J. Agric. Sci. Mansoura Univ., 34 (11): 10645 - 10661, 2009

THE ROLES OF ASCORBIC ACID AND A-TOCOPHEROL IN MINIMIZE OF SALT-INDUCED WHEAT FLAG LEAF SENESCENCE

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ABSTRACT

Experiments were conducted to study the α-tocopherol and ascorbic acid on modify the leaf senescence process of wheat grown under three salinity levels (0.8, 7.5, and 11.5 dSm^{-1}). The parameters analyzed were soluble protein content, chlorophyll content, electrolyte permeability, ions content, lipid peroxidation, hydrogen peroxide content, catalase and peroxidase activities, ascorbic acid content, phenol, and total carotenoids contents.

Salinity hastened the naturally-occurring senescence rate of wheat flag leaves, it decreased concentrations of chlorophyll, total carotenoids, ascorbic acid, total phenol, calcium, potassium, magnesium, K^+/Na^+ ratio and soluble protein contents as well as the activities of Catalase and peroxidase. On the other hand, it increased sodium and chloride and $chlorophyll_{a:b}$ ratio as well as membrane permeability, hydrogen peroxide content and malondialdehyde content.

Spraying with both antioxidants reduced the hydrogen peroxide accumulation, lipid peroxidation, membrane permeability, sodium and chloride content, whereas the antioxidants enzyme activities (catalase, and peroxidase) were increased. Enhanced accumulation of ascorbate, phenol, carotenoids, calcium, potassium and magnesium was recorded in antioxidants-sprayed plants at 65 days after sowing. Under moderate and sever salinity levels both antioxidants alleviated the harmful effects of salinity on leaf senescence related parameter.

The higher levels of antioxidants (ascorbic acid, phenol, carotenoids) and low level of H_2O_2 in flag leaf may be the prerequisite for delayed leaf senescence in antioxidants-sprayed plants. It can be concluded that ascorbic acid- sprayed plants can postpone the leaf senescence by peroxide/phenolic/ascorbate system which is involved in scavenging the ROS produced during leaf senescence.

Keywords: antioxidants, malondialdehyde, salinity, senescence, wheat

Abbreviation: Ascorbic acid (AsA), Ascorbic peroxidase (APx), Calcium (Ca^{+2}), Catalase (CAT), Chlorophyll (Chl), Electrical conductivity (EC), Electrolyte leakage percentage (ELP), Magnesium (Mg^{+2}), Malondialdehyde (MDA), Peroxidase (POX), Potassium (K^+), Reactive oxygen species (ROS), Sodium (Na^+), Thiobarbituric acid (TBA), Thiobarbituric acid reaction (TBAR), α-tocopherol (TOC),

INTRODUCTION

Photosynthesis is the primary source of dry matter production and grain yield in crop plants. Early senescence of leaf area seriously restricts the yield potential. Flag leaf photosynthesis in wheat contributes 30–50% of the needed for grain filling (Sylvester-Bradley et al. 1990). The onset and rate of leaf senescence are thus important factors for determining grain yield.

Leaf senescence usually occurs during the reproductive stage and it is correlated closely with the resistance/susceptibility to oxidative stress. The degradative processes due to leaf senescence are accompanied by chlorophyll loss, decreases in soluble protein contents, and changes in the $Chl_{a:b}$ ratios (Munne-Bosch, 2007). Moreover, leaf senescence is related to an increase in reactive oxygen species (ROS), lipid peroxidation and membrane leakiness (Navabpour et al., 2003). In plant cells, chloroplasts are one of the primary generators of ROS as singlet oxygen, superoxide radicals, hydrogen peroxide and hydroxyl radicals, which can cause damage proteins, chlorophyll, lipids, polysaccharides and DNA with numerous consequences (Møller et al., 2007). Moreover, salt-stress induced senescence is also related to osmotic component, accumulation of toxic ions (as sodium and chloride), and nutrient depletion; i.e. potassium and calcium (Leidi et al., 1991). Magnesium, by comparison, has received little attention, as a role-player in senescence-related processes, even though it is implicated in the regulation of protein synthesis and a decrease chlorophyll synthesis (Flowers and Dalmond 1992).

Plant cells possess a variety of defense strategies against oxidative injury. Those strategies can include detoxifying enzymes such as catalase (CAT), or peroxidase (POD); as well as non-enzymatic components, such as α-tocopherol, proline and ascorbic acid or phenol (Mittler 2002, Zabalza et al., 2007). Among the various non-enzymatic components the level and reduction state of ascorbate is widely used as an indicator of antioxidative stress in biological system (Kukavica and Jovanovic 2004). In addition, antioxidants and phenolics are able to act as ROS scavengers or ROS chain breaker, thus extinguishing strongly oxidative free radicals such as the hydroxyl radical yielding products with much lower oxidative capacities compared with the parent compounds. Hence, it is postulated that foliar spray of antioxidants (Conklin and Barth 2004, Krieger-Liszkay and Trebst 2006) can modify leaf senescence process in wheat flag leaves.

Ascorbic acid (AsA) fulfils essential metabolic functions in plants. It serves as a co-factor for many enzymes and contributes to the detoxification of ROS (Conklin and Barth 2004). The antioxidant activity of AsA is associated with longevity in plants and resistance to oxidative stress. Further, the endogenous level of AsA is suggested to be important to the regulation of developmental senescence (Pavet et al. 2005). α-Tocopherol (Toc) is a lipophilic membrane-located compound present in chloroplasts (Wise and Naylor 1987). Toc is believed to protect chloroplast membranes from photo-oxidation and to help provide an optimal environment for the photosynthetic machinery (Wise and Naylor 1987). Most of the proposed Toc functions are related to its antioxidant properties. The most prominent of which is protection of polyunsaturated fatty acids from lipid peroxidation (Krieger-Liszkay and Trebst 2006).

Much work has been done on antioxidants activity and the production of oxidants during senescence. However, little information is available on the actual role of the application of antioxidants under salinity stress in relation to the progression of leaf senescence. Hence, the present investigation evaluates the potential of both AsA and Toc-spray on altering ROS content,

antioxidant content, and antioxidant enzyme activities in senescing wheat flag leaves under salinity stress. The evaluation was made from 65 DAS by assessing chlorophyll and soluble protein content, $Chl_{a:b}$ ratio, ion content, hydrogen peroxide content, antioxidant content, membrane damage, and enzymatic activities.

MATERIALS AND METHODS

Two pot experiments were carried out in the green house of Agric. Bot. Dept., Fac. of Agric., Mansoura Univ during the two growing seasons of 2006 and 2007. A closed bottom plastic (pots 30 cm inner diameter) were filled with 15 kg clay loamy soil. The chemical analysis of the soil used are presented in Table (1).

Chemical analysis of the soil used according to Jakson (1973).

Anions meq/100 g soil				Cations meq/100 g soil			
Cl	**So_4**	**Co_3**	**HCo_3**	**Na**	**K**	**Ca**	**Mg**
0.51	0.786	-	0.27	0.45	0.006	0.38	0.60

Soil in the pots salinized with NaCl at different concentrations to recorded 0.8 (control), 7.5 and 11.5 dsm^{-1} levels. Before sowing, calcium super phosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O) fertilizers were added at the rate of 5 g P_2O_5 and 2 g K_2O/pot. Fifteen uniform wheat grains (Giza 168 cultivar) were sown on 10th and 15th November in the two growing seasons, respectively. Ammonium nitrate (33.5%) was added at the rate of 4 g N/pot in two equal portions; the first during the seedling stage and the second at the appearance of the flag leaf. At 40 days from sowing (DFS), the pots at each salinity levels were divided into three groups. The first group was sprayed twice with water (control), while the other two groups were sprayed twice (i.e. after 40 and 50 DFS) with aqueous solutions of either ascorbic or α-tocopherol at the rate of 100 mg/l until run-off, with Tween 20 as a wetting agent. At heading (65 DFS), four randomly selected plants were harvest per pot and the removed for determination of biochemical constituents.

Chlorophyll was extracted for 24 hour at room temperature in methanol after adding traces of sodium carbonate. Chlorophyll concentrations were determined spectrophotometrically according to Lichtenthaler and Wellburn (1985). Soluble protein concentration was measured at 595 nm using bovine serum albumin as standard according to the method of Bradford (1976). For ion content, dry flag leaf samples were digested with $HClO_3/H_2SO_4$ until the solution was clear, cooled, and brought to volume at 50 ml using deionized water. Potassium and sodium concentrations were determined using a flame photometer. Calcium and magnesium were determined using versenate methods according to Richard`s (1954). Chloride was extracted from dried plant materials using deionized water, then determined by volumetric titration with 0.001 N $AgNO_3$ using potassium dichromate as an indicator (Hanson and Munns 1988).

Lipid peroxidation was estimated as thiobibutric acid reactive substances (TBARS). Malondialdehyde content "MDA" was determined and calculated as µmoles/g of fresh weight by the method of Shao et al (2005). Dried leaf samples (0.5 g) were homogenized in 5 ml of distilled water. An equal volume of 0.5% thiobibutric acid (TBA) in 20% trichloroacetic acid solution was added and the sample incubated at 95°C for 30 min. The reaction was stopped by placing the reaction tubes in an ice bath. The samples were then centrifuged at 10,000×g for 30 min. The supernatant was removed, absorption read at 532 nm, and the amount of nonspecific absorption at 600 nm and subtracted from this value. The amount of MDA present was calculated from the extinction coefficient of 155 mM^{-1} cm^{-1}. Electrolyte leakage percentage measurement (ELP) was used to assess membrane permeability according to Goncalves et al. (2007), using an Electrical Conductivity Meter (EC). Flag leaf samples were placed in vials containing distilled water and incubated at room temperature for 24 h. Electrical conductivity of the resulting solution (EC1) was recorded after incubation. Samples were then placed in a boiling water bath for 30 min, cooled to room temperature, and the second reading (EC2) determined. The ELP was calculated as EC1/EC2 and expressed as percentage.

Hydrogen peroxide content was estimated by forming a titanium-hydro peroxide complex *via* methods outlined by (Rao et al. 1997). Catalase (EC 1.11.1.6) was extracted in a phosphate buffer and assayed by measuring the disappearance of H_2O_2 according to Teranishi et al. (1974). Peroxidase (EC1.11.1.7) was determined according to a modified method based on Reuveni and Reuveni (1995). Each enzyme activity was expressed as enzyme unit per gram fresh weight of leaf. Ascorbic acid was extracted from plant material and titrated using 2,6-dichlorophenol indophenole as described by Sadasivam and Manickam (1996). Total phenolic compounds were determined according to the method of Singleton and Rossi (1965) using Folin-Ciocalteau reagent.

Statistical analysis: The data were analyzed using Analysis of Variance (ANOVA) and mean separations adjusted by the Multiple Comparison test (Norman and Streiner 2003) using MSTAT-C v.1.2. statistically computer program. Significance between treatments was compared at the 0.05 probability level.

RESULTS

Chlorophyll and soluble protein concentration:

Data in Table (1) indicate that the concentration of total chlorophyll and soluble protein in flag leaf at two growing seasons were significantly decreased under salt stress. This decrease was more pronounced in 11.5 dSm^{-1} than in 7.5 dSm^{-1} NaCl. In the same time the ratio between chl a and chl b was increased by increasing salinity stress.

Under control, application of both antioxidants; AsA and Toc increased total chlorophyll and soluble protein content in the two growing seasons, whereas, decreased chla:b ratio. AsA was more effective than Toc

in this respect. Under moderate and sever NaCl salinity stress, application of antioxidants, especially ascorbic acid, nullifes the harmful effect of salinity levels on chlorophyll and soluble protein content as well as chla:b ratio in the two growing season (Table 1).

Ion contents: (K, Ca,Mg, Na, Cl)

Data in Tables (2, 3) show that sodium and chloride contents increased gradually with increasing salinity levels up to 11 dsm^{-1}, this increase was accompanied by a corresponding decline in potassium, magnesium and calcium concentration leading to a significantly decrease on the K^+/Na^+ ratio. Application of both antioxidants especially ascorbic acid, significantly increased the K^+/Na^+ ratio, potassium, calcium and magnesium, while decreased the content of sodium and chloride in flag leaf. The application of both antioxidants, in particular, AsA, partially reversed the negative effects of salinity in this respect at the two growing seasons.

Lipid Peroxidation, membrane permeability and hydrogen peroxide concentration:

One of the expected consequences of salt-induced cellular build-up of reactive oxygen species (ROS) is an increase in lipid peroxidation. The assay of cellular accumulation of lipid peroxidation products, in the form of thiobarbituric acid reactive substances (TBARS), can provide a comparative indication of such activity. In the present study, MDA content and membrane permeability were utilized as biomarkers for lipid peroxidation. Sodium chloride salt stress induced the accumulation of TBARS in flag leaf of salt-affected plants up to 11.5 dSm^{-1} followed by an increase in electrolyte leakage due to the hyper-accumulation of hydrogen peroxide in the two growing seasons (Table, 4). On the other hand, the application of antioxidants, particularly ascorbic acid, significantly decreased TBARS formation and membrane permeability as well as hydrogen peroxide content in flag leaf.

Regarding the combinations between sodium chloride salinity levels and antioxidants, the data clearly indicate that antioxidants, especially AsA, counteracted the harmful effect of salinity on TBARS formation and membrane permeability as well as H_2O_2 contents. Under the corresponding salinity levels, application of AsA or Toc significantly decreased TBARS, membrane permeability and hydrogen peroxide. Ascorbic acid was more effective than tocopherol in this aspect in both growing seasons.

Enzymatic and non enzymatic scavenging system:

Tables (5 and 6) shows that sodium chloride salinity reduced the activity of catalase (CAT) and peroxidase (POD), as well as ascorbic acid, total carotenoids and total soluble phenol up to 11.5 dSm^{-1}. Meanwhile, the application of both antioxidants significantly increased the activities of both CAT and POD enzymes, as well as the ascorbic acid, total carotenoids and total soluble phenol contents in the flag leaf in both seasons.

Exogenous application of antioxidants, in particular, AsA, counteracted the harmful effects of salinity on enzymatic and non-enzymatic scavenging systems (Tables 5 and 6) in both seasons.

1-2

3-4

5-6

Antioxidants stimulated the activities of CAT and POD besides leading to an accumulation of ascorbic acid, total carotenoids and total soluble phenol in flag leaves compared to untreated plants under the corresponding salinity levels.

DISCUSSION

Naturally-occurring stress induced senescence

Salinity-induced pre-senescence in flag leaves is associated with an increased production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide and its more toxic derivative hydroxyl radical (Breusegem and Dat, 2006). These toxic ROS in turn oxidize proteins, lipid and DNA when they reach certain threshold levels associated with nutrient relocation to the developing grains, prior to the death of the plants, resulting in lipid peroxidation, cellular damage and cell death as the antioxidants status of the leaf reduced (Kukavica and Jovanovic, 2004). Leaf senescence is most quantified by decreases in protein or chlorophyll concentration (Hameed et al. 2008) and by increases in membrane permeability (Vieira Santos et al., 2001) due to increasing membrane lipid peroxidation.

Among the different ROS, only H_2O_2 is relatively stable and able to penetrate the plasma membrane as an unchanged molecule. H_2O_2, in addition to being toxic in chloroplasts, being powerful inhibitors of the Calvin cycle, is now regarded as a signal molecule and a regulator of gene expression (Hung et al., 2005). The most potentially deleterious effect of H_2O_2 under these conditions is that at higher concentrations it can trigger genetically programmed cell suicide. Data presented suggest that increased oxidative stress as revealed by an increase in H_2O_2 concentration with increasing salinity levels, results in increased lipid peroxidation as reflected in an increase in MDA concentration, and further manifested by an increase in membrane permeability.

On the other hand, it was found that, MDA, a decomposition product of polyunsaturated fatty acid hydroperoxides, has been frequently described as a suitable biomarker for lipid peroxidation under salt stress (Seckin et al. 2009). Lipid peroxidation, which can be initiated by ROS, severely affects functionality and integrity of cell membranes. This increase in lipid peroxidation over the senescence period was seen as a high degree of membrane deterioration. The present investigation are in agreement with observations in a number of plants such as rice (Lutts et al. 1996), where lipid peroxidation was observed to increase in senesced tissues, accompanied by, an increase in electrolyte leakage. Such damage could result from various mechanisms including the oxidation and cross-linking of protein thiols, inhibition of key membrane proteins as H^+-ATPase, or changes to the composition and fluidity of membrane lipids. It seems that the increased solute leakage or membrane permeability during leaf senescence observed in the present study is a consequence of the increased lipid peroxidation (Table 4). Coupled with an increase in MDA and H_2O_2, AsA content, which is a non-enzymatic antioxidants and free radical scavenger, showed lower levels in

the flag leaf of plants grown under saline conditions as compared to control plants (Table 6). This is in agreement with a pervious study of Prochazkova et al. 2001 who found that a declination in ASA during leaf senescence in maize leaves ().

Specific effects of salt stress on leaf senescence have been related to the accumulation of toxic ions (sodium and chloride) or to potassium and calcium depletion (Leidi et al. 1991). Magnesium, by comparison, has received little attention, although it seems to play a central role in senescence-related processes. Magnesium is implicated in the regulation of protein synthesis (Flowers and Dalmond 1992). A decrease in magnesium absorption could also be responsible for decreased chlorophyll content (Leidi et al. 1991). It is known that an excessive amount of sodium and chloride under saline conditions decreased the tissue K^+/Na^+ ratio (Table 2) which in turn impairs the selectivity of the root membrane and results in the accumulation of sodium and chloride in plant organs (Bassuony et al. 2008). The promotion of Na^+ uptake by salinity was accompanied by a corresponding declines of K^+ and Ca^{2+} concentrations, showing an apparent antagonism between K^+, Mg^{2+} and/or Ca^{2+} and Na^+ (Alam 1994). Several mechanisms may be responsible by a decline in calcium and potassium content with increasing salinity, including the antagonism of sodium and both ions at the site of uptake in roots (Alam 1994).

Leaf yellowing is the first step in senescence-associated programmed cell death. The most convenient assay for the chloroplast senescence is the measurement of chlorophyll loss. The process of chlorophyll degradation is associated with a rapid and large accumulation of ROS such as H_2O_2 (Table 4). The decline in chlorophyll content might be partially due to lipid peroxidation of chloroplast membranes or due to the formation of hydroperoxides of fatty acids. Moreover salinity stress enhances the activity of chlororophyllase and interferes with the *de-novo* synthesis of proteins, such as those that bind chlorophyll (Jaleel et al. 2007). In leaves exposed to severe stress not only was the total chlorophyll content drastically reduced, but the $Chl_{a:b}$ ratio increased showing degradation of chl b at a higher rate than chl a (Table 1). This can be explained by the fact that the first step in chl b degradation involves its conversion to chl a (Fang et al. 1998). The increases in the ratio of $Chl_{a:b}$ has been linked with the change in pigment composition of photosynthetic apparatus that possesses lower levels of light harvesting chlorophyll proteins; LHCPs (Loggini et al. 1999).

Acquisition of salt tolerance:

Plant cells posses a variety of defense strategies against oxidative injury caused by salinity stress. Such strategies involve specific detoxifying enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) which decomposes superoxide radicals and hydrogen peroxide respectively, as well as various antioxidants quenchers including Toc and AsA as well as phenols (Mittler 2002). Reinforcement of plant defense mechanisms against oxidative damage, especially when plants are exposed to salinity stress, may be successful achieved by the exogenous application of antioxidants. Senescence associated parameters can thus effectively be retarded by antioxidants. The beneficial effects of antioxidants

addition to salt-affected wheat plants are associated with the maintenance of cell membrane integrity (Table, 4), reducing sodium and chloride contents (Table 2) and favoring potassium, calcium and magnesium absorption (Table, 3) which is reflected by a reduction in senescing-related parameters as indicated in the present study.

Several reports indicated that the beneficial effects of additional antioxidants on plant survival under different salt stress are associated with the partial inhibition of ROS formation and its effects. The lower level of lipid peroxidation in plant treated with antioxidants under normal or saline conditions (Table 4) suggested that it may provide better protection against oxidative damage. Although the inhibitory effect of AsA or Toc on lipid peroxidation appear, to be related, the actual mechanisms (s) are not yet clear. One possibility is that additional antioxidants activate the CAT and POD enzymes (Table 5), which are involved in the detoxification of H_2O_2 in plants. These results are in good agreement with those of Shalata and Neumann (2001) who found that CAT and POD activities increased in tomato plants treated with ascorbic acid. Moreover, application of antioxidants significantly increased carotenoids, ascorbic acid, and the total phenols content in flag leaf (Table 6). This is an adaptive significance, as it lowering the generation of free radicals and thus reduces the lipid peroxidation under salt stress (Alia et al. 1993). In this regard, phenols inhibit the oxidation of lipids, fats and proteins by the donation of a phenolic hydrogen atom to the free radical (Halliwell et al., 1995). The stereo-electronic effects of phenols are largely responsible for their reactivity with the radicals (Burton et al., 1985). The reaction mechanisms by which the hydrogen atoms of phenol is transferred to a radical can be in two distinct pathways hydrogen atoms transfer and proton–coupled electron transfer (Mayer et al., 2002). Jang et al (2007) also documented the antioxidant property of phenol. Thus, phenolics are able to act as radical scavenger or radical chain breakers, so extinguishing strongly oxidative free radicals.

It is now well documented that carotenoids are involved in the protection of the photosynthetic apparatus against photo-inhibitory damage by singlet oxygen ($^1O_2$), that is produced by the excited triplet state of chlorophyll. Carotenoids can directly deactivate singlet oxygen and can also quench the excited triplet stat of chlorophyll, thus indirectly reducing the formation of singlet oxygen species (Foyer and Harbinson 1994). Plant possesses well-defined antioxidant defense mechanisms that eliminate hazardous free radicals. Antioxidant protection involves compounds such as AsA, phenol, carotenoids, proline and an enzymatic system including, SOD, CAT and POD and the Halliwell-Asada pathway. High activities of enzymes involved in the H_2O_2-scavenging system have been described in several species. CAT, in co-operation with peroxidases and other enzymes destroys the H_2O_2 produced by SOD and other reactions (Foyer et al. 1994). Despite its restricted location in peroxisomes and glyoxysome, CAT can play a significant role in defense against oxidative stress, since H_2O_2 readily diffuse across membranes (Bowler et al. 1992). In the present investigation, CAT and POD activities were increased (Table 5) by application of either antioxidants under normal or salinity conditions. Hence, it is proposed that

CAT and POD may play an important role in the rapid defense responses of plant cells to oxidative stress (Zabalza et al., 2007). The higher inhibition of antioxidant enzymes under salinity stress as compared to antioxidant-treated plants indicates increased inactivation of all the antioxidant enzymes by ROS (Djanaguiraman et al., 2005). This might be due to the toxic effects of the high turnover rate of H_2O_2 or its harmful ROS, which impair enzyme activities (Noctor and Foyer, 1998). The result of the present study strongly suggests that antioxidant-sprayed plants have a stronger potential to eliminate ROS through higher CAT and POD activities because of increased phenol availability.

It is very clear that the application of ascorbic acid or tocopherol increased total chlorophyll either through the stimulation of its biosynthesis and/or delay of its degradation. This increase might be attributed to efficient scavenging of ROS by antioxidant enzymes and antioxidants; that would have destroyed the chlorophyll pigments. This view is further supported by the fact that chloroplast is a major source of the production of ROS in plants, but it lacks CAT to scavenge ROS, so that ascorbic acid acts as a substrate for ascorbate peroxidase (APX) to scavenge ROS produced in the thylakoid membranes (Davey et al. 2000). Generally, the stimulating effect of antioxidants on chlorophyll content may be due to stabilizing the enzyme active site and photosynthetic reactions. Application of antioxidants decreased the $chl_{a:b}$ ratio that plays a central role in stabilizing photosynthetic processes leading to increasing photo-assimilation and grain yield. Such a decrease in the $Chl_{a:b}$ ratio seems to be a conflict with the fact that Chl a is relatively stable during senescence, but chlorophyll b is almost labile (Thomas et al. 2002). There is a cycle of interconversion between Chl a and Chl b that is particularly significant in senescence (Metile et al. 1999). The decrease in the ratio of $Chl_{a:b}$ during leaf senescence, is still unclear due to contradictory results from different studies. Functionally, the decrease in $Chl_{a:b}$ ratio improves the capture of far-red radiation and helps to maintain an energy balance between PSI and PSII (Björkman 1981), resulting in optimal functioning. Therefore, the decrease in $Chl_{a:b}$ ratio may favour the plants before late senescence.

Application of both antioxidants alleviated the harmful effect of salinity on ion concentration due to reduction of sodium and chloride accumulation (Table, 2) as well as increasing calcium, potassium and magnesium (Table, 3) which lead to an increase in K^+/Na^+ (Table, 2), Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios when compared with non-saline-grown plants (El-Bassiouny 2005). Application of antioxidants led to an increase in the contents of ions in the flag leaf through their role in increasing osmotolerance and/or through regulating various processes including absorption of nutrients. The antagonistic relations between Na^+ and K^+ may be taken as an indication of the role played by antioxidants in modifying K^+/Na^+ selectivity under salt stress (Azooz 2004). This promotion effect may be due to its role in improving membrane permeability (Table 4) as well as increasing soluble protein content (Table 1) which protects the membrane and membrane bound enzymes.

It could be concluded that shoot treatments with antioxidants specially ascorbic acid can remarkably increases the capacity of wheat plant to survive under severe stress due to a partial inhibition of salt-induced leaf senescence.

ACKNOWLEDGMENT

I would like to thanks Prof. Dr. M. N. Helali (Faculty of Agriculture, Mansoura University), Prof Dr. Humberto Fabio Causin (F.C.E.N., Universidad de Buenos Aires, and Argentina), Prof Dr. Conceição Santos (University of Aveiro, Portugal) and Toler Heather (The Univ. of Tennessee, USA) for helpful insights and critical reviews of the manuscript.

REFERENCES

Alam S.M. (1994). Nutrient uptake by plants under stress conditions. In: Passarakli M (Ed) Handbook of plant and crop stress. Marcel Dekker, New York, Pp 227-246.

Alia P., P, Saradhi, and P. Mohanty (1993). Proline in relation to free radical production in seedlings of Brassica juncea raised under sodium chloride stress. Plant and Soil 156: 497–500.

Azooz M.M. (2004). Proteins, sugars and ion leakage as a selection criterion for the salt tolerance of three sorghum cultivars at seedling stage grow under NaCl and nicotinamide. Int. J. Agri. Biol. 6(1): 27-35.

Bassuony F.M., R.A. Hassanein, D.M. Baraka, and R.R. Khalil (2008). Physiological effects of Nictinamide and Ascorbic acid on *Zea mays* plant grown under salinity stress. II changes in nitrogen constituents, protein profiles, protease enzymes and certain inorganic cations. Austrailan J. Basic and applied science 2(3): 350-359.

Björkman O. (1981). Responses to different quantum flux densities. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Physiological Plant Ecology. Spring-Verlag, Berlin, pp 57-107.

Bowler C., M.V. Montague, and D. Inze (1992). Superoxide dismutase and stress tolerance. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:83-116.

Bradford M.M. (1976). A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

Breusegem F.V., and J.F. Dat (2006). Reactive oxygen species in plant cell death. Plant physiology 141:384-390.

Burton G.W., T. Doba, E.J. Gabe, L. Hughes, F.L. Lee, L. Prasad, and K.U. Ingold (1985). Autoxidation of biological molecules. 4. Maximizing the antioxidant activity of phenols. J. Am. Chem. Soc.107:7053-7065.

Conklin P.L., and C. Barth (2004). Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. Plant Cell and Environment 27: 959–971.

Davey M.W., M.V. Mantagu, I. Dirk, S. Maite, K. Angelos, N. Smirnoff, I.J.J. Binenzie, J.J. Strain, D. Favell, and J. Fletcher (2000). Plant ascorbic: acid chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food and Agri. 80:825-850.

Djanaguiraman M., J.A. Sheeba, D.D. Devi, and U. Bangarusamy (2005). Response of cotton to Atonik and TIBA for growth, enzymes and yield. J. Biol. Sci. 5:158-162.

El-Bassiony H.M. (2005). Physiological responses of wheat to salinity alleviation by nicotinamide and tryptophan. International Journal of Agriculture and Biology 653-659.

Fang Z., J. Bouwkamp, and T. Solomos (1998). Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *Phaseolus vulgaris* L. J. of Experimental Botany 49:503–510.

Flowers T. and D. Dalmond (1992). Protein synthesis in halophytes: the influence of potassium, sodium and magnesium in vitro. Plant and Soil 146:153-161.

Foyer C.H. and J. Harbinson (1994) Oxygen metabolism and the regulation of photosynthetic electron transport. IN: Foyer CH and Mullineaux PM (eds) Cause of photooxidative stress and amelioration of defense system in plants. CRC Press, Boca Raton, FL. pp 1-42.

Foyer C. H., M. Lelandais and K.J. Kunert (1994). Photoxidative stress in plants. Physiologia Plantarum 92: 696-717.

Goncalves J.F., AG Becker, D Crgnelutti, LA Tabaldi, LB Pereira, V Battisti, RM Spanevello, VM Morsch, FT Nicoloso and MRC Schetinger (2007) Cadmium toxicity causes oxidative stress and induces response of the antioxidant system in cucumber seedlings. Braz. J. Plant Physiol. 13(3):223-232.

Halliwell B, RI Asechbach, J Loliger, and OI Aruoma (1995).The characterization of antioxidants. J. Food Chem. Toxicol. 33:601-617.

Hameed A, S Naseer, T Iqbal, H Syed and M Ahsanulhaq (2008).Effects of NaCl salinity on seedlings growth, senescence, catalase and protease activities in two wheat genotypes differing in salt tolerance. Pak. J. Bot. 40(3):1043-1051.

Hanson EM and DN Munns (1988) Effect of $CaSo_4$ and NaCl on mineral content of *Leucaena leucocephata*. Plant and Soil 107:101-105.

Hung SH, CW Yu, and CH Lin (2005) Hydrogen peroxide functions as a stress signal in plants. Bot. Bull. Acad. Sin. 46:1-10.

Jackson, M. L. (1973). Soil Chemical Analysis. Prentice Hall of India, Private Limited, New Delhi, pp: 144-197.

Jaleel CA, P Manivannan and GMA Lakshmanan (2007).NaCl as a physiological modulator of proline metabolism and antioxidant *Phyllanthus amarus*. C R Biologies 2007; 330: 806-813.

Jang HD, KS Chang, YS Huang, CI Hsu, SH Lee, and MS Su (2007).Principal phenolic phytochemical and antioxidant activities of three Chinese medicinal plants. Food Chem 103:749-756.

Krieger-Liszkay A and A.Trebst (2006) Tocopherol is the scavenger of singlet oxygen produced by the triplet states of chlorophyll in the PSII reaction centre. J. Exp. Bot. 57(8):1677-1684.

Kukavica B and S.V. Jovanovic (2004). Senescence-related changes in the antioxidant status of ginkgo and birch leaves during autumn yellowing. Physiol. Plant. 122:321-327.

Leidi EO, R Nogales, and SH Lips (1991). Effect of salinity on cotton plants grown under nitrate or ammonium nutrition at different calcium levels. Field Crops Research 26: 35-44.

Lichtenthaler HK and AR Wellbum (1985). Determination of total carotenoids and chlorophylls A and B of leaf in Different Solvents. Biol. Soc. Trans 11: 591-592.

Loggini B, A Scartazza, E Brugnoli and F. Navari-Izzo (1999). Antioxidative defense system, pigment composition, and photosynthetic efficiency in Two wheat cultivars subjected to drought. Plant Physiology 119: 1091-1099.

Lutts S, JM Kinet and J. Bouharmont (1996). NaCL-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Annals of Botany 78: 389-398.

Mayer JM, DA Hrovat, JL Thomas, and WT Bordon (2002) Protein-coupled electron transfer vs. hydrogen atom transfer in enzl/tolune, methoxyl/methanol, and phenoxyl/phenol self exchange reactions. J. Am. Chem. Soc. 124:11142-11147.

Metile P, S Hortensteiner, and H Thomas (1999). Chlorophyll degradation. Annual Review of Plant Physiology and Plant Molecular Biology 50: 67– 95.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405-410.

Møller IM, PE Jensen and A Hansson (2007) Oxidative modifications to cellular components in plants. Annu. Rev. Plant Biol. 58: 459-481.

Munn´e-Bosch S (2007) Aging in perennials. Crit. Rev. Plant Sci. 26:123–138.

Navabpour S, K Morris, R Allen, E Harrison, SAH Mackerness, and V Buchanan-Wollaston (2003) Expression of senescence enhanced genes in response to oxidative stress. J. Exp. Bot. 54:2285-2292.

Noctor G and CH Foyer (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:249-279.

Norman GR and DL Streiner (2003) PDQ Statistics, 3rd Ed. BC Deckker Inc, London.

Pavet V, E Ollimos, G Kiddle, S Kumar, J Antonaiw, ME Alvarez, and CH Foyer (2005) Ascorbic acid deficiency activates cell death and disease resistance in *Arabidopsis thaliana*. Plant Physiol. 139: 1291-1303.

Prochazkova D., R K Sairam, GC Srivastava and DV Singh (2001) Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. Plant Science 161:765-771.

Rao MV, G Paliyath, DP Ormrod, DP Murr and CB Watkins (1997) Influence of salicylic acid on H_2O_2 production. Oxidative stress and H_2O_2 mtabolizing enzymes. Salicylic acid-mediated oxidative damage requires H_2O_2. Plant Physiol. 115:137-149.

Reuveni M and R Reuveni (1995) Efficacy of foliar application of phosphate in controlling powdery mildew fungus on field grown wine grapes: effects on cluster yield and peroxidase activity in berries. Journal of Phytopathology 143:21-25.

Richard LA (1954) Diagnosis and Improvement of saline and alkali soils.U.S. Dept. of Agric. Handbook No 60.

Sadasivam S and A Manickam (1996) Biochemical methods, Second edition, New age international. India.

Seckin B, A. H Sekmen and I Türkan (2009). An enhancing effect of exogenous mannitol on the antioxidant enzyme activities in roots of wheat under salt stress. J. Plant Growth Regul. 28: 12-20.

Shalata A and PM Neumann (2001). Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation. J. Experimental Botany 52(364): 2207-2211.

Shao HB, ZS Liang, MA Shao and BC Wang (2005) Changes of some physiological and biochemical indices for soil water deficits among 10 wheat genotypes at seedlings stage. Colloids and Surfaces B: Biointerfaces 42(2): 107-113.

Singleton VL and J A Rossi (1965) Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. Amer. J. Enol. Vitic. 16:144–158.

Sylvester-Bradley R, RK Scott and CE Wright (1990) Physiology in the production and improvement of cereals. Home-Grown Cereals Authority Research Review, vol. 18. HGCA, London.

Teranishi Y, A Tanaka, M Osumi and S Fukui (1974) Catalase activity of hydrocarbon utilizing candida yeast. Agr. Biol. Chem 38:1213-1216.

Thomas H, H Ougham, P Canter, I Donnison (2002) what stay-green mutants tell us about nitrogen remobilization in leaf senescence? J. Exp.Botany 53: 801– 808.

Vieira Santos CL, A. Campos, H. Azevedo and G Caldeira (2001). In situ and in vitro senescence induced by KCL stress: nutritional imbalance, lipid peroxidation and antioxidant metabolism. J. Exp. Bot. 52 (355): 351-360.

Wise RR and AW Naylor (1987) Chilling enhanced photo-oxidation. Plant Physiology 83:278-282.

Zabalza A, S Gaston, LM Sandalio, LA Del Rio, and M Royuela (2007) Oxidaive stress is not related to the mode of action of herbicides that inhibit acetolactate synthase. Environ. Exp. Bot. 59:150-159.

دور حامض الأسكوربيك والتوكوفيرول فى تقليل شيخوخه ورقه العلم بنباتات القمح المتأثرة بالملوحه

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الدور الذى يقوم به كل من حامض الأسكوربيك والتوكوفيرول فى تحسين نمو وإنتاجية النباتات المتأثرة بالملوحة غير مفهوم تماما. فى محاولة لتفهم ذلك الدور أجريت تجربتى أصص لدراسة تأثير الرش بكل من الأسكوربيك والتوكوفيرول فى تقليل المظاهر الفسيولوجية لشيخوخة أوراق العلم لنباتات القمح المتاثرة بثلاث مستويات من الملوحة (٠,٨، ٧,٥، ١١,٥ ديسيمينز/متر) مثل التغير فى محتوى البروتين الذائب، الكلوروفيل، نواتج أكسدة الدهون، نفاذية الأغشية للذائبات، فوق أكسيد الهيدروجين، نشاط الكتاليزوالبيروكسيديز،الأسكوربيك، الفينولات الكليه، الكاروتينيدات بالإضافة الى المحتوى الأيونى.

تزداد شيخوخة أوراق العلم بإرتفاع ملوحة التربه، حيث يقل محتوى لأوراق العلم من الكلوروفيل والكاروتينيدات، الأسكوربيك، الفينولات الكلية، الكالسيوم، البوتاسيوم، الماغنسيوم، نسبه البوتاسيوم للصوديوم، والبروتين الذائب بالإضافة لنشاط الكتاليز والبيروكسيديز، بينما يزداد محتوى أوراق العلم من الصوديوم والكلوريد ونسبه كلوروفيل أ الى ب ونفاذية الاغشية وفوق أكسيد الهيدروجين ونواتج أكسدة الدهون. إستخدام المواد المضادة للأكسدة وبصفة خاصة حامض الأسكوربيك يقلل من مظاهر شيخوخة الأوراق من خلال إنخفاض محتوى أوراق العلم من فوق أكسيد الهيدروجين، نواتج أكسدة الدهون، الصوديوم والكلوريد بالإضافه تقليل نفاذية الأغشيه للذائبات مقارنة بالكنترول، نتيجه لزيادة معدل تمثيل وتراكم عدد من المواد المضادة للأكسدة مثل الأسكوربيك، الفينول، والكاروتينيدات وتنشيط أنزيمى الكتاليز والبيروكسيديز، بالإضافة إلى إرتفاع اوراق العلم من الكالسيوم، البوتاسيوم، والماغنسيوم مقارنة بالكنترول بعد ٦٥ يوم من الزراعه. أما إستخدام المواد المضادة للأكسدة تحت مستويات الملوحه المتوسطه والعاليه يقلل من التأثيرات الضارة للملوحه على شيخوخه الاوراق من خلال تثبيطها للشواهد الداله على الشيخوخه.

المحتوى المرتفع من المواد المضادة للأكسدة والمحتوى المنخفض من فوق اكسيد الهيدروجين فى أوراق العلم نتيجه لإستخدام المواد المضادة للأكسدة يبين إمكانيه إستخدامهما لتقليل الشيخوخه بالإضافه الى دورهما كمواد مضادة للأكسدة من خلال تنشيطهما لنظام البيروكسيديز/الفينول/ الاسكوربيك والذى يتم من خلالة كسح الشوارد الأكسيجينية الحرة.

قام بتحكيم البحث

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Table (1).. Chlorophyll (mg/g FW) and soluble protein (mg/g FW) concentration of wheat flag leaf as well as $chl_{a:b}$ ratio as affected by salinity, antioxidants and their combinations in the two growing seasons.

Characters	First season											
	Antioxidants (B)											
	Total chlorophyll				$Chl_{a:b}$ ratio				Soluble protein content			
Salinity (dSm^{-1}) (A)	0	AsA	α-Toc	Mean of salinity	0	AsA	α-Toc	Mean of salinity	0	AsA	α-Toc	Mean of salinity
Control (0.12)	0.851	1.126	0.852	0.943	1.946	1.398	1.550	1.631	6.37	7.06	6.57	6.66
7.5	0.677	0.920	0.776	0.791	2.119	1.348	1.652	1.706	5.95	6.22	6.10	6.09
11.5	0.587	0.779	0.745	0.703	2.244	1.338	1.656	1.746	5.57	5.75	5.71	5.67
Mean of antioxidants	0.705	0.941	0.791		2.103	1.361	1.619		5.96	6.34	6.12	
LSD 5%	A 0.031	B 0.040	AB 0.054		A NS	B 0.555	AB NS		A 0.103	B 0.111	AB 0.179	
	Second season											
Control (0.12)	0.850	1.129	0.851	0.943	1.945	1.395	1.449	1.596	6.31	7.12	6.59	6.67
7.5	0.671	0.925	0.774	0.790	2.113	1.351	1.650	1.704	5.92	6.24	6.14	6.10
11.5	0.598	0.764	0.737	0.699	2.239	1.339	1.607	1.728	5.59	5.73	5.68	5.66
Mean of antioxidants	0.706	0.939	0.787		2.099	1.361	1.568		5.94	6.36	6.13	
LSD 5%	A 0.029	B 0.042	AB 0.055		A NS	B 0.557	AB NS		A 0.097	B 0.108	AB 0.174	

Table (2). Sodium and chloride concentration (mg/g DW) as well as K/Na ratio of wheat flag leaf as affected by salinity or antioxidants as well as their combinations in the two growing seasons.

Characters	First seasom											
	Antioxidants (B)											
	Sodium content				Chloride content				K/Na ratio			
Salinity (dSm^{-1}) (A)	0	AsA	α-Toc	Mean of salinity	0	AsA	α-Toc	Mean of salinity	0	AsA	α-Toc	Mean of salinity
Control (0.12)	7.63	4.98	5.95	6.20	20.15	14.00	17.12	17.09	2.71	6.10	4.01	4.27
7.5	12.65	7.41	8.19	9.40	28.55	19.32	20.64	22.84	1.55	3.00	2.48	2.34
11.5	15.30	8.77	9.54	11.20	35.87	21.01	22.30	26.39	0.98	2.15	1.79	1.64
Mean of antioxidants	11.88	7.08	7.89		28.19	18.11	20.02		1.74	3.75	2.76	
LSD 5%	A 0.452	B 0.482	AB 0.783		A 0.830	B 0.890	AB 1.439		A 0.108	B 0.121	AB 0.212	
	Second season											
Control (0.12)	7.62	4.96	5.97	6.18	20.22	14.55	17.02	17.26	2.71	6.11	4.03	4.28
7.5	12.22	7.33	7.99	9.18	28.28	19.45	20.77	22.83	1.58	2.97	2.53	2.36
11.5	15.22	8.74	9.45	11.13	37.37	20.77	21.58	26.57	0.96	2.14	1.80	1.63
Mean of antioxidants	11.68	7.01	7.80		28.62	18.26	19.79		1.75	3.74	2.79	
LSD 5%	**A 0.475**	**B 0.484**	**AB 0.837**		**A 0.947**	**B 0.938**	**AB 1.628**		**A 0.323**	**B 0.320**	**AB 0.555**	

Table (3). Potassium, Calcium and Magnesium (mg/g DW) concentrations of wheat flag leaf as affected by salinity or antioxidants as well as their combinations in the two growing seasons.

Characters	First season											
	Antioxidants (B)											
	Potassium content				Calcium content				Magnesium content			
Salinity (dSm^{-1}) (A)	0	AsA	α-Toc	Mean of salinity	0	AsA	α-Toc	Mean of salinity	0	AsA	α-Toc	Mean of salinity
Control (0.12)	20.88	30.35	23.87	25.03	1.02	2.71	1.39	1.70	0.437	0.680	0.543	0.553
7.5	19.58	22.49	20.36	20.81	1.04	1.29	1.17	1.16	0.373	0.460	0.443	0.426
11.5	14.87	18.92	17.16	16.98	0.64	0.93	0.88	0.81	0.267	0.383	0.357	0.346
Mean of antioxidants	18.44	23.92	20.46		0.90	1.64	1.14		0.359	0.508	0.448	
LSD 5%	A 0.451	B 0.481	AB 0.781		A 0.072	B 0.078	AB 0.125		A 0.030	B 0.033	AB 0.053	
	Second season											
Control (0.12)	20.74	29.99	24.14	24.95	1.03	1.78	1.40	1.40	0.360	0.657	0.533	0.517
7.5	19.25	21.84	20.33	20.47	1.02	1.29	1.15	1.15	0.350	0.517	0.467	0.444
11.5	14.75	18.77	17.13	16.88	0.71	0.92	0.83	0.82	0.260	0.420	0.363	0.348
Mean of antioxidants	18.24	23.53	20.53		0.92	1.33	1.12		0.323	0.531	0.454	
LSD 5%	A 0.525	B 0.531	AB 0.923		A 0.050	B 0.053	AB 0.095		A 0.026	B 0.024	AB 0.041	

Table (4). Lipid peroxidation (µmol/g FW) and Electrolyte leakage (%) and hydrogen peroxide (µM/g FW) of wheat flag leaf as affected by salinity or antioxidants as well as their combinations in the two growing seasons.

Characters	First season											
	Antioxidants (B)											
	Lipid peroxidation				Electrolyte leakage				Hydrogen peroxide			
Salinity (dSm^{-1}) (A)	0	AsA	α-Toc	Mean of salinity	0	AsA	α-Toc	Mean of salinity	0	AsA	α-Toc	Mean of salinity
Control (0.12)	518	353	457	443	75.26	74.74	74.38	74.80	14.60	12.51	12.53	13.21
7.5	759	574	639	657	88.17	80.30	85.00	84.49	20.45	15.93	18.82	18.40
11.5	922	719	788	810	90.31	83.36	86.26	86.64	23.09	16.33	19.39	19.60
Mean of antioxidants	732	549	628		84.58	79.47	81.87		19.38	14.92	16.91	
LSD 5%	A 13.63	B 13.93	AB 23.61		A 0.418	B 0.444	AB 0.724		A 0.201	B 0.194	AB 0.497	
	Second season											
Control (0.12)	795	378	448	440	75.65	73.99	74.49	74.71	13.91	11.54	13.00	12.82
7.5	822	570	652	681	88.91	80.44	84.59	84.65	19.26	16.80	18.14	17.79
11.5	942	700	869	837	92.31	53.36	86.23	77.30	22.08	17.57	18.66	19.44
Mean of antioxidants	753	549	656		85.62	69.26	81.77		18.42	15.21	16.60	
LSD 5%	A 15.14	B 15.16	AB 20.84		A 0.420	B 0.441	AB 0.721		A 0.365	B 0.377	AB 0.653	

Table(5). Catalase and peroxidase activities (unit/g FW) of wheat flag leaf as affected by salinity or antioxidants as well as their combinations in the two growing seasons.

Characters	First season							
	Antioxidants (B)							
	Catalase				Peroxidase			
Salinity (dSm^{-1}) (A)	0	AsA	A-Toc	Mean of salinity	0	AsA	A-Toc	Mean of salinity
Control (0.12)	9.260	11.393	8.380	9.678	28.97	33.18	31.15	31.10
7.5	6.030	10.80	7.693	8.176	26.56	32.36	28.87	29.25
11.5	5.417	9.800	7.017	7.411	25.41	30.55	27.27	27.74
Mean of antioxidants	6.902	10.66	7.697		26.98	32.03	29.08	
LSD 5%	A 0.218	B 0.228	AB 0.395		A 0.174	B 0.184	AB NS	
	Second season							
Control (0.12)	9.20	10.73	8.75	9.56	29.58	32.22	31.02	30.94
7.5	7.06	9.84	8.36	8.42	27.59	31.63	29.12	29.45
11.5	5.99	9.36	7.72	7.69	26.49	30.01	28.45	28.32
Mean of antioxidants	7.41	9.98	8.27		27.89	31.29	29.53	
LSD 5%	A 0.228	B 0.213	AB 0.398		A 0.329	B 0.335	AB 0.582	

Table (6). Ascorbic acid (mg/g FW), total soluble phenol (mg Catecol/100 g FW) and total carotenoids (mg/g FW) of wheat flag leaf as affected by salinity or antioxidants as well as their combinations in the two growing seasons.

Characters	Antioxidants (B)											
	Ascorbic acid				Total soluble phenol				Carotenoids			
Salinity (dSm^{-1}) (A)	0	AsA	α-Toc	Mean of salinity	0	AsA	α-Toc	Mean of salinity	0	AsA	α-Toc	Mean of salinity
Control (0.12)	4.713	9.770	6.257	6.913	18.47	16.92	13.74	20.25	0.073	0.068	0.039	0.080
7.5	3.613	6.540	5.783	5.312	22.76	19.57	16.31	17.98	0.085	0.082	0.079	0.075
11.5	2.433	6.470	5.170	4.691	19.52	17.44	15.69	15.24	0.082	0.075	0.060	0.059
Mean of antioxidants	3.587	7.593	5.737		16.38	19.55	17.55		0.060	0.082	0.072	
LSD 5%	A 0.328	B 0.332	AB 0.569		A 0.440	B 0.450	AB 0.762		A 0.009	B 0.012	AB NS	
	Second season											
Control (0.12)	4.64	9.56	6.41	6.87	18.59	22.37	19.50	20.15	0.074	0.067	0.049	0.063
7.5	3.64	6.82	5.72	5.39	16.68	19.49	17.56	17.91	0.084	0.080	0.081	0.081
11.5	2.61	6.39	5.34	4.78	12.68	17.42	15.56	15.22	0.081	0.075	0.065	0.073
Mean of antioxidants	3.63	7.59	5.82		15.98	19.76	17.54		0.079	0.074	0.065	
LSD 5%	A 0.415	B 0.326	AB 0.594		A 0.433	B 0.415	AB 0.742		A 0.009	B 0.011	AB NS	