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PHYSIOLOGICAL EFFECT OF STIGMASTEROL ON SALINITY CONTROL OF SUGAR BEET (*Beta vulgaris,* L.)

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ABSTRACT

The promotive role of stigmasterol under salinized soil level is well documented in this report to elucidate the physiological effect of stigmasterol (10^{-6} and 10^{-4} M) to overcome soil salinity (0.59, 0.67 and 0.96m mos) on growth aspects, yield, photosynthetic pigments and sugar contents in sugar beet organs (*Beta vulgaris,* L.) variety Rass poly. Stigmasterol was added during vegetative and root growth stages.

Growth measurements of sugar beet plant organs as top (leaf area, fresh and dry weights) and root (diameter and fresh weight) significantly reduced with increasing salinity levels of soil, while, root length was insignificantly increased during root growth and sugar accumulation stages.

Stigmasterol significantly increased top and root measurements of sugar beet plant specially 10^{-6} M at root growth and 10^{-4} M at sugar accumulation stages except leaf area and top fresh weight at sugar accumulation stage that were significantly reduced. These concentrations gave the same trend with salinity levels specially 0.69 m mos at root growth and 0.96 mmos.

Salinity levels with or without stigmasterol significantly increased chlorophyll (a and b) contents with increasing both, while carotene content with salinity levels or its interaction with stigmasterol was insignificantly increased, however it significantly increased with stigma sterol application alone. Leaves soluble sugar were significantly increased by salinity or stigmasterol compared with control at root growth stage and insignificantly at sugar accumulation stage, also with interaction especially with (10^{-6} M) of stigmasterol. Salinity levels insignificantly decreased nonsoluble sugar at both stages. However, it significantly increased with stigmasterol concentrations during both stages with salinity levels. So, total sugar content of leaves was significantly increased at root growth stage and reduced at sugar accumulation stage with salinity levels. While, stigmasterol or its interaction with salinity significantly increased it at all stages compared with control.

Increasing salinity levels increased sugar contents of root compared with control at both stages, sucrose and glucose at sugar accumulation stage were significantly increased. Stigmasterol had the same trend of salinity but there was significant effect on total sugars, sucrose, glucose and TSS at sugar accumulation stage. However, sucrose and glucose were significantly increased with increasing salinity levels with stigmasterol concentrations (10^{-4} and 10^{-6} M).

Stigmasterol concentration (10^{-4} M) was more effective to overcome salinity effect and increase sucrose content of sugar beet root.

Keywords: Stigmasterol, salinity, sugar beet, growth, sugars.

INTRODUCTION

Sugar beet is primarily a crop of temperate climates, but, in the last decades its cultivation has spread to sub tropical sugar crop to augment sugar cane production. The main problem that hinders its growth and production is its cultivation in lower soil fertility in Egypt. This return to salinity effect on plant growth and yield to various degrees depending on plant species, salinity level and ionic composition of the growth medium (Kent and lauchli, 1985). Salinity caused an increase of glucose, fructose, soluble carbohydrate and change in sucrose content, but, it reduced growth and elongated tissue of growing leaves. Nevertheless it had no effect on photosynthesis (Dey and Dixon, 1985). Root elongation is vital for the plants to survive salinity (Ashraf, *et al*., 1986). Salinity provoked a decrease in the hexose contents and also dramatically enhanced sucrose accumulation in all organs of the plants. It had fast changes in soluble carbohydrates, (Balibrea *et al.,* 1997). Stress may reduce root yield and the rate of photosynthesis, sugar juice content and sterols of sugar beet, but it increased content of certain melassigenic compounds (Rover, 1998 and Yahya, 1998). So, salility provoked a decrease in the hexose contents and it also dramatically enhanced sucrose accumulation in all organs of tomato plants to a greater extent in the salt sensitive variety (Balibrea *et al*., 1997). Additionally, the use of saline water to irrigate sugar beet decreased percentage sugar, sugar yield, (Kaffka *et al*., 1999) and growth Rajasekaran *et al*., (2000). So, cytological and physiological characters of sugar beet plants were accomplished with tolerant plant to salt treatment that were obtained through somatic embryogensis and organogensis processes by Moghaddam *et al*., (2001).

Stigmasterol is found as a free or compound in the cell. They are structural components of the lipid core of cell membranes and being precursor of numerous secondary metabolites including plant steroid hormones (Genus, 1978). Much less the biological functions of steryl conjugates such as fatty acid or glucoside esters and steryl acyl glucoside were as a sterol storage forms. Additionally, it has been suggest that the interaction of sterols with phospholipids stablizies membrane controls permeability (Grunwald, 1982). In this respect the role of sterols is of particular interest. It has been shown that membrane sterol composition seems to have an effect on the activity of H^+-ATP ases in plant (Cooke *et al*., 1989). Whereas, brassinosteroid induced inclination was accompanied by increasing lamina fresh weight, total water content, free water content, proton extrusion and decreased bound water content. Also, brassinosteroids play a role in plant developments including cell expansion, vascular differentiation, etiolation and reproductive development (Steven and Jenneth, 1998). In this respect Ozolina, *et al*. (1999) found that epibrassinosteroide was shown to relgulate the hydrolytic and transport activities of tonoplast phosphohydrolases. H^+-pyrophosphatase was sensitive during the dormancy period. Whereas H^+-ATPase was sensitive during growth and accumulation of metabolities. The transport function of the phosphohydrolases was stimulated to a grater extent than the hydrolytic function. The most effective

epibrassinolide concentration was in the range 10^{-13}-10^{-11}M. So, brassinolide stimulated mesocotyl, coleoptile and leaf sheath elongation and increased the number of leaves (Chon *et al*., 2000).

The aim of this investigation is to study the effects of stigmasterol on soil salinity level control, vegetative and root characters and their sugar contents.

MATERIALS AND METHODS

The experiment was conducted on salinized loamy soil at green house of National Research Centre, Cairo, Egypt in 1996/97 and 1997/98 seasons. Sugar beet (*Beta vulgaris,* L.) variety (Rass poly) was hand seeded on 29 and 20 November, in the two seasons respectively, at pots N. 30. Seedlings were thinned to two plant at each pot. Calcium superphosphate fertilizer (15.5% P_2O_5) was added pre sowing at 10 g/pot during vegetative stage. Also, nitrogenous fertilizer as urea (46% N) was applied at 15 g/pot divided into two equal doses after 21 and 35 days from sowing. Potassium sulphate (48% K_2O) was added at 10 g/pot after 21 and 35 days from sowing.

Soil Salinity levels:

Salinity treatments consisted of three levels (0.59, 0.67 and 0.96 m mos) of salinized soil compared with control (0.36 m mos). Soil salinity concentrations were obtained from treating loamy soil with different concentrations of saline water of Rashidy salt.

Stigmasteral treatments:

The treatments consisted of two concentration (10^{-4} and 10^{-6}M) of stigmasterol (stigmasta-5,22-diene-3 β-oL: (24 S)-24- ethylcholesta 5,22-dien-3 β-oL) and control with distilled water which was added as foliar application at towic spray with the previous concentrations at vegetative stage (45 days from planting) and root growth stage (75 days from sowing).

The experiment was arranged as split plot design keeping salinity levels in main plots and stigmasterol treatments in the sub plots replicated three times.

Growth and yield measurements:

The estimated root characteristics were diameter, length and fresh weight. Top characteristics were leaf area, fresh and dry weights. Three plants were taken to determine the measurements on root and leaves at root growth (after 75 days from sowing) and sugar accumulation stages (after 180 days from sowing).

Chemical constituents:

Photosynthetic pigments (Chl.a and Chl.b) were determined in fresh leaves according to Lichtenthalerand Wellburn (1983) and carotene according to (Booth , 1958). Root and leave sugars contents as total, soluble sugars, glucose and nonsoluble sugar were determined according to Dubois *et al*., (1956), sucrose content according to Frederick *et al*., (1949) and Frank (1952) and total soluble solids content in root juoice by hand refractometer.

Combined analysis of variance for data collected in both seasons were calculated as described by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect of salinity levels on top characteristics :

Data presented in Table (1) show that top characteristics (leaf area, fresh and dry weights) were significantly affected by salinity level of soil except top fresh weight at sugar accumulation stage. The lowest level of salinity (0.59 M mos) gave the least decreasing on all top characteristics at both root growth and sugar accumulation stages. These characters were insignificantly decreased with enhancing salinity level of soil. Salinity might seemed to cause a stress on the imbibition ability of water and nutrients requirements from the roots to top organs, thus it reduced growth and elongation of growing leaves tissue (Dey and Dixon, 1985). This might be due to the involvement of activated oxygen species in the mechanism with celluar toxicity of NaCl and pointed out differences in the induction of antioxidant defences in wheat (Meneguzzo *et al*., 1999). This effect was return to maintenance of cell turgor accomplishing through osmoregulation of solutes within the cells (Morgan, 1984). Whereas, brassinosteroid (24-epibrassinolide) promoted cell expansion in whole plants and excised organs, (Lee *et al*., 2000).

Table 1: Effect of salinity levels on the growth characters of sugar beet plants.

Top characteristics

Characters	Root growth stage			Sugar accumulation stage		
Salinity level M mos (1:5)	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)
Control	2233.0	152.95	12.25	9018.6	158.73	46.85
0.59	2651.4	171.15	14.2	6298.4	155.52	38.88
0.67	1929.3	144.75	12.8	5392.6	142.36	32.24
0.96	1842.9	140.70	13.13	5330.7	135.33	35.81
LSD at 5%	50.39	24.38	1.14	7.99	NS	3.33

Root characteristics

Characters	Root growth stage			Sugar accumulation stage		
Salinity level M mos (1:5)	Diameter (cm)	Length (g)	Fresh weight (cm)	Diameter (cm^2)	Length (cm)	Fresh weight (g)
Control	2.25	19.0	54.6	6.86	22.17	266.33
0.59	3.17	19.17	77.28	7.29	21.94	308.18
0.67	2.08	17.83	44.90	6.61	21.61	322.17
0.96	2.18	19.67	44.50	6.61	22.83	271.53
LSD at 5%	0.15	NS	9.13	NS	NS	NS

Effect of salinity levels on root characteristics :

Results presented in Table (1) showed that soil salinity levels had significant effect on root characteristics of sugar beet plant viz, diameter and fresh weight at root growth stage and insignificant effect at sugar accumulation stage and other characters. Root diameter and fresh weight were significantly decreased where salinity levels increased. While, root length gave the opposite trend in the two stages. Root fresh weight declined with enhancement of salinity levels. This effect might be due to decreased phospholipids, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylglcerol as a consequence of saline treatment as well as sistosterol: stigmasterol ratio in bean root (Cachorro *et al*., 1993). It seemed that these levels of salinity don't significantly affect root development. This might be due to biochemembranes plant plasma membrane, which contains ATP-ase as it's major in pump is a barrier controlling the process of transpority external salt into the cell (Brown and Dupont, 1989). Additionally, it generates an electric potential and pH gradient across the plasma membrane by extruding protons from the cell forms, the proton force that promote the secondary active transport across plasma membrane (Palmgren, 1991). In this connection, salinity caused a reduction in membrane potential, disturbed homeostasis, and loss of absorption capacity (Pakhomova, 1996). Increasing external concentrations of NaCl in media, root elongation was suppressed (Yermiyahu *et al*., 1997). So, water salinity did not affect overall clear root yield (Kaffka *et al*., 1999).

Effect of stigmasterol treatments on top characteristics :

Top characteristics viz, dry weight/ plant was significantly increased with stigmasterol application at root growth stage as shown in Table (2). While, leaf area and dry weight/plant these were significantly reduced compared with control at sugar accumulation stage. Magnitude values were obtained with the concentrations (10^{-6} and 10^{-4}M) at the two developmental stages. It might be due to brassinosteroid indication through increasing lamina fresh weight, total water, free water contents and proton extrusion. In addition to, vascular differentiation, etiolation and reproductive development (Steven and Jenneth, 1998), which might increase the translocation of plant requirements of soil to plant organs.

Effect of stigmasterol treatments on root characteristics :

Data presented in Table (2) showed that root characteristics as, diameter and fresh weight were significantly increased with stigmasterol application at (10^{-6}M) concentration during root growth stage. While, stigmasterol concentration (10^{-4}M) gave the same effect on root diameter and length during sugar accumulation stage. Root fresh weight/plant was insignificantly increased with stigmasterol application. The used concentrations seemed to be sufficient to provide sugar beet plant with it's needs of stigmasterol to consequent root growth. This positive effect might be due to brassionsteroide role on plant developmental including cell expansion, and etiolation (Steven and Jenneth, 1998). In this concern, Abd El-Wahed (2001) found that root fresh and dry weight and root number

increased with sitosterol application to maize plant.

Table 2: Effect of stigmasterol treatments on the growth haracters of sugar beet plants.

Top characteristics

Characters	Root growth stage			Sugar accumulation stage		
Stigmasterol treatments M	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)
Control	2233.0	152.95	12.25	9018.6	158.73	46.85
10^{-6}	2242.4	170.43	13.43	3862.5	121.43	27.64
10^{-4}	1948.1	133.22	14.45	5029.4	153.14	31.81
LSD at 5%	NS	NS	1.01	7.79	NS	3.58

Root characteristics .

Characters	Root growth stage			Sugar accumulation stage		
Stigmasterol treatments M	Diameter (cm)	Length (Cm)	Fresh weight (g)	Diameter (cm)	Length (cm)	Fresh weight (g)
Control	2.25	19.0	54.6	6.86	22.17	266.33
10^{-6}	3.05	20.33	66.55	7.33	22.00	324.57
10^{-4}	2.18	17.33	45.53	7.60	22.20	311.1
LSD at 5%	0.28	NS	11.59	NS	NS	NS

Effect of stigmasterol treatments under soil salinity levels on top characteristics:

The results presented in Table (3) showed that top characteristics (leaf area, fresh and dry weights) were significantly decreased with increasing soil salinity levels as well as stigmasterol concentrations during root growth stage. These characters were more affected with stigmasterol concentration (10^{-6} M) to protect sugar plant from soil salinity level injures. However, the maximal values of these characters were obtained with stigmasterol concentration (10^{-4} M) during sugar accumulation stage that significantly increased root dry weight.

Table 3: Effect of stigmasterol treatments under soil salinity levels on top characters of sugar beet plants.

Treatments	Stigmasterol M	Root growth stage			Sugar accumulation stage		
Salinity levels m mos (1:5)		Leaf area (cm^2)	Weight (g)		Leaf area (Cm^2)	Weight (g)	
			Fresh	Dry		Fresh	Dry
Control	00	2233.6	152.95	12.25	9018.6	158.73	46.85
0.59	10^{-6}	3141.5	188.35	13.60	5667.3	187.63	38.80
	10^{-4}	2579.0	172.15	16.75	4209.2	120.63	33.00
0.67	10^{-6}	1891.6	164.8	14.95	2600.9	85.73	20.27
	10^{-4}	1663.2	131.5	11.20	4558.4	182.60	29.60
0.96	10^{-6}	1694.0	158.15	11.75	3319.6	90.93	25.87
	10^{-4}	1601.6	111.0	15.40	6320.4	156.63	32.83
LSD at 5%		182.68	30.65	1.88	35.6	76.28	28.29

Stigmasterol effect on salinity levels might be due to increased sterol content in seedling. In addition, saline treatments caused a reduction in phospholipids, phosphatidylcholine, phosphatidyle thanolamine, phosphatidylserine and phosphatidylglycerol as well as sitosterol: stigmasterol ratio, (Cachorro *et al*., 1993). This effect could be recovered by stigmasterol application which is precursor of numerous secondary metabolites including plant steroid hormones (Genus, 1978).

Effect of stigmasterol treatments under soil salinity levels on root characters:

Root fresh weight was significantly decreased with increasing soil salinity levels as well as stigmasterol concentrations during root growth stage and root diameter at sugar accumulation stage as presented in Table (4). However, root length gave the opposite response to stigmasterol application under salinity levels. These results showed that stigmasterol concentrations (10^{-6}M and 10^{-4} M) were more effective on salinity injury of root diameter and fresh weight or length at root growth stage. While, stigmasterol concentration (10^{-4} M) gave the best control on soil salinity level at sugar accumulation stage. Length and fresh weight of root insignificantly increased with increasing stigmasterol concentration under salinity levels compared with control at sugar accumulation stage, while, it gave the opposite trend with root diameter. This effect might be due to a reduction in growth and elongation of tissues caused by salinity stress (Dey and Dixon, 1985 and Ashraf *et al*., 1986), which avoid by stigmasterol application. In this connection, sitosterol increased roots number, length, relative multiplication rate and relative extension rate of maize roots (Abd El-Wahed, 2001).

Table 4: Effect of stigmasterol treatments under soil salinity levels on root characters of sugar beet plants.

Characters treatments		Root growth stage			Sugar accumulation stage		
Salinity levels M mos (1:5)	Stigmarterol M	Diameter (Cm)	Length (Cm)	Fresh weight (g)	Diameter (Cm)	Length (Cm)	Fresh weight (g)
Control	00	2.25	19.00	54.60	6.86	22.17	266.28
0.59	10^{-6}	4.35	17.50	100.10	7.33	21.67	327.40
	10^{-4}	2.90	21.00	77.13	7.67	22.00	330.83
0.67	10^{6}	2.15	13.00	50.65	6.33	19.67	258.63
	10^{-4}	1.85	21.50	29.45	6.73	23.00	441.80
0.96	10^{-6}	2.65	18.50	48.90	6.27	21.00	204.4
	10^{-4}	1.65	21.50	30.00	6.80	25.33	343.87
LSD at 5%		NS	NS	51.62	1.4	NS	NS

Effect of salinity levels on leaves pigments and sugar contents:

Data presented in Table (5) showed that leaves chlorophyll a and b contents were significantly increased with soil salinity increment. The highest level of salinity (0.96 mmos) gave the highest chlorophyll contents. However, carotene content insignificantly increased as soil salinity level increased. In this respect, chlorophyll and carotenoid contents were reported to increase in

leave of plants subjected to salinity or drought or both (Hamada, 1996). This might be due to a slight enhancement of specific RNA observed in salt shocked and salt acclimated cells. High levels of flavodoxin protein were detected in cells acclimated to NaCl. which plays a role as an alternative electron carrier (Hagemann *et al*., 1999), that lead to accumulate large amounts of 5-aminolevulinic acid. Whereas, the protochlorophyllide is based on an increased formation of 5-aminolevulinic acid. (Hansson *et al*., 1997). Concerning the effect of salinity level of soil on leaf sugar contents, data presented in Table (5) showed that soluble and total sugar contents were significantly increased with increasing soil salinity level at root growth stage. The highest level of salinity (0.96 mmos) gave the highest values of leaf soluble and total sugar contents. Leaf total sugar content was significantly affected at sugar accumulation stage. Also, leaf nonsoluble sugar contents at both physiological stages were significantly decreased accomplishing with soil salinity level. Whereas, starch was degraded in response to high salt, to provide either low molecular mass osmotic component (Goyal *et al*., 1987) or sucrose (Rathert, 1985).

That was contribute to the activities of key enzymes of succinate metabolism and the activity of soluble acid invertase (Dorion *et al*., 1996), which led to decrease hexose contentes and it also, dramatically enhance sucrose accumulation in all organs of the plants (Balibrea *et al*., 1997).

Table 5: Effect of salinity levels on pigment and sugar contents of sugar beet.

Leaves pigments and sugar contents

Characters	Vegetative growth stage			Root growth stage			Sugar accumulation stage		
Salinity levels Mmos (1:5)	Pigments content mg/g			Sugars			Content %		
	Chl.a	Chl.b	Carotene	Soluble	Non-soluble	Total	Soluble	Non-soluble	Total
Control	1.06	0.80	5.34	5.03	8.99	14.02	4.85	2.32	7.17
0.59	0.94	0.64	5.83	6.00	11.73	17.74	3.77	9.75	13.51
0.67	1.09	0.78	6.64	7.88	10.18	17.23	5.27	8.23	12.67
0.96	1.29	1.07	6.81	10.47	8.29	18.74	4.52	6.71	11.22
LSD at 5%	0.11	0.17	NS	0.84	0.99	0.15	NS	1.33	1.07

Root sugar contents

Characters	Root growth stage		Sugar accumulation stage				
Salinity levels Mmos (1.5)	Soluble %	Total %	Soluble %	Sucrose %	Glucose %	Total %	TSS %
Control	30.44	54.22	50.00	54.42	19.07	19.02	11.33
0.59	29.63	53.20	52.78	19.82	15.90	63.62	12.66
0.67	27.94	54.90	53.33	19.21	17.59	66.38	12.88
0.96	42.86	57.14	54.00	23.20	20.54	68.67	12.78
LSD at 5%	4.61	1.08	NS	0.30	2.53	NS	NS

Effect of salinity levels on sugar content of root:

Data of root sugar contents in Table (5) showed that soluble and total sugar contents was significantly increased during root growth stage but it was insignificantly affected at sugar accumulation stage. So, sucrose and

glucose were significantly increased. Similar trend was accurred concerning TSS contents in the roots. These results showed that all sugar contents were related to soil salinity levels and sugar beet showed high response to (0.96 mmos) salinity level. It might be due to that salinity caused an increase of glucose, fructose, soluble carbohydrate and change in sucrose content. Nevertheless it had no effect on photosynthesis (Dey and Dixon, 1985). In this respect, salinity enhanced sucrose accumulation in all organs of tomato plants (Balibrea *et al*., 1997).

Effect of stigmasterol treatments on leaves pigments and sugar contents:

Leaves pigments (chlorophyll a and b) were significantly increased with stigmasterol application as presented in Table (6). Application of stigmasterol at concentration of 10^{-6}M gave the highest values of leaves chlorphyll components, which was sufficient to produce the best content of leaves pigments. The stigmasterol concentration (10^{-4}M) gave the highest value of carotene compared with the other treatment. In this trend carotene increase was related to stigmenterol concentration. This might be due to the presence of stigmasterol as a free or combined with fatty acids or sugars or both in the cell. In this connection, brassinosteroids stimulated a variety of physiological responses including changes in enzymatic activities, membrane potential, DNA, RNA and protein synthesis (Mandava, 1988 and Szekers and Konez, 1998). Whereas, protein complex is a small double electron carrier, that can functionally replace ferrodoxin for almost all of it's functions e.g. photosynthetic electron transport, nitrate reduction, fatty acid desaturation (Straus, 1994). A close binding of flavodoxin to PS_1 has been demonstrated by chemical crosslinking, in which binding to the PS_1 proteins (Muhlenhoff *et al*., 1996), that can transfer electrons.

Concerning stigmasterol effect on leaves sugar contents, data presented in Table (6) showed that soluble and nonsoluble sugars were contrasted as their significant response to stigmasterol at root growth and sugar accumulation stages. These contents were higher with concentrations (10^{-6} and 10^{-4} M) of stigmasterol at both stages. Concequantly total sugar content was significantly related to stigmasterol treatments during both physiological stages compared with control. The highest concentration (10^{-4} M) of stigmasterol was sufficient to produce the highest total sugar content. It might be due to epibrassinosteroide effect on regulation of the hydrolytic and transport activities of tonoplast photohydrolases, whereas, H^+-ATPase was sensitive during growth and accumulation of metabolites, (Ozolina *et al.,* 1999). Meanwhile, Triase-P and 3-phosphoglycerate formed during photosynthesis move out of the chloroplast in strict counter exchange with inorganic phosphate on the phosphate translocator. In the cytosol, a series of enzyme reactions results in the synthesis of sucrose which is either translocated away or temporarily transferred to the vacule by facilitated diffusion (Nelson and Spollen, 1987).

Effect of stigmasterol treatments on root sugar content:

Root total sugar content was significantly increased at root growth stage with stigmasterol application as presented in Table (6). While, sugar accumulation stage, all sugar contents were significantly enhanced compared with control except glucose content which was contrasted. The enhancement was positively accompanied with stigmasterol application. This showed that stigmasterol was effective on salinity control. It might be due to stigmasterol effect on numerous secondary metabolites including plant hormones (Genus, 1978) and biological functions of sterylglucoside esters and sterylacylglucoside which were a sterol storge forms (Grunwald, 1982). In this concern, it has been shown that membrane sterol composition seems to have an effect on the activity of H^+-ATPases in plant (Cook *et al*., 1989). Whereas, it affects triose 3 phosphoglycerate exchange with inorganic phosphate which produce sucrose in series of enzymatic functions (Nelson and Spollen, 1987).

Table 6: Effect of stigmasterol treatments on pigments and sugar contents of sugar beet.

Leaves pigments and sugar contents

Characters	Vegetative growth stage			Root growth stage			Sugar accumulation stage		
Stigmasterol treatments M	Pigments content mg/g			Sugars			Content %		
	Chl.a	Chl.b	Carotene	Soluble	Non-soluble	Total	Solu-ble	Non-soluble	Total
Control	1.06	0.80	5.34	5.03	8.99	14.02	4.85	2.32	7.17
10^{-6}	1.31	1.19	5.33	10.06	9.64	19.99	5.70	9.91	14.76
10^{-4}	0.93	0.49	8.61	9.22	11.61	19.73	3.01	12.46	15.76
LSD at 5%	0.01	0.13	0.13	1.05	1.35	2.03	1.52	3.53	0.48

Root sugar contents

Characters	Root growth stage		Sugar accumulation stage				
Stigmasterol treatments M	Soluble	Total	Soluble	Total	Sucrose	Glucose	TSS
Control	30.44	54.22	50.00	54.42	19.07	19.02	11.33
10^{-6}	34.26	54.58	53.22	72.93	20.85	18.35	12.60
10^{-4}	35.73	56.10	56.22	71.31	22.34	16.66	14.33
L.S.D at 5%	NS	1.35	NS	9.17	1.55	NS	2.11

Effect of stigmasterol treatments under soil salinity level on photosynthetic pigment and sugar contents of leaves:

Leaves chlorophylls (a and b) were significantly increased with stigmasterol concentration (10^{-6}M) under salinity level compared with control treatment. However, the increase in leaves carotene content was related to stigmasterol concentration (10^{-4} M) under salinity levels at vegetative stage as presented in Table (7).

Concerning leaves sugar contents, the results in Table (7) showed that stigmasterol concentration (10^{-6}M) under soil salinity level significantly increased soluble sugar content of leaves during root growth stage and sugar accumulation stage compared with control. While, nonsoluble sugar content gave the contrasted trend in the same stages. Total sugar content of leaves significantly increased during both physiological stages compared with control

with increasing stigmasterol concentrations and salinity levels. These results show that stigmasterol concentration (10^{-4} M) had more effect on sugar synthesis and translocation to storge organs under soil stress. It might be due to accumulation of protochlorophyllide (Hansson *et al*., 1997) and brassinosteroid effect on enzymatic activities membrane potential, DNA, RNA and protein synthesis (Mandava, 1988 and Szekers and Konez, 1998).

Table 7: Effect of stigmasterol treatments under soil salinity levels on pigment and sugar contents of sugar beet Leaves.

Characters Treatments	Stigmas-terol M	Vegetative stage			Root growth stage			Sugar accumulation stage		
		Pigment content mg/g			Sugar content %					
Salinity levels Mmos (1:5)		Chl..a	Chl.b	Carotene	Soluble	Non-soluble	Total	Soluble	Non-soluble	Total
Control	00	1.06	0.80	5.34	5.03	8.99	14.02	4.86	2.32	7.17
0.59	10^{-6}	1.34	0.95	4.66	6.78	12.45	18.54	3.72	11.46	15.19
	10^{-4}	0.86	0.59	7.49	6.17	13.99	20.66	2.72	15.47	18.19
0.67	10^{-6}	0.98	0.77	5.65	9.34	10.60	19.80	7.77	7.65	15.38
	10^{-4}	0.77	0.36	8.92	9.28	11.31	17.88	3.19	12.22	15.42
0.96	10^{-6}	1.62	1.87	5.67	14.05	7.56	20.77	5.60	8.12	12.82
	10^{-4}	1.18	0.528	9.42	12.32	8.67	21.47	3.12	9.70	13.67
LSD at 5%		0.16	0.25	NS	4.67	2.23	2.03	NS	4.14	2.44

Effect of stigmasterol treatments under salinity levels on root sugar content:

Root soluble sugar content was insignificantly changed with salinity level and stigmasterol combination during root growth stage compared with other treatments as presented in Table (8). Stigmasterol concentration (10^{-4} M) under salinity level (0.96 mmos) at root growth and (0.59 m mos) during sugar accumulation stages gave the highest values of soluble sugar. So, root sucrose and glucose contents were significantly increased with combined treatments compared with control.

Table 8: Effect of stigmasterol treatments under salinity levels on sugar percentage of sugar beet root.

Characters Treatments		Root growth stage		Sugar accumulation stage				
Salinity level Mmos (1:5)	Stigmasterol M	Soluble	Total	Soluble	Sucrose	Glucose	Total	TSS
Control	00	30.44	54.22	50.00	19.07	19.02	54.42	11.33
0.59	10^{-6}	30.90	52.12	47.67	18.93	12.36	59.93	12.33
	10^{-4}	27.56	53.26	60.67	21.46	16.31	76.50	14.33
0.67	10^{-6}	28.10	57.88	56.33	19.17	19.35	71.33	12.13
	10^{-4}	25.28	59.31	51.67	19.40	14.39	73.39	15.00
0.96	10^{-6}	43.79	52.60	55.67	24.20	23.33	64.04	13.33
	10^{-4}	54.36	56.86	56.33	26.40	19.26	87.54	13.67
LSD at 5%		26.57	5.99	NS	6.58	13.31	16.43	NS

However, their responses were contrasted. Whereas stigmasteral treatment (10^{-4} M) stimulate sucrose and (10^{-6} M) glucose synthesis. The total sugars were significantly increased with enhancement of both salinity level and stigmasterol concentration at both stages, in addition to its accumulation under saline stress (Balibrea *et al*., 1977). In this trend TSS was insignificantly increased with stigmasterol under salinity level combination. These results showed that stigmasterol concentration (10^{-4} M) was more effective to avoid salinity effect on total sugar content. Whereas steryl conjugates such as fatty acid glucoside esters and steryl acylyglucoside were a sterol storage forms (Grunwald, 1982).

REFERENCES

Abd El-Wahed, M.S.A. (2001). Sitosterol stimulation of root growth, yield and some biochemical constituents of maize. 17th International Conference on Plant Growth Substances, Brno, Czech Republic, July 1-6, p. 175.

Ashraf, M.; T. Mcneilly and A.D. Bradshaw (1986). The potential for evolution of salt (NaCl) tolerance in seven grass species. New Phytol., 103: 299-309.

Balibrea, M.E.; A.M. Rus-Alvarez; M.C. Bolarin and F. Perez-Alfocea (1997). Fastchanges in soluble carbohydrate and proline contents in tomato seedlings in response to ionic and non ionic iso-osmotic stresses. J. Plant Physiol., 151:221-226.

Booth, U.H. (1958). Extraction and estamination of carotene. Univ. of Combridge and Medical Rsearch Council Dunn. Nutritional Lab., Cambridge, England.

Brown, D.J. and F.M. Dupton (1989). Lipid composition of plasma membranes and endomenbranes prepared from roots of barley (*Hordeum vulgare* L). Plant Physiol., 90: 955-961.

Cachorro, P. Ortiz, A.and Cerda, A. (1993). Effect of saline stress and calcium on lipid composition in bean roots. Phytochem., 32(5): 1131-1136.

Chon, E.M.; K.N. Nishikawa; Y. Hirata; H. Saka and H. ABe (2000). Effect of brassinolide on mesocotyl, coleoptile and leaf growth in rice seedlings. Plant Pro. Sci., 3 (4): 360-365.

Cooke, D.T.; R.S. Burden; D.T. Clarkson and C.S. James (1989). Xenobiotic induced changes in membrane lipid composition: effects on the plasma membrane ATPase. In Mechanisms and regulation of transport processes. 18, Atkin, R. K. and Clifford, D.R., British plant Growth Regulator Group, Bristol, 41-53.

Dey, P.M. and R.A. Dixon (1985). Regulation of sucrose synthesis and storge. Biochemistry of storge carbohydrate in green plants, p. 37-45-Academic Press, London, Orlando, San Diegs, New York, Toronto, Montreal, Sydney, Tokyo.

Dorion, S.; S. Lalonde and H.S Saini (1996). Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a decline in invertase activity and changes in carbohydrate metabolism in anthers.

Plant Physiol., 111(1): 137-145.
Dubois, M.; K.S. Gilles; J. Hamiltion; R. Rebersand; F.Smith (1956). Colormetric methods for determination of sugar and related substances. Anal. Chem., 28: 350- 356.
Frank, A.L. (1952). Improvement in Anthrone method for determination of carbohydrates. Anal. Chem., 24: 219.
Frederick, J.; J.R. Viles and G.A. Leslie (1949). Determination of starch and cellulose with Anthrone. Anal. Chem., 21: 950.
Genus, J.M.C. (1978). Steroid hormones and growth and development. Phytochem; 17: 1-44.
Goyal, A.; A.D. Brown and H. Gimmler (1987). Regulation of salt starch degradation in *Dunaliella tertiolecta*. J. plant physiol., 127:77-96.
Grunwald, C. (1982). Steroids-secondary plant products. Encyclopedia of plant physiology New series, 8: 221-256.
Hagemann, M.; R. Jeajean; S. Fulda; M.Havaux; F. Joset and N. Erdmann (1999). Flavodoxin accumulation contributes to enhanced cyclic electron flow around photosystem in salt stressed cells of *synechocystis sp*. Strain PCC 6803. Physiol. Plant., 105:670-678.
Hamada, A.M. (1996). Effect of NaCl water stress or both on gas exchange and growth of wheat. Biologia plantarum, 38(3): 405-412.
Hansson, M.; S.P. Gough; B. Kannangora and D.V. Wettstein (1997). Analysis of RNA and enzymes of potential importance for regulation of 5-aminolevutionic acid synthesis in the protochlorophyllide accumulating barley mutant tigrina-d^{12}-Plant physiol. Biochem., 35 (11): 827-836.
Kaffka, S.; D. Dong and G. Petersons (1999). Saline water can be reused to irrigate sugarbeets, but sugar may be low. California Agriculture, 53 (1): 11-15.
Kent, L.M. and A. Lauchli (1985). Germination and seedling growth of catton: salinity-Calcium interaction. Plant cell Environ., 8: 155-159.
Lee, S.; B.J Woffenden; E. P.Beers and A.W. Roberts (2000). Expansion of cultured Zinnia mesophyll cells in response to hormones and light. Phyiol. Plant, 108 (2): 216-222.
Lichtenthaler, H.K. and A.R. Wellburn (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans., 11 (5): 591.
Mandava, N.B. (1988). Plant growth promoting brassinosteroids. Ann. Rev. Plant Mol., 39:23-52.
Meneguzzo, S.; F. Novari-Izzo and R. Izzo (1999). Antioxidative responses of shoots and roots of wheat to increasing NaCl concentrations. J. Plant physiol., 155:274-280.
Moghaddam, B.E.; M. Mesbah and N. Yavari (2001). Use of somaclonal variation for production of monogerm sugar beet salt tolerant plants. First International Conference on Biotechnology Applications for the Arid Regions, p. 39.
Morgan, J.M. (1984). Osmoregulation and water stress in higher plants Annu. Rev. Plant physiol., 35:299-319.

Muhlenhoff, U.; J. Zhao and D.A. Bryant (1996). Interaction between photosystem I and flavodoxin from the *cyanobacterium Synechococus* sp. PCC 7002 as revealed by chemical cross linking. Eur. J. Biochem., 235:324-331.

Nelson, C.J. and W.G. Spollen (1987). Fructans. Physiol. Plant. 71:512-516.

Ozolina, N.V., Pradedova, E.V.; Reuiskay, A.M. and Salyaev, R.K. (1999). Effect of brassinosteroids on the tonoplast proton pumps. Doklady Biochemistry, 367 (1/6) 138-139. (C.F. Crop Physiol. Abs., 2000).

Pakhomova, V. (1996). Electrogenic k^+/H^+ exchange in excised wheat roots. Biologia plantarum, 38(4): 495-499.

Palmgren, M.G. (1991). Regulation of plant plasma membrane H^+-ATPase activity. Physiol. Plant, 83: 314-323.

Rathert, G. (1985). The influence of high salt stress on starch, sucrose and degradative enzymes of two *glycine max* varieties that differ in salt tolerance. J. plant Nutr., 8:199-209.

Rajasekaran, L.R.; D. Aspinall and L.G. Paleg (2000). Physiological mechanism of tolerance of *Lycopersicon spp*. Exposed to salt stress. Candian J. of Plant Sci., 80(1): 151-159.

Rover, A. (1998). Description of important situations of stress uring the vegetative growth of sugar beet. Zuckerindustrie, 123 (9): 683-687 [C.F. Crop Physiol. Abs, 1999, 25(1): 16].

Snedecor, G.W. and W.G. Cochran (1980). Statistical methods-6th Ed., Iowa State Univ., Press, Ames Iowa, USA.

Straus, N.A. (1994). Iron deprivation: physiology and gene regulation In: Bryant DA (ed.) Molecular Biology of Cyanobacteria. Kluwer Academic Publishers. Dordrecht, pp 731-750. ISBN 0-7923-3273-3.

Steven, D. and M. Jenneth (1998). Brassinosteroids: Essential Regulator of plant Growth and Development. Annu. Rev. Plant Physiol. Plant Mol. Biol, 49:427.

Szekers, M. and C. Konez (1998). Effect of brassinosteroids on nodulation and nitrogenase activity in groundnut. (*Arachis hypogaea* L.) Plant Regulation, 28:125-167.

Yahya, A. (1998). Responses to salinity of sesame (*Sesamum indicum*, L.) and sugar beet (*Beta vulgaris*; L.) Acta Universities Agriculturae Sueciae-Agraria, 122 (90) 91-576 [C.F. Crop Physiol. Abs., 1999, 25(2) 244.

Yermiyahu, U.;0 S. Nir; G. Ben-Hayyim; U. Kafkafi and B. Thomas (1997). Root elongation in saline solution related to calcium binding to root cell plasma membranes. Plant and Soil, 191: 67-76.

التأثير الفسيولوجى للاستجماستيرول فى مقاومة الملوحة ببنجر السكر

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تم إجراء هذا البحث فى صوبة ا لمركز القومى للبحوث – دقى – جيزة – مصر. لدراسة تأثير الاستجماستيرول الفسيولوجى للتغلب على تأثير الملوحة فى بنجر السكر بدراسة التأثير على صفات النمو، المحصول، الصبغات (كلوروفيل أ، ب) والكاروتين وكذلك السكريات بأجزاء النبات.

تم زراعة بذور بنجر السكر للصنف Rass poly فى تربة مالحة بثلاث تركيزات (0.59، 0.67، 0.96 ومليموز) بالإضافة للكنترول (تربة غير مالحة 0.36 مليموز). وقد تم رش الاستجماستيرول بتركيز (10^{-6}، 10^{-4} مولر) تحت كل مستوى ملوحة مرتين بعد 45 يوم ، 75 يوم من الزراعة فى مرحلتى النمو الخضرى ونمو الجذور للبنجر وكان التصميم المستخدم هو القطع المنشقة مرة واحدة فى ثلاث مكررات.

وتتلخص النتائج كالآتى:

- لقد انخفضت صفات المجموع الخضرى (مساحة الأوراق/ نبات والوزن الغض والجاف للقمة بالنبات) وصفات الجذر (القطر ، الوزن) معنوياً وزاد طول الجذر غير معنوياً بزيادة مستوى الملوحة.
- زادت الصفات الخضرية (مساحة الأوراق/نبات والوزن الغض والجاف لقمة النبات) معنوياً بإضافة الإستجماستيرول بتركيز (10^{-6} مولر) فى مرحلة تراكم السكر وانخفضت الصفات الأخرى معنوياً.
- لقد زادت الصفات الخضرية لنبات البنجر زيادة معنوية بإضافة الاستجماستيرول بتركيز 10^{-6} مولر فى مرحلة نمو الجذر بينما كان التركيز 10^{-4} مولر فى مرحلة تراكم السكر بالجذر مع مستويات الملوحة المختلفة.
- لقد زادت صفات الجذر (القطر – الوزن الغض) زيادة معنوية بإضافة تركيز الاستجماستيرول (10^{-6}مولر) فى مرحلة نمو الجذر وزيادة غير معنوية فى مرحلة تراكم السكر بالجذر بإضافة (10^{-4} مولر).
- زادت مكونات الأوراق من الصبغات (كلوروفيل أ، ب) معنوياً والكاروتين غير معنوياً بزيادة تركيز الملوحة – كما زاد محتوى الأوراق من السكريات الذائبة والكلية معنوياً فى مرحلة نمو الجذر وانخفضت غير معنوياً فى مرحلة تراكم السكر بالجذر.
- لقد أدت زيادة الملوحة إلى زيادة السكريات الذائبة والكلية بالجذر فى مرحلة نمو الجذر زيادة معنوية وكذلك محتوى الجذر من السكروز والجلوكوز فى مرحلة تراكم السكر بالجذر مقارنة بالكنترول.
- زاد محتوى الأوراق من الصبغات (كلوروفيل أ،ب) بزيادة الملوحة وإضافة الاستجماستيرول بتركيز (10^{-6} مولر) فى مرحلة نمو الجذر بينما زاد محتوى السكريات الكلية مع التركيز 10^{-4} مولر فى مرحلة تراكم السكر بالجذر.
- زاد محتوى السكريات الكلية معنوياً فى مرحلة نمو الجذور بزيادة تركيز كل من الملوحة والاستجماستيرول. بينما كانت زيادتها معنوياً بإضافة الاستجماستيرول فى مرحلة تراكم السكر بالجذر.
- زاد محتوى الجذور من السكروز معنوياً بزيادة تركيز كلا من الملوحة والاستجماستيرول. وكذلك الجلوكوز بزيادة تركيز الملوحة فى مرحلة تراكم السكر بالجذر.