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Role of brassinolide on physio-biochemical traits of barley under salt stress

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Abstract
The study entitled "Role of Brassinolide on Physio-biochemical Traits of barley (*Hordeum vulgare* 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 barley cultivars namely RD 2035 (salinity susceptible) and RD 2794 (Salinity tolerant) were grown in cemented pots under salinity (0, 3, 6, 9 and 12 dSm^{-1}). Different concentrations of brassinolide (0.0, 0.5, 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 12 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 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. RD 2794 observed superior over RD 2035 on the basis of physio-biochemical analysis.

Keywords: Barley, salinity, brassinolide, physio-biochemical traits, RD 2794 and RD 2035

Introduction

Barley is the world's fourths important cereal crop after wheat, rice and maize. Being the most dependable crop in areas where alkali, frost or draught situation occur, it is cultivated in all most all parts of the world. The major barley producing countries are China, Russia, Germeny, USA, Canada, India, Turkey and Australia. The major use of barley grain is in brewing indurstries for manufacturing malt which is used for making beer, industrial alcohol, whisky, malt syrup, brandy malted milk, vinegar and yeast. Barley contains 12.5% moisture, 11.5% albuminoids, 74.0% carbohydrate, 1.3% fat, 3.9% crude fiber and 1.5% ash. Its surplus grains are used as concentrate for feeding livestock and poultry and as a base in mushroom production. Its straw and husk are good quality roughage for cattle. Barley straw is also used to prepare paper and card board.

Barley ranks next to wheat both in acreage and production among *rabi* cereals in India. It is so, as it requires lesser water and is fairly tolerant to salinity, alkalinity, frost and draught situations. Barley also does well even with brackish/saline water. It is also more suited to dry land and 'diara land'. Barley is generally grown on marginal and sub-marginal lands with low inputs. In Rajasthan, it is mostly grown on light textured soils, low in nitrogen and organic matter content with poor moisture retentive capacity.

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 (Atlassi *et al*., 2009) [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 (Yaycili and Alikamanoglu, 2012) [2]. Since salinity is complicated trait and genetically controlled, plants show different response when they grown under salinity stress according to their genes content (Gupta and Huang, 2014) [3].

Brassinosteroids (BRs) are a new type of polyhydroxy steroidal phytohormones with significant growth-promoting influence (Vardhini and Anjum, 2015) [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(Vardhini, 2013) [5]. BRs confer salt tolerance to plants by mitigating its negative effects on the physiological, biochemical and molecular processes in plants (Ashraf *et al*. 2010) [6]. Brassinolide improved the growth, yield and chemical composition of berseem (*Trifolium alexandrinum* L.) grown in saline soils (Daur and Tatar, 2013) [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 "Role of brassinolide on physio-biochemical traits of barley 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.120 pots for both cultivar RD-2794 (salinity tolerant) and RD-2035(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 3, 6, 9 and 12 dSm^{-1} Chloride and sulphate is used 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.

The plants were spraid with Brassinolides of following concentration for different treatment. The different concentrations of brassinolide 0.0, 0.5, 1.0 ppm and 1.5 ppm were sprayed at tillering stage (45 DAS) and anthesis stage (75 DAS). The observations were recorded 7 days after spray of brassinolides using Completely Randomized Design. The data of Membrane stability index were recorded by using the method of (Sullivan, 1972) [8]; Chlorophyll content by (Arnon, 1049) [9]; Determination of protein, proline and relative water content by the methods of (Lowry *et al*. 1951) [10], (Bates *et al*.1973) [11] and (Barr and Weatherley, 1962) [12]. 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 RD-2794 was found significantly more than RD-2035 under salinity conditions.

The per cent increase in chlorophyll "a" and "b" content of RD-2794 was recorded 14.41, 13.33 and 21.09, 15.0; relative water content was recorded 14.40 and 13.87 per cent; cell membrane stability 2.53 and 2.69 per cent; proline content was recorded 37.93 and 45.95 per cent; protein content was recorded 16.35 and 8.87 per cent; reducing sugar content was recorded 15.17 and 16.59 per cent more than RD- 2035 at 52 and 82 DAS, respectively.

Table 1: Effect of brassinolide on physiological and biochemical traits of barley 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								
RD-2794	1.35	1.55	1.19	1.15	77.91	77.81	74.34	74.54
RD-2035	1.18	1.28	1.05	1.00	75.99	75.77	64.98	65.46
S.Em ±	0.03	0.04	0.03	0.03	0.74	0.79	1.14	0.90
CD(P=0.05)	0.10	0.11	0.09	0.08	2.08	2.22	3.21	2.51
Salinity levels(dSm^{-1})								
Control	1.42	1.58	1.20	1.38	82.55	84.11	74.55	74.66
3.0	1.37	1.54	1.18	1.20	79.55	80.55	72.54	72.81
6.0	1.29	1.44	1.14	1.15	76.61	77.00	70.42	71.00
9.0	1.19	1.33	1.09	1.08	74.00	74.58	67.22	67.65
12.0	1.06	1.19	0.98	0.97	72.02	72.71	63.55	63.88
S.Em ±	0.05	0.06	0.05	0.05	1.17	1.25	1.81	1.42
CD(P=0.05)	0.15	0.17	0.14	0.13	3.29	3.51	5.07	3.97
Brassinolide								
0	1.04	1.24	0.99	0.95	71.89	73.32	64.86	65.13
0.5	1.26	1.37	1.09	1.06	75.63	76.18	67.63	68.09
1.0	1.34	1.49	1.17	1.13	78.66	79.41	70.72	71.23
1.5	1.41	1.57	1.21	1.16	81.61	82.25	75.42	75.56
S.Em ±	0.05	0.05	0.04	0.04	1.05	1.12	1.62	1.27
CD(P=0.05)	0.13	0.15	0.12	0.12	2.94	3.14	4.54	3.55

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

Treatments	Proline (mg/g fr. wt. of leaf)		Protein (mg/g fr. wt. of leaf)		Reducing Sugar (mg/g fr. wt. of leaf)	
	52 DAT	82 DAT	52 DAT	82 DAT	52 DAT	82 DAT
Varieties						
RD-2794	29	37	19.85	21.73	18.33	18.69
RD-2035	40	54	17.06	19.96	21.11	21.79
S.Em ±	3.8	3.1	0.26	0.31	0.32	0.36
CD(P=0.05)	10.6	8.7	0.73	0.87	0.89	1.02
Salinity levels						
Control	14	28	20.90	23.01	16.28	17.05
3.0	28	39	19.25	22.11	18.64	19.11
6.0	38	48	17.55	21.00	20.11	20.45
9.0	54	65	16.81	19.89	21.33	21.75
12.0	79	85	15.74	18.22	22.25	22.85
S.Em ±	3.2	2.9	0.41	0.49	0.50	0.58
CD(P=0.05)	8.9	8.2	1.15	1.38	1.40	1.62
Brassinolide						
0	29	32	16.72	19.17	17.53	18.38
0.5	31	38	17.99	20.39	19.07	19.69
1.0	40	46	19.17	21.48	20.55	20.84
1.5	52	56	19.93	22.34	21.73	22.06
S.Em ±	1.7	2.1	0.37	0.44	0.45	0.52
CD(P=0.05)	4.7	5.8	1.03	1.23	1.25	1.45

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 content due to application of brassinolide @1.5 ppm concentration was 35.58, 11.90 and 22.22, 11.01 per cent at 52 DAS, 26.61, 14.60 and 22.11, 9.43 per cent of chlorophyll "b" content at 82 DAS, respectively over control and 0.5 ppm concentration of brassinolide. Higher chlorophyll content in wheat by treatment of 28-homobrassinolide reported by results of (Sairam, 1994) [13].

The increase in relative water content due to application of brassinolide @ 1.5 ppm concentration was 16.28, 11.52 and 6.65 per cent at 52 DAS, 16.01, 10.97 and 6.08 per cent at 82 DAS, respectively over control, 0.5 and 1.0 ppm concentration of brassinolide. The increase in membrane stability due to application of 0.50, 1.0 and 1.50 ppm concentration of brassinolide was 13.52, 7.91, 3.75 at 52 DAS and 12.18, 7.97 and 3.58 per cent at 82 DAS over control, 0.50 and 1.0 ppm concentration of brassinolide, respectively.

The increase in proline content due to application of brassinolide at 0.50,1.0 and 1.50 ppm was obtained 84.61, 42.85; 130.76, 77.14 and 261.53, 114.28 per cent over that of control at 52 and 82 DAS. (Hayat *et al*.2010) [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.*

The increase in protein content due to application of brassinolide at 0.50 and 1.0 ppm was obtained 19.20, 10.78 and 16.54, 9.56 per cent over that of control at 52 and 82 DAS. Present investigation is in agreement with the results reported by (Bera *et al* 2006) [15]. The increase in reducing sugar due to application of brassinolide at 0.50 and 1.0 ppm was obtained 23.96, 20.02 and 13.95, 12.04 per cent over that of control at 52 and 82 DAS.

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 12.0 dSm^{-1} at 52 and 82 DAS.

The per cent decrease in chlorophyll "a" and "b" content at EC 3.0 dSm^{-1} was 3.65, 1.69, at EC 6.0 dSm^{-1} was 10.08, 5.26, at EC 9.0 dSm^{-1} was 19.33, 10.09 and at EC 12.0 dSm^{-1}, it was recorded 33.96 and 22.45 per cent at 52 DAS and EC 3.0 dSm^{-1} was 2.60, 15.00, at EC 6.0 dSm^{-1} was 9.72, 20.00, at EC 9.0 dSm^{-1} was 18.80, 27.78 and at EC 12.0 dSm^{-1}, it was recorded 32.77, 42.27 at 82 DAS over control, respectively. Chlorophyll contents are sensitive to salt exposure and a reduction in chlorophyll levels due to salt stress has been reported in wheat (Ashraf *et al* 2002) [16].

The data further revealed that relative water content decreased with increasing salinity levels up to EC 12.0 dSm^{-1}. The per cent decrease in relative water content at EC 3.0 dSm^{-1} was 2.77, at EC 6.0 dSm^{-1} was 5.86, at EC 9.0 dSm^{-1} was 10.90 and at EC 12.0 dSm^{-1}, it was recorded 17.31 per cent at 52 DAS and EC 3.0 dSm^{-1} was 2.54, at EC 6.0 dSm^{-1} was 5.15, at EC 9.0 dSm^{-1} was 10.36 and at EC 12.0 dSm^{-1}, it was recorded 16.88 per cent at 82 DAS over control, respectively.

The decrease in cell membrane stability at EC 3.0 was 3.77, 4.42 dSm^{-1}, EC 6.0 was 7.75, 9.23 dSm^{-1}, EC 9.0 was 11.55, 12.78 dSm^{-1} and EC 12.0 dSm^{-1} was recorded 14.62, 15.68 per cent over control at 52 and 82 DAS, respectively. The results are in accordance with the findings of (Sairam and Srivastava,2002)[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.

The decrease in proline content at EC 3.0 was 3.77, 4.42dSm^{-1}, EC 6.0 was 7.75, 9.23 dSm^{-1}, EC 9.0 was 11.55, 12.78 dSm^{-1} and EC 12.0 dSm^{-1} was recorded 14.62, 15.68 per cent over control at 52 and 82 DAS, 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 (Misra and Gupta,2005) [18].

The decrease in protein content at EC 6.0 was 19.09, 9.57dSm^{-1}, EC 9.0 was 24.33, 15.69 dSm^{-1} and EC 12.0 dSm^{-1} was recorded 32.78, 26.29 per cent over control at 52 and 82 DAS, respectively. Nuclic acid, protein level in NaCl treated rice seedling decreased with increase in salt concentration in comparison to control (Bera *et al* 2006) [19].

The significantly increase in reducing sugar at EC 12.0 dSm^{-1} and remained at par at EC 9.0 over control, EC 3.0 and 6.0 dSm^{-1}, respectively. The per cent increase in reducing sugar at EC 12.0 dSm^{-1} was 36.67, 34.02 and 19.37, 19.57 and 10.64, 11.74 over control, EC 3.0 and 6.0 dSm^{-1}, respectively at 52 and 82 DAS.

Interactive effect

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

Conclusion

RD-2794 was found to performed better in comparison to RD-2035 with respect to physio-biochemical parameters under salt stress. The adverse effects of salinity on physio-biochemical parameters of barley varieties were observed to reduce 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 12 dSm^{-1}.

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