Effect of salinity stress on *Vicia faba* productivity with respect to ascorbic acid treatment

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Abstract

A field experiment was carried out to study the response of presoaked faba bean seeds (*Vicia faba* cv. Misr 2) in freshly prepared ascorbic acid (50 ppm) or distilled water (control) for 4 hrs at natural environmental conditions, to salinity stress. NaCl treatments caused a reduction in all productivity and yield parameters as compared with control, particularly at high NaCl level (150 mM), whereas the number of pods per plant, fresh weight of pods per plant, number of seeds per pod and fresh weight of seeds per pod were reduced. In the mean time, ascorbic acid had no effect on the yield parameters. Salinity stress increased the protein content of seeds to some extent. Ascorbic acid treatment to salinized plants remarkably increased the protein content. Also, seed alkaloids were increased with salinity stress, but the response was more pronounced at 100 mM NaCl whether alone or combined with ascorbic acid. The results revealed that protein pattern showed considerable effects of salinity stress on the protein bands. Application of ascorbic acid induced the synthesis and increased the intensity of the original protein bands and caused the appearance of additional new bands.

Keywords: Vicia faba; ascorbic acid; NaCl; productivity; protein pattern; salinity; yield

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Introduction

The most common plant response to salt stress is a general reduction in growth and yield. As salt concentration increases above a threshold level, both the growth rate and ultimate size of crop plants progressively decrease (Neumann, 1997; Aly et al., 2003). Growth suppression seems to be a nonspecific salt effect that is directly related to the total concentration of soluble salts or osmotic potential of the soil water. Within limits, concentrations of different combinations of salts cause nearly equal reductions in growth. On the other hand, single salts or extreme ion ratios are likely to cause specific ion effects, ion toxicities or nutritional imbalances, which cause even further yield reductions. Since saline soils in the field generally consist of a mixture of different salts, specific ion effects are minimal and osmotic effects predominate, particularly on herbaceous crops (Levitt, 1980). Soil salinity has become a serious environmental problem which affects the growth and productivity of many crops. High salt content in the soil affects the soil porosity and also decreases the soil water potential that results in a physiological drought. High salt content also affects the physiology of plants, both at the

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cellular as well as whole plant levels (Murphy and Durako 2003). Some herbaceous crops, e.g. soybean, are also susceptible to ion toxicities, but most do not exhibit leaf injury symptoms even though some accumulate levels of Cl^- or Na^+ that cause injury in woody species (Levitt, 1980; Ebrahim, 2005).

Ascorbic acids (C6 H8 O6) is present in all living plant cells, the largest amounts being usually in the leaves and flowers, i.e., in actively growing parts (Smimoff et al., 2001 and Ebrahim, 2005). The fundamental role played by ascorbic acid in metabolic processes is not known.

The fact that it is very sensitive to reversible oxidation (ascorbic acid ↔ dehydroascorbic acid) suggests that it may be involved in cellular oxidation-reduction reactions, perhaps serving as a hydrogen-transport agent. 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 stimulate 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. Application of ascorbic acid can reduce the harmful effects of salt stress and may have stimulatory effects on plants; ascorbic acid is synthesized in the higher plants and improves 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; Athara et al., 2008).

Beans (*Vicia faba*) are considered the first legume crop in the arable area of Egypt. Total yield is consumed as green and dry seeds 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 (National program for pulses crops- Agricultural Research Center, Giza, Egypt, 2002).

So far, no references have indicated any hint for investigating the response of *Vicia faba* to ascorbic acid. Therefore, the present work aimed at: (1) investigating changes in crop yield, seed contents and protein pattern in NaCl-stressed bean plants, (2) assessing the role of ascorbic acid to alleviate these changes, (3) finding an explanation for such alleviatory role, and (4) finding the recommended dose of ascorbic acid for treating *Vicia faba*.

Materials and Methods

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

Plant material and growth conditions

Faba bean seeds (*Vicia faba* 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; El-Tayeb, 1995) or distilled water (control) for 4 hrs at natural environmental conditions.

Sand–clay soil ½ v/v (EC of 1:5 soil extract at 25°C = 0.58 m mohs 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}).

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

Measurements

At 5 months old, fresh seeds were dried in an aerated oven, at 70 °C, to constant weight. Carbohydrates and protein were extracted in borate buffer pH 8 [0.1 dry mass (10 cm^3 buffer)$^{-1}$]. Carbohydrates were estimated quantitatively using Nelson's method (1944) with some modifications suggested by Naguib (1963). Soluble proteins were assayed according to Bradford (1976). Alkaloids were measured quantitatively according to the method described by Harbone (1973).

Protein pattern in seeds - Electrophoretic detection of protein by sodium dodecyle sulphate, polyacrylamide gel electrophoresis (SDS-PAGE)

The method of Laemmili (1970) with slight modifications was adopted to use in the present study. The modifications included reducing TEMED from 30 µl to 25 µl and also reducing ammonium persulphate (APS) from 1.5 ml to 1.3 ml. The protein content in supernatant was estimated according to the method of Bradford (1976) by using bovine serum albumin as a standard protein. Protein content was adjusted to 2 mg/ml per sample.

Crop yield

The yield was obtained as number of pods/plant, number of seeds/pod, pods weight/plant, seed weight/pod and fresh weight of 100 seeds.

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

All experiments and analytical determinations were replicated three times at least. The presented data represent the mean values. Data obtained were analyzed statistically to determine the degree of significance of the differences between treatments. The method of two ways 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

NaCl treatments caused a reduction in all productivity and yield parameters as compared with control, particularly at high concentration (150 mM), where the number of pods per plant (Fig. I), fresh weight of pods per plant (Fig. II),

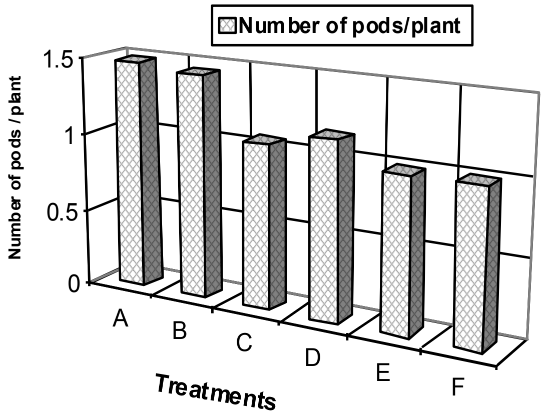

Fig.I. Number of pods per *Vicia faba* plant as affected by NaCl level (and 150 mM) and pre-soaking in 50 ppm ascorbic acid; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

number of seeds per pod (Fig. III) and fresh weight of seeds per pod (Fig. IV) were reduced. In the meantime, ascorbic acid had no effect on the yield parameters. Salinity stress increased the protein content of seeds to some extent (Fig. VI). Ascorbic acid treatment to salinized plants remarkably increased the protein content. Also, seed alkaloids were increased with salinity stress (Fig. VI), but the response was more pronounced at 100 mM NaCl whether alone or combined with ascorbic acid.

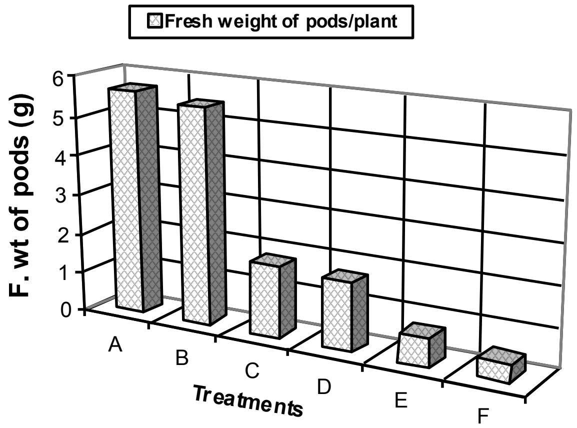

Fig. II. Fresh weight of pods per *Vicia faba* plant as affected by NaCl level (0.0, 100 and 150 and pre-soaking in 50 ppm ascorbic acid; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

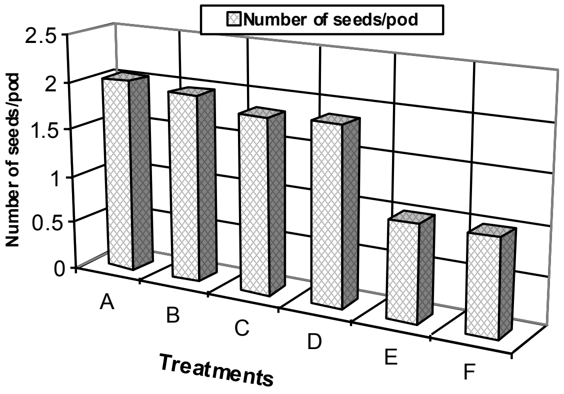

Fig. III. Number of seeds per *Vicia faba* plant pod as affected by NaCl level (0.0,100 and 150 mM) and presoaking in 50 ppm ascorbic acid; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

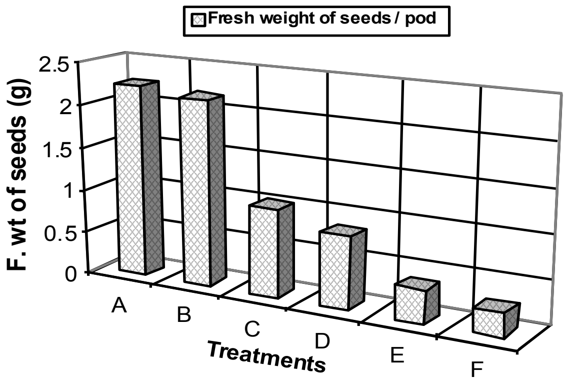

Fig. IV. Fresh weight of seeds per *Vicia faba* plant pod as affected by NaCl level (0.0,100 and 150 mM) and presoaking in 50 ppm ascorbic acid; ; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

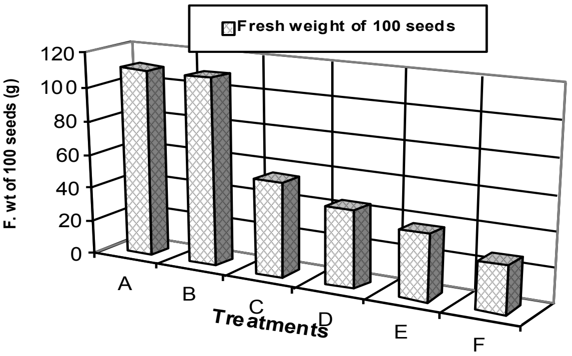

Fig. V. Fresh weight of 100 seeds of *Vicia faba* plant as affected by NaCl level (0.0,100 and 150 mM) and presoaking in 50 ppm ascorbic acid; ; A: 0.0 mM NaCl, B: 50 ppm Asc., C: 100 mM NaCl, D: 100 mM NaCl + Asc., E: 150 mM NaCl, F: 150 mM NaCl + Asc.

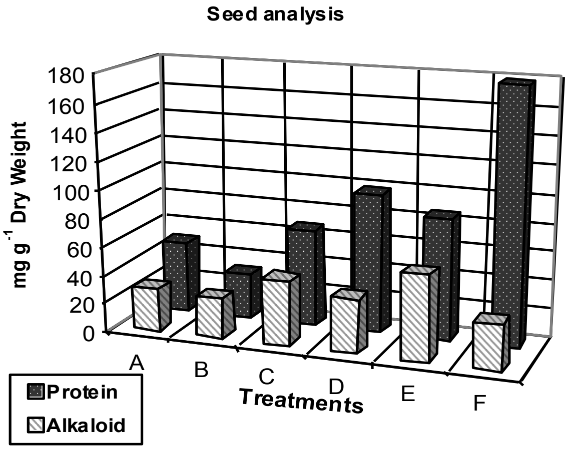

Fig. VI. Analysis of *Vicia faba* seed as affected by NaCl level (0.0,100 and 150 mM) and presoaking in 50 ppm ascorbic acid.

The plate of protein pattern (Fig. VII) showed considerable effects of salinity stress on the protein bands. Application of ascorbic acid induced the synthesis and increased the intensity of the original protein bands and caused the appearance of additional new bands. It appeared that protein metabolism is greatly affected in plants growing under saline condition.

The reduction of *Vicia faba* productivity may be attributed to this inhibitory effect of salt stress on the photosynthesis and the metabolic activities. Salinity stress can cause osmotic stress and salt toxicity in plants leading to a reduction in growth and ultimately in yield (Greenway and Munns, 1980; Ebrahim, 2005). Hussein et al. (2011) found that salt stress decreased all growth and yield parameters of wheat plant. Increasing water salinity from 3000 to 6000 ppm depressed plant spike length and dry weight of spikes compared to the unstressed plants (Wang, et al. 2007; Zheng, et al. 2009). This reverse effect may be due to the retarding effect on photosynthesis (Jampeetong and Brix, 2009), protein building (Parida and Das, 2005), mineral disturbances (Grattan and Grieve, 1998), hormonal balance (Shakirova, et al. 2003) and water adjustment (Shannon, 1997). Sharma (1995) cleared that *Vicia faba* was moderately salt tolerant with a salinity threshold of 4.56 ds/m and a 50 % reduction in seed yield observed at 9.5 / ds / m. Al Tahir and Al-Abdulsalam (1997) showed that water salinity significantly reduced the grain yield and grain number but did not affect grain weight. Pascale et al. (1997) found that salinity reduced mean pod weight by 15 % and the number of pods per plant by 48 %. Higher salinity stress decreased the seed yield by 67 % due to reduction in seed weight and number and also affected the product quality of broad bean**.** Sultana et al. (2000) found that the reduction in photosynthesis in the salinized plants led to low concentrations of assimilates in the leaves so that low levels and poor translocation of assimilates from the source reduced grain dry matter. Dragidevic et al. (2000) found that saline mineral water did not affect changes of lettuce yield. Shalata and Neumann (2001) found that in *Lycopersicon esculentum* Mill, the addition of anti-oxidant (0.5 mM ascorbic acid) to the root medium, prior to and during salt-treatment for 9 hours facilitated the subsequent recovery and long-term survival of 50% of the wilted seedlings. De Pascale et al. (2003) reported that irrigating with saline water containing sea salt up to4.4-dSm^{-1} (0.25% w/v) led to yield reduction (10%) in *Lycopersicon esculentum.* Maggio et al. (2004) showed that salinized tomato fruits were smaller than non-salinized ones. They also had higher acidity, increased soluble solids and higher sugar content. Colla et al. (2006) reported that increasing salinity resulted in a linear decrease in *Cucumis melo* yield compared with controls and that was due to reductions in the size and number of fruits. Garcia et al. (2006) reported that *Citrus reticulata* fruits yield was reduced by the salinity due to a decrease in the number of fruit per tree but not fruit size. High exogenous salt concentrations cause an imbalance of the cellular ions resulting in ion toxicity, osmotic

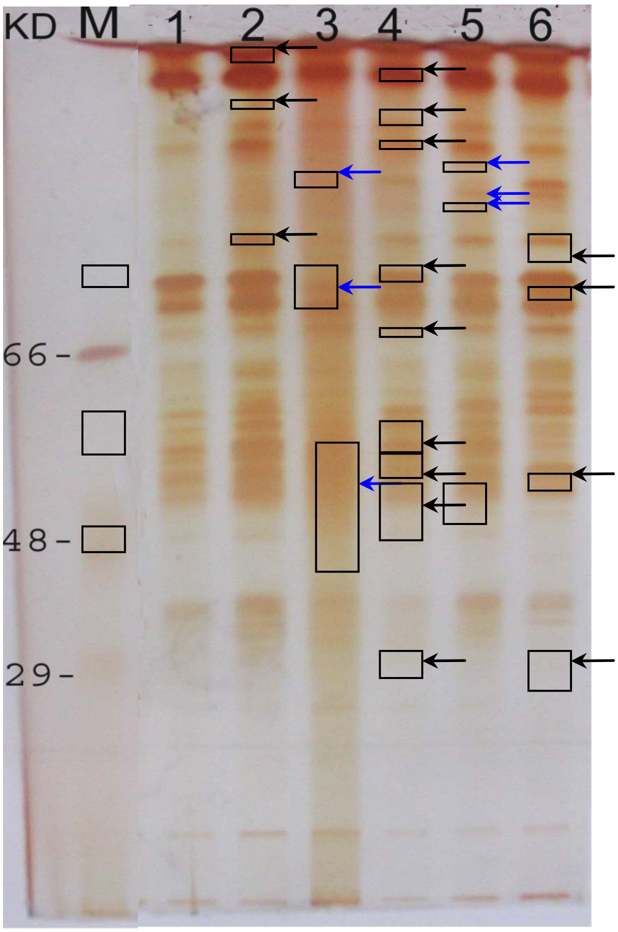

Fig. VII. Protein pattern

Lane 1: 0.0 mM NaCl
Lane 2: 50 ppm Asc.
Lane 3: 100 mM NaCl
Lane 4: 100 mM NaCl+Asc.
Lane 5: 150 mM NaCl
Lane 6: 150 mM NaCl +Asc.

stress and the production of ROS. Plant salt tolerance has generally been studied in relation to regulatory mechanisms of ionic and osmotic homeostasis (Ashraf and Harris, 2004).

Radi et al. (2001) and Ebrahim (2005) reported that vitamin treatments significantly increased dry matter yield of salt- stressed bean lines, resulting in the alleviation of the adverse effect of the high levels of salinity. The application of ascorbic acid caused significant increases in total yield of tomato plants (Abd El-Halim, 1995). El-Hifny and El-Sayed, (2011) reported that foliar application of ascorbic acid significantly increased yield and its components of pepper plants, i.e., fruit number, length, diameter and weight, as well as yield / plant and total yield / acre, in addition to fruit ascorbic acid content compared with untreated plants. The maximum values of yield and its components were obtained as a result of foliar spray with ascorbic acid at 400. Similarly, El-Greadly (2002) on cucumber, El-Banna et al. (2006) on pepper, Helal et al. (2005) on pea plants and El-Tohamy et al. (2008) on eggplant indicated that spraying with ascorbic acid had favorable effects on yield particularly with the higher concentration. This might be due to increasing of endogenous promoting hormones in plant (Abd El-Halim, 1995) in addition to stimulating of plant growth and CO_2 as well as microbial activity.

Ascorbic acid protects metabolic processes against H_2O_2 and other toxic derivatives of oxygen which affect many enzyme activities, minimizes the damage caused by oxidative processes through synergistic function with other antioxidants, and stabilizes membranes (Agarwal and Pandey, 2004; Sairam et al., 2005; Shao et al., 2008). Zeid et al. (2009) reported that exogenous ascorbic acid enhanced the productivity of wheat plants under salinity stress conditions. These effects may be attributed to the protective role of ascorbic acid in plant cells from the oxidative stress induced by salinity. Moreover, the increase in yield and its compounds might be due to the effect of the antioxidant role on enhancing protein synthesis and delaying senescence (Hammam et al., 2001). Foliar spray of ascorbic acid increased photosynthetic metabolites, which leads to the accumulation of different fractions of soluble sugars and nitrogen content in plant tissues under saline conditions. This evidence points to ascorbic acid as an important part of the plant defense system maintaining the integrity and normal function of the photosynthetic apparatus (Liu et al., 2008). Grain soaking in ascorbic acid had an inhibitory effect on the accumulation of sodium in different organs under various concentrations of NaCl (Al-Hakimi and Hamada, 2001). Concerning ascorbic acid, the mean values of spikes / m^2, spike length, grains number / spike, grains weight / spike and 1000- grain weight were significantly increased by sprayed the ascorbic acid on wheat plants at 1000 and 500 mg/L as compared with unsprayed wheat. These results may be due to sprayed vitamin C influencing many physiological process such as stimulating respiration activities cell division and many enzymes activities as reported by Oertil (1987), Abd-El-Hamed et al. (2004), Irfan et al. (2006) and Zewail (2007). Helsper et al.,(1982) observed that application of ascorbic acid at 1000 mg/L increased wheat grain yield by 15.35 and 15.64 % when compared with control plants.The positive response of wheat plants may be due to the effect of ascorbic acid on some enzymes which are important in regulation of photosynthetic carbon reduction. Hussein et al. (2011) reported that increasing the rate of spraying of ascorbic acid from 0 to 200 ppm and under different levels of salinity water led to increase in wheat spikes yield in most cases. Spraying 200 ppm ascorbic acid in most cases gave the highest value of yield parameters. Ascorbic acid could be used as a potential growth regulator to improve salinity stress resistance in several plant species (Gunes et al., 2005; Khan, et al., 2006 and Sheteawi, 2007). Also, ascorbic acid would inhibit stress-induced increases in the leakage of essential electrolytes following peroxidative damage to plasma membranes (McKersie, et al., 1999). In general, the effect of ascorbic acid in mitigating partially or completely the adverse effects of salt stress may be one aspect of the role of this compound in the activation of some enzymatic reactions. Among the positive effects of ascorbic acid in the counteraction of the adverse effects of salt stress are the stabilization and protection of the photosynthetic pigments and the photosynthetic apparatus from oxidization (Hamada 1998).

It is well documented that salinity promotes the synthesis of salt stress proteins (Ben–Hayyim et al., 1989), causing either a decrease in the level of total and soluble proteins or an increase in some other polypeptides. NaCl treatments enhanced protein synthesis to counteract the effects of salinity. It can be suggested that these new proteins may play an important role in triggering a special system helping the whole plant tolerance against NaCl stress. Studies revealed that salinity caused the disappearance of some protein bands, based on which it was concluded that the disappearance of polypeptides during salt stress compensates the synthesis of others (Sachs and Ho, 1990; Robinson et al., 1990) The results also showed that ascorbic acid induced the synthesis of new protein bands and this might be attributed to the synthesis of polypeptides or degradative product of proteins due to the effect of hydrolytic enzymes on high molecular weight proteins.

The polypeptides with molecular weights at 97, 82, 61, 58 and 34 KDa had disappeared under salinity and reappeared when the salinized plants were treated with ascorbic acid. This indicates that the inhibitory effect of NaCl stress on the synthesis of proteins could be overcome by vitamin C treatment. Singla and Grover (1994) found that the rate of protein biosynthesis decline during most stress conditions. However, cells preferentially synthesize a few specific proteins, which are termed stress proteins (De Rocher and Vierling, 1994; Moons et al., 1995; Han et al., 1997, Badr et al., 1998). It is apparent that one of the most important mechanisms involved in cell protection against salt stress is the induction of *de novo* synthetic protein groups (Kermode, 1997). Khattab (2001) found that in *Oryza sativa* L., some specific radicle emergence polypeptide bands disappeared under salt stress. Salt stress induced the synthesis of mid and high molecular weight new polypeptides (salt induced polypeptides). Bassuony et al., (2008) stated that four protein bands of molecular weights 69.37, 6.83, 4.51 and 2.67 KDa were *de novo* synthesized in *Zea mays* plants grown under salinity stress. It has been suggested that these proteins have an osmoprotection function **(**Dure et al., 1989 and Dure, 1993) or protected cellular structures (Close and Lammers, 1993). Also, Bassuony et al., (2008) stated that vitamin C treatments (grain soaking or shoot spraying) induced the synthesis of 2 new protein bands of molecular weights 15.86 and 1.01 KDa and increased the overexpression of protein band appeared at molecular weight 73.25 KDa in salinized *Zea mays* plant. These results suggested that these proteins may have a specific function to help maize plants to alleviate the harmful effects of salinity. Also based on the results of protein pattern it may be reasonable to assume that one of the multiple effects of vitamin C on stressed *Zea mays* plants is the *de novo* synthesis of a new proteins and the increased accumulation of certain existing proteins which may be involved in increasing the tolerance of maize plant.

Shukry and El–Bassiouny (2002) reported that salinization induced *de novo* synthesis of some salt responsive proteins of *Vicia faba* seeds during germination. The salt responsive proteins might be osmotin, dehydrin and ubiquitin, which were apparent in *Vicia faba* seeds. Ekmekçi and Karaman (2012) reported that *Silybum marianum* L. seeds germinated in 30% NaCl + water showed that NaCl + water salinity enhanced the synthesis of most original proteins which were already present in control seeds, especially, 55, 36, 29, 24, 20, 14 and 6.5 KDa polypeptides as well as synthesis of additional five new proteins with molecular weight of 205, 87, 84, 65 and 45 KDa. Soaking of *Silybum marianum* L. seeds in vitamin C elevated the levels of proteins in most bands of both seeds germinated either in 0 (control) or 30% NaCl + water. Further, in seeds germinated in 30% NaCl + water, vitamin C (lane 3) also resulted in appearance of five new proteins with molecular weight of 205, 87, 84, 65 and 45 KDa, respectively. In addition, the protein band 0 which had disappeared in seeds germinated in 0% NaCl + water and vitamin C reappeared. Similar results were reported by Azooz (2004), Kassim and Dowidar (2006) and Beltagi (2008). Ekmekçi and Karaman, (2012**)** stated that the changes in protein profile may be due to adaptation of *Silybum marianum* L. seeds to NaCl + water stress. The new bands of proteins in seedlings germinated in NaCl + water or in combination with vitamin C may be due to *de novo* synthesis of new protein (Gopala et al., 1987; Azooz, 2004). Bassuony et al. (2008) has shown that vitamin treatments induces a significant alterations in the enzymes related to protein metabolism. This indicates that vitamins might act as activators of protein synthesis. The new bands and the significant increase in the intensity of *Silybum marianum* L. as well as the original bands appearing in the control indicate that vitamin C has stimulatory effect on the protein component, which might be linked with the improvement of seed germination and growth.

Barakat (2003) stated that salinity induced a considerable variation in the protein pattern in *Triticum aestivum* L. These variations have been manifested in the novel expression of some polypeptides,in the absence of other and the overexpression of a third class of polypeptides. Bishnoi et al. (2006) found that, content of some proteins were decreased by boron treatment combined with NaCl in two *Cajanus cajan* L. genotypes, a salt tolerant and sensitive. Thus, 54.3 KDa protein disappeared in the plumule of salt sensitive seedlings, 68.4 KDa in the radicle of salt tolerant seedlings and 28.1 KDa in salt sensitive radicle. Cokuysal et al. (2006) stated that the *Nicotiana tabacum* (tobacco) cells growing in salt stress conditions showed distinct changes in their pattern of accumulation of total RNA and poly (A)+ RNA for the synthesis of salt stressed specific proteins.

Conclusion

The present wok demonstrated that presoaking *Vicia faba* seeds with 50 ppm ≈ 0.3 mM ascorbic acid for 4 hrs before sowing ameliorated the negative changes of NaCl- stress on productivity and yield parameters.

Acknowledgement

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References

Abd El-Hamed, A. M., S. H. Sarhan and **H. Z. Abd El-Salam.** 2004. 'Evaluation of some organic acids as foliar application on growth, yield and some nutrient contents of wheat'. *J. Agric. Sci.* Mansoura Univ. 29 (5): 2475-2481.

Abdel-Halim, S. M. 1995. 'Effect of some vitamins as growth regulators on growth, yield and endogenous hormones of tomato plants during winter'. *Egypt, J. Appl. Sci*. 10(12): 322-334.

Agarwal, S. and **V. Pandey.** 2004. 'Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia'*. *Biologia Plantarum* 48 (4): 555-560.

Al-Hakimi, A. M and **A. M. Hamada.** 2001. 'Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamin or sodium salicylate'. *Biol. Plant*. 44(2): 253-261.

Al-Hakimi, A. M. A. and **A. M. Hamada.** 2001. 'Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamine or sodium salicylate'. *Biol Plant.* 44: 253–261.

Al-Tahir, O. A. and **M. A. Al-Abdulsalam.** 1997. 'Growth of faba bean (*Vicia faba* L.) as influenced by irrigation water salinity and time of salinization'. *Agricultural-Water-Management*. 34 (2): 161-167.

Aly, M. M., S. M. El-sabbagh, W. A. El-shouny and **M. K. Ebrahim.** 2003. 'Physiological response of *Zea mays* to NaCl stress with respect to *Azotobacter chroococcum* and *Streptomyces niveus*'. *Biol. Sci*. 6(24): 2073-2080.

Ansari, S. A. and **F. A. Khan.** 1986. 'Effect of pre-sowing seed treatment with pyridoxine on growth and yield performance of summer moong'. *Indian Bot. Soc*. 65: 316-322.

Ashraf, M. and **P. J. C. Harris.** 2004. 'Potential biochemical indicator of salinity tolerance in plants'. *Plant Sci.* 166: 3–16.

Athara, H., A. Khanb and **M. Ashraf.** 2008. 'Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat'. *Env. Exp. Bot*. 63: 224-231.

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

Badr, A., A. S. Haider, S. F. Badr and **S. A. Radwan.** 1998. 'Genotypic variation within Egyptian wheat in response to salt stress and heat shock'. *Proceeding of the International Congress on Molecular Genetics.* 1:11-18.

Barakat, H. 2003. 'Interactive effects of salinity and certain vitamins on gene expression and cell division'. *Agric. And Biol*. 5(3): 219-225.

Bassuony, F. M., R. A. Hassanein, D. M. Baraka and **R. R. Khalil.** 2008. 'Physiological effects of nicotinamide and ascorbic acid on *Zea mays* plant grown under salinity stress' II-Changes in nitrogen constituent, protein profiles, protease enzyme and certain inorganic cations. *Aust. J. Appl. Sci*. ; 2: 350-359.

Beltagi, S. B. 2008. 'Exogenous ascorbic acid (vitamin C) induced anabolic changes for salt tolerance in chickpea (*Cicer arietinum* L.) plants'. *Afr. J. Plant Sci*. 2: 118-123.

Ben-Hayyim, G. Y. Vaadia and **G. B. Williams.** 1989. 'Protein associated with salt adaptation in citrus and tomato cell: Involvement of 26-KDa polypeptides'. *Physiol. Plant*. 77: 332-340.

Bishnoi, S. K., B. Kumar, C. Rani, K. S. Datta, P. Kumari, I. S. Sheoran and **R. Angrish.** 2006. 'Changes in protein profile of pigeon pea genotypes in response to NaCl and boron stress'. *Biol. Plant*. 50(1): 135-137.

Bradford, M. M. 1976. 'A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding'. *Anal. Biochem*. 72: 248-254.

Chapman, H. D. and **P. R. Pratt.** 1961. 'Methods of analysis for soils, plants and waters'. *Univ. California Division Agric. Sci.* 309.

Close, T. J. and **P. J. Lammers.** 1993. 'An osmotic stress protein of cyanobacteria is immunologically related to plant dehydrins'. *Plant Physiol.* 101: 773-779.

Cokuysal, B., H. Cakici and **E. Erbas.** 2006. 'Effects of salt stress on total RNA and poly (A) + RNA contents in tobacco suspension cultured cells at the different time course'. *Biol. Sci*. 9(1): 22-25.

Colla, G., Y. Rouphael, M. Cardarelli, D. Massa, A. Salerno and **E. Rea.** 2006. 'Yield, fruit quality and material composition of grafted melon plants grown under saline conditions'. *Hort. Sci. and Biotec*. 81(1): 146-152.

De Pascale, S., G. Angelino, G. Graziani and **G. A. Maggio.** 2003. 'Effect of salt stress on water relations and antioxidant activity in tomato'. *Acta Hort.* 613: 39-46.

De Rocher, A. E. and **E. Vierling.** 1994. 'Developmental control of small heat shock protein expression during pea seed maturation'. *Plant*. 5: 93-102.

Dragidevic, V., D. Prodanovic, S. Sredojevic and **B. Lazic.** 2000. 'Effects of salinity on lettuce (*Lactuca sativa* L.) quality'. *Acta Periodica Technologica*. **31: 225-229.**

Dure, L. 1993. 'Structural motifs in LEA Proteins. In: Plant Responses to Cellular Dehydration during Environmental Stress'. Current Topics in Plant Physiology Close T. J., Bray E. A. (eds.): Current topics in plant physiology, Vol.10 Rockville, M. D., Amer, Soci. *Plant Physiol*. pp: 91-103.

Dure, L., M. Crouch, J. Harada, T. H. Ho, J. Mundy, R. Quatrano, T. Thomas and **Z. R. Sung.** 1989. 'Common amino acid sequence domains among the LEA proteins of higher plants'. *Plant Mol. Biol*. 12: 475-486.

Ebrahim, M. K. 2005. 'Amelioration of sucrose-metabolism and yield changes, in storage roots of NaCl-stressed sugarbeet, by ascorbic acid'. *Agrochimica*. XLIX (3 - 4): 93–103.

Ekmekçi, B. A. and **M. Karaman.** 2012. 'Exogenous ascorbic acid increase resistance to salt of *Silybum marianum* (L.)'. *Afr. j. of Biotec.* 11(42):9932-9940.

El-Banna, E. N. S. A. Ashour and **H. Z. Abd-El-Salam.** 2006. 'Effect of foliar application with organic compounds on growth, yield and tubers quality of potato (*Solanum tuberosum* L.)'. *J. Agric. Sci. Mansoura Univ.* 31(2): 1165-1173.

El-Greadly, N. H. M. 2002. 'Effect of foliar application of ascorbic acid, ethrel and their combinations on growth, yield and endogenous hormones in cucumber plants'. *J. Agric. Sci. Mansoura Univ.* 27(8): 5269-5281.

El-Hifny, I. M. M. and **M. A. M. El-Sayed.**2011**.** 'Response of sweet pepper plant growth and productivity to application of ascorbic acid and biofertilizer under saline conditions'. *Aust. J. Basic and Appl. Sci*. 5(6): 1273-1283.

El-Kobisy, D. S, K. A. Kady, R. A. Medani and **R. A. Agamy.** 2005**.** 'Response of pea plant *pisum sativum* L. to treatment with ascorbic acid'. Egypt. J. *Appl. Sci*. 20: 36-50.

El-Tayeb, M. A. 1995**.** 'Effect of thiamin seed presoaking on the physiology of *Sorghum bicolor* plants grown under salinity stress'. *Egypt Bot*. 35(2): 201-214.

El-Tohamy, W. A, H. M. El-Abagy and **N. H. M. El-Greadly.** 2008**.** 'Studies on the effect of putrescine, yeast and vitamin c on growth, yield and physiological responces of Eggplant (*Solanum melongena* L.) under sandy soil conditions'. *Aust. J. of Basic and Appl. Sci.* 2(2): 296-300.

Garcia, S. F., J. G. Perez, P. Botia and **V. Martinez.** 2006**.** 'The response of young mandarin trees grown under saline conditions depends on the root stock'. *Agron*. 24(2): 129-139.

Gopala, R. P., C. D. Reddy and **J. K. Ramaiah.** 1987**.** 'Effect of B-vitamins on the protein component of clusterbeans *Cyamopsis tetragonoloba* L. *Taub*'. *Ann. Bot*. 59: 281-284.

Grattan, S. R. and **C. M. Grieve.** 1998**.** 'Salinity–mineral nutrient relations in horticultural crops. Review Article'. *Scientia Horticulturae* **;** 78 Issues 1-4, 30: 127-157.

Greenway, H. and **R. Munns. 1980.** 'Mechanism of salt tolerance in non-halophytes'. Ann. Rev. *Plant Physiol*. 31: 149-190.

Gunes, A. A. Inal, M. Alpaslan, F. Eraslan, E. G. Bagci and **N. Cicek.** 2005**.** 'Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity'. Original Research Article, *J of Plant Physiol*. 164, Issue 6: 728-736.

Hamada, A. M. 1998**.** 'Effect of exogenously added ascorbic acid, thiamine or aspirin on photosynthesis and some related activities of drought stress wheat plants. In Garab G (eds.): Photosynthesis: Mechanism and effects'. *Kluwer Academic Publishers, Dordrecht*, pp. 2581–2584.

Hammam, M. S., B. M. Abdalla and **S. G. Mohamed.** 2001**.** 'The beneficial effects of using ascorbic acid with some micronutrients on yield and fruit quality of hindy bisinnara mango trees'. *Assuit J Agric Sci*. 32: 181–193.

Han, B., P. Berjak, N. Pammenter, J. Farrant and **A. R. Kermode.** 1997**.** 'The recalcitrant plant species, *Castanospermum australe* and *Triticum dregena*, differ in their ability to produce dehydrin-related polypeptides during seed maturation and in response to ABA or water deficit-related stresses'. *Exp. Bot*. 48(314): 1717-1726.

Harbone, J. B. 1973**.** 'Phytochemical methods, a guide to modern techniques of plant analysis'. *Chapman and Hall*. London, pp. 185-186.

Helal, F. A, S. T. Farag and **S. A. El-Sayed.** 2005**.** 'Studies on growth, yield and its components and chemical composition under effect of vitamin C, vitamin B1, boric acid and sulphur on pea (*Pisum sativum* L.) plants'. *J. Agric. Sci. Mansoura Univ*. 30(6): 3343-3353.

Helsper, J. P., L. Kagan, J. M. Maynard and **F. A. Loewus.** 1982**.** 'L-Ascorbic acid biosynthesis in *Ochromonas danica*'. *Plant Physiol*. 69:485-468.

Hussein, M. M., K. M. Abd El-Rheem, S. M. Khaled and **R. A. Youssef.** 2011**.** 'Growth and nutrients status of wheat as affected by ascorbic acid and water salinity'. *Nature and Science* 9: 64-69.

Irfan, A., S. M. A. Basra, F. Mahammed and **A. Nawaz.** 2006**.** 'Alleviation of salinity stress in spring wheat by hormonal priming with ABA , Salisalic acid and ascorbic acid'. *Inter. J. Agric. Biology.* 8(1)23-28.

Jampeetong, A. and **H. Brix.** 2009**.** 'Effects of NaCl salinity on growth, morphology, photosynthesis and proline accumulation of *Salvinia natans'*. Original Research Article. *Aquatic Botany*. 91, Issue 3: 181-186.

Kassim, W. A. and **S. Dowidar.** 2006**.** 'Amino acids and soluble protein profile of radish seedlings under salt stress as affected by GA3 priming'. *Indian J. Plant Physiol*. 11: 75-82.

Kermode, A. R. 1997. 'Approaches to elucidate the basis of desiccation-tolerance in seeds'. *Seed Sci. Res.* 7: 75-93.

Khan, F. A. and **A. K. Zaidi.** 1985. 'On optimizing growth and yield of mustard by pre-sowing seed treatment with pyridoxine'. *Indian Bot. Soc*. 64: 91-94.

Khan, M. A., M. Z. Ahmed and **A. Hammed.** 2006. 'Effect of sea salt and L–ascorbic acid on the seed germination of halophytes'. *J. Arid Environ*. 67: 535–540.

Khattab, H. 2001. 'The interaction effects of salinity and certain bioregulators on the protein metabolism of two different rice cultivars'. *Biotec*. 9: 39-55.

Kudrev, T. and **S. Pandev.** 1965. 'Raising yield of swamp damaged wheat by vitamin B1 spraying'. C.R. *Acad. Bull. Sci*. 18: 555-557.

Laemmili, U. K. 1970. 'Cleavage of structural proteins during the assembly of the head of Bacteriophage T4'. *Nature* 227:680-685.

Levitt, J. 1980. 'Salt stresses. In: “Responses of Plants to Environmental Stresses” Vol. П. “Water, Radiation, Salt and Other stresses” '*Academic Press, New York* p. 365-454.

Liu, X., X. Hua, J. Guo, D. Qi, L. Wang, Z. Liu, Z. Jin, S. Chen and **G. Liu.** 2008. 'Enhanced tolerance to drought stress in transgenic tobacco plants overexpressing VTE1 for increased tocopherol production from Arabidopsis thaliana'. *Biotec Lett*. ; 30: 1275–1280.

Maggio, A., S. De Pascale, G. Angelino, C. Ruggiero and **G. Barbieri.** 2004. 'Physiological response of tomato to saline irrigation in long-term salinized soils'. *Agron*. 21(2): 149-159.

McKersie, B. D., S. R. Bowley and **K. S. Jones.** 1999. 'Winter survival of transgenic alfalfa over expressing superoxide dismutase'. *Plant Physiol.* 119: 839–847.

Moons, A., G. Bauw, E. Prinsen, M. V. Montagu and **D. V. Straeten.** 1995. 'Molecular and physiological responses to abscisic acid salt in roots of salt-sensitive and salt-tolerant indican rice varieties'. *Plant Physiol*. 107: 177-186.

Murphy, K. S. T. and **M. J. Durako.** 2003. 'Physiological effect of short term salinity changes on *Ruppia maritime. Aquat Bot.* 75: 293–309.

Naguib, M. I. 1963. 'Colorimetric estimation of plant polysaccharides'. *Zucker* 16: 15-18.

Nelson, N. A. 1944. 'Photometric adaptation of Somogi method for the determination of glucose'. *Biol. Chem*. 153: 275-280.

Neumann, P. 1997. 'Salinity resistance and plant growth revisited'. *Plant Cell Environ*. 20: 1193-1198.

Oertil, J. J. 1987. 'Exoenous application of vitamins as regulators for growth and development of plant'. Z.Pflanernahr. *Bodenr*. 150(6):375-391.

Oertli, J. J. 1987. 'Exogenous application of vitamins as regulators for growth and development of plants. A review'. Z. Pflanzenernähr. *Bodenk*. 150: 375-391.

Parida, A and **A. B. Das.** 2005. 'Salt tolerance and salinity effects on plants': a review Original Research Article. *Ecotoxic and Env. Safety*. 60, Issue 3:324-349.

Pascale, S., G. Barbieri and **S. De-Pascale.** 1997. 'Effect of soil salinity and top removal on growth and yield of broad bean as a green vegetable'. *Sci. Horticul*. 71(3-4): 147-165.

Radi, A. F., A. M. Ismail and **M. M. Azooz.** 2001. 'Interactive effect of some vitamins and salinity on the rate of transpiration and growth of some broad bean lines'. *Indian Plant-Physiol*. 6(1): 24-29.

Robinson, N. L., C. K. Tanaka and **W. J. Hurkman.** 1990. 'Time-dependent changes in polypeptide and translatable m RNA levels caused by NaCl in barley roots'. *Physiol. Plant*. 78: 128-134.

Sachs, M. M. and **T. H. Ho.** 1990. 'Alteration of gene expression during environmental stress in plants'. *Ann. Rev. Plant Physiol.* 37: 363-376.

Sairam, R. K., G. C. Srivastava, S. Agarwal and **R. C. Meena.** 2005. 'Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes'. *Biologia Plantarum* 49: 85-91.

Samiullah, S. A. and M. M. Afridi. 1988. 'B-vitamins in relation to crop productivity'. Indian Rev. *Life Sci*. 8: 51-74.

Shakirova, F. M., A. R. Sakhabutdinova, M. V. Bezrukova, R. A. Fatkhutdinova and **D. Fatkhutdinov.** 2003**.** 'Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity Original Research Article'. *Plant Science*. 164 Issue 3: 317-322.

Shalata, A. and **M. Neumann.** 2001**.** 'Exogenous ascorbic acid (Vitamin C) increases resistance to salt stress and reduces lipid peroxidation'. *Exp. Bot*. 52(364): 2207-2211.

Shannon, M. C. 1997**.** 'Adaptation of Plants to Salinity Original Research Article'. *Advances in Agronomy*. 60: 75-120.

Shao, H. B., L. Y. Chu, H. L. Zhao and **C. Kang.** 2008**.** 'Primary antioxidant free radical scavenging and redox signalling pathways in higher plant cells'. *Int J Biol Sci*. 4: 8–14.

Sheteawi, S. A. 2007**.** 'Improving growth and yield of salt-stressed soybean by exogenous application of ascobin'. *Int. J. Agri. Biol.* 9:473-478.

Shukry, W. M. and **H. M. El-Bassiouny.** 2002**.** 'Gibberellic acid effects on protein pattern, hydrolytic enzyme activities and ionic uptake during germination of *Vicia faba* in sea water'. *Acta-Botanica-Hungarica.* 44(1-2) 145-162.

Singla, S. L. and **A. Grover.** 1994**.** 'Detection and quantitation of a rapidly accumulating and predominant 104 KDa heat shock polypeptide in rice'. *Plant Sci.* 97: 23-30.

Smimoff, N., P. Conklin and **F. A. Loewus.** 2001**.** 'Biosynthesis of ascorbic acid in plants': a renaissance. Ann. Rev. *Plant Physiol. Plant Mol. Biol*. 52: 437-440.

Steel, R. G. and **J. H. Torrie.** 1980**.** 'Principles and Procedures of Statistics', *2nd ed, McGraw-Hill Book Company Inc.* New York, London.

Sultana, N., T. Ikeda and **R. Itoh.** 2000**.** 'Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains'. *Enviro. and Exp. Biol*. 43(2): 181-183.

Wang, Z. Q, Y. Z. Yuan, J. Q. Ou, Q. H. Lin and **C. F. Zhang.** 2007**.** 'Glutamine synthetase and glutamate dehydrogenase contribute differentially to proline accumulation in leaves of wheat (*Triticum aestivum*) seedlings exposed to different salinity'. Original Research Article. *J. of Plant Physiol*. 164, Issue 6: 695-701.

Zeid, F. A., O. M. El Shihy, A. M. Ghallab and **F. A. Ibrahim.** 2009**.** 'Effect of exogenous ascorbic acid on wheat tolerance to salinity stress conditions'. *Arab J. Biotech*. 12(1): 149-174.

Zewail, Y. M. R. 2007**.** 'Improvement of wheat productivity by using some biofertilizers and antioxdants'. *M.Sc. Thesis, Fac. Agric. Moshtohor ,Banha Univ*.

Zheng, C. D., F. Jiang, T. Liu, W. Dai, Q. Liu, Jing and **V. Cao.** 2009**.** 'Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity'. Original Research Article. *Env. and Exp. Bot*. 67: 222-227.