

BRIEF COMMUNICATION

Alleviation of salinity stress during germination in *Brassica juncea* by pre-sowing chilling treatments to seeds

P.C. SHARMA* and P. KUMAR**

Central Soil Salinity Research Institute, Karnal 132 001, India

Abstract

Influence of pre-sowing chilling treatments to seeds on seed germination and accumulation of osmotics in Indian mustard [*Brassica juncea* (L.) Czernj & Cosson] under NaCl stress was investigated. Germination was 100 % in water soaked non-chilled (control) seeds upto 100 mM NaCl, whereas it was upto 200 mM NaCl in chilling treated seeds. Pre-sowing chilling treatments to seeds for 5, 10 and 15 d also enhanced the dry mass of 6-d-old seedlings, and concentrations of saccharides and amino acids. The alleviation of NaCl stress by pre-sowing chilling treatments was also associated with decrease in Na⁺ and proline accumulation and a slight increase in K⁺ and Ca²⁺ contents of seedlings.

Additional key words: Indian mustard, ion accumulation, NaCl tolerance, amino acids, proline, saccharides.

Indian mustard [*Brassica juncea* (L.) Czernj & Cosson], one of the most important oilseed crops in semi-arid and arid regions, is most sensitive to salinity at germination stage compared to other developmental stages (Gill and Sharma 1990). It has been observed under field conditions that if soil salinity is kept low at sowing, then the crop is able to tolerate higher salinities at later growth stages.

An enhancement in growth and yield have been recorded in wheat (Singh and Banerji 1983) and *Phaseolus mungo* (Singh 1984) by pre-sowing chilling treatments to seeds. Taeb *et al.* (1992) showed that vernalization treatments decreases Na⁺ accumulation under salinity stress in wheat. Whether such treatments can improve

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*Fax: (+91) 184 200480, e-mail: cssri@X400.nic.in

**Present address: Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora 263 601, India.

salinity tolerance of crops at germination stage is not known. The aim of the present study was to determine whether pre-sowing chilling treatments to seeds has any alleviating effects on seed germination in *Brassica juncea* under salinity stress.

The seeds of Indian mustard [*Brassica juncea* (L.) Czernj & Cosson], cultivar Kranti (a standard check cultivar for salinity tolerance in All India Co-ordinated Research Project on Oil-seeds), from a lot of 100 % germinability, were surface sterilized with 0.1 percent HgCl_2 , thoroughly washed with distilled water and imbibed at room temperature (about 27 °C) for 8 h. The water soaked seeds were then subjected to pre sowing chilling treatments by keeping at 5 ± 1 °C for 5, 10 and 15 d in dark. These treatments were designated as C5, C10, and C15, respectively. Water soaked non-chilled seeds served as controls (C).

Fifty pre-treated seeds were placed in Petri dishes lined with *Whatman No. 1* filter paper saturated with 50, 100, 150, 200, 250 and 300 mM NaCl in 1/4 strength Hoagland nutrient solution and adjusted to pH 6.5. Three replicates were used for each treatment. The seeds were allowed to germinate in dark at 27 ± 1 °C. The germination counts in each treatment were recorded every 24 h upto a period of 6 d. In another set, 6-d-old seedlings were sampled for recording fresh and dry mass.

Approximately 50 mg of dried and powdered material was extracted with 1M HNO_3 at 95 - 100 °C. Na^+ and K^+ contents were determined by using flame photometer (*Evans Electroelenium*, Halstead, England) and Ca^{2+} and Mg^{2+} content by atomic absorption spectrophotometer (*952AA, GBC Scientific Equipment*, Dandenong, Australia).

Saccharides were analyzed in 6-d-old whole seedlings from alcohol soluble fractions using anthrone reagent method with glucose as standard (Loewus 1952). Total free amino acids were determined with ninhydrin reagent using glycine as reference (Lee and Takahashi 1966). Proline was estimated after extracting the mixture with toluene following the method of Bates *et al.* (1973).

Salinity delayed seed germination in Indian mustard but its overall percentage was not much affected by NaCl upto 150 mM; beyond this concentration, the germination percentage declined sharply and the germination was completely inhibited by 300 mM NaCl (Fig. 1A). Pre-sowing chilling treatments to seeds improved the germination rate and percentage. Faster germination was observed with increase in duration of pre-sowing chilling treatments under all NaCl concentrations (Fig. 1A). The 100 % germination was observed upto 200 mM NaCl in chilling treated seeds, whereas, it was only upto 100 mM NaCl stress under non-chilled (control) conditions. Further, some seeds germinated even under 300 mM NaCl salinity when the seeds were subjected to pre-sowing chilling treatments for 15 d.

An alleviation of salinity stress by pre-sowing chilling treatments to seeds was further noticed in increased fresh and dry masses of seedlings. The low NaCl concentration (50 mM) enhanced the dry mass by about 5 % compared to non-salinized controls. With the increase in NaCl concentration from 50 to 200 mM, seedling dry mass declined by 21 % in non-chilled controls and by 10 % in 15 d chilling treated seeds (Fig. 1B).

Enhancement in total soluble sugars and total free amino acids were observed in 6-d-old seedlings from chilling treated seeds and the increasing trend was maintained

even under salinity stress (Table 1). The increase in duration of pre-sowing chilling treatments to seeds had positive effect on the content of soluble sugars and total free amino acids in the seedlings (Table 1). NaCl stress caused an increase in proline content. The seedlings raised from chilling treated seeds were experiencing lower stress as evident from the lower accumulation of proline under increasing salinity (Table 1).

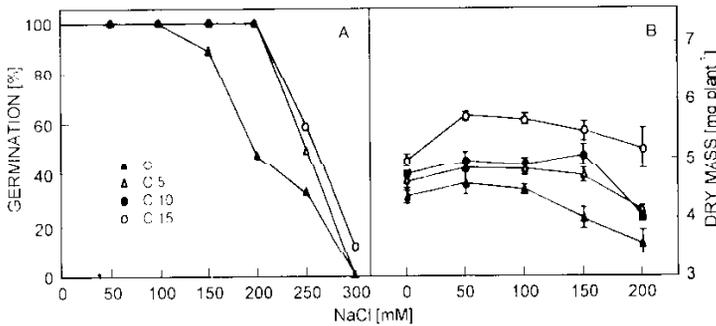


Fig. 1. Enhancement in germination percentage (A) and dry mass of 6-d-old *Brassica juncea* seedlings (B) under salinity stress (0 - 300 mM NaCl) by pre-sowing chilling treatments to seeds. (C - water soaked non-chilled controls; C5, C10 and C15 represents chilling treatments for 5, 10 and 15 d). Means \pm SE. $n = 120$.

Table 1. Effect of pre-sowing chilling treatments to seeds and NaCl concentrations on saccharides, total free amino acids and proline content [$\mu\text{g g}^{-1}$ (d. m.)] in 6-d-old seedlings of *Brassica juncea*.

| NaCl [mM] | | 0 | 50 | 100 | 150 | 200 |
|------------------------|----------|-------|----------|-------|----------------------------|-------|
| Saccharides | C | 1555 | 2405 | 3132 | 3875 | 3910 |
| | C5 | 3105 | 4015 | 4200 | 4485 | 4620 |
| | C10 | 3060 | 3150 | 3770 | 3890 | 4515 |
| | C15 | 3060 | 3435 | 3520 | 5010 | 5765 |
| CD ($P < 0.05$): | chilling | 166. | salinity | 186. | chilling \times salinity | 372 |
| Total free amino acids | C | 180 | 204 | 205 | 207 | 208 |
| | C5 | 197 | 209 | 230 | 276 | 234 |
| | C10 | 215 | 211 | 259 | 270 | 301 |
| | C15 | 239 | 243 | 277 | 287 | 246 |
| CD ($P < 0.05$): | chilling | = 4.3 | salinity | = 4.8 | chilling \times salinity | = 9.6 |
| Proline | C | 14 | 15 | 36 | 60 | 110 |
| | C5 | 16 | 15 | 18 | 32 | 116 |
| | C10 | 17 | 16 | 23 | 44 | 109 |
| | C15 | 16 | 13 | 22 | 66 | 113 |
| CD ($P < 0.05$): | chilling | 0.9 | salinity | = 1.1 | chilling \times salinity | = 2.1 |

The excessive accumulation of ions are toxic to developing seedlings under salinity. Na^+ increased in seedlings upto 150 mM NaCl treatment after which it either stabilized or declined (Fig. 2). On the other hand, K concentration decreased (Fig. 2) probably

due to the direct competition between K and Na at sites of uptake at the plasmalemma (Epstein 1966).

The enhancement in germination percentage and rate in chilling treated seeds was accompanied by lower Na⁺ content in the germinating seedlings under salinity (Fig. 2). Taeb *et al.* (1992), have shown that vernalization treatments decreases Na⁺ accumulation in wheat when grown in 175 mM NaCl salinity. The difference from the present study being the timing of the imposition of treatments; vernalizing treatments to 4-d-old seedlings in their study compared to pre-sowing chilling treatments to seeds in the present case.

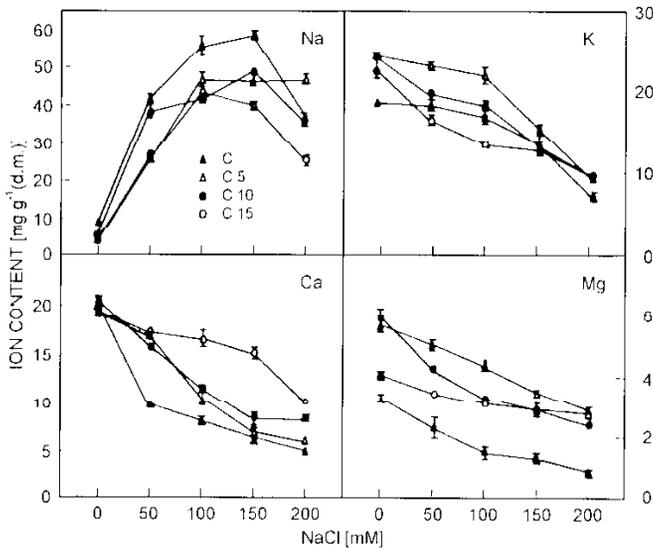


Fig. 2. Effect of pre-sowing chilling treatments to seeds on Na, K, Ca and Mg contents in 6-d-old *Brassica juncea* seedlings under salinity stress. Symbols as explained in Fig. 1. Means \pm SE, $n = 120$.

Further, pre-sowing chilling treatments to seeds for 5 and 10 d induced higher accumulation of K⁺ in seedlings, compared to controls. Thus Na/K ratio declined due to pre-sowing chilling treatments to seeds. The salt tolerant cultivars of mustard also accumulate comparatively less Na⁺ and maintained higher K⁺ content (Sharma and Gill 1994). Further, pre-sowing chilling treatments to seeds also enhanced Ca²⁺ uptake which play an important role in enhancing the salt tolerance of the seedlings (Fig. 2). In a study on salt tolerance of six *Brassica* species, He and Cramer (1992) associated the salt tolerance in *Brassica napus* to the higher Ca²⁺ concentration compared to the salt sensitive *B. carinata*. Mg²⁺ content also increased in chilling treated seeds compared to non-chilled controls and its accumulation was found to be affected with salinity (Fig. 2).

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