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# INFLUNCE OF SALINITY AND OSMOLYTES ON GROWTH, LIPID PEROXIDATION AND ELECTROLYTE LEAKAGE OF PRIMMED GERMINATING SALT-SENSITIVE AND SALT-TOLERANT BROAD BEAN SEEDS.

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## ABSTRACT

Interactive effects of (proline, ascorbic acid, reduced glutathione and salicylic acid) either alone or in combination with 50 and 300 mM NaCl on seedling growth, lipid peroxidation and electrolyte leakage of two phenotypes {salt-sensitive (cv. Giza 716) and salt-tolerant (cv. Giza 843)} of broad bean after priming were investigated. Treatment of the germinating broad bean seeds of both cultivars with 50 and 300 mM NaCl induced progressive significant decrease in fresh mass, dry mass, water content, relative growth index (RGI), relative growth rate (RGR), length of radicle and length of plumule but induced significant increase in lipid peroxidation and electrolyte leakage after priming, 7 and 14 days of germination .

On the other hand, addition of the optimum concentrations of proline (Pr), ascorbic acid (ASA), reduced glutathione (GSH) and salicylic acid (SA) either alone or in combination with 50 and 300 mM NaCl, induced significant increase in all growth parameters and showed a progressive significant decrease in electrolyte leakage determined in both cultivars after priming, 7 and 14 days of germination. Maximum significant improvement being operative with the optimum concentration of SA than the other used osmolytes. The present results are discussed in relation to applicability of the optimum concentrations of four osmolytes to two salt-sensitive and salt-tolerant broad bean seedlings.

**Keywords:** broad bean, salinity, proline (Pr), ascorbic acid (ASA), reduced glutathione (GSH), salicylic acid (SA), growth parameters, lipid peroxidation and electrolyte leakage.

## INTRODUCTION

Adverse plant responses to stress conditions depend on the osmotic and toxic effects of the stressful factors and on the level and duration of the stress (Shalata and Neumann, 2001). Responses range from germination and growth inhibition and accelerated leaf senescence under moderate stress to permanent wilting of shoots with subsequent plant death under severe stress (Yamaguchi and Blumwald, 2005). Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. Exogenous high salt concentrations affect seed germination, water deficit, cause ion imbalance resulting in ion toxicity and osmotic stress (Khan *et al.*, 2002; Khan and Panda, 2008).

Kurban *et al.* (1999) reported that total plant dry weight increased at low salinity (50 mM) but decreased at high salinity (100 and 200 mM) in *Alhagi pseudalhagi* seedling. Fresh and dry weights of *Salicornia rubra*, a halophyte, increased with the increase in salinity, with optimal growth at 200 mM NaCl, after which a decline in growth with a further increase in salinity

was reported (Khan, 2001). All growth, development and yield parameters appeared to remain unaltered, accelerated or, in most cases, suppressed with particular significance. Furthermore, in relation to water control levels, Younis *et al.* (2008) reported that administration of NaCl at low, medium or high concentration, in the growth medium of lettuce plants, induced significant decreases in growth components determined, at the vegetative and adult growth stages. Mao *et al.* (2008) show that NaCl application at 85 mM stimulates root growth of *Populus alba*. Furthermore, they confirm that the dry weight of roots and total plant dry weight in *P. alba* were stimulated by 85 mM NaCl and that net photosynthesis also increased significantly in 85 mM NaCl.

A metabolic response to salt stress is the synthesis of compatible osmolytes. These mediate osmotic adjustment and therefore protect sub-cellular structures and reduce oxidative damage caused by free radicals, produced in response to high salinity (Hare *et al.*, 1998). Osmolyte compounds include sugars, polyols, amino acids and tertiary and quarternary ammonium, and sulphonium compounds (Rhodes and Hanson, 1993).

Tolerance to abiotic stresses is very complex, due to the intricate of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Razmjoo *et al.*, 2008). Again; salinity is considered as a severe problem in agriculture since it results in a noticeable reduction in crop productivity. Lack of fresh water for irrigation, together with poor drainage of water from the cultivated soils, resulted in the accumulation of salts. In Egypt, the cultivated regions restricted to the Nile Valley, which depends on fresh water of the Nile River for irrigation, does not exceed 4 % of the total Egypt land area. Most of the newly reclaimed lands depend on underground water of various degrees of salinity for irrigation. In addition, progressive accumulation of salts became a serious problem in many cultivated areas of the Delta as a result of high ground water table, especially when accompanied by poor drainage.

In order to improve crop salt tolerance that will result into enhanced productivity on salt affected soils, different perspective strategies have been proposed by various plant scientists (Ashraf *et al.*, 2008). Of them, exogenous use of compatible organic solutes has gained a considerable ground as a shotgun approach to ameliorate the adverse effects of salt stress on plants (Ashraf and Foolad, 2007).

Pr is one of the most widely distributed osmolytes in stress conditions not only in plants but also in other organisms (Bartels and Sunkar, 2005). Pr has diverse functions, such as stabilization of subcellular structures (proteins and membranes), it functions as a hydroxyl radical scavenger and serves as a source of carbon and nitrogen (Kavi-Kishor *et al.*, 2005). In addition, Pr could act as a component of signal transduction pathways that regulate stress responsive genes (Khedr *et al.*, 2003).

One of the new plant growth regulators and osmoprotectant is SA and its derivatives like acetylsalicylic acid. They could be raised to the status of the above mentioned phytohormones because they have a significant impact on the various aspects of the plant life (Hayat and Ahmad, 2007). SA plays an important role in response to biotic and abiotic stresses. Thus, SA pre-

treatment provided protection against salinity in tomato plants, probably due to the increased activation of aldose reductase and APX enzymes and the accumulation of osmolytes, such as sugar, sugar alcohol or Pr (Szepesi *et al.*, 2005). The soaking of wheat (*Triticum aestivum* L.) seeds in 0.05 mM SA also reduced the damaging effects of salinity on seedlings growth and accelerated the growth processes (Shakirova *et al.*, 2003). Furthermore, pretreatment of barley seeds with SA caused a low level of oxidative stress, improving the oxidative capacity of the plants; SA can increase their tolerance to salt stress induced by 200 mM NaCl treatment (Cornelia and Bandicé, 2008).

Exogenous ASA has also been reported to protect plants under stress. Thus, Shaddad *et al.* (1990) found that presoaking seeds in either ascorbate or pyridoxine counteracted the adverse effects of salinity on germination and seedling growth as well as on some metabolic events of lupin and broad bean plants. Recently, Younis *et al.* (2009 and 2010) investigated the adverse effects of NaCl or mannitol on growth, nitrogen content, amino acids, protein patterns, nucleic acids and antioxidant system in *Vicia faba* seedlings. The role of exogenous ASA in increasing resistance to these assessors was also evaluated. Thus ASA appeared to ameliorate the observed damage effects induced by NaCl or mannitol; the magnitude of amelioration being a function of the type and the concentration of the stressful agent as well as the duration of treatment.

In plants, GSH and its analogs (7-glutamyl-cysteinyl-p-alanine and y-glutamylcysteineserine) are the most abundant storage and transport forms of the sulfur reduced in the chloroplasts. In addition to its nutritional role, GSH is also important for the protection of the plant. GSH, which is usually found in millimolar concentrations in a wide range of plants, is also involved in the reduction of proteins, in the destruction of $H_2O_2$ in chloroplasts, in the detoxification of xenobiotics (Martinoia *et al.*, 1993), and in protection against various stresses, such as irradiation (Meister and Anderson, 1983), heat (Nieto-Sotelo and Ho, 1986), oxidative stress (Alscher, 1989), and exposure to heavy metals (Grill *et al.*, 1985). Supply of GSH to suspension-cultured cells of bean stimulates the transcription of various defense genes, phenylalanyl ammonia-lyase, and chalcone synthase (Wingate *et al.*, 1988). These data led to the suggestion that GSH may function as a signal of redox perturbations induced by various biological stresses (Dhindsa, 1991).

From the above mentioned survey of literature, it was thought of interest to study further the possible effects of salinity and osmolytes on growth, lipid peroxidation and electrlyete leakage of primed germinating salt-sensitive and salt-tolerant broad bean seeds throughout the entire period of the experiment.

## MATERIALS AND METHODS

### Plant material

Two uniformly-sized lots of broad beans (salt-sensitive; Giza 716 and salt-tolerant; Giza 843) were selected and surface sterilized by immersion in 0.1 % $HgCl_2$ solution for 5 minutes. The sterilized seeds were thoroughly washed several times with distilled water thereafter each group of these

sterilized seeds was divided into several subgroups that were primed separately in aerated solutions of different concentrations of either NaCl or osmolyte maintained in 1/10 Hoagland solution for 24 hours at room temperature. Aeration was accomplished to avoid anaerobiosis as a complicating factor.

Each subgroup was divided into a number of sets; each of 35 seeds. These sets were allowed to germinate in plastic boxes (22 x 17 x 9 cm) furnished with Whatman No.1 chromatographic paper moistened by adding 20 $cm^3$ 1/10 Hoagland solution (for control samples) and NaCl or osmolyte each at an appropriate concentration maintained in 1/10 Hoagland nutrient solution, depending upon set of the preliminary experiments adopted. The germination boxes were incubated in the dark at 20 ± 0.1°C. During the experimental period of 14 days, when required, each box was supplied with 20 $cm^3$ of sterile distilled water or the specified NaCl or osmolyte solution.

Seeds were examined every day for germination and the percentage germination was calculated after 7 and 14 days from the start of the germination period. On these specified dates, seeds were examined for morphological appearance and for determination of length of radicle and plumule to the nearest cm using a ruler. Other growth parameters were also determined over the period of 14 days.

Since the seed coat, in general, is not being utilized in germination, the weight of the decoated seed represents the weight o f the living protein of the seed, i.e. embryo and cotyledons, in which resides the potential for growth. Thus after the desired period of germination, decoated seeds were taken, in triplicates, for determination of fresh weight before being died 80 °C in an aerated oven to constant dry weight ; water content was thus obtained by simple calculation.

It should be mentioned that the results obtained from the analyses of duplicate determinations and triplicate samples were remarkably close, thus the data presented in the corresponding tables and figures are the means of triplicate samples. The full data of the different stressed groups of germinating broad bean seeds were statistically analyzed using one-way analysis of variance (ANOVA) and comparison among means was carried out by calculating the Post Hoc L.S.D. with a significant level at * $P < 0.05$.

**Measurment of growth parameters**

The length of radicle and plumule were measured to the nearest cm. Immediately after sampling, the fresh weight of samples was recorded before being dried at 80 °C in an aerated oven to constant dry weight.

**Determination of electrolyte leakage**

To determine electrolyte leakage, fresh broad bean seedlings (100 mg) were cut into 5 mm length with a razor and placed in a test tube containing 10 $cm^3$ dist. water. The tubes were covered with plastic caps and placed in a water bath maintained at the constant temperature of 30 °C. After 2 h, initial electrical conductivity of the medium (EC1) was measured using a conductivity meter (HANNA Instrument, HI 8033). The samples were autoclaved at 120 °C for 20 min to completely kill the tissue and release all electrolytes. Samples were then cooled to 25 °C and the final electrical conductivity (EC2) was measured. The electrolyte leakage (EL) was

expressed following the formula EL= EC1 / EC2 × 100 (Dionisio-Sese and Tobita, 1998).

**Determination of lipid peroxidation**

Lipid peroxidation in broad bean seedlings was determined by estimating the malondialdehyde (MDA) content following the method of Heath and Packer (1968). One g of material was macerated in 5 $cm^3$ of 0.1 % TCA. The homogenate was centrifuged at 10,000 g for 5 min. For every 1 $cm^3$ aliquot of the supernatant, 4 $cm^3$ of 20 % TCA containing 0.5 % thiobarbituric acid (TBA) was added. The mixture was heated at 95°C for 30 min and then cooled quickly on ice bath. The resulting mixture was centrifuged at 10,000 g for 15 min and the absorbance of the supernatant was measured at 532 nm. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated by using extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$ and expressed as nmol MDA $g^{-1}$ fresh weight.

## RESULTS AND DISCUSSION

**1- Changes in growth parameters**

Except for the observed significant increase in fresh and dry mass accumulation, RGI, RGR as well as in water content, length of radicle and length of plumule of broad bean tolerant cultivars salinized with 50 mM NaCl, the other variously treatments, showed significant decrease in these growth parameters determined throughout the entire period of the experiment. The percent of decrease or increase in RGI, as an example, in response to salinity concentrations was as follows: 89.80 % for 50 mM NaCl and 81.22 % for 300 mM NaCl, after priming and 90.00 % for 50 mM NaCl and 0.0 % for 300 mM NaCl, after 7 days of germination 92.68 % for 50 mM NaCl and 0.0 % for 300 mM NaCl, after 14 days of germination, for salt-sensitive and was 103.23 % for 50 mM NaCl and 83.87 % for 300 mM NaCl for, after priming and 109.09 % for 50 mM NaCl and 90.91 % for 300 mM NaCl, after 7 days of germination and 110.42 % for 50 mM NaCl and 91.67 % for 300 mM NaCl, after 14 days of germination, for salt-tolerant, respectively (Tables 1 and 2).

Examination of tables 1 and 2, showed that treatment of germinating broad bean seeds of both cultivars with Pr, ASA, GSH and SA, as lone or in combination with the salinity levels, showed a progressive significant increase in all growth parameters as compared with control (1/10 Hoagland solution) or control salinized broad bean seeds throughout the entire period of the experiment. The following sequence of osmolytes (SA > ASA > Pr > GSH > control) showed the most pronounced concentration that caused the highest values in both cultivars as alone or in combination with salinity levels.

The percent of increase in RGI, as an example, in response to application of the optimum concentrations of SA osmolyte was as follows: 110.20 % for 0.09 mM SA, after priming and 120.00 % for 0.09 mM SA, after 7 days of germination and 134.15 % for 0.09 mM SA, after 14 days of germination, for salt-sensitive and was 111.29 % for 0.09 mM SA, after priming and 121.21 % for 0.09 mM SA, after 7 days of germination and 152.08 % for 0.09 mM SA, after 14 days of germination, for salt-tolerant,

respectively (Tables 1 and 2 ). The calculated percent of increase in RGI, in response to combination between salinity levels and the optimum concentration SA osmolyte was as follows: for salt-sensitive, 108.57 % for 50 mM NaCl + 0.09 mM SA and 92.24 % for 300 mM NaCl + 0.09 mM SA, after priming, and was 130.00 % for 50 mM NaCl + 0.09 mM SA and 100.00 % for 300 mM NaCl + 0.09 mM SA, after 7 days of germination, and 136.59 % for 50 mM NaCl + 0.09 mM SA and 102.44 % for 300 mM NaCl + 0.09 mM SA, after 14 days of germination (Table 1). For salt-tolerant, 108.87 % for 50 mM NaCl + 0.09 mM SA and 93.55 % for 300 mM NaCl + 0.09 mM SA, after priming and 130.30 % for 50 mM NaCl + 0.09 mM SA and 100.00 % for 300 mM NaCl + 0.09 mM SA, after 7 days of germination, and 139.58 % for 50 mM NaCl + 0.09 mM SA and 102.08 % for 300 mM NaCl + 0.09 mM SA, after 14 days of germination (Table 2).

In connection with the present results, Azooz *et al.* (2009) demonstrated that the dry matter production of the different organs (root, shoot & leaves) of the three maize cultivars differed in their response to salinity stress. Plants of cv. SC 129 and SC 13 tolerated salinity up to the level of 100 and 50 mM NaCl, respectively, and decreased gradually. However, cv. SC 155 displayed a highly significant reduction in dry matter of different organs at the most salinization levels as compared with the control. Generally, the growth of the salt-sensitive cultivar (SC 155) was extensively inhibited already at NaCl concentration lower than 250 mM NaCl. Moreover it was died at 250 mM NaCl. In contrast, the salt tolerant cultivars (SC 129 & SC 13) developed up to 250 mM NaCl. In the salt tolerant cultivar (SC 129) CAT activity increased sharply in relative to the control (Azooz *et al.*, 2009).

During growth, it was observed that root and shoot fresh and dry weights were increased at low salinity levels and decreased at high levels of salinity. Varma (1981) and Savvas and Lenz (2000) on eggplant has report the decreased in fresh and dry weight due to salts stress. Assimilar effect was also observed in *Zea mays* seedlings as stated by Al-Moaikal (2006). However, the increase in fresh and dry weights at low salinity levels may be due to more growth at those levels on uptake of more salts at that stage of growth (Basalah, 1991 and 2010).

Exogenous Pr was found to be very effective in detoxifying $H_2O_2$ by enhancing the activities of catalase and peroxidase in tobacco under salt stress (Hoque *et al.*, 2007). Salt stress brought about a reduction in the growth in *Pancratium maritimum* that was significantly increased by exogenous Pr. Salt stress (100 mM) reduced the biomass of both sorghum cultivars. However, exogenous application of Pr ameliorated the adverse effects of salt stress by keeping the growth, germination and pigmentation of sorghum to same level to some extent as compared to control (Nawaz *et al.*, 2010). It was concluded that exogenous Pr improves salt tolerance of plants by protecting the protein turnover machinery against stress damage and up-regulating stress protective proteins (Hoque *et al.*, 2007 ; Yazici *et al.*, 2003; Nawaz *et al.*, 2010).

**Table 1: Effects of increasing concentrations of NaCl as alone or in combination with the optimum concentrations of SA, ASA, Pr and GSH on % germination and growth parameters (fresh mass ; g seedling$^{-1}$, dry mass; g seedling$^{-1}$, water content; g seedling$^{-1}$, RGI; % of control, RGR mg g$^{-1}$ d$^{-1}$, length of radicle (cm seedling$^{-1}$) and length of plumule (cm seedling$^{-1}$) of germinating salt-sensitive broad beans after priming, 7 and 14 days.**

| Growth stages / Treatments | After priming | | | After 7 days | | | | | | After 14 days | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh mass | Dry mass | RGI | % germination | % change | Fresh mass | Dry mass | RGI | RGR | % germination | % change | Fresh mass | Dry mass | RGI | RGR |
| Control(1/10 Hoagland soln.) | 0.943 | 0.245 | 0.00 | 100 | 0.00 | 1.12 | 0.30 | 0.00 | 0.009 | 100 | 0.00 | 1.90 | 0.41 | 0.00 | 0.016 |
| 50 mM NaCl | 0.917* | 0.220* | 89.80 | 97* | -3.00 | 1.09 | 0.27 | 90.00 | 0.008 | 98* | -2.00 | 1.88* | 0.38* | 92.68 | 0.016 |
| 300 mM NaCl | 0.767* | 0.199* | 81.22 | 0.0* | -100.00 | 0.0 | 0.00 | 0.00 | -0.033* | 0.0* | -100.00 | 0.0* | 0.0* | 0.00 | 0.00* |
| 0.40 mM Pr | 0.988* | 0.260* | 106.12 | 100 | 0.00 | 1.26 | 0.33 | 110.00 | 0.012* | 100 | 0.00 | 2.22* | 0.50* | 121.95 | 0.024* |
| 50 mM NaCl+0.40 mM Pr | 0.965* | 0.254* | 103.67 | 97.44* | -2.56 | 1.19 | 0.35 | 116.67 | 0.016* | 98.86 | -1.14 | 2.07* | 0.53* | 129.27 | 0.026* |
| 300 mM NaCl+0.40 mM Pr | 0.790* | 0.220* | 89.80 | 4.22* | -95.78 | 1.05 | 0.28 | 93.33 | 0.010 | 5.25* | -94.75 | 1.80* | 0.39* | 95.12 | 0.016 |
| 4.0 mM ASA | 0.993* | 0.265* | 108.16 | 100 | 0.00 | 1.30 | 0.34 | 113.33 | 0.013* | 100 | 0.00 | 2.30* | 0.52* | 126.83 | 0.026* |
| 50 mM NaCl+ 4.0 mM AsA | 0.975* | 0.262* | 106.94 | 98.63 | -1.37 | 1.21 | 0.37 | 123.33 | 0.018* | 99.24 | -0.76 | 2.10* | 0.55* | 134.15 | 0.026* |
| 300 mM NaCl+4.0 mM AsA | 0.795* | 0.223* | 91.02 | 6.22* | -93.78 | 1.08 | 0.29 | 96.6* | 0.011* | 8.05* | -91.95 | 1.86* | 0.40 | 97.56 | 0.016 |
| GSH 1.0 mM | 0.980* | 0.253* | 103.27 | 100 | 0.00 | 1.24 | 0.32 | 106.67 | 0.011* | 100 | 0.00 | 2.18* | 0.49* | 119.51 | 0.024* |
| GSH 50 mM NaCl+1.0 mM | 0.960* | 0.250* | 102.04 | 97.23* | -2.77 | 1.17 | 0.33 | 110.00 | 0.013* | 98.15 | -1.85 | 2.05* | 0.52* | 126.83 | 0.027* |
| GSH 300 mM NaCl+1.0 mM | 0.787* | 0.218* | 88.98 | 3.11* | -96.89 | 1.03 | 0.27 | 90.00 | 0.009 | 4.86* | -95.12 | 1.76* | 0.37* | 90.24 | 0.014* |
| 0.09 mM SA | 0.999* | 0.270* | 110.20 | 100 | 0.00 | 1.33 | 0.36 | 120.00 | 0.015* | 100 | 0.00 | 2.41* | 0.55* | 134.15 | 0.027* |
| 0.09 mM SA 50 mM NaCl+ | 0.980* | 0.266* | 108.57 | 98.92 | -1.08 | 1.23 | 0.39 | 130.00 | 0.021* | 99.64 | -0.36 | 2.13* | 0.56* | 136.59 | 0.024* |
| 300 mM NaCl+ 0.09 mM SA | 0.800* | 0.226* | 92.24 | 7.55* | -92.45 | 1.11 | 0.30 | 100.00 | 0.012* | 8.15* | -91.85 | 1.91* | 0.42 | 102.44 | 0.017 |

*The mean difference is significant at the .05 level.

**Table 1: Continued**

| Growth stages / Treatments | After priming | | After 7 days | | | | | | After 14 days | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Water content | % change | Water content | % change | Length of radicle | % change | Length of plumule | % change | Water content | % change | Length of radicle | % change | Length of plumule | % change |
| Control (1/10 Hoagland soln.) | 0.698 | 0.00 | 0.820 | 0.00 | 1.31 | 0.00 | 1.20 | 0.00 | 1.49 | 0.00 | 3.92 | 0.00 | 3.56 | 0.00 |
| 50 mM NaCl | 0.697 | -0.14 | 0.820 | 0.00 | 1.20* | -8.40 | 1.12* | -6.67 | 1.50 | 0.67 | 3.70* | -5.61 | 3.48* | -2.25 |
| 300 mM NaCl | 0.567* | -18.77 | 0.00* | -100.00 | 0.00* | -100.00 | 0.00* | -100.00 | 0.0* | -100.00 | 0.0* | -100.00 | 0.0* | -100.00 |
| 0.40 mM Pr | 0.728* | 4.30 | 0.930* | 13.41 | 1.55* | 18.32 | 1.30* | 8.33 | 1.72* | 15.44 | 4.76* | 21.43 | 4.05* | 13.76 |
| 50 mM NaCl + 0.40 mM Pr | 0.711 | 1.86 | 0.840* | 2.44 | 1.25* | -4.58 | 1.15* | -4.17 | 1.54* | 3.36 | 3.85* | -1.79 | 3.48* | -2.25 |
| 300 mM NaCl + 0.40 mM Pr | 0.570* | -18.34 | 0.770* | -6.10 | 1.12* | -14.50 | 1.02* | -15.00 | 1.41* | -5.37 | 3.37* | -14.03 | 3.09* | -13.20 |
| 4.0 mM ASA | 0.728* | 4.30 | 0.960* | 17.07 | 1.58* | 20.61 | 1.32* | 10.00 | 1.78* | 19.46 | 4.78* | 21.94 | 4.28* | 20.22 |
| 50 mM NaCl + 4.0 mM AsA | 0.713* | 2.15 | 0.840* | 2.44 | 1.28* | -2.29 | 1.17* | -2.50 | 1.55* | 4.03 | 3.88* | -1.02 | 3.50* | -1.69 |
| 300 mM NaCl + 4.0 mM AsA | 0.572* | -18.05 | 0.790* | -3.66 | 1.15* | -12.21 | 1.05* | -12.50 | 1.46* | -2.01 | 3.47* | -11.48 | 3.12* | -12.36 |
| 1.0 mM GSH | 0.727* | 4.15 | 0.920* | 12.20 | 1.50* | 14.5 | 1.28* | 6.67 | 1.69* | 13.42 | 4.74* | 21.19 | 4.00* | 12.36 |
| 50 mM NaCl + 1.0 mM GSH | 0.710* | 1.72 | 0.840* | 2.44 | 1.22* | -6.87 | 1.14* | -5.00 | 1.53* | 2.68 | 3.82* | -2.55 | 3.46* | -2.81 |
| 300 mM NaCl + 1.0 mM GSH | 0.569* | -18.48 | 0.760* | -7.32 | 1.07* | -18.32 | 0.98* | -18.33 | 1.39* | -6.71 | 3.35* | -14.54 | 3.03* | -14.89 |
| 0.09 mM SA | 0.729* | 4.44 | 0.970* | 18.29 | 1.60* | 22.14 | 1.35* | 12.50 | 1.86* | 24.83 | 4.80* | 22.45 | 4.30* | 20.79 |
| 50 mM NaCl + 0.09 mM SA | 0.714* | 2.29 | 0.840* | 2.44 | 1.30* | -0.76 | 1.19 | -0.83 | 1.57* | 5.37 | 3.90* | -0.51 | 3.54* | -0.56 |
| 300 mM NaCl + 0.09 mM SA | 0.574* | -17.77 | 0.810* | -1.22 | 1.16* | -11.45 | 1.06* | -11.67 | 1.49 | 0.00 | 3.48* | -11.22 | 3.15* | -11.51 |

*The mean difference is significant at the .05 level.

**Table 2: Effects of increasing concentrations of NaCl as alone or in combination with the optimum concentrations of SA, ASA, Pr and GSH on % germination and growth parameters (fresh mass ; g seedling$^{-1}$, dry mass; g seedling$^{-1}$, water content; g seedling$^{-1}$, RGI; % of control, RGR mg g$^{-1}$ d$^{-1}$, length of radicle (cm seedling$^{-1}$) and length of plumule (cm seedling$^{-1}$) of germinating salt-tolerant broad beans after priming, 7 and 14 days .**

| Growth stages / Treatments | After priming | | | After 7 days | | | | | | After 14 days | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh mass | Dry mass | RGI | % germination | % change | Fresh mass | Dry mass | RGI | RGR | % germination | % change | Fresh mass | Dry mass | RGI | RGR |
| Control (1/10 Hoagland soln.) | 0.946 | 0.248 | 0.00 | 100.00 | 0.00 | 1.15 | 0.33 | 0.00 | 0.014 | 100 | 0.00 | 2.00 | 0.48 | 0.00 | 0.021 |
| 50 mM NaCl | 0.955* | 0.256* | 103.23 | 100.0 | 0.00 | 1.19* | 0.36* | 109.09 | 0.017* | 100.00 | 0.00 | 2.11* | 0.53* | 110.42 | 0.024* |
| 300 mM NaCl | 0.776* | 0.208* | 83.87 | 30.0 | -70.00 | 1.06* | 0.30* | 90.91 | 0.015 | 38.00 | -62.00 | 1.88* | 0.44* | 91.67 | 0.020 |
| 0.40 mM Pr | 0.995* | 0.266* | 107.26 | 100.0 | 0.00 | 1.32* | 0.39* | 118.18 | 0.021* | 100.00 | 0.00 | 2.38* | 0.60* | 125.00 | 0.030* |
| 50 mM NaCl + 0.40 mM Pr | 0.977* | 0.260* | 104.84 | 100.00 | 0.00 | 1.26* | 0.40* | 121.21 | 0.024* | 100.00 | 0.00 | 2.56* | 0.63* | 131.25 | 0.033* |
| 300 mM NaCl+ 0.40 mM Pr | 0.796* | 0.225* | 90.73 | 30.9* | -69.11 | 1.09* | 0.31* | 93.94 | 0.015 | 39.16* | -60.84 | 1.94* | 0.46* | 95.83 | 0.021 |
| 4.0 mM ASA | 0.999* | 0.270* | 108.87 | 100.0 | 0.00 | 1.35* | 0.39* | 118.18 | 0.021* | 100.00 | 0.00 | 2.51* | 0.64* | 133.33 | 0.036* |
| 50 mM NaCl + 4.0 mM AsA | 0.988* | 0.266* | 107.26 | 100.00 | 0.00 | 1.28* | 0.41* | 124.24 | 0.024* | 100.00 | 0.00 | 2.76* | 0.65* | 135.42 | 0.034* |
| 300 mM NaCl+ 4.0 mM AsA | 0.803* | 0.230* | 92.74 | 31.4* | -68.64 | 1.12* | 0.32 | 96.97 | 0.016* | 40.12* | -59.88 | 1.97* | 0.47 | 97.92 | 0.021 |
| GSH 0.01 mM | 0.988* | 0.263* | 106.05 | 100.0 | 0.00 | 1.30* | 0.37* | 112.12 | 0.020* | 100.00 | 0.00 | 2.33* | 0.58* | 120.83 | 0.030* |
| GSH 50 mM NaCl+ 0.01 mM | 0.973* | 0.259* | 104.44 | 100.00 | 0.00 | 1.25* | 0.40* | 121.21 | 0.024* | 100.00 | 0.00 | 2.49* | 0.61* | 127.08 | 0.030* |
| GSH 300 mM NaCl+ 0.01 mM | 0.792* | 0.223* | 89.92 | 30.4* | -69.56 | 1.06* | 0.30* | 90.91 | 0.013 | 38.41* | -61.59 | 1.89* | 0.44* | 91.67 | 0.020 |
| 0.09 mM SA | 1.02* | 0.276* | 111.29 | 100.0 | 0.00 | 1.38* | 0.40* | 121.21 | 0.021* | 100.00 | 0.00 | 2.63* | 0.73* | 152.08 | 0.047* |
| 0.09 mM SA 50 mM NaCl + | 0.997* | 0.270* | 108.87 | 100.00 | 0.00 | 1.30* | 0.43* | 130.30 | 0.027* | 100.00 | 0.00 | 2.77* | 0.67* | 139.58 | 0.034* |
| 300 mM NaCl + 0.09 mM SA | 0.806* | 0.232* | 93.55 | 32.2* | -67.78 | 1.14 | 0.33 | 100.00 | 0.017* | 40.34* | -59.66 | 2.02* | 0.49 | 102.08 | 0.023* |

* The mean difference is significant at the .05 level.

**Table 2 : Continued**

| Growth stages / Treatments | After priming | | After 7 days | | | | | | After 14 days | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Water content | % change | Water content | % change | Length of radicle | % change | Length of plumule | % change | Water content | % change | Length of radicle | % change | Length of plumule | % change |
| Control (1/10 Hoagland soln.) | 0.698 | 0.00 | 0.820 | 0.00 | 1.59 | 0.00 | 1.22 | 0.00 | 1.52 | 0.00 | 4.30 | 0.00 | 4.19 | 0.00 |
| 50 mM NaCl | 0.699 | 0.14 | 0.830* | 1.22 | 1.65* | 4.40 | 1.26* | 3.27 | 1.58* | 3.95 | 4.50* | 4.65 | 4.33* | 3.34 |
| 300 mM NaCl | 0.568* | -18.62 | 0.760* | -7.32 | 1.14* | -30.20 | 1.16* | -4.92 | 1.44* | -5.26 | 3.16* | -26.51 | 4.00* | -4.53 |
| 0.40 mM Pr | 0.729* | 4.44 | 0.930* | 13.41 | 2.18* | 37.11 | 1.56* | 27.87 | 1.78* | 17.11 | 5.90* | 37.21 | 5.46* | 30.31 |
| 50 mM NaCl + 0.40 mM Pr | 0.717* | 2.72 | 0.860* | 4.88 | 1.85* | 16.35 | 1.40* | 14.75 | 1.95* | 28.29 | 5.05* | 17.44 | 5.00* | 19.33 |
| 300 mM NaCl + 0.40 mM Pr | 0.571* | -18.19 | 0.780* | -4.88 | 1.47* | -7.55 | 1.18* | -3.28 | 1.48* | -2.63 | 4.11* | -4.42 | 4.12* | -1.67 |
| 4.0 mM ASA | 0.729* | 4.44 | 0.960* | 17.07 | 2.19* | 37.74 | 1.57* | 28.69 | 1.87* | 23.03 | 5.97* | 38.84 | 5.49* | 31.03 |
| 50 mM NaCl + 4.0 mM AsA | 0.722* | 3.44 | 0.870* | 6.10 | 1.90* | 19.50 | 1.44* | 18.03 | 2.11* | 38.82 | 5.15* | 19.77 | 5.11* | 21.96 |
| 300 mM NaCl + 4.0 mM AsA | 0.573* | -17.91 | 0.800* | -2.44 | 1.49* | -6.29 | 1.19* | -2.46 | 1.50* | -1.32 | 4.15* | -3.49 | 4.16* | -0.72 |
| 0.01 mM GSH | 0.725* | 3.87 | 0.930* | 13.41 | 2.16* | 35.85 | 1.56* | 27.87 | 1.75* | 15.13 | 5.88* | 36.74 | 5.44* | 29.83 |
| 50 mM NaCl + 0.01 mM GSH | 0.714* | 2.29 | 0.850* | 3.66 | 1.83* | 15.09 | 1.39* | 13.93 | 1.88* | 23.68 | 4.96* | 15.35 | 4.89* | 16.71 |
| 300 mM NaCl + 0.01 mM GSH | 0.569* | -18.48 | 0.760* | -7.32 | 1.41* | -11.32 | 1.17* | -4.09 | 1.45* | -4.61 | 4.08* | -5.12 | 4.10* | -2.15 |
| 0.09 mM SA | 0.744* | 6.59 | 0.980* | 19.51 | 2.21* | 38.99 | 1.59* | 30.33 | 1.90* | 25.00 | 6.00* | 39.53 | 5.51* | 31.50 |
| 50 mM NaCl + 0.09 mM SA | 0.727* | 4.15 | 0.870* | 6.10 | 1.95* | 22.64 | 1.46* | 19.67 | 2.10* | 38.16 | 5.29* | 23.02 | 5.22* | 24.58 |
| 300 mM NaCl + 0.09 mM SA | 0.574* | -17.77 | 0.810* | -1.22 | 1.50* | -5.66 | 1.20* | -1.64 | 1.53 | 0.65 | 4.18* | -2.79 | 4.17* | -0.48 |

*The mean difference is significant at the .05 level.

Azooz (2009) stated that the differential responses of two faba bean (*Vicia faba* L.) local Egyptian genotypes to salinity (0 or 140 mM NaCl) and seed priming with 0.2 mM SA were studied. Salinity caused no significant changes in dry weight and tissue water content of genotype 115, whereas they were significantly reduced in genotype 125. Application of SA not only mitigated the inhibitory effect of salt stress in both genotypes, but also in some cases induced a stimulatory effect greater than that estimated in the control plants. The results indicated that both faba bean genotypes can develop different mechanisms of adaptation to salt stress. The beneficial effect of SA could be used for improving their salt tolerance (Azooz, 2009).

Although salinity stress reduced the growth parameters of the two faba bean (*Vicia faba* L.) genotypes, there were major differences in their reduction. The genotype 115 seems to be the salt-tolerant and genotype 125 is the salt-sensitive. This was judged with the ability of genotype 115 to enhance its tissue water contents, whereas the opposite was appeared in genotype 125. Accordingly, plant salt tolerance is determined by genotypes and biochemical pathways that facilitate retention of water and synthesis of osmotically active metabolites (Azooz, 2004; Sarwat and El-Sherif, 2007). Differences due to salinity stress could be also, observed through variations in the criteria of osmotic solutes (soluble carbohydrate, protein & total free amino acids).

Khan *et al.* (2006) observed that salt stress caused a reduction in the growth of both wheat cultivars at the seedling stage. However, exogenously applied ASA as a foliar spray promoted the growth of both wheat cultivars under non-saline conditions. This growth promoting effect of ASA was more pronounced in S-24. These findings can be related to some earlier studies in which it has been observed that exogenous application of ASA promoted growth in wheat (Hamada and Al-Hakimi, 2001; Al-Hakimi, 2001), and tomato (Shalata and Neumann, 2001). ASA -induced increase in growth under non-saline may have been due to accelerated cell division and/or cell enlargement (Arrigoni, 1994).

El-shahawy *et al.* (2007) reported that Marjoram growth and development was seriously affected (up to 63% growth induction) at all levels of GSH application. The effect was more pronounced on increasing the elementary components of total sugar, total nitrogen and oil yield (up to 91%); irrespective of the rate and sort of application. The *in vitro* study of the allelopathic influence of marjoram oil extracts against seed germination (%) and seedling root and shoot length of certain broad and narrow-leaved weeds (e.g., *Amaranthus cruentus* and *Echinochloa crus-galli*) revealed of more superiority of the treated plants rather than the untreated one. It has been suggested that applying GSH might have a direct and/or indirect impact on increasing the allelopathic capacity of marjoram plants by increasing their content of the terpene and phenolic substances, which could be the key factor of the allelopathic influence of marjoram plants in their vicinity (see tables 1 and 2).

**2- Changes in lipid peroxidation.**

Perusal of the data presented in tables 3 and 4 revealed the following main points:

a- In relation to control (1/10 Hoagland solution), lipid peroxidation (MDA content) in both salt-sensitive and salt- tolerant broad bean seedlings salinized with increasing concentrations of NaCl, showed a progressive significant increase after priming, 7 and 14 days of germination and it was most pronounced with the highest salinity (300 mM NaCl) concentration in salt-sensitive cultivar than salt-tolerant one (Tables 3 and 4). MDA content in salt-tolerant cultivar appeared less affected by salinity concentrations than salt-sensitive one. The calculated percent of increase was as follows: 12.43 % for 50 mM NaCl and 32.67 % for 300 mM NaCl, after priming and 13.44 % for 50 mM NaCl and -100.00 % for 300 mM NaCl, after 7 days of germination and 14.46 % for 50 mM NaCl and -100 % for 300 mM NaCl, after 14 days of germination, for salt-sensitive and was 7.34 % for 50 mM NaCl and 27.24 % for 300 mM NaCl, after priming and 12.57 % for 50 mM NaCl and 29.37 % for 300 mM NaCl, after 7 days of germination and 13.61 % for 50 mM NaCl and 40.07 % for 300 mM NaCl, after 14 days of germination, for salt-tolerant, respectively (Tables 3 and 4).

b- For treatment of germinating broad bean seeds of both cultivars with the optimum concentrations of Pr, ASA, GSH and SA as alone or in combination with salinity levels, showed a progressive significant decrease in MDA content as compared with control (1/10 Hoagland solution) or control salinized broad bean seeds throughout the entire period of the experiment, which being more significant decrease with the optimum concentration of SA as alone or with 50 mM NaCl in salt-tolerant cultivar than salt-sensitive one . The following sequence of osmolytes (SA > ASA > Pr > GSH > control) showed the most pronounced concentration that caused the lowest values in both cultivars as alone or in combination with salinity levels. From tables 3 and 4 showed that MDA content in salt-tolerant cultivar treated with the optimum concentrations of osmolytes appeared less than that of salt-sensitive one. The percent of decrease in MDA content in response to application of the optimum concentration of SA, was as follows: -5.25 % for 0.09 mM SA, after priming and -2.26 % for 0.09 mM SA, after 7 days of germination and -1.16 % for 0.09 mM SA, after 14 days of germination, for salt-sensitive and was -5.01 % for 0.09 mM SA, after priming and -1.70 % for 0.09 mM SA, after 7 days of germination and -0.72 % for 0.09 mM SA, after 14 days of germination, for salt-tolerant, respectively (Tables 3 and 4).

c- Percent of decrease in MDA content in response to combination between salinity concentrations and the optimum concentration of SA, was as follows: for salt-sensitive, 10.42 % for 50 mM NaCl + 0.09 mM SA and 22.38 % for 300 mM NaCl + 0.09 mM SA, after priming, and 11.39 % for 50 mM NaCl + 0.09 mM SA and 23.21 % for 300 mM NaCl + 0.09 mM SA, after 7 days of germination, and 20.20 % for 50 mM NaCl + 0.09 mM SA and 23.82 % for 300 mM NaCl + 0.09 mM SA, after 14 days of germination (Table 3).

**Table 3: Effects of increasing concentrations of NaCl as alone or in combination with the optimum concentrations of Pr, ASA, GSH and SA osmoregulators on lipid peroxidation (mmole MDA / 100 g fresh mass) and electrolyte leakage (% ion leakage) of germinating salt-sensitive broad beans after priming, 7 and 14 days .**

| Growth stages / Treatments | After priming | | | | After 7 days | | | | After 14 days | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lipid peroxidation | % change | Electrolyte leakage | % change | Lipid peroxidation | % change | Electrolyte leakage | % change | Lipid peroxidation | % change | Electrolyte leakage | % change |
| Control (1/10 Hoagland soln.) | 40.22 | 0.000 | 50.72 | 0.000 | 48.21 | 0.00 | 60.3 | 0.00 | 52.61 | 0.00 | 66.14 | 0.00 |
| 50 mM NaCl | 45.22* | 12.43 | 54.51* | 7.47 | 54.69* | 13.44 | 70.46* | 16.85 | 60.22* | 14.46 | 80.22* | 21.29 |
| 300 mM NaCl | 53.36* | 32.67 | 60.26* | 18.81 | 0.0* | -100.00 | 0.0* | -100.00 | 0.0* | -100.00 | 0.0* | -100.00 |
| 0.40 mM Pr | 39.29* | -2.31 | 49.55* | -2.31 | 47.69* | -1.07 | 59.50* | -1.32 | 52.09* | -0.99 | 65.62* | -0.79 |
| 50 mM NaCl + 0.40 mM Pr | 45.12* | 12.18 | 54.05* | 6.57 | 55.41* | 14.93 | 64.42* | 6.83 | 66.56* | 26.51 | 73.77* | 11.54 |
| 300 mM NaCl + 0.40 mM Pr | 50.44* | 25.41 | 58.44* | 15.22 | 60.51* | 25.51 | 70.00* | 16.08 | 67.22* | 27.77 | 77.20* | 16.72 |
| 4.0 mM ASA | 38.89* | -3.31 | 49.12* | -3.15 | 47.66* | -1.14 | 59.46* | -1.39 | 52.04* | -1.08 | 65.51* | -0.95 |
| 50 mM NaCl + 4.0 mM AsA | 44.59* | 10.87 | 53.66* | 5.80 | 53.94* | 11.88 | 64.30* | 6.63 | 64.90* | 23.36 | 73.62* | 11.31 |
| 300 mM NaCl + 4.0 mM AsA | 50.13* | 24.64 | 57.26* | 12.89 | 60.11* | 24.68 | 69.35* | 15.01 | 65.70* | 24.88 | 77.11* | 16.59 |
| 1.0 mM GSH | 39.64* | -1.44 | 49.72* | -1.97 | 47.70* | -1.06 | 59.00* | -1.16 | 52.12* | -0.93 | 66.00* | -0.21 |
| 50 mM NaCl + 1.0 mM GSH | 45.19* | 12.36 | 54.21* | 6.88 | 56.30* | 16.78 | 65.14* | 8.03 | 66.67* | 26.72 | 74.21* | 12.20 |
| 300 mM NaCl + 1.0 mM GSH | 52.22* | 29.84 | 59.60* | 17.51 | 63.44* | 31.59 | 71.06* | 17.88 | 69.60* | 32.29 | 78.03* | 17.97 |
| 0.09 mM SA | 38.11* | -5.25 | 49.02* | -3.35 | 47.12* | -2.26 | 59.26* | -1.72 | 52.00* | -1.16 | 65.30* | -1.27 |
| 50 mM NaCl + 0.09 mM SA | 44.41* | 10.42 | 53.14* | 4.77 | 53.70* | 11.39 | 63.64* | 5.53 | 63.24* | 20.20 | 72.55* | 9.69 |
| 300 mM NaCl + 0.09 mM SA | 49.22* | 22.38 | 56.92* | 12.22 | 59.40* | 23.21 | 68.62* | 13.80 | 65.14* | 23.82 | 76.00* | 14.91 |

*The mean difference is significant at the .05 level.



**Table 4: Effects of increasing concentrations of NaCl as alone or in combination with the optimum concentrations of Pr, ASA, GSH and SA osmoregulators on lipid peroxidation (mmole MDA / 100 g fresh mass) and electrolyte leakage (% ion leakage) of germinating salt-tolerant broad beans after priming, 7 and 14 days.**

| Growth stages / Treatments | After priming | | | | After 7 days | | | | After 14 days | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lipid peroxidation | % change | Electrolyte leakage | % change | Lipid peroxidation | % change | Electrolyte leakage | % change | Lipid peroxidation | % change | Electrolyte leakage | % change |
| Control (1/10 Hoagland soln.) | 32.16 | 0.000 | 38.23 | 0.000 | 38.81 | 0.000 | 42.31 | 0.000 | 41.60 | 0.00 | 45.03 | 0.00 |
| 50 mM NaCl | 34.52* | 7.34 | 40.14* | 5.00 | 43.69* | 12.57 | 48.32* | 14.21 | 47.26* | 13.61 | 52.26* | 16.06 |
| 300 mM NaCl | 40.92* | 27.24 | 43.22* | 13.05 | 50.21* | 29.37 | 58.41* | 38.05 | 58.27* | 40.07 | 62.56* | 38.92 |
| 0.40 mM Pr | 31.66* | -1.55 | 37.44* | -2.07 | 38.53* | -0.72 | 41.95* | -0.85 | 41.48* | -0.29 | 44.92* | -0.29 |
| 50 mM NaCl + 0.40 mM Pr | 34.20* | 6.34 | 39.63* | 3.66 | 43.26* | 11.47 | 43.90* | 3.76 | 49.63* | 19.30 | 49.22* | 9.30 |
| 300 mM NaCl + 0.40 mM Pr | 40.21* | 25.03 | 42.66* | 11.59 | 48.66* | 25.38 | 48.44* | 14.49 | 52.20* | 25.48 | 52.11* | 15.72 |
| 4.0 mM ASA | 31.14* | -3.17 | 37.22* | -2.64 | 38.40* | -1.06 | 41.92* | -0.92 | 41.36* | -0.58 | 44.82* | -0.47 |
| 50 mM NaCl + 4.0 mM AsA | 33.58* | 4.42 | 39.55* | 3.45 | 43.15* | 11.18 | 43.88* | 3.71 | 48.86* | 17.45 | 48.66* | 8.06 |
| 300 mM NaCl + 4.0 mM AsA | 39.55* | 22.98 | 42.36* | 10.86 | 48.00* | 23.68 | 47.41* | 12.05 | 51.60* | 24.04 | 51.22* | 13.74 |
| 0.01 mM GSH | 31.70* | -1.43 | 37.80* | -1.12 | 38.60* | -0.54 | 41.99* | -0.76 | 41.51* | -0.23 | 44.96* | -0.16 |
| 50 mM NaCl + 0.01 mM GSH | 34.33* | 6.75 | 40.00* | 4.63 | 43.56* | 12.24 | 44.85* | 6.00 | 50.11* | 20.46 | 50.50* | 12.15 |
| 300 mM NaCl + 0.01 mM GSH | 40.45* | 25.78 | 43.02* | 12.53 | 48.85* | 25.87 | 48.08* | 13.64 | 52.39* | 25.94 | 52.40* | 16.37 |
| 0.09 mM SA | 30.55* | -5.01 | 37.00* | -3.22 | 38.15* | -1.70 | 41.80* | -1.21 | 41.30* | -0.72 | 44.63* | -0.89 |
| 50 mM NaCl + 0.09 mM SA | 33.42* | 3.92 | 39.24* | 2.64 | 42.22* | 8.79 | 43.60* | 3.05 | 45.72* | 9.90 | 48.02* | 6.64 |
| 300 mM NaCl + 0.09 mM SA | 39.16* | 21.77 | 41.15* | 7.64 | 47.36* | 22.03 | 47.32* | 11.84 | 51.30* | 23.32 | 51.14* | 13.57 |

*The mean difference is significant at the .05 level.

For salt-tolerant, 3.92 % for 50 mM NaCl + 0.09 mM SA and 21.77 % for 300 mM NaCl + 0.09 mM SA, after priming, and 8.79 % for 50 mM NaCl + 0.09 mM SA and 22.03 % for 300 mM NaCl + 0.09 mM SA, after 7 days of germination, and 9.90 % for 50 mM NaCl + 0.09 mM SA and 23.32 % for 300 mM NaCl + 0.09 mM SA, after 14 days of germination (Table 4).

In support of the present results, Hanaa *et al.* (2003) experimenting with three onion cultivars salinized with 2000, 4000 and 6000 ppm salts prepared from seawater they found that the salt stress increased the rates of lipid peroxidation ( as indicated by increasing the malonaldhyde (MDA) and their effect was increased gradually by increasing salts levels. The increasing values in MDA contents in salt stress plants treated at 2000, 4000 and 6000 ppm salts levels were 121.9, 248.6 and 290.3 % in Behary Red cv. and 126.4, 260.0 and 396.2% in Giza 20 cv., respectively of the control level (100%).

A tentative explanation of the present results was that salt damage to plants has been attributed to a combination of several factors, primarily the osmotic stress and accumulation of toxic ions. Water deficit caused by osmotic stress leads to stomata closure, thus compromising the CO2 supply for photosynthesis, and exposes chloroplasts to excess excitation energy (Tanaka *et al.* 1990). Prolonged (photo-) oxidative stress generates active oxygen species to unbalance the redox systems in favor of oxidized forms, inactivate enzymes, cause lipid peroxidation and potentially damage DNA (Scandalios, 1993). The peroxidation of lipid membranes in vivo further ensures a steady supply of free radicals. The peroxidation of lipid membranes is both a reflection and a measure of stress-induced damage at the cellular level. MDA content of the groundnut cell lines exposed to different levels of salinity stress, which represents indices of the extent of peroxidative damage, is documented (Jain *et al.*, 2001).

In order to have an insight into the ameliorative effects of Pr on salt stress-induced lipid peroxidation, we supplemented the amino acid exogenously to the salt-amended media (tables 3 and 4). Pr was able to alleviate the growth inhibitory effects of salt stress as seen by the fresh weight accumulation pattern of the salt-sensitive cell line (Jain et al., 2001). In the presence of 50 and 100 mM Pr, the salt concentration required to inhibit fresh weight increase by 50% (LD50) increased to 50 and 70 mM NaCl, respectively, in contrast to the 30 mM required for the controls (1.6- and 2.3-fold increase, respectively). Further, under similar conditions of salinity stress, the extent of lipid peroxidation, as reflected by the MDA content, was significantly reduced (Jain *et al.*, 2001). In the presence of 0.40 mM Pr in the growth medium the salt stress induced lipid peroxidation was reduced by 12.18 % for 50 mM NaCl + 0.40 mM Pr and 25.41 % for 300 mM NaCl + 0.40 mM Pr, after priming, and 14.93 % for 50 mM NaCl + 0.40 mM Pr and 25.51 % for 300 mM NaCl + 0.40 mM Pr, after 7 days of germination, and 26.51 % for 50 mM NaCl + 0.40 mM Pr and 27.77 % for 300 mM NaCl + 0.40 mM Pr, after 14 days of germination for salt-sensitive. For salt-tolerant, 6.34 % for 50 mM NaCl + 0.40 mM Pr and 25.03 % for 300 mM NaCl + 0.40 mM Pr, after priming, and 11.47 % for 50 mM NaCl + 0.40 mM Pr and 25.38 % for 300 mM NaCl + 0.40 mM Pr, after 7 days of germination, and 19.30 %

for 50 mM NaCl + 0.40 mM Pr and 25.48 % for 300 mM NaCl + 0.40 mM Pr, after 14 days of germination relative to the controls .

In addition to containing free radical damage, it has been suggested that the action of Pr involves effects on the hydration layer surrounding the phospholipids and possibly also its intercalation between phospholipid head groups (Rudolph *et al.* 1986). A synthesis of the circumstantial evidence obtained in this study (see tables 3 and 4) indicates a possible positive correlation between the ability to accumulate Pr and salinity tolerance. The salt stress-induced free radical damage to the lipid membranes could be effectively contained by Pr, thus providing evidence for the adaptive significance of Pr accumulation under stress conditions in addition to its role of a compatible osmolyte.

**2- Changes in electrolyte leakage.**

The changes in electrolyte leakage (% ion leakage) in salt-sensitive and salt-tolerant broad bean seedlings variously treated with increasing concentrations of NaCl as alone or in combination with the optimum concentrations of Pr, ASA, GSH and SA after priming, 7 and 14 days of germination are presented in tables 3 and 4.

a- There was a progressive significant increase in electrolyte leakage in both cultivars with increased salinity levels as compared with control (1/10 Hoagland solution), after priming, 7 and 14 days of germination (Tables 3 and 4). The percent of increase was most pronounced in 300 mM NaCl than 50 mM NaCl in salt-sensitive cultivar than salt-tolerant one. The percent of decrease in electrolyte leakage as affected by salinity concentrations, was as follows: 7.47 % for 50 mM NaCl and 18.81 % for 300 mM NaCl, after priming and 16.85 % for 50 mM NaCl and -100.00 % for 300 mM NaCl, after 7 days of germination 21.29 % for 50 mM NaCl and -100 % for 300 mM NaCl, after 14 days of germination, for salt-sensitive and was 5.00 % for 50 mM NaCl and 13.05 % for 300 mM NaCl, after priming and 14.21 % for 50 mM NaCl and 38.05 % for 300 mM NaCl, after 7 days of germination and 16.06 % for 50 mM NaCl and 38.92 % for 300 mM NaCl, after 14 days of germination, for salt-tolerant, respectively (Tables 3 and 4).

b- Treatment of both cultivars with the optimum concentrations of Pr, ASA, GSH and SA as alone or incombination with salinity levels, showed a significant decrease in electrolyte leakage as compared with control levels after priming, 7 and 14 days of germination. The following sequence of osmolytes (SA > ASA > Pr > GSH > control) showed the most pronounced concentration that caused the lowest values in both cultivars as alone or in combination with salinity levels. The percent of decrease was most pronounced with SA optimum concentration than the other osmolytes concentrations in salt-tolerant than salt-sensitive cultivar and it was as follows: -3.35 % for 0.09 mM SA, after priming and -1.72 % for 0.09 mM SA, after 7 days of germination and -1.27 % for 0.09 mM SA, after 14 days of germination, for salt-sensitive and was -3.22 % for 0.09 mM SA, after priming and -1.21 % for 0.09 mM SA, after 7 days of germination and -0.89 % for 0.09 mM SA, after 14 days of germination, for salt-tolerant, respectively (Tables 3 and 4 and Figs. 5, 6, 7 and 8).

c- The calculated percent decrease showed that combination of salinity with the optimum concentration of SA was most significant than the other osmolytes, as the following sequence: for salt-sensitive, 4.77 % for 50 mM NaCl + 0.09 mM SA and 12.22 % for 300 mM NaCl + 0.09 mM SA, after priming, and was 5.53 % for 50 mM NaCl + 0.09 mM SA and 13.80 % for 300 mM NaCl + 0.09 mM SA, after 7 days of germination, and 9.69 % for 50 mM NaCl + 0.09 mM SA and 14.91 % for 300 mM

NaCl + 0.09 mM SA, after 14 days of germination (Table 3). For salt-tolerant, 2.64 % for 50 mM NaCl + 0.09 mM SA and 7.64 % for 300 mM NaCl + 0.09 mM SA, after priming, and 3.05 % for 50 mM NaCl + 0.09 mM SA and 11.84 % for 300 mM NaCl + 0.09 mM SA, after 7 days of germination, and 6.64 % for 50 mM NaCl + 0.09 mM SA and 13.57 % for 300 mM NaCl + 0.09 mM SA, after 14 days of germination (Table 4).

In this connection ion leakage as percentage of electrical conductivity (EC%), MDA as lipid peroxidation and endogenous ascorbic acid as antioxidant in this work can asses the tolerance capacity of both genotypes to membrane damage induced by salinity stress. The lower of ion leakage (EC%) and lipid peroxidation (MDA) level in genotype 115 than in genotype 125, supported its salt tolerance and indicated that MDA might play important role in salt tolerance (Azooz, 2009). These results are confirmed by other investigators (Jaleel *et al.*, 2007; Khan and Panda, 2008; Azooz *et al.*, 2009).

SA treatment reduced the amount of MDA and ion leakage in treated plants as reported by Yildirim *et al.* (2008). The present results concerning electrolyte leakage (tables 3 and 4), showed that reduction of ion leakage might be related to the inductive responses of antioxidant enzymes that protect the plant from oxidative damage. Of specially interest, salt tolerant broad bean seeds treated with SA + 300 mM NaCl, which had the higher activity of antioxidant activity, which had the lower reduction in MDA content and ion leakage Thus, it can be concluded that the observed increase in dry weight of salt stressed faba bean genotypes in response to SA may be related to the induction of antioxidant response and protective role of membranes that increase the tolerance of plant to damage (Gunes *et al.*, 2007).

The GSH has a multifarious crucial positions attached in total with enhancing growth and development of growing plants. These include managing roots uptake for nutrients (i.e., sulphur), controlling cellular heavy metal concentration, managing protein synthesis and regulating cell division process (Lappartient and Touraine, 1996; Sanchez-Fernandez *et al.*, 1997). Talaat and Aziz (2005) referred that increasing the vegetative growth as well as oil productivity are two of the most distinguish features of applying GSH in aromatic plants e.g., Matricaria species. Chen *et al.* (2003) added that the role of GSH in protecting cells against toxic effects of free radicals is to keep the free radical scavenger ASA in its reduced form and hence active form (see tables 3 and 4).

In conclusion it has been demonstrated that both osmotic and ionic effects involved in NaCl salinity can limit photosynthesis and respiration leading to an increase in ROS generation, which are responsible for a secondary oxidative stress that can damage cellular structure and

metabolism. It is also known that plant responses to salt stress are multigenic, involving both osmotic and ionic homeostasis, as well as cell detoxification (Ahmed *et al.*, 2008). The efficiency of the latter process is dependent upon the plant antioxidant defense mechanisms. The scavenging system forms the primary defense line in protecting the broad bean cultivars against superoxide radicals (see tables 1, 2, 3 and 4).

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## تأثير الملوحة و المواد الاسموزية علي نمو ، المسار فوق التاكسدي للدهون و تسرب الالكتروليتات في بذور الفول المنقوعه لصنفي الفول المقاوم و الحساس للملوحة.

**محمود الباز يونس، محمد نجيب عبد الغني حسنين و شيماء محمد ناجي تركي**
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تم في هذا البحث إجراء تجربة لدراسة تاثير المواد الاسموزية و تشمل حمض البرولين، حمض الاسكوربيك، الجلوتاثيون و حمض السلسيليك علي نمو بادرات صنفي الفول البلدي المقاوم و الحساس للملوحة و ذلك عن طريق نقع البذور في المحاليل الاسموزية لمدة ٢٤ ساعة ثم إستكمال الانبات و نمو البادرات لمدة ١٤ يوم تحت ظروف الاجهاد الملحي.

أظهرت نتائج التجربة أن إنبات البذور في محلول الطعام بتركيزي ٥٠ و ٣٠٠ جزئ جرامي أدي إلي حدوث نقص معنوي في جميع دلالات النمو للبادرات فيما عدا إنبات البادرات للصنف المقاوم للملوحة عند تركيز ٥٠ جزئ جرامي و الذي أظهر زيادة معنوية في دلالات النمو و عند إضافة المواد الاسموزية إلي الاوساط الغذائية الملحية للبادرات أظهرت تحسن ملحوظ و زيادة معنوية في دلالات النمو و كان ذلك واضحا في الصنف المقاوم عن الصنف الحساس و الذي لم يعطي اي دلالات للنمو خصوصا عند تركيز ٣٠٠ جزئ جرامي من ملح كلوريد الصوديوم.

أدت معامله البذور النابته لصنفي الفول المقاوم و الحساس بملح كلوريد الصوديوم إلي زيادة معنوية في المسار فوق التاكسدي للدهون و كذلك تسرب الالكتروليتات من الخلايا و كانت الزيادة واضحة عند تركيز ٣٠٠ جزء جرامي من ملح كلوريد الصوديوم في الصنفين-إذا ما قورنت بمثيلاتها في الاوساط غير الملحية،و عند إضافة المواد الأسموزية إلي الاوساط المالحة لبذور الفول النابتة بصنفيها أدت إلي خفض مستوي المسار فوق التأكسدي للدهون في الخلايا النابتة و كذلك خفض معدل تسرب الالكتروليتات من الخلايا – عند مقارنتها بالبذور النابته في المحلول المغذي او المواد الاسموزية فقط.

و لقد تم تفسير النتائج المتحصل عليها في ضوء الميكانيكيات المختلفة للملوحة و أثر ذلك علي تكوين المواد الاسموزية سواء المسببة لحدوث الاتزان الاسموزي او الحالية للاسموزية و المقاومة لغنبعاث الشقوق الحرة نتيجة للاجهاد الملحي.

**قام بتحكيم البحث**

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