Phyton (Horn, Austria)	**Vol. 49**	Fasc. 1	43–62	17. 8. 2009

Hydroponic Treatment with Ascorbic Acid Decreases the Effects of Salinity Injury in two Soybean Cultivars

By

Afaf M. HAMADA*) and Abdel-Basset M. AL-HAKIMI**)

With 9 Figures

Received July 24, 2008

Accepted November 12, 2008

Key words: Ascorbic acid, membrane stability index, K^+ leakage, catalase, ascorbate peroxidase, guaiacol peroxidase and salinity.

Summary

HAMADA A.M. & AL-HAKIMI A.-B. M. 2009. Hydroponic treatment with ascorbic acid decreases the effects of salinity injury in two soybean cultivars. – Phyton (Horn, Austria) 49 (1): 43–62, with 9 figures.

The addition of 0.5 mM ascorbic acid (AsA) to the hydroponic growth solution of young soybean cultivars, cvs (*Glycine max* Exford, high sensitive and *G. max* Giza 21, low sensitive) under normal growth, conditions provided protection against subsequent salinity stress. This observation was confirmed by fresh and dry matter contents, dose of response, total water content, photosynthetic pigments, transpiration rate, AsA contents, membrane stability index, K^+ leakage and minerals (Na^+, K^+ content, translocation, uptake and K^+/Na^+ ratio). In addition, analysis of antioxidant enzymes showed that AsA pretreatment causes an increase in catalase (EC 1.11.1.6), ascorbate peroxidase (APX) (EC 1.11.1.11) and guaiacol peroxidase (EC 1.11.1.7) activities under salinity stress. The seedlings of two soybean cultivars differing in salt sensitivity were treated with 0.1, 0.2 and 0.4 M NaCl for 3 days.

Zusammenfassung

HAMADA A.M. & AL-HAKIMI A.-B. M. 2009. Hydroponic treatment with ascorbic acid decreases the effects of salinity injury in two soybean cultivars. [Ascorbinsäure

*) A. H. HAMADA, Botany Department, Faculty of Science, Assiut University, Assiut 71516, Egypt. Corresponding author: e-mail: afafhamada@yahoo.com

**) A.-B. M. AL-HAKIMI, Biology Department, Faculty of Science, Taiz University, Taiz, Yemen.

in Hydrokultur verringert Salzschäden bei zwei Sojabohnen-Kultivaren]. – Phyton (Horn, Austria) 49 (1): 43–62, mit 9 Abbildungen.

Zusätzliche Ascorbinsäure (AsA, 0,5 mM) zu Nährlösungen bewahrt junge Sojabohnen-Kultivare (*Glycine max* Exford, hoch empfindlich und *G. max* Giza 21, wenig empfindlich) unter normalen Wachstumsbedingungen vor Salzschäden (Behandlung mit 0,1, 0,2 und 0,4 M NaCl drei Tage lang). Insgesamt war die Stressantwort geringer. Dies wurde anhand Frisch- und Trockengewichtsbestimmungen, Ermittlung des Gesamtwasser- und des Fotosynthesepigmentgehalts, Messung der Transpirationsrate, dem Gehalt an AsA, dem Mebranstabilitäts- Index, K^+ und Mineralsalz Verlust („leakage") (Na^+ und K^+ Gehalt, Transport und Aufnahme sowie Bestimmung des K^+/Na^+ Verhältnisses) belegt. Zusätzliche Bestätigung erhalten diese Befunde durch den Anstieg der Aktivitäten der antioxidativen Enzyme durch die Asa-Behandlung bei Salzstress: Katalase (EC 1.11.1.6), Ascorbinsäure-Peroxidase (APX) (EC 1.11.1.11) und Gujacol-Peroxidase (EC 1.11.1.7) wurden bestimmt.

Introduction

Oxygen toxicity is an inherent feature of aerobic life since it has estimated that 1% of the oxygen consumed by plants is diverted to produce activated oxygen (ASADA & TAKAHASHI 1987) in various subcellular loci (DEL RIO & al. 1992). It has been proposed that salinity-stress condition, in particular, may trigger an increased formation of the superoxide radical and hydrogen peroxide, which can directly attack membrane lipids and inactivate antioxidant protective enzymes (CHEN & al. 1999). The oxidative burst phenomenon, caused by environmental challenges and pathogen attack in particular, oxidizes the apoplast. AsA, the major and probably the only antioxidant buffer in the apoplast, becomes oxidized in these conditions. The apoplastic enzyme, ascorbate oxidase (AO) also regulates the reduction/oxidation (redox) state of the apoplastic ascorbate pool (reviewed by PIGNOCCHI & FOYER 2003). Also, it is not only an important antioxidant but also has many other roles (NOCTOR & FOYER 1998). It is a cofactor of many enzymes (SMIRNOFF & WHEELER 2000) and a regulator of cell division and growth (KERK & FELDMAN 1995). Moreover, AsA is a signal-transducing molecule in plants (PASTORI & al. 2003). Application of AsA may help in improving growth of stressed plants by neutralizing the excessive superoxide radicals or singlet oxygen. The primary purpose of this investigation was to test the hypothesis that AsA pretreatment can completely or partially alleviate salt stress effects on plant growth.

Material and Methods

Plant Material

Preliminary experiments were carried out in order to detect the low NaCl-sensitive cultivar versus the high sensitive one. Soybean (*Glycine max* Exford, high sensitive, and *G. max* Giza 21, low sensitive) were grown hydroponically in half-strength Hoagland's solution in growth chamber (16/8 h light/dark at 22/20 °C, irra-

diance at leaf was 250μmolm^{-2} S^{-1}) periodicity for 2 weeks. Some of the plants pretreated with 0.5 mM AsA added to the hydroponic solution for 1 d under normal growth conditions. After that, the seedlings were treated with Hoagland solution containing 0.1, 0.2 and 0.4 M NaCl. Control plants were kept in Hoagland solution without NaCl or AsA. After treatment (3 days) fresh and dry matter yields of shoots roots were determined by drying in an aerated oven at 70 °C until constant dry mass. Total water content was calculated as dry weight subtracted from fresh weight.

Transpiration Rate Measurement

Transpiration rate was measured as described by BOZCUK 1975.

Photosynthetic Pigments Measurements

The contents of chlorophylls a and b and carotenoids were determined spectrophotometrically (METZNER & al. 1965).

Cell Membrane Stability Index

Cell membrane stability index was determined according to the method of BLUM & EBERCON 1981.

$$\text{Percentage Injury (\%)} = 1\text{- (T1/T2) / (1-C1/C2). 100}$$

Where T_1 and T_2 are the first (before autoclaving) and second (after autoclaving) conductivity measurements by conductimeter (YSI Model 35 Yellow Springs, OH, USA) of the salinization treatment, respectively, C_1 and C_2 are the first and second conductivity measurements of the control.

K^+ leakage, Na^+ and K^+ measurement

The flame photometric method (WILLIAMS & TWINE 1960) using Carl Zeiss flame photometer was used for the determination of potassium.

Ascorbic acid measurement

AsA content was determined spectrophotometrically at 524 nm (TONUMMURA & al. 1978).

Enzyme assays

For the analysis of catalase activity, 0.5g leaf tissue was homogenized in 2.5 ml ice-cold Tris buffer (0.5 M, pH 7.4). The enzyme activity of the extract was measured spectrophotometrically by monitoring the decrease in absorbance at 240 nm in Tris buffer (pH 7.4) containing 10mM H_2O_2. APX activity was measured in the presence of 0.25 mM AsA and 0.5 mM H_2O_2 by monitoring the decrease in absorbance at 290 nm in Tris buffer (pH 7.8). Guaiacol peroxidase activity was measured by ÁDÁM & al. 1995.

The total protein content in enzyme extract was determined by the method of LOWERY & al. 1951 using egg albumin as a standard.

Results

Fresh and dry matter yield as well as the water content and also dose of response (% of inhibition different of control) of the two soybean culti-

vars was substantially affected by NaCl supply and its interaction with the applied AsA (Figs. 1, 2 and 3). The results reveal that NaCl had an inhibitory effect on fresh, dry matter and water content of the two cultivars plant shoots and roots. The reduction was much lower in cv. Exford than cv. Giza 21.

The beneficial effect of 1 d of 0.5 mM AsA treatment in alleviating partially or completely the adverse effects of salt stress on growth, water content and dose of response were clearly exhibited by the two cvs plants (Figs. 1, 2 and 3). AsA not only alleviated the inhibitory effects of salinization treatments but also in most levels were of stimulatory effects where the fresh and dry matter gain and water content in shoots and roots showed marked increase as the concentration of NaCl in the culture media was decreased.

In addition, the response of the test cvs to salinity reflected in hampered transpiration rate, which gradually decreased as salinity, increased (Fig. 4). Furthermore, the data presented in Fig. 4 clearly demonstrate the effectiveness of AsA in alleviating partially or completely the depressive effects of salinzing the growth media on transpiration rate of the test cvs.

Membrane stability index clearly showed a significant decrease with increase of salinity levels (Fig. 4). The highest value of injury was recorded in plants subjected to the highest level of salinity (0.4 M NaCl). In the presence of AsA, the level of injury was significantly lowered i.e. membrane stability index is improved (Fig. 4).

One of the expressions of membrane damage is the leakage of some cell components. In this investigation, K^+ leakage of the two cvs exhibited a great accordance with membrane stability in response to salinity i.e. increased or decreased in accordance with the rhythm of membrane stability index (Fig. 4). Also, the lost of K^+ was proportional with the level of stress. In AsA pretreated plants, K^+ leakage generally lowered indicating that repairing of membrane after deteriorated by stress.

The effect of NaCl supply on the biosynthesis of photosynthetically active pigments (chlorophyll a, chlorophyll b and carotenoids) in the leaves of salt-stressed soybean cvs., in addition to interactive effects of salinity and AsA, are shown in Fig. 5. The data clearly show that all the investigated salinity levels had inhibitory effects on the biosynthesis of pigment fractions in soybean leaves. On the other hand, the effect of 1 d of 0.5 mM AsA pretreatment was generally effective in alleviating, partially or completely, the inhibitory effects of salinization treatments on pigment biosynthesis (Fig. 5).

The interactive effects of salinization and AsA treatment on the content of AsA in variously treated soybean cvs were tested (Fig. 5). It can be seen that the contents of AsA in leaves were significantly lowered with increasing NaCl salinization in the culture media. This reduction was more

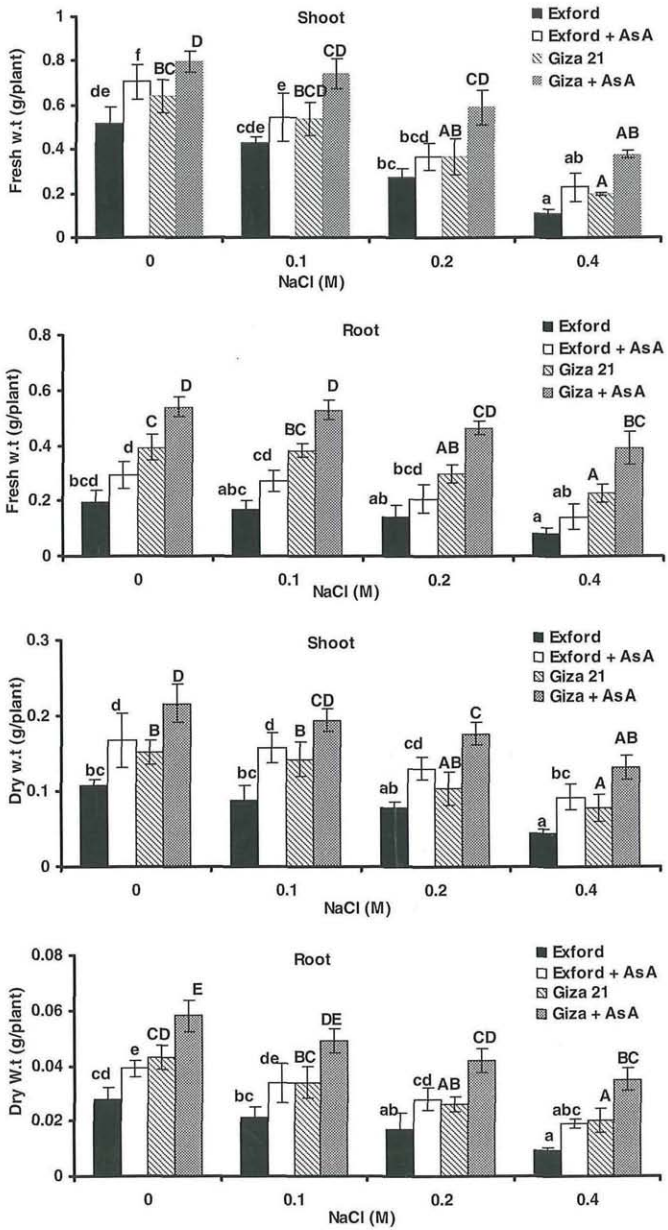

Fig. 1. Effect on young soybean cultivars of 1d of 0.5 mM hydroponic pre-treatment with AsA on fresh and dry weight of shoots and roots before 3d NaCl treatment. The values are means of 6 replicates (±SD). Bars carrying different litters are significantly different at $P < 0.05$.

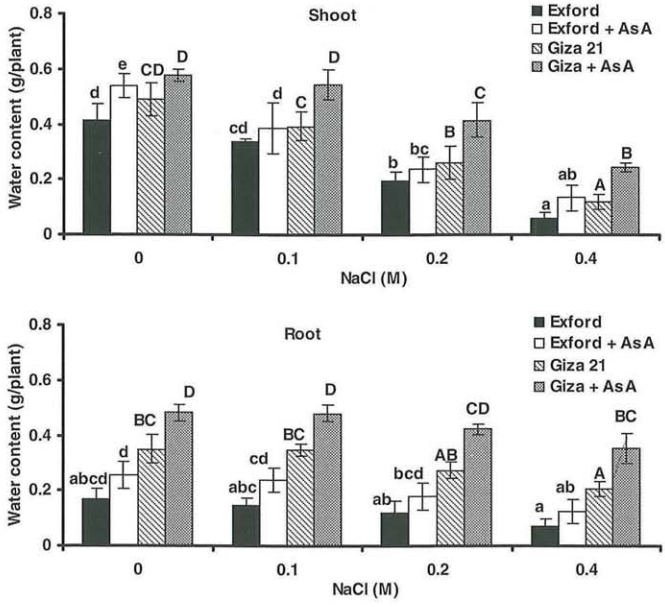

Fig. 2. Effect on young soybean cultivars of 1d of 0.5 mM hydroponic pre-treatment with AsA on water content of shoots and roots before 3d NaCl treatment. The values are means of 6 replicates (±SD). Bars carrying different litters are significantly different at $P < 0.05$.

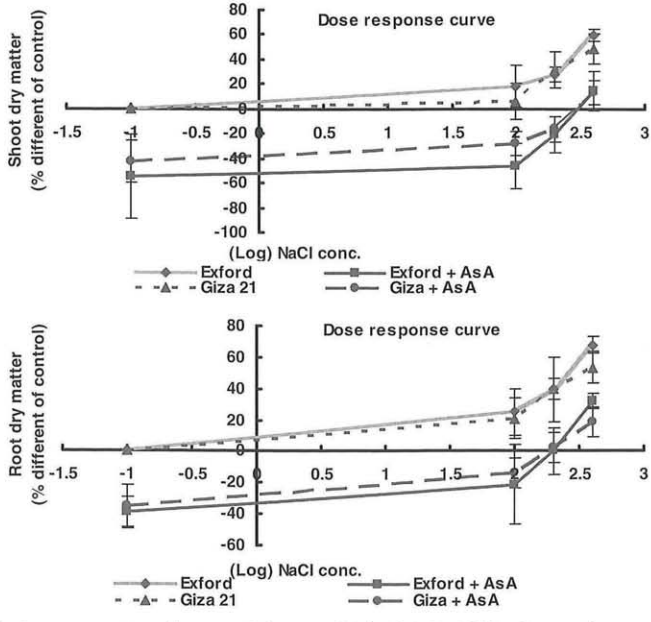

Fig. 3. Effect on young soybean cultivars of 1d of 0.5 mM hydroponic pre-treatment with AsA on dose of response of shoots and roots before 3d NaCl treatment. The values are means of 6 replicates (±SD).

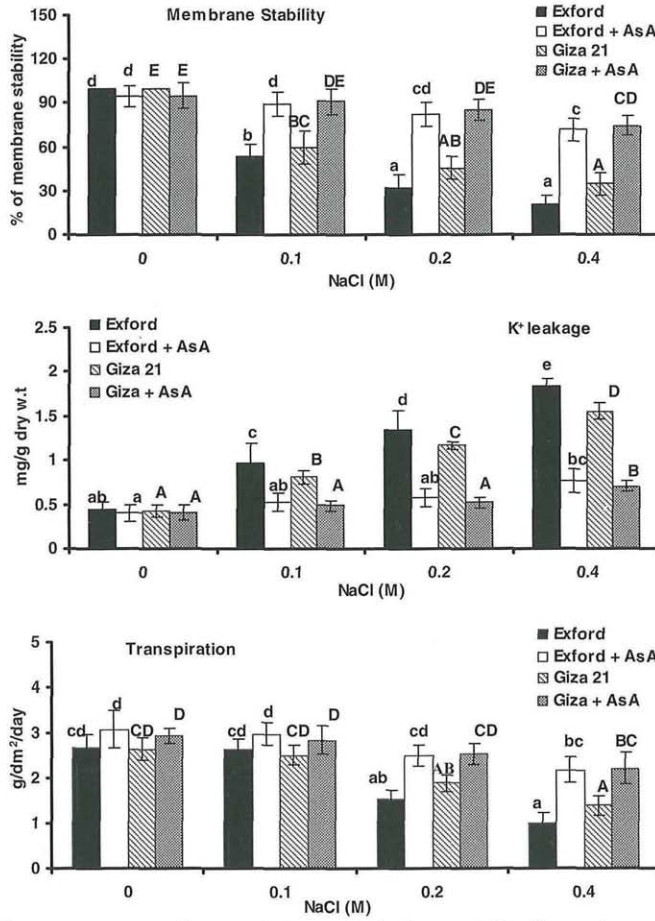

Fig. 4. Effect on young soybean cultivars of 1d of 0.5 mM hydroponic pre-treatment with AsA on transpiration rate, membrane stability and K^+ leakage before 3d NaCl treatment. The values are means of 6 replicates ($\pm$SD). Bars carrying different litters are significantly different at $P < 0.05$.

prominent at relatively higher salinization levels. Exogenous AsA for 1 d before salinity treatment induced a marked and progressive increase in the AsA content in leaves of soybean cvs., as compared with those of plants subjected only to the corresponding salinization levels. The inhibitory effect of salinity was completely eliminated at low and moderate salinization levels. Moreover, AsA treatment elevated the production of AsA in soybean leaves over those of the absolute control plants (00 NaCl), at the lower salinization levels.

Special attention was focused on the interactive effects of various levels of salinity and AsA treatment on catalase, APX and guaiacol perox-

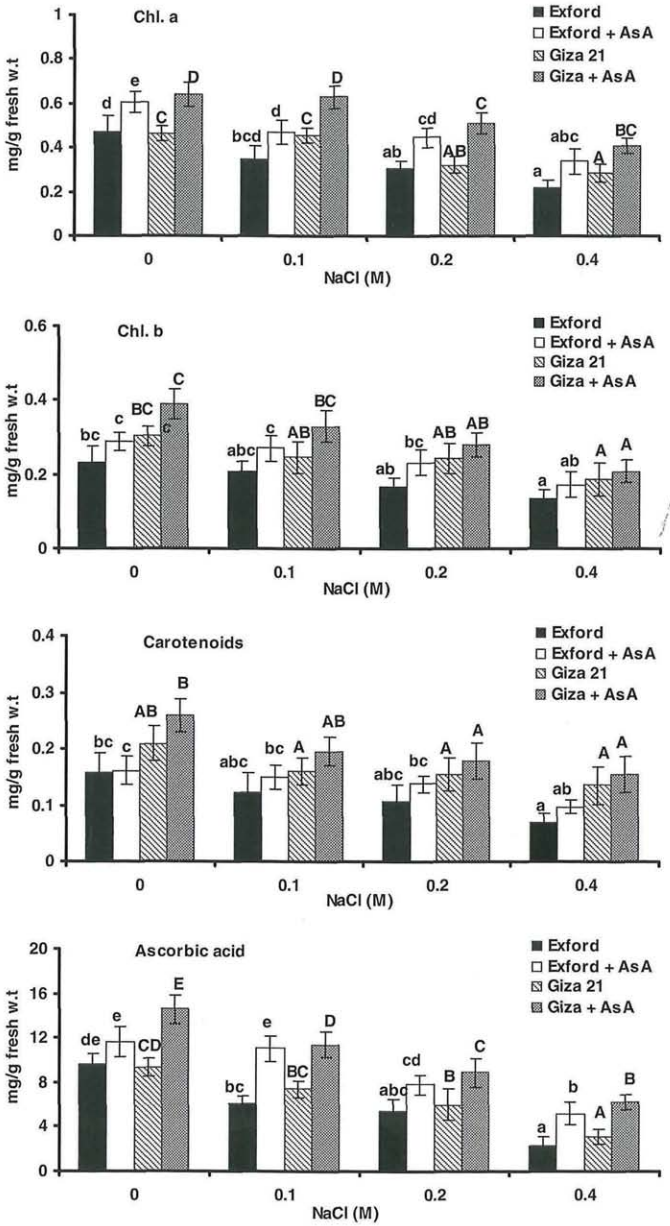

Fig. 5. Effect on young soybean cultivars of 1d of 0.5 mM hydroponic pre-treatment with AsA on chl.a, chl.b, carot. and ascorbic acid contents before 3d NaCl treatment. The values are means of 6 replicates (±SD). Bars carrying different litters are significantly different at $P < 0.05$.

idase activities in the test cvs. The results presented in Fig. 6 reveal that the enzymatic activities of the experimental plants were markedly affected by the salinization level and it decreased gradually as salinity increased. The presence of NaCl in the culture media at high concentration (0.4 M) greatly attenuated the enzymatic activities of the two-cultivar plants. The retarding effect of salinization on enzymatic activities of the test plants was partially alleviated as the concentration of NaCl in the culture media decreased. Furthermore, the data herein obtained clearly demonstrated the effectiveness of AsA in alleviating partially or completely the depressive effects of salinzing the growth media on enzymatic activities (catalase, APX and guaiacol peroxidase) of the test soybean cvs.

The data in Fig. 7 showed a considerable accumulation of sodium in either shoots or roots of the soybean cvs. This accumulation was more prominent at moderate and higher salinization levels (Fig. 7). AsA pretreatment retarded the accumulation of sodium in shoots and roots of salinized test plants (Fig. 7). This retarding effect was more pronounced at lower salinization levels as compared with those of the correspondingly salinized levels. The increase in salt level had generally a favourable effect on the accumulation of K^+ in shoot while in root the opposite trend was exhibited in the two soybean cvs. (Fig. 8). The maximum accumulation of K^+ in shoot and its minimum concentration in root were estimated at 0.4 M NaCl. AsA pretreatment resulted generally in a marked increase in K^+ contents in shoots and roots of the two cvs as compared with those of plants treated with NaCl only (Fig. 8).

Also, Na^+ and K^+ translocation increased with increasing salinization levels (Figs. 7 and 8). With respect to Na^+ and K^+ uptake, Na^+ uptake showed increasing with the increasing of salinity levels, and K^+ uptake showed non-significance differences between salinity levels. On the other hand, AsA pretreatment showed non significant effect on the Na^+ and K^+ translocation and uptake except, at levels 0.2 and 0.4 AsA decreased the Na^+ uptake (Figs. 7 and 8). In addition, the response of the test cvs to salinity was reflected in hampered K^+/Na^+ ratio in shoots and roots, which decreased as salinity, increased (Fig. 9). Furthermore, the data presented in Fig. 9 clearly demonstrate the effectiveness of AsA in alleviating partially the depressive effects of salinzing the growth media on K^+/Na^+ ratio of the test cvs.

Discussion

Down regulation of growth and development is a key feature of the plant response to stress. The retarded growth of salt-stressed plants may result from high internal concentration of toxic ions, impaired uptake of essential nutrients, disorganization or damage in cellular organelles or a combination of these (DE LACERDA & al. 2003).

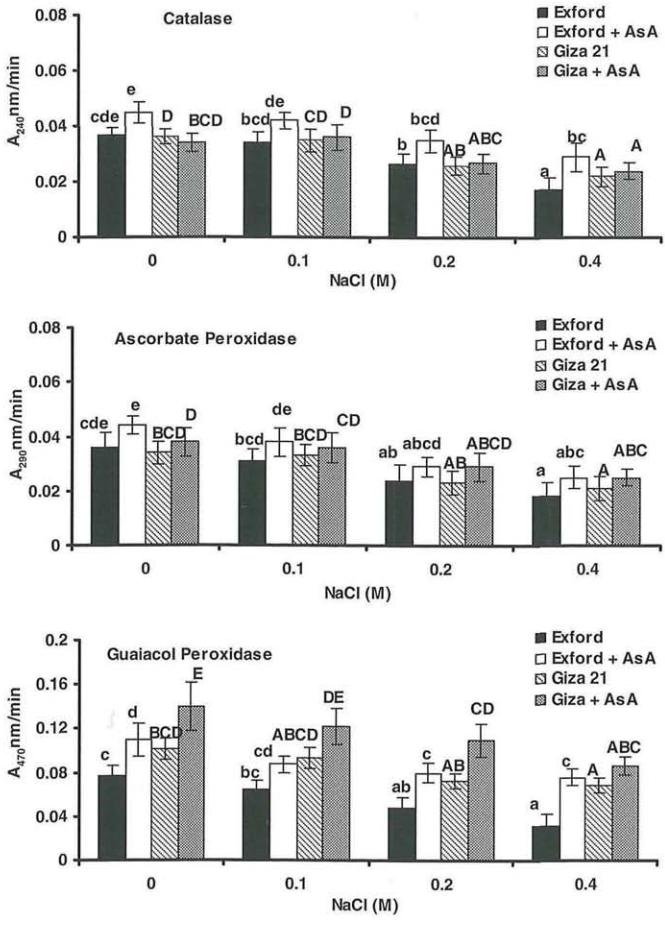

Fig. 6. Effect on young soybean cultivars of 1d of 0.5 mM hydroponic pre-treatment with AsA on catalase, ascorbate peroxidase and guaiacol peroxidase before 3d NaCl treatment. The values are means of 6 replicates ($\pm$SD). Bars carrying different litters are significantly different at $P < 0.05$.

The effectiveness of AsA treatment in relieving the inhibitory effects of salinity stress on the growth of the different organs of the plants is in agreement with the results obtained by KHAN & SRIVASTAVA 1998 that used AsA for ameliorating the retarding effects of NaCl on the early growth stage of maize. In addition, BORSANI & al. 2001 concluded that the presence of reactive oxygen-scavenging compounds (2mM AsA and 3mM reduced glutathione) in the germination media reversed the Arabidopsis wild-type necrotic phenotype seen under salt (100 mM NaCl) and osmotic (270 mM mannitol) stress. Moreover, the AO-generated oxidized forms of AsA (i.e.

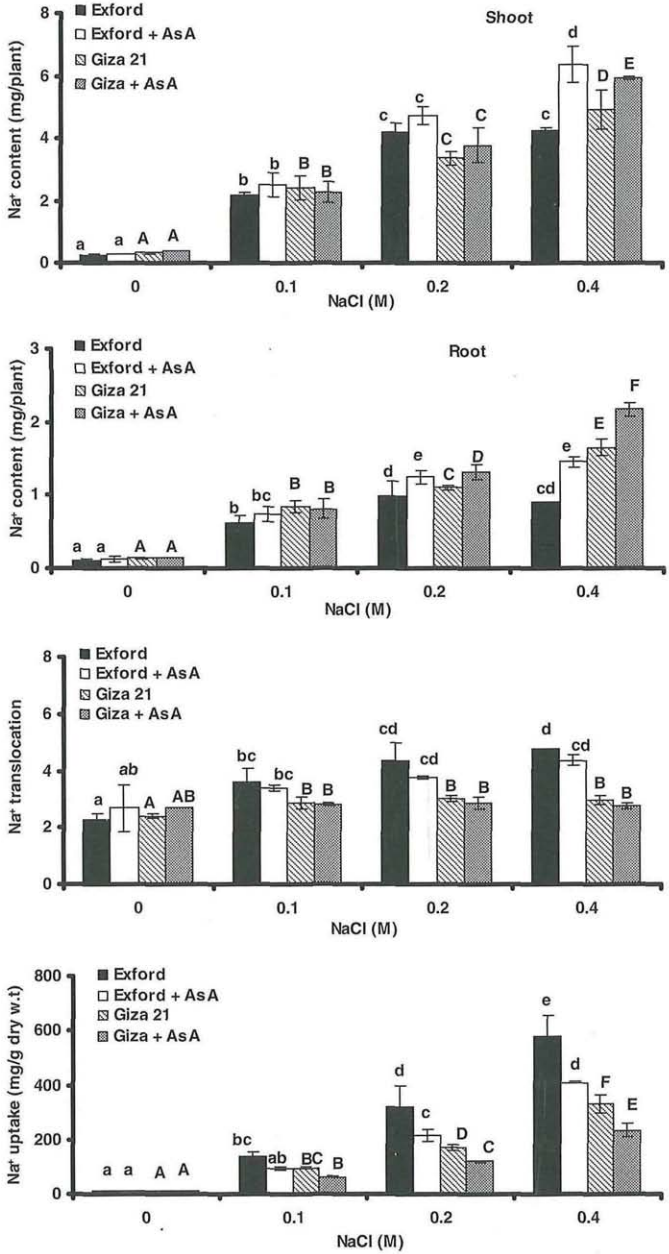

Fig. 7. Effect on young soybean cultivars of 1d of 0.5 mM hydroponic pre-treatment with AsA on Na^+ content in shoots and roots, Na^+ translocation and Na^+ uptake before 3d NaCl treatment. The values are means of 6 replicates ($\pm$SD). Bars carrying different litters are significantly different at $P < 0.05$.

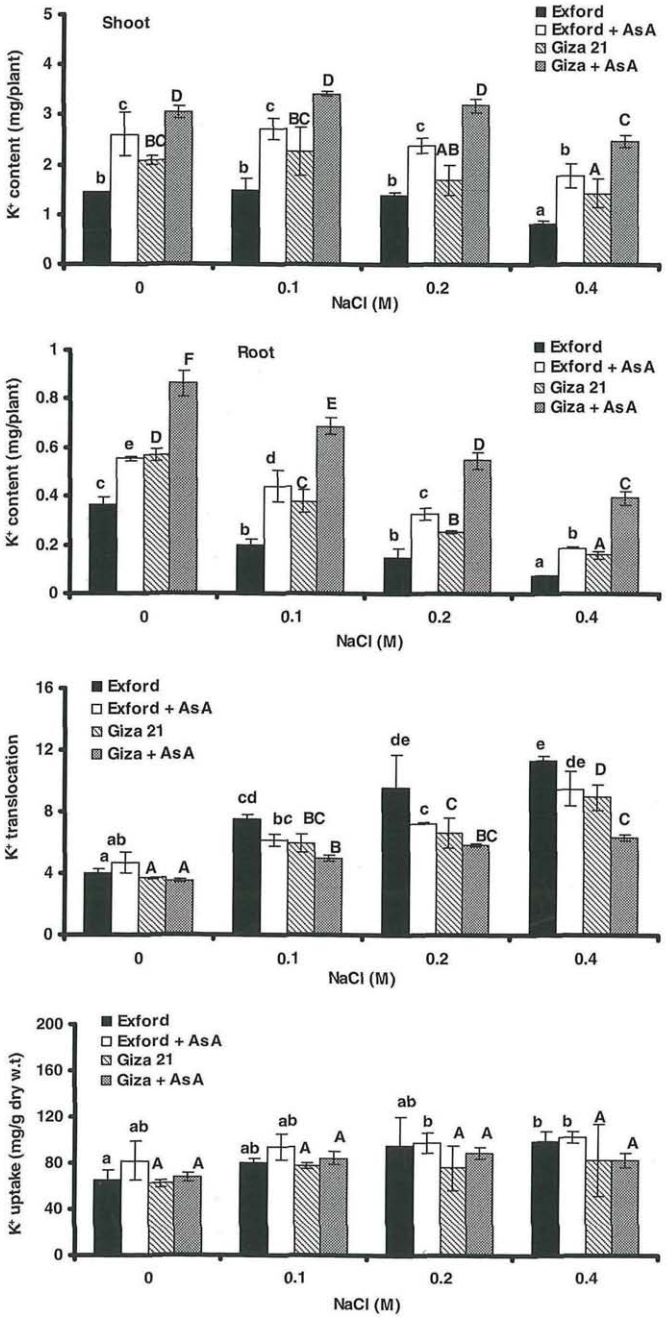

Fig. 8. Effect on young soybean cultivars of 1d of 0.5 mM hydroponic pre-treatment with AsA on K^+ content in shoots and roots, K^+ translocation and K^+ uptake before 3d NaCl treatment. The values are means of 6 replicates ($\pm$SD). Bars carrying different litters are significantly different at $P < 0.05$.

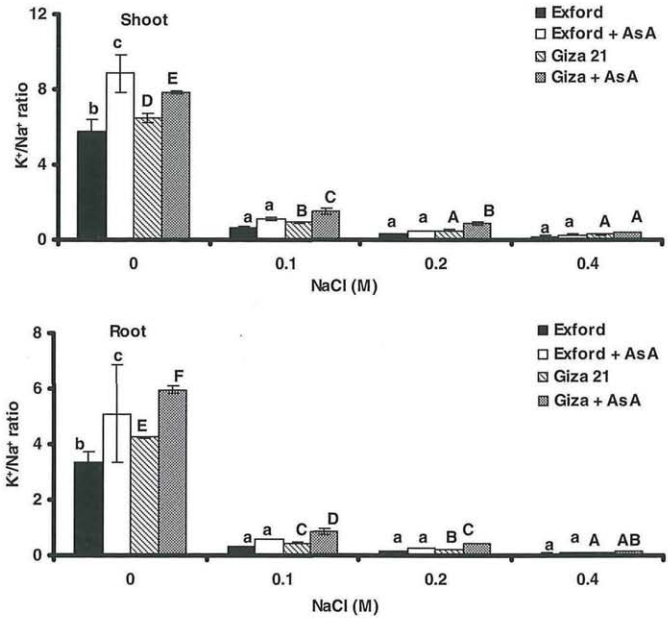

Fig. 9. Effect on young soybean cultivars of 1d of 0.5 mM hydroponic pre-treatment with AsA on K^+/Na^+ ratio in shoots and roots before 3d NaCl treatment. The values are means of 6 replicates ($\pm$SD). Bars carrying different litters are significantly different at $P < 0.05$.

DHA and MDHA) cause cell enlargement (GONZALEZ-REYES & al. 1994). Increases in apoplastic DHA leads to cell expansion by favouring cell wall loosening (LIN & VARNER 1991). In addition, increases in apoplastic MDHA lead to cell expansion through depolarization of the plasmalemma and vacuolation (HIDALGO & al. 1989).

The osmotic effect, resulting from salt stress may and very likely will cause disturbances in the water balance of the stressed plant, leading to a reduction of turgor, stomatal closure, reduction of photosynthesis and consequently an inhibition of growth (POLJAKOFF-MAYBER 1982). On the other hand, ERDEI & TALEISNIK 1993 suggested that bulk tissue turgor was not limiting growth under these conditions and emphasized the possible implication of alterations in the elastic condition of the cell wall in stress responses.

The inhibited transpiration activity with salt stress was attributed to a reduction in leaf area (WEST & al. 1979), to stomatal closure (BEHBOUDIAN & al. 1986) and/or ascribed in the first place to impairment of water uptake by roots (HAGEMEYER & WAISEL 1989) and to hinder the stomatal function and consequently the transpiration capacity is altered (HAMADA 1996). The mitigative effects of AsA on the inhibited transpiration capacity

of the test-salinized plants may be one aspect of the role of vitamin in hairy root growth, which should be considered helpful in water uptake and concomitant water loss via transpiration.

Membrane plays a central role in plant cell structure and metabolism as they participate in metabolic activities of plants directly or indirectly (RHODES 1987). In addition, in this work, leakage of K^+ was assessed under conditions of salinity. Under moisture stress, decreased membrane stability, chlorophyll content and chlorophyll stability index or *Triticum aestivum* were recorded (SAIRAM & al. 1997). Moreover, increased rates of solute leakage into non-electrolyte media are commonly associated with stress (NEORI & BOROCHOV 1991) and attributed to membrane modifications. During stress, the increase in Na^+ content and decrease in K^+/Na^+ ratio in leaves resulted in a rapid increase in electrolyte leakage, H_2O_2 and $O.^{-2}$ content (CHEN & al. 1998) and disruption in equilibrium of oxyradical metabolism (CHEN & al. 1994). Supplemental AsA can stimulate tonoplast H^+-ATPase and H^+-PPase activities under salt stress and suppress lipid peroxidation and membrane permeability, thus improving salt-tolerance (CHEN & al. 1999). In this context, GUO & al. 2005 found that L-galctono-γ-lactone and low concentrations of AsA (0.5 and 1 mM) decreased the electrolyte leakage of rice seedling subjected to chilling and water stress, indicating AsA and its precursor increased the resistance of rice seedlings to chilling and drought.

The reduction in pigments contents could be probably due to the inhibitory effects of the accumulated ions on the biosynthesis of different pigments (STROGONOV 1964). Moreover, the activity of the specific enzymes responsible for the syntheses of the green pigments was suppressed by salinity (STROGONOV & al. 1970). Also, it was found that salt stress induced important alterations in the chloroplast structure such as swelling of the thylakoids which might be related to the severe drop in chlorophyll contents (JEAN-PAUL & al. 1993). On the other hand, PELTZER & al. 2002 concluded that the changes in the photochemistry of chloroplasts in the leaves of water-stressed plants result in dissipation of excess light energy, thus, generation of active oxygen species (AOS), which are potentially dangerous under water stress conditions. Where, water deficit stress induces oxidative stress because of inhibition of photosynthetic activity due to imbalance between light capture and its utilization (FOYER & NOCTOR 2004). AsA was recorded to affect the chlorophyll content (CHOUDHURY & al. 1993) through promoting the capacity of chlorophyll by stabilizing and protecting these molecules from being oxidized. AsA has a central role in photosynthesis, since it acts as an antioxidant by removing hydrogen peroxide generated during photosynthetic processes in a group of reactions termed the 'Mehler peroxidase reaction sequence' (ASADA 1994). In addition, it is an essential cofactor for the synthesis of the energy quencher, zeaxanthin in the thylakoid lumen (PFÜNDEL & BILGER 1994).

Reduction in AsA concentrations in response to water stress were reported in *Vigna catjang* (MUKHERJEE & CHOUDHURI 1985), *Cohlearia atlantica* ans *Armenia maritime* (BUCKLAND & al. 1991), sorghum (ZHANG & KIRKHAM 1996) and *Triticum aestivum* (BARTOLI & al. 1999). AsA participates in the removal of H_2O_2 as a substrate of AsA peroxidase, directly reduces O^-_2, quench $^1O_2$ and regenerate reduced α-tocopherol (FOYER 1993). Any of the routes for ascorbate oxidation, as well as a slow synthesis rate of AsA or a decreased reduction rate of both oxidation products (monodehdroascorbate and dehydroascorbate), could lead to the decrease in AsA content in water stressed wheat (BARTOLI & al. 1999).

There is sporadic evidence in the literature to suggest that some environmental stresses, especially drought, toxic ions, organic compounds and salt alter the amounts and activities of enzymes involved in oxidative stress (GOSSETT & al. 1994). Suggestions include direct ion effects such as the interaction of Na^+ and Cl^- on enzyme structure and function, as well as dehydration, or a combination of both of these effects (NUI & al. 1995). Losses of catalase activity have previously been observed in salt-treated plants by KALIR & POLJAKOFF-MAYBER 1981 and by SINGHA & CHOUDHURI 1990. The apparent declines in catalase and PSII activity were attributed to repression of new protein synthesis by NaCl (HERTWIG & al. 1992). Most probably, the inhibition of protein synthesis in the salt-exposed rye leaves increasingly also retarded or prevented the increases in guaiacol-peroxidase in the excised leaf segments (STREB & FEIERABEND 1996). In addition, CHEN & al. 1999 reported that the activity of ascorbate peroxidase of barley leaves decreased after NaCl treatment. Supplementing AsA to the growth medium containing 300 mmol/l NaCl raised AsA content in leaves of barley (CHEN & al. 1999), which might provide enough substrate for ascorbate peroxidase, the crucial enzyme removing H_2O_2 in chloroplasts (CAO 1994); as a result ascorbate peroxidase activity was improved. Our findings on differential enzymatic activity of two plant cultivars are in agreement with those of NAYYAR & GUPTA 2006 who reported that maize had more amount of ascorbic acid and glutathione as well as higher activities of ascorbate peroxidase, glutathione reductase and dehydroascorbate reductase in its shoots. While wheat, comparatively had more of catalase (CAT) activity in its roots as well as shoots. The proportionately higher levels of AsA and GSH along with their related enzymes (APX, GR and DHAR) in maize reflect their greater involvement in C_4 (maize) plants to counter oxidative stress as compared to C_3 (wheat) plants, which had relatively more activity of CAT.

The applied NaCl induced Na^+ accumulation in shoots and roots of the tested plants; the highest Na^+ content was consistently displayed in plants subjected to the highest salinity level. High salinity disturbs intercellular ion homeostasis, leading to membrane damage, metabolic inactivation and

secondary effects that ultimately result in cell death. Sodium toxicity is primarily a cytosolic event but cellular adaptive responses to salt stress in plants are complex and remain poorly understood (FLOWERS 2004). In addition, high Na^+ levels lead to reduction in photosynthesis and production of reactive oxygen species (YEO 1998). Movement of salt into roots and to shoots is a product of the transpirational flux required to maintain the water status of the plant (YEO 1998). Increasing salinity in growth medium resulted in a considerable decrease in K^+ contents in roots of both soybean cvs. As common proteins transport Na^+ and K^+, Na^+ competes with K^+ for intracellular influx (BLUMWALD & al. 2000). Many K^+ transport systems have some affinity for Na^+, i.e., Na^+/K^+ symporters. Thus, external Na^+ negatively affects intercellular K^+ influx. However, salinity treatments promoted the accumulation of K^+ in cvs shoots. This accumulation may contribute in osmotic adjustment phenomenon (BOLARIN & al. 1995). The maintenance of adequate net uptake of K^+ at high Na^+ content is important, since the physiological functions, of K^+ in plants cannot be substituted by Na^+, except for the osmotic role of Na^+ in the vacuoles. It is therefore possible that K^+/Na^+ discrimination is associated with salt tolerance. On the other hand, AsA pretreatment induced, in most cases, a significant decrease in the accumulation of Na^+, while a promotion in the absorption of K^+ was recorded. This response may lead to the suggestion that AsA pretreatment may be involved in the maintenance of the ions in adequate amounts to enhance the metabolic processes. These nutrients may also lead to consider that the AsA treating could play an important role in osmoregulation, which could probably increase the efficiency of utilization of water under stress conditions maintaining salt tolerance of the experimental plants. Limiting Na^+ entry into the cell probably is one of the most important mechanisms to maintain a low Na^+ concentration in the cytosol.

The above results indicate that exogenous AsA (added to the growth solution 1 d before the salinity treatment) can stimulate catalse, ascorbate peroxidase and guaiacol peroxidase activities under salt stress and suppress lipid peroxidation and membrane permeability, thus improving salt-tolerance. In addition, we can conclude that AsA may be reestablished ion homeostasis by maintaining a relative high K^+ content and low Na^+ content in the cytosol of tested plants. Further research should be carried out to determine whether the increase in enzyme activity resulted from the change in protein content or configuration.

References

ÀDÁM A., BESTWICK C. S., BARNA B. & MANSFIELD J. W. 1995. Enzymes regulating the accumulation of active oxygen species during the hypersensitive reaction of bean to *Pseudomonas jringase* pv. *Phaseolicola*. – Planta 197: 240–249.

ASADA K. 1994. Production and action of active oxygen species in photosynthetic tissues. – In: FOYER C.H. & MULLINEUX P.M. (Eds.), Causes of oxidative stress and amelioration of defense systems in plants., pp. 77–104. – ISBN 0-84935-443-9, CRC Press Boda Raton, London.

ASADA K. & TAKAHASHI M. 1987. Production and scavenging of active oxygen in photosynthesis. – In: KYLE D.J., OSMOND C.B. & ARNTZEN G.J. (Eds.), Photoinhibition, pp. 227–287. – Elsevier, Amsterdam.

BARTOLI C. B., SIMONTACCHI M., TAMBUSSI L. & BELTRANES J. A. 1999. Drought and watering-dependent oxidative stress: effect on antioxidant content in *Triticum aestivum* L. leaves. – J. Exp. Bot. 50: 375–383.

BEHBOUDIAN M. H., TÖRÖKFAVY E. & WALKER R. R. 1986. Effects of salinity on ionic content, water relations and gas exchange parameters in lomnecitrus scion-rootstock combinations. – Sci. Hortic. 8: 105–116.

BLUM A. & EBERCON S. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. – Crop Sci. 21: 43–47.

BLUMWALD E., AHARAON G. S. & APSE M. P. 2000. Sodium transport in plant cells. – Biochem. Biophys Acta 1465: 140–151.

BOLARIN M. C., SANTA-CRUZ A., CAYUELA E. & PEREZ-ALFOCEA F. 1995. Short-term solute changes in leaves and roots of cultivated and wild tomato seedlings, under salinity. – J. Plant Physiol. 147: 463–468.

BORSANI O., VALPUESTA V. & BOTELLA M. A. 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. – Plant Physiol. 126: 1024–1030.

BOZCUK S. 1975. Effect of sodium chloride upon growth and transpiration in *Statice* sp. and *Pisum sativum* L. – In: Proceedings of third MPP meetings. pp. 37–42. – Ege University, Ismir.

BUCKLAND S. M., PRICE A. H. & HENDRY G. A. F. 1991. The role of ascorbate in drought-treated Cochearia atlantica Pobed, and Armeria maitima (Mill). Wild. – New Phytol. 119: 155–160.

CAO W. H. 1994. Ascorbate peroxidase as a key enzyme of the H_2O_2 scavenging system in chloroplasts. – Plant Physiol. Common. 30: 452–456. (in Chinese).

CHEN Q., LIU Y. L. & CHEN Y. H. 1998. Relationship between active oxygen damage and tonoplast H^+-ATPase activity in leaves of barley seedling under salt stress. – J. Nanjing Agric. Univ. 21: 21–25.

CHEN Q., ZHANG W. H. & LIU Y. L. 1999. Effect of NaCl, glutathione and ascorbic acid on function of tonoplast vesicles isolated from barley leaves. – J. Plant Physiol. 155: 685–690.

CHEN W. S., LIU H. Y., LIU Z. H., YANG L. & CHEN W. H. 1994. Gibberellin and temperature influence carbohydrate content and flowering in *Phalaenopsis*. – Physiol. Plant. 90: 391–395.

CHOUDHURY N. K., CHO H. T. & HUFFAKER R. C. 1993. Ascorbate induced zeaxanthin formation in wheat leaves and photoprotection of pigment and photochemical activities during aging of chloroplasts in light. – J. Plant Physiol. 141: 551–556.

DE LACERDA C. F., CAMBRAIA J., OLIVA M. A., RUIZ H. A. & PRISCO J. T. 2003. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. – Environ. Exp. Bot. 49: 107–120.

DEL RIO L. A., SANDALIO L. M., PALMA J. M., BUENO P. & CORPAS F. J. 1992. Metabolism of oxygen radicals in peroxisomes and cellular implication. – Free Radicals Biol. Med. 13: 557-580.

ERDEI L. & TALEISNIK E. 1993. Changes in water relation parameters under osmotic and salt stresses. – Physiol. Plant. 89: 381–387.

FLOWERS T. J. 2004. Improving crop salt tolerance. – J. Exp. Bot. 55: 307–319.

FOYER C. H. 1993. Ascorbic acid. – In: ALSCHER R.G. & HESS J.L. (Eds.), Antioxidants in higher plants. pp. 31–58. – CRC Press, 31–58, Boca Raton.

FOYER C. H. & NOCTOR G. 2004. Oxygen processing in photosynthesis: regulation and signaling. – New Phytol. 146: 359–388.

GONZALEZ-REYES J. A., HIDALGO A., CALER J. A., PALOS R. & NAVAS P. 1994. Nutrient-uptake changes in ascorbate free radicals-stimulated onion roots. – Plant Physiol. 104: 271–276.

GOSSETT D. R., MILLHOLLON E. P. & LUCAS M. C. 1994. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. – Crop Sci. 34: 706–719.

GUO Z., TAN H., ZHU Z., LU S. & ZHOU B. 2005. Effect of intermediates on ascorbic acid and oxalate biosynthesis of rice and in relation to its stress resistance. – Plant Physiol. Biochem. 43: 955–962.

HAGEMEYER J. & WAISEL Y. 1989. Influence of NaCl, $Cd(NO_3)_2$ and air humidity on transpiration of *Tamarix aphylla*. – Physiol. Plant. 75: 280–284.

HAMADA A. M. 1996. Effect of NaCl, water stress or both on gas exchange and growth of wheat. – Biol. Plant. 38: 405–412.

HERTWIG B., STREB P. & FEIERABEND J. 1992. Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. – Plant Physiol. 100: 1547–1553.

HIDALGO A., GONZALEZ-REYES J. A. & NAVAS P. 1989. Ascorbate free radical enhances vacuolization in onion root meristems. – Plant Cell Environ. 12: 455–460.

JEAN-PAUL D., RAIS L. B., MARIE-JOSEE A., BAHL J. & GUILLOT-SOLOMON T. 1993. Lipid and protein contents of jojoba in relation to salt adaptation. – Plant Physiol. Biochem. 31: 547–557.

KALIR A. & POLJAKOFF-MAYBER A. 1981. Changes in activity of malate dehydrogenase, catalase, peroxidase and superoxide dismutase in leaves of *Halimione portulacoides* (L.) Aellen exposed to high sodium chloride concentrations. – Ann. Bot. 47: 75–85.

KERK N. M. & FELDMAN L. J. 1995. A biochemical model for the initiation and maintenance of the quiescent centre: implication for organization of root meristems. – Develop. 121: 2825–2833.

KHAN M. G. & SRIVASTAVA H. S. 1998. Changes in growth and nitrogen assimilation in maize plants induced by NaCl and growth regulators. – Biol. Plant. 41: 93–99.

LIN L. S. & VARNER J. E. 1991. Expression of ascorbate oxidase in zucchini squash (*Cucurbita pepo* L.). – Plant Physiol. 96: 159–165.

LOWERY O. H., ROSEBROUGH N. J., FARR A. L. & RANDALL R. J. 1951. Protein measurement with the folin phenol reagent. – J. Bio. Chem. 193: 265–275.

METZNER H., RAU H. & SENGER H. 1965. Untersuchungen zur Synchronisierbarkeit einzelner Pigmentmangel Mutanten von Chlorella. – Planta 65: 186–194.

MUKHERJEE S. P. & CHOUDHURI M. A. 1985. Implication of hydrogen-peroxide-ascorbate system on membrane permeability of water stressed *Vigna* seedlings. – New Phytol. 99: 355–360.

NAYYAR H. & GUPTA D. 2006. Differential sensitivity of C_3 and C_4 plants to water deficit stress: Association with oxidative stress and antioxidants. – Environ. Exp. Bot. 58: 106–113.

NEORI H. B. & BOROCHOV A. 1991. Response of melon plants to salt. 1- Growth, morphology and root membrane properties. – J. Plant Physiol. 139: 100–105.

NOCTOR G. & FOYER C. H. 1998. Ascorbate and glutathione: keeping active oxygen under control. – Ann. Rev. Plant Physiol. Plant Mol. Biol. 49: 249–279.

NUI X., HASEGAWA P. M. & PARDO J. M. 1995. Ion homeostasis in NaCl stress environments. – Plant Physiol. 109: 735–742.

PASTORI G. M., KIDDLE G., ANTONIW J., BERNARD S., VELJOVIC-JOVANOVIC S., VERRIER P. J., NOCTOR G. & FOYER C. H. 2003. Leaf vitamin C contents modulate plant defense transcripts and regulate genes controlling development through hormone signaling. – Plant Cell 15: 1212–1226.

PELTZER D., DREYER E. & POLLE A. 2002. Differential temperature dependencies of antioxidative enzymes in two contrasting species. – Plant Physiol. Biochem. 40: 141–150.

PFÜNDEL E. E. & BILGER B. 1994. Regulation and possible function of the violaxanthin cycle. – Photosyn. Res. 42: 89–109.

PIGNOCCHI C. & FOYER C. H. 2003. Apoplastic ascorbate metabolism and its role in the regulation of cell signaling. – Curr. Opin. Plant Biol. 6: 379–389.

POLJAKOFF-MAYBER A. 1982. Biochemical and physiological responses of higher plants to salinity stress. – In: SAN PRIETO A. (Ed.), Biosaline research, a look to the future, pp. 245–270. – Plenum Press, New York.

RHODES D. 1987. Metabolic responses to stress. – In: DAVIES D.D. (Ed.), The biochemistry of plants, 12: 201–240. – Academic Press. San Diego. New York. Berkeley, Boston, Sydney, Tokyo, Toronto.

SAIRAM R. K., DESHMUKH P. S. & SHUKLA D. S. 1997. Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. – J. Agron. Crop Sci. Zeitschrift für Acker und Pflanzenbau 178: 171–177.

SINGHA S. & CHOUDHURI M. A. 1990. Effect of salinity (NaCl) on H_2O_2 mechanism in *Vigna* and *Orysa* seedlings. – Biochem. Physiol. Pflanz. 186: 69–74.

SMIRNOFF N. & WHEELER G. L. 2000. Ascorbic acid in plants: biosynthesis and function. – Crit. Rev. Plant Sci. 19: 267–290.

STREB P. & FEIERABEND J. 1996. Oxidative stress responses accompanying photoinactivation of catalase in NaCl-treated rye leaves. – Bot. Acta 109: 125–132.

STROGONOV B. P. 1964. Physiological basis of salt tolerance of plants. – Acad. Sci. USSR. 1962 (Ed.). – Israel Program for Scientific Translation, Jerusalem, Translation of the Russian

STROGONOV B. P., KABANOV V. V., SHEVAJAKOVA N. I., LAPINE L. P., KOMIZERKO E. I., POPOV B. A., DOSTONOVA R. K. & PRYKOD'KO L. S. 1970. Structure and function of plant celİ in saline habitats. – Nauka, Moscow. (In Russ).

TONUMMURA B., NAKATANI H., OHNISHI M., YAMAGUCHI-ITO J. & HIROMI K. 1978. Test reaction for a stopped-flow apparatus. Reduction of 2,6-dichlorophenolindiphenol and potassium ferricyanide by L-ascorbic acid. – Anal. Biochem. 84: 370–383.

WEST D. W., MERRIGAN I. F., TAYLOR J. A. & COLLINS G. M. 1979. Soil salinity gradients and growth of tomato plants under drip irrigation. – Soil Sci. 127: 281–291.

WILLIAMS C. H. & TWINE J. R. 1960. Flame photometric method for sodium potassium and calcium. – In: PAECH K. & TRACEY M.V. (Eds.), Modern methods of plant analysis, Vol. V, pp. 3–5. – Springer Verlag, Berlin.

YEO A. R. 1998. Molecular biology of salt tolerance in the context of whole-plant physiology. – J. Exp. Bot. 49: 915–929.

ZHANG J. & KIRKHAM M. B. 1996. Antioxidant responses to drought in sunflower and sorghum seedlings. – New Phytol. 132: 361–373.