Journal of Crop and Weed, 12(1):139-141(2016)

Variation in peroxidase banding pattern as response to boron deficiency in wheat (*Triticum aestivum* Thell am)

B. DAS, T. BANDOPADHYAY, [1]S. MUKHERJEE, [1]B. PRIYA AND [1]K. K. SARKAR

Tea Research Association, Tocklai, Jorhat, Assam ,[1]Department of Genetics and Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal-741252

Received: 02-06-2015, Revised:10-12-2015, Accepted: 05-05-2016

ABSTRACT

Widely used isozyme had been employed for identification of variations and estimation of genetic diversity within genotypes in a population and also successfully used in various crop improvement programme. Among the markers peroxidase had been reliably helpful in genetic studies and planning appropriate breeding strategies for improvement of many crops including wheat. The present study was conducted for characterization of some boron–deficiency tolerant and susceptible genotypes following PAGE technique. The tolerant genotypes Scholburgk, DBW 14, Halna and boron –deficiency susceptible genotypes PBW 343, WH 736, HD 2733 (Res.) were considered in the present investigation. Peroxidase profile revealed marked differences within tolerant and susceptible genotypes with respect to number of bands, Rm values and intensity of gels. Out of six bands, bands with Rm value 0.83 was absent in two susceptible genotypes WH 736 and HD 2733 and band with Rm value 0.283 was found absent in another susceptible genotypes PBW 343. Other remaining bands were present in all the genotypes irrespective of their tolerance or susceptibility and all the six bands were found present in the tolerant genotypes.

***Keywords*:** Boron deficiency, peroxidase, wheat

The '*terai*' zone of West Bengal with some advantageous agro climatic features like prolonged winter season, bright sunshine hour and high residual moisture content in soil provides opportunity for successful cultivation of wheat. Despite these natural advantages production and productivity as compared to other parts of state were found very low. Constraints concerned with poor productivity had been identified as deficiency of micronutrient, particularly 'boron' in its soil. In some boron-deficient part, boron concentration had been encountered as low as 0.27 ppm causing severe yield reduction in wheat (Chowdhury *et al.,* 2008). According to critical level soil containing extractable boron less than 1.0 mg Kg^{-1} indicated as boron deficient (Reisenauer *et al.,* 1973). Differential response to boron deficiency among wheat genotypes was reported earlier (Wimmer *et al.,* 2005). A wide range of genotypic variation in wheat being responsive to low boron in soil had been identified as Schomburgk, DBW 14 and Halna and such varieties were classified as tolerant (Das *et al.,* 2012) and susceptible PBN 343, WH 736 and HD 2733 considering extent of floral sterility in tolerant and susceptible genotypes under boron deficient soil (Das *et al.,* 2012). In the present investigation polyacrylamide gel electrophoresis technique was employed on isozymes of peroxidase to confirm the variable response among the genotypes due to boron deficiency based on distinct banding pattern shown by different genotypes. Isozyme profiles of peroxidase had been successfully employed as biochemical markers for identification of resilient genotypes against various biotic and abiotic stresses in many crops including wheat (Bakalova *et al.,* 2004).

The six genotypes HD 2733, WH 736, Schomburgk, DBW 14, Halna 3 and PBW 343 as parent 1,2,3,4,5,6 were grown in the *rabi* season of 2014 at the farm of Uttar Banga Viswavidyalaya, Pundibari, Coochbehar, West Bengal. The area was characterized by highly leached acid soil with low level in available boron with estimated concentration of less than 21 ppm. Leaf samples were collected from 28 days old seedlings from these genotypes. The samples were separately crushed in liquid nitrogen and isozymes were extracted with the help of a mixture containing 0.15 M Tris, 0.001 M EDTA, 0.3 PEG, 0.18 M sodium thioglycolate, 0.01 M dithioerythritol, 0.05 mM phenylmethyl sulphonyl fluoride and 12% glycerol (PH 7.91). 100 µl, of the enzyme extract of each of the sample was added to 10 µl of glycerol, 4 µl of bromophenol blue (solution) and mixed thoroughly. Electrophoresis was performed at 50 mA until the dye front reach the bottom of the gel. Incubated gel in 0.6 µl sodium acetate buffer (PH 5.4) containing 0.5 ml O-dianisidine HCl for 30 minutes at room temperature. The gel was transformed to 0.1 and Hydrogen peroxide until visible band developed.

The results showed variations among wheat genotypes with regards to number of bands as well as their locations on the gel (Rm value) and intensity (Table 1, 2 and Fig.1). The differences in band numbers as well

Short Communication

Email: subhrabckv@rediffmail.com

as their relative mobility (Rm) values within these varieties indicated their genetic distinctness at molecular level. The first band (Rm value 0.830) was present in four genotypes *viz*., Schomburgk, DBW 14, Halna and PBW 343 and common in other five genotypes. The 3rd, 4th, 5th and 6th bands with respective Rm values of 0.533, 0.708,0.758 and 0.817 were found in all the genotypes (Chun *et al.,* 1997). Varietal differences in wheat in banding pattern of peroxidase isozymes extracted from flag leaf following polyacrylamide gel electrophoresis were also reported earlier (Chun *et al.*, 1997).

Table 1: Presence and absence of peroxidase bands

Band No.	Lane No.						Rm value
	1	2	3	4	5	6	
1	A	A	P	P	P	P	0.830
2	P	P	P	P	P	A	0.283
3	P	P	P	P	P	P	0.533
4	P	P	P	P	P	P	0.708
5	P	P	P	P	P	P	0.758
6	P	P	P	P	P	P	0.817

Note: P= Presence of band; A= Absence of band

Table 2: Intensities of peroxidase bands

Band No.	Lane No.					
	1	2	3	4	5	6
1	-	-	M	M	L	L
2	L	L	M	M	M	-
3	D	D	D	D	D	L
4	L	M	D	M	L	L
5	M	D	D	D	M	M
6	L	L	M	M	L	L

Note: L= Light intensity; M= Medium intensity; D= Dark intensity

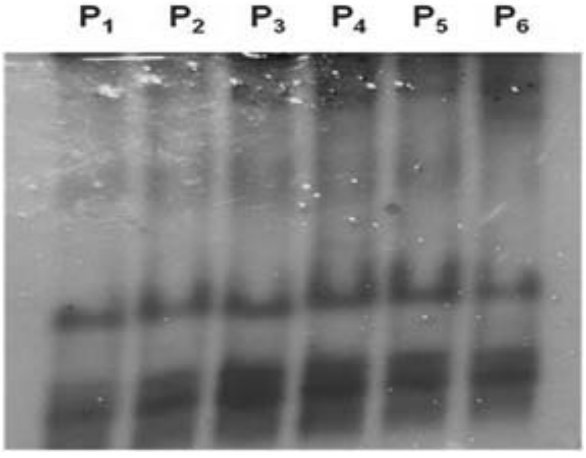

Fig. 1: Presence and absence of peroxidase bands

The intensities of bands were categorized as light, medium and dark and differences in band intensities could indicate differences in peroxidase activities. The first band had medium intensity in P3 and P4 and light intensity in P5 and P6. Second band had light intensity in P1 and P2 and medium intensity in P3, P4 and P5. The third band had dark intensity in all the genotypes except P6. The fourth band had light intensity for P1, P5 and P6, medium intensity in P2 and P4 and dark intensity in P3. The fifth band had dark intensity in P2, P3 and P4, medium intensity in P1, P5 and P6. The sixth band with light intensity was present in all the genotypes except P3 and P4 which exhibited light band. Variations with respect to number and intensity of bands of peroxidase profiles indicated diverse nature of peroxidase activity within the genotypes. Present findings corroborated the observation of (Babeanu and Ciobanu, 2007), (Zhu and Zhang, 2008) and (Hanif and Afshan, 2013). The present study identified on the basis of peroxidase isozymes profiles the boron deficiency tolerant and susceptible genotypes on the basis of induced floret sterility in wheat. The first band showed its presence in tolerant varieties like Schomburgk, DBW 14 and Halna and absent in susceptible varieties *i.e.* WH 736 and HD 2733 whereas second band was found absent- in another susceptible variety, PBW 343. Therefore, it could be suggested that presence of all six bands might be required to make genotypes tolerant against boron deficiency- induced sterility. Johnson *et al*. (2012) distinguished resistant genotypes from susceptible based on presence of two bands of peroxidase profile.

REFERENCES

Babeanu, C. and Ciobanu, G. 2007. Effects of cadmium on isoperoxidase pattern and antioxidant enzyme activities in germinating wheat seeds. *Bull. Univ. Agril. Sci. Vet. Med. Cluj Napoca Agric.*, **63**: 73-76.

Bakalova, S., Nikolova, D. and Nedeva, D. 2004. Isoenzyme profiles of peroxidase, catalase and superoxide dismutase as affected by dehydration stress and ABA during germination of wheat seeds. *Bulgarian J. Pl. Physiol.*, **30**:64-77.

Chowdhury, A.K., Singh G., Tyagi B.S., Bhattacharya P.M. and Singha Roy, A.K. 2008. Assessment of

wheat (*Triticum aestivum*) cultivars to boron deficiency-induced spike sterility and its impact on grain yield under Terai region of West Bengal. *Indian J. Agril Sci.,* **78**: 834-37.

Chun, J.Z., Zhen, L. and Sun, L.Z. 1997. A comparative study on peroxidase isozyme of K-type and V-type male sterile line of common wheat and their maintainer lines. *Acta Agric.*. **12**: 7-11.

Das, B., Mukherjee, S. and Sarkar, K.K. 2012. Heterosis estimation of wheat grown under boron deficient soil Terai region of West Bengal. *J. Crop Weed*, **8**: 22-25.

Hanif, F. and Afshan, S. 2013. Evaluating the response of wheat genotypes to salinity stress. *Asian J. Agril. Sci.,* **5**: 126-29.

Johnson, M., Janakiraman, N. and Irudayaraj, V. 2012. Isozyme analysis on different varieties of Sugarcane. *J. Stress Physiol. Biochem.,* **8**: 22-31.

Reisenauer, H.M., Walsh, L.M. and Hoeft, R.G. 1973. Testing soils for sulphur, boron, molybdenum and chlorine. In. *Soil Testing and Plant Analysis* (Eds.), Soil Science Society of America. Madison, Wisconsin, USA, pp. 173-200.

Wimmer, M.A., Bassil, E.S., Brown P.H. and Lauchli, A. 2005. Boron response in wheat is genotype-dependant and related to B uptake, translocation, allocation, phonological development and growth rate. *Func. Pl. Biol.,* **32**: 507-15.

Zhu, Y. and Zhang, R. 2008. Changes in activities of three defendant enzymes in different wheat cultivars infected by wheat blue dwarf phytoplasma. *J. Northwest A and F Univ. Natur.Sci.Edn.,* **36**:165-69.