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Research Paper :

Effect of brassinolide on biochemical constituents in rice (*Oryza sativa* L.) under salinity stress

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ABSTRACT

Brassinolide (BL) is a natural plant growth promoting substance that also exert anti-stress effects on plants. Five rice varieties *viz.*, GR-7, GR-11, GR-12, Dandi and Gurjari were treated with 100 mM NaCl , 200 mM NaCl, 4 µM BL, 8 µM BL concentrations with water as control and supplemented solutions of BL with different salinity levels such as 100 mM NaCl+4 µM BL, 100 mM NaCl+8 µM BL, 200 mM NaCl+4 µM BL and 200 mM NaCl+8 µM BL. Salt solutions were supplemented with brassinolide to observe the ameliorating effect of the phytohormone in the rice seedlings under salinity stress. Biochemical constituents such as the seed reserves, stress proteins and mineral ions were affected by NaCl salt stress. Decrease in soluble protein and increase in soluble sugar, free amino acids and proline, Na^+ ion content and decrease in K^+ ion content in the rice seedlings with salinity were observed with increasing levels of salt stress. Brassinolide was found to ameliorate the adverse effects of salinity stress and thereby increase the yield of the crop.

KEY WORDS : Ameliorate, Brassinolide, Phytohormones, Salinity stress, DAG (Days after germination)

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Abiotic stresses are the main reason for loss in crop yield and productivity. Among various abiotic stresses salinity is a major environmental stress especially for rice, which is mostly grown under irrigated conditions. Rice is a salt sensitive crop and salt stress has adverse effects on the growth of the plant. Attempts are being made to ameliorate environmental stress by using phytohormones (Kamuro and Takatsuto, 1999; Rao *et al.,* 2002). Brassinosteroids (BRs) are steroidal plant hormones with growth promoting activity (Mandava, 1988). This class of plant growth regulators is regarded as the sixth plant hormone (Abe, 1989). Brassinolide (BL) is an important BRs that has emerged as a new phytohormone with pleiotrophic effect (Sasse, 1997). In the present study, effect of salinity stress and its amelioration by brassinolide on biochemical constituents in rice plants was studied.

EXPERIMENTAL METHODOLOGY

An experiment was conducted during the year 2010 in the Department of Biochemistry, B. A. College of Agriculture, AAU, Anand under controlled environmental conditions. Five varieties of rice *viz.*, GR-7 (V_1), GR-11 (V_2), GR-12 (V_3), Dandi (V_4) and Gurjari (V_5) differing in degree of salt tolerance to salt stress were procured from Main Rice Research Station, AAU, Navagam, Gujarat. Seeds were surface sterilized with 0.1per cent$HgCl_2$ for 2-3 minutes and rinsed thoroughly with glass distilled water. Surface sterilized seeds were germinated in *Whatman no.1* filter paper lined glass petriplates containing different concentrations of NaCl (100 mM and 200 mM) alone, Brassinolide (4 µM and 8 µM) alone and NaCl solutions supplemented with BL concentrations. Controlled seeds were grown in distilled water. Three replications were maintained for each treatment. For biochemical analysis, seedlings were removed from glass plate on 5, 10 and 15 DAG, respectively. Total soluble sugar (TSS), soluble protein, free amino acids, proline and mineral ions (Na^+ and K^+) were estimated following the methods of Dubois *et al.* (1956), Lowry *et al.* (1951), Lee and Takahashi (1966), Malik and Singh (1980) and Jackson (1973), respectively.

EXPERIMENTAL FINDINGS AND ANALYSIS

The studies done on biochemical constituents indicated significant effects of brassinolide treatment. The data presented in Table 1 revealed that sugar and free

Table 1 : Effect of Brassinolide on NaCl stress induced soluble sugar, free amino acids and soluble protein content in seedlings of rice varieties

Variety	Total soluble sugar (µg/mg)			Free amino acids (µg/mg)			Soluble proteins (µg/mg)		
	5 DAG	10 DAG	15 DAG	5 DAG	10 DAG	15 DAG	5 DAG	10 DAG	15 DAG
GR-7	28.36	28.12	27.52	13.08	12.83	12.51	20.64	21.14	21.24
GR-11	29.31	29.01	28.18	12.92	12.78	12.49	20.17	21.09	21.25
GR-12	27.55	27.24	26.55	12.91	12.62	12.42	20.44	21.40	21.64
Dandi	29.94	29.66	28.96	13.99	13.75	13.40	20.82	21.35	21.50
Gurjari	20.93	20.64	19.85	16.15	15.94	15.51	21.99	22.66	22.95
Treatments									
Control-Water treatment	25.74	25.40	24.66	12.32	12.10	11.86	22.18	22.97	23.18
100 mM NaCl	26.60	26.30	25.55	13.30	13.08	12.81	19.63	20.36	20.58
200 mM NaCl	26.97	26.68	26.10	13.56	13.30	12.97	18.16	19.07	19.28
4 µM BL	27.84	27.62	26.93	14.21	13.89	13.62	22.86	23.52	23.70
8 µM BL	28.37	28.10	27.39	14.32	14.09	13.67	22.79	23.66	23.83
100 mM NaCl + 4 µM BL	26.99	26.68	26.03	13.99	13.81	13.51	21.10	21.53	21.71
100 mM NaCl + 8 µM BL	27.19	26.92	26.24	14.05	13.89	13.55	21.19	21.74	21.89
200 mM NaCl + 4 µM BL	27.55	27.28	26.51	14.22	14.01	13.68	19.69	20.27	20.48
200 mM NaCl + 8 µM BL	27.72	27.44	26.50	14.32	14.09	13.73	19.72	20.61	20.79
S.E.±	0.27	0.13	0.10	0.05	0.07	0.08	0.02	0.09	0.13
C.D. (P=0.05)	0.77	0.38	0.30	0.14	0.21	0.23	0.06	0.26	0.37

amino acids content showed an increased level with salinity treatment, Brassinolide alone and also with combinations of NaCl.

Among the five varieties studied, genotype Dandi recorded the highest sugar content and Gurjari which is susceptible variety showed the lowest sugar content at all the time intervals. Sugar content increased remarkably may be due to overcome the effects of excess salinity conditions and plants utilized their food reserves where Brassinolide helped to favour better starch accumulation and uptake of sugar.

Free amino acids content showed an increase level in susceptible variety under salinity stress. Gurjari recorded the maximum free amino acids content significantly and GR-12 recorded the minimum at all the time intervals. Brassinolide found to be beneficial in maintaining the free amino acids contents in seedlings under stress.

Opposite effect was observed in soluble protein content in seedlings under salt stress. Protein level in NaCl treated rice seedlings decreased with increase in salt concentration in comparison to control and the depression was less severe when Brassinolide was supplemented (Table 1). The reduction in protein content may be due to the diversion of some quantum of energy for growth and metabolism to overcome the stress situations. Gurjari which is a susceptible variety recorded significantly the highest protein content followed by Dandi where as GR-11 recorded significantly the lowest protein content at 5 DAG salinity as well as Brassinolide treatment period. At 10 DAG and 15 DAG treatment period varietal effects was found to be consistant except GR-7 was recorded the minimum protein content at 15 DAG.

Among the different treatment level 8 µM BL concentration showed quite positive effect under salt stress in all the varieties with different time intervals.

Proline which serve as osmoprotectants increased continuously up to 200 mM NaCl treatment and with Brassinolide treatment in all the varieties. The trend also remained similar with different time intervals (Table 2). Dandi recorded significantly the highest proline content followed by Gurjari and least was recorded in GR-7 in all the time intervals. Increase in proline content helped to reduce the water potential and change the osmotic gradient, assuring the water flow to the plant and helps in recovery during stress situations.

Development of salt tolerance in plants for adaptation to saline environment, however, is a complex phenomenon where apart from intracellular solutes (Garcia *et al*., 1997) certain inorganic ions K^+ and Na^+ also play intrinsic roles. Data from the Table 2 showed significant differences in Na^+ and K^+ content among the varieties with different treatment levels. Study on effects of ionic imbalance due to saline treatment revealed the highest sodium level in GR-11 and GR-12 and the potassium level in GR-12 and significantly the lowest was recorded in Gurjari at 5 DAG. But at 10 and 15 DAG, Dandi recorded the highest sodium level and potassium level followed by GR-12 Where as Gurjari remained the same to have significantly the lowest Na^+ and K^+ content except GR-7 recorded the lowest K^+

Table 2 : Effect of brassinolide on NaCl stress induced proline content and mineral ions (Na^+ and K^+) in seedlings of rice varieties

Variety	Proline content (µg/mg)			Na^+ (%)			K^+ (%)		
	5 DAG	10 DAG	15 DAG	5 DAG	10 DAG	15 DAG	5 DAG	10 DAG	15 DAG
GR-7	1.16	1.27	1.84	0.30	0.80	0.97	0.16	0.36	0.45
GR-11	1.34	1.43	2.09	0.36	0.79	0.96	0.17	0.39	0.53
GR-12	1.33	1.42	2.09	0.36	0.87	0.98	0.21	0.43	0.54
Dandi	1.39	1.51	2.15	0.28	0.88	1.13	0.16	0.41	0.55
Gurjari	1.38	1.46	2.07	0.22	0.74	0.96	0.13	0.32	0.49
Treatments									
Control-Water treatment	1.18	1.25	1.81	0.09	0.14	0.27	0.18	0.40	0.55
100 mM NaCl	1.23	1.32	1.93	0.40	1.13	1.36	0.15	0.31	0.44
200 mM NaCl	1.29	1.37	1.99	0.50	1.36	1.78	0.13	0.28	0.39
4 µM BL	1.32	1.44	2.06	0.14	0.22	0.57	0.19	0.46	0.59
8 µM BL	1.34	1.46	2.06	0.14	0.23	0.38	0.20	0.47	0.60
100 mM NaCl + 4 µM BL	1.35	1.46	2.11	0.36	0.92	1.06	0.17	0.41	0.53
100 mM NaCl + 8 µM BL	1.37	1.48	2.14	0.35	0.94	1.05	0.17	0.41	0.54
200 mM NaCl + 4 µM BL	1.39	1.48	2.15	0.38	1.21	1.26	0.15	0.35	0.48
200 mM NaCl + 8 µM BL	1.41	1.51	2.18	0.37	1.19	1.29	0.16	0.36	0.49
S.E.±	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C.D. (P=0.05)	0.01	0.01	0.02	0.01	0.01	0.01	0.00	0.01	0.01

content at 15 DAG. The results clearly indicated the increase in Na^+ ion content and decrease in K^+ ion content in all the varieties with salinity. Supplementation of NaCl with brassinolide significantly decreased Na^+, thereby toxicity level and increased K^+ in the salt stressed plants of all the varieties. From these results, it was cleared that a contrary relation existing between Na^+ and K^+ content during salinity stress and brassinolide application might have promoted the process of osmotic adjustment, which is achieved by more accumulation of organic compatible solutes, and lower accumulation of inorganic ions. The lower Na^+/K^+ ratio is a good indication of salt tolerance. It is cleared that both Na^+ and K^+ contributed significantly to the osmoregulatory process.

Conclusion:

Brassinolides are implicated in ameliorating the salinity stress effects in plants as it reflects higher content of the seed reserve mobiles and stress proteins and also mediate to keep the ionic balance thereby promote the process of osmotic adjustment under stress situations and finally the seed yield at harvest.

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