SHORT COMMUNICATION

EFFECT OF 28-HOMOBRASSINOLIDE ON SALINITY INDUCED CHANGES IN GROWTH, ETHYLENE AND SEED YIELD OF MUSTARD

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The seeds of *Brassica juncea* were soaked for 6 hours, in water, 50, 100 or 150 mM of NaCl and sown in pots. The plants were sprayed with water, 10^{-10}, 10^{-8} or 10^{-6} M aqueous solution of 28-homobrassinolide (HBL) at 15, 30 or 45 day after sowing (DAS) to the foliage of the plants. Sodium chloride retarded growth of root and shoot observed at day 60 (post-flowering) and lowered seed yield, at harvest (140 DAS). However, spray of HBL at 15, 30 or 45 DAS increased the values significantly for the above characteristics. 30th day spray was best among them. Moreover, the plants released more ethylene, under NaCl stress or HBL treatments. The ill effect of the lowest concentration (50 mM) of the salt was completely overcome by the spray of HBL (10^{-8}M) at 30 day stage.

***Key word*: Brassinosteroid, ethylene, salinity**

Brassica juncea is an important oil yielding crop, which accounts for approximately 80 % of the total production of rape seed and mustard in India (Prakash *et al.* 2004). Salinity affects at least 20 % of world's arable land and more than 40 % of irrigated land to various degrees (Rhoades and Loveday 1990). Salinity influences adversely several aspects of reproductive growth, including flowering, pollination, fruit development, yield and quality and seed production (Shannon *et al*. 1994). It also inhibits the activity of many enzymes (El-Shihaby *et al*. 2002).

Plant growth regulators are known to affect growth, assimilate translocation, flowering and ion transport (Arteca 1997). Brassinosteroids (BRs) are a new class of plant hormones possessing significant growth promoting activity. Due to their role in wide ranges of physiological responses, they are considered as essential regulators of plant growth and development (Clouse and Sasse 1998). Moreover, they have also been found to ameliorate the abiotic and biotic stresses (Sasse 2003). This experiment was undertaken to explore remedial measures for overcoming the ill effects of salinity, by improving the degree of resistance in the plants through BR-treatment.

The seeds of *Brassica juncea* Czern & Coss cv. Varuna, were procured from National Seed Corporation Ltd., New Delhi. The healthy seeds were surface sterilized with sodium hypochlorite solution (5%) followed by repeated washings with double distilled water. These seeds were soaked in 0, 50, 100 or 150 mM of NaCl solution, for 6 hours (duration is based on earlier experiment). Treated seeds were sown in earthen pots (25 cm diameter) filled with sandy loam soil and farmyard manure, in a ratio of 9:1. Aqueous solutions (100 ml) of 10^{-10}, 10^{-8} or 10^{-6}M of HBL (Godrej Agrovet Ltd., Mumbai) were sprayed at 15, 30 or 45 DAS. At the time of the sampling (60 DAS, post-flowering) three plants from each pot were used to determine the fresh and dry

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weight of root and shoot. For the estimation of ethylene, the leaf samples were incubated in a flask, sealed with an elastic septum for one hour at 25°C. Five ml of the gas phase was removed from the flask with a hypodermal syringe and ethylene was measured on a gas chromatograph (GC 5700, Nucon, India) equipped with 1.8 m Porapack N (80/100 mesh) column, a flame ionization detector and an integrator. Nitrogen was used as carrier gas. The flow rates of nitrogen, hydrogen and oxygen were 0.5, 0.5 and 5 mLS^{-1}, respectively. The oven temperature was 100°C and that of detector was 150°C. Ethylene identification was based on its retention time. Quantification was made by comparing the values with the peaks of a standard drawn by using graded concentrations of pure ethylene. Once the crop matured (140 DAS), the remaining plants were cut at the base to determine seed yield $plant^{-1}$. Oil from the seeds was extracted in soxhlet apparatus. Results were evaluated by adopting analysis of variance (Gomez and Gomez 1984). Least significant difference (LSD) was calculated at 0.05 level of probability.

Fresh and dry weight of both root and shoot of the plants, raised from the seeds soaked in the solution of NaCl were decreased, irrespective of its concentration (Table 1 and 2). Moreover, the relative rate of the loss was directly determined by the concentration of the salt. A maximum decrease in root fresh and dry weight and in shoot fresh and dry weight was recorded in the plants raised from the seeds soaked in 150 mM of NaCl, compared to water soaked control. However, the spray of the seedlings with HBL at 15, 30 or 45 DAS, enhanced the values for all the above characteristics. The 10^{-8} M of HBL completely neutralized the adverse effect of the lower concentration (50 mM) of the salt and the recorded values are comparable with those of the control at 30 day spray stage (Table 1 and 2). The foliage of the plants, raised from the seeds treated with the salt, released more ethylene than control and the values increased further if the same plants were sprayed with aqueous solution of HBL (Table 3). Maximum ethylene was evolved from the plants, raised from the seeds treated with the highest concentration of NaCl

Table 1. Effect of 28-homobrassinolide (15d, 30d and 45d stage) on the fresh and dry weight (g) of root of mustard at 60-d-stage subjected to saline stress

NaCl (mM)	15d					30 d					45d				
	Control	28-homobrassinolide (M)				Control	28-homobrassinolide (M)				Control	28-homobrassinolide (M)			
		10^{-10}	10^{-8}	10^{-6}	Mean		10^{-10}	10^{-8}	10^{-6}	Mean		10^{-10}	10^{-8}	10^{-6}	Mean
Fresh weight of root															
0	9.65	10.15	11.35	11.25	10.60	9.65	10.25	12.00	11.81	10.92	9.45	9.82	11.05	10.81	10.28
50	9.00	9.45	9.90	9.81	9.54	8.93	9.65	10.10	9.80	9.62	8.99	9.41	9.65	9.61	9.41
100	8.05	8.64	9.05	9.00	8.68	8.00	8.41	9.15	9.10	8.66	8.04	8.31	8.88	8.96	8.54
150	7.40	8.00	8.45	8.15	8.00	7.51	7.93	8.70	8.51	8.16	7.30	7.85	8.41	8.31	7.96
Mean	8.52	9.06	9.68	9.55		8.52	9.06	9.98	9.80		8.44	8.84	9.49	9.42	
L.S.D. at 5 %		NaCl = 0.51, HBL = 0.51 NaCl × HBL = 1.02					NaCl = 0.62; HBL = 0.62 NaCl × HBL = 1.24					NaCl = 0.58; HBL = 0.58 NaCl × HBL = 1.16			
Dry weight of root															
0	3.07	3.23	3.62	3.54	3.36	3.06	3.24	3.71	3.58	3.39	2.97	3.07	3.56	3.45	3.26
50	2.88	2.98	3.15	3.09	3.02	2.76	2.96	3.19	3.14	3.01	2.87	2.99	3.09	3.07	3.00
100	2.56	2.78	2.86	2.88	2.77	2.51	2.63	2.83	2.87	2.71	2.55	2.66	2.83	2.85	2.72
150	2.33	2.56	2.88	2.57	2.58	2.37	2.49	2.64	2.66	2.54	2.31	2.46	2.71	2.64	2.53
Mean	2.71	2.88	3.12	3.02		2.67	2.83	3.09	3.06		2.67	2.79	3.04	3.00	
L.S.D. at 5 %		NaCl = 0.25; HBL = 0.25 NaCl × HBL = 0.50					NaCl = 0.18; HBL = 0.18 NaCl × HBL = 0.36					NaCl = 0.19; HBL = 0.19 NaCl × HBL = 0.38			

Table 2. Effect of 28-homobrassinolide (15d, 30d and 45d stage) on the fresh and dry weight (g) of root of mustard at 60-d-stage subjected to saline stress

NaCl (mM)	15d					30 d					45d				
	Control	28-homobrassinolide (M)				Control	28-homobrassinolide (M)				Control	28-homobrassinolide (M)			
		10^{-10}	10^{-8}	10^{-6}	Mean		10^{-10}	10^{-8}	10^{-6}	Mean		10^{-10}	10^{-8}	10^{-6}	Mean
							Fresh weight of root								
0	19.00	19.65	22.15	21.85	20.66	19.13	20.00	26.00	25.70	22.70	19.10	20.10	24.10	24.00	21.82
50	17.93	18.41	19.16	19.00	18.62	18.00	18.80	20.40	20.60	19.45	18.15	18.60	19.40	19.80	18.98
100	15.15	16.30	17.40	17.20	16.51	15.46	16.10	17.40	17.00	16.49	15.39	15.80	17.00	16.90	16.27
150	13.05	13.90	16.10	15.80	14.71	13.13	14.00	16.80	16.40	15.08	13.10	13.70	15.90	16.40	14.77
Mean	16.28	17.06	18.70	18.46		16.43	17.22	20.15	19.92		16.43	17.05	19.10	19.27	
L.S.D. at 5 %		NaCl = 1.20; HBL = 1.20 NaCl × HBL = 2.40					NaCl = 1.48; HBL = 1.48 NaCl × HBL = 2.96					NaCl = 1.30; HBL = 1.30 NaCl × HBL = 2.60			
							Dry weight of shoot								
0	5.90	6.31	6.90	6.76	6.46	6.03	6.17	8.22	7.90	7.08	6.02	6.40	7.62	7.43	6.86
50	5.51	5.91	5.89	5.97	5.82	5.66	5.93	6.27	6.35	6.05	5.70	5.86	6.13	6.24	5.98
100	4.88	5.09	5.38	5.22	5.14	4.78	4.92	5.52	6.32	5.13	4.90	5.03	5.43	5.31	5.16
150	4.15	4.38	5.06	4.95	4.63	4.01	4.38	5.36	5.15	4.72	4.22	4.32	4.95	5.10	4.64
Mean	5.11	5.42	5.80	5.72		5.12	5.35	6.34	6.18		5.21	5.40	6.03	6.02	
L.S.D. at 5 %		NaCl = 0.31; HBL = 0.31 NaCl × HBL = 0.62					NaCl = 0.19; HBL = 0.19 NaCl × HBL = 0.38					NaCl = 0.16; HBL = 0.16 NaCl × HBL = 0.32			

(150 mM) and simultaneously sprayed with highest concentrations of HBL (10^{-6}M) at 30 day stage. The plants, developed from the seeds pre-treated with the salt gave poor seed yield than the control. The degree of the loss was proportionate to the concentration of NaCl. However, this loss could be reclaimed by the spray of HBL. The higher concentrations (10^{-8}M and 10^{-6}M) of the hormone proved most effective which not only counteracted the effect of the salt but significantly increased the values above the control (Table 3).

NaCl treatment significantly retarded growth rate both of root and shoot of *Brassica* plants (Table 1 and 2). On the other hand, HBL favoured plant growth and also reduced the inhibitory effect generated by NaCl, particularly at its lower concentration (Table 1 and 2). A positive response, in terms of ethylene production, was noted under NaCl and HBL treatments (Table 3). *Allenrolfea occidentalis* (Chrominski *et al*. 1989) and *Lycopersicon esculentum* (Feng and Barker 1992) also generated more ethylene under salt stress. Moreover, the plants grown from the seeds pre-treated with NaCl and augmented with HBL solution produced much more volume of ethylene than either of them alone (Table 3). Similarly, Brassinosteroid stimulation of ethylene biosynthesis has been reported in etiolated mungbean hypocotyls segments (Arteca 1997). This is possibly an expression of the genes which are known to be influenced by BRs (Kalinich *et al*. 1985) resulting in the synthesis/activation of some specific enzyme/s speeding up the conversion of S-adenosylmethionine to 1-amino cyclopropane-1-carboxylic acid, a precursor of ethylene (Schlagnhaufer 1985).

The lean vegetative growth of the plants, resulting from the seeds treated with NaCl, is naturally expected to lead to poor seed yield, at harvest. The important factor that may be responsible for such an observation may be lower level of rubisco (Soussi *et al*. 1999) and/or limited transport of assimilates to the sink (Sultana *et al*. 1999). However, HBL alone improved the seed yield and also neutralized the ill effect of the salt, if given in its combination (Table 3). The generation of such a response in the plants by the hormone was possibly a

Table 3. Effect of 28-homobrassinolide (15d, 30d and 45d stage) on the level of ethylene (nl g^{-1} fm h^{-1}) at 60-d-stage and on the seed yield (g $plant^{-1}$) of mustard at harvest subjected to saline stress

NaCl (mM)	15d					30 d					45d				
	Control	28-homobrassinolide (M)				Control	28-homobrassinolide (M)				Control	28-homobrassinolide (M)			
		10^{-10}	10^{-8}	10^{-6}	Mean		10^{-10}	10^{-8}	10^{-6}	Mean		10^{-10}	10^{-8}	10^{-6}	Mean
Ethylene															
0	8.15	8.49	9.23	9.26	8.78	8.20	8.61	9.35	9.41	8.89	8.16	8.59	9.41	9.41	8.89
50	9.26	9.69	9.93	10.04	9.73	9.16	9.71	10.11	10.05	9.75	9.20	9.71	10.31	9.99	9.80
100	11.23	12.13	13.15	13.13	12.41	11.15	12.26	13.41	13.29	12.52	11.20	12.21	13.30	13.19	12.47
150	11.54	12.48	13.65	13.61	12.82	11.48	12.51	13.61	13.59	12.79	11.51	12.61	13.91	13.65	12.92
Mean	10.04	10.69	11.49	11.51		9.99	10.77	11.62	11.58		10.01	10.78	11.73	11.56	
L.S.D. at 5 %		NaCl = 0.35; HBL = 0.35 NaCl × HBL = NS					NaCl = 0.36; HBL = 0.36 NaCl × HBL = NS					NaCl = 0.39; HBL = 0.39 NaCl × HBL = NS			
Seed Yield															
0	7.31	8.00	9.21	9.31	8.45	7.28	8.10	9.40	9.46	8.56	7.41	8.00	9.31	9.36	8.52
50	6.71	7.70	8.35	8.21	7.74	6.66	7.41	8.19	8.26	7.63	6.73	7.60	8.29	8.21	7.70
100	5.96	6.36	7.41	7.44	6.79	6.00	6.41	7.43	7.31	6.78	6.03	6.39	7.46	7.41	6.82
150	5.73	6.00	6.61	6.89	6.30	5.71	5.93	6.81	6.82	6.31	5.80	6.00	6.71	6.66	6.29
Mean	6.42	7.01	7.89	7.96		6.41	6.96	7.95	7.96		6.49	6.99	7.94	7.91	
L.S.D. at 5 %		NaCl = 0.42; HBL = 0.42 NaCl × HBL = 0.84					NaCl = 0.36; HBL = 0.36 NaCl × HBL = 0.72					NaCl = 0.41; HBL = 0.41 NaCl × HBL = 0.82			

cumulative expression of accelerated rate of nitrate assimilation (Mai *et al*. 1989), protein synthesis (Kalinich *et al*. 1985), net photosynthesis (Hayat *et al*. 2001), preferential translocation of photosynthates to the sink (Fujii and Saka 2001) and delayed leaf senescence (Iwahori *et al*., 1990). The healthy growth obviously had an impact on the productivity.

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