

African Journal of Biotechnology Vol. 10(32), pp. 5972-5978, 4 July, 2011
Available online at <http://www.academicjournals.org/AJB>
DOI: 10.5897/AJB10.2114
ISSN 1684-5315 © 2011 Academic Journals

Full Length Research Paper

Antioxidative and proline potentials as a protective mechanism in soybean plants under salinity stress

Mahmut Doğan

Harran University, Faculty Science, Department of Biology, Osmanbey Campus, Şanlıurfa, Turkey.
E-mail: dogan@harran.edu.tr.

Accepted 27 May, 2011

Stress can define as all negative factors affecting plant growth. One of the most important problems among stress factors is salt stress. Antioxidant responses are tested in Soybean (*Glycine max.* L.) cv., "A3935" grown under 0, 50, 100 and 150 mM NaCl in order to investigate the plants protective mechanisms against salt induced oxidative stress. The NaCl treatments are repeated in four different stages of that is 10, 20 and 30 days after sowing (DAS). The plants were uprooted randomly on 30 DAS and the enzymatic antioxidant potentials were analyzed. The fresh and dry weights and the chlorophyll content are reduced with increasing NaCl concentration. Proline concentration increased at high salinity compared to untreated plants. Moreover, salinity induces a significant decline in superoxide dismutase (SOD, EC 1.15.1.1), glutathione reductase (GR, EC 1.6.4.2), ascorbate peroxidase (APX, EC 1.11.1.11) and catalase (CAT, EC 1.11.1.6) activities may be of great importance in H₂O₂ intoxicating mechanism under oxidative stress. As expected Na⁺ and Cl⁻ concentrations in leaf and roots are higher at high salinity treatments compared to untreated plants. The concentrations of K⁺ are reduced with increasing NaCl concentrations.

Key words: Antioxidants, antioxidant enzyme, salinity, soybean, proline.

INTRODUCTION

All the factors that inhibit plant growth are defined as stresses. Drought, saltiness, excess irrigation, high or low temperature, pH and heavy metals are common sources of stress. Those stresses create social and economic problems, especially in developing countries. Environmental factors such as biotic and abiotic factors influence the characters, composition, growth and development of individual plants and plant communities. When any of these environmental factors exceeds the optimum tolerance of a plant, it produces stress to the plant influenced by the developmental, structural, physiological and biochemical processes of the plant. Soil salinity is one among these environmental stresses (Lawlor, 2002). Every year more and more land becomes non-productive owing to salt accumulation. The major effect of salinity is the inhibition of crop growth by the reduced hormone delivery from root to leaves (Azooz et al., 2004). Salt alters a wide array of metabolic processes culminating in stunted growth, reduced enzyme activities and biochemical constituents (Muthukumarasamy and Panneerselvam, 1997). High salinity is known to cause both hyperionic and

hyper osmotic effects in plants, leading to membrane disorganization and metabolic toxicity (Hasegawa et al., 2000). All the factors that inhibit plant growth are defined as stresses. Drought, saltiness, excess irrigation, high or low temperature, pH and heavy metals are common sources of stress. Those stresses create social and economic problems, especially in developing countries. Only 10% of the land that can be used for agriculture in the world is not under the effect of any environmental stress element. For the rest 90%, the most common stress element is drought with 26%, followed by salt stress by 20% (Blum, 1985; Ashraf, 1994). Researches on the solutions for the nutrition problems arising from the fast population increase in the world, the aim is to create plant types that can be grown under unfavorable environmental conditions.

Salt stress induces various biochemical and physiological responses in plants and affects almost all plant processes (Nemoto and Sasakuma, 2002; Megdiche et al., 2007). Salinity can cause hyperionic and hyper-osmotic effects in plants leading to membrane

Table 1. The composition of Hoagland and Arnon (1938) nutrient solution.

Macro mineral	g/l
Ca(NO ₃) ₂ · 4H ₂ O	0.821
KNO ₃	0.506
KH ₂ PO ₄	0.136
MgSO ₄ · 7H ₂ O	0.120
Micro minerals	mg/l
C ₆ H ₅ FeO ₇ · 5H ₂ O (Ferric citrat)	50.00
MnCl ₂ · 4H ₂ O	1.80
H ₃ BO ₃	2.90
ZnCl ₂	0.12
CuCl ₂ · 2H ₂ O	0.05

disorganization, increase in reactive oxygen species (ROS) levels, and metabolic toxicity (Jaleel et al., 2007). High-salt stress disrupts the homeostasis in water potential and ion distribution at both the cellular and the whole plant levels (Errabii et al., 2007). Excess of Na⁺ and Cl⁻ ions may lead to conformational changes in the protein structure, while osmotic stress leads to turgor loss and cell volume change (Errabii et al., 2007). However, the precise mechanisms underlying these effects are not fully understood because the resistance to salt stress is a multigenic trait (Errabii et al., 2007).

The major ROS scavenging activities include complex non-enzymatic (ascorbate, glutathione, α-tocopherol) and enzymatic (SOD, APX, GR and CAT etc.) responses (Prochazkova et al., 2001). The pathways include the water-water cycle in chloroplasts and the ascorbate-glutathione cycle (Asada, 1999). Antioxidant mechanisms may provide a strategy to enhance salt tolerance in plants.

Among the several approaches to solve the problem of saline soils, biological approach to identify and grow salt tolerant plants in such soils to bring about soil reclamation is promising. Some times these factors may act as stress leading to injury and in extreme cases the death of the plant (Jaleel et al., 2007a). Salinity effects are more conspicuous in arid and semiarid regions, where limited rainfall, high evapotranspiration and high temperature associated with poor water and soil management contributes to the salinity problem and is also of great importance to the agricultural production in these regions. Agricultural productivity is severely affected by soil salinity and the damaging effect of salt accumulation in agricultural soils has become an important environmental concern (Jaleel et al., 2007b). Salinity and drought are the most important abiotic stresses that adversely affect plant growth and productivity (Mahajan and Tuteja, 2005). They result in dehydration and osmotic imbalance of the cell. As the soil salinity increases, water is osmotically held in the soil and water becomes

less accessible to the plant (Marschner, 1986). Drought stress can occur for a variety of reasons, such as limited water availability or intense evaporation. Lots of work has already been carried out in this plant due to its medicinal importance however, the salinity effects and antioxidant potential attracted a little attention. So in this study, an attempt was made to understand the antioxidant potentials of proline in soybean by evaluating both enzymatic antioxidants to salinity stress.

MATERIALS AND METHODS

Plant culture and salt stress induction

Soybean (*Glycine max.* L) seedlings have been grown in a greenhouse with day/night temperature of 28/22°C and light intensity ranging from 700 to 1000 μmol m⁻² S⁻¹. The seeds are surface sterilized with 0.2% mercuric chloride (HgCl₂) solution for 5 min with frequent shaking and then thoroughly washed with deionizer water. The seeds were pre-soaked in 500 ml of deionizer water (control), 50, 100 and 150 mM NaCl solutions for 12 h. Seeds were sown in plastic pots (300 mm diameter) filled with 3 kg of soil mixture containing red soil, sand and farmyard manure at 1:1:1 ratio. Before sowing the seeds, the pots were irrigated with the respective treatment solutions and the electrical conductivity (EC) of the soil mixture was measured. Four seeds were sown per pot and the pots were watered to the field capacity with deionize water up to 30 DAS and every care was taken to avoid leaching. The initial EC level of the soil was maintained by flushing each pot with required volume of corresponding treatment solution 10, 20 and 30 DAS.

The position of each pot has been randomized at 4 days intervals to minimize spatial effects in the greenhouse, where the temperature was 28°C during the day and 22°C at night and the relative humidity varied between 60 to 70%. The seedlings are thinned to one per pot on the 10 DAS. Six plants per replicate are used and each treatment is replicated three times (18 plants/ treatment). Plants were harvested randomly on 30 DAS and analyzed for enzymatic antioxidant responses. Three plants were taken from each group for analysis.

Control plants were irrigated with half strength Hoagland solution (Table 1) and salt treated plants received same strength nutrient solution plus 150 mM NaCl (Bozcuk 1991). Hoagland and Arnon (1938) solution is twice diluted to prepare ½ strength nutrient solution. The pH of nutrient solution was adjusted to 5.7 to 5.8 by using minimum amount of 0.05 M KOH.

Antioxidant enzyme assays

Superoxide dismutase (EC 1.15.1.1) activity was assayed as described by Beauchamp and Fridovich (1971). The reaction mixture contained 1.17 × 10⁻⁶ M riboflavin, 0.1 M methionine, 2 × 10⁻⁵ M potassium cyanide (KCN) and 5.6 × 10⁻⁵ M nitroblue tetrazolium salt (NBT) dissolved in 3 ml of 0.05 M sodium phosphate buffer (pH 7.8). 3 ml of the reaction medium was added to 1 ml of enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes in a single row. Illumination was started to initiate the reaction at 30°C for 1 h, identical solutions that were kept under dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity was expressed in units. One unit (U) is defined as the amount of change in the absorbance by 0.1 h⁻¹ mg⁻¹ protein.

Glutathione reductase (GR, EC 1.6.4.2) contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2 and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25°C after which the reaction was terminated by adding 1 ml of 2.5 N H_2SO_4 . The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 N H_2SO_4 at zero time. The activity was expressed in $U\ mg^{-1}$ protein. One U is defined as the change in the absorbance by $0.1\ min^{-1}\ mg^{-1}$ protein.

Ascorbate peroxidase (EC 1.11.1.11) activity was determined as described by Asada and Takahashi (1987). The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 200 mM of enzyme extract. The absorbance was read as decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H_2O_2 (extinction coefficient $2.9\ mM^{-1}\ cm^{-1}$). The enzyme activity was expressed in $U\ mg^{-1}$ protein (U = change in 0.1 absorbance $min^{-1}\ mg^{-1}$ protein).

Catalase (EC 1.11.1.6) was measured according the method of Chandlee and Scandalios (1984) with small modification. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.4 ml of 15 mM H_2O_2 and 0.04 ml of enzyme extract. The decomposition of H_2O_2 is followed by the decline in absorbance at 240 nm. The enzyme activity is expressed in $U\ mg^{-1}$ protein (U = 1 mM of H_2O_2 reduction $min^{-1}\ mg^{-1}$ protein).

Chlorophyll determination

One plant per replicate was used for chlorophyll determination. Prior to extraction, fresh leaf samples were cleaned with deionized water so as to remove any surface contamination. Chlorophyll extraction was carried out on fresh fully expanded leaf material; 1 g leaf sample was ground in 90% acetone by using a pestle and mortar. The absorbance was measured with a UV/Visible spectrophotometer (Shimadzu UA-1208, Kyoto, Japan) and chlorophyll concentrations were calculated using the equation proposed by Strain and Svec (1966).

$$Chl. a\ (mg\ ml^{-1}) = 11.64 \times (A663) - 2.16 \times (A645)$$

$$Chl. b\ (mg\ ml^{-1}) = 20.97 \times (A645) - 3.94 \times (A663)$$

(A663) and (A645) represent absorbance values read at 663 and 645 nm wavelengths, respectively.

Ion determination

For ion measurements, tissues were first rinsed for 5 min with cool distilled water in order to remove free ions from the apoplasm without substantial elimination of cytosolic solutes. Tissues were oven-dried at 80°C for 48 h and then were ground. The dry matter obtained was used for mineral analysis. The major cations were extracted after digestion of dry matter with HNO_3 . The extract was filtered prior to analysis. Na^+ and K^+ concentrations were determined using a ICP spectrophotometer. For Cl^- content estimation, ions were extracted with hot distilled water (80°C) during 2 h. Chloride was determined (Buchler-Cotlove chloridometer) as described by Taleisnik et al. (1997).

Nutrient analysis and dry weight

Three randomly selected plants per replicate were divided into shoots and roots, and dried in a forced air oven for two days in

order to determine dry weights. Chemical analyses were carried out on dry weight basis. The dried samples were ground to powder using a pestle and mortar and stored in polyethylene bottles. Ground samples (ca. 0.5 g per replicate) were ashed at 550°C for 6 h.

Proline determination

Proline was determined according to the method described by Bates et al. (1973). Approximately, 0.5 g of fresh leaf material was homogenized in 10 ml of 3% sulfosalicylic acid and then this aqueous solution is filtered through Whatman's No. 2 filter paper and finally two ml of filtrated solution was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100°C. The reaction mixture is extracted with 4 ml toluene and the chromophore containing toluene is aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with a (Shimadzu UV 1208, Kyoto, Japan) Spectrophotometer. Appropriate proline standards are included for the calculation of proline in the sample.

Statistical analysis

Statistical analysis was performed by using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values were mean \pm SD for seven samples in each group. P values ≤ 0.05 were considered as significant.

RESULTS AND DISCUSSION

Plant growth

Fresh and dry weight of both shoot and root tissues are significantly inhibited by higher salinity concentration compared to low and no salinity as seen in Table 2. Similar results were reported in wheat plants by Datta et al. (1998). Salinity stress stimulates the accumulation of proline as depicted in Table 2. It has been widely reported that proline may play a role in stress adaptation within the cell example by Gilbert et al. (1998). Total chlorophyll contents decreased in response to salinity stress (Table 2). The decrease may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation (Sabater and Rodriguez, 1978) as well as damaging to the photosynthetic apparatus (Yasseen, 1983).

Antioxidant enzymes

The antioxidant enzymes such as SOD, GR, APX and CAT showed variations in their activities under salinity conditions. In the different parts of the plants, the enzyme activities vary greatly. High salinity reduces SOD activity in both roots (28% in high salinity) and in leaves (16% at low and 38% at high salinity). But low salinity leads to an increase SOD activity in root up to 2.85% (Table 4). SOD activity directly modulates the amount of ROS. In this

Table 2. Fresh weight (FW), dry weight (DW), Chlorophyll and proline content of soybean plants grown at various salinity treatments.

Concentration	FW (mg. plant ⁻¹)		DW (mg. plant ⁻¹)		Chlorophyll (mg. d ⁻¹ m ⁻²)	Proline (μmol g ⁻¹ dry mass)
	Root	Leaf	Root	Leaf	Root	Leaf
Control	33±0.6	36±0.3	18±0.2	10±0.4	45±0.5	8±0.5
50 mM NaCl	34±0.5	30±0.4	20±0.4	9±0.6	22±0.8	18±1.2
100 mM NaCl	22±0.4	21±0.6	7±0.8	7±1.2	20±2.1	22±2.3
150 mM NaCl	19±0.6	18±0.5	5±0.9	4±0.8	18±0.9	27±1.1

LSD (Concentrations x NaCl treatment)= 3.2 (fresh weight), 4.3 (dry weight), 4.1 (Chlorophyll) and 5.3 (Proline).

Values are given as mean±SD of seven experiments in each group.

Bar values not sharing a common superscript differ significantly at $P \leq 0.05$ (DMRT).

study, a reduced level in SOD activity is found in leaves under high salinity while a slight increase is found under low salinity in roots. According to Pastori et al. (2000), many stress situations caused an increase in the total foliar antioxidant activity. But in these results, only a reduction in foliar antioxidant activity was noticed. Reduced foliar SOD activity was reported in rice under salt stress (Dioniso-Sese and Tobita, 1998). In some of the previous works, SOD showed increase and in some, a reduction in SOD activity was also noted (Muthukumarasamy et al., 2000; Sreenivasulu et al., 2000; Rout and Shaw, 2001; Sairam and Srivastava, 2002; Pal et al., 2004). The reduction of foliar SOD activity under high salinity can also be a consequence of an altered synthesis and accumulation of less active enzymes (Dioniso-Sese and Tobita, 1998) and/or of a higher turnover of SODs (Chaparzadeh et al., 2004).

In leaves, the POX activity decreased by 20 and 57%, but in roots it showed a slight increase up to 10% in low salinity and 29% decrease in high salinity (Table 4) when compared to control plants. 150 mM salinity caused an increase in APX in roots (12%) when compared to untreated plants. Similarly, the treatment with 150 mM NaCl induced a significant increase in APX content of about 11% in leaves in comparison with control (Table 4). There was a significant ($P \leq 0.05$) increase in SOD, GR and APX activities of roots under low salinity than high salinity may suggest the existence of an effective scavenging mechanism to remove ROS because roots are the first organs that come in contact with salt and are thought to play a critical role in plant salt tolerance. Many variations are shown in previous works like in the pea (Hernandez et al., 2002), wheat (Sairam and Srivastava, 2002; Sairam et al., 2002), mulberry (Sudhakar et al., 2001) and tomato (Doğan et al., 2010a). One of the earlier works carried out by Muthukumarasamy et al. (2000) in radish showed a GR in activity. The low basal rate and reduction in GR activity of leaves seems to indicate that this enzyme does not take a crucial part in defense mechanisms against oxidative stress or that suffering GR for salt toxicity, a co-operation is activated

between different antioxidant enzymes for establishing a proper H₂O₂ homeostasis (Chaparzadeh et al., 2004). The results of this study did not observe any significant ($P \leq 0.05$) increase in CAT activity in leaves under high salinity injury. This result coincides with previous work in rice leaves under salinity stress (Lin and Kao, 2000).

On the contrary to other enzymes, CAT showed a minimum activity in leaves but high in roots. The increase in CAT activity was not significant in the leaves of plants grown at 100 mM NaCl. In the roots, the CAT activity decreased up to 55% at the highest concentration of NaCl (Table 3). Similar results were obtained in rice cultivars by Pal et al. (2004). The changes in CAT may vary according to the intensity of stress, time of assay after the stress and induction of new isozyme(s) (Shim et al., 2003; Kumar and Khan, 1982). The level of antioxidative response depends on the species, the development and the metabolic state of the plant, as well as the duration and intensity of the stress (Reddy et al., 2004). These results showed that the activity of antioxidant enzymes under salinity depends on kind, age, and organs of plants as well as on the salinity level.

Nutrient elements

Leaf blade Na⁺ concentrations of plants grown in non-saline solution were not significantly different for the three genotypes (Table 1). Na⁺ concentrations increased with increasing salinity ($P < 0.001$). Soybean had higher Na⁺ concentrations in the youngest fully-expanded leaf blade at all NaCl treatments, compared with the other two genotypes (Table 4). At 50 mM and 100 mM NaCl, leaf blade Na⁺ concentrations in soybean were 2-fold higher compared with the amphiploid, and 1000-fold and 1300-fold higher, respectively, at these two NaCl concentrations, compared with soybean (Table 4). For plants in 150 mM NaCl, the Na⁺ concentrations in the youngest fully-expanded leaf blade of soybean (leaf 5 and 6), were 1931, 1341, and 1115 μmol g⁻¹ dry mass, respectively. These data closely matches with those which have been

Table 3. Superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase APX and catalase (CAT) activities of soybean plants grown at various salinity treatments.

Concentration	SOD (U mg ⁻¹ protein)		GR (μg g ⁻¹ FW)		APX (μg g ⁻¹ FW)		CAT (μg g ⁻¹ FW)	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
Control	6.25±0.8	7.21±0.6	3.82±0.4	2.05±0.4	28.7±1.2	33.4±1.4	2.1±1.4	1.9±2.4
50 mM NaCl	6.45±0.6	6.15±0.7	3.98±0.6	1.95±0.6	29.9±1.3	35.6±1.3	1.1±2.2	1.9±2.3
100 mM NaCl	4.25±0.7	4.22±0.6	1.52±0.8	1.25±1.2	30.2±2.1	30.1±1.5	1.0±2.3	1.9±2.6
150 mM NaCl	3.92±0.3	3.75±0.8	0.98±0.9	1.05±0.8	29.8±2.2	35.1±2.4	0.9±2.1	1.7±2.4

LSD (Concentrations x NaCl treatment) = 4.3 (superoxide dismutase), 5.1 (glutathione reductase), 5.3 (ascorbate peroxidase) and 6.5 (catalase).

Values are given as mean ± SD of seven experiments in each group.

Bar values not sharing a common superscript differ significantly at $P \leq 0.05$ (DMRT).

Table 4. Sodium (Na⁺), Potassium (K⁺), and Chloride (Cl⁻) content of soybean plants grown at various salinity treatments.

Concentration	Na ⁺ (mmol kg ⁻¹)		K ⁺ (mmol kg ⁻¹)		Cl ⁻ (mmol kg ⁻¹)	
	Root	Leaf	Root	Leaf	Root	Leaf
Control	865±0.9	754±0.6	1424±0.8	1954±0.5	458±0.8	456±1.9
50 mM NaCl	1912±0.6	1789±0.8	1151±0.8	1854±1.2	1234±0.6	1229±2.3
100 mM NaCl	2348±0.5	2155±1.7	1721±0.6	1454±2.6	1487±2.2	1645±2.3
150 mM NaCl	2489±1.8	2235±0.9	1523±1.9	1123±0.8	1889±1.2	1789±3.5

LSD (Concentrations x NaCl treatment) = 3.23 (Na⁺), 3.1 (K⁺) and 3.3 (Cl⁻).

Values are given as mean ± SD of seven experiments in each group.

Bar values not sharing a common superscript differ significantly at $P \leq 0.05$ (DMRT).

produced by other workers for other crop species, for example, in rice (Asch et al., 1999). Salt stress is known to enhance the uptake and accumulation of toxic ions such as Na⁺ in crop species (Ashraf and O'Leary, 1996; Karadge and Gaikwad, 2003; Doğan et al., 2010b).

When plants were exposed to salinity, leaf blade Cl⁻ concentrations increased in both roots and leaves ($P < 0.005$) (Table 4). Cl⁻ concentrations in the youngest fully-expanded leaf blade of all three genotypes were lowest in plants grown in non-saline solution (Table 1). Leaf blade Cl⁻ concentrations were, however, 10-30-fold higher than those of Na⁺ for the plants grown in non-saline solution. Cl⁻ concentrations were lower in soybean than in the two other genotypes when grown in non-saline solution. When exposed to salinity, leaf blade Cl⁻ concentrations increased in all three genotypes ($P < 0.001$). The youngest fully-expanded leaf of soybean had 100-fold higher Cl⁻ compared with *soybean* and 80-fold higher compared with the amphiploid, in both 100 mM and 150 mM NaCl treatments (Table 4). For plants in 150 mM NaCl, the Cl⁻ concentrations in the youngest fully-expanded leaf blade of (leaf 5 and 6), were 110, 100, and 80 μmol g⁻¹ dry mass, respectively.

For all three genotypes, leaf blade K⁺ concentration was highest when grown in non-saline solution (Table 1)

and it increased with salinity ($P < 0.001$). K⁺ concentrations were higher in the youngest fully-expanded leaf blade of soybean compared with those in the other two genotypes, except at 150 mM NaCl (Table 4). It can be concluded that at high concentrations, NaCl leads to oxidative stress and in turn cause a significant increase in antioxidative responses.

This study have found that salinity stress increased the antioxidative mechanisms in roots, which are the immediate organs that have to be suffered from salinity more efficiently than leaves. Several defence strategies are functioning in roots and shoots against oxidative stress.

The enhancement in enzymatic antioxidants responses has an important role in the regulation of growth for the positive adaptation of plants to salt stress. The present data suggest that antioxidant potential operates in the defense mechanism of soybean (*Glycine max.* L.) plants against salinity stress and that the different effectiveness of the NaCl treatments in enhancing this potential may be related to induction of enzymatic antioxidants for better mitigation under stress conditions. The data may lessen or decrease NaCl stress conditions due to enzymatic antioxidant defense systems operating in soybean defense mechanisms of salt stress.

REFERENCES

- Asada K (1987). The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 50: 601-639.
- Asch F, Sow A, Dingkuhn M (1999). Reserve mobilization, dry matter partitioning and specific leaf area in seedlings of African rice cultivars differing in early vigor. *Field crops Res.* 62(2-3): 191-202.
- Ashraf M (1994) Breeding for salinity tolerance in plants. *Critical Reviews in Plant Sci.* 13(1): 17-42.
- Ashraf M, O'Leary JW (1996). Responses of some newly evolved salt-tolerant genotypes of spring wheat to salt stress. I. Yield components and ion distribution. *J. Agron. Crop Sci.* 176: 91-101
- Azooz MM, Shaddad MA, Abdel-Latef AA (2004). The accumulation and compartmentation of proline in relation to salt tolerance of three sorghum cultivars. *Indian J. Plant Physiol.* 9: 1-8.
- Beauchamp CO, Fridovich I (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal Biochem.* 44: 276-287.
- Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water-stress studies. *Plant Soil.* 39: 205-207.
- Bozcuk S (1991). Bazı kültür bitkilerinde tuzluluğun çimlenme üzerine etkisi ve tuz toleransı sınırlarının saptanması, *Doğa Trend J. Biol.* 15(2): 144-151.
- Blum A (1985). Breeding crop varieties for stress environments *CRC Crit. Rev. Plant Sci.* 2: 199-238.
- Chandlee JM, Scandalios JG (1984). Analysis of variants affecting the catalase development program in maize scutellum. *Theor. Appl. Genet.* 69: 71-77.
- Chaparzadeh N, Amico ML, Nejad RK, Izzo R, Izzo FN (2004). Antioxidative responses of *Calendula officinalis* under salinity conditions. *Plant Physiol. Biochem.* 42: 695-701.
- Datta K, Vasquez A, Tu J, Torrizo L, Alam MF, Oliva N, Abrigo E, Khush GS, Datta SK (1998). Constitutive and tissue-specific differential expression of the *cry1A(b)* gene in transgenic rice plants conferring resistance to rice insect pests. *Theor. Appl. Genet.* 97(1-2): 20-30.
- Dionisio-Sese ML, Tobita S (1998). Antioxidant responses of rice seedling to salinity stress. *Plant Sci.* 135: 1-9.
- Doğan M, Tıprıdamaz R, Demir Y (2010a). Effective salt criteria in callus-cultured tomato genotypes A, J. *Biosci. Contens V*, 65c: 613-618.
- Doğan M, Tıprıdamaz R, Demir Y (2010b). Salt resistance of tomato species grown in sand culture, *Plant Soil Environ.* 56(11): 499-507.
- Errabi T, Gandonou CB, Essalmani H, Abrini J, Idaomar M, Senhaji NS (2007). Effects of NaCl and mannitol induced stress on sugarcane (*Saccharum* sp.) callus cultures. *Acta Physiol. Plantarum*, 29: 95-102.
- Gilbert A, Gonzalez A, Evans-Freke I (1998). Corridors maintain species richness in the fragmented landscapes of a microecosystem. *Proc. R. Soc. Lond. B* 265: 577-582.
- Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000). Plant cellular and molecular responses to high salinity. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 51: 463-499.
- Hernandez JA, Almansa MS (2002). Short-term effects salt stress on antioxidant systems and leaf water relations of pea leaves *Physiolgya Plantarum* 115:251-257
- Hoagland DR, Arnon DI (1938). The water culture method for growing plants without soil. *Circ. Calif. Agric. Exp. Stat.*, pp. 347-461.
- Jaleel CA, Gopi R, Alagu Lakshmanan GM, Panneerselvam R (2006). Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. *Plant Sci.* 171: 271-276.
- Jaleel CA, Gopi R, Manivannan P, Panneerselvam R (2007a). Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) G. Don. plants under salinity stress. *Turkish J. Bot.* 31, 245-251.
- Jaleel CA, Gopi R, Manivannan P, Panneerselvam R (2007b). Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. *Acta Physiol. Plantarum*, 29, 205-209.
- Karadge BA, Gaikwad PV (2003). Influence of sodium chloride salinity on growth and organic constituents of *Catharanthus roseus* G. Don. *Indian J. Plant Physiol.* 8: 392-397.
- Kumar KB, Khan PA (1982). Peroxidase and polyphenol oxidase in excised ragi (*Eleusine coracana* cv. PR 202) leaves during senescence. *Indian J. Exp. Bot.* 20: 412-416.
- Lawlor DW (2002). Limitation to photosynthesis in water stressed leaves: stomata Vs. metabolism and the role of ATP. *Ann. Bot.* 89: 1-15.
- Lin CC, Kao CH (2000). Effect of NaCl stress on H₂O₂ metabolism in rice leaves. *Plant Growth Regul.* 30: 151-155.
- Mahajan S, Tuteja N (2005). Cold, salinity and drought stresses: An overview. *Arch. Biochem. Biophys.* 444: 139-158.
- Marschner H (1986). *Mineral Nutrition of Higher Plants*. Academic Press, London.
- Megdiche W, Ben Amor N, Debez A, Hessini K, Ksouri R, Zuily-Fodil Y, Abdely C (2007). Salt tolerance of the annual halophyte *Cakile maritima* as affected by the provenance and the developmental stage. *Acta Physiol. Plantarum*, 29: 375-384.
- Muthukumarasamy M, Panneerselvam R (1997). Amelioration of NaCl stress by triadimefon in peanut seedlings. *Plant Growth Regul.* 22: 157-162.
- Muthukumarasamy M, Dutta Gupta S, Panneerselvam R (2000). Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by triadimefon in NaCl stressed *Raphanus sativus* L. *Biol. Plantarum* 43: 317-320.
- Nemoto Y, Sasakuma T (2002). Differential stress responses of early salt-stress responding genes in common wheat. *Phytochemistry*, 61: 129-133.
- Pal M, Singh DK, Rao LS, Singh KP (2004). Photosynthetic characteristics and activity of antioxidant enzymes in salinity tolerant and sensitive rice cultivars. *Indian J. Plant Physiol.* 9: 407-412.
- Pastori G, Mullineaux P, Foyer CH (2000). Post transcriptional regulation prevents accumulation of glutathione reductase protein activity in the bundle sheath cells of maize. Implication on the sensitivity of maize to low temperature. *Plant Physiol.* 122: 667-675.
- Prochazkova D, Sairam RK, Srivastava GC Singh DV (2001). Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. *Plant Sci.* 161: 765-771.
- Reddy AR, Chiatanya KV, Vivekanandan M (2004). Draught induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.* 161: 1189-1202.
- Rout NP, Shaw BP (2001). Salt tolerance in aquatic macrophytic possible involvement of the antioxidative enzymes. *Plant Sci.* 160: 415-423.
- Sairam RK, Srivastava GC (2002). Changes in antioxidant activity in subcellular fractions of tolerant and susceptible wheat genotypes in response to long-term salt stress. *Plant Sci.* 162: 897-904.
- Sabater B, MT, Rodriguez (1978). Control of chlorophyll degradation in detached leaves of barley and oat through effect of kinetin on chlorophyllase. *Physiol. Plant*, 43: 274-276.
- Shim IS, Momose Y, Yamamoto AH, Kim DWA, Usui K (2003). Inhibition of catalase activity by oxidative stress and its relationship to salicylic acid accumulation in plants. *Plant Growth Regul.* 39: 285-292.
- Sreenivasulu N, Grimm B, Wobus U, Weschke W (2000). Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedling of foxtail millet (*Setaria italica*). *Physiol. Plantarum*, 109: 435-442.
- Sudhakar C, Lakshmi A, Giridarakumar S (2001). Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Sci.* 161: 613-619.
- Taleisnik E, Peyrano G, Arias C (1997). Response of *Chloris gayana* cultivars to salinity, 1. Germination and early vegetative growth. *Trop. Grasslands*, 31: 232-240.
- Yasseen BT (1983). An analysis of the effects of salinity on leaf growth in Mexican wheats. UK, The University of Leeds. Ph. D. thesis.