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The Impact of Stigmasterol on Growth, Productivity and Biochemical Response of *Vicia faba* L. Plants Grown Under Salt Stress

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SALINITY is one of major abiotic stress that reduces the yield of a wide variety of crops. We studied the effect of different salinity levels (0, 100, 150 and 200 mmol NaCl) and the addition of stigmasterol (500 µmol) on growth, yield and biochemical composition (carbohydrates, proline, potein, phenolic compounds and inorganic cations) of *Vicia faba* seeds. Increasing salinity up to 200 mmol caused significant decreases in the growth and yield of *V. faba*. Salt stress induced the accumulation of phenolics, proline and Na^+ in seeds, while the carbohydrate, total protein, K^+, Ca^{2+} and P^{3+} content of seeds declined. When seeds were soaked in stigmasterol, the adverse effects of salinity were negated and significant increases were observed in growth, carbohydrates content, total protein, and cation content (K^+, Ca^{2+} and P^{3+}) as compared to untreated plants. Moreover, a new set of proteins was induced in the seeds of stigmasterol-treated plants, and these proteins presumably function in salt tolerance. The results indicate that stigmasterol has a positive impact on growth, yield quantity and quality of *V. faba* plants subjected to salt stress.

Keywords: *Vicia faba*, Stigmasterol, Productivity, Salt stress, Phenols, Protein profile.

Faba beans are widely grown in the Mediterranean region as a source of protein for both human and animal nutrition. The nutritional value of faba beans has been attributed to their high protein content, which ranges from 25 to 35% of the total weight. The seeds are an excellent source of sugars, minerals and vitamins (Larralde and Martinez, 1991). Furthermore, the cultivation of faba beans increases the concentration of nitrogenous compounds in the soil (Hungria and Vargas, 2000). However; legumes such as faba bean (*Vicia faba* L.) are highly sensitive to salt stress (Mass, 1986).

Salt stress impedes vital aspects of plant growth and development, such as seed germination, seedling growth, seed vigor, vegetative growth, flowering and fruit set (Slathia *et al.*, 2012). Salinity affects plants by reducing water potential and causing ion imbalances, which can reduce growth and productivity (El-Hendawey *et al.*, 2004; Azooz, 2009).The length of plant shoots and roots and plant biomass is markedly reduced as salinity increases (El-Hendawey *et al.*, 2004). Consequently, improved tolerance to salinity is essential to improving productivity when water is limiting and NaCl concentrations are high. For

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example, wheat cultivars with salt tolerance showed increased yields during salt stress as compared to cultivars lacking salt tolerance (Asgari *et al*., 2012).

A variety of compounds, including phenolics, amino acids, and carbohydrates, may accumulate during salt stress (Parida *et al.*, 2003). Carbohydrates function in osmoprotection, carbon storage, and protection from free radicals (Abd El-Samed *et al.* 2004). Organic solutes, *e.g*. carbohydrates and amino acids, function as osmoprotectants by reducing the osmotic potential of the vacuole, thus preventing cytoplasmic dehydration. The increased carbohydrate content in salt-stressed plants may also be caused by the reduced distribution of these compounds in plant tissue (Dadkhah, 2010). In contrast, salinity decreases the concentration of soluble and insoluble sugars in *V. faba* (Gadallah, 1999). Many researchers have documented the inhibitory effects of salt on the biosynthesis of carbohydrates and amino acids; however, salt stress causes an increase in proline biosynthesis and accumulation (Ghassemi-Golezani, 2012). Salinity also promotes the synthesis of salt stress-specific proteins, which may protect cells against the adverse effects of NaCl (Ben-Hayyin *et al*. 1989). It is also important to note that plants may also synthesize and accumulate phenolics in response to salinity (Dkhil and Denden, 2010). Phenolics have antioxidant activity and are known to neutralize reactive oxygen species, which may be elevated during salt stress (Zheng and Wang, 2001).

Salt stress causes a considerable increase in sodium content and a decrease in potassium, calcium and magnesium ions. This is reflected in the decreased rations of K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+ (Ben-Hayyin *et al.*, 1989) and may be attributed to competition for sites that modulate cation influx (Jeschke and Wolf , 1998). Recently, Abbszadeh *et al*. (2012) demonstrated that the endogenous Na^+ concentration increased in various *Brassica napus* genotypes in response to salinity stress, whereas the K^+ concentration decreased.

Stigmasterol is a brassinosteroid, which is a class of growth regulators and signaling molecules essential for normal plant growth, along with auxins, cytokinins, gibberellins, abscisic acid and ethylene (Clouse, 1997). Brassinosteroids (BR) have regulatory functions in plant cell elongation and division, vascular differentiation and other developmental processes (Rao *et al*., 2002). A remarkable feature of brassinosteroids is their potential to increase plant resistance to a wide spectrum of biotic and abiotic stress conditions, such as temperature extremes, drought, high salt concentrations and pathogen attack (Gruszka, 2013). Despite this, only a few studies have been undertaken to understand how stigmasterol promotes stress tolerance.

The aim of the present work was to investigate changes in growth, productivity and the biochemical composition of *V. faba* seeds subject to salt stress. The potential role of stigmasterol in protecting *V. faba* from salt stress was also investigated.

Material and Methods

Vicia faba 'Sakha 1' was obtained from the Agriculture Research Center, Giza, Egypt. Stigmasterol was obtained from MP Biomedicals, LLC, France.

Growth conditions

The present study was conducted in November 2010 in the green house of Botany Department, Faculty of Science, Benha University. *V. faba* seeds were surface-sterilized with 0.1% mercuric chloride for 5 min. and washed thoroughly with several changes of sterile distilled water. The seeds were soaked overnight (12 hr) in either distilled water or in solution containing 500 µmol stigmasterol (prepared by dissolving stigmasterol in 50 µl of chloroform then complete to the total volume by distilled water as described by El Greedly and Mekki (2005). Ten seeds were sown in each pot at 3 cm depth. Ten plastic pots (diameter, 40 cm; depth 25 cm) were used per treatment. Each pot contained 15 kg of a mixture of clay and sand (2:1 w/w). Phosphorus and potassium were added before sowing at a rate of 6.0 and 3.0 g pot^{-1}, respectively, in the form of calcium superphosphate (155 g kg^{-1} P_2O_5) and potassium sulphate (480 g kg^{-1} K_2O). After emergence, seedlings (seven days old) were thinned to five per pot. Pots were maintained in a greenhouse with ambient light (approximately 16 hr light/8 hr darkness), and the mean temperature was 25 °C (day) and 10 °C (night) ± 3 °C.

Seedlings (20-day-old) that emerged after soaking seeds in water or 500 µmol stigmasterol were subjected to the desired salinity level (0, 100, 150 or 200 mmol NaCl). The plants were irrigated to maintain the water holding capacity at 70 g kg^{-1} throughout the experiment. The irrigation was every day. The plants were maintained at different salinity levels with or without stigmasterol until harvest. Ten replicates (pots) from each level of treatment were evaluated. Plant samples were collected at the fruiting stage (85-day-old plants) to measure several growth parameters including: number of leaves $plant^{-1}$; leaf area (cm^2 $plant^{-1}$); shoot and root length (cm $plant^{-1}$); and fresh and dry weight of shoots and roots (g $plant^{-1}$). At harvest (120-day-old plants), samples were collected to determine selected biochemical compounds and the following yield components: number of pods $plant^{-1}$, average length of pods $plant^{-1}$, pod diameter, dry weight of each pod, number of seeds pod^{-1}, number of seeds $plant^{-1}$, and dry weight of seeds $plant^{-1}$.

Determination of biochemical compounds of seeds

Measurement of carbohydrates content

Soluble sugars were extracted and determined by an anthrone sulfuric acid method (Whistler *et al*., 1962). Polysaccharides were determined in the dry residue remaining after extraction of soluble sugars (Whistler *et al.*, 1962). Total carbohydrates were calculated as the sum of the amounts of soluble sugars and polysaccharides in one sample. All data were calculated as g kg^{-1} dry weight.

Measurement of proline

Free proline was determined in bean seeds using acid ninhydrin reagent (Bates *et al.,* 1973). Proline concentration was determined using a standard curve and expressed as mg proline g^{-1} dry weight.

Measurement of total soluble protein

The total soluble protein concentration was determined spectrophotometrically using the Bio-Rad protein assay. Absorbance of samples was measured with a Carl Zeiss Spekol Spectrophotometer at A_{595} (Bradford, 1974).

Protein banding patterns

One-dimensional SDS-PAGE was carried out according to the method described by Studier (1973) in a linear polyacrylamide resolving gel (12%) with a stacking gel (4%). Samples were loaded into the wells and electrophoresed at 100 V until the dye front reached the bottom of the gel. The gel was removed from the plates and shaken in staining solution (400 ml methanol, 100 ml glacial acetic acid, 500 ml distilled water, one g Coomassie Brilliant Blue R-250) for 2 hr then transferred to a destaining solution (400 ml methanol, 100 ml glacial acetic acid, 500 ml distilled water) until protein bands appeared. Scans of the separated proteins were analyzed using the Gel Documentation System (S.N. 76 S/ 04069, Bio-RAD, Italy), which compares polypeptide maps, molecular protein markers, band intensity and molecular weight in relation to standard markers. The software used for analysis was Gel-Pro Analyzer *version 3 (MediaCybernetics Imaging Experts).*

Quantification of phenols and inorganic cations

Total phenols content was estimated using the Folin-Ciocalteau reagent (Malick and Singh, 1980) and expressed as g catechol kg^{-1} material. Na^+, K^+, Ca^{2+} and P^{3+} ions were extracted from dried seeds according to Chapman and Pratt (1978). Sodium and potassium were estimated by a flame emission technique as adopted by Ranganna (1977). Phosphorus and calcium were determined simultaneously by ICP spectroscopy according to the method of Soltanapour (1985).

Statistical analysis

The experiment utilized a completely randomized design. Mean values were calculated from measurement of three replicates and standard deviations of the mean were determined. All data were subjected to Duncan's multiple range test to discriminate significance (defined as $P \leq 0.05$). All data were analyzed statistically by one-way ANOVA using the Statistical Package for Social Science (SPSS, version 18.0).

Results

Changes in growth parameters at the fruiting stage

The results (Table 1) showed that most growth parameters *e.g*. shoot and root lengths, number of leaves and leaf area $plant^{-1}$, and fresh and dry weight of shoots and roots, were sharply reduced as salinity increased. Stigmasterol had a stimulatory effect on most growth parameters when compared to the corresponding control values. The highest shoot and root dry weights were obtained in plants treated with stigmasterol with no additional NaCl, followed by plants treated with stigmasterol and 100 μmol NaCl.

Table 1. Effect of different concentrations of NaCl (0, 100, 150 and 200 mmol) in control plants or in plants pretreated with stigmasterol on growth parameters and yield components of *Vicia faba* L. cv. 'Sakha 1' plants. Each value is a mean of 10 replicates.

Growth parameters (85 days after sowing)									
Treatment	**NaCl, mmol**	**Shoot length (cm)**	**Root length (cm)**	**No. of leaves plant^{-1}**	**Area of leaves plant^{-1} (cm^2)**	**Shoot weight (g)**		**Root weight (g)**	
						Fresh	**Dry**	**Fresh**	**Dry**
Reference controls	0	58.60 b	22.76 b	20.70 ab	348.50 c	15.03 b	2.83 c	3.62 a	0.27 b
	100	49.70 cd	17.40 c	18.70 b	278.80 d	12.23 cd	2.31 d	2.49 b	0.24 bc
	150	46.60 e	16.90 c	14.80 c	206.70 f	10.17 e	1.90 ef	2.00 bc	0.20 bc
	200	36.20 g	13.80 d	5.20 d	49.50 h	7.17 f	1.81 f	1.56 c	0.16 c
Stigmasterol (500 µmol)	0	62.50 a	24.66 a	21.86 a	467.20 a	16.59 a	3.38 a	3.64 a	0.36 a
	100	51.00 c	21.40 b	20.00 ab	367.00 b	12.73 c	3.06 b	2.52 b	0.31 a
	150	48.70 de	17.20 c	15.92 c	236.80 e	11.35 d	2.12 e	2.13 bc	0.23 bc
	200	42.00 f	17.16 c	6.80 d	89.70 g	10.38 de	2.02 e	1.77 bc	0.20 bc
LSD ($P \leq 0.05$)		2.16	1.78	2.41	2.21	1.02	0.18	0.92	0.09
Yield components (112 days after sowing)									
Treatment	NaCl, mmol	No. of pods plant^{-1}	Length of pods plant^{-1} (cm)	Pod diameter (cm)		Dry weight pod^{-1} (g)	No. of seeds pod^{-1}	No. of seeds plant^{-1}	Dry weight of seeds plant^{-1} (g)
Reference controls	0	3.40 b	7.84 b	4.96 b		2.062 b	3.30 c	11.22 c	6.95 c
	100	2.80 c	6.36 d	4.06 d		1.20 e	3.10 d	8.68 e	5.19 e
	150	2.80 c	4.87 f	4.00 d		1.15 e	2.90 e	8.12 e	3.08 g
	200	2.40 d	3.74 g	2.96 f		0.54 g	1.90 g	4.56 g	1.32 h
Stigmasterol (500 µmol)	0	3.80 a	8.96 a	5.50 a		2.30 a	3.90 a	14.82 a	12.74 a
	100	3.80 a	7.22 c	4.56 c		1.76 c	3.50 b	13.30 b	10.24 b
	150	3.20 b	5.78 e	4.08 d		1.47 d	3.30 c	10.56 d	6.23 d
	200	2.80 c	5.10 f	3.56 e		0.94 f	2.50 f	7.00 f	3.71 f
LSD ($P \leq 0.05$)		0.35	0.46	0.33		0.12	0.12	0.73	0.21

LSD, least significant difference. Values within a column with the same lower-case letters are not significantly different ($P \leq 0.05$)

Changes in yield components at harvest

The data presented in Table 1 revealed that the estimated V. faba yield components were significantly decreased with increasing amounts of NaCl as compared with control plants. Interestingly, the plants pretreated with stigmasterol exhibited a significant increase in all yield components compared with untreated controls. The maximum dry weight of seeds plant-1 was observed in plants treated with stigmasterol alone (12.74 g), followed by plants treated with stigmasterol and 100 mmol NaCl (10.24 g). The dry weight of seeds harvested from control plants was 6.95 g.

The positive impact of stigmasterol treatment on the dry weight of seeds was correlated with increasing salt stress. The greatest value (181.1% relative to plants treated with 200 µmol NaCl only) was observed in plants where seeds were pre-soaked in stigmasterol (500 µmol) and subjected to the highest level of salinity (200 mmol).

Biochemical changes of V. faba seeds

Changes in carbohydrates content

Sugar fractions (soluble and insoluble) as well as total carbohydrate content in seeds of *V. faba* treated with NaCl decreased significantly as salinity increased relative to control plants (Table 2). The application of stigmasterol resulted in significant increases soluble, insoluble and total carbohydrates contents in seeds of V. faba plants as compared to the corresponding controls. The carbohydrate content in seeds of plants subjected to 100 µmol NaCl and treated with stigmasterol and that of the control plants were almost the same.

TABLE 2. Effect of different concentrations of NaCl (0, 100, 150 and 200 mmol) on proline, total protein, total phenols and carbohydrates content of *Vicia faba* L. cv. 'Sakha 1' seeds of control plants or plants pretreated with stigmasterol. Each value is a mean of 3 replicates.

Proline, total protein and total phenols content					**Carbohydrates content (g glucose kg^{-1} DW)**		
Treatment	NaCl mmol	Proline (mg g^{-1} DW)	Total protein (g kg^{-1} DW)	Total phenols (g catechol kg^{-1} material)	Total soluble sugars	Insoluble sugars	Total carbohydrates
Reference controls	0	2.66 cd	153.01 c	5.36 b	21.39 b	110.26 c	131.66 b
	100	3.20 c	121.43 d	5.35 b	8.50 f	106.83 d	115.34 d
	150	4.85 b	73.99 g	6.39 a	8.27 f	98.24 g	106.51 f
	200	6.42 a	61.05 h	6.25 a	5.49 g	96.14 h	101.62 g
Stigmasterol (500 µmol)	0	2.17 d	191.27 a	5.23 b	22.72 a	118.31 a	141.03 a
	100	2.32 d	170.12 b	4.70 c	14.22 c	117.12 b	131.34 b
	150	4.55 b	115.21 e	4.89 c	11.15 d	105.65 e	116.80 c
	200	6.24 a	89.14 f	4.46 d	10.51 e	101.35 f	111.86 e
LSD ($P \leq 0.05$)		0.73	0.42	0.21	0.42	0.39	0.39

Values within a column with the same lower-case letters are not significantly different ($P \leq 0.05$)

Changes in total protein, phenols and proline

Salt stress had an inhibitory effect on total soluble protein content and a stimulatory effect on the proline level in seeds produced by salinized plants as compared with untreated controls. pretreatement with stigmasterol induced a significant increase in protein content in all treated plants but almost had no effect on proline levels as compared with control. Increases in total phenols were correlated with increasing concentrations of NaCl. The application of stigmasterol under various levels of salinity caused a significant decrease in phenols in seeds of V. faba as compared with controls.

Protein profiles

Variations were observed in the protein patterns of V. faba seeds; some proteins disappeared and others were synthesized de novo. Some of these responses were observed under both NaCl and stigmasterol treatments, while others were induced by either stigmasterol or salt (Fig. 1, Table 3). Six protein bands with molecular weight (MW) 341.6, 289.7, 155.6, 90.12, 75.73 and 16.67 kDa were synthesized de novo in seeds of faba beans grown under salt stress and are considered salt-induced proteins. The de novo synthesis of the 289.7, 155.6, 75.73 and 16.67 kDa proteins was observed in response to all concentrations of NaCl, while the 341.6 KDa protein was induced only in response to 100 and 200 µmol NaCl, and the 90.12 KDa protein was observed in plants treated with 150 or 200 µmol NaCl. Furthermore, the 312.26, 191.31, 117.39 and 72.31 kDa protein bands, which were observed in seeds of control plants, were not apparent in plants subjected to salt stress.

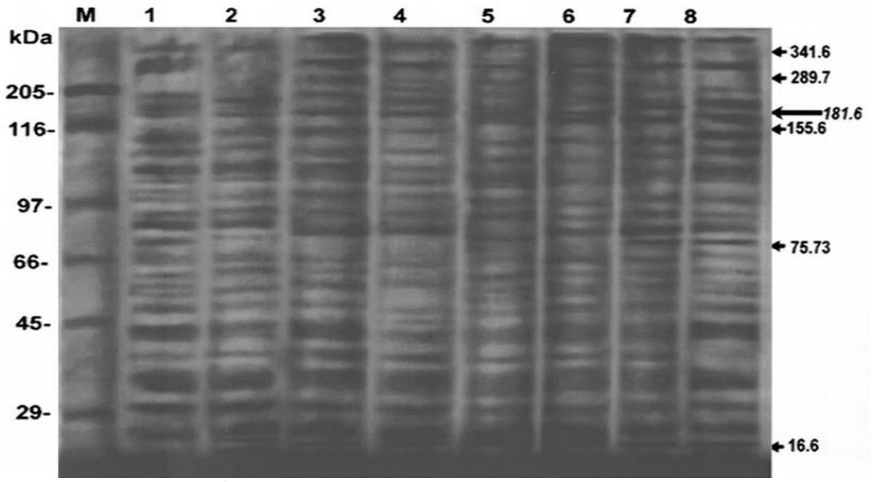

Fig. 1. Electrograph of soluble protein pattern obtained by one-dimensional SDS-PAGE, showing the changes in protein bands of *Vicia faba* seeds in response to salinity and/or stigmasterol treatment (salt-induced proteins marked by short arrows; stigmasterol-induced protein marked by long arrow). Each lane contained equal amounts of protein extracted from *V. faba* seeds.
Lane M = protein marker, Lane 1 = control (H_2O), 2 = 500 µmol stigmasterol, 3 = 100 mmol NaCl, 4 = 150 mmol NaCl, 5 = 200 mmol NaCl, 6 = 100 mmol NaCl + 500 µmol stigmasterol, 7 = 150 mmol NaCl + 500 µmol stigmasterol, 8 = 200 mmol NaCl + 500 µmol stigmasterol.

TABLE 3. Effect of different concentrations of NaCl (0, 100, 150 and 200 mmol) on protein banding patterns of *Vicia faba* L.cv. 'Sakha 1' seeds of control plants or plants pretreated with stigmasterol. Values represent the intensity of each protein band.

	NaCl (mmol)				500 µmol Stigmasterol + NaCl (mmol)			
MW kDa	0	100	150	200	0	100	150	200
362.4	0.0783	-	0.1075	-	-	-	-	-
341.6	-	0.1667	-	0.1071	-	0.0957	0.1083	0.1196
312.2	0.1293	-	-	-	0.1486	-	-	-
289.7	-	0.1690	0.1250	0.1187	-	0.10087	0.1119	0.1218
229.2	0.1321	-	-	0.1313	-	0.10938	0.1165	0.1203
191.3	0.1782	-	-	-	-	-	-	-
181.6	-	-	-	-	0.1755	-	0.1410	-
155.6	-	0.1957	0.1390	0.1535	-	0.1351	-	0.1778
117.3	0.1754	-	-	-	-	-	-	0.1494
107.0	0.1689	0.2018	0.1189	0.1461	0.1861	0.1321	0.1395	0.1677
98.4	0.2033	0.1918	0.1248	-	0.1737	-	0.133	0.1593
90.12	-	-	0.1511	0.1422	0.2099	-	0.1407	0.1915
83.29	0.1100	0.1667	0.12	-	0.1302	0.1271	-	0.1644
75.73		0.1286	0.0869	0.0857	-	-	0.0911	0.1354
72.31	0.0933	-	-	-	0.1432	0.0784	-	-
66.19	0.1345	0.1383	0.0896	0.1019	0.16914	0.0915	0.0918	0.1488
55.40	0.0715	0.1922	0.1294	0.1224	-	0.1054	0.1260	0.1719
45.05	0.1077		0.0740	0.0763	0.1492	0.0663	0.0655	0.1001
40.22	0.0677	0.1259	0.0906	0.0826	0.1243	0.0855	0.0831	0.0982
36.22	0.0532	0.1125	0.0795	0.0809	0.0902	0.0733	0.0763	0.0952
31.94	0.0333	0.0833	0.0519	0.0654	0.0764	0.0527	0.0504	0.0629
28.50	0.0610	0.1292	0.0656	0.0481	0.0974	0.0773	0.0778	0.0968
27.48	0.0819	0.1117	0.0832	0.0878	0.0879	0.0816	0.0722	0.0997
23.53	0.0397	0.0715	0.0513	0.0699	0.0429	0.0730	0.0367	0.0304
16.67	-	0.1760	0.0470	0.0996	0.1549	0.1058	0.0802	0.1054
11.26	0.2408	0.2555	0.1643	0.2006	0.2268	0.1577	0.2028	0.2772
Total no. of bands	19	17	19	18	17	18	19	21

Light gray indicates stigmasterol-induced protein. Dark gray indicates salt-induced protein. Black indicates protein induced by both salt and stigmasterol.

Lane M = protein marker, Lane 1 = control (H_2O), 2 = 500 µmol stigmasterol, 3 = 100 mmol NaCl, 4 = 150 mmol NaCl, 5 = 200 mmol NaCl, 6 = 100 mmol NaCl + 500 µmol stigmasterol, 7 = 150 mmol NaCl + 500 µmol stigmasterol, 8 = 200 mmol NaCl + 500 µmol stigmasterol.

Interestingly, two salt-induced proteins (90.12 and 16.67 kDa) were also induced in response to treatment with stigmasterol alone. Another protein band (181.6 KDa) was observed only in response to stigmasterol treatment. Finally, it should be noted that stigmasterol treatment increased the number of protein bands in seeds of salt-stressed plants as compared to the number detected in untreated salt-stressed plants.

Inorganic cations

As the salinity level increased, the Na+ content increased significantly, whereas K+, Ca2+ and P3+ decreased in V. faba seeds (Fig. 2); these changes were reflected in the decreased K+/Na+ and Ca2+/Na+ ratios as compared with control plants. Application of stigmasterol under various levels of salinity caused a reduction in the accumulation of Na+ and stimulated the accumulation of K+, Ca2+ and P3+, which led to an increase in the K+/Na+ and Ca2+/Na+ ratios compared with plants not treated with stigmasterol.

Discussion

Salt stress significantly reduced V. faba growth, and the reduction was correlated with increased salinization, reaching a maximum inhibition at 200 mmol NaCl . These reductions in growth might have resulted from the osmotic effect of the saline solutions, which caused disturbances in the plants' water balance, leading to reduction in photosynthesis and consequently a retarded growth rate (Chaparzadeh *et al*., 2004 and Mohsen *et al*., 2013). Application of stigmasterol (500 µmol) improved growth of V. faba plants by causing, in most cases, significant increases in the values of the measured growth parameters of the salt-stressed plants. The inhibitory effects of 100 µmol NaCl on the dry weight of shoots and roots were negated by stigmasterol treatment. This is probably due to increasing the efficiency of water uptake and utilization and/or acting as a growth stimulant, which could play a role in mitigating the adverse effect of NaCl on metabolic activities relevant to growth through enhancing cell division and/or cell enlargement (He *et al*., 2003).

Yield is a reflection of the integration of metabolic reactions in plants; consequently, any factor that influences this metabolic activity at any period of plant growth can affect the yield (Ibrahim and Aldesuquy, 2003). The present study demonstrated the negative effects of salt stress on multiple components of yield in V.faba as compared with control plants. Crop growth reduction in salinized plants might be related to the osmotic potential of the root-zone soil solution. This will lead to certain phonological changes and substantial reduction in productivity (Sohrabi *et al*. 2008). The results clearly indicated that application of stigmasterol was effective in alleviating the adverse effects of salt stress on the yield components of V. faba plants. In support of the above results, the increase in the number of seeds per plant of two sesame cultivars and the increase in number and weight of capsules as well as 1000-seed weight stigmasterol treatment were postulated to be due to an increased level of growth regulators, which improved photosynthetic activity and consequently benefited the number and weight of capsules and seed yield (El Greedly and Mekki, 2005).

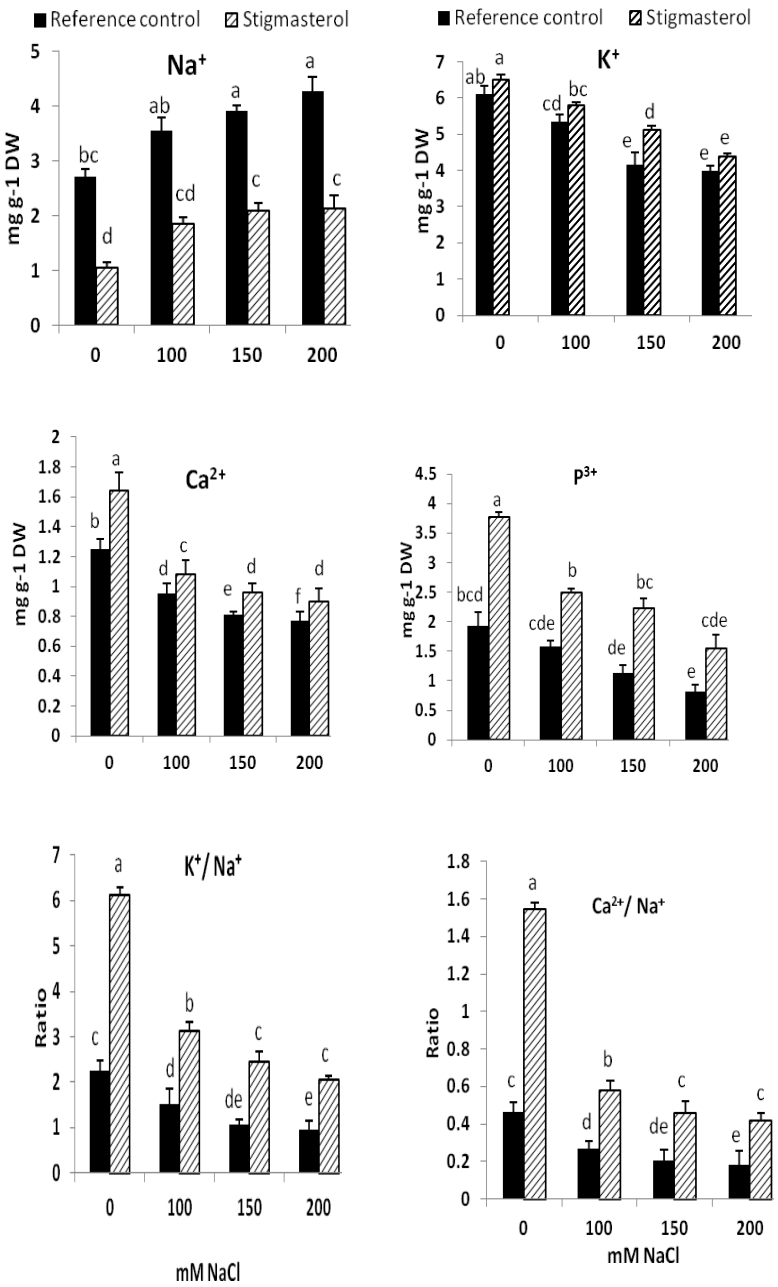

Fig. 2. Effect of different concentrations of NaCl (0, 100, 150 and 200 mmol) on mineral ion contents of *Vicia faba* L. cv. 'Sakha 1' seeds of control plants or plants pretreated with stigmasterol. Each value is a mean of 3 replicates. Columns with the same lowercase letters are not significantly different ($P < 0.05$).

The reduction in total carbohydrates in the seeds of faba bean plants under various levels of salinity concomitantly with a reduction in growth rate led to the conclusion that NaCl might inhibit photosynthetic activity and/or increased the utilization of carbohydrates by other metabolic pathways (Hassanein 2000). A significant increase in soluble, insoluble and total carbohydrate content of V. faba seeds of plants resulted when plants were pretreated with stigmaterol. Interestingly, stigmasterol treatment totally alleviated the inhibitory effect of 100 µmol NaCl on carbohydrate content (Table 2). Accumulation of carbohydrate plays a key role in alleviating salinity stress, either via osmotic adjustment or by conferring some desiccation resistance to plant cells (Srivastava *et al*., 1995). In addition, increasing the carbohydrate content of faba bean seeds improves their quality by increasing their nutritional value.

The data presented in Table 2 indicate that salt stress had an inhibitory effect on protein content and a stimulatory effect on proline level in seeds of salinized plants compared with controls. These results could be attributable to a decrease in protein synthesis and/or to an increase in protein degradation (El-Kallal *et al*. 2009). The degradation of protein under saline conditions was supported by our results, which revealed the accumulation of proline. Proline accumulation could also be the outcome of de novo synthesis. Proline has a protective response, not only due to its osmoprotectant role that prevents a salinity-induced water deficit stress, but also for its radical scavenging and protein stabilization properties (Kavi *et al*., 2005). Pretreatment of plants with stigmasterol (500 µmol) and growing them under different levels of salinity resulted in a highly significant increase in protein content but no significant change in proline level, except for the plants treated with 100 mmol NaCl + 500 µmol stigmasterol, which showed a significant decrease in proline content compared with plants treated with 100 mmol NaCl alone. These results suggest that the inhibitory effect of salinity stress was alleviated by stigmasterol treatment through inhibiting proline synthesis and/or enhancing the biosynthesis of other amino acids and their incorporation into protein (Abd El-Wahed *et al*., 2001).

The synthesis of phenolic compounds is generally affected in response to different biotic and abiotic stresses including salinity (Ayaz *et al*., 2000). Phenol accumulation in plants could be a cellular adaptive mechanism for scavenging free radicals of oxygen and preventing subcellular damage during stress (Ayaz *et al*., 2000). Application of stigmasterol under various levels of salinity caused a significant decrease in phenol content in seeds of V. faba plants compared with controls. These observations suggest that treatment with stigmasterol could alleviate the adverse effects of salinity on growth and metabolic activities through decreasing the build-up of reactive oxygen species (Abd El-Wahed *et al*. 2003). Recently, Gruszka (2013) stated that BR signaling regulates expression of genes, which are involved in the induction of antioxidant systems, which serve as protectors against deleterious effects of reactive oxygen species.

It has been suggested that the proteins synthesized de novo in the seeds of V.faba plants grown under salinity stress (MW 341.6, 289.7, 155.6, 90.12, 75.73 and 16.67 kDa) have an osmoprotection function or otherwise protect cellular structures (Abd El-Wahed *et al*., 2003). According to results obtained in this study, stigmasterol treatment induced the synthesis of new polypeptides (MW 181.6, 90.12 and 16.67 kDa; the latter two were also induced in response to salinity), indicating that stigmasterol might regulate the expression of salt stress inducible proteins as well as inducing de novo synthesis of specific polypeptides, which are anticipated to play an active role in salt resistance (Uma *et al*., 1995).

According to the present study (Table 2), the content of Na^+ was significantly increased, while the content of K^+, Ca^{2+} and P^{3+} decreased in the seeds of salt-stressed plants. Na^+ accumulation might lead to changes in essential ion uptake and ionic imbalance, reduced leaf expansion and limited growth (Jeschke and Wolf, 1998). Salt stress affects many metabolic and growth aspects, mainly due to the competition between Na^+ and K^+ for active metabolic sites, leading to depressed growth when expressed as dry matter production (Tester and Davenport, 2003). Application of stigmasterol under various levels of salinity improved the nutritional value of V. faba seeds by reducing the accumulation of Na^+ and stimulating the uptake of K^+, Ca^{2+} and P^{3+}, and this led to an increase in the K^+/Na^+ and Ca^{2+}/Na^+ ratios compared with plants untreated with stigmasterol. Also, an increase in K+ accumulation in seeds could be important for osmotic adjustment. It has been reported that K^+ takes part in maintaining a higher cytosolic K^+/Na^+ ratio, which is a key requirement for plant growth in high salt conditions (Kiarostami *et al*., 2010).

Conclusions

Stigmasterol can alleviate the adverse effects of salinity on V. faba plants, as indicated by its stimulatory effects on growth and development. These e ffects could be attributable to stigmasterol increasing the synthesis of protein, decreasing the levels of proline and phenolic compounds and correcting the nutritional disorders induced by salinity by decreasing Na^+ ion uptake and increasing K^+, Ca^{2+} and P^{3+} ion content in seeds relative to control and salinized seeds. In addition, as the nutritional value of faba beans has been attributed to their high protein content and being a good source of sugars and minerals, the results suggest that stigmasterol has a positive impact on the nutritional value of these seeds. Also noteworthy, the stimulatory effects persisted in plants pretreated with stigmasterol throughout their development as well as in the seeds they produced.

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تأثير للاستيجماستيرول على نمو، انتاجية و الاستجابة البيوكيميائية لنباتات الفول البلدى الناميه تحت الاجهاد الملحى

فردوس محمد بسيونى[1]، حنان احمد هاشم[2]، رئيفه احمد حسنين[2]، دينا محسن بركه[1] و رضوان رضوان خليل[1]
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الملوحه هي واحده من اهم أنواع إلاجهاد الغير حيوي التي تقلل من انتاجية العديد من المحاصيل. استهدف العمل في هذا البحث علي دراسة تأثير مستويات مختلفة من الملوحة (0، ، 100 ، 150 و 200 ملي مول كلوريد الصوديوم) مع أضافة الستيجماستيرول (500 ميكرومول) علي النمو، المحصول والتركيب البيوكيميائي (محتوى الكربوهيدرات، البرولين، البروتين الكلى، صورة البروتين و بعض المعادن) لبذور الفول البلدي. أظهرت النتائج ان زيادة الملوحه الى 200 مللي مول تسبب انخفاضا معنويا فى نمو ومحصول نبات الفول البلدي. كما تسبب الاجهاد الملحي في تراكم الفينولات والبرولين وايونات الصوديوم في البذور بينما انخفض محتوي البذور من الكربوهيدرات ، البروتين الكلي وايونات البوتاسيوم والكالسيوم والفسفور. عند المعاملة بنقع البذور فى الاستيجماستيرول ، تم ابطال التأثيرات الضارة للملوحة ولوحظ زياده معنوية في النمو، محتوي الكربوهيدرات ، البروتين الكلي وأيونات البوتاسيوم والكالسيوم والفسفورمقارنة بالنباتات الغير معاملة بالستيجماستيرول. بالأضافة الي ذلك تم تحديد مجموعه من البروتينات المستحثة في البذور الناتجة من نباتات سبق معاملتها بالستيجماستيرول وهذه البروتينات يفترض ان تعمل علي زياده تحمل الملوحة. وقد أشارت النتائج الي ان المعاملة بالستيجماستيرول لها تأثير ايجابي علي نمو وكمية وجودة المحصول لنبات الفول البلدي المتعرض لاجهاد الملوحة.