C.Ü. Fen-Edebiyat Fakültesi
Fen Bilimleri Dergisi (2007)Cilt 28 Sayı 1

Role of Abscisic Acid and Proline Treatment on Induction of Antioxidant Enzyme Activities and Drought Tolerance Responses of *Laurus nobilis* L. Seedlings

Lale YILDIZ AKTAŞ[1] Bengü TÜRKYILMAZ[1] Hülya AKÇA[2] Salih PARLAK[2]

[1]Ege University, Faculty of Science, Department of Biology, 35100 Bornova, İzmir
[2]Ege Forestry Research Institute, Zeytinalanı 35315 Urla, İzmir
e-mail: Lale.yildiz@ege.edu.tr

Received: 19.03.2007, Accepted: 22.05.2007

Abstract: The purpose of this study was to investigate exhibited physiological and biochemical drought tolerance responses of *Laurus nobilis* L. seedlings by abscisic acid (ABA) and proline treatment to dormant seeds. Abscisic acid and proline was applied to the dormant seeds by imbibitions in sterile water containing 100µM ABA, 1mM proline and ABA + proline combination and maintained at +4°C for 3 weeks. Seedlings were obtained from 4 week stratified seeds sown in pots and were watered regularly during six month. In the leaves of seedlings, the highest activities of superoxide dismutase and catalase were observed in ABA treated and the highest peroxidase activity was measured in the leaves of ABA + proline combination treated group. ABA caused induction of drought tolerance responses was determined by enhancement of paraquat tolerance, proline content, and decreasing of leaf area compared to control. The ABA treatment to dormant seeds can be use for providing drought tolerance ability to seedlings of laurel and its negative effect on growth can be reversed by proline treatment in combination with ABA.
Key words: *Laurus nobilis* L., abscisic acid, proline, drought, antioxidant enzyme.

Absisik Asit ve Prolin Uygulamasının *Laurus nobilis* L. Fidelerinde Antioksidant Enzim Aktivitelerinin ve Kuraklığa Tolerans Tepkilerinin Uyarılması Üzerindeki Rolü

Özet: Bu çalışmanın amacı, *Laurus nobilis* L. tohumlarına absisik asit (ABA) ve prolin uygulanması ile fidelerde beliren fizyolojik ve biyokimyasal kuraklığa tolerans tepkilerinin araştırılmasıdır. Absisik asit ve prolin uygulaması, tohumların 100µM ABA, 1mM prolin ve ABA + prolin kombinasyonu içeren steril su içerisinde +4°C'da 3 hafta süreyle tutulmasıyla yapılmıştır. Dört hafta katlanmış olan tohumlardan elde edilen fideler saksılara alınarak 6 ay süresince düzenli olarak sulanmıştır. En yüksek süperoksid dismutaz ve katalaz aktivitesi ABA uygulanan grupta, en yüksek peroksidaz aktivitesi ise ABA + prolin kombinasyonunun uygulandığı grubun yapraklarında belirlenmiştir. Absisik asitin kuraklığa tolerans tepkilerini uyarma etkisi; kontrole göre, paraquat toleransı ve prolin içeriğinin artması ile yaprak alanın azalması olarak belirlenmiştir. Tohumlara ABA uygulanması, defne fidelerin kuraklığa toleransının artırılması için kullanılabilir ve uygulamanın büyüme üzerindeki negatif etkisi prolinin ABA ile kombine kullanımıyla giderilebilir.

Anahtar kelimeler: *Laurus nobilis* L., absisik asit, prolin, kuraklık, antioksidant enzim.

Introduction

Plants are subjected to several biotic and abiotic stresses that adversely affect growth, metabolism and yield [1]. Among these, drought is a major abiotic stress, limiting crop production in arid and semi-arid climates. The Mediterranean climate shows a strong seasonality in water availability and temperature [2]. Irradiance and temperature are high during summer, but precipitation is minimal. Accordingly, the dry, hot and cloudless summer, with its high evaporative demand, is the most stressful period for the local flora [3].

Drought stress is known to inhibit photosynthetic activity in tissues due to an imbalance between light capture and its utilization [4]. Down regulation of photosystem II (PSII) activity results in an imbalance between the generation and utilization of electrons, apparently resulting in changes in quantum yield. These changes in the photochemistry of chloroplasts in the leaves of drought stressed plants result in the dissipation of excess light energy in the PSII core and antenna, thus generating reactive oxygen species (ROS-O_2^-, $^1O_2$, H_2O_2, OH,), which are potentially dangerous under drought stress conditions [5]. As a result of this accelerated production of active oxygen via chloroplast Mehler reaction [6], plants protect themselves increasing scavenging

capacity of ROS [7], by increasing activity of antioxidant enzymes / and or antioxidant molecules resulting in tolerance against the drought stress. Induction of oxidative stress in drought stressed plants has also been well known [7, 8, 9].

The plant hormone ABA is produced *de novo* under water deficit conditions and plays a major role in response and tolerance to dehydration [10]. ABA besides regulating stomatal opening [11] and root hydraulic conductivity [12, 13] has also been reported to induce tolerance to different abiotic stresses including drought, salinity and low temperature [10, 14, 15]. Exogenous application of ABA has been reported to significantly increase in the activities of enzymatic antioxidants; superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APOX) and glutathione reductase (GR) and the contents of non-enzymatic antioxidants; ascorbate, reduced glutathione, tocopherol and carotenoids [16]. Proline is a compatible solute involved in cell osmoregulation and protection of proteins during dehydration [17]. It also acts as a free radical scavenger and may be more important in overcoming stress than in acting as a simple osmolyte [10, 18].

Laurus nobilis L. (laurel) is a slow growing natural member of Mediterranean Region vegetation. De Lillis [19] reported to laurel as a drought tolerant species, but young seedlings have shallow root systems and can therefore be more sensitive to drought stress than adults like other members of the Mediterranean Region [20]. These species are vulnerable to drought stress in early phases of the development. Although ecological characteristics and hydraulic architecture of laurel were well established [21, 22], there is only research study conducted about antioxidant metabolites and enzymes to oxidative stress of the species caused by O_3 exposure reported by [23].

The leaves and fruits of *Laurus nobilis* have commercial importance and world consumption is increasing every year by 5% [24]. The plant drought tolerance potential must be improved until the young plants are established in the soil, for the purpose of reforestation of damaged land in South and West Coasts of Anatolia and to increase the production of laurel. To the best of our knowledge there is no previous study in which antioxidant enzymes and biochemical traits of tolerance mechanisms against drought have been measured in *L. nobilis*.

For this purpose, the research focused on induced exhibition of different physiological and biochemical drought tolerance responses and of *Laurus nobilis* L.

seedlings by exogenous abscisic acid and proline application to the dormant seeds by investigating photosynthetic characteristics, membrane stability, paraquat tolerance, proline accumulation and antioxidant enzymes SOD, CAT and POX.

Materials and Methods

Plant material and treatments

Laurus nobilis L seeds were obtained from natural growing population in Amberseki region in Karaburun – Izmir on the coast of Aegean Sea. Seeds were dried to a moisture content of 10% and stored at +4 °C in sealed jars. The pericarp was manually removed, and seeds were previously sterilized in 1% (v/v) sodium hypochlorite before imbibitions in sterile water containing 100µM abscisic acid, 1mM proline and 100 µM ABA + 1mM proline. Seeds were maintained in the different media at +4°C for 3 weeks. Seedlings were obtained from 4 week stratified seeds sown in pots containing a mixture of equal parts of peat, sand, forest soil and fertilizer in the Aegean Forestry Research Institute nursery. Treated and untreated seedlings were watered regularly during six month. At the end of growing period (6 month); harvested leaves were separated harvested for relative water content, electrolyte leakage analyses and a paraquat tolerance determination test, remaining leaves were frozen in liquid nitrogen and stored at – 20 °C for other analyses.

Paraquat treatment of leaf tissue

Paraquat treatment was conducted on the fully expanded second top leaf taken from the seedlings. Leaf samples were treated by floating the excised leaves on sterile water containing 100 µM PQ under a light intensity of 12.000 lux for 24 h [25]. Leaf extracts were then analyzed for the determination of the total chlorophyll content.

Leaf chlorophyll content

Paraquat treated and other leaf samples were extracted with 80% acetone and absorbance of supernatants were measured spectrophotometrically. Chlorophyll a was determined at wavelength 663 nm and b at 645 nm, and total chlorophyll at 652 nm and carotenoids were determined at 450 nm following the method reported by Linchtenthaler [26].

Leaf relative water content (RWC)

Leaf relative water content (RWC) was estimated according to the method of Ekanayake et al., [27]. Leaf material was weighed (4 leaves) to determine fresh weight and placed in distilled water at +4 °C for 19 h and turgid weight was recorded. Finally the samples were dried in an oven at 65-70 °C for 48 h and dry weights were recorded. RWC was calculated as:

RWC = [(fresh weight- dry weight) / (turgid weight- dry weight)] x 100

Leaf area

Using the fully expanded 2nd leaf from the top of seedlings, the leaf area was determined. The measurements were taken with a common desk-top scanner and software (Flaeche.exe) on the base of method of O'Neal et al., [28].

Proline assay

Proline content was determined according to the modified method of Bates et al., [29]. Five hundred milligrams of leaves were homogenized in 2 ml of 3% sulfosalicylic acid solution using tissue homogenizer. The homogenate was then centrifuged at 13.000 g for 10 min. One milliliter of the supernatant was then added into a test tube to which 1 ml of glacial acetic acid and 1 ml of freshly prepared acid ninhydrin solution were added (1.25 g ninhydrin dissolved in 30 ml of glacial acetic acid and 20 ml of 6 M orthophosphoric acid). Tubes were incubated in a water bath for 1 h at 100 °C and then allowed to cool to room temperature. Two milliliters of toluene was added and mixed on a vortex mixture for 20 s in a fume hood. The test tubes were allowed to stand for at least 10 min to allow the separation of toluene and aqueous phase. The absorbance of toluene phase was measured at 520 nm in a spectrophotometer. The concentration of proline was calculated from a proline standard curve. The concentration of proline was expressed as µmol/g FW.

Soluble protein and enzyme assays

For SOD activity measurements, one gram of leaves were homogenized in 5 ml of 0.05 M Na phosphate buffer (pH 7.8) including 1 mM EDTA and 0.2 g 1 x 8(200x 400 mesh) Dowex. Homogenate for peroxidase activity was prepared by grinding one

gram of leaf tissue in 3 ml of 0.05 M tris-glicine buffer (pH 8.3) containing 17% sucrose and 0.2 g 1 x 8 (200x 400 mesh) Dowex. For catalase activity measurements of one gram of leaves were homogenized in 3 ml of 0.05 M Na phosphate buffer (pH 7.6) including 1 mM EDTA and 0.2 g 1 x 8(200x 400 mesh) Dowex. The whole homogenization process was carried out in an ice bath. Homogenates were centrifuged at 13.000 x g for 40 min at +4 °C. Supernatants were used for enzyme activity and protein content assays. Total soluble contents of enzyme samples were determined according to Bradford [30] using BSA as a standard.

Superoxide dismutase activity (SOD) assay was based on the method of Beauchamp and Fridovich [31] which measures the inhibition in the photochemical reduction of *p*-nitroblue tetrazolium chloride (NBT) spectrophotometrically at 560 nm. One unit SOD activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT. The reaction mixture contained 50 mM Na phosphate buffer (pH 7.8), 33 μM NBT, 10 mM L-Methionine, 0.66 mM EDTA and 0.0033 mM Riboflavin.

Catalase activity (CAT) was measured according to the method of Bergmeyer [32] by the determination of the disappearance of H_2O_2 and by measuring the decrease in an absorbance at 240 nm. The reaction mixture contains 0.05 M phosphate buffer (pH 7.0) with 1mM EDTA and 3% H_2O_2. One enzyme unit was defined as μmol H_2O_2 destroyed per minute.

Peroxidase activity (POX) was determined according to the Herzog and Fahimi [33] method. The activity was measured by the increase in absorbance at 465 nm, by the rate of formation of oxidized diaminobenzidine-tetrahydrochoride dihydrate (DAB). The reaction mixture contained DAB solution and 0.6% H_2O_2. One enzyme unit was defined as μmol ml^{-1} destroyed H_2O_2 per minute.

Statistical analysis

The data presented are means of two different experiments, each including at least four replications. Statistical analysis was carried out with the SPSS statistical computer package (SPSS for WINDOWS, standard version, 6.1). Experimental data were analyzed with the protected least significant difference (LSD) test at $P<0.05$ level.

Results

PQ tolerance

The water stress effect on the physiology of plants is very similar to the stress caused by PQ (a bypridlium herbicide), which leads to the production of highly toxic free radicals generated by reaction of molecular oxygen with PQ radicals formed in the chloroplast during photosynthesis [34]. Therefore, a close correlation is expected between the plant's tolerance to stresses imposed by water and PQ. The level of PQ tolerance was estimated by measuring loss of chlorophyll after PQ treatment because reduction in the amount of chlorophyll after PQ application was reported to be a good indicator of PQ tolerance [35]. While, leaves of the 100 µM ABA + proline treated group showed the highest PQ tolerance, control leaves had the least PQ tolerance (0.79 ± 0.25 mg chlorophyll g^{-1} fresh weight) according to the total chlorophyll level (1.93 ± 0.5 mg chlorophyll g^{-1} fresh weight) (Table 1). Other treated leaves were significantly ($P<0.05$) less damaged by PQ treatment, chlorophyll content of proline and 100 µM ABA and treated group was over the control about 158%, 166% and 209%, respectively.

Leaf chlorophyll content

The total chlorophyll content of the leaves of control plants were significantly ($P<0.05$) higher than single treatment of ABA (Table 1). While, the proline and ABA + proline treatments to the seeds caused a slight decrease in leaves of seedlings, it was not significant ($P<0.05$). Carotenoid contents also decreased significantly in 100 µM ABA treated leaves of seedlings compared to control about 36.6%. Moreover, there were no significant differences among treatments of proline and ABA + proline and control.

Leaf relative water content (RWC)

Relative water content of leaves was calculated around 95.2 ± 2.1 showing no difference between control and treated plants (data not shown). In addition, there were no significant changes in fresh and dry weights of leaves of seedlings between the control and treated groups.

Leaf area

The highest leaf area was measured in the ABA + proline treated group as 8.7 ± 0.5 cm^2 (Table 1). The leaf area of seedlings in untreated control and proline treatment were less than ABA + proline treated group but this slight reduction was not statically significant ($P<0.05$). In contrast, 100 µM ABA treatments caused a significant decrease in leaf area of seedlings about 27% in comparison with control.

Leaf proline content

The highest value of leaf proline content was found in the proline treated plants as 0.52 ± 0.03 µmol g^{-1} fw, it was about 4.33-fold over the control (Table 1). ABA and ABA + proline treatments caused to increase in leaf proline content compared with the control, about 3.1-fold and 2,8-fold respectively.

Table 1. Effect of abscisic acid and proline treatment to dormant seeds of laurel, on paraquat tolerance as total chlorophyll content dependent of paraquat treatment, total chlorophyll (chl) and carotenoid content, leaf area and proline content of leaves of 6-month-old laurel seedlings. Means ± SE (n = 4).

Treatment	PQ tolerance (mg g^{-1} FW)	Total chl (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)	Leaf area (cm^2)	Proline content (µmol g^{-1})
Control	0.79 ± 0.25[a]	1.93 ± 0.50[a]	6.55 ± 0.65[a]	8.45 ± 0.45[a]	0,12±0,02[a]
Proline	1.25 ± 0.20[b]	1.47 ± 0.2[a]	4.56 ± 0.53[a]	8.20 ± 0.4[a]	0,52±0,03[b]
ABA	1.31 ± 0.15[b]	1.15 ± 0.2[b]	4.15 ± 0.40[b]	6.21 ± 0.7[b]	0,37±0,01[c]
ABA + Proline	1.65 ± 0.45[b]	1.72 ± 0.3[a]	5.1 ± 0.71[a]	8.7 ± 0.5[a]	0,34±0,05[c]

Different letters denote significant differences among treatments ($P < 0.05$)

Antioxidant enzyme activity

ABA and ABA + proline treatments to seeds of laurel caused significant increase in superoxide dismutase, catalase and peroxidase activities of the leaves of 6-month-old seedlings in comparison with control (Fig.1a,b,c).

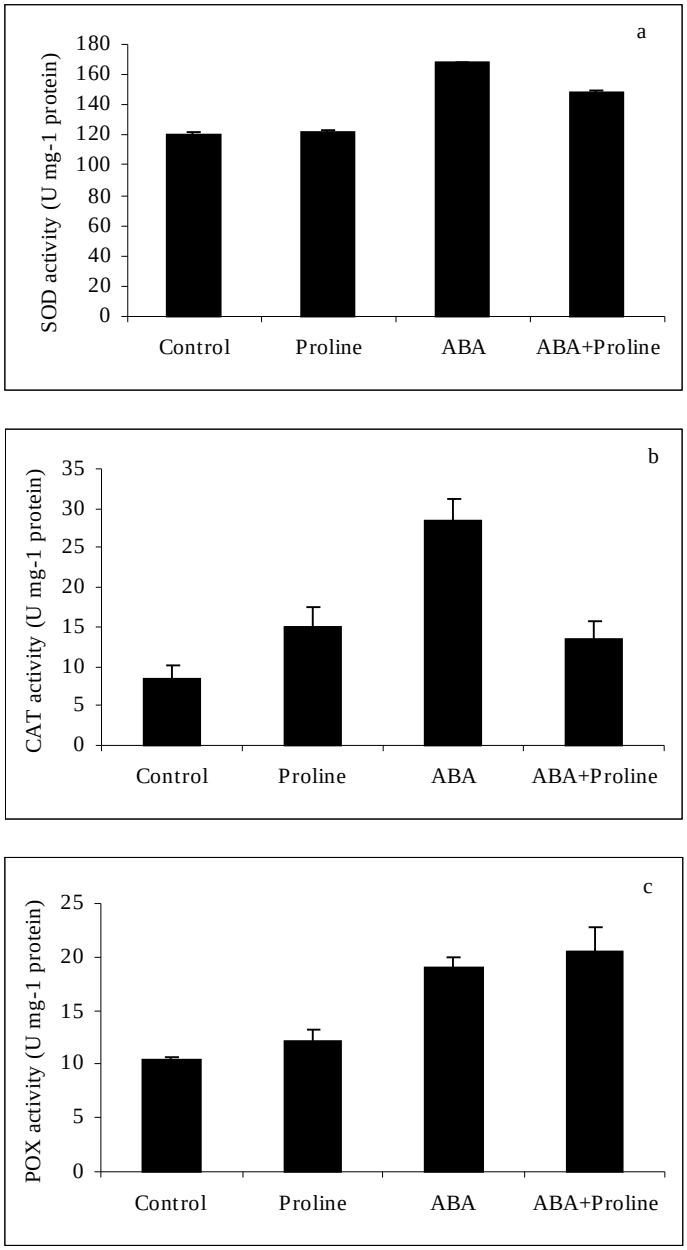

Figure 1. Effect of abscisic acid and proline treatment to dormant seeds of laurel on the activity of superoxide dismutase (a), catalase (b) and peroxidase (c) in leaves of 6- month-old laurel seedlings. Means ± SE (n = 4).

Although SOD activity was observed constitutively high in control leaves of *Laurus nobilis* seedlings as 120.6 ± 0.6 U mg^{-1} protein, ABA and ABA + proline combination caused significant increase in SOD activity compared with control about 1.39- and 1,23-fold, respectively (Fig. 1a). While the activity of SOD enzyme in proline treated group was higher than control, the difference was not statically significant.

ABA treatment was very effective treatment for inducing an increase in CAT activity about 3.38-fold compared with control (Fig. 1b). Although proline and ABA + proline combination led to higher CAT activity by about 1.77- and 1.60-fold than control leaves of laurel seedlings, this was not statically significant.

The highest values of POX activity were observed in leaves of ABA + proline treated group as 20.44 ± 2.28 U mg^{-1} protein (Fig. 1c). While the activity was significantly induced by single ABA treatment more than 1.8-fold, proline treatment was not caused significant change in POX activity in comparison with control.

Discussion

In this study, it has been shown that exogenous ABA and proline treatment to dormant seeds of *Laurus nobilis* L. significantly affected physiological and biochemical traits of 6-month-old seedlings, by inducing the responses against drought, in a well watered condition.

High photosynthetic pigment content was observed in control seedlings of laurel. Proline, ABA and ABA + proline combination provide drought recognition in seedlings exhibiting stress tolerance characteristics causing loss of chlorophyll pigment in treated groups even in well watered conditions. Results from the treated group were the same with drought effects in photosynthesis and are in agreement with the previous reports [4, 36]. This case obviously shows that the responses to drought have a tight connection to the recognition of ABA signals and accumulation of ABA in tissue.

Although photosynthetic characteristics were negatively affected in treated groups in unstressed conditions, this case caused an advantage in PQ treatment which was applied for mimicking drought stress conditions to plants, causing only slight damage in the same group. The ABA treatment to seeds induced drought tolerance of laurel seedlings evidenced by a reduction in PQ damage which was caused by the generation of active oxygen species. The results is correlative with results of Altinkut et al., [25] suggesting that enhanced tolerance to PQ could be used for selection criteria of drought tolerant genotypes.

The ABA induced PQ tolerance of seedlings was highly correlative with leaf area reduction and enhanced antioxidant enzymes SOD, CAT and POX activity. The results were supported by reports of Shikanai et al., [36] for transgenic tobacco and

Iturbe-Ormaetxe et al., [38] for pea plants demonstrated severe water deficit and PQ treatment led to identical results in protection against photooxidative stress with antioxidant enzyme. Agarwal et al., [39] reported a decrease in oxidative stress in ABA treated in wheat cultivars. Decreased oxidative stress by ABA treatment also reported in other research [40, 41, 42].

ABA treatment to dormant seeds caused leaf area reduction as a drought stress response, and the 100 µM ABA + proline combination acted synergistically for preventing both drought damage and growth retardation in laurel seedlings. Similar to our results, Yin et al., [43], have been pointed out that exogenous ABA application significantly affected morphological and physiological properties such as decreasing leaf area under both well-watered and water-stressed conditions in two poplar species.

In unstressed conditions (RWC, 95.2% ± 2.1), proline content was induced by the proline, ABA and ABA + proline combination. In this experiment, 100 µM ABA level was seemed sufficient for inducing proline accumulation and ROS scavenging enzymes of laurel seedlings. Although role of ABA in regulating proline accumulation has been matter of some confusion [44], it is demonstrated that ABA accumulation and changes in ABA sensitivity are important and / or required for the regulation of proline accumulation in ABA–deficient mutant and ABA insensitive-mutants of *Arabidopsis thaliana* [18]. This result was parallel to our results showing ABA-dependent proline accumulation in the leaves of laurel seedlings. Moreover, data from ABA + proline combination indicates that ABA and proline act synergistically for inducing proline accumulation is important during water limited conditions as well as recovery from the stress.

ABA at 100 µM, was the most effective treatment for inducing antioxidant enzymes SOD, CAT and POX activities. Among observed antioxidant enzymes, SOD and CAT showed maximum response to ABA, confined with the results of Agarwal et al., [39] reported the increase in activity of other enzymes was also observed, though not as marked as for SOD. Although many stress situations cause an increase in the total foliar antioxidant activity [45], several reports emphases enhanced stress tolerance related to overproduction of chloroplastic SOD [46, 47]. The effects of ABA for triggering adaptive responses have been previously reported [14, 10, 39]. ABA induced SOD, CAT; activities have also been reported by Jiang and Zhang [16, 41].

The combined action of CAT and SOD converts the toxic superoxide radical ($O_2.^-$) and hydrogen peroxide (H_2O_2) to water and molecular oxygen (O_2), thus averting the cellular damage under unfavourable conditions like water stress [8, 9]. Significantly high activities of antioxidant enzymes in the control group leaves clearly demonstrate that very efficient antioxidant system was functioning in laurel seedlings.

In conclusion, drought tolerance responses such as decreasing pigment contents, reduction of leaf area, proline accumulation and inducing antioxidant activity were demonstrated as ABA-regulated and / or dependent responses in *Laurus nobilis* L. The ABA treatment to dormant seeds can be use for providing drought tolerance ability to seedlings of laurel and its negative effect on growth can be achieved by proline treatment in combination with ABA.

References

[1] H.K. Lichtenthaler, *J. Plant Physiol.* 1996, 148, 4-14

[2] F. Di Castri, H.A. Mooney, *Mediterranean Type Ecosystems: origin and structure,* Springer, 1973, p. 150

[3] H.A. Mooney, *Mediterranean Type Shrublands-Ecosystems of the World*, In: F. Di Castri, D.W. Goodall, R.L. Specht, Elsevier, 1981, p. 249-255

[4] C.H. Foyer, G. Noctor, *New Phytol.* 2000, 146, 359-388

[5] D. Peltzer, E. Dreyer, E. Polle, *Plant Physiol.Bioc.* 2002, 40,141-150

[6] K. Asada, *Annu. Rev. Plant Phys.* 1999, 50, 601-639

[7] J. Mano, *Oxidative stress in plants*, In: D. Inze, M.V. Montago, Taylor and Francis, 2002, p. 217-245.

[8] A. Ramachandra Reddy, K.V. Chaitanya, D. Sundar, *J. Seri. Sci. Jpn.* 2000, 69,169-175

[9] K.V. Chaitanya, D. Sundar, S. Masilamani, A. Ramachandra Reddy, *Plant Growth Regul.* 2002, 36, 175-180

[10] A. Ramachandra Reddy, K.V. Chaitanya, M. Vivekanandan, *J. Plant Physiol.* 2004, 161, 1189-1202

[11] W. Hartung, S. Wilkinson, W. Davies, *J. Exp. Bot.* 1998, 49, 361-367

[12] E Hose, D.T. Clarkson, E. Steudle, L. Schreiber, W. Hartung, *J. Exp. Bot.* 2001, 52, 2245-2264

[13] S. Wilkinson, W.J. Davies, *Plant Cell Environ.* 2002, 25, 195-210

[14] J. Giraudat, F. Parcy, N. Bertauche, F. Gosti, J. Leug, P.C. Morris, M. Bouvier-Durand, N. Vartanian, *Plant Mol. Biol.* 1994, 26, 1557-1577

[15] V. Chandrasekar, R.K. Sairam, G.C. Srivastava, *J. Agron. Crop Sci.* 2000, 185, 219-227

[16] M.Y. Jiang, J.H. Zhang, *Plant Cell Physiol.* 2001, 4, 1265-1273

[17] D. Rontein, G. Basset, A.D. Hanson, *Metab. Eng.* 2002, 4, 49–56

[18] P.E. Verslues, E.A. Bray, *J. Exp. Bot.* 2006, 57, 201-212

[19] M. De Lillis, *Braun-Blanquetia.* 1991, 7, 127-139

[20] M.A. Lo Gullo, S. Salleo, R. Rosso, P. Trifilo, *Plant Soil*, 2003, 250, 259-272

[21] U. Niinemets, A, Cescatti, M. Rodeghiero, T. Tosens, *Plant Cell Environ.* 2005, 28,1552-1566.

[22] S. Salleo, M.A. Lo Gullo, F. Raimondo, A. Nardini, *Plant Cell Environ.* 2001, 24, 851-859

[23] C. Nali, E. Paoletti, R. Marabottini, G. Della Rocca, G. Lorenzini, A.R. Paolacci, M. Ciaffi, M. Badiani, *Atmos. Environ.* 2004, 38, 2247-2257

[24] Reports of Aegean Exporters' Associations, *http://www.egebirlik.org.tr* , 2005

[25] A. Altinkut, K. Kazan, Z. Ipekci, N. Gozukirmizi, *Euphytica*, 2001, 121, 81-86

[26] H.K. Lichtenthaler, *Method. Enzymol.* 1987, 148, 350–382

[27] I.J. Ekanayake, S.K. De Datta, P.L. Steponkus, *Ann. Bot.* 1993, 72, 73-80

[28] M.E. O'Neal, D.A. Landis, R. Isaacs, *J. Econ. Entomol.* 2002, 95, 1190-1194

[29] L.S. Bates, R.P. Waldern, I.D. Teare, *Plant Soil* 1973, 39, 205-207

[30] M. Bradford, *Anal. Biochem.* 1976, 72, 248-250

[31] C. Beauchamp, I. Fridovich, *Anal. Biochem.* 1971, 44, 276-287

[32] N. Bergmeyer, *Methoden der Enzymatischen Analyse,* Akademie Verlag, 1970, vol.1, p. 636-562

[33] V. Herzog, H. Fahimi, *Anal. Biochem.* 1973, 55, 554-562

[34] A.D. Dodge, *Endeavour* 1971, 30, 130-135

[35] I. Cakmak, H. Marschner, *Plant Cell Environ.* 1992, 15, 955-960

[36] C.M. Lu, J.H. Zhang, *J. Exp. Bot.* 1999, 50, 1199-1206

[37] T. Shikanai, T. Takeda, H. Yamauchi, S. Sano, K. Tomizawa, A. Yokota, S. Shigeoka, *FEBS Lett.* 1998, 428, 47-51

[38] I. Iturbe-Ormaetxe, P.R. Escuredo, C. Arrese-Igor, M. Becana, *Plant Physiol.* 1998, 116, 173-181

[39] S. Agarwal, R.K. Sairam, G.C. Srivastava, R.C. Meena, *Biol. Plant.* 2005, 49, 541-550

[40] M. Gong, Y.J. Li, S.Z. Chen, *J. Plant Physiol.* 1998, 153, 488-496

[41] M.Y. Jiang, J.H. Zhang, *Free Radical Res.* 2002, 36, 1001-1015

[42] J. Zhang, W. Jia, J. Yang, A.M. Ismail, *Field Crop. Res.* 2006, 97, 11-119

[43] C. Yin, B. Duan, X. Wang, L. Chunyang, *Plant Sci.* 2004, 167, 1091-1097

[44] P.D. Hare, W.A. Cress, J. Van Staden, *J. Exp. Bot.* 1999, 50, 413-434

[45] G. Pastori, P. Mullineaux, C.H. Foyer, *Plant Physiol.* 2000, 122, 667-675

[46] A.C.M. Arisi, G. Cornic, L. Jouanin, C.H. Foyer, *Plant Physiol.* 1998, 117, 565-574

[47] C.H. Foyer, *Oxidative stress in plants.* In: Inze, D., Montago, M.V., Taylor and Francis, 2002, p. 33-68.