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Effects of boron on biochemical parameters of wheat (*Triticum aestivum* L.)

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Abstract

Micronutrient deficiency is one of the major constraints to crop productivity. Agronomic interventions like nutrient seed priming (NSP) can significantly improve crop establishment in micronutrient deficient soils. However, the success of this technique will depend on the efficacy of the priming procedures. Hence the laboratory experiment was carried out to estimate the effect of different boron concentrations like 0 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm on wheat var. HUW-234 vs HUW-468 where, seeds were placed in petri plates with boron treated blotting papers and kept in growth chamber at 25°C for germination to take place. Amount of soluble sugar, starch and free amino acid content in shoot and root were found to be highest in T_0 (0 ppm or Control) in both the varieties. HUW-468 showed better results than HUW-234 in context to soluble sugar, starch and free amino acid content in both shoot and root. Whereas, proline and malondialdehyde is highest in T_4 (200 ppm) in both the varieties in both shoot and root. HUW-468 showed highest proline and malondialdehyde content for both shoot and root indicating that the variety is more tolerant to high salt toxicity than HUW-234.

Keywords: boron, wheat, *Triticum aestivum* L., soluble sugar, Starch, Free amino acid, proline, malondialdehyde

Introduction

Higher germination rate and vigorous seedlings are the key requirements for the success of stand and establishment of any crop plant. Many seed technologies such as seed enhancement are applied to improve germination percent and emergence of seedlings in many crops (Arif *et al*. 2008, Taylor *et al.* 1998) [3, 45]. Seed priming is a technique in which seeds are soaked in low water potential solutions to initiate pre-germinative metabolic activity but prevents radical protrusion (Bradford 1986, McDonald 2000, Ashraf and Foolad 2005, Farooq *et al.* 2006, Janmohammadi *et al.* 2008) [8, 33, 4, 16, 25]. But, the beneficial impact of seed priming has been well explained in many research-based data including early germination; improved germination rate (Dahal *et al.* 1990, Jett *et al.* 1996, Bradford 1986, Taylor and Harman 1990, Ghassemi-Golezani *et al.* 2008) [13, 27, 8, 46, 18], breaking of dormancy (Cantliffe *et al.* 1984, Wurr and Fellows 1984) [10, 47], vigorous seedlings (Harris 1996, Saber *et al.* 2012) [22, 43], better establishment (Khan *et al.* 1992, Jett *et al.* 1995, Arif *et al.* 2005, Ali *et al.* 2007, Diniz *et al.* 2009) [29, 26, 2, 1, 14] and overall increased yield (Rengel and Graham 1995a, 1995b, Yilmaz *et al.* 1998) [40, 41, 49]. Furthermore, it reduces leakage of the vital metabolites, repairs the damaged seed parts, improves RNA and protein synthesis (McDonald 2000) [33].

Seed enhancement is a novel technology that focuses on the development of macro or micronutrient enriched seeds (Rehman *et al.* 2012, Mirshekari 2012) [39, 35]. On a general basis, the nutrients are delivered to plants as soil applications, fertigation or foliar spray (Johnson *et al*. 2005, Robert 2008) [28, 42]. Application of these nutrients in the form of seed treatment, seed coating and seed priming, is another choice which eliminates many issues (Farooq *et al.* 2012) [39]. The utilization of the micronutrient-enriched seeds has been found as a better technique to overcome micronutrient deficiency (Musakhandov 1984, Harris *et al.* 1999) [36, 23]. Priming of the seeds with micronutrients enables them to rapidly imbibe water and revive metabolism and germination. This further results in a higher germination percent, better establishment, increased biotic and abiotic tolerance, and ultimately higher yields (Harris *et al.* 1999) [23].

Boron is a common micronutrient required by all the vascular plants. It is absorbed from the soil by plants in the form of borate. Since boron is not mobile in plants, hence the continuous supply from the soil or any other medium is a must in all the plant meristems. Deviation from the optimum boron concentration causes severe decrease of grain yield, which is because of the disruption of the metabolism involving boron (Brown *et al.* 2002) [9]. Boron has an important role in the formation of proteins, nitrogen metabolism, cell division, cell membrane integrity, cell wall formation, nucleic acids, and antioxidative systems (Parr and Loughman 1983, Gupta *et al*. 1985, Goldbach and Wimmer 2007, Bonilla *et al.* 2009, Koshiba *et al.* 2009) [38, 21, 19, 7, 31]. It also plays a significant role in the process of transpiration for the movement of potassium element to the stomata of the leaves. Boron also helps in maintaining a stable balance between sugars and starches, pollination and seed production (Gupta *et al.* 1985) [21].

Wheat is the major cereal crop of the world. Increased grain yield along with improved quality are of great significance for the today's increasing human population. As there is scope for the improvement of the germination percent, vigour of the seedlings and other biochemical process of germinating seedlings, the current investigation was performed to study the impact of various concentrations of boron element on the physiological as well as biochemical processes in wheat.

Materials and Methods

The present experiment was conducted in Laboratory of Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi in the *Rabi* season 2020. Wheat seeds of the varieties HUW-234 and HUW-468 were procured from the Department of Genetics and Plant Breeding of the same institute. Healthy, disease free and vigorous seeds of both the varieties were first washed with distilled water and kept in the middle of the sterilised Petri plates with blotting papers coated with different concentration of boron and all the Petri plates containing 4 treatments and control along with their five replications were placed in the growth chamber at a temperature of 25°C for germination. Various treatments were T_0, T_1, T_2, T_3 and T_4 representing different concentrations of boron as 0 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm respectively.

Biochemical parameters like soluble sugar content, starch content, free amino acid content, proline and malondialdehyde content were analysed in shoot and root at 3rd, 5th, 7th, 9th and 11th days of crop seed germination in each replication after 2 days of treatment. Soluble sugar content (mg glucose/ g), starch content (µg/g) in fresh shoot and root were determined by anthrone method given by Dubois *et al*. (1956). The free amino acids were determined through ninhydrin reagent method (Yemm and Cocking 1955) [48]. The proline content in the shoots and roots was estimated by the technique given by Bates *et al*. (1973) [5]. Melondialdehyde content in the samples was estimated by the method developed by Heath and Packer (1968) [24].

Data obtained from various observations at different stages of growth of wheat (*Triticum aestivum* L.) were subjected to statistical analysis by adopting the completely randomized block design (CRD) by Panse and Sukhatme (1967) [37].

Results and Discussion

Soluble sugar content (mg/g)

The shoot and root samples collected on 3rd, 5th, 7th, 9th and 11th days after treating different concentrations boron in each replication were analysed for soluble sugar content (Tables 1 and 2). The soluble sugar content ranged from 2.69 to 10.24 in var. HUW-234 and 4.12 to 10.23 in var. HUW- 468. Whereas in roots 1.35 to 3.85 in var. HUW- 234 and 2.48 to 5.87 in var. HUW-468. The soluble sugar content increased with increase in age of the seedlings and maximum in control and minimum in T_4 treatment. Maximum soluble sugar content in shoot is attributed to maximum photosynthesis and maximum translocation of the solutes compared to roots. Chakraborty and Bose (2020) [12] reported that soluble sugar content increases rapidly from 3rd day to 7th day which supports the present findings whereas soluble sugar content is high in boron (8 mM) treated seedlings which in contrast with our findings.

Table 1: Soluble sugar content in the shoots of wheat varieties HUW-234 and HUW-468 (*Triticum aestivum* L.) at different stages of growth.

Days after treatment	Huw-234					Huw-468				
	0 PPM	50 PPM	100 PPM	150 PPM	200 PPM	0 PPM	50 PPM	100 PPM	150 PPM	200 PPM
3	5.43	4.32	3.84	3.25	2.69	7.24	6.58	5.86	4.95	4.12
5	7.12	6.56	5.48	4.57	3.47	8.45	7.56	6.46	5.84	4.75
7	8.35	7.45	6.32	6.24	5.13	8.99	8.12	7.54	6.47	5.27
9	9.10	7.15	7.02	6.88	6.35	9.43	8.75	7.46	7.12	6.75
11	10.24	9.46	8.49	7.36	6.88	10.23	9.45	8.76	8.14	7.82
CD at 5%	2.483	2.477	2.324	2.294	2.418	1.501	1.476	1.493	1.630	2.030
SE (m)±	0.828	0.826	0.775	0.765	0.806	0.501	0.492	0.498	0.544	0.677
C.V.	23.010	26.437	27.818	30.231	36.768	12.621	13.604	15.430	18.693	26.372

Table 2: Soluble sugar content in the roots of wheat varieties HUW-234 and HUW-468 (*Triticum aestivum* L.) at different stages of growth.

Days after treatment	HUW-234					HUW-468				
	0 PPM	50 PPM	100 PPM	150 PPM	200 PPM	0 PPM	50 PPM	100 PPM	150 PPM	200 PPM
3	2.52	2.19	1.85	1.57	1.35	3.96	3.43	3.04	2.76	2.57
5	2.85	2.46	2.13	1.85	1.47	4.48	4.02	3.55	3.08	2.48
7	3.19	2.85	2.56	2.14	1.85	5.12	4.75	3.76	3.13	2.76
9	3.46	3.04	2.82	2.39	2.04	5.46	5.04	4.49	3.84	3.46
11	3.85	3.43	3.12	2.58	2.32	5.87	5.15	4.85	4.48	4.02
CD at 5%	0.694	0.652	0.687	0.544	0.537	1.024	0.983	0.977	0.931	0.886
SE (m)±	0.231	0.217	0.229	0.182	0.179	0.342	0.328	0.326	0.310	0.296
C.V.	16.308	17.392	20.524	19.278	22.178	15.340	16.370	18.509	20.077	21.618

Starch content (µg/g)

There is a significant difference between treatments *i.e.*, different concentration of boron (Tables 3 and 4). Starch content in shoot and root decreased progressively with advancement in plant growth. The starch content ranged from 0.45 to 3.31 in var. HUW-234 and 2.36 to 3.56 in var. HUW- 468 in shoots. Whereas, in roots it ranged from 0.11 to 1.12 in var. HUW-234 and 0.44 to 1.48 in var. HUW- 468. The maximum starch content in both shoot and root was observed in plants under T_0 treatment followed by T_1, T_2, T_3 and T_4 treatments at 3rd day indicating the highest photosynthetic rate at this stage compared to other. Lower concentrations of boron has been found to activate key enzymes that includes phosphorylase, amylase etc which are involved in the process of metabolism of starch. Higher concentrations of boron can be toxic according to Bonilla *et al*. (2004) [6].

Table 3: Starch content in the shoots of wheat varieties HUW-234 and HUW-468 (*Triticum aestivum* L.) at different stages of growth.

Days after treatment	**HUW-234**					**HUW-468**				
	0 PPM	**50 PPM**	**100 PPM**	**150 PPM**	**200 PPM**	**0 PPM**	**50 PPM**	**100 PPM**	**150 PPM**	**200 PPM**
3	3.31	2.03	1.82	1.58	1.43	3.56	3.42	3.28	3.02	2.73
5	2.73	2.06	1.88	1.66	1.45	3.35	3.23	3.11	2.92	2.75
7	1.95	1.78	1.56	1.47	1.28	3.22	3.10	3.04	2.96	2.45
9	1.86	1.59	1.45	1.42	1.35	3.16	2.94	2.65	2.58	2.36
11	1.12	1.93	1.08	0.83	0.45	3.05	3.14	2.96	2.76	2.55
CD at 5%	1.133	0.261	0.431	0.440	0.563	0.263	0.237	0.311	0.239	0.229
SE (m)±	0.378	0.087	0.144	0.147	0.188	0.088	0.079	0.104	0.080	0.076
C.V.	38.522	10.362	20.611	23.551	35.257	5.994	5.577	7.723	6.254	6.656

Table 4: Starch content in the roots of wheat varieties HUW-234 and HUW-468 (*Triticum aestivum* L.) at different stages of growth.

Days after treatment	**HUW-234**					**HUW-468**				
	0 PPM	**50 PPM**	**100 PPM**	**150 PPM**	**200 PPM**	**0 PPM**	**50 PPM**	**100 PPM**	**150 PPM**	**200 PPM**
3	1.12	1.03	0.86	0.65	0.57	1.48	1.27	1.12	0.97	0.81
5	1.04	0.95	0.76	0.58	0.46	1.29	1.23	1.08	0.88	0.72
7	0.92	0.79	0.65	0.55	0.33	1.15	1.02	0.88	0.71	0.63
9	0.78	0.61	0.54	0.46	0.24	1.04	0.95	0.76	0.62	0.48
11	0.65	0.45	0.41	0.23	0.11	0.92	0.88	0.64	0.67	0.44
CD at 5%	0.256	0.320	0.238	0.218	0.242	0.292	0.231	0.275	0.199	0.210
SE (m)±	0.085	0.107	0.079	0.073	0.081	0.097	0.077	0.092	0.066	0.070
C.V.	21.128	31.190	27.529	32.910	52.769	18.539	16.093	22.894	19.285	25.425

Free amino acid content (mg/g)

The significant difference was observed between treatments at all days of observation *i.e.*, at 3, 5, 7, 9 and 11 days (Tables 5 and 6). The free amino acid in both shoot and root increased continuously as per age of wheat seedlings. The amount of free amino acids in shoots had a range from 1.50 to 3.10 in var. HUW-234 and 1.62 to 3.45 in var. HUW- 468. Whereas, in roots 0.72 to 2.28 in var. HUW-234 and 1.04 to 2.97 in var. HUW- 468. The free amino acid content in seedling was found the highest in the Control, followed by T_1, T_2, T_3 and T_4 treatments during all days of observation. Maximum free amino acid content was found in shoot compared root of the wheat seedling indicating effect of stress resistance to adverse conditions and enzyme synthesis by DNA and mRNA will be maximum. Similar results were found by Khatun *et al*. (2013) [30].

Table 5: Free amino acid content in the shoots of wheat varieties HUW-234 and HUW-468 (*Triticum aestivum* L.) at different stages of growth.

Days after treatment	**HUW-234**					**HUW-468**				
	0 PPM	**50 PPM**	**100 PPM**	**150 PPM**	**200 PPM**	**0 PPM**	**50 PPM**	**100 PPM**	**150 PPM**	**200 PPM**
3	2.21	2.06	1.86	1.74	1.71	2.83	2.66	1.83	1.75	1.64
5	2.45	2.14	1.93	1.76	1.50	2.49	2.23	1.96	1.71	1.66
7	2.63	2.43	2.04	1.84	1.55	2.79	2.56	2.10	1.79	1.62
9	2.95	2.78	2.43	2.09	1.87	3.10	2.86	2.59	2.15	2.03
11	3.10	2.82	2.48	2.11	2.04	3.45	2.99	2.85	2.56	2.44
CD at 5%	0.486	0.471	0.386	0.240	0.301	0.485	0.393	0.583	0.486	0.479
SE (m)±	0.162	0.157	0.129	0.080	0.100	0.162	0.131	0.195	0.162	0.160
C.V.	13.577	14.372	13.410	9.400	12.938	12.327	11.022	19.202	18.213	19.007

Table 6: Free amino acid content in the roots wheat varieties HUW-234 and HUW-468 (*Triticum aestivum* L.) at different stages of growth.

Days after treatment	**HUW-234**					**HUW-468**				
	0 PPM	**50 PPM**	**100 PPM**	**150 PPM**	**200 PPM**	**0 PPM**	**50 PPM**	**100 PPM**	**150 PPM**	**200 PPM**
3	1.45	1.23	1.02	0.85	0.72	1.98	1.47	1.27	1.15	1.04
5	1.65	1.48	1.23	1.19	1.01	2.16	1.95	1.78	1.51	1.24
7	1.82	1.76	1.49	1.28	1.13	2.38	2.13	1.95	1.74	1.48
9	2.10	1.95	1.76	1.45	1.28	2.56	2.34	2.12	1.96	1.75
11	2.28	2.05	1.88	1.72	1.39	2.97	2.69	2.47	2.28	2.07
CD at 5%	0.449	0.454	0.480	0.431	0.348	0.512	0.609	0.594	0.577	0.547
SE (m)±	0.150	0.151	0.160	0.144	0.116	0.171	0.203	0.198	0.192	0.182
C.V.	17.989	19.969	24.252	24.782	23.479	15.860	21.459	23.104	24.896	26.910

Proline content (µg/g)

The proline content of wheat seedlings was estimated at 3, 5, 7, 9 and 11 days in both the wheat varieties (Tables 7 and 8). The maximum proline content was found to be highest in T_4 followed by T_3, T_2, T_1 and T_0. The proline content increased with increase in age of the seedlings in both shoot and root. Proline found to be synthesised more during salt toxicity indicating the resistance of the variety to salt stress. The variety HUW-468 said to be more salt tolerant compared to var. HUW-234. Higher content of proline in roots compared to shoots, help roots to go deeper into the soil and improves the osmotic regulation of salts in and out of the cell biological membranes in roots. The range of lower or higher levels of boron concentrations is very narrow in plants (Çelik *et al*. 1998) [11] and varies from crop to crop.

Table 7: Proline content in the shoots of wheat varieties HUW-234 and HUW-468 (*Triticum aestivum* L.) at different stages of growth.

Days after treatment	HUW-234					HUW-468				
	0 PPM	50 PPM	100 PPM	150 PPM	200 PPM	0 PPM	50 PPM	100 PPM	150 PPM	200 PPM
3	3.55	3.78	3.95	4.19	4.38	3.94	4.13	4.37	4.63	4.94
5	3.92	4.17	4.42	4.66	4.92	4.35	4.52	4.71	4.91	5.14
7	4.37	4.63	4.85	4.97	5.16	5.80	6.12	6.24	6.49	6.74
9	5.21	5.43	5.67	5.85	6.05	6.22	6.43	6.71	6.72	6.94
11	5.93	6.10	6.29	6.34	6.52	6.96	7.13	7.54	7.53	7.71
CD at 5%	1.300	1.263	1.266	1.178	1.162	1.707	1.723	1.801	1.662	1.613
SE (m)±	0.434	0.421	0.422	0.393	0.388	0.569	0.575	0.601	0.554	0.538
C.V.	21.092	19.539	18.754	16.887	16.033	23.347	22.681	22.713	20.473	19.109

Table 8: Proline content in the roots of wheat varieties HUW-234 and HUW-468 (*Triticum aestivum* L.) at different stages of growth.

Days after treatment	HUW-234					HUW-468				
	0 PPM	50 PPM	100 PPM	150 PPM	200 PPM	0 PPM	50 PPM	100 PPM	150 PPM	200 PPM
3	1.12	1.22	1.36	1.52	1.64	1.46	1.53	1.59	1.68	1.96
5	1.43	1.57	1.66	1.78	1.84	1.75	1.83	1.93	2.03	2.16
7	1.75	1.93	2.17	2.19	2.27	1.94	2.04	2.24	2.37	2.76
9	2.10	2.25	2.29	2.41	2.59	2.13	2.24	2.46	2.65	2.84
11	2.38	2.46	2.57	2.74	2.93	2.31	2.37	2.42	2.76	2.93
CD at 5%	0.677	0.673	0.658	0.653	0.709	0.443	0.448	0.491	0.598	0.589
SE (m)±	0.226	0.224	0.219	0.218	0.236	0.148	0.149	0.164	0.200	0.196
C.V.	28.736	26.608	24.406	22.885	23.461	17.235	16.677	17.213	19.416	17.351

Malondialdehyde (MDA) content (µmol/g)

The samples collected on 3rd, 5th, 7th, 9th and 11th days after treating different concentrations boron in each replication were analysed for Malondialdehyde content (Tables 9 and 10). The Malondialdehyde content ranged from 4.12 to 9.84 in var. HUW-234 and 5.37 to 9.85 in var. HUW- 468. Whereas in roots 7.15 to 12.05 in var. HUW-234 and 9.03 to 12.94 in var. HUW- 468. The MDA content increased with increase in age of the seedlings and found to be maximum in T_4 treatment indicating that its production increases with salt stress due to boron toxicity. Hence the var. HUW- 468 found salt tolerant compared to HUW-234. Similar results were recorded on malondialdehyde content, so that the present study is supported by previous findings of Gunes *et al*. (2007). It is found that salinity stress could significantly reduce the net photosynthesis rates, higher energy losses due to salt exclusion, lower nutrient mobilization, affecting the cell division and enlargement and hence overall decreased plant growth (Meiri and Poljakoff-Mayber 1970, Long and Baker 1986, Seeman and Sharkey 1986) [34, 44].

Table 9: Malondialdehyde (MDA) content in the shoots of wheat varieties HUW-234 and HUW-468 (*Triticum aestivum* L.) at different stages of growth.

Days after treatment	HUW-234					HUW-468				
	0 PPM	50 PPM	100 PPM	150 PPM	200 PPM	0 PPM	50 PPM	100 PPM	150 PPM	200 PPM
3	4.12	5.34	7.16	8.37	8.95	5.37	6.43	7.15	7.96	8.31
5	4.53	5.57	5.88	6.41	6.86	6.05	6.87	7.45	7.56	8.55
7	5.17	5.36	5.56	6.54	6.97	6.46	7.06	7.89	8.15	8.52
9	6.38	7.15	7.87	8.14	8.79	8.03	8.56	8.95	9.23	9.46
11	7.18	7.84	8.25	9.11	9.84	8.43	8.67	8.77	9.15	9.85
CD at 5%	1.715	1.561	1.594	1.592	1.758	1.757	1.378	1.065	0.997	0.905
SE (m)±	0.572	0.521	0.532	0.531	0.586	0.586	0.460	0.355	0.333	0.302
C.V.	23.365	18.620	17.126	15.392	15.830	19.081	13.672	9.877	8.844	7.555

Table 10: Malondialdehyde (MDA) content in the roots of wheat varieties HUW-234 and HUW-468 (*Triticum aestivum* L.) at different stages of growth.

Days after treatment	HUW-234					HUW-468				
	0 PPM	50 PPM	100 PPM	150 PPM	200 PPM	0 PPM	50 PPM	100 PPM	150 PPM	200 PPM
3	7.15	7.56	8.13	9.15	9.46	9.03	9.45	9.87	10.43	10.78
5	9.06	9.43	9.59	9.88	10.12	10.45	10.56	10.82	11.12	11.42
7	9.86	10.26	10.76	10.86	11.43	10.95	11.25	11.45	11.46	11.94
9	10.35	10.84	11.03	11.36	11.84	11.46	11.84	12.05	12.45	12.85
11	11.16	11.48	11.87	11.95	12.05	11.96	12.36	12.45	12.86	12.94
CD at 5%	2.047	2.034	1.945	1.511	1.519	1.507	1.524	1.370	1.325	1.242
SE (m)±	0.683	0.678	0.649	0.504	0.507	0.503	0.509	0.457	0.442	0.414
C.V.	16.043	15.302	14.119	10.592	10.321	10.438	10.251	9.023	8.475	7.730

Conclusion

In the present study, it was found that the higher concentrations of boron are toxic to seedling germination and growth. It was revealed that the amount of soluble sugar content in shoots was found higher than roots in both varieties of wheat, while HUW-468 showed better soluble sugar than HUW-234. Starch content in shoot of both varieties of wheat estimated more than the root, while, wheat variety HUW-468 was showed more starch content in shoot and root as compared to HUW-234. Free amino acid content in the shoot was found higher than content in the root of two varieties of wheat, while HUW-468 better than HUW-234. The proline content in shoots estimated more than its amount in roots in both varieties of wheat and similar trend has observed more in HUW-468 than HUW-234. MDA content in shoots was recorded less than amount of MDA content in roots in both varieties.

In short, reduction in nutrient uptake and translocation activities in both the wheat varieties showed decreased amount of soluble sugar and starch in seedlings treated with higher levels of boron concentration and synthesised higher Proline and Malondialdehyde (MDA) content due to boron stress. Wheat var. HUW- 468 is more tolerant to boron toxicity than var. HUW-234. Therefore, the future research programmes engaged in increasing boron tolerance should combat this issue via a multi-disciplinary approach by integrating physiological dissection of boron tolerance characters and the molecular tools. Further, the challenges in expanding the boron excess tolerance sources to plant breeders will depend on the identification and harness of new variations with different levels of tolerance to higher boron concentrations in the soil.

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