Indian Journal of Experimental Biology
Vol. 55, May 2017, pp. 321-328

Tolerance to combined boron and salt stress in wheat varieties: Biochemical and molecular analyses

Charu Lata[1], Ashwani Kumar[1]*, SK Sharma[1], Jogendra Singh[1], Shital Sheokand[2], Pooja[2], Anita Mann[1] & Babita Rani[2]

[1]ICAR-Central Soil Salinity Research Institute, Karnal (Haryana), India
[2]Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana) India

Received 05 December 2015; *revised 27 April 2016*

Plants' tolerance to stresses, particularly abiotic, is an important area of research, particularly in agriculture. Here, we studied the combined effect of excess boron (B) and salinity on three wheat varieties *viz*., KRL 35, KRL 210 and HD 2009. Root samples were collected 20 days after imposing different treatments, namely Control, 50 and 100 ppm B + 60 mM NaCl, respectively; and 50 and 100 ppm B + 100 mM NaCl, respectively in a hydroponic system. Results indicated that length, fresh and dry weight of root and shoot consistently decreased with increasing concentration of B and salt in the nutrient medium. These changes were accompanied by significant reductions in soluble sugars and proteins in roots, whereas proline content increased. The KRL 35 (salt tolerant variety) showed 5 specific polypeptides of 89.13, 53.4, 46.21, 32.35 and 31.10 kDa. Likewise, KRL 210 (moderately salt tolerant) showed *de novo* synthesis of 53.4 and 19.13 kDa, whereas three specific polypeptides (24.05, 19.13 and 17.52 kDa) appeared in HD 2009 (salt sensitive). Synthesis of the common polypeptide 25.12 kDa was observed with increase in stress treatments in all three varieties. Enhanced expression of 25.12 kDa proteins, particularly in the sensitive variety induced protein synthesis under excess boron and salt stress conditions. Thus, altered and enhanced expression of proteins might be responsible for the survival and growth of plants under excess B and NaCl affecting the functional capabilities of seeds in the stress environment. Appearance of new polypeptides or their disappearance might be related to the genotypic stress tolerance or sensitivity.

Keywords: Abiotic stress, Protein profile, Polypeptides, SDS-PAGE, Micronutrients, *Triticum aestivum*

Research into plant tolerance of abiotic stresses, including salinity, drought, heavy metal pollution, toxic concentrations of boron, aluminium or other elements and elemental deficiencies, is of fundamental importance for sustainable and secure agriculture. Stress tolerance of plants is a complex phenomenon that involves ecological, physiological, biochemical and molecular processes as well as morphological changes[1-4]. Globally, more than 900 million hectares of land, approx. 20% of the total agricultural land[5], are affected by salt, accounting for more than 6% of the world's total land area. In India, salt affected soils occupy an area of about 6.73 million ha, of which saline and sodic soils constitute about 40 and 60%, respectively[6]. Soil salinity is an increasing problem for agriculture, affecting the most productive crop areas of the world, those cultivated under irrigation in arid and semiarid regions; they represent less than 15% of global arable land, but produce more than 40% of world food[7].

*Correspondence:
E-mail: ashwanisharma107@gmail.com; ashwani.1@icar.gov.in

Though boron (B) is a micronutrient, it is present at toxic concentrations in soils and ground waters in arid and semi-arid environments worldwide[8,9]. B deficiency is a widespread agricultural problem, whereas salinity, another common agricultural problem, aggravates B toxicity in plants. Reduction in growth and yield are the most conspicuous physiological responses of plants exposed to excess of salt and boron[10]. Osmotic adjustment by accumulating compatible solutes has been considered an important physiological plant adaptation to increase salinity tolerance[11]. Soluble sugars and proline are the two important compatible plant metabolites[12-14], having significant role in adjusting soil water potential and regulating osmotic potential[15] to counter the adverse microclimatic situations. Besides their roles in osmotic adjustment, they may also protect membranes from damages and stabilize the structures and activities of proteins and enzymes[13,14,16]. Gnanaraj *et al*.[3] have reported phospholipase C (PLC) expression induced by drought, salinity and low temperature in mung beans and upregulated the same with salicylic acid. PLC has also been reported to have a crucial role in

phosphoinositide (PI) pathway that plays an important role in plant growth and development. Further, microorganisms including fungi also help crops overcome such stresses. *Neurospora discreta* strain SR8 from rhizospheric soil of *Sorghum bicolor* has been reported to support the latter's survival in high salinity condition and also in phosphate solubilization[4].

Interactions between salinity and B toxicity are complex and the mechanism of boron uptake under saline conditions is not well understood. Inadequate information on plant responses to combination of excess B and salinity on growth and yield is available with various reports showing antagonistic, additive or interactive effects. Bingham *et al*.[17] reported that wheat plants respond to B independently of soil salinity levels, whereas Holloway & Alston[18] and Grieve & Poss[19] reported that excess external B and salinity interact to limit growth and yield in wheat. However, they could not provide reasons for these interactions.

It is well known that alteration of gene expression is always involved in preparing plants for growth and survival under stress. Stresses induce quantitative and qualitative changes in protein content of the plant cells. Multiple stressed [salinity and heavy metal (Cd/Pb) stress] Indian mustard seedlings showed reduced germination, seedlings length and photosynthetic pigments but increased glutathione content. However, media supplementation with putrescine increased the protein contents in leaf under single or combined stress conditions[2].

Suppression of gene(s) responsible for certain proteins under stress might be one possible explanation for their disappearance[20]. Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions. Though protein biosynthesis generally declines under stress conditions, cells preferentially synthesize specific stress proteins also. Depressed protein synthesis and acceleration in their degradation in plants in response to salt stress has been reported by a number of workers[21,22]. Present study on the qualitative changes in protein profiles of three varieties of wheat differing in their tolerance to salinity was, therefore, undertaken to understand these mechanisms and delimit the combined effects of boron and salinity.

Materials and Methods

Three varieties of wheat (*Triticum aestivum* L.) differing in their tolerance to salinity *viz*. KRL 35 (salt tolerant), KRL 210 (moderately salt tolerant) and HD 2009 (salt sensitive) were evaluated for interactive responses to boron (B) and sodium chloride (NaCl) stresses in relation to germination. Seeds of each variety were surface sterilized using 0.1% bavistin, washed thoroughly and were pre-germinated in Petri dishes at 4ºC for 48 h and then transferred to the bench at room temperature (15-20ºC) for 24 h. These pre-germinated seeds were then placed in pots (capacity 2.0 L) covered with thick black sheet (to prevent exposure to sunlight) containing boron (50 and 100 ppm) along with two levels of salinity (60 and 100 mM NaCl). For each variety, there was control having no salt and 2.5 ppm boron and the seedlings were grown for a period of 20 days during the *Rabi* season of 2012-13 in the glasshouse of Division of Crop Improvement, ICAR-Central Soil Salinity Research Institute, Karnal, Haryana, India.

After pre-germination, the following treatments were imposed: control (2.5 ppm B, no salt), low boron and low salt (50 ppm B and 60 mM NaCl), high boron and low salt (100 ppm B and 60 mM NaCl), low boron and high salt (50 ppm B and 100 mM NaCl), high boron and high salt (100 ppm B and 100 mM NaCl). In order to avoid shock, NaCl was increased gradually in four equal doses over a period of four days. The nutrient solution was changed every five days. The pH of nutrient solution was maintained around 7.0 during the experiment. Using aquarium pumps and air stones, aeration of tanks (to prevent stagnation) was done. Experiment was laid in a complete randomized design with five replicates. Plants were harvested after 20 days of treatments, separated into roots and shoots and weighed. Root/shoot length was measured with the help of meter scale. Freshly harvested roots and shoots were oven-dried at 70ºC for 48 h for dry weight (DW) and then analyzed for total soluble sugars[23], proline[24] and protein content[25]. The data were analyzed statistically and the significance was tested using SAS 9.3 (SAS Institute Inc., Cary, NC, USA) software.

SDS-PAGE analysis

About 25 µL of crude protein extract containing 50 µg of protein extract was transferred to an equal volume of Laemmli's 2X sample buffer (0.5 M Tris-HCl, pH 6.8) containing 20% glycerol, 4% SDS, 0.5% bromophenol blue (w/v) and 10% β-mercaptoethanol and heated at 100°C for 3 min and cooled. Electrophoresis was carried out following the method

given by Laemmli[26]. The cooled samples were then loaded on SDS-discontinuous gel system with 0.1 mm thick stacking gel of 4% polyacrylamide in Tris-HCl buffer (pH 6.8) and a resolving gel of 10% polyacrylamide in Tris-HCl buffer (pH 8.8). The gels were run at 15mA in the stacking gel and 25 mA in the resolving gel. After electrophoresis, gels were fixed and stained with 0.25% (w/v) Coomassie Brilliant Blue R-250 in 40% (v/v) methanol with 7% glacial acetic acid (v/v) and then destained in 10% methanol (v/v) with 7.5% glacial acetic acid (v/v). After destaining, the gels were stored in 7% glacial acetic acid (v/v).

Results and Discussion

Boron toxicity symptoms (chlorosis developing to brown spots at the tips and the edges of leaves leading to necrosis) appeared at 50 ppm B and increased in severity at 100 ppm. Salinity aggravates toxicity symptoms of boron in wheat. Our earlier studies have shown significant interactive effects of B and salinity on plant growth parameters and mineral ion accumulation[27]. The main impact of B toxicity was on roots. As the concentration increased in the nutrient medium, the length and number of roots both reduced. Lower B concentration along with 60 and 100 mM NaCl had less pronounced effect on root growth in terms of length[28]. The root length showed significant variation with respect to treatment and variety (Table 1). At the highest levels of B + Salinity KRL 35 showed more than double root length (8.67 cm) compared to HD 2009 (Table 1). Root-shoot length consistently decreased with increasing concentration of B and salt in the nutrient medium. Lakra *et al*.[2], too observed similar decline in Indian mustard exposed to high salinity and heavy metal stress.

Root and shoot both play an important role in plant biology. When faced by environmental stresses, such as boron and salt toxicity, plants may not absorb the required amounts of essential minerals and water required for growth and development. When root growth is affected by toxic or different nutrient ions, overall growth and yield is affected[1]. Plants may also try to avoid contact with toxic elements, resulting in reduced or irregular root growth[29,30]. These results are in accordance with Hassan[29]; Ismail[31]; Alpaslan & Gunes[32] reporting reduced root and shoot length under B and salt. A noticeable point emerging from our results shows that the toxic effects of B and salinity would be more serious in the salt sensitive variety than the salt resistant or tolerant ones.

The combined effect of boron and salt on fresh and dry weight of roots and shoots revealed a response similar to that found for root and shoot architecture. Significant interaction between salt and boron concentration was found (Table 2). Plant biomass decreased in response to increase in concentrations of NaCl and B. KRL 35 showed minimum reduction in fresh and dry weights of root as well as shoot at high salt and boron levels relative to control. Whereas maximum reductions were observed in HD 2009 i.e. 98 and 94.38% reduction in root fresh and dry weight whereas 88.24 and 84.11% in shoot fresh and dry weight HD 2009 with respect to control (Table 2). Growth reductions caused by the boron and salinity treatments were accompanied by the appearance of toxicity symptoms on the leaves with the increase of boron in the growth media. Fresh weights were used

Table 1—Effects of B and salt on root length, shoot length and no. of primary roots in wheat varieties differing in their tolerance

Treatment ↓ / Varieties →	Root length (cm)			Shoot length (cm)			Number of roots		
	KRL 35	KRL 210	HD 2009	KRL 35	KRL 210	HD 2009	KRL 35	KRL 210	HD 2009
Control	45.63	37.23	41.67	25.37	16.07	18.73	8	7	7
50 ppm B + 60 mM NaCl	32.03	17.43	16.13	19.13	10.47	13.57	7	6	5
100 ppm B + 60 mM NaCl	9.27	6.93	6.37	15.37	8.40	5.73	7	5	5
50 ppm B + 100 mM NaCl	14.77	13.30	9.73	16.00	11.93	8.27	6	6	5
100 ppm B + 100 mM NaCl	8.67	5.43	3.43	11.13	6.97	4.67	6	4	4
Mean	22.07	16.07	15.47	17.40	10.77	10.19	6.8	5.6	5.2
LSD at 5%	Main plot (Treatment) – 2.33 Sub plot (Variety) – 0.98 Sub plot treatment means at same level of main plot treatment – 2.19 Main plot treatment means at same level of sub plot treatment – 2.94			Main plot (Treatment) – 0.61 Sub plot (Variety) – 0.52 Sub plot treatment means at same level of main plot treatment – 1.17 Main plot treatment means at same level of sub plot treatment – 1.13			Main plot (Treatment) – 1.10 Sub plot (Variety) – 0.63 Sub plot treatment means at same level of main plot treatment – N.S. Main plot treatment means at same level of sub plot treatment – N.S.		

Table 2—Effects of B and salt on fresh and dry weight of roots and shoots in wheat varieties differing in their tolerance

Treatment ↓ Varieties	Fresh weight of root (mg)			Dry weight of root (mg)			Fresh weight of Shoot (mg)			Dry weight of Shoot (mg)		
	KRL 35	KRL 210	HD 2009	KRL 35	KRL 210	HD 2009	KRL 35	KRL 210	HD 2009	KRL 35	KRL 210	HD 2009
Control	685	603	695	53.57	67.73	57.47	631	509	612	93.73	71.13	75.97
50 ppm B + 60 mM NaCl	625	164	175	55.83	18.33	17.3	484	208	178	65.67	25.8	34.9
100 ppm B + 100 mM NaCl	100	45	33	14.93	7.3	8.67	242	101	097	42.3	20.47	21.63
50 ppm B + 60 mM NaCl	221	140	83	29.4	16.27	12.77	302	170	114	51.47	17.8	31.5
100 ppm B + 100 mM NaCl	82	31	14	11.4	6.23	3.23	201	96	72	36.23	11.3	18.73
Mean	343	197	200	33.03	23.17	19.89	372	217	215	57.88	29.3	36.55
LSD at 5%	Main plot (Treatment) – 12.8 Sub plot (Variety) – 11.8 Sub plot treatment means at same level of main plot treatment – 26.38 Main plot treatment means at same level of sub plot treatment – 25.04			Main plot (Treatment) – 1.92 Sub plot (Variety) – 1.28 Sub plot treatment means at same level of main plot treatment – 2.86 Main plot treatment means at same level of sub plot treatment – 3.02			Main plot (Treatment) – 20.36 Sub plot (Variety) – 8.99 Sub plot treatment means at same level of main plot treatment – 20.09 Main plot treatment means at same level of sub plot treatment – 26.12			Main plot (Treatment) – 4.91 Sub plot (Variety) – 2.62 Sub plot treatment means at same level of main plot treatment – 5.87 Main plot treatment means at same level of sub plot treatment – 6.85		

to monitor the physiological response of each genotype. The decline in dry weight under salt stress could be attributed to the reduced mobilization of the reserve food materials from the endosperm (cotyledons) to the growing axis due to inhibitory effects of both, boron and salt, individually and in combinations[33,34].

Sugars are the source of energy and carbons needed for the adaptive or defensive responses to stresses. It was clear from the results that boron and salt affected the total soluble sugars content and a gradual decrease was noticed with increasing concentrations of boron and salt (Fig. 1A). KRL 35 showed highest accumulation of sugars (26.8 mg/g) at 100 ppm B + 100 mM NaCl. Carbohydrates such as sugars and starch get accumulated under stress, playing a leading role in osmo-protection, osmotic adjustment, carbon storage and radical scavenging[35,36]. There was a gradual rise in the level of proline in all the three varieties with increasing concentration of boron and salt. Even the lowest levels of boron and salt (50 ppm B + 60 mM NaCl) induced accumulation of proline (Fig. 1B). At the highest level (100 ppm B + 100 mM NaCl), maximum proline accumulation was observed in HD 2009 (18.6 mg/g) and minimum in KRL 35 (13.5 mg/g) as compared to their respective controls. Proline has been reported to increase the stress tolerance of the plants through functions as osmoregulation, the protection of enzymes against denaturation, and the stabilization of protein synthesis[37]. Accumulation of

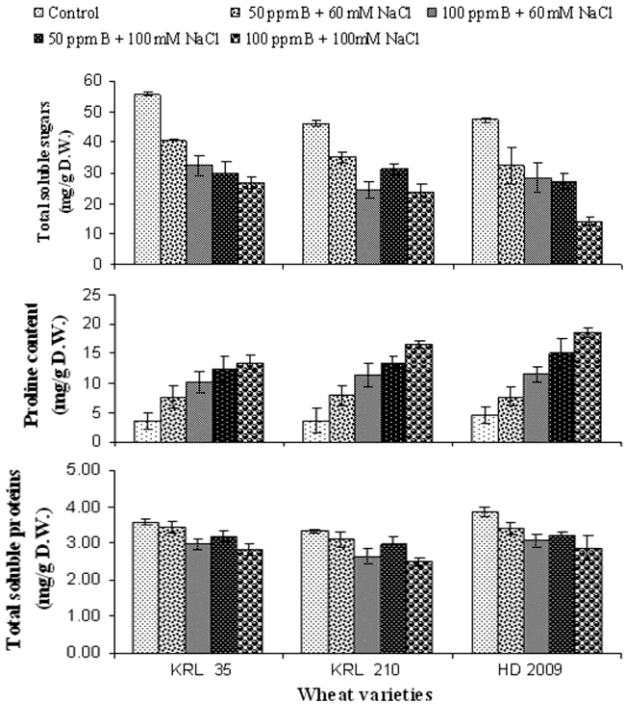

Fig. 1—Effects of boron and Salt on (A) total soluble sugars; (B) proline content; and (C) total soluble proteins (mg/g dry weight) in wheat varieties differing in their salt tolerance.

proline may supply energy to increase salinity tolerance and may occur through an increase in its synthesis with inhibition of its catabolism and revealed how much degree of stress a genotype is able to tolerate[38].

Excess boron and salt stresses induced quantitative and qualitative changes in protein content of the plant

cells. Total soluble proteins were determined by Bradford method[25] and the content decreased with increasing concentration of boron and applied salt. Minimum reduction in total soluble proteins was observed in KRL 35 (Fig. 1C). Excess B and salt reduced shoot fresh and dry weights, root fresh weight and biomass, protein synthesis, increased protein hydrolytic enzyme activity, decreased amino acid synthesis and interfered with tertiary and quaternary enzyme structures leading to decreased in soluble protein content[39,40]. The proteins that accumulate under stress conditions may provide a storage form of nitrogen that is re-utilized in post-stress recovery[41] and also play a role in osmotic adjustment.

Results of SDS-PAGE protein profile indicate that under B and salt stresses, qualitative and quantitative differences in polypeptide pattern exist between tolerant and intolerant varieties in comparison to control. Excess boron and salt stresses caused an induction or inhibition in the synthesis of some polypeptides in the roots of wheat varieties. Protein patterns, which were examined by SDS-PAGE gel electrophoresis (10%), showed differential expression of polypeptides in salt tolerant (KRL 35, Fig. 2A), moderately salt tolerant (KRL 210, Fig. 2B) and salt sensitive (HD 2009, Fig. 2C) varieties. Presence or absence of polypeptides could be potentially used as marker(s) to decipher the differential behaviour of varieties for boron and salinity. KRL 35 showed total number of 6 polypeptides under control condition and the number of polypeptide increased to 9 at 50 ppm B + 60 mM NaCl indicating *de novo* synthesis of 3 new polypeptide bands of MW 53.4, 46.21 and 25.12 kDa (Fig. 2A). Increased concentration of B to 100 ppm B + 60 mM NaCl caused appearance of two new polypeptide bands (89.13 and 31.10 kDa). At 50 ppm B + 100 mM NaCl one new polypeptide band of MW 32.35 kDa appeared whereas two polypeptides of 46.21 and 16.16 kDa disappeared. These proteins might be synthesized either *de novo* in response to salinity stress or may be present constitutively at low concentration and increase when plants are exposed to different stresses[42]. At higher salinity level of 100 mM NaCl with high boron, pattern of polypeptide resolution remained unaffected. This suggested that polypeptide expression varied depending upon the different developmental stages and the differential gene expression of concerned structural or regulatory genes. The timing of the sythesis of particular polypeptides coincides with the protein content during different stresses associating their involvement in tolerance[43].

Differences in polypeptide resolution of salt tolerant varieties indicate their cellular and molecular adaptive mechanism to osmotic stress. Differentially expressed polypeptides were observed in KRL 210 (moderately salt tolerant). It showed total number of 11 polypeptides in control which remained unaffected at 50 ppm B + 60 mM NaCl. But, increase in boron and salt was accompanied by appearnce as well as disappearance of polypeptide bands; 3 new polypeptide bands of MW 53.4, 25.12 and 19.13 kDa

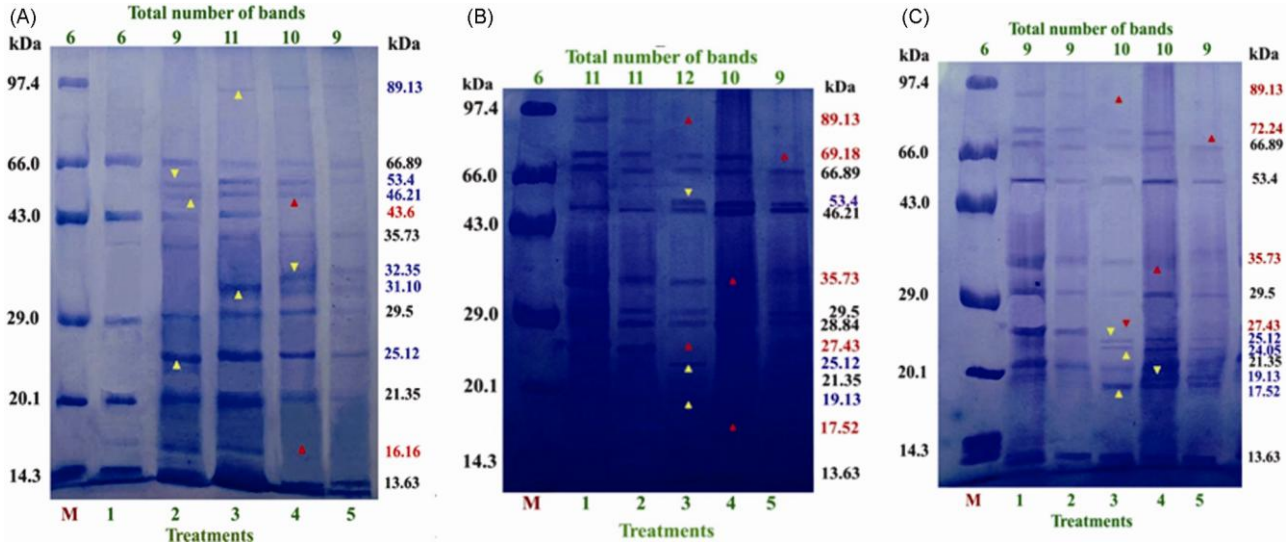

Fig. 2—Effects of boron and salinity on protein profile expression in roots of (A) KRL 35 (Salt tolerant); (B) KRL 210 (Moderately salt tolerant); and (C) HD 2009 (Salt tolerant) through SDS-PAGE (10 %). [Treatments/Groups: (I) Control; (II & III) 50 and 100 ppm B + 60 mM NaCl, respectively; and (IV & V) 50 and 100 ppm B + 100 mM NaCl, respectively]

appeared and 2 polypeptides of 89.13 and 27.43 kDa disappeared. At higher salinity level of 100 mM NaCl, disappearance of 2 (35.73 and 17.52 kDa) and appearance of one (69.18 kDa) polypeptide bands was observed at 50 and 100 ppm B, respectively (Fig. 2B). Depressed protein synthesis and acceleration of its degradation in plants in response to salt stress has been reported by number of workers[22,43,44].

While in HD 2009 (salt sensitive), total number of 9 bands were present in control and are not affected at 50 ppm B + 60 mM NaCl. Disappearnace of two polypeptides of 89.13 and 27.43 kDa MW with appearance of 3 new polypeptide bands of MW 25.12, 24.05 and 17.52 kDa was observed at 100 ppm B + 60 mM NaCl. Such modifications might lead to accumulation or depletion of certain metabolites resulting in an imbalance in the levels of a relatively small set of cellular proteins, which could increase, decrease, appear or disappear under stress. *De novo* synthesis of one new polypeptide of MW 19.13 kDa and disappearance of one polypeptide of 35.73 kDa was observed with increase in salinity (Fig. 2C). With the disappearance of one polypeptide band of 72.24 kDa, total 9 polypeptide bands were found at 100 ppm B + 100 mM NaCl in HD 2009. Disappearance of polypeptide bands may be interpreted as the "turning off" of protein synthetic genetic machinery "genes" in response to B and salt. The bands which were present in the treated seedling might be inherently associated with germination and growth processes[45,46]. Their disappearance might be affecting the functional capabilities of seeds to perform in the stress environment. This confirmed that varietal behaviour to salt (tolerant/sensitive) is dependent on the level and combination of stress involved. Amzallag and Lerner[47] reported 76.3 to 14.6 kDa polypeptide bands were apparently synthesized *de novo* that presumably are critical for plant adaptation to stress condition. Similar results were obtained in tobacco where a 26 kDa polypeptide was found due to combined stress and suggested its possible role in osmoregulation[48]. Bishnoi *et al.*[49], observed disappearance of 54.3 kDa protein in plumule of ICPL 88039, 68.4 kDa in radicle of Manak and 28.1 kDa in radical of ICPL 88039 varieties of pigeonpea under B and salinity stress. They reported that varieties tolerant to salinity are tolerant to B as well. These two stresses, thus seem to be associated with resolution of different polypeptides during the course of the given stress.

Results of our study further adduce support to the earlier findings and revealed the differential behavior of different varieties in response to the combined boron and salt stress. The criterion for a stress polypeptide/protein to be considered as a molecular marker is that it should show differential accumulation pattern with respect to the tolerant and sensitive cultivars. These polypeptides may be utilized for screening tolerant cultivars and further characterization of these proteins might help in identification of the exact genetic domain. Setter *et al.*[50] also reported that there are often single or dominant additive genes involved in tolerance to element toxicities e.g. for boron (B). James *et al.*[51] have also demonstrated differential expression of selected heat stress responsive genes (*hsp101* and *CRT*) in Pearl Millet and also suggested their utilization in improving thermotolerance of other food crops. Similarly, Singh *et al.*[52], have shown allelic diversity for salt stress responsive candidate genes among Indian rice landraces.

The present study reveals that the toxic effects of boron and salinity appear to be more detrimental in the salt sensitive varieties as compared to the salt tolerant varieties. It is more likely that the polypeptides disappearing in response to stresses are a result of denaturation and the timing of the synthesis or break down of particular polypeptides coincides with the protein content during stress, associating their involvement in tolerance process. Identification of differentially regulated polypeptide/proteins can lead to the identification of their corresponding genes involved in the physiology of stress resistance and could be possible to combine tolerance with other desirable traits so as to evolve new crop varieties better adapted to these abiotic stresses. Whether such factors may be involved in genotypic tolerance of wheat and other crops exposed to salinity remains the subject of continued research.

References

1 Krishnamurthy SL, Sharma PC, Sharma SK, Batra V, Kumar V & Rao LVS, Effect of salinity and use of stress indices of morphological and physiological traits at the seedling stage in rice. *Indian J Exp Biol*, 54 (2016) 843.

2 Lakra N, Tomar PC & Mishra SN, Growth response modulation by putrescine in Indian mustard *Brassica juncea* L. under multiple stress. *Indian J Exp Biol*, 54 (2016) 262.

3 Gnanaraj M, Udhayakumar N, Gandhi Rajiv R & Manoharan K, Isolation and gene expression analysis of Phospholipase C in response to abiotic stresses from *Vigna radiata* (L.) Wilczek. *Indian J Exp Biol*, 53 (2015) 335.

4 Sharma SB, Sayyed RZ, Sonawane M, Trivedi MH & Thivakaran GA, Neurospora sp. SR8, a novel phosphate solubiliser from rhizosphere soil of Sorghum in Kachchh, Gujarat, India. *Indian J Exp Biol*, 54 (2016) 644.

5 FAO, Agristat (www.fao.org) 2007.

6 Singh G, Bundela DS, Sethi M, Lal K & Kamra SK, Remote sensing and geographic information system for appraisal of salt-affected soils. *Indian J Environ Qual*, 39 (2010) 5.

7 Munns R & Tester M, Mechanisms of salinity tolerance. *Ann Rev Plant Biol*, 59 (2008) 651.

8 Nable RO, Banuelos GS & Paull JG, Boron toxicity. *Plant and Soil*, 193 (1997) 181.

9 Minhas PS & Samra JS, Quality Assessment of Water Resources in the Indo-Gangetic Basin Part in India, (Central Soil Salinity Research Institute, Karnal, India), 2003, pp. 68.

10 Sharma SK, Kumar A, Lata C, Singh M & Prasad RK, Personnel communication (2013).

11 Ashraf M & Harris PJC, Potential biochemical indicators of salinity tolerance in plants. *Plant Sci*, 166 (2004) 3.

12 Chaves MM, Maroco JP & Pereira JS, Understanding plant responses to drought – from genes to the whole plant. *Funct Plant Biol*, 30 (2003) 239.

13 Ben Ahmed C, Ben Rouina B, Sensoy S, Boukhris M & Ben Abdallah F, Changes in gas exchange, proline accumulation and anti oxidative enzyme activities in three olive cultivars under contrasting water availability regimes. *Environ Exp Bot*, 67 (2009) 345.

14 Hessini K, Martínez JP, Gandour M, Albouchi A, Soltani A & Abdelly C, Effect of water stress on growth, osmotic adjustment, cell wall elasticity and water-use efficiency in *Spartina alterniflora*. *Environ Exp Bot*, 67 (2009) 312.

15 Martin M, Miceli F, Morgan JA, Scalet M & Zerbi G, Synthesis of osmotically active substrates in winter wheat leaves as related to drought resistance of different genotypes. *J Agri Crop Sci*, 171 (1993) 176.

16 Villadsen D, Rung JH & Nielsen TH, Osmotic stress changes carbohydrate partitioning and fructose-2, 6-bisphosphate metabolism in barley leaves. *Func Plant Biol*, 32 (2005) 1033.

17 Bingham FT, Strong JE, Rhoades JD & Keren R, Effects of salinity and varying boron concentrations on boron uptake and growth of wheat. *Plant Soil*, 97 (1987) 345.

18 Holloway RE & Alston AM, The effect of salt and boron on growth of wheat. *Aust J Agri Res*, 43 **(**1992) 987.

19 Grieve CM & Poss JA, Wheat response to interactive effects of boron and salinity. *J Plant Nutr*, 23 (2000) 1217.

20 Elobeidy AA, Fayek MA & Mohmoud RA, Quantifying salt tolerance responses of some banana genotypes *in vitro*. 1st International Conference on Biotechnology Applications for the Arid Regions. (Eds. Shayji YA, Siolhu JS, Saleem M & Guerinik K; Kuwait), 2001, 135.

21 Chandershekhar V, Kar M & Chavan PD, Influence of salt stress on biochemical processes in chickpea. *Plant Soil*, 96 (1986) 439.

22 Lal RK & Bhardwaj SN, Yield-mineral uptake relation in field pea under saline environment. *Ann Agr Res*, 8 (1987) 110.

23 Yemm EW & Willis AJ, The estimation of carbohydrates in plant extracts by anthrone. *Biochem J*, 57 (1954) 508.

24 Bates LS, Waldren RP & Teare ID, Rapid determination of free proline for water stress studies. *Plant Soil*, 39 (1973) 205.

25 Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem*, 7 (1976) 248.

26 Laemmli UK, Cleavage of structural proteins during the assembly of the head of the bacteriophage Ty. *Nature*, 277 (1970) 680.

27 Sharma SK, Kumar A, Setter TL, Singh M, Lata C, Prasad KRK & Kulshrestha N, Boron tolerance in wheat varieties. *Vegetos*, 27 (2014) 322.

28 Lata C, Kumar A, Sharma SK, Setter TL, Singh M, Prasad KRK & Yaduvanshi NPS, Combined effects of excess boron and salinity in wheat varieties differing in tolerance to these stresses. *J Soil Salinity Water Quality*, 7 (2015) 107.

29 Hassan M, The transcriptional response of barley to boron toxicity. *Doctor of Philosophy Thesis*, University of Adelaide (2007).

30 Oyiga BC, Sharma RC, Shen J, Baum M, Ogbonnaya FC, Leon J & Ballvora A, Identification and characterization of salt tolerance of wheat germplasm using a multivariable screening approach. *J Agri Crop Sci*, 202 (2016) 472.

31 Ismail AM, Response of maize and sorghum to excess boron and salinity. *Biol Plant*, 47 (2003/04) 313.

32 Alpaslan M & Gunes A, Interactive effects of born and salinity stress on the growth, membrane permeability and mineral composition of tomato and cucumber plants. *Plant Soil*, 236 (2001) 123.

33 Sheoran IS, Effect of salinity on some important aspects of plant metabolism. *Ph.D. Thesis*, Haryana Agricultural University, Hisar, India (1975).

34 Mehta N & Bharti S, Growth and metabolism of gram seedling under salt stress. *Ind J Plant Physiol*, 26 (1983) 322.

35 Parida A, Das AB & Das P, NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves a true mangrove, *Bruguiera parviflora*, in hydroponic culture. *J Plant Biol*, 45 (2002) 28.

36 Khatar M & Mohammadi HM, Some physiological responses of wheat and bean to soil salinity at low matric suctions. *Int Agrophys*, 31 (2017) 83.

37 Darko E, Gierczik K, Hudák O, Forgó P, Pál M, Türkösi E, Kovács V, Dulai S, Majláth I, Molnár I, Janda T & Molnár-Láng M, Differing metabolic responses to salt stress in wheat-barley addition lines containing different 7H chromosomal fragments. *PLoS ONE*, 12 (2017) e0174170.

38 Annunziata M G, Ciarmiello L F, Woodrow P, Maximova E, Fuggi A & Carillo P, Durum wheat roots adapt to salinity remodeling the cellular content of nitrogen metabolites and sucrose. *Front Plant Sci*, 7 (2016) 2035.

39 Dubey R S & Rani M, Influence of NaCl salinity on growth and metabolic status of proteins and amino acids in rice seedlings. *J Agron Crop Sci*, 162 (1989) 97.

40 Adeyemi OA, Otusanya OO, Oloyede FA & Olabamiji TM, Comparative effects of boron toxicity and deficiency on the growth, chlorophyll, protein and some cations accumulation in *Zea mays* seedlings. *Int J Inno Sci Res*, 17 (2015) 316.

41 Singh NK, Bracken CA, Hasegawa PM, Handa AK, Buckel S, Hermodson MA, Pfankoch F, Regnier FE & Bressan RA, Characterization of osmotin: a thaumatin-like protein associated with osmotic adjustment in plant cells. *Plant Physiol*, 85 (1987) 529.

42 Pareek A, Singla SL & Grover A, Salt responsive proteins/ genes in crop plants. In:. *Strategies for improving salt tolerance in higher plants*. (Eds. Jaiwal PK, Singh RP & Gulati A; Oxford and IBH Publication Co., New Delhi), 1997, pp. 365.

43 Kumar A, Sharma SK, Lata C, Sheokand S & Kulshreshtha N, Combined effect of boron and salt on polypeptide resolutions in wheat varieties differing in their tolerance. *Indian J Agri Sci*, 85 (2015) 106.

44 Win KT & Oo AZ, Salt stress induced changes in protein profiles in two blackgram (*Vigna Mungo* L.) varieties differing salinity tolerance. *Adv Plants Agric Res*, 6 (6) (2017) 00239. http://medcraveonline.com/APAR/APAR-07-00239.pdf. As accessed on 20 April 2017.

45 Rani C, Bishnoi SK, Kumar B, Sheoran IS, Angrish R & Datta K S, Changes in protein profile under sodium chloride and boron toxicity stress in seedlings of two wheat cultivars. *Indian J Plant Physiol*, 12 (2007) 13.

46 Saad-Allah K M, Impact of sea salt stress on growth and some physiological attributes of some soybean (*Glycine max* L.) varieties. *Iran J Plant Physiol*, 6 (2015) 1559.

47 Amzallag GN, Lerner R, Physiological adaptation of plants to environmental stress. In: *Handbook of Plant and Crop Physiology*. (Ed. Pessarakli, M; Marcel Dekker, New York), 1994, 557.

48 Singh NK, Handa AK, Hasegawa PM & Bressan RA, Protein associated with adaptation of cultured tobacco cells to NaCl. *Plant Physiol*, 79 (1985) 126.

49 Bishnoi SK, Kumar B, Rani C, Datta KS, Kumari P, Sheoran IS & Angrish R, Changes in protein profile of pigeonpea varieties in response to NaCl and boron stress. *Biol Plant*, 50 (2006) 135.

50 Setter TL, Waters I, Sharma SK, Singh KN, Kulshreshtha N, Yaduvanshi NPS, Ram PC, Singh BN, Rane J, McDonald G, Khabaz-Saberi H, Biddulph TB, Wilson R, Barclay I, McLean R & Cakir M, Review of wheat improvement for waterlogging tolerance in Australia and India: the importance of anaerobiosis and element toxicities associated with different soils. *Ann Bot*, 137 (2009) 1.

51 James D, Tarafdar A, Biswas K, Sathyavathi TC, Padaria JC & Kumar PA, Development and characterization of a high temperature stress responsive subtractive cDNA library in Pearl Millet *Pennisetum glaucum* (L.) R.Br. *Indian J Exp Biol*, 53 (2015) 543.

52 Singh D, Singh B, Mishra S, Singh AK, Sharma TR & Singh NK, Allelic diversity for salt stress responsive candidate genes among Indian rice landraces. *Indian J Biotechnol*, 15 (2016) 25.