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Influence of Brassinosteroids on Wheat Plant (*Triticum aestivum* L.) Production under Salinity Stress Conditions
I- Growth Parameters and Photosynthetic Pigments

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Abstract: Green house experiment was conducted to alleviate the effect of irrigating saline water at (0.2000, 4000 and 6000 ppm NaCl solution) by using foliar spray of BRs "28-homoBL" at levels of 50, 100 and 200 mg/L on wheat plant (*triticum aestivum* L.). Brassinosteroids play an important role in growth, metabolic activities and productivity of wheat plants. BRs significantly increase all the growth parameters, photosynthetic pigments as compared with the untreated control plant. Foliar application of BRs also increased yield and yield attributes of treated plants and significantly overcome the depressive effect of saline irrigation water at all levels on crop productivity and photosynthetic pigments.

Key words: Brassinosteroids, wheat growth, salinity, photosynthetic pigments, yield.

INTRODUCTION

Salinity is a major environmental problem that cause a reduction in plant productivity (Irshad *et al*., 2002), especially in arid and semi-arid regions. Salinity stress is causing poor response of crops to fertilizer application as a result to decrease in photosynthesis in the presence of high osmotic pressure in root medium.

Wheat is a major food crop in most of the countries which face salinity problems especially in Egypt, as a part from arid and semi-arid region. Therefore, it is necessary to manage such soil for profitable agriculture by adopting proper in farm management practices.

Brassinostroids were isolated from extract *Brassica napus* pollen (Grove *et al*., 1979) because of their growth promoting properties and their potential use for enhancing crop production. Brassinosteroid (BRs) represent a new sixth class of plant hormones with wide occurrence in the plant kingdom. The application of biological active brassinosteroids cause remarkable growth responses in plants, including stem elongation, pollen tube growth, leaf bending, leaf unrolling, root inhibition, xylem differentiations and regulation of gene expression (Nomura *et al*., 2005). Recently Bajguz (2007) reported that brassinosteroids represent a class of plant hormones, more than 70 compounds have been isolated from plants.

In addition to the growth promoting effect, brassinosteroids stimulate a variety of physiological responses including changes in enzymatic activities, membrane potential, photosynthetic activity, DNA, RNA and protein synthesis and changes in the balance of other endogenous phytohormones (Krishna, 2003). Finally, Ryu *et al*. (2007) proposed that BRs is critical for signaling in plant growth and development.

The presented study were conducted to study the physiological role of different concentrations of salinity in irrigation water and foliar application with varied BRs concentrations on growth, photosynthetic pigments as well as yield of wheat plants grown on sandy soil.

MATERIAL AND METHODS

A greenhouse experiment was carried out at the National Research Center (Dokki, Cairo, Egypt). The aim of this study was to evaluate the response of wheat plant (*Triticum aestivum* L. Cultivar Giza 164) for irrigation with saline water (0.2000, 4000, and 6000 ppm NaCl) and the importance of using the growth regulator Brassinasteroids (28-homobrassinoloide) as a foliar application at different concentrations (0, 50, 100, and 200 mg/L).

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The investigated soil characterized by the following: pH 8.2; EC. 0.30 ds/m; Sand 87.7%, silt 10.0%; clay 2.3%, $CaCo_3$ 3.4%; organic matter 0.2%; Total N, (170); Available P, (8); K (670); Fe(6); Mn (2.4); Zn (0.45) ppm; Ca^{++} (2.8); Mg^{++} (0.1); Na^+ (2.2); K^+ (1.3); Cl^- (2.5) and SO_4 (2.8) mg/L. (standard methods were used as described by Page *et al*. (1984); Chapman and Pratt, 1978 and Lindsay and Norvell, 1978).

Twenty kilograms of the sandy soil was placed in each of 64 plastic pots (33 cm height and 24 cm wide), phosphorus as superphosphate (8 gm P_2 O_5/pot) was added to each pot thoroughly mixed with the soil during seed bed preparation. The amount applied of nitrogen as $(NH_4)_2SO_4$ and potassium as K_2SO_4 were 10 and 4 gm for each pot respectively applied as three equal doses after 15, 30 and 75 days from sowing. Ten seeds of wheat variety Giza 164, were sown in each pot in randomized application and then irrigated with tap water to keep wet enough to sustain a satisfactory seed germination. Then two weeks after sowing, seedlings were thinned out to five per pot. The saline water was used at seedling emergence. The pot experiment was carried out in a split-plot design, with a randomized complete blocks design. The 64 pots represented the experimental treatments, which were irrigated with different concentrations of NaCl (0, 2000, 4000 and 6000 ppm) and were foliarly sprayed with different concentrations, of brassinosteroid (28-homobrassinolide) at 0, 50, 100 and 200 mg/L. The combined 16 treatments with both BRs and NaCl solution, each treated pot with saline water (NaCl) after seedling emergence was treated with the different BRs concentrations foliarly, four replicates were considered for all treated pots, taking in consideration the control treatment using tap water. Tepole as surfactant was added to the spray solution of BRs at a rate of 1ml/L. The volume of the spraying solution was maintained just to cover completely the plants foliage till drip, after 25, 40 and 65 days from sowing date, tap water was sprayed on plants as control treatment.

Wheat samples were collected from each treatment at the elongation stage, 90 days from sowing, to determine the growth parameters (plant height; leaf area, tiller number, spike length and number of spikes/plant), photosynthetic pigments (chlorophyll a, b and carotenoids) were determined in the fresh leaves and calculated by means of Wellstein's formula (Wellstein, 1957).

At harvest time, after 120 days from sowing, Number of kernels per spike, weight of 1000 grains, straw and grain yield as well as biological yield per pot was estimated.

All the attained data were subjected to conventional methods of statistical analysis according to Snedecor and Cochran, (1990).

RESULTS AND DISCUSSION

Growth parameters:

A- Effect of Salinity (S):

Table (1) shows that irrigating wheat plants with different levels of saline water (2000, 4000 and 6000ppm NaCl) significantly reduced the growth parameters as compared with the control; this includes plant height, leaf area, tiller number, spike length and No. of spikes per plant, the effect was more pronounced with higher concentration of saline irrigation (6000 ppm). These effects might be due to salinity which inhibits the growth of wheat plants, through reduced water absorption and reduced metabolic activity due to Na^+ and Cl^- toxicity and nutrient deficiency caused by ionic interference (Aziz and Taalab, 2004). Our results are in agreement with those reported by Badr (2005) and Sherif *et al*., (2007) who stated that salinity treatments at all concentrations ranging from 2000 to 8000 ppm NaCl caused a significant decrease in plant growth parameters of faba bean and *Tagetes erecta* plants respectively.

Since under saline condition root pressure is reduced causing a decrease in water flow, that means less water is taken up by the roots and transported into shoot, consequently, less water is available for normal growth and development (El-Fouly *et al*., 2001; Tester and Darenport, 2003 and Mazher, 2008).

The decrease in fresh and dry weight of plant might be due to the inhibition of water absorption and/or distribution of mineral balance and/or absorption and utilization under salinity conditions (Abdel Aziz *et al*., 2006 and Mazher *et al*., 2006 a, b).

B- Effect of brassinosteroids (BRs):

Results indicate clearly that all the growth parameters under study significantly increased by increasing the concentration of sprayed brassinosteroids (50, 100 and 200 mg/L) as compared with control treatment. The obtained results are in good agreement with those reported by Fujii and Saka, (2001) on rice (*Oriza sativa* L.); by Youssef (2004) on *Pelargonium graveoes*; Balbaa (2007) on Fenugreek plants and Abdel Hamid (2008) on wheat who stated that foliar application of BRs showed a high significant increase in growth parameters as compared with untreated control plants.

Brassinosteroids (BRs) play an essential role in plant growth and development, have been implicated in many physiological responses (Sasse, 2003; Doust, 2007; and Bajguz and Hayat, 2009). Moreover, Vardhini and Rao (2003) stated that BRs, application also resulted in enhancement of seedling growth, which was evident in terms of seedling length, seedling fresh and dry weights and play pivotal role in wide range of developmental phenomena in plants, including cell division and cell elongation in stems and roots, photomorphogeneses, reproductive development, leaf senescence and stress responses.

C- Effect of interactions between (S) × (BRs):

Data presented in Table (1) show that the combined effect between salinity of irrigation water and foliar application with BRs at different concentrations significantly affected all the growth parameters except the leaf area which did not attain to the level of significance at 5%. The foliar application with BRs at concentration of 200mg/L for wheat plants irrigated with tap water produced the highest values of growth parameters whereas, spraying the wheat plants irrigated with the highest level of saline water (6000 ppm NaCl) gave the lowest values of the studied growth parameters.

In this context it is to be mentioned that the combined treatment with BRs and NaCl resulted in partial growth recovery as compared to the action of BRs and wheat germ agglutinin (WGA) in roots by 50%. It is probable that BRs could be involved in the hormonal control of the WGA level along with abcisic acid. BRs evidently exert a protective action on wheat seedling via a considerable decrease in the salt induced ABA and WGA accumulation in roots (Shakirova *et al.,* 2002).

Badr (2005) confirming the obtained results stated that interaction between salinity of irrigation water and osmo and bio regulators treatments significantly affect plant height and number of leaves/plant, while number of branches and flowers/plant, dry weight of stem, leaves and total faba bean plant were not significantly affected.

Table 1: Growth parameters of wheat plants after 90 days from sowing date as affected by saline irrigation water and BRs foliar application.

Treatments		Plant height (cm)	Leaf area (cm2)	Tiller No./ Plant	Spike length (cm)	No. of spikes/plant
Saline irrigation water (ppm)						
0		94.89	17.02	10.53	9.41	3.73
2000		89.48	15.47	9.99	8.84	3.34
4000		85.48	14.47	9.55	8.16	3.03
6000		79.66	14.29	8.78	7.46	2.59
LSD at 5%		1.68	0.22	0.35	0.45	0.27
BRs foliar application (mg/L)						
0		76.56	14.65	8.83	7.7	2.26
50		82.71	15.07	9.73	8.13	3.17
100		92.56	15.62	9.92	8.81	3.54
200		97.59	15.9	10.38	9.23	3.71
LSD at 5%		1.01	0.2	0.27	0.32	0.12
Saline water	BRs					
0	0	80.31	16.3	9.7	8.47	2.73
	50	89.32	16.8	10.4	9.12	3.83
	100	102.33	17.3	10.8	9.75	4.03
	200	107.6	17.66	11.2	10.3	4.33
2000	0	78.36	14.8	9.11	8	2.2
	50	84.11	15.34	10	8.61	3.5
	100	94.21	15.8	10.2	9.13	3.73
	200	101.25	15.93	10.67	9.63	3.92
4000	0	75.83	13.79	8.6	7.4	2.1
	50	81.2	14.18	9.7	7.78	3.1
	100	90.6	14.79	9.68	8.57	3.4
	200	94.29	15.11	10.23	8.87	3.5
6000	0	72.11	13.7	7.91	6.93	2
	50	76.22	13.97	8.81	7.01	2.23
	100	83.11	14.6	9	7.8	3.01
	200	87.2	14.9	9.4	8.11	3.1
LSD at 5%		2.02	NS	0.53	0.64	0.24

Photosynthetic pigments:

A- Effect of Salinity (S):

It is clear in Table (2) that increasing salt concentration in the irrigating water, gradually decreased the content of photosynthetic pigment (chlorophyll a, b and carotenoids). The highest values of chlorophyll were recorded from the control treatments while the lowest recorded in case of the highest level of salinity (6000 ppm NaCl). This effect has been attributed mainly to the excessive accumulation of Cl^- and Na^+ in the plant leaves, causing a nutritional imbalance. Salinity affects the carboxylation activity, decreased stomatal opening, changes in the photochemical processes and water stress and accelerated chlorophyll a breakdown (Sherif *et al*., 2007).

Generally, salinity caused decrease in chlorophyll synthesis as result of the sudden salinity effect. In this concern, Yamane *et al*., 2003 reported that swelling of thyllakoids is induced at the early stage of the damage when plants are affected by salt stress and leads to reduction in chlorophyll synthesis.

It could be concluded that salinity adversely affected the photosynthetic pigments. In addition, spraying salinity stressed-wheat plants with BRs can reduce the undesirable effect of salinity through improving growth and nutrients status of plants as well.

B- Effect of brassinosteroids (BRs):

In response to foliar application of BRs with its different concentrations (Table 2), chlorophyll pigments showed significant increases as compared with control. The magnitude of increase was more pronounced at 200 mg BRs/L. These results are in good agreement with those obtained by Mohamed (2005) and Cao and Zhao (2008), who stated that spraying *Nigella sativa* seeds and rice seedlings with BRs respectively caused highly significant increase in photosynthetic pigment content of plants. Also Dong *et al*., (1999) and Abdel Hamid (2008) found that spraying plant with brassinoloide significantly increased photosynthetic pigments in *Oryza sativa* and wheat plants respectively as compared with untreated control plants.

Yu *et al*., 2004, Morinaka *et al*., 2006, and Bajguz and Hayat (2009) stated that it has been shown BRs can regulate the initial carboxylation activity of ribulose 1,5-bisphosphate by influence photosynthetic CO_2 assimilation which is determined by electron transport efficiency in photosynthesis. Spraying BRs have been shown to affect on increasing the quantum yield of electron transport in photosystem II reaction and the maximum carboxylation of ribulose 1,5-bisphosphate, processes, they added application of homobrassinoloide resulted in increasing relative water content, nitrate reductase activity, chlorophyll content and photosynthesis under stress conditions, it also improved membrane stability. These beneficial effect resulted in higher leaf area, biomass production, and yield related parameters in treated plants. However, homo BL showed a higher activity in drought-tolerant wheat variety under water stress conditions. Generally Bajguz and Hayat (2009) stated that BRs removed the salinity induced inhibition of seed germination and seedling growth of rice (*Oryza sativa*). BRs application is most beneficial to growth under stress conditions and the favourable effect of BRs can be attributed to stimulate leaf elongation, photosynthetic pigments, fresh and dry weight of leaves and shoots of wheat and mustard plants (Braun and Wild, 1984).

C- Effect of interactions between (S) × (BRs):

The interaction between salinity of irrigation water and foliar application with BRs significantly affected Chl. a, Chl. b and Chl a+b, while carotenoids and total pigment were not affected. The highest values of all the photosynthetic pigments were obtained in wheat plants sprayed with 200 mg BRs/L and irrigated with tap water, while the lowest values were attained by plants irrigated with the highest salinity of irrigation water (6000 ppm) and sprayed with tap water. In this concern, Anuradha and Rao (2003) reported that BRs alleviate the inhibitory effect of salt stress on pigment levels and this could be one of the reasons for growth stimulation by BRs.

Table 2: Photosynthetic pigments of wheat plants after 90 days from sowing date as affected by saline irrigation water and BRs foliar application.

Treatments	Photosynthetic pigments (mg/g Fresh weight)				
	Chl. a	Chl. b	Chl. a+b	Carotenoids	Total pigments
Saline irrigation water (ppm)					
0	1.52	0.69	2.21	1.31	3.51
2000	1.47	0.61	2.08	1.36	3.43
4000	1.4	0.54	1.94	1.39	3.33
6000	1.33	0.47	1.8	1.45	3.25
LSD at 5%	0.04	0.03	0.06	0.04	0.09
BRs foliar application (mg/L)					
0	0.84	0.4	1.24	0.62	1.86
50	1.49	0.5	1.99	1.52	3.51
100	1.66	0.66	2.31	1.6	3.92
200	1.72	0.76	2.48	1.76	4.24
LSD at 5%	0.03	0.03	0.04	0.03	0.05

Table 2: Continue

Saline water	BRs					
0	0	0.91	0.48	1.39	0.55	1.94
	50	1.57	0.6	2.17	1.44	3.61
	100	1.76	0.78	2.54	1.53	4.07
	200	1.83	0.89	2.72	1.7	4.42
2000	0	0.89	0.41	1.3	0.6	1.9
	50	1.51	0.53	2.04	1.5	3.54
	100	1.7	0.7	2.4	1.58	3.98
	200	1.77	0.8	2.57	1.74	4.31
4000	0	0.81	0.37	1.18	0.64	1.82
	50	1.48	0.46	1.94	1.54	3.48
	100	1.61	0.63	2.24	1.61	3.84
	200	1.68	0.72	2.4	1.78	4.18
6000	0	0.75	0.33	1.08	0.68	1.76
	50	1.4	0.41	1.81	1.6	3.41
	100	1.56	0.52	2.08	1.69	3.77
	200	1.6	0.62	2.22	1.83	4.05
LSD at 5%		0.53	0.08	0.08	NS	NS

Yield components:

A- Effect of Salinity (S):

Presented results in Table (3) show that increasing the salinity levels in irrigation water of wheat plant exerted significant reduction on both yield and its components (no. of Kernels/spike; wt. of 1000 grains, straw, and grain yield gm/pot and the biological yield gm/pot) as compared with control treatment. Data proved that the highest values of the yield and its components were obtained in the control treatment which irrigated with tap water, while the lowest ones were attained by using the highest concentration of NaCl in the irrigation water (6000 ppm NaCl).

Salinity stress, decreased osmotic potential and increased turger potential which accompanied by an enhanced Na^+ and Cl^- concentration in leaves, and alteration of nutrient uptake. Chaparzadeh *et al*., (2003) stated that the growth and yield of individual leaves were found under salt stress and thus reduced the net productivity and crop yield.

Data indicated that increasing salinity levels of irrigation water exerted significant reductions in both grains and straw yields. The association between the depressive effect of salinity on grain yield and number of kernels per spike and wt. of 1000 grains is apparently related to the relation of metabolic processes and enzymatic reaction involved in nitrogen transport during the grain filling stage. These observation are in accordance with the data reported by El-Etreiby (2002) and Daoud (2005) they attributed the yield decrement of salt stressed wheat plants to the reduction in the number of the developed spikes.

Results in this study are in agreement with those obtained by Javed *et al*., (2003); El-Agrodi *et al*., (2005) and Hussein *et al*., (2008) who reported that salinity markedly decreased leaf growth which strongly influences tiller number leading to marked yield loss. They added that NaCl salt at different levels specially the higher ones gave significantly higher decrease in biological straw and grain yield of wheat plants.

B- Effect of brassinosteroids (BRs):

Data in Table (3) reveal clearly that the yield was significantly respond to foliar spraying with BRs at different concentration (50, 100 and 200 mg/L) as compared with control. The increased percentage as affected by foliar application with BRs was clear in grain rather than straw yield.

Such stimulation of plant growth and yield were attributed to several interpretations, that hypothesized by Willadino *et al*., (1996) who reported that the increases in growth may be due to BRs stimulation of various process associated with the synthesis of protein as well as promoting cell division. Abdel Hamid (2008) showed that BRs levels which are elevated in the leaves and probably in stem and roots have significant effect on seed weight. He stated that seed weight measurement resulted from enhanced filling of seeds with sucrose and other sugars which transported to them from leaves. Similar effects were obtained by Xu (2007) when spray application of epibrassinoide to sorghum plants at heading and grain filling stages.

The favorable effect of spraying BRs on wheat plants may be due to the genes controlling BRs hormone levels which were used to increase seed filling and grain yield in wheat (*Triticum aestivum*) and rice (*Oryza sativa*). The transgenic plants produced more tiller and seeds than wild type plants, seeds were heavier as well, especially seeds at the bases of the spikes, (Morinaka *et al*., 2006).

Came to the same results Hathout (1996) and Abdel Hamid (2008) on wheat, also Kerrit (2005) on maize, who stated that spraying wheat and maize plants with BRs significantly increased spikes length, spiklets number/spike, straw and grain yields, grain number and weight.

C- Effect of interactions between (S) × (BRs):

The interactions between the studied factors on all the measured parameters of wheat yield were significant (Table 3), it is clear that positive effect of both factors was at control treatment which irrigated with tap water and foliar sprayed with 200 mg BRs/L, in which the studied parameters were higher than the other treatments. Plants irrigated with the highest salinity level of 6000ppm NaCl and sprayed with tap water recorded the lowest values.

Hayat *et al*., (2006, 2007a and 2007b) revealed that plants showed increase in dry matter accumulation, together with an increase in the nitrate reductase and carbonic anhydrase activities, they added that 28 homobrassinoloide treated plants also possessed higher seed yield in comparison to the plants subjected to NaCl stress, at harvest. Similarly the spray of 28-homoBL to the foliage or splay through roots of *Brassica juneca* plants generated from the seeds soaked in NaCl enhanced the growth, nucleic acid content, ethylene and seed yield.

The above mentioned results confirm the beneficial effect of 28-homo BL foliar application in improving plant tolerance to salinity through minimizing the detrimental effects of salinity. BRs may influence the stress response of plants through stimulation of Jasmonic acid (JA) biosynthesis (Schaller *et al*., 2000).

Table 3: Yield and its parameters of wheat plants at harvest time as affected by saline irrigation water and BRs foliar application.

Treatments		No. of kernels/ spike	Wt of 1000 grain (gm)	Straw yield gm/pot	Grain yield gm/pot	Biological yield gm/pot
Saline irrigation water (ppm)						
0		45.73	27.73	41.47	18.89	60.7
2000		43.82	24.08	32.33	15.06	47.55
4000		42.33	23.13	30.9	14.31	47.63
6000		40.43	22.08	29.19	12.71	48.63
LSD at 5%		0.3	0.75	2.7	0.53	4.14
BRs foliar application (mg/L)						
0		40.98	17.3	22.14	9.23	31.49
50		42.51	21.95	29.93	13.59	43.52
100		43.92	26.33	37.64	17.05	57.15
200		44.91	31.43	44.19	21.09	72.36
LSD at 5%		0.25	0.3	1.19	0.39	3.64
Saline water						
BRs	0	43.31	20.1	28.3	12.2	40.83
	50	45.63	24.9	39.33	16.97	56.3
	100	46.95	30.1	45.6	20.8	66.4
	200	47.04	35.8	52.66	25.6	79.26
2000	0	42	17.3	21.53	9.5	31.1
	50	43.2	21.6	30.4	13.8	44.17
	100	44.5	26.7	35.33	16.4	51.73
	200	45.58	30.7	42.04	20.53	63.2
4000	0	40.71	16.4	20.11	9.1	29.21
	50	41.4	21	26.8	12.5	39.3
	100	43.01	25.1	35.06	15.8	60.86
	200	44.2	30	41.64	19.83	61.16
6000	0	37.88	15.4	18.6	6.13	24.8
	50	39.8	20.3	23.2	11.1	34.3
	100	41.22	23.4	34.57	15.2	49.6
	200	42.8	29.2	40.4	18.4	85.8
LSD at 5%		0.5	0.6	2.38	0.77	7.28

Conclusion:

It could be concluded that salinity adversely affected the growth of wheat plants expressed as yield parameters and crop productivity as will as photosynthetic pigments. The interaction between salinity levels and BRs treatments might protect the plants and enhanced growth criteria and could be useful for increasing grain yield and improving photosynthetic efficiency and pigments level. Hence it could be recommended to spray plants which grown in saline regions or irrigated with saline water with BRs to overcome the destructive effect of salinity. Thus BRs application is most beneficial to plant growth under stress condition.

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