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Full Length Article

Boron Seed Priming Improves the Seedling Emergence, Growth, Grain Yield and Grain Biofortification of Bread Wheat

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Abstract

Boron (B) deficiency is quite prevalent in wheat growing regions of Indo-Gangetic Plains. This study, consisted of three experiments, was conducted to evaluate the effect of B seed priming on the productivity and grain biofortification of bread wheat. In first experiment, wheat seeds were primed with 1, 0.5, 0.1, 0.05 and 0.01 *M* B solutions of boric acid (H_3BO_3) and borax ($Na_2B_4O_7.10H_2O$) while dry seeds and hydropriming were taken as control. Seed priming with 0.05 *M* B and 0.01 *M* B were better than other treatments of both B sources in improving germination and early seedling growth. In second experiment, wheat seeds primed with 0.01 and 0.05 *M* B solutions of both sources of B were sown in sand filled small pots. Seed priming with 0.01 *M* B solution of borax was better than other treatments in improving the germination and seedling growth. In third experiment, wheat seeds primed with 0.01 and 0.05 *M* B solution of borax only were sown. Seed priming with 0.01 *M* B solution increased the grain yield by 64% over control; however, increase in grain B contents was 27%. In conclusion, wheat seeds may be primed with 0.01 *M* B using borax to improve the grain yield and grain B contents in wheat.
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Keywords: Biofortification; Boron; Seed priming; Yield

Introduction

For proper growth and development of plants, supply of specific nutrients is necessary at appropriate time and in readily available form. To achieve higher yield, proper crop nutrition including both micro and macronutrients is essential (Arif *et al.,* 2006). For vascular plants, B is considered as an essential micronutrient. Deficiency of B affects vegetative as well as reproductive growth in plants thus causing cell expansion inhibition, death of meristematic cells and reduction in plant fertility (Marchner, 1995). Immediate B deficiency symptoms in plants include inhibition of root growth (Dell and Huang, 1997) and cessation of leaf elongation (Huang *et al*., 1996). However, all of physiological process are not equally affected by B deficiency in plants.

Boron is an important micronutrient that regulates various physiological processes in life cycle of vascular plants such as cell wall development, metabolism of carbohydrates and RNA (Herrera-Rodriguez *et al.,* 2010). Moreover, it also modulates the pollen tube growth and germination, integrity of plasma membrane, floret fertility, anther development and seed development as well (Oosterhuis, 2001). In wheat, floret sterility is one of the major causes of low grain yield (Anantawiroon *et al.,* 1997). Floret sterility is caused by a combination of factors including abiotic stresses such as drought and heat (Saini and Aspinall, 1982), and inadequate supply of B during reproductive phase of plant life cycle (Rerkasem, 1995). Plant has poorley developed anthers and pollens as it prone to B deficiency (Cheng and Rerkasem, 1993). Therefore the sterility induced by inadequate supply of B is of major concern in B deficient soils (Rashid *et al.,* 1997; Shorrocks, 1997). In wheat, due to sterility, grain setting is impaired, which results in more number of open spikelets with less number of grains (Rerkasem *et al.,* 1993). Deficiency of B may result in failure of grain setting without affecting its vegetative growth (Rerkasem and Loneragan, 1994). Hence, the amount of B which is sufficient for normal vegetative growth in wheat can cause improper development of anthers and pollens during reproductive growth (Rerkasem *et al.,* 1997). This deficiency can be cured by sufficient exogenous supply of B during reproductive growth of plant. Boron requirement for reproductive growth is more than vegetative growth as in leaves B concentration is 2 mg kg^{-1} dry matter which is about five times less than the B concentration in anther tissues (Rerkasem *et al.,* 1997).

Micronutrients may be delivered as soil application, foliar sprays and/or seed treatment. Each method helps in

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providing the required amount of nutrient to the crop and also enrich the seeds of progeny crop obtained from micronutrient treated crop (Johnson *et al.,* 2005). These both goals can be achieved by foliar application of micronutrients to the plants (Savithri *et al.,* 1999). However, this method requires additional application cost. For soil application of micronutrients, high quality fertilizers are not available and it is not easy to spread the small quantity of fertilizer evenly on the soil surface (Savithri *et al.,* 1999). Seed priming provides an effective and easy alternative for micronutrient application (Savithri *et al.,* 1999; Johnson *et al.,* 2005). Small amount of nutrient is required for seed priming hence can be an economical approach as compared with other methods. In rice for instance, seed priming with 0.001% B solution has been found an effective and pragmatic way of B application that improved yield related traits consequently better yield and grain B enrichment (Rehman *et al.,* 2012). In another study, B application methods (soil, foliar and seed priming) were compared and B seed priming proved economically the most viable option in improving rice yield (Rehman *et al.,* 2014).

However, to the best of our knowledge no study has been conducted to optimize the source as well as concentration of B for wheat seed priming. Therefore, this study was conducted to optimize levels and source of B for seed priming, and to monitor its potential in improving germination, seedling growth, grain yield and grain B contents of wheat.

Materials and Methods

This study was carried out in the Allelopathy Laboratory and Greenhouse, University of Agriculture, Faisalabad, Pakistan during 2012-2013. Seeds of wheat cultivars Faisalabad-2008 and Lasani-2008 were obtained from the Wheat Research Institute, Ayub Agriculture Research Institute, Faisalabad, Pakistan.

Experiments 1

For the 1st experiment, 50 g seeds of each wheat cultivars were soaked in aerated solution of 1.0, 0.5, 0.1, 0.05 and 0.01 *M* B [boric acid (H_3BO_3), B = 17% (MERCK, Purity = 99.5%) and borax ($Na_2B_4O_7$), B = 11% (MERCK, Purity = 98%)] or water (hydropriming) for 12 h at 25 ± 2°C and then dried back to their original weight. Aeration was provided with simple aquarium pump. Dry seeds and hydropriming were taken as control. Seeds were sown on November 16, 2012 in petri plates arranged according to three factor factorial completely randomized design with four replications. Ten seeds were sown in each petri plate (90 mm × 15 mm) between two layers of moist filter paper. Petri plates were placed at 25 ± 2°C throughout the experiment. After attaining the constant germination count, four seedlings were maintained in each petri plate. Seedlings were harvested on November 28, 2012.

Experiments 2

Seeds were primed with two best performing treatments from 1st experiment (0.01 and 0.05 *M* B solutions of both B sources) and water (hydropriming) for 12 h. Dry seeds and hydropriming were taken as control. Seeds of both wheat cultivars were primed with B by above mentioned procedure. Ten seeds of each treatment were sown manually in each sand filled pot on November 30, 2012. Pots were arranged by completely randomized design in factorial arrangement with four replications. After attaining the constant count, five seedlings of uniform size were maintained in each pot. Plants were harvested on December 20, 2012.

Experiments 3

Seeds were primed with best performing treatments and source (0.01 and 0.05 *M* B solutions of borax) and water (hydropriming) for 12 h. Seeds of both wheat cultivars were primed with B by above mentioned procedure while dry seeds and hydropriming were taken as control. Twenty seeds of each treatment were sown manually in each pot (45× 30 cm) filled (15 kg soil each) with sandy loam soil (having pH= 8.20. EC= 0.33 dS m^{-1} and B= 0.56 mg L^{-1}) on December 26, 2012. Pots were arranged according to completely randomized design in factorial arrangement with four replications. After attaining the constant count, ten seedlings of uniform size were maintained in each pot. Plants were harvested manually on April 24, 2013.

Observations

Seeds were scored as germinated when radicles reached 2 mm in length. The germination count was taken according to Association of Official Seed Analysts (AOSA, 1990) until a constant count was achieved. Mean germination/emergence time was calculated according to formula given by Ellis and Robert (1981). For final germination percentage (FGP), number of seeds germinated at constant were expressed in percentage. For final emergence count (FEC), number of seedling emerged at constant were expressed in percentage. Seedling length was measured with the help of a ruler. Seedlings were oven-dried at 70°C for 48 h to determine seedling dry weight by using weighing balance (USA, OHAUS, TS400S).

Five spikes were selected at random from each pot, and number of spikelets and grains in each spike were counted. The plants were harvested, and for grain yield, spikes were threshed manually and grains were weighed on a weighing balance. For 100-grain weight, a sub sample of 100 grains taken from each pot was weighed on weighing balance. For grain B contents, dry ashing of grain samples was done (Chapman and Pratt, 1961) and B was measured by azomethine-H method in the digested samples (Bingham, 1982). Data collected on all parameters were analyzed statistically by Statistical software Statistix 8.1.

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Least significance difference (LSD) test at 5% probability level was applied to compare the treatment means.

Results

Experiment 1

Seed priming treatments and B sources significantly ($p \leq 0.05$) affected mean germination time (MGT), final germination percentage (FGP), seedling length and seedling dry weight, however cultivars did not differ significantly for these traits (Table 1). Interaction of cultivars with B sources and treatments was also not significant ($p \leq 0.05$) for these traits (Table 1) and was significant between B sources and treatments (Table 1). Likely, interaction among cultivars, B sources and treatments was also not significant ($p \leq 0.05$) for each of emergence and seedling traits (Table 1). Less MGT and FGP was recorded in seeds primed with 1.00 and 0.50 *M* B solution of boric acid than rest of the treatments (Table 2). Seed priming with 0.01 *M* B solution of borax produced longer seedlings than control (Table 2). Similarly, maximum seedling dry weight was recorded in seed priming with 0.01 *M* B solution of borax and boric acid as well (Table 2).

Experiment 2

Seed priming treatments significantly ($p \leq 0.05$) affected mean emergence time (MET), final emergence percentage (FEP), seedling length and seedling dry weight while wheat cultivars differed significantly ($p \leq 0.05$) for FEP and seedling dry weight only (Table 3). Moreover, B sources also differed significantly ($P \leq 0.05$) for seedling length and seedling dry weight (Table 3). However, interaction between cultivars and B sources was not significant ($p \leq 0.05$) for MET, FEP, seedling length and seedling dry weight (Table 3). Interaction between cultivars and seed priming treatments was only significant ($p \leq 0.05$) for seedling dry weight (Table 3). Likely, interaction between B sources and seed priming treatments and among cultivars, B sources and seed priming treatments was also non-significant ($p \leq 0.05$) (Table 3). Seed priming with 0.01 *M* B solution and hydropriming took less time to mean emergence and had more FEP than control (Table 4). However, between cultivars, more FEP was recorded in cv. Faisalabad-2008 than cv. Lasani-2008 (Table 4). More seedling length was recorded in seed priming with 0.01 *M* B solution and hydropriming (Table 4), however borax gave more seedling length than boric acid (Table 4). While maximum seedling dry weight was recorded in cv. Faisalabad-2008 seeds primed with 0.01 *M* B solution (Table 4).

Experiment 3

Seed priming with B significantly ($p \leq 0.05$) affected MET, FEP, spikelets per spike, grains per spike, 100-grains weight, grain yield and grain B contents; while wheat cultivars differed significantly ($P \leq 0.05$) for 100-grains weight and grain yield (Table 5). Interaction between cultivars and seed priming treatments was non-significant ($p \leq 0.05$) for MET, FEP, spikelets per spike, 100-grains weight, grain yield and grain B contents (Table 5). Less MET was taken by seeds primed with 0.01 *M* B solution (Table 6). All treatments showed similar FEP except for seeds primed with 0.05 *M* B solution however maximum FEP was recorded in hydroprimed seeds while lowest in 0.05 *M* B solution (Table 6). Maximum spikelets per spike and grains per spike were recorded in seeds primed with 0.01 *M* B solution (Table 6). Maximum 100-grains weight was recorded in seeds primed with 0.01 *M* B solution and hydropriming (Table 6). More grain yield was recorded in seeds primed with 0.01 *M* B solution (Table 6). However, maximum grain B contents were recorded in seeds primed with 0.05 *M* B solution (Table 6).

Discussion

Seed priming with B, especially at low concentration, has potential to boost germination rate and seedling growth of wheat. Metabolic processes involved in early phases of germination are stimulated by seed priming thus seedlings produced from primed seeds with B emerged earlier and produce healthy seedlings (Tables 4, 6). Seed priming activates the hydrolytic enzymes and improve the physiology of embryos thus germination take place in less time (Bam *et al.*, 2006). Moreover, B facilitates the remobilization of seed nutrients stores during seed germination (Bonilla *et al.*, 2004). Boron affects the seed germination by controlling dormancy same as gibberellic acid (Cresswell and Nelson, 1972). In addition to that it also modulates the germination metabolism and translocation of sugars from endosperm to the developing embryo (Cresswell and Nelson, 1972). As only small amount of B is required to regulate the meristematic growth (Khan *et al.*, 2006), any excessive amount becomes toxic impeding the normal growth (Bonilla *et al.*, 2004). Therefore, germination and seedling growth was improved by seed priming with 0.01 *M* B solution, whereas it was suppressed by 1 *M* B solution (Tables 2 and 4). Toxic B concentration leads to different physiological effect during life cycle of vascular plants. Moreover, percentage of germinated seed is reduced under high concentration of B (Banuelos *et al.*, 1999) through inhibition of polyphenol oxidase activity in embryos and endosperm during germination process (Olcer and Kocacaliskam, 2007).

Early start of germination in seeds primed with 0.01 *M* B solution of borax gave rise to longer seedlings. Boron is required for elongation of cell wall (Martín-Rejano *et al.*, 2011) by providing mechanical support (O'Neill *et al.*, 2004) to newly synthesized polysaccharides due to its involvement in crosslinking of these polysaccharides (Kobayashi *et al.*, 1996).

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Table 1: Analysis of variance for influence of seed priming with boron on germination and seedling growth of wheat cultivars

SOV	DF	MGT	FGP	SL	SDW
Cultivars (C)	1	0.11 ^{ns}	22.3 ^{ns}	4.38 ^{ns}	2.43 ^{ns}
Boron sources (B)	1	6.92 **	12643.80 **	627.58 **	63.75 **
Treatments (T)	6	2.93 **	12782.70 **	1226.94 **	574.18 **
C×B	1	0.96 ^{ns}	72.30 ^{ns}	3.98 ^{ns}	2.43 ^{ns}
C×T	6	0.38 ^{ns}	49.40 ^{ns}	10.93 ^{ns}	3.77 ^{ns}
B×T	6	3.81 **	4691.70 **	204.34 **	22.00 **
C×B×T	6	0.47 ^{ns}	45.20 ^{ns}	6.05 ^{ns}	0.86 ^{ns}
Error	84	0.25	55.1	24.59	5.70
Total	111				

SOV=Source of variation; DF=Degree of freedom; ns=Non significant; *=Significant at $p \le 0.05$; **=significant at $p \le 0.01$; MGT=Mean germination time; FGP=Final germination percentage; SL=Seedling length; SDW=Seedling dry weight

Table 2: Influence of seed priming with boron on germination and seedling growth of wheat cultivars

Treatments	Mean germination time (days)			Final germination percentage (%)			Seedling length (cm)			Seedling dry weight (mg)		
	Boric acid	Borax	Mean	Boric acid	Borax	Mean	Boric acid	Borax	Mean	Boric acid	Borax	Mean
Control	2.78a	2.78a	2.78	98.75a	98.75a	98.75	30.27abc	30.27abc	30.27	18.62bc	18.62bc	18.62
HP	2.55a	2.55a	2.55	100.00a	100.00a	100.00	29.26a-d	29.26a-d	29.26	19.75b	19.75b	19.75
1.00 *M*	0.38c	2.79a	1.58	2.50e	70.00d	36.25	2.56f	19.25e	10.90	4.75f	10.54e	7.64
0.50 *M*	1.50b	2.76a	2.13	7.50e	82.50c	45.00	6.12f	19.60e	12.86	6.88f	10.15e	8.51
0.10 *M*	2.79a	2.67a	2.73	91.25b	95.00ab	93.13	25.01d	26.13cd	25.57	15.50d	16.75cd	16.12
0.05 *M*	2.67a	2.62a	2.64	98.75a	100.00a	99.38	27.22cd	28.14bcd	27.68	20.25b	19.12b	19.69
0.01 *M*	2.57a	2.55a	2.56	98.75a	100.00a	99.38	32.58ab	33.50a	33.04	22.75a	24.12a	23.44
Mean	2.67	2.18		71.07	92.32		21.86	26.59		15.50	17.00	

Figures sharing the same case letters do not differ significantly at $p \le 0.05$; HP= Hydropriming, 1.0, 0.5, 0.1, 0.05, 0.01 *M* are concentrations of boron solutions for seed priming; MGT= Mean germination time; FGP= Final germination percentage; SL= Seedling length; SDW= Seedling dry weight

Table 3: Analysis of variance for influence of seed priming with boron on seedling emergence and growth of wheat cultivars

SOV	DF	MET	FEP	SL	SDW
Cultivars (C)	1	0.56 ^{ns}	85.33 *	5.98 ^{ns}	99.19 **
Boron sources (B)	1	0.13 ^{ns}	9.19 ^{ns}	14.43 *	9.19 *
Treatments (T)	3	5.91 **	196.50 **	74.78 **	22.02 **
C×B	1	0.74 ^{ns}	1.69 ^{ns}	0.01 ^{ns}	0.52 ^{ns}
C×T	3	0.57 ^{ns}	10.28 ^{ns}	2.42 ^{ns}	5.08 *
B×T	3	0.26 ^{ns}	7.08 ^{ns}	5.31 ^{ns}	4.19 ^{ns}
C×B×T	3	0.04 ^{ns}	0.91 ^{ns}	0.47 ^{ns}	4.19 ^{ns}
Error	32	0.45	10.66	2.64	1.44
Total	47				

SOV= Source of variation; DF= Degree of freedom; ns= Non significant; *= Significant at $p \le 0.05$; **= significant at $p \le 0.01$; MET= Mean emergence time; FEP= Final emergence percentage; SL= Seedling length; SDW= Seedling dry weight

Table 4: Influence of seed priming with boron on seedling emergence and growth of wheat cultivars

Treatments	Mean emergence time (days)			Final emergence percentage (%)			Seedling length (cm)			Seedling dry weight (mg)		
	FSD-2008	LS-2008	Mean	FSD-2008	LS-2008	Mean	Boric acid	Borax	Mean	FSD-2008	LS-2008	Mean
Control	6.74	6.45	6.59A	93.33	90.00	91.67B	28.10	28.10	28.10B	13.67cd	12.67d	13.17
HP	4.88	5.42	5.15B	99.50	99.00	99.25A	32.50	32.50	32.50A	17.00ab	13.33cd	15.17
0.01 *M*	5.04	5.66	5.35B	98.67	96.67	97.67A	32.17	33.87	33.02A	18.33a	14.50c	16.42
0.05 *M*	6.29	6.29	6.29A	93.83	89.00	91.42B	27.52	30.22	28.87B	16.00b	13.00d	14.50
Mean	5.74	5.95		96.33A	93.67B		30.07B	31.17A		16.25	13.38	

Figures sharing the same case letters do not differ significantly at $p \le 0.05$ HP= Hydropriming; 0.01, 0.05 *M* are concentrations of boron solutions for seed priming

Deficiency of B also down regulates several enzymes, which are required for lossening of cell wall to facilitate the process of cell elongation (Cosgrove, 1999; Camacho-Cristóbal *et al.,* 2008). Furthermore, longer seedlings resulted in more seedling dry weight in seed priming with 0.01 *M* B solution of borax. However, higher concentrations of B beyond 0.05 *M* B were more toxic in case of boric acid as compared to borax. Similarly, early seedling growth and germination was improved in rice by seed priming with 0.001% B solution while higher concentration of B (0.5% B solution) proved to be toxic (Rehman *et al.,* 2012).

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Table 5: Analysis of variance for influence of seed priming with boron on stand establishment, yield related traits, grain yield and grain boron contents of wheat cultivars

SOV	DF	MET	FEP	SPS	GPS	GW	GY	GBC
Cultivars (C)	1	0.03^{ns}	225.78^{ns}	1.56^{ns}	9.74^{ns}	0.59**	3.06**	0.74^{ns}
Treatments (T)	3	2.71**	675.78**	6.71**	41.96**	0.49**	15.48**	0.57**
C×T	3	0.31^{ns}	19.53^{ns}	0.69^{ns}	4.30^{ns}	0.13^{ns}	0.12^{ns}	0.08^{ns}
Error	24	1.34	84.63	0.51	3.17	0.05	0.14	0.09
Total	31							

SOV Source of variation; DF= Degree of freedom; ns= Non significant; *= Significant at p 0.05; **= significant at p 0.01; MGT= Mean emergence time; FEP= Final emergence percentage, SPS= Spikelets per spike, GPS= Grains per spike, GW= 100-grains weight, GY= Grain yield, GBC= Grain boron contents

Table 6: influence of seed priming with boron on stand establishment, yield related traits, grain yield and grain boron contents of wheat cultivars

Treatments	MET	FEP	SPS	GPS	GW	GY	GBC
Control	13.00c	87.50a	16.38c	40.84c	3.94b	3.00c	1.33c
Hydropriming	12.68c	89.38a	17.51b	43.77b	4.34a	3.79b	1.34c
Seed priming (0.01 *M*)	13.49b	85.62a	18.56a	46.39a	4.50a	4.92a	1.70b
Seed priming (0.05 *M*)	14.01a	69.38b	17.19b	42.98c	4.10b	3.62b	1.84a

Figures sharing the same letters, for a parameter, do not differ significantly at $p \leq 0.05$, MET= Mean emergence time; FEP= Final emergence percentage, SPS= Spikelets per spike, GPS= Grains per spike, GW= 100-grain weight, GY= Grain yield, GBC=Grain boron contents

Improvement in germination and seedling growth by lower concentration of B priming solution (Tables 2, 4 and 6) enabled plants to grow better and produce more yield (Tables 2, 4 and 6). Deficiency of B reduces the grain setting in wheat owing to abnormality in pollen development and stigma function (Rerkasem *et al.*, 1993); whereas B application regulates the grain setting in cereals (Rehman *et al.*, 2012). Deficiency of B also impairs viability of pollen grains (Huang *et al.*, 2000). Pollen tube may also burst in the absence of B owing to the role of B in structure of cell wall of pollen tube (Brown *et al.*, 2002). Boron deficiency also inhibits cell expansion (Hu and Brown 1994). Thus, fertilization may not occur, which resulted in failure in grain setting and consequently low grain set in B deficient plants. As B is required for seed setting thus greater yield in B priming might be the result of more grain setting (Dear and Lipsett, 1987; Noppakoonwong *et al.*, 1997). Boron is also involved in translocation of assimilates from source to sink during grain development (Reddy *et al.*, 2003). Seed priming in 0.01 *M* B solution also increased the 100-grains weight in wheat. Boron-induced increase in assimilate supply (Reddy *et al.*, 2003) seems the main reason of this improvement in grain size. Significant increase in yield contributing traits by B priming (0.01 *M*) resulted in substantial increase in grain yield. It might also be due to role of B in enhancing the translocation of photo-assimilates from vegetative to reproductive parts (Reddy *et al.*, 2003), which might increase the grain yield in wheat.

Seed priming with B, 0.05 and 0.01 *M* B solutions, resulted in increase in grain B contents of wheat cultivars. Boron is mainly transported to the seed by xylem and to some extent by phloem (Brown and Shelp, 1997). In this study, the increase in grain B contents (Table 6) was due to its better availability to plants and translocation to the developing grain (Dvorak *et al.*, 2003). Principal mechanism of B transport from soil to plant is diffusion which governs by concentration gradient but under limited supply of B, other mechanisms of B transport are necessary which can transport B against concentration gradient (Dannel *et al.*, 2000). In Arabidopsis, a B transporter is observed to be upregulated under limited supply of B, which can transport B from soil to roots against concentration gradient (Takano *et al.*, 2002). However, this transporter of B only up-regulates in plants under limited B supply and adequate or luxuriant supply of B may result in degradation of this protein thus the up-regulation of this transporter is necessary to maintain the adequate supply of B during variable growing conditions (Takano *et al.*, 2005). In this study, the improvement in growth and yield (Tables 2, 4 and 6) at lower concentration of B solution (0.01 *M* B) might be due to up-regulation of B transporter which further enhance the supply of B to plant thus improved its performance. Seed priming with B at low concentration resulted in seedlings with longer shoots and roots and consequently able to acquire nutrients including B from more area and depth of soil. Thus seeds produced by these plants had more B contents as compared with control also reported earlier for rice (Rehman *et al.*, 2012).

In conclusion, wheat seeds may be primed with 0.01 *M* B using borax to improve the germination, early seedling growth, yield and grain B contents of wheat.

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