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BRIEF COMMUNICATION

Changes in protein profile of pigeonpea genotypes in response to NaCl and boron stress

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Abstract

Two pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes, a salt tolerant Manak and a salt sensitive ICPL 88039 were subjected to stress treatment of 3 mM boron, 60 mM NaCl and boron + NaCl at the seedling stage. Radicle and plumule proteins were analyzed by SDS-PAGE. Boron treatment increased 28.3 kDa proteins in plumule and 38.3 and 51.9 kDa proteins in radicle of Manak, however, there was no specific protein in ICPL 88039 either in plumule or in radicle. In NaCl treatment 95.6 kDa proteins appeared in plumule and 67.5 kDa proteins in radicle of Manak. Conversely content of some proteins decreased by boron treatment alone or in combination with NaCl although they were present in the controls. Thus, 54.3 kDa protein disappeared in ICPL 88039 plumule, 68.4 kDa in Manak radicle and 28.1 kDa in ICPL 88039 radicle.

Additional key words: boron toxicity, *Cajanus cajan*, salinity, SDS-PAGE.

Although boron (B) is a micronutrient, it is frequently found at toxic concentrations in soils and ground waters in arid and semi-arid conditions worldwide. B is now recognized as a toxic component of the saline milieu which interacts with salinity and further aggravates its toxic effects (Keren 1990, Mola-Doila *et al.* 1998, Wimmer *et al.* 2001, Ismail 2004). In view of the gravity of the problem, breeding for B tolerance has become an objective of some laboratories (Paull *et al.* 1991). For this an understanding of the mechanism of B toxicity particularly under saline conditions is considered vital (Wimmer *et al.* 2001, Läuchli 2002). It may be noted that in contrast to extensive studies on changes in protein expression as a result of various abiotic stresses such as heat, cold, salt and heavy metals (Grover *et al.* 2001), there is paucity of broad based information indicating qualitative changes in protein profiles in response to B toxicity particularly under saline conditions. Pigeonpea is a major crop of semi-arid tracts of India infested with the salinity-boron menace. Therefore, the examination of salinity tolerant and a salinity sensitive genotype of pigeonpea was undertaken to elucidate if any polypeptides whose levels are perturbed with B and/or salt are related to such tolerance.

Seeds of two pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes, a salt tolerant Manak (also numbered H77-216) and a comparatively salt sensitive ICPL 88039, were surface sterilized in 0.1 % sodium hypochlorite solution for 2 min and subsequently washed twice with distilled water. Ten healthy and uniform seeds were kept in plastic Petri plates of 9 cm diameter lined with filter paper. The desired treatments were given by adding the 10 cm^3 of aqueous treatment solutions [Water - control (C), 3 mM boron (B) as sodium tetra-borate, 60 mM NaCl (S) and 3 mM B + 60 mM NaCl (B+S)]. Petri plates were then incubated in a BOD incubator at 27 ± 1 °C in dark. The seeds produced dark coloured exudates which diffused in the medium and inhibited subsequent growth of seedlings. To overcome this problem, partially germinated seeds were transferred to other set of Petri plates with same treatment solution after 24 h. Sampling for various parameters was carried out after 7 d of sowing. Samples were prepared by grinding 500 mg plumule and radicle in 1 cm^3 Tris buffer (0.1 M, pH 7.5) containing 50 mg polyvinyl pyrroline. These were then centrifuged at 10 000 *g* at 4 °C for 15 min. The supernatant was used for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The

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protein quantification was done according to the method given by Bradford (1976). Electrophoresis on 12 % sodium dodecyl sulphate polyacrylamide gel was performed after Laemmli (1970) using vertical gel electrophoresis apparatus (*Model EC175, E-C Apparatus Corporation*, St. Petersburg, USA). The protein extract was transferred to an equal volume of sample buffer (Laemmli's 2× buffer), heated at 100 °C for three min, cooled and used for SDS-PAGE. An aliquot containing 50 µg of sample protein was loaded in each well and marker proteins (*Genei*, Bangalore, India) in a separate well. After completion of electrophoresis, staining and background destaining, relative mobility values were calculated for each of the marker proteins. Experiment was repeated twice and same trends of results were obtained

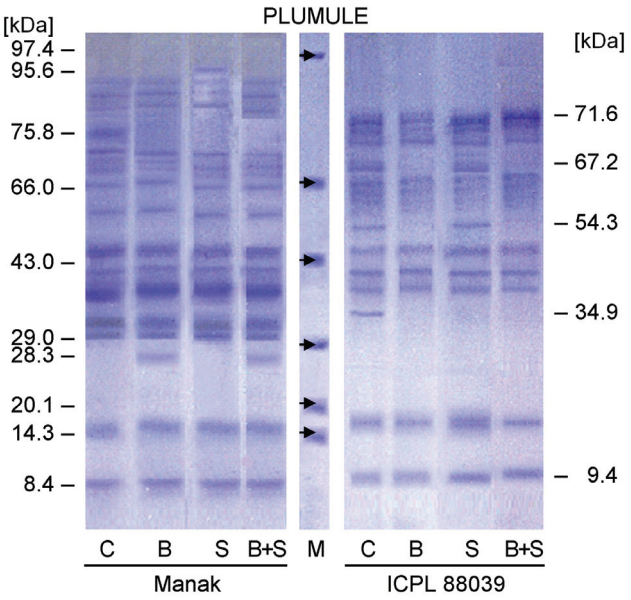

Fig. 1. SDS-PAGE resolved band pattern of proteins of plumule of Manak and ICPL 88039 cultivars of pigeonpea under C, B, S and B+S treatments. Position of molecular marker (M) proteins is indicated on the left side of the figure by *arrows*.

In plumule of tolerant genotype Manak, a maximum of 16 polypeptide bands were recorded with molecular masses ranging between 8.4 to 95.6 kDa in all the treatments (Fig. 1). As a result of B and B+S treatments, one new protein with molecular mass of 28.3 kDa appeared. However, this protein was absent in C and S treatments and thus seems to be B specific. Another 75.8 kDa protein was detected only in plumule of control but it disappeared in all treatments. Likewise, a 95.6 kDa protein was found in only S treatment though its expression disappeared in the B+S treatment indicating that the expression of this NaCl specific protein was curtailed by the B treatment. The remaining proteins were present in all the treatments including control. In plumule of ICPL 88039, a maximum of 14 bands were resolved with molecular mass ranging from 9.4 to 71.6 kDa (Fig. 1). The numbers of bands were lesser in plumule of the ICPL 88039 in comparison to Manak. Two 54.3 kDa and 67.2 kDa proteins were present in C and also in S treatment but disappeared in treatments B and B+S. Another 34.9 kDa protein was expressed only in C but disappeared in all other treatments. No B specific proteins were observed in the plumule of ICPL 88039. The remaining proteins were common to all the treatments.

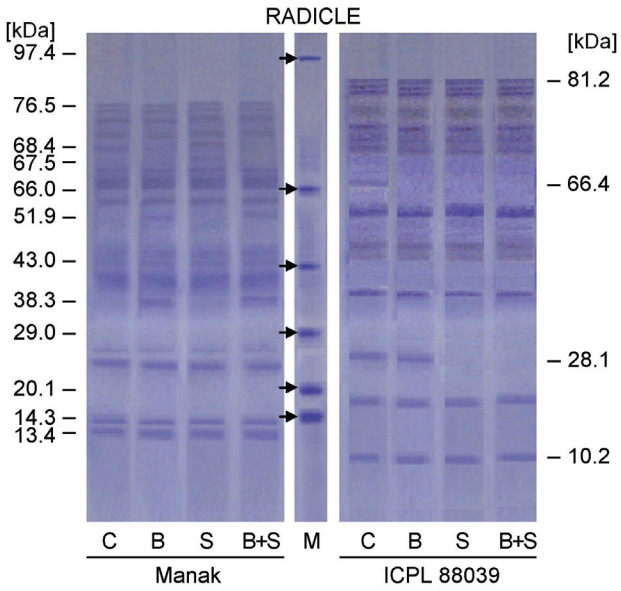

Fig. 2. SDS-PAGE resolved band pattern of proteins of radicle of Manak and ICPL 88039 cultivars of pigeonpea under C, B, S and B+S treatments. Position of molecular marker (M) proteins is indicated on the left side of the figure by *arrows*.

Protein profile of radicle of Manak (Fig. 2) depicted a maximum number of 16 proteins with molecular masses ranging from 13.4 to 76.5 kDa. A 68.4 kDa protein was present in radicle of C and S treated seedlings of Manak. Likewise, 38.3 and 51.9 kDa proteins were detected in radicle of B-treated seedling and were absent in all other treatments. One more 67.5 kDa protein was present only in radicle in case of S treatment but did not appear in any other treatment including control. Rest of the proteins were commonly found in radicle of the genotype Manak under all the treatments. The protein profile of radicle of ICPL 88039 seedlings included 16 bands with molecular mass ranging from 10.2 to 81.2 kDa. A band of 66.4 kDa was present in C treatment but absent in B, S and B+S treatments. Similarly, 28.1 kDa protein was expressed only in radicle of seedlings from C and B treatments. The remaining proteins were common to all the treatments. Sreenivasulu *et al.* (2000) reported that with 200 mM NaCl treatment tolerant cultivar Prasad of foxtail millet responded with the induction of 17 kDa and 22 kDa polypeptide bands which were not expressed in the sensitive cultivar Lepakshi. Elavumoottil *et al.* (2003) reported that salt tolerant calli of *Brassica oleracea* var.

botrytis exposed to 225 mM NaCl for six months synthesized a 27 kDa polypeptide which was absent in the control calli maintained on the non-saline stock culture medium. Disappearance of the protein bands may be interpreted as the "turning off" of protein synthetic genetic machinery (genes?) in response to salt and/or B treatments. It is more likely, however, that the "disappeared" proteins in response to stress are a result of their denaturation. Depressed protein synthesis and acceleration in its degradation in plants in response to salt stress has been reported by number of workers (Chandershekhar *et al*. 1986, Lal and Bhardwaj 1987). Sousa *et al*. (2004) reported that cowpea seedlings subjected to NaCl stress showed increased concentration of 9 proteins, decreased concentration of one and *de novo* synthesis of one 21.2 kDa protein. However no such studies on protein expression under salinity-B interaction appear to have been conducted. In this context, the expression of B and NaCl specific proteins in the tolerant pigeonpea genotype Manak when challenged with the B+S stress is an important feature of this investigation pointing to underlying molecular events right up to the genomics. It would be worthwhile to add that studies have revealed that wheat genotypes tolerant to salinity have also been found tolerant to B toxicity. Thus *Agropyron elongatum* having high sodic tolerance was also found to be B tolerant (Schuman 1969). In ongoing studies in our laboratory wheat cultivars Kharchia 65 and KRL 1-4 specifically bred for salinity tolerance were also found to be tolerant to B toxicity (Mola-Doila 2002). However, genes for salinity (Gorham *et al*. 1990) and boron (Jefferies *et al*. 2000) tolerance have different loci on the chromosomes. Further characterization of such salinity and B specific proteins would be worth investigation.

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