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INFLUENCE OF BORON NUTRITION ON PHYSIOLOGICAL PARAMETERS AND PRODUCTIVITY OF COTTON (*GOSSYPIUM HIRSUTUM* L.) CROP

SAGHEER AHMAD, NAZIM HUSSAIN*, NIAZ AHMED AND SYED MUHAMMAD ZAKA

Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan
**Corresponding author's email: nazimhussain@bzu.edu.pk*

Abstract

The productivity of arable crops is greatly impacted by imbalanced nutrient management. The deficiency of one or more mineral nutrients can cause substantial reduction in crop yields. Of the micronutrients, the deficiency of boron affects the yield potential of cotton to the greatest extent. This study was carried out to quantify the boron nutritional requirement of cotton. The treatments included three boron levels (0.0, 0.75 and 1.50 kg B ha^{-1}) and two application regimes (soil and soil plus foliar). Foliar application was done at 30, 60 and 90 days after sowing during peak growth stages. The results revealed that application of 1.50 kg B ha^{-1} along with foliar sprays increases crop growth rate (CGR) and net assimilation rate (NAR) by 15% and 31% respectively. While, the membrane leakage was reduced by 35% by addition of 1.50 kg B ha^{-1}, chlorophyll a and b contents improved by 2% and 5% through combined application of boron via soil and foliar spray. The water-use-efficiency was enhanced by 35% by application of B-fertilizer through soil as well foliar spray. Gas exchange parameters, i.e.net photosynthetic rate, transpiration rate and stomatal conductance increased by 66%, 47% and 62% respectively after application of B-fertilizer and there was a significant increase in number of bolls per plant, boll weight and an ultimate improvement in final seed cotton yield of 16%.

Key words: Cotton, *Gossypium hirsutum* L., Boron fertilizer, Soil application, Foliar spray, Seed cotton yield, Gas exchange characteristics.

Introduction

Cotton is one of the main cash crops of Pakistan and is an important source of foreign exchange for the country. Many soils in Pakistan are deficient in macro-and micro-nutrients (Sillanpaa, 1982). Out of the micronutrients, deficiency of boron (B) is widespread and affects 50-80% of soils in Pakistan (Sillanpaa, 1982; Gupta, 1993; Dell & Huang, 1997). The gravity of nutrient deficiency is exacerbated by the presence of calcareous soils and high pH (>7.0) combined with an arid and semi-arid environment (Shorrocks, 1997; Goldberg, 1997; Rashid & Ryan, 2004). The widespread deficiencies of micronutrients, particularly of boron (B), have now become an established phenomenon in cotton growing areas.

The current circumstances require efficient and balanced use of micronutrients in combination with NPK fertilizers to maximize cotton crop yield. Compared to other crop species, cotton, being dicot, is highly sensitive to deficiency of B and requires relatively large amounts to realize its yield potential. The prevalence of calcareous, high pH soils means that supply of the required amount of B to cotton is insufficient during vegetative and particularly reproductive growth (Gupta, 1993). B deficiency not only reduces growth and development but also causes reduction in cotton fiber quality (Dordas, 2006). B deficiency alters a number of physiological and biochemical processes which causes substantial reduction in vegetative and reproductive development (Yu and Bell, 1998; Ahmed *et al*., 2011). Boron is involved in translocation of sugars, in conserving the integrity of plasma membranes, in sustaining photosynthetic rates (Zhao & Oosterhuis, 2003), in cell division, nitrogen fixation and the metabolism of amino acids and nitrates (Shelp, 1993; Blevins & Lukaszewski, 1998). It is also involved in flower and fruit development, pollen germination and abscission (Dell *et al*., 2002). Han *et al*., (2008) reported that carbon dioxide assimilation, stomatal conductance and biological yield of sweet orange seedlings were influenced by boron. Other researchers (Miley *et al*., 1969; Murphy & Lancaster, 1971; Smithson, 1972; da Silva & de Andrade. 1980; Sun & Xu, 1986; Jiang *et al*., 1986; Dong, 1995; Howard *et al*., 1998, 2000; Dordas, 2006) reported that B fertilizer application in the soil as well foliar application caused an increase in cotton yield and fiber quality. However, other studies (Ohki, 1972; Oosterhuis & Venter, 1976; Heitholt, 1994) did not report any positive impact of B on production or fiber quality of cotton.

To establish whether foliar applied boron along with soil application during peak growth stages may improve cotton growth and development, an experiment was aimed to quantify the effectiveness of B-fertilizer on physical and physiological parameters of cotton plant.

Materials and Methods

A field oriented study was carried out at the Central Cotton Research Institute, Multan. The soils at the location are classified as Miani soil series (Hyperthermic Typic Haplocambid) consisting of silt loam with the following properties: moderately alkaline ($CaCO_3$, 5.7%), alkaline (pH, 8.3), soil organic matter (0.54%), extractable–P (8.5 mg P kg^{-1}), extractable-K (87 mg kg^{-1}), DTPA extractable–Zn (0.46 mg kg ha^{-1}) and HCl-extractable B (0.34 mg kg^{-1})[Ryan *et al*., 2001]. B treatments consisted of: (a) three boron levels (0.0, 0.75 and 1.50 kg B ha^{-1}) and (b) two application regimes (soil and soil plus foliar). Foliar application was done at 30, 60 and 90 days after sowing during peak growth stages. Plants were organized in split plot randomized complete block design with four replications. The cotton cultivar 'Cyto-178' was used as test crop. Borax (11%) was applied as boron source to cotton. The basal dose of fertilizers was 250 kg N, 100kg P_2O_5 and 100 kg K_2O ha^{-1} as urea, triple superphosphate and potassium sulphate, respectively. The planting geometry was 75 x 20cm and equivalent to 42000 plants per hectare. Phosphorus, potassium and various doses of B–along with 1/3rd of nitrogen were applied to soil by broadcast method before planting the crop. The plants from one square meter area were harvested and data on plant structure were recorded. While data on seed cotton yield were gathered by picking the crop at maturity. The determination of chlorophyll contents and membrane leakage were done according to Cornish *et al*., (1991) and Yan *et al*., (1996), respectively.

An open system LCA-4 ADC Portable Infrared Gas Analyzer was employed to measure various gas exchange characteristics. The measurements were recorded during peak flowering period, between 9.00 to 11.00 hours (Hajiboland & Farhanghi, 2010).The specifications of the instrument were: M flow of air/unit leaf area 403.3 $\mu molm^{-2}s^{-1}$; 99.0 k Pa pressure; pressure of water vapors inside chamber was maintained between 1120-1220 Pa; photosynthetically active radiation (PAR) at leaf surface was maximum up to 1711 $\mu molm^{-2}s^{-1}$; leaves temperature ranged between 36.7-38.8°C; ambient temperature ranged from 28.6 to 36.5°C and CO_2 concentration was 352 $\mu molmol^{-1}$. The conditions were replicated with each treatment (one leaf per replicate). Data were analyzed statistically by using Microsoft Excel 2007® and Statistix 8.1® (Analytical Software, Tallahassee, USA). The least significant difference (LSD) was done according to Steel *et al*., (1997).

Results and Discussion

Crop growth parameters: Crop growth rate (CGR) refers to dry matter produced by a crop in certain amount of time although it is affected by a variety of environmental and internal factors adequate levels of available micronutrients is one of them (Ahmed *et al*., 2018). Boron is an essential micronutrient required by plants for normal growth and development and its insufficiency results in growth impairments such as reduced plant height and biomass (Zhao & Oosterhuis, 2003). However when provided with the sufficient amounts of boron plants exhibit improved growth attributes including plant height at grain yields in wheat. This improved plant growth responses are probably owing to improved macronutrient uptake in response to boron application (Zafar *et al*., 2016). Similar results were found in this study where cotton crop showed significant improvement in crop growth rate (CGR) in response to B application as compared to control. Crop growth rate also differed significantly in response to the mode of B-fertilizer application and their interaction (Fig. 1) i.e. averaged across mode of application, CGR increased in parallel with increased B-fertilizer. The application of 1.50 kg B ha^{-1} caused an increase in CGR by 15% over the untreated control whereas it was increased by 10% by the addition of 0.75 kg B ha^{-1}. The data showed 3% increase of CGR in response to the addition of B-fertilizer through soil combined with foliar spray than applying B only through the soil. The date of B application also affected plant growth rates when plants were applied with 1.50 kg B ha^{-1}via the soil combined with three applications of 0.1% B at day 30, 60 and 90 after planting. Boron has multiple roles in plant physiology and its role for the improved plant growth responses is well documented. Niaz *et al*., (2013) showed improved plant height and number of nodes on main stem of cotton plants by 18.3% and 9.7% respectively in response to B application compared to untreated control. In another experiment Abid *et al*., (2007) 10.5% and 6.23% increased height and number of nodes in cotton treated with boron fertilizers @ 2kg/ha over control. The results from this study indicate that application of B- fertilizer causes an increase in cotton growth rate and net assimilate rates. This is supported by Zhao & Oosterhuis (2003) and Han *et al*. (2008), who found increased growth of cotton due to addition of B-fertilizer. Similarly Nadim *et al*., (2012) showed 23.58% higher crop growth rate in wheat in response to B application.

Net assimilation rates (NAR) is another parameter to measure plants abilities to produce dry weight and it reflects plants health and vigor in terms of its area of assimilatory surfaces (Ahmed *et al*., 2018). NAR is positively correlated by the application of variety of macro and micro nutrients. Boron application both by foliar and through soil residues resulted in 2.82% enhanced B accumulation in leaf tips of wheat and improved photosynthesis (Nadim *et al.*, 2012). NAR differed significantly ($p<0.05$) due to B-doses, mode of application and their interactive effects (Fig. 1). Averaged across mode of applications NAR increased successively with increased B-doses. The addition of 1.50 kg B ha^{-1} raised the level of NAR by 31% and by 15% after addition of 0.75 kg B ha^{-1}. Averaged across B-doses, the application of B-fertilizer through soil and foliar spray caused as increase in NAR of 5% over solely soil applied B-fertilizer. Similarly, Panhwar *et al.*, (2011) reported B and Zn combined soil application significantly increased the plant height, leaf area index, plants dry weight and chlorophyll content.

Cell membrane stability: B affects generic aspects of cellular physiology such as membrane integrity and our data show that electrolyte leakage was reduced significantly ($p\leq0.05$) with the addition of B fertilizer. Cell membrane leakage diverged significantly as a result of B-fertilizer addition, mode of application and their interactive effects (Fig. 2). Averaged across mode of application, the cell membrane leakage decreased linearly with increasing B-doses. The addition of 0.75 or 1.50 kg B ha^{-1} caused a reduction in cell membrane leakage by 12 and 35% respectively. Averaged across B-doses, the addition of B-fertilizer through soil in combination with foliar spray caused a reduction in membrane leakage by 8% compared to soil applied B-fertilizer. In plants, boron is considered to have important roles in cell wall synthesis, cell wall structure, membrane integrity and function (Nadim *et al*., 2012). It is proposed that role of B in membrane integrity is owing to its ability to cross link pectin a structural protein in the cell wall which is important for cell wall stability. The role of boron in improving plants stress tolerance is also well established owing to its role in inducing antioxidants in response to stress triggered production of reactive oxygen species (Uluisik *et al*., 2017). Membrane integrity may also be influenced by B in other ways: production of reactive oxygen species when plants face nutrient deficiencies cause damage to cell membranes and chloroplast structure (Parr & Loughhman, 1983; Cakmak & Romheld, 1997) which can manifest itself in augmented K^+ efflux (Hajiboland & Farhanghi 2010). For cotton, Loomis and Durst, (1991) reported that B–deficient tissues produce free radicals that damage the membrane.

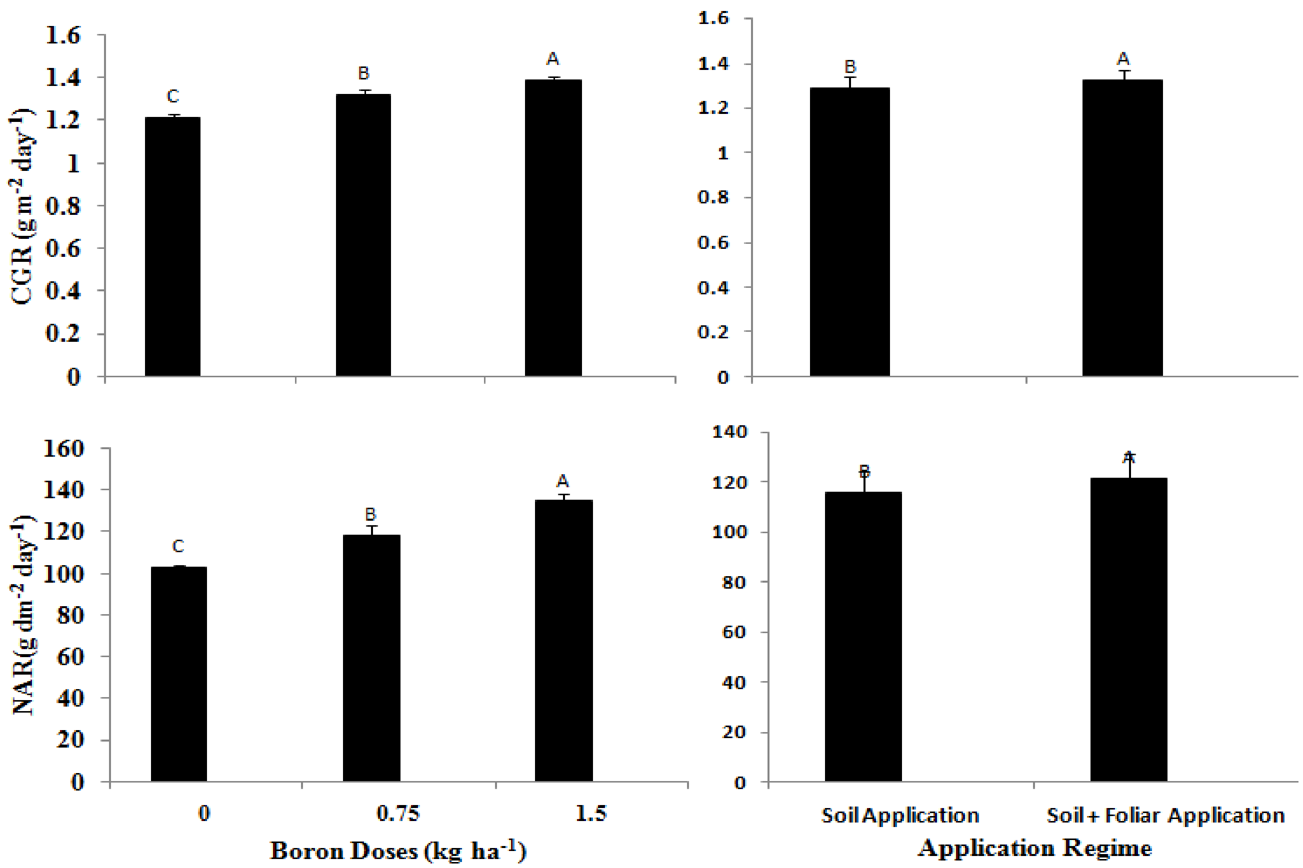

Fig. 1. Effect of different boron doses and application regime on CGR and NAR of cotton. Data represent the mean ±S.E. of four replications. The letters on different bars represent significance (p<0.05) of data.

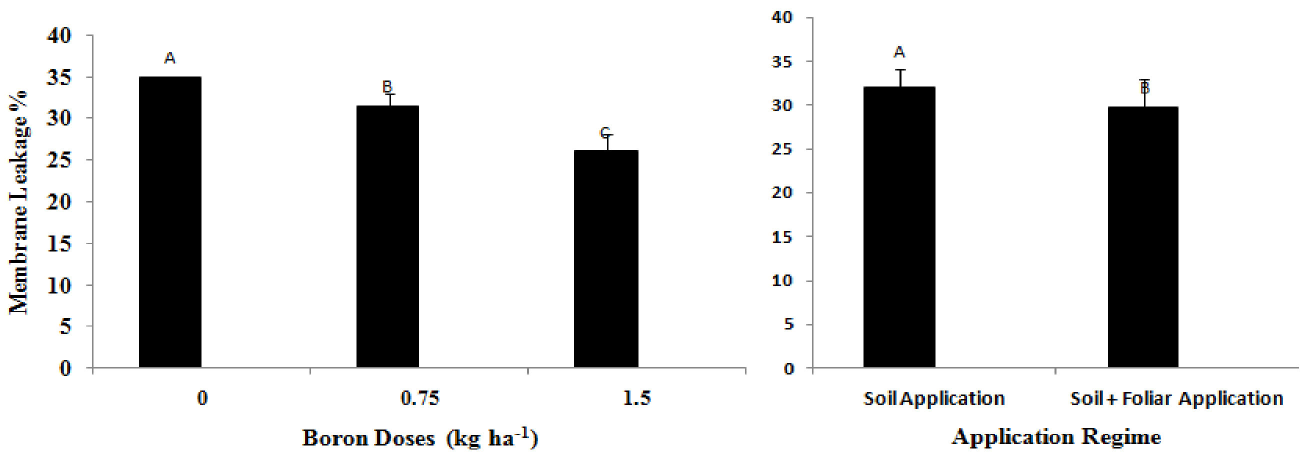

Fig. 2. Effect of different boron doses and application regime on membrane leakage % of cotton. Data represent the mean ± S.E. of four replications. The letters on different bars represent significance (p<0.05) of data.

Chlorophyll contents: Another generic plant health indicator is photosynthesis. B deficiency typically inhibits photosynthesis, possibly via reduced chlorophyll biosynthesis as suggested by Brown and Hu (1997) and Dordas (2006) in plum (*Prunussa licina* cv. 'Myrobalan') and cotton (*Gossypium hirsutum* L.) respectively. For example, Pinho *et al*., (2010) found that boron deficiency reduced chlorophyll contents in the leaflets of coconut (*Cocosnucifera*) by 30%. On the contrary, Nadim *et al*., (2012) suggested that B application significantly enhanced net assimilation rates in wheat seedlings which in turn is a measure of photosynthetic activity i.e. more photosynthetic activity in response to B application. Our findings are in line with these studies the chlorophyll content was significantly enhanced in response to addition of B-fertilizer, mode of application and their interactive effects (Fig. 3). Averaged across mode of application, the addition of 1.50 kg B ha^{-1} caused an increase in chlorophyll-a by 2%. Moreover, the application of B-fertilizer through soil and foliar spray caused a 0.4% increase in chlorophyll-a. Chlorophyll-b content was also significantly increased (Fig. 3). Thus, both chlorophyll a and b contents in cotton were enhanced by application of 1.50 kg B ha^{-1} through soil as well as foliar B application. In line with these data, the current study indicates that B nutrition can increase chlorophyll contents by 3-4% after addition of 1.50 kg B ha^{-1}along with three foliar applications. However, the increase was not statistically significant. Zhao and Oosterhuis (2003) showed that cotton crop grown in B-deficient conditions reduced photosynthetic rate, transpiration rate and stomatal conductance by around 40, 60 and 80%, respectively. Arif *et al*., (2012) also reported a significant increase in the chlorophyll content (a, b) in *Oryza sativa* when treated with B @3kg/acre compared to control and he also suggested this improved chl content is probably due to enhanced production of auxin in plants under B influence. However, this response to B application and correlated chlorophyll content is varied among species and is owing to varying agro-climatic conditions.

Gas exchange characteristics: Pinho *et al*., (2010) reported that there is linear correlation between stomatal conductance and B application. Application of B gave rise to a significant increase in photosynthetic rate (*PN;* Fig. 4). The application regime also significantly increased photosynthetic rate. Soil plus foliar applied B nutrition increased photosynthetic rate by 9 % as compared to soil application alone. Boron application gave rise to significant increases in transpiration rate (*E;* Fig. 4) and this depended on application regime. Similarly, stomatal conductance (g_s) increased after addition of B @ 0.75 kg ha^{-1} (Fig. 4) whereas inter-cellular CO_2 concentration (*Ci*) was reduced (Fig. 4) in an application regime dependent manner. B fertilization raised WUE by up to 36% in an application regime dependent manner (Fig. 4). Han *et al*., (2008) reported that adequate B supply to rooting medium of citrus seedling enhanced stomatal conductance and reduced intercellular CO_2 concentration. Results in Fig. 4 show that there was a significant increase in PN, g_s, E and WUE after application of1.50 kg B ha^{-1} along with three foliar sprays. Hajiboland & Farhanghi (2010) found similar improvements of these parameters in turnip (*Brassica rapa* L.). Although the relative importance of each of these photosynthetic parameters is hard to gage, overall CO_2 fixation is likely to be a main player. However, Pinho *et al*., (2010) showed reduced g_s and transpiration in B-deficit conditions resulted in over-heating of coconut leaflets.

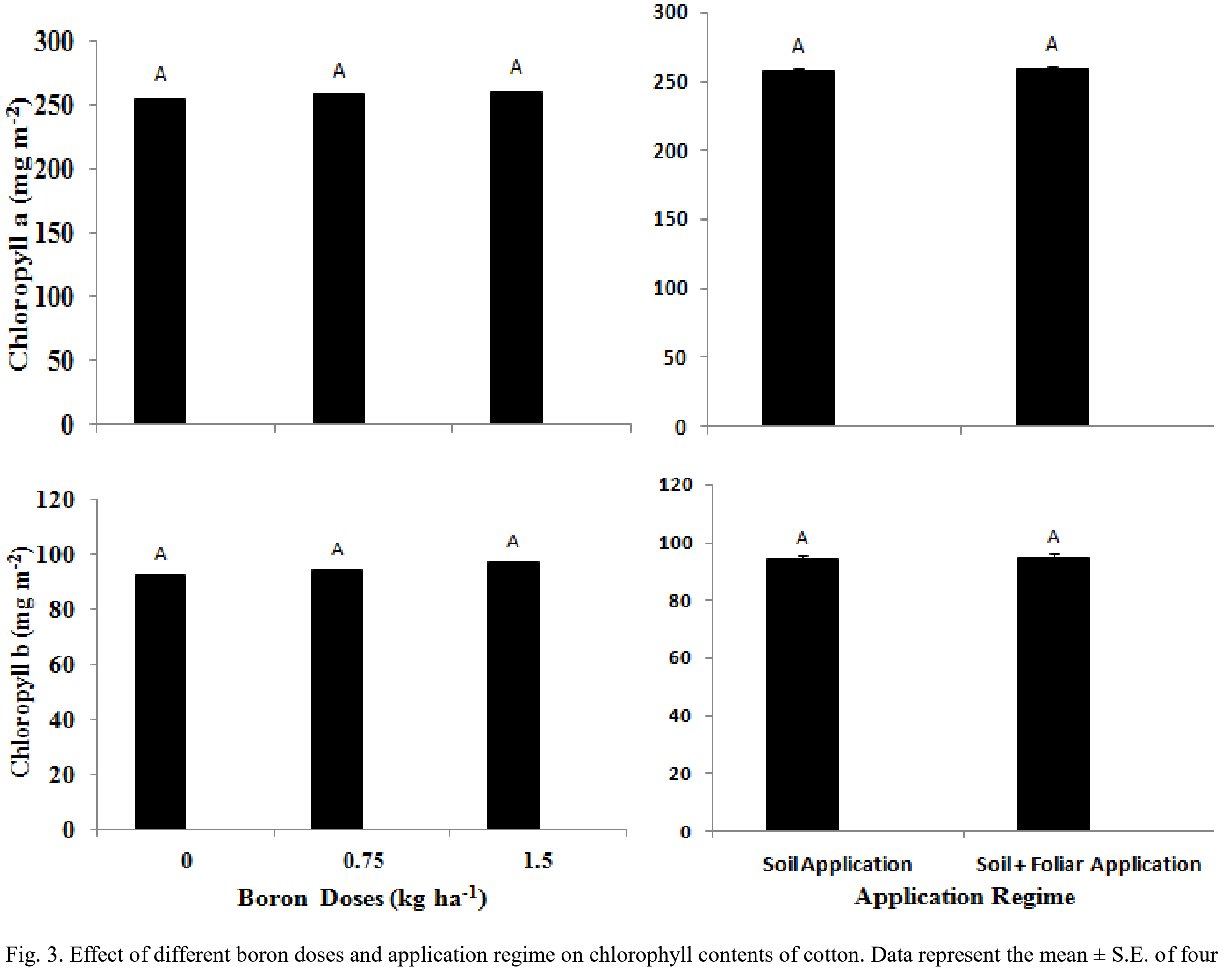

Fig. 3. Effect of different boron doses and application regime on chlorophyll contents of cotton. Data represent the mean ± S.E. of four replications. The letters on different bars represent significance (p<0.05) of data.

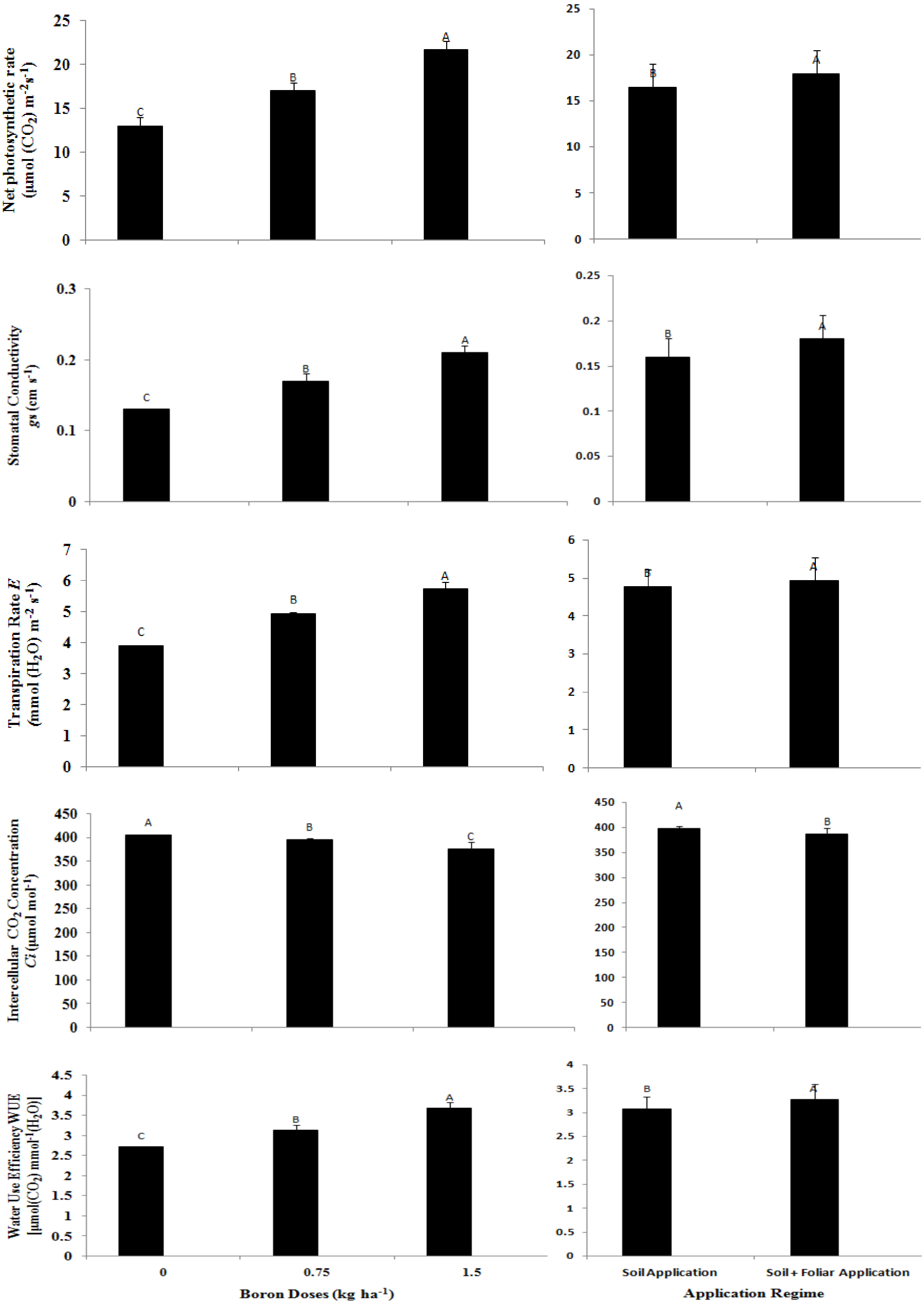

Fig. 4. Effect of different boron doses and application regime on gas exchange characteristics of cotton. Data represent the mean ± S.E. of four replications. The letters on different bars represent significance (p<0.05) of data.

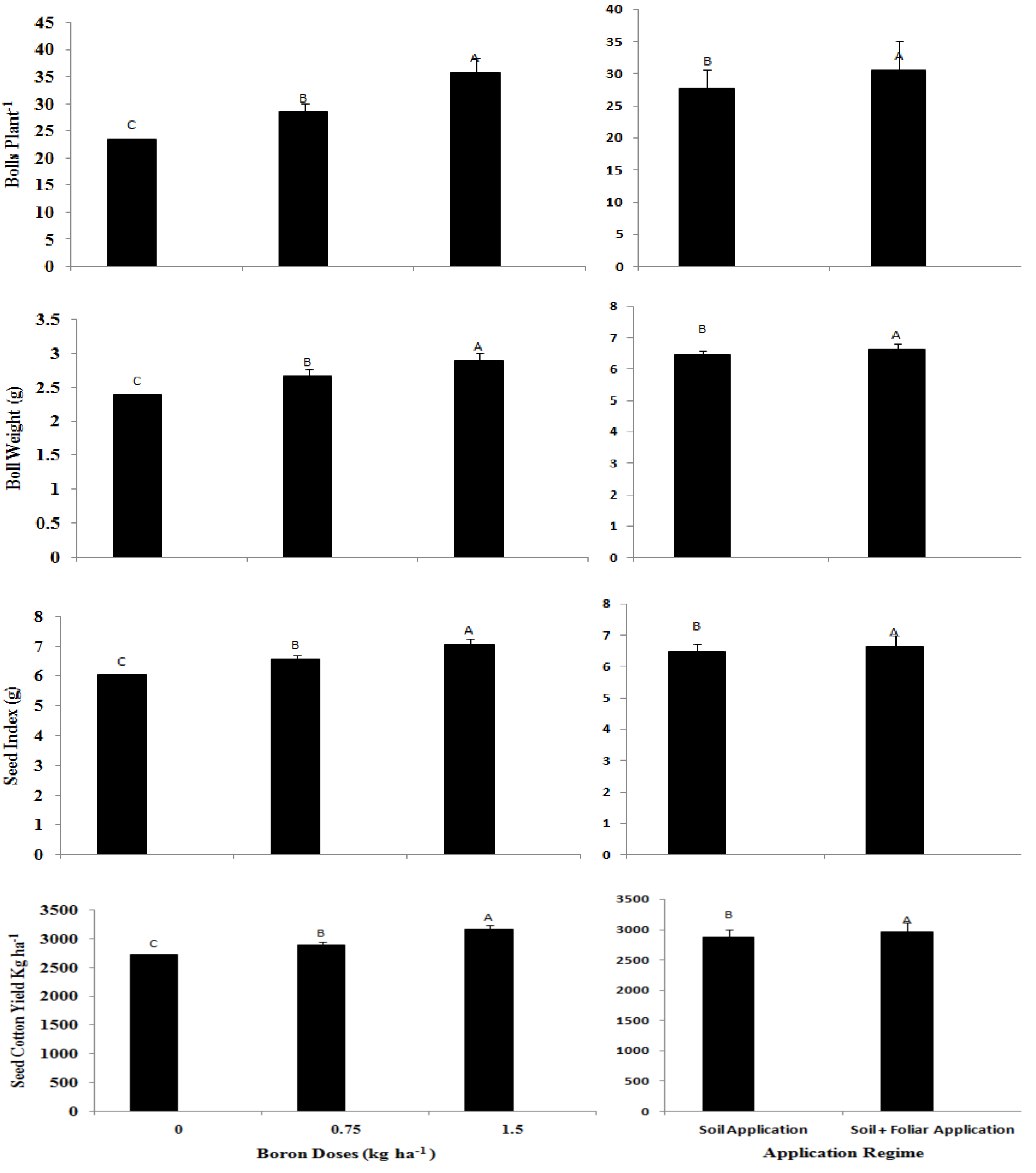

Fig. 5. Effect of different boron doses and application regime on seed cotton yield parameters. Data represent the mean ± S.E. of four replications. The letters on different bars represent significance (p<0.05) of data.

Seed cotton yield: Beneficial effects of boron application @ 1.0 kg/ha and 2.0 kg /ha have been reported to increase grain yields in wheat (Ahmed *et al*., 2018). B-doses of 0.75 and1.50 kg ha^{-1} led to an increase of 22 and 52% in number of bolls $plant^{-1}$ (Fig. 5). Similarly, application regime positively influenced total number of bolls $plant^{-1}$ while soil plus foliar applied B nutrition increased this parameter by11%. Boll weight was affected in a similar fashion (Fig. 5). The seed index, and therefore seed cotton yield, was also positively modulated by B fertilization (Fig. 5). Assuming a substantial inhibitory effect on photosynthesis when B is lacking, it is not surprising that B fertilization affects growth and ultimate yield. Reproductive stages are more prone to B deficiency than vegetative growth (de Oliveira *et al*., 2006) and poor fruit retention (Murphy & Lancaster, 1971) and small and deformed bolls (Roberts *et al*., 2000) in B deficient cotton plants have been reported frequently. In contrast, Rosolem and Costa (2000) did not observe any impact of temporary B deficiency on cotton fruit setting and changes may depend on soil type since Heitholt (1994) did not observe any positive effect of B-nutrition on cotton growth in alkaline typic dystrochrept soil. Reduced numbers and weights of fruits can be overcome by extra B provision. For example, in a Pakistani study by Rashid and Rafique (2002) a 12.5% gain in cotton boll bearing was reported in response to B fertilization when plants were grown on irrigated aridisols that contained less than 0.6 mg B kg^{-1}. In their extensive, multi-year experimentation, soil application and foliar feeding of B proved equally effective in enhancing cotton productivity. On calcareous soils, foliar B application increased boll weight (Dordas 2006).Our data (Fig. 5) showed a comparable (6%) improvement in seed index. Though some of these phenomena may derive from growth enhancement, for example via elevated photosynthetic rates, there is also evidence that B impacts on metabolite partitioning. Shorrocks (1992) and Dordas (2006) reported that B application in cotton crop raised leaf B concentration which resulted in more translocation of assimilates from source (leaf) to sink (boll), thereby increasing fruit retention.

Conclusions

The findings of the present study revealed that addition of B fertilizer to the soil and as foliar application had a positive influence on PN,E,gs and yield, whereas Ci and membrane leakage was reduced simultaneously. The increase in photosynthetic capacity and stomatal conductance under B-nutrition enhanced WUE and, combined with the increase in cell membrane stability, resulted in more retention of fruits and increased yield. The data show that when acid-extractable B drops below the critical value of 0.35–0.40 mg kg^{-1} soil, a B fertilizer regime of around1.50 kg B ha^{-1}will mitigate the most apparent deficiency symptoms and restore cotton harvest potential.

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