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Screening of wheat genotypes for their susceptibility to boron deficiency

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Abstract: Sand culture experiment was conducted in glass house to screen six wheat varieties for their relative susceptibility and tolerance to boron deficiency. Performance of wheat (*Triticum aestivum* L.) genotypes subjected to boron (B) deficiency were evaluated with respect to foliar symptoms of B deficiency, dry matter yield, tissue B concentration, grain yield, total chlorophyll, malondialdehyde (lipid peroxidation) concentration and polyphenol oxidase (DOPA oxidase) activity. Wheat genotypes responded differently to B deficiency. Wheat var. HD- 2874 was found to be least susceptible and var. DL-153-2 and HD-2733 were highly susceptible to B deficiency. The wheat varieties HD-2781and HD-2687 were moderately susceptible and var. HD-2868 was mildly susceptible to B deficiency. Based on their susceptibility to B deficiency the varieties could be arranged in the order DL-153-2>HD-2733>HD-2781>HD-2687>HD-2868>HD-2784.

Key words: Boron deficiency, Screening, Wheat genotypes

Introduction

Boron is an essential micronutrient element required for growth and development of vascular plants (Loomis and Durst, 1992). In plants B deficiency is the most wide spread of micronutrient deficiencies (Blevins and Lukaszewski, 1998). In many countries of the world, B deficiency is commonly found in light sandy coarse textured soils where leaching and heavy cropping have depleted soil B reserves and in soils which have been limed to correct soil acidity (Reisenauer *et al.,* 1973).

Boron requirements may vary among plant species and also among genotypes within a species (Rerkasem and Jamjod, 1997; Kataki *et al.*, 2001). Wheat is amongst the most important edible cereals, and world over wheat demand is estimated to increase approximately 40% by 2020. This will have to come predominantly from productivity increases rather than area expansion (Hakki et al. 2007). Deficiency of B causes large amount of grain yield loss of wheat through male sterility (Rerkasem *et al.*, 1989; Abedin *et al.*, 1994; Jahiruddin *et al.*, 1995; Subedi *et al*., 2001; Ahmed *et al*., 2007). Nachiangmai *et al.* (2002) reported that B efficiency may be dependent on greater ability of the efficient wheat variety 'Fang 60' to accumulate B from growing medium as compared to the inefficient variety 'SW 41'. Jamjod *et al*. (2004) reported genotypic variation among three wheat genotypes as B inefficient 'Bonza', moderately B inefficient 'SW 41', and B efficient 'Fang 60', and observed that the response to B could be accounted by two genes, Bo_d1 and Bo_d2. Ahmed *et al.* (2007) suggested that B deficiency of the wheat could be overcome in two ways either by addition of B fertilizer to soil or crop, or by the use of B efficient wheat varieties , the later being the most cost effective solution. Keeping this in mind the present study aims to screen wheat genotypes for their susceptibility to B deficiency. Earlier parameters like flag leaf B concentration, pollen viability, grain set index, grain yield / plant (Ahmed *et al*., 2007), and solution culture in filter paper (Chantachume *et al.,* 1995) have been used for screening of genotypes to B deficiency.

In the present study the susceptibility of the wheat varieties to B deficiency was determined by making a comparative study of the effect of B deficiency on the appearance of foliar symptoms of deficiency, growth attributes like dry matter yield and reproductive yield, tissue B concentration along with total chlorophyll, malondialdehyde (lipid peroxidation) concentration and polyphenol oxidase (PPO) activity.

Materials and Methods

Plant culture: Seeds of six wheat varieties (*i.e*., HD-2687, HD-2733, HD-2781, HD-2868, HD-2874 and DL-153-2) procured from Indian Agricultural Research Institute (IARI), Pusa, New Delhi, were surface sterilized by soaking in 5 % (v/v) mercuric chloride for 5 min followed by washing with deionised Manesty still water (MSW). Sterile seeds were soaked in MSW at 25^{0}C and after 48 hrs, ten uniform seeds were sown at the same depth of 1.5 cm in polyethylene pots containing 5 kg of purified sand (Sharma 1996). Entire study was conducted in glass house under controlled conditions. The experimental conditions during the experiment were: Light (PAR) ranged between 880 to 1080 µmol $m^{-2}s^{-1}$ at 12.00 noon, relative humidity (RH) ranged between 64-72% at 9.30 AM and maximum and minimum temperature ranged between 18-32 ^{0}C and 7-18 ^{0}C respectively, during the period of experiment. The composition of the nutrient solution was 4 mM Ca $(NO_3)_2$, 4 mM KNO_3, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 1 µM $ZnSO_4$, 0.1 mM Fe-EDTA, 10 µM $MnSO_4$, 1 µM $CuSO_4$, 0.1 µM Na_2MoO_4, 0.1 mM NaCl, 0.1 µM $CoSO_4$ and 0.1 µM $NiSO_4$ with two levels of B supply, deficient (0.066 mg B L^{-1}) and normal (0.33 mg B L^{-1}). The experiment was set up in the completely randomized design with 3 replicates of each treatment. Plants were maintained in culture till maturity (128 days).

Tissue B and chlorophyll: Tissue B was determined colorimetrically by complex formation with Azomethine–H in wet digest (HNO_3, $HClO_4$ 10:1 v/v) of oven dried plant material by method of Wolf (1971). Chloroplastic pigments were extracted in 80% acetone and assayed according to the methodology of Lichtenhaler (1987).

Lipid peroxidation: Lipid peroxidation was determined by the estimation of the malondialdehyde (MDA) content by method of Heath and Packer (1968). Fresh leaf material was homogenized with (0.1% TCA) and centrifuged at 10,000 g for 5 min. The supernatant was treated with 0.5% thiobarbituric acid (TBA) in 20% TCA and the mixture was incubated in boiling water for 30 min. MDA content was estimated spectrophotometrically by reading absorbance at 532 nm and adjusting for nonspecific absorbance at 600 nm by using the extinction coefficient of 155 m mol cm^{-1}.

Polyphenol oxidase: Polyphenol oxidase (PPO**)** was assayed by the method of Shenshi and Noguchi (1975). The enzyme was extracted by grinding 2g of fresh tissue in 10 ml of 0.01M phosphate buffer pH 7.5 containing 0. 2 M KCl, 0.001 M EDTA, 0.01 M sodium ascorbate and 1g of polvinylpyrrolidone. The leaf tissue was ground in a pestle mortar in an ice bath and was centrifuged at 10,000 xg for 20 minutes at 4 °C. The supernatant was used for the enzyme assay. The assay mixture for PPO contained 2 ml of 0.1 M phosphate buffer pH 6.5 and 1 ml of suitably diluted enzyme extract. The reaction was initiated by the addition of 0.5 ml 0.01 M DL-DOPA (3, 4-dihydroxy L-phenylalanine). The reaction was allowed to proceed for 30 min at 30 °C and 2 ml of 0.25 M lead acetate was added to stop the reaction. The contents were centrifuged and optical density of the supernatant was measured at 470 nm wavelenth. Corresponding blanks were run simultaneously.

Statistical analysis: All measurements were made on samples drawn in triplicate and the data were statistically analysed (ANOVA) for significance (LSD at p= 0.05).

Results

Visible symptoms: Within a fortnight of deficient B supply growth depression was observed to varying extent in different varieties. Wheat variety DL-153-2 showed maximum growth depression followed by varieties HD-2733, HD-2781, HD-2687 and HD-2868 respectively. The least growth depression was observed in variety HD-2874. The growth depression became perceptible after 10 days in variety DL-153-2, after 13 days in var. HD-2733 and HD-2781, after 14 days in variety HD-2868 and HD-2687 and after 17 days in variety HD-2874. In general B deficiency symptoms in wheat plants were typified by chlorosis in young leaves followed by development of minute chlorotic specks on the older leaves. The chlorotic specks later developed orange tints which enlarged and coalesced to form large, irregular, bright orange colored areas in the middle of leaves. The deficiency symptoms of B in the leaves appeared earliest, after 14 days of B treatment in wheat varieties DL-153-2, HD-2733 and HD-2781 which showed yellowing of younger leaves. While chlorosis was limited in upper half of leaves in varieties HD-2733 and HD-2781, in var. DL-153-2 entire leaf exhibited very conspicuous uniform yellowing. Besides this decrease in size of leaf lamina was observed in these three varieties. After 30 days deficient plants of var. DL-153-2 showed marked decrease in size of leaves and narrow upright leaves with necrosis at their terminal ends. The symptoms severity was less in variety HD-2687 and HD-2868. The leaves of these varieties showed mild chlorosis, which intensified after 25 days. Plant varieties HD-2868 exhibited chlorosis and inward rolling of leaf apices. In var. HD-2874, after 25 days mild chlorosis of leaves was observed. The onset and relative severity of symptoms have been summarized in Table 1.

Dry matter yield: Dry matter yield decreased in all the six varieties subjected to deficient B supply. The extent of depression in dry matter production due to B deficiency varied in different varieties. Up to 42 days of growth, except variety DL-153-2 and HD-2733, other varieties did not show much difference (>25%) in the dry matter yield. The maximum decrease in the dry matter (54%) due to B deficiency was observed in variety DL-153-2 followed by variety HD-2733 (52%). Least reduction in dry matter of B deficient plants was observed in variety HD-2874 (13%) followed by HD-2868 (17%) and HD-2687 (20%). At 105 days growth, the decrease in dry matter production ranged between 18 to 56%. At this stage decrease in dry matter production as a consequence of B deficiency was 56% in var. DL-153-2 and 51% in var. HD-2733. As compared to control the decrease in dry matter production of B deficient plants was least in varieties HD-2874 (18%) followed by var. HD-2868(26%) and var. HD-2687 (38%) (Table 2).

Tissue boron concentration: Tissue B concentration was determined in tops of all the six wheat varieties subjected to boron deficiency. The concentration of B decreased in all the varieties supplied with deficient B (Table 3). At 42 days the B concentration in tops of normal plants ranged from 8.6 µg B g^{-1} dry wt. (var. DL-153-2) to 11.25 µg B g^{-1} dry wt. (var. HD-2868) and from 4.53 µg B g^{-1} dry wt. (var. DL-153-2) to 7.32 µg B g^{-1} dry wt. (var. HD-2868) in deficient plants respectively. Maximum decrease in tissue B concentration in B deficient plants was found in var. DL-153-2 (47%) followed by HD-2733 (45%) and HD-2781 (43%). The decrease in tissue B was moderate in var. HD-2687 (40%) and HD-2868 (35%), and least in var. HD-2874 (30%).

Chloroplastic pigments: Chlorophyll content decreased in almost all the varieties grown with deficient (0.066 mg B L^{-1}) B supply. The reduction in chlorophyll content was least in var. HD-2874 (24%) followed by var. HD-2868 (26%), HD-2781 (29%), HD-2687 (31%), HD-2733 (49%) and DL-153-2 (55%) respectively. The maximum reduction in chlorophyll was found in variety DL-153-2 (55%). The decrease in chlorophyll a content was more compared to chlorophyll b in all the wheat varieties. The maximum decrease in chlorophyll a content was observed in var. DL-153-2 (59%) and the least decrease in chlorophyll a content was recorded in var. HD-2781 (33%). The least decrease in chlorophyll b content was

Table - 1: Onset and severity of B deficiency symptoms in six wheat (*Triticum aestivum* L.) varieties at 42 days of growth

Variety	Decrease in height (% of control)	Relative severity of the symptoms observed			Overall susceptibility
		Chlorosis in leaves	Decrease in leaf lamina	Development of necrotic lesions on younger leaves and stem	
HD-2687	21	++	+	+	M
HD-2733	34	+++	+++	++++	H
HD-2781	31	+++	++	++	M
HD-2874	11	+	+	-	LS
HD-2868	16	++	++	+	LS
DL-153-2	36	++++	++++	++++	H

H=High, M=Moderate, L=Low; ++++ =Very severe, +++ =Severe, ++ =Moderate, + =Mild

Table - 2: Effect of B deficiency on the dry matter yield of six varieties of wheat (*Triticum aestivum* L.)

Variety	Days of growth	Plant part	B supply: mg B L^{-1}	
			0.066	0.33
			g dry matter $plant^{-1}$	
HD-2687	42	Tops	0.386	0.436
	115	Top	3.294	4.228
		Root	0.281	0.362
		Whole plant	3.575	4.590
HD-2733	42	Tops	0.228	0.476
	115	Top	2.138	4.382
		Root	0.207	0.349
		Whole plant	2.345	4.731
HD-2781	42	Tops	0.365	0.486
	115	Top	3.193	4.492
		Root	0.351	0.429
		Whole plant	3.444	4.921
HD-2874	42	Tops	0.396	0.453
	115	Top	3.296	3.975
		Root	0.258	0.357
		Whole plant	3.554	4.332
HD-2868	42	Tops	0.348	0.485
	115	Top	3.501	4.871
		Root	0.389	0.475
		Whole plant	3.95	5.346
DL-153-2	42	Tops	0.244	0.512
	115	Top	2.015	4.722
		Root	0.269	0.473
		Whole plant	2.284	5.195

LSD (p=0.05) for tops at 42 days is 0.024 and for tops, roots and whole plant at 115 days is 0.019, 0.016 and 0.019 respectively

in variety HD-2874 (16%) and maximum decrease in chlorophyll b content was in var. HD-2733 (63%). Like the chlorophyll, carotenoid content also decreased in all the wheat varieties subjected to deficient B supply. The least reduction was in variety HD-2874 (20%) followed by HD-2868 (23%). The maximum decrease in carotenoids was noticed in var. DL-153-2 (68%) (Table 4).

Lipid peroxidation: Malondialdehyde concentration was measured at 42 days of growth in order to estimate the extent of lipid peroxidation. All the wheat varieties showed increase in lipid peroxidation under deficient B supply to a varying extent (Table 5). The highest accumulation of MDA was found in var. DL-153-2 (118%) followed by var. HD-2733 (47%) and var. HD-2687(41%). Wheat var. HD-2781 showing severe B deficiency symptoms exhibited lowest accumulation of MDA (15%) followed by HD-2868 (17%) and HD-2874 (29%).

Table - 3: Effect of B deficiency on the tissue boron concentration and content in tops of six varieties of wheat (*Triticum aestivum* L.*)* at 42 days of growth

Variety	Boron supply: mg B L^{-1}	
	0.066	0.33
	Boron concentration: m g g^{-1} dry weight	
HD-2687	5.88	9.83
HD-2733	5.33	9.55
HD-2781	6.12	10.65
HD-2874	5.66	8.15
HD-2868	7.32	11.25
DL-153-2	4.53	8.62

LSD (p=0.05) for boron concentration is 0.21

Table - 4: Effect of deficient B supply on the chloroplastic pigments of six wheat (*Triticum aestivum* L.) varieties at 42 days of growth

Variety	Boron supply: mg B L^{-1}	Chlorophyll			Carotenoids
		a	b	Total	
HD-2687	0.066	1.492	0.375	1.867	0.054
	0.33	2.101	0.604	2.705	0.077
HD-2733	0.066	1.092	0.215	1.307	0.048
	0.33	1.991	0.576	2.567	0.079
HD-2781	0.066	1.492	0.382	1.874	0.063
	0.33	1.922	0.726	2.648	0.082
HD-2874	0.066	1.608	0.713	2.321	0.068
	0.33	2.225	0.84	3.065	0.085
HD-2868	0.066	1.654	0.431	2.085	0.056
	0.33	2.19	0.609	2.805	0.072
DL-153-2	0.066	0.66	0.414	1.074	0.041
	0.33	1.279	1.066	2.345	0.131

LSD (p=0.05) for chl a is 0.201; chl b is 0.112; total chl is 1.011 and carotenoids is 0.011

Table - 5: Effect of B deficiency on the malondialdehyde concentration and activity of polyphenol oxidase in the leaves of six varieties of wheat (*Triticum aestivum* L.) at 42 days of growth

Variety	Boron supply: mg B L^{-1}	MDA: m mole 100 mg^{-1} FW	PPO: activity 100 mg^{-1} FW
HD-2687	0.066	8.52	0.041
	0.33	6.06	0.027
HD-2733	0.066	8.62	0.016
	0.33	5.88	0.009
HD-2781	0.066	5.43	0.024
	0.33	4.73	0.013
HD-2874	0.066	6.54	0.011
	0.33	5.06	0.010
HD-2868	0.066	8.2	0.011
	0.33	7.0	0.009
DL-153-2	0.066	16.6	0.023
	0.33	7.6	0.011

LSD (P=0.05) for MDA is 1.28 and PPO is 0.003

Table - 6: Effect of B deficiency on reproductive yield of six varieties of wheat (*Triticum aestivum* L.)

Variety	Boron supply: mg B L^{-1}	No. of spikelets $plant^{-1}$	No. of grains $plant^{-1}$	Wt. of 100 grains (g)	Seed wt. $plant^{-1}$
HD-2687	0.066	20	172	2.29	3.94
	0.33	24	228	2.89	6.58
HD-2733	0.066	19	171	2.25	3.85
	0.33	26	312	3.01	9.39
HD-2781	0.066	15	125	2.25	4.03
	0.33	24	268	3.34	8.95
HD-2874	0.066	16	140	2.44	5.02
	0.33	21	252	2.73	6.85
HD-2868	0.066	16	141	2.55	4.39
	0.33	20	220	2.98	6.56
DL-153-2	0.066	20	152	2.06	3.13
	0.33	24	288	3.29	9.48

LSD (p=0.05) for spikelets is 1.2, No. of grains $plant^{-1}$ is 2.8, for 100 grain wt. is 0.17and for grain wt. $plant^{-1}$ is 0.32

Polyphenol oxidase/DOPA oxidase: The activity of PPO increased in all the wheat varieties under deficient B supply (Table 5). The increase was highest in var. DL-153-2 (109%) followed by var. HD-2781 (84%) and var. HD-2733 (77%). The lowest increase was found in var. HD-2874 (10%) followed by var. HD-2868 (22%).

Reproductive yield: The reproductive yield decreased in all the wheat varieties subjected with deficient B supply (Table 6). The emergence and size of inflorescence and grain filling were inhibited by B deficiency in all varieties. The B deficient plants of var. DL-153-2 were smaller and bore very fewer spikelets and the grain formation was almost completely inhibited. Boron deficient plants of var. HD-2733 also contained very few and shriveled grains. The decrease in seed weight ranged between 36 to 67% in the different varieties. As in the case of vegetative yield the reproductive yield showed maximum decrease in var. DL-153-2 (67%) followed by var. HD-2733 (59%). Least decrease in reproductive yield was found in var. HD-2874 (36%).

Discussion

The B deficiency symptoms like chlorosis in young leaves followed by development of minute chlorotic specks on older leaves is in accordance with earlier reports (Agarwala and Sharma ,1979; Jamjod *et al*., 2004; Sharma, 2006) and has also been used as a criterion for screening wheat varieties (Agarwala and Sharma, 1979; Ahmed *et al*., 2007). Comparison of B deficiency symptoms (Table 1) categorizes variety DL-153-2 as most susceptible followed by varieties HD-2733 and HD-2781, varieties HD-2687 and HD-2868 as moderately to mildly susceptible and var. HD-2874 as least susceptible. A similar trend was also observed by the decrease in dry matter yield in the different wheat varieties. Wheat var. DL-153-2 showing most prominent B deficiency effects had lowest dry matter yield and var. HD-2874 which showed very mild B deficiency effects showed least decrease in dry matter yield. The decrease in dry matter yield in all the six wheat varieties under B deficiency was related to the visual deficiency symptoms and decrease in plant growth and its use for screening of wheat varieties is in accordance with the earlier reports in wheat by Bell (1997) and Rerkasem *et al.* (1993).

The wheat varieties studied here also exhibited decrease in tissue B concentration which was in consonance with the appearance of visible symptoms of deficiency. The variable decrease in tissue B concentration of wheat varieties coincides with the findings of Bellaloui and Brown (1998) who reported differences in sensitivity to B deficiency among cultivars and species as a consequence of either reduced B uptake, restriction in B translocation from roots to shoots or a combination of both processes. This was evident in varieties DL-153-2 and HD-2733 which showed 49 and 45% decrease in tissue B over control and exhibited severe symptoms of B deficiency while var. HD-2874 with lowest decrease in tissue B showed mild visible symptoms of B deficiency.

The decrease in chlorophyll and carotenoid concentration under B deficiency has earlier been reported by Pandev *et al.* (1981). The decrease in chlorophyll concentration may be due to B deficiency induced changes in chloroplast structure. Although the chlorophyll and carotenoid concentration decreased upto 54 and 71% respectivly in B deficient plants, but the levels did not coincide with the deficiency effects in the different varieties.

The lipid peroxidation, measured as MDA concentration is a good indicator of oxidative damage of the cell membrane (Mitter, 2002). Keeping in view the role of B on membrane stability we studied whether lipid peroxidation was correlated to B deficiency. Although an increase in MDA concentration under B deficiency was observed in all the six varieties but the increase was not reflected in the severity of deficiency effects of B in the different

varieties. Thus while in var. HD-2781 showing severe B deficiency effects the MDA concentration increased only by 15%, in var. HD-2874 which exhibited very mild deficiency symptoms, the MDA concentration was increased to 29% of control values.

Plant species with different sensitivity to B deficiency have been shown to differ in levels of PPO activity under B deficiency (Shkolnik, 1984). The enhanced PPO activity in leaves subjected to B deficiency is in accord with findings of Costeng and Lee (1987), Macheix *et al.* (1991) and Cakmak and Romheld (1997). The increase in activity of PPO followed the severity of deficiency symptoms being maximum in var. DL-153-2 (110%) while minimum activity was observed in var. HD-2874 (10%) which showed least B deficiency symptoms.

The marked decrease in reproductive yield as observed in present study has earlier been reported (Mozafar, 1993; Rawson ,1996a, b; Rerkasem and Jamjod, 1997; Huang *et al*., 2001; Ahmed *et al*., 2007). Earlier, screening of wheat genotypes at low B supply by Subedi *et al.* (1993) and Rerkasm *et al.* (1993) had shown responses to range from complete male sterility leading to no grain set in extremely inefficient genotypes to those moderately inefficient with mild symptoms and low grain yield to efficient genotypes which showed no deficiency symptoms. In our study also we observed that grain yield of the six varieties due to low B supply was significantly affected. Least decrease in grain yield (27% of control) was observed in var. HD-2874 showing very mild visible B deficiency effects while DL-153-2 exhibiting severe symptoms had profusely low grain yields (67%). The other varieties showed intermediate decreases in grain yield being 33% in HD-2868, 41% in HD-2687, 55% in HD-2781 and 59% in HD-2733.

From our study we conclude that symptom severity, tissue B concentration, grain yield as well as activity of PPO may be used as criteria for screening wheat varieties for B deficiency separately or in a combination. Although chloroplastic pigments and lipid peroxidation were also affected by B deficiency but they were not found suitable for screening of varieties. Based on the above criteria wheat var. DL-153-2 was found to be most susceptible and var. HD-2874 as least susceptible to B deficiency. Wheat variety HD-2733 was highly susceptible to B deficiency, where as var. HD-2781 and var. HD-2687 were moderately susceptible, and var. HD-2868 was mildly susceptible to B deficiency.

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