Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 2374-2381
Received: 15-05-2019
Accepted: 17-06-2019

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Quality assessment of wheat (*Triticum aestivum* L) grain under the influence of boron and liming materials in an acid soil of sub-Himalayan Terai region

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Abstract
Wheat owns high nutritive value with starch [60-80%], protein [8-15%], fat, sugar, cellulose, minerals and vitamins among the major nutrients. In Terai region, the performance of wheat cultivars hampers due to the presence of excess acidity and deficiency of Boron (B) in the soil. An experiment was undertaken for two years to study the combinatorial effect of lime and boron for evaluation of quality parameters in wheat. The findings revealed that doses of full lime (4.2 t ha^{-1}) and full boron0.5% (seed treatment) and 10 kg ha^{-1} (soil application as Borax) had a significant role on starch content of the grain compared to other quality parameters (gluten, total protein and moisture) under seed treatment and soil application to the crop. Strong positive correlation was observed between quality parameters and yield in both the cases. Micronutrient application (B) through seed treatment performed better than other method of application due to continuous supply of B right from the stage of seed germination. Hence, application of lime and B through seed treatment may be advocated in this area for improving the quality of wheat grain.

Keywords: Wheat, grain quality, lime, boron, seed treatment, soil treatment

Introduction
Wheat is highly nutritious cereal due to presence of starch constituting 60–70% of the mass of wheat grain, and 70–85% in wheat flour (Toepfer *et al*., 1972) [27] with a blending of two glucose polymers: amylose and amylopectin. It influences end-product quality and being the main source of dietary carbohydrate, is very important for human nutrition. Protein content is an important quality component which help to evaluate the suitability of wheat for a particular type including other key factors like mixing tolerance, loaf volume and water absorption capacity (Shah *et al*., 2008) [24]. On the contrary, both quality and quantity of protein are considered important in determining the flour for its end use quality (Farooq *et al*., 2001) [6]. The gluten fraction of protein makes the wheat to produce bread, other baked goods, noodles and pasta, and a range of functional ingredients. It offers ease of grain storage and converting grain into flour for making edible palatable, interesting and satisfying food such as bread, biscuits, cakes, noodles and is widely consumed as a chapattis. Estimation of biochemical quality parameters are necessary to produce flour which is attractive with high nutritional value and a competitive cost.

Boron, is the only micronutrient which present as non- ionized molecule in soil solution over different pH during the plant growth. It has major role in transportation of sugars across cell membrane, amino acid formation and synthesis of proteins (Tisdale *et al*., 2005) [26]. Micronutrients may be applied to the soil, foliar sprayed or added as seed treatments (Johnson *et al*., 2005) [11]. Seed priming is a well-established method to improve the speed and uniformity of germination and is better option from an economical prespective as less micronutrient is needed. (Singh *et al*., 2000) [23].

Soil acidity limits wheat production in many regions of the world including India. The soilsare acidic in reaction and pre-dominantly shallow and coarse-textured soils, poor in organic matters and plant nutrients (N). In the state of West Bengal, typical Terai (Duar) soils are present in Jalpaiguri, Coochbehar and Alipurduar districts with an area cover-age of about two million hacters of land. The soils of Sub-Himalayan Terai region of West Bengal being moderately acidic have a high content of Al, Fe and Mn but deficient in N, P and B.

In spite of possessing other advantages like suitable agroclimatic conditions, optimum photoperiod, superior genotypes. Wheat cultivation in Terai region of West Bengal suffers due to characteristic inherent acidic nature of the soil. The management of an acid soil is required for improving the potential of the soil for higher yield and productivity of wheat crop. Lime as an amendment to neutralize exchangeable Al^{3+} to a certain extent is quite effective as it is responsible for the poor yield of the crops.

Earlier a few studies were undertaken to evaluate effect of lime and boron on assessing the grain quality of wheat, but Limited studies were carried out to analyse the grain quality in response to individual and combined effect of lime and B applications (different sources and methods of application) particularly in acid soil. Based on the above perspectives, the experiment was conducted to assess the effect of lime and B on important quality parameters of wheat.

Materials and Methods

A field experiment was conducted (2014-15 and 2015-16)with wheat (Cv. K1009 @100kg ha^{-1}) following randomized block design (RBD) which was having 54 plots (4 m × 3 m)at the research farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India. (Table 1 and Table 2).

Boron (B) as boric acid for seed treatment @ 0.5% and 0.25% as full and half dose (soaking of seed in boric acid solution for 10-12 hrs. then sun dried) and borax as soil application @10kg ha^{-1} and 5 kg ha^{-1} (full and half dose respectively) were applied during the experiment. Likewise, lime, 15 days prior to sowing of crop @ 4.2 tons ha^{-1} and 2.1 tons ha^{-1} (full and half dose) were incorporated with the recommended doses of N, P and K.

The uptake of B by plants was measured by dry ashing and subsequent measurement of B by colorimetry using Azomethine – H and soil residual status of B by hot water extraction method followed by colorimetry measurement using Azomethine – H at CRI, flowering and maturity stages.

The crop was harvested at maturity after about 4 months of sowing and the grains were analysed for different biochemical parameters (viz. protein, gluten, moisture, starch and amylase). Soluble protein estimation of the wheat grain was done by Lowry's method using centrifugation by centrifugation unit followed by colour development (Lowry *et al*., 1951) [15] and reading was taken in a spectrophotometer at 660nm. Starch content of wheat flour was estimated by anthrone reagent method (Hodge *et al*., 1962) [10] at 630nm while Amylose content of the wheat grain was determined by the iodine method (McCready*et al*., 1950) [18] at 590 nm. The Gluten content of wheat flour was estimated by the wet and dry gluten method. (AOAC; 2016) [1]. Moisture content of wheat flour was determined by the hot air oven method (AOAC; 2016) [1].

The statistical analysis for the soil and plant data was computed by software (SPSS version 2017) while for the quality parameters the XL STAT (Addinsoft, Paris, France) was used. Further, principal component analysis (PCA) was conducted to evaluate the effects of different treatment combination on the quality parameters of wheat.

Results and Discussion

A research experiment was undertaken for two years (2014-15 and 2015-16), with an aim to study the combinatorial effect of different doses of lime and different mode of application of Boron including seed and soil application for evaluation of quality parameters in wheat. The residual status of B in soil under three growth stages of wheat plant namely CRI, flowering and at the time of harvest was determined. The available boron (kg ha^{-1}) in soils (Fig. 1 (a)) at different growth stages of the crop at the seed- treated plots varied from 1.91 to 2.42 (kg ha^{-1}) at CRI, 1.29 to 1.92 (kg ha^{-1}) at flowering and 0.67 to 1.46 (kg ha^{-1}) at harvest. In contrast, the B content for the soil- treated plots varied from 1.45 – 2.07 (kg ha^{-1}) at CRI, 1.05 – 1.63 (kg ha^{-1}) at flowering and 0.63 – 1.26 (kg ha^{-1}) at harvest respectively (Fig. 1(b)). Significant differences was found on extractable- boron among the soil and seed treated plots at different treatments which were quite higher in T_9, T_6 and T_3, where, full doses of Boron were applied irrespective of the application of lime. It was also observed that the extractable 'B' in seed- treated plots was relatively higher than the soil- treated plots in all the growth stages (CRI, flowering and harvest) of the crop (Fig.1).

In earlier studies (Barman *et al*., 2014) [2], it was revealed that there was increase in the extractable B in soil with the increasing level of applied B and lime as compared to control. It is worthwhile to mention that liming in an acid soil, increased the B adsorption capacity in soil due to formation of insoluble metaborate (Mikko, 1972) [19] coupled with higher adsorption of soluble B on the surface of the freshly precipitated amorphous Fe and Al oxides and hydroxides in soils at enhanced pH (Tisdale *et al*., 2005) [26]. In the present study, it was found that in both the mode of application of B, the maximum residual soil B was obtained in treatment receiving full dose of lime and boron, which means, Ca present in lime have synergistic effect with B availability in soil.

We tried to correlate this findings with the plant B content at the different growth stages of wheat plant. The boron content (mg kg^{-1}) at CRI and flowering and boron uptake (kg ha^{-1}) by the above ground part of the plants (Fig.2) showed that, the B content (mg kg^{-1}) at CRI and flowering stages, varied from 2.05 to 6.45 (mg kg^{-1}) at CRI, 2.12 – 7.34 (mg kg^{-1}) at flowering and the uptake (kg ha^{-1}) from 0.27 at 0.87 (kg ha^{-1}) at harvest in the seed- treated samples, the maximum being in the treatment T_9 in all the cases (NPK+LFBF). The variation (mg kg^{-1}) of the same was observed from 1.78 - 6.12 (mg kg^{-1}) at CRI, 1.82 – 6.92 (mg kg^{-1}) at flowering and uptake (kg ha^{-1}) from 0.24 – 0.68 (kg ha^{-1}) at harvest under the soil- application of nutrients. It was also found that, there were significant differences among the B content (mg kg^{-1}) and B uptake (kg ha^{-1}) corresponding to the different treatments, both at the seed - treated and soil- treated plots (Fig 2) and the average B uptake was quite higher in plants (0.63 kg ha^{-1}) under a seed treatment over that of (0.49 kg ha^{-1}) under soil application of nutrients. It was apparent that with the gradual increase of the doses of the B either in seed or in soil, the B uptake pattern also changed accordingly irrespective of the lime application.

These results are in agreement with Kizilgoz *et al*., 2010 [13] who reported that in wheat, with the increase in soil application of B there was an increase in shoot B concentrations. In another experiment, Kanval *et al*., 2008 [12] also demonstrated that, the application of both B and Ca increased shoot dry matter in maize which was under hydroponic study. Envas *et al*., (1994) [5] reported that in an acid soil the application of $CaCO_3$ increased the shoot growth in alfa-alfa and a linear relationship between plant B concentration and plant available B concentration in the soil was observed depending on Ca^{2+} addition.

The uptake of B (kg ha^{-1}) by wheat grain varied from 0.11– 0.39kg ha^{-1} (Fig.3) with an average of 0.26 kg ha^{-1} under the seed- treated plots while the same varied from 0.10– 0.35kg ha^{-1} in the plots treated with soil- application of nutrients. It was apparent that, under the seed- treated plots, the B uptake was significantly higher, over the soil treated plots. The maximum B uptake was at the treatment T_9 followed by T_6 and T_3 in accordance with the doses of lime and boron. Gomaa *et al*., 2015 [8] studied that application of micronutrients significantly increased concentration of micronutrients in grains. On the other hand, Debnath *et al*., 2011 [4] reported that B application had significant and positive effect on the B uptake by grain and straw. and in both the cases, the lowest B uptake was found under control.

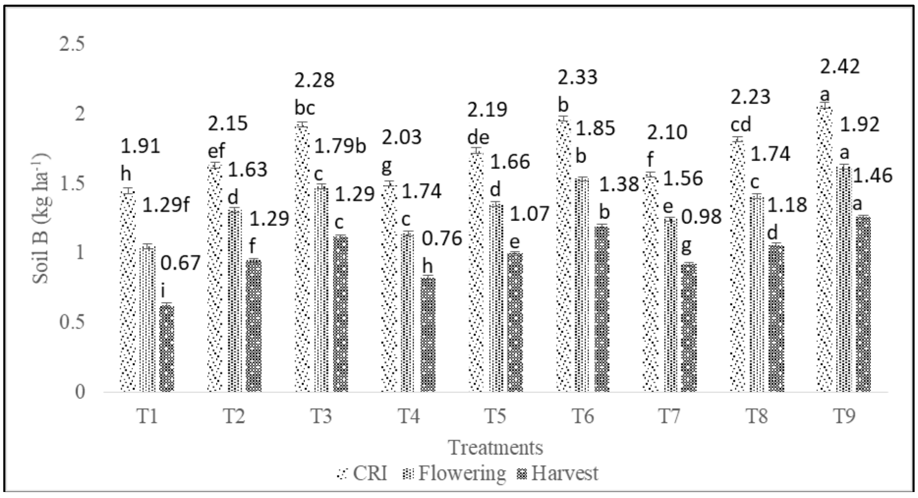
To correlate our earlier findings with Grain quality assessment we have estimated five biochemical parameters (Table 3) namely total protein content, gluten content, total starch content, amylose content and moisture content (%). In all the cases, maximum value was observed in (Table 3) T_9, i.e., 8.1%, 17.6%, 7.3%, 1.26% and 14.4% for protein content, gluten content, total starch content, amylose content and moisture content (%) respectively, whereas, for seed treated plots the parameters varied with an average of 6%, 13.5%, 4.6%, 0.88% and 12.9% respectively. Similarly, the maximum grain quality of wheat was observed in $T_{9,}$ i.e., 7.3%, 14.5%, 6.8% 1.01% and 13.37% under soil treated plots with an average of 5.7%, 11.9%, 4.05%, 0.74% and 10.81%. There was overall variation of 0.8%, 3.1%, 0.5% 025% and 1.03% in total protein, gluten, total starch, amylose and moisture content from seed treated plots *vis−a−vis* soil application of B.

When we tried to correlate between quality parameters (%) and yield data (kg ha^{-1}) (Table 4 and 5) under seed treated plots and soil treated plots a negative correlation was there between amylose content and yield in both cases. Under seed treated plots, except total starch content, other quality parameters were significant at 1% level with positive correlation (0.885**, 0.727** and 0.865**), while the total starch was significant at 5% level (0.456*). On the other hand, positive correlation with yield parameters was observed in which all the quality parameters were significant at 1%, i.e, 0.981**, 0.685**, 0.971** and 0.988** for soil treated plots. In an another correlation study between Grain B uptake (kg ha^{-1}) and the quality parameters (%) (Table 6 and 7), it was found that under seed treated plots, the correlation between grain B uptake and total protein, total starch, gluten and moisture were all significant at 1% with the values of 0.874**, 0.528**, 0.783** and 0.964**. Similarly, under soil treated plots, the quality parameters (total protein, total starch, gluten and moisture) were correlated with grain B uptake at 1% level of significance, viz. 0.908**,0.736**, 0.972** and 0.936**.

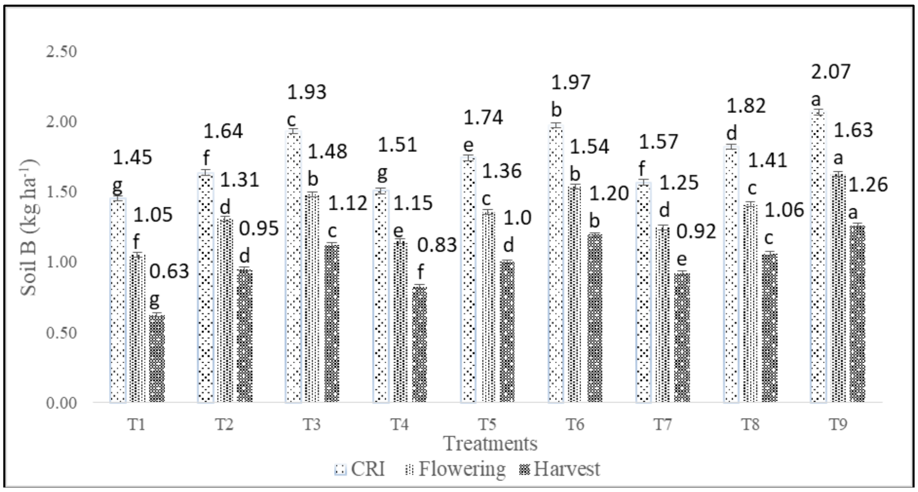
Principle component analysis (PCA) was performed to determine the ability of the effects of different treatment combination on the quality parameters of wheat. The first two PCs (Principle Components) constituted 90.86% and 88.62% [Fig -4(a) and (b)] of the total variation when seed treated with boron while that for 82.93% and 78.83% [Fig-5 (a) and (b)] when treated with soil application of Boron. It was further observed that the doses of full lime and full boron application had a significant role towards the starch content of the grain compared to other quality parameters (gluten, total protein and moisture) in both the cases.

Perten *et al*.,1992[21] found that the increase in protein content of the wheat flour results in an increase in gluten content, which is considered to be the most important parameter of wheat flour. The first four components were used to produce a scatter plot which was used to choose the number of PCs in the classification. The paired-wise scatter plots of the first two PCs were produced to generate the idea of the effect of lime and boron on the quality parameters of wheat. Information on the effects of microelement fertilizer on wheat grain yield and nutritional quality is still lacking. Boosting wheat production on low boron soils with B efficiency is an emerging issue especially in acid soils of developing worlds.(Rerkasen *et al*., 2004) [22].Our results showed that B fertilization can have positive effects on wheat yield and grain quality when applied with seed, but additional research is still needed in order to understand more about the most effective application methods.

In a study (Moeinian *et al*.,2011) [20], it was found that increased in the gluten percentage can be due to the role of B in making congenial conditions to increase the nitrogen and phosphorus uptake efficiency during grain and protein formation. According to the boron effect in increasing sugar and hydrocarbons transport through phloem, it has prominent role in enhancing the fruit quality. (Malakoti *et al*., 2008) [17].Similarly, Ferdoush *et al*., 2013[7] reported that B application has a significant increasing effect on the protein content of wheat grain. Buresova *et al*., 2010[3] observed that there was positive correlation between the starch content and amylose content in grain. The increase in gluten percentage of wheat grain may be due to the role of B in increasing the nitrogen and phosphorus uptake efficiency during grain and protein formation (Moeinian *et al*., 2011) [20].



(a)



(b)

Fig 1: Residual B status (kg ha^{-1}) in soil at different growth stages of wheat under different treatments (a) Seed- treated plots and (b) Soil- treated plots. Error bars indicate the SEM

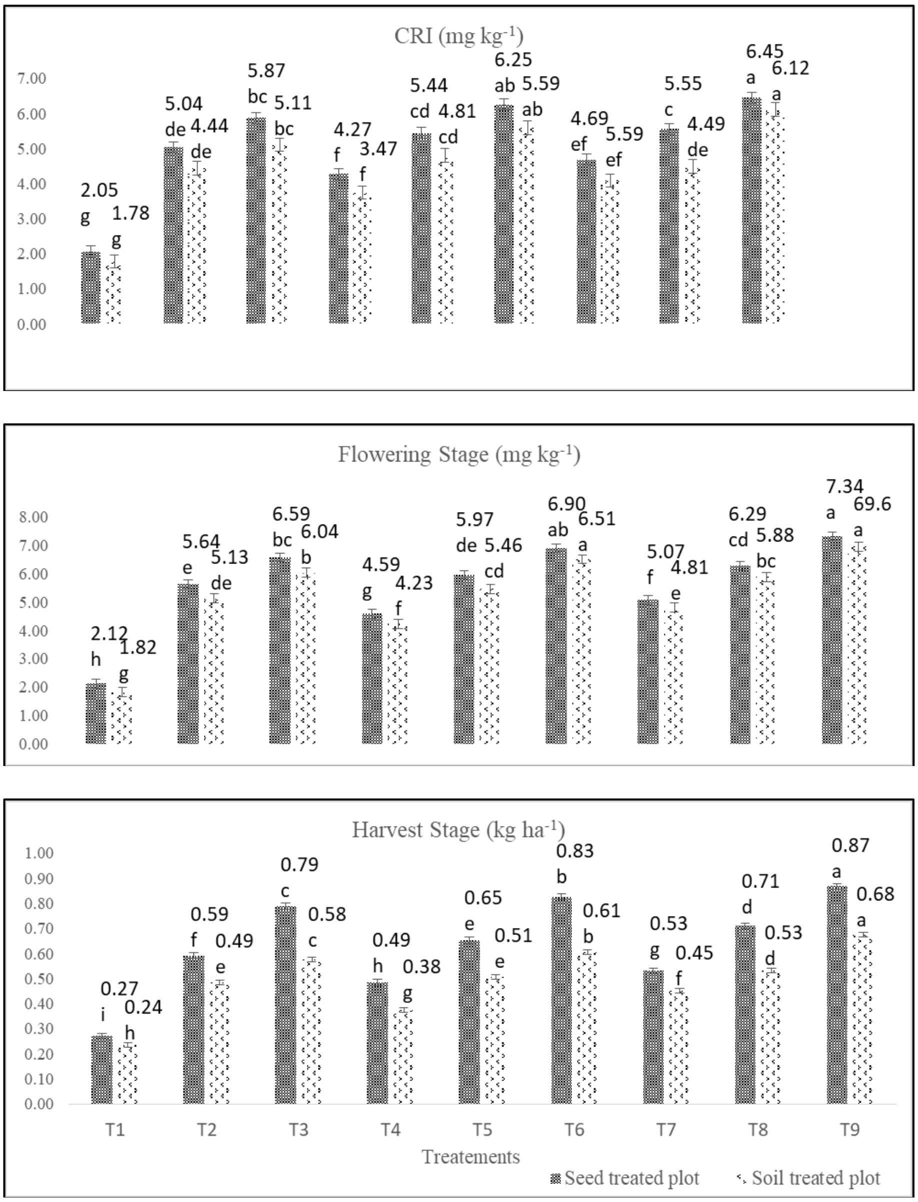


Fig 2: Plant B content in CRI (mg kg^{-1}), Flowering (mg kg^{-1}) and Uptakeat Harvest (kg ha^{-1}) of wheat under different treatments in Seed- treated plots and Soil- treated plots. Error bars indicate the SEM.

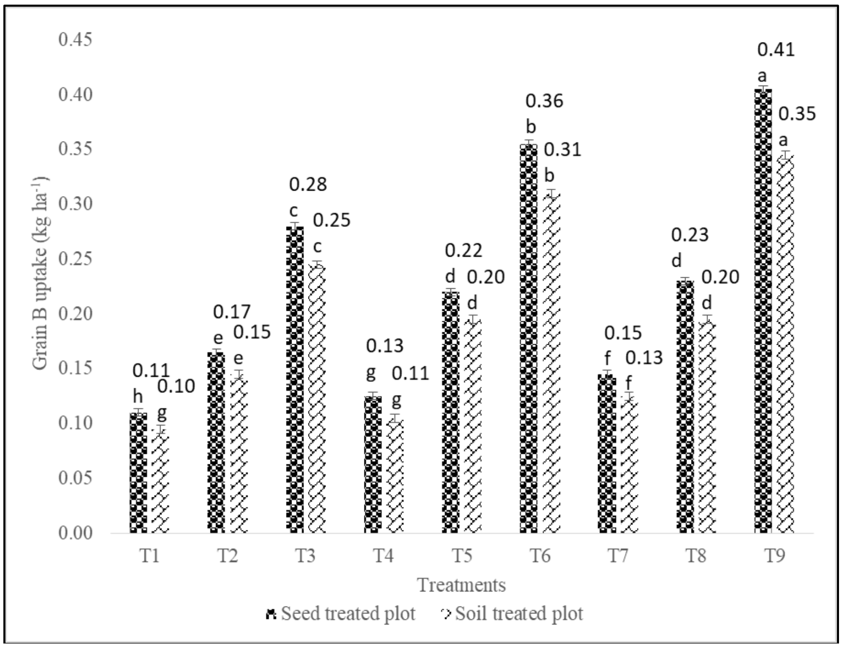


Fig 3: Grain B uptake (kg ha^{-1}) under Seed- treated plots and Soil- treated plots. Error bars indicate the SEM

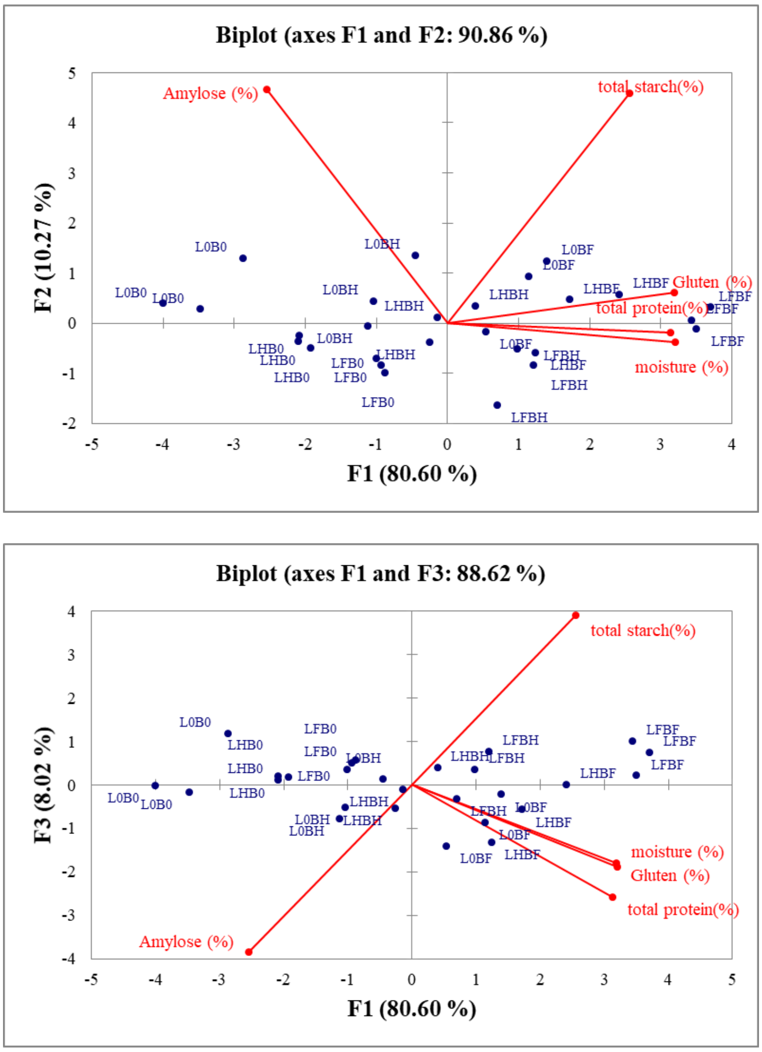


Fig 4: PC Biplots for quality parameters under Seed - treated plots of wheat grain

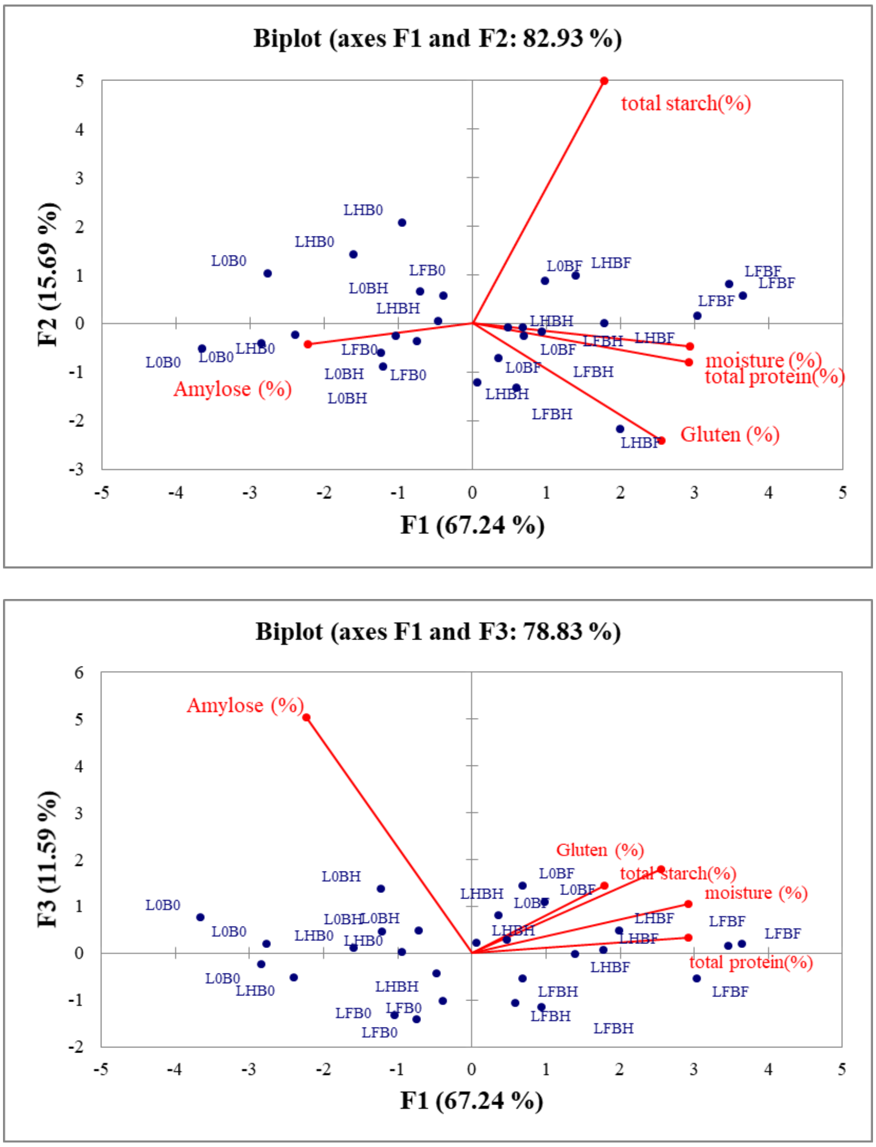


Fig 5: PCBiplots under soil - treated plots of wheat grain

Table 1: Details of treatment combinations

Treatment Combinations	
T_1	Control
T_2	NPK+L0BH
T_3	NPK+L0BF
T_4	NPK+LHBH
T_5	NPK+LHBH
T_6	NPK+LHBF
T_7	NPK+LFB0
T_8	NPK+LFBH
T_9	NPK+LFBF

L0	No lime	B0	No boron
LH	Half lime	BH	Half boron
LF	Full lime	BF	Full boron

Sources and Doses of fertilizers

	Source	Doses
Nitrogen	Urea	@ 100 kg ha^{-1}
Phosphorus	SSP (Single Super Phosphate)	@ 60 kg ha^{-1}
Potassium	MOP (Murate of Potash)	@40 kg ha^{-1}
Lime	$CaCO_3$	@4.2 t ha^{-1} (full dose), 2.1t ha^{-1} (half dose)
Boron	Boric acid (Seed treatment)	@ 0.5% (full dose), 0.25% (half dose)
	Borax (Soil treatment)	@ 10 kg ha^{-1} (full dose), 5 kg ha^{-1} (half dose)

Table 2: Details of Experiment

Season	*Rabi*
Tillage	Conventional
Variety	K1009
Plot Size	4m×3m
No. of Seed treated plots	27
No. of Soil treated plots	27
Spacing	23 cm

Table 3: Assessment of some quality parameters of wheat grain of the seed -treated and soil -treated plots

Treatments	Seed treatment					Soil Treatment				
	Total Protein (%)	**Gluten Content (%)**	**Total Starch (%)**	**Amylose content (%)**	**Moisture Content (%)**	**Total Protein (%)**	**Gluten Content (%)**	**Total Starch (%)**	**Amylose content (%)**	**Moisture Content (%)**
T_1	3.7	9.8	3.2	0.51	8.80	3.6	8.7	3.0	0.45	7.70
T_2	5.7	12.9	4.0	0.76	10.70	5.7	11.9	3.8	0.71	10.13
T_3	6.8	14.7	5.4	1.10	12.73	6.6	13.5	4.6	0.84	12.10
T_4	4.5	10.5	5.5	0.61	9.60	4.6	9.3	2.6	0.53	9.07
T_5	6.1	14.3	4.1	0.90	11.37	5.9	11.5	4.0	0.76	10.90
T_6	7.2	16.2	5.0	1.20	13.50	6.8	13.8	4.7	0.91	12.53
T_7	5.4	11.6	3.8	0.67	10.30	5.1	10.7	3.2	0.66	9.90
T_8	6.6	14.3	3.9	0.99	11.77	6.3	13.2	3.8	0.79	11.67
T_9	8.1	17.6	7.3	1.26	14.40	7.3	14.5	6.8	1.01	13.37
AVERAGE	6.0	13.5	4.6	0.88	12.9	5.7	11.9	4.05	0.74	10.81
S.E(±)	0.040	1.254	0.658	0.003	0.022	0.013	0.022	0.371	0.0002	0.015
LSD (P=0.05)	.119	3.761	1.971	.008	.067	0.040	0.067	1.113	0.0007	0.046

Table 4: Correlation matrix among Yield (kg ha^{-1}) and quality parameters under seed −treated plots

Variables	Amylose (%)	Total Protein (%)	Total Starch (%)	Gluten (%)	Moisture (%)	Yield (kg ha^{-1})
Amylose (%)	**1**	-.626**	-.352	-.440*	-.574**	-.681**
Total Protein (%)	-.626**	**1**	.443*	.775**	.938**	.885**
Total Starch (%)	-.352	.443*	**1**	.264	.499**	.456*
Gluten (%)	-.440*	.775**	.264	**1**	.785**	.727**
Moisture (%)	-.574**	.938**	.499**	.785**	**1**	.895**
Yield(kg ha^{-1})	-.681**	.885**	.456*	.727**	.895**	**1**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 5: Correlation matrix among Yield (kg ha^{-1}) and quality parameters under soil −treated plots

Variables	Amylose (%)	Total Protein (%)	Total Starch (%)	Gluten (%)	Moisture (%)	Yield (kg ha^{-1})
Amylose (%)	**1**	-.664**	-.495**	-.659**	-.715**	-.725**
Total Protein (%)	-.664**	1	.647**	.952**	.971**	.981**
Total Starch (%)	-.495**	.647**	**1**	.725**	.675**	.685**
Gluten (%)	-.659**	.952**	.725**	**1**	.978**	.971**
Moisture (%)	-.715**	.971**	.675**	.978**	**1**	.988**
Yield(kg ha^{-1})	-.725**	.981**	.685**	.971**	.988**	**1**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 6: Correlation matrix among Grain B Uptake (kg ha^{-1}) and quality parameters under seed− treated plots

Vatiables	Grain B uptake (kg ha^{-1})	Amylose (%)	Total Protein (%)	Total Starch (%)	Gluten (%)	Moisture (%)
Grain B uptake (kg ha^{-1})	**1**	-.516**	.874**	.528**	.783**	.964**
Amylose (%)	-.516**	**1**	-.626**	-.352	-.440*	-.574**
Total protein (%)	.874**	-.626**	**1**	.443*	.775**	.938**
Total starch (%)	.528**	-.352	.443*	**1**	.264	.499**
Gluten (%)	.783**	-.440*	.775**	.264	**1**	.785**
Moisture (%)	.964**	-.574**	.938**	.499**	.785**	**1**

******. Correlation is significant at the 0.01 level (2 tailed).
*. Correlation is significant at the 0.05 level (2- tailed).

Table 7: Correlation matrix between Grain B Uptake (kg ha^{-1}) and quality parameters under soil− treated plots

Variables	Grain B uptake (kg ha^{-1})	Amylose (%)	Total Protein (%)	Total Starch (%)	Gluten (%)	Moisture (%)
Grain B uptake (kg ha^{-1})	1	-.615**	.908**	.736**	.978**	.936**
Amylose (%)	-.615**	1	-.664**	-.495**	-.659**	-.715**
Total Protein (%)	.908**	-.664**	1	.647**	.952**	.971**
Total Starch (%)	.736**	-.495**	.647**	1	.725**	.675**

Gluten (%)	.978**	-.659**	.952**	.725**	1	.978**
Moisture (%)	.936**	-.715**	.971**	.675**	.978**	1

******. Correlation is significant at the 0.01 level (2 tailed).

*. Correlation is significant at the 0.05 level (2- tailed).

Conclusion

Liming increases B availability in an acid soil conditions which ultimately helps B in carbohydrate metabolism, transport of sugar, cell wall differentiation etc. In a given study, it was found that, with increasing level of lime and B the quality parameters like protein, starch, gluten etc. were increased. There was synergistic effect between lime and boron. Micronutrient application through seed treatment performed better than other method of application right from the seed germination, may be due to the supply of B. It is easy and cost effective method of application, which can be adopted by the resource poor farmers. Hence, application of lime and B through seed treatment may be advocated in this area for enhancing the quality of wheat grain.

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