed for an intraspecific crosses between the Uk-1 and Uk-3 accessions of Arabidopsis thaliana. The F1 hybrid progeny exhibited a constitutively active pathogen defense response that led to reproductive failure during growth at the ecologically relevant temperature of 16ºC. This incompatibility, termed hybrid necrosis, was due to an epistatic interaction between two loci associated with the pathogen defense response, suggesting that diversification of pathogen recognition loci could result in the restriction of gene flow between individuals within a species. Such a restriction could be reinforced or overcome, however, by the presence of alleles at additional loci that enhance or suppress hybrid necrosis. Our data indicate that the Dr-0, Er-0, Hl-0 and Is-0 accessions carry dominant suppressors of Uk-1/Uk-3 hybrid necrosis, while dominant enhancers are carried by the Bur-0 and Wc-1 accessions. Ongoing work towards identifying the causal loci using high-throughput DNA sequencing will provide insight into the evolutionary forces that lead to reproductive isolation and may uncover novel components of the plant defense response mechanism.

P09-008: REGENERATION OF THE MANGROVE FOREST AT THE PACIFIC COAST OF NICARAGUA AFTER TROPICAL STORM ALMA

Monreal, J.[1] - Dolmus, C.M.[2] - Gutierrez Chavarria, N. N.[2] - olina Mendoza, M. J.[2] - Loaisiga Zapata, M. I.[2] - Pastora de Velásquez, R. M.[2] - Dávila de Guerrero, M. I.[2] - Dolmus, J.R[2] - Esteban Terradillos, R.[3] - Fernández-Marín, B.[3] - García Plazaola, J. I.[3] - Morales Méndez, D.[3] - Jiménez Parrondo, M. S.[4] - Álvarez Morales, M. R.[1] - Gandullo, J.[1] - Rubio Casal, A. E.[1] - Echevarría, C.[1] - León Morillo, A.[1] - Jiménez Segovia, E. T[1] - García-Mauriño, S.[1] - Silva Maldonado, C. D.[2] -*
[1]Universidad de Sevilla
2Universidad Autónoma de Nicaragua-León
3Universidad del País Vasco
4Universidad de La Laguna
**Corresponding author, e-mail: monreal@us.es*

On May 2008, the tropical storm “ALMA” passed through the natural reserve “Isla de Juan Venado” at the Pacific coast of Nicaragua. About 70% of the mangrove forests were damaged by Alma, being the Rhizophora forests largely altered. Field trials were conducted in order to investigate environmental and antropic factors in mangrove establishment and early development, to identify the best approaches to regenerate the mangrove population. Five plots (20 x 20 m2) were established at the “Isla de Juan Venado”, near “Las Peñitas” (trials 1, 2, 3) and “Salinas Grandes” (trials 4, 5), on Agust 2008. Experimental variables were: area with dead forest debris (trial 1, 400 new R mangle propagules seeded); no intervention (trial 2, to evaluate natural regeneration of the forest), after removing vegetal debris (trial

3, 400 new propagules seeded), forest under high tidal influence (trial 4, 400 new propagules seeded), and an area where the original mangrove forest had been clear-cut (trial 5, 400 new propagules seeded). The survival and height of the new plants were evaluated once every two month.
The better results were shown in trials 5 and 3, were 100% of survival was reached, followed by trial 1. The maximum growth was measured in trial 3 (62.36 cm as a mean), trial 1 (47.7), and 5 (41.4). The main causes of seedling death were natural events (high tidal amplitude and current, crab herbivory) and antropic factors (sticks collection). The natural regeneration (trial 2) was scarce, these results highlighting the importance of reforestation, together with reduced antropic activity, to preserve the mangrove ecosystem.

P09-009: GENESIS OF GLANDULAR TRICHOMES AND BIOSYNTHESIS OF ESSENTIAL OILS IN LEAVES OF MINT: EFFECT OF TEMPERATURE AND LIGHT QUALITY

Kirichenko E[1] - Kurilov, D[2] - Olekhnovich, L.[1] - Tarakanov , I.[3] - Bidukova, G.[1] - Babosha, A.[1] - Enina, O.[1]- Smirnova I.[1] - Luu Dam, C.[4]*
[1]"N.V. Tsitsin" Main Botanical Garden of RAS
[2]"N.D. Zelinsky" Institut of Organic Chemistry of RAS
[3]K.A Timiryazev Agricultural Academy
[4]Vietnam National Museum of Nature
**Corresponding author, e-mail: evkir@list.ru*

The objective of this work was to elucidate the relationship between formation of glandular trichomes and biosynthesis of essential oils in plants related to genus Mentha L. Experiments were performed with varieties and wild forms, representing M.canadensis, M.longifolia, M. piperita and M. arvensis. Development of glandular trichomes was studied using scanning electron microscopy. Component composition of essential oils was analised by method of chromato-mass-spectrometry (Agilent Thechnologies). Identification of components was carred out using the library of mass-spectra NIST 05 and Kovach indices. We showed that secretory structures accumulating essential oils were genus-specific. The glandular apparatus was formed mainly during initiation of leaf primordia. In the growing leaf, the glandular trichomes were distributed irregularly along the surface of low and upper epidermis. We concluded that plant potential to produce essential oils was determined by the number of trichomes in a leaf, leaf number, duration of leaf ontogenesis.Temperature and light quality were shown to affect the genesis of glandular trichomes at the stage of initiation of leaf primordia. These factors changed the ratio between different types of secretory structures, their development and aging. Mature trichomes often collapsed and essential oils were volatilized. In total,we detected 78 individual components. This allowed us to determined the gradient of mentol content in the leaves of different storeis and the dynamics of major components in the stems of different years of vegetation as dependent on formation of glandular trichomes. Significance of longterm predetermination of morphogenetic phenomena in the life cycle of mint plant was appreciated.

P09-010: SECONDARY SUCCESSION OF THE ORIGINAL FLOODPLAIN FORESTS IN THE VÁH RIVER AREA (SLOVAKIA) RESULTED FROM HYDROLOGICAL REGIME CHANGES

Ábrahámová, A. - Kollár, J. - Šimonovic, V.*
Institute of Landscape Ecology, Slovak Academy of Sciences
**Corresponding author, e-mail: aniko.abrahamova@savba.sk*

In Slovakia, Váh river presents the most intensively utilized river. First of all, there was constructed a set of dams and hydropower plants resulting in essential changes in hydrological regime. In the article, we focus on its part between the cities of Sereď and Nové Mesto nad Váhom (western Slovakia). Decrease of underground water and flood frequency caused drying out of original floodplain forest vegetation (stands of Salici-Populetum association made of mainly hygrophilous species) and its successional change into the more xerophilous types, especially in the belt contacting the river (agradated area) made of gravel or sandy sediments. Such degrataged stage is provisionally named as Crataegus monogyna-Populus nigra community. Its floristic composition is typical by mixture of ecologically different species. The tree layer is usually poorly covered and made of Populus nigra, while shrub layer is often well-covered and made of Prunus spinosa, Crataegus monogyna etc. The herb layer is formed by combination of mainly meadow species (Galium mollugo agg., Poa angustifolia, Arrhenatherum elatius, Tithymalus cyparissias), ruderal species (Echinops sphaerocephalus, Elytrigia repens, Convolvulus arvense) and partly also species typical for floodplain forests (Rubus caesius, Urtica dioica, Galium aparine).
Key words: floodplain forest, secondary succession, Crataegus monogyna-Populus nigra community, Váh river, hydrological regime

P09-011: POSSIBLE CONTRIBUTION OF MITOTIC GENE CONVERSION IN THE CONSERVATION OF MATERNAL HAPLOTYPE OF PEROXIDASE GENES

Kupriyanova, E. - Ezhova, T.A.*
Department of Genetics, The M.V. Lomonosov Moscow State University
**Corresponding author, e-mail:* ekupriyanova@gmail.com

The distribution of variation in a genome is the result of an intricate interplay between mutation, recombination, selection, and demography and is influenced by the reproductive system and ecological constraints. Important observations have emerged from the analyses of several Arabidopsis loci that have been subjected to comparative sequencing in this cruciferous weed: (i) a number of genes have alleles that fall into two distinct classes of haplotypes, and (ii) there is more recombination than might be expected, given that Arabidopsis is a selfer. Molecular-genetic mapping of the area of localization of tandemly duplicated anionic peroxidase genes AtPrx53 and AtPrx54 was performed. It was stated that this area is the recombination hotspot where recombination frequency was 480 times higher than the V chromosome average. The cloning and DNA sequencing of recombinant chromosomes has shown that the alleles of peroxidase genes posess mosaic structure. This indicates that abnormal high frequency of recombination was the result of conversion mechanisms. The analysis of codominant DNA marker`s segregation demonstrated that conversion events lead to the elimination of heterozygosity in hybrid plants; a proportion of plants homozygous for the maternal alleles of AtPrx53 and AtPrx54 was increased. Thus, unlike other recombination hotspot, which generates polymorphism by creating new recombinant alleles, we identified conversion hotspot, which eliminate heterozygosity and restore the maternal haplotype. It can be suggest that mitotic gene conversion can be used by plants to preserve of maternal haplotypes which possess higher adaptive value in specific environmental conditions.
The study was supported by grants from RFBR program (09-04-01639).

P09-012: FLAVONOIDS IN SOME EUPHORBIA SPECIES OF SUBSECTION ESULAE

Kaveh, M.[1] - Noori, M.[2]
[1]Department of Biology, Azad University-Borujerd Unit-Ira, e-mailm: e.kaveh@gmail.com
[2]DepartmeonftBiology, Faculty of Science, University of Arak, Arak-Iran, e-mail:m-noori@araku.ac.ir

Flavonoids occur widely in plants and are a biologically major and chemically diverse group of secondary metabolites. They are also beneficial for the plant itself as physiological active compounds, as stress protecting agents, as attractants or as feeding

deterrents, and in general, by their significant role in plant resistance. Flavonoid characters of 11 collected Euphorbia species of subsection Esulae of Iran were studied. Aqueous-ethanolic extracts of fruit were examined to practice flavonoid detection, isolation and identification by 2-Dimentional Paper Chromatography (2-DPC), Thin Layer Chromatography (TLC) and available reference. Vouchers specimen of each sample was prepared for as reference for herbarium vouchers. Results showed all studied Euphorbia species contained flavonoid compounds in their fruit that their flavonoid profiles show a wide variety between the taxa. All of studied species contain flavone sulphate and flavone C-and C-/O-glucosides. Also all studied taxa have kaempferol. Our studies showed all collected Euphorbia populations are weed and grow in poor soils and destroyed pasture. Progress continues to be made in understanding the roles of flavonoids in stress protection, as well as in defining the mechanisms that control the amount and varieties of flavonoids that are produced in plants in response to diverse environmental use.
Key word: Euphorbia, Section Tithymalous, Flavonoid, stress protection

P09-013: STUDYING NATURAL FLOWERING TIME VARIATION IN ARABIDOPSIS LYRATA

Kemi, U. - Kuittinen, H. - Savolainen, O.*
Department of Biology, University of Oulu
**Corresponding author, e-mail: ulla.kemi@oulu.fi*

It is important for plants to synchronize flowering with the favorable seasons. Many genetic pathways mediate and combine signals adjusting flowering time: in Arabidopsis thaliana the photoperiod, vernalization and autonomous pathways are well known. Which pathways and loci affect flowering time differences between locally adapted populations is an interesting evolutionary question. Here we study flowering differences between four A. lyrata populations from different latitudes and examine the expression of candidate genes for flowering time variation. Traits related to flowering were studied in three photoperiods after vernalization. Different populations responded differentially to the studied photoperiods. Southern populations were able to flower in the shortest photoperiod, where as the longest photoperiod was long enough to induce flowering in all studied populations. However, the shortest photoperiod induced flower bud formation in all populations indicating that the plants responded to the vernalization treatment, but the photoperiod was too short for flowering. As variation in gene expression can contribute to local adaptation, expression data of flowering time pathway genes will be compared with the phenotype data on flowering.

P09-014: COMPLETE CHLOROPLAST GENOME FROM STRAWBERRY TREE (ARBUTUS UNEDO) COMPARED TO OTHER ASTERIDAE

Martínez-Alberola, F. - Lazaro-Gimeno, D. - Mezquita-Claramonte, S. - del Campo, E. - Casano, L. - Mateu, I. - Pedrola-Monfort, J. - Barreno, E.
Universitat De Valencia

Since the first report of a complete chloroplast genome (cpDNA) in 1984, only 86 complete cpDNA sequences from vascular plants are currently available. Moreover, nowadays there is not any complete cpDNA sequence from the order Ericales whitin the Asteridae clade. In this work we have sequenced (Prometeo 174/2008 and CGL2009-13429-C0200) the complete cpDNA of a wild population of Arbutus unedo L. from Montes de Toledo (western Spain). A. unedo is a shrub mainly distributed around the Mediterranean Basin. ITS phylogeny indicates a probable polyphyletic origin of the genus, showing a typical Madrean-Tethian range. After applying ultra-deep pyrosequencing technology (Roche 454) we obtained a total of 165.575 reads which were ensambled with bioinformatic tools (MIRA and Newbler). Results indicate that the sequenced cpDNA has a size of roughly 165.000 bp and is organized as in most of higher plants. It is divided in two single copy regions, the large single copy region (LSC) and the small single copy region (SSC), which are separated by two inverted repeats (IRa and IRb). Gene annotation and comparisons with the cpDNAs of several Asterids revealed that in spite of genes generally occurred in the same order; there are changes in gene position within the LSC region. Further analyses will be necessary in order to annotate the occurrence of post-transcriptional modifications such as intron splicing and editing.

P09-015: ABOVEGROUND BIOMASS OF A NORTH AFRICAN PASTURE SPECIES (CENCHRUS CILIARIS L.)

Mnif Fakhfakh L
Faculty of Sciences Sfax

In arid zone of Tunisia, over grazing pressure and unfavourable climatic conditions have influenced the degradation of the mains pastoral ecosystems of the country. Species selection is crucial to unsure the success of such initiatives. Buffel grass (Cenchrus ciliaris L.) is an African grass that has been widely introduced in subtropical arid regions of the world to improve rangelands for cattle production. Aboveground phytomass production within four Cenchrus ciliaris North African accessions (P1: Bou Hedma, P2: Tozeur, P3: Raas Jedir, P4: Sidi Toui) was observed after cultivation under uniform ecological conditions. Once established, all accessions have two reproductive periods: autumn and spring. Spike production peaks are in late spring and autumn. Spike production was highly variable between accessions during the growing season 2002/03.
Results obtained indicate clearly that accessions of this species exhibit a significant degree of variation with regard to aboveground biomass. Results showed differences in rain use efficiency (RUE) between accessions which appear to be, related to their productive performance. The P1 and P4 accessions originated from more arid habitats exhibited higher RUE as compared the P2 and P3 accessions indicating that the accessions are adapted to different rain use strategies. This variability between accessions is significantly determined by rainfall distribution. The differences in RUE and biomass production imply also that genetic differentiation in terms of drought tolerance exists between the accessions. Finally Cenchrus ciliaris shows a high intra-specific variability. Accession P1 coming from Bou Hedma was the accession of highest biomass and highest RUE in this study.

P09-016: BIOCHEMICAL EVENTS DURING LEAF SENESCENCE OF PHENOLOGICAL FORMS OF EUROPEAN BEECH

*Kraj, W.**
University of Agriculture in Cracow
**Corresponding author, e-mail: rlkraj@interia.pl*

The subject of study was biochemical changes in senescing leaves of early, intermediate and late phenological forms of European beech. Changes in photosynthetic pigments, soluble and insoluble proteins, aminoacids and membrane permeability were determined. Autumnal senescence of phenological forms started from September the 14th to the 24th and it did not depend on temperature changes, which suggest that the senescence process was initiated by photoperiod. The highest level of proteins in leaves of European beech forms was observed at the beginning of July, ie after their full development. Protein degradation linked to senescence process started on September the 10th. First period of the decrease of soluble proteins (until October the 10th) was quick, while the second period (which lasted until the end of senescence process) was slow. Degradation of insoluble proteins and increasing of cell membrane permeability were observed together. The insoluble proteins were degraded in two phases. The first one lasted from September the 14th to October the 1st, while the second one lasted from October the 15th until the end of senescence process. High level of remobilization of proteins

in late phase of senescence caused the increase of aminoacids content, which was the result of cessation of phloem transport. Phenological forms of European beech differed in initial data of senescence of the leaves as well as different periods of senescence phases. They also had different ability of protein compound remobilization. Early form of beech tree remobilized 74% of total proteins, while intermediate 73% and late form remobilized 68% of all proteins. The average level of remobilization of phenological forms of beech tree was 80% of soluble proteins and 64% of insoluble proteins.

P09-017: GENETIC DIVERSITY OF POLISH ISOLATES OF HYMENOSCYPHUS, THE TELEOMORPH OF CHALARA FRAXINEA, THE CAUSAL AGENT OF ASH DIEBACK

Zarek, M. - Kraj, W.*
University of Agriculture in Krakow
**Corresponding author, e-mail: emzarek@gmail.com*

Random Amplified Microsatellites (RAMS) markers were used to investigate the genetic structure and variation of Hymenoscyphus, the teleomorph of Chalara fraxinea, which causes dieback of Fraxinus excelsior in Europe. Ninety five isolates, obtained from ascospores, which represent six populations from lowland and upland parts of Poland were analyzed. 72 (89%) out of 81 bands generated with four RAMS primers were polymorphic. The lowland and upland groups of isolates were obtained by using PCA analysis.
Percentage of polymorphic loci was higher for upland (87.7) than for lowland (81.5) isolates. The genotypic diversity inferred from Shannon's index was higher for upland (0.422±0.028) than for lowland (0.390±0.028) isolates. Dice a similarity coefficient, which was the second measure of intrapopulation variation, also showed higher genetic differentiation of upland (0.74±0.002) than lowland (0.78±0.003) isolates. AMOVA partitioned the total variation into 77% intrapopulation,
19% between-population and 4% between upland and lowland isolates. This analysis and Nei genetic distance between pairs of populations showed that differentiation among populations was high and depended on population elevations. It appeared that the main factor which influences the genetic variation level is climatic conditions. As a result of greater differentiation of climatic conditions in upland region, the genetic variability of fungus was greater, which allows better toleration of varied external conditions.

P09-018: GENETICAL, MOLECULAR AND ECOLOGICAL ANALYSES OF FLOWERING VERNALIZATION RESPONSES IN ARABIDOPSIS THALIANA

Méndez-Vigo, B. - Ramiro, M. - Pozas, J. - Sánchez, E. - Martínez-Zapater, J.M. - Picó, X. - Alonso-Blanco, C.
Centro Nacional de Biotecnología (CSIC)
**Corresponding author, e-mail: bmendez@cnb.csic.es*

Wild genotypes of Arabidopsis thaliana collected from different natural populations show substantial variation for the acceleration of flowering initiation induced by long exposure to low temperatures, i.e. for their vernalization response (Alonso-Blanco et al., 2009). To determine the amount of quantitative variation existing for this response we have analysed flowering time in a collection of 183 genotypes from different populations of the Iberian Peninsula (Picó et al., 2008), grown with 0, 1, 2 or 3 months at 4 °C. This analysis shows that 17% of Iberian accessions have an obligate vernalization requirement, while several genotypes without such requirement present a stronger response than laboratory strains. We have selected Ll-0 and Ped-0 accessions with different extreme vernalization responses to obtain two new populations of recombinant inbred lines (RILs) derived from crosses with the reference strain Landsberg erecta (Ler).
To determine the genetic bases of natural variation for vernalization response we have measured the flowering time of these two RIL populations grown under different vernalization periods and we have carried out QTL mapping analyses of those data. On the other hand, to find out part of the molecular bases of this variation we have sequenced the FRIGIDA gene of the 183 accessions and we have carried out association analyses between FRI polymorphism and the flowering phenotypes of this collection. Finally, aiming to identify environmental factors that might drive FRI genetic variation we have compared FRI polymorphisms with geographic and climatic factors of the natural populations of origin. References
Alonso-Blanco et al. 2009 Plant Cell 21:1877-1896
Picó et al. 2008 Genetics 180:1009-1021.

P09-019: ASSOCIATION MAPPING: EXPLORING ALLELIC RESPONSES FOR COMPLEX TRAITS

Ishihara, H. - Sulpice, R. - Pyl, E-T. - Stitt, M.*
Max-Planck-Institute of Molecular Plant Physiology
**Corresponding author, e-mail: ishihara@mpimp-golm.mpg.de*

A. thaliana is a suitable model for QTL mapping of a wide range of simple or even complex traits. However, this approach is limited by the genetic and phenotypic diversity of the two parents comprising mapping populations. Association mapping is overcome these limits as it makes use of the variation existing in a large number of natural populations. However, full genome association mapping requires a very good coverage of the genome in a large number of accessions for the detection of associations to complex traits. SNP data generated in the frame of the 1001 genome project and the Arabidopsis thaliana "HapMap" project allow such coverage, but not definite identification of the responsible polymorphisms for an observed association. In the laboratory, we developed a strategy to identify candidate genes involved in the regulation of biomass and then make association mapping using their full sequence (Sulpice et al. 2009). Briefly, by determining other traits (metabolites, enzymes and transcripts) and analysing their connections together and with biomass, we could point the potential importance of two candidate genes. The two genes were then fully sequenced by Sanger method in >90 accessions and several associations could indeed be identified with biomass but also to some metabolic traits. However, to validate such approach, other genes should be tested. One of the major issues in our previous study appeared to be the necessity of getting high quality sequences for a large number of accessions. After selection of 31 additional genes potentially involved in the regulation of biomass, we are now sequencing them by 454 barcode sequencing. Based on the associations obtained, the validity of this strategy will be discussed.

P09-020: SCREENING FOR NITROGEN USE EFFICIENCY (NUE) IN HUNGARIAN POTATO CULTIVARS

Hoffmann, B. - Hoffmann, S. - Polgár, Z.*
University of Pannonia
**Corresponding author, e-mail: hoff-b@georgikon.hu*

Crop production is highly dependent on the supply of exogenous nitrogen (N) fertilizers. With increased fertilizer application rate the risks of N loss increase rapidly. The remaining N is lost as either surface runoff; leached nitrate in groundwater or by volatilization to the atmosphere; microbial denitrification, all of which pose environmental concerns. Although nitrate losses may be reduced through improved N fertilizer management practices, nitrate losses are still excessive under commercial production regions. Another approach may be to reduce nitrate loss by developing potato cultivars which utilize N more efficiently. Nitrogen use efficiency (NUE) is defined as dry matter production per unit N supply. Because of the critical role of N rate in achieving economic and environmental objectives, screening for genotypes with better NUE may reduce production costs and contamination of the environment by maximizing fertilizer utilization.

In this study variation in nitrogen use efficiency (NUE: dry matter production per unit crop N supply) characteristics of commercial potato cultivars of Hungarian origin were evaluated. Cultivars were grown with
(50-, or 100 kg N ha–1) or without application of N fertilizer. The experiment was set up as a split block design with fertilizer rates as main plots and the cultivars as sub-plots.

P10 Signalling And Gene Expression

P10-001: CYTOKININ AND LIGHT: THE WAYS TO REGULATE DE-ETIOLATION OF BARLEY

Kravtsov, A. - Zubo, Y. - Kulaeva, O. - Kusnetsov, V.*
Timiryazev Institute of Plant Physiology, Russian Academy of Sciences.
**Corresponding author, e-mail: KravtsovAK@rambler.ru*

De-etiolation is a set of physiological, biochemical, and morphological changes undergone by a seedling in response to transition from the growth in the dark to the light, which result in the chlorophyll accumulation and initiation of photosynthesis. We report here on thecytokinin(CK) and light effects on expression of plastid genes in the course de-etiolation of monocot plants (Hordeum vulgare L.).The leading factor in regulation of greening is light. However its effect on a plant is presumably determined by endogeneous factors, the main of which are phytohormones. To investigate hormonal regulation of greening, plant development was studied both in the light and at a preceding growth stage in the dark. As the time course of plant growth in the dark increased, the expression of plastid genes gradually declined, as well as the ability of a plant to green, and after 12 days of dark treatment the irreversible etiolation occurred. CK in the concert with light reduced duration of barley transition to autotrophic type of nutrition, enhanced pigment accumulation and activated expression of the genes the products of which participated in chloroplast biogenesis. For more rapid rates of plant greening both light and CK were required . Hence, our data proves CK involvement in regulation of plastome gene expression while greening. Along with the light-dependent regulation of gene transcription we demonstrate(for the first time) light independent CK activation of transcription for a number of plastid genes in etiolated barley seedlings. Thus, this study reveals for the first time cytokinin involvement in the de-etiolation process of monocot plants.

P10-002: THE REGULATION OF THE GENE OF PRIMARY RESPONSE TO CYTOKININ IN ARABIDOPSIS BY BRASSINOSTEROIDS

Efimova, M.[1] - Kudryakova, N.[2] - Karnachuk, R.[1] - Khripach, V.[3] - Kuznetsov, Vl.[4]*
[1]Tomsk State University
[2]Institute of Plant Physiology RAS
[3]Institute of Bioorganic Chemistry NAS of Belarus
[4]Timiryazev Institute of Plant Physiology RAS
**Corresponding author, e-mail: stevia555@mail.ru*

Brassinosteroids (BRs) are steroidal plant hormones known to play an essential role in a wide spectrum of physiological processes. It is assumed that BRs are integrated in a complex signaling networks via a modulation of levels and sensitivity of other phytohormones, though the precise function of BRsin these interactions is poorly understood. In the present study plants of Arabidopsis thaliana (L.) Heynh transformed with the PARR5::GUS consruct were used to estimate the influence of several BRs (brassinolide (BL), epibrassinolide (EBL) and homobrassinolide (HBL) on the expression of the ARR5 gene which belongs to the type A negative regulators of plant response to cytokinin (CK). Exogenous application of BRs to plant seedlings as well as to detached mature leaves in the dark but not under white light induced elevation of the GUS activity comparable with the shift of PARR5::GUS expression in plants treated with CK (benzyladenine). The levels of GUS activity induction differed ranging from the highest for BLand the lowest for HBL treatment. The activation of cytokinin primary response gene by BRs in darkness could be induced either by the direct effect of these hormones on the ARR5 gene promoter or indirect action through conversion of bound forms of CKs to free forms. The results further suggest that interaction between CKs and BRs is regulated by light which might alter responsibility of cells to BRs.
We are grateful to Prof. J.J. Kieber (University of North Caroline, NC, USA) for the generous gift of transgenic Arabidopsis seeds. This research was supported by The Federal Agency for Education (State Contract no. P1369) and Russian Foundation for Basic Research.

P10-003: MOLECULAR CHARACTERIZATION OF A BIOTIC AND ABIOTIC STRESS RESISTANCE-RELATED GENE RELA/SPOT HOMOLOGUE(PEPRSH) FROM PEPPER

*Kim , T. * - Tae-Ho, K.*
National Academy of Agricultural Science, RDA
**Corresponding author, e-mail: thkim1961@korea.com*

A gene encoding a putative guanosine 50-diphosphate (or 50-triphosphate) 30-diphosphate ((p)ppGpp)synthetase, designated PepRSH (Pepper RelA/SpoT homologue), was isolated from hot peppers. A genomic DNA gel blot analysis revealed that the pepper genome has at least a single copy of PepRSH. PepRSH transcripts were highly accumulated in non-host resistance response-induced leaves and in leaves following induction with salicylic acid, methyl jasmonate, wounding, hydrogen peroxide, and ultraviolet-B. The expression of PepRSH was also influenced by abiotic stresses, such as flooding and high salinity. The deduced PepRSH protein has a putative chloroplast-targeting transit peptide at its Nterminus, and immunolocalization studies verified the translocation of PepRSH to the chloroplast. The predicted PepRSH protein is markedly similar to known plant and bacterial RSH proteins. Expression of a putative (p)ppGpp synthetase domain in an Escherichia coli single mutant (RelAˇSpoT+) complemented growth of the mutant but not of an E. coli double mutant (RelAˇSpoTˇ), demonstrating that PepRSH has (p)ppGpp synthetase activity only in the (p)ppGpp synthetase domain. Site-directed mutagenesis of the conserved histidine and aspartic acid (HD) site in the putative HD domain of PepRSH revealed that the histidine and aspartic acid dual sites were critical residues for the (p)ppGpp synthetase activity of PepRSH protein. Mutation of the HD site limited the tolerance of bacteria to both salt and osmotic stress. Our results indicate that pepper plants have a (p) ppGpp regulatory system that is similar to that of bacteria and which may transduce stress-related signals through the regulation of (p)ppGpp by PepRSH localized in chloroplasts.

P10-004: THE NOVEL METHANOL INDUCED GENES OF N. BENTHAMIANA

Pozdyshev D[1] - Shvarts, A. M.[2] - Komarova T. V.[3]*
[1]Lomonosov Moscow State University
[2]Vavilov Institute of General Genetics, Russian Academy of Science
[3]Belozersky Institute of Physico-Chemical Biology, Moscow State University
**Corresponding author, e-mail: denispoz@gmail.com*

It is widely accepted idea that small highly volatile organic compounds (VOCs) released by pathogen-attacked neighbors may activate defenses before being attacked themselves. The

methanol (MeOH) is generally produced in the process of pectin demethylation by ubiquitous enzyme pectin methylestherase (PME). Here we studied effects of PME-generated MeOH on transcriptional activity of N.benthamiana genes. Our comparative study showed that quantity of MeOH generated at leaf tissue and emitted into air were increased at PME transgenes and mechanically wounded plants. Then, using subtractive hybridization approach we identified several genes up-regulated after MeOH treatment (MeOH-induced genes, MIGs). It has been shown also that transcriptional activity of MIGs such as pathogenesis-related 1,3-β-glucanase, PME inhibitor and 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase genes were increased respectively 400-, 10, - and 8-fold in leaves of N.benthamiana stored 24 h in MeOH vapors (733.0 ppm) of plant chamber. Moreover, RNA transcripts of these MIGs were increased in N. benthamiana receiver plants which stored in vapors of wounded neighbor N. benthamiana emitter plants. We suggested that MeOH may take part in plant-to-plant signaling.

P10-005: STUDYING OF THE "CHITIN-SPECIFIC" DOMAIN OF PLANT PEROXIDASES

Kuzmina, O. - Postrigan B. - Maksimov, I.*
Institute of Biochemistry and Genetics Ufa Scientific Centre Russian Academy of Sciences
**Corresponding author, e-mail: olya.kuzmina.ibg@gmail.com*

Studying of wheat origin as the important agricultural crop has the essential scientific and practical value caused by problems of selection. A variety of wild species of wheat and aegilops together with intraspecific polymorphism allow to assume the presence of the significant amount of stress genes induced in response to many disease agents in them. Among variety of protective genes the specific place is occupied with the genes coding pathogen-induced peroxidase isoforms among which concern and «chitin-specific» forms. Probably they are important in the lignification of pathogen - damaged plant tissues. The purpose of research is the molecular and genetic organisation analysis of the chitin-specific site of the peroxidase gene in wheat and aegilops. Comparison of sequenced peroxidase gene fragments with known a soft wheat nucleotide sequence Triticum aestivum TC151917 has revealed the 90 % homology with Tr. fungicidum and T. petropavlovskyi and 84 % homology with T. araraticum. The obtained data can be considered from an evolutionary position. So, T. fungicidum and T. petropavlovskyi, also as well as T. aestivum relate to subgenus Urartu. The three species are Au and B genomes carriers. Whereas, T. araraticum, T. militinae and T. boeoticum relate to Boeoticum subgenus and are Ab and G genomes carriers. It is shown a «chitin-specific» peroxidase domain possessed ability to bind a chitin. We have created a gene-engineering design composed of the dahlia mosaic virus 35S promoter and the wheat anionic peroxidase cDNA. It will allow us to find out a plant peroxidase role in protective reactions mechanisms against pathogenes.

P10-006: COMPARATIVE ANALYSIS OF THE TRANSLATION EFFICIENCIES BETWEEN RPS2 AND RPS16 MRNAS IN TOBACCO CHLOROPLASTS.

Nakamura, M. - Sugiura, M.*
Nagoya City University
**Corresponding author, e-mail: mnak@nsc.nagoya-cu.ac.jp*

The 20 amino acids, except for methionine and tryptophan, are codoed for by 2 to 6 codons called synonymous codons. Synonymous codons are not used with equal frequency in protein coding sequences. Although it has been thought that the codon usage is correlated with the translation efficiency, we found that translation efficiencies of synonymous codon groups are not always correlated with their usages in tobacco chloroplasts. This finding suggests that the inefficient codons in translation are selectively used in the several chloroplast mRNAs. Thus, we analyzed the codon frequencies of individual chloroplast mRNAs, and found that rps2 mRNA contains many translationally efficient codons, whereas rps16 mRNA is rich in inefficient codons. Coding region of rps2 and rps16 mRNA is 236 and 85 amino acids, respectively. This raises the question of which is faster in translation, long peptide with efficient codons or short peptide including many inefficient codons. To address this question, we measured the translation efficiencies of rps2 and rps16 mRNAs using tobacco chloroplast in vitro translation system.

P10-007: TREHALOSE-6-PHOSPHATE AND SUCROSE SIGNALLING IN ARABIDOPSIS THALIANA

Umesh Prasad, Y. - Feil, R. - Stitt, M. - Lunn, J.*
Max Planck Institute of Molecular Plant Physiology
**Corresponding author, e-mail: yadav@mpimp-golm.mpg.de*

Trehalose-6-phosphate "(Tre6P)", the intermediate of trehalose biosynthesis, plays an essential role in the control of plant metabolism and growth, although its precise functions are uncertain. It has been proposed that Tre6P acts as a signalling metabolite that reflects the availability of sucrose, and thereby regulates the growth and metabolism of the plant. The aims of the work were to test the hypothesis that Tre6P is a specific signal of sucrose status in plants, and to elucidate the upstream signal transduction pathway linking Tre6P to changes in sucrose levels, using Arabidopsis thaliana seedlings grown in liquid culture as the experimental system. Resupply of sucrose to C-starved seedlings led to rapid and massive "(up to 70-fold)" increases in the level of Tre6P. Addition of glucose, fructose or maltose also led to a rise in Tre6P. However, these three sugars also increased sucrose levels in the seedlings, and in all experiments Tre6P showed a stronger correlation with sucrose than with glucose or fructose, irrespective of which sugar was supplied. These results suggested that the rise in Tre6P was linked to changes in the level of sucrose, rather than directly to the other sugars. Inhibition of transcription by cordycepin had little effect on the sucrose-induced rise in Tre6P. In contrast, inhibition of protein synthesis by cycloheximide essentially blocked the Tre6P response to sucrose. The Tre6P response to sucrose is enhanced by treatment of the seedlings with MG132, which inhibits protein turnover via the ubiquitin-26S proteasome pathway. Based on these observations, it is postulated that sucrose induces synthesis of a short-lived regulatory protein that either activates TPS to increase the rate of Tre6P synthesis, or inhibits the hydrolysis of Tre6P by TPP.

P10-008: INFLUENCE OF LIGHT ON ACCUMULATION OF PHOTOSYNTHESIS-RELATED PROTEINS IN LOW LIGHT AND DARK-GROWN PICEA ABIES AND LARIX DECIDUA CALLI CULTURES

Balážová, R. - Blehová, A. - Demko, V. - Breznenová, K. - Hudák, J.*
Department of Plant Physiology, Faculty Of Natural Sciences, Comenius University in Bratislava
**Corresponding author, e-mail: balazovar@gmail.com*

Chloroplast development and chlorophyll (Chl) synthesis is not exclusively dependent on light in gymnosperms. Various conifers species display differences in their ability of Chl accumulation. Dark-grown seedlings of Picea abies (L) Karst. accumulate the highest amounts of Chl and its precursor protochlorophyllide (Pchlide) in all Pinaceae, but calli derived from 14-day-old green cotyledons of P. abies are completely white during the cultivation in the dark. At the other extreme, dark-grown Larix decidua Mill. seedlings synthesize Chl only in the early developmental stages. Calli derived from 14-day-old etiolated cotyledons often necrotized during cultivation in the dark. It is generally suggested, that plastids of dark-grown calli cultures do not contain developed thylakoid system, which is a prerequisite for the assembly of photosynthetic apparatus. Pchlide reduction is a key regulatory step in Chl biosynthesis, catalysed in the dark, by light-indepen-

dent protochlorophyllide oxidoreductase (DPOR). This enzyme complex consists of three protein subunits ChlL, ChlN and ChlB, encoded by three plastid genes chlL, chlN and chlB. Using semiquantitative RT-PCR, we observed low expression of chlLNB genes in dark-grown calli. It seems, that chlLNB expression and thus Chl accumulation could be modulated by light in P. abies and L. decidua calli cultures. This hypothesis is supported by the fact, that we observed lower levels of GluTR and FLP, which probably affected Chl biosynthetic pathway at the step of ALA formation. ChlB subunit was not detected in dark-grown P. abies calli cultures. Our results and the fact, that cells of dark-grown calli contain only trace amounts of photosynthetic pigments, indicate limited ability to synthesize Chl during cultivation in the dark.

P10-009: LOOKING FOR ARABIDOPSIS THALIANA HOMOLOGUES TO THE ZINNIA ELEGANS BASIC PEROXIDASE

Cuello, J.[1] - Herrero, J.[2] - Gómez-ros, L.[1] - Esteban Carrasco, A.[2] - Zapata, J.M.[2] - Ros Barceló, A.[1]*
[1]Universidad De Murcia
[2]Department of Plant Biology, University of Alcalá de Henares
**Corresponding author, e-mail: jcuello@um.es*

We have previously studied the effect of auxins and cytokinins on the basic peroxidase isoenzyme from Zinnia elegans (ZePrx), an enzyme involved in lignin biosynthesis. The results showed that auxins and cytokinins induce ZePrx, similarly to the way in which they induce xylem differentiation. This hormonal response was supported by the analysis of the ZePrx promoter, which contains cis-elements directly responsive to these hormones and cis-elements targets of the plethora of transcription factors, such as NAC, MYB, AP2, MADS and class III HD Zip, which are upregulated during the auxin- and cytokinin-induced xylem differentiation. Looking for Arabidopsis thaliana homologues to the ZePrx we have found that a high degree of homology at 1D, 2D and 3D between certain peroxidases from A. thaliana and ZePrx is not always accompanied by the presence of the same regulatory cis-elements in the respective promoters. We describe the attempts made to establish the minimal structural and regulatory elements contained in the promoter region that a peroxidase involved in lignification must fulfil.
This work was supported by a grant from the MEC (BFU2009-08151)-FEDER and Fundación Séneca (08610/PI/08).

P10-010: THE ACTION OF MIR169/NFY REGULATORY NETWORK IN ARABIDOPSIS THALIANA ROOT DEVELOPMENT

Sorin, C.[1]* - Declerck, M.[2] - Lelandais-Briere, C.[1] - Christ, A.[2] - Hudik, E.[2] - Todesco, M.[3] - Weigel, D.[3] - Crespi, M.[2] - Hartmann, C.[1]
[1]Université Paris Diderot-Paris France /Isv Cnrs Gif Sur Yvette France
[2]Isv, Cnrs Gif Sur Yvette, France
[3]Max Planck Institute For Developmental Biology Tübingen, Germany
**Corresponding author, e-mail:* Celine.Sorin@Isv.Cnrs-Gif.Fr

Roots are essential for water and nutrients acquisition in plants and root architecture is modulated by endogenous and environmental factors to optimize plant growth. microRNAs are major post-transcriptional regulators of various developmental pathways and stress responses and we have previously shown that miR169 regulation of NF-YA factors affected the formation of symbiotic nodules in legumes. To elucidate its role in root developmental plasticity in Arabidopsis, we have characterized miR169 overexpressing plants and lines with decreased miR169 activity (using a miR169 mimicry approach or mim lines, Nat. Genet. 39, 1033-7). Phenotypic analyses of mim lines suggest a role of at least one form of miR169 in root development. Expression patterns of some members of the NF-YA family of transcription factors are modulated in mim169 lines and miR169 overexpressing lines suggesting that slicing function of miR169 is partly or fully involved in the regulation mechanism. We are currently investigating NF-YA control and their role in root development.
Key words: microRNA, root development

P10-011: VALIDATION OF REFERENCE GENES FOR QUANTITATIVE REAL-TIME PCR DURING LEAF AND FLOWER DEVELOPMENT IN PETUNIA HYBRIDA

Mallona, I.[1] - Lischewski, S.[2] - Weiss, J.[1] - Hause, B.[2] - Egea-Cortines, M.[1]*
[1]Universidad Politécnica de Cartagena
[2]Leibniz-Institut für Pflanzenbiochemie
**Corresponding author, e-mail:* izaskun.mallona@upct.es

Background Identification of genes with invariant levels of gene expression is a prerequisite for validating transcriptomic changes accompanying development. Ideally expression of these genes should be independent of the morphogenetic process or environmental condition tested as well as the methods used for RNA purification and analysis.
Results In an effort to identify endogenous genes meeting these criteria nine reference genes (RG) were tested in two Petunia lines (Mitchell and V30). Growth conditions differed in Mitchell and V30, and different methods were used for RNA isolation and analysis. Four different software tools were employed to analyze the data. We merged the four outputs by means of a non-weighted unsupervised rank aggregation method. The genes identified as optimal for transcriptomic analysis of Mitchell and V30 were EF1α in Mitchell and CYP in V30, whereas the least suitable gene was GAPDH in both lines.
Conclusions The least adequate gene turned out to be GAPDH indicating that it should be rejected as reference gene in Petunia. The absence of correspondence of the best-suited genes suggests that assessing reference gene stability is needed when performing normalization of data from transcriptomic analysis of flower and leaf development.

P10-012: TERMINATION-DEPENDENT TRANSLATION OF CHLOROPLAST NDHK MRMA

*Sugiura M, Y.**
Nagoya City University, Graduate School of natural Sciences
**Corresponding author, e-mail: sugiura@nsc.nagoya-cu.ac.jp*

The chloroplast DNA of flowering plants is tightly packed and contains around 80 protein-coding genes. In tobacco chloroplasts, 79 protein-coding genes have so far been identified. Among them, eight genes are partially overlapped. The ndhC and ndhK genes are such examples. These genes are cotranscribed. The initiation AUG codon of ndhK mRNAs is located 4 nt upstream from the ndhC stop codon. Translational control is the major step of chloroplast gene expression.
Little is known how the second cistron of overlapping gene transcripts is translated. To study mechanisms of translation unique to chloroplasts, we have developed a highly active in vitro system from tobacco chloroplasts. Using our in vitro system, mutation of the ndhC stop codon arrested translation of the ndhK cistron. The result indicated that ndhK translation depends on termination of the preceding cistron. Surprisingly, removal of the ndhC 5'-UTR and its AUG still supported substantial translation of the ndhK cistron. This translation was abolished again by removing the ndhC stop codon.
Although translation of the downstream cistron of an overlapping mRNA is generally very low, we found that the ndhC/K mRNA produces NdhK and NdhC in similar amounts. Therefore, the ndhC/K mRNA is translated not only by translational coupling but also by a novel termination-dependent pathway. For the second pathway, free ribosomes are loaded on the middle of the ndhC-coding region, migrate to the ndhC stop codon and start to translate the ndhK cistron.

P10-013: NO PROMOTES THE ACTIVATION OF A GUANYLATE CYCLASE IN NYCTINASTIC CLOSURE OF ALBIZIA LOPHANTHA LEAFLETS

Bergareche, C. - Angelo, A.P - Chellik, S. - Moysset, L. - Simón, E.*
Universitat de Barcelona, Facultat de Biologia
**Corresponding author, e-mail: mbergareche@ub.edu*

NO forms complexes with plant metal containing proteins. In animals NO can initiate its biological effects through the activation of soluble guanylate cyclase (sGC); the interaction of NO with the heme ferrous iron of sGC triggers a conformational change that increases the catalysis of the second messenger cyclic GMP (cGMP) resulting in cell-specific downstream responses. Biochemical and pharmacological approaches had shown the ability of NO to induce cGMP synthesis in plant tissues. NO is involved in phytochrome mediated nyctinastic closure of Albizia lophantha leaflets. In our experimental system A.lophanta plants were maintained under 16 h light / 8 h dark cycles prior to experimental use. Pairs of leaflets were excised at 5 h of photoperiod and floated for 1 h in 10 mL control or test solutions, then irradiated with a 15 min pulse of red light (R) or a 5 min pulse of far red light (FR) and finally kept in darkness for 3 h. Exogenous application of NO donors to pairs of leaflets inhibits nyctinastic closure and NO effect is more apparent after R light irradiation. Pharmacological approaches supplying an inhibitor of nitric oxide synthase (150 mM L-NAME), results in the enhancement of nyctinastic closure, which suggests that a NOS-like enzyme may be involved in endogenous NO production response. Leaflet nyctinastic closure was not inhibited when an inhibitor of guanylate cyclase (50 mM Ly85583) was supplied, which indicates that NO increases cGMP levels. These results are corroborated by the supply of sildenafil, an inhibitor of phosphodiesterase type5 (PDE5) that produces an accumulation of cGMP and the subsequent inhibition of the nyctinastic closure.

P10-014: CYTOPLASMIC/NUCLEAR PROTEINS WITH CONSERVED EUONYMUS LECTIN-LIKE DOMAINS: EVIDENCE FOR A UNIVERSAL ROLE IN EMBRYOPHYTA.

Fouquaert, E.* - Plas, K. - Van Damme E. J. M.
University of Ghent
**Corresponding author, e-mail:* Elke.Fouquaert@UGent.be

In recent years evidence has accumulated that plants synthesize well-defined carbohydrate-binding proteins (lectins) upon exposure to stresses like drought, high salt, wounding, treatment with some plant hormones or pathogen attack (1). From recent research it could be concluded that proteins with an Euonymus europaeus lectin (EUL) domain represent a new family of inducible lectins (2). Searches in the publicly available databases revealed that proteins with (an) EUL domain(s) are expressed in all Embryophyta. The family of EUL proteins is rather heterogeneous, in that some proteins consist of one EUL domain, while others comprise two in tandem arrayed EUL domains (3). Originally, these EUL proteins have been identified in rice, where they are expressed in the roots after treatment with abscisic acid and after exposure to salt-stress. An in silico expression analysis for the EUL from Arabidopsis demonstrated that this putative lectin gene is upregulated by salt-stress and osmotic stress and upon treatment with abscisic acid, suggesting that this protein plays a role in the adaptive response of plants to adverse environmental conditions. Confocal microscopy of tobacco cells, expressing GFP-fusion constructs with some EUL proteins, confirmed the nucleocytoplasmic localization of EUL proteins from rice, Arabidopsis and Euonymus europaeus. The nuclear localization together with their inducible expression indicates that these proteins with an EUL domain play an important role in regulatory processes and/or cell signalling.

1) Van Damme EJM et al., 2004, Trends in Plant Science, 9: 484-489.
2) Fouquaert E et al., 2008. Plant Physiol., 147: 1316-1324.
3) Fouquaert E et al., 2009. BMC Plant Biol., 9: 136.

P10-015: AN UPSTREAM MINISATELLITE CAUSES RED APPLE FLESH COLOUR

Espley, R.* - Brendolise, C. - Chagne, D. - Volz, R. - Putterill, J. - Schouten, H. - Hellens, R. - Allan, A.
The New Zealand Institute of Plant & Food Research
**Corresponding author, e-mail:* richard.espley@plantandfood.co.nz

Mutations in the genes of the anthocyanin pathway or its regulators in plants have been linked to colour phenotypes. Generally, this is a loss of function with a reduction of anthocyanin or a change in patterning. Here we describe an insertion in the upstream regulatory region of the apple anthocyanin-regulating transcription factor MYB10. This modification results in a gain of function, producing an increase in anthocyanins throughout the plant and a striking phenotype that includes red foliage and red fruit flesh. The mutation comprises a 23 base pair sequence duplicated in five tandem repeats to form a minisatellite repeat unit. We show the association between the MYB10 minisatellite duplication and the red foliage and red fruit flesh phenotype found in all apple varieties tested. Our results show that the repeat-containing promoter can act in a way that is sufficient to account for the increased MYB10 transcript levels and subsequent ectopic accumulation of anthocyanin.

P10-016: STOMATOGEN, A PEPTIDE HORMONE POSITIVELY REGULATING STOMATA DENSITY

Sakagami, Y.[1]* - Irie, K.[2] - Kakimoto, T.[3] - Kondo, T.[1] - Takada, S.[3]
[1]Nagoya Univ.
[2]Kyoto Univ.
[3]Osaka Univ.
**Corresponding author, e-mail:* ysaka@agr.nagoya-u.ac.jp

Stomata are composed of a pair of guard cells and a pore between them, and their density and positions are regulated by developmental and environmental signals. When we overexpressed many genes coding for putative secretory proteins one-by-one in Arabidopsis, we identified a gene named STOMAGEN, which increases stomatal density when overexpressed. The STOMAGEN gene encodes a small peptide with a putative secretory-signal sequence at its N-terminus and is expressed preferentially in mesophyll cells. This peptide belongs to the EPIDERMAL PATTERNING FACTOR (EPF) family of the cysteine-rich-peptides superfamily. The mature form was a C-terminal 45-amino-acid peptide (stomagen) with three intra-molecular disulfide bonds. We chemically synthesized the stomagen using solid-phase Fmoc-based chemistry, and refolded under redox-equilibrated conditions. We confirmed that the structure of the natural stomagen was identical to that of the synthetic stomagen, and determined disulfide bond positions. Stomagen treatment at very low concentrations, as low as 10 nM, increased stomatal density of wild-type Arabidopsis plants. We propose that stomagen is a mesophyll-to-epidermis signaling molecule that positively regulates stomatal density. We also suggest that stomagen increases stomatal density by competing with negative regulators EPF1 and EPF2 for the receptor-like protein TOO MANY MOUTHS.

P10-017: ANALYSIS OF DROUGHT STRESS IN BARLEY BY EXPRESSION ANALYSIS AT GRAIN FILLING STAGE

Schweizer, G.[1]* - Diethelm, M.[1] - Hofmann, K.[1] - Albert, A.[2] - Winkler, J.B.[2] - Schmidhalter, U.[3] - Diego, R.P.[4] - Kleeßen, S.[4] - Lohse, M.[4] - Kersten, B.[4] - Herz, M.[2]
[1]LfL-Institut for Crop Science and Plant Breeding
[2]Helmhotz Center Munich, Institute of Biochemical Plant Pathology (BIOP)
[3]TUMunich, Department of Plant Science, Chair of Nutrition
[4]MPI for Molecular Plant Physiology , Department for Bioinformatics
**Corresponding author, e-mail:* guenther.schweizer@LfL.bayern.de

Climatic conditions are changing rapidly leading to less predictable rainfall during growth season, so the demand for barley varieties, tolerant to abiotic stresses, will increase. Drought tolerance therefore is a genetically complex plant adaption that involves multiple genes, regulation factors and pathways, so it is essential to find representative reference genes to understand their function and to use these candidate genes for an indirect selection assay. For this purpose three genotypes, differing in tolerance to drought stress and non parasitical leaf spots, were subjected to an experiment in the exposition chambers at the Helmholtz Center in Munich. In this experiment the plants were submitted to a specific drought stress at grain filling stage and/or increased UV radiation over the whole generation. Samples for RNA extraction were taken at ten dates throughout the experiment, in each case in dependence of the respective development al stage. Four of those sampling dates covering the whole drought stress period during grain filling stage were chosen for a transcriptome analysis with 44k barley Agilent Microarrays and 454 sequencing assays (Roche). The resulting data from the array analysis are currently being analyzed and contig construction from 454 sequencing is in progress. First expression data of the array experiment are visualized on barley signaling pathways using the MapMan tool and results of genotype specific gene expression at different sampling dates are shown in Venn diagrams and kinetic clusters. Aim of the project is the identification of candidate genes related to drought stress tolerance in barley and their validation for specific breeding strategies.

P10-018: FUNCTIONAL CHARACTERIZATION OF A TOMATO HOMOLOGUE OF ATGEM (GLABRA2-EXPRESSION MODULATOR)

Viron, N. - Bres, C. - Mounet, F. - Moise, A. - Petit, J. - Garcia, V. - Moing, A. - Rothan, C. - Lemaire-Chamley, M.*
Umr 619 Biologie Du Fruit, Centre Inra Bordeaux-Aquitaine
**Corresponding Author, E-Mail: Nviron@Bordeaux.Inra.Fr*

Tomato (Solanum lycopersicum) is a model plant to study fleshy fruit development. Tomato fruit size is mainly determined during early fruit development, before the onset of ripening, through a first phase characterized by intense cell divisions followed by a second phase characterized by cell expansion (Gillaspy et al., 1993).In order to identify new candidate genes potentially involve in the regulation of cell size during tomato fruit early development, we searched for correlations between cell size and regulatory gene expression level. For this, transcriptomic and cytological analyses of two fruit expanding tissues were performed (Mounet et al., 2009). A strong correlation was highlighted between mean cell size and transcript level of a tomato homologue of AtGEM (Glabra2-Expression Modulator). In Arabidopsis thaliana this protein plays a crucial role in cell division, fate and differentiation during root development (Caro et al., 2007). In silico analyses identify two GEM homologues in tomato genome subsequently named SlGEM1 and SlGEM2. Their respective expression profile in tomato organs and during fruit development has been studied by real-time RT-PCR. In addition, we investigated the role of SlGEM1 in tomato by generating transgenic lines (RNAi silencing and over-expression) and identifying EMS mutants by tilling. Phenotypic characterization of the different lines is underway with a special focus on fruit development and root hair / trichome differentiation.

Caro et al., 2007 Nature 447(7141): 213-217Gillaspy et al., 1993 The Plant Cell 5: 1439-1451Mounet et al.,
2009 Plant Physiology 149: 1505-1528

P10-019: INTEGRATION OF SHADE PERCEPTION AND HORMONE-MEDIATED GROWTH IN ARABIDOPSIS THALIANA BY HD-ZIP II TRANSCRIPTION FACTORS

Salla-Martret, M.1 - Bou-torrent, J.1 - Cifuentes-Esquivel, N.1 - Gallemí, M.1 - Sorin, C.2 - Palauqui, J.C.3 - Martínez-Garcia, J.F.1*
1Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB
2Institut des Sciences de Végétal
3French National Institute for Agricultural Research (INRA)
**Corresponding author, e-mail: msmgmg@cid.csic.es*

Plants sense the presence of competing neighbouring vegetation as a change in light quality: i.e. they sense the reduced ratio of red to far-red light (R:FR), detected by the phytochrome photoreceptors. The responses to shade are generally referred to as the shade avoidance syndrome (SAS), and involve various developmental changes intended to outgrow or outcompete the neighbouring plants. Despite its complexity, the SAS is a consequence of only one environmental signal, the reduction of R:FR ratio (simulated shade). A complex transcriptional network is initiated after simulated shade perception, rapidly and directly up-regulating the expression of PHYTOCHROME RAPIDLY REGULATED (PAR) genes. We showed that one of them, ATHB4, that encodes a homeodomain-leucine zipper (HD-Zip) class-II transcription factor, has a role in SAS signalling as a complex modulator in these responses, involving different hormones in its action. To go deeper, in our lab we have investigated the role of its close relatives (belonging to HD-ZipII subfamily) in SAS responses and in the control of hormone molecular and/or physiological responsiveness by using overexpressor and loss-of-function lines. On the other hand, we have investigated the role of ATHB4 as a regulator of plant development co-acting with HAT3 by using histological studies of the double mutant athb4hat3, shown to display strong morphological alterations. The latest results will be shown.

P10-020: SUBCELLULAR LOCALIZATION OF ARABIDOPSIS THALIANA BPM1 PROTEIN IN BY2 AND ONION CELLS AFTER TRANSIENT TRANSFORMATION

Bauer, N. - Leljak Levanic, D. - Juranic, M. - Jelaska, S.*
Department of Molecular Biology, Faculty of Science, University of Zagreb
**Corresponding author, e-mail: nbauer@zg.biol.pmf.hr*

Proteins with BTB/POZ and MATH domains recruit substrates for ubiquitination by largest E3 ligase Cul3 complex that play an essential role in the regulation of many biologic processes. A BTB/POZ-MATH gene family in Arabidopsis thaliana (BPM) comprises of 6 membersI.n these report dynamics of subcellular localization of eGFP labeled BMP1 after transient transformation of BY2 and onion cells wilbl e presented. Four hours after transformation BPM1:eGFP was localized exclusively in the cytoplasm. Within next 12 hours protein accumulates in nucleus but part of it still remains in cytoplasm. After 24 hours, protein is localized exclusively in nucleus, in speckle-type form. Two days after transformation BPM1:eGFP signal completely disappears from cells. In addition, A. thaliana plants were transformed with BPM1 gene under constitutive promoter, and although they transcribe transgene no protein was detected. The further characterization of BPM1 protein is under study and the future work will be focused on protein function.

P10-021: TRANSCRIPTOME ANALYSIS OF FATTY ACID DESATURASE MUTANTS REVEALS SPECIFIC DINOR-OXO-PHYTODIENOIC ACID (DN-OPDA) REGULATED

Andreu, V. - Lagunas, B. - Picorel, R. - Alfonso, M.
CSIC-EEAD

Jasmonic acid (JA) and 12-oxo-phytodienoic acid (OPDA) regulate diverse physiological processes in plants, including the response to wounding and defence signalling. All these compounds are derived from unsaturated octadecanoic (C18) fatty acids. dn-OPDA is a member of the jasmonate family that is detected at very high levels upon wounding and is derived from hexadeca-

noid (C16) unsaturated fatty acids. Allthough the gene and signalling pathways that are triggered by JA and OPDA have been well characterized; those regulated by dn-OPDA remain unidentified. In this work we have performed a transcriptome analysis on two different fatty acid desaturase mutants; the fad3/fad7-1/fad8-1 mutant which does not accumulate trienoic fatty acids and does not synthesize JA, OPDA and dn-OPDA while the fad5 mutant which does not accumulate 16:3 fatty acids and does not synthesize dn-OPDA but produces JA and OPDA at normal levels. Our data have identified 154 and 159 differentially regulated genes in the fad5 and the fad3/fad7-1/fad8-1 mutants, respectively when compared to the WT. We have identified 82 common genes that might correspond to dn-OPDA regulated genes. Among these genes we have identified S-locus protein kinases, proteins involved in the ubiquitin dependent protein degradation, disease resistance or hormonal responses. Among the 82 common genes, 9 have been identified as jasmonate regulated genes but any of the OPDA regulated genes identified previously in Arabidopsis merged in our analysis. This result suggested that JA and dn-OPDA might act sinergically or sharing common elements of their signalling pathways.

P10-022: ELEVATED EXPRESSION OF PATHOGEN-RESPONSIVE GENES IN NMD MUTANTS

Rayson, S.1 - Arciga-Reyes, L.1 - Wootton, L.1 - Truman, W.2 - Graham, N.3 - Grant, M.4 - Davies, B.1*
1Centre for Plant Sciences, University of Leeds
2School of Biosciences, University of Exeter
3Nottingham Arabidopsis Stock Centre
4School of Biosciences, University of Exeter
**Corresponding author, e-mail: s.j.broad@hotmail.com*

Genome-wide expression analyses of three independent mutant lines (upf1-5, upf3-1 and smg7-1), each deficient in a different nonsense-mediated mRNA decay (NMD) factor, reveal that many Arabidopsis genes are regulated, directly or indirectly, by NMD. Genes that are up-regulated in NMD-deficient mutant Arabidopsis plants are associated with diverse gene ontologies. A conservative list of 206 core NMD transcripts, up-regulated in all three mutant lines, is enriched for various features, the most strikingly overrepresented of which is a conserved upstream open reading frame. Gene ontologies related to two core processes are overrepresented amongst the core NMD transcripts. The first is amino-acid homeostasis, the control of which appears to be a conserved evolutionary function of NMD. The second is novel to plants. Highly specific plant-pathogen responses are activated in NMD-mutant plants, which do not support growth of Pseudomonas syringea pathovar tomato DC3000.

P10-023: MOLECULAR ANALYSIS OF CHANGES IN THE FLOWER AND LEAF ABSCISSION ZONE TRANSCRIPTOME - ROLE OF AUXIN DEPLETION

Meir, S.[1] - Lers, A.[1] - Philosoph-Hadas, S.[1]- Burd, S.[1] - Sundaresan, S.[1] - Selvaraj, V.[1] - Kochanek, B.[1] - Jiang, C.Z.[2] - Ried M.S.[2]*
[1]Agricultural Research Organization ARO
[2]University of California
**Corresponding author, e-mail: shimonm@volcani.agri.gov.il*

Abscission of organs from the plant is initiated by changes in the auxin gradient across the abscission zone (AZ) which sensitizes the AZ to ethylene. Changes in gene expression have been correlated with the ethylene-mediated execution of abscission, but there has been little study of the molecular and biochemical basis of the role of auxin depletion. After excising flowers or leaves from tomato (Solanum lycopersicum Mill.) inflorescences, leading to rapid pedicel or petiole abscission, respectively, we examined transcriptome changes in the flower and leaf AZs. Microarray analysis using the Affymetrix Tomato GeneChip revealed changes in expression, occurring prior to and during pedicel or petiole abscission, of many genes with possible regulatory functions. They included a range of auxin-related transcription factors (TFs) such Aux/IAA genes, ethylene biosynthesis related and ethylene signal transduction related genes. IAA application after flower or leaf removal, which prevented pedicel or petiole abscission, respectively, diminished these changes in the expression of the examined genes. The results support the suggestion that auxin depletion is an important mediator of the abscission response, which affects AZ sensitivity to ethylene. These results contribute to our understanding of the role of auxin in regulating flower and leaf abscission. (This work was supported by BARD grants No. IS-3815-05 and IS-4073-07).

P10-024: CHANGES IN PLANT PROTEIN TYROSINE PHOSPHORYLATION UNDER REDOX-AGENTS TREATMENT

Petrova, N. - Karimova, F.*
Kazan Institute of Biochemistry and Biophysics, the Russian Academy of Sciences
**Corresponding author, e-mail: npetrova@inbox.ru*

Posttranslational modifications (PTMs) of proteins are involved in signal transduction and cell metabolism regulation. Because the activity of many proteins is regulated by more than one type of PTMs the study of interaction of different types of PTMs is actual. Reactive oxygen species (ROS) involved in redox-regulation of cell activity and can modify the components of cellular signaling pathways including protein kinases, protein phosphatases and transcription factors. In literature, besides the revelation of redox-sensitive plant proteins it is absent data about redox-sensitive phosphotyrosine proteins (and changes in their tyrosine phosphorylation level (TPL)), which are critical for signal transduction and cell metabolism regulation by norm and adaptive reactions.
In this study we have used 2D-electrophoresis together with immunochemical approach to demonstrate changes in plant protein tyrosine phosphorylation by in situ altered cellular redox status in the roots of pea plants. More then 50 phosphotyrosine proteins were detected. Among revealed proteins, whose TPL was redox regulated, a part of proteinswere identified using MALDI-TOF mass-spectrometry (MS) or tandem mass-spectrometry (MS/MS). These proteins were associated with a variety of functions, including energy- and material metabolism and signal transduction.

P10-025: CYTOKININ HOMEOSTASIS AND ITS GENETIC REGULATION DURING VEGETATIVE AND REPRODUCTIVE DEVELOPMENT IN WHEAT

Song, J. - Jameson, P. E.*
School of Biological Sciences, University of Canterbury
**Corresponding author e-mail: jiancheng.song@canterbury.ac.nz*

The cytokinins are intimately involved in plant growth and development from the earliest stages of cell division and organ formation to the final stages of seed development and senescence. Cytokinin homeostasis during development of a specific tissue/organ is co-ordinately regulated by at least four multi-gene families. These include isopentenyl transferase (IPT), cytokinin oxidase /dehydrogenase (CKX), zeatin glucosyltransferase (ZOG) and β-glucosidase (GLU). Members of each of these four gene families were isolated using RT-PCR with degenerate or species specific primers. Full length cDNA sequences of selected genes that may be involved in seed yield determination have been obtained using the RACE strategy.
Real-time RT-PCR data showed that the expression patterns of individual members of the TaIPTs, TaCKXs, TaZOGs, and TaGLUs multi-gene families were tissue and developmentally specific during spike, ovule, seed and flag leaf development. Quantification of the mRNA levels of several type-A response regulators was used as a correlate
of bioactive cytokinin levels.

P10-026: EFFECTS OF CPPU TREATMENT ON CYTOKININ HOMEOSTASIS AND GRAIN YIELD IN WHEAT

*Yao, Z.*1 - Song. J2 - Jameson, P.E.2*
1College of Agronomy, Agricultural University of Hebei
2 School of Biological Sciences, University of Canterbury
**Corresponding author, e-mail: yzhj201@hebau.edu.cn*

Artificial disturbance of the endogenous cytokinin level dramatically affects economically important traits including plant architecture, organ size and life span, tolerance to biotic and abiotic stress, and particularly seed yield. Forchlorfenuron (CPPU), which is known to significantly increase the size of fruit, was applied to wheat at anthesis and/or two days after anthesis. The effect of CPPU on endogenous mRNA levels of cytokinin regulatory genes from four multi-gene families (isopentenyl transferase (IPT), cytokinin oxidase/dehydrogenase (CKX), zeatin glucosyltransferases (ZOG) and β-glucosidase (GLU)) was quantified using real-time RT-PCR.Substantial changes in the expression profiles of different family members were observed within four hours of spraying, and the disturbed expression of some gene family members was apparent up to seven days after spraying. Contrasting expression changes between leaf and grain were also detected. The effect of CPPU treatment on final seed yield and on yield components will also be reported.

P10-027: STUDYING OF THE «CHITIN-SPECIFIC» DOMAIN OF PLANT PEROXIDASES

*Kuzmina, O.**
Institute of Biochemistry and Genetics, Ufa Scientific Centre, Russian Academy of Sciences
**Corresponding author, e-mail: phyto@anrb.ru*

Studying of wheat origin as the important agricultural crop has the essential scientific and practical value caused by problems of selection. A variety of wild species of wheat and aegilops together with intraspecific polymorphism allow to assume the presence of the significant amount of stress genes induced in response to many disease agents in them. Among variety of protective genes the specific place is occupied with the genes coding pathogen-induced peroxidase isoforms among which concern and «chitin-specific» forms. Probably they are important in the lignification of pathogen - damaged plant tissues. The purpose of research is the molecular and genetic organisation analysis of the chitin-specific site of the peroxidase gene in wheat and aegilops. Comparison of sequenced peroxidase gene fragments with known a soft wheat nucleotide sequence Triticum aestivum TC151917 has revealed the 90 % homology with Tr. fungicidum and T. petropavlovskyi and 84 % homology with T. araraticum. The obtained data can be considered from an evolutionary position. So, T. fungicidum and T. petropavlovskyi, also as well as T. aestivum relate to subgenus Urartu. The three species are Au and B genomes carriers. Whereas, T. araraticum, T. militinae and T. boeoticum relate to Boeoticum subgenus and are Ab and G genomes carriers. It is shown a «chitin-specific» peroxidase domain possessed ability to bind a chitin. We have created a gene-engineering design composed of the dahlia mosaic virus 35S promoter and the wheat anionic peroxidase cDNA. It will allow us to find out a plant peroxidase role in protective reactions mechanisms against pathogenes.

P10-028: A NAC TRANSCRIPTION FACTOR WITH A ROLE IN ABA SIGNALING MODULATES NITRIC OXIDE LEVELS IN ARABIDOPSIS

Osuna, D.[1] - Fernández-Arbaizar, A.[1] - Albertos, P.[1] - Godoy, M.[2] - Franco, J. M.[2] - Solano, R.[2] - Carrasco, J.L.[3] - Vera, P.[3] - Lorenzo, O.[1]*
[1]Departamento de Fisiología Vegetal (CIALE). Universidad de Salamanca.
[2]Laboratorio de Genómica., Centro Nacional de Biotecnología, CSIC.
[3]Instituto de Biología Molecular y Celular de Plantas. Universidad Politécnica de Valencia
**Corresponding author, e-mail:* daniel.osuna@usal.es

The molecular basis of the abscisic acid (ABA) and nitric oxide (NO) crosstalk promoting seed germination and early development are currently unknown. The identification of the elements that participate in this response is, thus, essential to understand the NO perception and signalling by the plant. By means of a genetic screening in 3μM (+)-S-ABA that simulates the effect of NO scavenging by cPTIO, we have isolated the gap1 mutant (Nambara et al., 2002), showing an ABA- and cPTIO-insensitive phenotypes in the transition from dormancy to germination. GAP1 encodes a NAC TF nuclear localized, able to form homodimeric complexes, and it is also a potent transcriptional activator (Osuna et al., 2010). Whole genome transcriptional profiling of gap1 mutant versus Col-0 stratified Arabidopsis seeds revealed several hierarchical clusters with different function in germination and stress responses, highlighting the ABA and NO crosstalk. In addition, the DNA binding specificity of the NAC TF was provided by overexpression in both, Escherichia coli and Nicotiana benthamiana, followed by hybridization to oligonucleotide arrays, and complemented with gel shift assays, which has allowed us the identification of the consensus cis-regulatory sequences responsible of gene expression contained in NAC-regulated genes. Taken together, this data showed this NAC TF as a relevant ABA signaling pathway modulating NO levels during seed germination and stress responses. Finally, the identification of the cis-regulatory element recognized by NAC shed light on new molecular components downstream of this signaling network.

Acknowledgements: To Dr. Eiji Nambara group (Universidad de Toronto, Canada) for his collaboration in the mutant isolation.

P10-029: INTERACTION STUDIES OF PROTEINS INVOLVED IN OSMOSENSING AND CYTOKININ SIGNALING PATHWAYS IN POPULUS

Héricourt, F. - Chefdor, F. - Bertheau, L. - Larcher, M. - Depierreux, C. - Brignolas, F. - Carpin, S.*
Université d'Orléans, UFR-Faculté des Sciences, Laboratoire de Biologie des Ligneux et des Grandes Cultures
**Corresponding author, e-mail: francois.hericourt@univ-orleans.fr*

The osmosensing pathway in Arabidopsis thaliana is constituted by a multi-step phosphorelay similar to the one of Saccharomyces cerevisiae, involving a Histidine-aspartate Kinase (HK) osmosensor, AHK1, and a Histidine-containing Phosphotransfer (HPt) protein, AHP2. In Populus, we have identified a cDNA encoding a HK, named HK1, and five cDNA encoding HPt proteins, HPt1 to HPt5. Analysis of interaction tests performed with the cytoplasmic domain of HK1 and all HPts in a two-hybrid system revealed a strong interaction between HK1 and HPt2 and weak interactions between HK1 and other HPts. In *A. thaliana*, AHP2 interacts not exclusively with AHK1 but also with AHK2, AHK3 and AHK4, which are involved in the cytokinin signaling pathway. In order to determine the interconnectivity between these two different signaling pathways in Populus, we studied the interaction between HPts and cytokinin receptors.
Therefore, the homologous receptors of AHK2, AHK3 and AHK4 have been isolated from Populus and the cytoplasmic domain of these proteins has been tested in a two-hybrid system for their potential interaction with HPt1 to HPt5.
The results indicate that HPts are commonly used by the two signaling pathways as it is the case in *A. thaliana*, but clearly with distinct affinities. These results suggest also that HPt2 could be the HPt protein preferentially involved in the osmosensing pathway.

P10-030: GENE AND PROTEIN EXPRESSION OF CU TRANSPORTERS GMHMA8 IN SOYBEAN PLANTS: EFFECTS OF EXCESS CU

Del Castillo Garza, M.[1] - Bernal, M.[1] - Risueño, M.C.[2] - Picorel, R.[1] - Testillano, P.S.[2]*
[1]Estación Experimental de Aula Dei (CSIC)
[2]Centro de Investigaciones Biológicas (CSIC)
**Corresponding author, e-mail: delcastillomb@eead.csic.es*

The Cu transporter HMA8 is a member of the P1B-ATPase subfamily that plays an important role on Cu allocation and detoxification. We have identified two divergent genes in soybean, named GmHMA8-1 and GmHMA8-2, homologous to PAA2/HMA8 previously described in Arabidopsis thaliana. A previous work revealed their expression and chloroplastic localization in soybean suspension cultures (Bernal et al., 2007). Both genes are subject to alternative splicing by an intron retention mechanism leading to the formation of four transcripts: HMA8-1, HMA8-2 and the corresponding non spliced forms, NSP-HMA8-1 and NSP-HMA8-2. The intron retention yields a premature stop codon in the non spliced forms. Thus, four different putative GmHMA8 proteins with high homology between them may exist in soybean. Thegoal of this work was to analyze their expression in planta at a transcript and protein level by RT-PCR, in situ hybridization (FISH) and immunofluorescence techniques, and confocal analysis to investigate their global expression in each organ of the plant and whether their expression was tissue-specific. For this purpose, soybean plants were grown hydroponically in control conditions (0.12 μM CuSO4) and Cu-treated (10 μM CuSO4). Samples of different organs were frozen for RNA extraction, and fixed and cryoprocessed for FISH and IF essays. Results showed specific patterns of expression, with differences in control and Cu-treated plants. Transcripts were presents in the cytoplasm of mesophyll cells of leaves, whereas GmHMA8 proteins were localized in chloroplasts. These results give news insights of the role of GmHMA8 proteins on Cu homeostasis within the chloroplast in planta.

P10-031: WRKY III TRANSCRIPTION FACTOR FAMILY: A COMPLEX TRANSCRIPTIONAL REGULATORY NETWORK FOR DEFENSE AND SENESCENCE IN ARABIDOPSIS.

Besseau, S. - Li, J. - Palva, E. T.*
University of Helsinki - Viikki Biocenter
**Corresponding author, e-mail: sebastien.besseau@helsinki.fi*

Leaf senescence is the last stage of leaf development and leads to leaf death. Senescence is a way to remobilize nutrients from old leaves to new growing and reproductive organs. This step is therefore of pivotal importance for whole plant development. Senescence is an ordered sequence of events under genetic control consisting dismantling of cellular organelles, hydrolysis of macromolecules (such as proteins, nucleic acids, lipids, and chlorophyll), remobilization and transport of nutrients out of the senescing tissue. Various internal and external factors participate in triggering and modulating senescence, including age of leaves, nutrient availability, photoperiod, hormones, abiotic and biotic stresses. At the molecular level, senescence is regulated by a complex transcriptional regulatory network, involving massive reprogramming of gene expression. WRKY group III transcription factors (TF) family is known to be induced by SA and is related to plant defense. Some members of this WRKY III familywere already shown as crucial regulators of defense (e.g. WRKY70, 62, 38, 53) as well as regulators of senescence (e.g. WRKY70, 53). This dual function is explained by related physiological processes during defense and senescence. We have initiated a global and systematic study of WRKY III TF family members to address their contribution to the regulation of plant defense and senescence signaling in Arabidopsis. To this aim, expression patterns of the WRKY III TF genes were elucidated, the phenotypic effects of silencing of these genes characterized and the WRKY III interaction network identified by yeast 2-hybrid and co-immunoprecipitation. We report here that WRKY70 and 53 control senescence as part of a complex regulatory network with other WRKY III TFs.

P10-032: ROLE OF PP2C INTERACTING PROTEINS IN ABA SIGNALING AND STRESS RESPONSES

Rodriguez, D. - Modrego, A. - Saavedra, X. - Sanz, L. - Fresnillo, P. - Lorenzo, O.*
Universidad de Salamanca
**Corresponding author, e-mail: mdr@usal.es*

Protein phosphatases type 2C (PP2C) are major components of abscisic acid (ABA) signaling pathway. We have identified two functional PP2C from beechnut (Fagus sylvatica L.): FsPP2C1 and FsPP2C2, a negative regulator of the ABA pathway, and a positive regulator of ABA signaling, respectively. To further analyse the function of these PP2Cs, and by means of yeast two hybrid (Y2H) assay using an Arabidopsis thaliana cDNA library, two members of the recently described RCAR/PYR family, PYL8/RCAR3 and PYL7/RCAR2, were identified as putative interactors of FsPP2C1. Bimolecular fluorescence complementation (BiFC) assays in tobacco (Nicotiana benthamiana L.) plants confirmed the in planta interaction of both proteins and interestingly, resulted in a specific nuclear colocalization of this interaction. Gain-of-function assays by overexpressing PYL8/RCAR3 under a 35S promoter revealed increased ABA hypersensitivity of Arabidopsis transgenic seeds and consequently unability to germinate under osmotic or salt stress conditions. Furthermore, 35S:PYL8/RCAR3 plants showed increased tolerance to abiotic stress and higher expression levels of ABA responsive genes. Taken together, these results suggest that PYL8/RCAR3 positively regulates ABA signaling during seed germination and abiotic stress responses.Additionaly, three putative interactors of FsPP2C2 have been identified by Y2H assays, belonging to the small auxin up-RNA (SAUR) family, the large PPR family and an E3 ubiquitin ligase, respectively. Currenty, these interactions are being confirmed by BiFC assays and the corresponding functional tests are being performed. Interaction of FsPP2C2 orthologs in Arabidopsis (AtPP2Cs) with these new PP2C partners is also under study.

P10-033: TGA TRANSCRIPTION FACTORS MEDIATE GLUTAREDOXIN GRXC9 GENE ACTIVATION BY SALICYLIC ACID IN ARABIDOPSIS THALIANA

Herrera, A. - Carvallo, L. - Blanco, F. - Villarroel, E. - Holuigue, L.
P. Universidad Católica de Chile

Salicylic acid (SA) is one of the key signals involved in defense responses against biotic and abiotic stresses. GRXC9 gene, coding for a glutaredoxin with antioxidant function, is one of the genes rapidly activated by SA in Arabidopsis. We are interested in elucidate the mechanism of transcriptional activation of this gene by SA. In silico promoter analysis of GRXC9 gene identified two putative SA-responsive as-1-like elements in its proximal region. These elements have been previously described as targets for bZIP factors from the TGA family in promoters of model defense genes. In this work we used a combination of tools to elucidate the function of these elements in the SA-mediated transcriptional activation of GRXC9 gene. Mutants in the TGA 2/5/6 subclass of transcription factors showed impaired GRXC9 activation by SA. In vivo reporter assays, using constructs containing the full length, deletions and mutants of the GRXC9 promoter controlling the expression of GUS reporter gene, indicated requirement of both as-1-like elements for SA-mediated activation of GRXC9 gene. Also, we used yeast two- and one-hybrid assays to study the interaction between TGA transcription factors and their transactivation capacity. Finally, we assessed the association of TGA transcription factors, RNA polymerase II and modified histones to GRXC9 promoter by Chromatin Immunoprecipitation (ChIP) assays. Our results indicate that SA activates the transcription

of GRXC9 gene by a mechanism involving TGA transcription factors and as-1-like elements found in the promoter.
Supported by FONDECYT-CONICYT (1100656) and Núcleo Milenio de Genómica Funcional de Plantas (P06-009-F).

P10-034: STUDYING OF THE «CHITIN-SPECIFIC» DOMAIN OF PLANT PEROXIDASES

Kuzmina, O. - Maksimov, I.*
Institute of Biochemistry and Genetics Ufa Scientific Centre Russian Academy of Sciences
**Corresponding author, e-mail: olya.kuzmina.ibg@gmail.com*

Studying of wheat origin as the important agricultural crop has the essential scientific and practical value caused by problems of selection. A variety of wild species of wheat and aegilops together with intraspecific polymorphism allow to assume the presence of the significant amount of stress genes induced in response to many disease agents in them. Among variety of protective genes the specific place is occupied with the genes coding pathogen-induced peroxidase isoforms among which concern and «ñhitin-specific»forms. Probably they are important in the lignification of pathogen - damaged plant tissues. The purpose of research is the molecular and genetic organisation analysis of the chitin-specific site of the peroxidase gene in wheat and aegilops. Comparison of sequenced peroxidase gene fragments with known a soft wheat nucleotide sequence Triticum aestivum TC151917 has revealed the 90% homology with Tr. fungicidum and T. petropavlovskyi and 84 % homology with T. araraticum. The obtained data can be considered from an evolutionary position. So, T. fungicidum and T. petropavlovskyi, also as well as T. aestivum relate to subgenus Urartu. The three species are Au and B genomes carriers. Whereas, T. araraticum, T. militinae and T. boeoticum relate to Boeoticum subgenus and are Ab and G genomes carriers. It is shown a «chitin-specific» peroxidase domain possessed ability to bind a chitin. We have created a gene-engineering design composed of the dahlia mosaic virus 35S promoter and the wheat anionic peroxidase cDNA. It will allow us to find out a plant peroxidase role in protective reactions mechanisms against pathogenes.

P10-035: FUNCTIONAL IDENTIFICATION OF ARABIDOPSIS AND THELLUNGIELLA STRESS REGULATORY GENES

Abraham, E.[1] - Perez Salamó, I.[1] - Papdi, C.[1] - Joseph, M. P.[1] - Koncz, C.[2] - Szabados, L.[1]*
[1]Biological Research Center of the Hungarian Academy of Sciences, Institute of Plant Biology
[2]Max-Planck-Institut für Züchtungsforschung, Cologne, Germany
**Corresponding author, e-mail: abrahame@brc.hu*

Plants respond to environmental stresses by altering gene expression pattern via a complex signaling network. We developed a new genetic approach, a conditional overexpression system (COS) to identify regulatory genes involved in plant stress responses. Transformation ready cDNA library cloned in a plant expression vector under control of an inducible promoter was used to transfer into Arabidopsis, where activation of the inserted cDNA can lead to conditional phenotypes. Arabidopsis and Thellungiella cDNA libraries were used to produce transgenic lines which were tested in different screens (selecting for salt tolerance, ABA insensitive germination and activation of a stress responsive reporter gene construct). The Thellungiella library allowes large scale random interspecific gene transfer and subsequent identification of novel regulatory genes which control stress tolerance in halophyta species.
We could identify novel regulators of abiotic stress responses so application of inducible cDNA expression libraries provides an efficient tool. This work was supported by OTKA Grant F68598, EU FP6 Marie-Curie Training Program FP6-020232-2. Edit Abraham was supported by the Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences.

P10-036: SUBCELLULAR LOCALISATION OF HPT PROTEINS AND RESEARCH OF RESPONSE REGULATORS INVOLVED IN THE OSMOSENSING SIGNALING PATHW

Bertheau, L.[1] - Chefdor, F.[2] - Héricourt, F.[2] - Guirimand, G.[2] - Courdavault, V.[2] - Depierreux, C.[2] - Morabito, D.[2] - Brignolas, F.[2]- Carpin, S.[2]*
[1]Laboratoire de Biologie des Ligneux et des Grandes Cultures
[2]Université d'Orléans
**Corresponding author, e-mail: lucie.bertheau@univ-orleans.fr*

The osmosensing pathway in Saccharomyces cerevisiae is constituted by the multi-step phosphorelay SLN1-YPD1-SSK1. In Populus, we have identified a cDNA encoding a Histidine-aspartate Kinase (HK1), putative osmosensor similar to SLN1, and 5 cDNA encoding Histidine-containing Phosphotransfer (HPt) proteins, HPt1 to HPt5, similar to YPD1. A strong interaction between HK1 and HPt2 was revealed by a two-hybrid analysis.
In order to confirm this interaction, we studied the HK1 and HPts subcellular localisation and, secondly, their interactions in planta by the bimolecular fluorescence complementation system. Furthermore, to identify the complete multi-step phosphorelay in Populus, we decided to isolate cDNA encoding Response Regulators (RR) and to test their potential interaction with HPt2 in a two-hybrid system. HPt1 to 5 displayed a nucleocytoplasmic localisation and also highlighted a different behaviour of poplar HPts compared to YPD1 of yeast in normal condition of osmolarity. The interaction study between HK1 and HPt2 in planta is in progress. Furthermore, 7 cDNA encoding RR proteins have been isolated and the tests of their potential interaction with HPt2 are in progress.

P10-037: RIN3 IS AN ESSENTIAL COMPONENT OF THE ROS-MEDIATED RETROGRADE SIGNALLING PATHWAY

Barajas López, J.* - Kindgren, P. - Eriksson, M.J. - Strand, Å.
Umeå Plant Science Center
**Corresponding author, e-mail:* juan.de.barajas@plantphys.umu.se

Chloroplasts are major sites of the production of reactive oxygen species. An elaborate and highly sophisticated network, composed of scavenging enzymes, antioxidants and ROS-producing enzymes, is responsible for maintaining ROS levels under tight control.
This allows ROS to serve as signalling molecules that affects a wide range of diverse plant processes. However, little is known about how the ROS-mediated signal is transduced from the chloroplast and how the signal is perceived by the nucleus. In order to find components involved in the redox/ROS-mediated signalling pathway(s) we have designed a mutant screen for redox insensitive mutants.
We have used the LUCIFERASE reporter gene that was expressed under the control by the promoter of the redox-responsive gene LHCB2.4. A transgenic LHCB2.4::LUC reporter line was mutagenized with EMS, M2 seeds collected and seedlings screened for maintained LHCB2.4 expression following high light exposure (1000 mmol photons m-2 sec-1). Several plants that displayed high levels of luminescence under high light irradiance were selected and one line, rin3 also showed higher endogenous LHCB2.4 expression compared with LHCB2.4::LUC reporter line. Furthermore, the rin3 mutant accumulated significantly less anthocyanins. Expression of FER1 and BAP1, two marker genes responding selectively to superoxide/H2O2 and singlet oxygen, respectively, were not induced in the rin3 mutant following high light treatment.
The role of RIN3 in the ROS-mediated retrograde signalling pathway will be discussed.

P10-038: GENETIC EVIDENCE ON THE ROLE OF THE CLADE A PROTEIN PHOSPHATASE-2C HAB2 IN ABA SIGNALING

Fernandez-Arbaizar, A.[1] - Nonogaki, H.[2] - Lorenzo, O.[1]
[1]Centro Hispano-Luso de Investigaciones Agrarias (CIALE)-Dpto. Fisiología Vegetal. Universidad de Salamanca
[2]Department of Horticulture. Oregon State University

Seed biology is a highly topical subject in Plant Biology research. Essential regulatory molecules, such as abscisic acid (ABA) and jasmonates (JAs), have been related to seed germination although most of the molecular bases of the ABA and JAs action during this developmental cue are currently unknown.
It is well-known that alterations in a concrete signal transduction pathway may affect plant sensitivity to other hormonal signaling pathways. In this way, the JA-insensitive mutants coi1-16 and jar1-1 additionally show ABA-hypersensitive phenotypes being a good strategy to isolate mutants affected in ABA responses. Based on this observation, we have developed a screening strategy to find novel ABA mutants during germination using the JA insensitive background coi1-16. Screening of 105.000 M2 seedlings from 17 M1 EMS-mutagenized coi1-16 families, yielded 72 M2 new putative mutants able to supress the coi1-16 ABA-hypersensitive phenotype.
As a proof-of-concept, we have isolated the previously identified abi1-1 mutant and new alleles of the abi3 and abi4 mutants. In addition, we have isolated one hypermorphic mutation on higher arm of Chromosome I, affecting the protein phosphatase type-2C HAB2 that negatively regulates the ABA signaling pathway during seed germination. Double mutant hab2;coi1-16 shows aberrant seed development and suppresses coi1-16 hypersensitive phenotype to the pathogen Botrytiscinerea but not to Pythiumirregulare. Finally, this mutant is also insenstive to salt and osmotic stresses, highlighting a key role in the regulation of ABA responses.

P10-039: TRANSCRIPTIONAL REGULATION OF PHENOLIC COMPOUNDS BIOSYNTHESIS IN GRAPEVINE (VITIS VINIFERA L.)

Hichri, I.[1] - Bogs, J.[2] - Guillaumie, S.[1] - Mzid, R.[3] - Heppel, S.C.[2]- Pillet, J.[1] - Czemmel, S.[2] - Mechin, V.[1] - Leon, C.[1]
Destrac-Irvine, A.1 - Trossat-Magnin, C,1 - Delrot, S.1 - Lauvergeat, V.1
1UMR EGFV, France
2Heidelberger Institut Für Pflanzenwissenschaften, Germany
3Centre De Biotechnologie De Borj Cedria, Tunisia

Plant phenolics encompass several classes of molecules such as stilbenes, flavonoids and lignins, which are involved in many physiological processes during the plant development. Grape berry flavonoids, like anthocyanins and condensed tannins, play a key role in the quality of table fruits and wines. They also display potential human health benefits due to their powerful antioxidant activity.The flavonoid pathway is regulated by complexes of transcription factors belonging to MYB, bHLH and WDR families. We have identified a grape cDNA encoding a bHLH, VvMYC1, highly homologous to AN1 which is involved in the regulation of the anthocyanin pathway in Petunia. Transient promoter and yeast two-hybrid assays showed that VvMYC1 interacts with different grape MYB proteins to induce promoters of flavonoid pathway genes. The co-expression of VvMYC1 and VvMYBA1 in grape cells induces anthocyanin accumulation. In addition to VvMYC1 expression pattern in the berry during development, these results strongly suggest that VvMYC1 is part of the transcriptional cascade controlling the anthocyanin and condensed tannins biosynthesis in grapevine (Hichri et al., 2010).The regulation of lignin biosynthesis has also been investigated. In addition to impart strength and stiffness to the cell wall, lignins are essential components in waterproofing vascular cells, thus enabling the transport of water and solutes through the plant. We recently showed that overexpression of a WRKY gene, VvWRKY2, in tobacco, induces modifications of cell wall structure, xylem development and gene expression in stems and petioles. In situ hybridization and transient activation assays confirmed a role of this transcription factor in the regulation of lignification in grapevine (Guillaumie et al., 2010).

P10-040: TREHALOSE-6-PHOSPHATE AND SUCROSE SIGNALLING IN ARABIDOPSIS THALIANA

Yadav, U. - Feil, R. - Stitt, M. - Lunn, J.
Max Planck Institute of Molecular Plant Physiology

Trehalose-6-phosphate "(Tre6P)", the intermediate of trehalose biosynthesis, plays an essential role in the control of plant metabolism and growth, although its precise functions are uncertain. It has been proposed that Tre6P acts as a signalling metabolite that reflects the availability of sucrose, and thereby regulates the growth and metabolism of the plant. The aims of the work were to test the hypothesis that Tre6P is a specific signal of sucrose status in plants, and to elucidate the upstream signal transduction pathway linking Tre6P to changes in sucrose levels, using Arabidopsis thaliana seedlings grown in liquid culture as the experimental system. Resupply of sucrose to C-starved seedlings led to rapid and massive "(up to 70-fold)" increases in the level of Tre6P. Addition of glucose, fructose or maltose also led to a rise in Tre6P. However, these three sugars also increased sucrose levels in the seedlings, and in all experiments Tre6P showed a stronger correlation with sucrose than with glucose or fructose, irrespective of which sugar was supplied. These results suggested that the rise in Tre6P was linked to changes in the level of sucrose, rather than directly to the other sugars. Inhibition of transcription by cordycepin had little effect on the sucrose-induced rise in Tre6P. In contrast, inhibition of protein synthesis by cycloheximide essentially blocked the Tre6P response to sucrose. The Tre6P response to sucrose is enhanced by treatment of the seedlings with MG132, which inhibits protein turnover via the ubiquitin-26S proteasome pathway. Based on these observations, it is postulated that sucrose induces synthesis of a short-lived regulatory protein that either activates TPS to increase the rate of Tre6P synthesis, or inhibits the hydrolysis of Tre6P by TPP.

P10-041: INTERPLAY BETWEEN PHYTOCHROME A AND RETROGRADE SIGNALLING ESSENTIAL FOR PLASTID DEVELOPMENT

Kremnev D[1] - Piñas Fernandéz, A.[2] - Strand, Å.[1]*
[1]Umeå Plant Science Centre
[2]University of Edinburgh
**Corresponding author, e-mail: dmitry.kremnev@plantphys.umu.se*

Expression of the nuclear genes that encode components of the photosynthetic apparatus depends on the tightly regulated interaction between pathways mediated by PHYA and Mg-ProtoIX during plastid development.
To investigate the role of tetrapyrroles in modulating light signalling, phyA, gun5 and crd mutants were used. Three experimental conditions where LHCB1.1 expression in the gun5 mutant is uncoupled from the state of chloroplast were used to study the interaction between light and retrograde signalling pathways: Far-Red block of greening, exposure to continuous Far-Red light and seedling deetiolation in white light. Far-Red block of greening treatment is not lethal for the gun5 seedlings and under continuous Far-Red light the gun5 seedlings accumulate higher amounts of LHCB1.1 transcript compared to wild type and the crd mutant. During the first four hours of white light illumination, expression of LHCB1.1 is higher in gun5 and lower in crd compared to wild type. Similarly to the crd mutant, the phyA seedlings show a delay in LHCB1.1 transcript accumulation. Analysis of tetrapyrrole content revealed that Mg-ProtoIX acts as a negative regulator of PHYA driven expression of nuclear encoded photosynthesis genes. Taken together our results indicate

that Mg-ProtoIX accumulation initiates a signal that modulates PHYA mediated pathway during plastid development.

P10-042: H2O2 IN PLANT PEROXISOMES: AN IN VIVO ANALYSIS UNCOVERS A CA2+-DEPENDENT SCAVENGING SYSTEM

Costa, A.[1] - Drago, I.[1] - Behera, S.[1] - Zottini, M.[1] - Pizzo, P.[1] - Schroeder, J.I.[2] - Pozzan, T.[1] - Lo Schiavo, F.[1]*
[1] Università degli Studi di Padova
[2]University of California San Diego
**Corresponding author, e-mail: alex.costa@unipd.it*

Oxidative stress is a major challenge for all cells living in an oxygen-based world.
Among reactive oxygen species, H2O2, is a well known toxic molecule and, nowadays, considered a specific component of several signalling pathways.
In order to gain insight into the roles played by H2O2 in plant cells, it is necessary to have a reliable, specific and non-invasive methodology for its in vivo detection. Hence, the genetically-encoded H2O2 sensor HyPer was expressed in plant cells in different subcellular compartments such as cytoplasm and peroxisomes. Moreover, with the use of the new GFP-based Cameleon Ca2+ indicator, D3cpv-KVK-SKL, targeted to peroxisomes, we demonstrated that the induction of cytoplasmic Ca2+ increase is followed by Ca2+ rise in the peroxisomal lumen.
The analyses of HyPer fluorescence ratios were performed in leaf peroxisomes of tobacco and pre- and post-bolting Arabidopsis plants. These analyses allowed us to demonstrate that an intraperoxisomal Ca2+ rise in vivo stimulates catalase activity, increasing peroxisomal H2O2 scavenging efficiency.

P10-043: REGULATION OF TADHN DEHYDRIN GENE EXPRESSION BY CYTOKININ 6-BENZYLAMINOPURINE IN WHEAT PLANTS

Allagulova, CH. - Klyuchnikova, E. - Lastochkina, O. - Avalbaev, A. - Yuldashev, R. - Shakirova, F.*
Institute of Biochemistry and Genetics Ufa scientific centre Russian Academy of Sciences
**Corresponding author, e-mail: shakirova@anrb.ru*

Dehydrins are the II family of late embryogenesis abundant (LEA) proteins, accumulating in the seeds during their dehydration.
Their synthesis also induced in vegetative tissues in response to stress factors causing cell dehydration which is accompanied by increase in endogenous ABA level, playing key role in switching the vital programs to stressful. Therefore it is not surprising that expression of most dehydrin genes is controlled by ABA. However the data about the involvement of other phytohormones characterized by protective activity, in particular, cytokinins, in the regulation of dehydrin gene expression are few. Therefore the aim of this work was to investigate the influence of cytokinin 6-benzylaminopurine (BAP) in the low concentration 0.044 μM on gene expression of ABA-inducible TADHN dehydrin in wheat plants. In order to clarify the endogenous ABA role in BAP-induced dehydrin gene expression we used the ABA synthesis inhibitor fluridon.
The results showed that the BAP in the used concentration markedly increased the dehydrin gene expression already to 6 hours of treatment, reaching maximum to 9 hours.
The some decline in the dehydrin gene activity was observed to 24 hours of treatment, although the expression level was significantly higher than in control.
There was no effect of fluridon on BAP-induced TADHN dehydrin gene expression in wheat seedlings. This data might indicate on possible existence of ABA-independent pathway of TADHN dehydrin gene expression regulation by 6-benzylaminopurine in used concentration (0.044 μM) in wheat. This work is supported by Grants RFFI 08-04-01563 and MK-4081.2008.4.

P10-044: A NOVEL EXTRACELLULAR CYSTATIN FROM DACTYLIS GLOMERATA L. EMBRYOGENIC SUSPENSION CULTURES: CLONING, RECOMBINANT GENE EXPRESSION AND FUNCTIONAL ANALYSIS

Rakleova, G.[1] - Panchev, I.[1] - Atanasov, B.[2] - Dobrev, N.[1] - Tsacheva, I.[1] - Tchorbadjieva, M.[1]*
[1]Department of Biochemistry, Faculty of Biology, Sofia University
[2]Biophys. Chem. Proteins Lab, Institute of Organic Chemistry, Bulgarian Academy of Science
**Corresponding author, e-mail: grakleova@gmail.com*

Somatic embryogenesis is a unique phenomenon in the plant kingdom and its molecular mechanisms are still poorly understood. Somatic embryogenesis in cell suspension cultures provides a good model system for investigating early plant development. The conditioned medium harbors a complex array of molecules, which may exert a promotive or inhibitory effect on embryo development. Partial secretome analysis of the medium of Dactylis glomerata L. embryogenic suspension cultures revealed the presence of a novel extracellular cystatin. The inhibitor DgCYS1 contained the typical inhibitory motifs of cystatins. Interestingly, structural modeling of DgCYS1 showed that the N-terminal trunk contained an unstructured region (30aa), which upon certain conditions, could block the tripartite wedge that enters within the active site to cause inhibition. Hence, a possibility exists that the cystatin could be autoinhibited and thus, its activity to be controlled. DgCYS was cloned and expressed in its active form in E. coli BL21 (DE3) and current research is focused on identifying the target molecule(s) of the inhibitor from the culture medium of embryogenic suspension cultures.
This work was supported by grant BU-B-202/06 from the Bulgarian Ministry of Education and Science.

P10-045: THE MECHANISM OF REGULATION OF THE PHOTOMORPHOGENESIS BY BRASSINOSTEROIDS AND GREEN LIGHT

*I.F. Golovatskaya**
Tomsk State University
**Corresponding author, e-mail: Golovatskaya@Mail.Tomsknet.Ru*

The analysis of the hormone balance in the Arabidopsis thaliana 7-day-old seedlings treated with exogenous 24-epibrassinolide (EBL) showed that the directivity of the exogenous hormone action on dynamics of the ABA and IAA content in the Ler seedlings in the dark was similar to that of the green light (GL, 3.7 μmol quant/m2s, 60 min every day - de-etiolation). The lack of the cry1 modified the hormone response to the EBL action with respect to zeatin riboside level and the associated forms of the ABA. The deficit of the endogenic BRs in the seedlings of the det2 mutant changes the amount of the hormone response to the GL action as compared with the wild type Col, retaining the directivity of responses to the GL action.
Our experiments have shown that the disturbance of the hormone balance of the BR content is compensated to a lesser extent as compared with the cry1 photoreceptor deficiency. The latter indicates in favour of the BR participation as a link in the mechanism of the GL signal transduction. It is likely that the GL may use a transduction system lowering the deficit of one of receptors by means of a compensatory activation of other photoreceptors with overlapping functions. The deficit of the (BR) hormone lowers the compensatory reactions proper in whose realization it most likely participates itself.
This research was supported by Grant of the Federal target program “The scientific and scientific-pedagogical staff of innovational Russia” on 2009 - 2013 (contract P283 23.07.2009).

P10-046: FUCTION AND EXPRESSION OF THE ALPHA SUBUNIT OF RICE HETEROTRIMERIC G PROTEIN

Izawa, Y. - Takayanagi, Y. - Inaba, N. - Abe, Y. - Minami, M. - Fujisawa, Y. - Kato, H. - Ohki, S. - Kitano, H. - Iwasaki, Y.*

Fukui Prefectural University
**Corresponding author, e-mail: s0973002@s.fpu.ac.jp*

The rice mutant, d1 which is deficient for the heterotrimeric G-protein α subunit (Gα) gene, exhibites dwarfism, dark green leaves and small round seeds. Although studies of G-protein signaling were carried out using the d1 mutants, the cause of dwarfism was not known. To determine whether dwarfism in d1 is due to a reduction in cell number or to shortened cell length, the cell number of the leaf sheath, the internode, the root and the lemma was compared between Nipponbare, a wild-type rice and d1-5, a d1 allele derived from Nipponbare. In the d1-5 mutant, one base substitution from G to T at position 508 in Gα cDNA creates a premature stop codon that abolishes the function of Gα. As results, the cell number was reduced in all organs analyzed in d1-5. In addition, cell enlargement was found in roots and lemma of d1-5, although the organ length in d1-5 was shorter than that of wild-type rice.
We also analyzed the expression of the Gα gene in rice. Western blot analyses using anti-Gα antibody and RT–PCR analyses indicate that Gα is mostly expressed in the developing organs. The studies of Gα promoter activity using the GUS reporter gene confirmed that the expression of Gα was highest in developing organs. The promoter activity of the SAM was not high compared with that of developing organs. As results, we postulate that rice Gα may function mainly in developing organs after the differentiation was determined in SAM. We consider that rice Gα participates in the regulation of cell number in a developmental stage-dependent manner.

P10-047: FLAGELLIN GENE REGULATION MEDIATED BY MAPK PATHWAYS

Bueso, E. - Hirt, H.
INRA

The pathogen-associated molecular pattern flg22, a peptide corresponding to the most conserved domain of bacterial flagellin, is able to trigger MAPK cascades that induce innate immune responses. Flg22 is perceived by a protein complex containing several transmembrane receptor kinases, the leucine-rich repeat receptor kinases flagellin-sensitive 2 (FLS2), in association with BRI1-ASSOCIATED RECEPTOR KINASE (BAK1) and possibly with BAK1-LIKE (BKK1). MPK4 is thought to be part of the MEKK1-MKK1/MKK2 module whereby MKK4 and MKK5 contribute to MPK3 and MPK6 activation, but MPK6 can also be regulated by MKK1, MKK2, MKK3 and MKK9 (Pitzschke and Hirt, 2009). To determine which part of the cellular response to flg22 is mediated by the MPK3, MPK4 and MPK6 pathways, we carried out global transcriptome analyses using mutants of the MAPK signaling pathways. Our results show that flagellin-induced gene regulation is mediated by a complex choreography that is composed of specific and synergistic regulation of different subsets of response genes by all three MAPK pathways.

P10-048: NITRIC OXIDE INTERACTS WITH ABSCISIC ACID AND GIBERELLINS TO REGULATE EARLY DEVELOPMENT IN ARABIDOPSIS THALIANA

Lozano-Juste, J. - León Ramos, J
Ibmcp (Csic-Upv)

Nitric oxide (NO) regulates a wide range of plant processes. However, it remains unclear how NO is synthesized in plants. We have generated a triple nia1nia2noa1-2 mutant impaired in nitrate reductase (NIA/NR)- and Nitric Oxide-Associated 1 (AtNOA1)-mediated NO biosynthetic pathways. NO content in nia1nia2 and noa1-2 plants was lower that in wild-type plants and almost undetectable in the triple mutant. It has been reported that NO interacts with different phytohormones to regulate responses to stress and development. We have shown that NO negatively regulates ABA sensitivity and GA signalling in early development. The increasing deficiency in NO content of nia1nia2, noa1-2 and the triple mutant correlated well with increased seed dormancy and hypersensitivity to ABA. Similarly, we found an interaction of NO with GAs in regulating hypocotyl elongation under red light conditions. The increasing reduction of NO content in the different mutant backgrounds correlated well with longer hypocotyls. By contrast, NO treatments led to a reduction of hypocotyl elongation. We found that NO-dependent regulation of GA signalling involves NO-triggered DELLA accumulation and the subsequent reduction of hypocotyl elongation. Longer hypocotyls of NO-deficient mutants can be, in turn, explained by the reduced levels of DELLA proteins. Whether NO is regulating hypocotyl elongation by modulating DELLA abundance or controlling phytochrome interacting proteins (PIFs) expression or both is in progress. The NO-deficient mutants will be very useful tools to functionally characterize the role of NO in regulating different plant developmental processes and defence responses.

P10-049: BRM-CONTAINING SWI/SNF CHROMATIN REMODELING COMPLEX REGULATES GIBBERELLIN PATHWAY GENES IN ARABIDOPSIS

Archacki, R. - Buszewicz, D. - Halibart-Puzio, J. - Prymakowska-Bosak, M. - Jerzmanowski, A.*
University of Warsaw, Laboratory of Plant Molecular Biology
**Corresponding author, e-mail:rafa@ibb.waw.pl*

Eukaryotic cells are equipped with many factors that change chromatin structure, and thereby modulate DNA processing. Among these factors are SWI/SNF chromatin remodeling complexes which use the energy of ATP hydrolysis to change nucleosomal organization. SWI/SNF remodelers are evolutionary conserved from yeast to mammals and consist of 8-11 subunits, of which one is the central catalytic ATPase. In animals, SWI/SNF complexes play critical roles in the regulation of cell proliferation and diifferentiation, as well as in hormonal signaling. Here, we investigate involvement of BRAHMA (BRM), one of the Arabidopsis SWI/SNF ATPase, in gibberellin (GA) signaling. We found that inactivation of BRM causes misexpression of key GA pathway genes.
In brm null mutants, GA3ox1 biosynthetic gene is downregulated, while GA20ox genes and GID1B gene encoding a GA receptor are upregulated. Similar results were obtained when brm ga1-3 double mutant line (deprived of endogenous GAs) was compared to ga1-3 mutants, confirming that the misexpression of GA biosynthesis and signaling genes is a direct effect of the lack of BRM. To verify if these genes are regulated directly by BRM, we performed chromatin immunoprecipitation (ChIP) analysis using anti-BRM antibodies. We found that the promoter of GA3ox1 is enriched with the BRM protein, suggesting that at least this gene is a direct target of BRM. In conclusion, our results support a model in which SWI/SNF complex directly and indirectly regulates key genes of gibberellin signaling pathway.

P10-050: ANALYSIS OF BETA SUBUNIT OF RICE HETEROTRIMERIC G PROTEIN

Utsunomiya, Y. - Samejima, C. - Fujisawa, Y. - Kato, H. - Iwasaki, Y.
Fukui Prefectural University

Higher Higher plants have α, β, and γ subunit genes of the heterotrimeric G proteins as well as animals have. In rice, the deficient mutant for α subunit of heterotrimeric G protein, d1 was isolated in 1999. d1 exhibits typical phenotypes such as dwarfism, shortened seeds and deep-green leaves. The function of β and γ subunit is not studied because mutants were not isolated in rice plants. If function of β subunit is same as that of α subunit, the transgenic rice plants suppressing the expression of β subunit gene should show same abnormal phenotypes observed in d1. In order to estimate the function of β subunit, we produced the transgenic rice plants suppressing the expression of β subunit gene in wild type

(β RNAi / WT plants) and d1 (β RNAi / d1 plants) by RNAi method. β RNAi / WT plants showed the abnormal phenotype, namely dwarf, small seed, browning of lamina joint region and node, and decreased fertility. Almost all abnormality that β RNAi / WT plants showed was different from that of d1. Western blot analyses using anti-Gβ antibody indicated that the amount of the β subunit in some β RNAi / WT plants reduced about 30%, compared with wild type.β RNAi / d1 plants showed the abnormal phenotype, namely dwarf, small seed, browning of lamina joint region and node, and decreased fertility. Western blot analyses using anti-Gβ antibody indicated that the amount of the β subunit in some β RNAi / d1 plants reduced about 30%, compared with d1. RNAi / d1 plants showed more severe dwarfism, compared with d1. These results indicate that β subunit has some independent functions, compared with α subunit in rice heterotrimeric G protein.

P10-051: 24-EPIBRASSINOLIDE REGULATES CYTOKININ METABOLISM IN WHEAT SEEDLINGS

Avalbaev, A. - Yuldashev, R. - Shakirova, F. - Vysotskaya, L.*
Russian Academy of Sciences
**Corresponding author, e-mail: shakirova@anrb.ru*

Earlier in our research it was found that influence of 24-epibrassinolide (EB) on wheat seedlings caused rapid and stable double accumulation of cytokinins (CKs).
The important contribution to this process had the EB-induced inhibition of gene expression and activity of cytokinin oxidase (CO) which is responsible for cytokinin degradation. Meanwhile, rapid accumulation of isopentenyladenosine and zeatin nucleotide, the primary compounds in the cytokinin biosynthesis, in EB-treated plants suggests that EB might influence on CKs biosynthesis.
Probably our results about increase in EB-treated plants of concentration of O-glucosides, the storage forms of active cytokinins, might indicate in favour of this suggestion. Removing of EB from incubation medium of wheat seedlings led to the gradual return of CKs content to the control level.
This was accompanied by gradual increase both of CO enzyme activity and level of CO gene expression to the control level. Meanwhile, we have revealed the protective effect of EB on wheat seedlings in response to salinity, and this was connected with the return of salinity-induced decrease in the CKs content to the control level.
The received data indicate on the participation of EB in regulation of cytokinin metabolism and important role of endogenous CKs in realization of the physiological action of EB on wheat plants. This work is supported by Grant RFFI 08-04-01563.

P10-052: IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF A BHLH TRANSCRIPTION FACTOR INVOLVED IN THE RIPENING OF FLESHY FRUITS

Nicolas, P. - Lecourieux, F. - Lecourieux, D. - Gomes, E. - Delrot, S.*
UMR EGFV INRA
**Corresponding author, e-mail: Philippe.Nicolas@Bordeaux.Inra.Fr*

Grape berry development can be divided in two phases: a phase of “herbaceous” growth mediated by cell division and elongation leading to hard and acid green fruits.
The second phase corresponds to cell elongation and is characterized by the maturation of the berry, which is characterized by changes in texture and colour. This transition from green stage to ripening is called véraison.
From véraison on and during ripening, high amounts of sugars and phenolic compounds accumulate in the berry. In addition to their structural role, sugars can act as a signal able to affect fruit development and ripening. In this context, we identified a new transcription factor, VvbHLH1, which expression is sucrose-dependent and berry-specific. Its functional characterisation, supported by overexpression in grape and tomato, suggests that VvbHLH1 may play a key role during berry development and ripening by affecting berry size through modifications of the hormonal balance.

P10-053: SE5 SHEDS LIGHT ON THE ROLE OF PHYTOCHROMES IN PHOTOPERIODIC FLOWERING CONTROL IN RICE

Andres, F.[1] - Talón, M.[2] - Domingo, C.[2]
[1]Max Planck Institute for Plant Breeding Research
[2]Instituto Valenciano de Investigaciones Agrarias

A great number of plants synchronize flowering with day length. In rice (Oryza sativa), photoperiod is the primary environmental cue that triggers flowering. Heading is strongly influenced by daylength, promoting flowering under short days.
Two independent photoperiod pathways have been defined, one involving Hd1 and the other involving Ehd1, that control heading date by regulating Hd3a, the most important floral integrator. The s73 mutant, identified in a gamma irradiated Bahia collection, displays early flowering and photoperiodic insensitivity due to a null mutation in the SE5 gene, which encodes an enzyme implicated in phytochrome chromophore biosynthesis. Mutations in SE5 cause depletion in the phytochromes function. s73 mutant plants showed a number of alterations in the characteristic diurnal expression patterns of master genes involved in photoperiodic control of flowering, resulting in up-regulation of Hd3a. Molecular characterization of s73 provides new insight on the regulation of the photoperiodic control of flowering in rice by showing that phytochromes inhibit flowering affecting both Hd1 and Ehd1 flowering pathways.

P10-054: JUB1, A H2O2-REGULATED NAC TRANSCRIPTION FACTOR, NEGATIVELY CONTROLS SENESCENCE AND CONSTITUTES A CENTRAL ELEMENT IN H2O2 SIGNALING

Balazadeh, S.[1] - Wu, A.[2] - Allu, A.[3] - Xue, G.P.[4] - Mueller Roeber, B[1]
[1]University of Potsdam
[2]MPI of Molecular Plant Physiology
[3]MPI of Molecular Plant Physiology
[4]CSIRO Plant Industry

Senescence is a genetically controlled process occurring at late stages of development; it can also be induced by abiotic stresses including salinity.
A candidate signal mediating age-dependent or abiotic stress-induced senescence is hydrogen peroxide (H2O2). Many transcription factors (TFs) of the NAC family undergo expression changes upon leaf aging.
We started to analyze the gene regulatory networks (GRNs) controlled by NACs.
Our previous studies indicated that salt-triggered expression of positive senescence regulator ANAC092 and its downstream regulon may at least in part be mediated through a rise of cellular H2O2 level upon salt stress.
We now discovered another NAC TF, dubbed JUB1, which functions as a negative regulator of senescence. Its overexpression dampens the intracellular H2O2 level and increases life span accompanied by an increased resistance to oxidative stress. In contrast, precocious senescence and lowered tolerance against abiotic stresses were observed in a jub1-1 knock-down line. JUB1 expression is strongly and rapidly induced by external H2O2.
To explore the JUB1 GRN, we determined its preferred binding sites by in vitro binding site selection and performed microarray-based expression profiling using estradiol-inducible JUB1overexpression lines. Based on our results we hypothesize that JUB1 constitutes a central regulator of a finely tuned control system modulating cellular level of H2O2, regulating stress adaptation and the entry into senescence.

P10-055: FUNCTIONAL INTERPLAY OF SMALL RNA NODES AND HORMONES ON PLANT REPRODUCTIVE TRAITS

Rubio Somoza, I. - Detlef, W.*
Max Planck Institute for Developmental Biology
**Corresponding author, e-mail: ignacio.rubio@tuebingen.mpg.de*

Plant development relies on a natural succession of programs that require a delicate balance among cell division, elongation, differentiation and to a lesser extent cell death. Developmental programs enable the plant for organ patterning and mediate interactions with the surrounding environment.
These programs and their progression are orchestrated by a highly dynamic interplay between different plant hormones such as auxin, jasmonic acid (JA) and gibberellic acid (GA). Small RNAs (sRNA) have turn to be virtually involved in all levels of regulation of cellular information networks influencing from the chromatin state to the abundance of transcripts encoding direct regulators of gene expression and protein activity, such as transcriptional factors and F-box proteins. MicroRNAs (miRNAs), a special class of sRNAs, have been linked to the regulation of elements involved in the control of hormone synthesis, homeostasis or transduction in the JA, GA and auxins pathways. We have previously created a comprehensive collection of Arabidopsis lines (MIMIC lines) with specifically reduced activity of a different miRNA family allowing us to interrogate miRNA nodes and their connection within the dynamic hormone context.
We will show how two evolutionary unrelated miRNA/target nodes (GA and JA-related) regulate a third node targeting auxin signal transduction elements. This sRNA network mediates the hormone-dependent transition through developmental stages promoting inflorescence vasculature development and maturation of floral organs and establishes reproductive competence. These results show cellular and physiological importance of this network in a hormone context, further reinforcing the role of miRNAs in auxin gradation

P10-056: PROTEIN INTERACTORS OF KIN10

Rodrigues, A. - Duarte, L. - Martinho, C. - Baena-González, E.*
Instituto Gulbenkian de Ciência
**Corresponding author, e-mail: americo@igc.gulbenkian.pt*

The Arabidopsis SnRK1 protein kinases KIN10 and KIN11 are central regulators of the transcriptome in response to multiple types of stress. Sensing and signaling stress-associated energy deprivation, SnRK1s trigger changes in the expression of over 1000 genes that allow the re-establishment of homeostasis and the mounting of a more specific adaptive response.
Despite the importance of SnRK1s for plant growth and acclimation, our current knowledge on this signaling pathway is extremely limited. SnRK1s are the orthologs of the yeast SNF1 and mammalian AMPK, and so far most of the characterization of the SnRK1 system has relied on knowledge from these systems. However, although very valuable and successful, this approach is limited in its ability to uncover novel interactions unique to plants. In order to address this, we are undertaking Y2H screens with three major deviances from earlier screenings: i) we are using libraries generated from a wide range of stress treatments (kindly provided by Prof. John Cushman), ii) screening is performed in parallel also under low-glucose activating conditions and iii) it relies not only on the full-length (FL) SnRK1, but also on the non-conserved C-terminal domain To this end, using FL KIN10 as a bait and clone selection in medium with low and high glucose content, we have identified 59 clones with autotrophic growth in the absence of both adenine and histidine. We are currently confirming these interactions and further investigating the involvement of these components in the SnRK1 pathway.

P10-057: TRANSCRIPTOME PROFILING FOR BRASSICA NIGRA IN HEAVY METAL TOLERANCE

Cevher Keskin, B.[1] - Yildizhan, Y.[1] - Yuksel, B.[1] - Kulen, O.[1]*
Memon, A. R.[2]
[1]TUBITAK Marmara Research Center
[2]International University of Sarajevo
**Corresponding author, e-mail: birsen.keskin@mam.gov.tr*

There has been significant interest in the use of plants for the soil remediation contaminated with toxic heavy metals. This technology, termed phytoremediation provides environmentally friendly solutions to use plants to extract heavy metals from the soil and water to accumulate them in shoot tissue. Knowledge of the molecular mechanisms involved in phytoremediation may lead to the development of more efficient plants, and it has been suggested that better phytoremediation management practices could be accomplished by expressing selected genes in more favorable plant species. In this study, Affymetrix GeneChip Arabidopsis Genome Array (ATH1-121501 GeneChip, ATH1; Affymetrix, Santa Clara, CA, USA) was used for the transcriptomal profiling in response to 25 µM Copper. Root and leaf RNAs of B. nigra 6619 (non-tolerant) and B.nigra Diyarbakir (tolerant) ecotypes were hybridized with ATH chips.At the end of microarray experiments, we identified metal transport and accumulation related genes such as; MT1C (Metal Copper Binding Protein), MT2B (Metallothionein 2B, copper ion binding), MT3 (Metallothionein 3) and metal stress related genes (γ-glutamyl cysteine synthetase: γ-ECS, phytochelatin synthase: PC etc). The mRNA expression experiments by real time RT-PCR (qRT-PCR) showed that two enzymes of γ-ECS and PC were highly expressed when plants were subjected to 200 and 500 µM Cu in the growth medium. Quantitative Real Time RT-PCR experiments will be performed to identify other differentially expressed genes. It may be suggested that these genes will serve as molecular tools for the future expression in transgenic plants for the phytoremediation applications.

P10-058: MSP DOMAIN-CONTAINING PROTEIN REVEALS NEW LEVEL OF REGULATION OF STOMATAL SIGNALING IN ARABIDOPSIS

Paciorek, T. - Abrash, E. - Bergmann, D.*
Stanford University
**Corresponding author, e-mail: paciorek@stanford.edu*

Stomata are specialized epidermal structures that mediate gas exchange between plant and environment. The formation and patterning of stomatal complexes in Arabidopsis result from molecular interactions within a complex web of functionally interconnected regulators. To date, several components of signal transduction pathway including ligands, receptors and transcriptional factors involved in stomatal patterning have been identified. One of the most crucial regulators of stomatal patterning is the TOO MANY MOUTHS (TMM) gene. TMM encodes a receptor-like protein localized at the plasma membrane in stomatal lineage cells. TMM plays a central role in the deciding whether a cell will enter the stomatal pathway; mutations in this gene result in violation of major patterning rules governing stomatal development. The aim of this study was to identify specific modulators of the TMM receptor. We have designed and performed a forward genetic screen and identified specific suppressors of the tmm-1 mutant. Here, we present molecular characteristics of the MST1 (MSP domain-containing suppressor of tmm 1) gene which specifically modulates the phenotype of tmm but not other stomatal signaling mutants.

P10-059: EFFECTS OF THE SAMDC UORF PEPTIDE ON MRNA DEGRADATION AND PROTEIN DEGRADATION

Choi, Y. - Park, K.Y.*
Sunchon National University
**Corresponding author, e-mail: yj1730@sunchon.ac.kr*

S-adenosylmethionine decarboxylase (SAMDC), a key enzyme

for polyamines biosynthesis, was tightly regulated for homeostatic levels by translational inhibition of its own protein. When we measured degradation rate of downstream GUS mRNA in transgenic tobacco plants containing 35S promoter-driven uORF was clearly faster than in vector control plants. These data suggested that small uORF peptide accelerated the degradation of the downstream ORF mRNA. Also, we showed that degradation rate of GUS and SAMDC protein after treatment with cycloheximide and specific proteasome inhibitor MG115 was accelerated in the presence of small uORF peptide. But, the degradation of ADC protein, which was unrelated with uORF, was not affected by uORF peptide. These results implied that small uORF peptide might effectively acts as functional protein degradation regulator for only its main ORF, not only in cis but also in trans, in additional to a well-known function as translational inhibitor. Furthermore, translational level was completely retained in phosphorylated protein of Ser17 (P17, ser → ala), which is a putative site for protein kinase C. Also, each phosphorylation of Ser10 (P10) and Ser28 (P28), which were putative sites for cAMP/cGMP dependent kinase and casein kinase II respectively, decreased dramatically its downstream translation. Therefore, it is suggested that protein kinase C might have an important role for acting a translational inhibitor of downstream ORF.

P10-060: REGULATION OF ETHYLENE PRODUCTION BY PROLYL 4 HYDROXYLASES

Rishmawi, L. - Abu Zeineh, A. - Yamoune, A. - Heliel, O. - Mroueh, S. - Bensassi, K. - Fragkostefanakis, S. - Manioudaki, M. - Spano, T. - Kalaitzis, P.
Mediterranean Agronomic Institute of Chania

Prolyl 4 hydroxylases (P4Hs) belong to 2-oxoglutarate-dependent dioxygenases and catalyze the proline hydroxylation, a major post-translational modification, of hydroxyproline rich glycoproteins (HRGPs). There are 13 putative Arabidopsis P4Hs with differential expression in response to hypoxia, anoxia and mechanical wounding while two of them were characterized as recombinant proteins and shown to hydroxylate synthetic peptides representing extensin and AGP sequences. Arabidopsis P4H insertional mutants were characterized at the phenotypic and molecular level indicating alterations in ethylene regulation. Gene expression analysis showed differences in transcript abundance of ethylene biosynthetic genes indicating a correlation between ethylene production and prolyl 4 hydroxylase activity.

P10-061: THE PP2A REGULATORY SUBUNIT TAP46 CONTROLS GROWTH AND METABOLISM AS A COMPONENT OF TOR SIGNALING PATHWAY

Ahn, C.S. - Han, J.A. - Lee, H.S. - Pai, H.
Yonsei University Department of Biology

Tap42/α4, a regulatory subunit of protein phosphatases 2A (PP2A), is a downstream effector of the target of rapamycin (TOR) protein kinase that regulates cell growth in yeast in coordination with nutrient and environmental conditions. However, Tap42/α4 functions in higher eukaryotes differ from those in yeast, while the function of its homolog in plants, Tap46, is unknown. In this study, we have characterized the functions and phosphatase regulation of plant Tap46. Depletion of Tap46 in Nicotiana benthamania and Arabidopsis resulted in growth arrest and acute plant death with morphological markers of programmed cell death (PCD). Tap46 interacted with PP2A and PP2A-like phosphatases PP4 and PP6, and Tap46 deficiency dramatically decreased cellular PP2A activities. Immunoprecipitated mammalian TOR and the kinase domain of Arabidopsis TOR phosphorylated recombinant Tap46 protein in vitro, supporting a functional link between Tap46 and TOR. Tap46 depletion reproduced the signature phenotypes of TOR inactivation, such as dramatic repression of global translation and activation of autophagy and nitrogen mobilization, indicating that Tap46 may act as a positive effector of TOR signaling in controlling those processes. These findings suggest that Tap46, in conjunction with associated phosphatases, plays an essential role in plant growth and development as a component of the TOR signaling pathway.

P10-062: SUGAR AND ORGANIC ACID CONTENT IN TOMATO FRUITS OVEREXPRESSING AN ABA-REGULATED TRANSCRIPTION FACTOR

Bastías, A.[1] - Gómez Cadenas, A.[2] - Casaretto, J.A.[1]
[1]Universidad de Talca, Chile
[2]Universitat Jaume I

Fruit development is a complex process regulated by plant hormones and involves several coordinated metabolic and physiological changes. During these events, the phytohormone abscisic acid (ABA) is known to regulate the development and maturation of seeds. The AREB bZIP transcription factors mediate ABA-regulated gene expression involved in desiccation tolerance and are expressed mainly in seeds and in vegetative tissues under stress; however, they are also expressed in some fruits such as tomato. In order to understand the role of ABA signaling in fruit development, the expression of two AREB-like factors were investigated during different developmental stages. Moreover, tomato transgenic lines that over-express and down-regulate one AREB-like transcription factor, SlAREB1, were generated to determine its effects on the levels of some metabolites determining fruit quality. No significant changes were found in ethylene content in tomato fruits when analyzed by gas chromatography, which agrees with the normal ripening phenotype observed in transgenic fruits. Content of some organic acids and sugars was analyzed by capillary electrophoresis. Higher levels of citric acid, malic acid, glucose and fructose were observed in SlAREB1 over-expressing lines compared to those in antisense suppression lines in red-mature fruit pericarp. The higher hexose content correlated with increased expression of genes encoding a vacuolar invertase and a sucrose synthase. These results suggest that ABA affects the metabolism of these compounds during the fruit developmental program.

P10-063: ARABIDOPSIS BPMS INTERACT WITH MEMBERS OF THE ERF/AP2 FAMILY

Weber, H.[1] - Hellmann, H.[2]
[1]Applied Genetics, Freie Universität Berlin,Germany
[2]Washington State University, Pullman, WA, USA
**Corresponding author, e-mail: weberhenriette@gmail.com*

As sessile organisms plants are particularly exposed to environmental cues and consequently developed numerous mechanisms of regulation to acclimatize to environmental changes. The required variety in expression pattern is often achieved by rapid degradation of regulatory proteins like transcriptional activators and repressors via the ubiquitin-proteasome system. In all eukaryotes Cullin3 proteins function as scaffolding subunits of ubiquitin-E3 ligases, which target specific substrates for ubiquitination. In *Arabidopsis* CUL3 regulates plant growth and development, ehtylene biosyntehsis and red light signal transduction (Thomann et al., 2009).
In an earlier study we showed the assembling of CUL3 with members of the *Arabidopsis* BTB/POZ-MATH (BPM) family (Weber et al., 2005), and hyposthesized BPM proteins to be substrate adaptors for CUL3-based ubiquitin ligases. Here we give a detailed description of BPM expression and subcellular localization, and we provide strong evidence that BPM proteins use their MATH domains to interact with members of the plant specific ERF/AP2 transcription factor family. ERF/AP2 proteins are broadly involved in biotic and abiotic stress response as well as phytohormone signal transduction.Our findings provide first information on the interplay of ERF/AP2 transcription factors with CUL3-based E3 ligases, and a potentially novel regulatory mechanism of transcriptional control.

P10-064: FUNCTION OF UBIQUITINATION IN PHOSPHATE STARVATION SIGNALING IN ARABIDOPSIS

Rojas-Triana, M.[1] - Trigueros, M.[1] - Irigoyen, M. L.[1] - Puga, M. I.[1] - Bustos, R.[2] - Paz-Ares, J.[1] - Rubio, V.[1]*
[1]Centro Nacional de Biotecnología-CSIC
[2]Centro de Biotecnología y Genómica de Plantas U.P.M. - I.N.I.A.
**Corresponding author, e-mail: mrojas@cnb.csic.es*

Protein degradation is a post-transcriptional regulatory process that allows cells to respond rapidly to intracellular signals and to environmental conditions changes by adjusting the levels of key proteins. The Ubiquitin (Ub)/26S proteasome pathway (UPS) plays a fundamental role in this process in eukaryotes. This pathway involves E3-Ub ligases (E3s) that recognize target proteins and facilitates their covalent modification with Ub molecules, conferring specificity to the ubiquitination process.
In plants, the UPS pathway regulates diverse signaling processes and stress responses, including the control of phosphate starvation (-Pi) signaling, an essential nutrient for any organism growth. Analyzing the transcriptomic profile, using ATH1 arrays, of plants grown in different Pi conditions, we identified 104 -Pi responsive E3s (Phosphate starvation Controlled E3, PCE), which constitute 10% of the E3s represented in ATH1. Among these, we selected 4 genes (PCE1-4), which likely display functional redundancy, for further characterization.
Expression of these genes is rapidly induced by Pi (8h) and mutations on them cause alterations in the expression of -Pi responsive genes, suggesting a role as repressors of Pi signaling. PCE1-4 genes encode F-box E3 proteins that constitute a defined phylogenetic cluster featuring C-terminal kelch motifs and nuclear localization.
We aim to identify proteins that interact with PCE1-4, including their possible targets, by screening yeast two hybrid libraries. To date, we have confirmed 83 clones, using restrictive culture conditions, whose sequence will help us to identify PCE1-4 targets possibly involved in Pi signaling.

P10-065: IDENTIFYING DOWNSTREAM REGULATORS OF CYTOKININ SIGNALLING DURING THE CAMBIAL DEVELOPMENT

Elo, A. - Zhang, J. - Immanen, J. - Helariutta, Y.
University of Helsinki

Our knowledge of the regulation of vascular development, such as an establishment of procambial cell lines and the following differentiation into xylem or phloem has rapidly been expanding but still several major questions remain open. Even though cambial activity is instrumental for the plant secondary growth, the molecular control of the stem cell maintenance or the cell proliferation in cambium is largely unknown. We and the others have been able to show that cytokinin signalling induces cambial growth and cytokinins are major hormonal regulators required for cambial development (Nieminen et al 2008, Matsumoto-Kitano et al 2008). To identify genes involved in the cambium development and activity and to identify the components downstream of cytokinin signalling, we carried out a FACS based gene expression profiling experiment.
This experiment was carried out using a procambium/cambium expressed cell line marker ARR15::GFP. In this experiment we extracted RNA from the cambial cells representing three different developmental zones from either non-treated and cytokinin treated Arabidopsis roots. Based on our microarray profiling we could identify approximately 500 procambium/cambium enriched gene expressions, from which 100 genes seem to be regulated by cytokinin. Currently we are analysing the identified genes functionally.
Matsumoto-Kitano et al. Proc Natl Acad Sci U S A. 2008 105 (50) 20027 –20031. Nieminen K et al ,. Proc Natl Acad Sci U S A. 2008 Dec 16;105(50):20032-7

P10-066: EFFECT OF VARYING WATER SUPPLY ON STOMATAL RESPONSE DYNAMICS TO CHANGE IN [CO2]

Hladnik, J. - Kržan, K. - Eler, K. - Pintar, M. - Vodnik, D.*
**Corresponding author, e-mail: joze.hladnik@bf.uni-lj.si*

Stomatal regulation network integrates all environmental and internal stimuli that are essentially interdependent. This regulation provides an optimal stomatal conductance for any environmental conditions. In the present study we examined how plant water status affects stomatal sensitivity to the change of CO2 concentration ([CO2]). We investigated the short-term dynamic of stomatal response to a sudden [CO2] increase in maize supplied with different amounts of water. Gas exchange measurements were performed in short logging intervals and the response was monitored under two different levels of water vapour pressure deficit in order to observe the impact of air humidity. Generalized logistic curves were fitted to standardized stomatal response data, which enabled us to objectively estimate the level and the dynamics of the response. Soil water stress and high VPD significantly decreased relative stomatal closure in response to [CO2] rise, but simultaneously accelerated stomatal response to [CO2], as revealed by shorter half life. VPD significantly affected the response of well-watered plants. In contrast, a fast stomatal reaction of water-deprived plants was predetermined by a low xylem water potential of the leaf and the influence of air humidity was minor.

P11 Cell Biology

P11-001: EFFECTS OF THE ACTIVE CONSTITUENTS OF CROCUS SATIVUS L., CAROTENOIDS ON H1- SPECIFIC OLIGONUCLEOTIDE INTERACTION

Hooshyar, R. - Khanpour, N. - Zahra Bathaie, S.*
Tarbiat Modares University
**Corresponding author, e-mail: houshyar@modares.ac.ir*

Saffron carotenoides (crocin and crocetin), which have highly antitumor effect on different malignant cells, can be used in association with other antitumor drugs in the treatment and prevention of different kinds of cancers. But the molecular mechanisms of the saffron action are not known clearly. On the other side it is illustrated that transcriptional activation of genes occurs due to the H1 dissociation from linker DNA; hence, the effect of saffron components on the histone Hl-DNA complex, as a model of chromatin, is considered here. Since specific functions have been reported for crocin and crocetin, the present study aimed to investigate their interaction with H1-oligonucleotide complex. In addition, an oligonucleotide with high affinity for H1 is used for clarification of the mechanism of interaction. The circular dichroism (CD) spectra of these complexes changed due to the reduced interaction, after adding of the mentioned ligands. Our observations led to suggesting a mechanism in which the H1 depletion may affect transcription of some genes for example suppressing tumor genes. In conclusion, saffron various applications as an anti-genotoxic and anti-cancer agent are due to its secondary metabolites spatially carotenoides, which interact with H1- specific Oligonucleotide complexes and induce some conformational changes on them.

Keywords: saffron carotenoides, H1-DNA, interaction, circular dichroism

P11-002: ONTOGENETIC, ANATOMICAL AND HISTOCHEMICAL STUDY OF THE EXTRAFLORAL NECTARIES OF SAPIUM BIGLANDULOSUM MÜELL. ARG. (EUPHORBIACEAE)

Meira, R. - Coutinho, I. A. C. - Valente, V. M. M.*
Universidade Federal de Viçosa
**Corresponding author, e-mail:* rmeira@ufv.br

This paper aims to confirm the nature of the secretory structures found on the petiole and leaf margins of Sapium biglandulosum Müell. Arg. elucidating by this their role and functions. The anatomy, ontogenesis and histochemistry of these glands were studied by light microscopy techniques whereas mono and disaccharides in the exudates have been detected by High Performance Liquid Chromatography.
Twigs were collected and samples were fixed in FAA (formalin-acetic-50% ethanol, 1:1:18 v/v) and then stored in 70% ethanol. The FAA-fixed samples were dehydrated and embedded in paraffin + 8 % wax. The sections were stained with safranin and astra blue. Histochemical tests were performed, most of them in fresh material. High Performance Liquid Chromatography (HPLC) was used for disaccharides and monosaccharides detection. The exudate from the petiole had a total of 32.5 percent of sugar concentration (w/v), from which 38.1% was fructose, 43.7 glucose and 18.2 sucrose. The petiolar gland started its development from a group of meristematic cells which underwent asynchronous divisions. At the end of the ontogenesis, a well-structured vascularized gland made up
of a palisade secretory epidermis, secretory parenchyma and a secretory pore was observed. Leaf margin glands showed a similar anatomy. Histochemical tests revealed the presence of proteins, pectins, carbohydrates, tannins and anthocyanins. Based on the structural features of the secreting tissues, the position and the detection of glucose, fructose, and sucrose in the exudates, there is compelling evidence to characterize these glands as extrafloral nectaries. (FAPEMIG)

P11-003: ETHYLENE AND ABA AFFECT THE PROLIFERATION OF IN VITRO CULTIVATED A. THALIANA CELLS

Stepanchenko, N. - Nosov, A. - Novikova, G. - Moshkov, I.*
K.A. Timiryazev Institute of Plant Physiology Russian Academy of Sciences
**Corresponding author, e-mail:stepashka.nt@gmail.com*

Plant growth and development is determined by a strictly controlled balance between the cell proliferation and differentiation. The major goal is to evaluate a possible interaction between ethylene and ABA signaling routes in the control of cell cycle. Cell suspension cultures of A. thaliana Col-0 and ethylene insensitive mutants etr1 and ctr1 were used. In etr1 multiple tracheary elements were observed while in ctr1 and Col-0 they were almost absent. Thus, ethylene might inhibit tracheary elements formation, i.e. inhibits terminal differentiation. The effect of ABA was examined on the ethylene biosynthesis and the cell proliferation. ABA affected the ethylene synthesis only in ctr1. ABA caused significant decrease in DNA synthesis in Col-0, had no effect in ctr1, while in etr1 led to doubling DNA synthesis. These data correlate well to ABA influence on the rates of cell division revealed by mitotic index measuring. Proteomic approach has allowed us to suggest a number of potential candidates involved in ethylene-ABA cross-talk related to the cell proliferation. Possible molecular mechanisms underlying the effect of ethylene and ABA on the cell cycle will be discussed in the presentation.
The work is supported by RFBR, grant 08-04-000643.

P11-004: NITRIC OXIDE DONOR IMPACT ON PROLIFERATION OF NICOTIANA TABACUM BY-2 CELLS AND MICROTUBULES ORGANIZATION

Krasylenko, Y. - Blume Ya, B. - Yemets, A. I.*
Institute of Food Biotechnology and Genomics NAS of Ukraine
**Corresponding author, e-mail: j_krasylenko@ukr.net*

In plants nitric oxide (NO) functions as a secondary mediator in the control of such fundamental processes as growth and development as well as abiotic and biotic stress responses. Cytoskeleton and particularly microtubules are supposed to be downstream-effectors in NO-signalling cascades because of their involvement in plethora of processes in plants regulated by NO. α-Tubulin nitrotyrosination could realize direct NO-signalling via microtubules in plants, although its presence and functional role have to be elucidated. NO donor sodium nitroprusside (SNP) effects on cortical and endoplasmic microtubules, and also mitotic spindles, phragmoplasts and preprophase bands organization of Nicotiana tabacum ÂÓ-2 cells expressing GFP-MBD were studied in vivo. SNP treatment during 3 h in concentrations of 200 μM, 1 and 5 mÌ led to dose-dependent cortical microtubules stabilization. 200 μÌ SNP caused insufficient cortical microtubules stabilization comparing to the untreated control, whereas 1 mÌ SNP provoked more pronounced cortical microtubules stabilization, and 5 mÌ SNP, besides the stabilization, induced partial depolymerization of cortical microtubules. In cytoplasm of the majority of BY-2 cells treated by 1 and 5 mM SNP the tubulin clusters on the nucleus surface were observed that could indicate endoplasmic microtubules depolymerization. Slight increase of mitotic spindle number after 1 and 5 mM SNP treatment was revealed. Also the organization of mitotic figures became altered in BY-2 cells after

1 and 5 mM SNP treatment. These results indicate that microtubules could be NO-sensors in plant cell proliferation.

P11-005: ETHYLENE AND FUSICOCCIN-INDUCED STRESS RESPONSES IN ACER PSEUDOPLATANUS L. CULTURED CELLS

Malerba, M. - Crosti, P. - Cerana, R.*
Università degli Studi di Milano-Bicocca
**Corresponding author, e-mail: massimo.malerba@unimib.it*

Ethylene is an important gaseous plant hormone involved in many physiological and developmental processes as well as in responses to a variety of biotic and abiotic stresses (van Loon et al., Trends Plant Sci 11:184-191, 2006). Fusicoccin (FC), an activator of the plasma membrane proton pump, induces ethylene synthesis in sycamore (Acer pseudoplatanus L.) cultured cells (Malerba et al., J Plant Physiol;145:711-716, 1995). In these cells FC also induces a set of stress responses included cell death, that in a fraction of dead cells shows apoptotic features (specific DNA fragmentation and cytochrome c release from mitochondria), production of H2O2 and NO, accumulation of regulative 14-3-3 proteins in the cytosol and of HSP 70 molecular chaperone Binding Protein (BiP) in the endoplasmic reticulum. While the dependence of these responses on H2O2 and NO production has been extensively investigated (Malerba et al., Physiol Plant 133:449-457, 2008 and references therein), the possible signalling role of ethylene is still unknown. In this work, by means of Co2+, a specific inhibitor of ethylene biosynthesis, we investigate the possible involvement of ethylene in the above stress responses induced by FC and we compare the effect of FC with that of the ethylene-releasing compound ethephon (2-chloroethane phosphonic acid).

P11-006: TRAFFICKING AND TURNOVER OF THE ARABIDOPSIS IRT1 ROOT IRON TRANSPORTER

Barberon, M.[1] - Zelazny, E.[1] - Conéjéro, G.[1] - Robert, S.[2] - Curie, C.[1] - Frim,l. J.[2] - Vert, G.[1]*
[1]BPMP - UMR5004 - Montpellier
[2]VIB - Department of Plant Systems Biology - Ghent
**Corresponding author, e-mail: barberon@supagro.inra.fr*

Iron is an essential element for plants but toxic when present in excess, leading to a strict control of iron acquisition from the soil. IRT1, is the major root iron transporter, responsible for iron uptake from the soil under iron limitation in Arabidopsis. Previous work from our group showed that IRT1 is transcriptionally regulated by iron, resulting in a high IRT1 expression in iron-starved root epidermal cells. In addition, IRT1 was suggested to be controlled at the post-translational level, with iron affecting IRT1 protein stability, in a similar fashion with the yeast ZRT1 zinc transporter.
To shed light on two poorly-understood phenomena in plants, endocytosis and degradation of plasma membrane proteins, we studied the proposed post-translational regulation of IRT1 in Arabidopsis. Several complementary approaches led us to rule out the existence of an iron-dependent destabilization of IRT1. Rather, our work supports the model where IRT1 undergoes a constitutive internalization and degradation in the vacuole to ensure its proper turnover. We now use IRT1 as a model to decipher the mechanisms driving the internalization of plasma membrane proteins and the routes taken toward the vacuole. We are currently focusing on the role of ubiquitination and FYVE domain-containing proteins in IRT1 dynamics and sorting along the endocytic pathway.

P11-007: COMPLEXITY OF ARABIDOPSIS THALIANA LON1 PROTEASE DUAL TARGETING BY DIFFERENTIAL INITIATION OF TRANSLATION

Daras, G.* - Rigas, S. - Hatzopoulos, P.
Agricultural University of Athens
**Corresponding author, e-mail:* gdaras@aua.gr

Lon proteases control protein turnover and/or protein complex assembly in plant organelles modulating mitochondria biogenesis, a critical step during post-germinative growth and seedling establishment. Previous studies based on ESTs, organelle proteomes, in silico analysis, immunoassays and YFP-cell imaging revealed that Lon1 is targeted to mitochondria. Here, we report that Lon1 mRNA contains two in-frame translation initiation codons potentially encoding for two proteins: one starting at the 1st (Met1) and the other at the 46th (Met46) amino acid. Analysis in the vicinity of both start codons indicated that the sequence context around the second AUG is similar to the reported dicot consensus, while the first AUG context deviates extensively. Translational fusion of the fragment containing the two in-frame initiation codons to YFP led to simultaneous Lon1 targeting to mitochondria and chloroplasts. However, translational fusion of Met46 context revealed Lon1 localization in mitochondria. Intriguingly, substitution of the first AUG context with the second while retaining the second AUG context, targeted the fusion polypeptide to chloroplasts. According to the scanning model, we propose that even though the ribosome binds at the 5' of the Lon1 mRNA, the efficiency of translation initiation from Met1 is low. This AUG skipping allows the ribosome to efficiently recognize Met46-AUG containing a strong Kozak consensus sequence. The results indicate an alternative translation initiation mechanism that preferentially enhances the synthesis of the mitochondria-targeted isoform. This evidence reveals the vital role of Lon1 in mitochondria, in contrast to proteolysis in chloroplasts that is performed by plethora of proteases.

P11-008: INDUCTION OF ENDOREDUPLICATION CAUSED BY DNA DOUBLE-STRAND BREAKS

Umeda, M. - Adachi, S.*
Nara Institute of Science and Technology
**Corresponding author, e-mail:* mumeda@bs.naist.jp

The DNA damage checkpoint is a regulatory mechanism that is essential to precisely transmit genomic information to daughter cells. In yeast and animals, it leads to cell cycle arrest that allows cells to repair damaged DNA, or induces apoptosis. Plants are continuously threatened by various DNA-damaging stresses, thus the mechanism underlying genotoxic stress response is a kind of survival strategies. Here we show that DNA double-strand breaks (DSBs) induce endoreduplication in an ordered fashion in Arabidopsis. The transition to endoreduplication required signaling molecules responsible for DNA damage response, and was accompanied with overall downregulation of mitotic genes. Plants have two types of CDKs that directly control the cell cycle; a Cdc2/Cdc28 orthologue named CDKA and a plant-specific CDKB, which is further classified into two subtypes, CDKB1 and CDKB2. We found that the CDKB2 level was rapidly reduced upon DSBs, and this response was cancelled in DNA damage response mutants. Our results demonstrate that, unlike animals, endoreduplication is a default program in Arabidopsis to respond to DSBs.

P11-009: COMPLEX PLASMA MEMBRANE INVAGINATIONS ARE INVOLVED IN LOCAL PROTON EXTRUSION OF CHARA INTERNODAL CELLS

Foissner, I. - Schmölzer, P.*
Univ. Salzburg, Cell Biology/Plant Physiology
**Corresponding author, e-mail: ilse.foissner@sbg.ac.at*

Internodal cells of the characean green algae are able to generate alternating bands of high and low pH in the medium adjacent to their cell surface. There is a broad consensus that the higher rates of photosynthesis at the acid regions are brought about by an enhanced availability of CO2. There is, however, no agreement about the role of charasomes, complex elaborations of the

plasma membrane, in pH-banding and carbon acquisition. We have recently described plasma membrane domains that can be stained by the endocytic tracers FM1-43 and FM4-64 as well as by the sterol marker filipin. Here we show that these domains are also labelled by the plasma membrane specific dye NBD C6-sphingomyelin, suggesting plasma membrane invaginations, and by Lysotracker red, suggesting acidification. A comparison between the pH-banding pattern and the distribution of plasma membrane areas revealed that size, density and area fraction of plasma membrane domains are significantly higher at the acidic bands as compared with the alkaline regions. Furthermore, the plasma membrane domains are recognized by an antibody against a H+-ATPase which recognizes a 100 kDa band on SDS gels and which preferentially binds to charasomes on ultrathin sections from high pressure frozen cells. Our data suggest that charasomes provide regions separated from the bulk medium by a convoluted diffusion path. H+ exported to such regions will be slower to diffuse away and, hence will be more effective at generating a locally low pH at the cell surface. In such H+-extrusion areas carbonic anhydrases may mediate the dehydration of HCO3- and locally increase the availability of CO2.

P11-010: FREE POLYAMINES AND POLYAMINES CATABOLISM DURING SENESCENCE OF BARLEY LEAVES

Sobieszczuk-Nowicka, E.[1]- Bagniewska-Zadworna, A.[1] - Pietrowska- Borek, M.[2] - Legocka, J.[1]*
[1]Adam Mickiewicz University, Poland
[2]oznan University of LifeSciences
**Corresponding author, e-mail: evaanna@rose.man.poznan.pl*

Leaf senescence represents a key developmental phase in the life of plants. It is a period of massive mobilization of nitrogen, carbon and minerals from the mature leaf to other parts of the plant. Senescence of barley leaves is a highly regulated process and involves cessation of photosynthesis, disintegration of chloroplasts, breakdown of leaf proteins, loss of chlorophyll and removal of amino acids. Significant chromatin condensation, internucleosomal fragmentation of nuclear DNA and enhanced expression of cysteine proteases in senescing mesophyll prove that leaf senescence is a genetically defined process involving mechanisms of programmed cell death.
Changes in free polyamines and their catabolism have been shown to occur in leaf senescence of barley. A feature of this is an increase in diamine and polyamine oxidases expression and activity. The reduction of polyamines titer, mainly spermidine and spermine, throught the process suggests that it might be the process inducer. Hydrogen peroxide produced by polyamines oxidases may act as signal molecule or as cytotoxic agent. Besides, there is other possible role for free polyamines in senescence: regulation of the expression of senescence-related genes.
Acknowledgment: this work was supported by Polish Ministry of Science and Higher Education research grant N N303 418236.

P11-011: COMPARTMENT SPECIFIC LOCALIZATION OF GLUTATHIONE AND ITS PRECURSORS DURING ENVIRONMENTAL STRESS SITUATIONS

Müller, M. - Zechmann, B.*
Institute of Plant Sciences, University of Graz
**Corresponding author, e-mail: maria.mueller@uni-graz.at*

Glutathione as an antioxidant is involved in the detoxification of reactive oxygen species, which are commonly formed during various environmental stress situations. Glutathione metabolism involves highly compartment specific pathways and limitations in the ability of glutathione to protect the plant against stress situations can only be detected if glutathione contents are analyzed at the subcellular level. For this purpose an immunogold cytohistochemical approach was developed and adapted to different plant material in order to detect and quantify subcellular glutathione and its precursors with computer-supported transmission electron microscopy [1,2]. These studies showed that the distribution of glutathione is similar in different plant species (Arabidopsis thaliana, Cucurbita pepo, Nicotiana tabacum, Beta vulgaris). The accuracy of the glutathione-labeling method was supported by different observations. First pre-adsorption of the anti-glutathione antisera with glutathione reduced the density of the gold particles to background levels. Second, the overall glutathione-labeling density was reduced by about 90% in leaves of the glutathione-deficient Arabidopsis mutant pad2-1 and increased in plants with enhanced glutathione accumulation. Further studies showed changes in the compartment specific distribution of glutathione and its precursors during abiotic and biotic stress situations (e.g. heavy metal, virus infection) and demonstrate the compartment specific importance of glutathione metabolism for plant defense.
This work was supported by the Austrian Science Fund (FWF P16273, P18976, P20619).
1. Zechmann B. et al., J.Microscopy 55 (2006) p173.
2. Zechmann B. et al., J. Exp. Bot. 59 (2008) p 4017.

P11-012: TOWARDS A COMPREHENSIVE MODEL FOR SETTING UP ENDOPOLYPLOIDIZATION DURING TOMATO FRUIT DEVELOPMENT

Bourdon, M.[1] - Coriton, O.[2] - Cheniclet, C.[1] - Brown, S.[3] - Peypelut, M.[4] - Chevalier, C.[1] - Renaudin, J.P.[1] - Frangne, N.[1]*
[1] Biologie du Fruit, INRA et Université de Bordeaux, Centre INRA Bordeaux-Aquitaine, France
[2]Amélioration des Plantes et Biotechnologies Végétales, Plateforme Cytogénétique Moléculaire Végétale, INRA, Université de Rennes, France
[3]Institut des Sciences du Végétal, UPR 2355 CNRS, Gif-sur-Yvette, France
[4]Pôle Imagerie du Végétal, Bordeaux Imaging Center, Bordeaux, France
**Corresponding author, e-mail: matthieu.bourdon@bordeaux.inra.fr*

In the course of plant development, increased ploidy levels (referred to as Endopolyploidization) are frequently observed in vegetative and/or reproductive organs of many Angiosperm species. Endopolyploidization consists in a nuclear DNA duplication in the absence of subsequent mitosis and cell division. Despite its strong occurrence, little is known about its functional role [1, 2]. In tomato (Solanum lycopersicum), we have shown that most pericarp cells undergo Endopolyploidization duringfruit development [3]. To go further in the elucidation of the functional role of Endopolyploidization, we decided to address its onset in the course of fruit development in close relationship with cell expansion and differentiation. The use of FISH methodology allowed to consider Endopolyploidization at different levels. First, we analysed the spatial organization of chromosomes in endopolyploid cells and showed that the chromosomes are polytenic, the sister chromatids remaining attached to the same centromere. Then we established a model for ploidy distribution in the tomato pericarp tissue, by assessing the ploidy level of nuclei in their tissue context, thus opening the way towards a detailed description of pericarp development. Such a distribution, correlated with an acquisition of highly specific cell features (cell size, nuclear morphology and mitochondria distribution at the nucleus periphery) during fruit growth provided us with some essential clues in order to clarify the intricate relationship between Endopolyploidization and cell differentiation, in the context of fruit development.
1E. dgar and Orr-Weaver, Cell, 297-306, 105 (2001)
2J.oubès & Chevalier, Plant Mol. Biol., 735-745, 43 (2000)
3. Cheniclet et al., Plant Physiol., 1984-1994, 139 (2005)

P11-013: SHAPING A PROTUBERANCE - THE MECHANICS OF CELLULAR GROWTH

Chebli, Y.1 - Aouar, L.1 - Fayant2 - Anja Geitmann1*

[1]*Institut de Recherche en Biologie Végétale - Université de Montréal*
[2]*Ecole Polytechnique de Montréal*
**Corresponding author, e-mail: youssef.chebli@yahoo.ca*

Cellular growth in plant cells is driven by the turgor pressure but controlled by the mechanical properties of the cell wall. The formation of a cellular protuberance requires the spatially confined yielding of the wall. Using finite element modeling, a technique used in engineering, we established a theoretical model of tip growth as it occurs in pollen tubes and root hairs.
The model predicts that a characteristic spatial distribution of mechanical properties in the cell wall is required to produce the self-similar growth pattern that characterizes these cell types. The mechanical profile is characterized by a steep increase in cell wall extensibility in the transition zone between dome-shaped apex and cylindrical region of the cell.
To compare the theoretical requirements with the biological reality, we quantitatively assessed the spatial distribution of various cell wall components in the pollen tube wall of Lilium and Arabidopsis. We used immunofluorescence methods combined with quantitative image analysis to locate pectin, cellulose and callose. We found remarkable agreement between the expected gradient in cell wall extensibility and the distribution of de-esterified pectin polymers. Furthermore, we identified the orientation of cellulose microfibrils using scanning electron microscopy and found that their mechanical support is particularly important in the transition region. Callose on the other hand provides mechanical support to the cylindrical shank of the cell. Our data show that tip growth is produced by a highly controlled interplay of cell wall assembly processes and that each component is important for different aspects governing the shape and growth dynamics of the elongating cell.

P11-014: KUNITZ TRYPSIN INHIBITOR AS A PLANT CELL DEATH MODULATOR

Komarova, T. - Sheval, E. - Dorokhov, Y.*
Moscow State University, A.N.Belozersky Institute
**Corresponding author, e-mail: t.v.komarova@gmail.com*

Normally plant cell is adapted for producing large amounts of protein which is targeted and stored in special cellular compartments. But intensive uncontrolled and untargeted protein production is likely to result in programmed cell death (PCD). To study cell death of protein overexpressing plant cell we created specific vector system, TMV:GFP, allowing GFP production in huge amounts and resulted in cells death 3 days after agroinjection. Using microscopy and biochemical approaches we described the following features of the process: stages of cell and tissue death; GFP behavior in aggregates and its proper folding. Moreover we analyzed the pattern of mRNA expression in Nicotiana benthamiana tissues just before their death using subtractive hybridization. Several candidate genes including specific biotic cell death-associated protein (CDM) were found to be upregulated. NbCDM appeared to have strong homology with Kunitz trypsin inhibitors (KTI) family which is specific for serine proteases been implicated in PCD in plants. Joint expression of NbCDM under control of 35S promoter (35S-NbCDM) with TMV:GFP resulted in enhanced lesion development whereas 35S-NbCDM per se didn't induce necrotisation. Upregulation of NbCDM expression was also registered as a response to virulent bacterial pathogen Ralstonia solanacearum or even after its NLS-containing protein overexpression. Asit is well known that PCD requires a coordinate activation of different factors such as proteases and suppressors, NbCDM is likely to be one of such players inhibiting and modulating these processes.

P11-015: GENERATION OF NITRIC OXIDE UNDER ANOXIA IS INDEPENDENT OF NITRATE REDUCTASE ACTIVITY.

Kingston-Smith, A. - Eelen, I. - Davies, T.E. - Mur, L.A.J. - Aberystwyth University*
**Corresponding author, e-mail: ahk@aber.ac.uk*

Exposure of plants to abiotic stress causes changes in foliar protein which can affect crop yield and quality. This is also true of plant parts ingested by grazing ruminants whereby leaf cells are exposed to multiple stresses (heat, anoxia, microbial attack) in the hours following rumen entry causing autolysis. This has implications for the poor use of feed N by ruminants, which results in N deposition on land (as animal wastes) and contributes to N2O generation. As the cell signal NO has been implicated in cell death under biotic and abiotic stress this work investigated its potential role in control of autolysis in ingested plant cells. NO was detected in Arabidopsis thaliana Col-0 leaf discs after in vitro exposure to rumen-stress conditions. The source of NO generation in plants is currently unknown but could occur enzymically (catalysed by nitrate reductase; NR) or non-enzymically, an is enabled by an increase in intracellular nitrite under low oxygen conditions. The role of NR in NO generation under anoxia was determined by exposure of leaf discs from Col-0 and mutants Nia 1, Nia 2 and AtNos (which had 56%, 5% and 100% of NR activity of Col-0) to rumen-like conditions. NO was detectable by fluorescence microscopy in all lines after 1h. These results, plus no significant differences in foliar nitrite suggest that non-enzymic NO production in ingested plant cells could be responsible for autolysis.

P11-016: ASYNCHRONOUS DEATH OF STOMATA GUARD CELLS PREDETERMINED BY THE ASYMMETRIC OPEN MITOSIS

Selga, T. - Maija, S.*
Faculty of Biology, University of Latvia
**Corresponding author, e-mail: turs.selga@lu.lv*

The control of programmed cell death (PCD) by previous asymmetric cell division (ACD) in plants is far from clear. The stomatal cell lineage is a typical example of precisely planned series of ACDs, that finish with as if symmetrical division of the guard mother cell (GMC) to form identical stomatal guard cells (GC) (Hove and Heidstua 2008, Nodeau 2009, Dong et al 2009) We searched among several hundreds of stomata in various stages of their development and senescence from the young, full grown and senescing leaves of tobacco (Nicotiana tabacum L.) by use of several methods and techniques of cyochemistry and microscopy. Our data suggests that the stomatal GMCs in reality always divide spatially asymmetrically by the classic open mitosis and develop unequal GCs, that age and die differently by different alterations of their nuclei and other organelles. Perinuclear endoplasmic reticulum in one of sister cells disappears faster than in another. The GMCs and GCs always are structurally joined at least with one nucleus of adjacent cell and migration of variously stained nuclear substance among them is evident. The permanent plastid-nuclear complex (PNCs) that we have repeatedly demonstrated in photosynthesizing cells of different vascular plants exists in all GMCs and GCs during all their lifespan, and evidently are important control units.

P11-017: IDENTIFICATION OF PHOSPHORYLATED RESIDUES IN THE H+-ATPASE AND PHYSIOLOGICAL CONSEQUENCES OF THE EXPRESSION OF AN ACTIVATED FORM IN THE PLANT

Duby, G. - Piette, A.S. - Piotrowiak, D. - Salladini, A. - Boutry, M.*
Institut des Sciences de la Vie, Université Catholique de Louvain
**Corresponding author, e-mail: geoffrey.duby@uclouvain.be*

The plasma membrane H+-ATPase is building a proton electrochemical gradient that activates secondary transporters. It is involved, in cytosolic pH regulation, cell elongation and stomata aperture. It contains an auto-inhibitory C-terminal region and is

activated by phosphorylation of its penultimate residue, a threonine and the consecutive binding of regulatory 14-3-3 proteins to the enzyme C-ter. Mass spectrometry analysis of purified PMA2 (Plasma membrane H+-ATPases from N. plumbaginifolia) led to the identification of new phosphorylation sites. Phosphorylated Ser938 and Thr931, both located in the PMA2 14-3-3 binding site, were shown to act as negative regulators of the 14-3-3 binding and hence the enzyme activation whereas phosphorylated PMA2 Thr889, also located in the enzyme C-ter but outside the 14-3-3 binding site, seemed to be involved in an activation mechanism independent of the 14-3-3 protein binding. Altogether 4 phosphorylated sites concur to a complex regulation of the H+-ATPase. To identify the kinases involved, proteins co-purified with a His-tagged PMA2 isoform expressed in tobacco BY2 suspension cells were analyzed by mass spectrometry. Some putative kinases have been identified. To better characterize the physiological roles of the H+-ATPase in the plant, an activated enzyme was expressed in tobacco and Arabidopsis. Transgenic plants had a pleiotropic phenotype with, for example, a modified development and a better resistance to salt stress and basic pH.

P11-018: PATTERN FORMATION DURING SOMATIC EMBRYOGENESIS IN SCOTS PINE

Abrahamsson, M. - Valladares, S. - von Arnold, S.*
Swedish University of Agricultural Sciences, Department of Plant Biology and Forest Genetics
**Corresponding author, e-mail:* malin.abrahamsson@vbsg.slu.se

Somatic embryogenesis is an attractive method to propagate conifers vegetatively. However, in order to efficiently regulate the formation of plants via somatic embryos it is important to understand how the somatic embryos develop. The aim of this study has been both to elucidate the development of somatic embryos in Scots pine and to identify deviations from the normal plan leading to developmental arrest or to progressive accumulation of errors resulting in aberrant cotyledonary embryos. We have compared the developmental pathway of somatic embryogenesis in representative cell lines yielding cotyledonary embryos with normal and abnormal morphology. Embryogenic cultures of Scots pine are initiated from immature embryos during the cleavage phase, and proliferation by cleavage can also be observed in embryogenic cultures. In all cell lines a large proportion of the developing embryos degenerate but the degeneration pattern differs among cell lines. However, there were no fundamental differences in the early patterning of embryos between the cell lines except that the early somatic embryos in cell lines giving rise to abnormal embryos carried supernumerary suspensor cells, resulting in an unbalanced ratio between the embryonal mass and the suspensor, which partly can be explained by an aberrant polar auxin transport.

P11-019: ISOLATION AND CHARACTERIZATION OF ENDOSOMAL COMPARTMENTS IN ARABIDOPSIS

Sancho Andrés, G. - Groen, A.J. - Lilley, K.S. - Aniento, F.*
Universidad de Valencia
**Corresponding author, e-mail: gloria.sancho@uv.es*

The plasma membrane (PM) of plant cells undergoes dynamic changes in protein composition. Several PM proteins have been shown to cycle between the PM and endomembrane compartment(s), whereas other PM proteins are internalised and targeted to the vacuole for degradation. PM protein dynamics thus determines cell behaviour and affects plant performance. However, our current knowledge of the underlying mechanisms of these processes in plants is virtually non-existent. In animals, early/sorting endosomes are important sites for receptor signaling. Although this may also apply to plants, there are no markers to distinguish plant early/sorting from recycling endosomes. The endosomal compartments in which plant PM proteins are sorted for degradation or recycling to the PM are morphologically and functionally not defined, and their composition in terms of resident and cargo proteins is essentially unknown. In this work, we have carried out two proteomics methods to characterise endosomal compartments in Arabidopsis. Firstlyw, e have combined subcellular fractionation with LOPIT (Localization of Organelle Proteins by Isotope Tagging), a method which allows assignment of proteins to organelles using high throughput quantitative proteomics approaches. Secondly, in parallel, we have performed immunoisolation experiments, using as antigen the human transferrin receptor (hTfR), a model receptor for endocytosis in animal cells, heterologously expressed in transgenic Arabidopsis plants.

P11-020: NOVEL INSIGHTS INTO AQUAPORIN TRAFFICKING TO THE PLASMA MEMBRANE

Besserer, A. - Bienert Gerd, P. - Chevalier, A. - Zelazny, E. - Chaumont, F.
Institut des sciences de la vie, Université catholique de Louvain

The movement of water across plant plasma membrane (PM) depends on the amount and activity of aquaporins belonging to the Plasma membrane Intrinsic Proteins (PIP) subfamily. Recently, we showed that maize aquaporins belonging to PIP1 and PIP2 groups form hetero-oligomers when co-expressed in leaf mesophyll protoplasts. This physical interaction regulates their trafficking and triggers relocalization of ZmPIP1 from the endoplasmic reticulum (ER) to the PM (Zelazny et al., 2007;2009). This result suggests that ZmPIP1s carry ER retention signals which are inefficient upon hetero-oligomerization. Expression of mutated and chimeric PIPs indicates that the loop A of ZmPIP1;2 may contain an ER retention signal as its replacement with ZmPIP2,5 loop A leads to some extent to ZmPIP1;2 trafficking to the PM, but only if an additional ER export motif (N-terminal diacidic acid motif) is present. Regulation of PIP trafficking to the PM was further characterized in maize protoplast and in tobacco epidermal cells by co-expressing ZmPIP2;5 and the dominant negative mutant syntaxin SYP121-sp2. Our results showed that ZmPIP2;5 traffic to the PM is hampered by SYP121-sp2. Putative interaction between SYP121 and ZmPIP2;5 is under investigation. The membrane osmotic water permeability decreased in cells co-expressing ZmPIP2;5 and SYP121-sp2. Altogether data point toward a complex and highly integrated regulation of PIP trafficking in the maintenance of cellular water homeostasis.

P11-021: COMPARISION OF GALACTOGLUCOMANNAN OLIGOSACCHARIDES ACTION ON CELL ELONGATION IN HYPOCOTYL AND PRIMARY ROOT

Richterová, D. - Kollárová, K. - Lišková, D.*
Institute of Chemistry, Slovak Academy of Sciences
**Corresponding author, e-mail:* danica.richterova@savba.sk

Galactoglucomannan oligosaccharides (GGMOs) inhibit the auxins-induced elongation growth of stem segments and this effect is dependent on their chemical structure and concentration. GGMOs influenced induction and elongation of adventitious and lateral roots. The aim of this work was to answer the question: is the effect of GGMOs in elongating hypocotyls and roots related with the elongation or division of cells, and in which tissues? GGMOs were derived from spruce galactoglucomannan. Modified GGMOs - GGMOs-g were prepared by treatment of GGMOs with purified α-galactosidase. Uniform seedings of mung bean (Vigna radiata (L.) Wilczek) were transferred to hydroponic Hoagland solution containing GGMOs or GGMOs-g alone and/or in combination with IBA. Plants were grown 7 days in controlled conditions and then the length of hypocotyl and primary root was measured. For light microscopy the whole-mount procedure was used and the samples were stained with toluidine blue. The length of cells was determined by Lucia analysis system. The data were analyzed using ANOVA. GGMOs alone or in combination with IBA inhibited hypocotyl elongation, but they stimu-

lated primary root growth. However, distinct effect of GGMOs-g compared with GGMOs has been observed, and a different way of elongation in single tissues after GGMOs treatment has been determined (in the epidermis or rhizodermis, and in the primary cortex). The hypocotyl or root elongation growth induced by GGMOs was related to epidermal/rhizodermal cells length and primary cortical cells division. GGMOs didn't affect the length of primary cortical cells compared with IBA or the control.
Acknowledgement: This work was supported by grants - VEGA 1/0472/10, 2/0046/10 and APVT 51-013304.

P11-022: DISTINCT POPLAR DEFENSE STRATEGIES AGAINST HERBIVORES REQUIRE DIFFERENT TYPES OF EXTRAFLORAL NECTAR SECRETION

Escalante-Pérez, M.[1] - Reinders, J.[2] - Lautner, S.[3] - Fromm, J.[3] - Hedrich, R.[1] - Ache, P.[1]*
[1]University Würzburg, Biozentrum, Julius-von-Sachs-Institut für Biowissenschaften
[2]University Regensburg, Institute of Functional Genomics
[3]University Hamburg, Zentrum Holzwirtschaf
**Corresponding author, e-mail: escalante@botanik.uni-wuerzburg.de*

A large number of plant species grow extrafloral nectaries and produce nectar to attract predators such as ants to defend themselves against herbivores. We studied how insect feeding feeds back on nectary development and activity in Populus. Thereby we analyzed nectaries anatomy, ecology, gene expression and nectar chemistry from Populus trichocarpa and P. tremula x P. tremuloides (Ptt). Both vary widely in morphology and structure and thus differ in the type of nectar secretion which leads to different defense strategies. While in Ptt the presence of nectaries is constitutive, nectary appearance in P. trichocarpa is strictly inducible. Simulating insects foraging with P.trichocarpa we could demonstrate that wounding induces formation of non-secreting nectaries, while nectar production requires the involvement of an herbivore delivered elicitor. In line with these findings, microarray analysis of Ptt nectaries vs. leaves revealed up-regulation of genes involved in hormone action, stress, cell wall and sugar metabolism. Ptt nectar is very likely released via exocytosis. Consequently genes involved in lipid metabolism and secretion are also induced in nectaries.

P11-023: COMMON PLAYERS IN ORGANELLES DIVISION PROCESSES: MORPHOLOGICAL AND MOLECULAR ANALYSIS IN PLANT

Ruberti, C. - Costa, A. - Zottini, M.*
Dipartimento di Biologia, Università degli Studi di Padova, Italy
**Corresponding author, e-mail: cristina.ruberti@gmail.com*

Fiorella Lo Schiavo (Dipartimento di Biologia, Università degli Studi di Padova, Via U. Bassi 58/B, 35131 Padova, Italy.)
Mitochondria and peroxisomes are highly dynamic organelles with a large plasticity in their shape and morphology. In particular, recent reports show that mitochondrial morphology changes during plant senescence. Studies in mammals, fungi and plants have led to the finding that mitochondria and peroxisomes partially share components of their division machinery, such as dynamin-like and fission-like proteins. In Arabidopsis, dynamin-like proteins DRP3A and DRP3B and the fission like protein BIGYIN have been implicated in mitochondrial and peroxisome fission. In addition, another specific plant factor ELM1 has been recently reported to be involved in mitochondria fission. In order to gain inside the molecular mechanisms involved in the remodelling of organelles we analysed throughout the plant development the expression pattern and the subcellular localization of BIGYIN and ELM1. To this aim Arabidopsis transgenic plants expressing the GUS reporter gene under the control of the BIGYIN and ELM1 promoters, and plants transformed with pBIGYIN-YFP:BIGYIN construct have been generated. Moreover, BIGYIN and ELM1 fused to different fluorescent proteins (YFP and DsRed2) were transiently co-expressed in Arabidopsis mesophyll protoplasts. The same system was also employed to test the in vivo protein-protein interaction by means of bimolecular fluorescent complementation. Our data show that ELM1 and BIGYIN have a highly specific expression pattern in the plant, and that their subcellular localization is not restricted to the subcellular compartments previously described.

P11-024: RECRUITMENT OF GLUTATHIONE INTO THE NUCLEUS DURING CELL PROLIFERATION IN ARABIDOPSIS THALIANA

Foyer, C.H.[1] - Diaz Vivancos, P.[2] - Pellny, T.K.[3] - Markovic, J.[4] - Pallardó, F.V.[4]
[1]University of Leeds
[2]CEBAS-CSIC
[3]Rothamsted Research
[4]University of Valencia-CIBERER

Cellular redox homeostasis is considered to be important in the regulation of cell proliferation but little information is available on how redox control regulates the cell cycle or regarding the precise functions of key redox metabolites. The intracellular redox state of Arabidopsis cells is modulated during proliferation by interplay between the pyridine nucleotides, glutathione and ascorbate pools. Evidence of similarities in the redox control of cell proliferation in animals and plants will provided. For example, GSH is recruited into the nucleus early in cell proliferation in *Arabidopsis thaliana*. GSH accumulation in the nucleus was triggered by treatments that synchronize cells at G1/S as identified by flow cytometry and marker transcripts. Significant decreases in transcripts associated with oxidative signaling and stress tolerance occurred when GSH was localized in the nucleus. Increases in GSH1 and GSH2 transcripts accompanied the large increase in total cellular GSH observed during cell proliferation, but only GSH2 was differentially expressed in cells with high GSHn relative to those with an even intracellular distribution of GSH. Of the 7 Bcl-2 associated (BAG) genes in *A. thaliana*, only the nuclear-localized BAG 6 was differentially expressed in cells with high GSHn compared to GSHc. We conclude that GSHn is associated with decreased oxidative signaling and stress responses and that whole cell redox homeostasis is restored as the cell cycle progresses by enhanced GSH synthesis and accumulation in the cytoplasm.

P11-025: THE EFFECTS OF LOW AND HIGH TEMPERATURES ON ULTRASTRUCTURE OF BRASSICA CAMPESTRIS AND AMARANTHUS CAUDATHUS LEAVE CELLS

Klymchuk, D. - Vorobyova, T. - Kosakivska, I.*
M.G. Kholodny Institute of Botany, NAS of Ukraine
**Corresponding author, e-mail: microscopy@botany.kiev.ua*

The effects of low and high temperatures on the subcellular structure of mesophyll and bundle sheath cells in leaves of Brassica campestris var. olifera and Amaranthus caudathus L. belonging to plants with C3 and C4 carbon fixation, respectively, were evaluated. Plants were grown under regime 15 h light (5500±500 lx) and 9 h dark at a temperature 24±1ºC. 25-day-old plants during dark period were subjected to low positive (4ºC) and high (40ºC) temperatures for 2 h. Leaf samples taken from the middle region of the true leaves were fixed and cross sectioned (50-60 nm) for TEM analysis. Low and high temperatures decreased the volume of chloroplast starch granules, altered the amount of plastoglobuli per chloroplast and the amount cytoplasmic lipid drops in mesophyll and bundle sheath cells from both plants. Additionally, high temperature induced the alteration in mitochondrion inner and cytoplasmic membrane structures in mesophyll cells from both plants. These data suggest that short-term temperature stresses influenced first of all chloroplast starch deposition resulted the alteration in their metabolism. The probable roles of ultras-

tructural rearrangements in cell organelles during adaptation to temperature stresses are discussed.

P11-026: VESICLE DELIVERY IN POLLEN TUBES: PERFECTLY COORDINATED INTRACELLULAR LOGISTICS

Van Oostende, C.[1]- Guillet, D.[2] - Juillot, S.[1] - Geitmann, A.[1]*
[1]Institut de recherche en biologie végétale-Universite de Montreal
[2]Mc Gill University
**Corresponding author, e-mail: chloevanoostende@gmail.com*

Cellular growth in plants implies the continuous assembly of new cell wall surface to prevent the expanding wall from rupturing. Most plant cells are characterized by diffuse growth that requires cell wall assembly over large surfaces. In contrast, the generation of complex cell shapes necessitates spatially confined growth and assembly processes. An example for this is the pollen tube, which displays tip-growth, where the expansion occurs solely at the extreme end of the cell. Pollen tube growth plays a crucial function during sexual plant reproduction since it ensures the delivery of the male gametes to the ovules which are nestled deep in the pistillar tissues of the receiving flower.
Pollen tube growth is extremely fast and, therefore, cell wall assembly is the principal metabolic activity. The spatial confinement of the cell wall assembly requires an intracellular transport system that is precisely controlled in space and time. Hence, trafficking of vesicles and other elements of the endomembrane system (Golgi, endoplasmic reticulum, endosomes) must be subject to a sophisticated system of cellular transport logistics mediated by the cytoskeleton. By combining high temporal and spatial resolution laser scanning microscopy with advanced imaging techniques originally developed for molecular movements (STICS, spatio-temporal image correlation spectroscopy), we monitored the delivery of vesicles towards the tube apex, the movements of organelles and the dynamics of the cytoskeleton. We used these motion profiles to generate a mathematical model of intracellular trafficking that will help us to understand the logistic transport principles employed by growing plant cells.

P11-027: GENERATION OF ACETOLACTATE SYNTHASE INHIBITORS - TOLERANT ALFALFA

Feuillebois, P[1] - Schaller, A.[2] - Hain, R.[1]*
[1]Bayer CropScience, Herbicide Research
[2]University of Hohenheim, Institute of Plant Physiology and Biotechnology
**Corresponding author, e-mail: pauline.feuillebois@bayer-cropscience.com*

The cultivated alfalfa, Medicago sativa, is one of the most valuable legumes in the world. It is a perennial dicot with a tetraploid genome, rich in proteins, minerals and vitamins. Alfalfa is thus widely grown as forage for cattle. Chemical weed control is often necessary at an early stage since weeds in alfalfa fields reduce the yield and the crop quality. To enable better weed control in alfalfa, improving crop and seed production quality, our project aims to generate Acetolactate Synthase Inhibitors (ALSi) tolerant alfalfa using both GMO and non-GMO approaches. An in vitro alfalfa tissue culture has been set up by screening RA3 individuals (Medicago sativa L., cv Regen S, clone RA3) for their embryogenic response. Conditions have been optimized allowing regeneration cycles of fifty days on average. As the Acetolactate Synthase (ALS) enzyme is the target of ALSi herbicides, two ALS genes (MEDSA_ALSa and MEDSA_ALSb) have been identified in Medicago sativa and their sequences have been obtained. In the non-GMO approach, chemical mutagenesis is performed on somatic embryos to obtain putative ALSi tolerant mutants, screened using sulfonylurea herbicides (SUs). For the GMO approach, Agrobacterium tumefaciens - mediated alfalfa leaf disc transformations are carried out using an Arabidopsis thaliana mutated ALS gene conferring ALSi tolerance fused to a transit peptide. Somatic embryos obtained are selected with SUs to evaluate the potential herbicide tolerance. Positive tolerant plants will be tested for SUs tolerance in the greenhouse and fully characterized using molecular biology methods.

P11-028: MECHANISTIC FRAMEWORK FOR POLAR PIN AUXIN EFFLUX CARRIER TARGETING IN PLANT CELLS

Kleine-Vehn, J. - Jiri Friml*
VIB - Gent university
**Corresponding author, e-mail: jukle@psb.ugent.be*

Cell polarity is indispensable for differentiation, proliferation and morphogenesis of unicellular and multicellular organism. Most of our knowledge is derived from animal epithelial cells that organize cell polarity via the formation of tight junctions that separate distinct plasma membrane domains. Polarized plant cells lack tight junctions and the underlying mechanism that ensures cell polarity in plants is largely unknown.
PIN proteins are prominent polar cargos that determine the direction and rate of the cellular export of the phytohormone auxin. Here we provide molecular and mechanistic insights into polar PIN targeting. Quantitative life-cell imaging techniques revealed that plant cells facilitate spatially defined exo- and endocytosis by regulated endosomal trafficking and subsequent short range vesicle transport. These endosome-based mechanisms enable superpolar exocytosis to an inner core in the apical plasma membrane domain and the retrieval of non-polar PIN proteins by spatially defined endocytosis via a clathrin-dependent mechanism at the apical-lateral cell junction (adjacent to the polar domain). This interweaving mechanism for PIN polarity maintenance gets further stabilized by a sterol-dependent clustering of PIN proteins in the plasma membrane, largely abolishing their lateral diffusion.
Our findings provide the first mechanistic insight of how non-epithelial cells, such as plant cells, maintain polar plasma membrane domains.

P11-029: REQUIREMENTS FOR NUCLEAR AND NUCLEOLAR LOCALIZATION OF ARABIDOPSIS RIBOSOMAL PROTEIN L23A

Bonham-Smith, P. - Savada, R.P.*
University of Saskatchewan
**Corresponding author, e-mail: peta.bonhams@usask.ca*

The mechanism underlying the transport of plant r-proteins from the cytoplasm into, and their retention in, the nucleus/nucleolus is not understood. R-protein L23a is one of 81 r-proteins in Arabidopsis. It is present in two isoforms; L23aA that is essential for plant development under normal conditions and L23aB is not. Both isoforms have nine putative Nuclear Localisation Sites. Site Directed Mutagenesis of any one NLS has no effect on nuclear/nucleolar localization of L23aA. Simultaneous mutation of all nine NLSs has no effect on nuclear localization, however, nucleolar localization is completely disrupted. Combinatorial mutation studies show that, five (10KKAD13, 17KALK20, 86KK87, 121KK122, and 133KK134) NLSs are required for nucleolar localization. We are currently investigating: i) if nuclear localization of L23aA is mediated by the classic importin pathway and ii) what differentiates movement into and retention of L23aA in the nucleolus (rRNA binding) from nuclear localisation.

P11-030: THE ROLE OF REVERSIBLE ZEATIN GLUCOSYLATION IN THE HOMEOSTASIS OF CYTOKININS

Kiran, N. S. - Dubova, J. - Brzobohaty, B. -*
**Corresponding author, e-mail: kiran@mendelu.cz*

Reversible glucosylation of zeatin-type cytokinins is important for the homeostasis of active cytokinin forms at certain developmental stages. The sub-cellular location of this conversion

is proposed to affect the levels of the active cytokinin forms at particular locations and times during plant development. Using the zeatin-O-glucoside (ZOG)-specific ß-glucosidase Zm-p60.1, we have been able to disrupt the zeatin metabolic network during early tobacco seedling development and have shown that the vacuole is indeed the storage organelle for ZOG. We investigated the phenotypes of the progeny of crosses with tobacco plants over-expressing the glucosyltransferase ZOG1. During early development of the seedling (14 days after sowing - 9-10 days after germination), the different sub-cellular variants of Zm-p60.1 show divergent responses with respect to hypocotyl elongation on MS medium. Root lengths on medium containing zeatin are also divergent. The molecular and physiological changes underlying the observed phenotypes will be discussed.
Supported by grant Nos. LC06034 (Czech Ministry of Education, Youth and Sports) and 204/09/P289 (Czech Science Foundation)

P11-031: REBELOTE, ANOTHER LINK BETWEEN RIBOSOMAL PROCESSING AND ARABIDOPSIS DEVELOPMENT

de Bossoreille, S. - Morel, P. - Boltz, V. - Tréhin, C. - Negrutiu, I.*
ENS Lyon
**Corresponding author, e-mail: steve.de.bossoreille.de.ribou@ens-lyon.fr*

Bridges between nucleic acids sequences and proteins, ribosomes are central components and the "auletes" of living cells. Composed of ribosomal proteins and RNA, they move during their biogenesis from the nucleolus to the cytoplasm, where they translate RNA messengers into proteins. In the past years, some mutants of ribosomal-biogenesis-related proteins have shown the importance of these proteins during cell division and Arabidopsis development. The impact of ribosomal defects on development could be explained by dose effect (which could be important for cell fitness), specificity of ribosomes for some mRNA or multifunctional ribosomal proteins (Mary E. Byrne, 2009). Here I present our work on REBELOTE (RBL), one of the two Arabidopsis homologs of the yeast NOC2 protein, which act during the ribosomal 60S subunit biogenesis. Mutations in REBELOTE gene cause a range of phenotypes, from embryo lethality to growth defects (reduced plant size, altered leaf shape…). To have a better understanding of RBL-controlled processes, we first analyzed the ribosomal function of RBL, and searched for its protein partners. Our results shows that RBL act in two different nucleolar complexes supposed to regulate 60S ribosomal subunit biogenesis. Subsequently, we focused on the effects of rbl mutations on the cell division/elongation processes. Our work shows that defects observed at molecular and cellular levels could explain the slow down of cell divisions and growth delay in rbl mutants.

P11-032: NATURAL TOLERANCE AS A BASIS FOR THE DEVELOPMENT OF NEW HERBICIDE TOLERANCE TRAITS IN AGRICULTURE

Koehler, J.[1]- Porré, F.[2] - Schaller, A.[3]*
[1]University of Hohenheim, Stuttgart
[2]Bayer CropScience Frankfurt
[3]Plant Physiology and Biotechnology
**Corresponding author, e-mail:* judith.koehler@bayercropscience.com

Herbicides are chemicals used in agriculture to control unwanted weeds. Since the late 1940s, many herbicidal agents were discovered and extentsively used. As a consequence of extensive herbicide use, herbicide-resistant weed populations emerged over the past years. Modern agriculture has to face these problems and herbicide-tolerant crops are part of new weed control systems: they consist of a non-selective herbicide and a corresponding herbicide-tolerant crop. The underlying difficulty is the identification of traits leading to tolerance in plants and a better understanding of the mechanisms of tolerance is warranted.
It was recently shown the the green alga Chlamydomonas reinhardtii shows natural tolerance to some herbicides. Therefore, we are trying to identify the mechanism responsible for this tolerance. In the last decades, this unicellular alga has become a powerful model organism for the study of a number of fundamental topics in molecular biology and many tools are available that will help in the identification of the mechanism. Two species of the genus Callistemon have also been shown to be tolerant to herbicides. The mechanism of tolerance will be identified and compared to the one present in the algae.
A better understanding of herbicide tolerance and also the identification of traits are essential for agricultural purposes in modern weed control systems

P11-033: THE CONSERVED MEMBRANE PROTEIN BAB REGULATES BASL POLAR LOCALIZATION AND IS REQUIRED FOR ASYMMETRIC CELL DIVISIONS IN ARABIDOPSIS

Michniewicz, M. - Bergmann, D.*
Stanford University
**Corresponding author, e-mail: martap@stanford.edu*

Asymmetric cell division is fundamental for generation of cellular diversity during animal and plant development. The regulation of this process is mainly achieved by polarization of the cell along its axis and asymmetric segregation of cell fate determinants. The recently identified BASL protein is a novel regulator of asymmetric divisions in Arabidopsis. BASL exhibits a unique subcellular pattern of localization within stomatal-lineage cells: before asymmetric division BASL accumulates in a polarized crescent at the cell periphery and after division re-localizes to the nucleus and a peripheral crescent in self-renewing cells and their sisters. Acquisition and maintenance of such an intricate pattern of protein localization require involvement of complex regulatory systems. Here we will present characterization of the first component we have identified that controls subcellular distribution of BASL, the membrane protein BOCCA A BOCCA (BAB). We demonstrate that in the absence of BAB, BASL loses its polarized plasma membrane localization and appears diffuse within cells. BAB is conserved throughout evolution and is broadly expressed in the plant suggesting that it may be part of a general polarity mechanism. Notably, however, BAB is not required for subcellular localization of other polarized proteins (such as PIN1 and PIN2), suggesting high specificity of BAB activity.

P11-034: IDENTIFICATION OF HYPERACTIVE FORMS OF ARABIDOPSIS THALIANA MAP KINASES BERRIRI S (URGV)

Berriri S., Pateyron. S, Merlot S., Leung J., Hirt H. and Colcombet J.
URGV, France

Protein phosphorylations and dephosphorylations are common events occurring during intracellular signalling processes. Among plant kinases, Mitogen-Activated Protein Kinases (MAPKs) are more specifically involved in stress responses. However, despite an abundant literature, the exact roles and direct targets of the 20 Arabidopsis MAPKs are still not completely defined. Although many aspects of the activation mechanism of MAPKs have been unveiled, constitutive active MAPKs are difficult to generate. Classical strategies used to trigger kinase activation by mutation of the phosphorylated residues failed.
To bypass this problem, we built a screen based on functional expression in yeast in order to identify mutated MAPK which are active without upstream signal. We therefore aim to identify important residues involved in the activity control and will generate point mutants in other plant MAPKs. At the same time, these active MAPKs will be used to complement a MAPK mutant. Apart from studying the phenotypic consequences, our goal will be the identification of the molecular targets of the kinases using microarray-based transcriptome analysis.
Overall, the proposed work should provide direct information on

all MAPK targets and should be an important contribution to the overall understanding of signal transduction in a complex system of a high eukaryotic model system such as Arabidopsis thaliana.

P11-035: A NEW APPROACH TO VISUALIZE SECONDARY METABOLITES IN COFFEA PLANT

Conejero, G.[1] - Verdeil, J.l.[2]
1Inra
[2]Cirad

The autofluorescence of some secondary metabolites in plants can be used to determine their cellular localization by an original approach of spectral linear unmixing in multiphonic microscopy. So, the 5-caffeoyl quinic acid (chlorogenic acid, an abundant polyphenol in coffea plant), the caffeine and the complex formed by the two molecules can be to detect in the cells of leaf and seed of Coffea canephora, without staining or disturbance of the environment. Their localization allows to make hypotheses on their cellular function.

P11-036: REGULATION OF AUXIN HOMEOSTASIS BY CHANGES IN ACTIVITY OF AUXIN INFLUX AND EFFLUX CARRIERS

Hoyerova, K. - Lankova, M. - Pesek, B. - Kubes, M. - Petrasek, J. - Zazimalova, E.*
Institute of experimental botany, AS CR
**Corresponding author, e-mail: hoyerova@ueb.cas.cz*

The auxins are phytohormones widely involved in control of plant development and morphogenesis. Endogenous auxin content in relation to that of cytokinins is decisive for regulation of plant cell division, elongation and differentiation. Controlled auxin transport represents the unique regulatory mechanism of auxin action. Polar auxin transport machinery consists of a balanced system of passive diffusion combined with the activities of auxin influx and efflux carriers. We have revealed that the activities of putative auxin influx inhibitors regulate the overall auxin transport across plasma membrane depending on the plasma membrane dynamics. Furthermore, we observed the increase of endogenous free indolyl-3-acetic acid (IAA) level in tobacco BY-2 cells after 2-naphthoxyacetic acid and 3-chloro-4-hydroxyphenylacetic acid specific auxin influx inhibitors treatment. Our data imply that the induction of IAA biosynthesis after supression of auxin uptake is regulated indirectly via IAA level in the cell thus providing auxin homeostasis in the cell. Acknowledgements: This work is supported by the Ministry of Education, Youth and Sports of the Czech Republic, project: LC06034, and by the Grant Agency of the Academy of Sciences of the Czech Republic, project: KJB600380702.

P12 Epigenetics

P12-001: POSTTRANSLATIONAL MODIFICATIONS OF HISTONE H1 IN TOBACCO (NICOTIANA THABACUM L.

Kotlinski, M[1] - Dadlez, M.[2] - Jerzmanowski, A.[1]*
[1]Laboratory of Plant Mol. Biol. Warsaw University / Institute of Biochemistry and Biophysics, PAS
[2]Institute of Biochemistry and Biophysics, PAS
**Corresponding author, e-mail: mkotl@ibb.waw.pl*

Posttranslational modifications of core histones are well characterized and known to play significant role in regulation of transcription in Eucaryota. The knowledge about posttranslational modifications of linker (H1) histones is much more limited and so far restricted to mammals and Drosophila.
Here we present the first study of posttranslational modifications of plant (tobacco) H1. We optimized protocols of H1 purification and digestion for analyzes with mass spectrometry. The use of hybrid linear ion trap-orbitrap mass spectrometer allowed to obtain high mass accuracy spectra, for both the precursor and product ions. We utilized two different peptide fragmentation techniques: collision induced dissociation (CID) and electron transfer dissociation (ETD). The ETD technique provided good quality fragmentation spectra of multiply charged peptides frequently occurring in enzymatic digests of basic proteins such as histone H1. Plants are known to have many non-allelic H1 variants differing in domain structure and functions. We determined the patterns of posttranslational modifications for all of the 6 H1 variants occurring in tobacco, mapping numerous sites of acetylation, methylation and phosphorylation. We also observed differences in H1 modification patterns between different tissues.

P12-002: THE APPLICATION OF PEPTIDE PULL-DOWN TECHNIQUE FOR IDENTYFICATION OF ARABIDOPSIS HISTONE H3 – INTERACTING PROTEINS

Buszewicz, D.[1]- Palusinski, A.[2] - Prymakowska-Bosak, M.[3] - Jerzmanowski A [1]*
[1] (Laboratory of Plant Molecular Biology, Institute of Biochemistry and Biophysics, PAS)
[2](Warsaw University of Life Sciences)
[3](Laboratory of Plant Molecular Biology, University of Warsaw)
**Corresponding author, e-mail: dbuszewicz@gmail.com*

Posttranslational modifications of core histones are one of the most important mechanisms utilized by eukaryotic cells to regulate gene expression at transcriptional level. Modifications, such as acetylation, methylation or phosphorylation, can modulate chromatin state by altering interactions between histones and DNA or by recruitment of protein factors specifically recognizing modified residues. Many histone-binding proteins have been described in yeast and mammals, however, there is still poor understanding of such interactions in plants. One of the basic methods for identifying proteins that bind histones in a modification-dependent manner is peptide pull-down. This method utilizes synthetic histone peptides carrying a selected modification. After immobilization on solid support, peptides are used to precipitate interacting proteins from nuclear extract. Upon elution, bound proteins are resolved on SDS-PAGE, excised from gel and identified using mass spectrometry techniques. Method described above has been used for identification of histone H3 partners in yeast and mammals. In our study, we aimed at adapting this method for plant material. Optimalization of the extraction of native nuclear proteins from Arabidopsis thaliana, as well as the conditions of peptide pull-down, enabled identification of proteins interacting with histone H3 peptides methylated at lysine 4. Application of our protocol resulted in precipitation of AL1 and AL7, a known H3K4me2-binding, PHD-containing proteins and their close homologue AL6. This result indicates, that peptide pull-down can be applied for identification of Arabidopsis histone H3-interacting proteins.

P12-003: FUNCTIONAL ANALYSIS OF SLEZ2, A TOMATO ENHANCER OF ZESTE PROTEIN

Boureau, L.- How Kit, A. - Stammitti, L. - Moing, A. - Maucourt, M. - Deborde, C. - Bernillon, S. - Teyssier, E. - Gallusci, P.*
UMR 619 Biologie du fruit - Centre INRA Bordeaux-Aquitaine
**Corresponding author, e-mail: lisa.b33@hotmail.fr*

Polycomb (PcG) proteins, initially identified in Drosophila, have been characterized in plants and play essential functions in the control of plant development and reproduction. Among the different classes of PcG proteins, three have been analyzed in Arabidopsis: the Enhancer of Zeste (E(z)), the Extra Sex Com (ESC), the Suppressor of Zeste (12) (Su(z)12). These proteins are components of Polycomb Repressive Complexes 2 (PRC2) that maintain chromatin in a closed state and control the transition between plant developmental phases. In order to unravel the function of the E(z) protein in the control of tomato fruit and plant development, we have characterized three E(z) encoding genes, namely SlEz1, SlEz2 and SlEZ3. SlEZ21 and SlEZ2 encode functional proteins, whereas SlEZ3 is most likely a pseudo gene. Whereas both SlEZ1 and SlEZ2 tomato E(z) genes are similarly expressed in vegetative tissue, they present contrasted expression patterns in tomato flowers and fruits. Analysis of SlEZ2 RNAi lines indicates that this protein is involved in tomato plant growth but also participates to the control of flower and fruit development. Phenotypes include abnormal flowers, fruit development abortion, altered fruit colour and texture, reduced seed germination and plant of reduced size. Fruit metabolomic analyzes suggest an alteration of the metabolite content of fruits of transgenic plants.

P12-004: COORDINATED SILENCING AND REACTIVATION OF TWO TANDEM REPORTER GENES IN VEGETATIVELY PROPAGATED POTATO LINES

Fischer, L. - Nocarova, E.*
Charles University in Prague, Faculty of Science
**Corresponding author, e-mail: lukasf@natur.cuni.cz*

Instability of transgene expression inseparably accompanies preparation of transgenic plants. Although most changes in transgene expression occur early after transformation, the transgenes can be silenced long time after their integration, which complicates the use of transgenic plants in both basic research and agriculture. To study long-term changes in transgene expression in potato (Solanum tuberosum), we monitored the activity of two reporter genes, encoding green fluorescent protein (GFP) and neomycin phosphotransferase (NPTII), in a set of 17 transgenic lines during five years of vegetative propagation in vitro. Decrease in transgene expression was observed preferentially in lines with higher number of T-DNA insertions and higher initial GFP expression level. The silencing was observed in four lines, all of which successively silenced both reporter genes, furthermore the loss of GFP fluorescence preceded the loss of kanamycin resistance (silencing of NPTII gene) indicating interconnections between silencing of the two loci. This successiveness in silencing of the two genes was also reproduced in the silenced lines after reactivation of the silenced transgenes by a DNA demethylating drug 5-azacytidine. The possible mechanism of the coordinated silencing is discussed in the respect of a switch of GFP silencing from posttranscriptional to transcriptional level that was indica-

ted in all silenced lines by application of 5-azacytidine, that reactivates only genes silenced on transcriptional level.

P12-005: IBM1 – A PROTEIN WITH HOMOLOGY TO A HISTONE DEMETHYLASE CONTROLS THE SWITCH FROM VEGETATIVE TO REPRODUCTIVE DEVELOPMENT IN ARABIDOPSIS THALIANA

Klein, G. - Schubert, D.*
Institute for Genetics
**Corresponding author, e-mail: grigorij.klein@uni-duesseldorf.de*

The transition from vegetative to reproductive growth is important for successful reproduction. During the transition the identity of the shoot apical meristem needs to be reprogrammed. Instead of rosette leaves the meristem starts to produce a shoot which develops new inflorescences and flowers upwardly.
At this developmental point the meristem acquires a new identity which has to be stably inherited through mitotic divisions. As such changes of gene expression are not caused by mechanisms in the underlying DNA sequence, epigenetic mechanisms are likely involved in maintaining meristem identity. DNAand histone methylation are key epigenetic regulatory mechanisms. While Polycomb-group proteins, which harbor histone methyltransferase activity, are involved in the control of meristem identity, less is known about the relevance of Histone Demethylases for the development of Arabidopsis.
In a reverse genetics screen for mutants with meristem identity defects T-DNA insertions in Histone Demethylase genes were analyzed and resulted in the identification of novel alleles of ibm1 (increase in bonsai methylation 1) (Saze et al. 2008). ibm1 mutants exhibit reversion of the floral meristem to the inflorescence meristem and of the inflorescence meristem to a leaf producing meristem, which are both never observed in Arabidopsis wildtype. In accordance, meristem identity genes LEAFY and APETALA1 are downregulated in the shoot apex of ibm1. We suggest that IBM1 is a key regulator of shoot apical meristem identity in Arabidopsis, which activates meristem identity genes by demethylating histones.
Saze,H., Shiraishi,A., Miura,A., Kakutani,T. (2008) Control of genic DNA methylation by a jmjC domain-containing protein in Arabidopsis thaliana. Science 319: 452-465

P12-006: CHARACTERISATION OF EPIGENETIC MODIFICATIONS DURING PLANT DEVELOPMENT IN WILD AND CULTIVATED TOMATO SPECIES

Gallusci, P.[1] - Boureau, L.[1] - Raineri, M.[2] - Bertrand, A.[2] - Stammitti, L.[2] - Bernacchia, G.[2]*
[1]Université Bordeaux1 et 2, INRA
[2]University di Ferrara
**Corresponding author, e-mail: philippe.gallusci@bordeaux.inra.fr*

Rolin Dominique (Université Bordeaux1 et 2, INRA) Teyssier Emeline (Université Bordeaux1 et 2, INRA) Tomato is currently used as a model system to study fruit development and quality. Tomato fruit development can be divided in 3 distinct phases, namely cell division, cell elongation and fruit ripening. These developmental phases lead to massive changes of gene expression pattern.
To analyse the potential role of epigenetic mechanisms during the development of tomato plant and fruit, genomic DNA methylation analysis were performed demonstrating global and locus specific variations of CG and CXG methylation pattern in fruits of the cultivated tomato. The comparative analysis of DNA methylation in wild and cultivated tomato species indicate contrasted situations, characterized by different pattern of DNA methylation at repetitive loci such as the 5s rDNA and various retrotransposons. In addition, the molecular analysis of genes encoding the Polycomb (PcG) proteins demonstrates a genetic variability at these loci. The functional consequences of these observations are currently investigated.

P12-007: REGULATION OF SEED DEVELOPMENT BY POLYCOMB GROUP PROTEINS

Roszak P, Kohler, C.
Department of Biology, ETH Zurich, Switzerland

Seed development in flowering plants involves a complex set of developmental processes that are initiated by the double fertilization of the egg and the central cell, leading to the formation of the embryo and the endosperm, respectively. In the absence of fertilization, female gametophytes do not develop and abort. The repression of central cell development is ensured by the FERTILIZATION INDEPENDENT SEED (FIS) Polycomb group complex, which is composed of the four subunits MEDEA, FIS2, FERTILIZATION INDEPENDENT ENDOSPERM and MSI1. In any mutant defective in one component of this complex, seed development initiates without fertilization. However, the penetrance of this phenotype is strikingly different in different mutants. As subunits of the FIS complex are encoded by members of small gene families with partially overlapping expression profiles, one possible reason for this finding is genetic redundancy among different family members. This study aims at elucidating which PcG genes act redundantly with FIS genes in suppression of central cell proliferation. The results of these investigations and its implications will be discussed.

P12-008: NOVEL LINKS BETWEEN EPIGENETIC SIGNATURES AND SPECIFICATION OF DNA REPLICATION ORIGINS

Costas, C.[1] - Sanchez, M.P.[1] - Stroud, H.[2] - Yu, Y.[3] - Oliveros, J.C[4] - Feng, S. - Benguria, A.[4]. - Lopez-Vidriero, I.[4] - Zhang, X.[3] - Solano, R.[4] - Jacobsen, S.E.[2] - Gutierrez, C.[1]
[1]Centro de Biologia Molecular "Severo Ochoa", CSIC-UAM
[2]Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles
[3]Department of Plant Biology, University of Georgia, Athens
[4]Centro Nacional de Biotecnología, CSIC, Cantoblanco
[5]Howard Hughes Medical Institute, University of California, Los Angeles

Genomic integrity requires faithful chromosome duplication. Origins of replication are the genomic sites where DNA replication initiates in every cell cycle. There are multiple origins scattered throughout the eukaryotic genome whose genome-wide identification has been a hard challenge, especially in multicellular organisms. Thus, very little is known on the distinctive features of origins in terms of DNA sequence and chromatin context at a genomic scale. Here we have profiled origins in Arabidopsis thaliana by high-throughput sequencing of purified nascent DNA strands. We have identified 1543 replication origins, which were uniformly distributed across the Arabidopsis genome and enriched in binding signals of two replication initiation proteins, CDC6 and ORC1, as determined by CHIP-chip experiments. We have also analyzed novel epigenome maps of various histone modifications and found links between origins and epigenetic signatures, which differ from or have not been reported for other eukaryotic systems. Our data establish the basis for the understanding of the epigenetic specification of origins of replication on the Arabidopsis "originome" at a genome-wide scale and have implications for the mechanisms of origin specification and regulation in other eukaryotes.

P12-009: GENETIC ENGINEERING APPROACHES FOR SILENCING OF PLANT GENES OF DIFFERENT EVOLUTION ORIGINS

Matveeva, T. - Bogomaz, D. - Pavlova, O. - Lutova, L.
Saint-Petersburg State University

Genetic engineering approaches allow to make direct alteration of gene expression level. Special vectors, e.g, pKannibal (Wesley et al., 2001), can be used for effective gene silencing. Tobacco

plants were chosen as a model system for studying of silencing of different evolution origin genes influence on plant viability. Vectors based on pKannibal were constructed to silence dwf1 and mis genes. dwf1 controls one of the sterol metabolism step in plants and has a great influence on amount of end sterol products, including brassinosteroid level. Nicotiana spp. obtained mis gene during their evolution from Agrobacterium rhizogenes and it controls aminoacids derivatives (opines) synthesis. Our results revealed reduced viability of transgenic tobacco plants characterized by reduced dwf1 gene expression level and no changes in viability of transgenic tobacco plants transformed by vector for silencing of mis gene. That confirmed the great importance of sterol metabolism level for normal plant development and at the same time arises the question of evolutionary role of transferred mis gene. Some literary data indicated low level expression of mis gene in tobacco plants, but transfer from agrobacterium took place several times that may be an indication of some important role of transferred T-DNA part for tobacco plants. This work was supported by RFBR grant # 09-04-13671-ofi_c and RFBR grant # 08-04-01005-a, SC-6455.2010.4, FCP 2010-1.1-141-042-019.

P12-010: ARABIDOPSIS OCP1 MUTANT, A USEFUL TOOL TO DISSECT THE RNA-DIRECTED DNA METHYLATION (RDDM) PATHWAY IN PLANTS

López, A. - Vera, P.*
Instituto de Biología Molecular y Celular de Plantas (IBMCP-CSIC)
**Corresponding author, e-mail: alopesa@ibmcp.uvp.es*

Defense response in plants against pathogens involves reprogramming of transcription and Ep5C gene was used as a candidate gene to study such reprogramming. Ep5C gene expression is induced in response to different pathogen attacks. Transgenic Arabidopsis lines expressing the β–glucuronidase (GUS) gene under the control of Ep5C 5´promoter region were generated, one of those lines was selected and subsequently mutagenized with EMS. Mutants which showed misregulation of Ep5C gene expression were identified. In this work, we report the characterization of one of these mutants, named ocp1 that do not show any visible alteration in normal morphology and development. Map-based cloning has located the lesion responsible for the observed phenotype at NRPD2 gene which encodes the second largest subunit of RNA polymerases IV and V, specific to plants that seem to be involved in RdDM pathway. Loss of NRPD2 function leads to deficient methylation in different regions of the Arabidopsis genome, and methylation analysis in ocp1 reveals a strong hypomethylation of some of those elements. Furthermore, we analyzed the methylation degree of the promoter region of Ep5C in this mutant and found it significantly less methylated when compared with wild-type plants.
We are currently investigating the biology emerging from the RdDM pathway through phenotypic analysis of different mutants. Although loss-of-fuction mutants have been generated for this pathway, hardly any phenotype has been associated to them, remaining largely unknown the biological significance of the pathway. In this respect, the availability of the ocp1 mutant, representing a partial loss-of-funcion of NRPD2, may be used as a powerful tool to deepen our understanding of the regulation of the RdDM pathway in plants.

P12-011: ARABIDOPSIS LINKER HISTONE VARIANTS

Halibart- Puzio, J.[1] - Rutowicz, K.[1] - Puzio, M.[2] - Jerzmanowski, A.[2]*
[1]Institute of Biochemistry and Biophysics Polish Academy of Sciences-Asia
[2]University of Warsaw, Laboratory of Plant Molecular Biology

The central role of core histones in chromatin structural transitions that directly affect gene expression is now widely acknowledged. In contrast, little is still known about H1 linker histones role in these processes, despite numerous data from in vitro studies suggesting H1's critical function in determining regularity of higher order chromatin structures. Recently, a significant down-regulation by RNAi of genes encoding all three H1 variants in Arabidopsis thaliana has been shown to affect precise regulation of gene expression that was correlated with changes in specific DNA methylation pattern in many chromosome regions (Wierzbicki & Jerzmanowski, 2005). In both higher plants and animals numerous non-allelic H1 variants co-occur in the same cells. The wider biological meaning of this variability is unknown. Arabidopsis, in addition to two somatic H1 variants (H1-1 and H1-2) has a characteristic evolutionary conserved plant H1 variant designated H1-3, the expression of which is strongly upregulated by drought stress and ABA treatment.
We determined the tissue localization of H1-3 in Arabidopsis using transgenic plants expressing the H1-3:GFP fusion protein. The application of FRAP (Fluorescent Recovery After Photobleaching) technique showed that H1-3 has dramatically lower affinity to chromatin than the remaining two somatic variants H1-1 and H1-2. These data are consistent with the concept that drought-induced H1-3 variant in Arabidopsis may modulate chromatin structure for adaptation of transcription profile to stress conditions.

P13 Metabolism

P13-001: DYNAMICS OF PHOSPHOLIPIDS' CHANGES IN FRUITS OF COTTON

Zikiryayev, A. - Begimkulov, U.*
Tashkent State Pedagogical University
**Corresponding author, e-mail: AZikiryayev@yandex.ru*

In spite of the fact that phospholipids, glicolipids and sterins are contained in various bodies of cotton (seeds, fiber) in rather small quantities, their value in metabolic processes of plants is exclusively great. Last years the great attention is paid to lipid exchange as a factor of adaptation to environmental conditions. Lipids' participation in particular of phosphotidilglicerin, in stability of plants from cooling is supposed. According to F.I Roslyno, changes in lipid and fatty acid structure under the influence of water stress affect structurally functional peculiarities of membranes and consequently on photosynthetic activity of chloroplast of cotton leave. At the same time lipids play the important role in cellulose formation in fruits of cotton.
Study of phospholipids' structure in different periods of maturing of various parts of fruits of cotton has shown that phospholipids are important in their formation. The special attention is deserved natural change of general phospholipids' maintenance and their components in developing fibers. Change of phospholipids structure of fibers is connected with reducing of one components and increasing the others. In such way phosphotidilholin, phosphotidilcirin, phosphotidilinozit are able to be changed. Distinctions of phospholipids are connected with formation of cotton fibers. Justification of it is that the maintenance phospholipid is full during this period when there is an intensive growth and development of cells of fibers i.e. at the day age of 15-30.
Further study of phospholipids' accumulation and exchange in bottoms and especially in a fiber is of a huge theoretical and practical importance in connection with clarifying of the mechanism of cellulose biosynthesis in fruits of cotton.

P13-002: A SINGLE ACTIVE TREHALOSE-6-P SYNTHASE (TPS) AND A FAMILY OF PUTATIVE REGULATORY TPS-LIKE PROTEINS IN ARABIDOPSIS.

Rolland, F.[1]* - Vandesteene, L.[1] - Ramon, M.[2] - Le Roy, K.[1] - Van Dijck, P.[1]
[1]K.U.Leuven
[2]Massachusetts General Hospital, Harvard U.
**Corresponding author, e-mail:* filip.rolland@bio.kuleuven.be

The disaccharide trehalose is commonly found in bacteria, fungi and invertebrates, where it functions as a reserve carbohydrate and stress protectant with its unique physico-chemical properties. However, trehalose is not synthesized in vertebrates and while it accumulates in non-vascular and lower vascular plants like algae, liverworts, mosses and ferns (some of which are known as extremely drought-tolerant 'resurrection' plants), typically only minute amounts are produced in higher plants. Still, most higher plant genomes analyzed up till now contain large trehalose biosynthesis gene families and (heterologous) over-expression and mutation of trehalose biosynthesis genes or external trehalose feeding have marked effects on growth, stress tolerance, photosynthetic activity and carbon partitioning. An important regulatory role is emerging for the metabolic intermediate trehalose-6-P (T6P), which acts at least in part through inhibition of the SnRK1 protein kinases. Systematic gene expression and yeast complementation analyses of the entire family of T6P synthase (TPS) and T6P phosphatase (TPP) proteins suggests that in addition to a single TPS (TPS1, essential for embryo maturation and normal vegetative and reproductive growth) and a family of active TPP enzymes, Arabidopsis also encodes a whole family of catalytically inactive TPS/TPP-like proteins (TPS2-11) with putative tissue or cell type specific regulatory functions. We are using modeling and mutational analyses to study the putative conserved binding of substrate metabolites and associated functions of these proteins as metabolic sensors.

P13-003: BARLEY CYSTEINE PROTEASES OF C1A CLASS: MOLECULAR CHARACTERIZATION AND ROLES IN PLANT PROTEOLYTIC PROCESSES

Cambra, I. - Martínez, M. - Dader, B. - González-Melendi, P. - Diaz, I.
CBGP

Plant proteolysis is a complex process involving many different pathways and cellular compartments. Protease activities are regulated at the transcriptional level by differential expression and at the protein level by the activation of zymogens and by the binding of specific inhibitors and cofactors. We have focused our attention on the cysteine-protease class (CysProt) which comprises about 140 members among the 800 proteases encoded by angiosperm genomes. Particularly, we study the papain-like proteases (family C1, clan CA) from barley which includes 32 proteins. These CysProt participate in many physiological processes as protein degradation during senescence and abscission process, programmed cell death, accumulation and mobilization of storage proteins in seeds and tubers, or in protein processing.
We aim to understand their physiological roles by identifying the targets involved in specific proteolytic process, the fulfilment of their functions, their responses to different stimuli and the regulation of their activities. Up to now, we have molecularly characterised at least one member of each of the 8 main groups in which the 32 barley CysProt have been clustered. In addition, the whole cystatin gene family encoding cysteine-protease inhibitors from barley has also been characterised. Currently we are analyzing the protease-cystatin relationships: expression patterns, protein locations, protein-protein interactions, differential responses to abiotic and biotic treatments, and finally their implication in specific physiological processes.
This work is supported by the Spanish Ministerio de Educación y Ciencia (BFU2008-01166) and by the Agencia Española de Cooperación Internacional (A/017236/08).

P13-004: GLUCOSE 1-PHOSPHATE TRANSLOCATORS IN PLASMA- AND IN PLASTIDIAL ENVELOPE MEMBRANE FROM HIGHER PLANTS

*Fettke, J.**
University of Potsdam AG Plant Physiology
**Corresponding author, e-mail: fettke@uni-potsdam.de*

For several glucosyl transfer reactions glucose 1-phosphate is an essential metabolite that acts either immediately as glucosyl donor or as a substrate for the formation of the more general donors, ADPglucose and UDPglucose. We analyzed two glucose 1-phosphate related transport processes: the uptake by the cells and the import into intact plastids. Glucose 1-phosphate is taken up by both autotrophic and heterotrophic cells. The glucose 1-phosphate uptake is highly specific for the anomeric position of the phosphate ester as glucose 6-phosphate does not substitute the carbon 1 ester. Following uptake, glucose 1-phosphate is imported into the plastids and subsequently enters starch biosynthesis. As revealed by in situ and in vitro labeling experiments, at least two distinct starch synthesizing paths exist: First, in a single reaction (that is mediated by the plastidial phosphorylase isozyme) glucosyl residues are transferred from glucose 1-phosphate

directly to acceptors at the surface of native starch granules. The second path consists of two steps: first the conversion of glucose 1-phosphate to ADPglucose and, subsequently, the glucosyl transfer from ADPglucose to glucans of the starch granule that is catalyzed by various ADPglucose-dependent starch synthases. Amyloplasts from potato tubers utilize most of the imported glucose 1-phosphate for the direct glucosyl transfer. By contrast, the ADPglucose-dependent path is by far predominant in chloroplasts from mesophyll cells from Arabidopsis. However, the direct glucosyl transfer is detectable in mutants deficient in the plastidial phosphoglucomutase. This path results in a small yet significant starch level in the respective mutants.

P13-005: A FAMILY OF GLUCOSYLTRANSFERASES FROM C. SATIVUS STIGMAS

Gómez Gómez, L. - Trapero, A. - valero, Y. - Ahrazem, O. - Rubio-Moraga, A.*
University of Castilla-La Mancha
**Corresponding author, e-mail: marialourdes.gomez@uclm.es*

Plants produce tens of thousands of different natural products also referred to as secondary metabolites. These small organic molecules allow plants to cope with various types of stress, while also carrying out biological activities which are often of high interest to human industries. Although the true role of such metabolites in plants remains mostly unknown, it is evident that plants invest a great deal of resources in synthesizing, accumulating and sorting such metabolites, often produced through complex and highly regulated biosynthetic pathways operating in multiple cellular and sub-cellular compartments. Furthermore, some compounds are restricted to single species or related groups and are often generated only during a specific developmental period of the plant. All these particularities are found in Crocus sativus L. Saffron, the dried red stigmas of C. sativus, has been used as a flavouring and colouring agent since then and is currently considered the world’s most expensive spice. Saffron is made up of a complex mixture of volatile and non-volatile compounds that contribute to its overall aroma and flavour. Glucosylated carotenoids and flavonoids are the main compounds detected in saffron, the different glycosidic structures observed suggest the existence of different families of glycosyltransferases that act on these compounds. These enzymes are involved in defence, lignification, detoxification, floral development and pigmentation. Sugar analysis and glucosidase treatment of saffron confirm the presence of glucosyltransferases in the stigma tissue. Using a PCR approach, several glucosyltransferases have been isolated from saffron, expression analysis and phyogenetic relationships will be presented.

P13-006: GROWTH INHIBITION PROVOKED BY HERBICIDES THAT INHIBIT BRANCHED-CHAIN AMINO ACID BIOSYNTHESIS IS RELATED TO LIGNIN ACCUMULATION

Zulet González, A. - Igal, M. - Zabalza, A. - Royuela, M.*
Universidad Pública de Navarra
**Corresponding author, e-mail: amaia.zulet@unavarra.es*

Previous studies have shown that the herbicide imazethapyr (IM), an inhibitor of branched chain amino acid biosynthesis, produces ferulic acid (FER) accumulation in roots of treated plants. By other hand, FER has been shown to inhibit plant growth in relation to lignin production. In order to check if lignin accumulation is involved in the growth inhibition provoked by IM, it was evaluated the effect of this herbicide on lignin content and several enzymes of secondary metabolism comparing to the effect of supplying exogenous FER.Pea plants were grown in hydroponic tanks and when they were 12 days old were divided in three treatments: Control (C), FER and IM. FER and IM were added to the nutrient solution at a final concentration of 1 mM and 69 μM respectively. Lignin content and phenylalanine ammonia-lyase activity (PAL) were determined spectrophotometrically and 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAHPS) was monitored using immunological techniques. The study was conducted throughout 15 days.Root growth was arrested by both treatments after 24 h from the onset of the treatment. IM caused an increase in DAHPS protein expression while FER decreased it. PAL activity was not clearly affected by any of the treatments. FER only caused a transient increase in lignin content while of IM-treated plants, presented lignin accumulation in a constant way from day 7. These results suggest that, at least at the end of the period of study, growth inhibition provoked by IM was related to an accumulation of lignin, probably due to a greater carbon flow thought the shikimate pathway.

P13-007: ACYL-ACP DESATURASES FROM CASTOR BEAN (RICINUS COMMUNIS L.): THEIR IMPLICATION IN OLEIC ACID SYNTHESIS

Sanchez-Garcia, A. - Garcés, R. - Martínez-Force, E.*
Instituto de la Grasa, CSIC
**Corresponding author e-mail: asangar@cica.es*

Plant acyl-ACP desaturases are soluble enzymes that catalyze the intraplastidial desaturation step in the fatty acid biosynthetic pathway and are, therefore, localized in the plastids. They mainly convert stearoyl-ACP to oleoyl-ACP determining the monounsaturated fatty acid composition of vegetable oils. Castor (Ricinus communis L) is a non-food oil crop highly interesting for oleochemist industry. The stearoyl-ACP Δ9 desaturase (RcSAD1) from castor bean has been already studied and its crystal structure determined. However, three other genes have been sequenced and cloned from castor seeds coding for acyl-ACP desaturases, RcSAD2, RcSAD3 and RcSAD4. These three desaturases were heterologously expressed in E. coli to determine their substrate specificity for different acyl-ACP substrates by comparison with RcSAD1. In addition, the expression levels of the four genes were analyzed by QRT-PCR in developing seeds and vegetative tissues. The contribution of these enzymes to the oleic acid synthesis in castor will be discussed.

P13-008: LIPID CHARACTERIZATION OF AN ARABIDOPSIS MUTANT WITH LOWER EXPRESSION OF ACYL-ACP THIOESTERASE A GENES.

Moreno-Perez, A.[1] - Vaistij, F. E.[2] - Graham, I. A. [2] - Salas, J. J.[1] - Garcés, R.[1] - Martínez-Force, E.[1]*
[1]Instituto de la Grasa, CSIC
[2]Centre for Novel Agricultural Products, Department of Biology, University of York
**Corresponding author, e-mail: moreno@cica.es*

Acyl-acyl carrier protein (ACP) thioesterases are enzymes that terminate the intraplastidial fatty acid synthesis in plants by hydrolizing the acyl-ACP intermediates and releasing free fatty acids to be incorporated into glycerolipids. These enzymes are classified in two families, FatA and FatB, which differ in amino acid sequence and substrate specificity. In Arabidopsis thaliana the FatA proteins are encoded by two different genes, At3g25110 and At4g13050. In the present work, an Arabidopsis homozygous line with lower expression levels for both genes has been obtained crossing single mutant lines. The lipid profile (triacylglycerols, diacylglycerols, fatty acids and galactolipids) and acyl-CoA composition of this double mutant line has been characterized showing important differences with regard to wild type line. The importance of this line for expressing different thioesterases from other species will be discussed.

P13-009: CLONING AND MOLECULAR CHARACTERIZATION OF LPAAT AND DAGAT ACYLTRANSFERASES FROM SUNFLOWER SEEDS (HELIANTHUS ANNUUS L.)

Fernandez-Garcia, J. - Garcés, R. - Martínez-Force, E.*
**Corresponding author, e-mail: jdfernandez@cica.es*

Many plants accumulate triacylglycerols (TAG) in their seeds and fruits as lipid reserves. In our laboratory we are studying the sunflower (Helianthus annuus L.), whose oil has especial value for the food industry. However, the distribution of fatty acids in its different TAG species does not have the required best technological features.
In the present work we isolated, cloned and sequenced cDNAs coding for two isoforms of LPAAT (HaLPAAT1 y HaLPAAT2) as well as three isoforms of DAGAT (HaDAGAT1A, HaDAGAT1B y HaDAGAT2), enzymes involved in final steps of TAG biosynthesis.
The expression levels of these genes in vegetative tissues and seeds were examined by Q-PCR. All the studied acyltransferases are expressed ubiquitously, with differences in expression levels. The levels in vegetative tissues appear to be constant for each enzyme, with higher values for HaDAGAT2, however, that trend is broken in developing seeds by the appearance of peaks of expression at 12-15 days after flowering. This particular expression pattern is according to the oil accumulation period in seeds from the third week after flowering, suggesting a possible transcriptional regulation of these acyltransferases during the seed development, which HaDAGAT2 seems to have an important role due to his highest expression levels.

P13-010: AN OVERVIEW OF THE CARBON METABOLISM ACTORS THAT CONDITION THE SURVIVAL OF THE PARASITIC PLANT: PHELIPANCHE RAMOSA

Péron, T.[1] - Pouvreau, J.B.[1] - Draie, R.[2] - Véronési, C.[1] - Thoiron, S.[1] - Delavault, P.[1] - Simier, P.[1]*
[1]Laboratoire de Biologie et Pathologie Végétales (LBPV), IFR 149 Qualité et Santé du Végétal (QUASAV)
[2]Faculty of Agronomy, Tishreen University, Lattakia, Syria
**Corresponding author, e-mail: thomas.peron@etu.univ-nantes.fr*

Some broomrapes are harmful parasitic plants. They attach to the roots of the host plant establish vascular connections and then grow at the expense of the host plant's resources. The absence of chlorophyll in broomrape explains that it needs host-derived sucrose for growth. As a consequence, sucrose utilization is essential to entertain the sink strength and the development of the parasite. Nonetheless information on this topic remains poor. Our work shows at cellular and molecular levels the relative implication and the possible cross-talk between the key components of sucrose metabolism, including: invertase, sucrose synthase and sucrose transporters. The analyses were carried out on both broomrapes growing rapidly on a susceptible host genotype and broomrapes growing slowly on a tolerant host genotype. The data give some insights on the sink strength regulation in parasite and on the mechanisms of host tolerance which induce deregulation in broomrape growth.

P13-011: ANTIOXIDANT COMPOUNDS IN GREEN AND RED VARIETIES OF LETTUCE (LACTUCA SATIVA L.)

Baslam, M. - Morales, F. - Goicoechea, N.*
University of Navarra
**Corresponding author, e-mail: bmarouane@alumni.unav.es*

Lettuce is a major crop within the European Union and exhibits healthy properties due to its large supply of antioxidant compounds, mainly vitamin C, carotenoids and polyphenols. The beneficial effect of lettuce on both lipid metabolism and tissue oxidation could improve protection against cardiovascular diseases. Our objective was to study the levels of some antioxidants –carotenoids, phenolics, vitamin C and anthocyanines- and their distribution between the outer and the inner leaves of three varieties of lettuce consumed as salads – the green varieties Cogollos de Tudela and Batavia and the red variety Maravilla - Results showed that antioxidant compounds differed between the three lettuce varieties and, except for the higher amount of anthocyanins in the red variety, no relationship was found between the type and/or the amount of every antioxidant and the classification of each variety as "green" or "red". While the highest levels of carotenoids were detected in Cogollos de Tudela, the greatest amounts of soluble phenolics and vitamin C were measured in Batavia and Maravilla. In addition, within each variety the content of antioxidant compounds differed between the internal and the external leaves. The inner leaves had greater levels of anthocyanines, vitamin C and soluble phenolics than the outer leaves in Cogollos de Tudela, Batavia and Maravilla, respectively. In contrast, the highest contents of carotenoids and anthocyanins in Batavia and Maravilla were detected in the external leaves. The distribution of antioxidant compounds between the outer and inner leaves of each variety should be taken into account when consumed in the diet or when used as food crop for the called "Fourth Range" of vegetables.

P13-012: SUBCELLULAR COMPARTMENTATION OF PRIMARY METABOLISM IN ARABIDOPSIS THALIANA LEAVES – FOLLOWING THE ROUTE OF NEWLY FIXED CARBON

Vosloh, D. - Arrivault, S. - Feil, R. - Szecowka, M. - Fernie, A. - Lunn, J. - Stitt, M.*
Max-Planck-Institute of Molecular Plant Physiology
**Corresponding author, e-mail: vosloh@mpimp-golm.mpg.de*

Metabolism in plant cells is highly compartmented, with many pathways involving reactions in more than one compartment. For example, during photosynthesis in leaf mesophyll cells, carbon fixation and starch synthesis take place in the chloroplast, whereas sucrose is synthesized in the cytosol. Therefore, knowledge of compartmentation is essential for a proper understanding of how plant metabolism is regulated. Classical fractionation methods such as sucrose density gradient centrifugation can be used for enzyme localization, but the separation of organelles by these methods is generally too slow compared to the very short turnover times of pathway intermediates to provide useful information about the distribution of metabolites. To circumvent this problem we fractionate the cells under non-aqueous conditions, whereby the metabolic state is frozen at the time of harvest and held in stasis throughout the fractionation procedure. After analysing the distribution of marker enzymes, metabolites are measured using liquid or gas chromatography linked to tandem or single mass spectrometry, respectively. The combination of non-aqueous fractionation and sensitive mass spectrometry techniques (LC-MS/MS, GC-MS) allows us to determine the intracellular distribution of most photosynthetic intermediates and products. We also use LC-MS/MS and GC-MS to follow the labelling patterns of intermediates after pulse labelling with 13CO2. By combining all these data we can calculate the size and turnover times of the chloroplastic and cytosolic metabolite pools and determine the fluxes through the main photosynthetic pathways, providing an unprecedented level of understanding of photosynthetic carbon metabolism in leaves.

P13-013: VACUOLAR MONOSACCHARIDE IS CRITICAL FOR PLANT DEVELOPMENT AND SUGAR SENSING.

Neuhaus, E.
Technische Universität Kaiserslautern
**Corresponding author, e-mail: neuhaus@rhrk.uni-kl.de*

The vacuole represents the largest plant organelle and can occupy more than 90 % cell volume. We identified several solute carriers in the vacuolar membrane, e.g. a first malate carrier (Emmerlich et al. 2003) and a first glucose transporter (TMT; Wormit et al. 2006). Arabidopsis knock out mutants lacking all three TMT proteins show difficulties to accumulate monosaccharides upon cold stress. Interestingly, TMT overexpressor lines exhibit increased vacuolar monosaccharide levels and show substantially altered sugar signaling. These mutants also show altered growth pattern

and reveal for the first time that intracellular sugar compartimentation is critical for plant development. We will present the effects of TMT activities on gene expression, on altered source capacity of leaves and on sink strength of Arabidopsis seeds. It is concluded that the vacuolar sugar transporter TMT is a novel and important element of the cellular sugar sensing machinery in higher plants.

P13-014: STARCH SYNTHASE CLASS IV CONTROLS THE NUMBER OF STARCH GRANULES IN ARABIDOPSIS LEAVES.

Ragel de la Torre, P.[1] - Raynaud, S.[1] - Szydlowski, N. - Gámez Arjona, F.M.[1] - Lucas, M.M.[3] - D'Hulst, C.[2] - Mérida, A.[1]*
[1]Instituto de Bioquímica Vegetal y Fotosíntesis. CSIC-US.
[2]Université des Sciences et Technologies de Lille
[3]Instituto de Recursos Naturales. Centro de Ciencias Medioambientales. CSIC.
**Corresponding author, e-mail: paula@ibvf.csic.es*

Recent efforts have allowed to decipher the function of many of the elements involved in the synthesis of starch. However, the process of initiation of the synthesis of this polymer and the mechanisms that control the number of starch granules in the chloroplast remain to be elucidated. We have analyzed the starch synthesis in a knock-out mutant lacking the activity of the starch synthase Class IV (SSIV) (Roldán et al. 2007) as well as in other double and triple mutants lacking both SSIV activity and other SS activities: ssI-ssIV, ssII-ssIV, ssIII-ssIV, ssI-ssII-ssIII and ssI-ssII-ssIV (Szydlowski et al. 2009). These analyses indicate that SSIV is necessary to determine the correct number of starch granules (5-7) in chloroplasts of Arabidopsis leaves and its elimination leads to the accumulation of just a huge starch granule (two in some cases) in the chloroplast. We have identified SSIII as the enzyme necessary for the synthesis of starch in the absence of SSIV, and the elimination of both activities impair the synthesis of starch granules. However, this enzyme does not regulate the number of starch granules per chloroplast and its function in the initiation of the starch granule seems to be dependent on the presence of the other SSs. The function of starch synthases in the priming of starch and their mechanisms of action will be discussed.1. Roldán I, Lucas MM, Delvalle D, Planchot V, Jiménez S, Pérez R, Ball S, D'Hulst C, Mérida A. 2007. Plant J. 49: 492-504.2. Szydlowski N, Ragel P, Raynaud S, Lucas MM, Roldan I, Montero M, Munoz FJ, Ovecka M, Bahaji A, Planchot V, Pozueta-Romero J, D'Hulst C, Mérida A. 2009. Starch granule initiation in Arabidopsis requires the presence of either class IV or class III starch synthases. Plant Cell 21(8): 2443-2457.

P13-015: ENLIGHTING CROSS-TALK BETWEEN TERPENE PATHWAYS IN SPIKE LAVENDER: USE OF MEVALONATE TO RESTORE FOSMIDOMYCIN TOXIC EFFECT

Mendoza Poudereux, I. - Navarro-Marín, A. - Segura, J. - Arrillaga, I.*
Universidad de Valencia
**Corresponding author, e-mail: isabel.mendoza@uv.es*

Lavandula latifolia Medicus (spike lavender) is an aromatic shrub with economic interest for its content in essential oils, consisting mainly of monoterpenes. The biosynthesis of isopentenyl diphosphate (IPP), the basic C5 unit, occurs through the cytoplasmatic mevalonate (MVA), and/ or the plastidial methylerythritol-4-phosphate (MEP) pathways. This second route appears to be the main source of intermediates for the synthesis of monoterpenes. However, there is evidence of interconnection between MVA and MEP routes, so the relative contribution of each to the biosynthesis of the various classes of terpenes is still uncertain. In previous work we determined the appropriate concentrations of mevinolin (Mev), an inhibitor of the MVA pathway and fosmidomycin (FSM), an inhibitor of the MEP pathway that altered phenotypic traits on in vitro growth seedlings. The most important alterations observed were a decrease in root length at 1μM Mev and chlorosis at 30μM FSM concentrations.
In this work, we present the recovery of wild phenotype by the addition of increasing concentrations of mevalonate (the main intermediate of the cytoplasmatic pathway) alone or in combination with the selected inhibitors concentrations. The addition of 1.2 mM mevalonate to Mev-treated plants fully recovered the normal root-phenotype. On the other hand, 0.6 mM mevalonate applied to FSM-treated plants partially recovered the photosynthetic pigment content in spike lavender young leaves. These preliminary results indicate that at least a part of the IPP from the cytoplasm could be used to synthetize photosynthetic pigments in the chloroplasts.
Thiswork was funded by a FPU Research Fellowship from the Spanish Government (to I.M.-P.) and by the Generalitat Valenciana (Prometeo 2009/075).

P13-016: PYRIMIDINE DEGRADATION IS CRITICAL FOR SEED GERMINATION IN ARABIDOPSIS

Möhlmann, T.
Technische Universität Kaiserslautern
**Corresponding author, e-mail: moehlmann@biologie.uni-kl.de*

Pyrimidine degradation and salvage are important metabolic pathways but still hardly understood in plants. Plant nucleosidases are related to those of protozoan origin and catalyze the conversion of nucleosides to nucleobases. A first member of such an enzyme from Arabidopsis, AtURH1, was characterized in detail. The pure recombinant protein exhibited highest hydrolase activity for uridine, followed by xanthosine, inosine and adenosine. In addition, AtURH1 was able to cleave the cytokinin derivative isopentenyladenine-riboside. Mutants overexpressing the Arabidopsis enzyme provide clear evidence for a pivotal function of URH1 as regulative in pyrimidine degradation. Moreover, mutants with increased as well as decreased nucleosidase activity are delayed in germination, indicating that this enzyme activity must be well balanced in the early phase of plant development. A similar critical function for early seed germination of PYD1, catalyzing uracil degradation subsequent to URH1 will be presented. Furthermore, URH isoforms have been analysed, which become important under conditions of senescence and in extracellular purine degradation.
Jung et al. (2009) Plant Cell 2009 21: 876-891.

P13-017: ROS METABOLISM IN MITOCHONDRIA OF NITRATE- AND AMMONIUM- SUPPLIED ARABIDOPSIS THALIANA

Podgorska, A. - Lukawska, K. - Szal, B.*
University of Warsaw, Faculty of Biology
**Corresponding author, e-mail: apodgorski@biol.uw.edu.pl*

Ammonium nutrition results in toxicity symptoms (e.g. retardation of growth and chlorosis) in most plant species. Zhu et al. (2000) suggested that one of the reasons of the ammonium syndrome may be oxidative stress. When plants are cultured on NO3- a great part of electrons from the chloroplast ETC is consumed in N assimilation (Noctor and Foyer 1998) but under ammonium nutrition the surplus of reductants has to be exported and oxidized in the mitochondrial respiratory chain. According to this hypothesis increased mitochondrial respiration during plant growth on medium containing NH4+ as an exclusive N source may result in higher ROS formation (Guo et al. 2005).
The purpose of our study was to determine if ammonium as sole N source leads to oxidative stress due to a larger ROS production in Arabidopsis leaf mitochondria. Our analysis has shown that ammonium nutrition results in an increased H2O2 concentration in leaf tissues. Moreover, the enzymatic and non-enzymatic antioxidant systems were elevated in leaves of NH4+ -supplied plants. Mitochondrial antioxidant systems were characterized

using isolated organelles. The increased activities/protein level of mitochondrial enzymatic antioxidant system (including alternative oxidase) and changes in redox state of low-mass antioxidants together with cytochemical localization of H2O2 in plant cells suggest that mitochondria may have a significant role in ROS production during ammonium stress.

P13-019: MOBILIZATION OF STORAGE RESERVES IN THE OLIVE SEED DURING IN VITRO GERMINATION.

Zienkiewicz, A. - Zienkiewicz, K. - Jiménez-López, JC. - Alche JD. - Rodríguez-García M.I.*
Estación Experimental del Zaidín, CSIC.
**Corresponding author, e-mail: agnieszka.zienkiewicz@eez.csic.es*

We have investigated the mobilization of the main storage materials (proteins and lipids) present in the cotyledon cells of the olive seed during in vitro germination. For this purpose, a combination of cytological and biochemical methods has been used. In the mature embryo, seed storage proteins (SSPs) were accumulated in large, dense proteins bodies (PBs), surrounded by numerous, lipids storing oil bodies (OBs), filling up the cytoplasm. After seed imbibition, a gradual reduction in the pool of SSPs was detected by biochemical methods, which was accompanied by a decrease in the number of PBs, as a consequence of their fusion and by changes in the PBs matrix structure. Further mobilization of SSPs coincided with the transformation of several large PBs into a central vacuolar compartment with a few electro-dense inclusions inside its lumen, containing SSPs. The mobilization of lipids started after seed imbibition. At this time, a decrease in OBs number coincided with relatively high levels of lipoxygenase (LOX) activity. This enzyme was localized inside the PBs after immunocytochemistry. This localization, together with the close spatial relationship between OBs and the PBs boundary, suggests that PBs could be involved in the mobilization of lipids. We propose that PBs operate as multifunctional organelles. As germination proceeds, a strong decrease in both OBs number and LOX activity were observed simultaneously to the SSPs hydrolyses. All these cellular and biochemical changes strongly reflect an interconnected mobilization of storage proteins and lipids, which contributes to the differentiation process of the cotyledonary cells in the olive seed.

Acknowledgments. This work was supported by JA project (P06-AGR-01791) and the MICINN (project BFU2008-00629/BFI).

P13-020: P5BR2 IS A NOVEL DEFENSE-RELATED GENE INVOLVED IN DIGITALIS PURPUREA CARDENOLIDE BIOSYNTHESIS

*Gavidia, I. * - Pérez-Bermúdez, P.*
Universidad de Valencia
**Corresponding author, e-mail: isabel.gavidia@uv.es*

Although plant secondary metabolism is mostly related to defense mechanisms or interactions with the environment, interest in many plant secondary metabolites is mainly due to their biotechnological applications. The stereospecific 5β-reduction of progesterone is a required step for cardiac glycoside biosynthesis in foxglove plants. We recently isolated the gene P5βR (1) and here we investigate the function and regulation of P5βR2, a new progesterone 5β-reductase gene from Digitalis purpurea. P5βR2 cDNA was isolated from a D. purpurea cDNA library and further characterized at biochemical and structural level (see abstract in this Congress). Like P5βR, P5βR2 catalyzes the 5β reduction of the Δ4-double bond of several steroids. As in P5βR, the RNA transcripts of P5βR2 were present in all the organs tested, and were seen to be more abundant in leaves and flowers. A comparative study into the regulation of the P5βR and P5βR2 gene expressions showed that under stress conditions or upon treatment with chemical elicitors, the P5βR expression did not vary, whereas P5βR2 was highly responsive. The P5βR2 expression is regulated by ethylene and hydrogen peroxide but it was independent of SA and MJ (2). The correlation between P5βR2 expression and cardenolide formation (2) demonstrates the key role of this gene in cardenolide biosynthesis and, therefore, in the chemical defense of foxglove plants. Qualitative variations may play a role in plant defense which is as relevant as that of the quantitative changes observed.

(1) Gavidia I et al Phytochemistry 2007, 68: 853-864.
(2) Pérez-Bermúdez P et al. New Phytol 2010, 185: 687-700

P13-021: CROCINS TRANSPORT IN SAFFRON: FROM DEATH TO LIVE

Rubio Moraga, A. - Ahrazem, O. - Trapero, A. - Gómez Gómez, L.*
Universidad de Castilla La Mancha
**Corresponding author, e-mail: angelarubiotrabajo@yahoo.es*

Saffron, the desiccated stigmas of Crocus sativus, is highly appreciated by its peculiar colour, flavour and aroma. The main compounds that accumulated throughout stigma development in C. sativus, formed from the oxidative cleavage of b-carotene and zeaxanthin, are crocetin, its glucoside derivatives, crocins, and picrocrocin, all of which increased as stigmas reached a fully developed stage. After anthesis, and in the absence of fertilization, the flower enters in a senescence programme. Senescence represents the ultimate stage of floral development and results in wilting of whole flower. The programmed senescence of flowers allows the removal of a metabolically active tissue. Senescence is an active process and governed by a well defined cell death program. During the senescence of C. sativus flowers, changes in the composition of saffron apocarotenoids occurs and transport of crocins from the senescent stigma to the ovaries and the developing corm takes place. After, deglucosylation of crocins in these tissues results in crocetin accumulation. This mobilization mimics the export to storage cells (resorbed) of different compounds during leave senescence avoiding loss of nutrients in leaves that would otherwise be cycled back into the soil system through leaf litter decomposition. In C. sativus, the resorbed apocarotenoids are stored within the developing corm, and used for its active metabolism during early and active phases of corm development, where the glucose molecules from crocins would stimulate cell initiation and elongation.

P13-022: PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF THE GLYCOLYTIC PHOSPHOGLYCERATE KINASE (PGK1) OF ARABIDOPSIS THALIANA: NEW ROLES AND FUNCTIONS

Cascales-Miñana, B. - Muñoz-Bertomeu, J. - Anoman, AD. - Toujani, W. - Segura, J. - Ros, R.*
Universidad de Valencia
**Corresponding author, e-mail: borja.cascales@uv.es*

The phytohormone abscisic acid (ABA) controls the development of plants and plays a crucial role in their survival to adverse environmental conditions. Recently, central questions in the ABA signal transduction pathway has been resolved. A role of ABA in sugar regulation of plant development has also been well documented. However, the direct molecular connections between ABA and sugar signal transduction pathways are poorly defined. In the last years, new functions of the glycolytic enzymes in yeast and mammals have been described, providing new perspectives of the networks and connexion between metabolism and development. We have performed a functional genetic approach with the glycolytic phosphoglycerate kinase (PGK1) from Arabidopsis. Our results reveal that the loss-function of this enzyme causes a deep morphological phenotype characterized by serrated leaves, delayed flowering and short root. Physiological evidence also shows that the knock-out PGK1 mutants (pgk1) are hypersensitive to ABA in a germination assay. In contrast, pgk1 knock-on mutants show a strong insensitivity to ABA and an advanced development as compared to wild type controls. In this work, we report experimental evidence of putative new roles of this central

glycolytic enzyme, bringing new visions about the glycolytic enzymes in plants.
This work has been funded by the Spanish Government (BFU2009-07020), the Valencian Government (ACOMP/2009/328, PROMETEO/2009/075), by a research fellowship from the Spanish Government (FPU to B. Cascales-Miñana and AECI to A.D Anoman).

P13-023: DEVELOPMENTAL REGULATION OF UREIDE METABOLISM IN FRENCH BEAN (PHASEOLUS VULGARIS)

Díaz Leal, J. - Pineda, M. - Alamillo, JM*
Plant Physiology Department. Sciences Faculty.University of Córdoba
**Corresponding author, e-mail: b02dilej@uco.es*

The ureides, allantoin and allantoate, are key molecules in transport and storage of nitrogen in ureidic legumes. Ureides are produced through the oxidation of purines synthesized in root nodules and as a salvage pathway to remobilize nitrogen compounds in senescent or stressed tissues. In shoots and leaves from French bean plants using nitrogen-fixation as sole nitrogen source, ureide levels were low during the whole vegetative stage but they suffered a dramatic increase at the onset of flowering. This sudden increase suggests the involvement of regulatory signals governing the induction of ureide synthesis. Rise in ureide levels was accompanied by increases in allantoinase gene expression and activity, indicating that developmental stage regulates ureide levels mainly through induction allantoate synthesis catalysed by allantoinase. Comparison of nodulated, nitrogen fixing-plants, with plants using nitrate as main nitrogen source, revealed that transition from vegetative to reproductive stage occurred earlier in nitrogen-fixing plants, coupled to rise in ureides, than in nitrate-fed plants, in which ureide levels suffered only a moderate increase during late reproductive stage. Work supported by Grants BIO2006-09366 (MEC, Spain), AGR01283, P07-RNM-03307 and BIO-115 (JA-CICE) (Junta de Andalucía, Spain).

P13-024: PROFILING OF AROMATIC VOLATILE COMPOUNDS DURING FRUIT RIPENING OF DIFFERENT CITRUS SPECIES: A BIOCHEMICAL AND MOLECULAR APPROACH

Gurrea Martínez, A. - Zacarías, L. - Rodrigo Maria. J.*
Instituto de Agroquimica y Tecnología de Alimentos-CSIC
**Corresponding author e-mail: ciegur@iata.csic.es*

The main volatile compounds in citrus are mono- and sesquiterpenes, being the monoterpene limonene the major volatile in the essential oil fraction of citrus peel. Content and composition of essential oils in the peel of citrus fruits have been long studied but limited information is available about the emission on intact fruit. In the present study we report a comparative analysis of the profiling of volatile emission during ripening of fruits of 3 citrus species: Clemenules mandarin (Citrus clemetina), Navelate orange (Citrus sinensis) and the mandarin hybrid Ortanique (Citrus sinensis x Citrus reticulata). By headspace GC-MS analysis more than 50 volatiles were identified, being terpenes the main constituents at all stages and species, and limonene the most abundant. The emission of monoterpenes was similar in fruits of the 3 species, with a maximum emission of limonene, sabinene, pinene, ocimene and linalool in immature green fruits and decreasing thereafter. Interestingly, the emission of the main sesquiterpenes was different among the different species. Velencene and selinene were emitted at low levels or not detected in the aroma of Clemenules while in Ortanique and Navelate fruits increased significantly during ripening. Other sesquiterpenes as α-copaene, δ-cadidene, β-cubebene and germacrene D, showed a similar emission in the 3 species, decreasing during ripening. These results suggest that changes in the volatile emission during ripening may be associated with induction or repression of specific terpene synthase activities. The potential relationship between changes in the aromatic profile and the expression of selected mono- and sesquiterpene syntases genes will be presented and discussed.

P13-025: BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF CAROTENOGESIS DURING RIPENING OF PERSIMMON FRUITS (DIOSPYROS KAKI, CV ROJO BRILLANTE)

Rodrigo Esteve, M. - Garcia-Moreno, P. - Alquezar, B. - Carmona, L. - Zacarias, L.*
Instituto de Agroquimica y Tecnología de Alimentos-CSIC
**Corresponding author, e-mail: mjrodrigo@iata.csic.es*

Carotenoids are the pigments responsible of the colouration of persimmon fruit and also major determinants of the antioxidant and provitamin A activity. Changes in carotenoid profiling and expression of carotenoid biosynthetic genes during ripening of Rojo Brillante persimmon have been analyzed. In the peel of green-mature fruits, carotenoid profile was the characteristic of chloroplasts, being lutein the main carotenoid. During fruit ripening, the content of the phytoene, phytofluene, b-cryptoxanthin and lycopene increased. In the pulp, the level of carotenoids was lower than in the peel and at maturity the main carotenoid was b-cryptoxanthin followed by lycopene, zeaxanthin and antheranxin. The expression of phytoene synthase (PSY) and z-carotene desaturase (ZDS), genes of early steps of the pathway, b-lycopene cyclase (b-LCY), responsible of the formation of b-carotene, and b-carotene hydroxylase (b-CHX) which yields b-cryptoxanthin and zeaxanthin were analyzed. PSY and ZDS genes were induced during fruit ripening, in parallel with the massive accumulation of linear carotenes. b-LCY gene showed a dual pattern of expression during ripening; increasing at the time of fruit accumulated high levels of b-cryptoxanthin, followed by a dramatic decrease at full-coloured stage, which explains the accumulation of lycopene. b-CHX showed the highest level of expression in green-mature fruits, declining thereafter and maintained at low levels during the remaining ripening period. These results suggest that carotenogenesis in persimmon is mainly regulated by complex and coordinated transcriptional changes which correlated fairly well with the evolution of carotenoids content and composition.

P13-026: TREHALOSE 6-PHOSPHATE LEVELS AFFECT STARCH DEGRADATION IN LEAVES

Camara Mattos Martins, M.[1] - Fettke, J.[2] - Feil, R.[1] - Stitt, M.[1] - Lunn, J. E.[1]*
[1]Max-Planck-Institute of Molecular Plant Physiology
[2]University of Potsdam
**Corresponding author, e-mail: martins@mpimp-golm.mpg.de*

There is now considerable genetic and biochemical evidence that trehalose 6-phosphate (Tre6P), the intermediate of trehalose biosynthesis, is an essential signal metabolite in plants. It appears to be an indicator of sugar status that influences plant metabolism, growth and development, although its mode of action is uncertain. The aim of this project is to discover the signal transduction pathways that link Tre6P to its downstream targets. Arabidopsis thaliana plants have been transformed with constructs for ethanol-inducible expression of the enzymes that synthesize and hydrolyse Tre6P – trehalose-phosphate synthase (TPS) and trehalose phosphatase (TPP). Tre6P levels rose within 4 hours of TPS induction, leading to a pronounced inhibition of starch breakdown in the leaves and a starch excess phenotype at the end of the night, along with decreased levels of soluble sugars – sucrose, maltose, fructose and glucose. Western blotting and native gel electrophoresis analyses indicated that elevated Tre6P did not affect protein or activity levels of four enzymes involved in starch breakdown: glucan, water dikinase; phosphoglucan, water dikinase; cytosolic disproportionating enzyme and cytosolic glucan phosphorylase. Further enzymes (e.g. b-amylase) are being

tested, along with the possibility that Tre6P acts as an allosteric inhibitor of one or more of these enzymes. As a complementary approach, the inducible-TPS line has been chemically mutagenised, and the progeny are being screened for mutants that show loss of the starch excess phenotype. These complementary biochemical and genetic approaches should reveal the targets and mechanism of Tre6P signalling, and thus its role in control of starch degradation in leaves at night.

P13-027: SUBCELLULAR TRANSPORT IN TERPENE BIOSYNTHESIS

Ting, H. - Bouwmeester, H. - Van der krol, S. - Verstappen, F. - Schreuder, M.*
Lab. of Plant Physiology, Wageningen University
**Corresponding author, e-mail: jimmytinghm@gmail.com*

Terpenoids are the most structurally varied class of secondary metabolites in plants with signaling function in plant-plant and plant-insect interactions and which have important medicinal usages in humans.
The steps in the terpenoind biosynthesis, accumulation and emission occur in different subcellular compartments (e.g. plastids, ER, mitochondria, cytosol, vacuole, apoplast). Because many terpenoid intermediates are lipophilic, it is likely that specific transport mechanisms exist to transport these compounds within the cell and between cells. Terpenoid producing cells usually also contain high levels of specific lipid transfer proteins (LTP‘s) and we are investigating this suggestive link between terpenes and LTP‘s. For this purpose we studied how terpene accumulation changes in wt and LTP mutant plants using GC/LCMS (Arabidopsis and A. annua).
In a complementary approach we studied the changes in LTP expression in plants with manipulated terpene synthesis using microarray and RTPCR. Preliminary results will be presented.

P13-028: CHARACTERIZATION OF SEEDS OF A C4 PHOSPHOENOLPYRUVATE CARBOXYLASE-DEFICIENT MUTANT OF AMARANTHUS EDULIS

Alvarez, R.[1] - Gandullo, J.[1] - Vidal, J.[2] - Echevarria, C.[1]*
[1]Departamento de Biología Vegetal y Ecología. Facultad de Biología, Universidad de Sevilla
[2]Institut de Biotechnologie des Plantes, Université Paris-Sud.
**Corresponding author, e-mail: mrosario@us.es*

A mutant of Amaranthus edulis (line LaC4 2.16), deficient in leaf C4 PEPC has been isolated that is unable to carry out photosynthesis and develop in normal air, although the plant is able to grow to maturity in elevated CO2 (1).It has been used extensively to investigate the regulation and control of C4 photosynthesis (2, 3). However, no attempt has been made previously to examine whether the mutation in leaves may affect sink tissues.
In the present work, seeds produced by the mutant have been characterized further in terms of germination capacity, protein and energy (ATP) content, PEPC activity and regulatory phosphorylation. Our results show that PEPC activity, polypeptide, PEPC kinase activity and in vitro PEPC phosphorylation were not dramatically altered in seeds of the C4 PEPC-deficient mutant of Amaranthus edulis, when compared to the wild type. However, the PEPC in the dry seed was already phosphorylated and was not phosphorylated during germination as has been shown for all other seed PEPC analyzed to date The reduced soluble protein content and weight of mutant seeds reflect defects in the seed maturation process and may explain why the ATP concentrations were significantly reduced in the mutant seeds.
The lower ATP concentration would impact on general metabolism and regulatory processes, thus leading to a delayed germination rate.
References
(1)Dever et al (1995). Journal of Experimental Botany, 46, 1363-1376.
(2)Grisvard et al (1998). Gene, 213, 21-35.
(3)Bailey et al (2000). Journal of Experimental Botany, 51, 339-346.

P13-029: CARBON MANAGEMENT IN THE MAIZE LEAF - EXPLORING SPATIAL AND TEMPORAL DIMENSIONS

Czedik-Eysenberg, A.[1] - Wang, L.[1] - Arrivault, S.[1] - Tohge T[1]*
Nunes-Nesi, A.[1] - Fernie, A.[1] - Brutnell, T.[2] - Sulpice, R.[1] - Stitt M.[1]
[1]Max-Planck-Institute of Molecular Plant Physiology
[2]Boyce Thompson Institute, Cornell University
**Corresponding author, e-mail: czedik@mpimp-golm.mpg.de*

Plants are in constant struggle to balance their carbon resources, spatially (between sink and source regions) and temporally (day/night). The overall goal of this balance is to allow maintenance and growth.
In this study, we took advantage of certain features of the maize leaf to study how growth is regulated in response to the carbon availability. A maize leaf grows mainly longitudinal, which allows precise determination of leaf growth. Further, the leaf displays a clear spatial gradient between cell division, cell elongation, and mature photosynthising tissue (leaf base to tip).
Four week old maize plants were subjected to a normal diurnal cycle and a further extension of the night (carbon starvation). Leaf 8 growth was monitored during the full experiment at 5 min resolution and samples from mature and growing leaf zones were taken at 5 time points during the experiment. This allowed us to follow the reaction of leaf growth to carbon starvation and study the responses of primary metabolites. Analysis of gene expression, primary metabolism enzyme activities, translation rate and nucleus duplication status are ongoing to extend our understanding of carbon management. In a second experiment the developing leaf 3 of 7 day old maize plants was divided into 15 sections of 1 cm. A rapid quenching method in the light allowed preserving rapid-turnover Calvin-Benson cycle intermediates. Additionally, the sections are analyzed for primary and secondary metabolites and for gene expression by deep sequencing. This dataset will allow us to look at carbon distribution and sink-to-source transition in the developing maize leaf at very fine spatial resolution and will complement the first experiment where temporal aspects are considered.

P13-030: PEPC PROTEOLYSIS BY THE C19-PEPC-PROTEASE IS ACTIVATED BY PHOSPHATIDIC ACID.

Gandullo, J. - Álvarez, R. - Echevarría, C.*
Dpto. de Biología Vegetal y Ecología, Fac. de Biología, Univ. de Sevilla

The C19-PEPC-protease is a protein copurified with PEPC by several experimental methods including hydroxyapatite and anion-exchange chromatographies or immunochromatography with PEPC polyclonal antibodies. The C19-PEPC-protease is specifically activated by adding the C19 peptide, a conserved 19-amino acid peptide from carboxy terminus of PEPC (1,2). In this work we show that the C19-PEPC-protease is activated by phosphatidic acid (PA) in absence of the C19 peptide, and how the cleavage starts in the sequence of 18 amino acids in the N-terminal end of the protein that contains the phosphorylation motif. The same result was obtained when we added the C19 peptide (1). In addition, we found that PA promoted the exposition of 19 amino acids in the C-terminal end of the PEPC. This conserved C-terminal domain of the PEPC is normally buried in the subunit´s structure in a hydrophobic region. Finally, we demonstrated that the C19-PEPC-protease has preference by the non-phosphorilated N-terminal end of the PEPC when it is activated by PA. All these results suggest a new perspective about the regulatory phosphorylation of PEPC that could be implicated in the control of the degradation of PEPC by a mechanism including non-phosphorylated PEPC, C-terminal exposition and PA.

Reference: (1) Gandullo J et al (2008) Physiol Plant 133(3): P11-024 (2) Alvarez R et al (2003) Plant Physiol 132: 1097-1106

P13-031: CAROTENOID CLEAVAGE ACTIVITY OF RICE CCD GENE FAMILY IN E. COLI, ARABIDOPSIS AND RICE

Liang, Y.S.[1] - Song, M.H.[1] - Kim, J.K.[2] - Kim, J.K.[1] - Lim, S.H.[1] - Lee, J.Y.[1] - Suh, S.C.[1] - Ha, S.H.[1]
[1]National Academy of Agricultural Science
[2]Myongji University

Carotenoid cleavage dioxygenases (CCDs) play important roles in the generation of various bioactive apocarotenoids (hormones, pigments, flavors and defense compounds) by oxidative tailoring of carotenoids. Rice (Oryza sativa) has nine genes that constitute a family of putative CCDs. Three members (OsNCED1, 3 and 8) of the family are implicated in the synthesis of abscisic acid. Another three (OsCCD7, 8a and 8b) are related to control lateral shoot branching by forming novel phytohormone. However, the function of the other three (OsCCD1, 4a and 4b) are still unclear. The endogenous expression of rice CCDs was compared by RT-PCR and tiling chip analysis in diverse rice tissues. Their promoter activities were examined in transgenic rice plants. To confirm the carotenoid cleavage activity of four CCD genes (OsCCD1, 4a, 4b and AtCCD4), their cDNAs were introduced into the genetically engineered E.coli strains which produced carotenoid substrates of phytoene, lycopene, β-carotene, and zeaxanthin, respectively. According to substrate diversity by HPLC analysis, all four CCDs cleaved more actively zeaxanthin than lycopene and β-carotene. The highest specificity about lycopene and β-carotene was resulted in OsCCD4b and OsCCD4a among four CCDs. To examine carotenoid cleavage activity in Arabidopsis, four CCDs were transformed into Atccd4 knock-out mutant for functional complementation test as well as into Arabidopsis Col-0 wild-type. And currently, we are obtaining transgenic rice plants that were transformed with several vector constructs to intent over-expression of four CCDs or suppression of three rice CCDs. Transgene characterization in Arabidopsis and rice plants will be elucidated by the biochemical and molecular biological analyses.

P13-032: REGULATION OF C:N NUTRIENT BALANCE IN ARABIDOPSIS FATTY ACID AND CARBOHYDRATE METABOLISM MUTANTS

Bussell, J.D.- Smith, S.M.*
University of Western Australia
**Corresponding author, e-mail: bussell@cyllene.uwa.edu.au*

The Carbon-Nitrogen (C:N) balance plays a key role in regulation of plant growth to match the provision of carbon skeletons to the availability of nitrogen for assimilation or reallocation. For example, high C:N ratios in the growth medium retard progression of Arabidopsis seedlings from heterotrophic to photoautotrophic growth. Genetic studies have provided evidence for C- and N-sensing mechanisms, but very little is known about molecular mechanisms for sensing C:N or controlling growth under different C:N regimes. We present an analysis of some metabolic mutants, including mutants disrupted in fatty acid and carbohydrate metabolism. These mutants display phenotypes that are dependent on the C:N regime under which they are grown. Growth of kat2, a β-oxidation mutant, can be enhanced either by providing sugar or by removing nitrogen, implying that seedling growth is regulated by the C:N balance rather than by energy provision alone. We also report a new mutant that exhibits inhibited growth in response to both exogenously supplied sugar and increased photosynthesis. Moreover, the mutant accumulates starch when grown on media containing levels of N sufficient to repress starch accumulation in WT. Collectively these mutants point towards a metabolic switch regulating C-supply in response to available N.

P13-033: NUCLEASE ACTIVITIES DURING THE GERMINATION AND EARLY SEED DEVELOPMENT IN FRENCH BEAN

Lambert, R. - Cabello-Díaz, J.M. - Quiles, F.A. - Piedras Montilla, P.
Universidad de Córdoba

French bean (Phaseolus vulgaris) is a legume that accumulated ureides in cotyledons and embryonic axes during early seedling development that are synthesized from purines (Quiles et al., 2009). Nuclease activities were analysed to investigate if those ureides are the result of nucleic acid turnover. Nuclease activities were assayed in crude extracts obtained from cotyledons and embryonic axes from 0 to 6 days after imbibition (DAI) with DNA and RNA as substrates. Those activities were very low in cotyledon extracts (0 to 6 DAI). However, a major nuclease activity was induced after radicle emergence in embryonic axes which reached a maximum 3 DAI and remained high through the analysed time (up to 6 DAI). The levels of nuclease activity were lower in embryonic axes of other legumes analysed (chickpea, soybean). The activity induced in French bean corresponds to a protein with a molecular mass of about 39 kDa that used more efficiently ssDNA than RNA as substrate. Optimum pH was between 6.5 and 7.0 and maximum activity was found at temperatures above 60ºC, the enzyme being extremely resistant to heat denaturation. The activity was inhibited by Pi, PPi and inosine monophosphate, thus suggesting a feedback inhibition by the nucleic acid degradation products. The nuclease activity in French bean seedlings subjected to several growth and stress conditions will be also presented.

This work was supported by Ministerio de Ciencia e Innovación (AGL2009-11290) and Consejería de
Innovación, Ciencia y Empresa de la Junta de Andalucía (CVI1761).

P13-034: STUDY OF PARAMETERS ASSOCIATED WITH LEAF DEVELOPMENT IN SUNFLOWER PLANTS GROWN UNDER DIFFERENT IRRADIANCES

de la Mata, L. - de la Haba P. - Cabello, P. - Agüera E.*
Universidad de Córdoba, Área de Fisiología Vegetal
**Corresponding author, e-mail: b42matsl@uco.es*

Different parameters which vary during the leaf development in sunflower plants have been determined. For this purpose, plants were grown at different irradiances (350 and 125 mmol m-2 s-1) for a 50-day period, and the primary leaves were collected at 16, 22, 32, 42 and 50 days. Growth parameters and photosynthesis rate were determined in the leaves. The content of photosynthetic pigments, proteins, soluble sugars and starch were also measured. It was observed that young leaves (16 days) of plants grown at high irradiance showed 30% more dry matter than plants grown at low irradiance. In senescent leaves (42 and 50 days) a higher decrease on the leaf specific mass (7%) was observed at high irradiance. The content in photosynthetic pigments was considerably higher (30%) in plants grown at low irradiance, while the leaf development affected negatively the pigments content in both treatments. The leaf age diminished the photosynthesis rate, being the decrease of photosynthetic activity higher and more premature in plants grown at high irradiance. With regard to the content in carbohydrates, in both treatments the soluble sugar concentrations increased at the beginning of the leaf senescence while the contents in starch and soluble proteins decreased with leaf development. In conclusion, the changes in the parameters determined were more marked in plants grown at high light levels, and this suggests that the high irradiance may induce early leaf senescence, therefore shortening the growth cycle of the plant. This work was granted by Junta de Andalucía (Proyecto de Excelencia P07-CVI-02627, Grupo de Investigación: BIO-0159) and DGICYT (AGL2009-11290).

P13-035: ISOLATION OF ARABIDOPSIS MUTANTS IN THE EXPRESSION OF A GENE FOR OIL SYNTHESIS BY USING HIGH-THROUGHPUT REAL-TIME BIOLUM

Kawai, T.[1] - Onai, K.[2] - Matsumoto, T.[1] - Hashimoto, M.[1] - Maeo, K.[1] - Ishiura, M.[2] - Nakamura, K.[1]
[1] *Lab. Biochem. Grad. Sch. Bioagr. Sci., Nagoya University*
[2] *Cent. Gene Res., Nagoya Univ. and SENTAN, JST*

During the maturation of Arabidopsis seeds, a transcription factor WRI1/ASML1 coordinately activates many genes involved in fatty acid synthesis in the plastid. However, expression of genes for triacylglycerol (TAG) assembly in the endoplasmic reticulum is regulated differently. Arabidopsis plants with a luciferase (LUC) reporter gene under the promoter of a gene for diacylglycerol acyltransferase (DGAT1::LUC) showed strong LUC expression in maturing seeds and weaker expression in young leaves, that were similar to the expression patterns of DGAT1 mRNA. Mutants with altered LUC expression were screened with high-throughput real-time bioluminescence monitoring system, which can automatically monitor bioluminescence of 1,920 or 19,200 seedlings for a week. Seeds of a DGAT1::LUC plant were treated with EMS, and M2 seedlings which showed low LUC expression were further grown to obtain seeds. We then monitored bioluminescence of 32 to 48 individual M3 offsprings from each candidate. After screening ca 20,000 M2 seedlings, we obtained dozens of lines that consistently exhibit low LUC expression in M3 and at least two of them showed reduced LUC expression in developing seeds and reduced seed oil content. We also screened ca 10,000 T1 DGAT1::LUC plantsfurther transformed with enhancer T-DNA. Overexpression of one of a gene tagged with the enhancer under the 35S promoter caused enhanced LUC expression in seeds and increased oil content per seed.

P13-036: METABOLIC RESPONSES OF QUERCUS SUBER L. SEEDLINGS UNDER COMBINED ZINC AND DROUGHT STRESS

Disante, K.[1] - Fuentes, D.[3] - Cortina, J.[1] - Ljung, K.[2] - Lundgren, K.[2]*
[1]*Dept. de Ecología IMEM, Universidad de Alicante*
[2]*Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences*
[3]*Fundación Centro de Estudios Ambientales del Mediterráneo CEAM, Universidad de Alicante*
**Corresponding author, e-mail: kb.disante@ua.es*

Zinc (Zn) is an essential nutrient for higher plants that becomes toxic as its availability increases. In nature, stress factors seldom occur independently, but information on the interaction between Zn toxicity and other sources of stress is scarce. We have assessed the combined effect of Zn supply and low water availability on the metabolic profile of Quercus suber seedlings. The simultaneous application of two sources of stress triggered a strong metabolic response to maintain homeostasis. Seedlings were treated with four levels of Zn, ranging from 3 to 150 µM, while exposed to two contrasting irrigation regimes. Foliar and root Zn concentration increased with Zn availability, the increase being dependent on irrigation regime. The concentration of sugars, such as rhamnose and glucose, increased with Zn dose suggesting that these compounds may function as osmo-protectants. Observed differences in the organic acid profile (malic, aspartate and citric acid) in response to increasing Zn concentration may be related to tolerance. The relative concentration of α-Tocopherol, an antioxidant that deactivates photosynthesis-derived reactive oxygen species, increased at higher doses of Zn in well watered seedlings, but not in water-stressed seedlings. Low water availability resulted in an increase in the concentration of quinic acid and glucose, which may be related to protection against heavy metal and osmotic adjustment, respectively. In this study we relate metabolic changes with the physiological performance of Quercus suber seedlings under combined Zn and drought stress. Our results provide no evidence of synergism or antagonism between the two sources of stress.

P13-037: BIOCHEMICAL AND PHYSICOCHEMICAL IMPLICATIONS OF THE REVERSIBLE STARCH PHOSPHORYLATION

Hejazi, M. - Fettke, J. - Steup, M.
University of Potsdam AG Plant Physiology

The phosphorylation of starch is mediated by two dikinases that esterify glucosyl residues at the C6 and the C3 position. Deficiency in either of the two dikinases indicates that the transient phosphorylation of starch is essential for an efficient turn-over. Arabidopsis chloroplasts contain at least one dual specificity phosphatase (DSP) that counteracts the two dikinases. This phosphatase (designated as SEX4) is highly homogenous to the laforin of the mammalian system. Non-functional laforin is associated with a major neurodegenerative disease, epilepsy. Arabidopsis mutants lacking a functional SEX4 possess a starch-excess phenotype and accumulate phosphorylated maltodextrins. The reversible phosphorylation of starch-like α-glucans has been studied by using crystalline maltodextrins representing either the A- or the B-type allomorph. Phosphorylation has been quantified by monitoring the incorporation of labelled phosphate from [β-33P]ATP into α-glucans.
Dikinases possess a highly conserved pattern of amino acids. For a more detailed analysis of the reversible starch phosphorylation, a series of mutated dikinases has been generated in which single amino acids are exchanged. Catalytic features of the mutated dikinases have been studied both in the presence and in the absence of target glucans. The data obtained indicate an unexpected complexity of the biochemical action of the dikinases.

P13-038: ALLELOPATHIC POTENTIAL OF THREE NEPETA SPECIES: THE EFFECT OF NEPETALACTONE STEREISOMERS ON SEED GERMINATION AND ANTIOXIDATIVE CAPACITY OF LEPIDIUM SATIVUM

Nestorovic, J.[1] - Misic, D.[1] - Siler, B.[1] - Zivkovic, S.[1] - Stojic, A.2 - Perisic, M.[2] - Grubisic, D.[1]*
[1]*Institute for Biological research "Sinisa Stankovic"*
[2]*Institute of Physics*
**Corresponding author, e-mail: jakica81@gmail.com*

Allelopathic potential of three endemic Nepeta species, differing in their qualitative nepetalactone content, on the germination of Lepidium sativum L. seeds, was examined under in vitro conditions. Seeds of L. sativum were cocultivated with shoots of N. rtanjensis, N. sibirica and N. nervosa. The analyses of qualitative and quantitative nepetalactone content in the methanol extracts of in vitro grown shoots were performed by HPLC-DAD analysis. Trans-cis-nepetalactone was detected in shoots of N. rtanjensis Diklić & Milojević, while cis,trans-nepetalactone stereoisomer was present in N. sibirica L. No nepetalactone was observed in shoots of N. nervosa Royle & Bentham. The concentrations of nepetalactone in the atmosphere of culture vessels were detected by PTR-MS.
The inhibition of L. sativum seed germination, and subsequently of seedling growth, was pronounced during cocultivation with N. sibirica, and especially N. rtanjensis. The increase in nepetalactone content in the atmosphere of glass jars was strongly correlated with the decrease of L. sativum seed germination and seedling growth. No significant inhibition in seed germination was observed for seeds cocultured with shoots of N. nervosa. The content of total phenolics, as well as the total antioxidative capacity of L. sativum seedlings, increased with increasing nepetalactone concentration in the atmosphere of glass jars. Our results suggest that nepetalactone possess strong allelopathic potential, and indicate that trans,cis-nepetalactone is more active than cis,trans-nepetalactone.

P13-039: ENZYME AND METABOLITE PATTERN DURING POST-HARVEST STORAGE CONDITIONS IN BRASSICA RAPA L. CV. SYLVESTRIS

*Massaro, G. - Annunziata, M.G. - Iannuzzi, F - Nacca, F. - Carillo, P. - Fuggi, A.**
Dipartimento di Scienze della Vita, Seconda Università di Napoli, Caserta
**Corresponding author, e-mail: amodio.fuggi@unina2.it*

Keywords: broccoli, Brassica rapa, peroxidase, ascorbate, chlorophyll Vegetables after harvesting activate defence and senescent processes that affects also nutrtional, nutraceutic and organoleptic properties. They involve tissue wilting, yellowing and browning as well as the appearance of unpleasant tastes. In particular, chlorophyll degradation causing leaf yellowing and tissue browning are mainly due peroxidases and polyphenoloxidases.
In this work peroxidases from Brassica rapa cv. sylvestris have been studied. Their distribution has been determined in different storage conditions (10 °C, 4 °C in air and 4 °C in a modified atmosphere) up to 20 days from the harvest. The florets, stem and leaf blade were analysed separately. Peroxidase activities increased during post-harvest, mainly in plants stored at 10 °C in air. The isoenzyme distribution was different in the analysed tissues and also changed differently in the storage time. The pattern of chlorophyll and carotenoids, as well as of ascorbate, inhibitor of some peroxidase isoenzyme, was also followed. As strong antioxidant they were also important nutraceutics. In addition the changes of metabolites, like carbohydrates, proteins, free amino acids, glucosinolates, nitrate and nitrite were also determined.
Financial support was obtained by “Seconda Università degli Studi di Napoli”, “Ministero dell‘Università” and “Ricerca Scientifica e tecnologica” of Italy (PRIN 2006077008_005; 2008S9T3KK_003).

P14 Plant And Global Change

P14-001: DOES THE FUTURE CLIMATE (ELEVATED CO2 AND TEMPERATURE) CAUSE OXIDATIVE STRESS RESPONSES IN PLANTS?

Farfan Vignolo, E. - Rose, P. - Asard, H.*
University of Antwerp
**Corresponding author e-mail: evelyn.farfan@ua.ac.be*

Carbon dioxide concentrations (CO2) and other greenhouse gases, as well as average temperatures and the frequency of extreme drought and heat waves, are predicted to increase by 2100. Rising CO2 is generally considered to have a fertilizing effect, at least in C3 plants, thereby increasing biomass production. However, other climate factors such as increasing temperature and others abiotic stresses are likely to forfeit this beneficiary CO2 effect. Moreover, initial evidence indicates that elevated CO2 by itself may cause oxidative stress-like responses in plants.
We studied the oxidative stress responses of six grassland species (Lolium perenne, Poa pratensis, Medicago lupulina, Lotus corniculatus, Rumex acetosa and Plantago lanceolata), grown in sunlit field chambers with either ambient (Tair + 375 ppm CO2) or future climate (Tair+3°C + 620 ppm CO2) conditions. We analyzed stress levels (lipid peroxidation, protein carbonylation), antioxidant capacity (e.g. FRAP assay), molecular antioxidants (ascorbate, glutathione, tocopherols, polyphenols) and antioxidative enzyme activities (e.g. POS, MDHAR, GR). Our results show that different species respond different to the future climate, with no apparent overall oxidative damage to the plants. However, we did find significant changes in total antioxidant capacity, paralleled by significant changes in the ascorbate and glutathione pools. Therefore, it does appear that future climate conditions may increase lipid and protein oxidation, but this increased oxidative damage is effectively countered by increases in the antioxidative system. Further studies are needed in order to improve our knowledge of the molecular and biochemical mechanisms by which plants respond to climatic change.

P14-002: THE EFFECT OF A CHANGING CLIMATE ON GROWTH AND REPRODUCTIVE SUCCESS OF SPANISH JUNIPER (JUNIPERUS THURIFERA): SEARCHING FOR ITS ECOPHYSIOLOGICAL BASIS

Gimeno Chocarro, T.[1] - Granda, E.[1] - Pias, B.[1] - Camarero, J.J.[2] - Valladares, F.[1]*
[1]Instituto De Recursos Naturales Csic
[2]Araid Instituto Pirenaico EcoloGIA CSIC
**Corresponding author e-mail: Teresa.Gimeno@Ccma.Csic.Es*

Forests of Juniperus thurifera, endemic to the western Mediterranean basin are considered as priority for conservation. There has been an increasing interest in unravelling the dynamics of growth, regeneration and survival of these forests due to their potential vulnerability to global change Here we present the results of aM3-year study linking climate driven individual differences in growth with fecundity and physiological performance (gas exchange) of males, females and juveniles. Climate was monitored over the study period and completed with historic records from a nearby meteorological station. Wood cores were extracted for long-term analysis of growth and manual and digital dendrometers were installed to record growth phenological pattern. Cone production was recorded and gas exchange followed over the seasons in male, female and juveniles. We found that tree ring growth was favoured by wet springs and mild winters; we also found that the three sites differed in both long and short term growth patterns and that these differences could not be attributed exclusively to the climate. Female and male cone production differed in year to year variation. Males and females did not differ in gas exchange, or in their growth patterns. But adults and juveniles differed in their performance along time: juveniles had higher photosynthesis during favourable periods but this dropped more abruptly under harsh conditions. On the whole our results show that J. thurifera performance is sensitive to climate, but there are other factors affecting it beyond gender, which had a negligible influence here. Our results suggest that J. thurifera has great potential for coping with the current and future global change scenarios with leaf physiology playing a modest role in explaining it

P14-003: PHOTOSYNTHETIC ACCLIMATION IN ALFALFA AT ELEVATED CO2 DEPENDS ON GROWTH SEASON AND SINORHIZOBIUM STRAIN

Sanz, A. - Erice, G. - Irigoyen, J.J. - Aguirrolea, J. - Sánchez-Díaz, M.*
Universidad de Navarra
**Corresponding author e-mail: assaezdejau@alumni.unav.es*

Previous studies have shown that N availability may affect plant response to elevated CO2, leading to photosynthetic down-regulation and limiting potential plant production. The aim of the study was to analyze the effect of elevated CO2 (700 μmol mol-1) in interaction with the predicted temperature increase (+4ºC) in two different experimental dates (August and October) with similar degree-days accumulation (around 600). Three Sinorhizobium meliloti strains were used: 102F78, 102F34 and 1032GMI in plants grown in Temperature Gradient Greenhouses in Pamplona (42.80N, 1.66W; Spain). Despite the interaction between elevated CO2 and increased temperature enhanced plant production in the two different harvest dates, the most efficient strain was 102F78 in August and 102F34 in October. Elevated CO2 led to photosynthetic down-regulation regardless experimental date as shown by A/Ci curves and net photosynthesis. Most authors agree that leaf starch accumulation under elevated CO2 may induce photosynthetic down-regulation. However, in our study leaf starch was enhanced in October but not in August. Our data show that photosynthesis down regulation occurs with or without starch accumulation. Photosynthetic acclimation under elevated CO2 is often described as a consequence of carbohydrate accumulation (via gene repression). According to our results, another process like N availability may affect plant response to elevated CO2. Acknowledgements This work was supported by Ministerio de Ciencia e Innovación (MICINN BFU2008-01405), Fundación Universitaria de Navarra (PIUNA-2008) and Fundación Caja Navarra. The assistance of A. Urdiain and M. Oyarzun is appreciated.

P14-004: CLIMATE CHANGE AND GRAPEVINE CV. TEMPRANILLO: OXIDATIVE DAMAGE AND ANTIOXIDANT ENZYMES RESPONSE

Salazar Parra, C.[1] - Aguirreolea, J.[1] - Sánchez-Díaz, M.[1] - Irigoyen, J.J. 1 [1] - Morales, F.[2]
[1]Universidad de Navarra
[2]Estación Experimental Aula Dei (CSIC)

Photosynthesis (AN) and photosynthetic electron transport (ETR) are affected by different environmental stress factors, such as those associated with climate change. Under stress conditions, it can be generated an electron (e-) excess that cannot be consumed, which can react with O2 producing reactive oxygen

species. The aim of this work was to evaluate the influence of climate change (high CO2, high temperature -highT- and partial irrigation -PI-) on the antioxidant status in grapevine cv. Tempranillo leaves, from veraison to ripeness.
The lowest ratios between e- generated (ETR) and consumed (AN + respiration + photorespiration) were observed in high CO2-highT plants. In PI plants under ambient conditions, e- not consumed seemed to be diverted to alternative ways. Oxidative damage to chlorophylls was not observed. However, these plants had increases in MDA. These increases matched well with an early rise of H2O2 and antioxidant enzyme activities (SOD, APX, CAT). Enzymatic activities were maintained high until ripeness. In summary, plants under PI and ambient conditions were less efficient to cope with oxidative damage than did well-irrigated plants. Plants under high CO2 and highT were not affected by oxidative damage, mainly due to higher rates of e- consumed in photosynthesis.
Acknowledgements: MICINN (BFU2008-01405/BFI), Fundación Universitaria de Navarra, Caja Navarra, Gobierno de Aragón (A03 research group) and Asociación de Amigos de la Universidad de Navarra (CSP grant).

P14-005: HIGH NIGHT TEMPERATURES INFLUENCE GROWTH PERFORMANCE, METABOLISM, YIELD PARAMETERS AND GRAIN QUALITY OF RICE

Glaubitz, U.[1] - Erban, A.[1] - Kopka, J.[1] - Köhl, K. [1] - Fitzgerald, M.[2] - Hincha, D.K. [1] - Zuther, E.[1]*
[1]Max-Planck-Institute of Molecular Plant Physiology,
[2]International Rice Research Institute, Grain Quality, Nutrition, Postharvest Center (GQNPC)
**Corresponding author e-mail:glaubitz@mpimp-golm.mpg.de*

Global warming has an increasing influence on the productivity of crop plants. In the past century a stronger increase in daily minimum compared to maximum temperatures suggests an asymmetric global warming. However, only a few studies have investigated the influence of high night temperatures (HNT) on crop physiology.
We analyzed the effects of HNT on 13 rice cultivars and a DHL mapping population. A clear distinction between tolerant and sensitive cultivars was found based on chlorosis estimates. Growth was increased in tolerant in comparison to sensitive cultivars whereas photosynthetic yield and dark respiration remained unchanged. Also, the contents of soluble sugars and starch in the leaves did not vary between HNT and control conditions. GC-MS profiling of 10 cultivars indicated distinct metabolite patterns under HNT and control conditions. In sensitive cultivars amino acids were increased under HNT suggesting enhanced protein degradation. Tolerant cultivars showed a different pattern of metabolites already under control conditions, indicating the possibility to identify marker metabolites for breeding programs. HNT also resulted in a high degree of sterility and subsequent reduction in grain yield. Yield parameters together with a detailed analysis of seed quality traits will be presented.
Using chlorosis and necrosis data from 81 DH lines established by a cross of a tolerant and a sensitive parent, seven QTL could be identified.

P14-006: CULTIVAR-DEPENDENT EFFECT OF SALINITY AND ELEVATED CO2 ON MESOPHYLL CONDUCTANCE AND PHOTOSYNTHETIC PERFORMANCE IN HORDEUM VULGARE CULTIVARS

Pérez-López, U. - Robredo, A. - Lacuesta, M. - Mena-Petite, A. - Muñoz-Rueda, A.*
**Corresponding author e-mail: usue.perez@ehu.es*

The future environment may be altered by high concentrations of salt in the soil and elevated [CO2] in the atmosphere. These have opposite effects on photosynthesis: salt stress inhibits, and elevated [CO2] stimulates it.
Although both stomatal and mesophyll conductance limitations have been observed to regulate the response of photosynthesis to salt stress, the concentration of carbon in the chloroplast is more reliable estimator of assimilation than the intercellular carbon concentration. Thus, the study of the changes in mesophyll conductance caused by environmental constraints play a keystone role in our understanding of the response of photosynthesis to salinity. On the other hand, although elevated [CO2] can increase the intercellular carbon concentration it has also been observed a reduction in drawdown [CO2] between intercellular and chloroplast sites. In the present study we have analysed the mesophyll conductance and photosynthetic performance responses of two barley cultivars, Alpha and Iranis, to several salt concentrations under ambient or elevated CO2 concentrations.
Photosynthesis increased under elevated CO2 whereas it was reduced by salinity. Besides, cultivar dependent effects were detected. Growth in elevated CO2 caused reduction of mesophyll conductance while no effect of salinity was observed in this parameter. Examination of photosynthetic CO2 response curves showed that salinity caused a reduction of both Rubisco activity and ribulose-1,5-bisphosphate regeneration in Iranis, and only of ribulose-1,5-bisphosphate regeneration in Alpha. Elevated CO2 mitigated the salt effect.
This project was supported by grant MEC-BFU2007-60523/BFI, UNESCO 07/02, ETORTEK 07/44 and GRUPO UPV-GIU 07/43.

P14-007: ALTERED RESPONSE TO NITROGEN SUPPLY OF MIXED GRASSLAND COMMUNITIES IN A FUTURE CLIMATE

Van den Berge, J. - Naudts, K. - Janssens, I. A. - Nijs, I. - Ceulemans, R.*
University of Antwerp
**Corresponding author e-mail: joke.vandenberge@ua.ac.be*

Few studies have investigated whether responses to nutrient supply of mixed plant communities change under combined elevated CO2 and climate warming. In this study we analysed the response of constructed temperate grassland communities to five levels of nitrogen (N) supply, ranging from 0 to 150 kg N ha-1, under two climate scenarios. To this end grassland mesocosms, composed of three functional groups, were grown in sunlit, climate controlled chambers under either the present CO2 concentration and air temperatures, or a future climate with a higher CO2 concentration and a 3°C warming. Equal amounts of water were added to all of the mesocosms, so that warming could imply drier soils if evapotranspiration was higher. Biomass of the plant communities responded positively to N supply in the current climate, but was insensitive to N supply in the future climate. This altered response was not the result of a changing response from a single species, but all species seemed to contribute to it. The weaker response in the future climate was caused by changes in N uptake rather than by changes in nitrogen use efficiency, as community N stocks showed the same response pattern as community biomass. Climate change apparently modified the relation between fertilizer N addition and plant available N.

P14-008: CHAMAEROPS HUMILIS L. RESPONSE TO VARIOUS CLIMATE CHANGE SCENARIOS, USING DISTRIBUTION MODELS

Zapata Pérez, V. - Esteve Selma, M.A. - Martínez Fernández, M.J. - Robledano Aymerich, F. - Calvo Sendínm, JF.*
**Corresponding author e-mail: vmzapata@um.es*

Numerous studies have assessed the effects of climate change on biodiversity. Among other effects climatic change is expected to cause changes on the species distribution and the dominance of species ecosystems, leading even to local extinctions.
In Murcia Region (SE Spain), Chamaerops humilis is distributed mainly near the coast and in the pre-littoral mountains. In

its current distribution model, the climatic variables that affect the distribution of Chamaerops are winter precipitation and the number of days of frost. The aim of this work is to forecast the expected changes in the potential distribution of this species by means of species distribution modelling (SDM) and according to several regionalised climatic change scenarios (B2 and A2) and time slices.
Under the B2 and for the near future (2020-2050) expected a slight increase in the potential distribution area of the species but with a decrease in habitat quality. For the second projected time slice (2060-2090) the distribution area suffers an important fragmentation delivering three isolated populations of Chamaerops. For A2, the expected potential distributions are similar to those predicted for B2 but the initial expansion in the near future is absent.
The results can be interpreted in accordance to the theory developed by Neilson and Wullstein about the regional control of habitat size and niche specificity. The long term expected response makes this species a less useful indicator of the effects of global warming.

P14-009: PHOTOSYNTHETIC PERFORMANCE AND PHOTOPROTECTION IN TWO MEDITERRANEAN SPECIES AFTER EXPOSURE TO ENHANCED UVB RADIATION AND LOW WATER AVAILABILITY

Bernal Montolio, M. - Llorens, L. - Badosa, J. - Verdaguer, D. - Universitat de Girona*
**Corresponding author e-mail: meritxell.bernal@udg.edu*

UVB radiation levels reaching the earth surface have increased in the last decades. The aim of our study was to determine the effects of enhanced UVB radiation, low water availability and their interaction on photosynthetic performance and photoprotection of Laurus nobilis (Ln) and Pistacia lentiscus (Pl) seedlings. Plants were grown under three UV treatments from June to September: ambient UV (control), enhanced UVA and UVB (UVB treatment, 25% above ambient levels) and enhanced UVA (UVA treatment). At the same time, one half of the plants of each UV treatment (low-watered plants, LW) received half (the first month) and later one third of the water applied to the other half (well-watered plants, WW), which were irrigated to field capacity. In September, at midday and predawn, we measured chlorophyll fluorescence and collected leaves of Ln and Pl to analyze chloroplastic pigments by HPLC. Interestingly, LW plants of both species showed a higher maximum quantum yield of photosystem II (Fv/Fm) at predawn and a higher electron transport rate (ETR) in the early morning than WW plants. Ln and Pl plants grown in the UVA treatment showed a higher de-epoxidation state of the xanthophyll cycle (DPS) than those grown under enhanced UVB at midday, which suggest higher non-photochemical energy dissipation (NPQ). Although UVA treated plants also showed a higher zeaxanthin content, a higher NPQ was only observed in LW plants of Pl at midday, in accordance with a lower ETR. Thus,
at midday and/or under low water availability, enhanced UVB radiation might induce plant resistance to UVA increases in some species, since Pl plants in the UVB treatment (enhanced UVA+UVB) showed a lower DPS and NPQ than those exposed to the same UVA enhancement with no increase in UVB.

P14-010: EVIDENCES OF CROSS-TOLERANCE TO TWO ENVIRONMENTAL STRESSES (ENHANCED UVB RADIATION AND LOW WATER AVAILABILITY) IN LAURUS NOBILIS SEEDLINGS

Llorens Guasch, L - Bernal, M. - Badosa, J. - Verdaguer, D.*
Universitat de Girona
**Corresponding author e-mail: laura.llorens@udg.edu*

During the last decades, there has been an increase in the UVB radiation that arrives to the terrestrial surface. Taking into account that water availability is an important factor modulating plant responses to UVB and that drought is already limiting plant growth in the Mediterranean region, the aim of our study was to investigate whether plant exposure to enhanced UVB might lead to increased resistance to low water availability, a phenomenon known as cross-tolerance. Therefore, we grew seedlings of Laurus nobilis from May to September under three different UV radiation fluxes: ambient UV (control), enhanced UVA and UVB (UVB treatment, designed to simulate a 25% increase in UVB) and enhanced UVA (UVA treatment, designed as a control for the effects of UVA in the UVB treatment). In each UV treatment, half of the plants were watered to field capacity
(well watered plants, WW), whereas the other half received half (the first month) and later one third of the water supplied to WW plants (low watered plants, LW). In September, we did not find a significant effect of our treatments on leaf area, LMA and total phenol and flavonoid content of L. nobilis leaves. Interestingly, drier conditions decreased the leaf relative water content and stomatal conductance of L. nobilis only at ambient UV. Moreover, LW plants showed a higher above-ground biomass than WW plants under enhanced UVB. In general, while UV treatments did not affect WW plants, they affected LW plants, since, under drier conditions, plants showed thicker leaves and higher below- and above-ground biomass when grown under increased UVB than at ambient UV. Our results suggest that L. nobilis seedlings get benefits from the cross-tolerance when they are exposed to enhanced UVB and low water availability.

P14-011: 4F CROPS

*Margaritopoulou, T. * - Rigas, S. - Hatzopoulos, P. - Alexopoulou, E. - Milioni, D.*
Agricultural University of Athens, Greece
**Corresponding author e-mail: theonimarg@aua.gr*

Global changes are widely believed to be caused by a growing world population and increasing economic and industrial activities. 4F CROPS main objective is to survey and analyze parameters (environmental, technical, socio-economic and regulatory) that can contribute in the successful establishment of non-food cropping systems in the agriculture of EU27, alongside the existing food crop systems. Thisproject focuses on strengthening the knowledge base for selected non-food crops that are expected to become the new feedstock for biofuels and biomaterials.
Biotechnology has an enormous potential in developing non-food crops, either through improving biomass yield and quality or resistance to diseases, pests and environmental conditions, optimizing selected crop‘s processing characteristics and/or the end-product produced and by the creation of entirely new crops. Molecular technologies in agriculture are not a panacea but they can certainly contribute in the fight against the global change.
The 4F Crops project is expected to provide an overview of the current situation and trends in the European agriculture. It is intended as a basis of assessment for current and future efforts to introduce and establish - in combination with innovative technologies - non food crops such as switchgrass (Panicum virgatum), miscanthus (Miscanthus x giganteus), sweet sorghum (Sorghum bicolor), flax (Linum usitatissimum L.) and kenaf (Hibiscus cannabinus L).

P14-012: ANALYSING THE IMPACT OF HIGH TEMPERATURE IN ECOPHYSIOLOGICAL PARAMETERS OF TWO EUCALYPTUS GLOBULUS CLONES

Pinto, G.[1] - Silva, S.[1] - Dias, M. C.[1] - Costa, A.[1] -Correia, B.[1] - Branco, J.[1] - Gonçalves, P.[1] - Correia, C.[2]- Moutinho, J.[2] - Gonçalves, B.[2] - Santos, C.[1]*
[1]CESAM/ University of Aveiro
[1]Trás-os-Montes e Alto Douro/ CITAB
**Corresponding author e-mail: gpinto@ua.pt*

Among the environmental stresses, high temperatures can have a devastating effect on plant metabolism, disrupting cellular

homeostasis, and uncoupling major physiological processes. Preventing extensive production losses due to high temperature phenomenon in Eucalyptus plantations is an important issue considering the economical importance of this species. Although traditional genetic improvement has been concerned mainly with growth and wood properties, in the context of global change, breeders must select genotypes that can maintain growth performance under stress conditions and breeding programs requires detailed physiological information of the stress-response of the breeding material selected for nursery production To study this approach Eucalyptus globulus plants (six-month-old) were exposed to the following heat stress cumulative temperatures: 25ºC (control), 35ºC, 45ºC and 55ºC. Soil water content was maintained at field capacity in growth chambers under controlled environment conditions (light and relative humidity). All plants survive to the extreme temperature of 45 ºC but considerable losses were observed at 55ºC.
Several parameters were assessed to monitoring plant performance during stress treatment: RWC, cell membrane stability, photosynthesis, chlorophyll fluorescence, pigments, Calvin cycle enzymes (e.g. RuBisCO) and carbohydrates. Lipid peroxidation and proline were also determined.
These data will contribute to better understand Eucalyptus responses to heat and will add new insights to prevent expected disequilibrium coming from global climatic changes by selecting more heat tolerant forest genotypes.

P14-013: NITROGEN GASEOUS EMISSIONS IN TOMATO PLANTS (SOLANUM LYCOPERSICUM MILL.) UNDER NITRATE AND AMMONIUM NUTRITION

Setien, I. - Calleja Cervantes, ME. - Vega, I. - Menendez, S. - Gonzalez-Moro, MB.*
Universidad del País Vasco/EHU.
**Corresponding author e-mail: igor.setien@ehu.es*

Plants can exchange nitrogen oxides (NOx=NO+NO2), ammonia (NH3) and nitrous oxide (N2O) gases through the plant canopy, leading to serious environmental risks. This nitrogen gaseous exchange could be influenced by the N nutrition, and they mainly depends on the difference between atmospheric gas concentration and the gas compensation point of the plant. Thus the emission by plants take place when gaseous concentration in the atmosphere is lower than the plant compensation point, while the absorption takes place in the opposite case.
The aim of this work was to evaluate the effect of ammonium and nitrate nutrition on photosynthesis related gas-exchange parameters together with the nitrogen gaseous emissions.
Tomato plants were grown in perlite:vermiculite in a controlled environmental chamber under 10 mM NO3- or NH4+ nutrition. After six weeks of growth, CO2 and water vapour exchange were determined with an IRGA; NO, NO2 and NH3 by chemiluminiscence; and N2O by gas chromatography. Photosynthesis, stomatal conductance and transpiration rates were higher in NO3--than in NH4+ fed-plants. It was gas N2O that tomato plants emitted at highest rates with values between 5-45 nmol N2O m-2 s-1, being higher under ammonium nutrition. Emissions of NO and NH3 ranged between 0.45-0.65 nmol NO m-2 s-1 and 0.5-1.6 nmol NH3 m-2 s-1, respectively.
These emissions were higher under nitrate nutrition contrarily to those observed for N2O. Nitrogen gases missions were probably more related to underlying metabolic processes than to the stomatal conductance.
Acknowledgements: Research financially supported by MICINN (AGL2009-13339-CO2-01), GV/EJ and PA09/08. I. Setién holds a grant by UPV/EHU. Technical and human support provided by SGIker is gratefully acknowledged.

P14-014: DAILY VARIATION IN THE ACCUMULATION OF ULTRAVIOLET-ABSORBING COMPOUNDS IN AN AQUATIC LIVERWORT

Núñez Olivera, E. - Tomás, R. - Fabón, G. - Monforte, L. - Martínez Abaigar, J.*
Universidad de La Rioja
**Corresponding author e-mail: encarnacion.nunez@unirioja.es*

Bryophytes (mosses, liverworts and hornworts) are structurally simple and their protection against ultraviolet radiation (UVR) may be mainly based on the accumulation of ultraviolet-absorbing compounds (UVAC), whose daily changes are unknown in bryophytes. We studied the daily changes in the UVAC levels in the aquatic liverwort Jungermannia exsertifolia subsp. cordifolia under laboratory conditions. For this aim, we measured the bulk UV absorbance of acidified methanol extracts as the area under the absorbance spectrum curve in the interval 280-400 nm per unit of dry mass. The samples were exposed to three different radiation regimes: P (only PAR), PA (PAR + UV-A) and PAB (PAR + UV-A + UV-B). A simple extraction with acidified methanol was firstly conducted, presumably extracting UVAC from vacuoles, and, after this, a NaOH digestion of the residuum and a second extraction was performed to extract the cell-wall-bound UVAC. At every moment of the day, UVAC levels were higher in PAB than in PA and P samples, and in PA than in P samples. In general, UVAC levels were higher at midday than in the rest of the day, and this occurred in both cell compartments (vacuoles and cell walls) and in the three radiation regimes. Thus, UVAC levels showed clear daily changes and, although responding more strongly to UVR, responded also to high PAR. This response was much faster than previously suspected, and researchers should take into account the moment of the day in which samples are collected to interpret more adequately the responses of UVAC levels to UVR and to correctly evaluate the UVR protection of bryophytes.

P14-015: CHANGES IN THE STRESS SENSITIVITY OF PLANTS AND ECOSYSTEMS IN A FUTURE CLIMATE: INTERACTIONS BETWEEN STRESSORS.

Naudts, K. - Van den Berge, J. - Nijs, I. - Janssens, I. A - Ceulemans, R.*
University of Antwerp
**Corresponding author e-mail: kim.naudts@ua.ac.be*

In almost all ecosystems plants are subjected to stress. In the future, these stressors will occur under a modified atmospheric background, with higher CO2 concentrations and elevated air temperatures. Will this future climate alter the stress response of plant communities and their composing plant species? To answer this question grassland mesocosms were exposed to several stressors in an experimental platform consisting of 10 computer-controlled, air conditioned, sunlit growth chambers. Half of these chambers tracked ambient temperature at ambient CO2 concentration, the other half was exposed to a predicted climate scenario with elevated CO2 and temperature. The stressors included one acute stressor (drought) and two chronic stressors (nitrogen deficiency and zinc toxicity). The stressors were applied separately and in combination in order to examine the interactive impact and the effect of climate change on these interactions. An extensive set of measurements was conducted from leaf scale to ecosystem scale. Data collection included: gas exchange and leaf chemistry at leaf scale; above ground biomass at plant scale; below and above ground biomass at ecosystem scale. The results indicate a lower water consumption caused by a lower biomass production in the mesocosms subjected to chronic stressors. Therefore, these stressors are expected to have an antagonistic effect on drought stress. These and other results will be presented and discussed in relation to possible effects of a future climate.

P14-016: COMPARING LICHEN RECORDS OF HISTORICAL HERBARIUM WITH THEIR PRESENT DISTRIBUTION TO DETECT RECENT ENVIRONMENTAL CHANGES ON VESUVIUS VOLCANO

Aprile, G. G. - Migliozzi, A. - Catalano, I. - Di Salvatore, M. - Mingo, A -
DIARBOPAVE - Università degli Studi di Napoli Federico II

Lichens are among the most reliable biological indicators to assess direction and intensity of environmental changes. Vesuvius volcano was interest during last century by contrasting effects of both natural and anthropic disturbance. Volcanic eruption of 1943-44 opened a new fresh primary succession on lavic soils, but the impact of human activities lead to a heavy degradation of natural ecosystems. Since 1995, the volcano area is protected as a national reserve. With the aim of tracking the impact of such processes on biodiversity and environmental quality, Vesuvius lichen distribution was monitored over space and time. Past records by herbarium collections of nineteenth century were censed and reported to present criteria of classification. These data were entered in a GIS database and compared with two recent datasets, one preceding (1980) and the other following (last decade) the starting of protection regime over this territory. Multivariate techniques of analysis were employed to detect the different directions of change and to relate them to both natural and anthropic variables. About 270 taxa were recorded, most of which bark or rock crustose lichens from acid to sub-neutral substrata. A relative stability of poleophoby was observed, though indicators of eutrophication tended to increase in recent times. Other indicators, mainly related to aridity, successional stage and phytoclimatic range, are discussed in this work in relation to the changing environmental conditions of Vesuvius volcano.

P14-017: IMPACT CLIMATE CHANGE ON YIELD AND BIOCHEMICAL COMPOSITION OF WHEAT SEEDS

Abdumanon, A. - Kasimova, G.F. - Ergashev, A. - Karimov, Kh. H. - Saboiev, I.A. - Abdullaev, S. F. - Jumaev, K. - Rahimov, M.
Institute of plants physiology and genetics of Academy sciences Republic of Tajikistan

This paper presents the results of study effect of different climatic years on the parameters of the biological and economic productivity of plants and biochemical quality grain promising sorts of wheat. The analysis shows that January and November 2006 year, February and March 2007 year and February 1 - March 2009 year were periods with high monthly rainfall in the range. The results show that if in the period to September 2007 to August 2008 year annual precipitation was 358.7 mm, this value in the period September 2008-August 2009 year amounted to 905.6 mm, which is 2.53 times higher. During all these periods the monthly precipitation totals exceeded the norm from 1,3 to 1,73 times. Concerning sorts Kauz and Sham grown under normal climatic conditions, it may be noted that while the seed weight of wheat grown in 2008 year, slightly higher compared to the year 2009 year, but the protein content is lowered. The number of grains per ear and less in 2009, however, the number of seeds from year to year differences is minor. However, the mass of 1000 grains in 2009 for these plants was more favorable than in 2008 year. Comparative evaluation of biochemical parameters for sort Sham showed that the protein content almost does not change depending on climatic conditions in 2008 and 2009 year, despite significant differences in the sizes of seeds. The report discusses the impact of different climatic years to electrophoreticaly separation and identification of proteins in polyacrylamide gel.
This work was supported by the International Science and Technology Center (Project T-1635)

P14-018: THREE YEARS OF SEASONAL VARIATIONS IN ECOPHYSIOLOGICAL CHARACTERISTICS AND UV PROTECTION MECHANISMS IN TWO MOSSES FROM A SUBALPINE STREAM

Tomás, R. - Fabón, G. - Monforte López, L. - Martínez-Abaigar, J. Núñez-Olivera, E.
University of La Rioja

We studied the relationships between environmental variables and the physiology of two aquatic mosses (Bryum pseudotriquetrum and Fontinalis antipyretica) in a subalpine unforested stream over a three year period. Neither environmental or physiological variables showed significant interannual variations. Most environmental variables (water temperature, stratospheric ozone, and photosynthetic, UV-A and UV-B radiation) showed distinct seasonal variations, but only a few physiological ones did. In both species, photoprotection variables (the activity of the xanthophyll cycle and the bulk UV absorbance of the methanol-extractable UV-absorbing compounds, MEUVAC) varied more seasonally than variables related to physiological activity, such as the sclerophylly index and chlorophyll fluorescence parameters (Fv/Fm and FPSII). Changes in physiological activity would be attenuated by the buffering capacity of water with respect to the influence of environmental factors, and dynamic variables like Fv/Fm and FPSII would be little determined by cyclic environmental factors. In B. pseudotriquetrum, both MEUVAC and kaempferol 3,7-O-diglycoside (a potentially UV-protective flavonoid) were positively associated with radiation levels, whereas in F. antipyretica photoprotection mechanisms were not correlated with any environmental variable. In addition, MEUVAC was 3–4 fold higher in B. pseudotriquetrum than in F. antipyretica. Thus, different photoprotection mechanisms, with a different environmental regulation, can be suggested for these two species. DNA damage was not found in any sample, probably because both species displayed efficient DNA repair mechanisms.

P14-019: INTERACTIVE EFFECTS OF OZONE AND CO2 ON GRWOTH, TIELD AND PHYSIOLOGY OF POTATO

Hassan, I. - Hassan, I.A.
Alexandria University

Potato (Solanum tuberosum L. cv. Kara) was grown in open-top chambers (OTCs) in Northern Egypt at ambient (ca 350 ppm) or doubled CO2 (ca 690 ppm) either in charcoal-filtered air (15 nl l-1) or in non-filtered ambient air (78 nll-1 O3) to investigate the changes in physiology and yield under long-term elevated CO2 and/or O3 throughout 100 days.
Ambient O3 level reduced net photosynthetic rates, number and weight of tubers by 18, 41 and 21%, respectively, whereas elevated CO2 caused the opposite effect where it increased the same parameters by 44, 37 and 20%, respectively. Significant O3 x CO2 interactions were detected.
However, O3 caused an increase in GR and POD by 18 and 35%, respectively, while CO2 caused an increase in POD only by 46%, and there was no effect of O3 and/or CO2 on other enzymes.
The results of this study are discussed in relation to predicted atmospheric changes.
Key words:
Open-top chambers (OTCs) - Potato (Solanum tuberosum) – O3 – CO2 – photosynthesis – Stomatal Conductance – antioxidant enzymes – growth and yield.
Superoxide dismutase (SOD); Glutathione Reductase (GR); Ascorbate peroxidase (APX); Guaiacol peroxidase (POD)

P14-020: INFLUENCE OF MICROCLIMATE ON GRAPE-BERRY PROTEOME

Bordey, Y.[1] - Kappel, C.[1] - Magnin, N.[1] - Lapaillerie, D.[2] - Dupuy, J. W.[2] - Vilain, S.[2] - Bonneu, M.[2] - Gomes, E.[1] - Delrot, S.[1] - Trossat Magnin, C.[1]
[1]ISVV-UMR EGFV 1287-INRA, Bordeaux, France
[2]Plate-forme Protéomique, Université Bordeaux 2, Bordeaux, F

As many cultivated crops, grapevine may be particularly susceptible to climate change and hence, berry quality and production might be highly changed (White et al. 2006). Modification of the phenology timing and metabolite composition has been already observed (Pereira et al. 2006). In order to improve the knowledge on the impact of the microclimate on grape berries, we studied

changes in the proteome of field grown sun-exposed or shaded berries on the east or west side of the row and inside or outside the canopy. The proteome analysis from whole berries by 2-D PAGE revealed around 900 total proteins. Principal component analysis done with all protein spots ascertained three statistically different proteomes. This result suggested that microclimate conditions are significantly distinct according to berry position within the grape. Among proteins differentially expressed, some (rubisco, chalcone isomerase, abscissic stress ripening, 14-3-3 isoforms, thioredoxin-dependent peroxidise, ATP synthase and carotenoid dioxygenase) presented high variation of expression levels. The expression analysis by qPCR of several flavonoid gene showed that their transcript amounts were modified by berry microclimate. These results give particularly new insights in berry proteome and show microclimate-induced changes. Also, these data raise questions about the role in berries of proteins which abundance is modulated by microclimate changes.

P14-021: MONITORING EARLY WATER STRESS IN FORAGE MAIZE

Álvarez-Iglesias, L. - Sánchez-Moreiras, A. - Alonso-Salgueiro, I. - Barral, L. - Irisarri, J. - Mallo, D. - Martínez-Troncoso, O. - Reigosa, M.J. - Pedrol, N.*
University of Vigo
**Corresponding author e-mail: lorena.alvarez@uvigo.es*

Climatic change is a challenge for the sustainability of crop production. Agricultural rainfed areas in wet-temperate Atlantic zones are prone to more and more frequent episodes of early drought. Crops are more often exposed to water stress during seedling establishment, the most critical plant development phase. In the case of forage maize, sowing dates are limited by the preceding crop and the onset of adequate climatic conditions for soil tillage. Little research has been conducted to characterize the responses to water stress in the most broadly used cultivars in unirrigated lands. In this work, we tested some physiological tools for screening tolerance to early drought in forage maize and the establishment of recommendation criteria.
On the basis of previous screenings towards osmotic stress, three cultivars were selected because their contrasting tolerance to stress. Pre-grown seedlings were subjected to four levels of water availability, from field capacity to severe drought. Early physiological responses to water stress were monitored by means of chlorophyll a fluorescence-image analysis and IRGA, as well as some stress markers quantification (free proline, proteins, photosynthetic pigments, etc.).
Our results showed significant correlations between tolerance to growing levels of osmotic stress and drought,
as well as significant differences among cultivars with different physiological responses between susceptibility ranges. Tolerance to early drought was linked to osmoregulation capacity via free proline accumulation. Chlorophyll fluorescence-image analysis allowed describing contrasted early responses to water stress in forage maize cultivars, being a useful tool for screening seedling tolerance to early drought.

P14-022: DIRECT AND INDIRECT EFFECTS OF PHOTODEGRADATION ON CARBON TURNOVER IN TEMPERATE PLANT SPECIES OF SOUTH AMERICA

Austin, A. - Ballare, C.*
University of Buenos Aires
**Corresponding author e-mail: austin@ifeva.edu.ar*

Recent experimental evidence has shown that, in semiarid ecosystems, photodegradation can be important in controlling litter decomposition; however, plant species effects on decomposition through litter quality and its interaction with photodegradation is not known. Our objective in this study was to evaluate the susceptibility to photochemical mineralization in a range of temperate species in order to quantify the importance of abiotic photodegradation on mass loss and subsequent biotic decomposition. We harvested litter from 25 native and introduced species growing in temperate ecosystems in Argentina. While direct photodegradative losses were relatively small overall, the magnitude of the effect with attenuation of UV and visible light was 12% and 65% respectively. When these litter samples were then placed on the soil surface for biotic incubation, we observed a strong and consistent ($P<0.0001$) photofacilitation effect, with litter previously exposed to full solar radiation decomposing up to 120% more quickly. These results suggest that a wide variety of plant litter types could be susceptible to photodegradation, and that both direct and indirect effects of photodegradation could play an important role on carbon loss and nutrient release in a range of terrestrial ecosystems.

P17 Plant Microbe Interaction

P17-001: INFLUENCE OF MYCORRHIZAL FUNGI ON GROWTH, CHLOROPHYLL CONTENT AND K AND MG UPTAKE IN MAIZE UNDER DIFFERENT CONCENTRATIONS OF POTASSIUM AND MAGNESIUM

Khanpour Ardestani, N. - Reyhaneh, H. - Hassan Z.M.*
Tarbiat Modares University
**Corresponding author, e-mail: n.khanpour@modares.ac.ir*

Mycorrhizal fungi have a positive effect on growth and nutrition of host plant. In this research Influence of Vesicular-Arbuscular Mycorrhiza) VAM) on Growth, chlorophyll Content and K and Mg Uptake in Maize in pot culture were studied.This experiment was performed using natural soil containing spores of Glomus spp. Mycorrhizal spores were exposed to 4 concentrations of K solution, i. e. 240 (soil K content), 360 and 480 mg/L and 3 concentrations of Mg, i.e. 4.8 (soil Mg content), 7.2 and 9.6 meq/L concurrently. Plants were watered every 4 days for 16 days with 50 ml distilled water. A pot with sterilized soil was used as negative control. For study of mycorrhizal colonization, very thin longitudinal sections of plant roots (>1mm in diam.) were prepared manually and were stained with lactophenol-cottonblue. Mycorrhizal percentage was determined by the grid-line intersect method. Sampling from root and shoot of maize had done. The results have shown that mycorrhizal plant significantly had higher dry and fresh weight and chlorophyll content than non-mycorrhizal control plants (p £ 0.05). The concentrations 7.2 meq/L of Magnesium and 360 mg/L of Potassium concurrently with 7.2 meq/L of Mg had better effect on morphological characters (Dry and fresh weight of root and shoot). Mycorrhizal colonization increased Mg uptake but decreased K uptake.

Keywords: Magnesium and Potassium uptake, Growth, maize, Vesicular- Arbuscular Mycorrhiza (VAM)

P17-002: CHARACTERIZATION OF AN ALTERNARIA ALTERNATA EXTRACELLULAR LACCASE INVOLVED IN THE PATHOGENESIS OF CITRUS FRUIT

Del Río, J.* - Díaz, L. - Ortuño, A. - Pérez-Gilabert, M.
Universidad de Murcia
*Corresponding author, e-mail: jadelrio@um.es

Fungi of the genus Alternaria are respondible for substantial preharvest losses in Citrus. The susceptibility to Alternaria alternata depends on the Citrus species (1, 2). In this communication, an extracellular laccase from A. alternata, apparently involved in the infection process in several Citrus species, is described for the first time. The enzyme was isolated from the culture medium of this fungus and characterized using ABTS as substrate. It exhibits a pH optimum of 3.5 and a Km of 1.9 mM. The effect of temperature was also studied. The enzyme is active between 15 and 45ºC, the optimum temperature being around 35ºC. The thermostability of the enzyme at its optimum temperature is very low and 50% of the activity is lost in 35 min. Different inhibitor agents were also studied, with L-cysteine and caffeic acid found to be the most effective. Study of the substrate specificity of this enzyme reveals that Citrus flavonoids are substrates of A.alternata laccase. These results suggest that the enzyme could be involved in the degradation of flavonoids, observed in fruits infected with A. alternata. On the other hand, an increase of laccase activity was observed in the presence of Citrus fruit extracts. The possibility of considering laccase as a target to control the infection by Alternaria is also discussed.

(1)Peever et al. (2000). Phytopathology.90, 407-414.
(2) Ortuño et al. (2008). Physiological and Molecular Plant Pathology. 72, 162-166.

Acknowledgments: This work was funded by the Projects AGR/11621/08 of the Consejería de Agricultura, Agua y Medio Ambiente de la Región de Murcia, Spain, and 08676/PI/08 of the Consejería de Educación, Ciencia e Investigación de la Región de Murcia-Fundación Séneca, Spain.

P17-003: HOMOLOGY-DRIVEN PROTEOMICS REVEALS THE PROTEOME COMPLEXITY OF RESTING SPORES OF THE CLUBROOT PATHOGEN PLASMODIOPHORA

Ludwig-Müller, J.[1] - Knaust, A.[2] - Shevchenko, A.[2]*
[1]Technische Universität Dresden
[2]MPI-CBG, Dresden
**Corresponding author, e-mail: Jutta.Ludwig-Mueller@tu-dresden.de*

The clubroot disease is one of the most damaging diseases of the Brassicaceae, which is caused by the protist Plasmodiophora brassica. The study of the pathogen is hampered due to its obligate biotrophic nature and the organism does not belong unequivocally into a systematic group, even within the protists. P. brassicae forms a group with three other plasmodiophorids whose genomes are uncharacterized. Sequence databases contain 50 proteins and 96 ESTs of P. brassicae having low homology to other organisms. We employed a proteomics approach to characterize several stages of the life cycle of the pathogen outside and inside the host plant Arabidopsis thaliana. Protein extracts of the pathogen were separated on 1D SDS PAGE and the entire lane was cut into 9 slices that were separately digested with trypsin and analyzed by LC-MS/MS. Proteins were identified by MASCOT searches, while unmathed spectra were interpreted de novo and candidate sequences submitted to homology-based identifications by MS BLAST. We demonstrated that resting spores are not dormant, but contain many proteins, of which 20 proteins were identified with 120 unique peptides matched: 2 hits were P. brassicae ORFs, 2 hits were the known P. brassicae proteins polyubiquitins 1 and 2, as well as 16 cross-species hits to proteins from other pathogens and fungi. Among these were house-keeping proteins as well as proteins for which a function in the life cycle can be predicted. In conclusion, we were able to extract protein material from resting spores and identify a number of proteins with no genome of the pathogen available. Homology driven proteomics altogether matched more peptides, validated cross-species MASCOT hits and provided new protein identifications.

P17-004: THE STUDY OF BIOCHEMICAL FEATURES OF PATHOGEN-DEPENDENT ENZYMES (CA2+ - ATPASE) UNDER INFECTION OF POTATO WITH FUSARIUM SOLANI

Manadilova, A. - Tuleeva, G.T. - Duzgenbekova, B.*
Institute of Molecular Biology and Biochemistry
**Corresponding author, e-mail: man_Alija@mail.ru*

The important part of the defensive response at infection is the activation of pathogene-dependent enzymes, including ATPase. The plasma membrane fractions,have been isolated from the potato suspension cells (Tamasha, Santa) different in their resistance to the Fusarium Solani. Main structure-functional parameters of Ca2+ ATPase of potato under normal and infected conditions were studied; the optimums for ion concentration of Ca2+, pH incubation medium and kinetic characteristics were determined. The influence of Fusarium Solani metabolites over the activity of enzyme was studied. After the infection by the fungus cultural fil-

trate there is an increase of Ca2+ ATPase activity,but in resistant Tamasha variety, the enzyme activation is observed as quickly as one hour after infection, while the sensitive variety show the activation in 24 hours time. The infection leads to the change of physical and chemical parameters of the enzyme. It is seen from the changes of kinetic parameters; there is an increase of Km in infected potato cells. It is determined that carbohydrate and carbohydrate-lipidic fractions of Fusarium Solani mycelium are the suppressors of Ca2+ ATPase; protein fraction activates the enzyme. Naphthasarine, isolated from fungus mycelium by column chromatography, 2-3 times increases the activity of ATPase and identical to fusicoccin in terms of mechanism of action.

P17-005: FACTORS AFFECTING FUMONISIN BIOSYNTHESIS IN FUSARIUM VERTICILLIOIDES: A STUDY ON FUM1 EXPRESSION UNDER IN VITRO CONDITIONS AND DURING EARLY STEPS OF MAIZE COLONIZATION.

Visentin, I. - Montis, V. - Valentino, D. - Tamietti, G. - Cardinale, F.*
Department of Arboriculture, University of Turin
**Corresponding author, e-mail: ivan.visentin@unito.it*

Fumonisins are a family of mycotoxins (produced by Gibberella moniliformis, anamorph Fusarium verticillioides) that contaminate maize and maize-based products and cause great concern for human and animal health. The real role played by fumonisins in pathogenesis has been long controversial, although it is clear that they are phytotoxic. A better comprehension of the molecular mechanisms regulating their production could help clearing this point and preventing kernel contamination. In all eukaryotic organisms, acetylation of core histones and DNA methylation degree play a key role in the regulation of transcription, along the promoter regions. To assess the possibility that these epigenetic factors may be linked to fumonisin production, we observed the expression of a key biosynthetic gene (FUM1) in several strains of F. verticillioides after treatment with a histone deacetylase inhibitor, and investigated the methylation of FUM1 promoter under fumonisin-inducing and non-inducing conditions. Moreover we compared the activity of the endogenous FUM1 promoter (pFUM1) with that of the same region driving the ectopic expression of a transgene (GFP) after random insertion in other points of the genome in transgenic F. verticillioides strains (pFUM1::GFP). These are being tested for pFUM1 activity upon maize infection and in fumonisin-inducing and non-inducing conditions, to assess whether the FUM1 and GFP transcripts accumulate differently in dependence of the position of the corresponding coding and regulatory regions in the genome. Analysis of the data obtained from these assays could confirm the role of the epigenetic regulation in the fumonisin biosynthesis as already described for several other filamentous fungi.

P17-006: EXPANSINS ARE INVOLVED IN CONTROLLING ROOT GALL SIZE AFTER INFECTION OF ARABIDOPSIS THALIANA WITH THE PROTIST PLASMODIOPHORA BRASSICAE

Bretschneider, S. - Wieczorek, K. - Grundler, F. - Ludwig-Müller, J.*
Institute of Botany, Technical University of Dresden
**Corresponding author, e-mail: sabine.bretschneider@gmx.de*

Expansins are proteins that induce cell enlargement by loosening the matrix of the cell wall. They are regulated by symbionts and various pathogens as well as by auxin and mediate cell elongation via _acid-growth‘. Expansins are common in all land plants – from moss to flowering plants. In Arabidopsis there are 36 members of the expansin gene family (AtExp), in rice there are even 58. In this study we analysed whether expansins are specifically regulated during the clubroot disease of Arabidopsis thaliana. This disease is caused by the protist Plasmodiophora brassicae and affects only Brassicaceae. Symptoms are galls on the roots as well as wilted and stunted shoots. In a semi-quantitative RT-PCR expression analysis it was shown that most expansins are strongly up- or down-regulated in infected plants compared to healthy controls. In several promoter::GUS lines a direct correlation of expansin expression and infection structures was shown. Homozygous T-DNA mutants of AtExpA10 and AtExpA12 are more tolerant towards P. brassicae infection than wild type plants. Other possible candidates to be involved in clubroot formation are AtExpA1 and AtExpA15, based on expression studies, but this needs to be further examined. These studies potentially contribute to the development of highly tolerant or even resistant plants against P. brassicae infection.

P17-007: THE EFFECT OF FUSARIUM SOLANI INFECTION ON ANTIOXIDANT ENZYMES IN POTATO (SOLANUM TUBEROSUM) TUBERS

Sapko, O. A. - Utarbayeva, A. - Zhabayeva, DB. - Makulbek S, Baikara, B. - Kunayeva, R. M.*
M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry
**Corresponding author, e-mail: a.utar@mail.ru*

Plant have evolved complex of regulatory mechanisms in adapting to various environmental stresses. The oxidative stress is universal component of plant cell response reactions by pathogenesis. The antioxidant enzymes are effective components of protection to damage effects the reactive oxygen species. The fungus Fusarium solani cause the dry rot of potato tubers. The disease advance in tubers during storage.
Changes in activities of ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) and peroxidase (PO) were investigated in potato tubers. The potato with different tolerance (resistant and sensitive) and pathogenic and nonpathogenic strains of F.solani was used. Character and intensity of enzyme response depended on initial tolerance of analyzed tubers and pathogenicity of fungus. The infection has caused fast (in 3 hours) 12-15-times increase CAT activity in tubers of sensitive cultivar and decrease in initial activity (on 30-70 %) in tuber of resistant cultivar. The higher level of enzymes activity to effect of pathogenic strain was observed. The maximal induction SOD, APX and PO activities registered in 24 hours. Higher level of activity SOD and PO was registered in resistant cultivar infected pathogenic strain of fungus. Activity APX increased in sensitive tubers infected nonpathogenic stain. The received results confirm the important role of antioxidant enzymes in interaction F.solani - S.tuberosum.

P17-008:L-2-OXOTHIAZOLIDINE-4-CARBOXYLIC ACID (OTC) PARTIALLY PROTECTS PEACH PLANTS FROM SHARKA DISEASE: EFFECT IN ANTIOXIDATIVE METABOLISM AND PROTEIN EXPRESSION.

Hernandez, J. - Clemente-Moreno, M.J. - Diaz-Vivancos, P. - Barba-Espín, G. - Martínez-Gómez, P.*
Grupo de Biotecnología de Frutales CEBAS-CSIC
**Corresponding author, e-mail: jahernan@cebas.csic.es*

In this work, we investigate the effect of L-2-oxothiazolidine-4-carboxylic acid (OTC) pre-treatments and their interaction with Plum pox virus (PPV, Sharka disease) infection on the antioxidative metabolism of “GF305” peach (Prunus persica L. Batsch) at subcellular level. Experiments were carried out during 4 years (2007 to 2010) in controlled greenhouse conditions. In general, OTC increased the length of peach plants and decreased the percentage of branches showing Sharka symptoms in their leaves. This partial protection obtained by OTC treatments, correlated with a lower PPV contents evaluated using an ELISA-DASI test, mainly during the second cycle of growth in each year. At the same time, these plants showed a higher redox state of glutathione as well as an increase in GSH-related enzymes (GPX, GST and G6PDH) and POX activity at subcellular level.
Finally, the effect of OTC in the differential expression of proteins in healthy and PPV-infected leaves will be discussed.

This work was supported by CICYT AGL2006-01743/AGR and 11883/PI/09 (Fundación Séneca, Agencia de Ciencia y Tecnología de la Región de Murcia)

P17-009: AN AM FUNGUS AND A PGPR INFLUENCE PHYSIOLOGICAL AND MOLECULAR MECHANISMS INVOLVED IN ALLEVIATION OF LETTUCE PLANTS SUBJECTED TO ELEVATED CO2 AND DROUGHT

Kohler, J. - Alguacil M.M. - Caravaca, F. - Roldán, A.*
CEBAS-CSIC
**Corresponding author, e-mail: jkohler@cebas.csic.es*

Arbuscular mycorrhizal (AM) symbiosis and plant-growth-promoting rhizobacterium (PGPR) can alleviate the effects of water stress in plants but it is unknown whether these benefits can be maintained at elevated CO2. Therefore, we carried out a study where seedlings of Lactuca sativa were inoculated with the AM fungus (AMF) Glomus intraradices N.C. Schenk & G.S. Sm. or the PGPR Pseudomonas mendocina Palleroni and subjected to two levels of watering and two levels of atmospheric CO2 to ascertain their effects on plant physiological parameters and gene expression of one PIP aquaporin in roots. The inoculation with PGPR produced the greatest growth in lettuce plants under all assayed treatments as well as the highest foliar potassium concentration and leaf relative water content under elevated [CO2] and drought. However, under such conditions, the PIP2 gene expression remained almost unchanged. G. intraradices increased significantly the AMF colonization, foliar phosphorus concentration and leaf relative water content in plants grown under drought and elevated [CO2]. Under drought and elevated [CO2], the plants inoculated with G. intraradices showed enhanced expression of the PIP2 gene as compared to P. mendocina or control plants. Our results suggest that both microbial inoculation treatments could help to alleviate drought at elevated [CO2]. However, the PIP2 gene expression was increased only by the AMF but not by the PGPR under these conditions.

P17-010: A NEW APPROACH FOR THE COMPREHENSIVE ANALYSIS OF MICROCOMMUNITIES IN PLANT ECOLOGY

Moshynets, O.[1] - Shpylova, [1]. - Kosakivska, I.[1] - Kordium, V.[1] - Bogaerts, L.[2] - Potters, G.[2] - Koza, A.[3] - Spiers, A.[3]*
[1] Institute of Molecular Biology and Genetics NAS of Ukraine
[2] Department of Bioscience Engineering, University of Antwerp, Groenenborgerlaan 171 B-2020, Antwerp, BELGIUM
[3]The SIMBIOS Centre, University Abertay Dundee, Scotland, UK
**Corresponding author, e-mail: moshynets@gmail.com*

Plants are constantly interacting with a wide range of microbes which effect plant growth, development and response. However, comparatively little is known about ecological interactions in the phytosphere mostly due to the technical failing of current methods like the lack, or loss, of spatial informaton which is essential to the understanding of the interractions operating over the micrometer range. Here we offer a novel approach to assess the microflora in specific phytoenvironments. The QS (quasi-substratum) approach uses pieces of plastic film which are placed in the environment and allowed to develop microbial communities on their surfaces. X-ray microtomography can be used to determine the position of the film in situ with respect to local structures of the micro-environments. When recovered, the films can be stained to allow direct CLSM imaging of the surface-associated microcommunity while direct extraction of the microbial DNA enables the use of molecular methods. The QS approach was tested in the rhizosphere of Brassica napus where the interactions between the GFP-tagged rhizobacterium Pseudomonas fluorescens SBW25 and the native soil microbes were studied, as well as in the study of the endophytes of bamboo plants Phyllostachys atrovaginata. In the later case, endophytic micro-communities were observed microscopically and total microbial community 16S DNA was extracted. The subcloning and subsequent identification of species will be performed at later stage. The QS approach enables the use of modern imaging technologies coupled with molecular technologies, allowing comprehensive investigation of microbial diversity in the phytosphere, which allows more in-depth analysis of the interaction between microbes and plants in the future.

P17-011: REACTIVE ELECTROPHILE SPECIES ORCHESTRATE BACTERIAL-INDUCED STOMATAL CLOSURE IN ARABIDOPSIS THALIANA.

Montillet, J. - Leonhardt, N. - Mondy, S. - Tranchimand, S.*
Cea Cadarache-Ibeb-Lems
**Corresponding author, e-mail: Jean-Luc.Montillet@Cea.Fr*

Stomatal closure has been found as a part of plant innate immune response allowing restriction of bacterial invasion at leaf surface (Melotto et al. 2006). In this context, participation of different signalling molecules such as abscisic acid (ABA), salicylic acid (SA) and nitric oxide (NO) has been suggested. Our present work describes evidence indicating that reactive electrophile species (RES) originating from lipoxygenase activity are key players involved in this physiological process. The role of RES has been assessed in Arabidopsis challenged with strains of Pseudomonas syringae or treated with flg22 a pathogen-associated molecular patterns (PAMP). Our data show that steps at the upstream of this new signalling cascade differ from those triggered by ABA.
Reference: Melotto et al. 2006, Plant stomata function in innate immunity against bacterial invasion. Cell 126, 969-980.

P17-012: RHIZOBIAL ALDEHYDE OXIDASE AND LEGUME NODULATION

Mercedes Lucas, M. - Torres, MJ. - Paradela C. - Rincón, A. - Coba de la Peña, T. - Pueyo, J.J. - Delgado, MJ*
**Corresponding author, e-mail: mlucas@ccma.csic.es*

The symbiotic association between legumes and rhizobia leads to the development of a symbiotic organ, the nodule. Phytohormones such as the auxin indol acetic acid (IAA), synthesized by both symbionts, play an important role in legume nodulation. To date, little is known about the auxin biosynthesis pathways during root nodule development. In plants, the enzyme aldehyde oxidase (AO, EC 1.2.3.1) catalyzes the last step of IAA biosynthesis via the Trp-dependent indole-3-pyruvic acid pathway. We have previously described the presence of plant AOs in nodules of Medicago truncatula and Lupinus albus and proposed their involvement in the regulation of nodule development (1). In addition to plants, AOs have also been found in some microorganisms, including rhizobia (1, 2). We have performed a search in the genome sequence of Sinorhizobium meliloti 1021 by using the protein sequences corresponding to the small, medium and large subunits (AodS, AodM, AodL) of AO from Methylobacillus sp. KY4400 (2). We have identified three genes in S. meliloti 1021 clustered in the same transcriptional order found for aodS, aodM and aodL genes in Methylobacillus sp. In order to investigate the involvement of these genes in the symbiotic interaction S. meliloti-Medicago sativa, mutants in the first gene of the transcriptional unit have been constructed by marker exchange. The symbiotic phenotype of one of the strains obtained, mutated in the aodS-like gene, is being investigated.
(1) Fedorova et al. 2005. MPMI 18: 405–413.
(2) Yasuhara et al. 2005. Biosci. Biotechnol. Biochem. 69: 2435-2438.

P17-013: IMMEDIATE SUPPRESSION OF ROOT-BASED IMMUNITY GUARANTEES COLONISATION SUCCESS OF THE SYMBIONT PIRIFORMOSPORA INDICA

Schaefer, P.
Research Centre for BioSystems, Land Use and Nutrition; Justus Liebig University
**Corresponding author, e-mail: patrick.schaefer@agrar.uni-giessen.de*

Piriformospora indica is root-colonizing fungus that transfers several benefits to the colonised plants, e.g. enhanced biotic and abiotic stress resistance or tolerance, respectively. The fungus is member of the order Sebacinales, which was found to house various forms of mycorrhizal fungi. Several microorganisms are able to establish beneficial or pathogenic interactions with plants. In general, adaptation and specialisation evolved by beneficial or pathogenic microbes results in narrowed host range. This is most extremely demonstrated by the biotrophic lifestyle of certain leaf colonizing fungi that showplant species-dependent reproduction. Thorough epifluorescence and transmission electron microscopy-based studies in Arabidopsis roots defined an initial biotrophic preceding a cell death-associated colonisation phase of P. indica implicating a distinct host-microbe communication. Despite its biotrophism, P. indica colonises roots of an extraordinarily broad spectrum of monocot and dicot plants. Results presented indicate the importance of the suppression of early defense response, especially of the oxidative burst, to be significantly associated with its ability to establish a compatible root interaction. In addition, we will discuss the relevance of leaf defense determinants in root-based immunity.

P17-014: COMPARATIVE STUDY OF SOIL-BASED AND COMMERCIAL FORMULATIONS OF ARBUSCULAR MYCORRHIZAL FUNGI IN LETTUCE SUBJECTED TO SALT STRESS

*Garmendia López, I.**
Universidad de Alicante
**Corresponding author, e-mail: idoia.garmendia@ua.es*

Oltra Cámara M A (Oltra M A) Mangas Martín V J (Mangas V J) Arbuscular mycorrhizal fungi (AMF) can contribute to the salinity resistance of host plants by improving nutritional status, particularly of P and N, enhancing osmotic adjustment, increasing warter use efficiency and resucing oxidative damage. Our objective was to compare the effect of soil-based (Glomus intraradices and G. mosseae) and commercial AMF inocula (formulations 1 and 2) in salt stress tolerance of lettuce. No effect on shoot biomass was found with mycorrhizal inoculation under non-saline conditions and root biomass was significantly reduced. Only plants inoculated with formulation 1stimulated shoot dry weight not related with greater root AMF colonization. P and K concentration in leaves were improved bymycorrhizal association. Salinity did not reduce leaf relative water content and we observed no osmotic adjusment in leaves from non-mycorrhizal plants. However, root dry biomass and its starch content decreased, while leaf starch and root soluble sugar concentrations were enhanced in non-inoculated plants under saline conditions. Lettuce inoculated with formulation 2 and soil-based G. intraradices showed the highest root colonization percentages. Neverthelessn, one of the mycorrhizal treatments induced a significant improvement on the growth of lettuce subjected to salinity. The severity of the salt stress applied and the effectiveness of mycorrhizal inocula ameliorating its effect is discussed. This work was supported by Universidad de Alicante (UAUSTI09/04).

P17-015: IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF PUTATIVE PHOSPHORYLATION TARGETS OF SLCIPK INVOLVED IN IMMUNE RESPONSE IN PLANTS

Gutiérrez-Beltrán, E. - del Pozo, O. - de la Torre, F.*
IBVF-US/CSIC
**Corresponding author, e-mail: emgube@gmail.com*

Two novel components of the Pto-mediated hypersensitive response (HR) signal transduction pathway, a calcium sensor (calcineurin B-like; CBL) and a CBL-interacting protein kinase (CIPK) were identified in a random gene silencing screening (VIGS) in Nicotiana benthamiana. Recently, we have also demonstrated the participation of their tomato orthologs, SlCIPK and SlCBL during bacterial speck disease symptom development caused by Pseudomonas syringea pv tomato (P. syringae pv tomato) in tomato. Considering that SlCIPK is an active kinase that plays a role in R-gene signal transduction pathways, it might elicit a phosphorylation cascade in response to pathogen attack.
In order to identify putative phosphorylation targets and upstream regulation elements of SlCIPK, we conducted two parallel yeast two-hybrid screenings using SlCIPKWT and SlCIPKCA (a constitutive active mutant version) respectively as baits. A normalized prey library generated from tomato was used. From the screening we obtained several different interacting proteins. The selection of candidates was based on three different criteria:
(1) implication in plant immunity through reverse genetic approaches (VIGS), (2) capability to interact with SlCIPK in vivo and (3) putative biological function. Silencing susceptible tomato plants with one of these clones dramatically reduced P. syringae pv tomato growth four days after infection. Therefore, this clone named CIP29 (CIPK Interacting Protein) was selected for further in detail characterization. We have found that CIP29 is phosphorylated in vitro by SlCIPK. This clone represents a new phosphorylation target for the CIPK family, and the first identified substrate different of a membrane channel.

P17-016: TOWARDS IDENTIFICATION OF SYSTEMIC ACQUIRED RESISTANCE SIGNALLING PARTNERS DOWNSTREAM FROM ENHANCED DISEASE SUSCEPTIBILITY1

Vlot, C.[1] - Jordá, L.[2] - Colby, T.[2] - Wenig, M.[1] - Knappe, C.[1] - Rietz, S.[2]*
[1]Helmholtz Zentrum Muenchen; Institute of Biochemical Plant Pathology
[2]Max Planck Institute for Plant Breeding Research
**Corresponding author, e-mail: corina.vlot@helmholtz-muenchen.de*

Wittek, F (Helmholtz Zentrum Muenchen; Institute of Biochemical Plant Pathology) Breitenbach, H (Helmholtz Zentrum Muenchen; Institute of Biochemical Plant Pathology) Mackey, D (The Ohio State University; Department of horticulture and crop science) Parker, J E (Max Planck Institute for Plant Breeding Research) Systemic acquired resistance (SAR) is a long lasting, broad spectrum disease resistance that is induced in the systemic tissue of plants undergoing a localized immune response. Often, the primary immune reaction is accompanied by programmed cell death, the hypersensitive response (HR), of cells in and around the site of infection.
During the HR resistance-inducing signals are transmitted that can for instance be found in petiole exudates from Arabidopsis thaliana leaves that are infected with Pseudomonas syringae encoding the HR-inducing effector AvrRpm1. Petiole exudates from infected wild type (wt) A. thaliana trigger PR1 gene expression in healthy wt plants. By contrast, similar exudates from infected enhanced disease susceptibility1 (eds1) mutant plants do not, although the AvrRpm1-induced HR is intact in this mutant. This indicates that EDS1 is required for SAR signal generation and/or transmission.
We are now using conditional expression of AvrRpm1 to induce an HR in wt and eds1 mutant plants and could show that this induces SAR-like disease resistance in systemic non-AvrRpm1-expressing tissues of wt plants. Moreover, candidate SAR signalling components were identified by 2D gel and GC-MS analysis of apoplast extracts from AvrRpm1-expressing wt plants as compared to similar extracts from eds1 mutants. We are currently analyzing pathogen resistance and in particular SAR in Arabidopsis and tobacco over expressing the corresponding putative SAR signalling genes.

P17-017: MOLECULAR CLONING AND CHARACTERIZATION OF A PATHOGENESIS-RELATED PROTEIN CSPR10 FROM CROCUS SATIVUS

Ahrazem, O. - Gómez-Gómez, L. - Rubio Moraga, A. - Trapero-Mozos, A.*
E.T.S.I.Agrónomos, Universidad de Castilla-La Mancha
**Corresponding author, e-mail: oussama.ahrazem@uclm.es*

Pathogenesis-related proteins are produced as part of the active defences used to prevent attacks from microbial pathogens. In this study, a full length cDNA encoding the CsPR10 protein was identified in fresh saffron stigmas (Crocus sativus). The deduced amino acid sequence from the nucleotide sequence of the coding region showed its homology with PR10 proteins. The clone expressed as a protein in fusion with a GST tag had a 47 kDa protein in E. coli. CsPR10 had ribonuclease activity with features common to class II-type ribonuclease; its specific activity was quantified and found to be a 68.8 U mg-1 protein. CsPR10 inhibited F. oxysporum growth and its antifungal potency toward this fungus was reflected in the IC50 value of 8.3 μM. RT-PCR analysis revealed of CsPR10 the presence of high transcript levels in anther and tepal tissues, and low levels in stigmas and roots, whereas no signal was detected in leaves. This protein seems to be involved in the active defence response through activation of the jasmonic acid pathway.

P17-018: IDENTIFICATION OF NBS-LRR CANDIDATE RESISTANCE GENES IN WILD STRAWBERRY (FRAGARIA VESCA) EXPRESSED DURING INFECTION WITH PHYTOPHTHORA CACTORUM

Martinussen, I. - Elameen, A. - Chen, X. - Klemsdal, S. - Brurberg, M. B.*
Norwegian Institute for Agricultural and Environmental Research
**Corresponding author, e-mail: inger.martinussen@bioforsk.no*

Strawberry producers experience serious economical losses due to the development of diseases caused by fungal or oomycete pathogens. Phytophthora cactorum causes crown rot in strawberry which is characterized by a sudden wilt of the younger leaves and further collapse of the entire plant within a few days. In the past, the most efficient method of preventing disease in crop plants was through breeding for resistance using classical genetic approaches. However, breeding for a single disease resistance trait can take years and pathogens rapidly evolve mechanisms to overcome single resistance traits. In order to improve the ability to control and combat plant diseases, detailed insight in the molecular mechanisms of disease development is required. NBS-profiling is a powerful method developed to identify plant resistance (R) genes. The technique is based on amplification of DNA fragments, or as in this case c-DNA fragments, using adaptors and primers designed to anneal to the conserved regions in the P-loop and the kinase-2 motif in the NBS region of the R genes. Here we present a time course study on the expression of putative NBS-LRR genes from 0 to 8 days after infection with P. cactorum. Several new NBS resistance genes have been identified.

P17-019: SPATIAL VARIATION OF REACTIVE OXYGEN SPECIES AND ANTIOXIDANT ENZYMES IN STEMS OF TWO CAPSICUM ANNUUM VARIETIES INFECTED WITH PHYTOPHTHORA CAPSICI.

Candela, M.[1] - Requena, M. E. [1] - Gonzalez-Ramiro, L.[1] - Egea-Gilabert, C.[2]
[1]University of Murcia
[2]University Politechnic, Cartagena
**Corresponding author, e-mail:* mcandela@um.es

The response to the pathogenic oomycete, Phytophthora capsici, in plants of two varieties of pepper (Capsicum annuum L.), (SCM) resistant and (CW) sensible, was studied. Both varieties development a hypersensitive reaction and a potent signal associated with significant accumulation of reactive oxygen species (ROS) as superoxide anions (O2.-) and hydrogen peroxide (H2O2), and an interesting variation of antioxidant enzymes through stem. Both ROS increased much more in SCM stem tissue, whereas in CW, ROS generation was noticed lesser, and only in very few stem zones near inoculation. At similar level with these compounds, changes of lipid peroxidation, solutes leakage and the activity of the enzymes superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase, and oxidized, reduced and total glutathione were largely changed. The effects of inhibitors of endogenous Cu/Zn superoxide dismutase (Diethyldithio carbamate) and catalase (3-amino-1,2,4-triazole and salicylic acid) were also examined. Yields of ROS in the presence of the inhibitors diphenylene iodonium and hydroxamic acid suggest that ROS were generated in both host responses by more than one mechanism and which are in direct relation with resistant to pathogen.
This work has been partly supported by the Project BFU2004-4707-CO2-01 from CICYT, Spain

P17-020: CHEMICAL GENETICS - IDENTIFICATION OF NEW MICROBIAL ELICITORS OF PLANT DEFENSE

Steinhauser, C. - Fekete, A. - Schmitt-Kopplin, P. - Vlot, C. - Durner, J. - Von Rad, U.*
Helmholtz Center Munich
**Corresponding author, e-mail: clara.steinhauser@helmholtz-muenchen.de*

Defined bacterial or fungal surface molecules, called elicitors, induce a defense response in plants against disease. We aimed to identify new biological elicitors for defense from a library of 1150 bacterial extracts (provided by the German company Sourcon Padena). The extracts were screened in Arabidopsis thaliana cell suspension cultures with fluorescent dyes to specifically monitor the induction of two early plant defense signals, nitric oxide (NO) and reactive oxygen species. Furthermore, activation of genes related to inducible disease resistance signaling pathways was used as an indicator for defense on a genetic level. To this end, transgenic A. thaliana cell suspension cultures carrying promoter-GFP-constructs were used. Activation of salicylic acid-related signaling (systemic acquired resistance) was monitored with PR1::GFP and PR5::GFP construct, whereas jasmonic acid/ethylene-dependent signaling was monitored by using PDF1.2::GFP. As a result of the screening two bacterial extracts were identified that show strong NO and PR-gene induction in vitro. Subsequent studies with A. thaliana plants showed that the extracts induced lesions in vivo that are reminiscent of a Hypersensitive Response (HR) defense reaction. We are currently deploying various liquid chromatography approaches (HPLC and UPLC) combined with high resolution mass spectrometry (FTICR-MS) to isolate the active compounds, or elicitors, from the defense-inducing bacterial extracts.

P17-021: SULFUR SUPPLY INFLUENCES THE UP-REGULATION OF TOBACCO GENES ENCODING KEY ENZYMES OF CYSTEINE AND GLUTATHIONE BIOSYNTHESIS FOLLOWING TMV INOCULATION

Gullner, G.[1] - Künstler, A.[1] - Király, L.[1] - Müller M[2] · Zechmann B[2]*
1 Plant Protection Institute, Hungarian Academy of Sciences
2 (Institute of Plant Sciences, University of Graz)
**Corresponding author, e-mail: ggull@nki.hu*

Atmospheric sulfur depositions were strongly reduced in the 1980s so that soil sulfur deficiency became a nutrient disorder and some fungal infections became increasingly obvious. In agronomic field experiments a new form of disease resistance, the sulfur-induced resistance (SIR) was described. Sulfur is essential for the biosynthesis of cysteine (CYS), methionine and the tripeptide glutathione (GSH) in plants. GSH and other sulfur compounds play important roles in plant defense reactions against microbial pathogens. However, very little is known about the importance of CYS and GSH biosynthetic pathways in the

development of SIR. In this study, the molecular mechanisms underlying SIR were investigated in susceptible Samsun-nn tobacco plants in response to Tobacco mosaic virus (TMV) inoculation. Plants were cultivated with two different sulfur supplies. TMV coat protein mRNA level, and the transcript abundance of three genes encoding key enzymes of CYS and GSH biosynthesis: adenosine 5-phosphosulfate reductase (APR), gamma-glutamylcysteine synthetase (GSH1) and glutathione synthetase (GSH2) were studied in tobacco leaves following TMV inoculation. The expression of APR and GSH1 genes was markedly up-regulated by TMV inoculation, whereas that of GSH2 was not significantly affected. Higher sulfur supply resulted in stronger gene expression levels and
in a reduced amount of TMV coat protein mRNA. These results demonstrated the strong influence of sulfur supply on TMV resistance in tobacco.

P17-022: THE POTENTIAL ROLE OF PHOSPHATIDYLCHOLINE-HYDROLYSING PHOSPHOLIPASE C IN PLANT DEFENCE REACTIONS

Martinec, J.[1] - Pokotylo, I.[2] - Sasek, V.[1] - Burketova, L.[1] - Kocourkova, D.[1] - Kravets, V.[2] - Pejchar, P.[1]*
[1]Institute of Experimental Botany, Academy of Sciences of the Czech Republic
[2]Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine
**Corresponding author, e-mail: martinec@ueb.cas.cz*

Signalling involving phospholipid-derived second messenger molecules is emerging as one of central mechanisms of regulating cells functioning under both physiological and stress conditions. Many of the lipid components have been shown to be implicated in defence signalling during pathogen attack.
In presented study we report the rapid altering of diacylglycerol (DAG) level in tobacco and Arabidopsis cell suspension cultures treated by general elicitors, heat-killed cells of Pseudomonas syringae bacteria and lipopolysaccharides, by specific elicitor, flagellin-derived peptide flg22 or by substances involved in plant-pathogen interaction, salicylic acid and jasmonic acid. Similar results were obtained using two-month-old tobacco plants. We also report that DAG originated from the activity of phosphatidylcholine-hydrolysing phospholipase C (PC-PLC), an enzyme which was recently characterised in plants. Moreover, mutant plants with knocked-out PC-PLCs genes have been shown to exhibit significant decrease in resistance against bacterial attack. The potential role of PC-PLC, a new member of plant phospholipase signalling family in plant defence reactions is discussed.
This work was supported by grants of the Ministry of Education, Youth and Sports no. LC 06034 and no. ME09108.

P17-023: ANTIFUNGAL ACTIVITY OF A PINUS MONTICOLA – ANTIMICROBIAL PEPTIDE 1 (PM-AMP1) AND ITS ACCUMULATION IN CRONARTIUM RIBICOLA-INFECTED WESTERN WHITE PINE

Ekramoddoullah, A. - Zamani, A. - Liu, J.J.*
Natural Resources Canada
**Corresponding author, e-mail: aekramod@nrcan-rncan.gc.ca*

A 79 amino acid long, basic protein termed Pinus monticola-antimicrobial peptide 1 (Pm-AMP1) induced in cankered bark of western white pine (WWP) infected with the blister rust fungus Cronartium ribicola was found to show homology with other antimicrobial agents. Pm-AMP1 was over-expressed as a histidine-tagged fusion protein in E. coli BL21 Star cells, purified and used for antifungal assays against C. ribicola and other fungal pathogens. Application of 10 µg of Pm-AMP1 on the growing hyphal margins of C. ribicola, Phellinus sulphurescens, and Ophiostoma montium resulted in visible growth inhibition 3 to 12 days post-treatment. Spores of Fusarium oxysporum, Thielaviopsis basicola, Botrytis cinerea, Alternaria cucumerina, and Colletotrichum lagenarium were grown in 96-well plates, challenged with Pm-AMP1 and showed 44% to 97% growth inhibition 5 days post-treatment. An association between Pm-AMP1 accumulation in needles and quantitative partial resistance mechanisms such as bark reaction (BR) and Mechanism X (MX) was also investigated in seven WWP families. Following C. ribicola infection, BR family #1 showed the highest mean accumulation of Pm-AMP1 ($p<0.05$), whereas the most susceptible family (#10) showed the lowest ($p<0.05$). Family #1 showed a steady decline in Pm-AMP1 levels ($p=0.014$) as seedling health deteriorated from vigorous to dying. In families #2, #6 and #7 we observed a significant spike in Pm-AMP1 accumulation ($p<0.05$) as seedling health deteriorated from moderately to severely infected. Our results suggest that Pm-AMP1 is involved in the WWP defense response as observed by its inhibitory activity and through its association with different indicators of disease resistance.

P17-024: EFFECT OF CHITOSAN ON GUARD CELL PHOTOSYNTHESIS

Ördög, A.[1]* - Rózsavölgyi, T.[1] - Wodala, B.[1] - Hideg, E.[2] - Deák, Z.[2] - Ferhan, A.[2] - Tari, I.[1] - Horváth, F.[1]
[1] Department of Plant Biology, University of Szeged, Szeged, Hungary
[2] Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary
**Corresponding author, e-mail:* aordog@bio.u-szeged.hu

Stomata control gas exchange and transpirational water loss by turgor-driven volume changes, but they also provide the main entrance for pathogens. Guard cells (GC) respond to the presence of microbes by narrowing stomatal pores following perception of microbe-associated molecular patterns, such as chitosan (CHT), a deacylated derivative of a major fungal cell wall component chitin. Besides the inhibition of the light-induced stomatal opening CHT also induces stomatal closure, an action with distinct signalling pathways and transporters. Stomatal opening and closure is related to the H+-ATPase activity in the GC plasma membrane, as it affects the transport of osmotically active solutes, such as the passive movement of K+ via different sets of potassium channels, the activity of Cl-/H+ symporters and anion channels. ATP for proton pumping is supplied mostly from mitochondrial respiration; however, partial inhibition with DCMU implied a role of GC photosynthetic electron transport in the ATP supply. In order to ascertain whether CHT affects GC photosynthetic ATP production, the light-dependence of the photosynthetic electron transport rate of a single GC was assayed. Vicia faba epidermal peels were thus bathed in solutions containing different molecular weight CHT and chlorophyll fluorescence parameters were determined by Microscopy-PAM chlorophyll fluorometer (Walz, Germany). A possible role of nitric oxide molecule in CHT signalization, which may affect photosynthetic ATP production, was also investigated.
This work was supported by the Hungarian Scientific Research Fund (grant no. OTKA K 81471).

P17-025: PHYSIOLOGICAL PERFORMANCE OF CITRUS AURANTIFOLIA PLANTS INFECTED BY CITRUS TRISTEZA VIRUS

Pérez Clemente, R.- Montoliu, A. - de Ollas, C. - Arbona, V. - Lopez-Climent, M,F. - Argamasilla, R. - Gomez-Cadenas, A.*
Universitat Jaume I
**Corresponding author, e-mail: rosa.perez@uji.es*

Citrus tristeza virus (CTV) is the causal agent of the most economically important viral disease of citrus, the most widely grown fruit crop in the world, with a cultivated area of more than 7 million hectares distributed in about 100 countries. The aim of this work was to study the physiological performance of Citrus aurantifolia plants infected with two isolates of CTV with different aggressiveness. This genotype is sensible to CTV infection and

it has been traditionally used as an indicator plant for biological indexing. Leaf visible injure, oxidative damage in terms of malondialdehyde (MDA) concentration, leaf proline accumulation and the antioxidant enzyme activities ascorbate peroxidase and catalase were the parameters used to characterize the performance of plants under the biotic stress imposed by the virus. Characteristic symptoms of virus infection were evident in leaves 4 weeks after inoculation. The progression of the damage was more drastic in plants infected with the severe virus isolate. The high accumulation of MDA in leaves confirm the occurrence of oxidative damage in infected plants. The biotic stress imposed by CTV induced accumulation of the compatible osmolite proline, being this accumulation proportional to the virulence of the isolate used for plant inoculation. The studied enzymatic activities shown opposite trends, whereas ascorbate peroxidase activity increased as a consequence of viral infection, catalase reduced its activity under this biotic stress.

P17-026: BACTERIAL ELICITORS TRIGGER ISOFLAVONE METABOLISM IN GLYCINE MAX CELL CULTURES

Ramos Solano, B. - Algar, E. - García-Villaraco, A. - Lucas García ,J.A. - Gutiérrez Mañero, F.J.*
Universidad San Pablo CEU
**Corresponding author, e-mail: bramsol@ceu.es*

Benefits of isoflavones (IF) on human health have raised an interest to increase concentration in field conditions (Isanga & Zhang, 2008, Food Rev. Int., 24:252–276). However, due to the inducible nature of secondary metabolism, IF levels change according to environmental conditions (Berger et al, 2008, Crop Sci 48:700-708). This lack of reproducibility may be overcome using cell cultures under controlled conditions. Moreover, the use of elicitors of different nature appears as a challenging alternative (Poulev et al, 2003, J.Med.Chem., 46:2542-2547) so chemical and biotic elicitors from pathogenic microorganism have been used (Al-Tawaha et al, 2005, Ann.Appl.Biol., 146:303-310) but use of elicitors from free-living non-pathogenic microorganism are scant. The objective of this study was to obtain reproducible and increased levels of IF in three cell lines of Glycine max with different levels of IF production (Federici et al, 2003, Phytochem. 64:717-724) by the means of bacterial elicitors, from two PGPR strains, at different concentrations. Results show that only elicitors from Pseudomonas fluorescens N21.4 increased total IF levels, only in high and low yield cell lines revealing that IF metabolism was triggered and speaking of different molecular targets despite the low degree of cell differentiation. Acknowledgments: Nestlé Research Center for cell lines. Funded by AGL 2009-08324 and Universidad San Pablo CEU.

P17-027: COMPETITION FOR LIGHT COMPROMISES PATHOGEN DEFENSE

de Wit, M. - Voesenek, L. A. C. J. - Pierik, R.*
Institute of Environmental Biology, Utrecht University
**Corresponding author, e-mail: M.deWit@uu.nl*

Growth in high densities brings along competition for light with neigboring plants, but also an increased risk of pathogen attack; plants in close proximity facilitate plant-plant infection and the enclosed atmosphere of a canopy forms a microclimate favorable for pathogens. How plants cope with simultaneous stress from both competitors and pathogens remains largely unknown. Through physiological responses and gene expression studies using Arabidopsis we aim to gain insight into how plants can compete and defend themselves against pathogens at the same time. We show that shade avoidance induction through light signals suppresses both the SA- and JA-defense pathways. We have studied through which plant hormones this signaling crosstalk may occur and show that auxin and gibberellin do not mediate this crosstalk. Ethylene, on the other hand, is a candidate crosstalk regulator. Genome-wide gene expression studies are providing further insight into the mechanisms behind the interaction between shade avoidance and pathogen defense.

P17-028: CHANGES IN NITROGEN FIXATION AND ANTIOXIDANT ENZYMES ACTIVITY INDUCED BY SALICYLIC ACID AND ABSCISIC ACID IN MEDICAGO SATIVA UNDER SALT STRESS

Palma, F. - Iribarne, C. - García-Garijo, A. - Tejera, N.A. - Carmen, L.
Universidad de Granada

In the present work we investigated the effect of salicylic acid (SA) and abscisic acid (ABA) on nitrogen fixation and antioxidant metabolism of Medicago sativa root nodules under salt stress. Plants grew under controlled conditions and at the vegetative growth stage (49 days old) were treated with SA (0.1 and 0.5 mM) and ABA (1μM and 10 μM), 48 h later were exposed to the saline treatment (200 mM). Plants were harvested at 12 days after the saline treatment. Results showed that plant dry weight and nitrogen fixation decreased by SA, ABA and NaCl separately; however, the supply of hormonal treatments (SA and ABA) before the exposure to salt stress reduced the negative effect of NaCl. The activities superoxide dismutase (SOD) and peroxidase (POX) in plants treated with NaCl were significantly higher than in untreated plants, these enzyme activities increased with both hormones indicating that SA and ABA could protect plants under salt stress. The NaCl and SA treatments inhibited the activity catalase (CAT), however the application of ABA to salinized plants increased this activity, suggesting that ABA could mitigate salt stress. The lipoxigenase activity (LOX), used as stress indicator, increased with NaCl but decreased with hormones treatments in these stressed plants. Our results suggest that negative effects of salinity on Medicago sativa symbiosis can be alleviate by SA and ABA treatments.

P17-029: UNEXPECTED EFFECTS OF SILENCING THE VIRUS RESISTANCE GENE N IN NICOTIANA EDWARDSONII

Király, L.[1] - Künstler, A.[1] - Cawly, J.[2] - Schoelz, J.[2]*
[1]Plant Protection Institute, Hungarian Academy of Sciences
[2]Division of Plant Sciences, University of Missouri, USA
**Corresponding author, e-mail: lkir@nki.hu*

The resistance gene N determines host resistance to Tobacco mosaic virus (TMV). Our purpose is to clarify whether silencing of the N gene can influence non-host resistance to other viruses in Nicotiana edwardsonii. Transgenic plants silenced for N exhibit compromised host resistance to TMV (delay in the development of localized necrotic lesions, smaller/fewer lesions). Reduced lesion development indeed means compromised virus resistance because GFP expression from a TMV-30B-GFP construct used for virus inoculation is significantly higher in N-silenced plants. Furthermore, systemic necrosis characteristic of TMV-infected N. edwardsonii develops 3-5 days earlier in N-silenced plants. These results indicate a faster cell-to-cell and systemic movement of TMV in N-silenced N. edwardsonii as compared to wild type plants. Unexpectedly, however, transgenic N-silenced plants show enhanced non-host resistance to Tobacco necrosis virus (TNV), a virus unrelated to TMV: smaller/fewer lesions and significantly lower virus titers occur in inoculated leaves. Enhanced resistance to TNV does not result in enhanced expression of defense-related genes (NgPR1 and NtSGT) . Our results suggest that the product of the N resistance gene or a related gene is a susceptibility factor during TNV infection.
Research support: OTKA K61498

P17-030: POTATO PLANT RESPONSES TO THE COMBINATION OF TEMPERATURE DROP AND PHYTONEMATODE INVASION

Sherudilo, E. - Sysoeva, M. - Matveeva, E. - Lavrova, V.*
Institute of Biology, Karelian Research Centre RAS
**Corresponding author, e-mail: sherudil@krc.karelia.ru*

The aim of the study was to investigate a potato plant responses to short-term temperature drop combined with phytonematode invasion. Experiments were conducted with potato susceptible cultivar in growth chambers. Plants were subjected to temperature drop (5ºC x 2 h at the end of night) for 6 days. The nematodes were applied to potato plants (10 cysts per plant) before and after temperature drop. Subsequent growth conditions were optimal. Temperature drop applied before invasion increased plant chilling and nematode resistance, decreased the final nematode population and resulted in the expression of nematode resistance gene H1. Temperature drop applied after nematode invasion was ineffective: there were no differences of the developed cyst number between control and treated plants. The study was supported by RFBR (project 08-04-98833) and Federal Agency of Education.

P17-031: UREIDE ACCUMULATION IN WATER-STRESSED SOYBEAN PLANTS

*Gil-Quintana, E.**
Universidad Pública de Navarra
**Corresponding author, e-mail: erena.gil@unavarra.es*

Sanz-Corres X (Universidad Pública de Navarra) Larrainzar E (Universidad Pública de Navarra) Arrese-Igor C (Universidad Pública de Navarra) González EM (Universidad Pública de Navarra) In tropical legumes like soybean, most of the nitrogen fixed in nodules is used to synthesize ureides, the major long distance transport form of organic nitrogen in this species. Numerous studies suggest that the decline in nitrogen fixation (NF) during water deficit may be associated with increasing levels of nitrogen compounds in leaves and/or nodules of N2-fixing plants. The aim of the present work was to study the accumulation of ureides in the entire soybean plant subjected to a gradual water deficit to clarify their possible role in the regulation of NF.5 week-old soybean plants grown in symbiosis were separated randomly into two sets: control and drought. Transpiration and NF rates were measured at days 0, 1, 2, 4 and 7 and nodules, roots, stems and leaves were harvested for ureides determination. Our results show an allantoate accumulation in nodules previous to the decrease in NF. In addition, allantoate accumulates significantly in roots and stems when NF starts to show a significant decline. Leaf allantoate accumulation was only significant at the end of the experiment. The progressive accumulation of ureides in the whole plant suggests that its metabolism is regulated by drought at the plant level and that the regulation of NF should be studied in the entire plant and not as a result of a local ureide accumulation in nodules or leaves. Moreover, this general accumulation does not support the hypothesis that ureides have a role as a local signal inhibiting NF as previously hypothesized. Acknowledgements: AGL2008–00069/AGR; Government of Navarre 228/2008.

P17-032: IRON AND REACTIVE OXYGEN SPECIES IN INTERACTIONS OF PINUS SYLVESTRIS AND P, S AND F INTERSTERILITY GROUPS OF HETEROBASIDION ANNOSUM

Mucha, J.[1] - Guzicka, M.[1] - Lakomy, P.[2] - Karlinski, L.[1] - Zadworny, M.[1]*
[1] Institute of Dendrology, Polish Academy of Sciences
[2] Department of Forest Pathology, Faculty of Forestry, Poznan University of Life Sciences, Poland
**Corresponding author, e-mail: mucha_joanna@wp.pl*

Iron is reported to have an important role during infection e.g. promoting pathogen growth and enhancing tissue decay or evoking hypersensitive response reaction in plant cells. Presence of free iron in the living cells is also coupled with production of the most harmful reactive oxygen species, the hydroxyl radical. The aim of the study was to determine whether the changes in superoxide and hydrogen peroxide are dependent on two iron ions (ferric and ferrous) appearance in Scots pine seedling roots infected by strains from P, S and F groups of Heterobasidion annosum. Ferrous iron staining was associated with the nuclei of host cells and the fungal tissue of all tested strains during initial steps of interactions. Ferric iron was correlated with reddish-brown staining of H2O2 in roots inoculated with the strains of P group and they were localized around cell walls of cortex. In contrary, there was no correlation of hydrogen peroxide and ferric ion that was aggregated or spread in cytoplasm of the cortex cells of Pinus sylvestris inoculated by S strains. The principal component analysis of studied factors revealed a separation of P strains.
The research has been financially supported by the Ministry of Science and Higher Education (project no NN 309 136935)

P17-033: SYSTEMIC PLANT PROTECTION INDUCED BY PGPR IS NOT NECESSARILY ASSOCIATED TO AN INCREASE IN ISOFLAVONES IN GLYCINE MAX. VAR OSUMI.

Gutierrez Mañero, F. - Algar, E. - García Cristobal, J. - Lucas García, J.A - Ramos Solano, B. - Gutierrez Mañero, F.J.*
Universidad San Pablo CEU
**Corresponding author, e-mail: jgutierrez.fcex@ceu.es*

Soybean plays a crucial role in both the field of food and the pharmaceutical industry due to their input as plant protein and to the benefits of isoflavones (IF) for health. In addition, IF play a key role in nodulation and plant defense (Al-Tawaha et al, 2005, Ann.Appl.Biol. 146:303–310) and therefore, an increase in IF would be desirable for better field performance. Free living beneficial rhizobacteria Plant Growth Promoting Rhizobacteria (PGPR) have been used for systemic induction of plant’s secondary metabolism. The aim of this study was to increase IF content inoculating soybean with 4 PGPR that had a contrasted effect on IF metabolism (Ramos Solano et al, 2010, J.Agric. Food Chem. 58:1484-1492) and on their ability to protect plants against biotic and abiotic stresses (Barriuso et al, 2008, Phytopathol. 98:666-672) and establish the relation between IF increase and protection caused by the bacteria.
Soybean seedlings were inoculated with the PGPRs upon transplant and 20 days after; 7 d.a.i, seedlings were split in two groups, and one of them was challenged with the leaf pathogen Xanthomonas axonopodis pv. glycine. Plants were harvested 7 days after evaluating disease incidence and growth; IF were determined by HPLC.
All four strains protected soybean, ranging from 40% (Ps.fluorescens N21.4) to 80% (Curtobacterium sp. M84). On healthy plants, only N21.4 and N5.18 increased IF levels, but on pathogen challenged plants, IF increased on N21.4 and in M84 treated plants. Consistent with van Hulten’s (2006) studies, M84 was the only strain to prime the plant as revealed by the negative effect on growth associated to increased IF levels achieved only upon pathogen challenge. However, in view of the protection conferred by all four strains, it is evidenced that plants were all primed although growth was not negatively affected at this time point.
Funded by AGL 2009-08324, CM S0505 and Universidad San Pablo CEU.

P17-034: APPLICATION OF TOBACCO RATTLE VIRUS-BASED GENE SILENCING IN GERBERA HYBRIDA

Deng, X - Hytönen, T. - Valkonen, J. P. - Elomaa, P. - Teeri, T. H.*
Department of Agricultural Sciences, University of Helsinki
**Corresponding author, e-mail: xianbao.deng@helsinki.fi*

Gerbera hybrida, belonging to the large sunflower family, has attracted considerable attention as a new model plant for flower development study because of its particular flower organization in

the inflorescence. As a typical member of Asteraceae, gerbera tissues are rich in two glucosidic lactones gerberin and parasorboside, and many other secondary metabolites, which are important for the plants to protect against microbial attack and insect herbivores. During the recent years, high throughput genetic methods, such as EST sequencing and microarrays, have been adopted for identification of hundreds of candidate genes affecting gerbera flower development or secondary metabolism. In this highly heterozygous species, the functional studies need to be conducted through reverse genetic methods by producing transgenic lines. To facilitate these studies, our aim is to adapt virus-induced gene silencing (VIGS) method for gerbera. Tobacco rattle virus (TRV) has a very broad host range including gerbera. VIGS vectors based on TRV have been powerful in inducing gene silencing in many important plant species. Our initial studies show that the TRV-based vectors induce gene silencing also in gerbera leaf and flower tissues. Screening of 21 different gerbera genotypes showed large differences in VIGS response. Out of these varieties, 6 most sensitive gerbera genotypes were selected for further studies. Among different inoculation methods, vacuum inoculation induced most intensive silencing on gerbera leaf tissues, and stem wound scratching was more practical for inducing gene silencing on flowers. The silencing of gerbera phytoene desaturase gene (GPDS) and gerbera chalcone synthase (GCHS) gene induced typical gene knock-out symptoms on host gerbera plants.

P17-035: CA2+-PUMPING ATPASE IN THE PERIBACTEROID MEMBRANE OF BROAD BEAN ROOT NODULES: SUBSTRATE SPECIFICITY AND EFFECTS OF PH AND CALMODULIN

*Krylova V**
K.A.Timiryazev Institute of Plant Physiolgy Russian Academy of Sciences
**Corresponding author, e-mail: KrylovaVV@list.ru*

Ca2+-pumping ATPase in the peribacteroid membrane of broad bean root nodules:substrate specificity and effects of pH and calmodulin (K.A.Timiryazev Institute of Plant Physiolgy Russian Academy of Sciences) It is well known that the processes of ion transport through the peribacteroid membrane (PBM) of symbiosomes play an important role in the regulation of nitrogen fixing plant root-rhizobium symbiosis in legumes in the course of the interaction between pro- and eukaryotic cells in root nodules. Previously, we had shown that symbiosomes from yellow lupine and broad bean root nodules behave as Ca-storing compartments in infected cells and such a behavior is in part due to an active transport of Ca2+ through the PBM as a result of functioning of ATP-driven Ca2+-pump in this membrane. Here, some biochemical characteristics of the Ca2+-pumping ATPase in the PBM of broad bean root nodules were studied by monitoring the decrease in the external calcium concentration caused by ATP-dependent Ca2+ uptake by both isolated symbiosomes and the PBM vesicles with a metallochromic Ca2+-indicator arsenazo III. The rate of this process was shown to achieve a maximal value at pH 7.2 and to decline about six-fold at extreme chosen pH values (pH 6.0 and 8. 0). It was established that, although a predominant substrate for the Ca2+-ATPase is ATP, this enzyme is capable of utilizing other nucleotide triphosphates as well, such as ITP, GTP, UTP and CTP, as energy donors for providing Ca2+ translocation through the PBM. An efficiency of all these substrates was found to decline in the following series: ATP > ITP > GTP > UTP > CTP. An apparent Km of the Ca2+-ATPase for MgATP was shown to be in the range of 0.1 – 0. 2 mM. In the reaction medium not containing exogenous calcium, the rate of ATP-dependent Ca2+-pumping appeared to be markedly stimulated by exogenous calmodulin, a well-known Ca2+-sensor in animal and plant cells, from bovine brain achieving a maximal incre-ase of about two-fold at moderately high concentrations of the given protein. The results obtain-ned allow us to conclude that the characteristics revealed of the PBM Ca2+-ATPase under study share with those inherent in IIB type Ca2+-ATPases functioning in other plant cell membranes.

P17-036: ANALYSIS OF MICROTUBULE AND ACTIN FILAMENTS DYNAMIC OF THE PINUS SYLVESTRIS ROOTS TO INFECTION BY P, S AND F GROUPS OF HETEROBASIDION ANNOSUM

*Zadworny, M. *[1] - Guzicka, M.[1] - Lakomy, P.[1] - Samardakiewicz, S.[2] - Smolinski, D.J.[3] - Mucha, J.[1]*
[1]Institute of Dendrology, Polish Academy of Sciences
[2]Adam Mickiewicz University, Poznan, Poland
[3]Nicolaus Copernicus University, Torun, Poland
**Corresponding author, e-mail: zadworny@man.poznan.pl*

Cytoskeleton dynamic plays a crucial role in pathogen recognition and defence response during initial steps of interaction between invader and host in the plant cell. Extend of the response is linked to the host specialization and resistance status. The aim of our study was to characterize the cytoskeleton arrangement of Pinus sylvestris root cortex cell in reactions to infection by P, S and F intersterility group of Heterobasidion annosum and to enhance by this our knowledge about pathogen specialization. The cytoskeleton was visualized using immunofluorescence technique on Steedman‘s wax sections. P. sylvestris inoculation with non-adapted Fand S groupsfungal strains resulted in more intensive depolymerizationof host microtubule during early stages of interaction than was observed after inoculation with well-adapted P group. In S and F groups abnormal arrangements and deposition of the polymerized tubulin aggregates was seen. The analysis also showed polarisation of hostmicrofilaments towards the site of attempted penetration by specificfungal strains. Application of the cytoskeleton inhibitors enhanced fungal entry in the non-host cells. It suggests that cytoskeleton of P. sylvestris cell play a crucial role in restricting access by P, S and F intersterility groups of H. annosum showing different host preferences.
The research has been supported by the Ministry of Science and Higher Education (project no. NN 309377733).

P17-037: PRE-EXPOSURE OF ARABIDOPSIS TO CD2+ DOES NOT AMELIORATE THE PLANT RESPONSE TO THE INFECTION CAUSED BY BOTRYTIS CINEREA.

Cabot, C.* - Gallego, B. - Martos, M. - Sibole, J.V. - Barceló, J. - oschenrieder, C.
Universitat Autònoma de Barcelona
**Corresponding author, e-mail:* ccabot@uib.es

Plants aresimultaneously exposed to multiple stresses in nature. Different studies have shown that a stress exposure can prime the plant response to forthcoming unfavorable conditions, which are mediated by a cross-talk among multiple hormone signalling pathways. The interaction between jasmonic (JA) and salicilic (SA) acid has been studied using biotic stresses caused by a combination of biotrophic and necrotrophic organisms. While at very low phytohormone concentrations synergistic responses were reported, high SA concentration was found to suppress JA-induced gene expression. Little information is available on these phytohormone interactions in the combined response to biotic and abiotic stresses. In Arabidopsis, the JA-signalling pathway was found to participate in the plant response to Cd2+ and infections caused by the nectrofic pathogenic fungus Botrytis cinerea. In this work five week-old Arabidopsis thaliana Col 0 plants individually cultured in aereated 25% Hoagland solution were sumitted to the following treatments: 1,5 mM Na4SiO4; 1 µM CdCl2; 10 µM CdCl2; 1,5 mM Na4SiO4 + 1 µM CdCl2; 1,5 mM Na4SiO4 + 10 µM CdCl2. After 48 h, half of the plants of each treatment were inoculated with Botrytis cinerea. Different physiological parameters and gene expression of different markers for the ethylene, JA and SA gene signalling pathways were analyzed at different times. Only plants pre-exposed to Si showed an ame-

liorated growth response to Botrytis, with no significant effect found for either Cd-pretreated or Cd+Si-pretreated plants. The results are discussed in relavance to the different phytohormone signalling pathways.
*Supported by BFU2004-02237-C02-02

P17-038: BIOCHEMICAL CHARACTERIZATION OF CA2+-PUMPING ATPASE IN THE PERIBACTEROID MEMBRANE OF BROAD BEAN ROOT NODULES

Krylova, V. - Andreev, I. – Zartdinova, R. – Izmailov, S.*
K.A.Timiryazev Institute of Plant Physiology Russian Academy of Sciences
**Corresponding author e-mail: KrylovaVV@list.ru*

Ion transport through the peribacteroid membrane (PBM) of symbiosomes is known to play an important role in the regulation of nitrogen fixing plant root-rhizobium symbiosis related to the mutualistic interaction between proand eukaryotic cells in root nodules. Previously, we had shown that symbiosomes from yellow lupine and broad bean root nodules behave as Ca-storing compartments in infected cells and this is in part due to an active transport of Ca2+ through the PBM as a result of functioning in it of ATP-driven Ca2+-pump. Here, some biochemical charac-teristics of the Ca2+-pumping ATPase in the PBM of broad bean root nodules were studied by monitoring the decrease in the external calcium concentration caused by ATP-dependent Ca2+ uptake by both isolated symbiosomes and the PBM vesicles with arsenazo III. The rate of this process was shown to achieve a maximal value at pH 7.2 and to decline about six-fold at pH 6.0 and 8. 0. It was established that, although a predominant substrate for the Ca2+-ATPase is ATP, this enzyme is capable of utilizing other nucleotide triphosphates in the following series: ATP > ITP > GTP > UTP > CTP. An apparent Km of the Ca2+-ATPase for MgATP was shown to be in the range of 0.1 – 0. 2 mM. In the reaction added Ca-free medium, the rate of ATP-dependent Ca2+-pumping appeared to be markedly stimulated by exogenous calmodulin, a well-known Ca2+-sensor in animal and plant cells, from bovine brain achieving a maximal increase of about two-fold at moderately high concentrations of the given protein. These results allowed us to con-clude that the characteristics revealed of the PBM Ca2+-ATPase under study share with those which are inherent in IIB type Ca2+-ATPases functioning in other plant cell membranes.

P17-039: NONSYMBIOTIC AND TRUNCATED HEMOGLOBINS OF LOTUS JAPONICUS: DIFFERENTIAL GENE EXPRESSION IN PLANT ORGANS AND IN RESPONSE TO HORMONES

Bustos Sanmamed, M[1] - Tovar-Méndez, A.[2] - Crespi, M.[3] - Sato, S.4 - Tabata S.4 – Becana, M.[2]*
[1]Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas
[2]Estación Experimental Aula Dei (CSIC)
[3]Institute des Sciences du Végétale (CNRS)
[4]Kazusa DNA Research Institute (Japan)
**Corresponding author e-mail: pbustos@eead.csic.es*

In plants, three distinct types of hemoglobins (bs) are known: symbiotic, nonsymbiotic, and truncated.
Nonsymbiotic Hbs are further divided into class-1 and class-2 based on primary sequences and oxygen-binding properties. Symbiotic Hbs are well-characterized, whereas the functions of the other two types of Hbs are not fully understood. We have identified five genes encoding two class-1 (*GLB1-1, GLB1-2*), one class-2 (*GLB2*), and two truncated (*GLB3-1, GLB3-2*) Hbs in the model legume Lotus japonicus. Results show that *GLB1-2* and *GLB3-2* are expressed in all plant organs, whereas *GLB1-1, GLB2*, and *GLB3-1* are expressed abundantly only in nodules. Genes were differentially upregulated (*GLB1-1* with ethylene and cytokinins; *GLB1-2* with abscisic acid; *GLB2* with gibberellic acid, abscisic acid and polyamines; *GLB3-1* with polyamines) or downregulated (*GLB1-2* and *GLB2* with ethylene and cytokinins; *GLB3-1* with gibberellic acid; *GLB3-2* with cytokinins) in response to hormones. *GLB1-1* and *GLB1-2* mRNAs are localized in the cortex, vascular bundles, and fixation zone, whereas *GLB2* and *GLB3* mRNAs are mainly confined to the cortex. The data point out complex regulatory mechanisms of Hb expression in different plant tissues and hormone signaling pathways, and strongly suggest specific functions for each of the five proteins.

P17-040: ELUCIDATING IN PLANTA THE FUNCTION OF EFFECTORS FROM BROAD HOST-RANGE PATHOGENIC BACTERIA.

Schikora, A.[1] - Shaver, A.[1] - Kogel, K.H.[1] - Hirt, H.[2]*
[1]Institute of Phytopathology and Applied Zoology, IPAZ
[2]Plant Genomics Research Unit, URGV
**Corresponding author e-mail: adam.schikora@agro.uni-giessen.de*

The pathogenicity of *Enterobacteriaceae* depends on the ability of these bacteria to enter host cells.
Enteropathogens inject a cocktail of effector proteins that actively suppress the host's immune responses. *Salmonella* spp. present in food can cause salmonellosis, the most widespread cause of food poisoning worldwide. These bacteria are also known to infect plants. *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) contaminated fruits and vegetables are a significant source of human infections, however the interaction between pathogen and plant host cell is only partially understood. In this report we present a functional screen aiming to identify bacterial factors required for the infection of plant cells. S. Typhimurium virulence factors and effectors known from mammalian systems were cloned and expressed in various vectors.
This allowed for the investigation of multiple aspects of effector protein function and mode of action *in planta*. Sp ecifically, we focused on the localization, PAMP activity and HR promoting or suppressing action. Additionally we investigated the ability of *Salmonella effectors* to suppress the plant immune response. We further plan on expanding our study to include virulence assays of *Salmonella* mutants for effectors identified in this screen.

P17-041: INCREASE OF APOPLASTIC (EC)-POX ACTIVITIES IN OLEA EUROPAEA INTACT SEEDLING ROOTS IN CONTACT WITH GLOMUS INTRARRADICES

Caraballo-Sánchez, A.M. – Garrido, I. – Álvarez-Tinaut, M. – Espinosa, F.
Universidad Extremadura

The aim of this work was to study the oxidative reactions induced in olive roots after a few hours of contact with mycorrhizal compatible fungal hyphae, in comparison with those of MeJA elicited roots at the same times.
Axenically cultured isolate embryos from olive seed were used to obtain axenic intact seedling roots. A dual monoxenic culture of the compatible fungus Glomus intrarradices was used to perform the contact experiments.
O2.- generation, ECPOXs (DMAB-MBTH EC-POX and EC-GPOX) and SOD activities in the medium with roots were determined after 4, 6, 8, 10 and 12 hours of the treatments; at every time, simultaneous experiments were carried out with roots elicited by MeJA, by fungus contact, and controls (without any treatment).
The results obtained for all the activities measured in the controls carried out in every experiment, showed the same pattern characterized by homeostatic oscillations. No statistically significant differences (P=0.05) were observed for O2.- generation or SOD activity among roots elicited by MeJA or fungus contact and the respective controls, at any time. Neither ECPOX activities in roots induced by the fungus contact at every time were

significantly different of controls or MeJA treated roots at the same time. But when the total activities (after 12 h treatment) were compared, a remarkable statistically significant increase in roots induced by the fungus was observed. Our interpretation is that the elimination of H2O2 via ECPOX could be an efficient mechanism to attenuate the oxidative response in the apoplast of roots that contact with the compatible fungus.
Acknowledgements: This work was supported by the Spanish Ministry of Science and Innovation (CGL2009-12406) and the Extremadura Government (PRI09A023).

P17-042: HORMONAL PATHWAYS INVOLVED IN HEXANOIC ACID-INDUCED RESISTANCE AGAINST PSEUDOMONAS SYRINGAE IN TOMATO PLANTS.

Vicedo, B.[1] - Scalschi, L.[1] - Flors, V.[1] - Camañes, G.[1] - González-Bosch, C.[2] - Garcia-Agustín, P.[1]*
[1]Universitat Jaume I
[2]Universitat de València
**Corresponding author e-mail: bvicedo@camn.uji.es*

Plants are able to develop an enhanced defense mechanism so-called induced resistance (IR), in addition to basal defense upon appropriate stimulation.
We have demonstrated that tomato plants treated with hexanoic acid display enhanced resistance against the Pseudomonas syringae (Pst) DC3000 strain. After 72 hpi hexanoic acid treatment reduced the disease symptoms and the colony-forming units. To establish the involvement of the SA-, JA- ET- and ABA-signaling pathways in hexanoic acid induced-resistance, the expression of the PR1, LoxD, ACCOx and ASR1 marker genes was analyzed by RT-qPCR. No differences were found in the analysis of the ASR1 and ACCOx between infected plants with or without hexanoic treatment. However, a significant increase in the SA (PR1) and JA (LoxD) marker genes expression was observed in the plants treated with hexanoic acid and infected with Pst, 48 hpi. Several mutants relating to the different signaling pathways were studied to confirm these results. In addition, hormonal analysis of hexanoic acid-treated plants showed a faster and stronger accumulation of OPDA upon infection; nonetheless, JA and JA-Ile were not increased in these plants. In order to explain the increase in OPDA and the LoxD gene expression, we focused the analysis on the genes involved in the oxylipin synthesis pathway. The obtained results suggest that hexanoic acid-induced resistance against Pst could be mediated by activation of the oxylipin signaling pathway

P17-044: THE ANTIMICROBIAL ELECTROLYZED ACTIVATED WATER VERDEVIVA™ STIMULATES PLANT DEFENCES AGAINST PATHOGENS - MOLECULAR STUDIES.

Orlando, R. - Raineri, M. - Zarattini, M. - De Bastiani, M. - Ferro, S. - De Battisti, A. - Bernacchia, G.
Universita' di Ferrara

The fight against plant pathogens is of primary importance in agriculture to ensure that quali-quantitative features of fruits and vegetables fit the market requirements. Synthetic products can protect plants against pathogens but they can be toxic or environmental unfriendly therefore the use of less toxic and less persistent products is highly encouraged. It is known that the electrolysis of water enriched in salts can produce highly reactive oxidative species characterized by a short life span and a potent antimicrobial activity, even at low doses. In the last few years our group has developed the system for the production of electrolyzed activated water and has started to study the beneficial antimicrobial effect of Verdeviva on plants infected by different pathogens. Our research also focused on the effects of Verdeviva on plant cells at the molecular level to establish if it can prime plant defences against pathogens. Results will be presented showing that Verdeviva is indeed able to stimulate endogenous defences by activating several pathogen-related genes both in tobacco and apple plants.

P17-045: A NADPH OXIDASE IS INVOLVED IN MEDICAGO TRUNCATULA – SINORHIZOBIUM MELILOTI SYMBIOSIS.

Marino, D.[1] - Andrio, E.[1] - Danchin, E.G.J.[2] - Oger, E.[2] - Gucciardo, S.[2] - Lambert, A[2] - Puppo, A.[2] - Pauly, N.[2]
[1]Laboratoire Interactions Plantes Micro-organismes, Castanet Tolosan, France
[2]UMR Interactions Biotiques et Santé Végétale Centre INRA PACA, Sophia Antipolis, France

Reactive oxygen species such as anion superoxide (O2-) and hydrogen peroxide (H2O2) have been detected during the establishment and the functioning of legume - rhizobia symbioses. Moreover, a H2O2 threshold seems to be required to optimize the establishment of the Medicago sp – Sinorhizobium meliloti symbiosis.
Nevertheless, its origin remains largely unknown. In this framework, the functional characterisation of a M. truncatula NADPH oxidase has been performed. Plant NADPH oxidases (Rboh; respiratory burst oxidase homologue) are transmembrane redox chain proteins able to reduce O2 to O2-, which can be rapidly dismutated in H2O2. These proteins have been shown to play important roles in plant-microorganism interactions and developmental processes.
A in silico analysis leds to the identification of 7 complete rboh gene sequences in the M. truncatula genome (MtRbohs). Their expression in different M. truncatula tissues was analysed and one of these genes, MtRbohA, appeared to be significantly upregulated in Sinorhizobium meliloti - induced symbiotic nodules. MtRbohA expression was restricted to the nitrogen-fixing zone of the functional nodule. Specific RNA interference for MtRbohA provoked a decrease in the nodule nitrogen fixation activity, thus highlighting its role during nodule functioning.

P17-046: A PROTEOMIC APPROACH TO THE STUDY OF BOTRYTIS CINEREA INFECTION IN TOMATO (SOLANUM LYCOPERSICUM) FRUITS

Leyva, M.O.[1] - Vicedo, B.[2] - Finiti, I.[1] - García-Agustín, P.[2] - González-Bosch, C.[1] - Real, M.[3]
[1]Dep. Bioquímica y Biología Molecular, Universidad de Valencia IATA (CSIC)
[2]Dep. Ciencias Agrarias y del medio natural, Universitat Jaume I, Castellón
[3]Dep. de Genética, Universidad de Valencia

To gain a comprehensive understanding of the defense molecular mechanisms in tomato fruit against B. cinerea infection, a proteomic approach based on multiplexed isobaric tagging technology (iTRAQ) using tandem mass spectrometry was undertaken. MG4 fruit controls and B. cinerea challenged fruits (24 hpi) were analyzed. 30172 peptide MS/MS spectra were generated and 361 distinct proteins were assembled. Species distribution, terms, and pathway data retrieved from UniProt showed extensive protein coverage in terms of physicochemical properties and diversity in GO categories. The largest GO biological process categories were cellular and metabolic processes and response to stimuli. Differential proteins were selected based on the following cut-off criteria: i) p-value <0.05; ii) error factor <2; iii) average of at least 1.5-fold in abundance in response to B. cinerea infection in both replicates. Twelve proteins (3.3%) showed statistically significant changes (9 upregulated and 3 downregulated). For the remaining 96.7% of identified proteins, 16 (4.4%) although displayed differential expression in both replicates (p-value < 0.05) did not meet the 1.5-fold change cutoff, while 30 proteins (8.3%) did meet the 1.5-fold change at 90% confidence level. The main group of differential proteins corresponded to defense response proteins: 22 response to stress and stimulus; 9 response to biotic

stimulus (5 PR-10; 2 chitinase class II; 2 PR-P2; and 1 wound-induced proteinase inhibitor I); and 15 response to chemical stimulus (10 oxidative stress response; 4 hydric stress response; 1 herbicide response). Results indicate that pathogenesis-related proteins and antioxidant enzymes may play a role in the protection against B. cinerea tomato fruit infection.

P17-047: TRYPTOPHAN-DERIVED SECONDARY METABOLITES IN ARABIDOPSIS THALIANA CONFER NONHOST RESISTANCE TO NECROTROPHIC PLECTOSPHAERELLA CUCUMERINA FUNGI

Sanchez-Vallet, A.[*1] - Ramos, B.[1] - Bednarek, P.[2] - Lopez, G.[1] - Pislewska-Bednarek, M.[2] -*
Schulze-Lefert, P.[2] - Molina, A.[1]
[1]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid
[2]Max Planck Institut für Züchtungsforschung
** Corresponding author e-mail: andrea.sanchezv@upm.es*

A defence pathway contributing to nonhost resistance in Arabidopsis to biotrophic fungi involves the synthesis and targeted delivery of the tryptophan (trp)-derived metabolites indol glucosinolates (IGs) and camalexin at pathogen contact sites. We have examined whether these metabolites are also rate-limiting for colonization by necrotrophic fungi. Inoculation of Arabidopsis with adapted or nonadapted isolates of the ascomycete Plectosphaerella cucumerina triggers the accumulation of trp-derived metabolites. We found that their depletion in cyp79B2 cyp79B3mutants renders Arabidopsis fully susceptible to each of three tested P. cucumerina isolates. This assigns a key role to trp-derived secondary metabolites in limiting growth of both nonadapted and adapted necrotrophic fungi. However, 4-methoxy-indol-3-ylmethylglucosinolate, which is generated by the P450 monooxygenase CYP81F2 and hydrolyzed by PEN2 myrosinase, together with the antimicrobial camalexin play a minor role in restricting the growth of the nonadapted necrotrophs. This contrasts with a major role of these two trp-derived phytochemicals in limiting invasive growth of nonadapted biotrophic powdery mildew fungi, thereby implying the existence of other unknown trp-derived metabolites in resistance responses to nonadapted necrotrophic P. cucumerina.Impaired defence to nonadapted P. cucumerina,but not to the nonadapted biotrophic fungus Erysiphe pisi, on cyp79B2 cyp79B3 plants is largely restored in the irx1 background that shows a constitutive accumulation of antimicrobial peptides. Our findings imply differential contributions of antimicrobials in nonhost resistance to necrotrophic and biotrophic pathogens.

P17-048: DIFFERENTIAL REGULATION OF 3-AMINOMETHYLINDOLE/N-METHYL-3-AMINOMETHYLINDOLE N-METHYLTRANSFERASE AND GRAMINE IN BARLEY BY BOTH BIOTIC AND ABIOTIC STRESSES

Jonsson, L.[1] - Larsson, KAE.[1] - Saheed, SA.[2] - Gradin, T.[1] - Delp, G.[1] - Karpinska, B.[1] - Botha,
CEJ.[2]
[1]Södertörn University
[2]Rhodes University
**Corresponding author e-mail: lisbeth.jonsson@sh.se*

The expression of NMT (3-Aminomethylindole/N-Methyl-3-aminomethylindole N-methyltransferase; EC 2.1.1.), involved in the biosynthesis of the indole alkaloid gramine, was investigated in aphid-infested barley (Hordeum vulgare L.). NMT is induced by methyl jasmonate and it was hypothesized that the gene would be more strongly upregulated in aphid-resistant barley. Infestation by bird-cherry oat aphid (Rhopalosiphum padi, L.) and rose-grain aphid (Metopolophium dirhodum,Walker) resulted in higher NMT expression in the doubled haploid line 5172-28:4 (DH28:4), which has moderate resistance against R. padi, but not in other aphid-barley combinations. None of the aphid - plant combinations had however increased gramine. The increased abundance of NMT transcript in aphid-infested DH28:4 did not lead to higher amounts of NMT protein or NMT enzyme activity, neither did 200 times upregulation of NMT transcript in cotyledons incubated with methyl jasmonate. This illustrates that even large differences measured at transcript level may have no metabolic consequences. Drought stress or treatments with abscisic acid did lead to higher gramine concentrations in several barley cultivars, but without any concomitant increase of NMT transcripts. Thus, the regulation of the biosynthetic pathway to gramine at transcript and metabolite level diverges during two different stress conditions.

P17-049: EARLY DETECTION OF PLASMOPARA VITICOLA INFECTION IN GRAPEVINE LEAVES USING CHLOROPHYLL FLUORESCENCE IMAGING BOTH IN LABORATORY AND FIELD CONDITIONS.

Olejnicková, J.[1] - Vrchotová, N.[1] - Tríska, J.[1] - Sotolár, R.[2]*
[1]Institute of Systems Biology and Ecology ASCR
[2]Department of Viticulture and Viniculture, Mendel Univerzity in Brno
**Corresponding author e-mail: olejnickova@greentech.cz*

Plasmopara viticola is an important pathogen of grapevine. Our study aimed to determine whether chlorophyll fluorescence (Chl-F) imaging can be used to reveal early stages of *P. viticola* infection under conditions similar to those occurring in field, in vineyards.
Maximum (FV/FM) and effective quantum yield of photosystem II (ΦII) were identified as the most sensitive reporters of the infection both in lab and field conditions. Significant changes of FV/FM and ΦII were spatially coincident with spots of inoculation, where photosynthesis was impaired before occurrence of visible symptoms. Sensitive and resistant varieties differed in response to fungal attack: *in sensitive varieties*, ΦII images showed small spots with decreased PSII activity in early phase; in late phase of infection the affected area covered nearly the whole leaf area. *In resistant varieties* lowered PSII activity occurred only in place of fungal attack both in early and late phase. These spots were much larger; the infection was localized and not allowed to spread.
In order to look into plant defense reaction we analyzed content of phenolic comnpounds (resveratrol and pterostilben) that serve also as phytoalexins. In infected leaves these compounds were highly produced compared to healthy leaves. However, their production did not correlate with sensitiveness or resistance of the varieties.

P17-050: CDR1 POSITIONING WITHIN THE DISEASE RESISTANCE SIGNAL NETWORK

*Ortega-Galisteo, A.P.[1] - Creissen, G.[2] - Sandalio, LM.[1] - Romero-Puertas, M.[3] - Lamb, C.J.[2]**
[1]Departamento de Bioquímica, Biología Celular y Molecular de Plantas, Estación Experimental del
Zaidín, CSIC, Granada, Spain
[2]Disease and Stress Biology Department, John Innes Center, Norwich Research Park, Colney Lane,
UK
[3]Estación Experimental del Zaidín (CSIC)

Constitutive Disease Resistance 1 (CDR1) encodes an apoplastic aspartic protease that mediates a peptide signal system involved in the activation of inducible resistance mechanism (1). CDR1 over–expression causes dwarfing and resistance to virulent Pseudomonas syringae. These phenotypes reflect salicylic-acid-dependent activation of micro-oxidative burst and various defense-related genes. We have created and characterized transgenic plants containing CDR1 under the control of the dexamethasone inducible TA promoter (TApr::CDR1). Local dexamethasone induction allows determination of whether downstream com-

ponents are required for local and/or systemic effects of CDR1 and avoids possible secondary or pleiotropic effects arising from chronic over-expression of CDR1 throughout development. Furthermore, to explore how CDR1 is integrated within the disease resistance signal network and to position CDR1 relative to other key players such as small molecule signals like ROS and salicylic acid, and signal transduction proteins like NPR1, EDS1, PAD4 and DIR1, we have crossed TA:CDR1 plants with the following key mutant/transgenic phenotypes: npr1-1, eds1-1, pad4-5, dir1-1, rboh D, rboh F, rboh D/F and sid 2-2. ROS production, cell death and defence response have been analyzed to determine which signalling molecules are required for both, local and systemic effects of CDR1.

(1) Xia et al., Embo Journal, 2004, 23: 980-988

* Lamb CJ was sadly departed on August 2009

Funded by Marie Curie Actions-European re-integration Grants (FP7-PEOPLE-2007-2-2-ERG) Project nº 208146

P17-051: NITROGEN TRANSPORTS CAN ACT AS NATURAL SWITCHES FOR PLANT RESISTANCE SIGNALING

García-Agustín, P. - Camañes, G. – Pastor, V. – Vicedo, B. – Cerezo, M. – Flors, V.*
Universitat Jaume I
**Corresponding author e-mail: garciap@camn.uji.es*

Nitrogen levels have been associated to enhanced susceptibility of plants to different pathogens. However, a mutation in a high affinity nitrate transporter (HATS) codified by NRT2.1 enhances basal resistance and is not associated with different nitrate levels in normally fertilized plants. The nrt2 mutant shows lower susceptibility to Pseudomonas syringae with reduced disease rates and lower bacterial growth inside the plant tissue. The reduced susceptibility could be linked to a primed SA-dependent signaling together with a reduced CORONATIN sensitivity. Several bioassays have demonstrated that nrt2 is primed for the PR1 expression and SA levels upon Pst infection. In addition, nrt2 neither closes stomata at late time points after COR application nor enhances H2O2 production upon effector treatment. Coronatine less Pst DC3118 produces reduced symptoms on Ws background while it grows as Pst DC3000 in nrt2.1. Accordingly to SA priming, ABA and JA levels do not change in nrt2.1 during infection, but increase in wild-type plants. This establishes a possible link between nitrate transporters and plant responses to biotic stresses. Microarray analysis confirm the relevance of one of the branches of SA synthesis. Interestingly, the expression of many ribosomal proteins is also affected in the atnrt2 mutant upon Pst infection. Further genetic analyses show that AtNRT2.1 displays atypical regulation upon different stimuli and is tightly coordinated with AtNRT2.2 and AtNRT3.1.

P17-052: DIFFERENTIAL EXPRESSION OF PLANT GENES INVOLVED IN GIBBERELLINS METABOLISM DURING THE EARLY STEPS OF MEDICAGO TRUNCATULA / GIGASPORA MARGARITA INTERACTION

Ortu, G. – Küster, H. – Balestrini, R. – Bonfante, P.
Department of Plant Biology, University of Turin (Italy) – IPP-CNR

Keywords: arbuscular mycorrhiza, Medicago truncatula, Gigaspora margarita, gibberellins. Arbuscular mycorrhizas are symbioses established between the roots of most land plants and fungi belonging to Glomeromycota (Smith & Read 2008). While plants acquire minerals from the fungal partner improving their nutrient status, symbiotic fungi directly uptake organic carbon from their hosts. The improved nutrient status has a positive impact on whole plant physiology influencing growth, protection from diseases and cause important impact at the transcriptomic and cellular level (Hohnjec et al 2005). During colonization plant cells undergo strong modifications in order to accommodate the penetrating hyphae: in epidermal cells an ephemeral apparatus, the pre-penetration apparatus, is assembled (Genre et al 2005) at the moment of surface contact between the partners. The aim of the work was to identify plant genes which were differentially regulated during the early steps of M. truncatula-G. margarita interaction and to focus on genes potentially involved in gibberellins metabolism. A microarrays analysis on RNA extracted from M. truncatula transformed roots segments collected at the first contact with G. margarita appressoria showed an up regulation of 534 gene in the wild type respect to the mycorrhiza-defective dmi3-1 mutant. Out of them, 6 genes were found involved in different steps of gibberellins biosynthesis. Preliminary experiments in q-RT real time PCR confirm the upregulation of two genes involved in gibberelin pathway: GID1L3, a predicted gibberellin receptor and GA2oxydase7 involved in gibberellin catabolism.

P17-053: IDENTIFICATION AND CHARACTERIZATION OF A PUTATIVE MYB TRANSCRIPTION FACTOR DURING THE SYMBIOSIS BETWEEN LOTUS JAPONICUS AND GLOMUS INTRARADICES

Volpe, V.[1]– Güether, M.[1] - Dell´Aglio, E.[1] - Costa A.[2] - Ruberti, C.[2] - Lo Schiavo, F.[2] - Bonfante, P.[1]
[1]Department of Plant Biology, University of Turin (Italy) – IPP-CNR
[2]Department of Biology, University of Padova (Italy)

Keywords: arbuscular mycorrhiza symbiosis, Lotus japonicus, Glomus intraradices, transcription factors, RNA interference Arbuscular mycorrhizas (AMs) are very common symbioses established between land plants and soil fungi, where both partners benefit by nutritional exchanges. Transcriptome analysis of L. japonicus using microarray has identified a large number of genes whose expression is altered in mycorrhizal roots. Among them, 47 code for proteins involved in transport and 24 genes code for potential transcription factors (TF) (Guether *et al.*, 2009). One of them, a putative MYB-TF, is the second highest regulated gene of the whole array with 20,000 fold over-expression in mycorrhizal roots (Guether et al., 2009). The aim of this work is to analyze the role of the putative MYB-TF and to understand whether i) it is dispensable for arbuscular mycorrhiza symbiosis and ii) it is involved in the regulation of nutrient exchange processes.

To investigate the specific role of the LjMYB protein in mycorrhizal processes we first developed silenced lines using RNAi (RNA interference) technology.

We also generated transgenic hairy root lines, constitutively expressing the LjMYB gene, in order to obtain a deeper view of its functional meaning. The expression levels of LjMYB in two RNAi hairy root lines analyzed were reduced in respect to the control lines. The frequency of mycorrhizal colonization in RNAi-line1 was similar to the control lines, but the number of arbuscules was increased. As a further step, we analyzed the subcellular localization of MYB in Tobacco-leaf protoplasts expressing the chimeric p35S::EGFP::MYB gene: the signal was localized to the nucleus, indicating a role as a TF for the LjMYB protein.

P17-054: SULFUR STARVATION AND NITROGEN FIXATION IN NODULATED PEA PLANTS (PISUM SATIVUM L.)

Muñoz-Azcárate, O. – Aldasoro, J. - Arrese-Igor, C.
Universidad Pública de Navarra

Sulfur deficiency is receiving an increased attention as a limiting factor in cropping systems, although there is scarce information on its effect on biological N fixation (BNF). This can be an important field of study since nitrogen-fixing symbioses between legume plants and rhizobia are the largest natural source of nitrogen for agriculture and these symbioses are very sensitive to abiotic stresses.

A few studies have reported a decreased nodule performance

under S-deficient conditions. However, it is not well established yet whether this effect can be mainly attributed to an impairment of nodule development or nodule metabolism.Nodulated pea seedlings were grown in an aerated hydroponic solution in a controlled environment. A group of plants was transferred to a modified S-free solutionthe day of transplanting (S0 plants), and another group was transferred to the S-free solution seven days after transplanting (DAT) (S7 plants). BNF, photosynthesis, nodule biomass and root and shoot length and biomass were measured 28 DAT.Sulfur starvation resulted in a significant yield reduction of shoots, roots and nodules in S0 plants, but no significant effect was found in S7 plants. Also, BNF and photosynthesis in S0 plants showed a significant reduction when compared with control plants, while S7 plants showed no significant differences with control plants. Thus, it appears that S starvation is critical at the early stages of nodulation, being the negative effects mostly due to a lower nodule development.This work has been supported by the Ministry of Science and Innovation (Spain; AGL2008-0069/AGR). OMA is the recipient of a fellowship from MSI (FPI Programme).

P17-055: GENETIC STUDIES OF MAMP-TRIGGERED IMMUNITY IN ARABIDOPSIS

Tintor, N. – Reimer, EM. - Schulze-Lefert, P. – Saijo, Y.
Max Planck Institute for Plant Breeding Research

Plants recognize the presence of microbes through the perception of molecular structures typical of a microbial class, termed microbe-associated molecular patterns (MAMPs). In Arabidopsis, the Leu-rich repeat receptor-like kinases FLS2 and EFR recognize the bacterial MAMPs flagellin and EF-Tu (and their bioactive epitopes flg22 and elf18), respectively. Perception of these MAMPs triggers defense responses that restrict microbial invasion and growth. However, the molecular basis of MAMP-triggered immunity is still largely unknown. A forward genetic approach revealed priority in sweet life (psl) mutants that show de-repressed anthocyanin accumulation in the presence of elf18. Previously identified PSL genes encode for components of an ER-resident protein folding and maturation pathway that is recquired for the generation of functional EFR. psl25 plants are partially and differentially defective in elf18 but not flg22 responses and hypersusceptible to a bacterial phytopathogen, Pseudomonas syringae. Map-based cloning revealed a mutation in the PSL25 locus that encodes for a key enzyme in the ER N-glycosylation pathway. psl36 plants are impaired in responses to both flg22 and elf18, despite wild type-like accumulation and ligand-binding activity of FLS2 and EFR. This points to a role of PSL36 in post-recognition signaling of both receptors. Further characterization of psl25 and psl36 plants is underway.

P17-056: CELL WALL AND PLANT STRESS RESPONSES. THE ENDO-B-1,4- GLUCANASES ALTER THE RESISTANCE TO PATHOGENS IN ARABIDOPSIS

Finiti, I.[1] - Leyva, M.O.[1] - Vicedo, B.[2] - Real, M.D.[1] - García-Agustín, P.[2] - González-Bosch, C.[1]
[1]Universitat de Valencia, IATA (CSIC)
[2]Universitat Jaume I

Plant Endo-β-1,4-glucanases (EGases) depolymerize polysaccharides containing 1,4-β-D-glucan linkages and take part in cell wall editing processes such as elongation, fruit ripening and floral abscission. EGases are localized both in the membrane and secreted in the wall depending on their specific role.Plant cell wall modification is a critical component in the stress response and several cell wall modifying enzymes have been recently reported as important factors of resistance or susceptibility. In Arabidopsis the GH9 family consists of 25 members. A sequenced genome and ease of genetic manipulation is yielding information about the biological roles of many members of this family. Data from the Affymetrix microarray analyses revealed that some of them are differentially regulated upon several biotic and abiotic stresses. Here we show a linkage between the lack of EGase activity and the biotic stress response in Arabidopsis. Knockout plants lacking individual EGases showed altered resistance to *Botrytis cinerea* and *Pseudomonas syringae*. Both the redundancy of the EGase gene family and the complexity of the cell wall matrix degradation prevent severe phenotypes of single mutants. Nevertheless, the observed infection phenotypes were dependent on the mutated gene and were associated to changes of SA-, JA- and ABAsignaling pathways and callose deposition. In conclusion, our data support that EGase activity is part of the complex web of cell wall signaling and metabolism operating in response to plant–pathogen interactions.

P17-057: THE DEFENSOME MODEL FOR RICE INNATE IMMUNITY

*Ko Shimamoto**
Ikoma (Nara - Japan)
**Corresponding author e-mail: simamoto@bs.naist.jp*

We have been studying molecular signaling in rice innate immunity by studying the small GTPase OsRac1 and its interacting proteins by using a variety of exerimental methods. We have identified a number of OsRac1-interacting proteins and studied their functions and interactions with other proteins. We found that OsRac1 interacts withtwo types of receptors; membrane-boundreceptor-like kinases and NB-LRR type receptors. OsRac1forms a protein network with several cheperones and co-chaperones, SGT1, RAR1, Hsp90, Hsp70, and Hop/Sti1. A scaffloding protein, RACK1, also interacts with OsRac1. The OsRac1 network includes enzymes such as NADPH oxidase and CCR which are important for immune responses. Based on genetic, protein-protein interaction, and biochemical studies we propose thatthese proteins form acomplex termed ‘defensome’ which plays an important role in rice innate immunity. The defensome model proposes that proteins used in PTI and ETI are largely shared and OsRac1 acts as a molecular switch for both types of immune responses in rice. New data to support our model will be presented.

P17-058: BIPHASIC PRODUCTION OF ETHYLENE AND ROS IS AN IMPORTANT COMPONENT FOR DETERMINING STRESS TOLERANCE IN RESPONSE TO BIOTIC STRESS

Wi, S. - Park, K.Y.*
Sunchon National University
**Corresponding author e-mail: akrp@sunchon.ac.kr*

The relationship between ROS accumulation and ethylene production in response to biotic stress with the fungal pathogen, Phytophthora parasitica var. nicotianae, was investigated using stress-tolerant transgenic plants, in which ethylene biosynthesis or signaling and ROS production were impaired. It was observed that wild-type tobacco plants exhibited a gene-specific expression of NtACS members in response to Phytophthora infection. It seemed that pathogen treatment led to the peaked-expression of NtACS4 at 1 h for stress signaling and of NtACS1 at 72 h for necrosis during phase II. The profile of pathogen-induced ROS accumulation, determined by DAB and qRT-PCR for NADPH oxidase, RbohD and RbohF, was also showed a biphasic pattern which took place early. ROS accumulation was peaked twice at 1 h and 48 h after pathogen treatment. ROS accumulation was rapidly increased at post-inoculation from 36 h to 48 h, when transcript of RbohD was also increased. Pathogen-induced RbohD expression and ROS accumulation at phase I were significantly suppressed in stress-tolerant transgenic plants, in which ethylene biosynthesis and signaling were impaired. Biphasic ethylene production was also inhibited, especially at 1 h, in stress-tolerant transgenic plants with impairment of RbohD and F expression. Therefore, these results implied that ROS could act as a signal

upstream of ethylene biosynthesis in biphasic response to biotic stress.

P17-059: CYTOKININS PROMOTE PLANT IMMUNITY AGAINST A BACTERIAL PATHOGEN VIA ARR2-ELICITED TGA3 AND NPR1-DEPENDENT SALICYLIC ACID

*Hwang, I.**
POSTECH
**Corresponding author e-mail: ihwang@postech.ac.kr*

Cytokinins are known to affect plant immunity to various pathogens; however, the role of plant-derived cytokinins in pathogen responses has been elusive. Here, we found that cytokinins promote resistance of Arabidopsis to Pseudomonas syringae pv. tomato DC3000 (Pst DC3000). Modulated cytokinin levels or signaling activity in CKX- or IPT-overexpressing plants or in ahk2 ahk3 mutants correlated with altered resistance to Pst DC3000. Overexpression or knockout of the cytokinin-activated transcription factor ARR2, but not ARR1, affected resistance to Pst DC3000. TGA3 specifically interacted with ARR2, and mutation of TGA-binding cis-elements in the PR1 promoter abolished cytokinin- and ARR2-dependent PR1 activation. Cytokinin treatment did not increase pathogen resistance in npr1-1 and tga3 plants, as the cytokinin-dependent induction of PR1 was eliminated. Moreover, ARR2 binding to the PR1 promoter was enhanced by salicylic acid (SA). Taken together, these results show that cytokinins modulate the SA sinaling cascade to augment resistance against Pst, a process in which the interaction between TGA3 and ARR2 is important.

P17-060: FUNCTIONAL CHARACTERIZATION OF LYSM RECEPTOR KINASE GENES IN LOTUS JAPONICUS.

Nielsen, M.W. Lohmann, G.V.[1] - Shimoda, Y.[2] - Füchtbauer, W.S.[1] - Rasmussen, S.R.[1] - Sato, S.[2] - Tabata, S.[2] - Madsen, L.H.[1] - Stougaard, J.[1] - Radutoiu, S.[1]*
[1]CARB Center for Carbohydrate Recognition and Signalling, University of Aarhus
[2]Kazusa DNA Research Institute
**Corresponding author e-mail: mwn@mb.au.dk*

The nitrogen fixation symbiosis established between legumes and rhizobia is a highly controlled process that involves the exchange of signal molecules between the two symbiotic partners. In the model legume Lotus japonicus two LysM receptor kinases NFR1 and NFR5 mediate perception of rhizobial lipochitooligosaccharides (Nod-factors) signals and initiate a downstream signaling cascade leading to nodule development [1, 2]. In non-legume plants (Arabidopsis and rice) LysM receptor kinases are involved in chitin recognition and signaling in response to fungal and bacterial pathogens [3, 4, 5]. Here we present the identification and characterization of the LysM receptor kinase (Lys) gene family in L. japonicus. In silico searches in the L. japonicus genome sequence identified fifteen new LysM receptor kinase genes. A detailed expression analysis revealed that several Lotus Lys genes are regulated during the symbiotic interaction with Mesorhizobium loti and in response to chitin treatment. In order to get more insight into their function TILLING lines have been obtained for the Lys genes and they are currently being analyzed for their ability to form symbiosis with rhizobia and mycorrhizal fungi. Expression analysis showed that Lys3 transcription is induced several fold in response to inoculation with M. loti and we now focus on the phenotypic characterisation of Lys3 TILLING mutants. 1. Radutoiu et al. (2003) Nature 425:585-5922. Madsen *et al.* (2003) Nature 425:637-6403. Miya *et al.* (2007) PNAS 104: 19613-196184. Wan *et al.* (2008) Plant Cell 20:471-4815. Gimenez-Ibanez *et al.* (2009) Curr. Biol. 19: 423-429

P17-061: HORIZONTAL GENE TRANSFER FROM AGROBACTERIUM TO PLANT SPECIES DURING THEIR EVOLUTION.

Matveeva, T. – Bogomaz, D. – Pavlova, O. – Lutova, L.
St. Petersburg State University

Agrobacterium mediated transformation is the traditional way of genetically engineering plants. This method is based on natural vector system, Agrobacterium tumefaciens, or A. rhizogenes. These bacteria transfer fragments (T-DNA) from a large tumor-inducing (Ti) plasmid or root-inducing(Ri) plasmid respectively to their plant hosts. The evaluation of plant transformation has raised in our modern society question of the safety of transgenic foods. At the same time, a number of “untransformed” plants, such as some *Nicotiana* species, contain DNA sequences, homologous to the T-DNA from A. rhizogenes. Thus A.rhizogenes must have transferred T-DNA to *Nicotiana* species, and these genes have played a role in the evolution of the genus No one has reviewed a large number of plants to determine how wide spread is the transfer of T-DNA from *Agrobacterium* to plants. We developed methodology for effective and productive search for T-DNA-like sequences in plant genomes and screened about 200 plant species, belonging to 38 families of Dicotyledones for the presence of oncogenes, homologous to ones from *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. New examples of horizontal gene transfer from *Agrobacterium* to plants were found. These results demonstrate, that horizontal gene transfer of oncogenes from Agrobacrerium to plants is not a unique feature of Nicotiana. However, it is a rare event in the evolution of plants. Theresearch described in this publication was made possible in part by Award #ST-012-0 of the U.S. Civilian Research & Development Foundation for the Independent States of the Former Soviet Union (CRDF), Award # 08-04-01005-a of Russian Foundation for Basic Research and Grants of President of RF, MK-5352.2006.4 and SC-6455.2010.4, FCP 2010-1.1-141-042-019

P17-062: ELEVATED CYTOKININ LEVELS IN TOBACCO LEAVES CAUSE RESISTANCE AGAINST PSEUDOMONAS SYRINGAE PV. TABACI

Grosskinsky, D.[1] - Muhammad, N.[2] - Ramadan, U.[2] - Plickert, N.[2] - Engelke, T.[2] - Simon, U.[1] - Roitsch, T.[1]*
[1]Institute of Plant Sciences, University of Graz
[2]Institute of Pharmaceutical Biology, University of Wuerzburg
**Corresponding author e-mail: dominik.grosskinsky@uni-graz.at*

To address the role of the phyotohormone cytokinin in plant defense response the cytokinin biosynthetic gene ipt was fused with a pathogen inducible promoter. Transient expression of this construct in tobacco leaves resulted in resistance against a virulent strain of Pseudomonas syringae. The effect of stimulated endogenous cytokinin biosynthesis could be mimicked by a short pulse of exogenously supplied cytokinins. This resistance mechanism was shown to neither involve the classical salicylic acid, jasmonate or ROS dependant defense signaling pathways nor high sugar resistance. The increased level of cytokinins was shown to interfere with the establishment and spread of the pathogen via upregulation of the level of the antimicrobial phytoalexins.

P17-063: LIPID HYDROPEROXIDE REDUCTION IS CRUCIAL FOR THE, ESTABLISHMENT OF THE SINORHIZOBIUM MELILOTI – MEDICAGO SYMBIOSIS.

Gucciardo, S. - Coste, C.[1] - Fournol, A.[1] - Pagnotta, S.[2] - Benyamina, S.[1] - Puppo, A.[2] - Mandon, K.[2]*
[1]INRA Sophia-Antipolis
[2]Université de Nice
**Corresponding author e-mail: sebastien.gucciardo@sophia.inra.fr*

Sinorhizobium meliloti is a soil bacterium able to induce nodule formation on Medicago roots. Nodules contain differentiated endocytosed bacteria, named bacteroids, able to fix atmospheric dinitrogen. This symbiosis allows the plant to grow on poor nitrogen soil while bacteria are provided with carbon source. Previous

studies demonstrated that tight regulation of reactive oxygen species such as hydrogen peroxide (H2O2) and superoxide anion (O2-) is required for the establishment of a functional symbiosis. We characterized two S. meliloti peroxiredoxins belonging to a large family of proteins that used reactive cysteine thiols to reduce peroxides. ohr1 encodes an organic hydroperoxide resistant protein, and was expressed during symbiosis. In free-living cells and after induction under oxidative conditions, Ohr1 was able to reduce lipid hydroperoxides (LPs). This was the main enzyme involved in the protection of cells challenged with LPs. PrxC, which encodes a 1-Cys peroxiredoxin, showed a nodule specific expression; sequence analysis suggested that PrxC is able to reduce both LPs and H2O2.
S. meliloti ohr1 or prxC mutants did not exhibit significant nodulation phenotypes. However the ohr1/prxC double mutant was unable to form nitrogen-fixing nodules. These nodules contained normal endocytosed bacteria that seem to be rapidly degraded.
This study shows a major role of lipid hydroperoxides in the establishment of a functional symbiosis.

P17-064: IMPLICATION OF CALLOSE DEPOSITION IN THE OCP3-MEDIATED DISEASE RESISTANCE TO NECROTROPHIC PATHOGENS

García-Andrade, J.[*1] - Ramírez, V.[1] - Flors, V.[2] - Vera, P.[1]*
[1]IBMCP-UPV
[2]CAMN-UJI
**Corresponding author e-mail: jagarser@ibmcp.upv.es*

The phytohormones abscisic acid (ABA) and methyl jasmonate (MeJA) control the signaling pathways responsive of the plant adaptive responses to drought and pathogenic fungi infections, respectively. OCP3 is a transcriptional regulator of the Homeobox family and that has been previously demonstrated to be required for mediating specific aspects of plant responses as mediated by both ABA and MeJA. The ocp3 loss-of-function mutant shows a JA-dependent enhanced resistance towards Botrytis cinerea and Plectosphaerella cucumerina that is accompanied by an enhanced drought tolerance and increased sensitivity to ABA. This phytohormone has been proposed to be involved in the callose deposition surrounding the infection sites to restrain pathogen entry. In this work we show that the enhanced resistance of ocp3 plants towards B. cinerea and P. cucumerina goes along with an early and drastic increase of callose accumulation. These results suggest that OCP3 is implicated in the regulation of the rapid callose deposition involved in plant-pathogen interaction. To further study this observation we have generated a battery of mutants which allowed us to dissect the genetic requirements of the OCP3-mediated resistance towards necrotrophic fungi and the role played by ABA and JA in mediating the observed deposition of callose.

P17-065: A ROLE FOR ARABIDOPSIS DNA-BINDING PROTEIN PHOSPHATASE ATDBP1 IN POTYVIRUS INFECTION.

Castello, M.J. – Carrasco, JL. – Vera, P.
IBMCP-UPV

DBP factors (DNA-binding protein phosphatases) are a novel class of plant-specific proteins featuring sequence-specific DNA-binding and protein phosphatase activity with dual nucleo-cytoplasmic subcellular localization. To investigate the function of DBP factors, we performed a comparative analysis of the proteome of wild-type Arabidopsis thaliana plants against homozygous plants bearing a T-DNA insertion in the AtDBP1 gene. Looking at the protein level should enable us to identify both transcriptional and post-transcriptional putative targets of AtDBP1. In this analysis, the translation initiation factor eIF(iso)4E was found to accumulate at a lower level in the atdbp1 mutant when compared to Col-0 plants. The reduction in eIF(iso)4E abundance was verified by Western-blot and obeys to a post-transcriptional regulatory mechanism. eIF(iso)4E is a plant-specific isoform of eIF4E, has been implicated as a key factor in recessive resistance against potyviruses, so we analyzed the response of the atdbp1 mutant to potyvirus infection. Col-0 and atdbp1 plants were inoculated with a GFP-tagged version of Plum pox virus (PPV), and the progress of the infection was monitored at different time points. Viral accumulation was analyzed by RT-PCR, Western-blot and fluorescence microscopy in the inoculated and systemic tissue, and shown to be reduced and delayed in the atdbp1 mutant as compared to Col-0 wt plants.
Thus, here we identify AtDBP1 from Arabidopsis thaliana as a new player engaged in plant-potyvirus interactions and demonstrate that loss-of-function of AtDBP1 renders resistance to potyviruses, unveiling a new layer of biological and molecular complexity in the poorly understood recessive resistance to potyviruses.

P17-066: NOVEL AND SYMBIOSIS-RELATED MICRORNAS IN THE MODEL LEGUME LOTUS JAPONICUS

de Luis, A.[1] - Markmann, K.[2] - Cognat, V.[1] - Stougaard, J.[2] - Voinnet, O.[1]
[1]Institut de Biologie Moléculaire des Plantes (Strasbourg, France)
[2]Centre for Carbohydrate Recognition and Signalling (Aarhus, Denmark)

Root Nodule Symbiosis (RNS) is a complex biological process whereby legumes are able to fix atmospheric N2 through the association with bacteriae from the genus Rhizobium. Recent genome sequencing of several model legume species, including Lotus japonicus, has led to great advances in the understanding of the genetics underlying RNS. MicroRNAs (miRNAs) are 19-25 nt post-transcriptional gene regulators acting in a sequence-specific manner and whose key role in many aspects of plant biology has been widely demonstrated.
As a means to unravel the potential contribution of microRNAs to Root Nodule Symbiosis, we isolated and sequenced the small RNA fraction from L. japonicus roots and symbiotic nodules. First, we used the resulting data for the discovery of novel and conserved microRNA families in this plant species. Next, we obtained and compared miRNA profiles from root and nodules and observed that a discrete set of miRNAs is specifically upregulated in nodules, which was further confirmed by molecular analyses. Through the use of L. japonicus symbiosis mutants, we have shown that the expression of several of these miRNAs is dependent, not only on the development of nodules, but also on bacterial infection and the establishment of a fully competent nitrogen-fixing symbiosis. Our work reveals new players in the genetic interactions that take place in RNS, while setting the basis for an in-depth functional characterization.

P17-067: ISOLATION AND CHARACTERIZATION OF GENES ENCODING PECTIN METHYLESTERASE INHIBITOR PROTEIN IN WHEAT

Rocchi, V.[*1] – Janni, M.[1] - Kalunke, R.[1] - Castiglioni, C.[3] - Favaron, F.[3] - Bellincampi, D.4 – Giardina, T.[2] - D'Ovidio, R.[1]*
[1]Agrobiology and Agrochemistry Department, University of Tuscia,Viterbo-Italy
[2]Univ. P. Cézanne ISM2/BiosCiences, Marseilleç
[3]Univ.Padova Dept Te.S.A.F.
[4]Univ. Roma La Sapienza Dept. Chemistry
**Corresponding author e-mail: v.rocchi@unitus.it*

Pectin is an important components of the plant cell wall and its remodelling occurs during normal plant growth or following stress responses. Pectin degradation represent also an important step during the infection process of most pathogens. Cereal cell wall contains a lower amount of pectin compared to that present in dicot species, nonetheless pectinase activity has a primary role during pathogens infection of cereal tissues. Pectin is secreted into the cell wall in a highly methylesteried form and deme-

thylesterified in muro by pectin methylesterase (PME).
The activity of PME is controlled by the pectin methylesterase inhibitor protein (PMEI). Since an increased level or a blockwise distribution of cell wall pectin methyesterification can reduce disease symptom both in monocot and dicot species, we have isolated three wheat (Triticum durum L.) pmei genes, Tdpmei1, Tdpmei2 and Tdpmei3, and demonstrated that they encode functional PMEI. These genes represent the first PMEIs characterized in wheat and we showed that they are transcribed in roots, stems, leaves and spikelets with a different pattern of transcript accumulation. Tdpmei1 and Tdpmei2 are regulated during leaf expansion and accumulate mainly in the mature leave, whereas Tdpmei3 is more expressed in expanding leaves compared to the mature ones and accumulate strongly in the stem.

P17-068: THE ROLE OF REACTIVE OXYGEN SPECIES (ROS) IN THE NECROTROPH-BIOTROPH RESISTANCE TRADE-OFF CONFERRED BY DELLA IN WHEAT AND BARLEY.

Saville, R. – Nicholson, P. – Boulton, M.
John Innes Centre
**Corresponding author e-mail: robert.saville@bbsrc.ac.uk*

DELLA proteins are members of a family of putative transcription factors and are involved in gibberellic acid (GA) signal transduction[1]. *Arabidopsis* encodes five distinct DELLA proteins. Gain-of-function (GoF) mutant alleles such as the well characterised dwarf mutant *gibberellin insensitive (gai)* have reduced GA responsiveness. The 'Green Revolution' wheat semi-dwarfing alleles, *Rht-B1b* and *Rht-D1b* are orthologues of *Arabidopsis gai*[2] and are now used extensively in modern day cultivars to reduce plant height. Wheat is hexaploid and contains an *Rht* locus on each of its Group 4 chromosomes (4A, 4B and 4D). Barley is diploid containing one copy of the Rht orthologue *(Sln1).* There are both GoF and loss of function (LoF) *Sln1* mutant lines available for barley whereas only GoF *Rht* lines are available in wheat. Studies in *Arabidopsis* demonstrated that DELLAs promote susceptibility to virulent biotrophs and resistance to necrotrophs partly through their influence of the balance of salicylic acid and jasmonic acid signalling[3] and also through the modulation of ROS levels[4]. We have been investigating the necrotroph – biotroph resistance trade-off of the wheat *Rht* and barley *Sln1* mutants to cereal pathogens. We have shown that GoF (DELLA accumulating) mutants generally confer increased resistance to necrotrophs and increased susceptibility to biotrophs compared to the wild type and vice versa in LoF mutants, indicating a role for DELLA in controlling cell death processes. We discuss DELLAs' role in increasing tolerance to ROS-induced cell death.

[1]Peng et al. (1997), Genes & Dev., 11:3194-3205.
[2]Peng et al.(1999), Nature, 400:256-261
[3]Navarro et al. (2008), Current Biology, 18:650-655
[4]Achard et al. (2008), Current Biology, 18:656-660.

P17-069: METABOLOMIC ANALYSIS OF RAPESEED (BRASSICA NAPUS L.) SUSCEPTIBLE AND PARTIALLY RESISTANT TO THE TELLURIC PATHOGEN PLASMODIOPHORA BRASSICAE

Wagner, G.[1] - Charton, S.[1] - Marnet, N.[1] - Lugan, R.[2] - Lariagon, C.[1] - Bouchereau, A.[1] -*
Delourme, R.[1] - Manzanares-Dauleux, M.J.[1] - Gravot, A.[1]
[1]INRA, UMR 118 APBV INRA/Agrocampus Ouest/Université, Le Rheu, France
[2]C.N.R.S., Institut de Biologie Moléculaire des Plantes, Plateforme métabolomique, Strasbourg, France)
**Corresponding author e-mail: geoffrey.wagner@rennes.inra.fr*

Clubroot, caused by the obligate telluric biotroph *Plasmodiophora brassicae*, is one of the most devastating pathogens in *Brassicaceae*. The disease is characterised by the development of clubs on the root system due to cellular hypertrophy and hyperplasy. Our group focuses on partial quantitative resistance, under polygenic control, described as being more durable than qualitative resistance. Previous work in our team led to the identification of partial quantitative resistance to clubroot in the *Brassica napus* genotype Darmor-*bzh*, which was used as a model to perform a genetic analysis of the architecture of quantitative resistance. Cellular mechanisms underlying this resistance remain however poorly understood, particularly at the metabolic level. In this respect, we have undergone a metabolomic approach i) to characterize the metabolic differences between partial resistant and susceptible genotypes and ii) to identify differentially accumulated metabolites that could be used as reliable metabolic markers of resistance. A combination of targeted and untargeted metabolite profiles was performed in both root and shoot tissues of Darmor-*bzh* and Yudal (clubroot-susceptible genotype), at several time points after inoculation. We focused our analysis on primary and secondary metabolites: glucosinolates, flavonoids, carbohydrates and amino acids. The results are discussed in the light of current knowledge about source-sink relationships between shoots and roots during clubroot development.

P17-070: EVOLUTION AND VARIABILITY OF RESISTANCE AND VIRULENCE FACTORS INVOLVED IN THE INTERACTION BETWEEN GLOBODERA PALLIDA AND SOLANUM TUBEROSUM HARBORING THE RESISTANCE GENE GPA2

Carpentier, J. - Fouville, D. - Manzanares-Dauleux. M.J. – Kerlan, M.C. – Grenier, E.*
INRA, UMR 1099 Bio3P INRA/Agrocampus Ouest/Université, Le Rheu, France
**Corresponding author e-mail: jean.carpentier@rennes.inra.fr*

The cyst nematode *Globodera pallida* is one of the most damaging diseases of *Solanum tuberosum* causing serious losses in crops. This nematode is very difficult to control by using biological or chemical methods, these later being in addition toxic for environment and humans. Therefore, using resistant cultivars represents the best way to control endoparasitic nematodes like *G. pallida*. In *S. tuberosumh*, igh level of nematode resistance can be conferred by major genes isolate-specific.
The major gene *Gpa2* (NBS-LRR) confers resistance to the pathotype Pa2 of *G. pallida.* In this plant-nematode interaction, the resistance protein *Gpa2* needs the RanGTPase activating protein *RanGap2* to recognize the nematode avirulence gene *Rbp1*. In order to better understand the role of each factor in this interaction, we have characterized molecular variability of *RanGap2* and *Rbp1*, analyzed selection pressure which acted on *RanGap2* and *Rbp1* and detected nucleotidic sites of *RanGap2* and Rbp1under positive selection. The variability of these two genes was described by sequencing *RanGap2* from 56 plant genotypes belonging to 18 Solanum species and by sequencing Rbp1 from 200 nematode genotypes coming from 20 *G. pallida* populations. Then we have searched for hallmarks of selection pressure on *RanGap2* and *Rbp1* sequences using the ratio of nonsynonymous to synonymous substitution rates per site estimated with PAML package. The detected sites could have a major role in the recognition by *Gpa2*.
The variability study of both plant and nematode genes in its evolutive context would allow to better select the genetic factors to use for constructing varieties showing efficient and durable resistance.

P17-071: TOLERANCE TO TREHALOSE UNDERLIES ONE QTL FOR CLUBROOT PARTIAL RESISTANCE IN THE ARABIDOPSIS THALIANA ECOTYPE BUR-0

Gravot, A. - Grillet, L. – Wagner, G. – Jubault, M. – Bouguenec, V. – Lariagon, C. – Deleu, C. – Delourme, R. – Bouchereau, A. - Manzanares-Dauleux, M. J.*
INRA/Agrocampus Ouest/Université Rennes 1
**Corresponding author e-mail: antoine.gravot@univ-rennes1.fr*

Clubroot disease is caused by Plasmodiophora brassicae and leads to the development of galls in infected roots of Brassicaceae. This physiopathological process relies on the phytohormonal modulation of plant metabolic resource allocation. High accumulation of trehalose has been observed in clubroot infected plants. However, despite the central importance of the trehalose pathway in the regulation of plant primary metabolism, little is known about the implication of trehalose in the mechanisms of resistance / susceptibility to P. brassicae. The present work showed that the clubroot partially resistant Arabidopsis accession Bur-0 was tolerant to exogenous trehalose. A QTL analysis on a Bur-0 x Col-0 (susceptible to exogenous trehalose) segregating population led to the identification of one QTL involved in trehalose tolerance that co-localized with a previously identified QTL for quantitative resistance to P. brassicae. This result was confirmed by the analysis of near-isogenic lines (HIF). Accumulation of trehalose in tissues of trehalose treated Bur-0 was not drastically lower than in Col-0, suggesting that this tolerance could be related to contrasting downstream response to trehalose rather than to trehalose degradation. Trehalose was accumulated in both parental accessions during clubroot infection, but trehalase enzymatic activity was induced only in the susceptible Col-0 accession. We conclude that tolerance to trehalose in Bur-0 is likely involved in clubroot resistance, and that this tolerance relies on a trehalase-independent mechanism, supporting an original model where a partial resistance would rely on contrasting primary metabolism regulation

P17-072: PLANT HORMONES IN FUNCTIONING OF GLYCINE MAX – BRADYRHIZOBIUM JAPONICUM SYMBIOSIS

Volkogon, M. – Mamenko, P. – Kots, S.*
**Corresponding author e-mail: volkogon@ifrg.kiev.ua*

Biological nitrogen fixation via symbiotic systems of higher plants and nodule bacteria is the global process gaining its actuality nowadays at introduction of high yield farming, resources saving and environment protection means. Using of model systems with modified symbiotic characteristics is of a great interest for symbiosis physiology research uncovering role of biomolecules and the regulation pathways of symbiotic interactions.
We have studied the role of indoleacetic acid (IAA), zeatin (Z) and zeatin-riboside (ZR) in functioning of *Glycine max-Bradyrhizobium* japonicumsymbiotic systems under the seeds inoculation with strains and Tn5-mutants varying by their symbiotic properties. The study of auxin contents in roots and nodules of infected plants have indicated key role of host plants in IAA biosynthesis. It was shown that changes in IAA level are independent of inoculum strain activity – both high and non-active bacteria and their Tn5-mutants have promoted IAA level increase in the nodules. This confirms unspecific role of auxins in symbiosis formation laying in triggering of endoreduplication and mitoses in infected root cells.
As is known auxins act in complex with other plant hormones, cytokinins in particular, that control root nodules initiation and growth. Thus, Z and ZR levels in nodules had indicated direct relationships between their activity and nitrogen fixation ability. It was shown that inoculation with active strains and Tn5-mutants of B. japonicum had resulted in higher cytokinins biosynthesis. The results obtained expand our knowledge on hormonal control of symbiotic relationships and might be used as the theoretical and practical base for elaboration and improving of sustainable agriculture techniques.

P17-073: EXPRESSION OF SMALL GTP-BINDING PROTEINS IN ROOT NODULES OF MEDICAGO TRUNCATULA

Memon, A.R. - Wiebe, A. - Kliendt, C.K. - Yuzbasioglu, E. - Weisshaar, B. - Niehaus, K.
International University of Sarajevo, Genetics and Bioengineering programme, Bosnia & Herzegovina, Istanbul University, Turkey and CeBiTech, Univ.

Severasl tudies have shown that membrane associated small GTPases belonging to the Arf/Sar, Rab, and Rop/Rac families, along with their interacting proteins, playing a vital role in the diverse aspects of root nodule formation and development. The protein and gene expression studies could suggest a nodule-specific expression of small GTP binding proteins especially the expression of several Rabs and Rops.We previously made an attempt to retrieve the EST databases of of two model legume plants, Medicago and Lotus and searched the nodule specific expressed transcripts of small GTPases. As a result of this bioinformatic analyses, two ARL like genes, a group of 10 Rab GTPases and one or two ROP like GTPases were found to be mainly expressed in nodules. The sequences determined as a result of this study, we decided to commence detail expression analysis of these GTP-binding proteins at RNA and protein levels in rhizobium inoculated and non inoculated roots and nodules of Medicago truncatula. Our results show three to five times more Sar1 and Arf1 protein content in rhizobium infected roots compared to non inoculated one. qReal-time PCR experiments clearly showed about 5 to 10 times more expression of Sar1, Arf1, Arl1, Rab1, Rab4, Rab7, Rab11 and Rop2 and Rop4 in roots and nodules of inoculated plants compared to the roots of non inoculated ones.

P17-074: SEED MICROFLORA OF PLANT SPECIES AFTER CRYOCONSERVATION IN

Arapetyan, E. - Bilynska, I. – Usatenko, Y.*
Ivan Franko National University of Lviv
**Corresponding author e-mail: emarapetyan@gmail.com*

Seeds of wild plants have many different microorganisms on its coat. Influence of ultralow temperature on viability these microorganisms are showed. Seeds of 14 wild plant species from different family (Caryophyllaceae, Asteraceae, Brassicaceae) kept in the liquid nitrogen (-1960 C, experiment) and in the room conditions (control) during 6 months. After thawing of seeds its sowed on nutrient medium for microorganism.
Are finded out microorganisms presence on seed coat surface of all species as in control as in experiment variants. Quality microorganism composition were very different. Fungi dominated on surface of seeds after cryoconservation and bacterias - in control variant. The least contaminate are showed for seedsfrom Caryophyllaceae family.

P17-075: PARTIAL RESISTANCE OF POTATO TUBERS TO PECTOBACTERIUM ATROSEPTICUM: THE EFFECT OF PHYSIOLOGICAL AGE ON DEFENCE

Kröner, A. – Pusnel, R. – Hamelin, G. – Val, F.
UMR 1099 BiO3P (Biologie des Organismes et des Populations appliquée à la Protection des Plantes), INRA - Agrocampus Ouest - Université de Rennes I)

Pectobacterium atrosepticum is the causal agent of potential damage during storage of potato tubers. The extent of damage varies among cultivars showing different levels of resistance. Underlying mechanisms are still not completely understood, but could contribute to a better control of disease.
Our hypothesis is that the level of resistance could be explained by active induction of defence by potato tubers in response to Pectobacterium atrosepticum. It is supported by findings of (Kumar& Knowles, 2003) who studied the wound response of tubers during storage. They found wound healing ability to decrease in ageing tubers and propose that it could be caused by reduced inducible activity of phenylalanine ammonia-lyase (PAL) and reduced synthesis of phenolic compounds. These metabolic processes are also implicated in defence against pathogens.
The aim of the study is to show if levels of tuber resistance to Pectobacterium atrosepticum dependon tuber age and if it is

correlated to potential induction of defence related physiological changes occurring during tuber storage. Therefore, disease symptoms and defence related metabolic changes are analysed in seed tubersdiffering by physiological age after inoculation with Pectobacterium atrosepticum. Disease symptoms are evaluated by measuring the weight of soft rot. Activity of PAL is determined spectrophotometrically and total phenolic compounds are quantified by using a method described by Folin and Ciocalteu.
Results and conclusions will be presented during the conference.
Kumar GXM, Knowles NR. 2003. Wound-induced superoxide production and pal activity decline with potato tuber age and wound healing ability. Physiologia Plantarum 117(1)1: 08-117.

P17-076: ESSENTIAL OIL CONSTITUENTS OF PEROVSKIA ATRIPLICIFOLIA BENTH. - ANTIMICROBIAL EFFECTS

Zamfirache, M.[1] – Burzo, I.[2] - Gostin, I.N.[1] - Stefan, M.[1] - Badea M L.[2] - Olteanu, Z.[1] - Truta, E.[3]
[1]The Alexandru Ioan Cuza University of Iasi
[2]USAMV Bucharest
[3]ICB Iasi

The structure and micromorphology of the glandular trichomes, chemical composition and antimicrobial effects of the secreted essential oil of *Perovskia atriplicifolia* were studied. The volatile oils extraction was conducted using a Clevenger hydrodistillation system. The component separation was performed by gas chromatography using a *6890* Agilant GC-MS. The volatile compound identification was made using the NIST spectral bank and Kovats indexes. Antimicrobial effects were tested using a standard difusimetric method. SEM analysis revealed that leaves have numerous glandular trichomes of three morphological distinct types, all a unicellular foot and stalk: capitate hairs with bi-cellular head, peltate hairs with tetra-cellular head and peltate hairs multicellular (10-celullar) head. Qualitative and quantitative GC-MS analyses of the essential oils revealed limonene (18 %), γ – terpinene (16 %), β - caryophyllene (13 %), α - caryophyllene (12 %) and cymene (11%) as the main constituents. The investigated volatile oils have antimicrobial effects on *Staphylococcus aureus* (depending on concentration) and a mild influence on the growth and development of *Escherichia coli.*

P17-077: OXIDATIVE STRESS AND ANTIOXIDANT RESPONSE DURING THE INFECTION OF NICOTIANA BENTHAMIANA WITH PMMOV

Hakmaoui, J.[1] - García-Fontana, B.[1] - Jiménez, A.[2] - Camejo, D.[2] - Sevilla, F.[2] - Barón, M.[1]
[1]Estación Experimental del Zaidín. CSIC
[2]Centro Edafología y Biología Aplicada del Segura. CSIC

In some compatible virus–host plant interactions, an oxidative stress is produced manifested as an increase in different oxidative stress parameters (lipid peroxidation, protein oxidation and electrolyte leakage), accumulation of H2O2, as well as an imbalance in the antioxidative systems. Some authors have proposed that disturbances on the photosynthetic electron transport during viral pathogenesis induce an increased ROS generation. We have previously showed that the oxygen-evolving complex of photosystem II was the chloroplastidic target of the Pepper mild motle tobamovirus (PMMoV). In addition, PMMoV infection induced the down regulation of several chloroplastidic proteins involved in both the photosynthetic electron transport and the Benson–Calvin cycle (Pineda et al. 2010. Photosynth. Res. 103:31). The present study was carried out to link the viral-induced effect on the chloroplast to ROS generation during viral pathogenesis. N. benthamiana plants were infected with the Italian and Spanish strains of the PMMoV. Analysis of the oxidative stress biomarkers (lipid peroxidation, protein carbonylation, H2O2 production), evaluation of ROS-scavenging capacity by different enzymes (CAT, APX, SOD, MDHAR, GR) as well as determination of antioxidant compounds (glutation, ascorbate) were conducted at different post-infection times. The differences in virulence between the viral strains are reflecting in most of the analyzed parameters.
Work supported by grant MCINN AGL2008-00214.

P18 Water And Minerals

P18-001: WATER STRATEGIES OF FAGUS SYLVATICA L.

Potentini Couput, M.F. - Irigoyen Iparrea, J.
Universidad de Navarra, Facultad de Ciencias, Dpto. Biología Vegetal, Secc. Biología Vegetal, Unidad Asociada al CSIC, EEAD, Zaragoza e ICVV, Logroño

According to the literature Fagus sylvatica L. is described as sensitive drought species. However, drought response of beech under a warmer and drier climate, expected as a result of climate change, is currently being controversially debated. Beeches behave as a very sensitive plant to high vapour pressure deficit, and usually avoid large water deficits. Nevertheless, this species is described by some authors like more water-stress tolerant than is often assumed, therefore there is no consensus in the water strategies of beech. The aim of this work focus on investigates in situ, the strategies of beeches from two Navarra (Spain) stands, in the seasonal soil water shortage occurring during summer and autumn, which may constrain the tree physiology and growth. Pressure-volume curves were fitted in sun and shade leaves of two beech stands (Bértiz and Odéritz) of small and large trees in the growing seasons of 2009. Preliminary results showed an elevation in the rate of stomatal conductance in the large trees from Odéritz, while in those of Bértiz, a stomatal closure and a mayor rise in bulk elasticity modulus was observed (avoidance strategy). In both cases the leaf relative water content was optimum; hence, water state of leaves may not reflect the same plant or forest soil water status. These results were discussed in relation with other measured parameters as soil and stem water content, gaseous exchange (steady-state porometer) and leaf free proline.

P18-002: APPLICATION OF SANITIZED SEWAGE SLUDGES TO PEPPER PLANTS: EFFECTS ON YIELD, FRUIT QUALITY AND SEVERITY OF VERTICILLIUM WILT

Azcona, I.[1] - Pascual, I.[1] - Aguirreolea, J.[1] - Morales, F.[1] - Rellán-Álvarez, R.[2] - Sánchez - Díaz, M[1]
[1]Facultad de Ciencias. Universidad de Navarra - Dpto. Biología Vegetal. Sección Biología Vegetal (Unidad Asociada al CSIC, EEAD Zaragoza e ICVV Logroño)
[2]Dpto. Nutrición Vegetal. Estación Experimental de Aula Dei (EEAD). CSIC. Zaragoza, Spain

The objective of this study was to evaluate the influence of the application of two sanitized sewage sludges (Class A Biosolids, according to the USEPA) on yield and fruit quality of pepper plants, as well as its effect on the vascular wilt induced by Verticillium dahliae. Two experiments were conducted under controlled conditions. The treatments assayed were: ATAD sludge (aerobic thermophilic autothermal digestion) at doses of 15 and 30% v/v, composted sludge at doses of 15, 30 and 45% v/v, and control (without amendment). Both ATAD and composted sludge stimulated fruit yield. The concentration of heavy metals in fruit remained far below the limits set by the European Union for vegetables. The sludge application did not increase the level of capsaicinoids (degree of pungency), and the concentration of vitamin C (considered as a biomarker for nutritional quality of fruits and vegetables) remained at high levels. Concerning the effect of sludge on Verticillium-wilt, ATAD sludge did not affect the disease severity, whereas the effect of composted sludge depended on the dose applied. The highest dose (45%) enhanced the disease severity, while the lowest dose (15%) attenuated it significantly, which resulted in higher plants with increased photosynthetic activity (net photosynthesis and electron transport rate) and a higher fruit production. Acknowledgements: NILSA (Navarra de Infraestructuras Locales S.A.). Navarra Government. Asociación de Amigos de la Universidad de Navarra. Aragon Government (A03 research group).

P18-003: IMPACT OF LIGHT QUALITY ON LEAF HYDRAULIC CONDUCTANCE: AN EXPERIMENT IN BETULA PENDULA

*Sellin, A.**
University of Tartu
**Corresponding author e-mail: arne.sellin@ut.ee*

Responses of leaf hydraulic capacity to light quality were examined on detached shoots of silver birch, taken from lower (shade leaves) and upper thirds of the crowns (sun leaves). Hydraulic conductance of leaf blades (Klb), petioles (Kp) and branches (i.e. leafless stem; Kb) was determined after the shoots were exposed to PPFD of 200-250 μmol m-2 s-1 for 1, 3 or 5 h, using blue (spectral emission maximum at 450-460 nm), white (wide spectral band) or red light (>600 nm). Klb depended significantly on light quality ($P<0.001$) and canopy position ($P<0.001$), Kb on canopy position ($P<0.001$) and exposition time ($P=0.014$), none of the three factors had effect on Kp. The highest values of Klb were recorded in the blue light (3.63 and 3.13x10-4 kg m-2 MPa-1 s-1 for the sun and shade leaves, respectively), medium values in the white light (3.37 and 2.46x10-4 kg m-2 MPa-1 s-1, respectively) and the lowest values in the red light (2.83 and 2.02x10-4 kg m-2 MPa-1 s-1, respectively). In leaves, where the dominant system involved in energy transduction is photosynthesis, light response of Klb has been suggested to involve photophosphorylation-induced opening of the plasma membrane aquaporins. Our experiment indicates that light quality has a significant impact on leaf hydraulic properties, as quantum energy is directly proportional to the frequency of light waves.

P18-004: REACTIONS OF FOUR SPECIES BELONGING TO FAMILY BRASSICACEAE TO EXCESS NI

Maksimovic, I. - Gani, I. - Putnik-Delic, M. – Zeremski, T. - Marjanovic-Jeromela, A.*
Faculty of Agriculture, Novi Sad (Serbia)
**Corresponding author e-mail: ivanam@polj.uns.ac.rs*

Nickel (Ni) is heavy metal that can be present in excessive amounts in the soil. On one hand, it is important to prevent the accumulation of Ni in edible plant parts, and on the other it is of interest to find plant species/genotypes that are suitable for phytoremediation of Ni-contaminated sites. To test the Ni accumulation capacity and Ni impact on different species belonging to the family Brassicaceae, experiments were carried out under semi-controlled conditions, with plants grown in water cultures in the glasshouse. Winter and spring variety of rapeseed (Brassica napus, L.), white mustard (Brassica alba, L.), black mustard (Brassica nigra, L.) and turnip (Brassica rapa, L.) were grown in the presence of Ni during the entire experiment. Before sowing, seeds were imbibed in deionized water containing 10-5 M Ni, overnight. The same final Ni concentration was present in the ½ strength Hoagland nutrient solution used to grow plants. Root and shoot biomass declined in the presence of Ni (1.28 to 3.3 times and 1.5 to 6 times, respectively). Ratio between shoot and root dry mass did not change in black mustard but it was over 50% lesser in white mustard in the presence of Ni. Percentage of dry matter declined, both in leaves and stems (from 9 to 52%) and roots (0 to 67%). Transpiration intensity increased in all genotypes, from 9% in turnip to 101% in white mustard. Concentration of Mg and Zn increased in all genotypes in the presence

of Ni while concentrations of K, P, Ca, Cu, and Fe did not change in the same manner in all species and tissues. Ni concentration increased in treated plants and there was no statistically significant difference in Ni concentration between different genotypes.
Research was financed by Ministry of Science and Technology of the Republic of Serbia (TR 20083).

P18-005: ALUMINIUM HYPERRESISTANCE IN BRACHIARIA SPECIES

Arroyave, C. - Tolrà, R. – Barceló, J. – Poschenrieder, C.*
Universidad Autónoma de Barcelona
**Corresponding author e-mail: catyaq@gmail.com*

In recent years Brachiaria species (Poaceae), have gained increasing importance as pasture in tropical areas.
Good feeding quality and high resistance to multiple environmental stress factors makes these plants with C4 type photosynthesis especially attractive and further breeding efforts are ongoing. High acid soil tolerance is a common feature of Brachiaria species. Here we report on the differences in Al tolerance of two Brachiaria species, B. decumbens and B brizantha. The hydroponic study using low ionic nutrient solution without Al supply or containing 100 to 400 μM Al (pH 4.2) revealed high Al tolerance in both species. However at 200 μM Al or higher clear species differences in Al tolerance were observed. After 72 hours in 200 μM Al (31.7 μM Al^{3+} activity), *B. brizantha* exhibited a 43% inhibition of root elongation and alteration of root structure. Contrastingly, in *B. decumbens* inhibition of root elongation was only 20%. Root staining with haematoxylin, morin, or lumogallion revealed higher Al accumulation in the root tips of less Al tolerant *B. brizantha* than in the hypertolerant B. decumbens. Mineral analysis also confirmed better Al exclusion from roots in *B. decumbens.* However, this species translocated proportionally more Al to the shoots than *B. brizantha*. Phosphorus, Fe, and Mn concentrations were considerably more affected by Al in *B. brizantha*.
In conclusion, both species are highly tolerant to both low pH and Al toxicity. The better performance of *B. decumbens* seems due to more efficient Al exclusion from the roots. Nonetheless this species also is quite tolerant to higher Al shoot concentrations.
The mechanisms of Al tolerance in Brachiaria deserves further investigation. Acknowledgements: Supported by the Spanish Government BFU2007-60332/BFI.

P18-006: K+ FOLIAR FERTILIZATION INCREASES NA+ EXCLUSION IN SUNFLOWER PLANTS

Benlloch González, M. - Fournier, J.M. – Benlloch, M.*
Agronomy Department. Cordoba University
**Corresponding author e-mail: g72begom@uco.es*

Sodium is a toxic element for glycophytes. Most of glycophytes avoid sodium accumulation in the shoot by the exclusion mechanism. In sunflower plants it has been described that its capacity to exclude Na+ strongly depends on their potassium nutritional status: in normal K+ plants the exclusion mechanism operates properly.
However, in starved K+ plants the root shows a lower capacity of Na+ exclusion. On the other hand it has been recently suggested that in the leaves of sunflower plants may exist a mechanism which enables the perception of the K+ status of the plant and triggers transduction processes governing water and K+ transport in the xylem.
The aim of this study was to elucidate whether the lower capacity to exclude Na+ observed in sunflower plants when they are starved in K+ is regulated by the K+ nutritional status of the shoot. The plants were watered regularly with nutrient solutions containing different concentrations of KCl. In this way, two types of plant were obtained: plants with normal K+ status and plants starved in K+ respectively. At the end of the growth period the KCl of the nutrient solution was replaced by NaCl (25 mM) and a foliar treatment of KCl was applied to 50 % of both types of plants. The salinity treatment favoured the accumulation of Na+ in the root of all plants, whereas in the shoot it only favoured its accumulation in those starved in K+. The K+ foliar treatment did not modified significantly the K+ content of the leaves, while it inhibited the accumulation of Na+ in the leaves of K+ starved plants, restoring partially the Na+ exclusion character in K+ starved sunflower plants.
These results suggest that the perception of the K+ status of the plant by the leaves is involved in the regulation of the Na+ unload in the xylem of the root.

P18-007: MOBILITY AND HYPERACCUMULATION OF MINERALS AND HEAVY METALS IN WILD MUSHROOMS

Stihi, C. - Radulescu, C. – Busuioc, G. – Popescu, V.I. – Gheboianu, A.*
Valahia University of Targoviste, Romania
**Corresponding author e-mail: cstihi@yahoo.com*

The minerals and heavy metals play an important role in the metabolic processes, during the growth and development of mushrooms, when they are available in appreciable concentration. Inthis work the concentrations of Mg, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Se and Pb were analyzed using the Graphite Furnance Atomic Absorption spectrometry (GFAAS) together with Energy Dispersive X-ray Fluorescence spectrometry (EDXRF) in 8 wild mushrooms and their growing soil, collected from various forestry fields in Dambovita County, Romania. The analysed mushrooms were: *Amanita vaginata, Amanita rubescens, Amanita phalloides, Armillariella mellea, Armillariella tabescens, Agaricus campestris, Hypholoma fasciculare, Hypholoma pudorinus.* The accumulation coeficients (AC) and mobility coeficients (MC) were calculated to asses the mobility of minerals and heavy metals from soil to mushrooms through different levels: level 1 (Soil-Stem) and level 2 (Stem-Cap). The data on accumulation and mobility of heavy metals such Cd, Cu, Fe, Ni, Pb and Zn (in ascending order of acumulation) from soil to cap through stems, suggested that all the metals were highly mobile from soil to mushrooms components.

P18-008: EFFECT OF EPIBRASSINOLIDE ON PIGMENT CONTENT IN EXCISED CUCUMBER COTYLEDONS

*Gören Sağlam, N.**
İstanbul University
**Corresponding author e-mail: gorenn@istanbul.edu.tr*

Brassinosteroids (BRs) are a new group of plant hormones with significant growth-promoting activity. BRs as plant hormones with pleiotropic effects as they influence varied developmental processes such as growth, seed germination, rhizogenesis, senescence, flowering, abscission, maturation and also confer resistance to plant against varies abiotic stresses.
The most important role played by the brassinosteroids is their stimulation of the growth of coleoptile in monocotyl plants, and growth in the stem, petiole and flower stalks in dicotyl plants. The promotion of growth by BRs is due to both cell division and cell elongation. Brassinosteroids increase chlorophyll breakdown but inhibit anthocyanin biosynthesis.
Brassinosteroids are steroidal plant hormones that influence varied growth and development processes such as germination of seeds, flowering, senescence and abscission. In this study its effect was investigated on excised cotyledons of cucumber seedlings.
The cucumber seedlings were grown in dark conditions for 9 days and then their etiolated cotyledons were harvested. Then, they were transferred into Petri dishes containing 10-7, 10-9 and 10-11 M epi-BL. Cotyledons were incubated for 14 hours in the dark at room temperature, then they were incubated in light period for 3 hours. Chlorophyll, carotenoid content, protein amount and peroxidase (POD) activity in the cotyledons was examined.

P18-009: THE SIGNAL ROLE OF NITRATE IMPROVING THE GROWTH OF AMMONIUM-FED WHEAT PLANTS INVOLVES THE ROOT TO SHOOT REDISTRIBUTION OF THE MAIN PHYTO-REGULATORS.

Grcia-Mna, J. - Garnica, M. - Houdusse, F. - Zamarreño, AM. - Yvin, JC.*
R&D Roullier Group
**Corresponding author e-mail: gmina@timacagro.es*

Some studies reported that the presence of nitrate in the nutrient solution was able to trigger specific metabolic events favouring growth promotion in plants fed with ammonium and urea (1). On the other hand, other studies related the plant growth- promoting effect of nitrate, even a very low concentration (signal effect), to the enhancement of the root to shoot translocation of certain active cytokinins (CK) (2,3). We have investigated whether the positive effect of very low nitrate-concentrations (signal role) on ammonium-fed wheat plants is associated with significant changes in the plant distribution of those phyto-regulators related to stress (ethylene, ABA and polyamines) and development (CK and IAA). The results showed that very low concentrations of nitrate (100 mM) promoted both root and shoot growth of wheat seedlings fed with ammonium. This action of nitrate was associated with an increase in the shoot concentration of active forms of CK and a decrease in the relative shoot concentration of CK-inactive forms. The presence of nitrate also enhanced IAA shoot content and tended to lower ABA concentration and ethylene root production. Nitrate supply also induced changes in polyamines distribution. These results present further evidence that the possible signal effect of nitrate involved in its beneficial effect on the growth of wheat plants fed with ammonium could be mediated by a coordinated action of different phytoregulators; increasing growth-promoting phytoregulators (active CK and IAA), and decreasing stress-related phytoregulators (ABA and ethylene).

(1) Houdusse F, et al. (2005). Funct. Plant Biol, 32:1057-67.
(2) Rahayu YS, et al. (2005). J Exp Bot 2005;56:1143-52.
(3) Sakakibara H. J Plant Res 2003;116:253-7.

P18-010: STUDY OF NITRATE ASSIMILATION AND ACCUMULATION IN "BABY LEAF" READY TO EAT SPINACH LEAVES

Cabassi, G.[1] - Antonacci, S. - Marino Gallina, P.[2] - Ferrante, A.[2]*
[1]CRA-FLC
[2]DiProVe - University of Milan
**Corresponding author e-mail: g_cabassi@katamail.com*

In order to study the behavior of nitrate absorption and assimilation in ready to eat spinach production a experiment was carried out in greenhouse twice, according a randomised block design, in order to study metabolite levels and the expression of six genes involved in nitrate assimilation: NTR (high affinity nitrate transporter) NR (nitrate reductase) NiR (nitrite reductase) GS1 and GS2 (glutamine synthase citosolic and plastidial respectively) and GLU (glutammate synthase). Spinach plants were grown in big pots on soil substrate whit four different levels of NO3-N supply (10, 30, 75, 150 kg/ha). Spinach leaves were harvested at 3 sampling dates (7 days before commercial harvest, commercial harvest, corresponding to four fully expanded leaves, and 7 days after). For each experiment the expression of candidate genes was analyzed using qRT-PCR and the main agronomic parameters were also measured: aerial biomass, fresh and dry matter production, leaf area index and nitrate content. The nitrogen metabolites were also analised: ammonium, stach and sucrose content were analized using enzymatic methods. The concentration of the main amino acids (Glu, Asp, Gln, Asn, Gly, Ser, Ala) was measured by HPLC. The theses fertilized using 10 and 30 kg/ha of N showed low nitrate accumulation and low biomass production. The thesis fertilised with 75 kg/ha showed a high biomass production and nitrate concentration below 500 ppm at the 3rd sampling date as a result of vacuolar nitrate remobilization, whereas the thesis supplied with 150 kg/ha of N always showed high nitrate accumulation. NR and NIR expression were upregulated acoording to nitrate supply, GS2 was upregulated in the theses fertilised with 75 kg/ha and with 150 kg/ha of N until commercial harvest date.

P18-011: REGULATION OF FERRIC CHELATE REDUCTASE AND H+-ATPASE ENZYMES BY IRON NUTRITION IN CITRUS AND EFFECTS OF PH, BICARBONATE AND MICROELEMENTS

Martinez Cuenca, M. - Forner Giner, Ma. - Primo Millo, E.- Legaz, F.*
Instituto Valenciano de Investigaciones Agrarias
**Corresponding author e-mail: martinez_mru@gva.es*

The aim of this work was to study the regulation of iron uptake in citrus by the enzymes implicated in iron reduction (FCR: Ferric Chelate Reductase) and acidification of the rizosphere (H+-ATPase: Proton-pumping ATPase). Also, the effects of some factors (pH, bicarbonate and microelements) affecting these enzyme activities were determined. For this purpose, two-month-old Citrange Carrizo seedlings (a hybrid of Citrus sinensis x Poncirus trifoliata) were grown under glass-house conditions in individual pots filled with coarse sand and irrigated with a nutrient solution, either with or without 20 mM Fe-EDDHA. Activities of FCR or H+-ATPase were tested at different solution pH and after adding 10 mM HCO3- or 3 mM of either Zn2+, Mn2+, Cu2+ to the uptake solution. Fe-deficient plants showed higher FCR and H+-ATPase activities than Fe-sufficient plants (2.99- and 2.11-fold, respectively). Both enzymes presented their optimum activity near the neutral pH range. Addition of HCO3- to the uptake solution (10 mM for 5 minutes) inhibited H+-ATPase activity in Fe-sufficient plants by a 28.7% relative to the control, while FCR activity was increased in a 66.3%.
Short-time treatments (5 minutes) with either Zn2+, Mn2+, Cu2+ (at 3 mM) added to the uptake solution enhanced both activities. The order of effectiveness of microelements in increasing FCR activity was Mn2+>Cu2+>Zn2+ whereas with H+-ATPase activity was Cu2+>Mn2+>Zn2+.

P18-012: PHYSIOLOGICAL RESPONSE OF LETTUCE BABY LEAF UNDER LIMITED NITROGEN CONDITIONS

Ferrante, A.[1] - Ferrarese, M.[1] - Podetta, N.[1] - Antonacci, S.[1] - Cabassi, G.[2]*
[1] University of Milan
[2]CRA-FLC
**Corresponding author e-mail: antonio.ferrante@unimi.it*

Baby leaf vegetables cultivation and commercialization have been continuously increasing in the horticultural markets. The growing cycle of these vegetables is very short depending from the season. It usually ranges from 20 to 60 days. They are harvested when plants reach 10-15 cm and with 4-5 fully expanded leaves. In this stage the plants are in active growth, therefore their physiological behavior is not completely studied. Among nutrient the nitrogen and in particularly nitrate assimilation is extremely important since nitrate content in leaves cannot overcome the EU thresholds. The experiments were performed on lettuce (Lactuca sativa L.). The soil was opportunely chosen with low nutrition content and organic matter. The nitrogen doses were 0, 5, 10 or 15 g m-2 and organic matter levels were increased from 0.5 to 1.5%. Plants were grown until reaching the baby leaf development stage. The nitrate and macronutrients content were monitored in leaves and roots. Growth indexes such as absolute growth rate, relative growth rate and leaf area index were calculated. Chlorophyll a fluorescence parameters and fresh biomass were determined in response of nitrogen doses. Nitrate content in limited nutrient availability and low organic matter content did not increase by increasing the nitrogen doses.

Plant growth linearly increased with nitrogen supply with a R2 of 0.997.
Acknowledgment: research funded by AZORT project.

P18-013: UTILIZATION OF NITROGEN 15N FROM FERTILIZERS BY CEREALS IN A MIX STAND WITH FIELD PEA

*Rutkowska, A.**
Institute of Soil Science nad Plant Cultivation-National Research Institute
**Corresponding author e-mail: agrut@iung.pulawy.pl*

The objective of two factorial pot experiment was quantitative estimation of nitrogen derived from fertilizers by wheat, barley and oats in a mix stand with field pea under differential mixtures composition: 20, 40, 60 and 80% of cereals (2, 4, 6, 8 cereal plant + 4, 3, 2, 1 pea plant/pot respectively). Nitrogen fertilizer (NH4NO3) was applied in the rates: 0,3, 0,6, 0,9 and 1,2 g N/pot, accordingly to cereal percentage in the mixtures. The control – unfertilized pure stand of pea (5 plants) and fertilized with 1,5 g N/pot pure stand of cereals (10 plants) were included. The amount of 15N stored in grain by wheat was 12% less in comparison with barley and oats however total quantity of 15N derived from fertilizers by plant biomass was comparable between species and increased together with N rate from 0,16 to 0,62 g N/pot on the average what consist 50 to 67% of total N-labeled taken up by the mixtures. The coefficient of nitrogen utilization wasn't differ significantly between cereal species and ranged from 52% (the lowest N rate) to 73% (the highest one). On the contrary, coefficient of nitrogen utilization by pea strongly depended on N fertilization and decreased from 56% in 0,3 g N/pot to 9% in 1,2 N/pot treatment. Transfer of nitrogen biologically fixed by pea to cereals was closely related to proportion of cereal : pea plants in the mixture and constituted from about 5% of total nitrogen accumulated by cereal biomass in treatment with the ratio 1 : 8 to 90%under 4 : 2 ratio.

P18-014: APPLICATION OF MICRO-PROTON INDUCED X-RAY EMISSION (MICRO-PIXE) IN PLANT BIOLOGY

Pongrac, P.[1] - Vogel-Mikuš, K.[2] - Regvar, M.[2] - Vavpetic, P.[1] - Grlj, N.[1] - Pelicon, P.[1]
[1]Jožef Stefan Institute
[2]Department of Biology, Biotechnical Faculty, University of Ljubljana

Plant performance depends on dynamics of both essential and non-essential elements, their concentrations and spatial distribution in tissues. Traditionally, digestion or chemical fixation of tissues is required to perform such studies. However, such sample preparation results in destruction of plant material and/or elemental redistribution. In contrast, micro-proton induced X-ray emission (micro-PIXE) technique can provide quantitative lateral element distribution mapping of plant tissues prepared by rapid freezing, cryo-sectioning and freeze-drying [1,2]. In micro-PIXE the limits of detection range from 0.1 to 1 ppm for elements from Ca to Zr and are generally well bellow 10 ppm elsewhere in the element range, from Na to U [3]. When element concentrations exceed 50 ppm maps provide excellent allocation information. However, when the concentrations are lower than 1 ppm, the corresponding maps might not provide useful information on the localization, due to poor spectral statistics. Using an off-line analysis of the spectra extracted from distinctive morphological structures, the resulting data can provide reliable information on the element concentrations in the selected tissue. Transnational access to micro-PIXE facility at Jožef Stefan Institute is granted upon approved short scientific proposal [4]. Examples of application of the micro-PIXE technique in plant tissues of different plant species from various environmental conditions will be demonstrated.
[1] Vogel-Mikuš et al. 2008. Plant Cell Environ 31:1484-1496
[2] Schneider et al. 2002. Int J PIXE 12:101-107
[3J]ohansson & Campbell 1988. PIXE: A novel technique for elemental analysis. John Wiley & Sons, New York.
[4w] ww.spirit-ion.eu

P18-015: COMPARISON OF NUTRITIONAL STATUS BETWEEN INTEGRATED, ORGANIC AND CONVENTIONAL KIWIFRUIT FARMING SYSTEMS

Martínez Núñez, L. - Barreal, M.E. – Otero, V. – Gallego, PP.*
Universidad de Vigo
**Corresponding author e-mail: lourdesmn@gmail.com*

This study was conducted, in southern Galicia (Spain), on three Kiwifruit Actinidia deliciosa var. deliciosa (A.Chev.) C.F Liang et A. R. Ferguson (cv Hayward) plantations exposed to three different management systems: conventional, integrated and organic. Integrated and organic management are regulated by specific laws in Galicia region, trying to preserve the environment. Organic farming plantation was fertilized by applying solid fertilizers and compost generated from plant residues and poultry slurry in the farm; however, with conventional and integrated systems, fertilization was achieved by fertigation with mineral fertilizers. The objective of this study was to compare the nutritional status and soil fertility of kiwi plants in the three farming systems. Mineral composition of soil, leaves and fruits were determined during different phenological stages of the plant over two years period. The results obtained in this study suggest that the management system used affect the nutritional levels of the plant in fact, organic and integrated systems increase the pH of these acid soils, improve the availability of nutrients for the plant, without exhausting the soil.

P18-016: XYLEM HYDRAULIC CHARACTERISTICS AND WATER RELATIONS IN STEMS OF HOPS (HUMULUS LUPULUS L.)

Gloser, V.[1] - Balaz, M.[1] - Svoboda, P.[2]*
[1]Masaryk University, Department of Experimental Biology
[2]Hop Research Institute
**Corresponding author e-mail: VitGloser@sci.muni.cz*

Thin stems of vines frequently transport water on long distance to large leaf area. Therefore, transport efficiency and mechanisms preventing a failure of transport need to be particularly well balanced in xylem. We examined relationships between structural and functional traits related to water transport stem xylem of hop plants. These stems are only 6 to 12 mm thin and serve to rapid water transport along shoot axis up to 12 m long to total leaf area of 1-2 square meters. Hydraulic conductivity measured along the stem axes correlated well with hydraulic conductivity calculated from anatomical traits measured in stem cross sections. Calculated conductivity using Hagen-Poiseuille equation was higher than measured namely due to difficulties in identification of functional conduits. Leaf specific conductivity did not change along stem axes and was similar to that previously found in some lianas. Leaf water potential of well watered plants at midday was found as low as -1.5 MPa with no visible signs of wilting. Also the vulnerability to embolism expressed as 50 % loss of conductivity (PLC50) was approx.
-1.8 MPa, higher that in some others previously examined vines. We discuss, why are the herbaceous stems of hop more efficient in water transport than previously examined woody vines and if the properties of hop stems may be beneficial under water limitation.

Auth
no

Author Index

CORPORATE SUPPORTERS

GOLD SPONSOR

Hansatech Instruments

SILVER SPONSOR

Agrisera
Antibodies for plant sciences

American Society of Plant Biologists

BERTHOLD TECHNOLOGIES

WILEY-BLACKWELL

BRONZE SPONSOR

CLF Plant Climatics

FINNZYMES
Part of Thermo Fisher Scientific

SPEX SamplePrep

Taylor & Francis
Taylor & Francis Group

CONGRESS PARTNERS

CSIC
Consejo Superior de Investigaciones Científicas

epso

FESPB

Gobierno de España
Ministerio de Ciencia e Innovación

Generalitat Valenciana

sociedadespañola FISIOLOGIA VEGETAL

Universidad Politécnica de Valencia

Universitat de València

GEYSECO
GRUPO GEYSECO SL

ORGANIZING SECRETARIAT:
GRUPO GEYSECO. C/ Universidad, 4 46003 Valencia - Spain
Tel.: +34 96 352 48 89 - Fax: +34 96 394 25 58 - www.geyseco.es - fespb2010@geyseco.es