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Effect of zinc and boron fertilization on biochemical constituents of wheat (*Triticum aestivum* L.) grain under temperate conditions of Kashmir

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Abstract
A two-year field experiment (2014 and 2015) was conducted at the experimental farm of SKUAST-Kashmir to evaluate the effect of soil-applied zinc (Zn) and boron (B) on the biochemical quality of wheat (*Triticum aestivum* L.) cv. HS-240 grain 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 factorial randomized complete block design with three replications on a silty clay loam Alfisol (pH 6.54). Pooled analysis over the two years showed that crude protein, lysine and tyrosine contents increased significantly with Zn and B up to 15 kg Zn ha^{-1} and 1.5 kg B ha^{-1}, respectively, and declined at the highest rates. The Zn × B interaction was significant for crude protein, lysine and tyrosine. The combination of 15 kg Zn and 1.5 kg B ha^{-1} ($Zn_{15}B_{1.5}$) recorded the highest crude protein (13.62%), gluten (14.13%), total soluble sugars (4.32%), carbohydrate (64.42%), lysine (3.18%) and tyrosine (5.29%), against 10.62, 10.35, 2.66, 51.41, 1.93 and 4.17%, respectively, in the unfertilized control. Combined application of 15 kg Zn ha^{-1} and 1.5 kg B ha^{-1} is therefore recommended for improving the nutritional quality of wheat grain on similar soils of the temperate Kashmir valley.

Keywords: Wheat, zinc, boron, crude protein, gluten, lysine, tyrosine, grain quality

Introduction

Wheat (*Triticum aestivum* L.) is the principal staple cereal of the temperate regions and supplies a large share of the dietary energy and protein consumed worldwide (Shewry *et al.*, 2015) [1]. Global wheat production in 2023 was estimated at approximately 785 million tonnes (FAO, 2023) [2]. In India, wheat ranks next only to rice as a staple food grain, and its straw is an important source of fodder for livestock. Besides starch, wheat grain provides appreciable quantities of protein, B-group vitamins, dietary fibre and minerals, and its nutritive value compares favourably with that of most other cereals (Zohary *et al.*, 2000; Shewry *et al.*, 2015) [3, 1].

The end-use and nutritional quality of wheat grain is determined largely by its carbohydrate and protein fractions. During grain development, starch accumulates in the kernel at the expense of free reducing and non-reducing sugars (Abou-Guendia *et al.*, 1973) [4]. The storage proteins of the endosperm, the gliadins and glutenins, together form gluten, which governs dough strength, extensibility and baking performance (Brites *et al.*, 2001) [5]. Grain protein content in cultivated wheat varies widely with genotype and environment, and wild relatives carry genes for still higher protein concentration (Nevo *et al.*, 1986) [6], indicating considerable scope for improving grain protein through management.

Among the management factors, micronutrient supply strongly influences grain composition. Zinc is a structural and catalytic component of numerous enzymes and is required for protein synthesis, carbohydrate metabolism and auxin production; Zn deficiency depresses soluble protein and causes free amino acids to accumulate in plant tissues (Cakmak *et al.*, 1989; Marschner, 1995) [7, 8]. Boron is involved in cell-wall formation, membrane integrity, sugar translocation and reproductive development (Gupta, 1979; Marschner, 1995) [9, 8]. Cereal grains are inherently low in Zn, particularly on soils of low Zn availability, and fertilizer application offers a rapid means of improving grain Zn and grain quality (Cakmak, 2008) [10]. Information on the combined effect of Zn and B on the biochemical composition of wheat grain under the temperate conditions of Kashmir is, however, scarce. The present study was therefore undertaken to evaluate the individual and interactive effects of Zn and B fertilization on the protein, gluten, total soluble sugar, carbohydrate, lysine and tyrosine contents of wheat grain.

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Materials and Methods

Experimental site and design

A field experiment was conducted during two consecutive seasons (2014 and 2015) at the experimental farm of Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K) to study the response of wheat (*Triticum aestivum* L.) to Zn and B nutrition under the temperate conditions of the Kashmir valley. The experiment comprised 16 treatment combinations of 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}), laid out in a factorial randomized complete block design (RCBD) with three replications. 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 N, P_2O_5 and K_2O (100:60:30 kg ha^{-1}) was applied through urea, diammonium phosphate (DAP) and muriate of potash (MOP). Zinc and B were supplied through zinc sulphate and borax, respectively, and were applied to the soil by broadcasting during field preparation before sowing. The soil of the experimental site was an Alfisol of silty clay loam texture; its initial physico-chemical properties are presented in Table 1.

Table 1. Initial physico-chemical properties of the experimental soil

Parameter	Value	Parameter	Value
Soil order / texture	Alfisol / silty clay loam	Available S (mg kg^{-1})	10.54
pH (1:2.5)	6.54	Exchangeable Ca (mg kg^{-1})	1810.05
EC (dS m^{-1})	0.16	Exchangeable Mg (mg kg^{-1})	240.65
Organic carbon (%)	0.76	Available Zn (mg kg^{-1})	0.82
Available N (kg ha^{-1})	283.56	Available Cu (mg kg^{-1})	1.00
Available P_2O_5 (kg ha^{-1})	17.15	Available Fe (mg kg^{-1})	12.65
Available K_2O (kg ha^{-1})	141.45	Available Mn (mg kg^{-1})	1.54
		Available B (mg kg^{-1})	0.67

Biochemical analyses

Crude protein. Total nitrogen in grain was determined by the Kjeldahl method (McKenzie *et al.*, 1954) [11], and crude protein content was calculated by multiplying the nitrogen content by the factor 6.25, on the assumption that protein contains 16% nitrogen.

Total soluble sugars and carbohydrate. Total soluble sugars in grain were estimated following the procedure described by Malik *et al.* (1980) [12], and total carbohydrate was estimated by the anthrone method (Thimmaiah, 1999) [13].

Gluten. Flour was kneaded with water into a dough, the starch and water-soluble fractions were washed out, and the residual gluten was dried at 100 °C to constant weight. Gluten content was expressed as a percentage of the flour weight.

Lysine. Lysine was estimated by the colorimetric method of Tsai *et al.* (1972) [14]. The seed protein was hydrolysed with the proteolytic enzyme papain, and the α-amino groups of the liberated amino acids were complexed with copper. The free ε-amino group of lysine, which does not complex with copper, was reacted with 2-chloro-3,5-dinitropyridine to form the ε-dinitropyridyl derivative of lysine. Excess reagent was removed with ethyl acetate, and the absorbance of the derivative was read at 390 nm.

Tyrosine. Tyrosine content was determined by the standard procedure described by Thimmaiah (1999) [13].

Zinc and boron. Oven-dried grain 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 (v/v), and the digestion was accelerated in a microwave digestion system. Zinc in the digest was determined following the method of Verdier *et al.* (1957) [15], and B was determined by the azomethine-H spectrophotometric (colorimetric) method (Gaines *et al.*, 1979) [16].

Statistical analysis

Data for each year were subjected to analysis of variance for a factorial RCBD, and pooled analysis was performed over the two years following Gomez *et al.* (1984) [17]. Treatment means were compared using the critical difference (CD) at the 5% level of probability.

Results and Discussion

Crude protein

Crude protein content of wheat grain was significantly influenced by Zn, B and their interaction in both years and in the pooled analysis (Table 2; Fig. 1). In the pooled data, protein content increased from 10.78% at 0 kg Zn ha^{-1} to 12.46% at 15 kg Zn ha^{-1} and then declined to 11.93% at 22 kg Zn ha^{-1}. Similarly, B application raised protein content from 11.45% in the control to 12.10% at 1.5 kg B ha^{-1}, beyond which it fell to 11.51% at 2.0 kg B ha^{-1}. The interaction was significant: protein content increased from 10.62% in the control (Zn_0B_0) to 13.62% under $Zn_{15}B_{1.5}$, an improvement of 28.2%. The same trend was observed in both years, although protein values in 2015 were consistently higher than those in 2014.

The increase in protein with Zn application was attributed to the central role of Zn in protein synthesis. Cakmak *et al.* (1989) [7] showed that Zn deficiency lowered soluble protein and caused free amino acids to accumulate, and that re-supply of Zn restored protein levels; an adequate Zn supply therefore favoured the incorporation of amino acids into protein. Boron improved nitrogen utilization and protein synthesis, which raised protein concentration in the grain (Marschner, 1995) [8]. The positive response of grain protein to Zn fertilization agreed with the findings of Schmidt *et al.* (2007) [18] in wheat. The decline at the highest rates of Zn and B was probably due to nutrient imbalance; boron in particular has a narrow range between deficiency and toxicity (Gupta, 1979) [9].

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Table 2. Effect of zinc and boron on crude protein content (%) of wheat grain

Zn (kg ha^{-1})	2014					2015					Pooled				
	Boron (kg B ha^{-1})					*Boron (kg B ha^{-1})*					*Boron (kg B 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	10.00	10.18	10.62	10.43	10.30	10.74	11.18	11.43	11.25	11.15	10.62	10.78	10.93	10.80	10.78
7.5	11.25	11.43	11.74	11.31	11.43	11.90	12.30	12.73	12.00	12.23	11.23	11.57	11.80	11.76	11.59
15.0	11.56	11.91	13.12	11.50	12.02	12.53	13.00	14.12	12.24	12.97	12.04	12.45	13.62	11.75	12.46
22.0	11.43	11.50	11.55	11.24	11.43	12.43	12.50	12.55	12.09	12.39	11.93	12.00	12.05	11.74	11.93
Mean	11.06	11.25	11.75	11.12		11.90	12.24	12.70	11.89		11.45	11.70	12.10	11.51	
CD ($p \leq 0.05$)															
Zn			0.355					0.396					0.289		
B			0.348					0.357					0.293		
Zn × *B*			0.711					0.791					0.578		

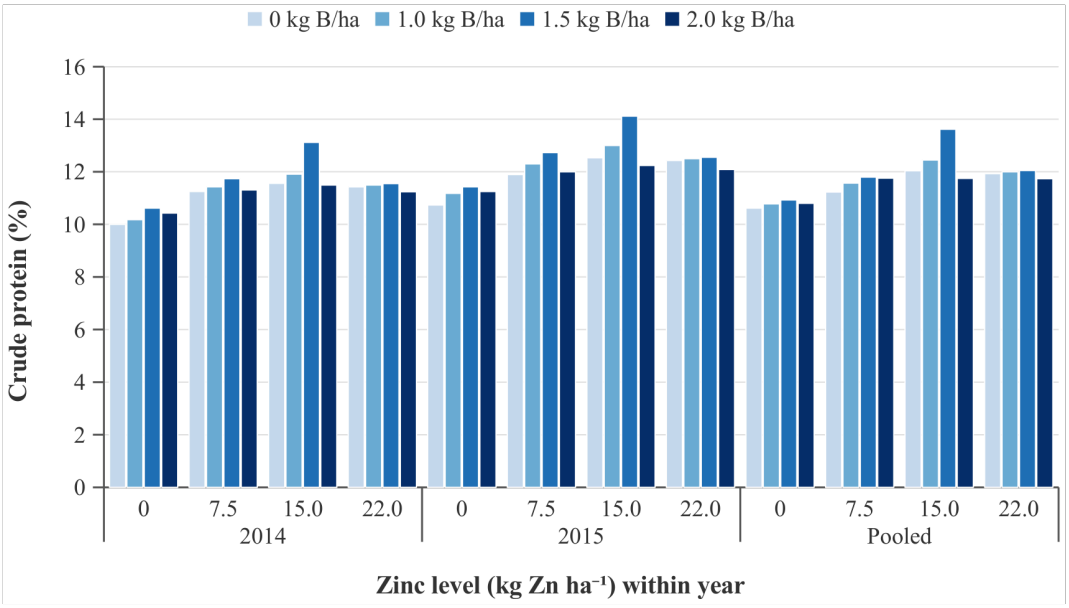


Figure 1. Effect of zinc and boron levels on crude protein content (%) of wheat grain in 2014, 2015 and pooled over years. CD ($p \leq 0.05$) values are given in Table 2.

Total soluble sugars

Total soluble sugar (TSS) content of the grain increased with Zn application (Table 3; Fig. 2). In the pooled data, TSS increased from 2.91% at 0 kg Zn ha^{-1} to a maximum of 3.47% at 15 kg Zn ha^{-1}, an increase of 19.2%, and decreased slightly to 3.39% at 22 kg Zn ha^{-1}. Boron raised mean TSS from 2.91% in the control to 3.66% at 1.5 kg B ha^{-1}; however, the main effect of B and the Zn × B interaction were non-significant in both years and in the pooled analysis. Numerically, the highest TSS (4.32%) was recorded under $Zn_{15}B_{1.5}$, compared with 2.66% in the control.

Zinc is required for the activity of carbonic anhydrase and of enzymes involved in carbohydrate metabolism, and an adequate Zn supply sustained photosynthesis and the production of soluble sugars (Marschner, 1995) [8]. Boron facilitated the translocation of sugars across membranes and their movement to developing sinks (Gupta, 1979; Mengel *et al.*, 1982) [9, 19], which accounted for the higher sugar content at moderate B levels.

Table 3. Effect of zinc and boron on total soluble sugar content (%) of wheat grain

Zn (kg ha^{-1})	2014					2015					Pooled				
	Boron (kg B ha^{-1})					*Boron (kg B ha^{-1})*					*Boron (kg B 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	2.80	2.93	3.18	3.10	3.00	2.53	2.74	3.05	2.82	2.78	2.66	2.83	3.11	3.04	2.91
7.5	3.02	3.11	3.28	3.23	3.16	2.78	2.87	2.94	2.90	2.87	2.76	2.98	3.12	3.07	2.98
15.0	3.12	3.27	3.45	3.31	3.28	3.06	3.10	4.30	3.20	3.41	3.12	3.21	4.32	3.24	3.47
22.0	3.10	3.23	3.40	3.26	3.24	3.15	3.24	4.20	3.16	3.43	3.10	3.17	4.11	3.18	3.39
Mean	3.01	3.13	3.32	3.22		2.88	2.98	3.62	3.02		2.91	3.04	3.66	3.13	
CD ($p \leq 0.05$)															
Zn			0.921					0.917					0.919		
B			NS					NS					NS		
Zn × *B*			NS					NS					NS		

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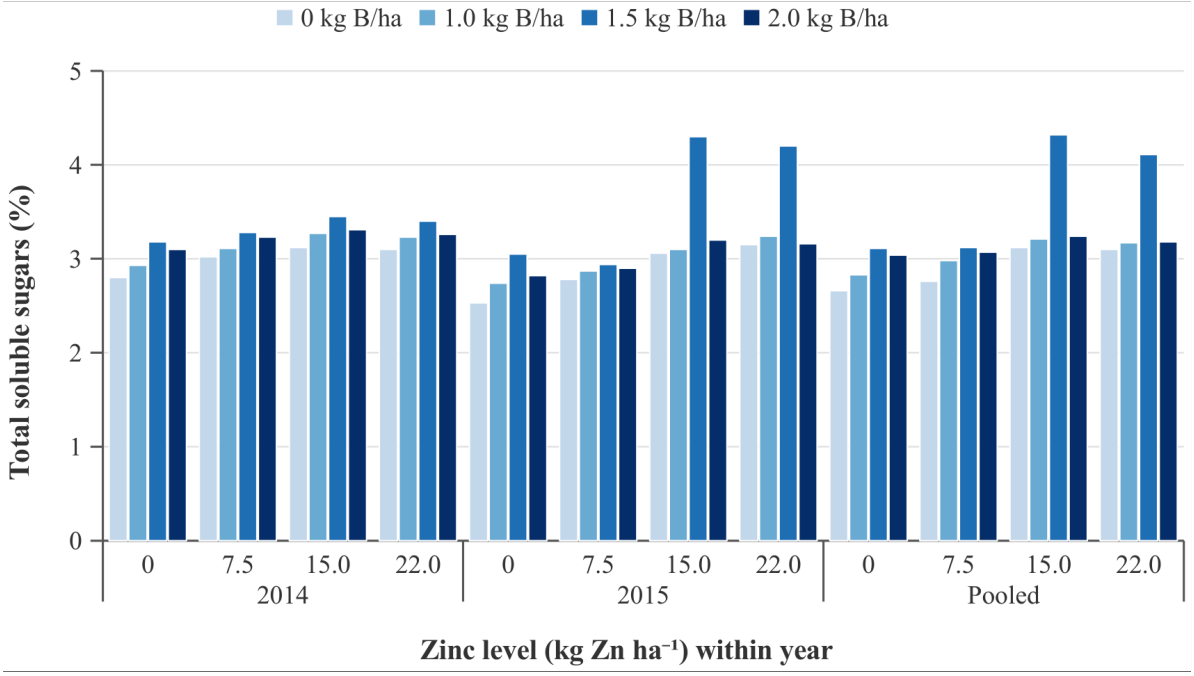


Figure 2. Effect of zinc and boron levels on total soluble sugar content (%) of wheat grain in 2014, 2015 and pooled over years. CD ($p \leq 0.05$) values are given in Table 3.

Gluten

Gluten content of the grain increased with Zn application (Table 4; Fig. 3). In the pooled data, gluten increased from 11.13% at 0 kg Zn ha^{-1} to 12.53% at 15 kg Zn ha^{-1} (12.6% increase) and remained at a similar level (12.41%) at 22 kg Zn ha^{-1}. Application of 1.5 kg B ha^{-1} raised mean gluten from 11.48% to 12.83%, although the effect of B and the Zn × B interaction were non-significant. The highest gluten content (14.13%) was recorded under $Zn_{15}B_{1.5}$, against 10.35% in the control.

Gluten is formed mainly by the gliadin and glutenin storage proteins, whose composition determines dough strength and extensibility (Brites *et al.*, 2001) [5]. Since Zn is required for RNA metabolism, ribosome integrity and protein synthesis (Cakmak *et al.*, 1989; Marschner, 1995) [7, 8], a better Zn supply during grain filling favoured the accumulation of these storage proteins, which explained the higher gluten content in Zn-fertilized plots.

Table 4. Effect of zinc and boron on gluten content (%) of wheat grain

Zn (kg ha^{-1})	2014					2015					Pooled				
	Boron (kg B ha^{-1})					*Boron (kg B ha^{-1})*					*Boron (kg B 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	10.30	11.28	11.42	11.39	11.09	10.40	11.34	11.46	11.45	11.16	10.35	11.31	11.44	11.42	11.13
7.5	11.75	11.89	12.15	12.00	11.94	11.93	11.95	12.23	11.49	11.90	11.84	11.92	12.19	11.74	11.92
15.0	11.95	12.11	14.10	12.30	12.61	12.06	12.23	14.16	11.87	12.58	11.98	12.15	14.13	11.87	12.53
22.0	11.78	12.00	13.58	12.25	12.40	11.98	12.12	13.61	11.53	12.31	11.76	12.09	13.59	12.22	12.41
Mean	11.44	11.82	12.81	11.98		11.59	11.91	12.87	11.58		11.48	11.86	12.83	11.81	
CD ($p \leq 0.05$)															
Zn			1.553					1.602					1.574		
B			NS					NS					NS		
Zn × *B*			NS					NS					NS		

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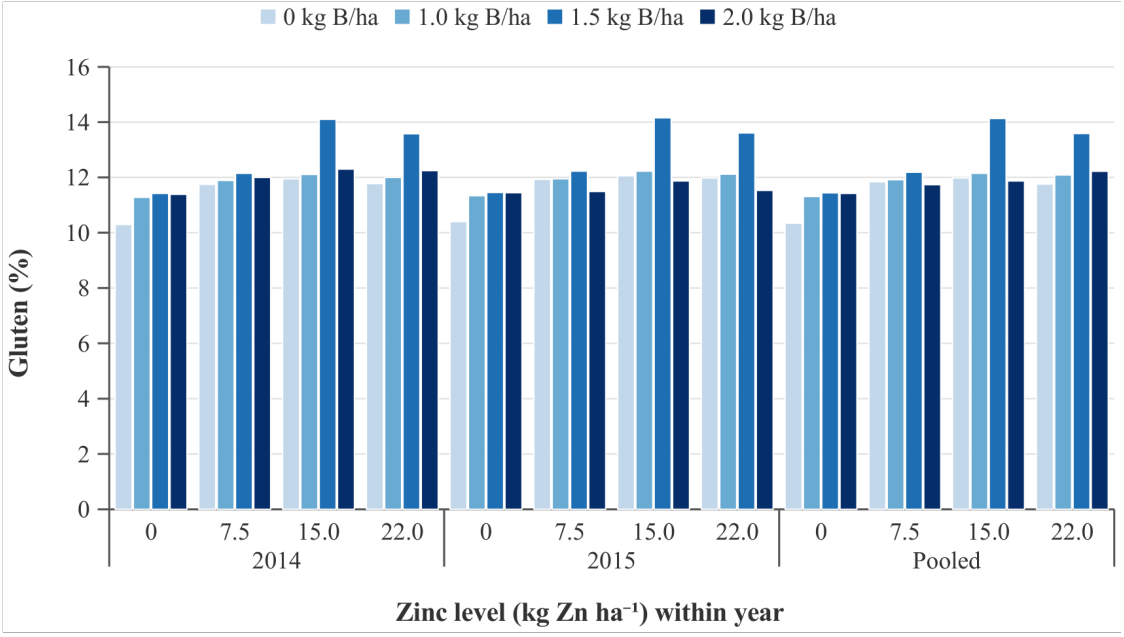


Figure 3. Effect of zinc and boron levels on gluten content (%) of wheat grain in 2014, 2015 and pooled over years. CD ($p \leq 0.05$) values are given in Table 4.

Carbohydrate

Carbohydrate content of wheat grain increased from 52.18% at 0 kg Zn ha^{-1} to 57.39% at 15 kg Zn ha^{-1}, and from 53.29% at 0 kg B ha^{-1} to 58.17% at 1.5 kg B ha^{-1}. The combined application of Zn and B raised carbohydrate content from 51.41% in the control to 64.42% under $Zn_{15}B_{1.5}$. The increase with Zn was attributed to improved photosynthesis and carbonic anhydrase activity, which enhanced the supply of assimilates for starch formation, whereas B improved the translocation of sugars to the developing grain (Gupta, 1979; Marschner, 1995) [9, 8]. In contrast, Schmidt *et al.* (2007) [18] reported a lower carbohydrate content in wheat following Zn fertilization; the difference was probably related to soil Zn status and the rates of application.

Lysine

Lysine content was significantly influenced by Zn, B and their interaction (Table 5; Fig. 4). In the pooled data, increasing Zn from 0 to 15 kg ha^{-1} raised lysine content from 2.16% to 2.61%; the value at 15 kg Zn ha^{-1} was on par with that at 22 kg Zn ha^{-1} (2.48%). Boron increased lysine from 2.13% in the control to 2.71% at 1.5 kg B ha^{-1}, which was significantly superior to all other B levels. The interaction effect was significant, and the highest lysine content (3.18%) was recorded under $Zn_{15}B_{1.5}$, against 1.93% in the control.

Zinc deficiency has been shown to impair protein synthesis and to cause free amino acids to accumulate (Cakmak *et al.*, 1989) [7]. An adequate Zn supply therefore favoured the incorporation of amino acids, including lysine, into grain protein. The parallel increase in crude protein and lysine in the present study supported this interpretation.

Table 5. Effect of zinc and boron on lysine content (%) of wheat grain

Zn (kg ha^{-1})	2014					2015					Pooled				
	Boron (kg B ha^{-1})					*Boron (kg B ha^{-1})*					*Boron (kg B 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	2.02	2.12	2.23	2.17	2.13	2.00	2.15	2.23	2.17	2.13	1.93	2.18	2.31	2.23	2.16
7.5	2.15	2.23	2.26	2.19	2.20	2.28	2.41	2.56	2.42	2.41	2.12	2.26	2.42	2.32	2.28
15.0	2.25	2.32	3.31	2.24	2.53	2.54	2.65	3.21	2.48	2.72	2.29	2.43	3.18	2.54	2.61
22.0	2.23	2.30	3.12	2.21	2.46	2.45	2.60	2.85	2.43	2.58	2.19	2.37	2.95	2.43	2.48
Mean	2.16	2.24	2.73	2.20		2.31	2.45	2.71	2.37		2.13	2.31	2.71	2.38	
CD ($p \leq 0.05$)															
Zn			0.195					0.196					0.226		
B			0.187					0.179					0.224		
Zn × *B*			0.389					0.392					0.443		

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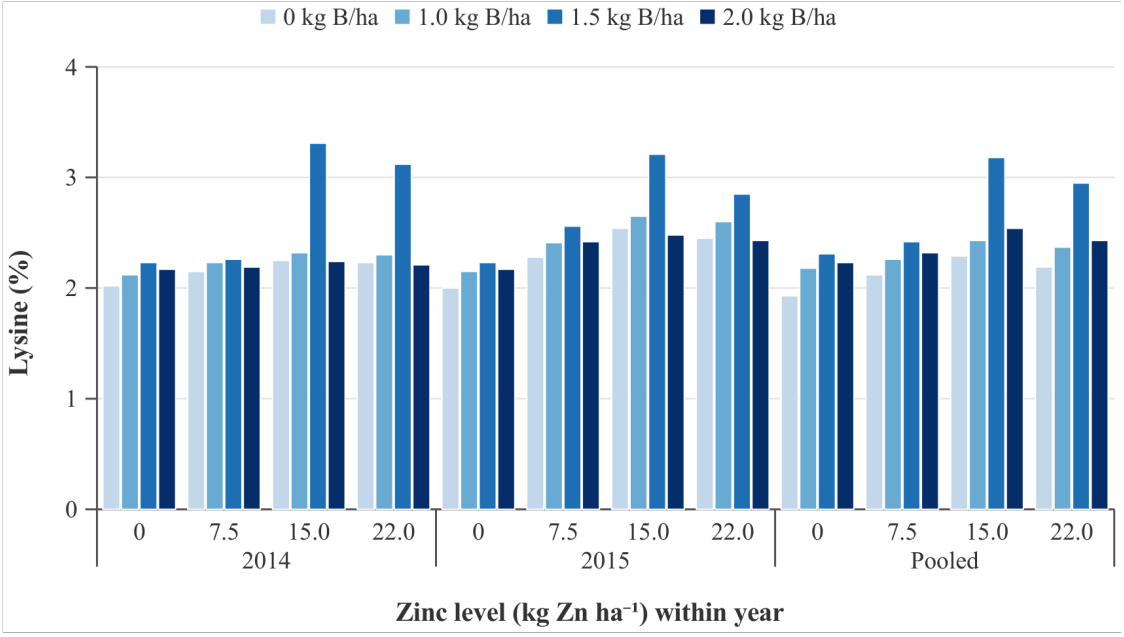


Figure 4. Effect of zinc and boron levels on lysine content (%) of wheat grain in 2014, 2015 and pooled over years. CD ($p \leq 0.05$) values are given in Table 5.

Tyrosine

Tyrosine content increased significantly with Zn and B (Table 6; Fig. 5). In the pooled data, the maximum tyrosine content due to Zn (4.70%) was obtained at 15 kg Zn ha^{-1}, which was significantly superior to the control (4.24%) and on par with 7.5 and 22 kg Zn ha^{-1}. Boron increased tyrosine from 4.34% in the control to 4.81% at 1.5 kg B ha^{-1}, which was significantly higher than all other B levels. The Zn × B interaction was significant, and tyrosine content increased from 4.17% in the control to 5.29% under $Zn_{15}B_{1.5}$.

The increase in tyrosine followed the same pattern as crude protein and lysine, and was attributed to improved nitrogen metabolism and amino acid synthesis under adequate Zn and B supply (Cakmak *et al.*, 1989; Mengel *et al.*, 1982) [7, 19]. The decline at 2.0 kg B ha^{-1} indicated that B in excess of the optimum level did not benefit amino acid accumulation.

Table 6. Effect of zinc and boron on tyrosine content (%) of wheat grain

Zn (kg ha^{-1})	2014					2015					Pooled				
	Boron (kg B ha^{-1})					*Boron (kg B ha^{-1})*					*Boron (kg B 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	3.50	4.51	4.97	4.86	4.46	3.47	4.44	4.87	4.76	4.38	4.17	4.25	4.30	4.24	4.24
7.5	4.64	4.75	5.12	4.97	4.87	4.73	5.12	5.29	4.54	4.92	4.38	4.50	4.54	4.50	4.48
15.0	5.32	5.42	5.53	5.25	5.38	4.94	5.24	6.44	4.67	5.32	4.43	4.51	5.29	4.60	4.70
22.0	5.10	5.37	5.42	5.18	5.26	5.33	5.39	5.48	4.58	5.19	4.39	4.44	5.13	4.54	4.62
Mean	4.64	5.01	5.26	5.06		4.61	5.04	5.52	4.64		4.34	4.42	4.81	4.47	
CD ($p \leq 0.05$)															
Zn			0.198					0.223					0.226		
B			0.187					0.215					0.228		
Zn × B			0.396					0.445					0.443		

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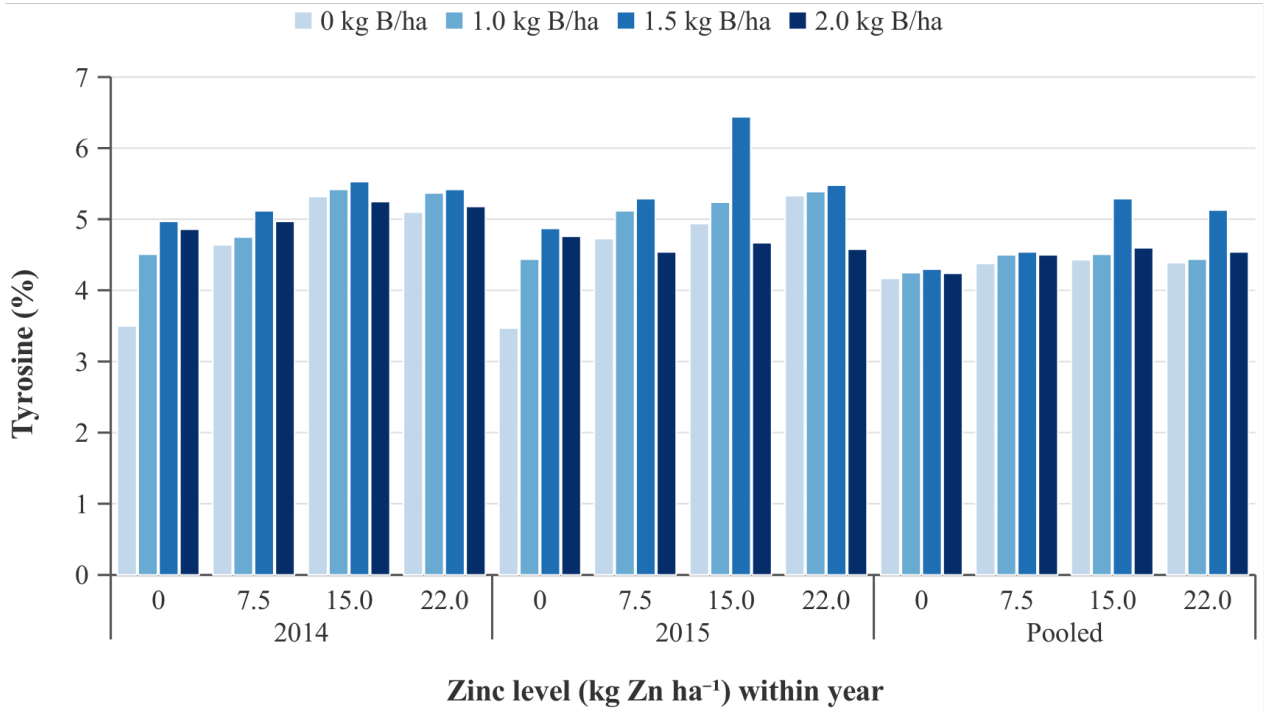


Figure 5. Effect of zinc and boron levels on tyrosine content (%) of wheat grain in 2014, 2015 and pooled over years. CD ($p \leq 0.05$) values are given in Table 6.

Conclusion

Application of Zn and B improved the biochemical quality of wheat grain under the temperate conditions of Kashmir. The combination of 15 kg Zn ha^{-1} and 1.5 kg B ha^{-1} recorded the highest crude protein, gluten, total soluble sugar, carbohydrate, lysine and tyrosine contents, while higher rates of Zn (22 kg ha^{-1}) and B (2.0 kg ha^{-1}) did not bring any further improvement. Combined application of 15 kg Zn and 1.5 kg B ha^{-1} is therefore recommended for producing nutritionally superior wheat grain on similar soils of the Kashmir valley.

Conflict of interest

The authors declare that they have no conflict of interest.

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