Biotechnology & Biotechnological Equipment

ISSN: 1310-2818 (Print) 1314-3530 (Online) Journal homepage: https://www.tandfonline.com/loi/tbeq20

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To cite this article: N. Gören & S. Çağ (2007) The Effect of Indole-3-Acetic Acid and Benzyladenine on Sequential Leaf Senescence on *Helianthus Annuus* L. Seedlings, Biotechnology & Biotechnological Equipment, 21:3, 322-327, DOI: 10.1080/13102818.2007.10817467

To link to this article: https://doi.org/10.1080/13102818.2007.10817467

THE EFFECT OF INDOLE-3-ACETIC ACID AND BENZYLADENINE ON SEQUENTIAL LEAF SENESCENCE ON *HELIANTHUS ANNUUS* L. SEEDLINGS

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ABSTRACT

The effect of IAA and BA on the mechanism of senescence which occurs in the cotyledons of Helianthus annuus L. seedlings showing epigenic germination has been investigated. In this study 0,01μM and 10μM IAA solutions were sprayed to the leaves and BA solutions with the same concentrations - to the cotyledons of 16 days-old seedlings. We have determined chlorophyll content, protein amount, peroxidase activity and the changes in the membrane permeability in the cotyledons of the treated and control seedlings. Compared to the control cotyledons IAA in the concentrations of 10μM and 0,01μM caused a breakdown of chlorophyll at the ratio of 64% and 54%, respectively. Soluble protein levels in the IAA at 10μM treatment decreased in the ratio of 12%. IAA treatments with 10μM and 0,01μM stimulated POD activity with about 11 and 8 times, respectively. On the other hand, it has been observated that 10μM and 0,01μM BA treatment retarted senescence, whereas 0,01μM BA and 0,01μM IAA when were applied together enhanced senescence. It has been discussed that IAA which is known as an allosteric activators of cytokinin oxidase stimulates the cytokinin oxidations so it increases senescence that occurs in the cotyledons. In this research, according to the results it has been thought that the senescence signal may be a substance in the nature of IAA.

Keywords: Sequential leaf senescence, auxin, cytokinin, cytokinin oxidase, sunflower (*Helianthus annuus* L.)

Abbreviations: IAA – Indole-3-acetic acid; BA – N^6-Benzyladenine; POD – peroxidase

Introduction

Senescence is an important developmental process in plants that eventually leads to whole plant, organ, tissue and cell death through highly regulated, endogenously controlled degenerative processes (9). Senescence generally occurs without simultaneous growth, following organ maturity. It is influenced by environmental or endogenous (e.g. hormonal) perturbations by initiating or accelerating the different steps of the process: initiation, degeneration, and terminal phase (4, 26). Senescence is controlled by nuclear genes (26, 40). New proteins are synthesized, for example, to mediate degradation and transport, and to maintain cell metabolism (37).

Leaf senescence involves degradation of protein, chlorophyll, nucleic acid, membrane, and subsequent transport of some of the degradation products to other parts of the plant. The yellowing of the leaves due to chlorophyll degradation is the most obvious visible symptom (11). Cotyledon senescence is not fundamentally different from leaf senescence (16). The cotyledons go through the same stages as true leaves – germination, growth, and desiccation – but in a shorter amount of time than it takes true leaves. This aspect of cotyledons makes them a model system for the study of plant development (12, 45).

The first symptoms of senescence are a decline in the photosynthetic rate and an increase in the respiration rate. Other changes follow and include a breakdown of chloroplast membrane, loss of chlorophyll, and metabolism of protein and lipid (41). Chloroplasts are rich reservoirs of proteins, Rubisco, chlorophyll a/b-binding proteins, and membrane lipids. Thus, chloroplasts are degraded first, while the mitochondria and peroxisomes remain functional. Nuclei also remain functional and transcriptionally active, and, although ribosomal degradation occurs, it is not completed until late in senescence (41).

Senescence is regulated by the developmental program of the plant, but it can be modulated by several hormones. Gibberellins, cytokinins and brassinosteroids have been implicated in retarding senescence, whereas ethylene, abscisic acid, and jasmonates are reported to enhance senescence related changes (8, 41). On the other hand there are studies that claim Brassinosteroids promote senescence (15, 49).

This study inspects the senescence process that take place when auxins and cytokinins are applied separately and together.

Materials and Methods

Plant material and hormone treatments: *Helianthus annuus* seedlings have been used as material in the experiments comprising this study. Seedlings were germinated in a moist incubator at 25^0C for 48 hours in the dark and were transferred to soil and placed in a growth chamber which is exposed to 12 hours' photoperiod at 27 ± 2^0C during the day with 10.000 luxes, and 22 ± 2^0C at night. The grade of the senescence in the cotyledons was scored that it was adapted from Lindoo and Noodén (Plastocron Index) (19). The course of the senescence was observed and analyses were made upon application of 10μM and 0,01μM IAA (Sigma) on the leaves, and of 10μM and 0,01μM BA (Sigma) on the cotyledons of the seedlings

both respectively and together. The analyses were made on the day when the Average Green Areas of the cotyledons in the control group of plants reached 50%.

Chlorophyll determination. Pigment was extracted by grinding the cotyledons of sunflower in 80% acetone (v/v) and the total chlorophyll content determined spectrophotometrically (Shimadzu 1601) (1).

Extraction of protein. The cotyledon samples of sunflower seedlings were homogenized with ice-cold 0.1mM sodium phosphate buffer (pH 6.8). The homogenates were then centrifuged at 13,000 rpm for 30 min at 4°C and supernatants were used for determination of total soluble protein content and total POD enzyme assays. Protein content of the extracts was determined according to Bradford (5) using bovine serum albumin as standard.

Peroxidase activity assay. The reaction mixture consisted of 0.25% (v/v) guaiacol in 1ml 0.1M sodium phosphate buffer, pH 7.0, containing 0.1% hydrogen peroxide. 60µl of the crude enzyme extract were added to initiate the reaction which was measured spectrophotometrically at 470nm due to the guaiacol oxidation was recorded for 2min and defined quantitatively as unit mg^{-1} protein (2).

Changes in the permeability of the membrane. In order to measure the changes in the permeability of the membrane, fresh weights of all cotyledons under senescence and green-health cotyledons of (control) seedlings, cotyledons were placed in petri dishes lined with filter paper and containing 8ml of distilled water. Then they were placed in an oven at 25°C for 24, 48 and 72 hours. Absorption values of samples which were removed from the liquid medium in petri dishes at the end of these periods were measured with spectrophotometer at 280nm (32).

Statistical analysis. Each treatment was analyzed with at least three replicate tissue samples bulked at least 20 plants. The data presented here are the mean values ± SE of three independent experiments. Comparison with $P<0.05$ were considered significantly different.

Results and Discussion

Hormones either accelerate or delay senescense in various plant species. For instance, it was determined that GA delayed chlorophyll loss in the leaves, and inhibited RNA and protein destruction (25). Physiological studies showed that cytokinins could inhibit leaf senescence and that internal cytokinin levels

TABLE 1.

The effect of IAA and BA on % of Average Green Area of cotyledons of *Helianthus annuus* seedlings

	AVERAGE GREEN AREAS OF COTYLEDONS (%)								
DAYS	**CONTROL**	**10^{-8} M BA**	**10^{-8} M IAA**	**10^{-8} M IAA-10^{-8} M BA**	**10^{-5} M BA**	**10^{-5} M IAA**	**10^{-5} M IAA-10^{-5} M BA**	**10^{-5} M IAA-10^{-8} M BA**	**10^{-8} M IAA-10^{-5} M BA**
16	100	100	100	100	100	100	100	100	100
17	100	100	100	100	100	100	100	100	100
18	100	100	100	100	100	98.7	100	100	100
19	100	100	98.7	100	100	98.7	100	100	100
20	96.2	100	96.2	97.2	100	92.5	100	100	100
21	95	100	95.9	93	100	85	100	97.2	100
22	90	100	92.5	86.1	100	58.7	98.9	94.4	100
23	88.7	98.7	85	76.2	100	40	98.9	70.8	100
24	85.4	97.5	78.5	60.9	100	23.7	98.9	65.3	100
25	75	72.2	52.7	50.8	100	10.8	93.7	62.7	97.2
26	54.6	70.3	23.4	26.3	95.7	7.5	90.2	55.5	95.8
27	50	62.5	15.6	13.8	93.9	2.5	88.3	40.2	87.5
28	40	52.9	10.9	8.7	89.1	0	79.1	28.4	81.2
29	28.7	45.8	5.2	5	79.3		59.3	16.3	70.9
30	16.2	41.6	0	0	65.2		50	9.8	66.2
31	5	34.7			52.7		37.5	0	45.4
32	0	19.4			41.6		29.1		28.7
33		4.1			23.9		18.7		11.2
34		0			15.2		13.5		10
35					6.5		4.1		2.5
36					3.2		1		0
37					0		0		

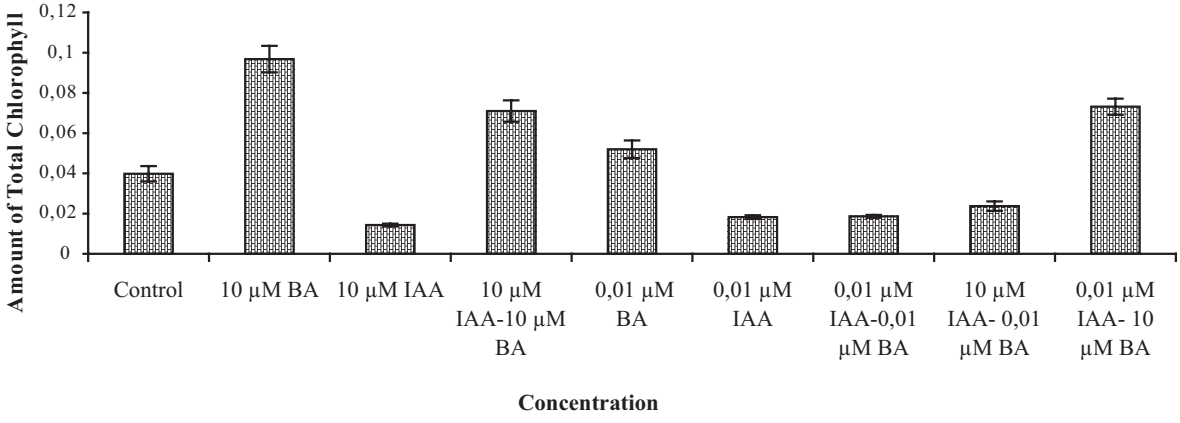

Fig. 1. Comparasion of total chlorophyll content in cotyledons of seedlings that they treated with same and different IAA and BA concentration

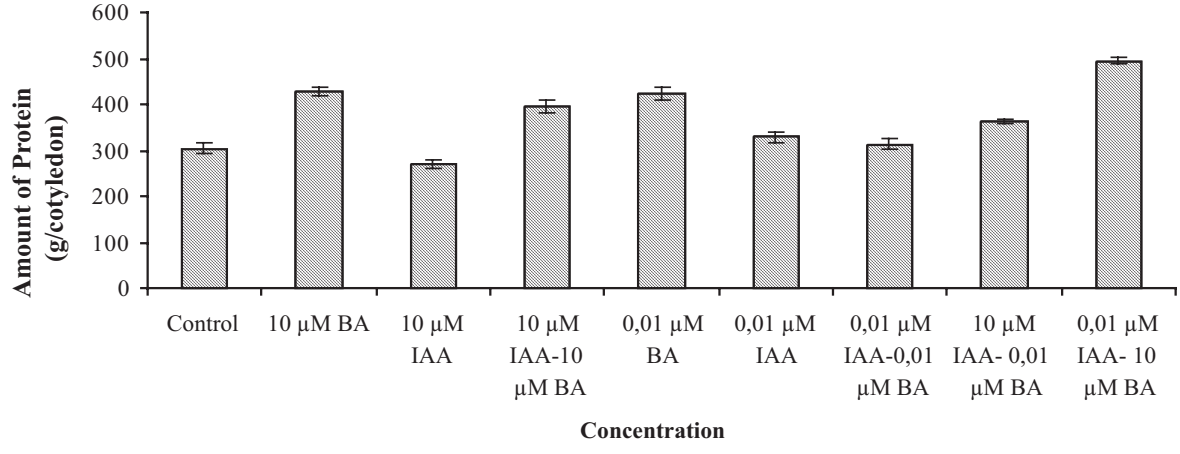

Fig. 2. Comparasion of total protein amount in cotyledons of seedlings that they treated with same and different IAA and BA concentration

decreased as leaf senecesence progressed (13). Synthetic and natural auxins delay senescence in a major part of the tissues (3, 8, 24).

In this study, when 16-days-old seedlings were sprayed with 0,01µM BA on their cotyledons or with 0,01µM IAA on their leaves, there was a delay in the onset and at the end of senescence in the cotyledons of BA-sprayed seedlings. It was observed that senescence accelerated in the cotyledons of seedlings with IAA-sprayed leaves. It was seen that senescence that occurred in the case of co-applied BA and IAA was similar to the case in which IAA was applied on its own. It was understood that co-application of these concentrations did not delay senescence. This is significant. When the same applications were made with 10µM IAA and 10µM BA, it was observed that BA delayed senescence and IAA accelerated the process. In the case of their co-application, it was seen that BA slowed down the accelerative effect of IAA on senescence, and that senescence started later compared to the control group. Resting on these findings, we decided to inspect the interaction between BA and IAA in different concentrations. Upon co-application of 10µM IAA on the leaves and 0,01µM BA on the cotyledons and 0,01µM IAA on the leaves and 10µM BA on the cotyledons, it was seen that auxins and cytokinins in the higher concentration were more effective. These experiments established the accelerative impact of 10µM IAA and the retarding impact of 10µM BA on senescence. These results are shown the **Table 1**.

The total chlorophyll and soluble protein amounts in the experiments mentioned above were determined and shown in **Fig. 1** and **Fig. 2**. The results were seen to be concordant with the above-mentioned observations. An increase of 45% in the chlorophyll amounts and 11% in the protein amounts was observed in the cotyledons which were sprayed with 0,01µM BA compared with the control group. Only with the seedlings which were sprayed with 0,01 µM IAA, and with seedlings to which IAA and BA were co-applied we observed no change and a decrease of 51-52% in the chlorophyll amounts and 15% in the protein amounts were determined compared to the control group. While chlorophyll content in the cotyledons of seedlings which were sprayed with 10µM IAA decreased by 64% and protein amount - by 12% compared to the control group, there was an increase of 143% and 41% in seedlings that were sprayed with 10µM BA in the chlorophyll and protein amounts respectively. In the case of co-application this increase

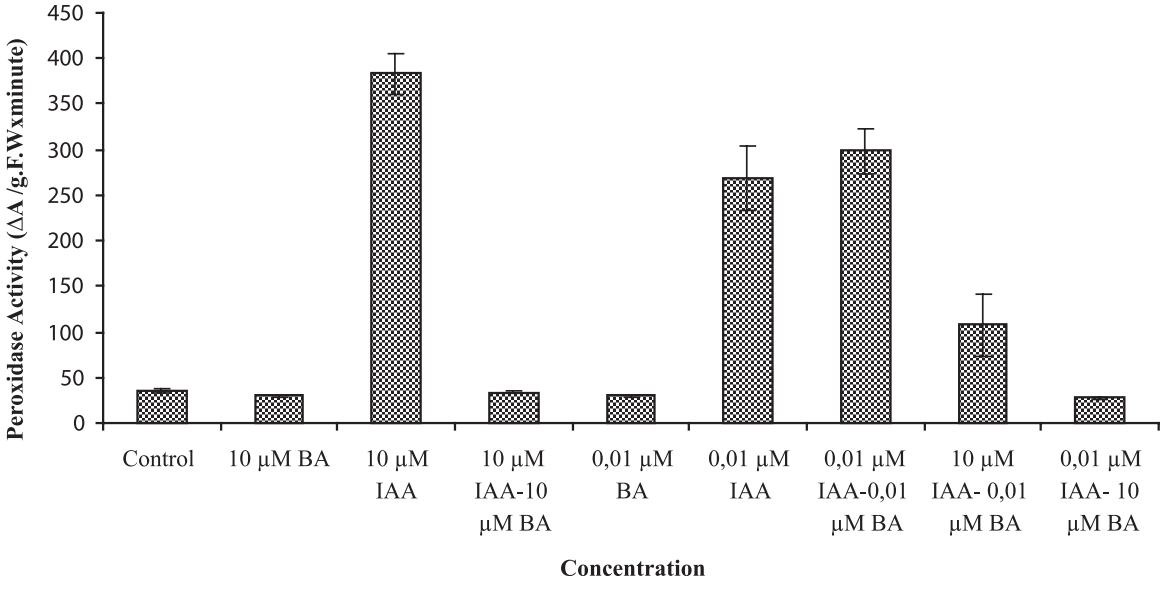

Fig. 3. Comparasion of peroxidase activity in cotyledons of seedlings that they treated with same and different IAA and BA concentration

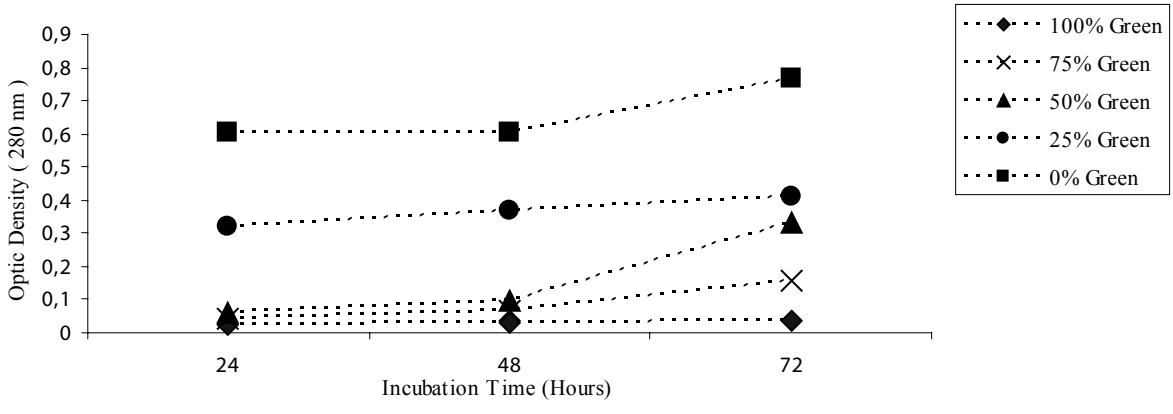

Fig. 4. Comparasion of leaked subtances into the incubation medium from cotyledons of different senescence stage of *Helianthus annuus* seedlings

was 78% for the chlorophyll amount and 30% for the protein amount compared to the control group. While co-application of 10µM IAA and 0,01µM BA resulted in a decrease of 34% in the chlorophyll amount compared to the control, there was an increase of 104% in the chlorophyll and 34% in the protein amounts compared to the control in the cotyledons of seedlings to which 0,01µM IAA and 10µM BA were co-applied. These results have been contrasted with literatures which are expressed that auxins are decreased the chlorophyll loss and protein destruction besides delayed the senescence (10, 29). On the other hand, there are some literatures about auxins increase the senescence (22, 38).

Changes were determined in POD (EC 1.11.1.7) activity during senescence (**Fig. 3**). While some researchers accept the changes in the POD activity as senescence parameter (23, 33), another think on the contrary and does not accept these changes as a senescence parameter (48). As it can be seen from the figure, chlorophyll amount decreased in the cotyledons of seedlings that were sprayed with 0,01µM IAA, while enzyme activity was high (8 times). Similar was the increase in POD activity (9 times) in the cotyledons upon co-application of 0,01µM BA and 0,01µM IAA on the seedlings. While the application of 10µM IAA increased POD activity in the cotyledons 11 times compared to the control group, their co-application brought similar results with the control. Upon co-application of the solutions of 10µM IAA and 0,01µM BA, it was determined that the peroxidase activity in the cotyledons were 3.1 times higher than in the cotyledons of the control group. It seems possible that the POD has functional significiance during the senescence process on the cotyledons of sunflower seedlings. The results obtained from the experiments which were made to determine whether the chlorophyll in the cotyledons, protein destruction and the increase in POD activity were concordant with Plastocron Index are shown in **Table 2**.

Values from the spectrophotometer measurement of dissolved substances in the water medium in which there were

TABLE 2

Plastocron Index

Plastocron Indeks	Chlorophyll Amount (mg / Cotyledon)	Protein Amount (µg/ Cotyledon)	Peroxsidase Activity (ΔA/g.F.W.xMinute)
100	100	100	9
75	70	97	9
50	43	82	10
25	26	73	21
0	18	64	100

cotyledons classified according to their degree of senescence show that there was not a significant change in the amount of substances that leak from the cotyledons with the highest rate of green area (100% green) to their environment, and that it was almost a straight line (**Fig. 4**). There were not substantial changes in all cotyledons in the first 48 hours, but there was a significant increase at the 72nd hour. On the other hand, it was seen that as the percent of green area decreased, there was a rapid increase in the amount of substances that leaked to the incubation medium (at 24th, 48th, and 72nd hour). These increases were attributed to the impairment to the integrity of membrane during senescence.

Membrane damage is characteristic feature of senescence (44). This feature results in the increase of permeability, loss of ionic gradients, and a decrease in the function of important membran proteins such as ion pumps (44).

The substances that leak from the cell membrane to outer medium is deemed to be a good parameter in senescence (14). The increase in leakage is even faster in the last phases of senescence (25). Pigments, sugars, and electrolytes were observed to have leaked during senescence (42). In this study it was determined that the biggest increase in the leakage occurred in cotyledons with 0% green area.

That these experiments show that IAA stimulates senescence contradicts with the retarding effect of auxin on senescence. Besides, Sağlam and Okatan (36) observed that the lower cotyledons of sunflower seedlings which were laid flat had senescence before the upper cotyledons, and this might be because of IAA which is known to have a basipetal movement (17, 27). Taking into consideration that zinc provided IAA stability (39, 43), it was determined that senescence were delayed in the cotyledons of seedlings that were grown without zinc (34). Further, it was determined that physical factors like Triiodobenzoic acid (TIBA) or horizontal klinostat effect that inhibits the bazipetal transport of IAA caused the delay of senescence in the cotyledons (28, 46).

In this study it was seen that BA could not delay senescence in spite of its low concentration upon co-application of 0,01µM IAA and BA. This result contradicted with the other results. Literature studies showed that cytokinins in low concentrations increased etylene biosynthesis (6, 7, 47). In the mentioned experiments, in addition to the IAA solution, the increase of ethylene, the biosynthesis of which is stimulated by the 0,01µM BA, increased senescence. This condition eradicates the contradiction.

A group of researchers stated that a signal is produced in the leaves and moved to the roots which play a major role in senescence (18). This leads us to think that IAA plays a major part in senescence. In a previous study (35) it was determined that ^{14}C-IAA was moved from the stem to the root and then transmitted to the leaves by way of xylem, and that ^{14}C-IAA was not transmitted to cotyledons at an advanced stage of senescence. IAA may accelerate senescence by activating the oxidase enzyme of cytokinin. Likewise, Palni et al. (30) and Lu et al. (21) stated that oxidases played a major part in the regulation of auxins and cytokinins. For the initiation of the senescence, it is required not only a signal, or a hormone, but also a certain receptor to be present.

During senescence the mRNA levels of genes that coding for the proteins that are deemed to be involved in the senescence program increase, while mRNA levels of genes that coding for proteins involved in photosynthesis decrease (20). Likewise, in this study the changes in the protein content of the cotyledons among the control and experiment materials were not as significant as the changes in the chlorophyll content. This was interpreted as the possible synthesis of new proteins during senescence.

Consequently, it was determined that the senescence that occurred in the cotyledons of *Helianthus annuus* L. seedlings was accelerated by the application of 10µM and 0,01µM IAA. It has been discussed that IAA which is known as an allosteric activators of cytokinin oxidase stimulates the cytokinin oxidations so it increases senescence that occurs in the cotyledons. This study show that auxin works as an antagonist with cytokinin in terms of its effect on senescence.

Acknowledgements

The present work was supported by the Research Fund of Istanbul University. Project No. T289-18062003

REFERENCES

1. **Arnon D.I.** (1949) Plant. Physiol., **24**, 1-15.
2. **Birecka H., Briber K.A., and Catalfamo J.L.** (1973) Plant Physiol., **52**, 43-49.

3. **Biswas A.K. and Choudhuri M.A.**(1981) Indian J. Exp. Biol., **16,** 434-436.
4. **Borras L., Maddoni G.A., and Otegui M.E.** (2003) Field Crops. Res., **82,** 13-26.
5. **Bradford, M.** (1976) Anal. Biochem., **72,** 248-254.
6. **Carimi F., Zottini M., Formentin E., Terzi M., and Schiavo F.L.** (2003) Planta, **216,** 413-421.
7. **Cary A.J., Liu W., and Howell S.H.** (1995) Plant Physiol., **107,** 1075-1082.
8. **Chamarro J., Ostin A., and Sandberg G.** (2001) Phytochemistry, **57,** 179-187.
9. **Chandlee J.M.** (2001) Physiol. Plant., **113,** 1-8.
10. **Even-Chen Z., Atsom D., and Itai C.** (1978) Physiol. Plant., **44,** 377-382.
11. **Fang Z., Bouwkamp J.C., and Solomos T.** (1998) J. Exp. Bot., **49,** 503-510.
12. **Fridlender M., S. Lev-Yadun I., Baburek K.A. and Levy A.A.** (1996) Planta, **199,** 307-313.
13. **Gan, S. and Amasino R.M.** (1997) Plant Physiol., **113,** 313-319.
14. **Halevy A.H. and Mayak S.** (1979) Hortic. Rev., **1,** 204-236.
15. **He Y., Tang W., Swain J., Green A., Jack T. and Gan S.** (2001) Plant Physiol., **126,** 707-716.
16. **Kadıoğlu A. and Durmuş N.** (1997) Plant Peroxidase Newsletter, **10,** 51-57
17. **Kerk N.M., Jiang K., and Feldman L.J**.(2000) Plant Physiol., **122,** 925-932.
18. **Lejeune P., Bernier G., Requier M.C., and Kinet J.M.** (1994) Physiol. Plant., **90,** 522-528.
19. **Lindoo S.S., and Noodén L.D.** (1976) Bot. Gaz., **137,** 218-223.
20. **Lohman K.N., Gan S., John C.M., and Amasino R.** (1994) Physiol. Plant., **92,** 322-328.
21. **Lu I.L., Sutter E., and Burger D.** (2001) Plant Growth Regulation, **35,** 161-170.
22. **Mishra S.D., and Gaur B.K.** (1980) Indian J. Exp. Biol., **18,** 297-298.
23. **Mukherjee D., and Rao K.V.M.** (1993) Indian J. Plant Physiol., **36,** 13-16.
24. **Noodén L.D., Kahanak G.M., and Okatan Y.** (1979) Science, **206,** 841-843.
25. **Noodén L.D. and Leopold A.C.** (1988) Senescence and Aging in Plants, Academic Press, San Diego, Ca, 0-12-520920-7.
26. **Noodén L.D., Guiamet J.J., and John I.** (1997) Physiol. Plant., **101,** 746-753.
27. **Normanly J.** (1997) Physiol. Plant., **100,** 431-442.
28. **Okatan Y., ve Ünal M.** (1996) XIII. National Biology Congress, Istanbul, **1**, 22-31 (in Turkish)
29. **Osborne D.J.** (1959) Nature, **183,** 1459-1462.
30. **Palni L.M.S., Burch L., and Horgan R.** (1998) Planta, **174,** 231-234.
31. **Patra H.K., and Mishra D. (**1979) Plant Physiol., **63,** 318-323.
32. **Poovaiah B.W. and Leopold A.C.** (1976) Plant Physiol., **58,** 182-185.
33. **Sağlam-Çağ S.** (2007) Biotechnol.&Biotechnol.Eq., **1**(21), 63-65
34. **Sağlam-Çağ S.** (1999) XVI. International Botanical Congress, St. Lois, USA
35. **Sağlam-Çağ S.** (1997) Ph.D. Thesis. Istanbul University, Institute of Science and Engineering, Istanbul (In Turkish)
36. **Sağlam S., ve Okatan Y.** (1990) X. National Biology Congress, Erzurum, 1, 249-257 (in Turkish)
37. **Sillanpaa M.** (2003) Ph.D. Thesis,University of Helsinki
38. **Singh S., Letham D.S., and Palni M.S.** (1992) Physiol. Plant., **86,** 388-397.
39. **Skoog F.** (1940) Amer. J. Bot., **27,** 939-951
40. **Smart C.M., Hosken S.E., Thomas H., Greaves J.A., Blair B.E., and Schuch W.** (1995) Physiol. Plant., **93,** 673-682.
41. **Srivastava L.M.** (2002) Plant Growth and Development. Hormones and Environment, Academic Press, California, 0-12-660570-X
42. **Suttle J.C., and Kende H.** (1978) Plant Physiol., 62, 267-271.
43. **Takaki H. and Kushizaki M.** (1970) Plant and Cell Physiol., **11,** 793-804.
44. **Thompson J.E., Froese C.D., Madey E., Smith M.D., and Hong Y.** (1998) Prog. Lipid Res. **37,** 119-141
45. **Tsuyaka H., Tsuge T., and Uchimiya H.** (1994) Planta, **195,** 309-312
46. **Ünal M., ve Okatan Y.(**1996) XIII. National Biology Congress, Istanbul,**1**, 32-41 (in Turkish)
47. **Vogel J.P., Schuerman P., Woeste K.E., Brandstatter I., and Kieber J.J.** (1998) Genetics, **149,** 417-427.
48. **Yanova P., Guleva E., Zozikova E., and Kotseva E.** (2001) Bulg. J. Plant Physiol., **27,** 54-7.
49. **Zhao Y.J., Xu R.J., and Luo W.H.** (1990) Chin. Sci. Bull., **35,** 928-931.