



AMELIORATING EFFECTS OF EXOGENOUSLY APPLIED ASCORBIC ACID ON SEAWATER IRRIGATION - INDUCED OXIDATIVE STRESS IN *ROSELLE* SEEDLINGS

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SUMMARY

The objective of this work was to evaluate the ameliorating effects of ascorbic acid (AsA) to stress imposed by seawater irrigation on roselle (*Hibiscus sabdariffa* L. with red sepals variety) seedlings. Irrigation of seedlings with seawater caused a reduction of growth and water content. On the other hand, ion leakage, lipid peroxidation, activities of some antioxidant enzymes, endogenous AsA, total free amino acids and proline were enhanced. Exogenously application of 200 ppm AsA through seeds presoaking ameliorating the inhibitory effects of seawater stress, which were accompanied by increase of soluble carbohydrates and proteins and antioxidant enzymes. However, seawater stress induced accumulation of proline, total free amino acids, ion leakage and lipid peroxidation was lesser in ASA treated as compared with AsA untreated seedlings. The results suggested that, application of AsA may help in improving the tolerance of *Hibiscus sabdariffa* L. seedlings to seawater stress by neutralizing the excessive reactive oxygen species (ROS).

Key words: Antioxidant enzymes, chlorophyll content, *Hibiscus*, ion leakage, lipid peroxidation

INTRODUCTION

Salinity is a widespread environmental stress for crop plants. It has reached 19.5% of the irrigated land and 2.1% of the dry-land agriculture existing on the globe (FAO 2000). Soil salinity is one of the significant abiotic stresses for crop plants worldwide. Up to 20% of the irrigated arable land in arid and semi-arid regions is already salt affected and it is still expanding (Mühling and Läuchli 2003). In those environments, seawater infiltration can occur or provides the only source of water for irrigation. Today, there is increasing use of saline water in agriculture in arid and semiarid regions, where the availability of fresh water is scarce. Under salt stress, plants have to cope with water stress imposed by the low external water potential and with

ion toxicity due to accumulation of ions inside the plants (Romero-Aranda *et al.* 2006). In addition to its known components of osmotic stress and ion toxicity, salt stress is also manifested as an oxidative stress, which contributes to its deleterious effects (Teixeira and Pereira 2007).

Growth inhibition in plant exposed to salinity stress is associated with damage caused by reactive oxygen species (ROS) like superoxide, hydrogen peroxide and hydroxyl radicals (Wahid *et al.* 2007). In order to avoid the harmful effects of these ROS, plants evolved an effective scavenging system composed of non-enzymatic antioxidants, such as ascorbic acid and enzymatic antioxidants like catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase

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(GR). Scavenging system having potential to quench ROS in stress tolerance plants (Sairam and Srivastava 2002, Koca *et al.* 2007).

Ascorbic acid (Vitamin C) has been proposed for a long time as a biological antioxidant protecting plants from the oxidative stress resulting from biotic and abiotic stresses (Smirnoff 1996 and 2005). Shalata and Neumann (2001) reported that stress increased the accumulation of lipid peroxidation products produced by interactions with damaging reactive oxygen species; additional ascorbic acid inhibited this response. Thus, high endogenous ascorbic acid in plants is necessary to counteract oxidative stress and regulating processes of plant metabolism. Endogenous ascorbic acid can be increased by exogenous application of ascorbic acid through foliar spray (Heikal *et al.* 2000), seed presoaking (Khan *et al.* 2006) or addition to root medium (Athar *et al.* 2008).

Roselle (*H. sabdariffa* L.) is one of the most important medicinal plants. Several authors have observed variations in antioxidative defences in plants grown under NaCl (Koca *et al.* 2007 and Athar *et al.* 2008), but there is a lack of information about the effect of salt stress in plants irrigated with seawater. Thus, the aim of this work was to provide additional information about the effects of seawater irrigation and ascorbic acid treatments in trying to ameliorate the inhibitory effects of seawater stress on *Hibiscus sabdariffa* L. seedlings.

MATERIALS AND METHODS

The seeds of roselle (*Hibiscus sabdariffa* L. with red sepals variety) were obtained from the Faculty of Agriculture, South Valley University, Egypt. Homogenous seeds were surface - sterilized using 0.1% HgCl_2 solution for 3 min, then rinsed 5 times with sterile distilled water, and divided into two groups. One group was soaked in distilled water (0.0 ascorbic acid), and the other group was soaked in 200 ppm (this concentration was suggested after some preliminary experiments) freshly prepared ascorbic acid (AsA) for 6 h, after which they thoroughly air dried. The treated seeds either by distilled water or 200 ppm ascorbic acid were divided into 4 treatments and sown in plastic pots (12 cm in diameter and 10 cm in height, 10 seeds in each pot) lined

with polyethylene bags and filled with soil composed of clay and sand (1:1 by volume). The pots of first treatment were irrigated with normal water to serves as control (0.0% seawater), and the other three treatments were irrigated with different concentrations (7, 14 and 21%) of seawater (seawater /normal water). Three replicates from each treatment were prepared. Then, the pots were kept in growth chamber maintained at $32/28 \pm 2^\circ\text{C}$ day/night (12 h) temperature cycles, light intensity of $900 \text{ mmol m}^{-2} \text{ s}^{-1}$, and relative humidity 70%. The concentrations of seawater were maintained constant through the experimental period by adding distilled water. After 18 d of seawater treatment the seedlings were harvested, washed with deionized water, measured and analyzed.

Chlorophylls (chl *a* and chl *b*) contents were estimated in 80% acetone extracts according to Linchtenthaler and Wellburn (1983). The contents of chlorophyll *a* and *b* and chlorophyll *a/b* ratios were calculated. Dry matter yields of the seedlings were determined after drying the washed freshly seedlings in an aerated oven at 80°C to constant weight. The samples were ground into fine powder and stored in sealed glasses at room temperature for the chemical analysis.

Carbohydrate fractions were extracted from the plant tissues and determined according to the anthrone sulphuric acid method (Fales 1951, Schlegel 1956, Badour 1959). The soluble and insoluble proteins were determined according to Bradford (1976). Proline was determined according to Bates *et al.* (1973). Total free amino acids were determined according to the method of Lee and Takahashi (1966). Ion leakage (membrane permeability) of the excised leaves was measured as described by Yan *et al.* (1996). Lipid peroxidation level was measured as the content of malondialdehyde (MDA) using the thiobarbituric method (Zaho *et al.* 1994). It was expressed as n mol of MDA formed using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$. Ascorbic acid concentration was estimated according to the method of Mukherjee and Choudhuri (1983).

Enzyme activity: Fresh leaves (250 mg) were frozen in liquid nitrogen and finely ground and extracted in extraction buffer (50 mM potassium phosphate buffer,

pH 7.0) containing 1 mM EDTA and 1% soluble PVP. The homogenate was centrifuged at 15,000 g for 20 min at 4 °C. The resulting supernatant was used for POD, CAT, APX and GR assays.

The activity of peroxides was determined using guaiacol reaction solution (Maehly and Chance 1954), the increase in absorbance due to the formation of tetraguaiacol was monitored at 470 nm (Klapheck *et al.* 1990). Catalase activity was assayed as described by Aebi (1984). It was estimated by the decrease of absorbance at 240 nm as a consequence of H₂O₂ consumption (Havir and Mellate 1987). Ascorbate peroxidase activity was determined from the decrease in the absorbance of ascorbic at 290 nm as ascorbic acid oxidized (Asada and Chen 1992). The activity of glutathione reductase was measured according to Foyer and Halliwell (1976), which depends on the rate of decrease in the absorbance of NADPH at 340 nm. All data were analyzed statistically by one-way ANOVA. Results are presented as means ± SD based on three independent determinations.

RESULTS

Seawater irrigation caused a significant reduction on fresh weight, dry matter and water contents (Fig. 1) of *Hibiscus sabdariffa* L. seedlings. However, low concentration had no effect. The presoaking of seeds in 200 ppm AsA solution resulted a significant increase in growth parameters and water content as compared with AsA untreated seedlings. Moreover, water contents of seedlings irrigated with seawater were higher than those of seedlings irrigated with normal water (control).

Low concentration (7%) of seawater exerted a favourable effect on the contents of chlorophyll *a* + *b* of *H. sabdariffa* L. seedlings, whereas at the higher concentrations of seawater, pronounced decrease was observed (Fig. 2C). The reduction in chlorophyll *b* (Fig. 2B) was more pronounced than for chlorophyll *a* (Fig. 2A), resulting in a higher chl. *a/b* ratio (Fig.2D), as compared to the control. Ascorbic acid treatments show a stimulatory effects on the biosynthesis of chlorophyll contents, especially at higher levels of seawater. Moreover, at all seawater concentrations used, AsA

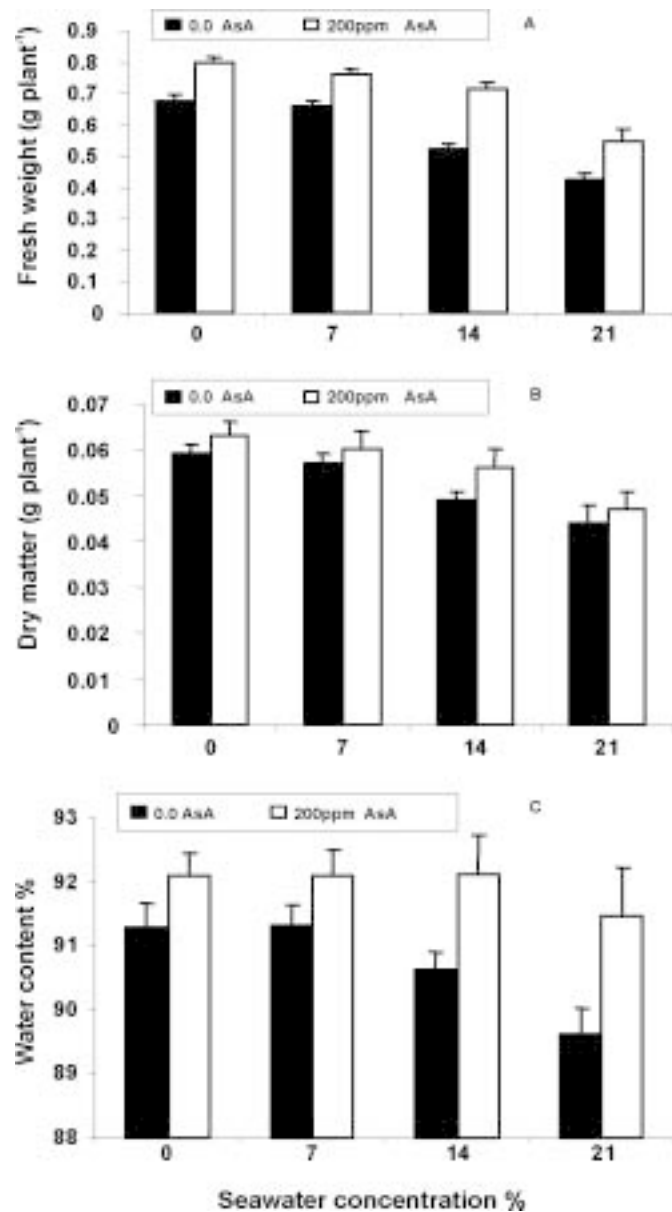


Fig. 1. Effect of seawater concentration (%) and/or ascorbic acid 200 ppm on fresh weight (A), dry matter (B) and water content% (C) of *Hibiscus sabdariffa* L. seedlings. Each value is the mean of three replicates. Vertical bars represent SD (*n*=3)

treatments resulted in greater chlorophyll contents than the control seedlings (0.0% seawater).

Generally, the results of this investigation show that, seawater stress increases the contents of carbohydrates (soluble, insoluble and total) and soluble protein. However, insoluble and total proteins were markedly

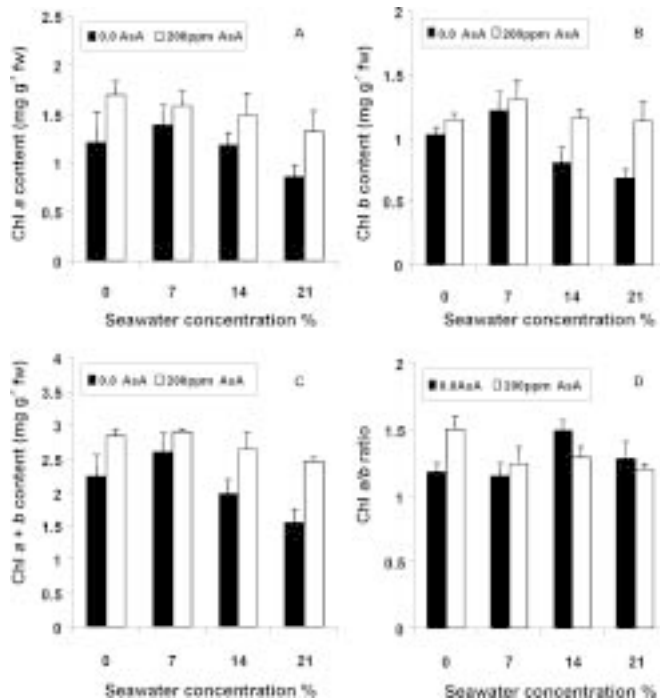


Fig. 2. Effect of seawater concentration (%) and/or ascorbic acid 200 ppm on chl *a* (A), chl *b* (B), chl *a* + *b* (C) and chl *a* / *b* (D) of *Hibiscus sabdariffa* L. seedlings. Each value is the mean of three replicates. Vertical bars represent SD ($n=3$).

decreased with increasing of seawater concentration (Fig. 3). The increase in soluble carbohydrates (about 250%) caused by seawater treatments was more obvious than soluble protein (about 170%) at the highest concentration (21%). Ascorbic acid enhanced the production of carbohydrates and proteins in all seawater treatments.

The contents of total free amino acids (Fig. 4A), proline (Fig. 4B), ion leakage (Fig. 4C) and MDA concentrations (Fig. 4D) of *H. sabdariffa* L seedlings, were increased as result of seawater irrigation. This increase was insignificant at low seawater treatments, while it was significant at the higher levels. Ascorbic acid treatments resulted a pronounced decrease in the total free amino acids and proline contents. In addition, the data show that ascorbic acid treatments reduced the values of ion leakage and inhibited the level of MDA content, whatever seawater level used, as compared with the corresponding AsA untreated seedlings.

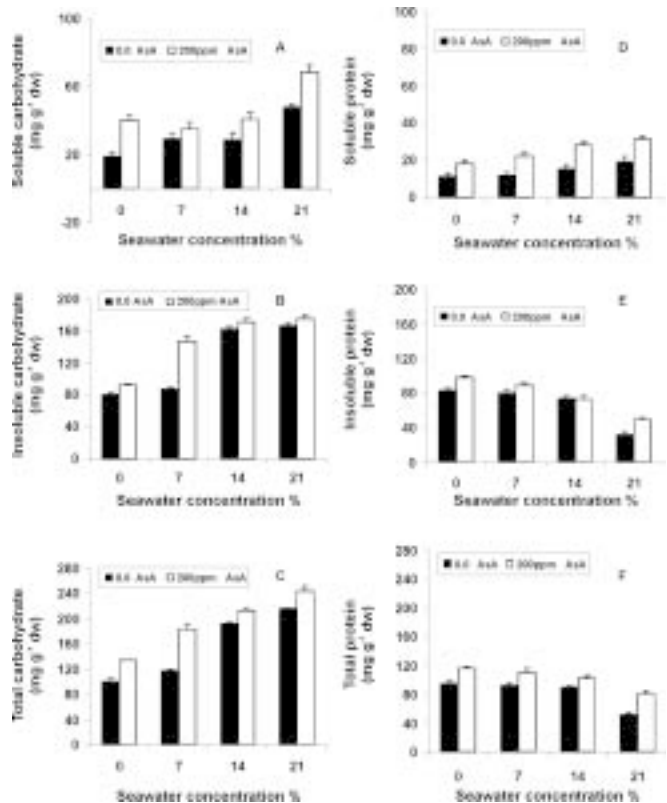


Fig. 3. Effect of seawater concentration (%) and/or ascorbic acid 200 ppm on soluble, insoluble and total carbohydrate (A, B and C) and protein (D, E and F) contents respectively, of *Hibiscus sabdariffa* L. seedlings. Each value is the mean of three replicates. Vertical bars represent SD ($n=3$).

In seedlings subjected to seawater stress (Fig. 5A, B, C and D), the activities of antioxidant enzymes (CAT, POD, APX and GR respectively) were significantly increased by increasing seawater levels. Catalase and peroxidase activities were increased significantly than the other enzymes at all applied seawater concentrations. The content of endogenous ascorbic acid (Fig. 5E) was significantly increased by seawater in comparison with control. The data also show a positive relationship between the activities of APX (Fig. 5C), GR (Fig. 5D) and the endogenous AsA (Fig. 5E) with seawater concentration. Presoaking of seeds in AsA generally increased the activity of these enzymes as compared with AsA untreated seedlings. Further, the endogenous AsA content was significantly increased by exogenous AsA treatment.

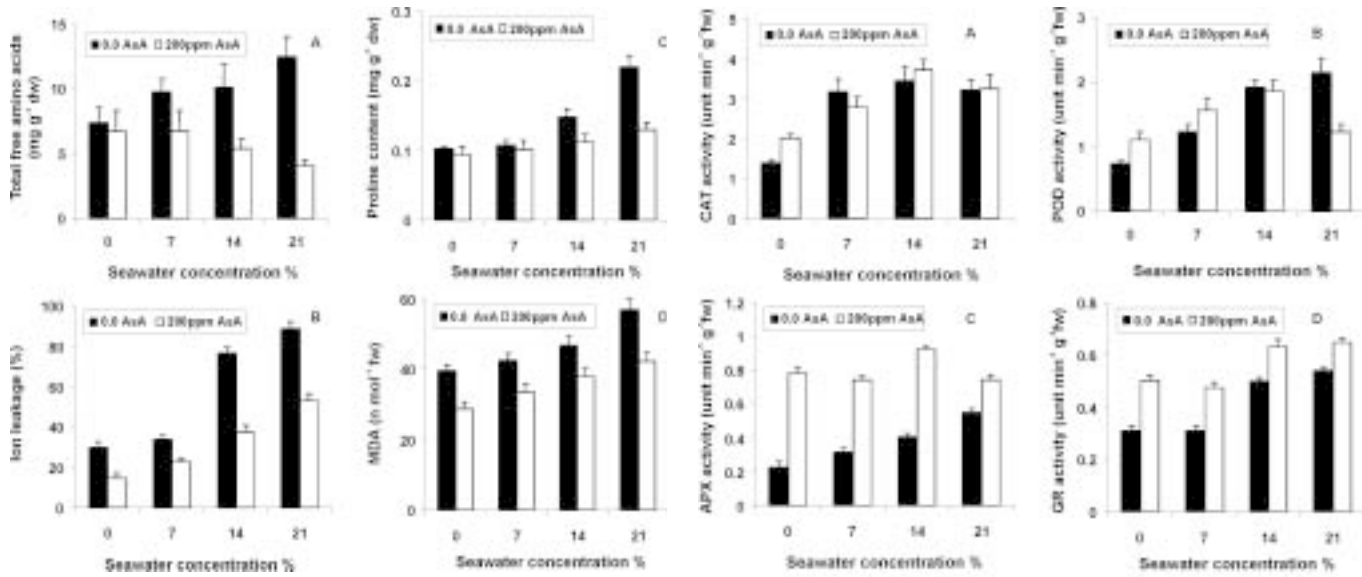


Fig. 4. Effect of seawater concentration (%) and/ or ascorbic acid 200 ppm on total free amino acids (A), proline (B), electrolyte (ion) leakage % (C) and MDA contents (D) of *Hibiscus sabdariffa* L seedlings. Each value is the mean of three replicates. Vertical bars represent SD ($n=3$).

DISCUSSION

The inhibitory effects of seawater salt on growth may be due to the metabolic disorders induced by seawater stress. The effect of seawater salt is disturbances in growth and it reflected in decreased productivity (Kant *et al.* 2007). The insignificant effect of seawater at low concentration on the growth of *H. sabdariffa* L. seedlings confirming the relatively tolerance of these seedlings to seawater stress, and the osmo-regulatory role of organic solutes in increasing the ability of seedlings to absorb water, and consequently maintain a constant pigments and tissue water content (Shaddad *et al.* 1990). These results were linked with water content and total chlorophyll of *H. sabdariffa* L seedlings, and are in agreement with those reported by Shabala *et al.* (2000) and Koca *et al.* (2007). The significant reduction in growth at higher concentrations of seawater may be attributed to the harmful osmotic effect of the higher seawater concentrations on soil moisture stress and nutrient balance disorder in the root media (Salter *et al.* 2007).

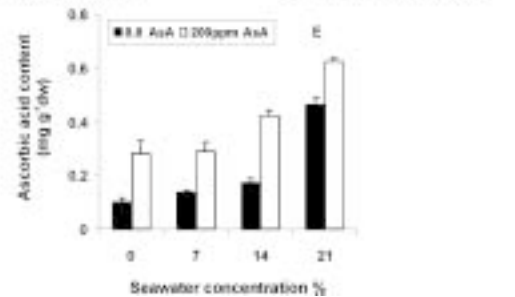


Fig. 5. Effect of seawater concentration (%) and/ or ascorbic acid 200 ppm on the activities of catalase (CAT) A, peroxidase (POD) B, ascorbate peroxidase (APX) C, glutathione reductase (GR) D and the content of endogenous ascorbic acid E (AsA) of *Hibiscus sabdariffa* L. seedlings. Each value is the mean of three replicates. Vertical bars represent SD ($n=3$).

The favourable effect of seawater on the content of chl. *a* and *b* could be due to chloroplast development or to increased thylakoid number (Garcia *et al.* 2005). In addition, compatible solutes play an important role in preventing thylakoid membrane damage during dehydration (Schwab and Heber 1984). The inhibitory effects of seawater stress on leaf chlorophyll contents could be due to suppression of specific enzymes responsible for the synthesis of green pigments (Garg and Singla 2004). De La Rosa and Maiti (1995) suggested that the tendency to decrease the chlorophyll content might be due to the synthesis of nitrogen compounds. This suggestion is in agreement with our

results, which showed that, the decrease in the biosynthesis of chlorophylls was accompanied by increase the synthesis of protein and proline. Ascorbic acid improves the growth of *H. sabdariffa* L seedlings recorded under non-saline or saline conditions, this may be due to increase in cell division and/or cell enlargement as suggested by Cabo *et al.* (1996) and Athar *et al.* (2008). Ascorbic acid also induces the synthesis of leaf chlorophyll contents, this was parallel with results obtained by Gopala Rao and Sastry (1972).

The accumulation of soluble carbohydrates and proteins under seawater irrigation could be accounted for the osmotic adjustment that could have contributed to the maintenance of water balance of *H. sabdariffa* L. seedlings under sea salt stress conditions. The pattern of changes of total carbohydrate was opposite to that obtained for changes in protein. This gives a considerable reason to believe that salt tolerance seems to be linked with an equilibrium and interconversion between carbohydrates and nitrogen metabolism in the stressed plant (Shaddad *et al.* 1990, Azooz *et al.* 2002). The enhancement effect of AsA on the production of carbohydrates and proteins might be attributed to increase of photosynthetic rate as reported by Athar *et al.* (2008).

Total free amino acids and proline concentration significantly accumulated as a result of seawater stress. Proline accumulation might be used as an indicator in selection for withstanding saline stress through the involvement in osmoregulation (Udeda *et al.* 2007). The reduction of total free amino acids and proline as a result of AsA treatments was closely associated with the obvious increase in soluble protein contents. This means that AsA treatments could induce the incorporation of amino acids into protein as reported by Heikal *et al.* (2000).

The present study showed a positive correlation of ion leakage and MDA with the concentration of seawater. This correlation cleared that the insignificant increase of ion leakage and MDA content at low seawater concentration is important sign of higher oxidative damage limiting capacity under seawater stress, and the significant increase at the highest level of seawater was as a results of plasmalemma injury caused

by ROS (Yin *et al.* 2008). However, exogenous AsA inhibited the ion leakage and accumulation of MDA content. This is in accordance with the results obtained by Shalata and Neumann (2001). The additional ascorbic acid might inhibit stress-induced increases in the leakage of essential electrolytes following peroxidative damage to plasma membrane (Molassiotis *et al.* 2006).

The activity of antioxidant enzymes (CAT, POD, APX and GR) and the endogenous AsA content significantly increased by increasing seawater concentration. The increases in the activities of these enzymes are related to the salt tolerance of *H. sabdariffa* L. seedlings. The tolerance of many plants has been reported as a response to salt stress due to the increase in the activities of antioxidant enzymes (Parida and Das 2005, Koca *et al.* 2007 and Athar *et al.* 2008). Catalase is the main scavenger of strong oxidant H_2O_2 and converts it to water and molecular oxygen (Willekens *et al.* 1995). The higher activity of CAT and POD at all seawater concentrations is a good indication of ability of *H. sabdariffa* L. seedlings to cope with reactive oxygen species. Similar results were found by Azevedo Neto *et al.* (2006) and Koca *et al.* (2007). The positive relationships between the activities of APX, GR and AsA in our study, support the hypothesis that GR catalyzes reactions that maintain large pools of glutathione and ascorbic acid in the H_2O_2 scavenging pathway in chloroplast (Smith *et al.* 1989). The enzymatic action of APX reduces H_2O_2 using the ascorbic acid as an electron donor. The higher AsA contents that we observed at high concentration of seawater could suggest that ascorbic acid synthesis was stimulated or ascorbic acid catabolism was inhibited. In some plants acclimated to high salinity condition, a significant increase in ascorbic acid was found (Shalata *et al.* 2001, Chaparzadeh *et al.* 2004). Application of AsA increased the activity of antioxidant enzymes and the content of endogenous AsA. This may be reduce the accumulation of ROS in salt stress seedlings (Athar *et al.* 2008).

In conclusion, this report shows that, exogenous application of ascorbic acid increased the capacity of *Hibiscus sabdariffa* L. seedlings to survive the inhibitory effects of severe seawater stress. This increase was associated with the high constitutive activity of

antioxidant enzymes, decrease of ion leakage and lipid peroxidation levels. Being an endogenous non-enzymatic antioxidant, ascorbic acid is effective in scavenging ROS. Finally, in future, the beneficial effects of ascorbic acid may be used for improving plant growth and yield in saline areas or by using diluted seawater for irrigation of plant.

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REFERENCES

- Aebi, H. (1984). Catalase *in vitro*. *Methods Enzymol.* **105**: 121-126.
- Asada, K. and Chen, G. (1992). On activation of ascorbate peroxidase by thiols requires hydrogen peroxide. *Plant Cell Physiol.* **33**: 117-123.
- Athar, H., Khan, A. and Ashraf, M. (2008). Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environ. Exp. Bot.* **63**: 224-231.
- Azevedo Neto, A.D., Perico, J.T., Eneas-Filho, J., Braga de Abreu, C.E. and Gomes-Filho, Y. (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environ. Exp. Bot.* **56**: 235-241.
- Azooz, M.M., Hassanein, M.A. and Faheed, F.A. (2002). Riboflavin (Vitamin B2) treatments counteracting the adverse effects of salinity on growth and some physiological responses of *Hibiscus sabdariffa* L. *Bull. Fac. Sci. Assuit Univ.* **31** (2-D): 295-303.
- Badour, S.S.A. (1959). Analytisch-chemische untersuchung des Kaliummangles bei *Chlorella* im Vergleich mit anderen Mangelzuständen *Ph.D. Dissertation* Göttingen.
- Bates, L.S., Waldren, R.P., and Tear, L.D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil* **39**: 205-207.
- Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248-254.
- Cabo, R.C., Gonza-lez-Reyes, J.A., Cordoba, F. and Navas, P. (1996). Rooting hastened in onions by ascorbate and ascorbate free radical. *Plant growth Regul.* **15**: 53-56.
- De La Rosa-Ibarra, M. M. and Maiti, R.K. (1995). Biochemical mechanism in glossy sorghum lines for resistance to salinity stress. *Plant Physiol.* **146**: 515-519.
- Fales, F.W. (1951). The assimilation and degradation of carbohydrate by Yeast cells. *J. Biol. Chem.* **193**: 113-124.
- FAO (2000). Globale network on integrated soil management for sustainable use of salt-affected soils. Available in: <http://www.fao.org/ag/AGL/agll/spush/intro.htm>.
- Foyer, C.H. and Halliwell, B. (1976). The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. *Planta* **133**: 21-25.
- Garcia, X. V., Garcia, E.M., Rascon, Q.C., Herrera, L.E. and Aguado, G.A.S. (2005). Chlorophyll accumulation is enhanced by osmotic stress in graminaceous chlorophyllic cells. *J. Plant Physiol.* **162**: 650-661.
- Garg, N. and Singla, R. (2004). Growth, photosynthesis, nodule nitrogen and carbon fixation in the chickpea cultivars under salt stress. *Braz. J. Plant Physiol.* **16**: 137-146.
- Gopala Rao, P. and Sastry, K.S. (1972). B-group vitamins during the seedling growth of late and early varieties of groundnut (*Arachis hypogea* L.) *J. Indian Bot. Soc.* **51**: 155-161.
- Havir, E.A. and Mellate, N.A (1987). Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiol.*, **84**: 450-455
- Heikal, M.M., Ismail, A.M. and Azooz, M.M. (2000). Interactive effects of salinity and vitamin B6 on some metabolic processes in two broad bean lines. *Acta Agron. Hung.* **48**: 327-336.
- Kant, S., Kant, P., Lips, H. and Barak, S. (2007). Partial substitution of NO₃⁻ by NH₄⁺ fertilization increases ammonium assimilating enzyme activities and reduces the deleterious effects of salinity on the growth of barley. *J. Plant Physiol.* **164**: 303-311.

- Klapheck, S., Zimmer, I. and Cosse, H. (1990). Scavenging of hydrogen peroxide in endosperm of *Ricinus communis* by ascorbate peroxidase. *Plant Cell Physiol.* **31**: 1005-1013.
- Koca, H., Bor, M., Ozdemir, F. and Turkan, I. (2007). Effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ. Exp. Bot.* **60**: 344-351.
- Linchtenthaler, H.K., and Wellburn, A.R. (1983). Determination of total carotenoids and chlorophyll *a* and *b* of leaf extract in different solvents. *Biochem. Soc. Trans.* **11**: 591-592.
- Lee, Y.P. and Takahashi, T. (1966). An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem.* **14**: 71-77.
- Maehly, A.C. and Chance, B. (1954). The assay of catalase and peroxidase. *Methods Biochem. Anal.* **1**: 357-424..
- Molassiotis, A., Sotiropoulos, T., Tanou, G., Diamantidis, G. and Therios, I. (2006). Boron-induced oxidative damage and antioxidant and nucleolytic responses in shoot tips culture of the apple rootstock EM9 (*Malus domestica* Borkh). *Environ. Exp. Bot.* **56**: 54-62.
- Mühling, K.H. and Läuchli, A. (2003). Interaction of NaCl and Cd stress on compartmentation pattern of cations, antioxidant enzymes and proteins in leaves of two wheat genotypes differing in salt tolerance. *Plant Soil* **253**: 219-231.
- Mukherjee, S.P. and Choudhuri, M.A. (1983). Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Plant Physiol.* **58**: 166-170.
- Parida, A.K. and Das, A.B. (2005). Salt tolerance and salinity effects on plants. *Ecotoxicol. Environ.* **60**: 324-349.
- Romero-Aranda, M.R., Jurado, O. and Cuartero, J. (2006). Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status. *J. Plant Physiol.* **163**: 847-855.
- Sairam, R.K. and Srivastava, G.C. (2002). Changes in antioxidant activity in sub-cellular fractions of tolerant susceptible wheat genotypes in response to long term salt stress. *Plant Sci.* **162**: 897-907.
- Salter, J., Morris, K., Bailey, P.C.E. and Boon, P.I. (2007). Interactive effects of salinity and water depth on the growth of *Melaleuca ericifolia* Sm. (Swamp paperbark) seedlings. *Aquatic Bot.* **86**: 213-222.
- Schlegel, H.G. (1956). Die verwertung organischer Sauren durch *Chlorella* im licht. *Planta* **47**: 510-526.
- Schwab, K., Heber, U. (1984). Thylakoid membrane stability in drought tolerant and drought- sensitive plants. *Planta* **161**: 37-45.
- Shabala, S., Babourina, O. and Newman, I. (2000) Ion specific of osmoregulation in bean mesophyll cells. *J. Exp. Bot.* **51**: 1243-1253.
- Shaddad, M.A., Radi, A.F., Abdel-Rahman, A.M. and Azooz, M.M. (1990). Response of seeds of *Lupinus termis* and *Vicia faba* to the interactive effect of salinity and ascorbic acid or pyridoxine. *Plant Soil* **122**: 177-183.
- Shalata, A. and Neumann, P.M. (2001). Exogenous ascorbic acid (Vitamin C) increases resistance to salt tolerance and reduced lipid peroxidation. *J. Exp. Bot.* **364**: 2207-2211.
- Shalata, A., Mittova, V., Volokita, M., Guy, M. and Tal, M. (2001). Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt-dependent oxidative stress: the root antioxidative system, *Physiol. Planta* **112**: 487-94.
- Smirnoff, N. (1996). The role of active oxygen in the response of plant to water deficit and desiccation. *New Phytol* **125**: 27-58.
- Smirnoff, N. (2005). Ascorbate, tocopherol and carotenoids: metabolism, pathway engineering and functions. In: N. Smirnoff (ed.), *Antioxidants and Reactive Oxygen Species in Plants*, pp. 53-86. Blackwell Publishing Ltd., Oxford, UK.
- Smith, I.K., Vierheller, T.L. and Thorne, C.A. (1989). Properties and functions of glutathione reductase in plants. *Physiol. Planta.* **77**: 449- 456.
- Teixeira, J. and Pereira, S. (2007). High salinity and drought act on an organ-dependent manner on potato glutamine synthetase expression and accumulation. *Environ. Exp. Bot.* **60**: 121-126.

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- Udeda, A., Yamamoto-Yamane, Y. and Takabe, T. (2007). Salt stress enhances proline utilization in the apical region of barley roots. *Biochem. Biophys. Res. Comm.* **355**: 61-66.
- Wahid, A., Perveen, M., Gelani, S. and Basra, S.M.A. (2007). Pretreatment of seed with H₂O₂ improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *J. Plant Physiol.* **164**: 283-294.
- Willekens, H., Inze, D., Van Montagu, M. and Van Camp, W. (1995) Catalase in plants. *Mol. Breed.* **1**: 207-228.
- Yan, B., Dai, Q., Liu, X., Huang, S. and Wang, Z. (1996). Flooding induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. *Plant Soil* **179**: 261-268.
- Yin, H., Chen, Q. and Yi, M. (2008). Effect of short-term heat stress on oxidative damage and responses of antioxidant system in *Lilium longiflorum*. *Plant Growth Regul.* **54**: 45-54.
- Zaho, S.J., Xu, C.C. and Zou, Q. (1994). Improvements of the method for measurement of malondialdehyde in plant tissue. *Plant Physiol. Commun.* **30**: 207-210.