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Protein, Amino Acids and Yield of Lentil (*Lens culneris* L.) Expressed Differential Response to Incremental Doses of Sulphur and Boron in Rice Fallow Soil of Mirzapur

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ABSTRACT

Background: Lentil is an important legume crop and plays an important role in human nutrition and soil fertility improvement. It contains 57-60% carbohydrate, 24-26% protein, 3.2% fiberand only 1.3% fat with the full complement of amino acids. It is a potential crop for about 10 million hectares of rice-fallow areas of eastern India. This study aimed to standardized Sulphur and Boron requirements of Lentil in Eastern India.

Methods: The experiment was conducted in a glass house during 2018-19 and 2019-20 in a factorial completely randomized design with three replications and 16 treatment combinations of sulphur and boron. The treatment combinations were product of 4 levels each of sulphur and boron as 0, 15, 30 and 45 kg ha^{-1} (0, 0.067, 0.134 and 0.201 g pot^{-1}) and 0, 1, 2 and 3 kg ha^{-1} (0, 0.004, 0.009 and 0.013 g pot^{-1}), respectively. Lentil grain yield was observed at harvest and sulphur-containing amino acids, as well as seed nutrient content, were analyzed followingstandard operating procedures.

Result: Lentil grain yield was enhanced by 1.85 times with a combined application of 45 kg S and 2 kg B ha^{-1} with a recommended dose of NPK. The sulphur-containing amino acids namely methionine, cystine and cysteine content showed significant improvement with increasing levels of sulphur application however were not significantly affected with boron levels. The protein content in lentil seed increased considerably with application of sulphur levels as compared to boron levels and it was highest with 30kg S ha^{-1} + 2kg B ha^{-1} along with recommended dose of NPK fertilizers.

Key words: Borax, Cysteine, Cystine, Gypsum, Methionine, Protein, Red soil, Rice-fallow.

INTRODUCTION

Lentil (*Lens culneris* L.) is a nutritious food legume that is particularly a rich source of protein for millions around the world. It is especially a low fat containing nutritive source of energy for vegetarian people. Besides nutritious miracle, lentil also synthesizes N with rhizobia in symbiosis and enriches soil fertility and health (Quddus, 2014).

Micro and secondary nutrients have been neglected in Indian agriculture, despite being very necessary for crops, especially pulses and oilseeds. Boron (B), an often-deficient micronutrient, reduces the flower drop, increases the pod setting in pulses and also increased nodulation in pulses. Boron has a direct or indirect role in the absorption of N, P, Kand its deficiency has modified the optimal balance of these three macronutrients (Abou Seeda *et al*., 2021). Sulphur (S) is a secondary essential macronutrientfor plants as it is present in all major metabolic compounds such as amino acids (methionine and cysteine), glutathione, proteinsand sulpho-lipids in oil seeds and pulses (Sexton *et al*., 1998).

Soils of eastern India are deficient in sulphurand micronutrients which hinders pulse production, especially in rice-fallow lands which are the potential area for lentil production (Kumar *et al*., 2019).

This study provides insights into the roles played by sulphur and boron fertilization on lentil yield and quality.

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MATERIALS AND METHODS

Site description and soil characteristics

For two years (2018-19 and 2019-20), the pot experiment was conducted in net house, Department of Soil Science and Agricultural Chemistry, Banaras Hindu University, Varanasi (25°15′53″N and 82°59′27″E, ~80 m above MSL), soil was collected from the Rajiv Gandhi South Campus, BHU, Barkchha, Mirzapur, Uttar Pradesh which is situated at 25°2′25″ latitude and 82°32′24″ longitude in Vindhyan region. The soil is sandy loam in texture with slightly acidic (pH 5.83) soil reaction with low available Nitrogen (181.06 kg ha^{-1}), Phosphorus (9.23 kg ha^{-1}), Sulphur (8.52 mg kg^{-1}), Boron (0.36 mg kg^{-1}) and oxidisable organiccarbon content (3.74 g kg^{-1}).

Experimental setup

The experiment was conducted in a factorial completely randomizeddesign with sixteen treatmentcombinations replicated thrice in pots (10 kg soil per pot). The sowing of lentil seeds (var.HUL-57) and three well established seedlings were allowed per pot for maintaining uniformity and reducing errors. The general recommended dose of fertilizers for the Varanasi region for lentil *i.e*. N-P_2O_5-K_2O 40-60-20 kg ha^{-1} (0.179-0.268-0.089 g pot^{-1}) were used. Urea (source of N), di-ammonium phosphate (source of N and P) and muriate of potash (source of K) were applied in soil in each treatment.

Treatment details

Sulphur and Boron each at four levels *viz*. 0, 15, 30 and 45 kg ha^{-1} (0, 0.067, 0.134 and 0.201 g pot^{-1}) and 0, 1, 2 and 3 kg ha^{-1} (0, 0.004, 0.009 and 0.013 g pot^{-1}) respectively were tested in 16 combinations with RDF (Table 1.). Sulphur and boron were applied as gypsum and borax, respectively.

Chemical analysis

Protein content in lentil seeds was determined by Kjeldahl method (Bremner, 1965). Methionine content was estimated spectrophotometrically by Horn *et al* (1946) method and the cystine and cysteine were analysed calorimetricallyby following Goa (1961) procedure.

Statistical analysis

Recorded data were statistically analyzed by applying the analysis of variance technique (Gomez and Gomez, 1984). The critical difference (5% level of probability) was computed for comparing treatment means in cases where the effect came out to be significant by F-test.

RESULTS AND DISCUSSION

Effect of sulphur and boron application on yield of lentil crop

Seed yield

A perusal of data indicated that sulphur and boron application gave significantly higher grain yield than the application of a recommended dose of NPK alone (Table 2). The results showed that the maximum grain yield (6.86 g pot^{-1}) was recorded with the application of 45 kg sulphur and 2 kg boron ha^{-1} with RDF which was significantly superior to all other treatment combinations. The combined application of Sulphur and Boron with the level S_3B_2 with RDF produced 16.24% more yield as compared to the application of 45 kg S ha^{-1} singly with RDF and 67.15% more as compared to the application of 2 kg B ha^{-1} + RDF without Sulphur. The increasing level of S increased the seed yield but B application beyond 2.0 kg B ha^{-1} caused a reduction in seed yield. This suggests that a negative interaction existed between S and B when boron was applied at a higher rate. The improvement in yield due to higher sulphur levels may be due to its crucial role in energy conversion, enzyme activationand carbohydrate metabolism (Juszczuk and Ostaszewska, 2011).

Stover yield

The pooled data illustrated that among all the treatment combinations, the maximum stover yield (12.74 g pot^{-1}) was recorded in 45 kg sulphur and 2 kg boron ha^{-1} with the recommended dose of NPK which was statistically at par with 45 kg sulphur and 1 kg boron ha^{-1} as well as 45 kg

Table 1: Treatments detail.

Treatments	Notation	Treatment's detail	
		Per ha	Per pot
T_1	S_0B_0	RDF (40: 60: 20, N: P_2O_5: K_2O kg ha^{-1})	RDF (0.179:0.268:0.089 g pot^{-1})
T_2	S_0B_1	RDF + 0 kg S + 1 kg B ha^{-1}	RDF + 0 kg S + 0.004g B pot^{-1}
T_3	S_0B_2	RDF + 0 kg S + 2 kg B ha^{-1}	RDF + 0 kg S + 0.009 g B pot^{-1}
T_4	S_0B_3	RDF + 0 kg S + 3 kg B ha^{-1}	RDF + 0 kg S + 0.013 g B pot^{-1}
T_5	S_1B_0	RDF + 15 kg S + 0 kg B ha^{-1}	RDF + 0.067 g S + 0 kg B pot^{-1}
T_6	S_1B_1	RDF + 15 kg S + 1 kg B ha^{-1}	RDF + 0.067 g S + 0.004 g B pot^{-1}
T_7	S_1B_2	RDF + 15 kg S + 2 kg B ha^{-1}	RDF + 0.067 g S + 0.009 g B pot^{-1}
T_8	S_1B_3	RDF + 15 kg S + 3 kg B ha^{-1}	RDF + 0.067 S + 0.013 g pot^{-1}
T_9	S_2B_0	RDF + 30 kg S + 0 kg B ha^{-1}	RDF + 0.134 g S + 0 kg B pot^{-1}
T_{10}	S_2B_1	RDF + 30 kg S + 1 kg B ha^{-1}	RDF + 0.134 g S + 0.004 g B pot^{-1}
T_{11}	S_2B_2	RDF + 30 kg S + 2 kg B ha^{-1}	RDF + 0.134 g S + 0.009 g B pot^{-1}
T_{12}	S_2B_3	RDF + 30 kg S + 3 kg B ha^{-1}	RDF + 0.134 g S + 0.013 g B pot^{-1}
T_{13}	S_3B_0	RDF + 45 kg S + 0 kg B ha^{-1}	RDF + 0.201 g S + 0 kg B pot^{-1}
T_{14}	S_3B_1	RDF + 45 kg S + 1 kg B ha^{-1}	RDF + 0.201 g S + 0.004 g B pot^{-1}
T_{15}	S_3B_2	RDF + 45 kg S + 2 kg B ha^{-1}	RDF + 0.201 g S + 0.009 g B pot^{-1}
T_{16}	S_3B_3	RDF + 45 kg S + 3 kg B ha^{-1}	RDF + 0.201 g S + 0.013 g B pot^{-1}

sulphur and 3 kg boron ha^{-1} with RDF (Table 2). It could be due to more plant height and branches $plant^{-1}$. Sulphur and boron were found to increase N-fixation, resulting in higher plant growth and development (Parry *et al*., 2016).

Seed sulphur content

The graded simultaneous application of sulphur (S) and boron (B) significantly influenced the sulphur content in lentil seeds (Table 3). Sulphur content in seeds ranged from 0.210 to 0.247 % at varying levels of S and B. Statistical analysis showed, that B application did not significantly influence the S content in seeds. Studies on interaction effects suggest that the treatment combination S_2B_2 (30 kg S ha^{-1} + 2 kg B ha^{-1}) recorded maximum S content of 0.241%, which signifies the positive interaction between two nutrients. Significant improvement in the content of sulphur in lentil seed due to S, Band their interaction could be attributed to easy and enhanced availability of S with an extended root system as a result of external supply of S (Karthikeyan and Shukla, 2008).

Seed boron content

Two years of data analysis revealed that the maximum boron content of 21.09 mg kg^{-1} was recorded with S_3 (45 kg S ha^{-1}) among all other S levelsand maximum B content of 21.53 mg kg^{-1} was found with 3 kg B ha^{-1} (B_3), which was superior to other B levels. The combined application of 45 kg S ha^{-1} plus 3 kg B ha^{-1} (S_3B_3) resulted in maximum boron content of 21.98 mg kg^{-1} followed by S_2B_2 (30 kg S ha^{-1} + 2 kg B ha^{-1}). The little improvement in the boron content in lentil seed due to S, B and their interaction could be attributed to the addition of boron in soil through fertilizer (Chander *et al*., 2010).

Nitrogen content in seeds

The nitrogen content in lentil seeds ranged from 3.49 % (S_0B_0) to 3.94 % (S_3B_2). Among the S levels, maximum N-content of 3.90 % in lentil seeds was recorded with S_2 (30 kg S ha^{-1}) and was found superior to other sulphur levels. Among boron levels, B_2 (2 kg B ha^{-1}) registered a maximum N content of 3.79% in lentil seed but was at par with B_1 (1 kg B ha^{-1}) and B_3 (3 kg B ha^{-1}). Treatment combination S_2B_2 (30 kg S ha^{-1} + 2 kg B ha^{-1}) recorded the maximum N-content of 3.92% in lentil grain whereas lower dose of sulphur with higher dose of boron resulted in lower N content in lentil seed. The increase in the content of nitrogen in lentil seed due to S, Band their interaction could be attributed to increased root activity and bacterial nitrogen fixation (Longkumer *et al*. 2017).

Seed protein content

The protein content of seeds is a very important quality characteristic. Sulphur fertilization was found to have a significant effect on lentil seed protein content than boron. Application of 30 $kgha^{-1}$ of sulphur had a positive effect on protein content (9.77 % higher), however, different levels of boron had no significant effect on protein content (Table 4). Combined application of 30 kg S ha^{-1} + 2 kg B ha^{-1} (S_2B_2) recorded significantly higher protein content of 24.63%. Sulphur helps in N-fixation in legumes and further its better utilization in plants for protein development, this might be

Table 2: Effect of sulphur and boron application on lentil yield (Pooled data for season 2018-19 and 2019-20).

Treatment	Seed yield pot^{-1} (g)					Straw yield pot^{-1} (g)				
	B_0	B_1	B_2	B_3	Mean	B_0	B_1	B_2	B_3	Mean
S_0	3.70	3.93	4.11	4.06	3.95	7.82	8.06	8.39	8.28	8.14
S_1	4.49	4.58	4.87	4.80	4.69	8.97	9.01	9.50	9.42	9.22
S_2	5.22	5.43	5.84	5.82	5.58	10.19	10.51	11.21	11.19	10.77
S_3	5.92	6.47	6.86	6.48	6.43	11.16	12.01	12.74	12.05	11.99
Mean	4.83	5.10	5.42	5.29	-	9.53	9.90	10.46	10.23	-
		S	0.23					0.68		
CD (P =0.05)		B	0.23					0.68		
		S × B	0.45					1.36		

Table 3: Effect of sulphur and boron application on total sulphur and boron content in lentil seed (Pooled data for season 2018-19 and 2019-20).

Treatment	Total sulphur content (%) in lentil seed					Total boron content (mg kg^{-1})in lentil seed				
	B_0	B_1	B_2	B_3	Mean	B_0	B_1	B_2	B_3	Mean
S_0	0.210	0.212	0.214	0.214	0.213	19.19	19.73	20.69	21.10	20.18
S_{15}	0.223	0.225	0.228	0.229	0.226	19.26	20.05	20.95	21.39	20.41
S_{30}	0.239	0.241	0.247	0.246	0.243	19.48	20.91	21.30	21.68	20.84
S_{45}	0.231	0.233	0.236	0.237	0.234	19.58	21.26	21.57	21.98	21.09
Mean	0.226	0.228	0.231	0.232	-	19.37	20.49	21.13	21.53	-
		S	0.012					NS		
CD (P =0.05)		B	NS					1.02		
		S × B	NS					NS		

the reason behind enhanced protein content in seed (Shock *et al*., 1998).

Methionine content

The application of S was found significant however, the impact of B and interaction effects remained non-significant (Table 5). Sulphur applied @30 kg S ha^{-1} (S_2) and 45 kg S ha^{-1} (S_3) registered maximum and similar methionine content of 1.08 g per 16 g N. Among boron levels, B_2 (2 kg B ha^{-1}) and B_3 (3 kg B ha^{-1}) was recorded at 1.02 gmethionine per 16 g N. Interaction between sulphur and boron has been found non-significant to influence methionine content in lentil seeds. Pandurangan *et al*. (2015), also stated that adequate sulphur nutrition is required to maximize the concentration of sulphur amino acids and sulfate fertilization might become necessary for legumes (Szulc *et al*.2014).

Cystine content

The application of sulphur significantly increased the cystine content but boron application does not (Table 5). Thus, maximum cystine content (0.60 g per 16 g N) was found with S_2 (30 kg S ha^{-1}) as compared to other sulphur levels. Among different boron levels, B_2 (2 kg B ha^{-1}) recorded maximum cystine content of 0.56 g per 16 g N. Among different treatment combinations, S_2B_2 (30 kg S ha^{-1} + 2 kg B ha^{-1}) recorded maximum cystine content of 0.63 g per 16 g N. The result is supported by Klikocka *et al*. (2016), who reported that S significantly increased the content of cysteine (by 6.0%) in spring wheat seeds.

Cysteine content

Results indicate that with the increasing level of sulphur, cysteine content also increased up to 30 kg ha^{-1} after that it decreases (Table 5). Among different levels of sulphur, S_2 (30 kg S ha^{-1}) recorded maximum cysteine content of 0.44 g per 16 g N followed by S_3(3 kg S ha^{-1}), however boron didn't showed significant effect. The treatment combination S_2B_2 (30 kg S ha^{-1} + 2 kg B ha^{-1}) recorded maximum cysteine content of 0.47 g per 16 g N. The lower cysteine content was found where sulphur doses were not applied with boron and RDF.

Sulphur is an integral part of S containing amino-acids which are the source of proteins while boron plays an important role in protein and nucleic acid metabolism. The quality improvement in crops due to sulphur and boron application has also been reported by Karthikeyan and Shukla (2008).

Correlation studies

Pearson's correlation coefficients between total nitrogen, sulphur and boron content in seeds with protein, methionine, cystineand cysteine contents in seedare presented in Fig 1. The correlation coefficients were 0.95, 0.97, 0.78 and 0.97 for methionine, cysteine, cystineand protein content concerning Seed sulphur content, respectivelyand are significant at <0.01 P value. Similarly, all the S-containing amino acids and protein content significantly and positively correlated to N content in lentil seeds. However, the positive weak correlation of amino acids *viz*. methionine (0.47),

Table 4: Effect of sulphur and boron application on nitrogen and protein content in lentil seed (Pooled data for season 2018-19 and 2019-20).

Treatment	Nitrogen content (%) in lentil seed					Protein content (%) in lentil seed				
	B_0	B_1	B_2	B_3	Mean	B_0	B_1	B_2	B_3	Mean
S_0	3.49	3.57	3.59	3.57	3.55	21.81	22.31	22.44	22.28	22.21
S_{15}	3.66	3.69	3.72	3.69	3.69	22.84	23.03	23.25	23.03	23.04
S_{30}	3.86	3.90	3.94	3.92	3.90	24.09	24.34	24.63	24.47	24.38
S_{45}	3.82	3.86	3.89	3.87	3.86	23.84	24.13	24.33	24.19	24.12
Mean	3.70	3.75	3.79	3.76	-	23.15	23.45	23.66	23.49	-
		S	0.08					0.52		
CD (P = 0.05)		B	NS					NS		
		S × B	NS					NS		

Table 5: Effect of sulphur and boron application on sulphur-containing amino acids in lentil seed (Pooled data for season 2018-19 and 2019-20).

Treatment	Methionine content (g per 16g N)					Cystine content (g per 16g N)					Cysteine content (g per 16g N)				
	B_0	B_1	B_2	B_3	Mean	B_0	B_1	B_2	B_3	Mean	B_0	B_1	B_2	B_3	Mean
S_0	0.82	0.83	0.83	0.85	0.83	0.48	0.48	0.49	0.47	0.48	0.27	0.28	0.27	0.28	0.27
S_{15}	1.00	1.01	1.05	1.04	1.03	0.56	0.56	0.59	0.58	0.57	0.37	0.36	0.39	0.40	0.38
S_{30}	1.04	1.08	1.10	1.09	1.08	0.60	0.61	0.63	0.56	0.60	0.43	0.44	0.47	0.43	0.44
S_{45}	1.06	1.10	1.08	1.09	1.08	0.52	0.54	0.54	0.52	0.53	0.38	0.39	0.40	0.39	0.39
Mean	0.98	1.00	1.02	1.02	-	0.54	0.55	0.56	0.53	-	0.36	0.37	0.38	0.38	-
		S	0.05					0.03					0.02		
CD		B	NS					NS					NS		
(P =0.05)		S													
		S × B	NS		NS			NS					NS		

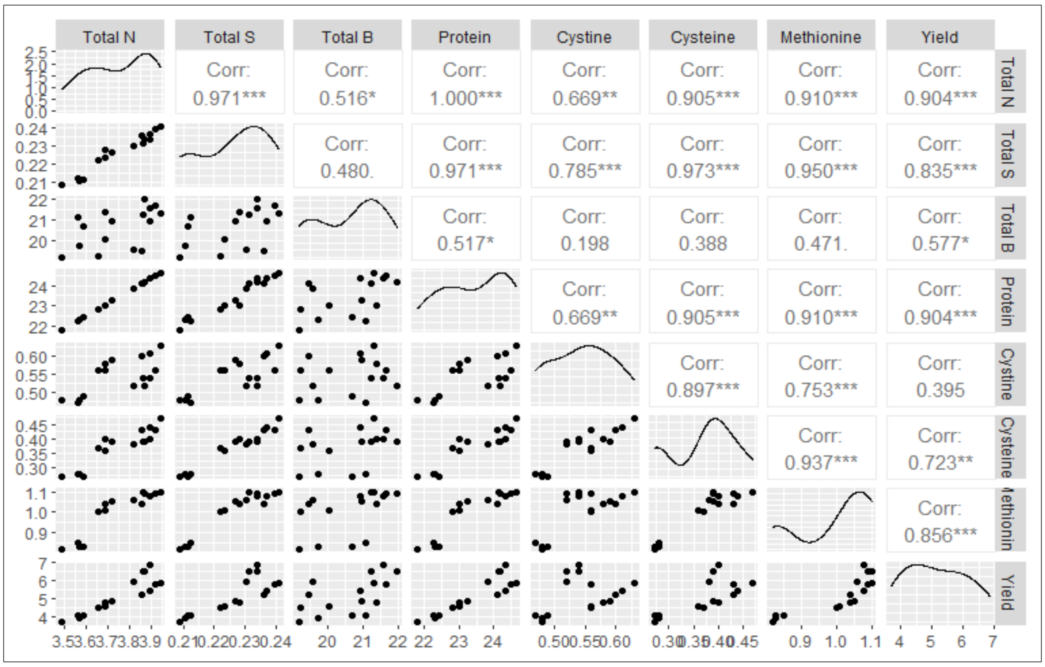

Fig 1: Pearson's Correlation coefficients among nutrient content with sulphur-containing amino acids and grain yield.

cysteine(0.39), cystine(0.20)and protein (0.52) was found with the boron content inlentil seed. It showed the positive impact of sulphur on lentil seed quality whereas, the lesser influence of boron.

CONCLUSION

Based on the present investigation, it canbe concluded that both S and B fertilization have a decisive role in the yield and quality of lentil grown in red soils of eastern India. Results indicated the major role of sulphur in yield and quality improvement which was further supported by the application of boron. The most beneficial effects were obtained when both sulphur and boron were used in fertilization with RDF. Based on these results, the application of 30 kg S + 2 kg B ha^{-1} in red soils of eastern India for rice-fallows areas, would be beneficial in terms of production as well as the quality of pulses.

Conflict of interest: None.

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