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Effect of brassinolide on physio-biochemical traits of wheat under salt stress

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Abstract
The study entitled ‘’Effect of Brassinolide on Physio-biochemical Traits of Wheat (*Triticum aestivum* L.) Under Salinity’’ was conducted in the cage house at Department of Plant Physiology, S.K.N. College of Agriculture Jobner during *rabi* season of 2015-2016 under pot culture experiments. Two wheat cultivars namely Raj-1482 (salinity susceptible) and Raj-3077 (Salinity tolerant) were grown in cemented pots under salinity (0, 5 and 10 dSm^{-1}). Different concentrations of brassinolide (0.0, 1.0 and 1.5 ppm) were sprayed at 45 and 75 days after sowing. Control plants were provided normal water. Physio-biochemical observations were recorded at 52 and 82 days after sowing in pot conditions. A significant decrease were recorded in Chlorophyll, protein, relative water content, cell membrane stability, with increase in salt stress up to EC 10 dSm^{-1}.Whereas the foliar spray treatment with brassinolide up to 1.5 ppm significantly increased chlorophyll, proline, protein, reducing sugar, relative water content, cell membrane stability, potassium and calcium content in both the cultivars at 52 and 82 DAS under salt stress as well as non stress conditions. The 1.5 ppm concentration of brassinolide was found most effective under salt stress and non stress conditions. Raj-3077 observed superior over Raj 1482 on the basis of physio-biochemical analysis.

Keywords: Wheat, salinity, brassinolide, physio-biochemical traits, Raj-1482 and Raj-3077

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 nowadays, 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 grown under salinity stress according to their genes content [3].

Brassinosteroids (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 stress, 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]. Problem of salinity is increasing day by day; 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. The objective of this study was to observe the effect of exogenous application of brassinolide as foliar spray in amelioration of harmful effects of salinity on growth and yield of wheat.

Materials and methods

Plant materials and experimental details: A pot experiment were conducted at cage house located in the Department of Plant Physiology, S.K.N. College of Agriculture, Jobner during *Rabi* season 2015-15, to investigate "Effect of brassinolide on physio-biochemical traits of wheat under salt stress". The pots were filled with 15kg of loamy sandy soil having a bulk density of 1.5 g cm^{-3}, electric conductivity (EC) 0.4 dSm^{-1}, P^H 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.54 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 EC 5 and 10 dSm^{-1}; Chloride and sulphate in 3:1 ratio by using following salts; NaCl, $NaSO_4$, CaCl and $MgCl_2$. 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 per treatment up to maturity.

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

The plants were spraid with Brassinolides of following concentration for different treatment. The different concentrations of brassinolide 0.0 (control), 1.0 ppm and 1.5 ppm were sprayed at tillering stage (45 DAS) and anthesis stage (75 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 [8]; Chlorophyll content by [9]; Determination of protein, proline and relative water content by the methods of [10], [11] and [12]. Sodium and potassium content in seed was estimated by analysis of suitable aliquot of acid digested plant material (extract-I) by flame photometer. Calcium content in seed was determined by useing of suitable aliquot of acid digested plant material (extract-I). Statistical analysis of data was processed using completely randomized block design. The standard error of each means values were also calculated for presentation with bar diagram.

Results and Discussion

Varietal response

It is evident from the data in Table 1and 2 that the increase in chlorophyll "a" and "b", relative water content percent, cell membrane stability, proline, protein, reducing sugar content in leaf samples of Raj-3077 was found significantly more than Raj-1482 under salinity conditions. The per cent increase in chlorophyll "a" and "b" content of Raj-3077 was recorded 16.41, 19.33 and 18.10, 12.17; relative water content was recorded13.21 and 12.19 per cent; cell membrane stability 4.41 and 5.26 per cent; proline content was recorded 73.68 and 9.09 per cent; protein content was recorded 4.96 and 5.72 per cent; reducing sugar content was recorded 2.76 and 1.39 per cent than Raj-1482 at 52 and 82 DAS, respectively.

Data from Table 2, showing that the cultivar Raj-1482 registered significantly higher Na and K content over Raj-3077. The per cent increase in Na and K content in grain samples of Raj-1482 was 3.23 and 5.12 than Raj-3077 whereas Raj-3077 registered significantly higher Ca content over Raj-1482. The per cent increase in Ca content in grain samples of Raj-3077 was 16.79 than Raj-1482.

Effect of brassinolide

A study of the data in the above table 1 indicated that spray treatment with brassinolide up to 1.5 ppm concentration significantly increased chlorophyll "a" and "b" content, relative water content percent, cell membrane stability, proline content, protein content, reducing sugar content over its preceding levels at 52 and 82 DAS. The increase in chlorophyll "a" and "b" content in leaves due to application of 1.0 and 1.50 ppm concentration of brassinolide was 23.07,15.38;29.80,27.47 and 12.19,15.78,18.69;31.57 per cent at 52 and 82 DAS, respectively over that of control under saline conditions. Higher chlorophyll content in wheat by treatment of 28-homobrassinolide reported by results of [13].

A further study of the data in the above table 1 indicated that brassinolide significantly increased relative water content percent over its preceding levels. The increase in relative water content percent content was 10.08, 18.04 and 11.41, 21.55per cent at 52 and 82 DAS, respectively over that of control under saline conditions.

A further study of the data in the above table1 indicated that brassinolide significantly increased cell membrane stability over its preceding levels. The increase in cell membrane stability was 11.80, 21.24 and 9.01, 15.52 per cent at 52 and 82 DAS, respectively over that of control under saline conditions.

A further study of the data in the above table 2 indicated that brassinolide significantly increased proline content over its preceding levels. The increase in proline content was 36.84, 121.05 and 21.95, 73.17 per cent at 52 and 82 DAS, respectively over that of control under saline conditions [14]. They reported that application of brassinosteroids increases the accumulation of proline and enhances activities of antioxidant enzymes in salt stressed *Cicer arietinum* and *Vigna radiata.*

A further study of the data in the above table 2 indicated that brassinolide significantly increased protein content over its preceding levels. The increase in protein content was 8.05, 25.47 and12.23, 24.01 per cent at 52 and 82 DAS, respectively over that of control under saline conditions. Present investigation is in agreement with the results reported by [15].

A further study of the data in the above table 2 indicated that brassinolide significantly increased reducing sugar content content over its preceding levels. The increase in reducing sugar content content was 9.58, 15.23 and 12.19, 18.67 per cent at 52 and 82 DAS, respectively over that of control under saline conditions.

The increase in sodium, potassium and calcium content were recorded in seed due to use of 1.0 and 1.50 ppm concentration of brassinolide was 2.31, 6.94 per cent; 11.17, 15.47 per cent and13.36, 18.37 per cent over that of control after harvesting.

Effect of salinity

Data presented in the above table 1 further revealed that salt stress caused significant reduction in chlorophyll "a" and "b" content up to EC 10.0 dSm^{-1} at 52 and 82 DAS. The decrease in chlorophyll "a" and "b" content at EC 5.0 and EC 10.0 dSm^{-1} was recorded 3.87, 10.84; 4.72, 10.26 and 10.25, 17.94; 7.62, 14.40 per cent. Chlorophyll contents are sensitive to salt exposure and a reduction in chlorophyll levels due to salt stress has been reported in wheat [16].

Salinity caused significant reduction in RWC percent up to EC 10.0 dSm^{-1} at 52 and 82 DAS. The decrease in RWC content at EC 5.0 and EC 10.0 dSm^{-1} was recorded 6.27, 14.66 and 6.25, 14.87 per cent over control at both the stages, respectively.

Salt stress caused significant reduction in cell membrane stability percent up to EC 10.0 dSm^{-1} at 52 and 82 DAS. The decrease in cell membrane stability content at EC 5.0 and EC 10.0 dSm^{-1} was recorded 5.84, 19.37 and 5.79, 19.31per cent over control at both the stages, respectively. The results are in accordance with the findings of [17] they found that salinity caused to decrease membrane stability index in two wheat genotypes but the reduction was more pronounced in susceptible one (Raj-1482) than tolerant (K-65) genotype.

Under salinity a significant increase in proline content percent was noticed up to EC 10.0 dSm^{-1} at 52 and 82 DAS. The decrease in proline content content at EC 5.0 and EC 10.0 dSm^{-1} was recorded 85.71, 200.05 and 21.95 and 73.17per cent over control at both the stages, respectively. This is because proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell, which is reported in salt tolerant and salt sensitive cultivars of many crops [18].

Salinity caused significant reduction in protein content percent up to EC 10.0 dSm^{-1} at 52 and 82 DAS. The decrease in protein content content at EC 5.0 and EC 10.0 dSm^{-1} was recorded 9.24, 21.57 and 4.36, 18.71 per cent over control at both the stages, respectively. Nuclic acid, protein level in NaCl treated rice seedling decreased with increase in salt concentration in comparison to control [19].

Under salinity a significant reduction in reducing sugar percent was recorded up to EC 10.0 dSm^{-1} at 52 and 82 DAS. The decrease in reducing sugar content at EC 5.0 and EC 10.0 dSm^{-1} was recorded 3.81, 13.10 and 7.74, 14.38 per cent over control at both the stages, respectively.

Further examination of data given in above table 2 revealed that a significant increase in Na, K and Ca content in grains was recorded due to salinity up to EC 10.0 dSm^{-1} after harvesting at EC 5.0 and EC 10.0 dSm^{-1}. The increase in Na, K and Ca content to the extent of 3.46, 3.98 per cent; 7.35, 6.87 per cent and 16.66 and 23.77 per cent over control at the stages of investigation, respectively.

Interactive effect

The interactive effect of variety and salinity; and variety and brassinolide on sodium content was found to be non significant.

Conclusion

Raj-3077 was found to performed better in comparison to Raj-1482 with respect to physio-biochemical parameters, growth, yield and yield attributes under salt stress. The adverse effects of salinity on growth, physio-biochemical parameters, yield and yield attributes of wheat varieties were observed to reduced by the use of brassinolide up to 1.5ppm concentration as foliar spray.It may be concluded from this investigation that the 1.5ppm concentration of brassinolide may be recommended to farmers for the cultivation of wheat under salt stress up to EC 10 dSm^{-1}.

Table 1: Effect of brassinolide on physiological and biochemical traits of wheat under salinity

Treatments	Chlorophyll 'a' (mg/g fr. wt. of leaf)		Chlorophyll 'b' (mg/g fr. wt. of leaf)		Cell membrane stability (%)		Relative water content (%)	
	52 DAT	82 DAT	52 DAT	82 DAT	52 DAT	82 DAT	52 DAT	82 DAT
Varieties								
Raj-3077	1.34	1.50	1.16	1.15	68.16	73.68	75.65	74.88
Raj-1482	1.12	1.21	0.95	1.01	65.15	69.80	65.65	65.75
S.Em ±	0.05	0.05	0.02	0.04	1.05	0.78	1.38	1.73
CD(P=0.05)	0.14	0.14	0.07	0.11	3.00	2.24	3.96	4.95
Salinity levels								
Control	1.29	1.48	1.17	1.17	72.77	78.29	75.72	75.64
5.0 dSm^{-1}	1.24	1.41	1.05	1.09	68.52	73.75	70.97	70.91
10.0 dSm^{-1}	1.15	1.18	0.96	1.00	58.67	63.17	64.62	64.39
S.Em ±	0.06	0.06	0.03	0.05	1.28	0.96	1.69	2.12
CD(P=0.05)	0.17	0.17	0.08	0.14	3.67	2.74	4.86	6.06
Brassinolide								
0	1.04	1.23	0.91	0.95	60.12	66.30	63.18	61.85
1.0	1.28	1.38	1.05	1.10	67.22	72.28	69.55	68.91
1.5	1.35	1.46	1.16	1.25	72.83	76.59	74.58	75.18
S.Em ±	0.06	0.06	0.03	0.05	1.28	0.96	1.69	2.12
CD(P=0.05)	0.17	0.17	0.08	0.14	3.67	2.74	4.86	6.06

DAT=Days after Treatment

Table 2: Effect of brassinolide on physiological and biochemical attributes of wheat under salinity

Treatments	Proline (mg/g fr. wt. of leaf)		Protein (mg/g fr. wt. of leaf)		Reducing Sugar (mg/g fr. wt. of leaf)		Na content in grain (%)	K content in grain (%)	Ca content in grain (%)
	52 DAT	82 DAT	52 DAT	82 DAT	52 DAT	82 DAT	After harvesting	After harvesting	After harvesting
Varieties									
Raj-3077	19	55	18.13	22.04	16.65	17.17	0.247	0.390	0.506
Raj-1482	33	60	17.23	20.78	17.11	17.41	0.255	0.370	0.421
S.Em ±	0.07	0.14	0.32	0.53	0.26	0.27	0.026	0.030	0.024
CD(P=0.05)	0.21	0.41	0.91	1.51	0.74	0.76	0.074	0.085	0.069
Salinity levels									
Control	19	41	19.70	23.19	16.17	17.02	0.260	0.408	0.408
5.0 dSm^{-1}	26	50	17.88	22.18	17.90	18.34	0.251	0.378	0.476
10.0 dSm^{-1}	42	71	15.45	18.85	18.61	19.88	0.241	0.352	0.505
S.Em ±	0.12	0.19	0.39	0.65	0.32	0.33	0.032	0.036	0.029
CD(P=0.05)	0.33	0.56	1.12	1.85	0.91	0.93	0.099	0.104	0.084
Brassinolide									
0	12	35	15.90	19.20	16.28	16.81	0.259	0.349	0.419
1.0	23	49	17.18	21.55	17.84	18.86	0.253	0.388	0.475
1.5	30	61	19.95	23.81	18.76	19.95	0.241	0.403	0.496
S.Em ±	0.12	0.19	0.39	0.65	0.32	0.33	0.032	0.036	0.029
CD(P=0.05)	0.33	0.56	1.12	1.85	0.91	0.93	0.091	0.104	0.084

DAT=Days after Treatment

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