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CHANGES IN PROTEIN PROFILE UNDER SODIUM CHLORIDE AND BORON TOXICITY STRESS IN SEEDLINGS OF TWO WHEAT CULTIVARS

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SUMMARY

Two genotypes of wheat (*Triticum aestivum* L.), a salt tolerant KRL 1-4 and a salt sensitive HD 2329 were subjected to stress treatments of control, 10 mM B, 120 mM NaCl and 10 mM B+ 120 mM NaCl at the seedling stage in a BOD incubator at 26±1 °C for 96 h. Proteins from coleoptile and radicle were resolved by SDS-PAGE. In tolerant cultivar (KRL 1-4), two proteins of 83.6 and 28.1 kDa appeared in the coleoptile and a 35 kDa protein appeared in the radicle in the seedlings treated with B and B+NaCl. Similarly, 46.6 kDa protein was observed in the radicle of KRL 1-4 only in B treated seedlings. These results clearly indicate characteristically B induced proteins. Two proteins of 74.2 and 48.8 kDa in HD 2329 coleoptile and a 64.6 kDa protein in the radicle of both KRL 1-4 and HD 2329 seedlings were seen specific to salt treatment and were absent in boron treated seedlings. Likewise, a 70 kDa protein in the coleoptile profile of KRL 1-4 seedlings was expressed only with salt treatment, but was absent in boron treatment.

***Key words*: Boron toxicity, salinity, SDS-PAGE, stress, wheat.**

INTRODUCTION

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. It is now recognized that B is a toxic component of the saline milieu which interacts with salinity and further aggravates its toxic effects (Keren 1990, Mola-Doila *et al.* 1998, Ismail 2004). Salinity and B toxicity problems co-exist. Toxic levels of B and salinity inhibit the seed germination, early seedling growth, vegetative growth and reproductive development in crop plants. When B is superimposed on salinity even in moderate concentrations that are not toxic *per se*, it accentuates the deleterious effects of salinity manifold. 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, Lauchli 2002).

For further advances in our understanding of the mechanism of B toxicity under saline conditions subtle changes in the stress induced protein changes need to be looked into. Mahboobi *et al.* (2000) studied the changes in protein profile of barley cultivars in response to toxic B concentration. The changes in polypeptide composition due to B toxicity were more abundant in leaf tissues than in roots. These changes especially at shoot level may form the basis of elucidation of the tolerance mechanism to B toxicity at molecular level. Therefore, the present investigation has been undertaken to study the qualitative changes in protein profiles of coleoptile and radicle to delimit effects both under B excess and salinity alone as well as in combination with each other in a tolerant (KRL 1-4) and a sensitive cultivar (HD 2329) of wheat.

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MATERIALS AND METHODS

Seeds of two cultivars of wheat, i.e. KRL 1-4 (salt tolerant) and HD 2329 (salt sensitive) were first surface sterilized with 1% solution of sodium hypochlorite for 2 min. Petri plates (9 cm diameter) were sterilized with absolute alcohol and lined with filter paper at the bottom. Ten healthy and uniform seeds were sown in each petriplate. The desired treatments were given by adding 10 cm^3 of aqueous treatment solutions [water- control(C), 10 mM boron (B) as sodium tetra-borate, 120 mM NaCl (S) and 120 mM NaCl + 10 mM B (B+S)]. Petriplates were incubated in a BOD incubator at 26 ± 1°C in dark and the observations were recorded after 96 h separately in coleoptile and radicle.

Samples for protein profiles were prepared by macerating 500 mg sample tissue in 1 cm^3 chilled tris buffer (0.1 M, pH 7.5) containing 50 mg polyvinyl pyrrolidine (PVP). 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 protein quantification was done according to the method given by Bradford (1976). Electrophoresis on 12% SDS polyacrylamide gel was performed after Laemmli (1970) using vertical gel electrophoresis apparatus (Model EC-175, 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 minutes, cooled and used for SDS-PAGE (Laemmli 1970). An aliquot containing 50 µg of sample protein was loaded in each well with marker proteins (Genei, Banglore, India) in a separate well. After completion of electrophoresis, staining was done with coomassie brilliant blue G -250 dye. The relative mobility values were calculated for each of the marker protein. Experiment was repeated twice and same trends of results were obtained. R_f values of marker proteins were plotted against log of molecular weights of marker proteins using semi logarithmic paper. Molecular weights of different proteins were estimated by matching their R_f values with appropriate point on the standard curve.

RESULTS AND DISCUSSION

In the coleoptile protein profile of KRL 1-4 seedlings (Fig. 1), a total of 22 protein bands were seen with molecular weight ranging between 16.1 to 101 kDa. A 70 kDa protein band was expressed only in NaCl treatment though its expression disappeared in Salt + B treatment indicating that the expression of this NaCl specific protein was curtailed by B treatment. In other words, B causes denaturation of this protein band induced with salinity. Similarly, Sousa *et al.* (2004) also reported that cowpea seedling subjected to NaCl stress showed increased concentration of 9 proteins, decreased concentration of one and *de novo* synthesis of one 21.2 kDa protein. Two protein bands with molecular weight 83.6 and 28.1 kDa were present only in B and salt + B treatment and were absent in control and NaCl alone. It shows that the expressions of these protein bands require B, *i.e.* B induced these proteins. The appearance of these bands specifically with B did not disappear even when B was used in combination with NaCl. These results are in accord with Bishnoi *et al.* (2006) who have also observed 28.3 kDa protein in plumule and 38.3 and 51.9 kDa proteins in radicle of Manak (a salt tolerant genotype of pigeonpea) with B treatment. A protein band of 40.6 kDa was observed only in control seedlings and was

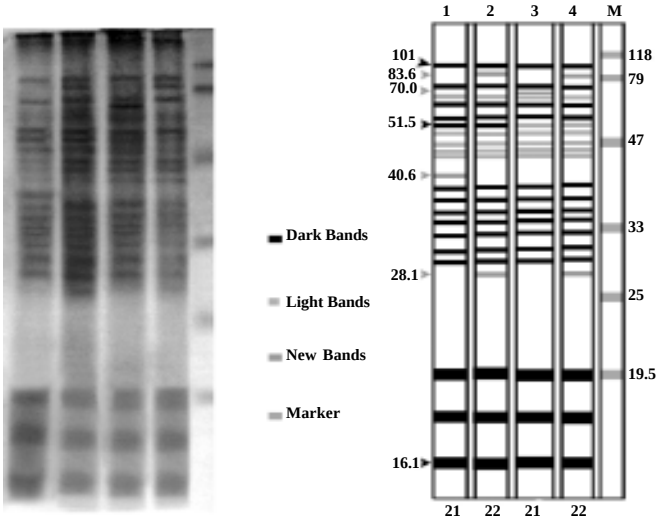

Fig. 1. Changes in protein profile of coleoptile of wheat seedlings (cv. KRL 1-4) under NaCl-Boron toxicity.
[Lane 1= Control, Lane 2= Boron (10 mM), Lane 3= NaCl (120 mM), Lane 4= NaCl (120 mM)+Boron (10 mM)]

absent in all other treatment lanes. This protein band might have denatured at toxic level of salt and B concentration. Remaining 20 protein bands were present in all treatment seedlings and may be inherently associated with germination and growth processes of seedlings. Their disappearance might somehow be affecting the functional capabilities of seeds to germinate and their further growth processes involving healthy seedling formation.

Likewise, in the coleoptile profile of HD 2329 seedlings (Fig. 2), a total of 19 protein bands were resolved with molecular weight ranging from 16.1 kDa to 101 kDa. Two bands with molecular weight 74.2 kDa and 48.8 kDa were present in control seedlings and salt (NaCl) treated seedlings but were absent in B and salt + B treated seedlings. These proteins may be denatured at toxic B levels. The results clearly indicate that expression of 'salt stress' proteins are related to the adaptation process of seeds to salinity as well as to the genetic constitution of a selected salt-tolerant genotype as was observed by Dell-Aquilla and Spada (1993). A 39.5 kDa protein was observed in coleoptile profile of control seedlings and was absent in salt and B treated seedlings, *i.e.* this protein might have denatured or not expressed at toxic salt and B concentrations. Depressed protein synthesis and acceleration in its degradation in plants in response to salt stress has been reported by a

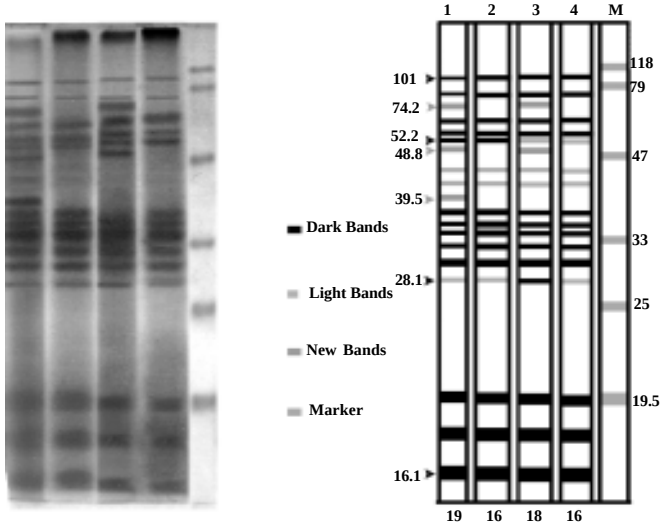

Fig. 2. Changes in protein profile of coleoptile of wheat seedlings (cv. HD 2329) under NaCl-Boron toxicity.
[Lane 1= Control, Lane 2= Boron (10 mM), Lane 3= NaCl (120 mM), Lane 4= NaCl (120 mM)+Boron (10 mM)]

number of workers (Garg and Garg 1982, Chandershekar *et al*. 1986, Lal and Bhardwaj 1987). In HD 2329 coleoptile, the expression of a 28.1 kDa protein was increased with salt treatment. Although the band is present in all the treatments including control but in salt treatment the band got darkened, i.e. its expression might be increased with salt treatment. It indicated that the protein might be salt induced. These results are in consonance with our similar investigations in pigeonpea genotypes, where a 54.3 kDa protein disappeared in plumule and 28.1 kDa in radicle of the salt sensitive genotype ICPL 88039 and a 68.4 kDa protein disappears in radicle of salt tolerant genotype Manak (Bishnoi *et al.* 2006).

In the coleoptile profile of KRL 1-4 seedlings (Fig. 1) a 51.5 kDa protein and in HD 2329 seedling a 52.2 kDa protein was expressed only in control and B treatment but their expression is decreased under salt and salt + B interaction, *i.e.* this protein might have denatured partially under salt and salt + B treatment. Daskalyuk *et al.* (1992) detected a similar protein band of 52.0 kDa in wheat seedlings under NaCl treatment. The synthesis of this protein band is decreased when incubated for 24 hours in 0.4M NaCl solution. Pareek *et al.* (1998) has given evidence for the accumulation of a 55.0 kDa polypeptide in rice, wheat, soybean, *Pisum sativum, Zea mays* and *Brassica juncea* seedlings with respect to high temperature.

A total of 20 protein bands were resolved in the protein profile of radicle of wheat cultivar KRL 1-4 seedlings (Fig. 3). A 46.6 kDa protein was present in the radicle of B treated seedlings and was absent in all other treatments. Another 35.0 kDa protein was expressed in B and salt + B treated seedlings and disappeared in control and salt treated seedlings. The expression of these proteins may be related to the acclimation process of seeds to B as well as to the genetic constitution of a selected B-tolerant genotype. The appearance of 35.0 kDa protein in the radicle profile of tolerant cultivar is supported by the observations made by Mahboobi *et al.* (2000), who also identified a 35.0 kDa protein in the root profile of tolerant cultivar as a result of B toxicity. This protein failed to show up in the root profile of sensitive cultivar. These results also suggest that in salt tolerant

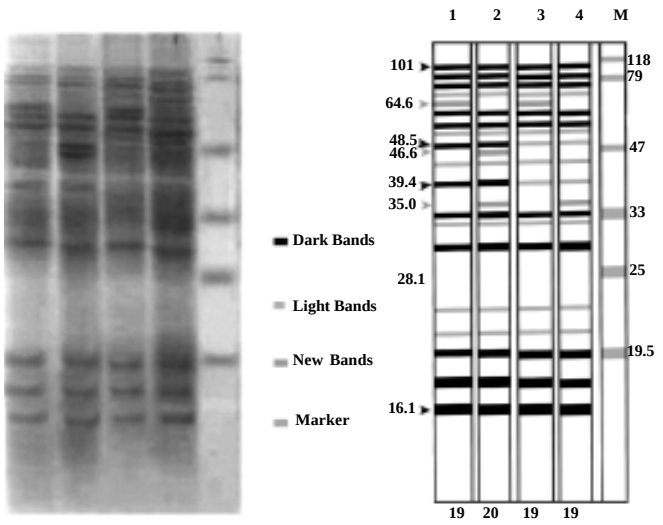

Fig. 3. Changes in protein profile of radicle of wheat seedlings (cv. KRL 1-4) under NaCl-Boron toxicity.
[Lane 1= Control, Lane 2= Boron (10 mM), Lane 3= NaCl (120 mM), Lane 4= NaCl (120 mM)+Boron (10 mM)]

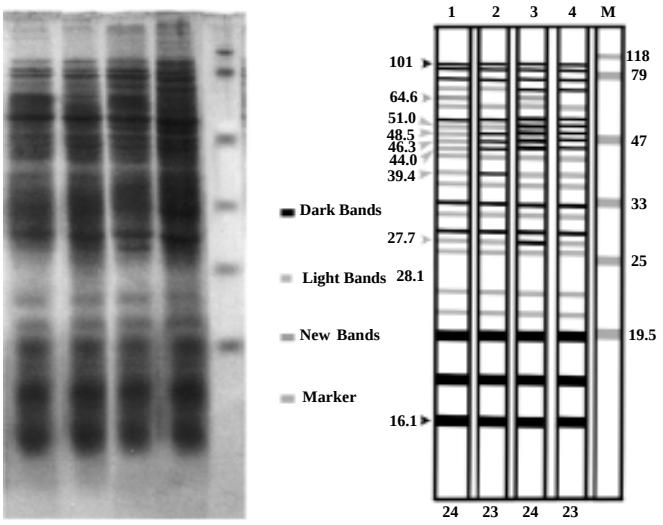

Fig. 4. Changes in protein profile of radicle of wheat seedlings (cv. HD 2329) under NaCl-Boron toxicity.
[Lane 1= Control, Lane 2= Boron (10 mM), Lane 3= NaCl (120 mM), Lane 4= NaCl (120 mM)+Boron (10 mM)]

(KRL 1-4) cultivar, many proteins may be associated with resistance mechanism of this cultivar to B toxicity. A 64.6 kDa protein was present in control and salt treated seedlings in the radicle of both KRL 1-4 and HD 2329, but was absent in B and salt + B treated seedlings, which shows the denaturation of protein at toxic B level. However, 2 protein bands with molecular weight 48.5, and 39.4 kDa were present in the radicle protein profile of control and all salt and B stressed seedlings, but their expression is reduced in salt and salt + B treated seedlings. Remaining protein bands were present in all seedlings, *i.e.* control as well as salt and B stressed seedlings.

In the radicle protein profile of HD 2329 seedlings (Fig. 4), a total number of 24 protein bands were resolved with molecular weight ranging between 16.1 to 101 kDa. A 64.6 kDa protein was present only in control and salt treated seedlings but was not present in B treated and salt + B treated seedlings. This protein band might have denatured under toxic B concentration. Four bands of molecular weight 51.0, 48.5, 46.3 and 44.0 kDa were present in the radicle profile of control as well as in salt and B treated seedlings, but their expression was, in general, increased particularly in salt and salt + B treated seedlings. Similarly, the expression of a 39.4 kDa protein was increased in B treated seedlings. Although the protein is present in all treatments including control but the band got darkened with B treatment. Likewise, a 27.7 kDa protein expression was increased with salt treatment. Remaining proteins remain unchanged in salt and B treatments. Disappearance of the proteins may be interpreted as the "turning off" of protein synthetic genetic machinery (genes?) in response to salt and/or excess B treatment. It is more likely, however, that the "disappeared" proteins in response to stresses are a result of denaturation (Elavumoottil *et al.* 2003).

An important feature of this investigation is the expression of B and salt specific proteins in tolerant wheat cultivar (KRL 1-4) when challenged with Salt + B interaction. It can be concluded from the present studies on wheat and others reported on pigeonpea from our laboratory (Bishnoi *et al.* 2006) that the genotypes tolerant to salinity are tolerant to B as well, the two stresses seem to be associated with different protein profiles. There are other reports as well which indicate that genotypes tolerant to salinity are also tolerant to B (Schuman 1969, Paull *et al.* 1991). Thus *Agropyron elongatum* having high sodic tolerance was also found to be B tolerant (Schuman 1969). Ongoing studies in our laboratory on 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 that of B (Jefferies *et al.* 2000) tolerance have different loci on chromosomes. More intensive characterization of such salinity and B specific proteins would be worthwhile and shall aid in evolving breeding techniques involving biotechnological interventions to develop B tolerant crop cultivars as is being done in some laboratories (Paull *et al.* 1991).

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