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Boron bioavailability enhanced by foliar applied fulvic acid to improve grain yield and quality of fine basmati rice

Atif Kamran[1]✉, **Irzam Naveed**[1], **Summera Jahan**[1], **Lubaba Komal**[1], **Manzer H. Siddiqui**[2], **Saud Alamri**[2] & **Arij Khalil**[3]

Boron is a critical yet poorly understood micronutrient, especially regarding its transport within plant leaves. Little research has been done to enhance the bioavailability of Boron in rice using organic compounds like fulvic acid for better nutrient quality and yield. While fulvic acid (FA) is well-known for enhancing the mobility of metallic nutrients like iron (Fe) and zinc (Zn), however, its role in facilitating Boron, a metalloid, remains unclear. Therefore, this study aimed to evaluate the potential of FA in mediating Boron transport to improve grain quality and yield in Basmati rice. A field trial was conducted using a randomized complete block design (13 × 24 ft²), applying foliar treatments of Boron alone and Boron combined with FA at critical growth stages (tillering and panicle formation). The study assessed the impact of these treatments on rice morpho-physiological parameters, grain yield and leaf Boron content. The results revealed that both foliar treatments significantly improved yield and leaf Boron content over control. However, the promising results were obtained in response to boron's only application. Boron application (T2) significantly increased number of tillers (41%), chlorophyll content (55%), 1000 grain weight (32%), Boron content (131%) and yield per plot (43) as compared to control (T1). While combined application Boron + FA (T3) substantially enhanced number of tillers (34%), fertile florets per panicle (23%), Boron content (46%) and yield per plot (33%) over control (T1). The present findings suggest that Boron foliar application enhances grain weight, yield, and rice quality while reducing panicle sterility. However, FA did not significantly mediate Boron uptake in rice, indicating limited interaction between FA and this non-metallic micronutrient.

Keywords Fertile florets per panicle, Fulvic acid, Leaf Boron content, Panicle sterility, Tillering

Rice (*Oryza sativa* L.) is an important food source as 50% of world's inhabitants depend on rice as a staple food and the demand is increasing continuously[1,2]. It has nutritional and agronomic value globally, providing energy (21%) and protein (15%) required by human population[3]. Almost 90% of the world rice production occurs in Asian countries[4]. Rice ranks second among cereal cash crops after wheat, covers 11% of the total cropped area, and commonly cultivated in wet environments[5]. Rice is cultivated on approximately 167 million hectares worldwide, producing an annual yield of 782 million tons[6]. According to United Nation (UN) predictions, from 2020 to 2025 the world's inhabitants will increase from six to eight billions and 40% more rice will be needed[7]. This substantial increase in food requirement is directly associated with mineral nutrition of human diet received from several food crops[8].

Mineral nutrition of plants is necessary for controlling physiological and biochemical processes of plants. They are not only crucial for plant growth but also the basic need for human health. Boron is a member of metalloid elements and is essential micronutrient in plants which leads to better pollination with improved seed setting as well as grain formation[9]. Although, it is required in low amounts but has a primary role in cell membrane integrity and cell wall biosynthesis. Its presence has an important role in improving plant height and chlorophyll content. It is also required for protein synthesis, nitrogen metabolism and antioxidative systems[10]. The deficiency of Boron influences the productivity of 132 crops in 80 countries approximately[11]. Reduction in pollen fertility and grain filling causes severe crop loss just because of Boron deficiency[12]. Its deficiency in plants

[1]Institute of Botany, University of the Punjab, Lahore 54590, Pakistan. [2]Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia. [3]College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, China. ✉email: atif.botany@pu.edu.pk

hinders pollen tube development as well as pollen germination. In rice, Boron deficiency causes reduction of pollen fertility because of poor pollen and anther development, reduced pollen germination and deforms cell wall pectin in pollen tube. It results in decreased number of grains per panicle as a result rice yield decreased[13]. In Pakistan, approximately 35% of 2.6 Mha rice areas suffer from deficiency of Boron[14]. Boron also affects zinc uptake and transport in plants. It has been shown that Boron deficiency can lead to reduced zinc uptake, while excessive Boron can inhibit zinc transport. Boron also plays a role in regulating iron uptake and transport in rice. About 33% acute boron deficit rice fields have been reported in India[15]. Given Boron's essential role in cell wall biosynthesis and reproductive development, its deficiency can severely impact rice yield and grain quality.

To deal with Boron deficiency, it can be applied to soil as well as foliar application. Foliar application of Boron not only provides a way to apply Boron at specific growth stage but also removes deficiency of Boron[11,16]. Humic substances are natural organic compounds including humin, humic and fulvic acid (FA). In these substances, FA has comparatively less molecular weight along with high amount of oxygen rich and carbon poor functional groups[17]. The FA provides increased drought resistance, enhanced nutrient uptake capacity, stabilized soil pH and decreased fertilizer leaching[18]. Humic substances (humic and FA) attract positive ions, form the chelate with micronutrients and provide slowly when required to the plants[19]. The FA is also involved in Zn transport and metabolism. It helps to regulate Zn uptake, absorption, and utilization in cells. The FA also interacts with Fe, enhancing its absorption and transport[20]. The FA deficiency can lead to impaired Fe uptake in rice and increased risk of chlorosis. Foliar application of fulvic acid proves to be more efficient as compared to humic acids based on its solubility at lower pH values.

Given FA's ability to enhance micronutrient bioavailability, this study investigates whether FA similarly improves Boron transport and uptake. In this study, the combined application of boron and Fulvic acid was applied to investigate Boron bioavailability, its uptake and transport. The objectives of the current study involved evaluating the effect of Boron foliar application on morpho-physiological attributes of rice and to understand the interaction of combined application of Boron and FA in improving grain yield and quality.

Materials and methods

Experimental site

A field trial was conducted at Seed Center, University of the Punjab, Lahore (N31°30 × 3.70008´´ E74°18 × 1.7028´´), to assess the Boron bioavailability mediated by Fulvic acid and its impacts on morpho-physiological as well as yield contributing parameters of rice. The experiment was carried out during 2022–2023 rice growing season.

Sampling and field Preparation

Seeds of three rice cultivars i.e. Kainat, Super and Super Chanab were received from Kala Shah Kaku, Rice Research Institute, Lahore. The field was prepared for cultivation and solution of Boron (2–3 Kg ha^{-1}) and FA (0.5 L $acre^{-1}$) were made for application. Urea, DAP, and SOP were supplied to soil for N: P: K @ 100:30: 30 Kg ha^{-1}. The required amount of these fertilizers was calculated as:

$$\text{Required amount of fertilizer} = \text{fertilizaer application rate} \times \text{area to be fertilized}$$

Experimental design and treatments

The experiment layout comprised of Randomized Complete Block Design (RCBD) having three replicates with three treatments and three rice cultivars. The total experimental area was (13 × 24) ft^2 divided into two equal blocks with subsequent division into nine equal plots of size 2 × 2 ft^2. These nine plots represented each replicate of three treatments applied on three cultivars. The treatments included T1 (Control), T2 (foliar applied borax as Boron solution) and T3 (Boron + FA foliar solution as10% w/v).

Treatment plan and growth conditions

The treatments were given at the tillering and panicle formation stage in the form of foliar spray. Treatment T2 was prepared as 26 g of Borax dissolved in 1080 mL of deionized water. On the other hand, T3 foliar solution was prepared by dissolving 108 g of FA in 1080 mL of water. For T3, we dissolved 13 g of Boron in stock solution. Then at the time of spray 7 mL from each stock solution was diluted to 100 mL and used for each plot. The foliar treatments were applied at two stages i.e. tillering (43 days after sowing (DAS)) and panicle formation (58 DAS).

Data collection

Measurements of vegetative parameters of rice, three plant samples, being representatives of treatments per plot, were taken after 10 days of foliar spray. Flag leaf from each plant sample was collected for chlorophyll, carotenoid and Boron content estimation. Data for reproductive and proximate parameters of rice were taken after maturity.

Estimation of photosynthetic pigments

Pigment analysis in leaves of rice was estimated by Arnon's method[21]. Chlorophyll a and chlorophyll b contents were observed by recording absorbance at the wavelength of 663 nm and 645 nm respectively. The following equation was then used to calculate total chlorophyll content in each sample.

$$\text{Total chlorophyll content}\left(\frac{\text{mg}}{\text{g}}\right) = 2.02\,(\text{A645}) + 8.02\,(\text{A663})\ \text{V}/1000 - \text{W}$$

The total carotenoids were estimated by recording the absorbance at 480 nm[22]. Using the following equation, total carotenoids were calculated.

$$\text{Carotenoid content}\left(\frac{\text{mg}}{\text{g}}\right)=\frac{1000\,(A_{480})-3.7\,(A_{663})-104\,(A_{645})}{227}$$

Protocol for boron content

Leaf Boron content was analyzed by colorimetric method using azomethine-H reagent[23] From acid extract of rice plant leaf tissue ash. Samples (100 mg rice leaves) were dry ashed in convection oven at 100℃ for 3 days and ash extracted in 10 mL of 0.36 N H_2SO_4 for 1 h at room temperature. Several acidic extractants like buffer solution, masking solution, azomethine H, Boric acid stock solution and extracting conditions were compared for the accurate values after heating and readjusting the final volume. Ammonium acetate (100 g) was dissolved in a beaker containing 160 mL acetic acid and 20 mL of water (pH 4.5). The masking solution was prepared by dissolving disodium salt of EDTA i.e., ethylene diamine tetra acetic acid (50 g) and trisodium salt of NTA i.e. nitrilotriacetic acid (20 g) in 300 mL of water. The pH of the solution was adjusted to 4.5 with acetic acid and then dilute to 1000 mL. The azomethine H (1.00 g) and ascorbic acid (2.00 g) was dissolved in 50 mL of water by heating gently and then made up to 100 mL. The Boric acid standard stock solution was prepared as Solution I (1.00 g L^{-1} B_2O_3) and Solution II. For solution I, the boric acid was dried to constant mass in a desiccator, weighed 1.776 g, dissolved in water and transferred to a volumetric flask and made up to 1000 mL with water. For solution II, the working standard solution of B_2O_3 was prepared from solution I by diluting 20 mL of this solution to 1000 mL with distilled water to obtain a standard solution containing 20 mg L^{-1} B_2O_3. This solution was transferred to a clean, dry polyethylene flask.

Calibration graph

Calibrations were made using aliquots of standard Boron solution II which was prepared to contain concentration ranging from 0 to 100 µg B_2O_3 in 25.0 mL volumetric flask. Each reagent was then added to the flask with vigorous swirling, followed by the addition of 5 mL of the buffer, masking, and azomethine H solutions. Lastly it was diluted to mark with distilled water. The solutions were allowed to stand for at least 2 h, the absorbance at 415 nm of each solution was then measured against reagent blank. The calibration curve was constructed from absorbance bar chart of the concentration of B_2O_3 against the net absorbance of the standard solutions.

Proximate analysis

Proximate analysis of rice grains involves determining the composition of grains in terms of crude protein, crude fat, ash, crude fiber, and dry matter mass. Rice grains were ground into a fine powder using a mill. It was ensured that the particle size remained uniform throughout the sample, which is critical for reducing variation in results during subsequent analysis. Following the grinding exercise, the powdered samples were put in relevant airtight containers. Crude protein estimate was done through Kjeldahl Method in 10 g of the rice sample, 0.5 g portion was taken, added concentrated sulfuric acid and a catalyst (selenium)[24]. After digestion, the mixture was again neutralized with sodium hydroxide. The released ammonia was collected in a distillation of boric acid solution. After the distillation of ammonia, the ammonia solution was further titrated with a standard acid solution. Nitrogen content was calculated and multiplied by a conversion factor (6.25 for rice) to determine the crude protein content:

$$\text{Crude protein }(\%)=\text{Nitrogen content }(\%)\times\ 6.25$$

The acid-based digestion approach was used to determine crude fiber. First, 1.25% sulfuric acid and then 1.25% sodium hydroxide were used to digest 2 g of rice. After that, the residue underwent filtering, washing, drying, and weighing. Residue was heated at 550 °C in a muffle furnace to figure out the fiber content. The crude fiber percentage was calculated by subtracting the weight of the ash from the weight of the residue, calculated as:

$$\text{Crude fiber }(\%)=\frac{\text{weight of residue}-\text{weight of ash}}{\text{Sample weight}}\ \times\ 100$$

Dry matter mass was determined by the drying process. A representative sample of 50 g of rice grains was used to assess dry matter mass and it was ensured that rice grains were clean from dust, husks and other impurities. These grains were ground into fine powder and its weight was recorded as the initial weight (W_ initial). A drying oven was used to dry the rice sample. Its temperature was set at 105 ℃ and rice samples were dried in oven for 24 h to ensure the complete moisture removal. Then re-weigh the dried rice samples to assess final dry mass (W_ dry).

$$\text{Dry matter }(\%)=\frac{\text{W\_dry}}{\text{W\_initial}}\ \times\ 100$$

$$\text{Dry matter mass (g)}=\text{W\_dry}$$

Crude fat was determined by the Soxhlet Extraction method[25]. The 2 g of the ground rice sample was weighed and placed in a Soxhlet apparatus thimble. Fat was extracted using petroleum ether in the Soxhlet extractor over a period of 6–8 h. The solvent evaporated, and the extracted fat was dried in an oven at 105 °C for 1 h. After cooling in a desiccator, the extracted fat was weighed to determine the fat content using the following calculation:

$$\text{Fat }(\%) = \frac{\text{Weight of extracted fat}}{\text{Sample weight}} \times 100$$

Ash content was determined by the muffle furnace method. The 5 g of the rice sample was added into a pre-weighed crucible. The sample was incinerated in a muffle furnace at 550 °C until a consistent light gray ash was obtained for 5 h. After incineration, the crucible was cooled in a desiccator and reweighed. The ash content was calculated as a percentage of the initial sample weight using the following formula:

$$\text{Ash content }(\%) = \frac{\text{Weight of ash}}{\text{Initial weight}} \times 100$$

Statistical analysis

The data was analyzed through the IBM SPSS Statistics 20 software using one-way ANOVA and Post hoc Duncan's test for inter-treatment variation. For estimating the means of each treatment, a model was formulated in which treatments, cultivars and treatments × cultivars were used as variables whereas replications and blocks were used as random factors. GraphPad Prism 9.0.0 and Orign Pro ver. 2023 were employed for graphical presentation of the results obtained.

Results

Vegetative parameters

Results revealed that T2 and T3 considerably enhanced the vegetative metrics compared to the control (T1). Data analysis depicted that T3 foliar application substantially improved vegetative parameters (Table 1). Statistical analysis of plant height in rice revealed that maximum increase was observed at T2 (343.44 cm) followed by T3 treatment (334.54 cm) and control (291.63 cm) where no foliar treatment was applied. Number of tillers exhibited maximum increase (41%) with T2 followed by T3 (34%). At control the number of tillers was recorded to be 9.94. The number of leaves in rice, however, showed non-significant differences at foliar treatments that varied significantly from control. Number of leaves was recorded to be 78, 77 and 54 from T2, T3 and T1 respectively. These results depicted that T2 and T3 substantially improved morphological attributes of rice (Table 1).

Maximum increase in leaf area (263.04 cm^2) was observed fromT2 that is non-significantly different from 262.59 cm^2 recorded at T3. Leaf area (197.00 cm^2) was observed at control is significantly lower from foliar treatments. Mean values of panicle length exhibited a significant difference by T2. Maximum panicle length (89.60 cm) was observed at T2 followed by T3 (86.21 cm) and T1 (73.67 cm). Panicle weight also displayed a significant difference with T2. Maximum panicle weight (4.41 g) was observed at T2 followed by T3(3.98 g) than T1 (3.03 g). These results demonstrated that T2 resulted in a significant increase in panicle length and panicle weight as compared to T3 where half the amount of Boron was given in foliar spray along with FA (Table 1).

Yield contributing parameters of rice

Maximum spikelets per panicle (14.67) were recorded at T2 followed by T3 and T1 with 13.82 and 10.44 spikelets per panicle respectively. Florets per panicle were maximum (144.45) at T2 followed by 136.29 from T3 treatment. Control (T1) exhibited minimum florets per panicle (111.06). Fertile florets per panicle were found to be significantly different by being maximum (131.57) from T2 followed by 123.58 from T3 which is 23% higher than T1 (100.42). These results depicted that individual and combined application with FA, foliar applied Boron significantly improved agronomical attributes of rice (Table 1).

Foliar applied Boron resulted in maximum yield per plant (46.67 g) with T2 treatment. In T1, yield per plant was 25.78 g followed by T3 treatment where it was found to be 38 g. Similarly, a significant increase in yield per plot (427.67 g) was recorded from T2 treatment, which is 43% higher than T1, followed by T3 treatment with 397 g (33% higher than T1) and T1 gave 299.56 g yield per plot. The 1000 grain weight also exhibited a significant

Vegetative parameters of rice												
	Plant height (cm)		Number of tillers		Number of leaves		Leaf area (cm^2)		Panicle length (cm)		Panicle weight (g)	
Treatments	Mean	PDT	Mean	PDT	Mean	PDT	Mean	PDT	Mean	PDT	Mean	PDT
Control	291.63	c	9.94	c	54.17	c	197	b	73.67	c	3.03	c
Boron	343.44	a	13.97	a	77.56	a	263.04	a	89.6	a	4.41	a
Boron + FA	334.54	b	13.3	b	73.63	b	262.59		86.21	b	3.98	b

Yield contributing parameters of rice												
	Spikelets $panicle^{-1}$		Florets $panicle^{-1}$		Fertile florets $panicle^{-1}$		Yield $plant^{-1}$		Yield $plot^{-1}$		1000 grain weight (g)	
Treatments	Mean	PDT	Mean	PDT	Mean	PDT	Mean	PDT	Mean	PDT	Mean	PDT
Control	10.44	c	111.06	c	100.42	c	25.78	c	299.55	c	25.28	c
Boron	14.67	a	144.45	a	131.57	a	46.67	a	427.67	a	33.27	a
Boron + FA	13.82	b	136.29	b	123.58	b	38	b	397	b	29.56	b

Table 1. Duncan multiple range test of vegetative and yield contributing parameters of rice under different treatments. Alphabets present relative grouping based on PDT (Post hoc Duncan's Test).

difference with maximum grain weight (33.27 g) from T2 as compared to 29.56 g from T3 treatment followed by 25.28 g from T1. These results demonstrated that T2 resulted in significant increase in yield per plant, yield per plot and 1000 grain weight (32%) when compared to T1, where T3 has been observed to moderately enhanced these attributed as compared to T1 (Table 1).

Physiological parameters of rice

Chlorophyll content (mg g^{-1}), carotenoid content (mg g^{-1}) and Boron content (mg L^{-1})

Maximum value (0.45 mg g^{-1}) of chlorophyll content was recorded from T2 followed by 0.36 mg g^{-1} from T3 than 0.28 mg g^{-1} which was observed from T1. T2 increased chlorophyll content by 55% as compared to T1. Carotenoid content in leaves of rice exhibited significant difference and was highest (9.19 mg g^{-1}) from T2than a decreased order of carotenoid content was observed from T3 than T1 with respective values of 7.94 mg g^{-1} and 5.77 mg g^{-1}. Boron content was recorded to be maximum (18.70 mg L^{-1}) in plants treated with T2 (which is 131% higher than T1) followed by T3 (49% higher than T1) and least was observed from control (T1) with 8.09 mg L^{-1}. It was observed that application of Boron combined with FA significantly enhanced chlorophyll and carotenoid content, as well as Boron accumulation in plants as compared to control, even when using a half-dose of Boron in foliar treatments but the most significant results was depicted by individually applied Boron (Table 2).

Crude protein (%), crude fiber (%), fat (%) and Ash (%)

In contrast to T1, both treatments significantly enhanced crude protein and crude fiber. Mean values (12.20% and 12.19%) of crude protein were obtained from T2 and T3, respectively. While mean values (1.69% and 1.60%) of crude fiber were obtained from T2 and T3 treatment, respectively. However, mean values (10.71% and 1.38%) of crude protein and crude fiber were less with T1. Fat and ash percentages significantly increased in response to foliar treatments. Around 6.27% and 6.37% increase in fat were obtained in response of T2 and T3 treatment, respectively. While 6.30% and 6.70% increase in ash were obtained in response of T2 and T3, respectively. However, mean values of fat and ash (5.61% and 5.16%) were less at control (T1). Both foliar treatments, T2 and T3, significantly enhanced the physiological attributes of rice, indicating their efficacy in improving crop nutritional values (Table 2).

Statistical significance of cultivars, treatments and their interaction

The statistical analysis of variation among cultivars, treatments, and their interaction on various rice traits revealed the following patterns:

Cultivars and treatments

Plant height, number of leaves, leaf length, panicle weight, yield per plot, and crude protein exhibited significant effects due to both cultivars and treatments (p-values ≤ 0.01 or 0.05). These results suggest that the genetic differences among cultivars and the applied treatments substantially influenced these traits, highlighting their importance in rice cultivation and breeding programs (Table 3).

Cultivars × treatments interaction

Significant effects of the interaction between cultivars and treatments were observed for panicle length, florets per panicle, and yield per plot (p-values ≤ 0.01 or 0.05). This indicates that the combined influence of specific cultivars and treatments produced unique effects on these traits, which proved important for optimizing rice production under varying conditions (Table 3).

Physiological parameters of rice								
	Chlorophyll content (mg g^{-1})		Carotenoid content (mg g^{-1})		Boron content (mg L^{-1})		Fat content (%)	
Treatments	Mean	PDT	Mean	PDT	Mean	PDT	Mean	PDT
Control	0.29	c	5.77	c	8.09	c	5.62	b
Boron	0.45	a	9.19	a	18.7	a	6.27	ab
Boron + FA	0.37	b	7.94	b	11.8	b	6.37	a
	Crude protein (%)		Crude fiber (%)		Dry matter mass (%)		Ash (%)	
	Mean	PDT	Mean	PDT	Mean	PDT	Mean	PDT
Control	10.71	b	1.38	b	86.71	b	5.16	b
Boron	12.21	a	1.69	a	89.56	a	6.31	ab
Boron + FA	12.19	a	1.6	ab	89.56	a	6.7	a

Table 2. Duncan multiple range test of physiological parameters of rice under different treatments. Alphabets present relative grouping based on PDT (Post hoc Duncan's Test).

Traits	Cultivars	Treatments	Cultivars × treatments
Plant height (cm)	0.00**	0.00**	0.15^{NS}
Number of tillers	0.48^{NS}	0.00**	0.03*
Number of leaves	0.00**	0.00**	0.34^{NS}
Leaf length (cm)	0.00**	0.03*	0.38^{NS}
Leaf width (cm)	0.00**	0.24^{NS}	0.94^{NS}
Leaf area (cm^2)	0.00**	0.00**	0.09^{NS}
Panicle length (cm)	0.54^{NS}	0.00**	0.00**
Panicle weight (g)	0.00**	0.00**	0.22^{NS}
Spikelets panicle^{-1}	0.08^{NS}	0.00**	0.19^{NS}
Florets panicle^{-1}	0.00**	0.00**	0.03*
Fertile florets panicle^{-1}	0.00**	0.00**	0.06^{NS}
Yield plant^{-1} (g)	0.04*	0.00**	0.19^{NS}
Yield plot^{-1} (g)	0.00**	0.03*	0.01**
1000 grain wt. (g)	0.49^{NS}	0.00**	0.11^{NS}
Chlorophyll content (mg g^{-1})	0.631^{NS}	0.000**	0.107^{NS}
Carotenoid content (mg g^{-1})	0.08^{NS}	0.00**	0.01**
Boron content (mg L^{-1})	0.41^{NS}	0.00**	0.00**
Crude protein (%)	0.00**	0.00**	0.23^{NS}
Crude fiber (%)	0.62^{NS}	0.00**	0.01**
Dry matter mass (%)	0.01**	0.00**	0.08^{NS}
Fat (%)	0.00**	0.00**	0.02**
Ash (%)	0.00**	0.00**	0.07*

Table 3. Probability of F value for vegetative parameters and yield components of rice with respect to cultivars and different Boron & Boron + FA treatments.

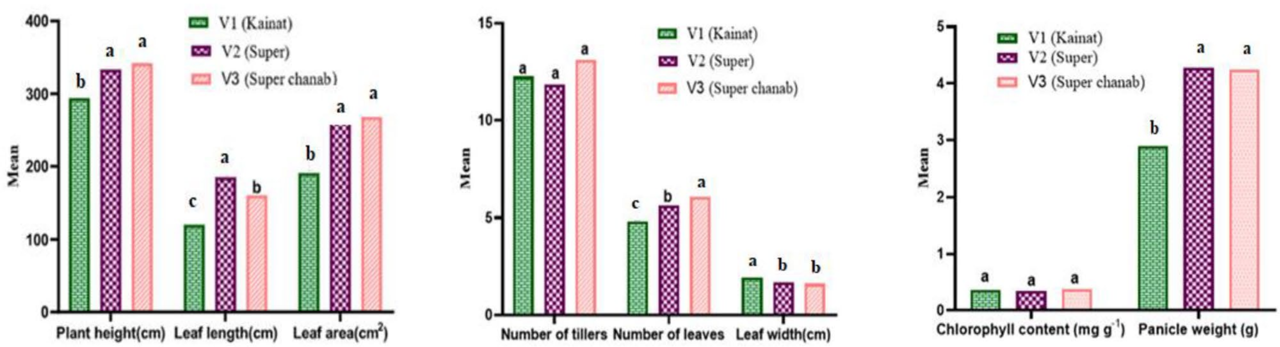

Fig. 1. Illustration of mean comparison of different vegetative parameters among three cultivars of rice.

Non-significant effects
Factors with p-values reported as "NS" (Not Significant) did not exhibit significant impacts on the traits tested. These findings suggest that observed variations for these traits are likely due to random variation rather than the tested factors (Table 3).

Hence, the analysis demonstrated that both genetic and treatment factors play significant roles in influencing rice traits, with specific interactions between cultivars and treatments affecting certain traits uniquely.

Mean comparison of morpho-physiological attributes among three cultivars of rice
Our analysis of the number of leaves among different cultivars revealed significant variations. Specifically, Super Chanab exhibited a statistically significant increase in leaf number compared to both Kainat and Super. This suggests that Super Chanab has a superior capacity for leaf production relative to the other cultivars tested.

Regarding chlorophyll content and number of tillers, no significant differences were observed among the cultivars (Fig. 1). All varieties, including Super Chanab, Super, and Kainat, showed similar levels of chlorophyll content. This lack of significant variation suggests that the chlorophyll levels in these cultivars are generally consistent, and the cultivars have comparable photosynthetic capabilities.

In terms of plant height, panicle weight and leaf area, Super and Super Chanab exhibited a statistically significant increase compared to Kainat (Fig. 1). Both Super and Super Chanab were significantly taller than

Kainat, highlighting their superior growth in height. The observed difference in height impact other agronomic traits and overall crop performance.

Mean comparison of reproductive attributes of rice under three treatments

The results demonstrate that T2 significantly enhances several key agronomic parameters, including spikelets per panicle, panicle weight, panicle length, and 1000 grain weight, compared to both T3 and T1 (control) treatments. On the other hand, T3 provides notable benefits over control (Fig. 2). The consistent increase in yield per plant and yield per plot with Boron treatment further underscores its efficacy in optimizing rice productivity. These findings suggest that Boron is a crucial element for improving rice yield, while the addition of FA offers substantial additional benefits in transportation, translocation and accumulation of Boron.

Principal component analysis

To elucidate the underlying factors affecting rice morphological and yield characteristics, Principal Component Analysis (PCA) was practiced making data less dimensional and explore the correlations among different dependent and independent variables.

The PCA was represented in a biplot (Fig. 3), which visually depicts the positioning of various morphological and biological parameters of rice under different treatments. Two main components were identified by the PCA:

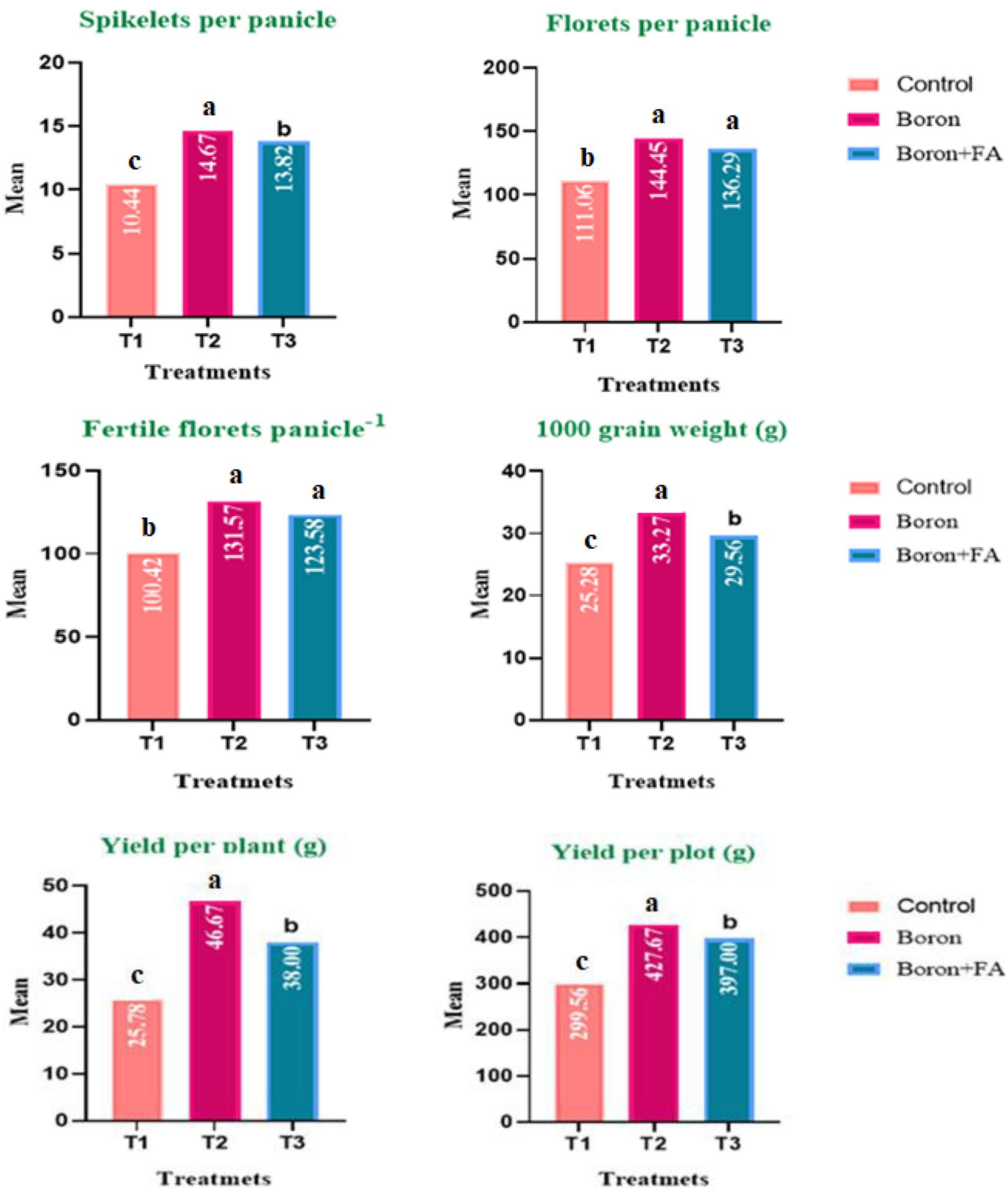

Fig. 2. Illustration of mean comparison of different yield parameters of rice under three treatments.

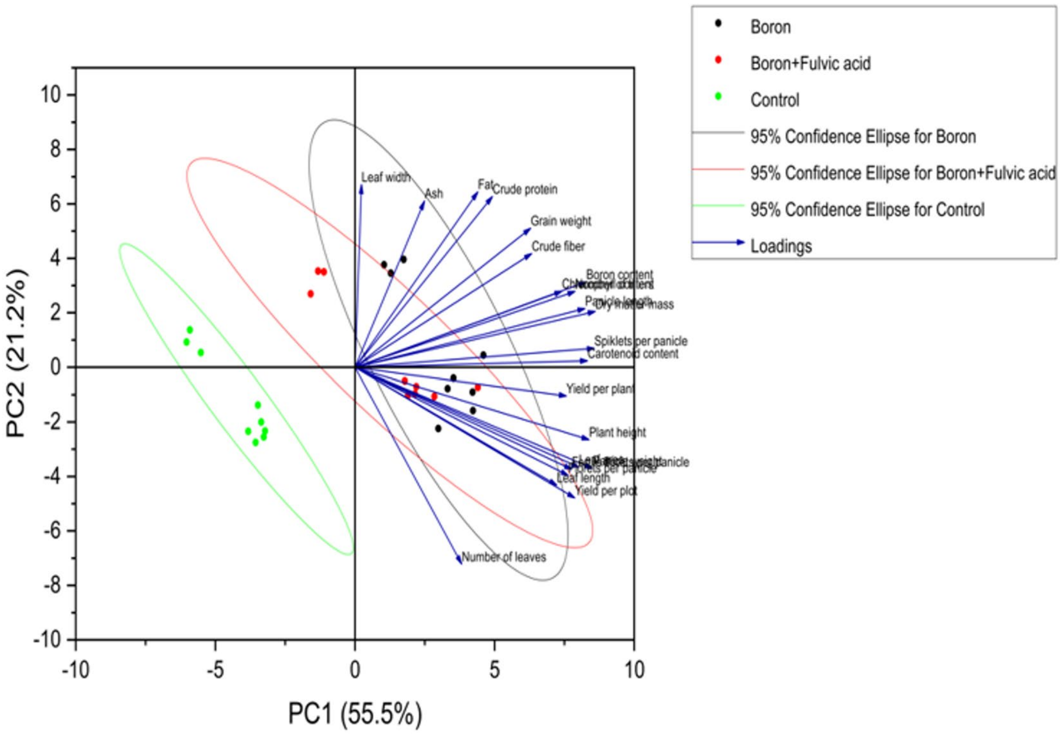

Fig. 3. Demonstration of the relationship between treatments and morpho-physiological attributes of rice under Principal Component Analysis.

PC1 accounted for 55.5% of the variance, while PC2 explained 21.2%. Together, these components accounted for 76.7% of the total variance in the data.

The biplot demonstrated that small angles between vectors indicate strong relationships among dependent variables. For instance, vectors like grain weight, crude fiber, and crude protein show strong correlations, indicating that these traits tend to vary together. While vectors pointing in opposite directions suggest a negative correlation among variables e.g., number of leaves and grain weight indicate a negative correlation, meaning as one trait increases, the other tends to decrease. Our analysis revealed a significantly positive correlation between foliar treatments and growth and yield characteristics of rice.

The T2 (Boron) with black dots and T3 (red dots) treatments are clustered close to each other and aligned with vectors representing positive agronomic traits like grain weight, crude protein, and crude fiber. This indicates that these treatments have a beneficial effect on these traits. Control (green dots) is distinctly separated from the other treatments and aligned opposite to many important agronomic traits, indicating a negative impact on these parameters. This separation clearly shows that the absence of treatment results in significantly poorer performance in key traits compared to the treated groups.

Furthermore, the ellipses represent the 95% confidence intervals for each treatment group. The distinct non-overlapping ellipses for the control and treated groups (Boron (T2), Boron + FA (T3)) indicate statistically significant differences in their effects on the measured traits. The overlap between T2 and T3 ellipses suggest some level of similarity in their effects, though each treatment still had unique impacts on certain traits (Fig. 3).

Pearson's correlation analysis

Pearson's correlation analysis ($p = 0.05$) assessed relationships between leaf Boron content and key yield traits (Fig. 4). Yield showed a strong positive correlation with tiller number (ϒ = 0.69), fertile florets per panicle (ϒ = 0.75), and plant height (ϒ = 0.90), indicating that increased tillering and panicle fertility significantly enhance rice production. Panicle length (ϒ = 0.65) and panicle weight (ϒ = 0.82) also contributed to higher yields, emphasizing the role of panicle structure in grain formation.

Leaf Boron content was positively correlated with spikelets per panicle (ϒ = 0.67), 1000-grain weight (ϒ = 0.91), chlorophyll (ϒ = 0.85), and carotenoid content (ϒ = 0.76). These findings suggest that Boron supports spikelet development, grain weight, and photosynthetic efficiency, ultimately improving yield quality. Additionally, the strong correlation between Boron content and panicle length (ϒ = 0.90) reinforces Boron's role in enhancing yield potential (Fig. 4). Pearson's correlation analysis identified yield to be strongly positively correlated with tiller number, fertile florets per panicle, and plant height, pointing toward their vital contribution towards grain yield. Panicle weight and panicle length also made noteworthy contributions towards yield. Leaf Boron content was highly correlated with spikelet number, 1000-grain weight, and chlorophyll content, highlighting its contribution toward grain quality and photosynthesis. These results emphasize the interactive impact of major agronomic characteristics and Boron availability on rice yield.

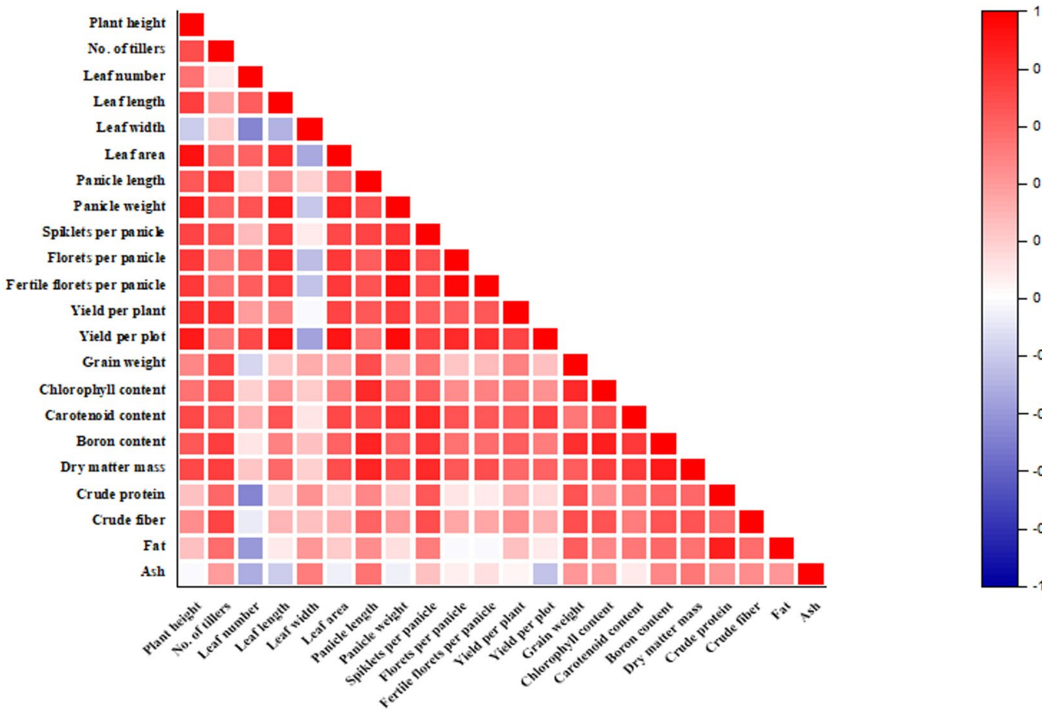

Fig. 4. Depiction of the interrelationship of different morphological and yield parameters of rice under Correlation Analysis.

Discussion

The experimental study aimed to investigate the role of fulvic acid (FA) as a chelating agent in combination with Boron foliar application on rice yield-contributing parameters. To the best of our knowledge, this is the first study to report on the combined foliar application of Boron and FA, and its effects on rice growth and yield traits. Our findings demonstrated that the inclusion of FA in the foliar application significantly enhanced nutrient uptake, transportation, and provided protection against micronutrient fixation and leaching. The combined application of Boron and FA resulted in notable improvements across several agronomic traits, including an increase in plant height, number of tillers, leaf area, florets per panicle, and fertile florets per panicle. However, despite these encouraging results, the improvements observed with the combined Boron and FA application were not as significantly higher than the Boron foliar application alone. This suggests that FA enhances the effectiveness of Boron to a certain level, however, it is not solely responsible for making Boron bioavailable. The reduced leaf Boron content from Boron + fulvic acid treatment can be caused by plant physiological restrictions at a particular growth stage rather than chemical incompatibility or soil interactions because fulvic acid increases Boron mobility and redistributes it within the plant instead of accumulating it in leaves [26,27].

The foliar application of Boron has been shown to enhance vegetative growth in rice, leading to significant improvements in several yield components and increased Boron content within the plant. Boron application significantly increases plant height, an effect attributed to Boron's role in carbohydrate and protein synthesis[28]. Boron is essential for sugar transport, cell wall structure, root development, and other physiological processes that contribute to increased plant height[29]. Boron is absorbed by the roots and transported through plant tissues, primarily driven by transpiration[30]. In the absence of Boron treatment[13]the reduced plant height in control treatments might be due to an insufficient supply of nutrients.

The combined application of Boron and fulvic Acid (FA) exhibited positive effects on plant height and leaf area, comparable to the effects observed with Boron foliar treatment alone[31]. These results are attributed to the enhanced bioavailability of Boron mediated by FA. As a potent chelating agent, FA increases the solubility and stability of Boron in the soil, preventing its precipitation and leaching. This enhanced bioavailability facilitates a more efficient uptake of Boron by the rice plant's roots[32].

Once absorbed, FA further aids in the translocation of Boron within the plant, ensuring its movement from the roots to the shoot system, particularly to the leaves. This improved translocation process is critical for optimizing Boron's physiological roles in the plant, including its involvement in cell wall formation, carbohydrate transport, and protein synthesis[29]. The enhanced availability and movement of Boron within the plant system led to increased plant height and expanded leaf area, both of which are vital for maximizing photosynthetic efficiency and overall plant vigor[33].

In the context of improving grain yield and quality in Fine Basmati rice, the increased vegetative growth supported by the Boron + FA combination directly contributes to better grain filling and development. The improved plant architecture, resulting from greater plant height and leaf area, allows for more efficient light interception and nutrient utilization, ultimately enhancing grain yield and quality[34]. Thus, the combined use of

Boron and FA represents an encouraging agronomic strategy to optimize Boron bioavailability, which is crucial for achieving higher productivity and superior quality in Fine Basmati rice.

The application of Boron, both alone and in combination with fulvic acid (FA), significantly enhanced several key agronomic traits over the control treatment, including yield per plant, yield per plot, chlorophyll content, carotenoid content, Boron content, and total soluble protein. These improvements in rice yield can be largely attributed to the increased 1000-grain weight and reduced panicle sterility resulting from Boron application[35]. Boron is crucial for various aspects of plant development and reproduction, playing an indispensable role in carbohydrate metabolism and translocation[29]cell wall formation, membrane integrity, pollen tube development, and the promotion of pollination and seed formation[36]. These physiological roles of Boron led to reduced panicle sterility and, consequently, increased yield per plant and yield per plot.

Moreover, the foliar application of Boron has been shown to enhance the phloem mobility of Boron to floral parts, which is vital for improving grain partitioning and overall yield[37,38]. highlighted that Boron deficiency indirectly affects photosynthesis by reducing chlorophyll and soluble protein levels in leaves, which in turn restricts the activity of photosynthetic enzymes, hindering the Hill reaction and decreasing net photosynthesis. Therefore, appropriate foliar application of Boron and Boron + FA results in increased chlorophyll and carotenoid content, as well as efficient Boron accumulation in the leaves and grains of rice plants, thereby enhancing photosynthetic efficiency and overall grain yield.

The number of tillers, florets per panicle, and fertile florets per panicle also showed significant increases under Boron and Boron + FA treatments compared to the control. Previous studies by have reported that the number of tillers per plant may be enhanced due to the proper differentiation and development of plant tissues, which is facilitated by Boron's role in cell wall deposition and membrane function[39–41]. Consequently, the foliar application of Boron maximized the production of, florets per panicle, tillers, and fertile florets per panicle, consistent with the findings of[42].

Regarding florets per panicle and fertile florets per panicle, the increase in the number of filled grains per panicle observed under Boron and Boron + FA treatment could be due to improved grain filling and reduced pollen sterility, as suggested by[12]. The inclusion of FA, with its auxin-like activity, further enhances this effect by stimulating hormonal activity, increasing cell permeability, and improving dry matter yield[43]. Consequently, the Boron + FA treatment likely exhibits similar positive effects on the number of tillers, florets per panicle, and fertile florets per panicle, reinforcing the potential of this combined treatment to improve grain yield and quality of Fine Basmati rice.

Conclusion

Boron bioavailability in rice grain is crucial to grain quality and yield. Current study aimed to enhance Boron mineral content in rice by foliar application of Boron alone and combined with fulvic acid. The findings of this study demonstrated that Boron individual application significantly improved key agronomic and quality parameters, such as 1000-grain weight, grain size, milling recovery, paddy yield, and cooking quality in fine grain Basmati rice. The enhancements in yield and nutritional composition are primarily linked to the reduction in panicle sterility, highlighting Boron's essential role in reproductive growth and grain development. The application of fulvic acid aids together with Boron suggested that its application at critical growth stages, particularly during tillering and panicle formation, is most effective in maximizing yield and quality outcomes as compared to control. The foliar applied FA with Boron enhances nutrient uptake, its role in Boron bioavailability remains unexplored. Therefore, further research is required to understand the role of FA based on biochemical and molecular attributes of rice, involved in enhancing Boron bioavailability.

Data availability

The data analyzed and used in this article can be availed from corresponding author on reasonable request.

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Author contributions
AK; conducted all laboratory tests, supervision, data analysis, computer graphics, conception, methodology, and the original draft's authoring, review, and editing. IN; was responsible for all data analysis, hand sketches, laboratory tests, and helped write the initial draft. SJ; conceived the study, review, and revised the final draft. LK; reviewed and revised the paper. All authors read the manuscript. MHS and SA; played role in writing final draft, reviewed and revised the manuscript. AK; reviewed and revised the paper.

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Declarations

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to A.K.

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