Indian J. Plant Physiol., Vol. 11, No. 2, (N.S.) pp. 182-189 (April-June, 2006)

BRASSINOLIDE AMELIORATES ADVERSE EFFECTS OF SALT STRESS ON GERMINATION AND SEEDLING GROWTH OF RICE

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Received on 8 Dec., 2005, Revised on 5 June, 2006

SUMMARY

Rice in general is a salt sensitive crop. Seeds of two cultivars, viz. Kamini (salt susceptible) and Pusa 2-21 (salt tolerant) were allowed to germinate and grow in glass distilled water (control), 150 mM and 300 mM NaCl solutions, 4 µM brassinolide solution, 150 mM and 300 mM NaCl solutions supplemented with 4 µM brassinolide. Seed germination, seedling growth and hydrolytic enzymes (amylase and protease) associated with seedling development were adversely affected by NaCl salt stress. Reduction in DNA, RNA and soluble proteins and increase in peroxidase and free proline in rice seedlings were observed with increasing levels of salt stress. The effect was more conspicuous in Kamini than Pusa 2-21. Brassinolide, a steroidal component of plant origin was found to counter the adverse effect of salt stress irrespective of tolerant (kamini) and susceptible (Pusa 2-21) cultivars. Ameliorative effects of brassinolide were associated with increase in the levels of nucleic acids, soluble proteins, peroxidase and free proline content under salt stress.

***Key words*: Amelioration, brassinolide, germination, rice, salinity stress, seedling growth.**

INTRODUCTION

Among various plant growth processes, seed germination and early seedling growth are considered critical for raising a successful agricultural crop, as they indirectly determine the crop stand and density and affect consequently the yield of the crop (Gelmond 1978). Salinity adversely affects seed germination, seedling growth and different metabolic activities in plants (Narayana and Rao 1987, Begum *et al.* 1997). It reduces DNA, RNA and protein synthesis and differentially influences the activities of the hydrolytic enzymes (Dubey 1983, Kumar *et al.* 1996).

Several studies showed wide variabilities among genotypes of different crops in their tolerance to several stress factors like drought and salinity (Maity *et al.* 1984, Dingkuhn *et al.* 1991). Attempts are being made to ameliorate environmental stress by using phytohormones (Kamuro and Takatsuto 1999, Rao *et al.* 2002). Brassinolide has emerged as a new phytohormone with pleiotropic effect (Sasse 1997), and influences varied physiological processes like germination, growth, flowering, senescence and confers resistance to plant against various abiotic stresses. In the present investigation, effect of salinity stresses and its amelioration by brassinolide was studied on seed germination, seedling growth and some biochemical constituents in two rice cultivars differing in salt tolerance.

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MATERIALS AND METHODS

An experiment was conducted in the Department of Plant Physiology, Bidhan Chandra Krishi Viswavidyalaya, West Bengal during November – December, 2004 and again during April – May, 2005 with two cultivars of rice (*Oryza sativa* L.) namely Kamini (salt susceptible) and Pusa 2-21 (salt tolerant) at the temperature ranging between 15^0C to 24^0C. Seeds were surface sterilized with 0.1% $HgCl_2$ for 2 – 3 minutes and washed thoroughly with glass distilled water. Surface sterilized seeds were soaked for 24 hours in glass distilled water (control), 150 mM and 300 mM NaCl solutions, 4 µM brassinolide solution, 150 mM and 300 mM NaCl solutions supplemented with 4 µM brassinolide. Twenty five seeds from each treatment were placed in 9 cm diameter petridishes lined with Whatman No. 1 filter paper and moistened with respective solution. Seeds were then allowed to germinate at 28±1 ^{0}C in a BOD incubator. Three replicates were maintained for each treatment and germination percentage was recorded after 48 hours. Simultaneously, rice seedlings were developed for twelve days using standard glass plate technique (Mishra and Bera 1996) where pre-soaked seeds were arranged in a row over 30 cm × 20 cm glass plates lined with Whatman No. 1 filter paper. The whole set was then placed in transparent polythene bag containing respective solution for continuous supply of ingredients during the experimentation period. Surface sterilized seeds treated similarly with glass distilled water served as control.

On 4th and 12th day, the seedlings were removed from glass plate and seedling growth in terms of total seedling length (cm), fresh and dry weight were recorded. For biochemical analysis, seedlings were separated into endosperm and embryonic axis (the term axis denotes combination of growing plumule and radicle). Amylase and protease assay were made from endosperm and embryonic axis, whereas all other biochemical observations were made with embryonic axis only. Amylase, protease and peroxidase enzyme activities were estimated following the methods of Punjabi and Basu (1978), Mathur *et al.* (1988) and Lobenstein and Linsey (1961) respectively. DNA and RNA were extracted following the method of Smillie and Krotkov (1960) with some modifications and estimated colorimetrically at 600ηm and 660ηm after colour development with diphenylamine reagent (Burton 1956) and orcinol (Mejbaum 1939), respectively using Bausch and Lamb Spectronic-20 genesys Spectrophotometer. Soluble proteins were extracted with Tris-HCl buffer pH 8.0, precipitated with 20% (w/v) TCA and the precipitate was dissolved in 1N NaOH and quantified following the method of Lowry *et al*. (1951). Proline was extracted and estimated adopting the procedure of Bates *et al*. (1973).

RESULTS AND DISCUSSION

The data presented in Table 1 revealed that germination percentage of salinity susceptible (Kamini) and tolerant (Pusa 2-21) rice cultivars distinctly differed with increase in level of salt stress. At 0.15M NaCl concentration, 46.51% and 55.56% seed germination were achieved in susceptible and tolerant cultivars respectively compared to control. However, at 0.3M NaCl concentration, no seed germinated in cv. Kamini but at the same concentration 20% seed germination was noticed in cv. Pusa 2-21. Supplementing NaCl with 4µM brassinolide substantially reduced inhibitory effect of NaCl stress on seed germination (Table 1). Similar to germination, seedling growth in terms of length, fresh and dry weight were adversely affected with increase in the concentration of salt. However, supplementation of 4µM brassinolide in the saline medium countered the adverse effects of salt, indicating alleviating influence of brassinolide on salinity stress induced inhibition of seedling growth irrespective of cultivars tested. The inhibition of seed germination and seedling growth of rice which were normally observed under salinity stress might be attributed to the inhibition of the hydrolysis of endosperm reserves (Dubey 1983) and to the translocation of food reserves from endosperm to embryonic axis (Sheoran and Garg 1978). The exposure of plants to salinity results in metabolic perturbances and lowered physiological processes, which ultimately reflects in growth inhibition (Amzallag 1997).

Hydrolytic enzymes like amylase and protease decreased considerably with increase in salinity in both endosperm and embryonic axis during seedling growth

Table 1. Effect of brassinolide on NaCl stress induced inhibition of seed germination and seedling growth of rice cultivars

Cultivars	Treatments	Germination (%)	Seedling growth					
			4 Days			12 Days		
			Length (cm)	Fw. (mg seedling$^{-1)}$	Dw. (mg seedling$^{-1)}$	Length (cm)	Fw (mg seedling$^{-1)}$	Dw. (mg seedling$^{-1)}$
Kamini	Control	86	2.60	236.58	28.48	10.07	780.54	90.64
	0.15M NaCl	40	1.75	125.60	15.24	7.85	605.44	74.80
	0.3M NaCl	0.0	0.96	40.92	6.56	6.02	418.60	50.90
	4µM BR	94	3.18	265.14	30.12	11.68	812.40	95.66
	0.15M NaCl + 4µM BR	56	2.36	187.88	21.62	8.72	642.68	78.34
	0.3M NaCl + 4µM BR	24	1.64	106.34	13.56	6.56	478.12	56.58
Pusa 2-21	Control	90	2.95	250.40	29.88	12.37	860.65	109.64
	0.15M NaCl	50	2.28	176.16	20.06	11.04	704.80	101.12
	0.3M NaCl	20	1.34	72.58	9.78	8.72	552.66	78.60
	4µM BR	96	3.42	268.72	30.36	15.16	896.82	117.36
	0.15M NaCl + 4µM BR	76	2.84	212.84	25.84	11.64	754.36	110.48
	0.3M NaCl + 4µM BR	50	2.40	176.46	20.00	9.48	610.44	85.65
	S.Em (±)							
	Cultivar (C)	0.263	0.070	0.211	0.159	0.228	0.466	0.290
	Treatment (T)	0.455	0.121	0.365	0.275	0.394	0.808	0.502
	C×T	0.644	0.172	0.516	0.389	0.557	1.143	0.710
	CD at 5%							
	Cultivar (C)	0.771	0.205	0.619	0.466	0.669	1.367	0.851
	Treatment (T)	1.335	0.355	1.071	0.807	1.156	2.370	1.472
	C×T	1.889	NS	1.513	1.141	NS	3.353	2.082

of two cultivars studied (Table 2). Amylase and protease were consistently higher in the salt tolerant cv. Pusa 2-21 than susceptible one. Crop cultivars have been known to differ in their amylolytic activity and imposition of salinity has been reported to depress amylase activity in wheat (Sairam *et al.* 2002). Lowering of amylase would lead to lower availability of sugar leading to inhibited respiration and finally the seedling growth (Sarin 1961). Supplementing NaCl with 4µM brassinolide alleviated inhibitory effect of NaCl stress on these enzymes. Phytohormones are known to influence a number of physiological processes including enzyme activation. Mayer and Shain (1974) suggested that the effect of growth regulators was not

Table 2. Effect of brassinolide on NaCl stress induced total amylase and protease activity in seedlings of rice cultivars.

Cultivars	Treatments	4 Days				12 Days			
		Total amylase (Unit/mg protein)		Protease (Unit/mg protein)		Total amylase (Unit/mg protein)		Protease (Unit/mg protein)	
		Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo
Kamini	Control	16.12	2.34	1.06	0.88	10.46	2.84	1.68	0.84
	0.15M NaCl	11.42	1.80	0.84	0.60	8.28	2.06	1.48	0.62
	0.3M NaCl	6.84	1.45	0.46	0.32	3.46	1.66	0.96	0.34
	4µM BR	17.66	2.86	1.12	1.02	10.52	3.14	2.00	1.08
	0.15M NaCl + 4µM BR	13.58	2.00	1.00	0.76	9.36	2.52	1.68	0.92
	0.3M NaCl + 4µM BR	9.60	1.50	0.82	0.44	5.60	1.94	1.16	0.62
Pusa 2-21	Control	17.00	2.84	1.58	1.06	12.54	3.10	1.82	1.36
	0.15M NaCl	12.48	2.16	1.12	0.82	9.36	2.76	1.58	1.18
	0.3M NaCl	8.64	1.92	0.68	0.50	5.42	2.18	1.02	0.58
	4µM BR	18.12	3.24	1.66	1.28	14.86	3.34	2.26	1.76
	0.15M NaCl + 4µM BR	14.58	2.66	1.34	1.10	11.94	2.96	1.72	1.52
	0.3M NaCl + 4µM BR	11.24	2.16	0.96	0.84	8.68	2.72	1.48	1.04
	S.Em (±)								
	Cultivar (C)	0.009	0.020	0.008	0.005	0.011	0.009	0.005	0.014
	Treatment (T)	0.016	0.034	0.013	0.009	0.020	0.016	0.010	0.022
	C×T	0.023	0.048	0.019	0.013	0.028	0.025	0.015	0.036
	CD at 5%								
	Cultivar (C)	0.026	0.059	0.023	0.015	0.032	0.026	0.015	0.041
	Treatment (T)	0.047	0.010	0.038	0.026	0.059	0.047	0.029	0.065
	C×T	0.067	NS	0.056	0.038	0.082	0.073	0.044	0.106

due to *de novo* synthesis of hydrolytic enzymes but due to activation of pre-packed enzymes and other cellular components.

Contrary to amylase and protease, peroxidase activity increased in response to salt stress in both the cultivars compared to control (Table 3). Higher peroxidase activity

Table 3. Effect of brassinolide on NaCl stress induced peroxidase activity (unit/mg protein) in seedlings of rice cultivars.

Cultivars	Treatments	4 Days	12 Days
Kamini	Control	175	320
	0.15M NaCl	205	480
	0.3M NaCl	340	525
	4µM BR	210	410
	0.15M NaCl + 4µM BR	316	516
	0.3M NaCl + 4µM BR	360	584
Pusa 2-21	Control	190	362
	0.15M NaCl	260	503
	0.3M NaCl	390	570
	4µM BR	265	440
	0.15M NaCl + 4µM BR	370	566
	0.3M NaCl + 4µM BR	475	624
	S.Em (±)		
	Cultivar (C)	0.232	0.822
	Treatment (T)	0.402	1.423
	Cultivar (C)	0.232	0.822
	C×T	0.569	2.013
	CD at 5%		
	Cultivar (C)	0.680	2.411
	Treatment (T)	1.179	4.174
	C×T	1.669	5.904

was obtained in tolerant cultivar as compared to susceptible one (Kamini). Peroxidase enzyme plays an important role in scavenging mechanism of plants. Higher peroxidase activity particularly in tolerant cultivar is an indication to prevent degradation of membrane integrity of the cells against free radicals formed under salt stress.

Salt stress adversely affected nucleic acids metabolism in developing rice seedling. Although, both DNA and RNA content increased with increase in the age of seedling, decline in DNA and RNA content in NaCl salt treated plant was noticed in comparison to control (Table 4). Similar effect of salt stress was observed by Sheoran and Garg (1978) in mungbean. Nucleic acids play important role in seedling growth and elevation of DNA and RNA in rice seedling by brassinolide treatment could be correlated with enhanced seedling growth (Table 1). The present results report ameliorating effect of brassinolide, as it reflects higher content of these metabolites (DNA and RNA) in NaCl salt treated rice seedlings supplemented with brassinolide.

Nucleic acid and protein metabolism are very closely related processes. Hence, similar to nucleic acid, protein level in NaCl treated rice seedling decreased with increase in salt concentration in comparison to control and the depression was less severe when brassinolide was supplemented (Table 5). The protein content of tolerant cv. Pusa 2-21 was found to be higher than susceptible cv. Kamini, which might be attributed to higher nucleic acid content in the former. Decreased protein content in the rice seedling in response to NaCl salt salinity stress was considered for its decreased de-novo synthesis as salt stress caused a mark change in protein synthesizing apparatus of plant tissue and decrease considerably the capacity for protein synthesis (Shah and Loomis 1975).

Proline level in rice seedling showed a tendency to increase with salinity concentration and supplementing NaCl with brassinolide further enhanced the proline content (Table 5). Moreover, increase in proline content was more pronounced in tolerant than susceptible cultivar. This study also coincides with that of Weimberg *et al*. (1982) and Reddy and Vora (1983) who reported an increase in proline content in sorghum plants under increasing saline stress. Rosa Ibarra and Maiti (1995) in their observations with salinity stress, were of opinion that increase in proline is probably due to the capacity of some plants to accumulate organic (sucrose, fructose and glucose) and inorganic (Na, K and Cl) metabolites in the cytoplasm to reduce the water potential and change the osmotic gradient, assuring the water flow to the plant and thereby increase tolerance.

Table 4. Effect of brassinolide on NaCl stress induced DNA and RNA content in seedlings of rice cultivars

Cultivars	Treatments	DNA (mg g^{-1} fw)		RNA (mg g^{-1} fw)	
		4 Days	12 Days	4 Days	12 Days
Kamini	Control	0.95	2.45	0.52	1.26
	0.15M NaCl	0.60	2.08	0.33	1.08
	0.3M NaCl	0.45	1.54	0.21	0.62
	4µM BR	1.20	2.85	0.75	1.48
	0.15M NaCl + 4µM BR	1.15	2.15	0.59	1.26
	0.3M NaCl + 4µM BR	0.75	1.74	0.41	0.88
Pusa 2-21	Control	1.15	2.72	0.76	1.52
	0.15M NaCl	1.00	2.18	0.64	1.10
	0.3M NaCl	0.85	1.86	0.36	0.76
	4µM BR	1.65	3.08	0.91	1.61
	0.15M NaCl + 4µM BR	1.35	2.56	0.72	1.30
	0.3M NaCl + 4µM BR	1.00	2.04	0.52	1.04
	S.Em (±)				
	Cultivar (C)	0.020	0.008	0.041	0.010
	Treatment (T)	0.034	0.014	0.070	0.018
	C×T	0.048	0.020	0.099	0.025
	CD at 5%				
	Cultivar (C)	0.059	0.023	NS	0.029
	Treatment (T)	0.100	0.041	NS	0.053
	C×T	NS	0.059	NS	0.073

Higher germination percentage, enzymic activities and metabolic contents in salt tolerant Pusa 2-21 than susceptible Kamini helps to survive better than the latter under salinity stress. The present study also revealed ameliorating effect of brassinolide on salinity stress induced inhibition of seed germination and seedling growth of rice. It is concluded that brassinolide can ameliorate adverse effects of salinity stress by influencing various metabolic constituents and enzymic activities in this crop.

Table 5. Effect of brassinolide on NaCl stress induced soluble proteins and proline content in seedlings of rice cultivars.

Cultivars	Treatments	Soluble proteins (mg g^{-1} fw)		Proline (mg g^{-1} fw)	
		4 Days	12 Days	4 Days	12 Days
Kamini	Control	1.04	4.65	0.12	0.36
	0.15M NaCl	0.76	3.26	0.14	0.54
	0.3M NaCl	0.38	2.75	0.21	0.61
	4µM BR	1.46	4.80	0.28	0.64
	0.15M NaCl + 4µM BR	1.12	4.44	0.36	0.68
	0.3M NaCl + 4µM BR	0.76	3.16	0.41	0.72
Pusa 2-21	Control	1.32	4.76	0.20	0.46
	0.15M NaCl	1.10	3.65	0.34	0.62
	0.3M NaCl	0.86	3.08	0.49	0.82
	4µM BR	2.12	5.16	0.36	0.78
	0.15M NaCl + 4µM BR	1.68	4.25	0.54	0.84
	0.3M NaCl + 4µM BR	1.14	3.84	0.62	0.96
	S.Em (±)				
	Cultivar (C)	0.047	0.102	0.010	0.014
	Treatment (T)	0.081	0.177	0.018	0.023
	C×T	0.115	0.250	0.025	0.033
	CD at 5%				
	Cultivar (C)	0.138	NS	0.029	0.041
	Treatment (T)	0.238	0.519	0.053	0.067
	C×T	NS	NS	0.073	NS

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