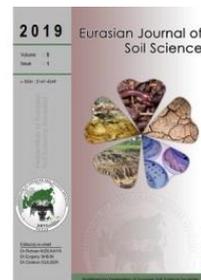




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## Interactive effect of phosphorus and boron on plant growth, nutrient accumulation and grain yield of wheat grown on calcareous soil

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### Abstract

Most of the arable soils in Pakistan are deficient in plant available phosphorus (P) and boron (B) primarily due to alkaline and calcareous nature along with low organic matter. A combined deficiency of these nutrients may intensify the plant growth suppression by reducing their efficient utilization. A pot experiment was conducted to investigate the interactive effect of P and B on growth, nutrient accumulation and grain yield of wheat grown on calcareous soil. Wheat crop was grown at three P levels (45, 90 and 135 kg P ha<sup>-1</sup>) in combination with five B levels (0, 0.5, 1.0, 1.5 and 2.0 kg B ha<sup>-1</sup>) following completely randomized design. The results revealed that yield and yield related attributes increased linearly with the addition of B at each P level. Nonetheless, the significant interactive effect of both nutrients was most pronounced in the treatment having 90 kg P ha<sup>-1</sup> and 1.5 kg B ha<sup>-1</sup>. Applied B rates resulted in relatively higher P concentration in grains and straw at P level of 90 kg ha<sup>-1</sup> contrarily to 45 and 135 kg P ha<sup>-1</sup>. The B concentration in grains and straw increased with corresponding addition of B at each P level but at variable rate, with the maximum response at higher P level. Grain and straw yield illustrated positive correlation with total P uptake ( $R^2 = 0.96$  and  $0.81$ ) and total B uptake ( $R^2 = 0.95$  and  $0.70$ ) respectively. Likewise, positive correlation ( $R^2 = 0.94$ ) between total P uptake and total B uptake under combined application of P and B indicated their synergistic relationship. Overall, the treatment combination of 90 kg P ha<sup>-1</sup> with 1.5 kg B ha<sup>-1</sup> was found as the most suitable dose for better plant growth, nutrient accumulation and grain yield of wheat.

**Keywords:** Boron nutrition, grain yield, nutrient interaction, synergism, wheat.

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### Introduction

Phosphorus (P) is the second essential macronutrient after nitrogen required for normal plant growth and development (Brady and Weil, 2008). It is the key constituent of nucleic acids, phospholipids and ATPs and play role in array of plant cellular processes such as cell division, energy storage and transfer, respiration, photosynthesis and enzymatic regulation (Watanabe et al., 2006; Lambers and Plaxton, 2015). It is involved in seedling development, growing of early roots, early heading formation and accelerates maturity of crops (Alinajoati et al., 2011). Boron (B) is also an essential micronutrient having crucial role in multiple physiological and biochemical processes within plant body such as cell division and enlargement, cell wall formation, sugar translocation, carbohydrate metabolism, nitrogen metabolism and water relations (Oyinola, 2007; Marschner, 2012). On plant level, the key role of B includes floral organs development, flower male fertility and pollen tube growth (Gupta and Solanki, 2013).

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Deficiencies of P and B are among the leading soil fertility problems in Pakistan where most of the cultivated soils are alkaline and calcareous nature characterized by low organic matter and high base saturation (Niaz et al., 2016). Although many soils have large reserves of total P, but the bio-available fraction is often 100-times less than the total P (Hinsinger et al., 2011). Added P may form secondary minerals of calcium and magnesium or bound to surfaces of  $\text{CaCO}_3$  and clay minerals in alkaline calcareous soils thereby reducing its availability to plants (Akhtar et al., 2008, Irfan et al., 2016; Abbas et al., 2018a). Phosphorus deficiency reduces cell division, carbohydrate metabolism, soluble protein contents and dry matter accumulation (Lambers and Plaxton, 2015). Boron is the second most deficient micronutrient in Pakistan after zinc and is reported to influence the growth of major field crops like wheat, cotton, maize, rice, sugarcane, potato, rapeseed and mustard (Rashid, 2006). Its deficiency impaired the biomass production by manipulating relative concentration of individual element as well as the balance among certain nutrient elements within plants (Tariq and Mott, 2007).

Various soil factors influence the availability of B to plants i.e., soil pH, native soil B contents, organic matter, carbonates, clay mineralogy, soil moisture, temperature and soil texture (Matula, 2009). The rapid fixation of applied B on calcareous soils is a serious problem to maintain adequate B concentration in soil solution (Majidi et al., 2010). Interaction of B with other nutrients could be antagonistic or synergistic depending upon soil type, crop species, growth stages, plant tissues and environmental conditions (Tariq and Mott, 2007). Type of B interaction with other nutrients results in shifting of internal physiological balance between certain elements causing secondary alterations in the absorption and accumulation of other ions (Bonilla et al., 2004). Huang et al. (2012) have reported the increase in P uptake by plants under B application indicating their synergistic relationship. Positive interaction of adequate P and B levels has been found in *brassica* resulting in enhanced biomass production (Lei et al., 2009).

Wheat (*Triticum aestivum* L.) is an important cereal crop having a central significance in agriculture sector of Pakistan. During 2016-17, area under wheat cultivation in Pakistan was 9.05 million hectares with total production of 25.75 million tonnes. It contributed 9.6% in value addition of agriculture and 1.9% in GDP of Pakistan (GOP, 2017). Cereal crops are relatively tolerant to B deficiency during vegetative growth phase. Nonetheless, during reproductive phase, B deficiency may cause severe yield losses through sterility (Uraguchi and Fujiwara, 2011). The yield losses in wheat by sterility can be retrieved by ensuring adequate B supply to plants during reproductive phase. Several earlier investigations revealed that combined application of P and B significantly enhanced the growth, yield and quality of various field crops (Huang et al., 2012; Kabir et al., 2013; Chowdhury et al., 2015; Muhlbachova et al., 2017). The current study was therefore conducted to investigate the interactive effect of P and B on growth, nutrient accumulation and grain yield of wheat grown on calcareous soil.

## Material and Methods

### Plant material and site description

Healthy and uniform seeds of wheat genotype TD-1 were kindly provided by wheat section of Agriculture Research Institute, Tandojam – Pakistan. The study was carried out during Rabi, 2016-17 under natural conditions in a net-house at Nuclear Institute of Agriculture (NIA), Tandojam – Pakistan (Latitude  $25^{\circ} 25' 19.8''$  North and Longitude  $68^{\circ} 32' 27.8''$  East). The climate of the research area is arid with mean annual precipitation of 136 mm. During the study period, the average daily maximum and minimum temperatures were 27 and 9.8 °C respectively, while average evaporation was 2.7 mm day<sup>-1</sup>, average sunshine was 8.0 hours day<sup>-1</sup>, and average relative humidity was 54.9 %. The maximum total rainfall (3.0 mm) was recorded in the month of January, 2017 (NAMC, 2017). Bulk soil (15 cm surface layer) was collected from experimental farm area of NIA, Tandojam. Then a composite sample of the selected soil was air dried and grinded to pass through 2 mm sieve and analyzed for basic soil physico-chemical properties (Table 1). In brief, the respective soil was silt loam in texture characterized by alkaline in reaction, low in organic matter, nitrogen, available phosphorus and boron while high in available potassium and calcium carbonate ( $\text{CaCO}_3$ ) contents.

### Pot experiment

Plastic pots (19 cm diameter, 30 cm depth) inner lined with polythene sheet were filled with seven kilograms of thoroughly mixed soil. Experiment was conducted according to completely randomized design with factorial arrangements having three replications. Canal water was used in each pot prior to sowing of seeds for attaining appropriate moisture for seed germination. In each pot, five seeds were sown manually

while three plants were maintained after seedling emergence and allowed to grow till maturity. Fifteen treatment combinations were formulated using three P levels (i.e. 45, 90 and 135 kg P ha<sup>-1</sup>) and five B levels (i.e. 0, 0.5, 1.0, 1.5, and 2.0 kg B ha<sup>-1</sup>). Each treatment was also fertilized with nitrogen (N) and potassium (K) at the rate of 120 kg N ha<sup>-1</sup> and 60 kg K ha<sup>-1</sup> respectively. Description of treatments detail is presented in Table 2. The required quantities of P and B according to treatment plan and potassium were applied at the time of wheat sowing while, N was applied in three equal splits viz; at sowing, two, and five weeks after sowing. Urea (46% N), triple super phosphate (TSP, 46% P<sub>2</sub>O<sub>5</sub>) and sulphate of potash (SOP, 50% K<sub>2</sub>O) were used as the source of N, P and K, respectively. All pots were irrigated during the entire crop period according to plant requirements. Plants were harvested at maturity, threshed manually to separate grains from straw. After recording yield and yield related attributes, samples were dried at 70°C for 72 hours in a forced air-driven oven till further analysis.

Table 1. Basic physico-chemical properties of collected soil used in experiment (0-15 cm surface soil)

Soil properties	Unit	Value	Method/ Reference
<b>Physical properties</b>			
Sand	%	22.22	Bouyoucos (1962)
Silt	%	55.83	~
Clay	%	21.95	~
Textural class	-	Silt loam	~
<b>Chemical properties</b>			
pH <sub>(1:2.5)</sub>	-	8.10	Mclean (1982)
EC <sub>(1:2.5)</sub>	dS m <sup>-1</sup>	0.56	Richards (1954)
Total calcium carbonate	%	6.25	Estefan et al. (2013)
Organic matter	%	0.83	Nelson and Sommers (1982)
Oxidizable organic carbon	%	0.36	~
Total organic carbon	%	0.48	~
Kjeldahl nitrogen	%	0.04	Jackson (1962)
Available phosphorus	mg kg <sup>-1</sup>	2.23	Soltanpour and Workman (1979)
Available potassium	mg kg <sup>-1</sup>	250	~
Available zinc	mg kg <sup>-1</sup>	0.99	~
Available iron	mg kg <sup>-1</sup>	40.24	~
Available boron	mg kg <sup>-1</sup>	0.65	Estefan et al. (2013)

Table 2. Detail of treatments used in experiment

Treatment	Treatment abbreviation	Phosphorus applied		Boron applied	
		kg P ha <sup>-1</sup>	mg P kg <sup>-1</sup> soil	kg B ha <sup>-1</sup>	mg B kg <sup>-1</sup> soil
T <sub>1</sub>	P <sub>45</sub> - B <sub>0.0</sub>	45	22.5	0.0	0.00
T <sub>2</sub>	P <sub>45</sub> - B <sub>0.5</sub>	45	22.5	0.5	0.25
T <sub>3</sub>	P <sub>45</sub> - B <sub>1.0</sub>	45	22.5	1.0	0.50
T <sub>4</sub>	P <sub>45</sub> - B <sub>1.5</sub>	45	22.5	1.5	0.75
T <sub>5</sub>	P <sub>45</sub> - B <sub>2.0</sub>	45	22.5	2.0	1.00
T <sub>6</sub>	P <sub>90</sub> - B <sub>0.0</sub>	90	45.0	0.0	0.00
T <sub>7</sub>	P <sub>90</sub> - B <sub>0.5</sub>	90	45.0	0.5	0.25
T <sub>8</sub>	P <sub>90</sub> - B <sub>1.0</sub>	90	45.0	1.0	0.50
T <sub>9</sub>	P <sub>90</sub> - B <sub>1.5</sub>	90	45.0	1.5	0.75
T <sub>10</sub>	P <sub>90</sub> - B <sub>2.0</sub>	90	45.0	2.0	1.00
T <sub>11</sub>	P <sub>135</sub> - B <sub>0.0</sub>	135	67.5	0.0	0.00
T <sub>12</sub>	P <sub>135</sub> - B <sub>0.5</sub>	135	67.5	0.5	0.25
T <sub>13</sub>	P <sub>135</sub> - B <sub>1.0</sub>	135	67.5	1.0	0.50
T <sub>14</sub>	P <sub>135</sub> - B <sub>1.5</sub>	135	67.5	1.5	0.75
T <sub>15</sub>	P <sub>135</sub> - B <sub>2.0</sub>	135	67.5	2.0	1.00

Each treatment was also fertilized with nitrogen and potassium at the rate of 120 kg N and 60 kg K ha<sup>-1</sup>. The 120 kg N, 90 kg P and 60 kg K ha<sup>-1</sup> are the general recommended rates of N, P and K for wheat crop in the region

### Phosphorus and boron assay

Oven dried samples (grains and straw) were grinded using Wiley's mill (3383L10, Thomas Scientific, USA) fitted with stainless steel blades to pass through a 0.42 mm screen. Total P concentration was determined

following yellow color method as described by [Chapman and Pratt \(1961\)](#). Briefly, the plant material (0.2 g each) was wet digested using 10 mL of di-acid mixture [nitric acid: perchloric acid (5:1, v/v)] in a conical flask, kept overnight and then placed on a hot plate until a clear solution was obtained. After digestion, volume was made upto 100 mL with distilled water and then filtered. The 10 mL of clear filtrate and 10 mL of ammonium-vanadomolybdate reagent was used to develop yellow color. For B determination, samples (grains and straw) were ashed at 550°C for six hours and subsequently ash was treated with 10 mL of 0.36 N H<sub>2</sub>SO<sub>4</sub> on a steam bath for 20 minutes. Azomethine-H was used for developing color for B determination by taking 1 mL sample aliquot into 10 mL polypropylene tube ([Bingham, 1982](#)). Total P and B concentration in the samples was determined by reading light absorption at 470 nm wavelength using spectrophotometer (U-2900UV/VIS, Hitachi, Japan). Phosphorus and boron uptake by grains and straw of wheat plants was calculated as described by [Irfan et al. \(2017\)](#).

$$\text{Phosphorus uptake (mg plant}^{-1}\text{)} = \text{Phosphorus concentration (mg g}^{-1}\text{)} \times \text{Dry matter (g plant}^{-1}\text{)}$$

$$\text{Boron uptake (\mu g plant}^{-1}\text{)} = \text{Boron concentration (\mu g g}^{-1}\text{)} \times \text{Dry matter (g plant}^{-1}\text{)}$$

### Statistical analysis

The data regarding plant growth, yield and related attributes, and nutrient uptake by wheat plants under combined P and B fertilization was statistically analyzed using computer software STATISTIX 8.1 (Analytical Software, Inc., Tallahassee, FL, USA) following the methods of [Steel et al. \(1997\)](#). Correlations among various parameters were carried out using Microsoft Excel (Redmond, WA, USA). A two factorial completely randomized design was employed for analysis of variance while least significant difference test at  $p \leq 0.05$  was used to determine the differences among treatment means.

## Results

### Yield and yield related attributes

The data regarding yield and yield related attributes (i.e. plant height, number of tillers per plant (NTP), number of grains per spike (NGS) and 100-grain weight) of wheat crop is presented in Table 3.

Table 3. Interactive effect of phosphorus and boron on yield and yield attributes of wheat grown on calcareous soil

Treatments	Plant height (cm)	No. of tillers plant <sup>-1</sup>	No. of grains spike <sup>-1</sup>	100-grain weight (g)	Grain yield (g plant <sup>-1</sup> )	Straw yield (g plant <sup>-1</sup> )
P <sub>45</sub> – B <sub>0.0</sub>	65.1 g	4.0 d	37.0 e	2.57 d	3.58 e	6.34 i
P <sub>45</sub> – B <sub>0.5</sub>	66.4 fg	4.0 d	38.7 e	2.64 d	3.72 e	6.77 hi
P <sub>45</sub> – B <sub>1.0</sub>	66.9 fg	4.2 cd	40.0 de	3.78 c	3.94 e	6.80 hi
P <sub>45</sub> – B <sub>1.5</sub>	67.7 e-g	4.3 b-d	41.3 c-e	3.89 bc	4.07 e	7.23 g-i
P <sub>45</sub> – B <sub>2.0</sub>	68.4 d-g	4.4 b-d	43.3 b-e	3.96 bc	4.13 e	7.45 g-i
P <sub>90</sub> – B <sub>0.0</sub>	70.4 c-g	5.1 ab	49.0 a-d	4.26 a-c	5.83 b-d	8.61 f-h
P <sub>90</sub> – B <sub>0.5</sub>	70.6 c-f	5.2 a	49.7 a-d	4.53 ab	5.39 d	9.17 fg
P <sub>90</sub> – B <sub>1.0</sub>	71.8 b-f	5.1 ab	50.7 a-c	4.61 ab	5.68 cd	10.19 ef
P <sub>90</sub> – B <sub>1.5</sub>	73.0 a-e	5.3 a	53.7 a	4.77 a	6.13 a-c	12.14 de
P <sub>90</sub> – B <sub>2.0</sub>	73.8 a-d	5.7 a	53.7 a	4.87 a	6.14 a-c	13.24 cd
P <sub>135</sub> – B <sub>0.0</sub>	75.6 a-c	5.0 a-c	51.0 a-c	4.78 a	5.81 b-d	14.60 bc
P <sub>135</sub> – B <sub>0.5</sub>	76.6 ab	5.1 ab	51.7 ab	4.88 a	6.38 ab	16.36 ab
P <sub>135</sub> – B <sub>1.0</sub>	77.3 ab	5.1 ab	53.3 a	4.79 a	5.76 b-d	16.58 ab
P <sub>135</sub> – B <sub>1.5</sub>	77.7 a	5.7 a	53.7 a	4.87 a	6.61 a	16.64 a
P <sub>135</sub> – B <sub>2.0</sub>	76.2 ab	5.6 a	52.3 ab	4.81 a	5.92 b-d	16.79 a
LSD <sub>0.05</sub> (P)	2.45	0.36	4.42	0.33	0.30	0.90
LSD <sub>0.05</sub> (B)	3.17	0.46	5.70	0.43	0.39	1.16
LSD <sub>0.05</sub> (P × B)	5.48	0.80	9.87	0.74	0.68	2.01

Treatment explanations are in Table 2. P = phosphorus levels; B = boron levels; P × B = interaction between P and B levels. Treatment means not sharing similar letter(s) in the same column differ significantly from each other (LSD test,  $p \leq 0.05$ ). Values are means of three replications (n = 3)

The P and B levels influenced significantly ( $p \leq 0.05$ ) the plant height, NTP and NGS. Nonetheless, the interaction between P and B levels was non-significant. Plant height increased linearly with corresponding increase in B levels at each P level. Maximum plant height (77.7 cm) was recorded in treatment P<sub>135</sub> – B<sub>1.5</sub> which was statistically at par to treatment P<sub>90</sub> – B<sub>1.5</sub> and onward all other treatments. The effect of B levels was most pronounced for NTP at lower P level as compared to medium and higher P levels. Minimum NTP (4.0) were observed in treatment P<sub>45</sub> – B<sub>0.0</sub> while maximum NTP (5.7) were recorded in treatments P<sub>90</sub> – B<sub>2.0</sub>

and P<sub>135</sub> – B<sub>1.5</sub>. The NGS showed increasing trend to applied B rates with relatively higher response at lower P level (i.e. 45 kg P ha<sup>-1</sup>) than at higher P levels (i.e. 90 and 135 kg P ha<sup>-1</sup>). The maximum NGS (53.7) were counted in treatment P<sub>135</sub> – B<sub>1.5</sub>, while the treatment P<sub>45</sub> – B<sub>0.0</sub> produced minimum NGS (37.0). Phosphorus and B interaction had significant ( $p \leq 0.05$ ) effects on 100-grain weight, grain yield and straw yield (Table 3). The treatment P<sub>135</sub> – B<sub>0.5</sub> produced highest 100-grain weight of 4.88 g among all treatments. The treatment P<sub>135</sub> – B<sub>1.5</sub> produced maximum grain yield (6.61 g plant<sup>-1</sup>) which was at par to grain yield at P<sub>135</sub> – B<sub>0.5</sub> (6.38 g plant<sup>-1</sup>), P<sub>90</sub> – B<sub>2.0</sub> (6.14 g plant<sup>-1</sup>) and P<sub>90</sub> – B<sub>1.5</sub> (6.13 g plant<sup>-1</sup>). Straw yield illustrated increasing trend in relation to B addition at each P level. Boron application at the rate of 2.0 kg ha<sup>-1</sup> showed maximum response for straw yield at all P levels. The treatment P<sub>135</sub> – B<sub>2.0</sub> produced maximum straw yield of 16.79 g plant<sup>-1</sup> while minimum grain yield of 6.3 g plant<sup>-1</sup> was noticed in P<sub>45</sub> – B<sub>0.0</sub> treatment.

### Phosphorus accumulation

Phosphorus concentration [P] in grains of wheat plants was increased significantly ( $p \leq 0.05$ ) in response to combined application of increasing P and B levels (Table 4). The maximum grain [P] was estimated in treatment P<sub>135</sub> – B<sub>2.0</sub> while minimum was determined in treatment P<sub>45</sub> – B<sub>0.0</sub> (4.17 vs. 2.70 mg g<sup>-1</sup>). Significant main effects of P and B levels were observed for straw [P] of wheat plants. The straw [P] enhanced linearly in relation to increasing P and B levels with maximum value of 1.43 mg g<sup>-1</sup> in P<sub>135</sub> – B<sub>2.0</sub> treatment. Phosphorus uptake by grain, straw and total (grain + straw) was increased significantly with the additional inputs of P and B fertilizers (Table 4). Overall, the response of higher P level was most pronounced in terms P uptake by grains and straw as compared to lower levels. The treatment P<sub>90</sub> – B<sub>1.5</sub> exhibited grain P uptake of 22.59 mg plant<sup>-1</sup>, which was statistically at par to all other subsequent treatments. Nonetheless, maximum grain P uptake (24.63 mg plant<sup>-1</sup>) was recorded in P<sub>135</sub> – B<sub>2.0</sub> treatment. Likewise, highest straw P uptake was noticed in treatment P<sub>135</sub> – B<sub>2.0</sub> while minimum was observed in treatment P<sub>45</sub> – B<sub>0.0</sub> (5.40 vs. 24.12 mg plant<sup>-1</sup>). The maximum total P uptake (50.11 mg plant<sup>-1</sup>) by wheat plants was recorded in pots with treatment P<sub>135</sub> – B<sub>1.5</sub> showing statistically similarity to treatments P<sub>135</sub> – B<sub>2.0</sub> (48.75 mg plant<sup>-1</sup>), P<sub>135</sub> – B<sub>0.5</sub> (46.84 mg plant<sup>-1</sup>), and P<sub>135</sub> – B<sub>1.0</sub> (45.63 mg plant<sup>-1</sup>).

Table 4. Interactive effect of phosphorus and boron on phosphorus concentration and uptake by grains and straw of wheat plants grown on calcareous soil

Treatments	P concentration (mg g <sup>-1</sup> )		P uptake (mg plant <sup>-1</sup> )		
	Grain	Straw	Grain	Straw	Total
P <sub>45</sub> – B <sub>0.0</sub>	2.70 d	0.84 g	9.69 e	5.40 i	15.10 f
P <sub>45</sub> – B <sub>0.5</sub>	2.93 cd	0.95 fg	11.03 e	6.45 hi	17.48 f
P <sub>45</sub> – B <sub>1.0</sub>	3.03 cd	1.01 e-g	12.30 e	6.85 hi	19.15 f
P <sub>45</sub> – B <sub>1.5</sub>	3.11 cd	1.03 d-g	12.62 e	7.42 hi	20.04 f
P <sub>45</sub> – B <sub>2.0</sub>	3.12 cd	1.08 c-g	12.89 e	8.00 g-i	20.89 f
P <sub>90</sub> – B <sub>0.0</sub>	3.28 b-d	1.11 b-f	19.09 cd	9.62 g-i	28.71 e
P <sub>90</sub> – B <sub>0.5</sub>	3.33 b-d	1.19 a-f	17.87 d	10.87 f-h	28.74 e
P <sub>90</sub> – B <sub>1.0</sub>	3.62 a-c	1.25 a-e	20.53 b-d	12.81 e-g	33.34 de
P <sub>90</sub> – B <sub>1.5</sub>	3.68 a-c	1.32 ab	22.59 a-c	15.91 d-f	38.50 cd
P <sub>90</sub> – B <sub>2.0</sub>	3.94 ab	1.24 a-e	24.24 ab	16.45 c-e	40.69 bc
P <sub>135</sub> – B <sub>0.0</sub>	3.98 ab	1.26 a-d	23.08 a-c	18.46 b-d	41.54 bc
P <sub>135</sub> – B <sub>0.5</sub>	4.00 ab	1.29 a-c	25.53 a	21.32 a-c	46.84 ab
P <sub>135</sub> – B <sub>1.0</sub>	4.01 ab	1.36 a	23.08 a-c	22.54 ab	45.63 ab
P <sub>135</sub> – B <sub>1.5</sub>	4.03 ab	1.39 a	26.70 a	23.41 ab	50.11 a
P <sub>135</sub> – B <sub>2.0</sub>	4.17 a	1.43 a	24.63 ab	24.12 a	48.75 a
LSD <sub>0.05</sub> (P)	0.35	0.11	2.10	2.32	3.11
LSD <sub>0.05</sub> (B)	0.45	0.14	2.72	2.99	4.02
LSD <sub>0.05</sub> (P × B)	0.78	0.24	4.71	5.19	6.96

Treatment explanations are in Table 2. P = phosphorus levels; B = boron levels; P × B = interaction between P and B levels. Treatment means not sharing similar letter(s) in the same column differ significantly from each other (LSD test,  $p \leq 0.05$ ). Values are means of three replications (n = 3)

### Boron accumulation

The data pertaining to boron concentration [B] and B uptake by grains and straw of wheat plants is presented in Table 5. Phosphorus and B levels had significant ( $p \leq 0.05$ ) effects on [B] in both grains and straw. At each P level, increase in B levels resulted in enhanced [B] of aboveground plant parts. The minimum grain [B] of 9.20 µg g<sup>-1</sup> was determined in P<sub>45</sub> – B<sub>0.0</sub> treatment while maximum (19.57 µg g<sup>-1</sup>) was

recorded in P<sub>135</sub> – B<sub>2.0</sub> which was at par to 18.98 µg g<sup>-1</sup> under P<sub>135</sub> – B<sub>1.5</sub>. Overall, the magnitude of straw [B] was comparatively higher than grain [B]. The highest straw [B] of 29.40 µg g<sup>-1</sup> was observed in treatment P<sub>135</sub> – B<sub>2.0</sub> followed by 25.80 µg g<sup>-1</sup> (P<sub>135</sub> – B<sub>1.5</sub>). Significant ( $p \leq 0.05$ ) main and interactive effects of P and B levels were observed for grain B uptake, straw B uptake and total (grain + straw) B uptake (Table 5). The average straw B uptake of wheat plants was about three fold higher than grain B uptake under each P level. The maximum and minimum grain B uptake was recorded in treatments P<sub>135</sub> – B<sub>1.5</sub> and P<sub>45</sub> – B<sub>0.0</sub> respectively (125.81 vs. 32.88 µg plant<sup>-1</sup>). Numerically, the highest value of straw B uptake (492.90 µg plant<sup>-1</sup>) was recorded in treatment P<sub>135</sub> – B<sub>2.0</sub> which was statistically identical to 433.35 µg plant<sup>-1</sup> (P<sub>135</sub> – B<sub>1.5</sub>) and 416.92 µg plant<sup>-1</sup> (P<sub>135</sub> – B<sub>1.0</sub>). Increasing B rates at each P level resulted in enhanced total B uptake by wheat plants and exhibited maximum value of 608.74 µg plant<sup>-1</sup> in treatment P<sub>135</sub> – B<sub>2.0</sub> followed by 559.16 µg plant<sup>-1</sup> (P<sub>135</sub> – B<sub>1.5</sub>) and 516.66 µg plant<sup>-1</sup> (P<sub>135</sub> – B<sub>1.0</sub>), while minimum (117.87 µg plant<sup>-1</sup>) was recorded in P<sub>45</sub> – B<sub>0.0</sub> treatment.

Table 5. Interactive effect of phosphorus and boron on boron concentration and uptake by grains and straw of wheat plants grown on calcareous soil

Treatments	B concentration (µg g <sup>-1</sup> )		B uptake (µg plant <sup>-1</sup> )		
	Grain	Straw	Grain	Straw	Total
P <sub>45</sub> – B <sub>0.0</sub>	9.20 h	13.62 e	32.88 h	84.99 g	117.87 i
P <sub>45</sub> – B <sub>0.5</sub>	9.83 gh	15.14 de	36.69 h	102.75 g	139.43 hi
P <sub>45</sub> – B <sub>1.0</sub>	10.40 gh	16.03 de	41.59 gh	108.74 g	150.33 hi
P <sub>45</sub> – B <sub>1.5</sub>	11.97 fg	17.48 de	48.73 gh	126.51 fg	175.25 hi
P <sub>45</sub> – B <sub>2.0</sub>	13.05 ef	19.48 cd	54.00 g	145.21 fg	199.21 g-i
P <sub>90</sub> – B <sub>0.0</sub>	12.94 ef	15.84 de	75.32 f	135.43 fg	210.75 g-i
P <sub>90</sub> – B <sub>0.5</sub>	14.45 de	16.77 de	78.19 f	152.13 fg	230.32 gh
P <sub>90</sub> – B <sub>1.0</sub>	14.57 de	20.23 b-d	82.81 ef	205.22 ef	288.03 fg
P <sub>90</sub> – B <sub>1.5</sub>	15.88 cd	25.45 ab	97.19 c-e	308.83 cd	406.02 de
P <sub>90</sub> – B <sub>2.0</sub>	17.12 bc	26.24 a	105.04 bc	350.43 b-d	455.46 c-e
P <sub>135</sub> – B <sub>0.0</sub>	14.66 de	19.20 cd	84.91 d-f	282.84 de	367.75 ef
P <sub>135</sub> – B <sub>0.5</sub>	16.33 cd	24.01 a-c	103.73 bc	393.48 bc	497.21 b-d
P <sub>135</sub> – B <sub>1.0</sub>	17.28 bc	25.24 ab	99.74 cd	416.92 ab	516.66 a-c
P <sub>135</sub> – B <sub>1.5</sub>	18.98 ab	25.80 a	125.81 a	433.35 ab	559.16 ab
P <sub>135</sub> – B <sub>2.0</sub>	19.57 a	29.40 a	115.84 ab	492.90 a	608.74 a
LSD <sub>0.05</sub> (P)	0.97	2.46	7.09	41.75	42.58
LSD <sub>0.05</sub> (B)	1.25	3.17	9.16	53.89	54.97
LSD <sub>0.05</sub> (P × B)	2.17	5.49	15.86	93.35	95.20

Treatment explanations are in Table 2. P = phosphorus levels; B = boron levels; P × B = interaction between P and B levels. Treatment means not sharing similar letter(s) in the same column differ significantly from each other (LSD test,  $p \leq 0.05$ ). Values are means of three replications (n = 3)

## Discussion

Most of the cultivated soils in Pakistan have moderate to severe deficiencies of macronutrient P (Irfan et al., 2018) and micronutrient B mainly due to alkaline and calcareous nature of soils accompanied with low organic matter (Niaz et al., 2016). The rapid fixation and low availability of applied P and B fertilizers on such soils is a serious problem to maintain their adequate concentration in soil solution (Abbas et al., 2018b). Interaction among nutrients might be synergistic or antagonistic which causes a shift in the internal physiological balance between certain elements resulting in secondary alterations in the absorption and accumulation of other ions (Bonilla et al., 2004). Hence, the prime objective of the present study was to investigate the interactive effect of P and B on plant growth, nutrient accumulation and yield of wheat grown on calcareous soil.

In current study, significant effect of increasing P and B rates were observed regarding plant height, number of tillers per plant, spike length and number of grains per spike. Nonetheless, the effect of B was most prominent at lower P level as compared to medium and higher P levels. Similarly, Kabir et al. (2013) have reported that application of P in combination with B significantly increased the plant height and number of branches per plant in groundnut. The increase in plant height by P might be due to enhanced photosynthetic rate thereby encouraging the vegetative growth (El-Habbasha et al., 2007). Furthermore, B is essential for cell division and cell elongation resulting in enhanced plant growth and plant height (Camacho-Cristóbal et al., 2015). Boron has positive role in transporting carbohydrates from source to sink while its deficiency

retards the synthesis of nucleic acids, carbohydrates metabolism and ultimately reduce biomass (Rashid et al., 2004). According to Han et al. (2008), B deficiency influence plant growth by reducing enzymatic activities and lowering stomatal conductance and CO<sub>2</sub> assimilation in plant leaves. On the other hand improved B nutrition lowers the sterility in bread wheat from 42.6 – 4.5% thereby increasing grain yield (Subedi et al., 1997). Combined deficiencies of P and B generate a chain reaction to inhibit protein synthesis leading to impaired growth and dry weight (Hewitt, 1983). Alam et al. (2010) have observed the increase in 1000-grain weight of summer mungbean under the combined fertilization of P and B. Positive interaction of adequate P and B levels has been found in *brassica* to enhance biomass yield (Lei et al., 2009).

Grain and straw yield of wheat plants exhibited increasing trend to B addition under each P level. A highly positive correlation of grain and straw yield was also observed with total P uptake ( $R^2 = 0.96$  and  $0.81$ ) and total B uptake ( $R^2 = 0.95$  and  $0.70$ ). Likewise, positive correlation ( $R^2 = 0.94$ ) between total P uptake and total B uptake under combined application of P and B indicating their synergistic relationship (Figure 1). The enhanced B contents in wheat plants in relation to increasing P levels also suggested the improvement of metabolic functioning of plants. Muhlbachova et al. (2017) have found positive correlation between dry matter yield and B contents in Barley ( $R^2 = 0.3273$ ,  $p \leq 0.001$ ) under P fertilization. In contrast (Kaya et al., 2009) reported that B toxicity in tomato plants could be mitigated by the supplemental P fertilizer. But the soils in Pakistan are deficient in available B contents so it is possible to assume that increased B contents in wheat was induced by increasing P levels. However, a possible competition between B and P cannot be excluded as both nutrients are available to plants as anions i.e.,  $B(OH)_4^-$  and  $H_2PO_4^-$  (Matula, 2009).

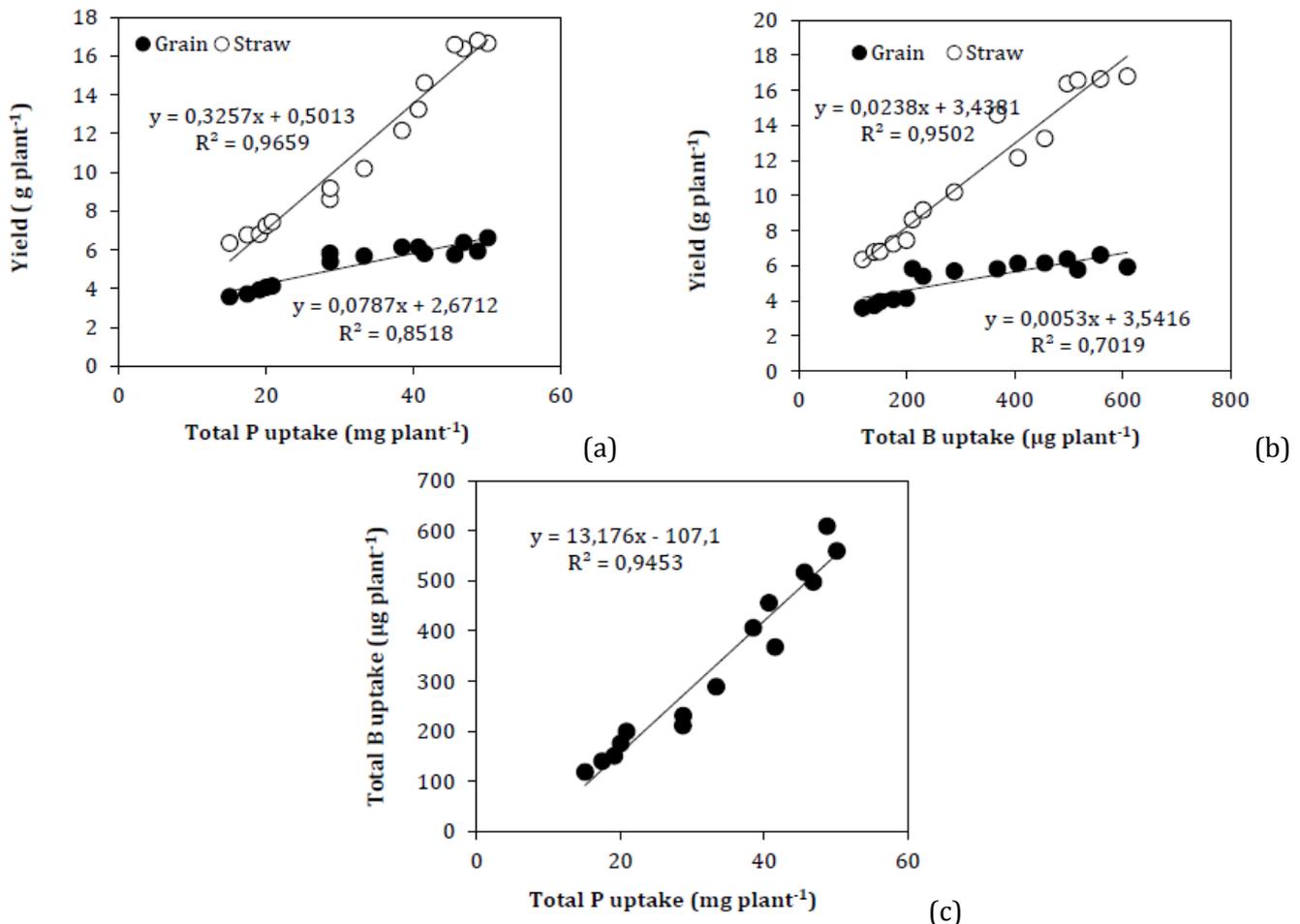


Figure 1. Correlation between yield and total P uptake (a), yield and total B uptake (b), and total P uptake and total B uptake (c) of wheat plants grown on calcareous soil

Phosphorus and B accumulation in grains and straw of wheat plants enhanced considerably at each P level in relation to increasing B levels. Ali et al. (2015) have reported the positive effect of B addition on the P concentration in tobacco leaves. In barley, increased B, N, P, K and Na contents and decreased Ca and Mg contents under B application have been observed by Singh and Singh (1983). In contrast, lower concentrations of P, K and Ca has been observed in healthy plants in comparison to severally B deficient

plants which is perhaps due to the dilution effect occurred in healthy plants. According to [Pollard et al. \(1977\)](#), B deficiency reduces the capacity for the absorption of phosphate due to the reduced ATPase activity, which could be rapidly restored by the B addition. The evidences regarding the function of B in the regulating plant membranes and ATPase as a component of transport process are well documented. The possible mechanism behind this control is the elevation of endogenous levels of auxins and the interaction of B with polyhydroxyl components of the membranes.

The synergistic effect of P and B was found for most of the growth and yield parameters of wheat crop in present study. Plant growth suppression was relatively more under the combined deficiency of both nutrients which might be due to the retardation of their efficient utilization. As P is the integral part of nucleic acids and B is also required for the synthesis of some components of nucleic acids, so their deficiency induces degradation of nucleic acids ([Rashid et al., 2004](#)). Boron deficiency during grain filling results in poor anther and pollen development, causing significant reduction in grain yield ([Uraguchi and Fujiwara, 2011](#)). Combined deficiencies of P and B may intensify these responses in plants. Plant biomass production can be predicted by the available P and B contents, as stated by [Davies et al. \(2011\)](#). Several earlier investigations revealed that combined application of P and B significantly enhanced the growth, yield and quality of various field crops ([Huang et al., 2012](#); [Kabir et al., 2013](#); [Chowdhury et al., 2015](#); [Muhlbachova et al., 2017](#)). Therefore, an adequate and consistent supply of P and B is necessary to achieve better nutrient balances and more biomass production.

## Conclusion

Phosphorus and Boron have crucial roles in array of physiological, biochemical and metabolic functions within plant body. As most of the cultivated soils in Pakistan are deficient in both nutrients, so the combined deficiency of these nutrients may adversely affect plant growth and their utilization efficiency. A synergistic effect of combined application of P and B was found for most of the growth and yield attributes in present study. However, the significant interactive effect of both nutrients was most pronounced in the P<sub>90</sub> – B<sub>1.5</sub> treatment. Thus the treatment combination of 90 kg P ha<sup>-1</sup> with 1.5 kg B ha<sup>-1</sup> was the most suitable dose for better plant growth, nutrient accumulation and grain yield of wheat. However, further verification of results is warranted under field conditions in relation to crop species, growth stages, analyzed plant organs and soil type.

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