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Impact of Brassinolide on Physiology, Growth and Yield of Wheat (*Triticum aestivum* L.) under Salinity

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Abstract An experiment was conducted to investigate the role of brassinolide in amelioration of salinity harmful effects on physiological variables, growth and yield of wheat cv namely Raj-3077 (salinity tolerant) and Raj-1482 (salinity susceptible) in the laboratory and cage house during *rabi* season of 2014-2015. The seeds were sown in petriplates and in cemented pots containing 15 kg soil in which saline irrigation (tap water, 4 and 8 dSm^{-1}) were applied as and when required. Different concentrations of brassinolide (0.0, 0.5, 1.0 and 1.5 ppm) were added in the saline set of petridishes whereas, in the pot experiment, plants were sprayed with brassinolide at 52 and 82 days after sowing. Control plants were provided normal water whenever needed. Seedling growth parameters were recorded at 8 days after treatment in the laboratory and physiological, biochemical observations were recorded at 52 and 82 days after sowing in pot conditions. Yield parameters were recorded at harvest. Analysis of data revealed that a significant decrease were recorded in germination percentage, root length, shoots length, fresh weight, dry weight of root and shoot, plant height, leaf area, number of spikes, length of spike per plant, number of seeds per spike, chlorophyll, relative water content, cell membrane stability, protein content and grain yield with increase in proline and carotenoid content up to 8 dSm^{-1} whereas a significant increase were recorded in these parameters by the use of brassinolide up to 1.5 ppm concentration. On the basis of the above findings Raj-3077 observed superior over Raj-1482 with respect to traits studied under laboratory and pot experiment.

Keywords Wheat, Brassinolide, Physiology, Growth, Salinity.

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Introduction

Wheat is an important staple cereal crop throughout the world. It is eaten in various forms by more than thousands million human beings in the world. Its straw is used as the feed for large population of animals. In India, it is the second staple food crop following the rice. It contains about 8–15% protein and its gluten is especially important for bakery and bread making. Wheat is an important *rabi* cereal crop which is grown throughout the temperate, sub-tropical and tropical regions and ranks only next to rice in area and production.

Scarcity of food and water deficit are the greatest problem discussed now a days, and it is linked to both with population growth and water allocation to different sectors, such as domestic, agronomic and industrial uses. According to the FAO Land and Plant Nutrition Management Service, over 6% of the world's land is affected by either salinity or sodicity. Moreover the low water quality and the poor drainage systems are the greatest causes of these stresses and this problem is more acute with higher evaporation, especially in arid and semi arid zones, where saline soils are widespread that induced the decreasing of land productivity in many countries over the world [1]. Furthermore salinity affects soil fertility and due to these situations some solutions were taken to reduce this problem through soil reclamation or growing tolerant species; however, soil reclamation is a very expensive process and then the selection of tolerant varieties of crops is still the most practical solutions when salinity is low. Salinity has negative impact on water and nutrient uptake because of osmotic and ionic imbalance. This will produce plants with reduced height, less leaves and tillers as well as reduced yield [2]. Since salinity is complicated trait and genetically controlled, plants show different response when they growth under salinity stress according to their genes content [3].

Brassino steroids (BRs) are a new type of polyhydroxy steroidal phytohormones with significant growth-promoting influence [4] BRs played important roles in monitoring the stress-protective properties in plants against a number of abiotic stresses like low temperature/chilling/freezing, salt, high temperature/heat sress, water/drought/water logging, heavy metals and biotic stresses [5]. BRs confer salt tolerance to plants by mitigating its negative effects on the physiological, biochemical and molecular processes in plants [6]. Brassinolide improved the growth, yield and chemical composition of berseem (*Trifolium alexandrinum* L.) grown in saline soils [7, 8]. Problem of salinity is increasing day by day, one of the best solutions is to use saline soils effectively for improved salt toletance in crops. For this purpose different approaches, were adopted, among those one is the exogenous application of plant growth regulators. The objective of this study was to observe the effect of exogenous aplication of brassinolide as foliar spray in amelioration of harmful effects of salinity on growth and yield of wheat.

Table 1. Composition of salts for preparing different levels of saline irrigation water.

Salinity levels	$NaSO_4$ (mg/l)	NaCl (mg/l)	$MgCl_2$ (mg/l)	CaCl (mg/l)
Control	Tap water	Tap water	Tap water	Tap water
EC4 dSm^{-1}	621.14	717	534	643
EC8 dSm^{-1}	1243	1434	1068	1286

Materials and Methods

Plant materials and experimental details

A laboratory and pot experiment were conducted at cage house located in the Department of Plant Physiology, SKN College of Agriculture, Jobner during *rabi* season 2014-15, to investigate impact of brassinolide on physiology, growth and yield of wheat (Triticum aestivum L.) under salinity. The pots were filled with 15 kg of loamy sandy soil having a bulk density of 1.5 g cm^{-3}, electric conductivity (EC) 0.4 dSm^{-1}, pH 8.2, sodium absorption ratio 12.5 and $CaCO_3$ 0.14%. The field capacity and permanent wilting point of the soil were 11.8 and 2.8%, respectively. 72 pots for both cultivar Raj-3077 (salinity tolerant) and Raj-1482 (salinity susceptible) were used for growing of wheat up to harvesting. The recommended doses of manures, fertilizers and other inputs were provided at the appropriate time. Salts used to prepare saline irrigation water of EC4 and 8 dSm^{-1}; Chloride and sulfate in 3:1 ratio by using following salts; NaCl, $NaSO_4$, CaCl and $MgCl_2$ (Table 1). One liter of the saline water was provided to each pot having three plants as and when required. The control plants were irrigated with tap water. The plants were irrigated with saline water as

Table 2. Effect of salinity and brassinolide on germination %, root and shoot length, fresh and dry weight of seedling root and shoot of wheat. DAT=Days after treatment.

Treatments	Germination (%) 8 DAT	Root length (cm) 8 DAT	Shoot length (cm) 8 DAT	Fresh weight of root (mg/seedling) 8 DAT	Dry weight of root (mg/seedling) After oven drying	Fresh weight of shoot (mg/seedling) 8 DAT	Dry weight of shoot (mg/seedling) After oven drying
Varieties							
Raj-3077	85.74	3.38	6.56	7.83	1.88	29.79	5.55
Raj-1482	76.47	3.76	5.26	8.91	2.25	26.40	4.70
SEm±	0.72	0.11	0.16	0.19	0.05	0.34	0.07
CD (p=0.05)	2.03	0.32	0.45	0.55	0.13	0.96	0.21
Salinity levels							
Control	86.50	4.10	6.74	9.30	2.41	31.80	7.11
4.0 dSm^{-1}	82.44	3.58	5.98	8.40	2.09	26.90	4.31
8.0 dSm^{-1}	74.33	3.04	5.01	7.30	1.69	24.40	3.95
SEm±	0.88	0.14	0.20	0.24	0.05	0.41	0.09
CD (p=0.05)	2.49	0.40	0.56	0.68	0.16	1.17	0.26
Brassinolide							
0	74.00	2.60	4.72	6.79	1.66	23.60	4.08
0.5	76.60	3.25	5.50	7.86	1.91	25.30	4.72
1.0	82.44	3.82	6.20	8.89	2.21	28.50	5.49
1.5	88.75	4.40	7.08	9.95	2.48	31.80	6.22
SEm ±	1.01	0.16	0.23	0.28	0.06	0.48	0.11
CD (p=0.05)	2.87	0.46	0.64	0.78	0.18	1.35	0.30

per treatment up to maturity.

The plants were sprayed with brassinolides of following concentration for different treatment. The different concentrations of brassinolide 0.0 (control), 0.5 ppm, 1.0 ppm and 1.5 ppm were sprayed at vegrtative stage (52 DAS) and pre-anthesis stage (82 DAS). The observations were recorded 10 days after spray of brassinolides using completely randomized design. The data of membrane stability index were recorded by using the method of Sullivan [9]; chlorophyll content by Arnon [10]; determination of proline and protein by the methods earlier [11, 12]. Determination of seedling growth and yield parameters; seed germination percentage was calculated by counting the number of seeds germinated at 8 DAT. The root length and shoot length were measured with the help of scale at 8 DAT. Five washed seedling from each petri-dish were separated into root and shoot for the determination of fresh weight (8th DAT) and dry weight of seedling root. Dry weight was determined after oven drying the root samples at 70°C until constant weight was obtained. The height of three randomly selected plants were measured from base to the top of the plant with the help of meter scale in cm at 60 and 90 DAS. Leaf area was measured with the help of leaf area meter (LICOR 3000 USA). Three plants were selected randomly and average green leaf area was calculated at 60 and 90 DAS. The total number of spike per plant were counted in each pot and then the average was calculated. Length of the main spike excluding awns was measured with the help of scale. The total number of seeds per spike was counted in each pot and then the average was calculated. After harvest, plants were air dried and the grain yield was taken and calculated as per plant basis.

Results and Discussion

Seedling parameters

Varietal response

Table 2 showed that Raj-3077 (salinity tolerant) ex-

Table 3. Effect of salinity and brassinolide on germination %, root and shoot length of wheat. DAT=Days after treatment.

Treatments	Plant height 60 DAT	Plant height 90 DAT	Leaf area 60 DAT	Leaf area 90 DAT	Number of spikes/plant After harvesting	Length of spike/plant After harvesting	Number of seeds/spike After harvesting	Grain yield (g/plant) After harvesting
Varieties								
Raj-3077	38.60	60.90	84.88	103.57	2.86	8.90	48.60	4.98
Raj-1482	34.13	54.90	75.36	90.52	2.40	8.11	41.35	3.86
SEm ±	0.38	0.46	0.65	0.77	0.033	0.06	0.38	0.04
CD (p=0.05)	1.07	1.31	1.85	2.18	0.093	0.21	1.09	0.12
Salinity levels								
Control	42.49	60.70	90.10	108.88	3.00	9.20	52.01	5.20
4.0 dSm^{-1}	36.32	54.25	80.80	98.20	2.57	8.54	45.41	4.59
8.0 dSm^{-1}	30.28	49.50	69.35	84.00	2.26	7.77	37.50	3.46
SEm ±	0.46	0.57	0.80	0.94	0.040	0.09	0.47	0.05
CD (p=0.05)	1.31	1.61	2.27	2.67	0.114	0.26	1.33	0.14
Brassinolide								
0	30.32	48.90	68.25	85.12	2.22	8.00	38.81	3.62
0.5	35.47	54.22	78.15	93.72	2.49	8.35	41.87	4.25
1.0	38.66	58.60	85.29	103.00	2.79	8.67	48.77	4.66
1.5	41.00	65.08	88.79	106.35	3.08	9.05	50.45	5.14
SEm ±	0.53	0.66	0.92	1.09	0.047	0.11	0.54	0.06
CD (p=0.05)	1.51	1.86	2.62	3.09	0.132	0.30	1.54	0.17

hibited significant increase in germination percentage (10.81% more than Raj-1482). A significant increase in root length (10.1 more than Raj-1482 at 8 DAT), fresh weight (12.1% more than Raj-1482) and dry weight (16.4% more than Raj-1482) of seedling root more in Raj-1482 than Raj-3077 whereas an increase was recorded in shoot length (19.8% more than Raj-1482 at 8 DAT), fresh weight (11.3% more than Raj-1482) and dry weight (15.3% more than Raj-1482) of seedling shoot more in Raj-3077.

Effect of salinity

Data further revealed that salinity levels (4 and 8 dSm^{-1}) significantly reduced the seedling traits as compared to control this includes germination percentage (4.69 and 14.0% more than control), root length (12.68 and 25.8% more than control at 8 DAT), shoot length (11.2 and 25.6% more than control at 8 DAT), fresh weight of root (9.6 and 21.5% more than control), dry weight of root (13.2 and 29.8% more than control), fresh weight of shoot (15.4 and 23.2% more than control) and dry weight of shoot (15.6 and 22.7% more than control) at 4 and 8 dSm^{-1}. Higher salt concentration hampers vital processes such as germination percentage and seedling growth [13]. The harmful effects of salt stress includes the reduction in germination rate and seedling growth [14]. A decline in root and shoot length may be due to NaCl toxicity and disproportion in nutrient absorption by the seedlings [15]. As salt concentration increases in the medium, plants absorb lesser water causing physiological desiceation and may be responsible for decrease in fresh and dry weight of seedlings [16].

Effect of brassinolide

Table 2 further showed that a significant increase were recorded in seedling parameters; these involves germination percentage (3.3, 10.2 and 16.6% more than control at 8 DAT), root length (20.0, 31.9 and 40.9% more than control at 8 DAT); shoot length (14.1, 23.8 and 33.3% more than control at 8 DAT); fresh weight of root (13.6, 23.6 and 31.7% more than control at 8 DAT); dry weight of root (13.0, 24.8 and 33.0% more than control); fresh weight of shoot (6.7, 17.1 and 25.7% more than control at 8 DAT) and dry weight of shoot (13.5, 25.6 and 34.4% more than control after oven drying) at 0.5, 1.0 and 1.5 ppm concentration. Vardhini and Rao [17] reported that 28-brassinolide and 24-epibrassinolde are very effective in increasing germination percentage and seedling growth rate of sorghum under osmotic stress. Generally [18] stated that BRs removed the salinity induced inhibition of seed germination and seedling growth of rice (*Oryza sativa*).

Growth and yield parameters

Varietal response

Table 3 showed that Raj-3077 showing significantly higher plant height (11.5 and 9.8% at 60 and 90 DAT), leaf area (11.2 and 12.6% at 50 and 65 DAT), number of spikes/plant (16.0%), length of spike/plant (8.8%), number of seeds/spike (14.9% more than Raj-1482) and grain yield/plant (22.4% more than Raj-1482).

Effect of salinity

Data further revealed that the increasing level of salinity up to 6 dSm^{-1} significantly reduced the growth and yield traits as compared to control; this includes plant height (14.5 and 10.6; 28.7 and 18.4% more than control at 60 and 90 DAT); leaf area (10.3 and 9.8; 23.0 and 22.8% more than control at 60 and 90 DAT); number of spikes/plant (14.3 and 24.6% more than control); length of spike/plant (7.1 and 15.5% more than control); number of seeds/spike (12.6 and 27.8% more than control) and grain yield/plant (11.7 and 33.4% more than control) at 4 and 8 dSm^{-1}. These effects might be due to salinity which inhibits the growth of wheat plant through reduced water absorption and reduced metabolic activity due to Na and Cl toxicity and nutrient deficiency caused by lonic interference [19]. 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 [20].

Effect of brassinolide

Table 3 further showed that a significant increase were recorded in growth and yield traits, this includes plant height (14.5, 21.5 and 26.0; 9.8, 16.5 and 24.8% at 60 and 90 DAT); leaf area (12.6, 19.9 and 23.1; 9.1, 17.3 and 19.9% at 50 and 65 DAT); number of spikes/plant (10.8, 20.4 and 27.9%); length of spike/plant (4.1, 7.7 and 11.6%); number of seeds/spike (7.3, 20.4 and 23.0%) and grain yield/plant (14.8, 22.3 and 29.5%) as compared to control at 0.5, 1.0 and 1.5 ppm concentration. Foliar application of brassinolide 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. The obtained results are in good agreement with those reported by Abdel and Ebitahal [21] on wheat who stated that foliar spray of brassinolide showed a high significant increase in growth parameters as compared with un-treated control plants.

Physiological parameters

Varietal response

Table 4 showed that Raj-3077 indicating significantly higher chlorophyll a content (12.5 and 19.2% at 52 and 82 DAT), chlorophyll b (12.2 and 11.7% at 52 and 82 DAT), carotenoid content (13.8 and 12.9% at 52 and 82), relative water content (12.7 and 12.1% at 52 and 82), cell menbrane stability (4.4 and 5.27%), proline content (20.0 and 14.5%) and protein content (4.9 and 5.7%) more than Raj-1482.

Effect of salinity

Data further revealed that the increasing level of salinity 4 and 8 dSm^{-1} significantly reduced the physiological variables as compared to control; this includes chlorophyll content a (5.8 and 13.2, 5.1 and 20.1% at 52 and 82 DAT); chlorophyll content b (10.8 and 18.3; 5.8 and 14.1% at 52 and 82 DAT); cell membrane stability (5.8 and 19.3; 5.7 and 18.6% at 52 and 82 DAT); relative water content (6.3 and 14.6; 6.25 and 14.8% at 52 and 82 DAT); protein content (9.2 and 21.5; 4.36 and 18.7% at 52 and 82 DAT) with increase in proline content (25.0 and 45.4; 21.4 and 30.1 at 52 and 82 DAT) and carotenoid content (8.8 and 21.9; 10.0 and 22.0 at 52 and 82 DAT).

Chaves et al. [22] reported that the reduction in crop production in various plant species exposed to

Table 4. Effect of salinity and brassinolide on germination %, root length and shoot length of wheat. DAT=Days After Treatment.

Treatments	Chlorophyll 'a' (mg/g fw) 52 DAT	82 DAT	Chlorophyll 'b' (mg/g fw) 52 DAT	82 DAT	Carotenoid content (μg/ml) 52 DAT	82 DAT	Relative water content (%) 52 DAT	82 DAT	Cell membrane stability (%) 52 DAT	82 DAT	Proline (mg/g fw) 52 DAT	82 DAT	Protein (mg/g fw) 52 DAT	82 DAT
Varieties														
Raj-3077	1.36	1.56	1.16	1.19	1.06	1.14	73.85	73.54	70.69	76.60	0.35	0,62	18.31	21.54
Raj-1482	1.19	1.26	1.01	1.05	1.53	1.71	64.45	64.57	67.57	72.56	0.20	0.53	19.26	22.85
SEm±	0.05	0.04	0.02	0.03	0.03	0.04	1.09	1.33	0.88	0.76	0.008	0.013	0.27	0.41
CD (p=0.05)	0.15	0.10	0.05	0.09	0.07	0.10	3.10	3.77	2.50	2.15	0.023	0.038	0.75	1.17
Salinity levels														
Control	1.36	1.54	1.20	1.20	1.03	1.13	74.33	74.29	75.48	81.17	0.14	0.44	20.94	24.04
4.0 dSm^{-1}	1.28	1.46	1.07	1.13	1.33	1.49	69.67	69.64	71.07	76.53	0.24	0.56	19.00	22.99
8.0 dSm^{-1}	1.18	1.23	0.98	1.03	1.52	1.65	63.44	63.24	60.85	66.04	0.44	0.73	16.42	19.54
SEm±	0.07	0.04	0.02	0.04	0.03	0.04	1.34	1.63	1.08	0.93	0.010	0.016	0.32	0.50
CD (p=0.05)	0.19	0.13	0.06	0.11	0.09	0.13	63.44	63.24	3.06	2.64	0.029	0.046	0.92	1.43
Brassinolide														
0	1.05	1.21	0.97	0.99	1.01	1.11	74.58	75.18	61.88	67.18	0.12	0.37	16.12	19.66
0.5	1.29	1.39	1.07	1.11	1.25	1.43	79.12	80.56	66.41	71.88	0.22	0.53	17.07	21.15
1.0	1.35	1.47	1.13	1.17	1.39	1.53	83.58	85.62	71.68	76.15	0.29	0.63	19.84	23.41
1.5	1.40	1.58	1.17	1.21	1.53	1.63	88.28	90.98	76.55	83.11	0.48	0.77	22.11	24.55
SEm±	0.08	0.05	0.03	0.04	0.04	0.05	1.55	1.88	1.25	1.07	0.012	0.019	0.37	0.58
CD (p=0.05)	0.20	0.15	0.07	0.13	0.11	0.15	4.38	5.33	3.53	3.04	0.033	0.053	1.06	1.65

salt stress linked to the decline in photosynthesis. The binding between chlorophyll and chloroplast proteins depend upon the ion content of cells under high salinity, such links are loosened and as a result more chlorophyll can be destroyed [23]. Free proline is known to accumulate in response to biotic and abiotic stresses and has been shown to protect plants against free redical induced damage [24], this is because proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell. The data of protein content are in accordance with the findings of Bera et al. [25]. It could be concluded that salinity adversely affected the photosynthetic pigments. In addition, spraying salinity stressed-wheat plants with brassinolide can reduce the undesirable effect of salinity through improving growth and nutrients status of plants as well.

Effect of brassinolide

Table 3 revealed that a significant increase were recorded in physiological traits; this includes chlorophyll a content (18.6, 22.2 and 25.0; 12.9, 17.6 and 23.4% at 52 and 82 DAT); chlorophyll b content (9.3, 14.1 and 17.0; 10.8, 15.3 and 18.1% at 52 and 82 DAT); cell membrane stability (6.8, 13.6 and 19.1; 6.5, 11.7 and 19.1% at 52 and 82 DAT); relative water content (5.7, 10.7 and 15.5; 6.6, 12.1 and 17.3% at 52 and 82 DAT); carotenoid content (19.2, 27.3 and 32.6; 16.5, 22.9 and 31.9% at 52 and 82 DAT); proline content (20.0, 38.4 and 31.5; 27.4, 37.2 and 44.7% at 52 and 82 DAT) and protein content (5.5, 18.7 and 27.0; 7.0, 16.0 and 19.9% at 52 and 82 DAT) as compared to control. Brassinolide detoxified the stress generated by Na and Cl significantly improves the values for growth and photosynthetic parameters [8]. Abdel and Ebitahal [21] found that spraying plant with brassinoloide significantly increased photosynthetic pigments in *Oryza sativa* and wheat plants respectively as compared with untreated control plants. These results are in good agreement with those obtained by Cao and Zhao [26] who stated that spraying *Nigella sativa* seeds and rice seedlings with BRs respectively caused highly significant increase in photosynthetic pigment content of plants. The results of cell membreane stablity are in accordance with the findings of Gograj et al. [27]. The results of proline content are in accordance with the findings of Gograj et al. [27]. The results of protein content are in accordance with the findings of Bera et al. [25], as compared to control at 0.25.0.50 and 1.00 ppm concentration over control.

Conclusion

In this study, salinity induced variations in grain yield and quality were larger among sensitive genotypes than salt tolerant ones. These results suggested that the use of salt-tolerant wheat cultivars might be the most promising strategies for harvesting higher grain yield of best quality under saline conditions. One of the best solutions is to use saline soils effectively for improved salt tolerance in crops. For this purpose different approaches, were adopted, among those one is the exogenous application of plant growth regulators.

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