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# Foliar-applied boron improves flag leaf reserves translocation, pollen viability and yield of aromatic monsoon rice

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# **1 Introduction**

Bangladesh has high diversity in rice varieties with a stalk of more than 8,000 rice germplasm of which nearly 100 are aromatic [\(Siddique et al.,](#page-11-0) [2017;](#page-11-0) [Khale](#page-10-0)[quzzaman et al.,](#page-10-0) [2012\)](#page-10-0). The locally adapted landraces and some released modern rice varieties are smallgrained with a pleasant aroma and preferred by the consumers despite their high prices [\(Ahmad et al.,](#page-9-0)

[2005\)](#page-9-0). Aromatic rice production is gaining momentum in Bangladesh and other Asian countries since it offers a comparatively higher market price than coarse-grained ones [\(Routray and Rayaguru,](#page-11-1) [2017;](#page-11-1) [Sikdar et al.,](#page-11-2) [2008\)](#page-11-2). It also shows high export potentials after meeting the local demand. The adoption of modern aromatic rice varieties may increase farmers' net income by 23 percent [\(Shrestha et al.,](#page-11-3) [2002\)](#page-11-3). Despite such attractive traits, the yield potential of aromatic rice is much lower due to many production constraints, such as several climatic and management factors [\(Chakraborty,](#page-9-1) [2020\)](#page-9-1).

The planting time influences the productivity of aromatic rice. In many regions of South and Southeast Asia, the planting time of monsoon rice may be delayed due to heavy rainfall or late recession of floodwater. The late-planted rice used to experience low temperatures during the reproductive stage. It causes failure of panicle initiation and consequently increases the spikelet sterility resulting in poor grain yield [\(Zhou et al.,](#page-12-1) [2012;](#page-12-1) [Shimono et al.,](#page-11-4) [2007\)](#page-11-4). The decrease in mean temperature below 11 °C caused severe grain sterility [\(McKenzie et al.,](#page-10-1) [1994;](#page-10-1) [Terres](#page-11-5) [et al.,](#page-11-5) [1994\)](#page-11-5). Generally, high spikelet sterility occurs when plants are exposed to temperatures 9-12 °C [\(de Souza et al.,](#page-9-2) [2017\)](#page-9-2). Low temperature caused a higher reduction in grain yield during pre-heading than that of warm temperatures during post-heading [\(Huang et al.,](#page-10-2) [2016\)](#page-10-2). [Gunawardena et al.](#page-9-3) [\(2003\)](#page-9-3) critically observed the evidence of spikelet sterility at the microspore development stage of rice at low temperatures. The most critical time of reducing seed viability was reported 14 to 7 days after anthesis due to high or low temperatures in rice [\(Martínez-Eixarch and Ellis,](#page-10-3) [2015\)](#page-10-3). These problems can be solved by foliar application of B as advocated by different studies [\(Rehman](#page-11-6) [et al.,](#page-11-6) [2014;](#page-11-6) [Ahmad et al.,](#page-9-4) [2012\)](#page-9-4).

Boron deficiency restricts rice productivity and reduces grain quality [\(ur Rehman et al.,](#page-11-7) [2018\)](#page-11-7). Exogenous application of B triggers extensive growth and improves yield-related traits of rice [\(Suman and](#page-11-8) [Raj,](#page-11-8) [2018\)](#page-11-8). It also enhances pollination, grain set, and grain formation in rice [\(Fatima et al.,](#page-9-5) [2018;](#page-9-5) [ur Rehman](#page-11-9) [et al.,](#page-11-9) [2012\)](#page-11-9). Rice growers mostly use nitrogen, phosphorus, and potassium fertilizers, but B application is limited. Sometimes B is soil-applied in the rice-wheat system [\(Khan et al.,](#page-10-4) [2006\)](#page-10-4). However, the method and rate of B application are vitally important, particularly in aromatic rice, where higher doses may cause toxicity. Foliar applied B was found to be most effective due to the rapid availability of nutrients [\(Firdous et al.,](#page-9-6) [2017\)](#page-9-6) and retains high phloem mobility from senescing leaves to reproductive organs in plants. Researches on the response of either foliar or soil applied B on the tillering, growth, grain yield and quality with a simultaneous decrease in panicle sterility in aromatic rice are hardly available. In Pakistan, [Fatima et al.](#page-9-5) [\(2018\)](#page-9-5) and [Rehman et al.](#page-11-6) [\(2014\)](#page-11-6) worked considerably details and suggested that foliar application of B could be a potential practice for correcting B deficiency in aromatic rice resulting in yield improvement through increasing the number of tillers and grain weight and reducing panicle sterility. However, the role of foliar-applied B following environmental consequences associated with low temperature is rarely observed. It is important because

minimum temperatures below 18 °C at the booting stage may result in spikelet sterility as well as delay in heading of rice [\(Dingkuhn et al.,](#page-9-7) [1995\)](#page-9-7). Even understanding the physiological basis of yield improvement resulting from assimilates translocation and reducing spikelet sterility through foliar-applied B is limited. Therefore, the study aimed at evaluating the foliar-applied B on pollen viability, flag leaf reserve mobilization, and productivity of late-sown aromatic monsoon rice cv. Kataribhog experiencing low temperature at the reproductive stage in early December under Bangladesh conditions.

# **2 Materials and Methods**

#### **2.1 Site description**

This study was conducted at the field laboratory of the Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur from September 2014 to January 2015. The site belongs to Agro-Ecological Zone (AEZ) 28 Madhupur Tract at  $24^{\circ}2'20.34''$  N latitude and  $90^{\circ}23'53.30''$  E longitude with an elevation of 14.9 m above the mean sea level. The textural class of both top and subsoil was identified as silty clay loam. Soil is acidic in reaction having medium (1.9%) organic matter and phosphorus, and low potassium and boron contents. In the study area, the prevailing average minimum temperatures were 17.6 °C and 11.5 °C in November and December. Due to late planting, the plants experienced low temperatures during the reproductive phase from mid-November to mid-December. The area is also characterized by 79-86% relative humidity and having no rainfall in November and December, but 193.7 mm rainfall was recorded during other months of the growing period.

#### **2.2 Experimental treatment and design**

The six treatment variables are as follows: B0-no B applied, B50-B applied at 50 ppm (0.2 kg B ha<sup>-1</sup>), B100 - B applied at 100 ppm (0.4 kg ha<sup>-1</sup>), B150-B applied at 150 ppm (0.6 kg ha<sup>-1</sup>), B200-B applied at 200 ppm (0.8 kg ha<sup>-1</sup>), and B250-B applied at 250 ppm  $(1.0 \text{ kg ha}^{-1})$ . The doses of B were selected based on the findings of [Shah et al.](#page-11-10) [\(2011\)](#page-11-10), who reported that foliar applied 0.5-1.0 kg B ha<sup>-1</sup> at panicle initiation had a positive response in rice. All the treatments were applied as a foliar spray at 33 and 47 days after transplanting (DAT). The size of the experimental unit was  $4.0 \times 2.5$  m, and the planting configuration was  $25 \times 20$  cm. The adjacent blocks and plots were separated from one another by 1.0 m and 0.75 m, respectively. The experiment was carried out in a Randomized Complete Block Design (RCBD) with three replications.

## **2.3 Crop production and management**

The crop tested in the experiment was fine aromatic rice (*Oryza sativa* L.) cultivar Kataribhog, a popular local cultivar of Bangladesh. Seeds were sown manually on puddled soil in a nursery bed, and all management practices were given for the proper establishment of seedlings. Thirty-days old seedlings were transplanted on 17 September 2014 with 2/3 seedlings hill<sup>-1</sup> on well-puddled soil. After transplanting, 2-4 cm standing water was maintained up to the hard-dough stage, and supplementary irrigation water was applied whenever necessary. In Bangladesh, the first week of August is the optimum time for transplanting aman rice [\(Kabir et al.,](#page-10-5) [2017\)](#page-10-5). So, the plants used to experience optimum temperature during panicle initiation and grain formation. During final land preparation, cowdung (5 ton ha<sup>-1</sup>), one-third urea (76 kg N ha<sup>-1</sup>), triple superphosphate  $(30 \text{ kg } P_2O_5 \text{ ha}^{-1})$ , muriate of potash  $(42 \text{ kg K } \text{ha}^{-1})$ , and gypsum (17 kg S ha<sup>-1</sup>) were applied as per the recommendation of [BARC](#page-9-8) [\(2012\)](#page-9-8). Different boron dozes were applied as foliar spray from Borax (20% B). The rest amount of the urea was top-dressed in two equal installments at 25 and 45 DAT. Stem borer infestation at the active tillering stage were effectively controlled using Furadan 5G at 10 kg ha $^{-1}$ .

## **2.4 Observation and data collection**

After final land preparation, topsoil samples of 20 cm depth were collected from the field following standard procedure for determining soil boron content. The days to the visualization of a single flower from seeding were counted as days to first flowering. The days from seeding to fully headed panicles of about 50% tillers in each plot were treated as days to 50% flowering. The days from seeding time to changing the base of a panicle color of about 50% tillers from green to brown color were recorded as days to maturity. The plants under different treatments were harvested at variable dates depending on physiological maturity.

#### **2.4.1 Pollen viability test**

The anthers were collected using a needle before 8 am, and pollens were kept in the slides and then enclosed by coverslips. Iodine-potassium iodide (I2/KI) test was performed as described by [Rathod et al.](#page-11-11) [\(2018\)](#page-11-11) for pollen viability. Potassium iodide (1 g) and iodine (0.5 g) were dissolved in 100 mL of distilled water. Two drops of the solution were put over pollen and then enclosed by a coverslip. After 5-10 min, the pollen grains darkly stained were counted under a microscope. The pollen viability (%) was calculated as the percent ratio of the number of stained pollen grains to the total number of pollen grains on the slide.

#### **2.4.2 Measuring leaf chlorophyll index**

Leaf chlorophyll index was expressed in term of leaf greenness or SPAD value. Chlorophyll Meter (SPAD-502, Minolta Camera Co., Japan) was used to determine the SPAD value. The SPAD values were recorded for selected plants in each treatment. The outermost and fully expanded mature leaf of each plant was selected. The SPAD readings were taken in the morning, usually at 11.00 am from the tip, midway, and base of the leaf and averaged. The flag leaf SPAD values were also taken at 62 DAT until 86 DAT at three-day intervals.

#### **2.4.3 Determining yield and yield attributes**

The selected ten plants were harvested during maturity for recording data on yield and yield-related traits. Panicle length was measured from the base of the panicle to the tip of each panicle. The filled and unfilled grains per panicle were counted using a Multi Auto Counter (Model DCI, Tokyo, Japan). Accordingly, weight of 1000 grains were counted from the filled grains and were weighed. Grain yield was recorded from  $1 \text{ m}^2$  areas of the middle of each plot for avoiding border effects. The grains were threshed, cleaned, dried, and converted to t ha<sup> $-1$ </sup> after adjusting 14% grain moisture. The straw yield was recorded after oven drying at 70 °C for three days. Harvest index (HI) was calculated using the following formula:

$$
HI\,(\%) = \frac{Y_e}{Y_b} \times 100\tag{1}
$$

*HI* = Harvest index (%),  $Y_e$  = Economic yield (grain yield), and  $Y_b$  = Biological yield (grain + straw).

#### **2.5 Boron concentration determination**

At physiological maturity, three plant samples were collected randomly from each treatment. The roots were discarded from the plants, and plants were partitioned into leaf, stem, and grain. The plant samples were dried at 60 °C for 48 hours. The grain bran and endosperm were removed from the grain and separated using a grinding machine after passing through a 20-mesh sieve. The grain and straw samples were analyzed for the determination of B concentration. Plant and soil samples were analyzed by following Curcumin methods [\(Piper,](#page-10-6) [1942\)](#page-10-6).

#### **2.6 Statistical analysis**

Software Statistix 10 was used to analyze the data. Grain yield and yield-related traits were analyzed using an analysis of variance (ANOVA). The basis of all analysis was a randomized complete block design. A least significant difference (LSD) was used to perform posthoc multiple comparisons and separate

the treatment means, where  $P \leq 0.05$  was appropriate. The correlation matrix of trait relationships was performed by SPSS 16.

# **3 Results and Discussion**

## **3.1 Crop phonology**

Boron deficient control plants delayed the various phenology events like days to first flowering, 50% flowering, and maturity of cultivar Kataribhog [\(Ta](#page-4-0)[ble 1\)](#page-4-0). In general, days to phenological events decreased with the increase of boron level up to 200 ppm, but further increase in boron level tends to decrease days to those events. For instance, B deficient control plants took 106 days to mature, and 200 ppm B reduced to maturity duration 100 days. Then, there was a tendency to take a longer time to maturity duration with the increased amount of boron. [Rashid et al.](#page-11-12) [\(2000\)](#page-11-12) also observed delaying flowering and maturity of four days in rice. Under B-deficient conditions, sink capacity decreased, and translocation of assimilates from source to sink reduced [\(Marschner,](#page-10-7) [1995\)](#page-10-7). Consequently, floral development, anthesis, fertilization, and grain formation are affected, which leads to delayed maturity duration [\(Nadeem and Farooq,](#page-10-8) [2019;](#page-10-8) [Pandey,](#page-10-9) [2010\)](#page-10-9).

#### **3.2 SPAD value**

The chlorophyll meter value (SPAD value) is proportional to the amount of chlorophyll and nitrogen present in rice leaf [\(Gholizadeh et al.,](#page-9-9) [2017;](#page-9-9) [Wakiyama,](#page-12-2) [2016;](#page-12-2) [Yang et al.,](#page-12-3) [2014\)](#page-12-3), and a higher value indicates better nutritional condition and healthier plant. The Irrespective of B concentration, SPAD value increased with plant age up to 60 DAT and then declined [\(Fig. 1\)](#page-4-1). [Islam et al.](#page-10-10) [\(2014\)](#page-10-10) also observed an increase in leaf chlorophyll at an early stage and its subsequent decrease in the late age of crop growth and development. The SPAD value varied significantly for various B levels at 40 DAT, and it was more conspicuous at 60 DAT. Generally, the higher the concentration of B, the higher was the SPAD value. However, this trend was not continued after 75 days and showed a decreasing trend of SPAD value for increased B levels. [Kumar](#page-10-11) [et al.](#page-10-11) [\(2015\)](#page-10-11) stated that boron increased total chlorophyll content in rice genotypes and the responses varied with levels of boron. Foliar applied B showed a remarkable increase in chlorophyll contents in super Basmati rice [\(Rehman et al.,](#page-11-6) [2014\)](#page-11-6).

#### **3.3 SPAD value of flag leaf**

The flag leaf is a major source of carbohydrates stored in the leaves. In general, the B doses had no significant effect on the SPAD value of the flag leaf. The SPAD value of the flag leaf gradually decreased from

62 DAT to 86 DAT [\(Fig. 2\)](#page-4-2). Initially, their variation was wider but narrowed down towards the end. The reduced SPAD value during the reproductive stage indicated that assimilates had diverted to other plant organs, most likely in the grains. However, there was an indication of reducing the SPAD value of flag leaf applied with a higher B dose. A range of leaf chlorophyll increments was also observed for different levels of foliar-applied B [\(Rehman et al.,](#page-11-6) [2014\)](#page-11-6). The decrease of chlorophyll content at higher B concentration has been reported by many researchers and the causes were explained by the fact that a higher dose of B increased phenolic contents that resulted in chlorophyll enzyme degradation [\(Seth and Aery,](#page-11-13) [2014;](#page-11-13) [Yamauchi and Watada,](#page-12-4) [1994\)](#page-12-4).

#### **3.4 Flag leaf and panicle dry matter**

The flag leaf is the end source of assimilates contributing to grain formation. Therefore, it is imperative to comprehend the assimilate reserves translocation of flag leaves to panicles for different boron levels during the grain filling stage. [Fig. 3](#page-5-0) demonstrated that flag leaf dry matter (DM) decreased with increasing B concentration. In contrast, the DM increased with a decrease in flag leaf DM. This decrease and increase of DM were almost linear up to 200 ppm B. This linearity breaks due to a further increase in B concentration, which might of B toxicity. It indicates that B improves rice panicle, but excess B application may not be favorable due to impairing assimilates translocation from flag leaf.

[Fig. 4](#page-5-1) illustrates the relationship between flag leaf DM and panicle DM as influenced by foliar-applied B. The decrease of flag leaf DM and subsequent increase of panicle weight showed a significant strong polynomial relationship ( $r = 0.98$ <sup>\*\*</sup>). The decrease in flag leaf DM and subsequent increase in panicle DM due to increased foliar-applied B are the indications of phloem mobility from flag leave to reproductive organs. It is in agreement with the findings of [Hanson](#page-10-12) [\(1991\)](#page-10-12), who observed that the application of B to the leaves of several fruit trees increased B contents of flower buds and enhanced fruit set. The function of the flag leaf is to act as the source of producing photoassimilates, and grain formation mainly depends on source activity instead of sink capacity [\(Shahruddin](#page-11-14) [et al.,](#page-11-14) [2014\)](#page-11-14). The phloem mobility from leaves of a plant is related to photosynthate translocation, and B is transported as B-sorbital complexes [\(Brown,](#page-9-10) [1996\)](#page-9-10). Foliar applied B increased stem dry matter accumulation at the post-anthesis period and then showed a gradual decrease with a parallel increase of siliqua dry weight in *Camelina sativa* [\(Khan et al.,](#page-10-13) [2016\)](#page-10-13). Such information on phloem B mobility might be useful in improving the B content in rice production.

B dose (ppm)	Days to first flowering	Days to 50 % Flowering	Days to Maturity	
$\overline{0}$	76 a	83 a	106 a	
50	75 ab	81 b	104 b	
100	74 bc	80 <sub>bc</sub>	103 <sub>b</sub>	
150	73 с	79 c	102c	
200	71 d	77 d	100d	
250	73 c	79 c	101 cd	
<b>LSD</b>	1.1	1.47	1.4	

<span id="page-4-0"></span>**Table 1.** Effect of foliar applied boron on phenology in aromatic rice cultivar Kataribhog

<span id="page-4-1"></span>Means followed by the uncommon letter (s) within column are significantly different from each other (P<0.05).



<span id="page-4-2"></span>**Figure 1.** SPAD value at different growth stages of aromatic rice as influenced by B application



**Figure 2.** Flag leaf SPAD value at different growth stages of aromatic rice as influenced by B application

<span id="page-5-0"></span>

<span id="page-5-1"></span>**Figure 3.** Effect of foliar-applied B on flag leaf dry matter (DM) and panicle DM in aromatic rice cultivar Kataribhog



<span id="page-5-2"></span>**Figure 4.** Relationship between flag leaf DM and panicle DM as influenced by foliar applied B in aromatic rice cultivar Kataribhog



**Figure 5.** Effect of foliar applied B on pollen viability in aromatic rice cultivar Kataribhog

## **3.5 Pollen viability**

The application of B had a significant impact on percent pollen viability [\(Fig. 5\)](#page-5-2). At a low dose of B (50 ppm), pollen viability improvement was much less, but higher doses exhibited remarkable improvement. Generally, pollen viability increased with the increase in B concentration up to 200 ppm, and the addition of B slightly declined pollen viability. A profound effect of B began above 50 ppm. The viability increased by 32.1% at 100 ppm that rose to 72.9% at 200 ppm. Boron deficiency hinders anther development during microsporogenesis and consequently affects pollen viability, which is the main reason for panicle sterility [\(Huang,](#page-10-14) [2000\)](#page-10-14). Foliar-applied B enhanced the germination of the pollen tube and grain setting through protein and carbohydrate synthesis [\(Moeinian et al.,](#page-10-15) [2011\)](#page-10-15). The low water status of panicle in rice during anthesis is responsible for panicle sterility [\(Farooq](#page-9-11) [et al.,](#page-9-11) [2011;](#page-9-11) [He and Serraj,](#page-10-16) [2012\)](#page-10-16) and poor grain quality [\(Rashid et al.,](#page-11-15) [2004\)](#page-11-15). Boron application was also found to a substantial increase in grain yield for reduced panicle sterility in their studies. An adequate supply of B may ensure grain setting by decreasing panicle sterility [\(Rehman et al.,](#page-11-6) [2014\)](#page-11-6). They also found that B application improves leaf water status that contributes to reducing the panicle sterility. Adequate B may also help the continuous supply of assimilates to the developing grains [\(Dixit et al.,](#page-9-12) [2002\)](#page-9-12).

## **3.6 Yield and yield attributes**

Foliar applied B significantly increased the productive tillers and improved yield and yield-related pa-rameters [\(Table 2\)](#page-7-0). The effective tillers, panicle length, filled grain, grain weight, grain yield, and harvest index increased significantly with the increasing levels of B applications up to 200 ppm, thereafter, all the characters were depressed with a further increase in B level. The effective tillers increased by 52.9% over control for the 200 ppm B level. It implied that B has a profound effect on the tiller number in the tested variety of rice. The foliar-applied B may increase the number of tillers per hill in fine-grain basmati rice [\(Rehman et al.,](#page-11-6) [2014\)](#page-11-6). However, [Hussain et al.](#page-10-17) [\(2012\)](#page-10-17) did not find any significant effect of B on the tiller formation at tillering and flowering stages. The highest panicle length was 24.05 cm for the 200 ppm B that accounted for 39.1% higher compared to the control treatment. The increase in panicle length might be associated with better uptake of B. The highest percentage of filled-grain was 95.9 for 200 ppm B application, while the lowest was 88.1 without B. At 200 ppm of B, grain weight gained a 14.0% increase over control, although grain weight gained a much higher amount (20.3%) for 150 ppm B. The highest grain yield was recorded as 2.66 t ha $^{-1}$  for 200 ppm B that was 47.0% higher than the control. The B is

an essential element for better utilization of macronutrients by plants and thereby enhances the translocation of photo-assimilates from source to sink during the growth period [\(Stangoulis et al.,](#page-11-16) [2010\)](#page-11-16). Rice grain production is attributed to effective tillers, panicle length and filled grains panicle−<sup>1</sup> , and individual grain weight [\(Kumar et al.,](#page-10-11) [2015;](#page-10-11) [Rehman et al.,](#page-11-17) [2015\)](#page-11-17). This study suggests that the increase in yield was the combined effect of a higher number of tillers per unit area, and the number of grains per panicle, 1000-grain weight, and enhanced growth and development of rice variety. Recently, the three spray applications of Si and B also proved significantly superior for yield and yield attributes of rice [\(Nagula et al.,](#page-10-18) [2015\)](#page-10-18). The harvest index did not follow any regular trend and was not significant due to treatment.

#### **3.7 Boron content in leaf and grain**

Boron levels had a significant effect on its accumulation in leaf and grain of rice plants, where the highest accumulation was observed for 200 ppm B accounting 32.4% increase in leaf and 40.9% in grain compared to control [\(Table 3\)](#page-7-0). Generally, higher the B levels, higher were the concentrations of B in leaf and grain indicative to better uptake of B due to foliar-applied B. Foliar applied B also showed an increase of B contents in leaf and grain with an increase in B levels [\(Rehman et al.,](#page-11-6) [2014;](#page-11-6) [Rashid et al.,](#page-11-18) [2007\)](#page-11-18). Limited information is available on the effects of foliar B application on B uptake patterns by leaves and grains of aromatic rice. Several earlier studies demonstrated that foliar-applied B increased its accumulation in plant parts of different crop species [\(Bellaloui et al.,](#page-9-13) [2013;](#page-9-13) [Asad et al.,](#page-9-14) [2003\)](#page-9-14). However, foliar-applied B might have significant accumulation in the shoot and root system but not in pods [\(Viçosi et al.,](#page-12-5) [2020\)](#page-12-5). The study revealed that B concentration slightly increased in grains compared to leaves. Generally, B concentration in reproductive parts increased with increasing B levels [\(Rashid et al.,](#page-11-19) [2005\)](#page-11-19). Boron has limited phloem mobility in crops, and thereby its supply is needed for healthy reproductive growth and development [\(Cak](#page-9-15)[mak,](#page-9-15) [1994\)](#page-9-15). It is worth noting that foliar-applied B is quickly absorbed by the leaves, and thereby successive applications are necessary to get better benefits from the spray [\(Bogiani et al.,](#page-9-16) [2013\)](#page-9-16).

#### **3.8 SPAD value** *vs* **grain yield**

There was a strong significant positive correlation (*r* = 0.96\*\*) between grain yield and SPAD values at 40, 50, and 60 DAT [\(Table 4\)](#page-8-0). This correlation matrix indicates that the application of born at 33 and 47 DAT had a positive impact on grain yield. [Rao](#page-11-20) [et al.](#page-11-20) [\(2013\)](#page-11-20) observed that foliar-applied B had a significant relation with grain yield in rice. In earlier studies, SPAD chlorophyll meter-based leaf chloro-

B dose (ppm)	No. of effective tillers hill-1	Panicle length (cm)	of filled No. grain panicle-1	filled $\%$ grain	WTS(g)	Grain yield $(t \text{ ha-1})$	Harvest index
$\overline{0}$	17 e	17.29 e	76 d	88.1 d	15.11 d	1.81 f	0.23c
50	19d	18.53 d	80c	91.3 c	15.59d	1.91e	0.26c
100	$21 \text{ cd}$	19.75c	84 bc	93.3 bc	16.58c	2.00 <sub>d</sub>	0.26c
150	23c	21.89 b	86 b	91.8 c	18.18 b	2.33c	0.27 <sub>b</sub>
200	26a	24.05a	94 a	95.9a	17.22a	2.66a	0.28a
250	25 <sub>b</sub>	21.13 b	85 b	94.4 ab	17.16 bc	2.45 <sub>b</sub>	0.26 <sub>b</sub>
<b>CV</b>	5.23	2.06	2.27	1.54	2.98	3.98	7.86
Sig.	**	$**$	$**$	$**$	$***$	$***$	$***$

<span id="page-7-0"></span>**Table 2.** Effect of different levels of foliar applied B on yield components and grain yield of aromatic rice cultivar Kataribhog

Different letters in each column are significantly different from each other. \*\* and \*indicate significant differences at  $p \le 0.05$  and 0.01, respectively. CV = Co-efficient of variation

B dose (ppm)		Boron in leaf $(\mu g/mL$ leaf)	Boron in grain $(\mu g/mL)$		
	Mean $\pm$ SD	$%$ increase	Mean $\pm$ SD	% increase	
$\theta$	$10.36 \pm 0.79$ d	$\overline{\phantom{0}}$	$11.43 \pm 1.10c$		
50	$11.64 \pm 0.29$ cd	11	$12.96 \pm 0.20$ bc	11.8	
100	$12.35 \pm 0.60$ cd	16.1	$13.89 \pm 0.72$ bc	17.7	
150	$12.69 \pm 0.90$ bc	18.4	$15.18 \pm 0.55b$	24.7	
200	$15.32 \pm 1.50a$	32.4	$19.35 \pm 0.77a$	40.9	
250	$13.34 \pm 1.28$ ab	22.3	$17.48 \pm 0.58$ ab	34.6	

**Table 3.** Boron content in leaf and grain after foliar applied different levels of B in aromatic rice

Values are means  $\pm$  standard deviation (n=3). The different letters indicate statistically significant differences among the mean values at P≤0.05 using Tukey's post hoc test. The percent increase is relative to control (B0).

phyll measurement showed a close relationship with grain yield [\(Maiti et al.,](#page-10-19) [2004;](#page-10-19) [Blackmer and Schepers,](#page-9-17) [1995\)](#page-9-17). However, the SPAD values showed consistent positive relations with grain yield at different growth stages in wheat [\(Islam et al.,](#page-10-10) [2014\)](#page-10-10). From the study, it was also observed that the relationship was also remarkable even at 70 DAT but it dramatically changed onward and showed a strong negative correlation and became significant ( $r = -0.93$ <sup>\*\*</sup>) at 90 DAT. The decrease in chlorophyll content for the higher concentration of foliar-applied B and such a negative relationship between SPAD values and grain yield indicated that leaf assimilates were probably translocated into the grains. The SPAD values at 80 and 90 DAT were negatively related to the early stages of plant SPAD values at different DAT, affirming the loss of chlorophyll or nitrogen content in leaf at the reproductive growth stages.

# **3.9 Flag leaf SPAD value** *vs* **grain yield**

The SPAD values of the flag leaf had negative correlations with the grain yield [\(Table 5\)](#page-8-0). The relationships were less negative from 62 to 83 DAT but significantly more negative ( $r = -0.98$ <sup>\*\*</sup>) at 86 DAT. It indicated that translocation of assimilates from flag leaf to grain

was more pronounced during the grain-filling period. Similar is the case at the beginning of the panicle initiation phase (71 DAT), where the relationship was significantly negative. As the flag leaf SPAD values decreased for higher doses of B during this period, flag leaf reserve translocation to the grain was highly evident for higher B doses. In the previous studies, it was observed a close relationship between flag leaf chlorophyll measured from SPAD meter and grain yield [\(Islam et al.,](#page-10-10) [2014;](#page-10-10) [Maiti et al.,](#page-10-19) [2004\)](#page-10-19). However, a significant strong relationship prevailed at the reproductive phase of crops. [Ramesh et al.](#page-10-20) [\(2002\)](#page-10-20) noticed a substantial correlation between SPAD value and grain yield of direct-seeded rice.

## **3.10 Grain yield** *vs* **plant B content**

The correlation coefficient between grain yield and B content leaf showed a strong relationship (*r* = 0.84\*\*) [\(Fig. 6\)](#page-8-1). The relationship between B content in grain and grain yield was more positively correlated (*r* = 0.92\*\*). This relationship indicates that foliar-applied B accelerated B accumulation in plant leaves that eventually translocated into the grains and increased the grain yield in aromatic rice.

Parameter	Υ1	X1	X <sub>2</sub>	X3	X4	X5	X6	X <sub>7</sub>
Y1								
X1	0.62							
X <sub>2</sub>	$0.96**$	0.64						
X3	$0.97**$	0.61	$0.93**$					
X4	$0.96**$	0.63	$0.99**$	$0.97**$				
X5	0.72	0.7	0.67	$0.84*$	0.76			
X6	$-0.6$	$-0.77$	$-0.75$	$-0.65$	$-0.76$	$-0.55$		
X <sub>7</sub>	$-0.93*$	$-0.45$	$-0.95**$	$-0.84*$	$-0.91*$	$-0.44$	0.64	

<span id="page-8-0"></span>**Table 4.** Correlation matrix between SPAD values and grain yield in aromatic rice as influenced by B application

Y1=grain yield (t/ha) and X1, X2, X3, X4, X5, X6and X7 are the SPAD readings at 30, 40, 50, 60,70, 80 and 90 DAT respectively. \*\*indicates significance at  $P \le 0.01$ ; \*indicates significance at  $P \le 0.05$ 

**Table 5.** Correlation matrix between flag leaf SPAD values and grain yield in aromatic rice as influenced by B application



Y1=grain yield (t/ha) and X1, X2, X3, X4, X5, X6, X7, X8 and X9 are the flag leaf SPAD readings at 62, 65, 68, 71, 74, 77, 80, 83 and 86 DAT respectively; \*\*indicates significance at  $p \le 0.01$ ; \*indicates significance at  $p \le 0.05$ 



<span id="page-8-1"></span>**Figure 6.** Correlation co-efficient between grain yield and B content of leaf and grain in aromatic rice cultivar Kataribhog

# **4 Conclusion**

The study reveals that foliar-applied B is beneficial in reducing yield loss of aromatic monsoon rice experiencing low temperatures during the reproductive stage. However, B dose to be applied as foliar needs careful selection. The plant response to the low amount of B (50 ppm) was not remarkable, whereas a high dose (250 ppm) might cause toxicity in rice plants. Our study suggests that B in soluble form from borax at 200 ppm B (0.8 kg ha<sup>-1</sup>) is effective and could be applied for reducing pollen sterility and attaining optimum grain yield in aromatic rice cv. Kataribhog if plants are exposed to low temperatures during the reproductive stage. A detailed study on the crop responses to foliar-applied B under variable sowing dates might provide a clear understanding of the effectiveness of B application in aromatic monsoon rice associated with low temperature.

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# **Conflict of Interest**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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