Ameliorative Effect of Salicylic Acid on Growth, Minerals and Nitrogenous Compounds of *Vicia faba* L. Plants under Salt Stress

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> HIS STUDY aimed to identify the response of three broad bean genotypes (*Vicia faba* L. namely Wardy, Assiut125 and Assiut 84/4) to different levels of NaCl (0.0, 50, 100, 150 mM). Also, to elucidate the stimulatory role of salicylic acid at 0.5 mM as a foliar spray on the studied bean genotypes under salt stress. Growth, photosynthetic pigments, minerals, membrane integrity and some nitrogenous compounds were evaluated. The three studied broad bean genotypes varied in their salt tolerance and in their response to the salicylic acid application. Salinity reduced the shoot growth, the photosynthetic pigments, K⁺ contents and total proteins while it increased proline, total amino acids, soluble proteins and Na⁺ contents. Increasing the NaCl-salt level caused more cell membrane injury and electrolyte loss. The Na⁺/K⁺ ratio increased with increasing salt concentration in old leaves than in young leaves of bean genotypes. Exogenous salicylic acid succeeded to ameliorate the oxidative stress in bean salt-stressed genotypes especially at the most sensitive genotype Assiut 84/4. It can be concluded that broad bean plants could be possibly cultivated in moderate saline soils due to its capacity for osmotic adjustment. Moreover, salicylic acid could be used as a promising compound in broad bean cultivated under salt stress conditions.

> Keywords: Salicylic acid, Bean, Salinity, Electrolyte leakage, Proline.

Salinity is a major abiotic stress seriously threatens plant growth and resulted in about one-third of world's cultivated area affected by salinity (Kaya *et al.*, 2002). *Vicia faba* L. is a crop of great importance in the Mediterranean region. Cultivation of broad bean became more important globally and this may be related to its high protein content and excessive costs of protein-rich food in order to meet the increasing human demands (Gueguen and Cerletti, 1994). In Egypt, broad bean (*Vicia faba* L.) is one of the main cultivated legumes. Currently, climate change has become major constraints in crop production. Salt stress widely affects the semi-arid and irrigated areas (Drevon and Sifi, 2003).

Abiotic stress resulting from elevating salinity leads to inhibition of photosynthesis and other biochemical processes related to the plant growth, development and crop production (Tiwari *et al.*, 2010). Furthermore, abiotic

stress causes oxidative stress in the plant cell resulting in excessive electrons leakages which lead to enhancement of reactive oxygen species (ROS) generation (Asada, 2006). The cell is considered as the major site of salinity injury (Mansour, 1997) where salt stress changes the composition of the plant plasma membrane.

A metabolic adaptation which enables plants to cope with osmotic stress, involves an increase generation of osmoprotectants, as proline and total free amino acids. Compatible osmolytes not only provide osmoregulation but they may also protect the structure (Hare *et al.*, 1998) or act as free-radical scavengers that protect DNA from damaging effects of ROS (Ashraf and Foolad, 2007). Proline accumulation has been reported to balance the deleterious effects of salinity. Moreover, it considered as organic nitrogen reserve that used during stress recovery (Sairam and Tyagi, 2004). In addition, proline is larger accumulated amino acid in the plants under abiotic stress (Irigoyen *et al.*, 1992). Moreover, K⁺ is essential for enzymes activation, osmotic pressure regulation, and membrane polarization (Kaya *et al.*, 2007). As the K⁺ is involved in multiple previous plant activations, the Na⁺/K⁺ ratio is a good indicator for salt tolerance in wheat (Zheng *et al.*, 2008). However, excessive production of ROS under salt stress usually result in lipid peroxidation and enhance K⁺ leak from the cell by stimulating K⁺ efflux channels (Cuin and Shabala, 2007).

Salicylic acid (SA) is a hormone-like substance plays an important role in photosynthetic rate, stomatal conductance, elevating antioxidative protection (Xu *et al.*, 2008), and reducing the accumulation of Na⁺ and Cl⁻ (Gunes *et al.*, 2007). The effect of SA on plant growth and metabolism is still a matter of controversy in consideration of different plant species, salinity intensity and SA doses (Horvath *et al.*, 2007).

The aim of this work is to evaluate the difference in salt tolerance of three broad bean genotypes and to determine the efficiency of SA in the alleviation of the harmful effects of salt stress on those broad bean plants. Growth, photosynthetic pigments, electrolyte leakage, accumulation and distribution of proline, proteins and total free amino acids were evaluated. In addition, the change in Na⁺/K⁺ ratio in the old and young leaves due to salt treatments was also evaluated.

Material and Methods

Plant material and treatments

In this investigation seeds of three broad bean genotypes (*Vicia faba* L.): Wardy, Assiut125 and Assiut 84/4 were provided by Faculty of Agriculture, Assiut University, Egypt. Seven seeds were sown in each pot containing 5 kg of clay soil. All pots were watered to the soil water content with tap water until the appearance of third true leaves. Pots were placed in an open air under natural

conditions. Three replicates treatments were classified into two groups; the first group represents the salinity stress treatment at four levels (0.0, 50, 100, 150 mM) and the second group represents the interaction between salinity stress at different levels and SA at 0.5 mM as follows: (0.0+SA, 50+SA, 100+SA, and 150+SA mM). Plants in each pot were sprayed with 20 ml SA at the age of 35 days however plants in the first group were sprayed with distilled water only. At 55 days after sowing (DAS), the plants were harvested to determine their NaCl salt-tolerance and to clarify further insights on their physiological responses.

Shoot Dry Weight and Height

The shoot dry weight and height were expressed as g plant⁻¹ and cm shoot⁻¹, respectively.

Electrolyte Leakage

Relative electrolyte leakage or the membrane integrity index was calculated as a percentage of :

[REC = $(EC_1/EC_2) \times 100$] Where EC₁ and EC₂ are the electrolyte

conductivities measured before and after boiling respectively (Shi et al., 2006).

Photosynthetic Pigments

Contents of Chlorophyll and chlorophyll b , total carotenoids were estimated by the spectrophotometric method recommended by Lichtenthaler (1987) and were expressed as mg g^{-1} FW.

Determination of Proteins

Soluble protein and total protein content of root, stem and leaves were determined according to Lowry *et al.* (1951) and expressed as mg g^{-1} DW.

Total Free Amino Acids

Free amino acids were determined according to Moore and Stein (1948) as mg g^{-1} DW.

Determination of Proline

Free proline content of root, stem and leaves were determined according to (Bates *et al.*, 1973) and was calculated as mg g^{-1} DW.

Measurments of Minerals

 K^+ and Na⁺ content was estimated by Williams and Twine (1960) using Carl Zeiss flame photometer as mg g⁻¹ DW.

Statistical Analysis

Data were analyzed using SPSS. Significance between means among different treatments and the \pm SE were exhibited by Duncan's test at 5% (Gomez and Gomez, 1984).

Results

Growth Parameters

Shoot length and shoot dry matter decreased by increasing salinity level. The reduction percent in shoot length was 13%, 13.5% and 31% in Wardy, Assiut 125 and Assiut 84/4 respectively at the highest salt level (Fig. 1). The percentage of reduction in shoot dry weight at 150 mM was about 33%, 40% and 48% in Wardy, Assiut 125 and Assiut 84/4 respectively (Fig. 2).

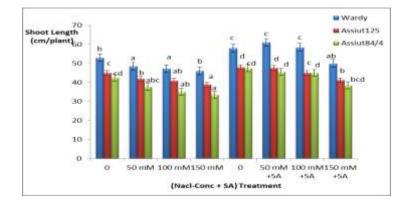


Fig. 1. Changes in shoot length (cm/plant) of the three broad bean genotypes at the vegetative stage as a result of salinity stress alone or in combination with SA. Data are means of three replicates \pm SE. Data labeled with different letters are significantly different at *P*< 0.05.

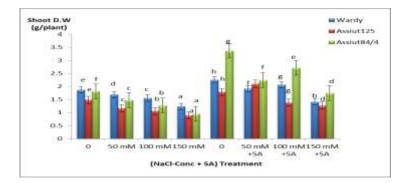


Fig. 2. Changes in dry weight (g/plant) of the three broad bean genotypes at the vegetative stage as a result of salinity stress alone or in combination with SA. Data are means of three replicates \pm SE. Data labeled with different letters are significantly different at *P*< 0.05.

SA treatment induced marked increase in both shoot length and shoot dry matter at the different NaCl levels. The stimulation percentage in the shoot dry matter at 150 mM level was 9%, 25% and 43.7% comparing with their corresponding untreated Wardy, Assiut 125 and Assiut 84/4 plants respectively.

Electrolyte Leakage

Electrolyte leakage of the three broad bean genotypes showed significant increase by increasing Nacl salt stress. The higher degree of membrane injury caused by salinity was recorded at the highest salinity level used (150 mM). There were marked variations among the different broad bean genotypes response, the cell permeability increased by about 28.5%, 59.6% and 118.5% in Wardy, Assiut125 and Assiut 84/4 genotypes respectively at the highest salt stress used (Fig. 3).

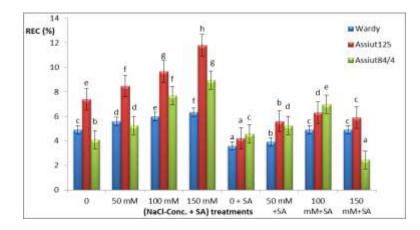


Fig. 3. Changes in electrolyte leakages (EC) of the three broad bean genotypes at the vegetative stage as a result of salinity stress alone or in combination with SA. Data are means of three replicates \pm SE. Data labeled with different letters are significantly different at *P*< 0.05.

SA obviously inhibited the electrolyte conductivity under the different salinity levels comparing to their corresponding non-treated stressed plants.

Photosynthetic Pigments

The different fractions of photosynthetic pigments (Chl.a, Chl.b, Carotenoids) markedly decreased by increasing salinity level (Table 1). The highest reduction occurred at the highest salt stress dose. The reduction in Chl.a at 150 mM was about 12%, 33% and 37.5% in Wardy, Assiut 125 and Assiut 84/4 respectively. SA treatment enhanced the photosynthetic pigments content under the different salinity levels in the three tested bean plants.

 TABLE 1. Changes in contents of chlorophyll a, chlorophyll b and carotenoids (mg/g FW) of three broad bean genotypes at the vegetative stage as a result of salinity stress alone or in combination with SA. Data are means of three replicates ±SE. Data labeled with different letters are significantly different at P< 0.05.</th>

Treatment	Genotype		Wardy		Assi	ut125		Assiut84/4		
	NaCl level	Chl.a	Chl.b	Caro.	Chl.a	Chl.b	Caro.	Chl.a	Chl.b	Caro.
Salinity	0	1.90±0.03 ^{abc}	0.56±0.029 ^{ab}	1.97±0.075 ^{ab}	10.2 ± 0.18^{d}	2.72±0.05°	6.18±0.1°	2.20±0.17 ^d	0.88±0.04°	$1.16 {\pm} 0.029^{\mathrm{ef}}$
alone	50	1.83±0.06 ^{abc}	0.50±0.028ª	1.78±0.057ª	9.05±0.34°	2.10±0.063 ^b	5.67±0.07 ^b	1.70±0.05 ^{bc}	0.59±0.023 ^b	0.90±0.023 ^{bc}
	100	$1.76{\pm}0.127^{\text{ab}}$	$0.49{\pm}0.014^{a}$	1.81±0.023 ^a	7.77±0.07 ^b	$1.93{\pm}0.075^{ab}$	5.43±0.02 ^b	1.50±0.03 ^{ab}	0.37±0.011ª	$0.79 {\pm} 0.017^{b}$
	150	1.67±0.06ª	0.48±0.04ª	1.69 ± 0.028^{a}	6.80±0.05ª	1.69±0.08ª	4.84±0.08ª	1.37±0.02ª	0.31±0.011ª	0.66±0.023ª
Salinity	0	$2.18{\pm}0.08^{d}$	0.68±0.04c	2.28±0.069 ^b	11.57±0.3°	3.69±0.29 ^e	6.54±0.14 ^d	2.30±0.17 ^d	1.00±0.144 [°]	$1.25 {\pm} 0.086^{ m f}$
$+\mathbf{SA}$	50	1.99±0.12 ^{bcd}	0.66±0.017 ^{bc}	$2.00 \pm 0.31 b^{b}$	14.25±0.58 ^f	3.24±0.023 ^d	7.99 ± 0.092^{f}	2.45±0.03 ^d	0.96±0.029°	1.09±0.034 ^{de}
	100	$2.07{\pm}0.04^{\text{cd}}$	0.58±0.03 ^{abc}	2.02±0.04 ^{ab}	$13.47 \pm 0.23^{\rm f}$	3.1 ± 0.057^{cd}	7.67±0.17 ^e	1.91±0.02°	1.26 ± 0.023^{d}	$1.11{\pm}0.03^{\text{de}}$
	150	1.95 ± 0.02^{bcd}	0.58±0.05 ^{abc}	2.32±0.04 ^b	12.18±0.1 ^e	$5.00 {\pm} 0.07^{\rm f}$	6.35±0.01 ^{cd}	1.84±0.02°	1.55±0.023°	$1.00{\pm}0.02^{cd}$

Chl.a; chlorophyll a, Chl.b; chlorophyll b, Caro; carotenoids.

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Protein

Soluble proteins markedly accumulated among the different plant parts of Wardy genotype by increasing salinity. In Assiut 125, soluble proteins increased in roots and leaves with little reduction in stem by increase salt levels. In Assiut 84/4, soluble proteins markedly decreased in stem while it gradually increased by increasing salinity level in leaves and to some extent in the root (Table 2).

SA treatment induced different responses at the different plant organs among the three tested broad bean plants. In Wardy genotype, SA-induced a significant reduction in the soluble protein of root, stem, and in leaves of low salinity levels. Contradictory, SA-induced an increase in soluble protein in leaves in the higher salt levels in relation to the corresponding untreated Wardy genotype. SAinduced an increase in the soluble proteins of stem and leaves but caused a decrease it in the root of Assiut 125 genotype under different salt stress levels used comparing to their control ones. SA-caused a reduction in the soluble proteins of leaves and roots from their relative controls while it caused slight changes in a stem at the different salt levels in Assiut 84/4.

Total proteins accumulations were varied among the studied organs of each genotype. In Wardy genotype the total protein content markedly decreased in stem and leaves with a slight tendency to increase in roots by increasing salinity level. In Assiut 125 broad bean genotype, the total protein content obviously decreased in stem while it increased in root and leaves by increasing salt stress. Total protein content decreased in leaves of Assiut 84/4 but it increased in root and stem by increasing salinity up to 150 mM where it dramatically decreased. Generally, SA treatment enhanced the total protein accumulations in the different organs among the three tested broad bean genotypes under the different salinity levels used comparing to the control plants.

Total Free Amino Acids

Total free amino acids (Table 3) markedly enhanced in stem and leaves of Wardy genotype by increasing the salinity level of the expense of root amino acids which reduced by increasing the salinity level. In Assiut 125 total free amino acids decreased in root with a little reduction in stem at the high salt levels. This response was accompanied by marked accumulation of the total amino acids in leaves by increasing salinity. However, Assiut 84/4 exhibited a marked increase in total free amino acids at the different plant parts. SA treatment induced total free amino acids accumulations at the different salt doses used comparing with the control plants.

TABLE 2. Changes in the soluble and total protein contents (mg/g DW) of the three broad bean genotypes at the vegetative stage as a result of salinity stress alone or in combination with SA. Data are means of three replicates \pm SE. Data labeled with different letters are significantly different at P < 0.05.

Protein fraction	Treatment -	Genotype		Wardy			Assiut 125				Assiut 84/4		
Hacton	mannent	NaCl level	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves		
		0	49.41 ± 0.64^{d}	73.03±0.57°	$128.67{\pm}0.38^{\textrm{b}}$	$49.40{\pm}0.23^{d}$	57.62±0.35°	63.20±0.29ª	36.31 ± 0.17^{d}	58.21±0.12°	$98.42{\pm}0.24^{ m b}$		
	Salinity	50	$50.68 {\pm} 0.29^{d}$	$86.06{\pm}0.21^{\rm f}$	$153.55 {\pm} 0.61^{\circ}$	48.96 ± 0.4^{d}	52.90±0.51°	70.39±0.22 ^b	$60.96{\pm}0.28^{\rm h}$	70.68±0.39 ^g	$154.26{\pm}0.44^{\rm f}$		
	alone	100	62.61±0.65°	106.05 ± 0.58^{h}	$137.95{\pm}0.43^{d}$	74.06±0.29 ^e	47.65±0.57 ^b	74.13±0.58°	37.18±0.1 ^e	49.55±0.2°	175.81±0.57 ^g		
Soluble		150	$98.27 {\pm} 0.176^{ m f}$	131.81±0.69 ^g	$132.86{\pm}0.46^\circ$	78.55±0.57	43.25±0.34ª	78.96 ± 0.46^{d}	30.14 ± 0.17^{a}	30.48±0.4 ^ª	$184.36{\pm}0.51^{\rm h}$		
proteins		0	39.30±0.17°	58.65±0.29°	154.62±0.18 ^e	37.52±0.28°	$69.95 {\pm} 0.29^{ m f}$	$97.68 {\pm} 0.29^{\rm f}$	32.24 ± 0.13^{b}	$54.39{\pm}0.58^{d}$	$126.30 {\pm} 0.17^{d}$		
	Salinity	50	37.52±0.28 ^b	37.52±0.24ª	$132.75 {\pm} 0.26^{\circ}$	33.56±0.17	$69.80{\pm}0.23^{\rm f}$	$102.24{\pm}0.14^{ m g}$	$54.25{\pm}0.14^{ m g}$	40.82±0.23 ^b	82.13 ± 0.29^{a}		
	+ SA	100	33.56±0.81 °	$42.07{\pm}0.34^{\textrm{b}}$	121.01 ± 0.17^{a}	35.32 ± 0.18^{b}	56.01 ± 0.17^{d}	88.58±0.23°	43.31 ± 0.18^{f}	$67.24{\pm}0.14^{\rm f}$	129.38±0.18°		
		150	35.32±0.51 ª	59.09±0.46°	$161.66 {\pm} 0.57^{\rm f}$	35.46 ± 40^{b}	$70.24{\pm}0.58^{\rm f}$	$135.99 {\pm} 0.57^{ m h}$	34.58±0.33°	$78.90{\pm}0.57^{\mathrm{h}}$	$100.77{\pm}0.44^{\circ}$		
	-	0	229.46±0.58 ^b	209.29±1.15°	313.05 ± 1.15^{d}	106.19 ± 0.63^{a}	168.79±0.75 ^d	119.40 ± 0.2^{b}	132.27 ± 0.75^{b}	$93.97 {\pm} 0.58^{b}$	$336.53 {\pm} 0.58^{d}$		
	Salinity	50	244.56±0.69°	$202.58{\pm}0.87^{\textrm{b}}$	236.17±0.28°	108.40±0.23 ^b	158.79±0.86°	131.88±0.29 ^c	151.36±0.33°	$159.86{\pm}1.4^{\rm f}$	$337.42{\pm}0.81^{\text{d}}$		
	alone	100	255.87±0.47°	196.08±0.6 ^{ab}	196.84±0.8 ^b	119.40±0.29°	140.30±0.4 ^b	138.63 ± 0.63^{d}	$193.71 {\pm} 0.86^{ m f}$	$105.42{\pm}0.24^{\circ}$	$289.87{\pm}0.63^{\textrm{b}}$		
Total		150	161.37±0.58ª	165.96 ± 0.57^{a}	$183.50 {\pm} 0.29^{a}$	127.33±0.19 ^e	118.29±0.58ª	115.15±0.62ª	89.13±0.65ª	$65.19 {\pm} 0.29^{a}$	246.80±0.46ª		
proteins	-	0	$265.69 {\pm} 0.97^{g}$	238.85±0.87°	369.11 ± 0.38^{g}	121.93 ± 0.58^{d}	183.76±0.29°	191.87±0.29°	157.54 ± 0.75^{d}	129.34 ± 0.77^{d}	355.02±1.15°		
	Salinity	50	$247.20{\pm}0.35^{d}$	$267.91{\pm}0.52^{\circ}$	334.78 ± 1.7^{f}	127.55±0.29 ^e	$167.03 {\pm} 0.29^{d}$	$253.08 \pm 0.51^{ m f}$	189.68±0.39°	168.37±1.37 ^g	$449.23{\pm}0.58^{\rm f}$		
	+ SA	100	298.37 ± 1.2^{h}	336.21 ± 0.69^{d}	329.12±0.59 ^e	161.66 ± 0.22^{g}	159.99±1.15°	280.67 ± 0.58^{g}	$197.85 {\pm} 0.58^{ m g}$	155.17±0.29 ^e	326.85±0.49°		
		150	261.66 ± 0.46^{f}	258.22±0.46°	382.32±1.15 ^h	146.83±0.58 ^f	254.19 ± 0.81^{f}	281.84 ± 0.92^{h}	134.91±0.69 ^b	184.22±0.34 ^h	468.16±1.27 ^g		

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 TABLE 3. Changes in total free amino acids content (mg/g DW) of the three broad bean genotypes at the vegetative stage.as a result of salinity stress alone or in combination with SA. Data are means of three replicates ±SE. Data labeled with different letters are significantly different at P< 0.05.</td>

Treatment	Genotype		Wardy			Assiut 125		Assiut 84/4			
Salinity alone	NaCl level	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves	
-	0	6.18±0.1 ^e	$4.01{\pm}0.18^{a}$	8.89 ± 0.17^{a}	6.18±0.10 ^e	15.65±0.29ª	36.65±0.23ª	4.55±0.29ª	12.87 ± 0.58^{b}	23.58±0.64ª	
~	50	5.55±0.17 ^d	3.51 ± 0.12^{a}	8.09±0.05ª	5.55±0.11 ^d	20.90 ± 58^{cd}	47.87±0.29 ^{de}	4.62±0.17ª	10.13±0.29 ^a	23.61±0.29 ^a	
	100	3.85±0.29 ^{bc}	5.00±0.14 ^b	17.69±0.57°	3.85±0.06 ^{bc}	15.49±0.23ª	46.6±0.4 ^{cd}	4.87 ± 0.12^{a}	10.81 ± 0.24^{a}	35.8±0.24 ^c	
	150	3.26 ± 0.14^{a}	5.13±0.07 ^b	13.35±0.2 ^b	3.26±0.15ª	15.45 ± 0.18^{a}	45.85±0.58 ^{bc}	7.65±0.34 ^b	21.47 ± 0.58^{d}	34.06±0.57 ^{bc}	
Salinity +SA -	0	4.28±0.12 ^c	7.93±0.23°	22.26±0.15 ^d	4.27±0.11°	19.49±0.23 ^b	48.40±0.23 ^e	23.89±0.63 ^e	16.10±0.29 ^c	35.24±0.41°	
TSA -	50	3.52±0.18 ^{ab}	5.7±0.04 ^b	18.43±0.24 ^c	$3.51 {\pm} 0.17^{ab}$	19.78±0.17 ^b	$51.55 {\pm} 0.87^{\rm f}$	18.73±0.12 ^e	20.61 ± 0.18^{d}	54.26±1.16 ^d	
	100	3.89±0.11 ^{bc}	8.64±0.29 ^d	17.50±0.57°	3.95±0.14 ^{bc}	20.08±0.29 ^{bc}	$52.89{\pm}0.58^{\mathrm{f}}$	15.24±0.14°	16.31±0.11 ^c	35.84±0.29 ^c	
	150	3.73±0.11 ^{ab}	$8.89{\pm}0.29^{d}$	21.48 ± 0.58^{d}	3.72±0.31 ^{bc}	21.70±0.4 ^d	44.83±0.58 ^b	12.07±0.58°	23.30±0.63 ^e	33.13±0.65 ^b	

Proline

Proline changes varied among the three broad beans genotypes. In Wardy genotype, little enhancement was obtained in proline content in different plant parts however; marked accumulation of proline was recorded at 100 mM NaCl especially in the leaves. SA-induced a reductive response in proline in all tested organs except a slight increase was detected in leaves under excessive salinity levels. Proline accumulation in Assiut 125 was commonly increased by salinity. The highest proline content was recorded at 150 mM (Table 4). SA decreased the proline content in root and stem but enhanced it significantly in leaves comparing to the salt stressed plants. In Assiut 84/4 proline decreased in root but significantly increased in stem and leaves especially at 150 mM NaCl, the highest accumulation was recorded in leaves. SA decreased the proline content markedly at the different salt doses on comparing with their corresponding untreated plants.

Minerals Distribution

The distribution pattern of K^+ in leaves was opposite to that of Na^+ and the younger leaves had greater K^+ concentration than the older leaves under the different NaCl treatment. K^+ content was commonly enhanced by salt stress. While Na⁺ content was low in young leaves and high in old leaves of Wardy broad genotype, K^+ content was contrary. In Assiut 125 and Assiut 84/4 genotypes, Na⁺ accumulation markedly increased by salt stress and the absolute Na⁺ content was higher in old leaves than in young ones. K^+ content in Assiut 125 and Assiut 84/4 genotypes was lesser than in Wardy genotype leaves (Table 5).

SA application caused marked decrease in Na⁺ content under the different salinity levels and this behavior was more obvious in young leaves of the three studied bean genotypes. However, K⁺ content markedly increased by SA treatment under the different NaCl levels in both old and young leaves of the different broad bean genotypes. SA treatment resulted in marked inhibition in the Na⁺/K⁺ ratio.

Discussion

NaCl-salt stress is a limiting factor in plant growth. In order to improve the salt stress deterioration effects, plants exhibited some mechanisms to overcome salt toxicity and decreased water potential in the soil which caused by salinity (Munns and Tester, 2008).

Salinity markedly inhibited the growth of plants and this may be due to stomatal closure, and the increased generation of ROS in the chloroplasts (Daneshmand *et al.*, 2010). Salinity, in this study, significantly led to a remarkable decrease in shoot dry weight, plant height and photosynthetic pigments contents. Salt stress generally reduces the plant availability of water leading to a reduction in growth, photosynthesis as well as in some biochemical processes.

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TABLE 4. Changes in proline content (mg/g DW) of the three broad bean genotypes at the vegetative stage.as a result of salinity stress
alone or in combination with SA. Data are means of three replicates \pm SE. Data labeled with different letters are significantly
different at P< 0.05.</th>

Treatment	Genotype		Wardy			Assiut 125		Assiut 84/4		
	NaCl level	Roots	Stem	Leaves	Roots	Stem	Leaves	Roots	Stem	Leaves
Salinity	0	$0.41 \pm 0.017^{\rm bc}$	$0.33{\pm}0.011^{\text{cde}}$	2.012±0.03 ^c	0.16 ± 0.011^{ab}	$0.71 {\pm} 0.005^{b}$	$2.63{\pm}0.07^{a}$	$1.49 \pm .009^{de}$	$0.60 {\pm} 0.005^{b}$	3.90±0.05 ^e
alone	50	0.40 ± 0.005^{bc}	$0.30 {\pm} 0.028^{\rm bc}$	2.99±0.046 ^e	0.24±0.01 ^c	$0.51 {\pm} 0.023^{a}$	2.49±0.034ª	1.54±0.07 ^e	0.69±0.023 ^c	2.73±0.001 ^b
	100	$0.54{\pm}0.028^{d}$	0.38±0.017 ^e	$5.91{\pm}0.088^{f}$	$0.15{\pm}0.017^{\text{ab}}$	0.90±0.051°	2.54±0.058ª	1.46±0.02 ^{de}	1.11±0.029 ^e	8.38±0.06 ^g
	150	0.45±0.017°	$0.37{\pm}0.011^{de}$	1.94±0.023 ^c	$0.61{\pm}0.03^{d}$	$1.01{\pm}0.034^{d}$	3.10±0.04 ^b	1.23±0.05°	$1.21{\pm}0.023^{f}$	$9.20{\pm}0.11^{h}$
Salinity	0	0.38±0.011 ^b	0.26±0.017 ^{ab}	$2.28{\pm}0.028^d$	$0.16{\pm}0.005^{ab}$	0.46±0.011 ^a	4.79±0.06 ^d	$1.39{\pm}0.029^{d}$	$0.00{\pm}0.005^{a}$	$0.00{\pm}0.005^{a}$
+ SA	50	0.28±0.017ª	0.24±0.005ª	1.56±0.017 ^b	$0.20{\pm}0.01^{\rm bc}$	$0.45 {\pm} 0.017^{a}$	5.75±0.08 ^e	0.83±0.01ª	0.59±0.02 ^b	$4.34{\pm}0.12^{f}$
	100	$0.54{\pm}0.028^{d}$	0.23±0.017ª	$1.37 {\pm} 0.005^{a}$	0.16±0.017 ^{ab}	$0.45 {\pm} 0.029^{a}$	4.68±0.056 ^d	0.76 ± 0.017^{a}	$0.77 {\pm} 0.01^{d}$	3.20±0.09°
	150	0.23±0.011ª	$0.32{\pm}0.011^{cd}$	2.20±0.051 ^d	0.14±0.011ª	0.530.011ª	3.79±0.051°	1.05±0.06 ^b	1.09±0.051 ^e	3.50±0.13 ^d

Mineral	Treatment	Genotype	Wa	rdy	Assiu	it 125	Assiut 84/4		
		NaCl level	Young	Old	Young	Old	Young	Old	
		0	1.80 ± 0.023^{a}	1.31 ±0.035 ^b	0.80 ± 0.029^{d}	$0.59\pm\!0.023^{\text{cd}}$	1.20 ± 0.02^{c}	1.05 ±0.026 ^c	
	Salinity	50	1.91 ± 0.0233^{b}	1.25 ± 0.044^{ab}	0.70 ± 0.037^{c}	0.50 ± 0.023^{b}	1.00 ± 0.02^{b}	0.90 ± 0.026^{b}	
	alone	100	$2.00\pm\!0.037^{b}$	1.25 ± 0.028^{ab}	0.55 ± 0.017^{b}	0.43 ± 0.02^{a}	1.00 ± 0.012^{b}	0.56 ± 0.029^{a}	
K ⁺ Content		150	$2.30 \pm 0.034^{\circ}$	$1.20\pm\!0.028^a$	0.43 ± 0.023^{a}	0.39 ± 0.031^{a}	0.60 ± 0.017^{a}	0.55 ± 0.028^{a}	
	Salinity	0	$2.00\pm\!0.031^{b}$	1.50 ± 0.029^{cd}	0.91 ± 0.034^{e}	0.71 ± 0.02^{cf}	1.27 ± 0.017^{d}	1.70 ± 0.028^{d}	
	+ SA	50	$2.60\pm\!0.017^{\texttt{d}}$	1.44 ± 0.031 ^c	$1.20 \pm 0.026^{\rm f}$	$0.70 \pm 0.023^{\mathrm{ef}}$	1.80 ± 0.02^{e}	2.60 ±0.023 ^e	
		100	$2.00\pm\!0.043^{\texttt{b}}$	$1.60\pm\!0.04^{\text{de}}$	$1.50 \pm 0.026^{\text{g}}$	$0.63 \pm 0.023^{\text{de}}$	$2.00\pm\!0.03^{\rm f}$	$2.70\pm\!\!0.02^{\rm f}$	
	-	150	2.99 ± 0.021^{e}	1.63 ± 0.034^{e}	$2.30\pm\!0.026^h$	$0.55 \pm 0.014^{\text{bc}}$	$2.80 \pm 0.02^{\text{g}}$	$2.77 \pm 0.043^{\mathrm{f}}$	
		0	1.81 ± 0.029^{e}	3.79 ± 0.3^{a}	0.90 ± 0.014^{d}	$1.60 \pm 0.02^{\circ}$	1.30 ± 0.023^{b}	1.10 ± 0.029^{b}	
Na ⁺ Content	Salinity	50	$1.90 \pm 0.031^{\mathrm{f}}$	3.57 ± 0.031 a	1.40 ± 0.026^{e}	$2.21 \pm 0.035^{\mathrm{f}}$	$1.70 \pm 0.028^{\circ}$	1.60 ± 0.026^{d}	
	alone	100	$2.10 \pm 0.52^{\rm g}$	4.30 ± 0.017^{b}	$1.90\ {\pm}0.037^{\rm f}$	$2.80\pm\!0.024^{g}$	$4.85 \pm 0.029^{\rm f}$	2.50 ± 0.026^{e}	
		150	$2.30 \pm 0.26^{\rm h}$	$4.86 \pm 0.02^{\circ}$	$2.20 \pm 0.031^{\text{g}}$	3.50 ± 0.023^{h}	$6.00 \pm 0.029^{\text{g}}$	$3.50 \pm 0.027^{\rm f}$	
		0	0.70 ± 0.26^{a}	3.57 ± 0.029^{a}	0.50 ± 0.017^{a}	1.22 ± 0.026^{a}	$1.00{\pm}0.029^{a}$	0.60 ± 0.011^{a}	
	Salinity	50	$0.99\pm 0.034^{\texttt{b}}$	$3.68\pm\!0.023^{\text{a}}$	0.60 ± 0.02^{b}	1.50 ± 0.023^{b}	$1.30 \pm 0.026^{\text{b}}$	0.56 ± 0.006^{a}	
	+ SA	100	$1.33 \pm 0.31^{\circ}$	$3.68\pm\!0.035^{\text{a}}$	0.75 ± 0.017^{c}	1.80 ± 0.029^{d}	$2.90 \pm 0.023^{\text{d}}$	0.56 ± 0.009^{a}	
		150	1.50 ± 0.027^{d}	5.09 ±0.049°	0.88 ± 0.026^{d}	2.00 ± 0.032^{e}	4.55 ±0.028 ^e	1.40 ±0.014 ^c	

TABLE 5. Changes in mineral (K^+ , Na^+) content (mg/g DW) of the young and old leaves the three broad bean genotypes at the vegetative stage.as a result of salinity stress alone or in combination with SA. Data are means of three replicates ±SE. Data labeled with different letters are significantly different at P < 0.05.

Salinity reduced the chlorophyll content in leaves of many crops (Parida and Das, 2005). The reduction percent of the different studied growth parameters at 150 mM salinity was high in Assiut 84/4 genotype while it was low in Wardy genotype. According to the decrease in the growth parameters, the salt tolerance of the studied bean genotypes ranked the Wardy genotype as the most salt tolerant broad bean genotype followed by Assiut 125 genotype and the Assiut 84/4 broad bean genotype proved to be the most salt sensitive one.

SA foliar application enhances the resistance toward salt stress of many crops (Noreen and Ashraf, 2008). SA acts as an internal signal molecule that could enable physiological adaptation to the abiotic stress including oxidation damage (Borsani *et al.*, 2001).

As the salinity injury begins firstly from the cell, the disturbance in cell membrane permeability takes place before other visual deterioration signs of salinity (Mansour, 1997). In this study, the cell electrolyte loss increased by salt stress among the different tested broad bean genotypes. In turn, the highest level of leakage achieved at 150 mM salt level. The most electrolyte leakage occurred in Assiut 84/4 and the least one detected in Wardy genotype. From these results it can be detected that this parameter has been used as a good and suitable criterion for recognizing NaCl-salt tolerance, this is agreed with (Farooq and Azam, 2006).

Exogenous application of SA succeeded to significantly overcame the cell injury among the different treatments in this study. SA successfully enhanced the different studied plant growth parameters (shoot length, shoot dry weight), leaf chlorophyll, carotenoid contents and membrane injury of plants under NaCl-stress among the three studied bean genotypes. There were marked differences in SA-stimulation among the different studied bean genotypes. The highest SA-enhancement in these attributes was detected in Assiut 84/4 the most salt sensitive genotype. The increase in dry weight of the three bean genotypes under salinity in response to SA foliar application may have a protective role of membranes resulting in an enhancement in the salt-tolerance of these genotypes to damage. This stimulatory effect of SA is agreed with (Gunes *et al.*, 2007). SA played an essential role in repairing the cell membrane damage and decreasing electrolyte leakage under the different salinity levels among the three broad beans, agreed with (Idrees *et al.*, 2011).

In this study, total free amino acids especially proline increased under salinity even at low NaCl-salt concentrations. Proline accumulations tended to balance the deleterious effects of salinity. Accumulation of proline and total free amino acids considered a significant biological parameter in salinity tolerance. Proline accumulations in NaCl-salt tolerant plants could be related to the stress adaptation. Soluble protein, proline and amino acids are the most important compatible solutes, play an important role in salt tolerance (Azooz, 2009).

Salinity resulted in a significant accumulation of proline in the different organs of the studied broad bean genotypes. The highest proline accumulation was detected in leaves and this confirms good translocation of proline from the root and stem towards leaves this may be due to proline participation in the osmotic potential in leaves and consequently in the osmotic adjustment. Low proline content was detected in the most salt tolerant; Wardy genotype under the higher salinity level. Proline accumulation may play a role in plant adaptation within the cell under abiotic stress (Sperdouli and Moustakas, 2012). Proline accumulation in salt-stressed plants expreses the reduced proline oxidation to glutamic acid which inhibited utilization of proline in protein synthesis and stimulated proteins turnover (Tuna *et al.*, 2008).

Exogenous application of SA negatively affects the proline accumulation; commonly SA caused a reduction in the proline content along with the total amino acids content of the *Vicia faba* L genotypes organs by increasing the salt stress. SA acts as an organic nitrogen source that is important during the stress recovery (Sairam and Tyagi, 2004). SA enhances the uptake of K^+ under salinity conditions, potassium is important in protein synthesis. Salinity also leads to the generation of ROS inside the plant; this could be responsible for losing the protein and nucleic acids (Kim *et al.*, 2005).

In this study marked changes in the soluble protein content of the tolerant and sensitive broad bean genotypes against salt stress were exhibited. It was detected that soluble protein content of roots stem and leaves was higher in Wardy genotype (NaCl-tolerant genotype) than in Assiut 125 and Assiut 84/4 (NaCl-sensitive genotype) bean genotypes, agreed with (Ashraf and Tufail, 1995) in sunflower and (Pareek *et al.*, 1997) in rice. The soluble proteins markedly increased with salinity in the different plant organs, especially in leaves.

With respect to the production of total protein, salinity stress resulted in slight reductions in the accumulation of total proteins in the roots, stems and to some extent in leaves of the most sensitive genotypes (Assiut 125 and Assiut 84/4) than in the most tolerant bean genotype (Wardy). In Wardy broad bean the soluble proteins markedly accumulated by salt stress on the expense of the total proteins that significantly decreased by salinity. This is an adaptation manner of increasing the soluble proteins by hydrolysis of the total proteins in order to share in the cell osmotic adjustment. In this study , few detections of total protein accumulation in plants grown under saline conditions were found and it may act as a storage form of nitrogen that can be used to overcome stress. The reduction in protein under salt treatments has been related to the decline in protein synthesis, accelerated proteolysis, lower availability of amino acids and degradation of protein synthesis enzymes (Jaleel *et al.*, 2007 and Lakhdar *et al.*, 2008). The enhanced protein carbonization has been used as a good trait of oxidative damage in plants under several abiotic stresses (Miller *et al.*, 2007).

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Commonly, SA enhanced the soluble and total proteins of the different plant parts from their control ones under salt stress especially in leaves; the increase in the total proteins were more obvious. The higher stimulation was detected at the leaves especially in Assiut 84/4 at 150 mM salt concentration, confirming the more stimulatory effect of SA by the most sensitive broad bean genotype under salt stress.

In this study, K^+ and Na^+ ions accumulation and distribution among the old and young leaves confirmed the variation degrees of NaCl-salt tolerance of the broad bean genotypes. Both salt-sensitive and salt-tolerant genotypes had a higher Na^+ accumulation than control. Because the plants keep Na^+ ions in old leaves, the transportations of them into young leaves were limited. Moreover, K^+ ion was higher in young leaves than in old leaves with little elevation by increasing salt level, this balance was achieved by transporting K^+ from old leaves to young leaves through phloem (Wolf *et al.*, 1991), this mechanism is more obvious in the Wardy genotype; the salt tolerant one which has a greater K^+ accumulation capacity than the other two salt sensitive broad bean genotypes (Assiut 125 and Assiut 84/4). K^+ accumulation is important in membrane potential balance and in regulating the osmotic potential (Kaya *et al.*, 2007). According to (Blumwald *et al.*, 2000), the decrease in K^+ concentration due to NaCl may be attributed to a high concentration of exogenous Na^+ leading to an increase in the Na^+/K^+ ratio.

Application of SA reduced the adverse effects of salt stress on Na⁺ and K⁺ accumulation and distribution. The ameliorative role of SA on membrane integrity and regulation of ion uptake has also been reported (Erasalan *et al.*, 2007; Gunes *et al.*, 2007). This protective effect of SA resulted in a decrease in the ratio of Na⁺/K⁺, which is a critical marker under salt stress. The reduction in Na⁺ and enhancement of K⁺ in stressed broad bean plants in response to application of SA may cause maintenance of the photosynthesis. According to (Khafaga *et al.*, 2009), SA treatment and its interactions helped in mitigating the harmful effect of salinity stress on *Vicia faba* L. by reducing the water loss caused by stress and/or enhancing the water and ions uptake.

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التأثير التحسينى لحامض الساليسيليك على النمو والمعادن والمركبات النيتر وجينية لنباتات الفول البلدى تحت الإجهاد الملحى

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استهدفت هذه الدر اسة التعرف على المقاومة الملحية لثلاث سلالات من الفول البلدى وهم الواردى وأسيوط ١٢٥ وأسيوط ٨٤/٤ وايضا توضيح دور حامض السالسيليك في تحسين سلالات الفول محل الدراسة تحت تأثير الإجهاد الملحي بتركيزات مختلفة (٠، ٥٠، ١٠٠و ١٥٠مل مولر) .(وقد تم تقييم النمو، أصباغ التمثيل الضوئي، المعادن، سلامة الغشاء الخلوي وبعض المركبات النيتروجينية وقد وجد أن هناك اختلاف واضح بين الثلاث سلالات من الفول من حيث المقاومة الملحية لهم وايضا من حيث استجابتهم للمعاملة بحامض السالسيلك . حيث تسببت الملوحة في خفض نمو المجموع الخضري , الأصباغ النباتية ، محتوي البوتاسيوم و البروتينات الكلية في حين تسبب الاجهاد الملحي في رفع مستويات البرولين ، البروتينات الذائبة ، الأحماض الأمينية الكلية و محتوى الصويوم داخل النبات . أوضحت الدراسه ان زيادة مستوى ملح كلوريد الصوديوم تسبب في المزيد من الضرر لغشاء ونفاذيه الخلية. وقد زادت نسبة الصوديوم إلى البوتاسيوم مع زيادة تركيز الملح في الأوراق المسنة عنه في الأوراق الحديثة لأصناف الفول المختلفة . ونتج عن رش النباتات تحت المستويات الملحية المختلفة بتركيز ٥ مللي مول حامض الساليسليك الى تحسين واضح لجميع الصفات السابقة وقد نجحت المعاملة الخارجية لحامض الساليسليك في تحسين الاجهاد الناتج عن الأكسده في نباتات الفول المجهده ملحيا خاصبة السلالة الاكثر حساسية وهي اسيوط ٤/٨٤ . ويمكن أن نخلص إلى أن نباتات الفول ذات قابلية لان تزرع في التربة متوسطة الملوحة نظر ا لمقدرتها على التعديل الاسموزى داخل خلاياه بالاضافة الى امكانية استخدام حامض السالسيليك على صورة الرش الورقى كمركب واعد عند زراعة الفول تحت تأثير الملوحة .