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Impact of Exogenous Ascorbic Acid on Antioxidant Activity and Some Physiological Traits of Common Bean Subjected to Salinity Stress

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Abstract

The role of ascorbic acid as a key molecule antioxidant involves in biotic and abiotic stress has already been well described. Recent study indicates that it also participates in the detoxification of generated reactive oxygen species due to abiotic stresses. Thus, In order to study effects of salt stress and ascorbic acid on antioxidant enzyme activity, chlorophyll content, lipid peroxidation and abscisic acid accumulation of bean an experiment was conducted. The exogenous application of ascorbic acid in nutrition solution of bean plants indicated that later is involve in reduction of antioxidant enzyme activity and increase of resistance to salt stress. We observed that antioxidant enzyme activity increased due to salt stress and ascorbic acid via eliminating of reactive oxygen species decreased activities. Moreover, abscisic acid and lipid peroxidation were affected of salt stress. Increase of ascorbic acid concentration at 100 mM NaCl treatment prevented of lipid peroxidation and decreased generation of malondialdehyde as final product of peroxidation of membrane lipids. However, at high level of salinity, ascorbic acid did not significant effect on reduction of lipid peroxidation. Whereas, chlorophyll content decreased by reactive oxygen species. Ascorbic acid increased chlorophyll content at all of stressed and non-stressed treatments. Abscisic acid assay showed that, accumulation of this hormone increased under salt stress treatments and raise of ascorbic acid concentration in nutrition solution of stressed plants prevented of abscisic acid accumulation. Our data provide strong support to the hypothesis that exogenous of ascorbic acid reduces the harmful effects of salinity and increases resistance to salinity in bean plant.

Keywords: abscisic acid, antioxidant enzyme, ascorbic acid, lipid peroxidation, salt stress

Introduction

High salt concentrations in soils inhibiting crop growth and yield are a frequent constraint to agriculture in arid regions. Irrigation with poor quality water is one of the main factors resulting in salt accumulation and decrease of agricultural productivity. Improving plant resistance to salt, although not a final solution, may provide yield stability in subsistence agriculture and limit solemnization due to irrigation by reducing inputs (Flowers and Yeo, 1995).

Osmotic stresses, ion imbalances, and the direct toxic effects of ions on the metabolic process are the most important and widely studied physiological impairments caused by salt stress (Munns *et al.*, 2006). Salt stress, like many abiotic stress factors, also induces oxidative damage to plant cells catalyzed by reactive oxygen species (ROS) (Azevedo-Neto *et al.*, 2006). The Plants exposed to salt stress adapt their metabolism in order to cope with the changed environment. Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals and instigate biochemical changes that adjust the metabolism accordingly (Hasegawa *et al.*, 2000).

Reactive oxygen species such as superoxide radical ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) are also produced during salinity stress, and are responsible for the damage to membranes and other essential macromolecules such as photosynthetic pigments, proteins, DNA and lipids (Fahmy *et al.*, 1998). In plant cells chloroplasts, mitochondria and peroxisomes are important intracellular generators of ROS (Salin, 1991). Reactive oxygen species produced, as results of various abiotic stresses need to be scavenged for maintenance of normal growth. The primary scavenger is superoxide dismutase (SOD) (EC 1.15.1.1), which converts $O2^{\cdot}$ to H_2O. Which is eliminated by ascorbate peroxidase (APX) (EC 1.11.1.11) in association with dehydroascorbate reductase (EC 1.8.5.1) and glutathione reductase (GR) (EC 1.6.4.2), and regenerate the ascorbic acid (AsA) (Asada 1994). Hydrogen peroxide is also scavenged by catalase (EC 1.11.1.6) (Comba *et al.* 1998), and POX though the enzyme is less efficient than ascorbate peroxidase-glutathione reductase system. Changes in activities of various antioxidant enzymes under salinity stress have been reported (Hernandez *et al.*, 2000).

Ascorbic acid is an abundant small molecule in plants. Ascorbic acid is a key substance in the network of antioxidants that include ascorbate, glutathione, a-tocopherol, and a series of antioxidant enzymes. Ascorbic acid has also been shown to play multiple roles in plant growth, such as in cell division, cell wall expansion, and other developmental processes (Smirnoff, 1996; Asada, 1999; Conklin, 2001;

Pignocchi and Foyer, 2003). Ascorbic acid, as an antioxidant, detoxifies H_2O_2, which is formed by the dismutation of O^-_2 AsA functions co-ordinately with glutathione and several enzymatic antioxidants to counteract O^-_2, which is produced by the Mehler reaction and photorespiration (Noctor and Foyer, 1998). Ascorbic acid is also believed to detoxify $^1O_2$ and $OH^·$ (Smirnoff, 1996; Noctor and Foyer, 1998; Asada, 1999).

However, little information is available on the effects of salt stress on the activated oxygen species metabolism and effect of AsA on antioxidant enzymes activity in bean plants. This knowledge can supply information on the possible involvement between of antioxidants as a defense against ROS and AsA in the response of salt stress. Hence, the objective of the present investigation was to study the effect of three levels of salt stress and four levels of AsA on various antioxidant enzymes activity, chlorophyll and abscisic acid (ABA) content and lipid peroxidation of bean plants.

Materials and methods

Plant material and growth conditions

Seeds of bean (*Phaseolus vulgaris* L. c.v. Naz) were surface sterilized for 5 min in sodium hypochlorite solution and then in 96 % ethanol for 30 s. Bean seeds was germinated on several layers of moistened tissue paper in the dark at 23˚C. Two-day-old seedlings were transferred to plastic pots. Pots containing 300-cm^3 perlite were used in the experiments. The plants of bean were grown hydroponically in half-strength Hoagland solution in growth chamber with 12 h photoperiod under a photon flux density of 450-μmol photons m^{-2} s^{-1} at 28/25 ˚C.

Ascorbic acid application and stress induction

For the AsA feeding experiment, 0, 25, 50 and 100 mM AsA solution was added in half-strength Hoagland solution. In order to salt stress induction, NaCl was dissolved in distilled water and the plants were watered to drip with 0, 100 and 400 mM NaCl solution after the plants were grown for 3 weeks. After 48 h, the fully expanded leaves were collected for the experiments. The leaves were frozen in liquid N_2 and then stored at -80˚C pending biochemical analysis.

Crude extract preparation

For enzyme extracts and assays, tissues were frozen and then ground in 4 mL solution containing 50 mM phosphate buffer (pH 7.0), 1% (w/v) polyvinylpolypyrrolidone. The homogenate was centrifuged at 15000 × g for 30 min and supernatant was collected and used for enzyme assays.

Antioxidant enzymes

Catalase activity was analysed using Native-polyacrylamide gel electrophoresis (Native- PAGE) and spectrophotometry method. For Native-PAGE of antioxidant enzymes, total soluble proteins extracted for SDS-PAGE were separated on 11% acrylamide Native gels without SDS and mercaptoethanol. Electrophoretic separation was performed at 4°C using a mini protean 3-electrophoresis unit (BioRad Laboratories, Hercules, CA, USA). Ten mg protein samples in native sample buffer (without SDS and mercaptoethanol) were loaded onto the gel. Staining for CAT was performed using the method described by Woodbury *et al.* (1971). Gels were briefly washed in distilled water followed by incubation in 0.003% (v/v) H_2O_2 for 10 min. After a brief rinse, CAT was detected by incubating the gels in 1% ferric chloride and 1% potassium ferricyanide until the bands appeared (a few seconds).

Spectrophotometry activity was estimated by the method of Cakmak and Horst (1991). The reaction mixture contained 100 μL crude enzyme extract, 500 μL 10 mm H_2O_2 and 1400 μL 25 mm phosphate buffer. The decrease in the absorbance at 240 nm was recorded for 1 min.

The activity of SOD was measured according to the method of Giannopolities and Ries (1977). The assay medium contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM p-nitro blue tetrazolium chloride, 2 mM riboflavin, 0.1 mM EDTA and 5 mL enzyme extract. Glass test tubes containing the assay medium were illuminated with a fluorescent lamp (120 W); identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of enzyme activity was determined as the amount of the enzyme to reach an inhibition of 50% nitro blue tetrazolium reduction rate by monitoring the absorbance at 560 nm.

Peroxidase activity was determined by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm (Ghanati *et al.* 2002). The reaction mixture contained 100 μL crude enzyme, 500 μL H_2O_2 5 mm, 500 μL guaiacol 28 mm and 1900 μL phosphate buffer 60 mm (pH 7.0). Peroxidase activity of the extract was expressed as μmol tetraguacial min^{-1} mg^{-1} protein.

Ascorbate peroxidase activity was measured according to Aono *et al.* (1995). Tissue was homogenized in 1 mL of 50 mM phosphate buffer (pH 7.0) containing 5 mM ascorbate, 5 mM DTT, 5 mM EDTA, 100 mM NaCl and 2% (w/v) polyvinyl pyrrolidone (PVP). The homogenized materialwas centrifuged at 15,000 × g for 15 min at 4˚C. The reaction was initiated by adding H_2O_2 to a final concentration of 44 μM as described by Nakano and Asada (1981). The reaction rate was monitored by decrease in absorbance at 290 nm.

Glutathione reductase activity was determined by the method of Halliwell and Foyer (Halliwell and Foyer, 1978). Its activity was assayed in a 1 mL reaction mixture containing 0.25 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.05 mL of 10 mM oxidized glutathione, 0.12 mL of 1mM NADPH, 0.48 mL of distilled water, and 0.1

mL of enzyme extract. The resultant decrease in NADPH was observed at 340 nm.

Chlorophyll

Chlorophyll content was determined using methods developed by Moran and Porath (1980). Leaves were soaked in 5 mL of dimethyl formamide for 24 h (in the dark) and absorbance read at 664 and 647 nm and leaf CHL content calculated by: $((ABS_{664} \times 7.04) + (ABS_{647} \times 20.27)) \times 5$/sample weight (g) = µg chlorophyll g^{-1} FW.

Maximum photochemical efficiency of PSII

When salt stress was initiated, maximum photochemical efficiency was calculated each 12 h once. Maximum photochemical efficiency was determined by a portable fluorometer (PAM-2000, Walz, Effeltrich, Germany) connected with a leaf-clip holder (2030-B, Walz) and with a trifurcated fibre-optic (2010-F, Walz). Data acquisition software (DA-2000, Walz) was used in a computer to dispose data. Before measurement, the leaves were dark-adapted for 30 min. The maximum photochemical efficiency of PSII was determined from the ratio of variable (*Fv*) to maximum (*Fm*) fluorescence.

Malondialdehyde content

Malondialdehyde (MDA) was measured by modifying the method discussed by McCue and Shetty (2002). In a test tube, 200 mL of the tissue homogenate was mixed with 800 mL of water, 500 mL of 20% (w/v) trichloroacetic acid and 1 mL of 10 mM thiobarbutyric acid. The test tubes were incubated for 30 min at 100°C and then centrifuged at 13,000 × g for 10 min. The absorbance of the supernatant was measured at 532 nm and the concentration of MDA was calculated from its molar extinction coefficient 156 $mmol^{-1}$ cm^{-1} and expressed as mmol g^{-1} FW.

Endogenous abscisic acid

abscisic acid was extracted, purified and assayed following the procedure described by Li et al. (1992) with some modifications using GC–MS technique as reported earlier (Nayyar *et al*., 2005).

Statistical analysis

All data were analyzed using MSTATC software. Each treatment was analyzed in three replications. When analysis of variance (ANOVA) showed significant treatment effects, Duncan's Multiple Range Test was applied to compare the means at $P<0.05$.

Results and discussion

Catalase activity increased because of salt stress. However, there was not significant effect in non-stressed plant (). Total CAT activity mostly increased in salt stressed plants, but it was higher at 400 mM NaCl than at 100 mM

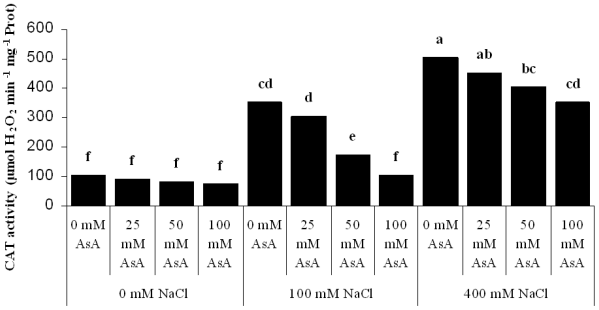

Fig. 1. The effect of NaCl salinity and ascorbic acid on catalase activity in the leaves of bean plants. Ascorbic acid with different concentration was added to nutrition solution. After growing for 3 weeks, the bean plants were irrigated with 0, 100 and 400 mM NaCl 48 h, and then the fully expanded leaves were collected for the assay of catalase activity. Within each figure, bars with different letters were significantly different at the 0.05 level.

NaCl. Data on CAT activity showed that with increase of AsA concentration there was significant decrease in activity at all the plants exposure to salinity. Activity gels showed bands of CAT in treated plants with salt stress and AsA. The band intensity is measure of activity (Fig.2). An increase in the activity of antioxidative enzymes under salt stress could be indicative of an increased production of ROS and a build-up of a protective mechanism to reduce oxidative damage triggered by stress experienced by

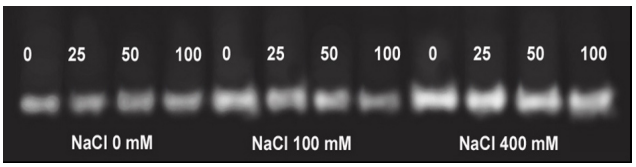

Fig. 2. Activity gels of bean leaves under different concentration of NaCl and ascorbic acid, 0, 25, 50, 100 mM. The band intensity is measure of enzyme activity.

plants. Catalase activity is also associated with the scavenging of H_2O_2. Superoxide dismutase activity, on the other hand, increased in salt stressed plants in response to salt stress (Fig.3). Leaf-SOD activity from stressed plants was more than non-stressed plants. Superoxide dismutase activity also decreased significantly under applied of AsA, at different concentration in nutrition solution. To overcome the effects of salinity-induced oxidative stress, plants make use of a complex antioxidant system. Relatively higher activities of ROS-scavenging enzymes have been reported in tolerant genotypes when compared to susceptible ones, suggesting that the antioxidant system plays an important role in plant tolerance against environmental stresses. In the present study, a significant increase in leaf CAT and SOD activity was observed in bean plants under saline conditions, suggesting that SOD may function as a ROS scavenger, by converting O_2^- to H_2O_2 (Alscher *et al*., 2002). Superoxide dismutase is responsible for the scavenging of toxic $O_2^{\cdot}$ in different cell organelles (Salin,

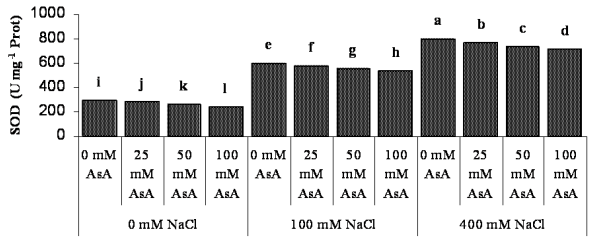

Fig. 3. The effect of NaCl salinity and ascorbic acid on superoxide dismutase activity in the leaves of bean plants. Ascorbic acid with different concentration was added to nutrition solution. After growing for 3 weeks, the bean plants were irrigated with 0, 100 and 400 mM NaCl during 48 h, and then the fully expanded leaves were collected for the assay of superoxide dismutase activity. Within each figure, bars with different letters were significantly different at the 0.05 level.

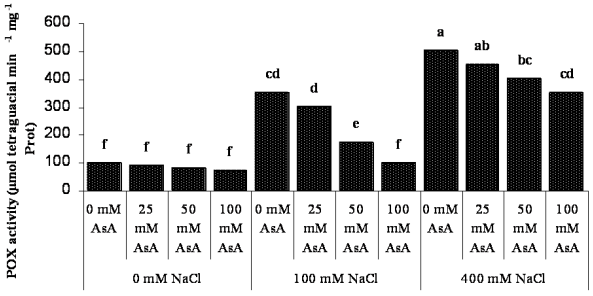

Fig. 4. The effect of NaCl salinity and ascorbic acid on peroxidase activity in the leaves of bean plants. Ascorbic acid with different concentration was added to nutrition solution. After growing for 3 weeks, the bean plants were irrigated with 0, 100 and 400 mM NaCl during 48 h, and then the fully expanded leaves were collected for the assay of peroxidase activity. Within each figure, bars with different letters were significantly different at the 0.05 level.

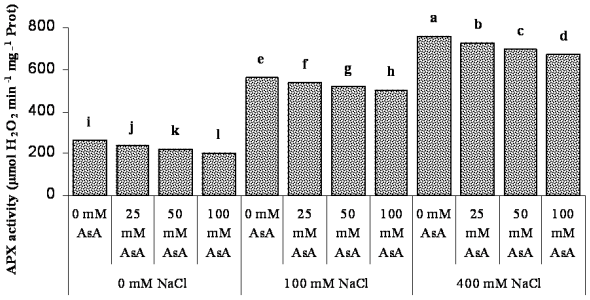

Fig. 5. The effect of NaCl salinity and ascorbic acid on ascorbate peroxidase activity in the leaves of bean plants. Ascorbic acid with different concentration was added to nutrition solution. After growing for 3 weeks, the bean plants were irrigated with 0, 100 and 400 mM NaCl during 48 h, and then the fully expanded leaves were collected for the assay of ascorbate peroxidase activity. Within each figure, bars with different letters were significantly different at the 0.05 level.

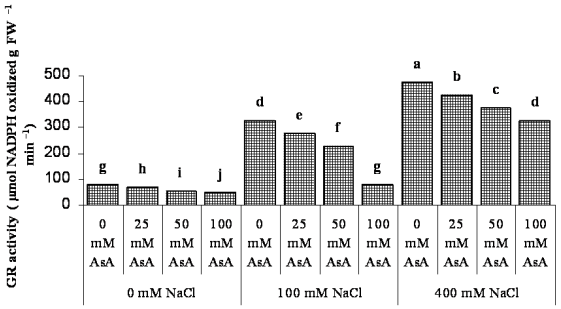

Fig. 6. The effect of NaCl salinity and ascorbic acid on glutathione reductase activity in the leaves of bean plants. Ascorbic acid with different concentration was added to nutrition solution. After growing for 3 weeks, the bean plants were irrigated with 0, 100 and 400 mM NaCl during 48 h, and then the fully expanded leaves were collected for the assay of glutathione reductase activity. Within each figure, bars with different letters were significantly different at the 0.05 level.

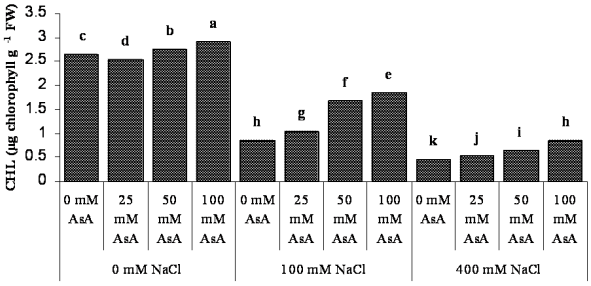

Fig. 7. The effect of NaCl salinity and ascorbic acid on chlorophyll content of bean plants. Ascorbic acid with different concentration was added to nutrition solution. After growing for 3 weeks, the bean plants were irrigated with 0, 100 and 400 mM NaCl during 48 h, and then the fully expanded leaves were collected for the assay of chlorophyll content. Within each figure, bars with different letters were significantly different at the 0.05 level.

1988). Salinity induced increase in SOD activity has been reported by various workers (Hernandez *et al.* 2000). It may also increase the level of H_2O_2 by increasing the activities of SOD, in consequence CAT activity increase. Even though a high SOD activity protects the plant against the superoxide radical, it cannot be considered solely responsible for membrane protection against peroxidation because it converts O_2^- to H_2O_2, which is also a ROS. Other enzymes, such as CAT and POX, should then scavenge this ROS. The fact that leaf CAT and SOD activity of the salt stressed plant was higher than control treatment However, when the plants were subjected AsA activities were decreased. Our results do show that leaf CAT and SOD activities increase with salt stress similar results have been observed when Beta maritime (halophyte) and the non-halophyte Beta vulgaris were studied (Bor *et al.*, 2003). Ascorbic acid is an antioxidant, which can scavenge O_2^-

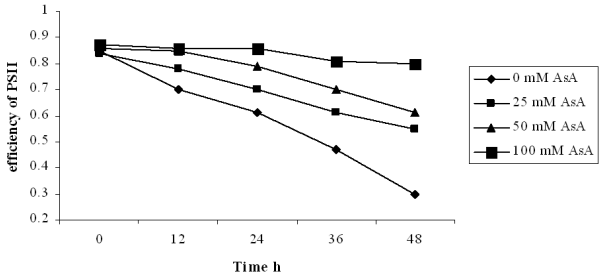

Fig. 8. The effect of 400 mM NaCl and ascorbic acid on maximum photochemical efficiency of bean plants. Ascorbic acid with different concentration was added to nutrition solution. After growing for 3 weeks, the bean plants were irrigated with 400 mM NaCl during 48 h, and maximum photochemical efficiency was calculated during 48 h.

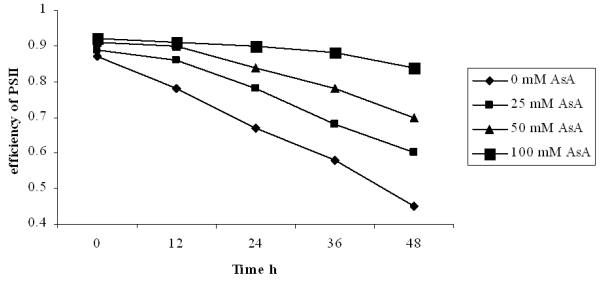

Fig. 9. The effect of 100 mM NaCl and ascorbic acid on maximum photochemical efficiency of bean plants. Ascorbic acid with different concentration was added to nutrition solution. After growing for 3 weeks, the bean plants were irrigated with 100 mM NaCl during 48 h, and maximum photochemical efficiency was calculated during 48 h.

and H_2O_2 non-enzymatically, and takes part in APX mediated scavenging of H_2O_2 (Asada, 1992). Ascorbic acid decreased the activity of these enzymes maybe by elimination of free radicals. When AsA was applied in nutrition solution, there was an obvious decrease in CAT and SOD activities in the leaves. A role of AsA in the ascorbate–glutathione cycle in mitochondria and peroxisomes has been described (Jime´- nez *et al.*, 1997). It also plays a protective role against ROS that are formed during biotic and abiotic stress (Noctor and Foyer 1998). Ascorbate is oxidized by oxygen free radicals and dehydroascorbate is generated (Noctor and Foyer, 1998). This leads to a decline in antioxidant activities.

Salt stress caused an increase in POX activity at all salt stress treatments. Peroxidase activity also decreased significantly under applied of AsA in stressed plants, at different concentration of AsA (Fig. 4). Under normal conditions (without salinity stress), POX activity at all treatments was same. It seems that, AsA as non-enzymatic antioxi-

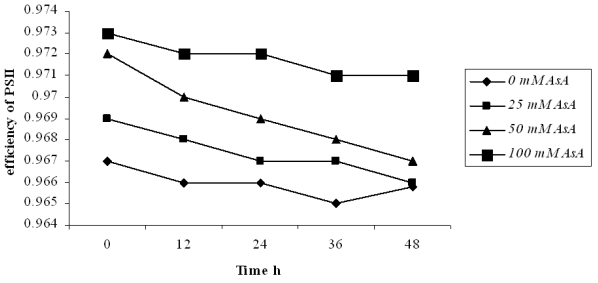

Fig. 10. The effect ascorbic acid on maximum photochemical efficiency of bean plants. Ascorbic acid with different concentration was added to nutrition solution. After growing for 3 weeks, the bean plants were irrigated with 0 mM NaCl (distilled water) during 48 h, and maximum photochemical efficiency was calculated during 48 h.

dants neutralized ROS and prevented of O_2 and H_2O_2 generation in consequence POX activity decreased.

Ascorbate peroxidase and GR activity strongly increased under salt stress specifically at 400 mM NaCl. (Fig. 5 and 6) The activity decreased at all treatments when AsA concentration was raised. Since APX and GR are key enzymes of the ascorbate glutathione cycle (Noctor and Foyer, 1998), this pathway could be a potential mechanism

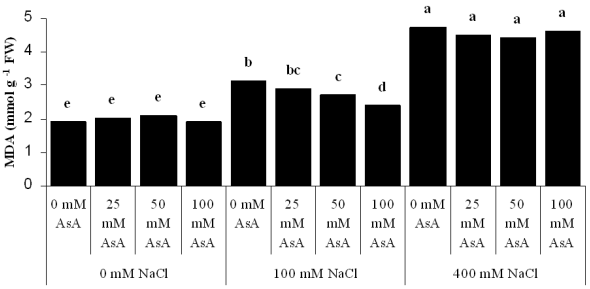

Fig. 11. The effect of NaCl salinity and ascorbic acid on malondialdehyde content of bean plants. Ascorbic acid with different concentration was added to nutrition solution. After growing for 3 weeks, the bean plants were irrigated with 0, 100 and 400 mM NaCl during 48 h, and then the fully expanded leaves were collected for the assay of malondialdehyde content. Within each figure, bars with different letters were significantly different at the 0.05 level.

for acclimation or adaptation to salt stress. Even though in some species, salt tolerance was associated with increases in both APX and GR activities (Bor *et al.*, 2003). The role of GR and glutathione in the H_2O_2 scavenging in plant cells has been well established in ascorbate–glutathione cycle (Halliwell-Asada pathway) (Bray *et al.*, 2000). The ascorbate–glutathione cycle is very efficient in regenerating the reduced forms of ascorbate. In this cycle NAD (P)-dependent monodehyroascorbate and glutathione

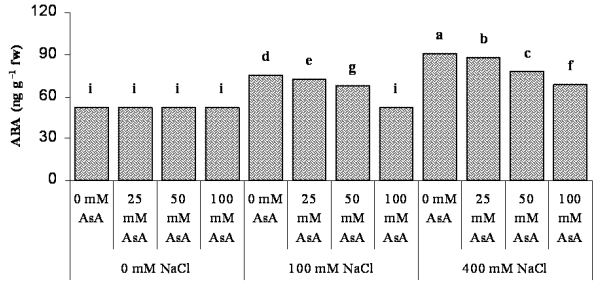

Fig. 12. The effect of NaCl salinity and ascorbic acid on abscisic acid accumulation in the leaves of bean plants. Ascorbic acid with different concentration was added to nutrition solution. After growing for 3 weeks, the bean plants were irrigated with 0, 100 and 400 mM NaCl during 48 h, and then the fully expanded leaves were collected for the assay of abscisic acid accumulation. Within each figure, bars with different letters were significantly different at the 0.05 level.

dependent dehyroascorbate, respectively, reduces monodehyroascorbate radical and dehyroascorbate to ascorbate. The oxidative form of glutathione is reduced by GR (Noctor and Foyer, 1998). Under salt stress, the activity of GR increased and the glutathione contents accumulate. The ascorbate–glutathione cycle, may play an important role in scavenging ROS. In plant cells, AsA is a major antioxidant which is involved in the ascorbate-glutathione cycle (Noctor and Foyer, 1998) The regeneration of reduced ascorbate in this cycle is achieved by the enzyme monodehydroascorbate reductase (EC 1.6.5.4).

Total CHL content under control condition was higher in compared to salt stressed treatments. Chlorophyll content decreased when plants were subjected to salt stress. However, maximum reduction in CHL content was observed at 400 mM NaCl (Fig. 7). Under normal condition, highest CHL content obtained at 100 mM AsA treatment. Hernandez *et al.* (1995) observed higher CHL degradation in sodium chloride sensitive pea cultivar as compared to tolerant one. Delfine *et al.* (1999) reported no changes in the CHL content in 20 days salt stressed spinach (*Spinacia oleracea* L.) plants. In plants, oxidative stress and signs of senescence include loss of chlorophyll and protein and decline in membrane permeability, all of which lead to a progressive reduction in photosynthetic capacity. Salt stress leads to an increase in free radicals in chloroplasts and destruction of chlorophyll molecules by ROS, which results in reduction of photosynthesis and growth. Singlet oxygen atoms and O_2^- radicals predominantly attack double-bond-containing compounds, thus damaging the chloroplast membrane system and photosynthetic reaction centers (Zhang *et al.* 2003). This in turn may result in the release of chlorophyll from the thylakoid membranes. The decrease in CHL content may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for CHL Degradation (Sabater and Rodriquez, 1978). The maximal efficiency of PSII decreased in both levels of salt stress. After 48 h, *Fv / Fm* decreased from 0.85 to 0.30 at 400 mM NaCl and without exogenous AsA whereas, *Fv / Fm* decreased from 0.87 to 0.80 at 400 mM NaCl and 100 mM AsA (Fig. 8). Similar this result was observed, at 100 mM NaCl stress (Fig. 9). The maximal efficiency increased with increasing of AsA concentration in both of group stress. There was not any change; noticeably in *Fv / Fm* ratio in normal condition (without stress), nevertheless AsA enhanced efficiency of PSII (Fig. 10). An increase in reactive oxygen species generation due to abiotic stress such as salt stress would result in further damage to PSII. Reactive oxygen species can also cause serious damage to organelles such as chloroplasts, mitochondria and plasma membranes. In consequence, efficiency of PSII decreases. This in turn may result in decrease damage to chloroplasts and PSII via scavenging of ROS by ascorbic acid. Delfine *et al.* (1999), however, found that the photochemical efficiency of the salt stressed leaves of spinach reduced after 50 days salt stress, indicating that high salt concentrations started to affect leaf photochemistry.

Maximum lipid peroxidation, assessed through MDA content was observed at 400 mM NaCl treatment. AsA treatment did not any positive effect on decrease of lipid peroxidation at highest level of salt stress, but at 100mM NaCl stresses, later had significant effect on decrease of lipid peroxidation. In normal condition, there was not any different among levels of AsA (Fig. 11). The data showed that, lipid peroxidation in leaves increased as the stress level raise. These results agree with those of Bor *et al.* (2003) who found that salt stress increases the lipid peroxidation in the leaves of two beet species. It has been demonstrated that, salinity induces oxidative stress in plant tissues, and lipid peroxidation has frequently been used as an indicator of oxidative stress when plants are subjected to salinity. This has been shown for *Oryza sativa* (Vaidyanathan *et al.*, 2003) and *Gossypium hirsutum* (Meloni *et al.*, 2003). Dolatabadian *et al.* (2008) reported similar inhibitory effects of exogenous AsA on lipid peroxidation in canola seedlings exposed to salt stress. Our results in this work suggested that, AsA prevented of lipid peroxidation by scavenging of ROS.

Abscisic acid assay showed that, salt stress increased ABA content in compared to none-stress plants. AsA feeding prevented of enhancement of ABA in treated plants with NaCl. In normal condition (without stress) we do not observed any changes in ABA content due to AsA application (Fig. 12). Evidence has accumulated that connects ABA accumulation to the endogenous AsA content and its redox status with regard to the plant response to drought (Hu *et al.*, 2005). Therefore, it could be predicted that this elevation in ABA levels via alteration of AA would lead to an early induction of senescence. It is well known that senescence correlates with loss of antioxidant capacity and consequently with an increase in ROS (Zimmermann

and Zentgraf, 2005). According to the 'free radical theory of ageing' proposed by Harman in 1956 (Harman, 1956), it would be predicted that low levels of AsA result in a pronounced production of ROS compared with the wild type regardless of the growth conditions. As a result, damage of the photosynthetic apparatus would be increased, leading to a faster decline in photosynthetic activity in AsA deficient tissue, and thus accelerate senescence. The plant hormones such as ABA and ethylene are known to promote senescence especially at stress conditions. Salt stress induces senescence and ABA is commonly known to induce senescence. Moreover, elevation of ABA is also found to increase the generation of antioxidants, and hence, to reduce the impact of oxidative injury in water-stressed maize leaves (Jiang and Zhang, 2002). According to our results, ABA content was raised due to salt stress. Ascorbic acid decreased ABA content via delay in senescence appearance by scavenging of ROS as promote senescence.

Conclusions

Results of our study demonstrated that salt stress significantly increased antioxidant enzyme activity and enzymes activity decreased due to AsA application. It seems that, AsA via neutralize or scavenging of ROS lead to resistance increase. According to these results, it can be suggested that usage of AsA can reduce the harmful effects of ROS and improves plant resistance, especially in hydroponics culture that increase of electrical conductivity and salinity are important problems.

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