Response of salt-stressed *Vicia fava* plant to application of ascorbic acid on the growth and some metabolites

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Abstract

The present work aimed at investigating changes in growth and some metabolic activities in NaCl-stressed bean plants, and assessing the role of ascorbic acid to alleviate these changes. The field experiment was carried out to study the response of presoaked fava bean seeds (*Vicia fava* cv. Misr 2) in freshly prepared ascorbic acid (50 ppm ≈ 0.3 mM; as recommended dose as described by El-Tayeb, 1995) or distilled water (control) for 4 hrs at natural environmental conditions, to salinity stress during different growth periods. At vegetative stage, the fresh and dry weights were decreased with salt treatment. The shoot length was hardly, if at all, affected by salinity stress either in the plants treated with ascorbic acid or not. The pigment biosynthesis was substantially affected by salt treatment. Addition of ascorbic acid to stressed plants reduced the inhibitory effect of NaCl on pigment content. Salinity enhanced the accumulation of reducing sugars in both root and shoot of *Vicia fava,* particularly at the high level of NaCl during the vegetative stage of growth. Whereas, the salt stress caused a decrease in the sugars content of both plant organs during the flowering and fruiting stages. NaCl treatment caused a reduction in sucrose content of *Vicia fava* root at the high level of NaCl during vegetative stage. In addition, the polysaccharide content of roots and shoots gradually increased with the progress of age, except at fruiting stage. These findings suggest that ascorbic acid achieved better results during growth stages.

Keywords: ascorbic acid; growth; minerals; NaCl; pigments; salinity; *Vicia fava*

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Introduction

The most common plant response to salt stress is a general reduction in growth and yield. Neumann (1997) and Aly et al. (2003) stated that when the salt stress increased, the growth rate and the size of crop plants dramatically were affected. This growth suppression was related to osmotic potential of the soil water caused by soluble salts. Within limits, concentrations of different combinations of salts cause nearly equal reductions in growth. On the other hand, Levitt (1980) found that excess of ions lead to yield reduction caused by ion toxicities or nutritional imbalances. The herbaceous crops are exposed to osmotic effects as a result of saline soils in the field generally consisting of a mixture of different salts. Soil salinity has become a serious environmental problem which affects the growth and productivity of many crops. The soil water

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potential is dramatically decreased by rising of salt levels in the soil. High salt content also affects the physiology of plants, both at the cellular as well as whole plant levels (Murphy et al., 2003). Levitt (1980) and Ebrahim (2005) said that soybean is very sensitive to ion toxicity as it is a herbaceous crop which does not exhibit leaf injury symptoms, but in contrast woody species exhibit leaf injury when Cl^- or Na^+ ions are accumulated.

Ascorbic acids ($C_6 H_8 O_6$) is present in all living plant cells, the largest amounts being usually in the leaves and flowers, i.e., in actively growing parts (Smirnoff et al., 2001; Ebrahim, 2005). Ascorbic acid serves as a hydrogen transport agent which is involved in cellular oxidation reduction reactions. Attempts have been made to employ active vitamins to overcome the drastic effects of salinity on seed germination and seedling growth as well as on some metabolic mechanisms (Khan and Zaidi, 1985; Ansari and Khan, 1986; Samiullah and Afridi, 1988). Presowing seed treatment of responsive cultivars with vitamins could thus be exploited to enhance grain yield at harvest (Kudrev and Pandev, 1965). Moreover, Oertli (1987) reported that under certain conditions, the exogenous application of vitamins to plants stimulates their growth, thus, apart from their main role as coenzymes, it is not improbable that vitamins may also play other independent roles in the biochemical processes of plants, repairing the injurious effects of unfavorable conditions. Ascorbic acid can scavenge the reactive oxygen species which are very harmful to the plant growth. It is a product of D-glucose metabolism which affects some nutritional cycle activities in higher plants and plays an important role in the electron transport system (El-Kobisy et al., 2005). Several studies have shown that ascorbic acid plays an important role in improving plant tolerance to abiotic stress (Shalata and Neumann, 2001; Al-Hakimi and Hamada, 2001; Athar et al., 2008).

Beans (*Vicia fava*) are considered the first legume crop in Egypt of the arable area. Total yield as green and dry seeds are consumed in human feed because the plant has high levels of protein (18 %), carbohydrates (58 %), vitamins and other minerals. In addition to the improvement of soil texture and its fertility, the plant seeds are considered as a valuable source for energy and proteins (El-Greadly, 2002).

So far, the literary references indicated no hints for investigating the response of *Vicia fava* to ascorbic acid. Therefore, the present work aimed at firstly investigating changes in crop yield, seed contents, and protein pattern in NaCl-stressed bean plants, secondly, assessing the role of ascorbic acid to alleviate these changes, thirdly, finding an explanation for such alleviatory role and finally, finding a recommended dose for treating *Vicia fava* with ascorbic acid.

Materials and Methods

A pot experiment was conducted in the Experimental Farm of the Faculty of Science, Tanta University, Tanta, Egypt [geographical coordinates 30° 47' N (Lat.), 31° 0' E (Long.)] during 2003 and 2004 to increase the salt tolerance of *Vicia fava* by ascorbic acid.

Plant material and growth conditions

Fava bean seeds (*Vicia fava* cv. Misr 2) were obtained from Gemmiza Agricultural Research Station, Gharbia, Egypt. The seeds were selected for uniformity of size and shape and surface sterilized (2.5% clorox for 5 min.) and rinsed thoroughly in distilled water. The seeds were then soaked in freshly prepared ascorbic acid (50 ppm ≈ 0.3 mM as the recommended dose described by El-Tayeb, 1995) or distilled water (control) for 4 hrs at natural environmental conditions.

Sand-clay soil 1/2 v/v (EC of 1:5 soil extract at 25° C = 0.58 mmohs cm^{-1}, pH of 1:5 soil suspension = 7.8) was used, the soil was mixed thoroughly to assure complete and uniform distribution (25 cm diameter, 35 cm depth, 5.5 Kg soil pot^{-1}).

Fava seeds were divided into 3 groups (0.0, 100 and 150 mM NaCl). Each was classified into 2 subdivisions (0.0 and 50 ppm ascorbic acid). Fifteen seeds were sown per pot and then gradually thinned to five before the end of the season. The sowing date was Nov., 2003 and the experiment was conducted for about 5 months. Pots were irrigated with the above NaCl

concentrations, to slightly lesser than the field capacity level, whenever they needed, but with equal amounts.

Nitrogen-Phosphorus fertilizers were applied at rates of one g urea/pot and 1.7 g super-phosphate/pot, respectively. Phosphorus was added during soil preparation (i.e., before sowing). Nitrogen was applied after 6 weeks of sowing.

Measurements

At three growth stages (vegetative, flowering and fruiting) the plant root and shoot organs were dried in an aerated oven, at 70°C, to constant weight. Carbohydrates were extracted in borate buffer pH 8 [0.1 dry mass (10 cm^3 buffer)$^{-1}$]. Carbohydrates were estimated quantitatively using Nelson (1944) with some modifications as suggested by Naguib (1963). After extracting in borate buffer, 10 mg of the dry plant residue were mixed with 0.2 ml of 0.1% (w/v) amylase and 0.1 ml acetate buffer (6 ml 0.2 N acetic acid + 4 ml 0.2 N Na- acetate), completed to 3 ml with distilled water, left for 24 hrs at room temperature, then centrifuged for 15 min at 3000 rpm to measure the starch content. Soluble proteins were assayed according to Bradford (1976). Alkaloids were also measured quantitatively according to the method described by Harborne (1973).

Photosynthetic pigments were estimated at vegetative and flowering stages by using fresh leaves which were homogenized immediately in 5 ml 85% cold acetone, centrifuged for 15 minutes at 3000 XG, then kept overnight in a refrigerator. The acetone extract was diluted to the appropriate volume and then its color intensity was measured at 663, 644 and 452.5 nm (Metzner et al., 1965).

Plant samples were analyzed for calcium, magnesium, copper, and manganese concentrations by means of the Atomic Absorption Flame Emission Spectrophotometer (Model Perkin Elmer 2380 Atomic Absorption Spectrophotometer).

Sodium and potassium were estimated by the flame photometer (Corning Scientific Instruments, model 410) as described by Johnson and Ulrich (1959).

Phosphorus was estimated by Allen et al. (1974). Two ml of the digested plant sample was taken in a test tube and 0.7 ml ammonium molybdate solution (Ammonium molybdate 25 g + Conc. H_2SO_4 250 ml completed to one liter) was added and shaken vigorously. Then 0.5 ml ascorbic acid solution (Ascorbic acid 0.3 g + H_2SO_4 20% 50 ml) was added and the solution was completed to 10 ml distilled water, shaken and then put into water bath for 15 min. at 70° C to determine phosphorous color and the density using spectrophotometer at 650 nm.

Analytical procedures of mineral ion concentrations were carried out according to Allen et al. (1974). Mixed acid digestion method was used in preparing the sample solution. A known weight of the oven dried plant material was transferred to 150 ml digestion flask and then 4 ml of conc. sulphuric acid were added. The mixture was heated gently until charring. Thereafter, 2 ml of 30% perchloric acid was added to the residue and then heated until the disappearance of fumes and the whole mixture turned into clear pale green solution. This indicates that all organic compounds were oxidized completely to carbon dioxide and the excess of perchloric acid was reduced to chlorine. The solution was then diluted to a certain volume with distilled water.

Soil analysis

Soil pH and electrical conductivity (EC) were measured from soil saturation extracts according to Chapman and Pratt (1961) method. 1:5 soil extracts were prepared for the determination of soil conductivity (EC) using an electric EC meter. Soil pH was determined for the same extract by using an electric pH meter, supplied with a glass electrode with a calomel reference electrode.

Statistical analysis

The obtained data were analyzed statistically to determine the degree of significance between treatments. Two-way analysis of variance (ANOVA; factorial) was applied for all data. The least significant

difference (LSD) at 5% was used to compare means (Steel and Torrie, 1980).

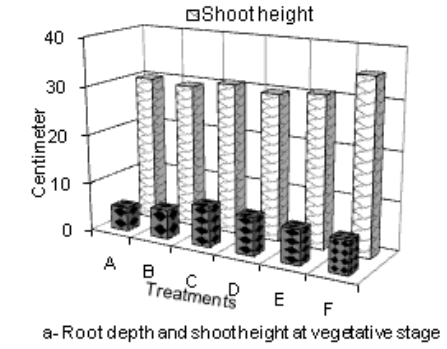
Results

Analysis of data revealed that, in all treatments both root depth and shoot height were progressively increased with progress of age (Fig. I). During vegetative and flowering stages, the root depth was increased with salinity. In contrast, at fruiting stage a reduction of root depth was observed comparing with control. However, ascorbic acid application to salt-treated plants slightly enhanced the root depth at all growth stages, but the response was more obvious at the vegetative stage.

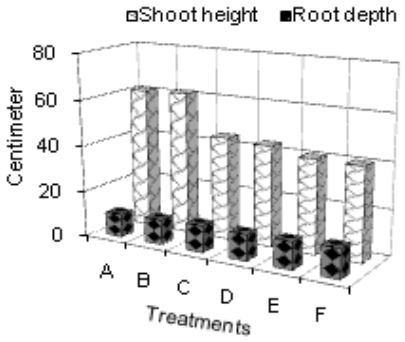
Concerning shoot height, data revealed that, at vegetative stage the two test levels of NaCl hardly affected shoot height of plants either treated or not with ascorbic acid. By contrast, at flowering and fruiting stages, salinity induced a drastic reduction on shoot height. At the same time, ascorbic acid application furthered the inhibitory effects of salinity stress.

Statistical analysis indicated that the effect of stages, salinity, ascorbic acid and the interaction between them on root depth and shoot height were highly significant. In Fig. II (a, b) data revealed that, at vegetative stage fresh and dry weights of root were increased with 100 mM NaCl, but hardly if at all any effect was observed with 150 mM NaCl. However, ascorbic acid application had no effect on root fresh weights at low level, but a slight decrease was observed at the high salinity level. At the same time, no effect was noticed at all on root dry weight. In contrast, at flowering and fruiting stages, salinity decreased the fresh and dry weights of roots either in plants treated or not with ascorbic acid. Root fresh weight was increased with progress of age from vegetative to flowering stage, but a noticeable decrease was recorded at fruiting stage and the same trend was observed with ascorbic acid application.

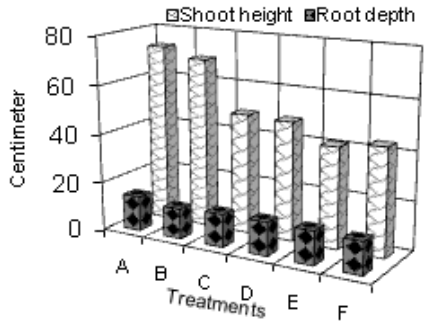
Concerning shoot weight in Fig. II (a, c, e), the data showed that at all growth stages salinity caused a reduction in shoot fresh weight of plants either treated or not with ascorbic acid but the response was more pronounced at fruiting stage. Shoot fresh weight also exhibited a dramatic drop



a- Root depth and shoot height at vegetative stage



b- Root depth and shoot height at flowering stage



c- Root depth and shoot height at fruiting stage

Fig. I. Root depth and shoot height of Vicia fava at vegetative, flowering, and fruiting stages as affected by NaCl levels (0.0, 100, and 150 mM) and presoaked seeds in 50 ppm ascorbic acid. a- root depth and shoot height at vegetative stage, b- root depth and shoot height at flowering stage, c- root depth and shoot height at fruiting stage; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

with the high level of salinity even in the presence of ascorbic acid.

Regarding the dry weight of shoots, it is clear from Fig. II (b, d, f) that NaCl treatments caused a slight increase in shoot dry weight at

vegetative stage. In the meantime, ascorbic acid insignificantly inhibited dry matter accumulation. On the other hand, at flowering and fruiting stages, salinity caused a highly significant decrease of shoot dry weight in stressed plants either treated or not with ascorbic acid.

Generally, in all treatments the shoot dry weight was highly significantly increased with age. Results showed that NaCl caused a highly significant decrease in chlorophyll a (Chl a) and

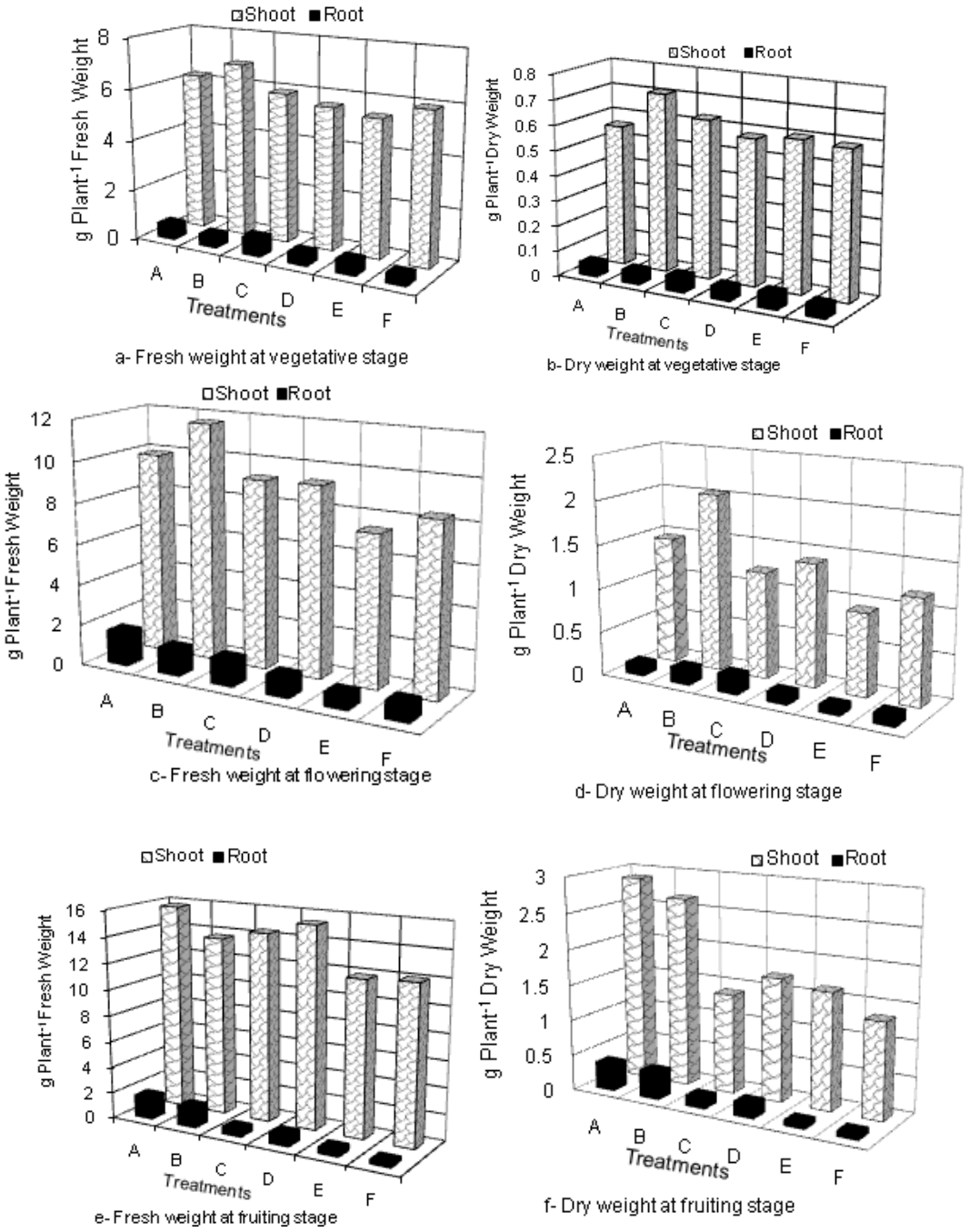


Fig. II. Fresh and dry weights of both root and shoot of *Vicia fava* at vegetative, flowering, and fruiting stages as affected by NaCl levels (0.0, 100, and 150 mM) and presoaked seeds in 50 ppm ascorbic acid. a- Fresh weight at vegetative stage, b- dry weight at vegetative stage, c- fresh weight at flowering stage, d- dry weight at flowering stage, e- fresh weight at fruiting stage, f- dry weight at fruiting stage; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

chlorophyll b (Chl b) contents at vegetative stage (Fig. III). In the mean time, ascorbic acid mitigated the salt inhibitory effect of Chl a and Chl b. On the other hand, at flowering stage NaCl decreased Chl a and Chl b contents, whereas ascorbic acid application to salt-treated plants furthered the inhibitory effects of NaCl on both Chl a and Chl b.

At vegetative stage, NaCl caused a noticeable effect in reducing sugar contents of root and shoot of *Vicia fava* plants (Fig. IV). In the meantime, application of ascorbic acid to salt-treated plants furthered the stimulatory effects of salinity and enhanced the accumulation of reducing sugars in both root and shoot to a great extent which was more prominent with high level of NaCl.

At flowering stage, NaCl treatments tended to lower root reducing sugars to a great extent whereas, a remarkable accumulation of shoot reducing sugars were observed. Ascorbic acid application to salt treated plants counteracted the inhibitory effects of salinity and increased the accumulation of reducing sugars in both root and shoot.

At the end of plant growth and development (fruiting stage), the salinized plants either treated or not with ascorbic acid showed a drastic decrease in reducing sugar contents of root, as shoots salinity decreased monosaccharide and starch contents but ascorbic acid application increased monosaccharide and sucrose contents.

It is interesting to note that, at all growth stages, the high dose of NaCl applied to plants either treated or not with ascorbic acid was more effective to accumulate reducing sugars. Also, shoots accumulated a large amount of reducing sugars more than roots, particularly with the high dose of NaCl. Statistical analysis indicated that the effects of salinity, ascorbic acid and the interaction of salinity and ascorbic acid on reducing sugars contents were highly significant.

Fig. (V) reveals that, at vegetative stage the sucrose content of roots was increased with NaCl concentration reaching the minimum value

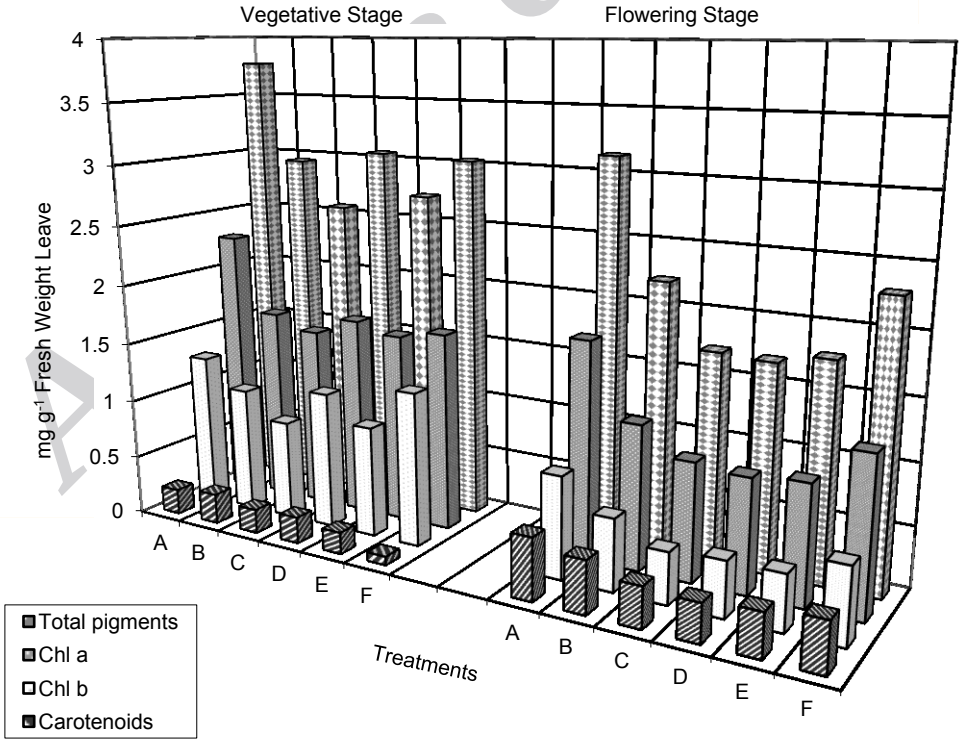


Fig. III. Pigments content of *Vicia fava* leaves at vegetative and flowering stages as affected by NaCl levels (0.0, 100, and 150 mM) and presoaked seeds in 50 ppm ascorbic acid ; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

with the high level of salinity. On the contrary, at flowering and fruiting stages, NaCl caused a remarkable accumulation in root sucrose content compared with control. Generally, ascorbic acid application to salt-treated plants induced a highly significant accumulation of sucrose in roots at all stages of growth. Statistical analysis indicated that, the effects of salinity and the interaction of salinity and ascorbic acid on sucrose content of root were highly significant.

Concerning accumulation of sucrose in shoot, findings suggested that at all stages, the sucrose content was gradually increased with increasing NaCl concentration. Ascorbic acid application caused an increase in the shoot sucrose contents compared with control (0.0 ascorbic acid), but less than its corresponding salt-treated control. Statistical analysis indicated that the effect of salinity, ascorbic acid and their interaction were highly significant on shoot sucrose content.

Fig. VI shows that, at all stages of growth NaCl treatments decreased accumulation of root polysaccharide content. Whereas, ascorbic acid application to salt-treated plants slightly enhanced the root polysaccharide more prominently under low in comparison with high NaCl dose. At all stages, NaCl treatments increased accumulation of shoot polysaccharide as compared with control. Generally, the shoot polysaccharide was decreased at the end of fruiting stage. Ascorbic acid application at vegetative and flowering stage increased shoot polysaccharide to a great extent, but at fruiting stage it caused an apparent decreased in shoot polysaccharide.

Statistical analysis indicated that the effects of salinity, ascorbic acid and their interaction were highly significant on root and shoot polysaccharide.

At all stages, the soluble protein contents of root and shoot were decreased with NaCl treatment (Fig. VII). Ascorbic acid application to salt-treated plants at vegetative and flowering stages caused a highly significant and gradual increase in the soluble protein contents of root and shoot. However, at fruiting stage ascorbic acid caused a decrease in the soluble protein contents of the root and shoot but it was more prominent in roots. Statistical analysis indicated

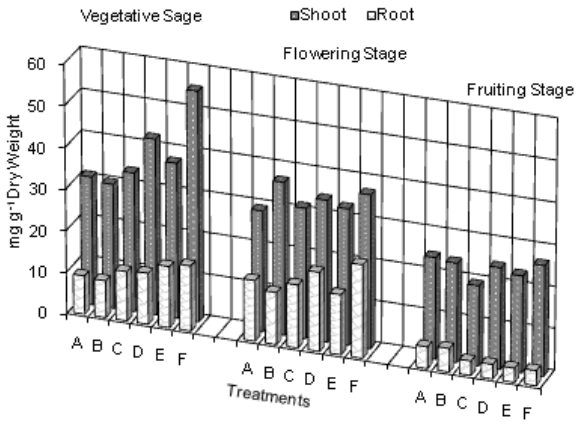


Fig. IV. Reducing sugars content of both root and shoot of *Vicia fava* at vegetative, flowering, and fruiting stages as affected by NaCl level (0.0, 100, and 150 mM) and presoaking in 50 ppm ascorbic acid ; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

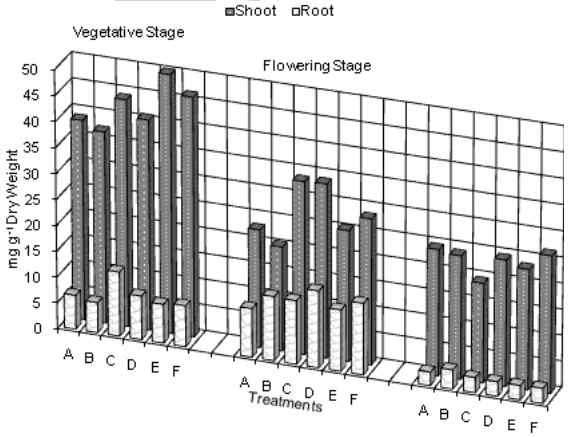


Fig. V. Sucrose content of both root and shoot of *Vicia fava* at vegetative, flowering, and fruiting stages as affected by NaCl level (0.0, 100, and 150 mM) and presoaked seeds in 50 ppm ascorbic acid; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

that, the effect of salinity, ascorbic acid and their interaction were highly significant and increased both root and shoot soluble protein content.

Fig. (VIII) shows at all stages, NaCl treatments caused a highly significant increase in the shoot alkaloid content. At the same time, ascorbic acid had no effect to non-salinized seeds, whereas coupling ascorbic acid achieved a highly significant increase in alkaloid contents of shoots compared with its corresponding controls. Statistical analysis indicated that the effects of

salinity, ascorbic acid and their interaction were highly significant in shoot alkaloids.

Concerning the effect of salinity on mineral contents, Fig. (IX b) reveals that salinity stress induced an increase in phosphorous contents in roots and shoots of *Vicia fava* plants either treated or not with ascorbic acid. However, ascorbic acid increased the phosphorus contents of salt- treated plants.

With respect to Fig. (IX b), salinity lowered the root sodium content, but raised sodium accumulation in shoot to a great extent which was more obvious at the high NaCl level. Ascorbic acid treatment for salt-treated plants furthered the same effect of salinity stress, i.e., decreased root sodium and increased shoot sodium. However, sodium was accumulated in shoots more than roots. On the other hand, potassium content in Fig. (IX c) decreased with salinity in both roots and shoots compared with control. Ascorbic acid caused an increase in potassium root and shoot compared with the untreated plants. However, salinity stress lowered the sum of sodium and potassium contents in roots either treated or not with ascorbic acid and raised it in shoots, particularly at the high level of NaCl.

Regarding the effect of salinity stress on divalent cations, Fig. (IX d) reveals that salinity stress increased calcium accumulation in both roots and shoots. Adding ascorbic acid to untreated seeds seemed to have no effect compared with control, whereas coupling ascorbic acid with salt-treated plants lowered calcium content in both roots and shoots as compared with the untreated plants.

Also, salinity stress induced an increase in magnesium content of both root and shoot of *Vicia fava* plants which was more obvious at the high salinity level (Fig. IX e). Ascorbic acid treatment to untreated seeds increased magnesium accumulation in both roots and shoots more than the control, whereas ascorbic acid treatment to salt-treated plants lowered root as well as shoot magnesium contents.

The sum of divalent cations (calcium + magnesium) of each root and shoot was increased by increasing NaCl concentration. This stimultaneous effect was more prominent at the high level of salinity. Ascorbic acid treatment

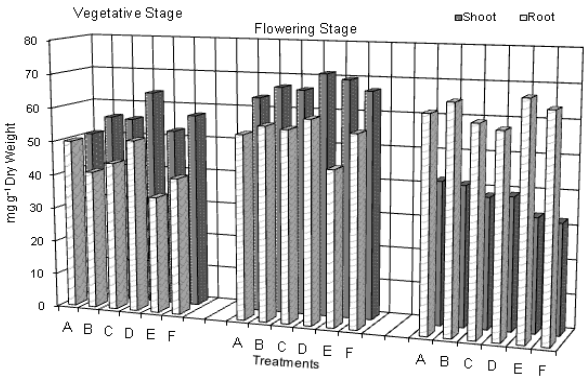


Fig. VI. Polysaccharide content of both root and shoot of *Vicia fava* at vegetative, flowering, and fruiting stages as affected by NaCl level (0.0, 100, and 150 mM) and presoaked seeds in 50 ppm ascorbic acid ; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

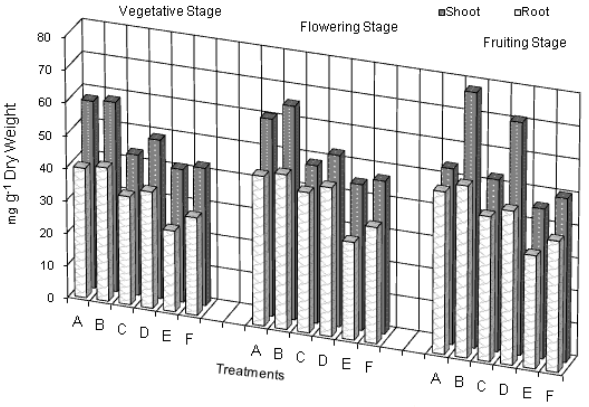


Fig. VII. Total soluble proteins content of both root and shoot of *Vicia fava* at vegetative, flowering, and fruiting stages as affected by NaCl levels (0.0, 100, and 150 mM) and presoaked seeds in 50 ppm ascorbic acid; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

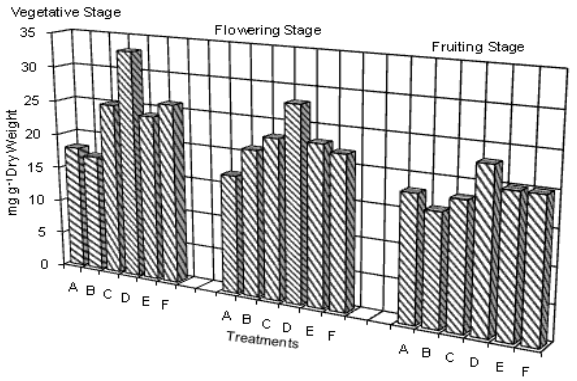


Fig. VIII. Total alkaloids content of *Vicia fava* shoot at vegetative, flowering, and fruiting stages as affected by NaCl levels (0.0, 100, and 150 mM) and presoaked seeds in 50 ppm ascorbic acid; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

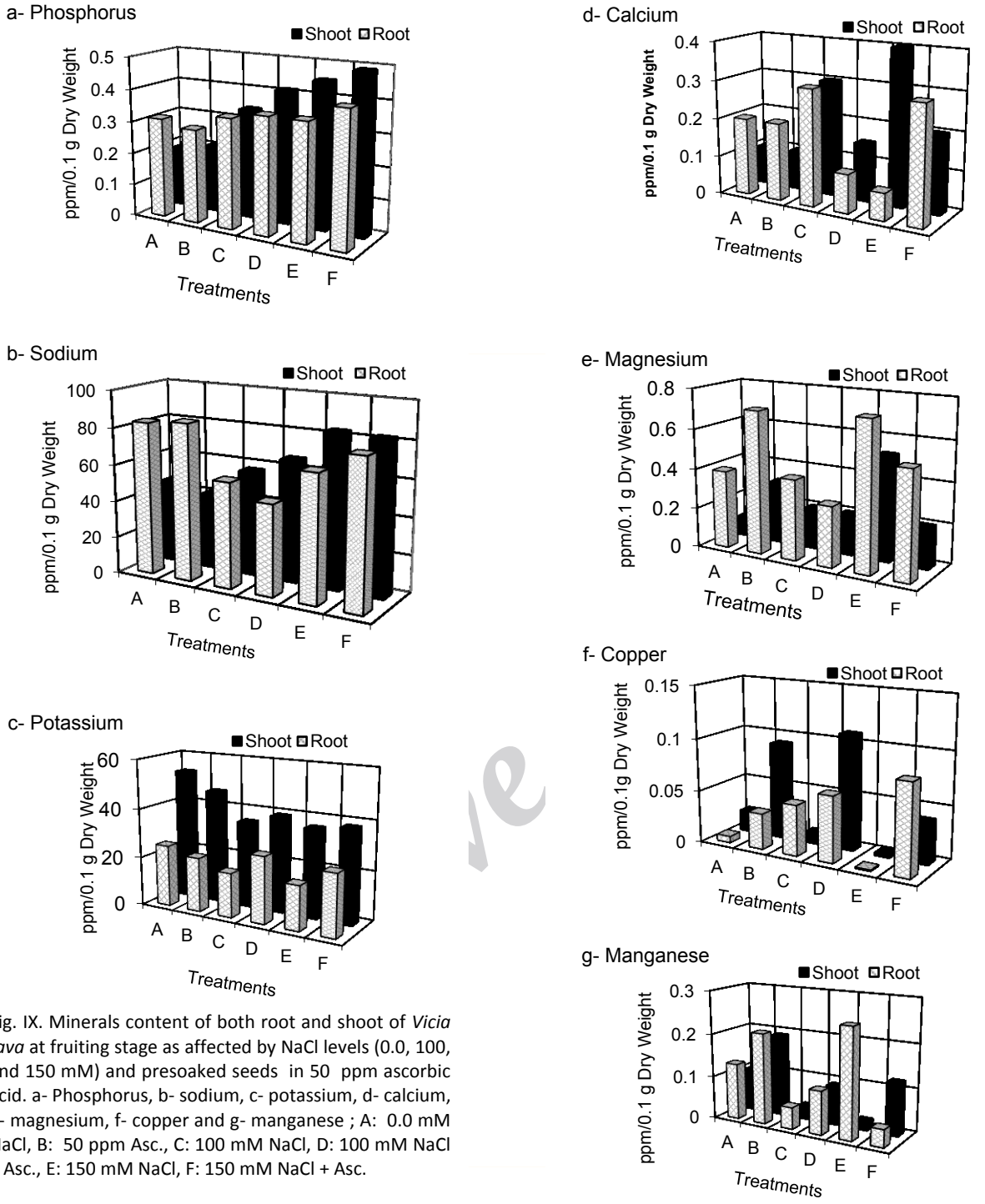


Fig. IX. Minerals content of both root and shoot of *Vicia fava* at fruiting stage as affected by NaCl levels (0.0, 100, and 150 mM) and presoaked seeds in 50 ppm ascorbic acid. a- Phosphorus, b- sodium, c- potassium, d- calcium, e- magnesium, f- copper and g- manganese ; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

increased calcium and magnesium accumulation through increasing magnesium content than calcium. Coupling ascorbic acid with salt-treated plants decreased the calcium and magnesium accumulation in both root and shoot.

Salinity induced an increase in the root-copper content at low level of NaCl and decreased it at the high salinity level (Fig. IX f). Ascorbic acid caused a highly significant and gradual increase in the root copper contents. Shoot-copper content was decreased with different NaCl concentrations, as very small copper contents were observed at the high salinity level. Ascorbic acid increased shoot-copper content at both levels of salinity. In Fig. (IX g), salinity decreased the root manganese content at the low NaCl concentration and increased it at the high dose. In case of ascorbic

acid, manganese content was gradually decreased with NaCl concentration, but a severe drop was observed at the high NaCl level.

In shoot, manganese content was gradually decreased with salinity compared with control. Ascorbic acid application decreased manganese content at low NaCl concentration, but at the high NaCl concentration ascorbic acid caused a highly significant increase in the shoot manganese contents.

Discussion

The results revealed that at vegetative stage, salinity stress decreased root fresh and dry weights. Also, salinity caused a suppressive effect on the shoot fresh and dry weights as compared with control. The suppression was more pronounced at high salinity level (150 mM NaCl) either in the plants presoaked in ascorbic acid or not. The fresh and dry matters yield of *Vicia fava* were markedly suppressed by salinization stress and they were lowered gradually with the rise of salinity level. Ekmekçi and Karaman (2012) reported a reduction in *Silybum marianum* plant growth as a result of Na Cl + water stress. This was also reported in studies on several other plants (Hajer et al., 2006; Alqurainy, 2007; Long et al., 2008). Increase in NaCl + water level reduced the absorption of water leading to a drop in water content of tested plants. Thus, the inhibitory effect of NaCl + water on growth parameters could be attributed to the osmotic effect of NaCl + water salinity (Salter et al., 2007). In addition, the changes in water status under NaCl + water stress may cause a reduction in meristem activity as well as cell elongation (Shah, 2007). Also, reduction in the rate of plant growth under salt stress is probably due to the accumulation of high amounts of toxic salts in the leaf and other tissues, which leads to dehydration and turgor loss, and eventually death of leaf cells and tissue (Munns, 2002). Also, the reduction in growth could be attributed to a reduction in cell division and/or in cell enlargement (Terry et al., 1971). Schwarz (1985) stated that reduced plant growth, under water stress conditions, has been considered to result from various factors; the most important of them is the physiological drought which was induced by the low water potential of the soil solution and osmotic adjustment in plants as a result of increased ionic concentration in their cells. The adverse effects of salt accumulation on plant may lead to disturbance in plant metabolism. Under such conditions, the metabolic processes of plant cells are frequently altered. These alterations, consequently, lead to a reduction in plant growth and productivity (Greenway and Munns, 1980; Sharma and Hall, 1991). Hamada and El-Enany (1994) found that increasing salinity of soil up to 160 mM NaCl reduced growth and transpiration in pot grown *Vicia fava* cv. Bunyard's Exhibition and peas cv. Progress No. 9. Barakat (2003) reported that salinity resulted in progressive reduction in *Triticum aestivum* L. growth rates as indicated by values of root and shoot lengths. Demiral and Türkan (2006) studied NaCl stress in two types of rice (*Oryza sativa* L.) cultivars differing in salt tolerance (salt tolerant Pokkali and salt sensitive IR – 28). They found that shoot fresh weight of Pokkali, shoot and root dry weights of IR – 28 showed a decrease under salinity. Roussos et al. (2006) studied the effect of four levels of NaCl salinity (0.0, 56.4, 112.8 and 169.2 mM) on jojoba explants during the proliferation stage. They reported that the fresh and dry weights of explants as well as their mean shoot length increased up to the medium salt concentration, while the mean shoot number decreased. Salinity enhanced the length and thickness of leaves as well as the thickness of shoot. This may be attributed to adverse inhibitory effect of salt stress on the growth and metabolic activities of the plant. Hartung (2004) reported that salinity affect meristimatic cell division and elongation as well as root penetration and these negatively affect plant height and root length. Younis et al. (2003) reported that the reduction in growth caused by salinity stress is due to inhibited apical growth in plants as well as internal hormonal imbalance. In both cases, reduction could have been caused by the toxic effects of ions (Na^+ and Cl^-) on metabolism or from adverse water relations. Moreover, the retardation in plant growth caused by salinity may be attributed mainly to the osmotic stress, which reduces availability and uptake of water and essential nutrients (Neumann, 1997), as well as the excessive

accumulation of both toxic ions i.e. Na^+ and intermediate compounds such as reactive oxygen species (Rodriguez et al., 2004) which cause damage to DNA, lipid, and proteins and consequently a decrease in plant growth.

Soaking *Vicia fava* seeds in ascorbic acid did not alleviate the inhibitory effects of salinity, where the fresh and dry matters gain in roots and shoots showed slight or no decrease or increase. These results may be due to exposure of the plant to long term stress which mainly causes sodium toxicity and water deficit (Munns et al., 1982; Ahmed et al., 1980; Shaddad, 1990 and El-Tayeb, 1995). The presoaking seeds in ascorbic acid solution had no effect on growth parameters particularly at high level of salinity and at the last stages of growth. In contrast, Amin et al., found that ascorbic acid at 100, 200 and 400 mg L^{-1} increased vegetative growth of wheat plants at milky and softy-dough stages. The increment in growth characters (i.e. plant height, number of tiller, number of spikes/plant, flag leaf area (cm), blades area/plant (cm) and dry weight/plant (g) reached maximum values at 400 mg/L of ascorbic acid compared to control plants at two physiological stages of growth. Ejaz et al. (2012) stated that maximum increases in sugarcane shoot length and diameter were recorded respectively at 0.5 mM ascorbic acid applied through irrigation and at 1.0 mM ascorbic acid applied as foliar spray, under both salt stressed and non-stressed conditions. Ejaz et al. (2012) found that salinity had a detrimental effect on shoot fresh/dry weights of sugarcane plants. Ascorbic acid application with different levels (0.1, 0.5 and 1.0 mM) improved the sugarcane biomass production. The effect of ascorbic acid treatments either through irrigation or foliar spray on shoot fresh weights was almost the same but in the case of dry weights the effect of irrigation was quite pronounced as compared to foliar spray at all the tested concentrations. But, under salinity stress, the root biomass was decreased as compared with control. Irrigation of salt-treated plants with 0.5 and 1.0 mM ascorbic acid hardly improved the root biomass as well as by foliar application. Ekmekçi and Karaman (2012) stated that the adverse effects of NaCl + water on the growth parameters were mitigated by irrigation of seeds with 100 ppm vitamin C. Also, Azooz (2004), Alqurainy (2007), and Athar et al. (2008) suggested that vitamin C could accelerate cell division and cell enlargement of treated plants. Shoot spraying with vitamin C was more effective in improving growth parameters of the treated plants, which was associated with increasing the water content and relative water content of leaves and reduction in transpiration rate. Khafagy et al. (2009) stated that the interactions between salinity levels and pre-soaking in ascorbic acid decreased plant height and root length of sweet pepper when compared with untreated plants. Ascorbic acid plays an important role in preserving the activity of enzymes (Padh, 1990). Smirnoff (1996) pointed out that the beneficial effect of ascorbic acid on root length may be attributed to the fact that ascorbic acid is involved in the regulation of root elongation, cell vacuolation and cell expansion.

The data of photosynthetic pigments clearly demonstrated that the biosynthesis of pigments was substantially affected by salt treatment. The reduction in leaf photosynthetic pigments in stressed *Vicia fava* plants might be attributed to the toxic effect or action of accumulated NaCl in leaves which inhibit the pigment biosynthesis and increase their degradation, and/or due to the damage of chloroplast structure. The reduction in photosynthetic pigments was attributed to enhance activity of the chlorophyll degrading enzyme chlorophyllase (Mishra and Sharma, 1994). Moreover, the high salinity caused a disturbed chloroplast structure, number, and size which affected chlorophyll content (El-Banna and Attia, 1999) and/or caused disruption of chloroplasts by oxidative stress which causes a decrease in chlorophyll content as well as a decrease in the photosynthetic reactions (Rahman et al ., 2000). Ekmekçi and Karaman (2012), Shah (2007), and Beltag (2008) stated that photosynthetic pigments of *S. marianum* (L) Gaertner leaves were substantially affected under NaCl + water irrigation. The content of chl. a and chl. b was more or less unchanged under 10% NaCl + water level while at higher levels of NaCl + water, a significant decrease was observed. On the other hand, the content of carotenoid was increased at low and moderate NaCl + water levels as compared with control.

The reduction in chl. b was higher (about 44%) than chl. a (about 30%) below the control at the highest. The reduction observed in chlorophyll content under NaCl + water irrigation could be as a result of inhibition of chlorophyll biosynthesis or increase in its degradation (Khan et al., 2006). Furthermore, under NaCl + water stress, an oxidative stress could result, which causes deterioration in chloroplast structure. This leads to a decrease in chlorophyll content while carotenoid content is increased (Khosravinejad and Farboondia, 2008). Carotenoids are known to act as efficient quenchers of free radical caused by reactive oxygen species. Thus, increasing carotenoids in plants treated with NaCl + water and/or vitamin C could enhance the capacity of these plants to minimize the damage caused by reactive oxygen species. Therefore, chlorophyll content of plants treated with vitamin C was increased, which could result from the protection effect of vitamin C and carotenoids to the photosynthetic apparatus from NaCl + water induced oxidative stress (Khan et al., 2006). Khafagy et al. (2009) stated that at 6000 ppm NaCl led to a decrease in chlorophylls a and b in sweet pepper leaves, and this effect increased consistently and rapidly with increasing salinity level as compared to non-stressed treatment.

The reduction in chlorophyll content concurrently with the increase in soluble protein contents led to the suggestion that nitrogen may be shifted to the synthesis of protein instead of chlorophyll. This is one of the adaptive responses to salt stress. In this connection, Hamada and El-Enany (1994) found that the concentrations of chlorophyll and carotenoids were increased in most cases in Vicia fava leaves under salinity while in pea plants they remained more or less unchanged by up to 50 mM NaCl. At higher concentrations, a significant decrease in these contents was observed. Gadallah (1999) stated that salinity decreased the content of chlorophyll of *Vicia fava* cv. Cabvor 103. Also, Sultana et al. (2000) reported that reduction in photosynthesis in the salinized plants of rice depended not only on the reduction of available CO_2 by stomatal closure, but also on the cumulative effects of leaf water and osmotic potential, stomatal conductance, transpiration rate, relative leaf water content, and biochemical constituents such as photosynthetic pigments, soluble carbohydrates, and proteins. Dragidevic et al. (2000) found that saline mineral water did not affect lettuce chlorophyll content. Sairam and Srivastava (2002) found that NaCl salinity caused a decrease in chlorophyll of wheat plant. De Pascale et al. (2003) reported that the concentrations of total carotenoids were gradually increased until 4.4-dSm^{-1} and decreased at higher salinity levels in *Lycopersicon esculentum* Mill. In addition, Demiral and Türkan (2006) studied NaCl stress in two types of rice (*Oryza sativa* L.) cultivars differing in salt tolerance (salt- tolerant Pokkali and salt- sensitive IR-28). They found that salinity decreased chlorophyll b and carotenoid contents of cultivar IR-28 and no effect on cultivar Pokkali. Addition of ascorbic acid to salt treated plants reduced the inhibitory effect of NaCl on pigment content whereas it slightly increased the total pigment contents at the vegetative stage. Amin et al. (2008) reported that there was a gradual increase in chl. a, chl. b, and carotenoids with increasing concentration of ascorbic acid up to 400 mg/l over their corresponding control at two stages of wheat plant growth. Similarly, Shaddad et al. (1990) and Abdel-Wahed et al. (2006) on maize, Hathout et al. (1993) on tomato, Salem et al. (2000) on sugar beet, Hanna et al. (2001) on wheat, and El-Gabas (2006) on sunflower plants found that ascorbic acid increased chlorophyll a, b, total chlorophylls, and attributed this to stimulation of biosynthesis of chlorophylls and delay in leaf senescence. Khafagy et al. (2009) reported that application of ascorbic acid significantly increased both chlorophyll a and b concentrations as compared with control.

Salinity enhanced the accumulation of monosaccharides in both root and shoot of *Vicia fava*, particularly at the high level of NaCl during the vegetative stage of growth. Whereas the salt stress caused a decrease in the monosaccharide contents of both organs during the flowering and fruiting stages. NaCl treatment caused a reduction in sucrose content of *Vicia fava* root at the high level of NaCl during vegetative stage, but slightly affected the sucrose content during the two last stages of growth. In contrast, the sucrose content of shoot increased during the different growth stages. In addition, the starch contents of

roots and shoots gradually increased with the progress of age, except at fruiting stage. In this connection, Imamul-Huq and Larher (1983) observed that plant leaves subjected to water stress often show a decrease in starch, which was generally accompanied by an increase in reducing sugar content. Yang et al. (1990) and Heuer (1991) have reported increased sucrose levels under saline conditions in Sorghum and sugar beet, respectively. Also Abd El–Samad (1993) found that salinity increased the carbohydrate content in roots and decreased in shoots of *Vicia fava* plants. Karadge and Gaikwad (2003) observed the accumulation of starch in the young and mature leaves of *Catharanthus roseus* G. plant at the lower salt concentrations (25 and 50 mM NaCl). While there was a decrease at the higher salinity levels (100 and 200 mM NaCl), the level of starch was increased in the stem and root linearly with increasing salt level in the medium.

The presence of ascorbic acid with NaCl appeared to alleviate the inhibitory effect on the function of the photosynthetic pigments and the biosynthesis of carbohydrate fractions of the stressed plants. The results appear to suggest that the disturbance of carbohydrate fractions might be attributed to the reduction of photosynthesis process concurrently with the transport of soluble sugars to the roots and their condensation into starch and/or might be due to the diversion of sugars into amino acids and proteins. Salt and drought stresses have been reported to favor the accumulation of soluble sugars in the leaves of sugar beet and other plants (Shehata et al., 1994; Mostafa, 1996; Ashmaye, 1998; Hamada and Garab, 1998; Heikal et al., 1999; Hamada, 2000; De Guang et al., 2001; Aly et al., 2003), but to reduce the levels of total carbohydrates (Ashmaye, 1998; Heikal et al., 1999). However, Mahgoub (1997) reported that salt stress sharply decreased the carbohydrate fractions, but vitamin treatments generally stimulated the accumulation of carbohydrates in the different organs of salt-affected bean lines and the inhibitory effect of salt stress was partially or completely alleviated. On the other hand, Sallam (1999) and Ebrahim (2005) found that contents of soluble sugars (total, reducing, non-reducing and sucrose) were increased in *Vicia fava* and sugar beet plants, but total and non- soluble carbohydrate were decreased with increasing salinity level. On the other hand, Al–Hakimi and Hamada (2001) showed that soluble sugars of shoot in wheat plants were lowered with the rise of NaCl concentration while the soluble sugar contents of roots and starch of shoots were raised. They founded that soaking grains in ascorbic acid could counteract the adverse effects of NaCl on the wheat seedlings by alleviating the salt stress. Amin et al. (2008) found that foliar application of ascorbic acid significantly increased total carbohydrates content in wheat grains up to 400 mg L^{-1} relative to their untreated controls. Similarly, the highest values of total carbohydrates and crude protein content of wheat grains were obtained by foliar application of ascorbic acid at 500 ppm compared with plants treated with 1000 ppm ascorbic acid or control (Hanna et al., 2001). Azza et al. (2011) observed that total carbohydrates percentage as affected by ascorbic acid in all *Codiaeum variegatum* plant organs, followed the same trend obtained previously on vegetative growth and gradually increased by increasing the level of ascorbic acid treatments used. The promoting effect of amino acids on total carbohydrates may be due to their important role of biosynthesis of chlorophyll molecules which in turn affected total carbohydrates content (Azza et al., 2011). Also, similar results were reported by Talaat et al.(2005) on *Catheranthus roseus* L., Attoa et al. (2000) on *Iberis amara* L., Nahed and Balbaa (2007) on *Salvia forinacea* plants, and Abdel Aziz et al. (2009) on *Antirrhinum majus*.

The soluble protein content was gradually increased with the progress of age, while NaCl treatments caused a decrease in soluble proteins of roots and shoots. Ejaz et al. (2012) stated that sugarcane soluble protein contents were increased by salt stress but application of ascorbic acid has no effect. Munns et al. (1979) reported that soluble proteins could play an important role in osmotic adjustment in water-stressed plants. Salt and drought stresses have been reported to favor the accumulation of soluble proteins in the leaves of sugar beet and other plants (Shehata et al., 1994; Mostafa, 1996; Ashmaye, 1998; Hamada, 1998; Heikal et al., 1999; Hamada, 2000; De Guang et al., 2001; Aly et al., 2003), but to reduce the levels of total

proteins (Ashmaye, 1998; Heikal et al., 1999). Skriver and Mundy (1990) and Chandler and Robertson (1994) assumed that stress-induced proteins play a role in stress tolerance, which may be essential for the survival of plants under extreme stress conditions. El-Khawas (1999) reported that application of two levels of NaCl (100 and 200 mM) induced an increase in the soluble protein contents of *Trigonella foenum* graecum. Gadallah (1999) found that salinity decreased the soluble proteins content of *Vicia fava*. Ebrahim, (2005) recorded a decrease in total soluble proteins of sugar beet exposed to salt stress. Under salinity the plants produce stress-responsive proteins that are involved in detoxification of ROS and thus play a role in adaptation to stress (Witzel et al., 2009 and Bandehagh et al., 2011).

Application of ascorbic acid stimulated the accumulation of soluble proteins in roots and shoots, so that it could be concluded that salinity increased the protein activity of hydrolyzing enzymes or increased the synthesis of new salt-induced proteins to counteract the effect of salinity. It has been widely reported that the protein contents were increased after exogenous application of ascorbic acid in potato (Sajid and Aftab, 2009) and chickpea (Beltagi, 2008). Stress conditions affect the cellular protein contents of plants (Guo and Song, 2009 and Mohammadkhani and Heidari, 2008). Wimmer et al. (2003) reported that according to the plant species or cultivar the stress conditions make changes in soluble protein contents due to the cell structural modifications (Ashraf and Harris, 2004). Heikal et al. (1999) found that vitamin C induced a simultaneous effect on the production of soluble proteins in root and shoot systems of salt-stressed *Cicer arietinum*. Similar results were reported on sugar beet (Ebrahim, 2005). Al-Hakimi and Hamada, 2001) revealed that soluble proteins of wheat shoots rose with the rise of NaCl concentration. Grain soaking in ascorbic acid, thiamin or sodium salicylate could counteract the adverse effects of NaCl in wheat seedlings by suppression of salt stress. Amin et al. (2008) reported that the highest values of crude protein content of wheat grains was obtained by foliar application of ascorbic acid at 500 ppm compared to plants treated with 1000 ppm ascorbic acid or control (Hanna et al. (2001).

Total alkaloid content revealed that NaCl treatments enhanced the accumulation of alkaloids in *Vicia fava* shoots, particularly with increasing the level of NaCl. Shain (1987) reported increased alkaloid content in *Atropa belladonna*, *Catharanthus roseus* and *Solanum laciniatum* when subjected to water stress. Saenz et al. (1993) observed that under severe drought, the alkaloids content of mature leaves of *Catharanthus roseus* plant increased highly significantly. Karadge and Gaikwad (2003) found that total alkaloid content was increased in both root and shoot of *Catharanthus roseus* G. up to 100 mM NaCl salinity.

Application of vitamin C (ascorbic acid) markedly increased alkaloid content. This may be due to the role of vitamin C to alleviate the inhibitory effect of salinity protecting the plant from stress damage. The literature dealing with the effect of salinity and ascorbic acid on the content of alkaloids is not plentiful. However, Ashraf and McNeilly (2004) reported that glucosinolate content of the seed meal in cauola generally increases with an increase in salt level of the growth medium.

The levels 100 and 150 mM NaCl increased phosphorus and magnesium of root system of *Vicia fava*, but decreased the sodium and calcium contents. It appeared that NaCl treatment caused an accumulation of phosphorus, sodium, calcium, and magnesium in shoot. Potassium content decreased in both organs of the tested plants. Application of ascorbic acid slightly affected the mineral contents of both organs. It increased the magnesium content of root, but decreased that of shoot system. The alteration in distribution and accumulation of mono and divalent cations in the different organs of salt-stressed plants may be an indication of the role of these cations in regulating the physiological activities of the plants. It appears that the decrease in potassium ion content was replaced by an increase in sodium ions as shown by Lutts et al. (1996) using *Oryza sativa* plants. Also, NaCl increased the calcium content in shoots of beans as an important factor that provides a degree of salt tolerance in plants. Calcium ions are required in

maintaining membrane integrity and transport of other ions (Salisbury and Ross, 1992). Hamada and El–Enany (1994) found that the content of sodium in the roots and shoots of *Vicia fava* and pea plants was increased with increasing salinity. In *Vicia fava*, calcium concentration in shoots, and potassium and calcium contents of roots increased with increasing salinity, while in pea plants, the contents of potassium and calcium were almost unaffected by salinity. Salinity induced an increase in the content of these ions in pea roots. Magnesium content in shoots of both *Vicia fava* and peas and in roots of *Vicia fava* decreased with an increase in salinity. Cordovilla et al. (1995) showed that the higher nitrogen rate moderates the adverse salinity effects on growth and increases the uptake of potassium and calcium of fava bean plants. Sallam (1999) found that soaking seeds in 1.5% NaCl was better than other treatments in increasing potassium in shoots of fava bean plants. Dragidevic et al. (2000) stated that saline mineral water did not affect content of phosphorus, potassium and sodium in Lettuce. Salinity led to the increase in magnesium. Shalata and Neumann (2001) reported that addition of anti-oxidant (0.5 mM ascorbic acid) to the root medium partially inhibited the response, but did not significantly reduce sodium uptake or plasma membrane leakiness.

Tammam (2003) revealed that the sodium content in both roots and shoots of *Vicia fava* plants increased with increasing salinity, whereas potassium and calcium were decreased. Garcia et al. (2006) stated that leaf concentrations of phosphorus and potassium were decreased with increasing salinity levels in *Citrus reticulata*. Khafagy et al. (2009) found a gradual increase in Na^+ concentration in either sweet pepper shoots or roots with increasing salinity levels in nutrient solution. Moreover, root system accumulated high concentration of Na^+ with increasing salinity levels in nutrient solution as compared to shoot. However, K^+ concentration decreased in the root system more than in shoot. Na^+ concentration significantly decreased while K^+ concentration increased with application of ascorbic acid as compared to non-salinized plants. The preferential accumulation of sodium in root over shoots may be interpreted as a mechanism of tolerance in at least two ways: firstly, maintenance of a substantial potential for osmotic water uptake into the roots, and secondly, restricting the spread of Na^+ to the shoots (Renault et al., 2001). On the other hand, significant decrease in K^+ concentration occurred with increasing salinity levels.

Seed soaking in ascorbic acid had an inhibitory effect on the accumulation of sodium in root system under various concentrations of NaCl. Furthermore, application of ascorbic acid ameliorated the inhibitory effects of NaCl on potassium and magnesium accumulation in the different organs. Amin et al. (2008) found that foliar application of ascorbic acid significantly increased P and K content in wheat grains up to 400 mg L^{-1} relative to their untreated controls. Also, ascorbic acid significantly increased N, P, and K content in leaves and grains of Ber (Rajpal et al., 2001), cotton, (El-Shazly and El-Masri, 2003), wheat (Abdel-Hameed et al., 2004), and sunflower plants (El-Gabas, 2006) compared with their controls. Raafat et al. (2011) stated that foliar application of ascorbic acid significantly increased P and K contents in wheat grains at 150 mg L^{-1} relative to their untreated controls. Also, ascorbic acid significantly increased P and K contents in leaves and grains of Ber (Rajpal et al., 2001), wheat (Abdel-Hameed et al., 2004), and sunflower plants (El-Gabas, 2006) compared with their controls.

Conclusion

The present wok demonstrated that presoaking *Vicia fava* seeds with 50 ppm ≈ 0.3 mM ascorbic acid for 4 hrs before sowing ameliorated the negative changes of NaCl- stress on growth criteria, metabolites and mineral content.

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