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ORIGINAL ARTICLE

Ascorbic Acid and α-Tocopherol Minimize Salt-Induced Wheat Leaf Senescence

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Background
Leaf senescence is an oxidative process, and most of the catabolic events involved in senescence are propagated irreversibly once initiated.

Results
Salinity hastened the senescence of wheat flag leaves, decreased the concentrations of chlorophyll, total carotenoids, ascorbic acid, total phenol, calcium, potassium, magnesium, K^+/Na^+ ratio and soluble proteins, as well as the activities of catalase and peroxidase. Conversely, salinity increased sodium, chloride, and the $chlorophyll_{a:b}$ ratio, as well as membrane permeability, hydrogen peroxide, and malondialdehyde synthesis.
Both antioxidants application reduced the hydrogen peroxide accumulation, lipid peroxidation, membrane permeability, sodium and chloride content over control plants. The antioxidants enzyme activities were significantly increased by antioxidant spray. Enhanced accumulation of ascorbate, phenol, carotenoids, calcium, potassium and magnesium was seen in antioxidants-sprayed plants compared with control plants at 65 days after sowing. Under moderate and sever salinity levels application of both antioxidants alleviated the harmful effects of salinity on leaf senescence related parameter. The higher levels of antioxidants and low level of H_2O_2 in flag leaf may be the prerequisite for delayed leaf senescence in antioxidants-sprayed plants.

Conclusions
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.

Key words: Antioxidants, Malondialdehyde, Salinity, Senescence, Wheat

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Photosynthesis is the primary source of dry matter production and grain yield in crop plants. However, because of the close correlation between leaf area and grain yield in wheat, early senescence seriously restricts the yield potential. Flag leaf photosynthesis in wheat contributes 30–50% of the carbon 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, decrease in soluble protein contents, and changes in the ratio of chlorophyll a:b (Munne-Bosch 2007). Leaf senescence is also 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 degrade all almost cell components including membrane lipids, proteins, chlorophyll, polysaccharides and DNA (Mшller et al. 2007). Finally, senescence is also related to salt stress and the 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 (Flowers and Dalmond 1992).

Plant cells possess a variety of defense strategies to overcome salt-mediated oxidative stress. Those strategies can include antioxidant compounds (ascorbic acid, α-tocopherol, proline and carotenoids or phenol) and detoxifying enzymes like catalase (CAT), and peroxidase (POD) (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 systems (Kukavica and Jovanovic 2004). In addition, antioxidants and phenolics are able to act as ROS scavengers or ROS chain breakers, thus extinguishing strongly oxidative free radicals. Hence, it is postulated that foliar spray of antioxidants such as AsA and Toc (Conklin and Barth 2004, Krieger-Liszkay and Trebst 2006) could modify leaf senescence process in wheat flag leaves.

Ascorbic acid (AsA) 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 in 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). The most prominent function of Toc is protection of polyunsaturated fatty acids from lipid peroxidation (Krieger-Liszkay and Trebst 2006).

Although, there is an abundance of information concerning the effects of NaCl on inducing senescence processes (Breusegem and Dat 2006, Zhao et al. 2007, Hameed et al. 2008) and the antioxidant defense systems, relatively little information is available on the role of antioxidants in improving leaf longevity or delaying leaf senescence. In addition to this, most of the available reports on the interaction between salinity and antioxidants focus on growth, yield and some physiological changes (Abd El-Aziz et al. 2006, Hussein et al 2007, Athar et al. 2008). To our knowledge, little is known about the actual role of antioxidants in delaying leaf senescence. Hence, the present investigation evaluates the potential of both AsA and Toc as foliar sprays in altering ROS content, antioxidant content, and antioxidant enzyme activities in senescing wheat flag leaves under

salinity stress in order to provide a basis for developing strategies for reducing the risks associated with salinity and maintaining sustainable plant production. The evaluation was made 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

Fifteen uniform wheat grains (Giza 168 cultivar) were sown on 1st December, 2005 and 15th November, 2006 (first and second season, respectively), in closed-bottom plastic pots containing 15 kg clay loam soil, (containing 0.786 meq sulphate, 0.27 meq bicarbonate, 0.51 meq chloride, 0.38 meq calcium, 0.60 meq magnesium, 0.006 meq potassium and 0.45 meq sodium each per 100 g soil) with or without additional salinity. Soils were salinized prior to sowing by adding sodium chloride (NaCl) solution to adjust salt concentrations to 0.12, 0.35 and 0.70% NaCl of oven dry soil. Actual salinity levels expressed as ECe (dSm^{-1}) were determined at three times before and during cultivation. The means of salinity levels in soil were 0.8, 7.5 and 11.5 dSm^{-1}.

Two weeks after sowing, the seedlings were thinned to 10 uniform seedlings per pot. Phosphorous and potassium fertilizers were added to the soil before sowing at the rate of 5 g P_2O_5 in the form of calcium super phosphate (15.5% P_2O_5) and 2 g K_2O in the form of potassium sulphate (48%). 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.

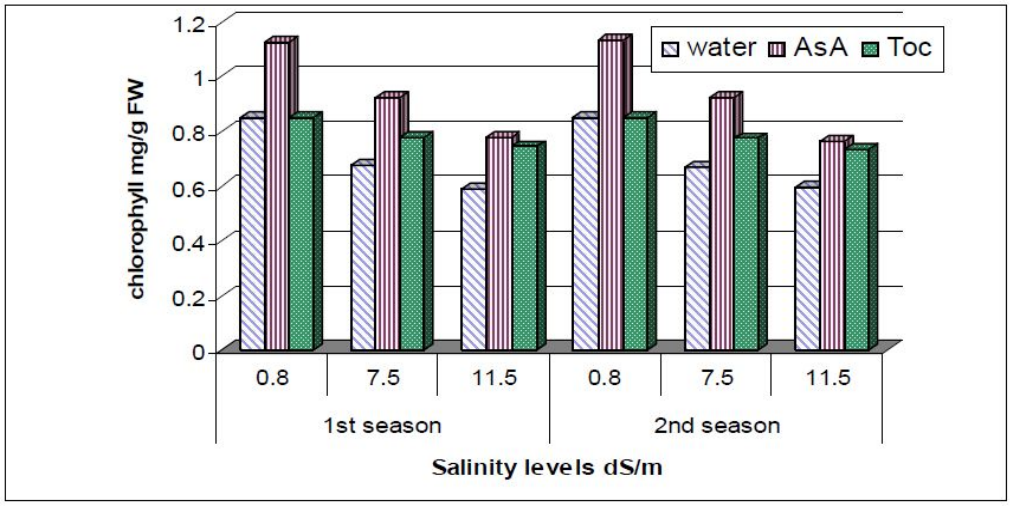
At 40 days from sowing (DFS), the pots at each salinity levels were divided into three groups. The first group was sprayed twice with water (control), while the other two groups were sprayed twice (i.e. after 40 and 50 DFS) with aqueous solutions of either ascorbic or α-tocopherol at the rate of 100 mg/l until run-off, with Tween 20 as a wetting agent. At heading (65 DFS), four randomly selected plants were harvested per pot and removed for determination of biochemical constituents.

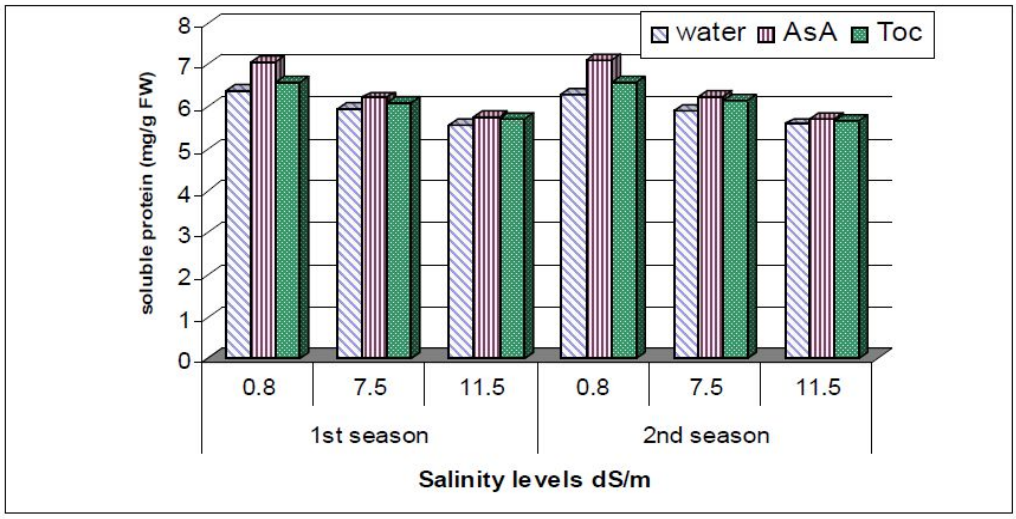
Table 1a. Flag Chlorophyll (mg/g FW) and soluble protein (mg/g FW) contents of wheat flag leaf as well as $chl_{a:b}$ ratio as affected by soil salinity in the two growing seasons.

Salinity (dSm-1)	Total chlorophyll		Chla:b ratio		Soluble protein content	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
0.8	0.943	0.943	1.631	1.596	6.66	6.67
7.5	0.791	0.790	1.706	1.704	6.09	6.10
11.5	0.703	0.699	1.746	1.728	5.67	5.66
LSD at 0.05	0.031	0.029	NS	NS	0.103	0.097

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

Antioxidant	Total chlorophyll		Chla:b ratio		Soluble protein content	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Water	0.705	0.706	2.103	2.099	5.96	5.94
AsA (100 mg/L)	0.941	0.939	1.361	1.361	6.34	6.36
Toc (100 mg/L)	0.791	0.787	1.619	1.568	6.12	6.13
LSD at 0.05	0.040	0.042	0.555	0.557	0.111	0.108





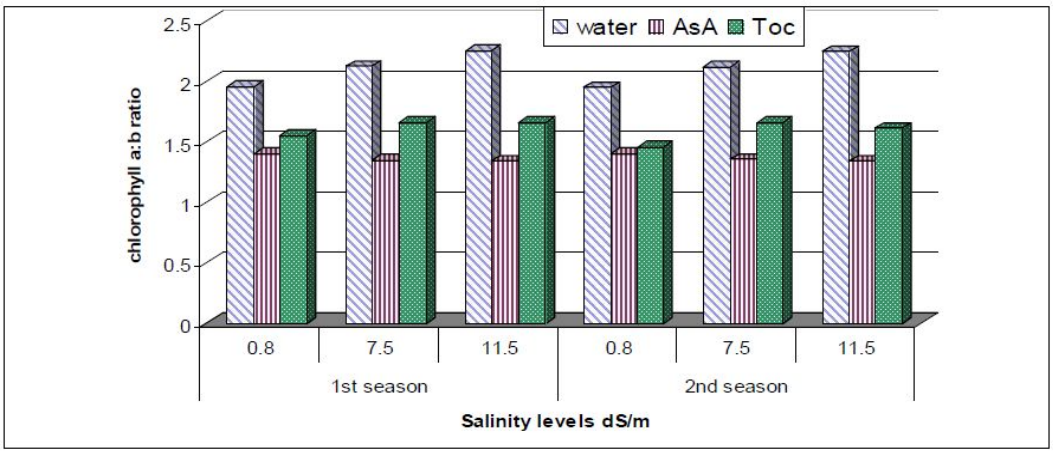


Figure 1. Chlorophyll, Soluble protein contents and Chlorophyll A:B ration in wheat flag leaf as affected by the Interaction of soil salinity and antioxidants application in the two growing season.

Chlorophyll was extracted for 24 hour at room temperature in methanol after adding traces of sodium carbonate. Chlorophyll concentrations were determined spectrophotometrically (Spekol 11, Uk) 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 (Cornell, UK). Calcium and magnesium were determined using versenate methods according to Richard (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).

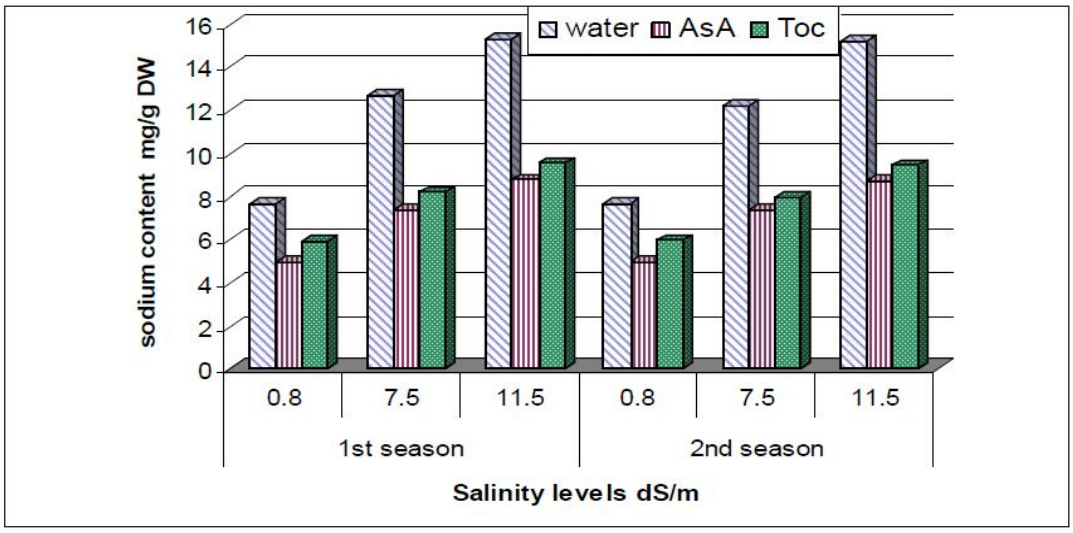
Lipid peroxidation was estimated as thiobarbituric acid reactive substances (TBARS). Malondialdehyde content "MDA" was determined and calculated as µmoles/g of fresh weight by the method of Shao et al (2005). Leaf samples (0.5 g) were homogenized in 5 ml of ethanol. An equal volume of 0.5% thiobarbituric 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 10,000Чg for 30 min. The supernatant was removed, absorption read at 532 nm, and the amount of nonspecific absorption at 600 nm read and subtracted from this value. The amount of MDA present was calculated from the extinction coefficient of 155 mM^{-1} cm^{-1} .

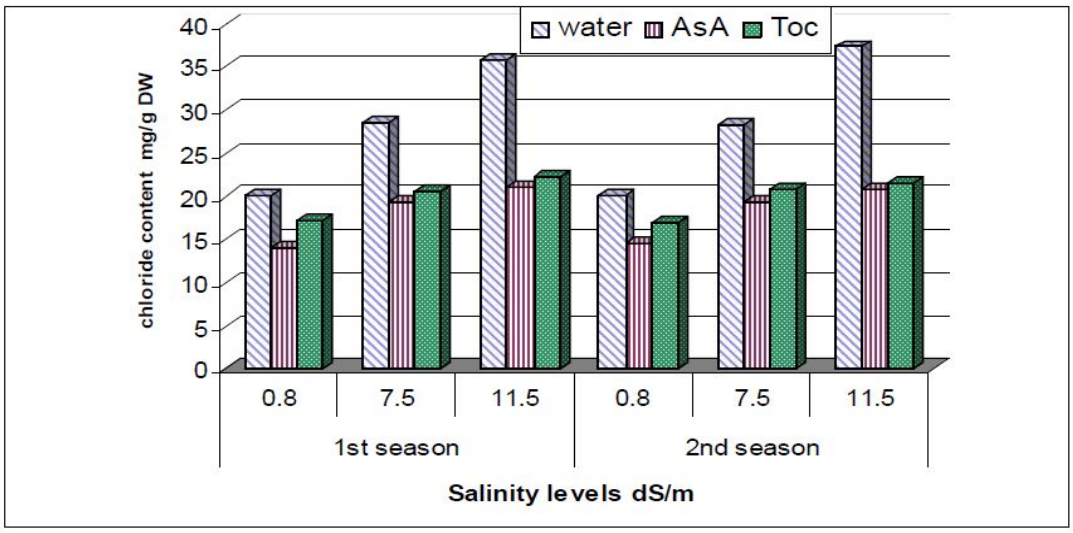
Table 2a. Sodium and chloride content (mg/g DW) as well as K/Na ratio of wheat flag leaf as affected by soil salinity in the two growing season.

Salinity (dSm-1)	Sodium		Chloride		K/Na ratio	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
0.8	6.20	6.18	17.09	17.26	4.27	4.28
7.5	9.40	9.48	22.84	22.83	2.34	2.36
11.5	11.20	11.13	26.39	26.57	1.64	1.63
LSD at 0.05	0.452	0.475	0.830	0.947	0.108	0.323

Table 2b. Sodium and chloride content (mg/g DW) as well as K/Na ratio of wheat flag leaf as affected by antioxidants application in the two growing season.

Antioxidant	Sodium		Chloride		K/Na ratio	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Water	11.88	11.68	28.19	28.62	1.74	1.75
AsA (100 mg/L)	7.08	7.01	18.11	18.26	3.37	3.74
Toc (100 mg/L)	7.89	7.80	20.02	19.79	2.76	2.79
LSD at 0.05	0.482	0.484	0.890	0.938	0.121	0.320





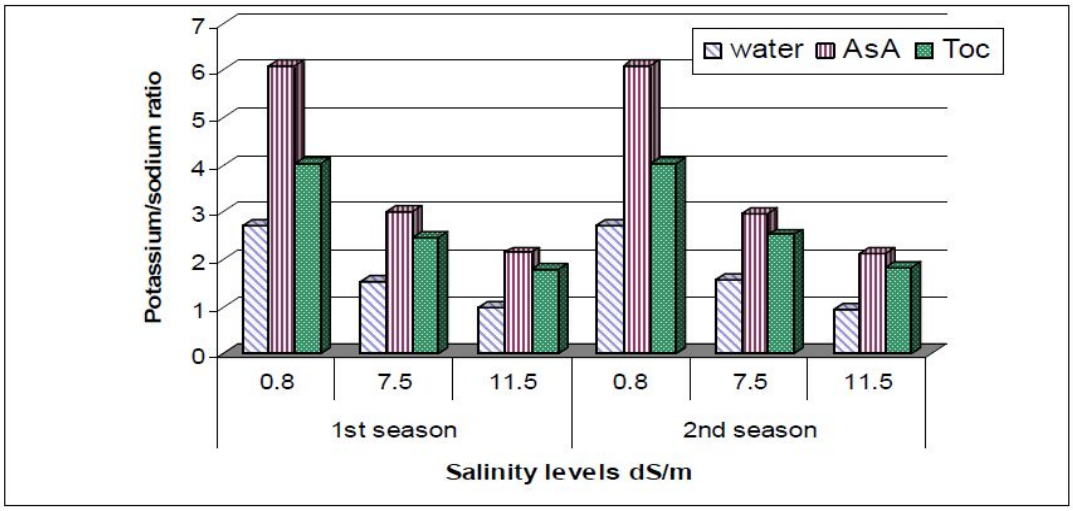


Figure 2. Sodium, Chloride content and Potassium Sodium ratio in wheat flag leaf as affected by the Interaction of soil salinity and antioxidants application in the two growing season.

Electrolyte leakage percentage measurement (ELP) was used to assess membrane permeability according to Goncalves et al. (2007), using an Electrical Conductivity Meter (Hanna, UK). Flag leaf samples were placed in vials containing distilled water and incubated at room temperature for 24 h. Electrical conductivity of the resulting solution (EC_1) was recorded after incubation. Samples were then placed in a boiling water bath for 30 min, cooled to room temperature, and the second reading (EC_2) determined. The ELP was calculated as EC_1/EC_2 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

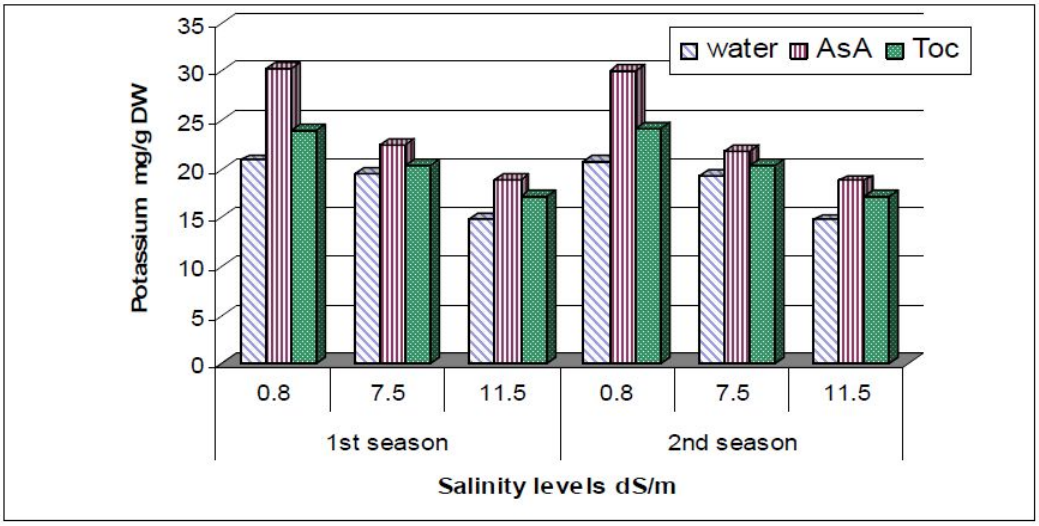
Statistical analysis: The data were analyzed using Analysis of Variance (ANOVA) and mean separations adjusted by the Multiple Comparison test (Norman and Streiner 2003) using MSTAT-C v.1.2. statistically computer programme. Significance between treatments were compared at the 0.05 probability level.

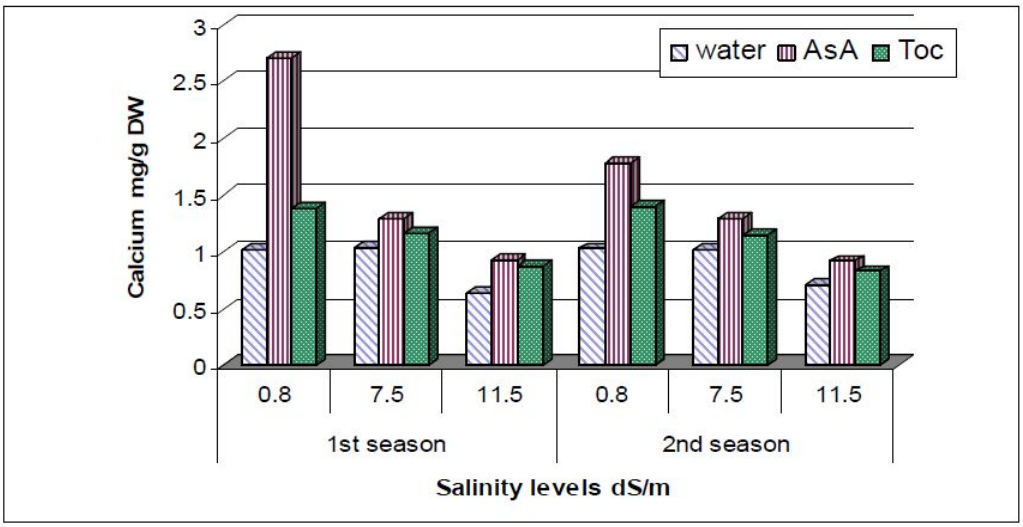
Table 3a. Potassium, Calcium and Magnesium (mg/g DW) contents of wheat flag leaf as affected by soil salinity in the two growing season.

Salinity (dSm-1)	Potassium		Calcium		Magnesium	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
0.8	25.03	24.95	1.70	1.40	0.553	0.517
7.5	20.81	20.47	1.16	1.15	0.426	0.444
11.5	16.98	16.88	0.81	0.82	0.346	0.348
LSD at 0.05	0.451	0.525	0.072	0.050	0.030	0.026

Table 3b. Potassium, Calcium and Magnesium (mg/g DW) contents of wheat flag leaf as affected by antioxidants application in the two growing season.

Antioxidant	Potassium		Calcium		Magnesium	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Water	18.44	18.24	0.90	0.92	0.359	0.323
AsA (100 mg/L)	23.92	23.53	1.64	1.33	0.508	0.531
Toc (100 mg/L)	20.46	20.53	1.14	1.12	0.448	0.454
LSD at 0.05	0.481	0.531	0.078	0.053	0.033	0.024





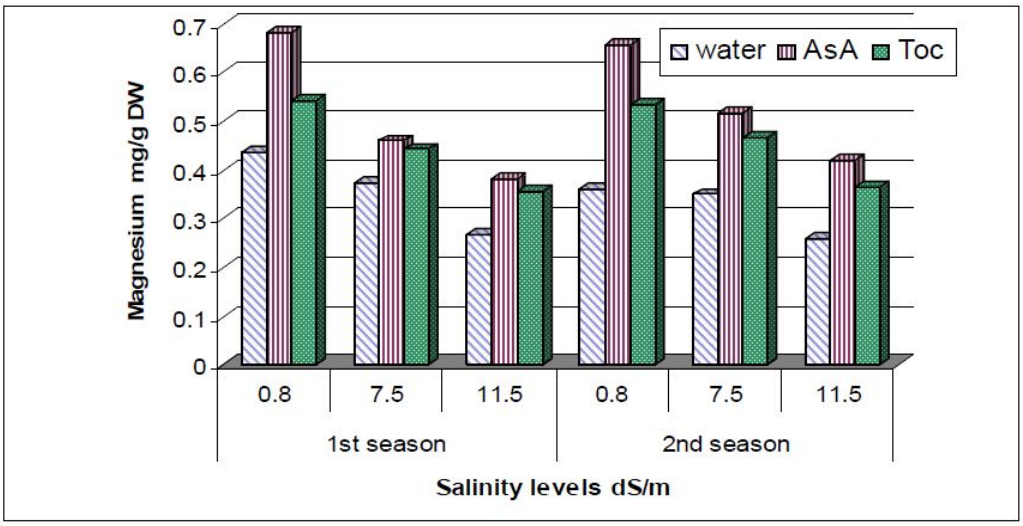


Figure 3. Potassium, Calcium and Magnesium content in wheat flag leaf as affected by the Interaction of soil salinity and antioxidants application in the two growing season.

RESULTS

CHLOROPHYLL AND SOLUBLE PROTEIN:

Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity. Data in Table 1a indicate that the contents of total chlorophyll and soluble protein in flag leaf were significantly decreased under NaCl salt stress. This decrease was more pronounced in 11.5dSm^{-1} than in 7.5 dSm^{-1} NaCl. However Chl $_{a:b}$ ratio was increased.

Under control, application of both antioxidants; AsA and Toc increased total chlorophyll and soluble protein content, whereas, decreased chla:b ratio (Table 1b). AsA was more effective than Toc. Under moderate and sever NaCl salinity stress, application of antioxidants, especially ascorbic acid, nullifies the harmful effect of salinity levels on chlorophyll and soluble protein content as well as chl$_{a:b}$ ratio Figure (1).

ION CONTENTS:

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

LIPID PEROXIDATION, MEMBRANE PERMEABILITY AND HYDROGEN PEROXIDE:

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.

Table 4a. Lipid peroxidation (µmol/g FW) and Electrolyte leakage (%) and hydrogen peroxide (µM/g FW) of wheat flag leaf as affected by soil salinity in the two growing season.

Salinity (dSm-1)	Lipid peroxidation		Electrolyte leakage		Hydrogen peroxide	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
0.8	443	440	74.80	74.71	13.21	12.82
7.5	657	681	84.49	84.65	18.40	17.79
11.5	810	837	86.64	77.30	19.60	19.44
LSD at 0.05	13.63	15.14	0.418	0.420	0.201	0.365

Table 4b. Lipid peroxidation (µmol/g FW) and Electrolyte leakage (%) and hydrogen peroxide (µM/g FW) of wheat flag leaf as affected by antioxidants application in the two growing season.

Antioxidant	Lipid peroxidation		Electrolyte leakage		Hydrogen peroxide	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Water	732	753	84.58	85.62	19.38	18.42
AsA (100 mg/L)	549	549	79.47	69.26	14.92	15.21
Toc (100 mg/L)	628	656	81.87	81.77	16.91	16.60
LSD at 0.05	13.93	15.16	0.444	0.441	0.194	0.377

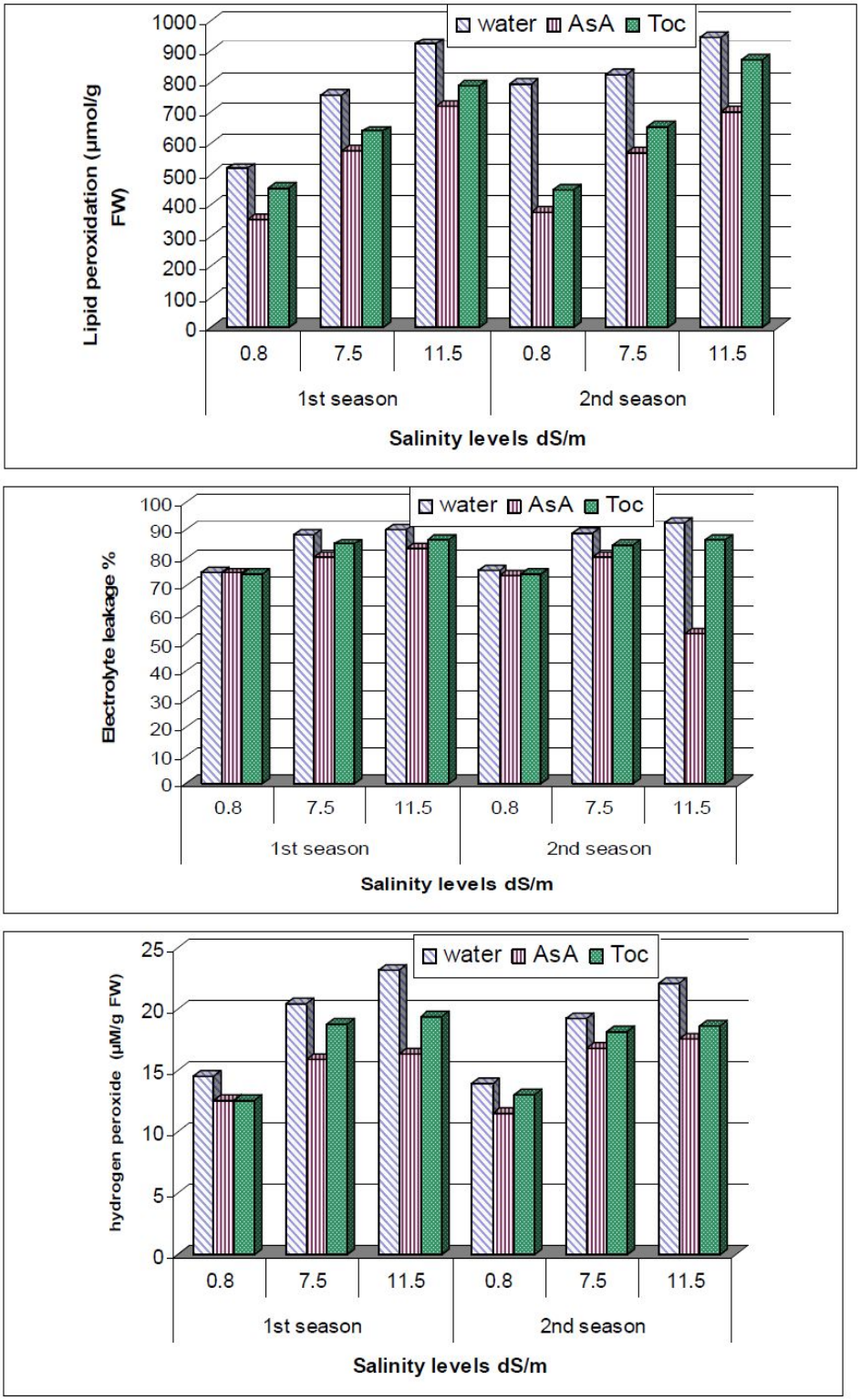


Figure 4. Lipid peroxidation, Electrolyte leakage percentage and Hydrogen peroxide content in wheat flag leaf as affected by the Interaction of soil salinity and antioxidants application in the two growing season.

Table 5a. Catalase and peroxidase activities (unit/g FW) of wheat flag leaf as affected by soil salinity in the two growing season.

Salinity (dSm^{-1})	Catalase		Peroxidase	
	1st season	2nd season	1st season	2nd season
0.8	9.67	9.56	31.10	30.94
7.5	8.17	8.42	29.25	29.45
11.5	7.41	7.69	27.74	28.32
LSD at 0.05	0.218	0.228	0.174	0.329

Table 5b. Catalase and peroxidase activities (unit/g FW) of wheat flag leaf as affected by antioxidants application in the two growing season.

Antioxidant	Catalase		Peroxidase	
	1st season	2nd season	1st season	2nd season
Water	6.90	7.41	26.98	27.89
AsA (100 mg L^{-1})	10.6	9.98	32.03	31.29
Toc (100 mg L^{-1})	7.69	8.27	29.08	29.53
LSD at 0.05	0.228	0.213	0.184	0.335

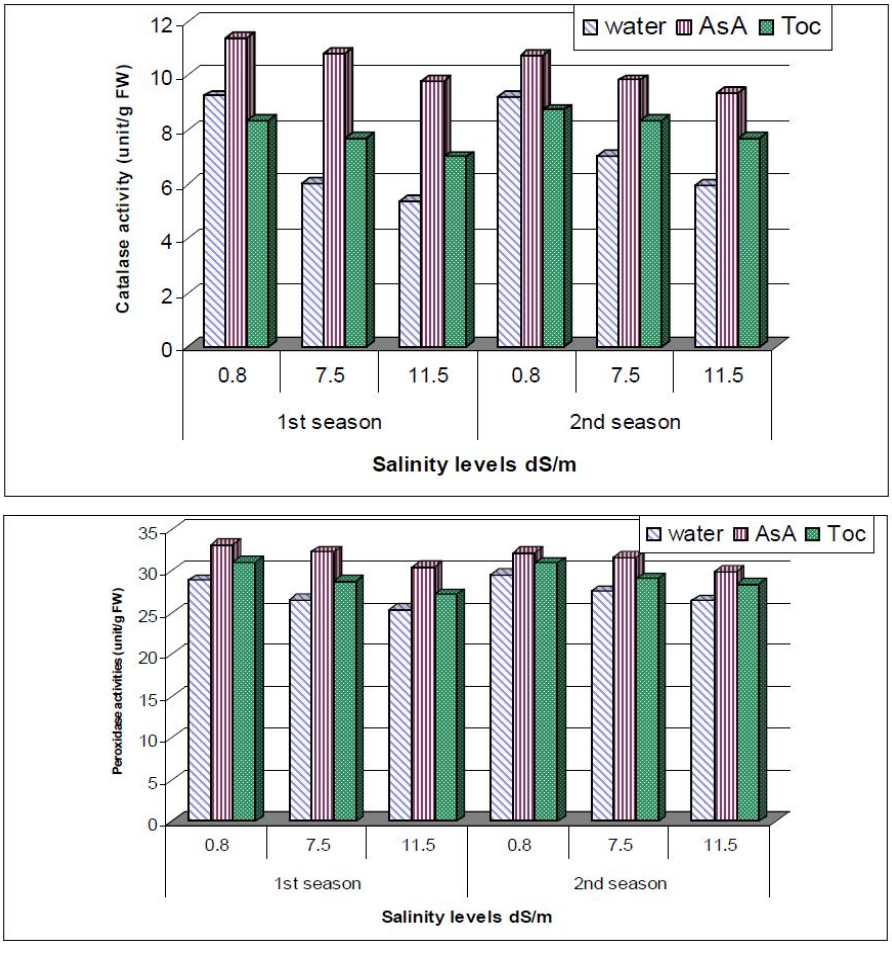


Figure 5. Catalase and Peroxidase activities in wheat flag leaf as affected by the Interaction of soil salinity and antioxidants application in the two growing season.

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 (Table, 4a). On the other hand, the application of antioxidants, in particular, ascorbic acid, significantly decreased TBARS formation and membrane permeability as well as hydrogen peroxide content in flag leaf (Table 4b).

Regarding the combinations between sodium chloride salinity levels and antioxidants, the data illustrated in Figure (4) clearly indicate that antioxidants, especially ascorbic acid, counteracted the harmful effect of salinity on TBARS formation and membrane permeability, as well as H_2O_2 content. Under the corresponding salinity levels, application of ascorbic acid or tocopherol significantly decreased TBARS, membrane permeability and hydrogen peroxide.

ENZYMATIC AND NON ENZYMATIC SCAVENGING SYSTEMS:

Tables 5a and 6a shows the time courses of enzymatic and non-enzymatic antioxidant in flag leaf of wheat treated with or without antioxidants under normal or salinity conditions. In general, the activity of catalase (CAT) and peroxidase (POD), as well as ascorbic acid, total carotenoids and total soluble phenol, were characterized by a gradual reduction with increasing sodium chloride salinity levels 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 (Tables 5a, 6a) compared to untreated plants.

Exogenous application of antioxidants, especially ascorbic acid; counteracted the harmful effects of salinity on enzymatic and non-enzymatic scavenging systems (Figures 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.

Table 6a 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 soil salinity in the two growing season.

Salinity (dSm-1)	Ascorbic acid		Total soluble phenol		Carotenoids	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
0.8	6.91	6.86	20.25	20.15	0.080	0.063
7.5	5.31	5.39	17.98	17.91	0.075	0.081
11.5	4.69	4.78	15.24	15.22	0.059	0.0736
LSD at 0.05	0.328	0.415	0.440	0.433	0.009	0.009

Table 6b 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 antioxidants application in the two growing season.

Antioxidant	Ascorbic acid		Total soluble phenol		Carotenoids	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Water	3.58	3.63	16.38	15.98	0.060	0.079
AsA (100 mg^{L-1})	7.59	7.59	19.55	19.76	0.082	0.074
Toc (100 mg^{L-1})	5.73	5.82	17.55	17.54	0.072	0.065
LSD at 0.05	0.332	0.326	0.450	0.415	0.012	0.011

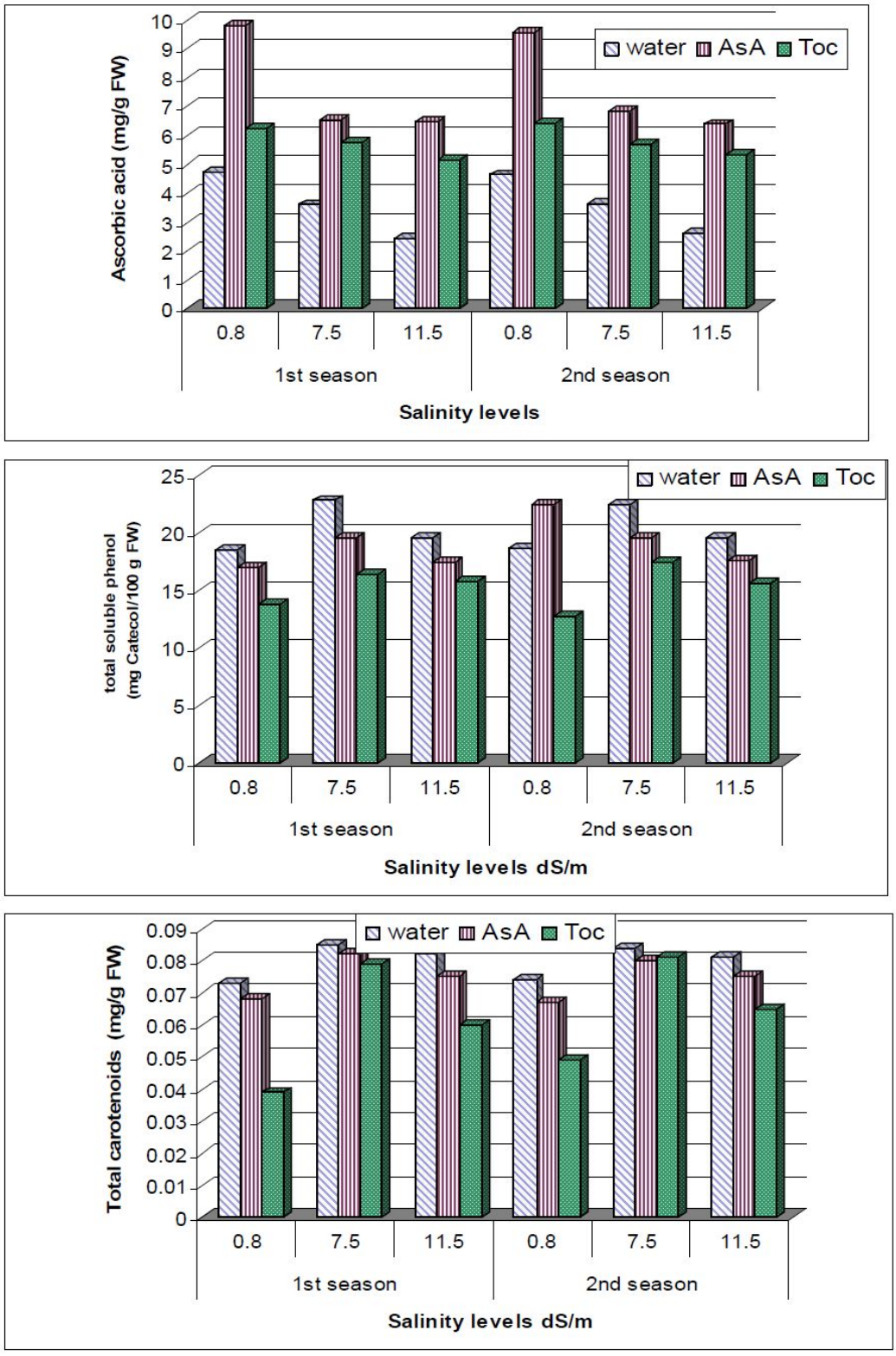


Figure 6. Ascorbic acid, Total soluble phenol and Total carotenoids contents in wheat flag leaf as affected by the Interaction of soil salinity and antioxidants application in the two growing season.

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 is reduced (Kukavica and Veljovic-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 (Zhao et al. 2007).

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 salinity stress induced H_2O_2 production and result in acceleration of leaf senescence through lipid peroxidation and other oxidative damage. H_2O_2 being a strong oxidant can initiate localized oxidative damage in leaf cells leading to disruption of metabolic function and loss of cellular integrity resulting in leaf senescence promotion. It also changes the redox status of surrounding cells where it initiate an antioxidative response by acting as a signal of oxidative stress (Sairam and Srivastava 2000).

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. The present investigation is 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 4a). Coupled with an increase in MDA and H_2O_2, AsA content, which is a non-enzymatic antioxidant and free radical scavenger, showed lower levels in the flag leaf of plants grown under saline conditions as compared to control plants (Table 6a). This is in agreement with a pervious study where AsA was found to decline during leaf senescence in maize leaves (Prochazkova et al. 2001).

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 2a) which in turn impairs the selectivity of the root membrane and results in the passive accumulation of sodium and chloride in plant organs (Table 2a; 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 4a). 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 chlorophyllase 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 that chl b was degraded at a higher rate than chl a (Table 1a). 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 increase 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 decompose superoxide radicals and hydrogen peroxide respectively, as well as various antioxidants quenchers including vitamins E and C 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, 4b), reducing sodium and chloride contents (Table 2b) and favoring potassium, calcium and magnesium absorption (Table, 3b) 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 4b, Figure 4) suggested that it may provide better protection against oxidative damage.

Although the inhibitory effect of ascorbic acid or tocopherol 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 5b), 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 6b). This has an adaptive significance, as it lowers 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 our experiments, CAT and POD activities were increased (Table 5b, Figure 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. However, 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 active site of enzyme 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). One possible reason seems to be that there is a cycle of interconversion between Chl a and b that is particularly significant in senescence (Metile et al. 1999). The physiological significance of the decreased 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 (Bjцrkman 1981), resulting in optimal functioning. Therefore, the decrease in $Chl_{a:b}$ ratio may favor the plants before late senescence.

Application of both antioxidants alleviated the harmful effect of salinity on ion content due to reduction of sodium and chloride accumulation (Figure 2) as well as increasing calcium, potassium and magnesium content (Figure 3) which leads to an increase in K^+/Na^+ (Figure 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 from soil solution. 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 4b and Figure 4) as well as increasing soluble protein content (Table 1b and Figure 1) which protects the membrane and membrane bound enzymes.

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

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