INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
09–039/CAJ/2009/11–4–343–350
http://www.fspublishers.org

Full Length Article

Salt Stress Mitigation by Seed Priming with Salicylic Acid in Two Faba Bean Genotypes Differing in Salt Tolerance

M.M. AZOOZ[1]
Department of Botany, Faculty of Science, South Valley University, 83523 Qena, Egypt
1Corresponding author's e-mail: azzozm@yahoo.com

ABSTRACT

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 salicylic acid (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. Genotype 115 exhibited higher accumulation of osmotic solutes, carotenoids and antioxidant enzymes activity (CAT, POD, APX & GR) than genotype 125. In contrast, ion leakage and lipid peroxidation were lower in genotype 115 than in genotype 125. Salinity induced high selectivity of K^+/Na^+ ratio in genotype 115 than genotype 125 and in shoot than in root for both genotypes. 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.

Key Words: Antioxidant enzymes; Ion leakage; Lipid peroxidation; K^+/Na^+ ratio; Osmotic solutes; Photosynthetic pigments

INTRODUCTION

Salt tolerance in plants is a complex trait, which varies widely among closely related species and between different varieties (Sreenivasulu *et al.*, 2000; Ashraf, 2002). Differences between closely related plants are particularly interesting to identify a small number of factors responsible for salt tolerance (Gehlot *et al.*, 2005). Salinity stress has been studied in relation to regulatory mechanisms of osmotic and ionic homeostasis (Ashraf & Harris, 2004). The response of plants to a salinity stress may vary with the genotype; nevertheless some general reactions occur in all genotypes. Salinity can affect plant physiological processes resulting in reduced growth and yield (Yamaguchi & Blumwald, 2005). Increased tolerance to salinity stress in crop plants is necessary in order to increase productivity with limited water supplies and high salinity. Tolerant genotypes respond to salinity stress with complex changes in their physiological and molecular status (Morsy *et al.*, 2007). During the course of salinity stress, active solute accumulation of osmotic solutes such as soluble carbohydrates, proteins and free amino acids is claimed to be an effective stress tolerance mechanism. The adaptability of plant species to high salt concentrations in soil by lowering tissue osmotic potential was accompanied by accumulation of these osmotic solutes (Zhu, 2002; Jaleel *et al*., 2008). Studies of Heikal *et al*. (2000), Ismail and Azooz (2002) revealed that salt tolerance of *Vicia faba* L. was correlated with higher accumulation of ionic and osmotic solutes in salt-tolerant than that of salt-sensitive plants.

Differences in the accumulation patterns of Na^+ and K^+ were found under salinity stress. The salt tolerant plants maintained a high K^+ content and higher K^+/Na^+ ratio compared with the salt sensitivity plants (Azooz *et al*., 2004; Rejili *et al.*, 2007). High K^+/Na^+ ratio is more important for many species than simply maintaining a low concentration of Na^+ (Cuin *et al.*, 2003).

Salinity stress is known to trigger oxidative stress in plant tissues through the increase in reactive oxygen species (Apel & Hirt, 2004). Chloroplasts are the major organelles producing the reactive oxygen species (ROS) such as, the superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and singlet oxygen (O^-_1) during photosynthesis (Asada, 1992). Salt stress induces a significant reduction in photosynthesis. This reduction depends on photosynthesizing tissue (leaf area) and photosynthetic pigments (Dubey, 2005; Raza *et al*., 2006). The production of ROS can be particularly high, when plants are exposed to salinity stress (Athar *et al*., 2008; Ashraf, 2009). ROS cause chlorophyll degradation and membrane lipid peroxidation. So, malondialdehyde (MDA) accumulation as product of lipid peroxidation and chlorophyll retention are two oxidative stress indicators that are tested tools for determining salt tolerance in plants (Yildirim *et al.*, 2008). To scavenge ROS, plants posses specific mechanisms, which include activation of antioxidant enzymes (Jaleel, *et al*., 2006) and non-

To cite this paper: Azooz, M.M., 2009. Salt stress mitigation by seed priming with salicylic acid in two faba bean genotypes differing in salt tolerance. *Int. J. Agric. Biol.*, 11: 343–350

enzymatic antioxidants such as, carotenoids and ascorbic acid (Mittler, 2002).

Salt tolerance has been found to be positively associated with a more efficient antioxidant system (Mittler, 2002; Noreen & Ashraf, 2008). A correlation between the antioxidant enzyme activities and salinity tolerance was demonstrated by comparison of tolerant cultivar with sensitive cultivar in several plant cultivars. These activities were reported to increase under salinity stress and closely related to salt tolerance of many plants (Azevedo Neto *et al.*, 2006; Athar *et al.*, 2008).

Faba bean is an important legume crop as a major source of protein and occupies large area of cultivated land in Egypt. Cultivation of faba bean leads to increase of soil nitrogenous compounds (Hungria &Vargas, 2000). Salicylic acid (SA) is considered as a hormone-like substance, which plays an important role in regulating a number of physiological processes and provide protection against biotic and abiotic stresses in plant. The protective function of SA includes the regulation of ROS and antioxidant enzymes (Khan *et al.*, 2003; Shi & Zhu, 2008). The role of SA in defense mechanism to alleviate salt stress in plants was studied (Afzal *et al*., 2006; Eraslan *et al*., 2007; Hussein *et al*., 2007). The mitigation effect of SA to abiotic stresses was investigated through its application either by foliar spray of maize (Khodary, 2004), seed soaking of wheat genotypes (Al-Hakimi, 2006) or through rooting medium of wheat (Arfan *et al*., 2007). The effect of salicylic acid on the physiological processes is variable, promoting some processes and inhibiting others depending on its concentration, plant species and environmental conditions (El-Mergawi & Abdel Wahed, 2004).

Comparing the response between genotypes of the same species to salinity provides a convenient and useful tool for un-veiling basic mechanisms involved in salt tolerance. The mechanism of salt tolerance is still not fully understood (Ghars *et al*., 2008). Therefore, the objective of this work was conducted to compare the effect of salt stress on growth parameters, physiological and antioxidant responses of two faba bean (*Vicia faba* L.) genotypes differing in salt tolerance and whether seed priming with salicylic acid (SA) could mitigate the adverse affect of salt stress.

MATERIALS AND METHODS

Seeds of both faba bean (*Vicia faba* L.) genotype 115 and 125 (local Egyptian faba bean genotypes) were obtained from the Faculty of Agriculture, Assuit University, Egypt. Homogenous seeds of both genotypes were surface sterilized using 5% sodium hypochlorite solution for 5 min and then rinsed 3 times with sterile distilled water. The seeds of each genotype were subjected to seed priming, first group was hydroprimed (soaked in distilled water), while the second group was osmoprimed [soaked in 0.2 mM salicylic acid (SA) for 10 h, this concentration was suggested after some preliminary experiments]. After soaking period the seeds were air dried. Seeds of the two groups were sown in plastic pots (10 seeds in each pot) lined with polyethylene bags and filled with soil composed of clay and sand (1:1 by volume). The pots of each group were further divided into two sub-groups; the pots of the first sub-group were irrigated with normal water only to serve as control, while the pots of second sub-group were irrigated with 140 mM NaCl. Four replicates from each treatment were prepared. The pots were kept in growth chamber maintained at 22/20±2°C day/night (10/14 h) temperature cycles and relative humidity 60±5%. The concentration of NaCl was maintained constant through the experimental period (20 days) by adding distilled water.

Harvesting. The plants were uprooted 20 days after planting and split up into the root and shoot system. They were rinsed with deionized water and blotted on paper towels before being weighed (fresh weight).

Growth measurement. Leaf area was measured by Digital Planimeter (Placom KP-90). To determine dry weight; the freshly harvested roots and shoots were dried in an aerated oven (Program oven, MOV-313P, Sanyo, Japan) at 80°C until constant weight. The samples were ground into fine powder and stored in sealed glasses at room temperature for the chemical analysis.

Determination of relative water content, ion leakage and photosynthetic pigments. Leaf relative water content (RWC) was determined according to Smart (1974). Ion leakage was measured as electrical conductivity (EC%) according to Yan *et al*. (1996); the washed leaves were cut into 1 cm pieces and placed in a glass beaker containing 10 mL deionised water. The beakers were kept at 30°C for 3 h and the conductivity of solution was measured by a conductivity meter. The same samples were boiled for 2 min and then their conductivity was measured again, when the solution was cooled to room temperature. The percentage of electrolyte leakage was calculated as follows, EC (%) = (C1/C2) X 100. Where C1 and C2 are the electrolyte conductivities measured before and after boiling, respectively.

Photosynthetic pigments (chlorophyll *a*, chlorophyll *b* & carotenoids) contents were estimated in 80% acetone extracts using the spectrophotometric method according to Lichtenthaler and Wellburn (1983).

Determination of osmotic solutes, Na^+ and K^+. Soluble carbohydrate was extracted from the plant tissues and determined according to the anthrone sulphuric acid method (Badour, 1959); the dried tissue of roots and shoots was extracted by distilled water. One mL of the carbohydrate extract was mixed with 9 mL of anthrone sulphuric acid reagent in a test tube and heated for 7 min at 100°C. The absorbency was read spectrophotometrically (*Spectronic Genesys* ZPC, Rochester, NY, USA) at 620 nm against blank containing only distilled water and anthrone reagent. Soluble protein was determined according to Bradford (1976). Free amino acids were determined according to the

method of Lee and Takahashi (1966). Na^+ and K^+ were determined by the flame photometric method (Williams & Twine, 1960).

Assays of Some Antioxidant Enzyme Activities

Enzyme extraction. The samples were prepared as described by Mukherjee and Choudhuri (1983). A leaf sample (0.5 g) was frozen in liquid nitrogen and finely ground by pestle in a chilled motor, the frozen powder was added to 10 mL of 100 mM phosphate buffer (KH_2PO_4/K_2HPO_4) pH 7.0, containing 0.1 mM Na_2EDTA and 0.1 g of polyvinylpyrrolidone. The homogenate was filtered through cheese cloth then centrifuged at 15000 g for 10 min at 4°C. The supernatant was re-centrifuged at 18000 g for 10 min and then the resulted supernatant was collected and stored at 4°C for catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutatione reductase (GR) assays.

Assay of catalase activity. Catalase (EC 1. 11. 1. 6) activity was assayed according to Aebi (1984). The activity of catalase was estimated by the decrease of absorbency at 240 nm for 1 min as a consequence of H_2O_2 consumption (Havir & McHale, 1987).

Assay of peroxidase activity. Peroxidase (EC 1. 11. 1. 7) activity was determined according to Maehly and Chance (1954) by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance due to formation of tetraguaiacol was recorded at 470 nm (Klapheck *et al.*, 1990).

Assay of ascorbate peroxidase activity. The activity of ascorbate peroxidase (EC 1. 11. 1. 11) was assayed according to (Chen & Asada, 1992), by measuring the decrease in absorbance at 290 nm for 1 min of ascorbic as ascorbic acid oxidized.

Assay of glutathione reductase activity. The activity of glutathione reductase (EC 1. 6. 4. 2) was measured according to Foyer and Halliwell (1976), which depends on the rate of decrease in the absorbance of NADPH at 340 nm.

All the enzyme activates were calculated and expressed as unit min^{-1} gm^{-1} fresh weight.

Determination of lipid peroxidation. Lipid peroxidation level was measured as the content of malondialdehyde (MDA) using the thiobarbioturic method (Zhao *et al.*, 1994). It was expressed as nmol of MDA formed using an extinction co-efficient of 155 mM^{-1} cm^{-1} and the results expressed as nmol (MDA) g^{-1} fresh weight.

Ascorbic acid determination. It was determined according to the method of Mukherjee and Choudhuri (1983). The absorbance was read at 525 nm. Its concentration was calculated from a standard curve plotted with known concentrations of ascorbic acid.

Statistical analysis. All data were analyzed statistically by one-way ANOVA using the Statistical Package for Social Science (SPSS) program. Values in the tables indicate mean values of four independent determinations. The least significant difference (L.S.D.) was used to test the difference between treatments; $p \leq 0.05$ and $p \leq 0.01$ were considered statistically significant and highly significant, respectively.

RESULTS

Growth parameters (dry weights of root, shoot & leaf area) and water relations (plant water content & leaf relative water content) were reduced in both faba bean (*Vicia faba* L.) genotypes as response to salinity stress (Table I). The reduction values were much higher ($P \leq 0.01$) in genotype 125 than genotype 115 in compared to control plants. The reduction percentage in dry weight was higher in root (about 24 & 12%) than of shoot (about 19 & 4%) in genotype 115 and 125, respectively as compared with control. However, salicylic acid (SA) treatments had stimulation effects on such parameters under both saline or non-saline (control) conditions. SA treatments were not only alleviated the inhibitory effect of salt stress on shoot dry weight and leaf relative water content of genotype115, but also induced a great stimulating effect than those of control plants.

Salinity stress reduced total chlorophyll (chl. *a*+*b*) contents in genotype 125, whereas they were un-changed in genotype 115. On the other hand, carotenoids content was significantly increased ($p \leq 0.01$) in both genotypes. It was increased by 51.7% and 26.8% in genotype 115 and 125, respectively over control plants (Table II). Consequently, total chlorophyll/carotenoids ratio was significantly reduced ($p \leq 0.01$). Seed priming with SA improved all fractions of photosynthetic pigments in both genotypes, especially in plants subjected to salt stress.

The responses of osmotic solutes (soluble carbohydrate, protein & free amino acids) to salinity were greatly varied (Table III). Soluble carbohydrate was increased in root and shoots of genotype 115, while it was un-affected in shoot and increased in root of genotype 125 in response to salinity stress compared with control. Soluble protein was significantly reduced in root and shoot of both genotypes. Total free amino acids were significantly decreased in root, while they were un-affected in shoot of genotype 125. Meanwhile, total free amino acids were significantly increased ($p \leq 0.01$) in genotype 115. It is worthy to mention that the contents of osmotic solutes were generally higher in genotype 115 than in genotype 125 especially in salt-stressed plants. Application of SA resulted in higher contents of soluble carbohydrate and free amino acids, whereas soluble protein was decreased as compared with SA untreated plants especially in plants subjected to salt stress.

Contents of Na^+, K^+ and K^+/Na^+ ratio (Table IV) reveal that, salinity increased the level of Na^+ in root and shoot of both genotypes. K^+ content was un-affected in genotype 115, while it was significantly reduced in genotype 125. K^+/Na^+ ratios in root and shoot of both genotypes were significantly reduced ($P \leq 0.01$) in salt-stressed plants compared to control. The reduction percentage was higher (52.6 & 53.2%) in genotype 125 than in genotype 115 (22.2 & 16%) in both root and shoot, respectively. Considerable variations in Na^+ and K^+ contents within both genotypes demonstrated that Na^+ content was

Table I. Dry weight (DW) of root and shoot (g plant^{-1}), percentage water content (WC%), leaf area (cm^2) and percentage relative water content (RWC%) of two faba bean (*Vicia faba* L.) genotypes in response to the interactive effect of 140 mM NaCl salinity (S) and seed priming with 0.2 mM salicylic acid (SA)

Treatments		Root		Shoot		Leaf	
		DW	WC%	DW	WC%	Area	RWC%
Genotype 115	Control	0.059	90.8	0.181	90.5	18.2	75.8
	SA	0.071 B	91.6	0.212 B	90.2	20.0	81.7 A
	S	0.052	89.8	0.173	89.4 A	16.5	72.2
	S + SA	0.055	90.1	0.186	89.6 A	17.9	76.4
L.S.D.	at 5%	0.008	1.1	0.010	0.8	2.1	4.3
	at 1%	0.011	1.5	0.015	1.2	3.1	6.3
Genotype 125	Control	0.051	89.2	0.174	89.7	16.3	66.1
	SA	0.058 A	90.1 A	0.179 A	90.6 A	17.4	67.4
	S	0.039 B	87.3 B	0.141 B	86.9 B	12.6 B	52.0 B
	S + SA	0.045 A	88.4	0.152 B	87.8 B	14.8 A	56.3 B
L.S.D.	at 5%	0.005	0.9	0.005	0.8	1.2	2.8
	at 1%	0.008	1.3	0.008	1.1	1.8	4.1

Means values in each column, which are significantly different ($P \leq 0.05$) are followed by A letter and the highly significantly different ($P \leq 0.01$) are followed by B letter as compared with control (0.0 NaCl)

Table II. Photosynthetic pigments (mg g^{-1} FW) of two faba bean (*Vicia faba* L.) genotypes in response to the interactive effect of 140 mM NaCl salinity (S) and seed priming with 0.2 mM salicylic acid (SA)

Treatments		Chl a	Chl b	Chl a+b	Carotenoid	Chl a+b/ Carotenoid
Genotype 115	Control	0.779	0.289	1.068	0.058	18.4
	SA	0.805 A	0.309 A	1.114	0.071 B	15.7 A
	S	0.748 B	0.272	1.020	0.088 B	11.6 B
	S + SA	0.903 B	0.328 B	1.231 A	0.107 B	11.5 B
	L.S.D. at 5%	0.039	0.019	0.104	0.008	2.1
	L.S.D. at 1%	0.057	0.027	0.146	0.012	3.0
Genotype 125	Control	0.708	0.254	0.962	0.056	17.2
	SA	0.723 A	0.268 A	0.991	0.064 A	15.5 A
	S	0.582 B	0.210 B	0.792 B	0.071 B	11.2 B
	S + SA	0.665 B	0.247	0.912	0.085 B	10.7 B
	L.S.D. at 5%	0.013	0.014	0.077	0.007	1.4
	L.S.D. at 1%	0.019	0.021	0.116	0.011	1.9

Means values in each column, which are significantly different ($P \leq 0.05$) are followed by A letter and the highly significantly different ($P \leq 0.01$) are followed by B letter as compared with control (0.0 NaCl)

lower in shoot than in root of both genotypes, whereas K^+ was much higher in genotype 115 than in genotype 125. SA treatments resulted in reducing Na^+ and increased K^+ content, which in turn increased the ratio of K^+/Na^+ in both genotypes. K^+/Na^+ ratio was higher in genotype 115 than in genotype 125 either under salt stress or when treated with SA. Moreover, in shoot of genotype 115, SA increased K^+/Na^+ ratio over control, when plants subjected to salinity stress.

The activity of antioxidant enzymes such as CAT, POD, APX and GR was differentially affected by salinity stress (Table V). Salinity stress did not induce CAT and POD activity in leaves of genotype 125. However, the activity of both enzymes was significantly increased (about 87% & 48%, respectively) over control in leaves of genotype 115. On the other hand the activity of APX and

Table III. Soluble carbohydrate, protein and total free amino acids (mg g^{-1} DW) two faba bean (*Vicia faba* L.) genotypes in response to the interactive effect of 140 mM NaCl salinity (S) and seed priming with 0.2 mM salicylic acid (SA)

Treatments		Root			Shoot		
		Soluble carbo-hydrate	Soluble protein	Free amino acids	Soluble carbo-hydrate	Soluble protein	Free amino acids
Genotype 115	Control	29.5	112.8	10.5	24.8	210.2	21.0
	SA	31.3	93.4 B	17.3 B	23.2	194.8 B	22.6
	S	36.5 B	89.6 B	16.9 B	35.4 B	204.1	25.8 B
	S + SA	41.8 B	91.6 B	17.8 B	53.9 B	218.2	28.3 B
L.S.D.	at 5%	3.0	4.9	2.1	4.2	8.4	2.8
	at 1%	4.4	7.2	3.0	6.1	12.2	4.1
Genotype 125	Control	20.4	71.7	8.4	22.8	143.7	16.9
	SA	25.1 B	74.4	7.7	25.6	142.1	17.5
	S	25.5 B	56.1 B	6.0 B	20.2	113.2 B	15.5
	S + SA	29.5 B	73.5 B	8.1	25.0	157.5 B	13.1 B
L.S.D.	at 5%	3.2	4.0	0.9	3.1	6.5	2.1
	at 1%	4.7	5.8	1.2	4.6	9.5	3.0

Means values in each column, which are significantly different ($P \leq 0.05$) are followed by A letter and the highly significantly different ($P \leq 0.01$) are followed by B letter as compared with control (0.0 NaCl)

Table IV. The contents of Na^+, K^+ and Na^+/K^+ ratio (mg g^{-1} DW) of two faba bean (*Vicia faba* L.) genotypes in response to the interactive effect of 140 mM NaCl salinity (S) and seed priming with 0.2 mM salicylic acid (SA)

Treatments		Root			Shoot		
		Na^+	K^+	K^+/Na^+	Na^+	K^+	K^+/Na^+
Genotype 115	Control	12.4	32.4	2.61	5.4	34.0	6.30
	SA	10.1 A	40.0 B	3.996 B	3.9A	37.1	9.51 B
	S	14.6 A	29.7	2.03 B	6.2	32.8	5.29 A
	S + SA	12.1	38.9 B	3.21 B	5.1	39.0 A	7.65 B
L.S.D.	at 5%	1.8	2.8	0.37	1.1	3.7	0.77
	at 1%	2.6	4.1	0.53	1.6	5.3	1.13
Genotype 125	Control	9.2	24.8	2.70	6.7	32.2	4.81
	SA	10.4	24.2	2.33	5.2 A	33.6	6.46 B
	S	16.6 B	21.3 A	1.28 B	11.8 B	26.5 B	2.25 B
	S + SA	15.3 B	26.9	1.76 B	9.2B	28.0 A	3.04 B
L.S.D.	at 5%	1.7	3.1	0.38	1.5	3.1	0.45
	at 1%	2.5	4.5	0.55	2.2	4.5	0.66

Means values in each column, which are significantly different ($P \leq 0.05$) are followed by A letter and the highly significantly different ($P \leq 0.01$) are followed by B letter as compared with control (0.0 NaCl)

GR was significantly increased ($p \leq 0.01$) in both genotypes under saline conditions. It is noticeable that the activity of APX and GR was greater in genotype 115 than 125. Seed priming with SA markedly stimulated the activity of these antioxidant enzymes in both faba bean genotypes, either under saline or non-saline conditions.

The results (Table VI) revealed that, MDA, EC% and ascorbic acid were increased as a result of salinity stress in both genotypes. These increases were higher (about 210, 73 & 55%) in genotype 125 than those of genotype 115 (about 26, 10 & 37%), respectively over control plants. Further increases were observed in MDA, EC% and ascorbic acid as a result of SA treatments, particularly in plants subjected to salinity stress.

Table V. Antioxidant enzyme activities (unit min^{-1} g^{-1} FW) in leaves of two faba bean (*Vicia faba* L.) genotypes in response to the interactive effect of 140 mM NaCl salinity (S) and seed priming with 0.2 mM salicylic acid (SA)

Treatments	Genotype 115				Genotype 125			
	CAT	POD	APX	GR	CAT	POD	APX	GR
Control	1.80	10.0	0.48	0.44	1.24	8.5	0.40	0.28
SA	2.12 B	10.9	0.60 B	0.52 B	2.48 B	12.2 B	0.54 B	0.34 A
S	3.36 B	14.8 B	0.68 B	0.56 B	1.54	9.4	0.56 B	0.40 B
S + SA	3.68 B	15.8 B	0.96 B	0.64 B	2.92 B	21.5 B	0.76 B	0.52 B
L.S.D. at 5%	0.41	2.5	0.07	0.05	0.31	1.7	0.06	0.05
L.S.D. at 1%	0.60	3.6	0.10	0.08	0.46	2.6	0.09	0.07

Means values in each column, which are significantly different ($P \leq 0.05$) are followed by A letter and the highly significantly different ($P \leq 0.01$) are followed by B letter as compared with control (0.0 NaCl)

Table VI. MDA (nm g^{-1} FW), (EC%) and ascorbic acid (AsA) contents (mg g^{-1} DW) in leaves of two faba bean (*Vicia faba* L.) genotypes in response to the interactive effect of 140 mM NaCl salinity (S) and seed priming with 0.2 mM salicylic acid (SA)

Treatments	Genotype 115			Genotype 125		
	MDA	EC%	AsA	MDA	EC%	AsA
Control	56.9	57.7	4.2	24.8	47.8	3.5
SA	39.6 B	45.6 B	4.7	21.1 A	43.4 A	3.6
S	71.8 B	63.3 B	6.5 B	76.8 B	82.9 B	4.8 A
S + SA	47.0 B	55.7	7.4 B	53.3B	72.3B	5.2 B
L.S.D. at 5%	3.9	3.9	0.82	2.8	3.7	0.8
L.S.D. at 1%	5.7	5.6	1.20	4.1	5.3	1.1

Means values in each column, which are significantly different ($P \leq 0.05$) are followed by A letter and the highly significantly different ($P \leq 0.01$) are followed by B letter as compared with control (0.0 NaCl)

DISCUSSION

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 & 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), which were accumulated more in genotype 115 compared with those of genotype 125. Furthermore, the osmotic adjustment in genotype 115 was associated with an increase in soluble carbohydrate and free amino acids, whereas it was associated with increase of root soluble carbohydrate only in genotype 125. No consistent relationship has been found between salt tolerance and soluble protein (Sarwat & El-Sherif, 2007). Present results indicated that, the increase in free amino acids content in genotype 115 under salt stress may be related to the breakdown of protein. The ability of genotype 115 to absorb more water from the saline soil than genotype 125 was linked with its ability to stimulate the synthesis of such osmotic solutes. The accumulation of osmotic solutes and their roles under salinity stress has been discussed by many studies (Kerepesi & Galiba, 2000; Azooz, 2002 & 2004).

Photosynthetic pigments remained mostly un-changed in genotype 115, while their values declined in genotype 125 as plants subjected to salinity stress. Such genotype variations in the biosynthesis of photosynthitacally active pigments, which grown under salinity stress are consistent with the results of Sarwat and El-Sherif (2007) and Yildirim *et al*. (2008). The un-changed effect of salinity stress on chlorophyll contents in genotype 115 may be attributed to its higher level of antioxidant content and may be responded as protection of the genotype against chlorophyll degradation. In this regard, Sairam *et al*. (2002) and Yildirim *et al.* (2008) suggested that chlorophyll content is one of the important indicators of salt tolerance in crop plants.

It is worthy to mention that, carotenoids content was significantly higher in both genotypes under salinity stress as compared to control. Consequently, there was a progressive decline in total chlorophyll/carotenoids ratio. This means that chlorophylls are more sensitive to salt stress than carotenoids. Carotenoids might play a role as a free radical scavenger. Therefore, increasing of carotenoids in genotypes treated with salinity and/or SA could enhance their capacity to reduce the damage caused by ROS, which in turn increased chlorophyll content of such plants. This could be due to the protection effect of SA and carotenoids to the photosynthetic apparatus from salinity-induced oxidative stress. The same findings were reported by Eraslan *et al*. (2007).

SA treatment had a pronounced ameliorative as well as, growth promoting effect under both saline and non-saline conditions. The ameliorative effect of SA might be linked to the observable increase in WC, RWC and photosynthetic pigments as well as, leaf area. Consequently the efficiency of the photosynthetic apparatus was increased due to SA treatments (Khan *et al*., 2003; Yildirim *et al*., 2008), which in turn considerably increased the biosynthesis of osmotic solutes under salinity stress. These osmolytes might increase the osmotic pressure of cytoplasm and enhance water flow into the different plant organs and tissues. This may indicate that SA might alleviate the imposed salt stress, either via osmotic adjustment or by conferring desiccation resistance to plant cells as reported by other investigators (Khodary, 2004; Hussein *et al*., 2007; Gunes *et al*., 2007).

The higher content of K^+, Na^+ and K^+/Na^+ ratio in root than in shoot in genotype 115 than in genotype 125 has been considered a physiological trait indicator of salt tolerance in plants (Morsy *et al*., 2007). These results confirm those obtained by Chartzoulakis *et al*. (2002) and Kaya *et al*. (2007). The ability of plant to limit Na^+ transport into the shoot is critically importance for the maintenance of high

growth rates and protection of the metabolic processes in elongation cells from the toxic effects of Na^+ (Razmjoo *et al*., 2008). This could be attributed to the ability of root to exclude Na^+ from the xylem sap flowing to the shoot, which would imply the better growth of shoot than root (Kaya *et al*., 2007). The selectivity of high K^+/Na^+ ratio in plants is important control mechanism and a selection criterion for salt tolerance (Wenxue *et al*., 2003). Cuin *et al*. (2003) concluded that, high K^+/Na^+ ratio is more important for many species than simply maintaining a low Na^+ concentration. SA treatments reduced Na^+, while increased K^+ and K^+/Na^+ ratio in both genotypes. This indicates that seed priming with SA induced a reduction of Na^+ absorption and toxicity. This could explain the mitigation effect of SA on growth of both genotypes. Further, the antagonistic relation between Na^+ and K^+ as a result of SA treatment indicates that, SA could play a role in modifying K^+/Na^+ selectivity under salt stress, which is reflected in lowering membrane damage and higher water content in both genotypes especially under salinity stress.

The differences in the activity of antioxidant system among the two faba bean genotypes revealed that salinity stress did not induce CAT and POD activity in genotype 125, whereas their activities were significantly increased in genotype 115 over control plants. Thus, it could be concluded that there was a strong correlation between salt tolerance and CAT and POD activity. Similar results were obtained by Costa *et al*. (2005) and Azooz *et al*. (2009). The higher activities of APX and GR in genotype 115 than genotype 125 were in agreement with those reported by Mandhania *et al.* (2006), who concluded that under salinity stress, the stimulation of APX and GR activity was much higher in salt-tolerant than those of salt-sensitive cultivar. They also reported that the greater GR activity in salt stressed tolerant cultivar indicated that these plants exhibit more active ascorbate-glutathione cycle than the non-tolerant cultivar. These results give a considerable reason to believe that salt tolerance of genotype 115 seems to be linked with increase in the activity of antioxidant enzymes.

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. These results are confirmed by other investigators (Jaleel *et al*., 2007; Khan & 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 reduction of ion leakage might be related to the inductive responses of antioxidant enzymes that protect the plant from oxidative damage. Of specially interest, the genotype 115, 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).

Ascorbic acid as an antioxidant could scavenge ROS and H_2O_2 and take part in ascorbate peroxidase (APX) mediated scavenging of H_2O_2 (Asada, 1992). The stimulation effect of salinity or in combination with SA on the endogenous ascorbic acid might play an important role as an antioxidant and protect the faba bean plants from the oxidative damage by scavenging ROS that are generated during salt stress conditions (Arrigoni & De Tullio, 2000; Athar *et al.*, 2008).

In conclusion, both faba bean genotypes have different mechanism to adapt to salt stress. Based on the responses and the ability of the experimental faba bean genotypes to cope with salinity stress, genotype 115 could be regarded as more salt-tolerant than genotype 125. The tolerance of genotype 115 to salinity stress seems to be related to its ability to enhance higher accumulation of osmotic solutes, selectivity of K^+/Na^+ ratio, carotenoids, antioxidant enzyme activities and lower of ion leakage and lipid peroxidation than those of genotype 125 under salt stress. Seed priming with SA can be used for enhancing the salt tolerance potential of both genotypes.

Acknowledgment. I greatly appreciate M.A. Shaddad, Prof. of Plant Physiology, Faculty of Science, Assuit University, Egypt for his suggestions and comments on this manuscript.

REFERENCES

Aebi, H., 1984. Catalase *in vitro*. *Methods Enzymol*., 105: 121–126

Afzal, I., S.M.A. Basara, M. Faooq and A. Nawaz, 2006. Alleviation of saslilnity stress in spring wheat by hormonal priming with ABA, salicylic acid and ascorbic acid. *Int. J. Agric. Biol*., 8: 23–28

Al-Hakimi, A.M.A., 2006. Counteraction of drought stress on soybean plants by seed soaking in salicylic. *Int. J. Bot.,* 2: 421–426

Apel, K. and H. Hirt, 2004. Reactive oxygen species: Metabolism, oxidative stress and signal transduction. *Annu. Rev. Plant Biol*., 55: 373–399

Arfan, M., R. Athar and M. Ashraf, 2007. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *J. Plant Physiol.,* 164: 685–694

Arrigoni, O. and M.C. De Tullio, 2000. The role of ascorbic acid in cell metabolism: between gen-directed functions and un-predictable chemical reactions. *J. Plant Physiol.,* 157: 481–488

Asada, K., 1992. Ascorbate peroxidase-a hydrogen peroxide-scavenging enzyme in plants. *Physiol. Planta*, 85: 235–241

Ashraf, M., 2002. Salt tolerance of cotton: Some new advances. *Cri. Rev. Plant Sci.,* 21: 1–30

Ashraf, M., 2009. Biotechnological approach of improving plant salt tolerance using antioxidants markers. *Biotech. Adv*., 27: 84–93

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

Athar, H., A. Khan and M. Ashraf, 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environ. Exp. Bot.,* 63: 224–231

Azevedo Neto, A.D., J.T. Prisco, J. Enéas-Filho, C.E.B. Abreu and E. Gomes-Filho, 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *J. Environ. Exp. Bot*., 56: 87–94

Azooz, M.M., 2002. Physiological responses of seedlings of two wheat cultivars (cv. Seds-1 & cv. Banyswif-3) to salt stress tolerance. *J. Union Arab Biol. Cairo Physiol. Algae*, l0: 39–55

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

Azooz, M.M., M.A. Shaddad and A.A. Abdel-Latef, 2004. Leaf growth and K^+/Na^+ ratio as an indication of the salt tolerance of three sorghum cultivars grown under salinity stress and IAA treatment. *Acta Agron. Hungarica*, 52: 287–296

Azooz, M.M., A.M. Ismail and M.F. Abou Elhamd, 2009. Growth, lipid peroxidation and antioxidant enzyme activities as a selection criterion for the salt tolerance of three maize cultivars grown under salinity stress. *Int. J. Agric. Biol*., 11: 21–26

Badour, S.S.A., 1959. Analytisch-chemische untersuchung des Kaliummangles bei *Chlorella* im Vergleich mit anderen Mangelezustanden *Ph.D. Dissertation,* Göttingen

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

Chartzoulakis, K., M. Loupasaki, M. Bertaki and I. Androulakis, 2002. Effects of NaCl salinity on growth, ion content and CO2 assimilation rate of six olive cultivars. *Sci. Hort*., 96: 235–247

Chen, G. and K. Asada, 1992. Inactivation of ascorbate peroxidase by thoils requires hydrogen peroxide. *Plant Cell Physiol*., 33: 117–123

Costa, P.H., A.D. Azevedo Neto, M.A. Bezerra, J.T. Prisco and E. Gomes-Filho, 2005. Antioxidant-enzymatic system of two sorghum genotypes differing in salt tolerance. *Brazil J. Plant Physiol.*, 17: 353–361

Cuin, T.A., A.G. Miller, S.A. Laurie and R.A. Leigh, 2003. Potassium activities in cell compartments of salt-grown barley leaves. *J. Exp. Bot*., 54: 657–661

Dubey, R.S., 2005. Photosynthesis in plants under stressful conditions. *In:* Pessarkli, M. (ed.), *Photosynthesis Handbook,* 2nd edition, pp: 717–718. C.R.C. Press, New York, USA

El-Mergawi, R.A. and M.S.A. Abdel-Wahed, 2004. Diversity in salicylic acid effects on growth criteria and different indol acetic acid forms among faba bean and maize. *Egyptian J. Agron*., 26: 49–61

Eraslan, F., A. Inal, A. Gunes and M. Alpaslan, 2007. Impact of exogenous salicylic acid on the growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. *Sci. Hort*., 113: 120–128

Foyer, C.H. and B. Halliwell, 1976. The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. *Planta*, 133: 21–25

Gehlot, H.S., A. Purohit and N.S. Shkhawat, 2005. Metabolic changes and protein patterns associated with adaptation to salinity in *Sesamun indicum* cultivars. *J. Cell Mol. Biol*., 4: 31–39

Ghars, M.A., E. Parre, A. Debez, M. Bordenave, L. Richard, L. Leport, A. Bouchereau, A. Savouré and C. Abdelly, 2008. Comparative salt tolerance analysis between *Arabidopsis thaliana* and *Thellungiella halophila*, with special emphasis on K^+/Na^+ selectivity and proline accumulation. *J. Plant Physiol*., 165: 588–599

Gunes, A., A. Anal, M. Alpaslan, F. Eraslan, E.G. Bagci and N. Cick, 2007. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutration in maize (*Zea mays* L.) grown under salinity. *J. Plant Physiol*., 164: 728–736

Havir, E.A. and N.A. McHale, 1987. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiol*., 84: 450–455

Heikal, M.M., A.M. Ismail and M.M. Azooz, 2000. Interactive effects of salinity and some vitamins on some metabolic processes in two broad bean lines. *Acta Agron. Hung.*, 48: 327–336

Hussein, M.M., L.K. Balbaa and M.S. Gaballah, 2007. Salicylic acid and salinity effect on growth of maize plants. *Res. J. Agric. Biol. Sci*., 3: 321–328

Hungria, M. and M.A.T. Vargas, 2000. Environmental factors affecting nitrogen fixation in grain legumes in the topics, with an emphasis on Brazil. *Field Crops Res.*, 65: 151–164

Ismail, A.M. and M.M. Azooz, 2002. Response of *Vicia faba* to salinity and vitamins. *Indian J. Plant Physiol*., 7: 303–306

Jaleel, C.A., R. Gopi, G.M. Alagu Lakshmanan and R. Panneerselvam, 2006. Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. *Plant Sci.,* 171: 271–276

Jaleel, C.A., R. Gopi, B. Sankar, P. Manivannan, A. Kishorekumar, R. Sridharan and R. Panneerselvam, 2007. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. *South Africa J. Bot*., 73: 190–195

Jaleel, C.A., P. Kishorekumar, A. Manivannan, B. Sankar, M. Gomathinayagam and R. Panneerselvam, 2008. Salt stress mitigation by calcium chloride in *Phyllanthus amarus*. *Acta Bot. Croat*., 67: 53–62

Kaya, C., A.L. Tuna, M. Ashraf and H. Altunlu, 2007. Improved salt tolerance of melon (*Cucumis melo* L.) by the addition of proline and potassium nitrate. *Environ. Exp. Bot*., 60: 397–403

Kerepesi, I. and G. Galiba, 2000. Osmotic and salt stress induced alteration in carbohydrate content in wheat seedlings. *Crop Sci.,* 40: 482–487

Khan, M.H. and S.K. Panda, 2008. Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. *Acta Physiol. Plant*, 30: 81–89

Khan, W., B. Prithiviraj and D.L. Smith, 2003. Photosynthetic response of corn and soybean to foliar application of salicylates. *J. Plant Physiol*., 160: 485–492

Khodary, S.E.A., 2004. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. *Int. J. Agric. Biol*., 6: 5–8

Klapheck, S., I. Zimmer and H. Cosse, 1990. Scavening of hydrogen peroxide in endosperm of *Ricinus communis* by ascorbate peroxidase. *Plant Cell Physiol*., 31: 1005–1013

Lee, Y.P. and T. Takahashi, 1966. An improved coloremeteric detrmination of amino acids with the use of ninhydrin. *Anal. Biochem*., 14: 71–77

Lichtenthaler, H.K. and A.R. Wellburn, 1983. Determination of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochem. Soc. Trans,* 11: 591–592

Maehly, A.C. and B. Chance, 1954. The assay of catalase and peroxidase. *Meth. Anal. Biochem*., 1: 357–424

Mandhania, S., S. Madan and V. Sawhney, 2006. Antioxidant defense mechanism under salt stress in wheat seedlings. *Biol. Plant*, 50: 227–231

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*., 7: 405–410

Morsy, M.R., L. Jouve, J. Hausman, L. Hoffmann and J. McD Stewart, 2007. Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance. *J. Plant Physiol.,* 164: 157–167

Mukherjee, S.P. and M.A. Choudhuri, 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Plant Physiol*., 58: 166–170

Noreen, S. and M. Ashraf, 2008. Alleviation of adverse effects of salt stress on sunflower (*Helianthus Annus* L.) by exogenous application of salicylic acid: Growth and photosynthesis. *Pakistan J. Bot*., 40: 1657–1663

Raza, S.H., H.R. Athar and M. Ashraf, 2006. Influence of exogenously applied glycine betine on photosynthetic capacity of differently adapted wheat cultivars under salt stress. *Pakistan J. Bot.,* 38: 341–351

Razmjoo, K., P. Heydarizadeh and M.R. Sabzalian, 2008. Effect of salinity and drought stresses on growth parameters and essential oil content of *Matricaria chamomile. Int. J. Agric. Biol*., 10: 451–454

Rejili, M., A.M. Vadel, A. Guetet and M. Neffatti, 2007. Effect of NaCl on growth and the ionic balance K^+/Na^+ of two populations of *Lotus creticus* (L) (Papilionaceae). *South Africa J. Bot*., 73: 623–631

Sairam, R.K., K.V. Rao and G.C. Srivastava, 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci.,*163: 1037–1046

Sarwat, M.I. and M.H. El-Sherif, 2007. Increasing salt tolerance in some barley genotypes (*Hordeum vulgare*) by using Kinetin and benzyladenin. *World J. Agric. Sci.,* 3: 617–629

Shi, Q. and Z. Zhu, 2008. Effects of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. *Environ. Exp. Bot*., 63: 317–326

Smart, R.E., 1974. Rapid estimation of relative water content. *Plant Physiol*., 53: 258–260

Sreenivasulu, N.B., Grimm, U. Wobus and W. Weschke, 2000. Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). *Physiol. Planta*, 109: 435–442

Wenxue, W., P.E. Bilsborrow, P. Hooley, D.A. Fincham, E.Lombi and B.P. Forster, 2003. Salinity induced differences in growth, ion distribution and partitioning in barley between the cultivar Maythorpe and its derived mutant Golden Promise. *Plant Soil*, 250: 183–191

Williams and Twine, 1960. Flame photometric method for sodium, potassium and calcium. *In*: Peach, K. and M.V. Tracey (eds.), *Modern Methods of Plant Analysis,* Vol. 5, pp: 3–5. Springer-Verlag, Berlin

Yamaguchi, T. and E. Blumwald, 2005. Developing salt-tolerant crop plants: Challenges and opportunities. *Trends Plant Sci*., 12: 615–620

Yan, B., Q. Dai, X. Liu, S. Huang and Z. Wang, 1996. Flooding induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. *Plant Soil,* 179: 261–268

Yildirim, B., F. Yaser, T. Ozpay, D.T. Ozpay, D. Turkozu, O. Terziodlu and A. Tamkoc, 2008. Variations in response to salt stress among field pea genotypes (*Pisum sativum* sp. arvense L.). *J. Anim. Veter. Adv*., 7: 907–910

Zhao, S.J., C.C. Xu, Q. Zhou and Q.W. Meng, 1994. Improvements of the method for measurement of malondialdehyde in plant tissue. *Plant Physiol. Commun*., 30: 207–210

Zhu, J.K., 2002. Salt and drought stress signal transduction in plants. *Annl. J. Plant Biol*., 14: 267–273

(Received 04 February 2009; Accepted 19 March 2009)