J. Plant Production, Mansoura Univ., Vol. 2 (4): 597 - 615, 2011

AMELIORATIVE EFFECT OF STIGMASTEROL ON THE PRODUCTIVITY OF *Vicia faba* PLANTS GROWN UNDER SALT STRESS.

Bassuony, Fardos M.*; Hanan A. Hashem; Raeifa A. Hassanein**; Dina M. Baraka* and R. R. Khalil***

*** Botany Dept., Fac. Science, Benha Univ., Benha, Egypt.**

**** Botany Dept., Fac. Science, Ain Shams Univ., Cairo, Egypt.**

ABSTRACT

The present investigation was carried out to study the effect of post-emergence soil treatment with different salinity levels (0.0, 100, 150 and 200 mM NaCl) on growth, endogenous phytohormones, yield components and some biochemical activities of the yielded seeds of *Vicia faba* plants. The results indicated that increasing of salinity up to 200 mM showed significant decreases in growth parameters at fruiting stage and yield components. On the other hand, the levels of IAA, GA_3 decreased, while ABA level increased with salt stress. Biochemical activities of the yielded seeds such as carbohydrate contents and total protein significantly decreased as compared with non-salinized plant, while salt stress induced the accumulation of the total phenol and proline contents. In addition, the content of Na^+ increased significantly under salinity stress, while K^+, Ca^{+2} and P^{+3} contents were decreased, when compared with those of the control. Application of stigmasterol by seed soaking, counteracted the adverse effects of salinity through inducing significant increases in all growth criteria at fruiting stage, yield components, levels of IAA, GA_3, soluble, insoluble and total carbohydrates, total protein concomitantly with decreases in total phenols, proline content and level of ABA as compared with those of the reference plants. Also, treatment with stigmasterol resulted mostly in a decrease of Na+ accumulation concurrently with significant increases of K^+, Ca^{+2}, P^{+3} contents, K^+/Na^+ and Ca^{+2}/Na^+ ratio when compared with those of the reference controls. These results indicate the role of stigmasterol in increasing the tolerance of *Vicia faba* plants to salt stress. Three prominent types of modifications were observed in the protein patterns of yielded seeds, some proteins were disappeared, other proteins were selectively increased and synthesis of a new set of protein was induced, some of these responses were observed under stigmasterol and salinity, while others were induced by either stigmasterol or salinity.

Keywords: *Vicia faba*, growth, yield, stigmasterol, phytohormons, proline, total phenol, protein profile, carbohydrate contents, cations

INTRODUCTION

Faba bean is widely used in the Mediterranean region as source of protein in both human and animal nutrition (Crépon *et al.*, 2010). The nutritional value of field bean has bean attributed to its high protein contents, which ranged from 25 to 35%. It is also a good source of sugars, minerals and vitamins (Larralde and Martinez, 1991). In addition Cultivation of faba bean leads to increase of soil nitrogenous compounds (Hungria and Vargas, 2000). In Egypt faba bean occupies a large area of cultivated land. Egypt is one of the countries that suffer from severe salinity problems. (El-Hendawy *et al.*, 2004).

Salt stress has threefold effect; viz. it reduces water potential and causes ion imbalance or disturbances in ion homeostasis and toxicity. This altered water status leads to initial growth reduction and limitation of plant productivity (Hagemann and Erdmann, 1997 and Hayashi and Murata, 1998). Under salt stress, shoot and root length, fresh and dry masses, of leaves, stems and roots were markedly decreased by increasing salinity level (Hassanein *et al.*, 2009a and Azooz, 2009). 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; Azooz, 2009). Stresses inhibit plant growth through their effects on the hormonal balance (Lerner and Amazallag, 1994). The decrease in growth promoting substances concomitantly with an increase in ABA content in response to salinity stress were observed by Hassanein *et al.*, (2009b). In this respect, El-Khallal *et al.*, (2009b) cleared that salt stress led to sharp changes in the balance of endogenous hormone levels through decreasing IAA, GA_3 and Zeatin significantly and increasing ABA level greatly.

Saccharides such as glucose, fructose, sucrose, and starch were accumulated under salt stress (Parida *et al.*, 2003). Their major functions are osmoprotection, osmotic adjustment, carbon storage and radical scavenging (Singh *et al.*, 2000; Abd El-samed, *et al.* 2004). On the other hand, in *Vicia faba*, salinity decreased soluble and insoluble sugars (Gadallah, 1999). Azooz *et al.*, (2002); Hassanein *et al.*, (2009a) and Hassanein *et al.*, (2009b) found that, salinity induced inhibitory effects on the biosynthesis of carbohydrate and free amino acid, but opposite effects were observed on the biosynthesis of protein and proline.

Plant can synthesize and accumulate phenolic compounds in response to different biotic and abiotic stresses including salinity (Dkhil and Denden 2010). Furthermore, Hichem *et al.*, (2009) reported that such variation in concentration of leaf phenolics within a plant under salt stress in relation to leaf age may be due to the reflection of different requirements for counteracting abiotic stresses at different growth stages. The antioxidant activity of phenolic compounds can play an important role in neutralizing ROS (Zheng and Wang, 2001).

Environmental stress cause important modification in gene expression (Soussi *et al.*, 2001). Gene expression is manifested by the appearance of new protein, which is not present before the stimulation. Salinity promotes the synthesis of salt stress- specific proteins; many of these proteins were suggested to protect the cell against the adverse effect of salt stress (Ben-Hayyin *et al.*, 1989, Hassanein *et al.*, 2009a). Azooz, (2004) proved that salinity stress led to the appearance of 67 and 26 KDa polypeptide (in cv. Dorado) and 45 KDa (in cv. Hagen shandawil). While, Hassanein *et al.* (2009b) observed that, five protein bands (M.wts. 94, 82, 73, 56 and 46 KDa) disappeared and also two protein bands (M.Wts. 31.97 and 20.5KDa) were *de novo* synthesized in response to salt stress.

Salinity stress caused a considerable increase in sodium content and decrease in potassium, calcium and magnesium ions content, which in turn reflected in the decrease in K^+/ Na^+, Ca^{++}/ Na^+, and Mg^{++}/ Na^+ ratios

(Hassanein *et al.*, 2009b and El-Khallal *et al.*, 2009a), and this may be due to the competition for sites through which influx of both cations occurs (Jescke and Wolf, 1998). Similar results were obtained by Azooz *et al.*, (2002) who showed that the content of Ca^{++} and K^{+} decreased significantly under salinity stress, and Na^{+} contents were intensively accumulated.

Stigmasterol belongs to brassinosteroids which are of the growth regulators activity and signaling molecules essential for normal plant growth along with auxins, cytokinins, gibberellins, abscisic acid and ethylene (Clouse, 1997). Steroids as a group of plant hormones had a regulatory significant function in cell elongation and division, vascular differentiation and other diverse developmental programs (Rao *et al.*, 2002 and Sasse, 2003).A remarkable feature of brassinosteroids is their potential to increase resistance in plants to a wide spectrum of stresses, such as low and high temperatures, drought, high salt and pathogen attack. Despite this, only a few studies aimed at understanding the mechanism by which brassinosteroids promote stress resistance have been undertaken (krishma, 2003).

The aim of this work was to investigate the changes in growth, endogenous phytohormones, productivity and some biochemical activities of yielded seeds of *Vicia faba* plant and the possible role played by stigmasterol in regulating salt-induced changes in these parameters.

MATERIALS AND METHODS

Pure strain of *Vicia faba* (Sakha 1) was obtained from Agriculture Research Center, Giza, Egypt. Stigmasterol was obtained from MP Biomedicals, LLC. France.

Experimental technique: Pot experiment was carried out under natural condition, in the green house of Benha university faculty of science. Plastic pots (40 cm in diameter) were used and containing 15 kg of a mixture of clay-sand (2:1 w/w) soil. Phosphorus and potassium fertilization were added before sowing at a rate of 6.0 and 3.0 g/pot in the form of calcium superphosphate (15.5 % P2O5) and potassium sulphate (48 % K2O), respectively. *Vicia faba* seeds were divided into two groups, seeds of the first group were soak in tap water, while those of the other group were soaked in stigmasterol solution (200ppm) for 12 hours at room temperature the soaked seeds were sown in the plastic pots. Ten seeds were sown in each pot at 3 cm depths. The pots arranged in completely randomized design after emergence, the seedlings (7 day-old) were thinned to 5 healthy seedlings per pot. Pots were kept in a green house under natural conditions of light with a 16 hours photoperiod and average 25/10 °C ± 3 day / night temperature.

Seedlings (15 days from sowing) resulted from soaking in water or stigmasterol solution were subjected to the desired salinization levels (0, 100, 150 and 200 mM NaCl). The test plants were irrigated with water (70 % water holding capacity). The plants were left to grow under the different salinization levels and stigmasterol treatments until the harvest. Ten replicates (planted pots) from each level of treatments were considered.

Plant samples were collected at vegetative stage (after 40 days) to extract and determinate the growth hormones, at fruiting stage (after 85 Days) to measure the growth parameters (number of leaves/ plant, leaf area (cm^2/plant), shoot and root length (cm/plant), fresh and dry weight (g/plant)) and finally at harvest stage (after 120 Days) to determine yield components (number of pods/plant, length of pod/plant, pod diameter, dry weight of each pod, number of seeds/pod, number of seeds/plant, and dry weight of seeds/ plant) and some biochemical activities of yielded seeds.

Determination of growth hormones: Acidic hormone (IAA, GA_3 and ABA) were carried out by the method described by Wasfy *et al.*, (1974).

Determination of biochemical activities of yielded seeds: Soluble sugar was extracted by using 80 % ethanol according to the method described by Homme *et al.* (1992) and determined by the anthrone sulphuric acid method described by Whistler *et al.* (1962).Polysaccharides were determined in the dry residue left after extraction of soluble sugars (Whistler *et al.*, 1962).

Free proline was determined in the yield seeds according to the methods described by Bates *et al.* (1973).

Total protein in the yield seeds was determined spectrophotometrically according to the method described by Bradfored (1976).

Electrophoretic determination of total protein was estimated according to their molecular weight by denatured sodium dodcyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by Laemmli (1970) as modified by Studier (1973).

Estimation of total phenol was carried out in the yield seeds with the Folin-Ciocalteau reagent (Malick and Singh, 1980).

Inorganic cations 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 flame emission technique as adopted by Ranganna (1977). Phosphorus and calcium were determined simultaneously by ICP spectroscopy according to the method of Soltanapour (1985).

The results were statistically analyzed using L.S.D. at 5% and 1% levels, of probability according to SAS program (1982). Three replicates were used in each parameter.

RESULTS AND DISCUSSION

Changes in endogenous phytohormones: The data given in table1 clearly indicate that salt stress caused marked significant decreases in both IAA and GA_3 contents and increases in ABA level of *Vicia faba* plant, as compared with those of the control. These results are in agreement with those of Mulholland *et al.* (2003); El-Khallal *et al.* (2009b) they observed that, Phytohormones play critical roles in regulating plant responses to salinity stress, through decreasing the endogenous growth hormones IAA, GA_3 and zeatin content and increasing ABA level markedly.

In this regard, the amount of ABA is determined by the dynamic balance between biosynthesis and degradation, and these two processes are influenced by environmental factors such as light, water stress, and other growth regulators (Cutler and Krochko, 1999; Wang *et al.*, 2001).

Application of stigmasterol in this study under the various levels of salinity led to high significant increases in the values of GA_3 and IAA concurrently with decrease in ABA level. In this respect, Shunquan *et al.* (2001) and El-Greedly and Mekki (2005) showed that stigmasterol treatment enhanced the biosynthesis of phytohormones which can play an important role as signals and regulators of growth and development of plants. The increase in IAA and GA_3 in *Vicia faba* plants concurrently with the increase in growth rate suggested the role of the endogenous hormones in stimulating the cell division and/or the cell enlargement and subsequently growth (El-Bassiouny, 2005 and Hassanein *et al*, 2009b).

Table (1): Interactive effect of salinity and stigmasterol on hormone contents (mg/100g F.Wt) of shoot of *Vicia faba* plants at the vegetative stage. Values are the mean of 3 replicates.

Treatment	NaCl mM	IAA	%	GA_3	%	ABA	%	IAA+GA3 / ABA
Reference control	00	6.80	100.0	62.0	100.0	9.70	100.0	7.09
	100	3.38	100.0	45.8	100.0	15.61	100.0	3.15
	150	3.05	100.0	27.0	100.0	20.37	100.0	1.47
	200	1.89	100.0	15.9	100.0	20.64	100.0	0.861
Stigmasterol 200 ppm + NaCl mM	00	6.90	101.4	66.0**	106.4	7.30**	75.2	9.98
	100	5.17**	152.9	58.0**	126.6	11.20**	71.7	5.64
	150	3.88*	127.2	33.4**	123.7	18.85**	92.53	1.97
	200	3.16**	167.1	25.7**	161.6	19.63**	95.1	1.47
L.S.D. at 5 %		0.625		2.199		0.462		-
L.S.D. at 1 %		0.889		3.713		0.611		-

*** Significant differences**

**** Highly significant differences as compared with reference controls**

Changes in growth parameters in fruiting stage:

The results of the present work (Table 2 and 3) show that most of the growth parameters as height of shoot, root length, number of leaves and their area/ plant, fresh and dry weights of shoots and roots of *Vicia faba* plants were highly significantly reduced with increasing the salinity level. The reduction in plant growth of saline stressed *Vicia faba* plant, in the present work might be attributed to the inhibitory effect of ABA which was induced by salinity on cell division and /or cell expansion (Hassanein, 2000) and /or resulted from the osmotic effect of salinity which caused disturbances in water balance of stressed *Vicia faba* plant leading to reduction in photosynthesis and consequently a retarded growth rate (Chaparzadeh *et al.*, 2004). Furthermore, Jaleel *et al.* (2008) found that, salinity affected all the morphological parameters and decreased growth performance of *Catharanthus roseus* treated with NaCl salinity. The deleterious effects of salinity on plant growth are associated with: (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress) or (4) a combination of these factors (Ashraf, 1994).

Application of stigmasterol in the present work improved growth of *Vicia faba* plants by causing, in most cases, significant increases in the values of the above growth parameters of salt stressed plants. The inhibitory effects of high levels of salinity were partially alleviated. This is probably by increasing the efficiency of water uptake and utilization as well as protecting the thylakoid membranes, and the photosynthetic apparatus.
It appears from these results that the stigmasterol used in this work might act as growth stimulants which could play a role in mitigation the adverse effect of NaCl on metabolic activities relevant to growth through enhancing cell division and/or cell enlargement (He *et al.*, 2003).

Table (2): Interactive effect of salinity and stigmasterol on shoot length, root length, no. of leaves, area of leaves, of *Vicia faba* Plants at harvest stage. Values are the mean of 3 replicates.

Treatment	NaCl mM	Height of shoot (cm/plant)	%	Root length (cm/plant)	%	No. of leaves per plant	%	Area of leaves (Cm^2 / plant)	%
Reference control	00	58.60	100.0	22.76	100.0	20.70	100.0	348.5	100.0
	100	49.70	100.0	17.40	100.0	18.70	100.0	278.8	100.0
	150	46.60	100.0	16.90	100.0	14.80	100.0	206.7	100.0
	200	36.20	100.0	13.80	100.0	5.20	100.0	49.5	100.0
Stigmasterol 200 ppm + NaCl mM	00	62.50**	106.6	24.66*	108.3	21.86	105.6	467.2**	134.0
	100	51.00	102.6	21.40**	122.9	20.0	106.9	367.0**	131.6
	150	48.70	104.5	17.20	101.7	15.92	107.5	236.8**	114.6
	200	42.00**	116.0	17.16**	124.3	6.8	130.7	89.7**	181.2
L.S.D. at 5 %		2.164		1.783		2.409		2.214	
L.S.D. at 1 %		2.980		2.456		3.319		3.050	

* Significant differences
** Highly significant differences as compared with reference controls.

Table (3): Interactive effect of salinity and stigmasterol on fresh and dry weights of shoot and root of *Vicia faba* Plants at harvest stage. Values are the mean of 3 replicates.

Treatment	NaCl mM	Shoot weight (g/plant)				Root weight (g/plant)			
		Fresh	%	Dry	%	Fresh	%	Dry	%
Reference control	00	15.03	100.0	2.83	100.0	3.616	100.0	0.271	100.0
	100	12.23	100.0	2.306	100.0	2.49	100.0	0.240	100.0
	150	10.17	100.0	1.902	100.0	1.998	100.0	0.198	100.0
	200	7.17	100.0	1.81	100.0	1.562	100.0	0.164	100.0
Stigmasterol 200 ppm + NaCl mM	00	16.59**	110.3	3.38**	119.4	3.64	100.6	0.362*	133.5
	100	12.73	104.0	3.06**	132.6	2.518	101.1	0.309	128.7
	150	11.35*	111.6	2.12*	111.4	2.13	106.6	0.231	116.6
	200	10.38**	144.7	2.015*	111.3	1.766	113.0	0.199	121.3
L.S.D. at 5 %		1.019		0.178		0.923		0.087	
L.S.D. at 1 %		1.404		0.246		1.271		0.120	

* Significant differences
** Highly significant differences as compared with reference controls.

Yield Parameters:

Changes in yield components: Data presented in table 4 reveal that the yield components as number of pods per plant, length of pod per plant, pod diameter, dry weight of each pod, number of seeds per pod, number of seeds per plant, and dry weight of seeds per plant of *Vicia faba* were significantly decreased with increasing salinity levels as compared with non-salinized plant.

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). So, The reduction in yield of stressed plants might be attributed to the decrease in photosynthetic pigments, carbohydrates accumulation (polysaccharides) and nitrogenous compounds (total nitrogen and protein) as well as the reduction in the rate of translocation of photo-assimilates from source (leaf) toward the sink (seeds) across the conductive canals (phloem) (Shah, 2007; Aldesuquy *et al.*,2009). Furthermore, crop growth reduction due to salinity is generally related to the osmotic potential of the root-zone soil solution. This will lead to certain phonological changes and substantial reduction in productivity (Abou-Hadid, 2003; Sohrabi *et al.*, 2008).

Table (4): Interactive effect of salinity and stigmasterol on yield components of *Vicia faba* plants at harvest stage. Values are the mean of 3 replicates.

Treatment	NaCl mM	No. of pods per plant	Length of pod per plant(cm)	Pod diameter (cm)	Dry wt. of each pods (g)	No. of seeds per pods	No. of seeds per plant	Dry weight of seeds/ plant
Reference control	00	3.4	7.84	4.96	2.062	3.3	11.22	6.95
	100	2.8	6.36	4.06	1.198	3.1	8.68	5.19
	150	2.8	4.87	4.0	1.146	2.9	8.12	3.08
	200	2.4	3.74	2.96	0.540	1.9	4.56	1.32
Stigmasterol 200 ppm + NaCl mM	00	3.8*	8.96**	5.5**	2.296**	3.9**	14.82**	12.74**
	100	3.8**	7.22**	4.56**	1.756**	3.5**	13.3**	10.24**
	150	3.2*	5.78**	4.08	1.474**	3.3**	10.56**	6.23**
	200	2.8*	5.10**	3.56**	0.938**	2.5**	7.0**	3.71**
L.S.D. at 5 %		0.346	0.455	0.328	0.124	0.123	0.729	0.205
L.S.D. at 1 %		0.477	0.626	0.452	0.170	0.165	1.004	0.282

*** Significant differences**

**** Highly significant differences as compared with reference controls**

Stigmasterol treatments generally induced a highly significant increase in the values of the yield components, as compared with those of the reference controls. The results clearly indicated that application of stigmasterol was significant in alleviating the adverse effects of salt stress on yield and yield components of *Vicia faba* plants. In support of the above result, Nassar (2004) at soy bean and El-Greedly and Mekki (2005) stated that, the increase in seed/plant of two sesame cultivars and the increase in

number and weight of capsules as well as 1000-seed weight at high stigmasterol concentration (200 ppm) might be due to the increment of growth regulators which improved photosynthetic activities, consequently this beneficially affect number and weight of capsules and seed yield.

Biochemical activities of the yielded seeds.

Changes in carbohydrate contents: The data recorded in the present work (Table 5) clearly indicate that carbohydrate fractions (soluble and insoluble) as well as total carbohydrate content in the yielded seeds of *Vicia faba* plant treated with NaCl were highly significantly decreased with increasing salinity level as compared with the non-salinized plants.

The reduction in total carbohydrates in the seeds of broad bean plants under the various level of salinity concomitantly with the reduction in growth rate led to the conclusion that sodium chloride might inhibit the photosynthetic activity and/or increased partial utilization of carbohydrates into other metabolic pathways (Hassanein, 2000). In this respect, Plaut *et al.* (1990) found that, the decrease in net CO2 assimilation was attributed to NaCl effect on plant water status. Moreover, Brugnoli and Lauteri (1991) and singh and Dubey (1995) attributed the overall reduction of growth parameters to higher sensitivity of photosystem II and Hill reaction activity to salinity stress which resulted in reduction of photosynthetic capacity in different saline stressed plants.

Table (5): Interactive effect of salinity and stigmasterol on carbohydrate contents (mg glucose/100g D.Wt) of *Vicia faba* seeds at harvest stage. Values are the mean of 3 replicates.

Treatment	NaCl mM	Total soluble sugar		Insoluble sugar		Total carbohydrate	
Reference control	00	2139.4	100.0	11026.2	100.0	13165.6	100.0
	100	850.28	100.0	10683.4	100.0	11533.6	100.0
	150	827.42	100.0	9823.9	100.0	10651.3	100.0
	200	548.57	100.0	9613.6	100.0	10162.1	100.0
Stigmasterol 200 ppm + NaCl mM	00	2271.9**	106.1	11830.8**	107.2	14102.7**	107.1
	100	1421.7**	167.2	11711.9**	109.6	13133.6**	113.8
	150	1115.4**	134.8	10564.5**	107.5	11679.9**	109.6
	200	1051.4**	191.6	10134.8**	105.4	11186.2**	110.0
L.S.D. at 5 %		42.029		38.952		39.006	
L.S.D. at 1 %		57.889		53.650		53.725	

*** Significant differences**

**** Highly significant differences as compared with reference controls**

Application of stigmasterol resulted in a highly significant increase in the contents of soluble, insoluble and total carbohydrates in seeds of *Vicia faba* plant as compared with those of the reference controls. Where the maximum contents of soluble, insoluble and total carbohydrates were about 191.6%, 109.5% and 113.8%, respectively. These positive results in the accumulation of carbohydrate constituents particularly the soluble sugars in

the seeds of salt affected *Vicia faba* plants either via increasing endogenous levels of certain phytohormones or by acting as activators of carbohydrate synthesis in leaves, which induced significant promotive effect on growth parameters of fruiting stage (Abd-El Wahed, *et al.*, 2000 and 2001; Nassar, 2004). Moreover, accumulation of carbohydrate play a key role in alleviating the salinity stress, either via osmotic adjustment (Ackerson, 1985) or by conferring some desiccation resistance to plant cells (Srivastava, *et al.*, 1995).

Changes in total protein, total phenol and proline contents.

The data presented in table 6 indicate that salt stress induced inhibitory effect on the production of protein content and stimulatory effect on the proline content in the yielded seeds of salinized plant as compared with control (Azooz, 2004). These results can be attributed to the decrease in protein synthesis and/or to the increase in its degradation. The degradation of protein under salinity condition was supported by our results which revealed the accumulation of proline contents. The accumulation of proline with the rise of salinity level are in agreement with the results obtained by Kavi *et al.* (2005), Mekki and Orabi (2007) and Mahmoud and Mohamed (2008) they reported that, accumulation of proline due to salinity stress, was to protect the cell by balancing the osmotic strength of cytosol with that of vacuole and external environment. Proline and total polyamine accumulation could be a protective response, not only due to the osmoprotectant role that prevents a salinity water deficit stress, but also for the radical scavenger and protein stabilization properties. The synthesis of proline is a gene-regulated process that involves the activation of genes of its biosynthesis and down regulation of genes of its degradation (Sumithra and Reddy, 2004).

Table (6): Interactive effect of salinity and stigmasterol on proline, total protein and total phenol contents of *Vicia faba* seeds at harvest stage. Values are the mean of 5 replicates.

Treatment	NaCl mM	Proline (mg/g D.Wt.)		Total protein (mg/100g D.Wt)		Total phenol (mg phenol/100g material)	
Reference control	00	2.657	100.0	15300.7	100.0	536.45	100.0
	100	3.197	100.0	12143.1	100.0	535.03	100.0
	150	4.854	100.0	7398.5	100.0	638.70	100.0
	200	6.420	100.0	6104.9	100.0	624.63	100.0
Stigmasterol 200 ppm + NaCl mM	00	2.170	82.60	19127.0**	125.0	523.17**	97.52
	100	2.315*	72.41	17011.6**	140.0	470.23**	87.88
	150	4.552	93.77	11520.8**	155.7	489.51**	76.64
	200	6.236	97.13	8914.2**	146.0	446.17**	71.42
L.S.D. at 5 %		0.731		42.029		21.2	
L.S.D. at 1 %		1.062		57.889		29.2	

* Significant differences

** Highly significant differences as compared with reference controls

Application of stigmasterol under the various levels of salinity resulted in a highly significant increase in protein contents and a non-significant change in proline level, except for the plants treated with 100 mM NaCl which induced a significant decrease as compared with those of the reference controls. In addition the maximum contents of protein were about 155.7% and minimum contents of proline were about 72.41% when compared with reference controls (Abd El-Wahed *et al.,* 2001; Ali *et al.,* 2002). Thus, it could be suggested that the inhibitory effect of salinity stress was alleviated by stigmasterol treatments through inhibiting proline synthesis and/or enhancing the biosynthesis of other amino acids and their incorporation into protein.

Total phenol: results of the present work (Table 6) showed accumulation in the total phenol contents with the increase in salinity levels as compared with those of the control (Ayaz *et al.,* 2000). The synthesis of phenolics is generally affected in response to different biotic and abiotic stresses including salinity (Parida *et al.,* 2004), Dostanova *et al.* (1979) and Latha *et al.* (1989) found that, phenol accumulation in tolerant genotype could be a cellular adaptative mechanism for scavenging the free radicals of oxygen and preventing subcellular damage during stress. Perhaps high accumulation of phenolics at the reproductive stage occurs due to their putative role in reproduction (Bravo, 1998). Furthermore, Hichem *et al.* (2009) reported that variation in concentration of leaf phenolics within a plant under salt stress in relation to leaf age may be due to the reflection of different requirements for counteracting a biotic stresses at different growth stages.

Application of stigmasterol under the various levels of salinity caused a significant decrease in phenol content in seeds of *Vicia faba* plants, as compared with those of reference controls. Therefore, one could suggest that treatment with stigmasterol could alleviate the adverse effect of salinity on growth and metabolic activities through decreasing the build-up of active oxygen species and thereby increasing resistance to salt stress (Abd El-Wahed *et al.,* 2003).

Protein profiles: in the present work (plate 1) two types of modification were observed in the protein patterns of *Vicia faba* seeds, some proteins were disappeared and certain of other proteins were *de novo* synthesized, some of these responses were observed under salinity and stigmasterol treatments, while others were induced by either stigmasterol or salinity.

Six protein bands of molecular weights 341.6 KDa (at 100 and 200 mM NaCl), 289.7, 155.6, 90.12 KDa (at 150 and 200 mM NaCl), 16.62 KDa (at 100 and 150 mM NaCl), 75.73 and 16.67 KDa were *de novo* synthesized in yielded seeds of *Vicia faba* plant grown under salinity stress. It has been suggested that these protein have an osmoprotection function or protected cellular structures (Zorb *et al.* 2004 and Bassuony *et al.,* 2008). In addition protein bands of molecular weights 312.26, 191.31, 117.39 and 72.31 KDa disappeared in seeds of salinized *Vicia faba* plant. Stigmasterol treatments induced the synthesis of both 117.39 and 72.31 KDa in salinized *Vicia faba* seeds. Also stigmasterol treatments induced the synthesis of three new polypeptides of molecular weights 181.61, 90.12 and 16.67 KDa and disappearance of four protein bands, their molecular weights are 362.48,

229.24, 191.31 and 117.39 KDa in yielded seeds of non-salinized *Vicia faba* plants. These results added support to the result obtained by Esaka *et al.*, (1992), Uma *et al.*, (1995), Bassuony *et al.*, (2008), Hassanein *et al.*, (2009a).

Protein profile of broad bean seeds indicated that stigmasterol might regulate the expression of salt stress inducible proteins as well as induced *de novo* synthesis of specific polypeptides, which are anticipated to play an active role in salt resistance.

Brassinolide altered gene expression involved in the stimulation of protoplasmic drought tolerance in leaf cells and induced changes in protein profile in response to water and salt stress (Ghasepour *et al.*, 1998, El-Khallal and Nafic, 2000). The formation of specific protein bands in response to NaCl, brassinlide and salicylic acid appeared to be a reflection in alteration of gene expression machinery along the genomic make up DNA (El-Khallal *et al.*, 2009b).

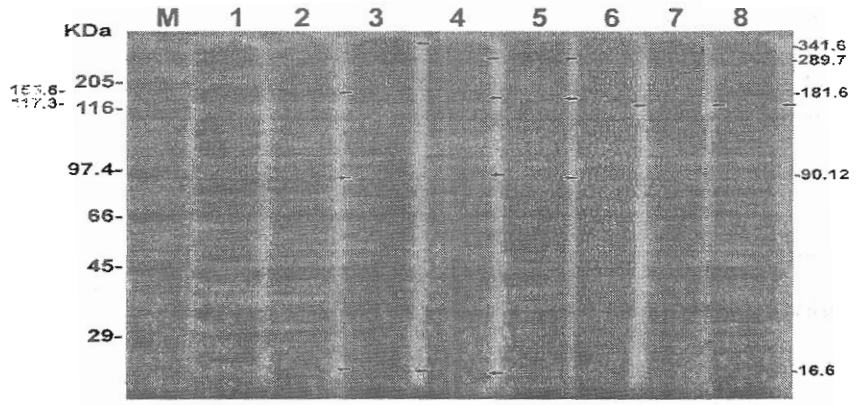

Plate (1): Electrograph of soluble protein pattern by one dimensional SDS-PAGE showing the change of protein bands (marked by arrowheads) in response to salinity and/ or stigmasterol of *Vicia faba* seeds at yield. Each lane contains equal amounts of protein extracted from *Vicia faba* seeds.

Lane M protein markers
Lane 1 control (H_2O)
Lane 2 200ppm stigmasterol
Lane 3 100 mM NaCl
Lane 4 150 mM NaCl
Lane 5 200 mM NaCl
Lane 6 100 mM NaCl + 200 ppm stigmasterol
Lane 7 150 mM NaCl + 200 ppm stigmasterol
Lane 8 200 mM NaCl + 200 ppm stigmasterol

Inorganic cations: the contents of Na^+ gradually increased, while the contents of K^+, Ca^{+2} and P^{+3} decreased in yielded seeds of *Vicia faba* plant with increasing salinity level (Table 7), which in turn reflected in the decrease in K^+/Na^+ and Ca^{+2}/Na^+ ratio (Table 7) as compared with non-salinized plants

(Zaho and Ren, 2007 and Kiarastami *et al.,* 2010). Sairam *et al.* (2002) and El-Khallal *et al.* (2009a) have suggested that Na^+ accumulation in salt stressed plants led to low water potential, change in essential ion uptake and ionic imbalance and reduces leaf expansion, photosynthetic rate and limit growth. Results revealed that salt stress affects many metabolic and growth aspects mainly due to the competition between Na^+ and K^+ on active metabolic sites leading to depressed growth expressed as dry matter production (Rivelli *et al.,* 2002; Tester and Davenport, 2003; Parida *et al.,* 2004).

Application of stigmasterol under the various levels of salinity caused a reduction in the accumulation of Na^+ and stimulated the accumulation of K^+, Ca^{++} and P^{+++} and this led to increase in K^+/Na^+ and Ca^{+2}/Na^+ ratio, as compared with that of untreated plant with stigmasterol. Our results supported by Shabala *et al.* (2005) and El-Khallal *et al.* (2009a) who found that, presoaking and spraying with brassinosteroids were effective in counteracting the toxic level of Na^+ by increasing mineral uptake and utilization. Also, increase in K^+ uptake in shoots could be important for osmotic adjustment and enzyme activity. It had been reported that K^+ takes part in maintaining a higher cytosolic K^+/Na^+ ratio, which is a key requirement for plant growth and high salt conditions

Table (7): Interactive effect of salinity and stigmasterol on inorganic cation of *Vicia faba* seeds at harvest stage. Values are the mean of 3 replicates.

Treatment	NaCl mM	Mineral Content (mg/g dry weight)								Ratio of mineral	
		Na+	%	K+	%	Ca++	%	P+++	%	K+/Na+	Ca++/Na+
Reference control	00	2.71	100.0	6.12	100.0	1.25	100.0	1.93	100.0	2.258	0.461
	100	3.55	100.0	5.33	100.0	0.953	100.0	1.58	100.0	1.501	0.268
	150	3.92	100.0	4.16	100.0	0.812	100.0	1.13	100.0	1.061	0.207
	200	4.28	100.0	4.00	100.0	0.773	100.0	0.816	100.0	0.934	0.180
Stigmasterol 200 ppm + NaCl mM	00	1.06*	39.11	6.50	106.2	1.64**	131.2	3.77**	195.3	6.132	1.547
	100	1.85*	52.11	5.78	108.4	1.08**	113.3	2.49*	157.5	3.124	0.583
	150	2.09*	53.31	5.10*	122.5	0.962**	118.4	2.22**	196.4	2.440	0.460
	200	2.14**	50.0	4.38	109.5	0.901**	116.5	1.54	188.7	2.046	0.421
L.S.D. at 5 %		1.025		0.572		0.068		0.907			
L.S.D. at 1 %		1.981		1.118		0.093		1.051			

*** Significant differences**
**** Highly significant differences as compared with reference controls**

In conclusion, stigmasterol can mitigate the adverse effect of salinity through increasing the contents of IAA and GA_3 and decreasing ABA level which may be involved in protecting the photosynthetic machinery and there by increasing the carbohydrate contents and the growth rate. Also stigmasterol could alleviate the adverse effect of salinity through increasing the synthesis of protein and decreasing proline & phenolic compounds and

corrects the nutritional disorders induced by salinity by decreasing Na^{+} ions and increasing K^{+}, Ca^{+2} and P^{+3} ions contents in the yielded seeds over those of control and salinized ones.

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التأثير الايجابى للاستيجماستيرول على انتاجية الفول البلدى النامى تحت الاجهـــاد الملحى

فردوس محمد بسيونى*، حنان احمد هاشم**، رئيفه احمد حـــسنين**، دينـــا محـــسن بركه* و رضوان رضوان خليل*

* قسم النبات – كلية العلوم – جامعة بنها

** قسم النبات – كلية العلوم – جامعة عين شمس

استهدف العمل فى هذا البحث على دراسة تأثير معالجة التربه بعد الانبات مع مستويات ملوحه مختلفة (٠،٠، ١٠٠، ١٥٠، ٢٠٠ ملليمول كلوريد صوديوم) على النمو، الهرمونات النباتية، صفات المحصول وبعض الانشطة البيوكيميائية فى محصول البذور الناتج متمثل فى (محتوى الكربوهيدرات، البرولين، البروتين الكلى، صورة البروتين، الكاتيونات الغير عضوية) فى الفول البلدى. لقد اشارت النتائج الى ان زيادة الملوحه الى ٢٠٠ ملليمول اظهرت انخفاضا فى صفات النمو فى مرحلة الاثمار وصفات المحصول. من ناحية اخرى حدث انخفاضا فى محتوى اندول حمض الخليك وحمض الجبريليك فى حين زاد مستوى حمض الابسيسيك مع الاجهاد الملحى. حققت الانشطه البيوكيميائيه فى محصول البذور الناتج مثل محتويات الكربوهيدرات والبروتين الكلى انخفاضا بشكل ملحوظ مقارنة مع النباتات الغير معامله بالملوحه. بينما حث الاجهاد الملحى على تراكم محتوى البرولين. بالاضافة الى ذلك، زيادة محتوى الصوديوم بشكل كبير تحت اجهاد الملوحه ، فى حين انخفضت محتويات البوتاسيوم، الكالسيوم والفوسفور بالمقارنة مع النباتات غير معامله (الكنترول). وعند المعاملة بنقع البذور فى الاستيجماستيرول(بتركيز ٢٠٠ جزء من المليون) لتخفيف الضرر الناتج عن التاثير العكسى للملوحة ادى الى زيادة معنويه فى كل صفات النمو فى مرحلة الاثمار، صفات المحصول الناتج، مستويات اندول حمض الخليك، حمض الجبريليك، السكريات الذائبه وغير الذائبه والكلية، والبروتين الكلى وانخفاض فى الفينول الكلى، البرولين ومستوى حمض الابسيسيك بالمقارنة مع النباتات المعامله بدون الاستيجماستيرول. كذلك، ادت معظم المعاملات مع الاستيجماستيرول الى انخفاض فى الصوديوم وزيادة معنويه فى محتويات البوتاسيوم، الكالسيوم والفوسفور، وذلك بالمقارنة مع النباتات المعامله بالملوحه فى غياب الاستيجماستيرول. وقد لوحظ ثلاثة تغيرات اساسية فى صورة البروتين، حيث ان بعض البروتينات اختفت والبعض الاخر زادت كثافته كماظهرت بعض البروتينات الجديدة نتيجة للاستيجابه للمعامله بالاستيجماستيرول والملوحه معا بينما البعض الاخر تكون نتيجة للمعامله اما بالاستيجماستيرول او الملوحه كلا على حده.

قام بتحكيم البحث

أ.د / محمود محمد درويش — كلية الزراعة – جامعة المنصورة

أ.د / هاله محمد صفوت البسيونى — مركز البحوث الزراعية