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THE EFFECT OF Zn, Cu AND Mn ON SENESCENCE IN EXCISED COTYLEDONS OF *Eruca sativa* L.

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SUMMARY

The mineral nutrient concentrations in soil are affected by factors such as pH, organic matter, clay and redox potentials. The solubility of such micronutrients as Zn, Cu and Mn is increased by lower pH, or vice versa. The alterations in concentration of these nutrients may affect the growth and development of plants, but also lead to their senescence and programmed cell death related to age and mineral nutrient concentrations. This study aimed to explore the senescence rate due to lack of some essential micronutrients, such as Zn, Cu and Mn, associated with indole acetic acid (IAA). In addition to peroxidase and protease activity, total chlorophyll, nitrogen and protein values were determined. Decreased catabolic reactions and delayed senescence were indicated in excised cotyledons from lack of Zn.

KEYWORDS: Senescence, zinc, copper, manganese, mineral nutrient deficiency.

INTRODUCTION

Zinc (Zn), copper (Cu) and manganese (Mn) are some of the micronutrients or trace elements essential for most living organisms. Trace metals in geological sense are the main source of metal toxicity problems in the environment, since most organisms are not adapted to deal with them when they occur locally at high concentrations [1, 2]. In geological terms, trace elements are defined as those occurring at 1000 ppm or less in the earth's crust. They may become phytotoxic when in excess in plants.

Metals in the soil solution are the only soil fraction directly available for plant uptake. Hence, factors which affect the concentration and speciation of metals in the soil solution will affect the bioavailability of metals to plants. Soil factors, which have an effect on the metal bioavailability, include the total metal concentration in the soil, pH, clay and content of hydrous oxides, organic matter and redox conditions [2]. The availability of most micronutrients tends to decrease with increasing pH, because the solubility of these elements decreases. Zn, Cu and Mn deficiency also may be induced by increase in soil pH, which stimulates their absorption to surfaces of various soil constituents, such as metal oxides and clay minerals [1-3]. In accordance with these pH-related changes in metal bioavailability, many studies have found that plant uptake rates of Mn and Zn increase with decrease in soil pH [2].

Zn deficiency is occurring in different climate regions and almost all countries [4]. Of these, it especially occurs more frequently in calcareous soils with high pH, such as those found in arid and semiarid regions, and in the Mediterrenean region. Zn deficiency has also been identified as the most widespread micronutrient deficiency in Turkey [5]. High pH values decrease desorption of Zn, Cu and Mn from soil surfaces, thus limiting Zn availability to plants [2, 6].

Deficiencies of nutrients in plants have various visual symptoms that are usually similar regardless of the species. Zn, Cu and Mn deficiency in plants lead to severe reduction in growth and development [7]. Catabolic and anabolic events related to senescence define it as a special metabolism [8]. Senescence mechanisms are generally examined by using whole plants, but many researchers are known to prefer excised organs and tissues to study the affecting external and internal factors [9-12], characterized by arrest of photosynthesis, degradation of organelle structure, nutrient deficiency [14], rapid decline of chlorophyll [13-15] or protein contents [16], and dramatic increase in lipid peroxidation [16, 17]. But those factor studies resulting in the onset of senescence are quite considerable [18, 19].

Plants can grow in soils containing highly variable amounts of mineral nutrients, such as the essential Zn^{2+}, Cu^{2+} and Mn^{2+}, though the mechanisms of adaptation are poorly understood. Sixteen elements are known to be required for both synthesis and activity of enzymes responsible for catabolic reactions of senescence. Zn is

involved in chlorophyll formation and inhibits its degradation. Chlorosis occurs from Zn defficiency and Mn plays a structural role in membrane system in the chloroplasts activating many enzymes. Chlorosis and necrotic lesions are symptoms of lack of Mn. As to Cu, it is involved in the synthesis of certain enzymes and proteins, and acts as an electron transporter.

The purpose of this study is to find a possible relationship between senescence and the above-mentioned elements.

MATERIALS AND METHODS

11-day-old seedlings of rocket (*Eruca sativa* L.) were grown on sawdust under climate room conditions (12-h photoperiod, 8,000 lux light, 65-75% humidity, and 25 ± 2°C) and used as experimental material. Five pairs of cotyledons were harvested on the 11th day, exposed to a dark period of 12 h, then transferred into petri dishes of 7 cm in diameter filled with 7 ml of Hoagland solution as control group and different test solutions [Hoagland solutions contained no Zn (H^{-Zn}), Cu (H^{-Cu}) or Mn (H^{-Mn})] and incubated for 4 h in light.

Following incubation, the chlorophyll content of cotyledons was determined according to Arnon Method [20]. For determination of total nitrogen, a method combining Kjeldahl and spectrometry was used [21]. Amounts of soluble proteins were measured according to Bradford [22] using Bovine Serum Albumin (BSA). Peroxidase activity (POD) was measured spectrophotometrically (Shimadzu UV 160) at 470 nm by using 0.1 M phosphate buffer (pH 5.8), 15 mM guaiacol and 5 mM H_2O_2 [23]. 50 mM citrate phosphate buffer adjusted to two different pHs (pH 4.2 for acidic protease, pH 6.6 for neutral protease) was employed with Azocoll (Calbiochem) as substrate to determine protease activity and absorbance of supernatants was measured at 520 nm [24].

RESULTS

The present study demonstrated that the fresh harvested cotyledons of 11-day old rocket seedlings incubated in the experimental solutions showed an increase in weight in different ratios compared to initial fresh weights prior to treatment (Figure 1). Fresh weights of cotyledons incubated in control solutions without Mn and Cu decreased by 8%, whereas fresh weights of Zn-deficient ones remained unchanged, compared to those incubated in Hoagland medium only.

Since chlorophyll is degraded in the course of senescence [13-15], its measurement required to monitor senescence in case of lack of these minerals (Figure 2). While chlorophyll content of excised cotyledons incubated in H^{-Cu} and H^{-Zn} solutions decreased by 30% in comparison to the initial, that of the ones with H^{-Zn} incubation did not decrease, on the contrary elevated by 23%, according to the ones in Hoagland solution, a result which is quite difficult to understand.

Nitrogen amount in cotyledons, in which chlorophyll was measured, also was determined (Figure 3). As can be seen, declines in nitrogen levels are parallel to those of chlorophyll contents. Yet, the highest level of nitrogen was found in the cotyledons incubated in the solution without Zn, compared to that before treatment. Results of peroxidase (POD) enzyme activity, considered to be a senescence parameter, are given in Figure 4.

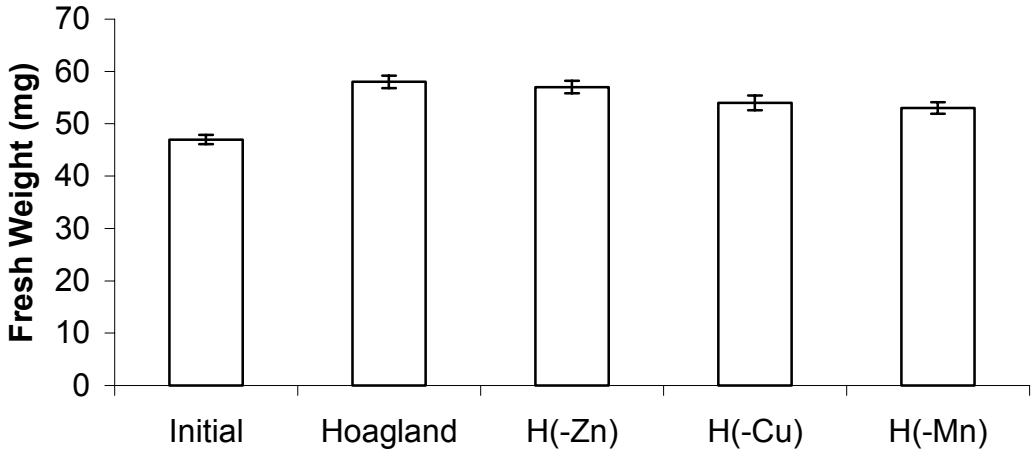

FIGURE 1
Comparison of fresh weights of the cotyledons before (as control) and after incubation in different experimental solutions.

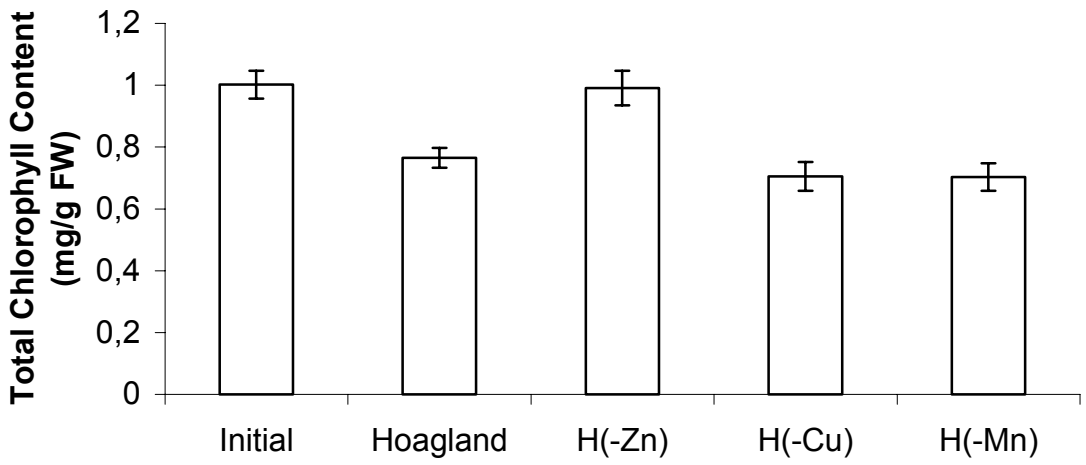

FIGURE 2
Chlorophyll amounts of the cotyledons before and after incubation in different solutions.

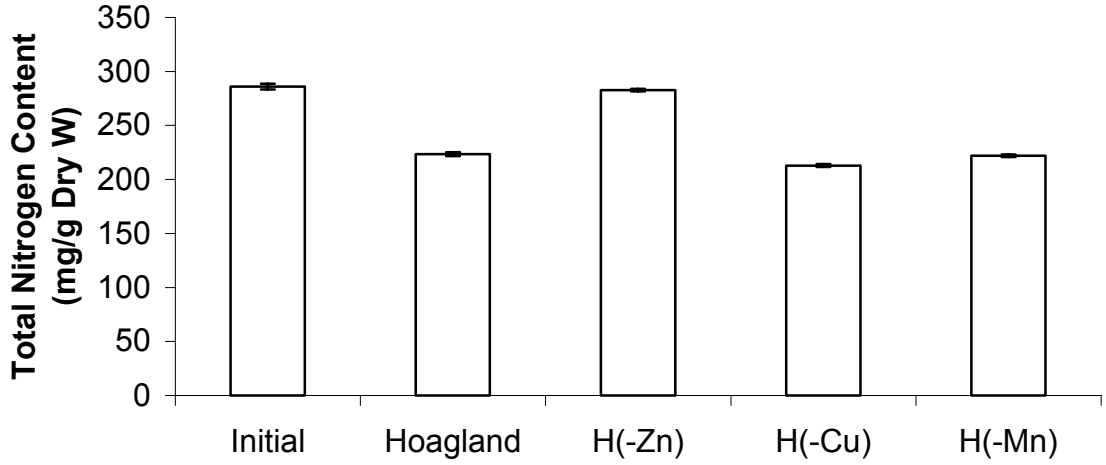

FIGURE 3
Nitrogen amounts of the cotyledons before and after incubations in different solutions of the micronutrients tested.

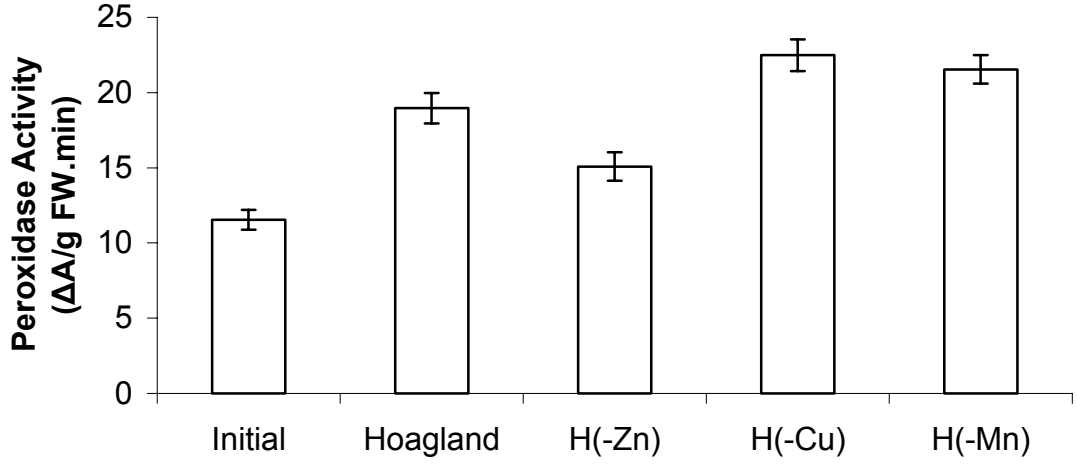

FIGURE 4
POD activities of the initial and incubated cotyledons in the solutions.

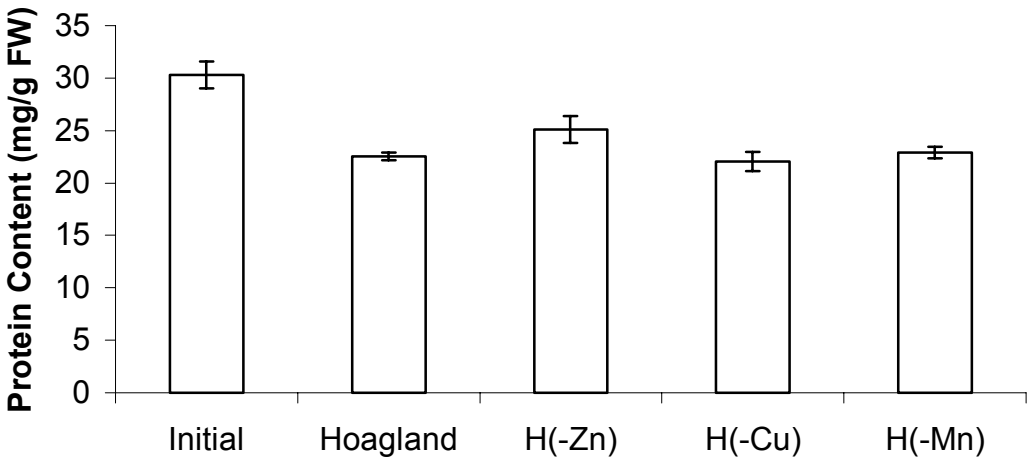

FIGURE 5
Protein amounts of the experimental cotyledons.

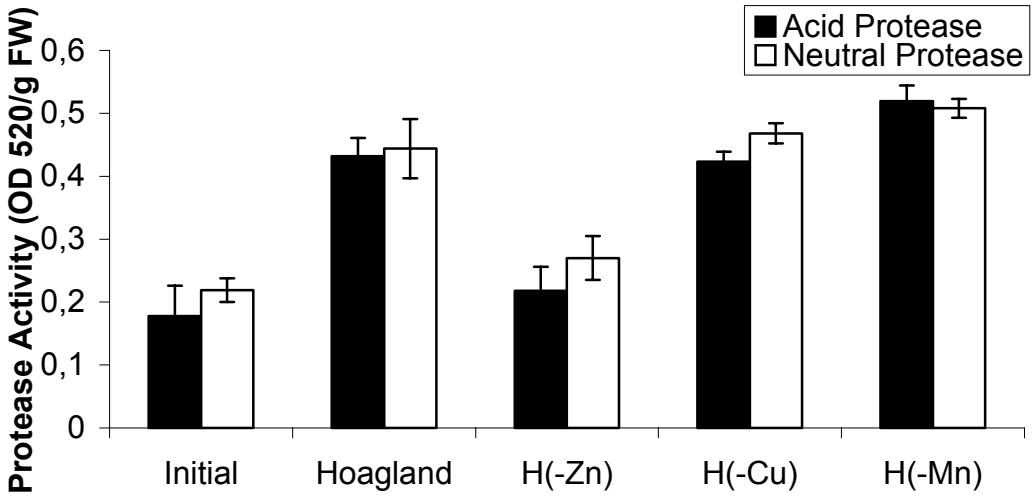

FIGURE 6
Protease activities of the cotyledons before and after incubation.

POD activities were seen to be elevated before treatment and the lowest elevation was found in cotyledons incubated without Zn. Because it is known that protein amount decreases while protease activity elevates during senescence, we measured protein amount and protease activity in the cotyledons. As the protein amount declined, the acid and neutral protease activities elevated after incubation in different solutions, with the exception of H^{-Zn}, which inhibited protease activity (Figures 5 and 6).

Cotyledons incubated in H^{-Mn} showed maximal acid and neutral protease activity. However, the inhibition ratios of acid and neutral protease activities in H^{-Zn} were 58% and 47%, respectively. In addition, the initial protease activity was quite low. These findings reveal that proteases are synthesized for protein degradation.

In conclusion, all catabolic reactions were slowed down in the excised cotyledons from solutions lacking in Zn, considering Zn deficiency supported senescence.

DISCUSSION

The mineral nutrient concentrations of soil are affected by several factors, such as pH, organic matter, clay and redox potentials [1, 2]. Mineral nutrient deficiency is one of the most crucial factors for plant development and

researchers have studied the relationship between this deficiency and senescence [14, 15], known as programmed cell death [25-27]. Although a lot of were conducted to explore the mechanisms of senescence, the primary cause has not been understood yet. But, researchers continue their investigations to obtain some clues. This study aims to put some light in this complex subject.

Alterations in membrane systems of the cell, especially membrane degradation, was detected [28-30]. As a matter of fact, decrease in fresh weight of cotyledons was parellel to a decrease in chlorophyll content (Figure 1). Buttler [31] reports about the degradation of tonoplast and chloroplast membranes occurring in close succession, degradation of the former starting later, but completing earlier than the latter. Fresh weight of the cotyledons began to decrease earlier and prior to incubation, followed by a reduction in chlorophyll content, which means that the tonoplast membrane degrades rapidly resulting in water loss.

Unlike the situation in mineral deficiencies, higher fresh weight of the cotyledons incubated in full Hoagland solution, which does not affect cholorophyll content, revealed that degradation of tonoplast membrane starts later than that of chloroplast membrane in the presence of the minerals tested. This is in concordance with Buttler's findings [31], and from this, one can conclude that minerals in Hoagland solution preserve integrity of tonoplasts, which is not affected by lack of Zn.

Likewise, integrity of choloroplast membranes can be said to be maintained in Zn deficiency, since chlorophyll content is very close to the initial level, indicating retaining senescence. In contrast, a decrease in chlorophyll content occurred in cotyledons incubated in both full Hoagland and the solutions lacking Cu and Mn.

If the same case is considered for total nitrogen and protein content, one can say that certain substances were degraded as a result of metabolic reactions in the course of 4-h incubation of harvested rocket cotyledons under 12-h photoperiod, the degradation being less in medium lacking Zn. Protein synthesis failed to function in harvested cotyledons incubated in the solutions, and the resulting effect was seen to be less in the cotyledons in the solution lacking Zn. Cu and Mn deficiencies did not affect biochemical reactions selectively, because they were not different to those in the full Hoagland solution cotyledons.

Thayer et al. [8] emphasized that proteases must be synthesized for protein degradation. Acceleration of protease activity versus decline of protein amount has long been known to occur during senescence [32-35]. Protein degradation was detected in harvested cotyledons incubated in the solutions, but being less in the solution lacking Zn. Negative correlation between protease activity and protein amount is quite significant for senescence. However, there was no prominent difference between the cotyledons incubated in full Hoagland and the solutions lacking Cu and Mn. Definite decrease in protease activity in the cotyledons incubated in Zn-lacking solution may be interpreted that Zn delays senescence.

Negative correlation between POD activity increment and chlorophyll value in Zn deficiency reveals that POD activity may be a parameter for senescence in this study. However, there are workers claiming opinions contrary to this [12, 36].

It is clear from the results of this study that Zn is effective on senescence, and the results are of value on the basis of physiological functions of Zn. It has long been known that Zn stabilizes IAA in plant tissues, and IAA amount decreases in Zn deficiency [37, 38]. In another study, cotyledons of sunflower seedlings grown under lack of Zn showed delayed senescence, and higher chlorophyll and nitrogen amount, in comparison to those grown in the presence of Zn [39]. Moreover, carbohydrate synthesis was indicated to have decreased Zn deficiency [40]. The fact of elevated chlorophyll, nitrogen and protein amount versus reduced carbohydrate synthesis under Zn-deficient conditions may be a clue to the question of a relationship between senescence and carbohydrate metabolism.

The effects of Mn^{2+} on auxin levels, which is oxidized by Mn^{2+}, have been reported previously [41, 42]. A recent study reported that Mn oxidizes IAA and Cu causes IAA degradation [43].

The statement of Sağlam and Okatan [44] that senescence signal is a IAA-like substance is in accordance with our results, which indicate decreased IAA and delayed senescence from Zn deficiency, and also elevated IAA in tissues in connection with IAA oxidation in Mn and Cu deficiencies. In conclusion, IAA can be considered as a factor which triggers senescence.

REFERENCES

[1] Duffus, J.H. (1983) Environmental Toxicology. Edward Arnold Ltd. London.

[2] Reichman, S.M. (2002) The responses of plants to metal toxicity: A rewiew focusing on Copper, Manganese and Zinc. Melbourne, Australia.

[3] Barrow, N.J. (1993) Mechanism of reaction of zinc with soil and soil components. In: Zinc in Soils and Plants. A.D. Robson (ed.). Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 15-31.

[4] Sillanpää, M. and Vlek, P.L.G. (1985) Micronutrients and the agroecology of tropical and Mediterreanean regions. Fert. Rest.151-167.

[5] Cakmak, I., Yılmaz, A., Kalaycı, M., Ekiz, H., Torun, B., Erenoglu, B. and Braun, H.J. (1996) Zinc deficiency as a critical problem in wheat production in Central Anatolia. Plant and Soi. 180, 165-172.

[6] Dang, Y.P., Edwards, D.G., Dalal, R.C. and Tiller, K.G. (1993) Identification of an index tissue to predict zinc status of wheat. Plant and Soil.154, 161-167.

[7] Brown, P.H., Cakmak, I. and Zhang, Q. (1993) Form and function of zinc in plants. In: Zinc in Soils and Plnats. A.D. Ronson (ed). Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 93-106

[8] Thayer, S.S., Choe H.T., Tang, A. and Huffaker, R.C. (1987) Protein turnover during senescence. Plant senescence: Its biochemistry and physiology. William W. Thomson, Eugene A. Nothnagel and Ray C. Huffaker (eds.) 1987. The American Society of Plant Physiologists.

[9] Grover, A. and Sinh, S.K. (1985) Senescence of detached leaves in pigeon pea and chick pea: Regulation by developing pods. Physiol. Plantarum. 65, 503-507.

[10] Mukherjee, D. and Rao, K.V.M. (1993) Alteration patterns of hill activity, peroxidase acticity and sugars of pigeon pea during maturation and senescence. Indian J. Plant Physiol. 36, 13-16.

[11] Kar, M. and Mishra, D. (1975) Inorganic pyrophosphatase activity during rice leaf senescence. Can. J. Bot. 53, 503-511.

[12] Yonova, P., Guleva, E., Zozikova, and Kotseva, E. (2001) Senescence-dependent changes in some metabolic processes affected by 1,1'-polymethylenebis (3-arylsubstıtuted) ureas in barley (*Hordeum vulgare* L.) leaf segments. Bulg. J. Plant Physiol. 27(1-2), 5 4-71.

[13] Thimann, K.V. (1978) Senescence in controlling factors in plant development. Special Issue of the Botanical Magazine, Tokyo. 1, 19-43.

[14] Thomas, H. and Stoddart, J.L. (1980) Leaf senescence. Ann. Rev. Plant Physiol. 31, 83-111.

[15] Noodén, L.D., Guiamét, J.J. and John, I. (1997) Senescence mechanisms. Physiol. Plantarum. 101, 746-753.

[16] Matile, P. (1998) Leaf senescence and the breakdown of chloropyll. J. Exp. Bot. (supplement), 49, p. 45.

[17] Parish, R.W. (1968) Studies on senescing Tobacco leaf disks with special reference to peroxidase. I. The effect of cutting and inhibitors of nucleic acid and protein synthesis. Planta. 82, 1-13.

[18] Frang, Z., Bouwkamp, J.C. and Solomos, T. (1998) Chylorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *Phaseolus vulgaris* L. J. Exp. Bot. 49(320), 503-510.

[19] Buchanan-Walloston, V. (1997) The Molecular biology of leaf senescence. J. Exp. Bot. 48(307), 181-199.

[20] Arnon, D.I. (1949) Copper enzymes in isolated chloroplast. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24, 1-15.

[21] Lindoo, S.S. and Noodén, L.D. (1976) The interrelation of fruit development and leaf senescence in Anoka Soybeans. Bot. Gaz. 137, 218-223.

[22] Bradford, R. (1976) A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein Dye-binding. Anal. Biochem. 72, 248-254.

[23] Birecka, H., Briber, K.A. and Catalfamo, J.L. (1973) Comparative studies on Tobacco pit and sweet potato root isoperoxidases in relation to injury, indolacetic acid, and ethylene effects. Plant Physiol. 52, 43-49.

[24] Kaur-Sawhney, R., Shih, L.M., Cegielska, T. and Galston, A.W. (1982) Inhibition of protease activity by polyamines. Relevance for control of leaf senescence. FEBS letters. 145, 345-349.

[25] He, Y. and Gan, S. (2002) A gene encoding an acylhydrolase is involved in leaf senescence in Arabidopsis. The Plant Cell. 14, 805-815.

[26] Önder, N. and Yentür, S. (1999) Bitkilerin Büyüme Gelişme Farklılaşma ve Hareket Fizyolojisi. İstanbul Üniversitesi Fen Fakültesi Yayınları. İstanbul.

[27] Yentür, S. (2003) Bitki Anatomisi. İstanbul Üniversitesi Fen Fakültesi Yayınları. İstanbul.

[28] Cherry, J.H., Chroboczek, H., Carpenter, W.J.G. and Richmond, A. (1965) Nucleic acid metabolism in peanut cotyledons. Plant Physiol. 40, 582-587.

[29] Draper, S.R. (1969) Lipid changes in senescing cucumber cotyledons. Phytochem. 8, 1641-1647.

[30] Camp, P.J., Burke, J.J., Huber, S.C. and Moreland, D.E. (1981) Biochemical changes during senescence of wheat leaves. Plant Physiol. 67 supp. 382.

[31] Buttler, R.D. (1967) The fine structure of senescing cotyledons of cucumber. J. Exp.Bot. 18, 535-543.

[32] Martin, C. and Thimann, K.V. (1972) The role of protein synthesis in the senescence of leaves. I. The formation of protease. Plant Physiol. 49, 64-71.

[33] Wittenbach, V.A. (1979) Ribulose Biphosphate Carboxylase and proteolitic activity in Wheat leaves from anthesis through senescence. Plant Physiol. 64(5), 884-887.

[34] Palavan-Ünsal, N., Çağ ,S., Çetin, E. and Büyüktunçer, D. (2002) Retardation of senescence by *meta*-topolin in Wheat leaves. J. Cell and Mol. Biol. 1, 101-108.

[35] Wagstaff, C., Leverentz, M.K., Griffiths, G., Thomas, B., Chanasut, U., Stead, A.D. and Rogers, H.J. (2002) Cysteine protease gene expression and proteolytic activity during senescence of *Alstromeria* petals. J. Exp.Bot. 53(367), 233-240.

[36] Patra, H.K. and Mishra, D. (1979) Pyrophosphatase, peroxidase and polyphenoloxidase activities during leaf development and senescence. Plant Physiol. 63, 318-323.

[37] Skoog, F. (1940) Relationships between zinc and auxin in the growth of higher plants. Amer. J. Bot. 27, 939-951.

[38] Takaki, H. and Kushizaki, M. (1970) Accumulation of free triptophan and triptamine in zinc defficient maize seedlings. Plant and Cell Physiol. 11, 793-804.

[39] Sağlam-Çağ, S. (1997) *Helianthus annuus* L.'da sırasal yaprak senesensinin düzeni üzerine mineral besinlerin ve büyüme regülatörlerinin etkileri. Doktora Tezi. İ.Ü. Fen Bilimleri Enstitüsü. İstanbul.

[40] Pearson, J.N. and Rengel, Z. (1997) Genotypic differences in the production and partitioning of carbohydrates between roots and shoots of wheat grown under zinc or manganase deficiency. Annals of Bot. 80, 803-808.

[41] Mathers, H. (1999) Common toxicity of woody ornamentals. http:// hcs.osu.edu/basicgreen/nutrition/toxicities.htm

[42] Savitsky, P.A., Gazarian, I.G., Tishkov, V.I. and Langrimini, L.M. (1999) Oxidation of indole-3-acetic acid by dioxygen catalysed peroxidases: specificity for the enzyme structure. Biochem. J. 340, 579-583.

[43] Tsai-Chi Li, Teng-Yung Feng, Wen-Shaw Chen and Zin-Huang Liu. (2001) The acute effect of copper on the levels of indole-3-acetic acid lignin in peanut roots. Australian Journal of Plant Physiology, 28(4), 329-334.

[44] Sağlam, S. and Okatan, Y. (1990) Bazı Epigeik Fidelerde Sırasal Yaprak Senesensi Üzerine İncelemeler. X. Ulusal Biyoloji Kongresi, 18-20 Temmuz, Erzurum, 249-257.

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