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Effect of brassinolide in alleviating the adverse effects of salinity on physiological, biochemical and yield attributes of wheat

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Abstract

A pot experiment was conducted during *rabi* season, 2013 in the cage house at Department of Plant Physiology, S.K.N. College of Agriculture, Jobner, Rajasthan to study the effect of brassinolide in alleviating the adverse effects of salinity on physiological, biochemical and yield attributes of wheat. Wheat cultivars namely HD-2687 (salinity susceptible) and Raj-3777 (Salinity tolerant) were investigated in pot conditions, desired salinity levels were obtained by mixing $NaSO_4$, NaCl, $CaCl_2$, and $MgCl_2$ in irrigation water in 3:1 ratio of chloride and sulphate. Different concentrations of brassinolide (0.0, 0.25, 0.50 and 1.0 ppm) were sprayed at 45 and 60 days after sowing. Control plants were provided normal water whenever needed. Analysis of data revealed that salinity stress decreased photosynthetic rate, transpiration rate, stomatal conductance, total chlorophyll content, cell membrane stability and protein content in both the cultivars up to EC 8 dSm^{-1} at 50 and 65 DAS, but except in proline content which was increased, whereas, brassinolide spray up to 1.0 ppm concentration significantly increased all these traits at 50 and 65 DAS in both the cultivars. Length of spike per plant and test weight reduced significantly by salinity at EC 4 and 8 dSm^{-1}. The 1.0 ppm concentration of brassinolide was found most effective in increasing the parameters studied. Reduction in physiological-biochemical and yield contributing parameters on account of salt stress was more in cultivar HD-2687. On the basis of the above findings genotype Raj-3777 observed most salt tolerant and the tolerance was mediated by physiological-biochemical characteristics.

Keywords: Salinity, transpiration, proline, brassinolide, stomatal conductance, wheat

1. Introduction

Soil salinity is one of the most important abiotic stress and limiting factor for worldwide conventional agriculture. Up to 20% of the irrigated arable land in arid and semiarid regions is already salt affected and is still expanding (Muhling and Lauchli, 2003) [15]. Under salt stress, plants have to cope with stress imposed by the low external water potential and with ion toxicity due to accumulation of ions inside the plants (Romero-Aranda *et al.,* 2006) [18]. Salt stress is known to affect the seed germination, growth, water deficit, ion imbalance and cause several biochemical lesions in various plants (Promila and Kumar, 2000 and Dash and Panda, 2001) [16, 7]. Salt stress is a serious problem in crop production in India.

Wheat (*Triticum aestivum* L.) is an important staple cereal crop throughout the world. In India, it is the second staple food crop following the rice. 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. There are certain chemicals which may be useful for protecting crop plants against abiotic stresses like salinity. Brassinolide has emerged as a new phytohormone with pleiotropic effect (Sasse, 1997) [21], and influences varied physiological processes like germination, growth, flowering, senescence and confers resistance to plant against various abiotic stresses. As the first steroidal plant growth regulator was isolated from the pollens of rape (*Brassica napus*), a generic name “Brassinosteroids” has been given to this new group of phytohormones. Brassinosteroids improve the resistance of plants against environmental stresses such as water stress, salinity stress, low temperature stress and high temperature stress (Rao *et al.,* 2002) [17]. Hence, the present work was planned to find out some useful physiological and biochemical parameters for screening tolerant/ susceptible wheat genotypes against salinity stress and to understand the mechanism of salt tolerance in wheat during seedling growth to elucidate the basis for improvement of the genotypes under brassinolide treatment.

2. Materials and Methods

The effect of brassinolide in alleviating the adverse effects of salinity on physiological, biochemical and yield attributes of wheat cultivars namely Raj-3777 salinity tolerant and HD-2687 salinity susceptible will be screened out in pot conditions. Seeds were raised in seventy two cemented pots filled with about 10 Kg of well-mixed FYM soil in each pot. The crop will be irrigated with saline irrigation water one liter to each pot of EC 0 (Tap water), 4 and 8 dS m^{-1} prepared by mixing of $NaSO_4$, NaCl, $CaCl_2$, and $MgCl_2$ salts in 3:1 ratio of chloride and sulphate up to maturity. The different concentrations of brassinolide were sprayed at Vegetative stage (45 DAS) and Pre-anthesis stage (60 DAS). Observations were recorded at 50 and 65 DAS (5 days after spray of brassinolides). The rate of photosynthesis, Transpiration rate, Stomatal conductance were measured by using Infra-Red Gas Analyzer (CID 301, USA) on the flag leaf and measurements were taken between 10.00 to 11.30 AM (Indian Standard Time). Total chlorophyll content as mg g^{-1} fresh weight of leaf (The sum of chlorophyll ‘a’ and chlorophyll ‘b’) were estimated according to the method of Arnon (1949) [3]. Free proline (mg g^{-1} fresh weight of leaf) was determined using the method of Bates *et al.* (1973) [5]. Membrane stability (%) was calculated by taking the electrical conductivity of leaf leachates in double distilled water at 40° and 100° C by following the method of Sai ram (1994). Protein (mg g^{-1} fresh weight of leaf) was measured by method of Lowry *et al.*, (1951) [12] at 50 and 65 DAS. After harvest, length of the main spike excluding awns was measured with the help of scale in cm, plants were air dried and the grain was taken as per plant basis and 1000-grains were counted and their average weight (g) was recorded. The experiment was laid out in factorial based on Completely Randomized Design with three replications.

3. Results and discussion

Data shown in Table 1 revealed that the photosynthetic rate differed with increasing level of salinity of irrigation water up to EC 8.0 dSm^{-1} (32.24 and 29.39 per cent) at 50 and 65 DAS over control (normal water irrigation). Variety Raj-3777 (salinity tolerant) performing better than HD-2687 (salinity susceptible) in stress as well as non-stress conditions. This may be due to the direct effect of salts on stomatal conductance via. a reduction in guard cell turgor and intercellular CO_2 to inhibit photosynthesis (El-Hendawy *et al.* 2005) [8]. However, spray treatment with brassinolide up to 1.0 ppm significantly increased, 73.61 and 61.19 per cent photosynthetic rate over control at 50 and 65 DAS under normal and stress conditions (Table 1). Shamsul hayat *et al.* (2010) [22] stated that treatment with homobrassinolide detoxified the stress generated by NaCl and significantly improved the values for growth and photosynthetic parameters. Generally, transpiration rate tended to decline with increasing salinity in present study. This may be due to the fact that lowered water potentials in the root can trigger a signal from root to shoot (such as abscisic acid, which has been suggested to be the operating mechanism), Zhang and Davies (1991) [25]. Variable responses with the salt tolerant and sensitive genotypes showed a relatively greater decline in transpiration rate and stomatal conductance (Table 1). A higher transpiration rate, 29.67 and 25.77 per cent was recorded by genotype Raj-3777 than HD-2687 under non stress and salt stress conditions. Transpiration rate and stomatal conductance decrease significantly with increasing salinity levels and increased with spray treatment of brassinolide up to 1.0 ppm concentration significantly at 50 and 65 DAS over its preceding levels, Table 1. Similar reports were made by earlier workers, Gograj Jat *et al.* (2012) [9] they reported that use of brassinolide up to 1.0 ppm was observed to increase significantly photosynthetic rate, transpiration rate (at flowering stage) and stomatal conductance of clusterbean cultivars in salinity stress. The results in the study showed that the decline in stomatal conductance due to salinity was minimum in salt tolerant cultivar Raj-3777 than in comparison to salt susceptible cultivar HD-2687 at 50 and 65 days after sowing. This is because of salt accumulation in the mesophyll cells may inhibit carbon assimilation resulting in an increase in internal CO_2 concentration, with eventual reduction in stomatal conductance (Mexwell and Johnson, 2000) [13]. The per cent increase in stomatal conductance of Raj-3777 was observed 6.07 and 5.95 than HD-2687 due to brassinolide spray. The 1.0 ppm concentration of brassinolide increase 42.30 and 27.00 per cent stomatal conductance over control which was found maximum at non stress and salt stress conditions. Further, the total chlorophyll content decreased in both genotypes with the increase in salt stress and decrease being more in HD-2687 (susceptible genotype). The higher chlorophyll amounts in tolerant cultivars may be related to their ability in repairing salt-dependent damage. The decrease in chlorophyll content at EC 4.0 and EC 8.0 dSm^{-1} was found 10.43, 7.19 and 26.08, 21.22 per cent at both stages over normal water irrigation (Table 1). Similar study made by earlier workers the chlorophyll contents significantly reduced under elevated salt stress, as the chlorophyll contents are sensitive to salt exposure and a reduction in chlorophyll levels due to salt stress has been reported in wheat plants (Ashraf *et al.*, 2002) [4].

Table 1: Effect of salinity and brassinolides on photosynthetic rate, transpiration rate, stomatal conductance and total chlorophyll content of wheat

Treatments	**Photosynthetic rate (μ mol CO_2 m^{-2} s^{-1})**		**Transpiration rate (m mol H_2O m^{-2} s^{-1})**		**Stomatal conductance (m mol m^{-2}sec^{-1})**		**Total Chlorophyll (mg/g f.w.)**	
Varieties	**50 DAS**	**65 DAS**	**50 DAS**	**65 DAS**	**50 DAS**	**65 DAS**	**50 DAS**	**65 DAS**
Raj-3777	33.52	37.28	1.82	2.25	47.11	51.56	2.12	2.63
HD-2687	21.18	24.65	1.28	1.67	44.25	48.49	1.92	2.40
S.Em. +	0.39	0.42	0.020	0.024	0.58	0.60	0.026	0.030
C.D. (P=0.05)	1.11	1.20	0.057	0.068	1.64	1.69	0.074	0.086
Salinity levels (dSm^{-1})								
0	32.38	36.20	1.88	2.34	50.57	54.86	2.30	2.78
4	27.73	31.14	1.55	1.95	45.24	49.68	2.06	2.58
8	21.94	25.56	1.22	1.60	41.25	45.54	1.70	2.19
S.Em. +	0.48	0.52	0.025	0.029	0.71	0.73	0.032	0.037
C.D. (P=0.05)	1.36	1.47	0.070	0.083	2.01	2.07	0.091	0.106
Brassinolides (ppm)								
0	19.44	23.14	1.14	1.52	37.47	43.99	1.70	2.24
0.25	25.51	29.10	1.46	1.85	42.44	47.14	1.94	2.40

0.50	30.71	34.33	1.73	2.15	49.50	53.10	2.14	2.60
1.00	33.75	37.30	1.87	2.34	53.32	55.87	2.30	2.82
S.Em. +	0.55	0.60	0.028	0.034	0.82	0.84	0.037	0.043
C.D. (P=0.05)	1.57	1.70	0.080	0.096	2.32	2.39	0.105	0.122

Maximum chlorophyll content was obtained due to spray treatment with 1.0 ppm concentration of brassinolide at both the stages of investigation. Our results are supported by findings of Sharma *et al.* (2008) [24] they showed that increase in chlorophyll content along with other yield attributes in wheat with foliar spray of bio-regulators. Membrane stability decreased under salinity stress in investigation however, which was recorded significantly higher in Raj-3777 than HD-2687 under non stress conditions (Table 2). The obtained results are in good agreement with Sairam and Srivastava (2002) [20] they reported that salinity caused to decrease membrane stability index in two wheat genotypes but the reduction was more pronounced in susceptible one (HD-2687) than tolerant (K-65) genotype. Cell membrane stability decrease significantly with increasing level of salinity up to EC 8.0 dSm^{-1} at 50 and 65 DAS. Further, cell membrane stability increased due to application of 0.25, 0.50 and 1.0 ppm concentration of brassinolide which was recorded 5.80, 5.90; 13.81, 12.86 and 21.15, 19.20 per cent over control in investigation. Brassinosteroid induce plants resistance to environmental stresses such as drought, salt, low or high temperatures etc., through brassinosteroid induced effects on membrane stability and osmoregulation, this is likely to suggest that brassinosteroid had an important role in membrane stability and osmoregulation (Rao *et al.*, 2002) [17]. Proline level in wheat leaves showed a tendency to raised with salinity concentration and supplementing salt with brassinolide further enhances the proline content showed in Table 2. Enhancement of proline under stress could be due to prevention of feedback inhibition of all biosynthetic enzymes by sequestering proline away from its site of synthesis (Kavikishore *et al.* 1995) [10], reduction in rate of catabolism or decreased activity of enzymes involved in degradation of proline. In addition the maximum increase in proline content was recorded 261.53, 114.28 per cent due to use of 1.0 ppm concentration of brassinolide under stressed as well non stressed plants at both the stages of study. Proline content was more pronounced in tolerant cultivar Raj-3777 (41.66 and 15.00 per cent) than susceptible cultivar at 50 and 65 DAS in the above table. The increase in leaf proline was relatively greater in the tolerant genotypes than the susceptible one are in accordance with the findings of Sharma *et al.* (2003) [23]. Application of saline irrigation water up to EC 8 dSm^{-1} bring a significant reduction in protein content in wheat plants (21.58, 18.70 per cent) over control whereas, supplementing brassinolide enhanced the protein content (Table 2). Different results have been reported in case of effect of salt stress on protein content. Bera *et al.* (2006) [6] stated that nucleic acid, protein level in NaCl treated rice seedling decreased with increase in salt concentration in comparison to control but the depression was less severe when brassinolide was supplemented. The protein content recorded 7.64, 4.57 per cent more in tolerant cultivar (Raj-3777) at 50 and 65 DAS than susceptible cultivar in experiment. A further study of data in Table 2 indicated that variety Raj-3777 achieved 9.74 per cent more spike length per plant than HD-2687 under normal and stress conditions. The yield per plant, fertility percentage, and number of productive tillers, panicle length and number of primary braches per panicle were reduced by salinity also reported by Ali, Y. *et al.* (2004) [2]. The significantly higher values for length of spike per plant was obtained with spray treatment of brassinolide up to 1.0 ppm under saline and normal conditions and maximum spike length was found with 1.0 ppm concentration of brassinolde spray which was recorded 11.97 per cent over control in this study. This is because, 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 photosynthetic pigments and crop productivity as reported by, Mona E. Eleiwa *et al.*, (2011) [14]. The test weight of both wheat genotypes showed a considerable variation and decreased with an increase in salt concentration of irrigation water (Table 2). The highest test weight was obtained by variety Raj-3777, 7.63 per cent more than HD-2687 in controlled as well as stress conditions. Results were indicated that test weight of wheat plants were declined as the salinity of irrigation water increased but the reductions were steeper at the highest salt concentration of EC 8 dSm^{-1} (13.78 per cent over control) and could be counteracted through successive ascending the level of brassinolide over control in this study. Test weight increased significantly with exogenous brassinolide treatment at appropriate stage of their development results in increase of crop yield and quality are in similar with findings of Khripach *et al.* (2000) [11]. In cereals, brassinosteroides promote the number of ears and the number and weight of kernels per ear are also contributed this study (Ali *et al.*, 2008) [1].

Table 2: Effect of salinity and brassinolides on proline content, cell membrane stability, protein content, length of spike and test weight of wheat

Treatments	Proline (mg/g fr.wt.)		Cell membrane stability (%)		Protein (mg/g fr.wt.)		Length of spike/plant (cm)	Test weight (g)
	50 DAS	65 DAS	50 DAS	65 DAS	50 DAS	65 DAS		
Varieties								
Raj-3777	0.36	0.60	71.51	77.37	19.62	22.73	8.93	40.48
HD-2687	0.21	0.51	67.07	73.29	18.12	21.69	8.06	37.39
S.Em.+	0.009	0.015	0.73	0.89	0.22	0.24	0.07	0.30
C.D. (P=0.05)	0.026	0.042	2.07	2.52	0.61	0.68	0.19	0.86
Salinity levels (dSm^{-1})								
0	0.15	0.42	75.65	81.99	21.03	24.06	9.24	41.78
4	0.25	0.54	71.23	77.30	19.08	23.01	8.53	39.00
8	0.46	0.70	60.99	66.70	16.49	19.56	7.71	36.02
S.Em.+	0.011	0.018	1.09	1.09	0.26	0.30	0.08	0.37
C.D. (P=0.05)	0.032	0.052	2.53	3.08	0.75	0.84	0.23	1.06
Brassinolide (ppm)								
0	0.13	0.35	62.88	68.80	16.35	19.78	8.02	35.80

0.25	0.24	0.50	66.53	72.86	17.57	21.10	8.33	37.44
0.50	0.30	0.62	71.57	77.65	19.54	23.37	8.65	40.60
1.00	0.47	0.75	76.18	82.01	22.01	24.60	8.98	41.90
S.Em.+	0.013	0.021	1.26	1.26	0.31	0.34	0.09	0.43
C.D. (P=0.05)	0.037	0.060	2.92	3.56	0.87	0.97	0.26	1.22

4. Conclusion

It is concluding that brassinolide increases plant adaptation to salt stress by stimulating the physiological, biochemical process which would help to minimize yield reduction in wheat plants. Further, the results concluded that cultivar Raj-3777 (Salinity tolerant) withstands more effectively than cultivar HD-2687 (Salinity susceptible) under salinity. We believe that cultivar Raj-3777 may be very promising to farmers for cultivation in saline areas up to EC 8 dSm^{-1}.

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