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Response of wheat (*Triticum aestivum* L.) to zinc and boron nutrition: Grain yield, nutrient concentration and uptake under temperate conditions of Kashmir

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Abstract
A field experiment was conducted during two consecutive years (2014 and 2015) at the experimental farm of SKUAST-Kashmir to study the response of wheat (*Triticum aestivum* L.) to soil-applied zinc (Zn) and boron (B) under the temperate conditions of the Kashmir valley. Sixteen treatment combinations, comprising four levels of Zn (0, 7.5, 15 and 22 kg Zn ha^{-1}) and four levels of B (0, 1.0, 1.5 and 2.0 kg B ha^{-1}), were evaluated in a randomized complete block design with three replications. The soil was an Alfisol of silty clay loam texture with pH 6.54, 0.76% organic carbon, 0.82 mg kg^{-1} available Zn and 0.67 mg kg^{-1} available B. On a pooled basis, grain yield increased significantly with both nutrients. Among individual levels, 15 kg Zn ha^{-1} (35.77 q ha^{-1}) and 1.5 kg B ha^{-1} (34.88 q ha^{-1}) produced the highest grain yields, which were 14.06 and 9.44% higher than the respective controls. The combination $Zn_{15}B_{1.5}$ recorded the maximum grain yield (36.02 q ha^{-1}) against 29.47 q ha^{-1} in the control. Grain Zn and B concentrations were also highest at 15 kg Zn ha^{-1} and 1.5 kg B ha^{-1} and declined at the highest rates of either nutrient. Zinc application raised grain Zn uptake significantly (165.72 g ha^{-1} at 15 kg Zn ha^{-1}), whereas the effect of B on Zn uptake was non-significant. Boron uptake responded significantly to both nutrients and reached 147.93 g ha^{-1} at 1.5 kg B ha^{-1}. The Zn × *B* interaction was significant for grain yield only. Combined application of 15 kg Zn ha^{-1} and 1.5 kg B ha^{-1} was found most suitable for wheat under the conditions of the study.

Keywords: Wheat, Zinc, Boron, Grain yield, Nutrient uptake, Temperate Kashmir

1. Introduction

Wheat is a staple cereal across South Asia, and its grain supplies a large share of the daily energy and protein intake of the population. Cereal grains are, however, naturally low in Zn, and the problem becomes more serious when the crop is grown on soils with low plant-available Zn. Zinc deficiency in soils therefore lowers both the yield and the nutritional value of the grain, and the application of Zn fertilizers has been proposed as a rapid way to raise grain Zn while research on Zn-efficient cultivars continues (Cakmak, 2008) [1].

Zinc takes part in a wide range of plant processes, including enzyme activation, carbohydrate metabolism, protein synthesis and the maintenance of membrane integrity. Field studies have shown consistent yield responses of wheat to Zn on deficient soils. Torun *et al.* (2001) [2] observed that Zn fertilization increased the grain yield of all 25 wheat cultivars they tested, by about 37 to 40% on average, and Seilsepour (2007) [3] related the yield response of wheat to the level of available Zn in soil.

Boron is required for cell wall formation, carbohydrate metabolism, pollen germination and seed development (Vitosh *et al.*, 1997) [4]. It is also involved in the movement of water and nutrients from roots to shoots, and a shortage of B impairs anther development and leads to poor seed set and lower grain yield (Rehm *et al.*, 1998) [5]. Boron deficiency has been reported on many crops and soils worldwide (Shorrocks, 1997) [6]. Wheat is particularly sensitive because of its high B requirement for flower fertilization and grain set, and B application has raised wheat yields on deficient soils (Rashid *et al.*, 2011) [7]. Boron supply has also been linked with higher levels of essential amino acids in wheat protein (Iqtidar *et al.*, 1984) [8] and with improved nutrient uptake by cereals (Singh *et al.*, 1984) [9].

The two micronutrients do not act independently. Their combined supply can influence growth and the concentration of nutrients in plant tissue (Hosseini *et al.*, 2007) [10], and combined application of Zn and B has increased the number of grains per spike and the grain yield of wheat (Ali *et al.*, 2009) [11]. In the temperate Kashmir valley, where wheat is grown as a winter crop, information on the combined response of the crop to Zn and B is limited. The present study was therefore undertaken to assess the effect of Zn and B, applied singly and in combination, on the grain yield of wheat and on the concentration and uptake of Zn and B in grain.

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2. Materials and Methods

2.1 Experimental site and soil

The field experiment was conducted during 2014 and 2015 at the experimental farm of Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K) under the temperate conditions of the Kashmir valley. The soil of the experimental site was an Alfisol of silty clay loam texture. Before sowing, it had pH 6.54, electrical conductivity 0.16 dS m^{-1}, organic carbon 0.76%, available N 283.56 kg ha^{-1}, available P_2O_5 17.15 kg ha^{-1} and available K_2O 141.45 kg ha^{-1}. The available S, Ca, Mg, Zn, Cu, Fe, Mn and B contents were 10.54, 1810.05, 240.65, 0.82, 1.00, 12.65, 1.54 and 0.67 mg kg^{-1}, respectively.

2.2 Treatments and experimental design

The experiment comprised 16 treatment combinations of four Zn levels (0, 7.5, 15 and 22 kg Zn ha^{-1}) and four B levels (0, 1.0, 1.5 and 2.0 kg B ha^{-1}) arranged in a factorial randomized complete block design with three replications. The wheat variety HS-240 was sown during the *rabi* season at a seed rate of 100 kg ha^{-1} with a row-to-row spacing of 23 cm in plots measuring 5.0 m × 2.5 m. The recommended dose of 100 kg N, 60 kg P_2O_5 and 30 kg K_2O ha^{-1} was supplied through urea, diammonium phosphate (DAP) and muriate of potash (MOP). Zinc and B were applied as zinc sulphate and borax, respectively, by broadcasting on the soil surface during field preparation before sowing.

2.3 Plant analysis and nutrient uptake

Grain yield was recorded plot-wise at harvest and expressed in q ha^{-1}. Grain samples were oven-dried, ground and analysed for Zn and B. The samples were wet-digested with a di-acid mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in the ratio 9:3, and the digestion was accelerated using a microwave digestion system. Zinc in the digest was determined following the method of Verdier *et al.* (1957) [12], and B was determined by the azomethine-H spectrophotometric (colorimetric) method described by Gaines *et al.* (1979) [13]. Uptake of each nutrient by grain was calculated as follows:

$$\text{Nutrient uptake (g ha}^{-1}) = [\text{Nutrient concentration in grain (mg kg}^{-1}) \times \text{Grain yield (q ha}^{-1})]/10$$

2.4 Statistical analysis

Data for each year and the pooled data over the two years were subjected to analysis of variance appropriate to a factorial randomized complete block design (Gomez *et al.*, 1984) [14]. Treatment means were compared using critical difference (CD) values at the 5% level of probability.

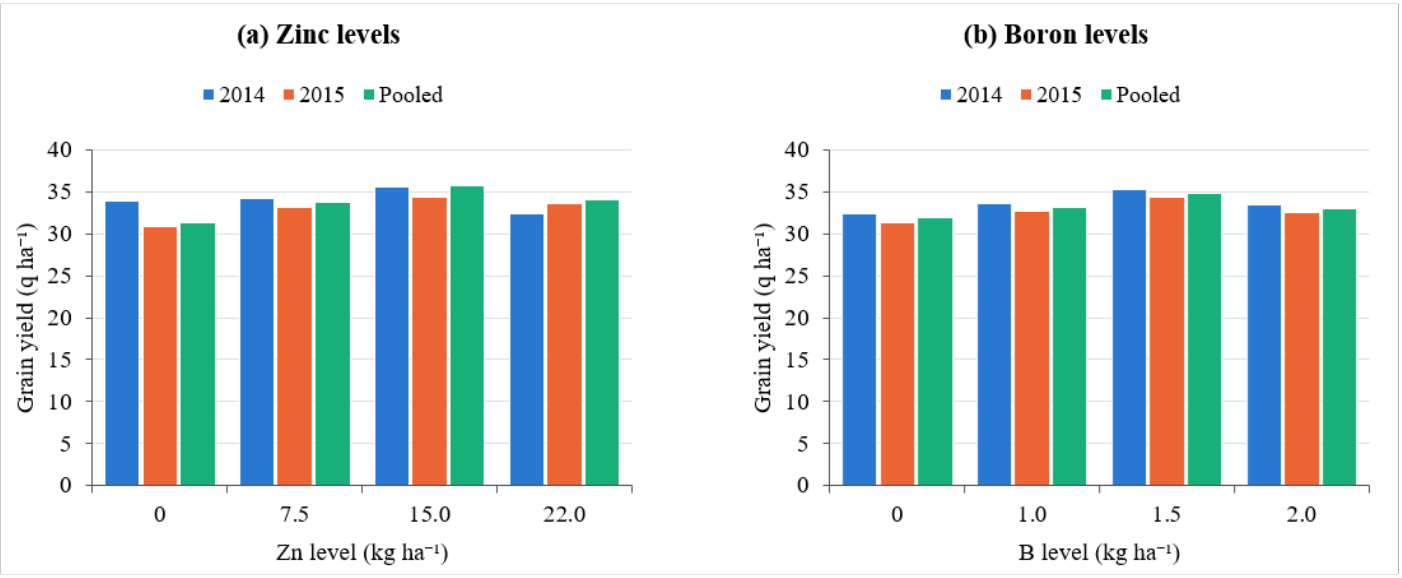
3. Results and Discussion

3.1 Grain yield

Grain yield increased significantly with Zn application up to 15 kg Zn ha^{-1} (Table 1; Fig. 1a). On a pooled basis, mean grain yield rose from 31.36 q ha^{-1} without Zn to 33.69, 35.77 and 34.04 q ha^{-1} with 7.5, 15 and 22 kg Zn ha^{-1}, which corresponded to increases of 7.43, 14.06 and 8.55% over the control. The yield at 15 kg Zn ha^{-1} was significantly higher than that at 22 kg Zn ha^{-1}, which indicated that the Zn requirement of the crop was met at the intermediate rate. The response was attributed to the role of Zn in enzyme activity, carbohydrate metabolism and protein synthesis, which supported better growth and grain filling. Positive yield responses of wheat to Zn were also reported by Torun *et al.* (2001) [2], El-Majid *et al.* (2000) [15] and Seilsepour (2007) [3].

Among the B levels (Fig. 1b), 1.5 kg B ha^{-1} produced the highest mean grain yield (34.88 q ha^{-1}), which was significantly higher than that at all other levels. Grain yield increased by 4.11, 9.44 and 3.39% over the control with 1.0, 1.5 and 2.0 kg B ha^{-1}, respectively. The lower yield at 2.0 kg B ha^{-1} showed that the range between adequate and excess B supply was narrow. Improved pollen viability, seed set and grain formation under adequate B supply probably accounted for the yield gain (Rehm *et al.*, 1998; Shorrocks, 1997) [5, 6]. Rashid *et al.* (2011) [7] likewise recorded higher wheat yields with B application on B-deficient soils.

The Zn × *B* interaction for grain yield was significant (Fig. 1c). Pooled grain yield ranged from 29.47 q ha^{-1} in the control to 36.02 q ha^{-1} under $Zn_{15}B_{1.5}$, an increase of 22.23%. Combinations involving the highest rates of the two nutrients gave lower yields, the lowest among them being 30.73 q ha^{-1} under $Zn_{22}B_{2.0}$. The higher yield under the combined application of moderate rates pointed to a complementary effect of the two nutrients. Ali *et al.* (2009) [11] found that combined Zn and B application raised the number of grains per spike and the grain yield of wheat, and yield gains with combined micronutrient application were also reported by Arif *et al.* (2006) [16], Islam *et al.* (1999) [17] and Asad *et al.* (2000) [18].



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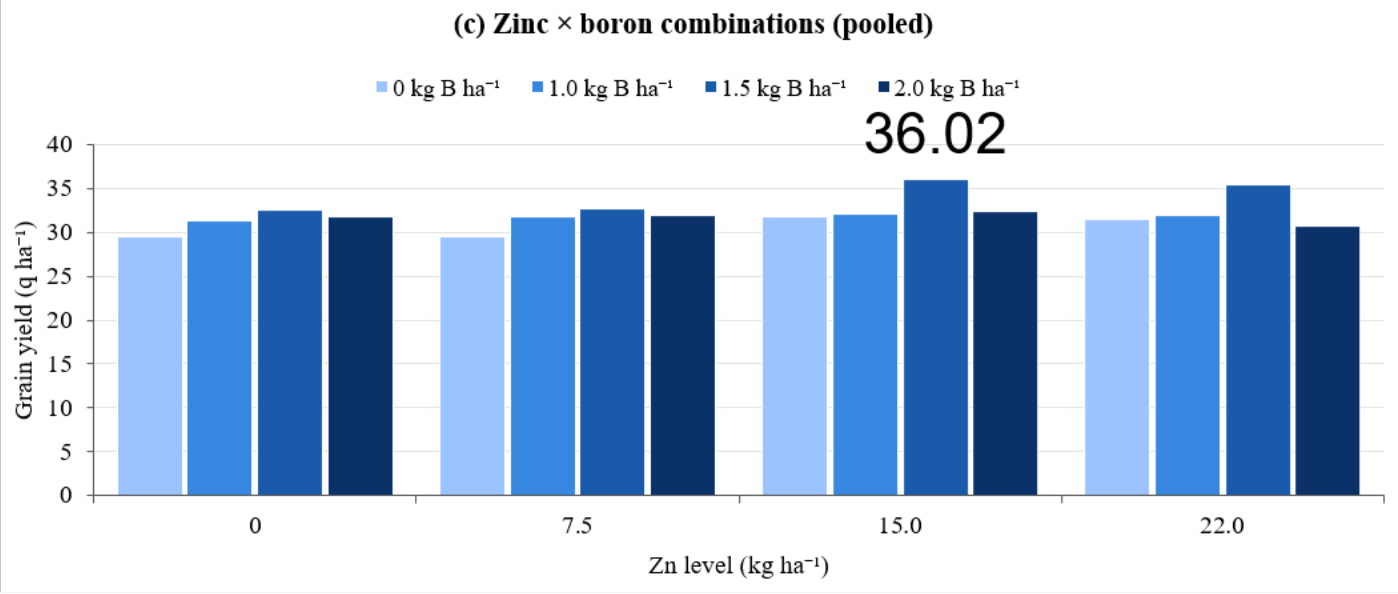


Figure 1. Effect of zinc and boron on grain yield (q ha^{-1}) of wheat: (a) zinc levels and (b) boron levels in 2014, 2015 and pooled over the two years; (c) zinc × boron combinations (pooled data). Values are the means given in the corresponding table.

3.2 Zinc concentration in grain

Grain Zn concentration increased significantly with Zn application up to 15 kg Zn ha^{-1} (Table 2; Fig. 2a). The pooled value rose from 42.15 mg kg^{-1} in the control to 50.00 mg kg^{-1} at 15 kg Zn ha^{-1}, an increase of 18.62%, and then declined significantly to 48.51 mg kg^{-1} at 22 kg Zn ha^{-1}. The decline at the highest rate coincided with the lower grain yield at that rate and may reflect a nutrient imbalance at the higher dose; this requires further study. Boron application also raised grain Zn (Fig. 2b), from 44.50 mg kg^{-1} in the control to 49.31 mg kg^{-1} at 1.5 kg B ha^{-1} (10.81%), beyond which it fell to 44.70 mg kg^{-1} at 2.0 kg B ha^{-1}. The positive effect of moderate B on grain Zn suggested a synergistic relationship between the two nutrients.

Table 1. Effect of zinc and boron on grain yield (q ha^{-1}) of wheat

Zn levels (kg ha^{-1})	2014					2015					Pooled				
	B levels (kg ha^{-1})					B levels (kg ha^{-1})					B levels (kg ha^{-1})				
	0	1.0	1.5	2.0	Mean	0	1.0	1.5	2.0	Mean	0	1.0	1.5	2.0	Mean
0	30.37	31.84	33.04	32.25	33.87	29.37	30.84	32.04	31.18	30.85	29.47	31.34	32.54	31.71	31.36
7.5	31.50	32.00	32.98	32.50	34.19	30.50	31.50	32.13	33.51	33.20	29.50	31.77	32.75	31.90	33.69
15.0	32.00	32.56	36.52	33.50	35.63	31.75	32.06	35.52	32.23	34.32	31.75	32.12	36.02	32.36	35.77
22.0	32.25	32.50	35.80	31.23	32.38	31.25	31.28	34.30	30.23	33.55	31.50	31.87	35.46	30.73	34.04
Mean	32.40	33.68	35.34	33.42		31.34	32.68	34.42	32.48		31.87	33.18	34.88	32.95	
CD ($p \leq 0.05$)															
Zinc (Zn)			1.471					1.584					1.480		
Boron (B)			1.465					1.454					1.542		
Zn × *B*			2.943					3.168					2.960		

CD: critical difference at $p \leq 0.05$; NS: non-significant. Pooled values are over 2014 and 2015.

The Zn × *B* interaction for grain Zn was non-significant (Fig. 2c), although the highest concentration (55.20 mg kg^{-1}) was recorded under $Zn_{15}B_{1.5}$ and the lowest (39.84 mg kg^{-1}) in the control. An adequate Zn supply improves root growth and the absorption of Zn from soil and favours its transfer to the developing seed (Grewal *et al.*, 1997; Cakmak, 2008) [19, 1]. Increases in grain Zn with applied Zn were also reported by Dewal *et al.* (2004) [20], Singh *et al.* (2004) [21] and Jain (2004) [22].

Table 2. Effect of zinc and boron on zinc concentration (mg kg^{-1}) in wheat grain

Zn levels (kg ha^{-1})	2014					2015					Pooled				
	B levels (kg ha^{-1})					B levels (kg ha^{-1})					B levels (kg ha^{-1})				
	0	1.0	1.5	2.0	Mean	0	1.0	1.5	2.0	Mean	0	1.0	1.5	2.0	Mean
0	40.37	42.96	43.32	43.00	42.41	39.32	41.96	43.00	42.32	41.65	39.84	42.46	43.50	42.82	42.15
7.5	43.15	43.30	43.50	44.75	43.67	42.15	42.30	44.50	43.75	43.17	42.65	42.80	45.00	44.25	43.67
15.0	45.26	46.23	55.30	47.67	48.62	46.85	47.23	54.23	47.63	48.99	48.50	49.73	55.20	46.46	50.00
22.0	45.18	45.76	53.70	46.10	47.69	45.26	47.00	52.60	44.34	47.30	47.02	48.23	53.52	45.25	48.51

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Zn levels (kg ha^{-1})	2014					2015					Pooled				
	B levels (kg ha^{-1})					B levels (kg ha^{-1})					B levels (kg ha^{-1})				
	0	1.0	1.5	2.0	Mean	0	1.0	1.5	2.0	Mean	0	1.0	1.5	2.0	Mean
Mean	43.49	44.56	48.96	45.38		45.65	44.65	48.58	44.51		44.50	45.81	49.31	44.70	
CD ($p \leq 0.05$)															
Zinc (Zn)			1.770					1.623					1.352		
Boron (B)			1.637					1.546					1.446		
Zn × *B*			NS					NS					NS		

CD: critical difference at $p \leq 0.05$; NS: non-significant. Pooled values are over 2014 and 2015.

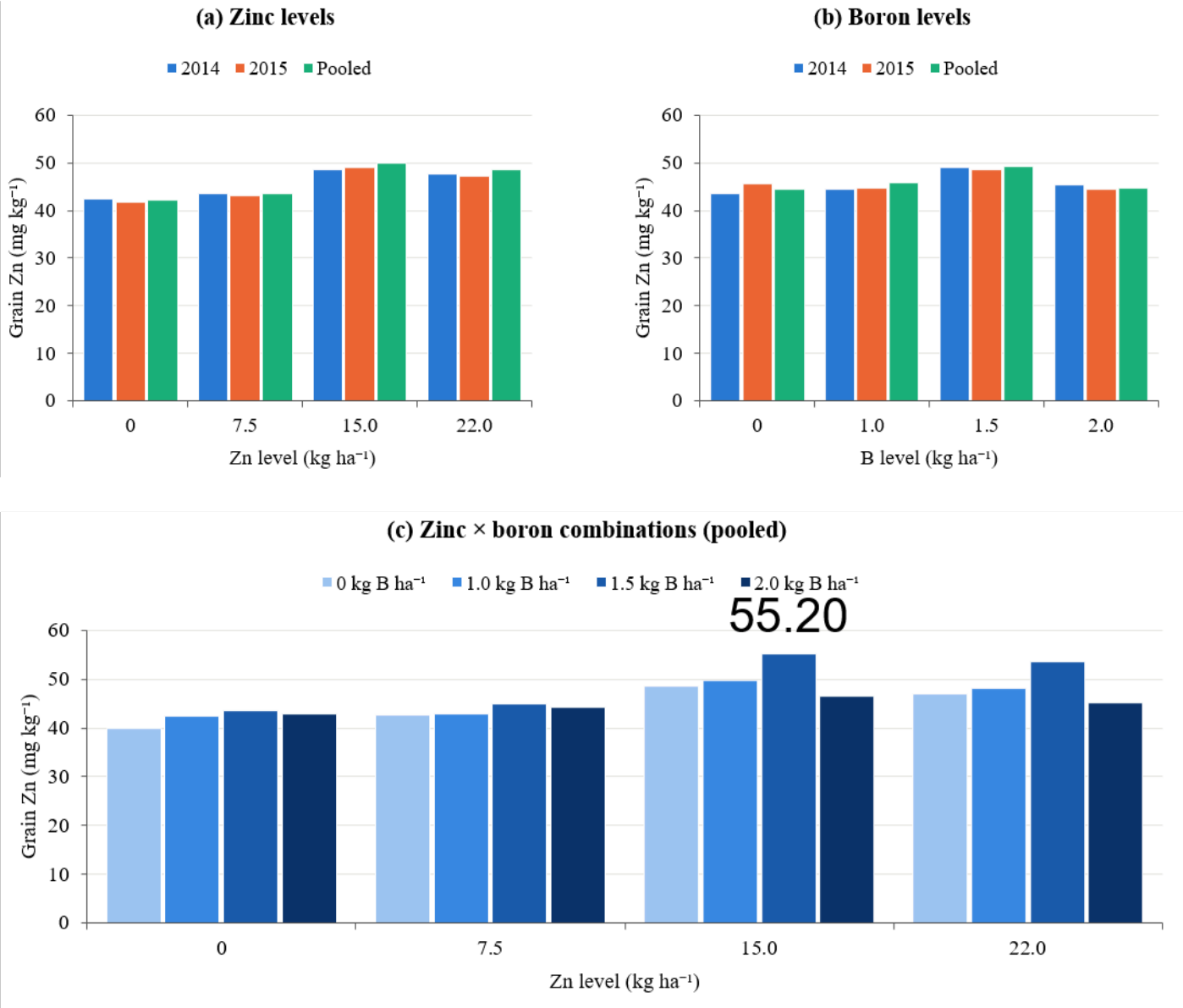


Figure 2. Effect of zinc and boron on zinc concentration (mg kg^{-1}) in wheat grain: (a) zinc levels and (b) boron levels in 2014, 2015 and pooled over the two years; (c) zinc × boron combinations (pooled data). Values are the means given in the corresponding table.

3.3 Boron concentration in grain

Grain B concentration varied significantly with both Zn and B (Table 3; Fig. 3a, b). Zinc application increased grain B from 37.61 mg kg^{-1} in the control to 42.03 mg kg^{-1} at 15 kg Zn ha^{-1} (11.75%), after which it decreased significantly to 40.96 mg kg^{-1} at 22 kg Zn ha^{-1}. Boron application increased grain B from 37.00 mg kg^{-1} in the control to 43.15 mg kg^{-1} at 1.5 kg B ha^{-1} (16.62%), and the value declined to 41.19 mg kg^{-1} at 2.0 kg B ha^{-1}. The higher grain B with B application reflected the greater availability of B in soil. The Zn × *B* interaction was non-significant (Fig. 3c); the highest grain B (44.74 mg kg^{-1}) was recorded under $Zn_{15}B_{1.5}$ and the lowest (33.75 mg kg^{-1}) in the control.

The increase in grain B with Zn observed in this study differed from the findings of Torun *et al.* (2001) [2], who reported that Zn fertilization did not change shoot B concentration when averaged over 25 wheat cultivars grown on a B-toxic soil. The low B status of the present soil, as against the B-toxic soil in that study, may explain the difference. The relationship between Zn and B in plant tissue is known to depend on the supply of both nutrients (Hosseini *et al.*, 2007) [10].

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Table 3. Effect of zinc and boron on boron concentration (mg kg^{-1}) in wheat grain

Zn levels (kg ha^{-1})	2014					2015					Pooled				
	B levels (kg ha^{-1})					B levels (kg ha^{-1})					B levels (kg ha^{-1})				
	0	1.0	1.5	2.0	Mean	0	1.0	1.5	2.0	Mean	0	1.0	1.5	2.0	Mean
0	33.42	37.96	40.33	39.00	37.67	32.08	34.80	39.33	37.83	36.01	33.75	36.46	41.41	38.83	37.61
7.5	34.25	38.80	41.72	39.25	38.50	35.25	35.96	40.72	38.25	37.54	35.75	37.30	42.22	40.75	39.00
15.0	37.26	43.50	45.70	43.50	42.49	38.26	43.50	44.24	41.70	41.92	39.70	42.33	44.74	41.35	42.03
22.0	35.20	43.20	44.20	42.75	41.34	36.20	42.16	42.75	41.45	40.64	38.76	39.00	44.25	41.85	40.96
Mean	35.03	40.80	43.00	41.12		35.44	39.10	41.76	39.80		37.00	39.02	43.15	41.19	
CD ($p \leq 0.05$)															
Zinc (Zn)			1.070					1.321					0.758		
Boron (B)			1.114					1.210					1.187		
Zn × *B*			NS					NS					NS		

CD: critical difference at $p \leq 0.05$; NS: non-significant. Pooled values are over 2014 and 2015.

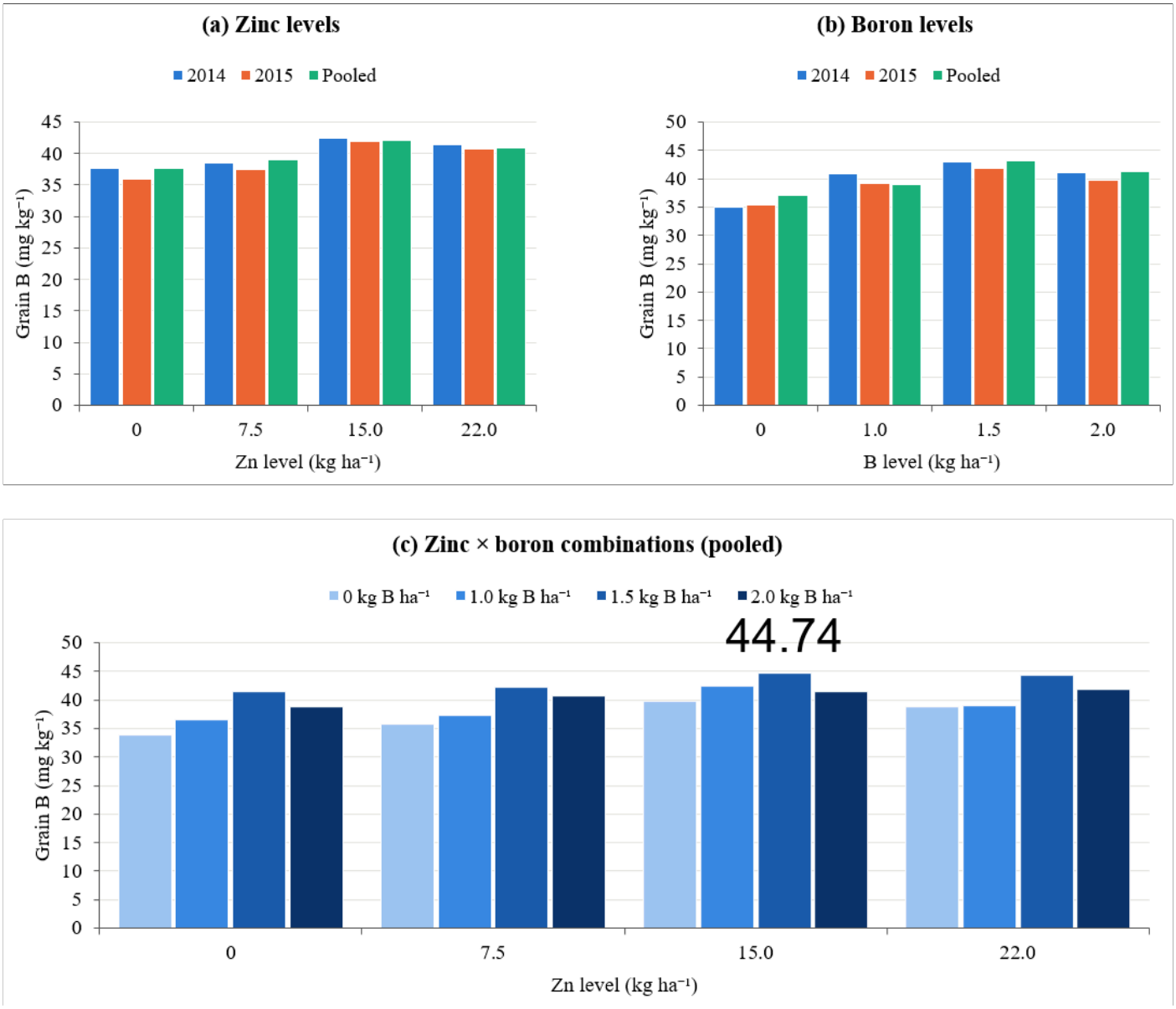


Figure 3. Effect of zinc and boron on boron concentration (mg kg^{-1}) in wheat grain: (a) zinc levels and (b) boron levels in 2014, 2015 and pooled over the two years; (c) zinc × boron combinations (pooled data). Values are the means given in the corresponding table.

3.4 Zinc uptake by grain

Zinc application significantly increased Zn uptake by grain (Table 4; Fig. 4a). On a pooled basis, uptake rose from 131.95 g ha^{-1} in the control to 165.72 g ha^{-1} at 15 kg Zn ha^{-1} (25.59%), which was statistically at par with 22 kg Zn ha^{-1} (157.66 g ha^{-1}). Boron levels did not affect Zn uptake significantly (Fig. 4b), although the highest uptake (169.38 g ha^{-1}) was recorded at 1.5 kg B ha^{-1}. The Zn × *B* interaction was also non-significant (Fig. 4c), with the maximum uptake of 198.83 g ha^{-1} under $Zn_{15}B_{1.5}$. The same trend was observed in both years. Because uptake is the product of grain yield and grain Zn concentration, the increase reflected gains in both. Higher Zn uptake by

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wheat with Zn application was also reported by Dewal *et al.* (2004) [20], Parihar *et al.* (2005) [23] and Jain *et al.* (2006) [24].

Table 4. Effect of zinc and boron on zinc uptake (g ha^{-1}) by wheat grain

Zn levels (kg ha^{-1})	2014					2015					Pooled				
	B levels (kg ha^{-1})					B levels (kg ha^{-1})					B levels (kg ha^{-1})				
	0	1.0	1.5	2.0	Mean	0	1.0	1.5	2.0	Mean	0	1.0	1.5	2.0	Mean
0	122.60	136.78	143.13	138.68	135.29	115.48	129.40	137.77	131.95	128.65	117.41	133.07	141.55	135.78	131.95
7.5	135.92	138.16	143.46	145.44	140.74	128.56	133.25	142.98	146.61	137.85	125.82	135.98	147.38	141.16	137.57
15.0	144.83	150.12	201.96	159.69	164.15	148.75	151.42	192.62	153.51	161.57	153.99	159.73	198.83	150.34	165.72
22.0	145.71	148.72	192.25	143.97	157.66	141.44	147.02	180.42	134.04	150.73	148.11	153.71	189.78	139.05	157.66
Mean	137.26	143.44	170.20	146.94		133.55	140.27	163.44	141.52		136.33	145.62	169.38	141.58	
CD ($p \leq 0.05$)															
Zinc (Zn)			8.52					8.54					9.19		
Boron (B)			NS					NS					NS		
Zn × *B*			NS					NS					NS		

CD: critical difference at $p \leq 0.05$; NS: non-significant. Pooled values are over 2014 and 2015.



Figure 4. Effect of zinc and boron on zinc uptake (g ha^{-1}) by wheat grain: (a) zinc levels and (b) boron levels in 2014, 2015 and pooled over the two years; (c) zinc × boron combinations (pooled data). Values are the means given in the corresponding table.

3.5 Boron uptake by grain

Boron uptake by grain was significantly influenced by both nutrients (Table 5; Fig. 5a, b). Zinc application increased B uptake from 117.81 g ha^{-1} in the control to 139.23 g ha^{-1} at 15 kg Zn ha^{-1} (18.18%), which was at par with 22 kg Zn ha^{-1} (132.98 g ha^{-1}). Among the B levels, 1.5 kg B ha^{-1} recorded the highest uptake (147.93 g ha^{-1}), 30.61% more than the control (113.26 g ha^{-1}) and significantly higher than the other levels. The Zn × *B* interaction was non-significant (Fig. 5c), and the maximum uptake (161.15 g ha^{-1}) was recorded under $Zn_{15}B_{1.5}$. A similar trend was observed in both years. The higher B

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uptake followed from the combined increase in grain yield and grain B concentration at these levels.

Table 5. Effect of zinc and boron on boron uptake (g ha^{-1}) by wheat grain

Zn levels (kg ha^{-1})	2014					2015					Pooled				
	B levels (kg ha^{-1})					B levels (kg ha^{-1})					B levels (kg ha^{-1})				
	0	1.0	1.5	2.0	Mean	0	1.0	1.5	2.0	Mean	0	1.0	1.5	2.0	Mean
0	101.50	120.86	133.25	125.77	120.34	94.21	107.32	126.01	117.95	111.37	99.47	114.26	134.74	123.12	117.81
7.5	107.89	124.16	137.60	127.56	124.30	107.51	113.27	130.83	128.17	119.94	105.46	118.50	138.92	130.00	123.22
15.0	119.23	141.63	166.90	145.72	143.37	121.47	139.46	157.14	134.40	138.11	126.04	135.96	161.15	133.80	139.23
22.0	113.52	140.40	158.23	133.50	136.41	113.12	131.87	146.63	125.30	129.23	122.09	124.29	156.91	128.60	132.98
Mean	110.53	131.76	148.99	133.13		109.07	122.98	140.15	126.45		113.26	123.25	147.93	128.88	
CD ($p \leq 0.05$)															
Zinc (Zn)			8.72					8.24					6.70		
Boron (B)			8.25					8.43					7.15		
Zn × *B*			NS					NS					NS		

CD: critical difference at $p \leq 0.05$; NS: non-significant. Pooled values are over 2014 and 2015.

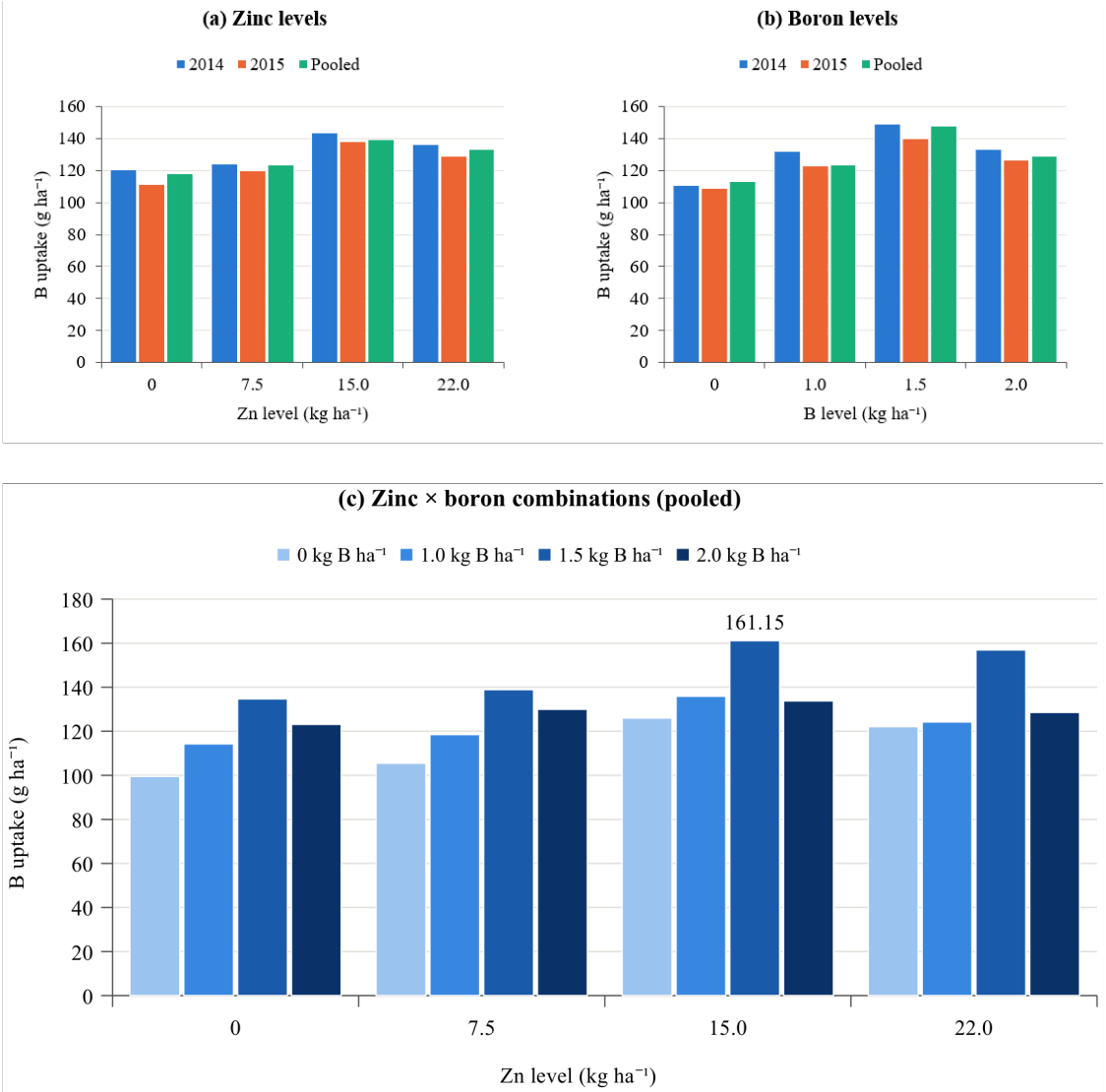


Figure 5. Effect of zinc and boron on boron uptake (g ha^{-1}) by wheat grain: (a) zinc levels and (b) boron levels in 2014, 2015 and pooled over the two years; (c) zinc × boron combinations (pooled data). Values are the means given in the corresponding table.

4. Conclusion

Soil application of Zn and B significantly improved the grain yield of wheat and the concentration and uptake of both nutrients in grain under the temperate conditions of Kashmir. Grain yield and grain Zn and B concentrations were highest at 15 kg Zn ha^{-1} and 1.5 kg B ha^{-1} and declined at the higher rates of either nutrient. The combination of 15 kg Zn ha^{-1} with 1.5 kg B ha^{-1} gave the highest grain yield (36.02

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q ha^{-1}) together with the highest grain Zn and B concentrations. This combination can be suggested for wheat on similar soils of the valley. Multi-location trials are needed before a general recommendation is made for the different agro-climatic zones of the region.

Conflict of interest

The authors declare that they have no conflict of interest.

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