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Growth, yield and fiber quality characteristics of Bt and non-Bt cotton cultivars in response to boron nutrition

MEHRAN Muhammad[1], ASHRAF Muhammad[1*], SHAHZAD Sher Muhammad[2], SHAKIR Muhammad Siddique[3], AZHAR Muhammad Tehseen[4,7], AHMAD Fiaz[5] and ALVI Alamgir[6]

Abstract

Background Boron (B) deficiency is an important factor for poor seed cotton yield and fiber quality. However, it is often missing in the plant nutrition program, particularly in developing countries. The current study investigated B's effect on growth, yield, and fiber quality of Bt (CIM-663) and non-Bt (Cyto-124) cotton cultivars. The experimental plan consisted of twelve treatments: Control (CK); B at 1 mg·kg^{-1} soil application (SB1); 2 mg·kg^{-1} B (SB2); 3 mg·kg^{-1} B (SB3); 0.2% B foliar spray (FB1); 0.4% B foliar spray (FB2); 1 mg·kg^{-1} B + 0.2% B foliar spray (SB1 + FB1); 1 mg·kg^{-1} B + 0.4% B foliar spray (SB1 + FB2); 2 mg·kg^{-1} B + 0.2% B foliar spray (SB2 + FB1); 2 mg·kg^{-1} B + 0.4% B foliar spray (SB2 + FB2); 3 mg·kg^{-1} B + 0.2% B foliar spray (SB3 + FB1); 3 mg·kg^{-1} B + 0.4% B foliar spray (SB3 + FB2). Each treatment has three replications, one pot having two plants per replication.

Results B nutrition at all levels and methods of application significantly ($P \leq 0.05$) affected the growth, physiological, yield, and fiber quality characteristics of both cotton cultivars. However, SB2 either alone or in combination with foliar spray showed superiority over others, particularly in the non-Bt cultivar which responded better to B nutrition. Maximum improvement in monopodial branches (345%), sympodial branches (143%), chlorophyll-a (177%), chlorophyll-b (194%), photosynthesis (169%), and ginning out turn (579%) in the non-Bt cultivar was found with SB2 compared with CK. In Bt cultivar, although no consistent trend was found but integrated use of SB3 with foliar spray performed relatively better for improving cotton growth compared with other treatments. Fiber quality characteristics in both cultivars were improved markedly but variably with different B treatments.

Conclusion B nutrition with SB2 either alone or in combination with foliar spray was found optimum for improving cotton's growth and yield characteristics.

Keywords Boron, Cotton, Fiber length, Fiber strength, GOT, Micronaire value, Seed cotton yield

*Correspondence:
ASHRAF Muhammad
mashraf@bzu.edu.pk; mashraf_1972@yahoo.com
[1] Department of Soil Science, Bahauddin Zakariya University, Multan, Pakistan
[2] Department of Soil and Environmental Sciences, College of Agriculture, University of Sargodha, Sargodha, Pakistan
[3] Pesticide Residue Laboratory, Kala Sha Kaku, Pakistan
[4] Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan
[5] Physiological/Chemistry Section, Central Cotton Research Institute, Multan, Pakistan
[6] Soil and Water Testing Laboratory, Jhelum, Pakistan
[7] School of Agricultural Science, Zhengzhou University, Zhengzhou, 450000, China

Introduction

Cotton (*Gossypium hirsutum* L.) is an important commercial crop grown in various environments for its high-quality fiber and oil. It is globally playing a leading role in the agricultural and industrial economy by providing raw materials, particularly for the textile industry, and employment (Rana et al. 2020). Globally, it was grown on 33.1 million hectares, yielded 136 million bales, and produced about 35% of the total fiber during the year 2020 (FAO 2021). Pakistan is ranked in the 3rd position in the world for cotton exports, the 4th in terms of area under cotton cultivation and the 39th in average productivity. Around 26% of farmers in Pakistan are growing cotton on 1937 thousand hectares, and producing 8.3 million bales. It provides raw materials for the textile industry which is the largest agro-industrial sector of Pakistan, employs 17% of people, earns 60% of foreign exchange, and contributes 0.6% to GDP and 2.4% of the value added in agriculture (Economic Survey of Pakistan 2022).

An adequate plant nutrition program for cotton should be comprised of macronutrients including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) as well as micronutrients such as copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), boron (B), chlorine (Cl), nickel (Ni) and molybdenum (Mo) (White and Brown 2010). However, the current nutrient management program for cotton in Pakistan is based mainly on the use of N, P, and K, while neglecting micronutrients (Khan et al. 2016). An inadequate and imbalanced supply of plant nutrients might be the major cause of low seed cotton yield and fiber quality of cotton in Pakistan (Ashraf et al. 2017). The excessive vegetative growth, poor flower, and fruit setting, as well as retention, and increased susceptibility to insects and pests, might be the result of poor and imbalanced crop nutrition (Rodrigues et al. 2022). Intensive cultivation, high yielding targets, soil alkalinity, and inadequate use of chemical fertilizers result in the deficiencies of multiple nutrients (Yaseen et al. 2013). The deficiency of macro and micronutrients is declining cotton productivity and fiber quality in current years which will become worse in the future if not addressed appropriately (Kumar et al. 2018).

B is considered the most important micronutrient required in all stages of cotton growth, particularly during flowering and boll formation (Rashid et al. 2002; Yeates et al. 2010). In various areas of the world where cotton is being grown, B deficiency is widespread (Zhao and Oosterhuis 2003; Ahmed et al. 2011). Boron deficiency affects 50% of Pakistan's cotton-growing regions (Ahmed et al. 2013). It has been found that tropical soils with their low levels of organic matter and clay are frequently deficient in B (Communar and Keren 2008; Arif et al. 2012). The soil B concentration of 0.60 $mg \cdot kg^{-1}$ extracted with hot water has been considered as the threshold for general crops (Aitken and McCallum 1988) while 0.4~0.55 $mg \cdot kg^{-1}$ for cotton (Oosterhuis 2001). Ahmad et al. (2019) reported that sandy texture, high pH, and low organic matter content could be the main reasons for low B availability for cotton. Furthermore, high calcium carbonate not only increases the soil pH to reduce B availability but also serves as the binding site for the adsorption of soluble B (Shaaban et al. 2004; Shaaban and Helmy 2006).

B is essential for several metabolic processes in cotton including carbohydrate metabolism, sugar transfer, respiration, flower, and fruit development, cell division and elongation, as well as membrane stability (Blevins and Lukaszewski 1998; Zhao and Oosterhius 2002; Ali et al. 2011; Mengel et al. 2012). B contents above 16 $mg \cdot kg^{-1}$ in recently matured leaves of cotton are considered sufficient for growth and yield (Rosolem et al. 1999). B deficiency may produce small and deformed bolls, poor flower and fruit setting as well as retention, and consequently reduced seed cotton yield and fiber quality (Roberts et al. 2000; Brown et al. 2002; Fontes et al. 2008). According to Sankaranarayanan et al. (2010), B deficiency at the maturity stage of cotton increases the shedding of flowers and bolls, which eventually lowers the seed cotton yield. Cotton is found to be more sensitive to B deficiency at the reproductive phase which might the major factor of low seed cotton yield on B-deficient soils (Rosolem and Costa 2000; de Oliveira et al. 2006). The boll formation and retention in cotton is greatly affected by carbohydrate contents in plants which depends on the B-driven movement of photo-assimilates from leaves to fruits (Bogiani and Rosolem 2012). The reduction in carbohydrate translocation due to B deficiency may cause increased boll shedding, and less seed cotton yield (Zhao and Oosterhuis 2003). Furthermore, water transport, Ca absorption, hormone biosynthesis, and root growth in plants are severely affected by B deficiency, reducing cotton growth and development (Abdulnour et al. 2000; Lou et al. 2001).

Plant response to B nutrition may vary greatly depending upon crop species, varieties within species, level and method of B application, nature of the soil, and climatic conditions (Ahmad et al. 2009). Rosolem et al. (1999) reported that cotton varieties may behave differentially to B nutrition due to the variations in these varieties' potential for carbohydrate transport, B storage and utilization, and associated mechanisms. B application methods including seed dressing, soil, and foliar application may perform differently depending upon many soil, plant, and climatic factors (Kumar et al. 2018).

An adequate supply of B is required for optimum crop yield and quality. However, the differential response of crop species and varieties within species, and a narrow range between deficiency and toxicity levels of B in soil necessitate the choosing of the optimum B dose for achieving quality crop production. It is considered that Bt and non-Bt cotton cultivars are greatly different in their growth and yield behavior as well as nutritional requirements. The present research was planned to evaluate the effect of different levels and methods of B application on growth, yield, physiological, and fiber quality characteristics of Bt and non-Bt cotton cultivars. The research was based on the hypothesis that a combination of soil and foliar application of B might be more effective to achieve optimum seed cotton yield and fiber quality characteristics.

Materials and methods

Experimental site description

The experiment was conducted under natural conditions in an open wirehouse having GPS values 30.10° N, 71.25° E, and 128.3 m elevation at Faculty of Agricultural Sciences & Technology, Bahauddin Zakariya University, Multan, Pakistan. During the experimental period, the minimum monthly temperature ranged from 19.8 to 28.9 °C while the maximum temperature was in the range of 36.2~42.2 °C. Relative humidity changed from 35% to 58%, precipitation was 12~24 mm, evapotranspiration was 3.1~9.6 mm, the wind speed was 0.83~2.77 $m·s^{-1}$, and sunshine was 8.5~10.5 h per day during this period. The soil was collected from a cultivated field under a cotton-wheat cropping system. The soil was air-dried, pulverized, and passed through a 2-mm sieve prior to filling the pots. Earthen pots having a volume of 25 × 20 × 20 cm^3 were used in experimentation. Each pot was lined with a polythene sheet and filled with 20 kg of prepared soil. Selected physicochemical characteristics of soil analyzed prior to experimentation are presented in Table 1.

Table 1 Pre-sowing analysis of experimental soil

Soil characteristic	Values
Soil texture	Loam
Electrical conductivity	0.73 $dS·m^{-1}$
Sodium adsorption ratio	2.30 $(mmol·L^{-1})^{1/2}$
pH	8.22
Organic matter	0.71%
Saturation percentage	28.45%
Total N	0.068%
Available P	7.14 $mg·kg^{-1}$
Extractable K	198 $mg·kg^{-1}$
Hot water extractable B	0.295 $mg·kg^{-1}$

Experimental details

The experimental plan comprised of twelve treatments: Control (CK); B at 1 $mg·kg^{-1}$ soil application (SB1); 2 $mg·kg^{-1}$ B (SB2); 3 $mg·kg^{-1}$ B (SB3); 0.2% B foliar spray (FB1); 0.4% B foliar spray (FB2); 1 $mg·kg^{-1}$ B + 0.2% B foliar spray (SB1 + FB1); 1 $mg·kg^{-1}$ B + 0.4% B foliar spray (SB1 + FB2); 2 $mg·kg^{-1}$ B + 0.2% B foliar spray (SB2 + FB1); 2 $mg·kg^{-1}$ B + 0.4% B foliar spray (SB2 + FB2); 3 $mg·kg^{-1}$ B + 0.2% B foliar spray (SB3 + FB1); 3 $mg·kg^{-1}$ B + 0.4% B foliar spray (SB3 + FB2). Each treatment was replicated thrice, and each replication has one pot having two plants. Measurements were made separately for each plant and then averaged to get the mean value for each replication. Boric acid (H_3BO_3) from Sigma Aldrich Chemicals was used as a B source. Soil application of B was done prior to sowing by incorporating the required amount of H_3BO_3 into respective pots. While the foliar spray was made at 35 and 70 days after germination using 30 mL solution for each plant per spray. Two cotton cultivars CIM-663 and Cyto-124 were used in the experimentation. CIM-663 was a Bt cultivar having high yield potential, heat tolerance, big boll, and tolerance to pest incidence. It was developed by Central Cotton Research Institute, Multan, Pakistan in the year 2020. It has a fiber length of 28.8 mm, a ginning out turn (GOT) of 38.8%, and a micronaire value of 4.4 $\mu g·inch^{-1}$. CYTO-124 was a non-Bt high-yield cultivar that possessed resistance against the leaf curl virus. It was also developed by Central Cotton Research Institute, Multan, Pakistan in the year 2016. It has a fiber length of 30.3 mm, a GOT of 43%, and micronaire value of 4.4 $\mu g·inch^{-1}$.

The sowing was done on April 22, 2021. Ten dehulled cottonseeds of each cultivar were sown in each pot and thinned to two seedlings per pot 15 days after germination. The uprooted plants were incorporated into the same pot. Recommended amounts of N 80 $mg·kg^{-1}$ soil as urea, P_2O_5 50 $mg·kg^{-1}$ soil as single superphosphate, and K_2O 50 $mg·kg^{-1}$ soil as potassium sulfate were added. The whole of P, K, and 1/3 N were added at the time of sowing while the remaining N was added in two splits, 40 and 75 days after germination. For plant protection against different insects and pests, Bifenthrin, Pyriproxyfen, Acephate, and Novastar were sprayed when required. Weeding was done manually throughout the experimentation.

Physiological characteristics

Physiological characteristics in terms of chlorophyll contents, membrane stability index (MSI) and relative water content (RWC) were determined during active boll development (80 days after germination). Chlorophyll-a and chlorophyll-b were measured by the methods of Arnon (1949) and Davies (1976) using the 4th top most leaf from each plant. For this purpose, 0.5 g of leaf samples was treated overnight in 80% acetone. Absorbance readings of the supernatant was recorded at 645 (A) and 663 (B) nm with spectrophotometer (Beckman Coulter DU 730 UV–Vis Spectrophotometer, USA), and used the following formula to determine the chlorophyll content:

$$\text{Chlorophyll} - \text{a}\left(\text{mg}\cdot\text{g}^{-1}\right) = \{[(0.0127\times\text{B}) - (0.00269\times\text{A})\times\text{V}]/\text{W}\};$$

$$\text{Chlorophyll} - \text{b}\left(\text{mg}\cdot\text{g}^{-1}\right) = \{[(0.0229\times\text{A}) - (0.00468\times\text{B})\times\text{V}]/\text{W}\};$$

where A and B are absorbance, V is the volume of sample extract, and W is the weight of the sample.

For MSI estimation, 100 mg of leaf material (the 5th topmost leaf) was divided into two sets and each placed in test tubes containing 10 mL of double distilled water. One set of leaf samples was heated in a water bath at 40 °C for 30 min, and the conductivity of the solution (C1) was measured with a conductivity meter (Elico, CM 183 EC-TDS analyzer, India). The second set of leaf samples in test tubes was heated in a water bath for 20 min at 100 °C, and its conductivity was measured (C2). The MSI was calculated in accordance with method of Blum and Ebercon (1981).

$$\text{MSI} = [1-(\text{C1}/\text{C2})]\times 100.$$

For determining RWC, the 5th topmost leaf from each plant (after measuring MSI) was weighed to record the fresh weight. After that, leaf segments were soaked in distilled water for four hours and reweighed for turgid weight. The leaf segments were then dried at 70 °C for constant weight in an oven (SLN 32, POL-EKO-APARA TURA). RWC was calculated according to Barr and Weatherley (1962).

$$\text{RWC}(\%) = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}}\times 100$$

Gas exchange characteristics

Measurements of net photosynthetic rate, transpiration rate, and stomatal conductance were made on the fully expanded 3rd topmost leaf of each plant using an open-system portable infrared gas analyzer (LCA-4 ADC, Analytical Development Company, Hoddesdon, England). Measurement was made at 9.0 am with the following specifications/adjustments; maximum leaf surface PAR was 1 711 $\mu mol\cdot m^{-2}\cdot s^{-1}$, the air molar flow per unit leaf area was 403.3 $\mu mol\cdot m^{-2}\cdot s^{-1}$, the atmospheric pressure was 99.9 kPa, the water vapor pressure into the chamber was 6.0∼8.9 mbar, the leaf temperature was 30.7∼42.0 °C, the ambient temperature was 28.6∼38.5 °C, and the ambient CO_2 concentration was 352 $\mu mol\cdot mol^{-1}$.

Leaf boron content

Leaf samples (the 6th and 7th leaves from the top) were collected at 80 days after germination. The leaves were washed with distilled water, air-dried and then oven dried at 72 °C till constant weight in an oven (SLN 32, POL-EKO-APARATURA). Dry ashing was used to determine leaf B content in accordance with Chapman and Pratt (1961). Spectrophotometer (Beckman Coulter DU 730) was used to obtain absorbance measurements of samples, blanks, and standard solutions at 420 nm. B content was calculated using the calibration curve (Bingham 1982; Ho et al. 1986; Malekani and Cresser 1998). The following formula was used to compute the B content;

$$\text{B}\left(\text{mg}\cdot\text{kg}^{-1}\right) = \text{B}\left(\text{mg}\cdot\text{kg}^{-1}, \text{from calibration curve}\right)\times\text{V}/\text{W}$$

where V is the total volume of the plant digest (mL) and W is the weight of dry plant (g).

Plant growth and yield characteristics

Data regarding plant height, monopodial and sympodial branches per plant, and leaf area per plant were recorded at 120 days after germination. Plant height was measured with a meter rod, and leaf area with a leaf area meter (LI-3100, LI-COR, Lincoln, NE), while other characteristics were recorded manually. At maturity, yield characteristics including the number of bolls per plant, boll size, and boll weight were measured. Boll size was measured with a Vernier caliper. Seed cotton yield and lint yield were measured after picking and ginning. For the measurement of ginning out turn (GOT %), lint weight was divided by seed cotton weight and multiplied by 100.

Fiber quality characteristics

Fiber length was measured using Fibrograph (ASTM 1994a). Pressley Fiber Bundle Tester was used to measure fiber strength (ASTM 1994b), while Micronaire Tester (ASTM 1994c) for fiber fineness.

Statistical analysis

The statistical analysis was done in accordance with a completely randomized design with two factors, one factor being the cultivar and the other factor being the B application (Steel et al. 1997). The least significant difference (LSD) test was performed to compare the mean values of different treatments.

Results

Plant growth characteristics

Plant growth characteristics of Bt and non-Bt cotton cultivars in terms of plant height, monopodial branches, sympodial branches, and leaf area were significantly ($P \leq 0.05$) affected by different B levels and methods of application. When comparing the B levels, a mixed trend was observed. Overall, soil + foliar application performed best followed by soil and foliar application in descending order (Table 2). Among the soil application levels, the tallest plants were found with SB3 for Bt and SB2 for the non-Bt cultivar. In the case of foliar application, FB1 showed superiority over FB2. When combined use of soil and foliar application was done, SB1 + FB1 caused maximum improvement in plant height of both Bt and non-Bt cultivars. The highest increase in monopodial branches plant^{-1} of Bt cultivar was 377% with SB3 + FB2 compared with CK. However, non-Bt cultivar performed optimally with SB2. Sympodial branches plant^{-1} were highest with SB1 + FB1 for Bt and SB2 for the non-Bt cultivar. Leaf area was improved with B nutrition, highest improvement of 82.9% in the Bt cultivar with SB3 + FB2 compared with CK. The non-Bt cultivar showed the highest leaf area with SB2 + FB2, indicating that it required a relatively lower level of B than the Bt cultivar.

Physiological characteristics

B levels and methods of application had a significant ($P \leq 0.05$) effect on the physiological characteristics of both Bt and non-Bt cotton cultivars. The highest improvement was found with SB2 either alone or in combination with foliar spray (Table 3). Chlorophyll-a contents were found highest with SB3 + FB2 in Bt while with SB2 in the non-Bt cultivar. The highest increase of 140% in chlorophyll-b was observed with SB2 + FB1 in Bt and 194% with SB2 in the non-Bt cultivar compared with CK. B nutrition with FB1 showed superiority over others for improving MSI in both Bt and non-Bt cultivars. Relatively, a slight increase in RWC was found with B nutrition, highest improvement with SB3 + FB2 in Bt while with SB2 in the non-Bt cultivar compared with CK.

Gas exchange characteristics

Gas exchange characteristics such as photosynthetic rate, stomatal conductance, and transpiration rate were significantly ($P \leq 0.05$) affected by different levels and methods of B application in both Bt and non-Bt cotton cultivars (Table 4). Overall, integrated use of soil and foliar application caused higher improvement in gas exchange characteristics of both cultivars compared with the sole application. The highest photosynthetic rate was found with SB2 + FB2 for Bt and with SB2 for the non-Bt cultivar. Stomatal conductance was improved with B nutrition, with higher improvement in

Table 2 Growth characteristics of Bt and non-Bt cotton cultivars grown with different levels and methods of B application

Treatments	Plant height /cm		Monopodial branch numbers per plant		Sympodial branch numbers per plant		Leaf area /m^2	
	Bt	Non-Bt	Bt	Non-Bt	Bt	Non-Bt	Bt	Non-Bt
CK	45.1^{f}	51.0^{ef}	1.3^{de}	1.1^{e}	7.0^{ef}	6.3^{e}	69.0^{g}	72.9^{g}
SB1	49.4^{de}	61.8^{d}	4.4^{b}	2.1^{cd}	10.3^{cd}	14.2^{a}	94.5^{d}	88.0^{de}
SB2	51.4^{d}	72.8^{bc}	2.1^{d}	4.9^{a}	11.9^{c}	15.3^{a}	110.2^{bc}	97.4^{c}
SB3	54.9^{cd}	70.1^{c}	3.2^{c}	3.2^{bc}	14.0^{b}	9.4^{cd}	92.8^{d}	103.2^{b}
FB1	64.5^{a}	67.4^{cd}	3.4^{c}	3.3^{bc}	11.4^{c}	12.0^{b}	86.2^{e}	74.8^{g}
FB2	57.8^{bc}	69.2^{c}	3.2^{c}	2.4^{cd}	14.2^{ab}	13.2^{ab}	85.4^{e}	88.4^{de}
SB1 + FB1	65.2^{a}	61.5^{d}	2.9^{cd}	1.9^{d}	15.9^{a}	12.1^{b}	94.8^{d}	82.7^{e}
SB1 + FB2	63.7^{a}	85.3^{a}	3.9^{bc}	2.2^{cd}	12.2^{bc}	13.4^{ab}	80.0^{ef}	89.1^{d}
SB2 + FB1	59.4^{ab}	65.9^{cd}	3.1^{c}	2.1^{cd}	14.1^{ab}	13.0^{b}	97.3^{d}	89.3^{d}
SB2 + FB2	60.3^{a}	59.8^{de}	3.3^{c}	3.3^{bc}	11.4^{c}	11.4^{bc}	111.0^{bc}	111.3^{a}
SB3 + FB1	57.7^{bc}	64.5^{cd}	5.1^{ab}	1.9^{d}	12.3^{bc}	11.3^{bc}	113.4^{b}	100.4^{bc}
SB3 + FB2	60.4^{b}	59.2^{de}	6.2^{a}	2.1^{cd}	11.9^{c}	9.5^{cd}	126.2^{a}	76.5^{g}

Values are means of three replicates with two plants per replicate (n = 3). Values with the same letter in a column do not differ significantly at $P \leq 0.05$. CK: Control; SB1: 1 mg·kg^{-1} B through soil application; SB2: 2 mg·kg^{-1} B; SB3: 3 mg·kg^{-1} B; FB1: 0.2% B foliar spray; FB2: 0.4% B foliar spray; SB1 + FB1: 1 mg·kg^{-1} B + 0.2% B foliar spray; SB1 + FB2: 1 mg·kg^{-1} B + 0.4% B foliar spray; SB2 + FB1: 2 mg·kg^{-1} B + 0.2% B foliar spray; SB2 + FB2: 2 mg·kg^{-1} B + 0.4% B foliar spray; SB3 + FB1: 3 mg·kg^{-1} B + 0.2% B foliar spray; SB3 + FB2: 3 mg·kg^{-1} B + 0.4% B foliar spray

Table 3 Physiological characteristics of Bt and non-Bt cotton cultivars grown with different levels and methods of B application

Treatments	Chlorophyll-a /(mg·g^{-1} FW)		Chlorophyll-b / (mg·g^{-1} FW)		MSI /%		RWC /%	
	Bt	Non-Bt	Bt	Non-Bt	Bt	Non-Bt	Bt	Non-Bt
CK	0.52^{e}	0.40^{f}	0.42^{f}	0.34^{fg}	68.7^{e}	64.8^{d}	75.2^{f}	68.6^{g}
SB1	0.70^{c}	0.75^{cd}	0.53^{e}	0.61^{c}	74.8^{cd}	69.7^{c}	83.4^{c}	78.7^{c}
SB2	0.71^{c}	1.11^{a}	0.72^{c}	1.00^{a}	83.9^{a}	76.2^{ab}	84.4^{bc}	84.1^{a}
SB3	0.83^{b}	0.83^{c}	0.54^{e}	0.62^{c}	64.8^{f}	57.4^{f}	78.6^{de}	78.4^{c}
FB1	0.82^{b}	0.62^{de}	0.65^{d}	0.60^{c}	76.5^{c}	73.1^{bc}	77.8^{e}	71.7^{ef}
FB2	0.90^{a}	0.60^{de}	0.74^{c}	0.63^{c}	71.8^{d}	65.9^{d}	86.1^{b}	73.8^{c}
SB1 + FB1	0.93^{a}	0.74^{cd}	0.83^{bc}	0.54^{d}	77.5^{bc}	74.4^{b}	78.8^{de}	72.4^{ef}
SB1 + FB2	0.85^{b}	0.63^{de}	0.90^{ab}	0.85^{b}	74.8^{cd}	75.3^{b}	85.7^{b}	71.5^{f}
SB2 + FB1	0.84^{b}	0.61^{de}	1.01^{a}	0.55^{d}	84.6^{a}	80.5^{a}	88.5^{a}	74.6^{de}
SB2 + FB2	0.91^{a}	0.96^{b}	0.72^{c}	0.72^{bc}	83.2^{a}	71.8^{bc}	78.2^{de}	78.7^{c}
SB3 + FB1	0.80^{b}	0.62^{de}	0.70^{c}	0.52^{d}	67.9^{e}	64.3^{de}	77.6^{e}	71.6^{ef}
SB3 + FB2	0.95^{a}	0.46^{f}	0.63^{d}	0.44^{de}	62.4^{fg}	57.8^{f}	89.3^{a}	73.5^{e}

Values are means of three replicates with two plants per replicate (n = 3). Values with the same letter in a column do not differ significantly at $P \le 0.05$. CK: Control; SB1: 1 mg·kg^{-1} B through soil application; SB2: 2 mg·kg^{-1} B; SB3: 3 mg·kg^{-1} B; FB1: 0.2% B foliar spray; FB2: 0.4% B foliar spray; SB1 + FB1: 1 mg·kg^{-1} B + 0.2% B foliar spray; SB1 + FB2: 1 mg·kg^{-1} B + 0.4% B foliar spray; SB2 + FB1: 2 mg·kg^{-1} B + 0.2% B foliar spray; SB2 + FB2: 2 mg·kg^{-1} B + 0.4% B foliar spray; SB3 + FB1: 3 mg·kg^{-1} B + 0.2% B foliar spray; SB3 + FB2: 3 mg·kg^{-1} B + 0.4% B foliar spray

Table 4 Gas exchange characteristics of Bt and non-Bt cotton cultivars grown with different levels and methods of B application

Treatments	Photosynthetic rate /(μmol (CO_2)·m^{-2}·s^{-1})		Stomatal conductance/(mol·m^{-2}·s^{-1})		Transpiration rate / (mg (H_2O)·m^{-2}·s^{-1})	
	Bt	Non-Bt	Bt	Non-Bt	Bt	Non-Bt
CK	4.73^{f}	5.64^{fg}	0.13^{f}	0.17^{e}	2.43^{f}	2.73^{e}
SB1	7.96^{c}	13.18^{b}	0.15^{ef}	0.21^{c}	3.20^{e}	5.64^{ab}
SB2	8.22^{c}	15.19^{a}	0.20^{de}	0.24^{b}	4.07^{d}	6.05^{a}
SB3	6.16^{de}	10.34^{d}	0.20^{de}	0.22^{c}	5.01^{c}	6.28^{a}
FB1	7.09^{d}	13.30^{b}	0.26^{c}	0.19^{d}	4.99^{c}	5.76^{ab}
FB2	7.98^{c}	11.79^{c}	0.31^{b}	0.18^{de}	5.13^{bc}	5.15^{b}
SB1 + FB1	9.18^{b}	11.42^{c}	0.24^{cd}	0.18^{de}	6.17^{ab}	6.44^{a}
SB1 + FB2	10.85^{a}	9.16^{de}	0.19^{de}	0.23^{bc}	5.73^{b}	6.23^{a}
SB2 + FB1	10.95^{a}	12.28^{bc}	0.19^{de}	0.27^{a}	6.07^{ab}	6.23^{a}
SB2 + FB2	11.04^{a}	11.04^{cd}	0.19^{de}	0.19^{d}	5.27^{bc}	5.27^{b}
SB3 + FB1	9.25^{b}	9.80^{d}	0.37^{a}	0.19^{d}	6.75^{a}	4.71^{bc}
SB3 + FB2	9.39^{b}	9.03^{de}	0.31^{b}	0.25^{ab}	5.96^{ab}	6.11^{a}

Values are means of three replicates with two plants per replicate (n = 3). Values with the same letter in a column do not differ significantly at $P \le 0.05$. CK: Control; SB1: 1 mg·kg^{-1} B through soil application; SB2: 2 mg·kg^{-1} B; SB3: 3 mg·kg^{-1} B; FB1: 0.2% B foliar spray; FB2: 0.4% B foliar spray; SB1 + FB1: 1 mg·kg^{-1} B + 0.2% B foliar spray; SB1 + FB2: 1 mg·kg^{-1} B + 0.4% B foliar spray; SB2 + FB1: 2 mg·kg^{-1} B + 0.2% B foliar spray; SB2 + FB2: 2 mg·kg^{-1} B + 0.4% B foliar spray; SB3 + FB1: 3 mg·kg^{-1} B + 0.2% B foliar spray; SB3 + FB2: 3 mg·kg^{-1} B + 0.4% B foliar spray

the Bt cultivar compared with the non-Bt one. Among different treatments, SB3 + FB1 performed best for Bt while SB2 + FB1 for the non-Bt cultivar in improving stomatal conductance. When comparing the B application methods for stomatal conductance, the Bt cultivar responded better to foliar application, while the non-Bt cultivar to soil application. In the case of transpiration, SB2 and SB3 performed better than SB1 either alone or in combination with foliar application. Among B application methods, integrated use of soil and foliar application performed best followed by foliar and soil application in descending order.

Leaf boron content

Leaf B was significantly ($P \le 0.05$) increased with the increasing level of B application. Soil application of B caused a higher increase in leaf B content compared with foliar application. Overall, SB3 + FB2 caused the highest increase of 295% in leaf B content of Bt and 269% in the non-Bt cultivar compared with CK (Fig. 1).

Yield characteristics

Seed cotton yield and yield characteristics including the number of bolls per plant, boll size, boll weight, and GOT were significantly ($P \leq 0.05$) affected by different levels and methods of B application in both Bt and non-Bt cotton cultivars (Fig. 2). The minimum number of bolls per plant was found in CK which improved with B nutrition, the highest improvement with SB3 + FB2 in Bt and SB2 + FB2 in the non-Bt cultivar compared with CK (Fig. 2a). Boll size was maximally improved by 71.6% in Bt cultivar with SB2 + FB1 while 36.5% in a non-Bt cultivar with SB2 compared with CK. Soil application caused a higher increase in boll size compared with foliar (Fig. 2b). The highest boll weight was found with SB3 in Bt and SB2 in the non-Bt cultivar (Fig. 2c). Seed cotton yield was improved with all levels and methods of B application, the highest improvement with SB2 in the Bt and SB1 + FB1 in the non-Bt cultivar (Fig. 2d). Minimum GOT was found in CK which improved maximally with SB1 in the Bt and with SB2 in the non-Bt cultivar (Fig. 2e).

Fiber quality characteristics

Fiber quality characteristics in terms of fiber length, fiber strength, and fiber fineness were relatively less affected by different levels and methods of B application in both Bt and non-Bt cultivars compared with growth and yield characteristics (Fig. 3). The highest improvement in fiber length was found with SB1 + FB2 in the Bt and with FB2 in the non-Bt cultivar (Fig. 3a). Fiber strength was least affected among the fiber quality characteristics by B nutrition. It was maximally improved by 13.2% in the Bt cultivar with SB2 + FB2, while 11.5% in the non-Bt cultivar with SB1 compared with CK (Fig. 3b). The highest improvement of 78.6% in fiber fineness of Bt cultivar was found with FB2 while 79.4% of non-Bt cultivar with SB3 compared with CK (Fig. 3c).

Discussion

B-mediated improvement in cotton growth of both cultivars was attributed to its involvement in the synthesis of photosynthetic pigments and photosynthesis (Liu et al. 2000; Karaman et al. 2012; More et al. 2018). According to Dordas (2006), a higher photosynthetic rate at adequate B supply could be the main mechanism for improving cotton growth and development. B-induced improvement in plant height was associated with its role in cell division, cell elongation, and the distance increase between main stem nodes and internodes (Ahmed et al. 2013). B deficiency might inhibit the development of petiole and peduncle cells, resulting in lower cotton growth and productivity (de Oliveira et al. 2006). The mixed trend to change the growth characteristics of both cotton cultivars by different levels and methods of B application was due to the reason that cotton required relatively lower B at the vegetative growth stage (Sagheer et al. 2019). The higher efficiency of soil + foliar application was associated with quick B supply by foliar spray

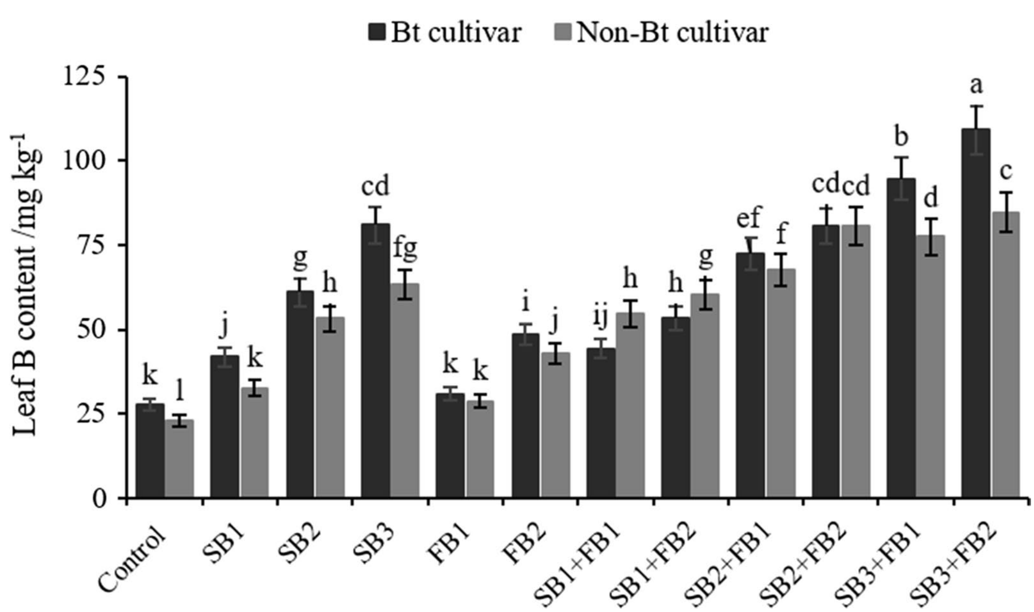

Fig. 1 Leaf B content of Bt and non-Bt cotton cultivars grown with different levels and methods of B application

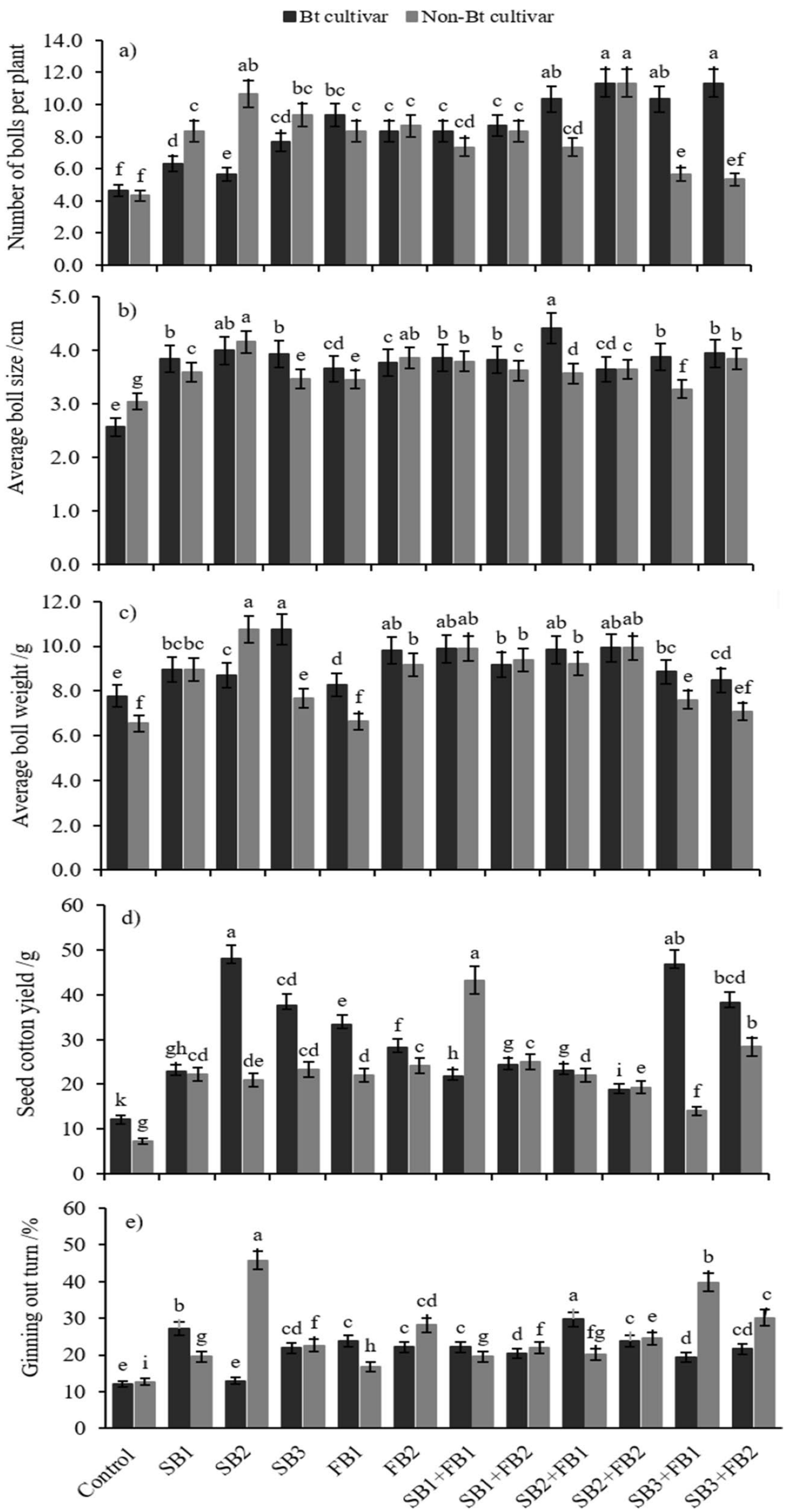

Fig. 2 Seed cotton yield and yield characteristics of Bt and non-Bt cotton cultivars grown with different levels and methods of B application

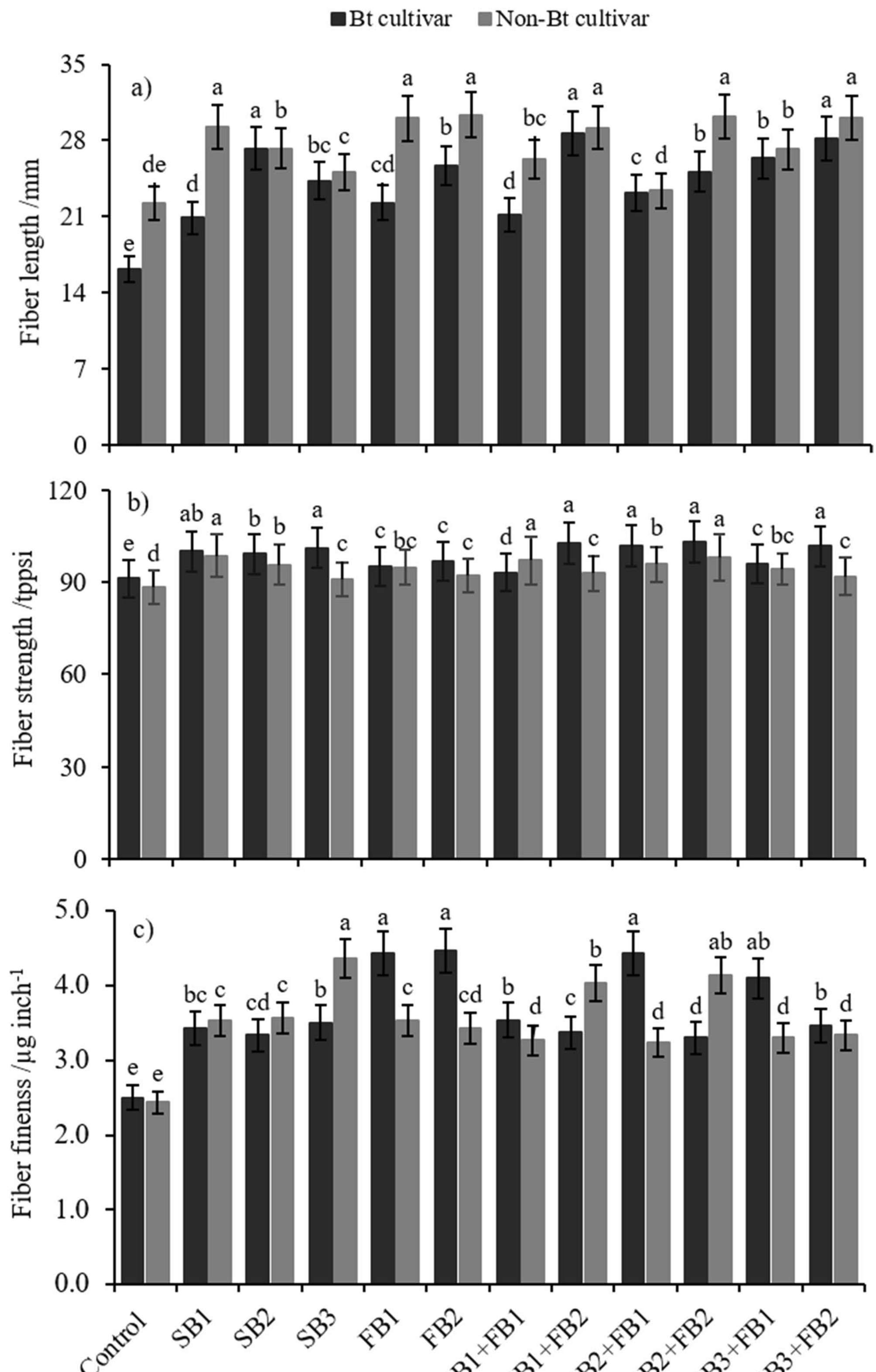

Fig. 3 Fiber quality characteristics of Bt and non-Bt cotton cultivars grown with different levels and methods of B application

and its persistent availability by soil application (Kumar et al. 2018; Atique-ur-Rehman et al. 2020).

B-mediated improvement in chlorophyll synthesis, MSI, and RWC could be due to its role in the structural stability of chloroplast and cell membrane (Nadim et al. 2012). B involvement in membrane integrity was associated with the synthesis of pectin which is a structural protein improved membrane structure stability (Hu et al. 1996; Wu et al. 2017). Furthermore, B deficiency enhanced the production of reactive oxygen species (ROS) which damaged the structure of chloroplast and cell membrane, resulting in lower chlorophyll content, photosynthesis, and MSI (Hajiboland and Farhanghi 2010; de Souza Júnior et al. 2022).

Improvement in photosynthesis, stomatal conductance, and transpiration with B nutrition was associated with increased leaf area (Li et al. 2012), higher chlorophyll synthesis (Dordas 2006), increased assimilation rate (Nadim et al. 2012), and translocation of photosynthates from source to sink (More et al. 2018). B deficiency could cause leaf yellowing, dieback, brittleness, leaf thickening, veins swelling, and leaf rupturing, all of which led to reduced chlorophyll contents and photosynthetic rate (Liu et al. 2014). B deficiency could decrease the stomatal density and chloroplast contents which led to lower chlorophyll and photosynthesis (Wei et al. 2022)). Boron deficiency might damage the vascular bundles which restricted the transport of water, carbohydrates, and nutrients, leading to lower photosynthesis, transpiration, and stomatal conductance (Li et al. 2017).

The marked increase in seed cotton yield and yield characteristics of Bt and non-Bt cultivars in response to B nutrition could be associated with its role in pollen production and pollen viability, germination and development of pollen tubes, flowering, fruit setting, and retention (Silva et al. 2003; Wang et al. 2003; de Oliveira et al. 2006; Qamar et al. 2020). Increased membrane integrity, photosynthetic rate, and stomatal conductance with B nutrition resulted in an increase in the number of bolls per plant, boll size, and weight (Ahmad et al. 2009). Higher yield characteristics at a high level of B application indicated that the B requirement was more critical at the reproductive phase in cotton (Wei et al. 2022). Furthermore, adequate B application increased the B content in the leaf which could also contribute to the improved cotton yield by improving chlorophyll synthesis, photosynthesis, enzyme activities, flowering, and boll development (Rashid and Rafique 2002). The higher B requirement Bt cultivar was related to its genetic makeup and higher yield potential (Shah et al. 2015).

The improvement in fiber quality with B nutrition might be associated with its role in cell division and differentiation, cell enlargement, photosynthesis, and photosynthates translocation from leaves to bolls (Liu et al. 2000; Zhao and Oosterhuis 2003; de Oliveira et al. 2006; Karaman et al. 2012; Bogiani et al. 2013). The role of B in improving fiber quality was also related to its involvement in enzymatic activities, hormonal balance, protein synthesis, and metabolism (Camacho-Cristobal et al. 2004; Martın-Rejano et al. 2011; Ahmed et al. 2013; Wei et al. 2022). Seilsepour et al. (2013) reported that B could improve the fiber quality of cotton by producing strong and well-developed fibers. B was found to speed up the fiber maturity and thus improving the fiber quality characteristics (Rashidi and Seilsepour 2011).

Conclusions

Cotton growth, physiological, yield, and fiber quality characteristics in Bt and non-Bt cultivars were improved by different levels and methods of B application. Among different treatments, SB2 either alone or in combination with foliar spray showed superiority over other treatments. B-mediated improvement in leaf area, chlorophyll synthesis, photosynthesis, stomatal conductance, and MSI could be the principal mechanisms for increased cotton productivity.

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Author contributions

Mehran M executed the experiment. Ashraf M and Shahzad SM helped in planning and writing the manuscript. Shakir MS, Ahmad F, and Alvi A helped in research planning and analytical work. Azhar MT helped with data analysis. All authors read and approved the final manuscript.

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Availability of data and materials

Mean data are provided in table and figure files. Replication data are available and can be provided on request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

None of the authors have any conflict of interest.

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