

1 **Cotton growth, yield, quality and boron distribution as**
2 **affected by soil-applied boron in calcareous saline soil**

3

4

5 Atique-ur-Rehman^{1*¶}, Rafi Qamar^{2ψ}, Abid Hussain^{1¶}, Hassan Sardar^{3ψ}, Naeem Sarwar^{1§},
6 Hafiz Muhammad Rashad Javeed^{4§}

7 ,

8 ¹Department of Agronomy, Bahauddin Zakariya University, Multan, Punjab, Pakistan

9 ²Department of Agronomy, College of Agriculture, University of Sargodha, Sargodha,
10 Punjab, Pakistan.

11 ³Department of Horticulture, Bahauddin Zakariya University, Multan, Punjab, Pakistan

12 ⁴Department of Environmental Sciences, COMSATS University Islamabad, Vehari
13 Campus, Vehari 61100, Pakistan

14 *Corresponding author

15 Email: atiqjugg@gmail.com; dr.atiqe@bzu.edu.pk

16 ¶This author supervised the experiment, supply inputs and approved the manuscript

17 ψ This author done the statistical analysis and write the manuscript complete

18 § Improved manuscript

19 Abstract

20 Boron (B) is deficient in the calcareous, Typic Haplocambid soils of cotton growing
21 belt of Pakistan, and thus is a vital reason for less cotton yield in the region. In order to
22 investigate the growth and quality alterations associated with soil applied B on cotton (*c.v.*
23 *CIM-616 and CIM-600*) an experiment was conducted. Boron was applied at 0.00, 2.60, 5.52,
24 7.78 and 10.04 mg B kg⁻¹ of soil using borax (Na₂B₄O₇.10H₂O), in a complete randomized
25 design with factorial arrangement with four replications. Results revealed that soil applied B
26 @ 2.60 mg B kg⁻¹ of soil significantly (P≤0.05) improved cotton growth, yield, quality and B
27 distribution among different parts. Different growth and yield parameters like plant height,
28 leaf area, number of bolls, boll size and weight, seed cotton yield, photosynthesis, transpiration
29 rate, stomatal conductance, water use efficiency, GOT, staple length and fiber fineness and
30 strength except B uptake by roots, seed, leaves and stalk plant body which was significantly
31 increased with B (10.04 mg B kg⁻¹) in both cultivars of cotton, but the degree of effects was
32 varied between cultivars. The results indicated that studied traits of both cultivars were
33 significantly (P≤0.05) decreased in B-deficient stressed treatments. Between hybrids, CIM-
34 600 produced significantly (P≤0.05) maximum recorded parameters under 2.60 mg B kg⁻¹
35 application compared than CIM-616. Our findings confirm that the adequate level of B (2.60
36 mg B kg⁻¹) had pronounced effects on various growth, yield, physiological and fiber quality
37 associated traits, as compared to B uptake traits of cotton cultivars.

38 **Keywords:** Cotton, soil application; photosynthesis, fiber quality, boron

39

40 Introduction

41 Pakistan ranked 4th in share of production and consumption and 3rd in export of cotton
42 (*Gossypium hirsutum* L.) in the world [1]. However, yield stagnation (752 kg ha⁻¹) and poor

43 fiber quality are serious problems in cotton production [2]. Certain factors are particular for
44 these problems, include poor fertilization especially lack of certain micronutrients like boron
45 (B), which could improve the cotton yield and quality [3]; [4]. Boron deficiency has been
46 prominent in cotton growing regions of the world including Pakistan, where 50% cotton
47 growing area is deficient in B [5]; [6] and [7]. Its deficiency is common in tropical soils,
48 where organic matter and clay content are lower [8] which are responsible for its leach
49 down through the soil profile [9]; [10]. Degree of B adsorption onto the soil surfaces depend
50 on the soil characteristics such as structure, pH, organic matter and clay content, iron and
51 aluminum oxide and hydroxyl content and salinity [11]; [12]. However, its balanced
52 application needs more consideration due to its narrow range between deficiency and
53 toxicity which significantly retard the cotton production and physiological traits without
54 any visible symptoms [13]. Moreover, B use improvement is difficult due to its low
55 mobility in phloem vessels and result in low degree of its reutilization in cotton plant [14];
56 [15]. Due to low mobility in phloem vessels the required concentration of photosynthate and
57 carbohydrate are not reached from leaves to fruits [15] that increases the rate of squares and
58 bolls shedding at maturity, which finally affect the fiber quality [16]. Boron temporary
59 deficiency can cause irreversible damage in cotton plants and thus significantly affect cotton
60 yield [17]. Moreover, B withdrawal for a short period, establish deficiency and disturb the
61 reproductive structures [18]; [19]. Likewise, boll retention depends on carbohydrates
62 concentration in the plant body that mainly influenced by photoassimilate translocation from
63 leaves to fruits, which under B deficiency decreases with increase in abortions [6]. Boron
64 deficiency indirectly affects the metabolism of proteins and nucleic acids [20]; [21], and also
65 mediates the levels of hormones and phenolic substances in the plant body [22]; [23] and [24].
66 Boron low mobility may cause temporary deficiency in cotton, although excess amount of B
67 present in the soil solution. Due to critical role of B and lower mobility in cotton, continuous
68 fertilization of B is needed throughout the plant's life.

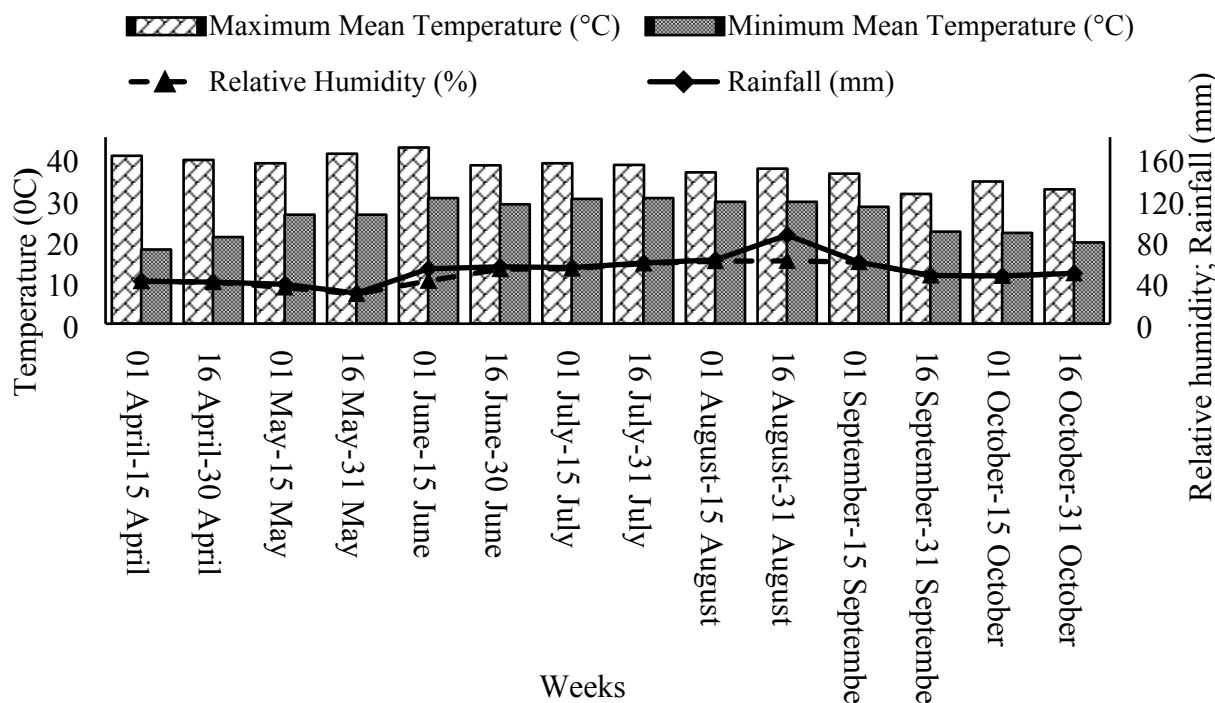
69 Boron fertilization not only improved the establishment and progress of reproductive
70 organs [25]; [26] but also plays a crucial role in the vegetative growth of cotton plants [27];
71 [28]. However, continuous application of B without any soil test can also generate the toxicity

72 in the soil [29], which disturb various physiological processes in cotton plants, such as
73 reduction of chlorophyll contents, photosynthetic rates, lower cell division in root portion and
74 lignin contents [30]. Boron uptake and transport in the new developing tissues depends on the
75 transpiration stream, which may be reduced due to low evaporation rate, stomatal conductance
76 in tropical region. However, there are some contradictory findings about B mobility within
77 cotton plants, with respect to the most suitable rates and application forms [31]. A critical level
78 of B concentrations in matured cotton leaf is 53 mg B kg⁻¹, which should be 15-20 mg B kg⁻¹
79 [4]. Due to narrow range of B concentration, plant analysis is not reliable technique for
80 estimating of B nutritional status. Furthermore, new cotton genotypes response to B generally
81 vary, although no significant differences were recorded among old cotton cultivars [32]; [31].
82 The variation in their abilities of carbohydrate transport, use and storage of B related
83 mechanisms might be the reason [14]. Boron demands in cotton plants are relatively high
84 compared to other crops [6] and it requires an average of 340 g B ha⁻¹ from which about 12%
85 is to be accumulated in seed [33]. Therefore, its slight excess may deteriorate the fiber quality
86 [34]. In cotton growing regions, it is need of the time to find solution for B deficiency
87 especially for the newly developed genotypes. The present study is therefore, conducted for
88 improvement of yield and quality of cotton fiber in hyperthermic, sodic haplocambids, haplic
89 Yermosols of cotton belt of Pakistan.

90 **Materials and methods**

91 The experiment was conducted in earthen pots placed in wire-house at Bahauddin Zakariya
92 University, Multan (30.10 °N, 71.25 °E and 421 ft. altitude above sea level) during cotton
93 growing season 2018. Earthen pots (25 x 40 cm⁻²) filled with 20 kg soil and covered with
94 polyethylene sheet having bulk density ≈ 1.04 mg m⁻³ [35]. Soil in the pots was equilibrated
95 before 7 days of sowing [36]. Before conducting experiment, soil was air-dried, crushed
96 and pass through 2 mm sieve for performing different physico-chemical properties.
97 Hydrometer techniques was used for determination of soil textural class and it was silty clay
98 loam belongs to Sindhalianwali soil series, and was hyperthermic, sodic haplocambids/Haplic
99 Yermosols according to USDA and FAO classification, respectively. Soil pH and EC were 8.3

100 and 12 dS m⁻¹ that were measured by a pH meter (Beckman 45 Modal, US) and EC meter
101 (VWR Conductivity Meter DIG2052) respectively. Soil organic matter content was 0.78%,
102 while total N 0.035%, NaHCO₃-DTPA available-P 7.65 mg kg⁻¹ and NH₄OAc-extractable-
103 K 162 mg kg⁻¹. From soil analysis it is clear that B was deficient (0.43 mg B kg⁻¹). Summary
104 of weather data during the crop growth period is depicted in Fig. 1.



105

106 **Fig. 1.** Meteorological data recorded at Bahauddin Zakariya University, Multan during 2018

107 The experiment was laid out according to completely randomized design (CRD) with
108 factorial arrangement. There were two cotton cultivars viz. CIM-616 and CIM-600 tested with
109 different B treatments viz. control or 0, 2.6, 5.52, 7.78 and 10.04 mg B kg⁻¹ soil and each
110 treatment was replicated five times. Boric acid (H₃BO₃) (17.5% B) was used as source of B. A
111 total of 10 delinted cotton seeds of both cultivars were sown in each pot on 1st May, 2018
112 and plants were thinned to two at 15 days after sowing (DAS). Soil in the pots were
113 monitored and maintained at soil moisture up to 70% by weighing regularly. Recommended
114 doses of N, P and K @ 200, 100 and 70 kg ha⁻¹ were uniformly mixed thoroughly into the soil.
115 When plants reached at maturity their bolls were separated and lint was detached manually

116 from seed. Standard production practices were adopted and plants were kept free of insect-
117 pests through using pesticide sprays.

118 For measuring different parameters, standard procedures were adopted. Leaf area of
119 selected plants was determined with a leaf area meter (CI-202. Portable Laser Leaf Area
120 Meter). Measurements of photosynthesis, stomatal conductance and transpiration rate of
121 fully expanded leaves were taken between 9h00 and 11h30 with a portable photosynthesis
122 measuring system (IRGA - LI-6400, LICOR). Water use efficiency was determined by
123 dividing photosynthesis with transpiration rate. The plants were uprooted carefully at
124 maturity and divided into roots, shoots, leaves and seed after removing lint. For
125 determination of B concentration, different plant parts were separately washed with de-
126 ionized water then dried in a thermo-ventilated oven at $65 \pm 5^\circ\text{C}$ up to constant weight.
127 Then dried material was ground in a John Wiley mill and passed through a 40 mesh screen.
128 The ground material was dry ashed at 550°C for 6 hours in a muffle furnace. Then the ash
129 was taken in 0.36N H_2SO_4 and the B concentration was determined by spectrophotometer at
130 420 nm wavelength using azomethine-H method [37]. The samples of seed-cotton were
131 separated into lint using single roller laboratory gin and then ginning out turn (GOT) was
132 calculated. Fiber quality traits viz. fiber length, fiber fineness and fiber strength were
133 analyzed on High Volume Instrument (HVI), manufactured by M/S Zellweger Uster Ltd.,
134 Switzerland. The instrument was calibrated as per the instruction manual [38] followed by
135 the standard procedure as described by [39].

136 Data collected were statistically analyzed by Fisher's analysis of variance and treatment means
137 were compared using least significant difference at 5% probability level [40].

138 **Results**

139 Boron application to cotton had significant effects on cotton growth, yield and fiber
140 quality (Table 1 to 4). However, plant height was affected by application of B and among
141 different treatments, 2.6 mg B produced taller plants than other treatments (Table 1). From
142 both cultivars, CIM-600 expressed 1% more plant height than CIM-616 (Table 1).
143 Likewise, application of 2.6 mg B expressed higher leaf area than other treatments.
144 Different yield contributing parameters were also improved more by 2.6 mg B application

145 and an increase of 38.5% in number of bolls per plant was recorded with this treatment.
146 Likewise, number of bolls per plant of CIM-600 was recorded 13% higher than CIM-616
147 (Table 1). An increase of 46% in boll size was also recorded with 2.6 mg B and it was
148 remained well than all other treatments. The cultivar CIM-600 showed 16% bigger boll size
149 than CIM-616 under different doses of soil applied B (Table 1). Soil applied 2.6 mg B in
150 CIM-600 and CIM-616 produced 52% heavier boll weight CIM-600 and CIM-616 (Table
151 1). Similarly, maximum seed cotton yield per plant was recorded by 2.6 mg B that was 51%
152 more than control. Between two cultivars, a 2% higher seed cotton yield per plant was
153 recorded in CIM-600 than CIM-616 (Table 1).

154 **Table 1: Influence of soil applied boron on number of bolls per plant, boll size and weight and seed**
155 **cotton yield per plant of cotton cultivars**

156

Treatments (mg B kg ⁻¹ soil)	Plant height (cm)			Leaf area (cm)			Number of bolls per plant			Boll size (cm)			Boll weight (g)			Seed cotton per plant (g)		
	CIM- -616	CIM- 600	Mean	CIM- -616	CIM- 600	Mean	CIM- -616	CIM- 600	Mean	CIM- -616	CIM- 600	Mean	CIM- -616	CIM- 600	Mean	CIM- -616	CIM- 600	Mean
0.0	86.8 d	87.7c 91.3a	87.3B 91.0A	143.6 f	144.2 ef	143.9 C	15i	18g	16E	1.8f	2.3de f	2.1D	2.07e d	2.83c 4.4a	2.45D 3.9A	220.25g 4.30a	225.54g 4.50a	222.89 4.40A
2.6	90.7 b	91.3a 146.5	91.0A 147.6	147.1 b a	147.6 a	147.1 A	24c	29a	26A	3.4b	4.4a	3.9A	4.30a	4.50a	4.40A	448.90b 448.90b	460.52a 450.52a	454.71 454.71
5.52	86.4 d	88.1c 144.5	87.3B 145.6	145.0 de c	145.6 c	145.0 B	23d	26b	24B	3.1bc	3.5b	3.3B	3.20b c	3.50b 3.16b	3.35B 2.95C	418.19c 308.12e	426.92c 322.45d	422.55 315.28
7.78	83.3 f	84.2e 143.5	83.7C 145.2	144.3 f cd	145.2 cd	144.3 C	21e	24c	22C	2.6cd e	2.8cd 2.7cd	2.7C	2.73d 2.16e	3.16b 2.30e	2.95C 2.23D	315.28 238.42f	322.45d 245.58f	315.28 242.00
10.04	80.4 h	81.3g 143.7	80.9D 144.6	144.2 f de	144.6 de	144.2 C	17h	21e	19D	2.1ef D	2.7cd 2.4C	2.4C	2.16e 2.89B	2.30e 3.1A	2.23D 3.26A	242.00 327.16	245.58f 332.82	242.00 B
Mean	85.5 B	86.5A B	87.3B A	144.4 B	145.4 A	144.4 B	20B	23A		2.6B	3.1A		2.89B	3.26A		327.16 B	332.82 A	
LSD at 5 %	Cultivars (C): 0.086; Boron levels (B): 0.28; C x B: 0.47	Cultivars (C): 0.24; Boron levels (B): 0.46; C x B: 0.77	Cultivars (C): 0.14; Boron levels (B): 0.35; C x B: 0.59	Cultivars (C): 0.05; Boron levels (B): 0.33; C x B: 0.56	Cultivars (C): 0.05; Boron levels (B): 0.26; C x B: 0.45	Cultivars (C): 4.22; Boron levels (B): 3.76; C x B: 6.33												

157 Different physiological traits were significantly improved with B application and
158 among different treatments, 2.6 mg B produced 45% higher photosynthesis than control,
159 and was significantly higher than other B treatments. From both cultivars, CIM-600
160 produced 8.2% higher photosynthesis than CIM-616 (Table 2). Almost same trend was
161 recorded for transpiration rate. Likewise, application of 2.6 mg B produced 9% more

162 transpiration rate than control, which was significantly higher than other B treatments
163 (Table 2). Between two cultivars, transpiration rate of CIM-600 was 0.50% more than
164 CIM-616 (Table 2). Regarding stomatal conductance, 37% higher stomatal conductance
165 than control was recorded application of 2.6 mg B, which was significantly higher than
166 other treatments. From both cultivars, CIM-600 recorded 1% higher stomatal conductance
167 than CIM-616 (Table 2). Similarly, application of 2.6 mg B improved water use efficiency,
168 that was 40% higher than control and other treatments. From both cultivars, 9% higher
169 water use efficiency was recorded in CIM-600 than CIM-616 (Table 2).

170 **Table 2: Influence of soil applied boron on photosynthesis, transpiration, stomatal conductance**
171 **and water use efficiency of cotton cultivars**

Treatments (mg B kg ⁻¹ soil)	Photosynthesis (μmol CO ₂ m ⁻² s ⁻¹)			Transpiration rate (mmol m ⁻² s ⁻¹)			Stomatal conductance (μmol m ⁻² s ⁻¹)			Water use efficiency (μmol CO ₂ mol ⁻¹ H ₂ O day ⁻¹ m ⁻²)		
	CIM-616	CIM-600	Mean	CIM-616	CIM-600	Mean	CIM-616	CIM-600	Mean	CIM-616	CIM-600	Mean
0.0	5.01j	5.42i	5.22E	13.20h	13.40g	13.30E	2.36e	2.40e	2.38E	0.38h	0.40g	0.39E
2.6	9.12b	9.92a	9.52A	14.61b	14.68a	14.64A	3.74a	3.77a	3.76A	0.62b	0.68a	0.65A
5.52	7.92d	8.32c	8.12B	13.92d	13.97c	13.94B	3.60b	3.62b	3.61B	0.57d	0.59c	0.58B
7.78	6.42f	7.82e	7.12C	13.78e	13.81e	13.79C	2.91c	2.95c	2.96C	0.47e	0.57d	0.52C
10.04	6.12h	6.22g	6.17D	13.50f	13.52f	13.51D	2.83d	2.87d	2.85D	0.45f	0.46ef	0.45D
Mean	6.92B	7.54A		13.80B	13.87A		3.09B	3.12A		0.49B	0.54A	
LSD at 5 %	Cultivars (C): 0.001; Boron levels (B): 0.007; C x B: 0.01			Cultivars (C): 0.01; Boron levels (B): 0.17; C x B: 0.02			Cultivars (C): 0.01; Boron levels (B): 0.03; C x B: 0.05			Cultivars (C): 0.01; Boron levels (B): 0.01; C x B: 0.01		

172

173 Boron contents in different parts of cotton was increased with increase in B
174 application and there was an increasing trend of B in cotton roots, leaves, stalk and seed
175 from lower to higher level (Table 3). For example, 74% higher B was recorded in roots
176 with 10.04 mg B than control (Table 3). Similarly, 86% higher B contents were noted in
177 leaves of CIM-600 with 10.04 mg B than other treatments (Table 3). The cultivar CIM-600
178 produced 2% higher B uptake by leaves than CIM-616 under different B treatments (Table
179 3). Similarly, maximum concentration of B in stalk was recorded at 10.04 mg B that was

180 67% higher than control (Table 3). Between two cultivars, a 4% higher B uptake by stalk
181 was recorded in CIM-600 than CIM-616 (Table 3). Among different soil applied B, 10.04
182 mg had 40% higher B uptake by seed cotton than control (Table 3). The cultivar CIM-600
183 showed 3% higher B uptake by seed cotton than CIM-616 under different doses of soil
184 applied B (Table 3).

185 **Table 3: Influence of soil applied boron on boron uptake by roots, seed, leaves and stalk of cotton**
186 **cultivars**

Treatments (mg B kg ⁻¹ soil)	Boron uptake by roots (mg kg ⁻¹)			Boron uptake by seed cotton (mg kg ⁻¹)			Boron uptake by leaves (mg kg ⁻¹)			Boron uptake by stalk (mg kg ⁻¹)		
	CIM-616	CIM-600	Mean	CIM-616	CIM-600	Mean	CIM-616	CIM-600	Mean	CIM-616	CIM-600	Mean
0.0	10.75j	12.08i	11.41E	1.12j	1.16i	1.14E	37.78j	38.12i	37.95E	21.85j	24.20i	23.02E
2.6	28.72h	30.25g	29.48D	1.32h	1.35g	1.34D	93.81h	95.45g	94.63D	39.58h	41.12g	40.35D
5.52	35.74f	37.12e	36.43C	1.43f	1.48e	1.46C	192.81f	196.87e	194.84C	58.34f	61.12e	59.73C
7.78	39.67d	41.82c	40.74B	1.65d	1.70c	1.68B	212.78d	222.42c	217.60B	64.78d	66.88c	65.83B
10.04	43.56b	45.02a	44.29A	1.87b	1.90a	1.89A	268.78b	269.82a	269.30A	69.34b	72.12a	70.73A
Mean	31.68B	33.25A		1.48B	1.52A		161.19B	164.54A		50.77B	53.08A	
LSD at 5 %	Cultivars (C): 001; Boron levels (B): 0.003; C x B: 0.005			Cultivars (C): 0.002; Boron levels (B): 0.002; C x B: 0.003			Cultivars (C): 0.001; Boron levels (B): 0.002; C x B: 0.003			Cultivars (C): 0.03; Boron levels (B): 0.02; C x B: 0.04		

187

188 Fiber quality traits were also significantly affected by B application (Table 4). Soil
189 applied 2.6 mg B produced 6% higher GOT than control and other B levels. CIM-600
190 recorded 1.5% higher GOT than CIM-616 under different soil applied B (Table 4). Boron
191 application significantly affected staple length and 2.6 mg B gave 3.5% more staple length
192 than control (Table 4). From the cultivars, CIM-600 presented 5.4% higher staple length
193 than CIM-616 (Table 4). Regarding fiber fineness, among different B concentrations,
194 application of 2.6 mg and 5.52 mg B exhibited 17% higher staple length than control. The
195 cultivar CIM-600 produced 15.5% higher fiber fineness than CIM-616 (Table 4). Likewise,
196 5% higher fiber strength was recorded with 2.6 mg B as compared to control while other B

197 treatments were statistically at par with each other. Cotton cultivars CIM-600 attained 1.8%
198 more fiber strength than CIM-616 (Table 4).

199 **Table 4: Influence of soil applied boron on GOT, staple length, fiber fineness and**
200 **strength of cotton cultivars**

Treatments (mg B kg ⁻¹ soil)	GOT (%)			Staple length (mm)			Fiber Fineness (µg inch ⁻¹)			Fiber Strength (G tex ⁻¹)		
	CIM-616	CIM-600	Mean	CIM-616	CIM-600	Mean	CIM-616	CIM-600	Mean	CIM-616	CIM-600	Mean
0.0	39.73	40.40	40.06D	27.73	29.16	28.45D	3.40	4.16	3.78B	27.16	27.73	27.45C
2.6	42.50	42.83	42.66A	28.60	30.40	29.50A	4.40	4.70	4.55A	28.60	29.16	28.88A
5.52	40.83	41.73	41.28B	28.30	29.83	29.06B	4.06	4.70	4.38A	28.20	28.70	28.45B
7.78	40.73	41.50	41.11B	28.03	29.70	28.86BC	3.50	4.50	4.00B	28.20	28.56	28.38B
10.04	40.26	40.73	40.50C	27.83	29.16	28.61CD	3.50	4.26	3.88B	27.83	28.40	28.11B
Mean	40.81B	41.44A		28.10B	29.70A		3.77B	4.46A		28.00B	28.51A	
LSD at 5 %	Cultivars (C): 0.05; Boron levels (B): 0.28; C x B: NS			Cultivars (C): 0.08; Boron levels (B): 0.31; C x B: NS			Cultivars (C): 0.23; Boron levels (B): 0.38; C x B: NS			Cultivars (C): 0.20; Boron levels (B): 0.39; C x B: NS		

201 GOT: Ginning out turn

202 Discussion

203 Boron application has significant effects on growth, yield, physiological and fiber
204 traits of cotton. Significant improvement in cotton plant height (11.1%) and leaf area
205 (2.2%) with 2.6 mg B (Table 1) shows that appropriate dose of B plays role in different
206 physiological, biochemical, metabolic and enzymatic activities of plant [41], thus its
207 deficiency declines (4.1%) in plant height [42]. Moreover, lower plant height at higher B
208 may be due to its narrow range between deficiency and toxicity which may damage the
209 plant structure that limit the cotton growth without any visible symptoms [13]; [31].
210 Improvement in plant growth might be due to improved macronutrient uptake in response
211 to B application [43]. Its deficiency severely declines various physiological and growth
212 parameters like leaf area and seed cotton yield [42]. Boron insufficiency results in growth
213 impairments such as reduced plant growth [6]. Higher number of bolls per plant and boll

214 size and weight might be as result of increase in sugar translocation, membrane
215 permeability, photosynthetic rate and migration of photosynthate from source to sink. [44]
216 postulated that enhanced B supply to plants promotes flower development, pollen
217 germination, fertilization, and seed development and, thus, reduces fruit shedding, which
218 resulted in an increase in the number of bolls per plant and boll weight [45]; [19]. Boron
219 deficiency causes significant shedding of square and boll shedding [4]; [46]. Seed cotton
220 yield enhanced with the increase of boll size and weight, bolls per plant and B uptake
221 (Table 3) and water use efficiency [6]; [47]; [4] and [46]. Abortion of reproductive parts
222 occurred because of B deficiency that impairs the formation of the peduncle vascular
223 system, impairing carbohydrate transport to the ovary [19]; [15]. Furthermore, higher
224 concentrations of B have adverse effects on plant metabolic activities related to chlorosis
225 and necrosis, loss of photosynthetic capacity and eventually reduction in plant productivity
226 [48]. Necrotic areas developed on leaves between veins and loss of leaves due to toxicity,
227 which inhibited photosynthetic process and exerted a negative impact on cotton growth
228 [48].

229 Soil applied has significantly improved photosynthesis and other related parameters
230 of cotton (Table 2). Earlier it is reported that photosynthesis improved 45.2% at 2.6 mg B/
231 Kg of soil in cotton (Table 2) which might be due to enhance in net assimilation rates which
232 in turn is a measure of photosynthetic activity [49]. Significantly lower photosynthesis rate
233 was possibly due to reduced chlorophyll biosynthesis as suggested by [46] in cotton. Our
234 results supported the findings of [50] who reported that photosynthetic rate were lowered in
235 plants under B deficiency leading to growth inhibition. The reduction in leaf area (Table 1)
236 is mainly responsible for the lower photosynthetic rate in cotton plants under control

237 conditions. Additionally, it has also been reported that B deficiency (control) reduces
238 photosynthetic efficiency by changing stomatal density and stomatal conductance to
239 decrease the conductivity of CO_2 [51]. Higher rate (above 2.6 mg B) showed 35.2% lower
240 photosynthesis, leaf chlorophyll contents, root cell division and lignin and suberin levels
241 [30]. Present study showed 9.2%, 36.7% and 40% lower transpiration rate, stomatal
242 conductance and water use efficiency in control than 2.6 mg B respectively. Our results
243 validate the findings of [52] who concluded that significant decreased in stomatal
244 conductance, photosynthetic rate and transpiration rate in the functional leaves of cotton
245 after the formation of brown rings on the petiole are due to destruction of vascular bundle
246 of petiole. [53] also reported that B deficiency deformate the phloem sieve which affects the
247 transport of carbohydrates, water and nutrients that results in reduction of stomatal
248 conductance and transpiration rate. Therefore, the destruction of petiole vessels impairs the
249 transport of photosynthetic products of the leaves to the other parts. Significant reduction of
250 transpiration rate and stomatal conductance in control also reduced the water use efficiency
251 (WUE) (Table 2). Boron application raised WUE by up to 40% as compared to control
252 (Table 2). Owing to other improvements in physiological performance, [51] reported that B
253 enhanced stomatal conductance and reduced intercellular CO_2 concentration and resulting a
254 significant increase in photosynthesis, transpiration rate, stomatal conductance and WUE.
255 Boron deficiency cause plant morphological changes, especially in the leaves [19] with a
256 decrease in the number and functioning of stomata [54], which impairs transpiration rate
257 [6].

258 Boron partitioning in different parts of cotton was varied among tissues. The B
259 contents in roots, stem, leaves and seeds were increased with increase in B concentration in

260 soil. It was noted that more B was in leaves than stalk that might be due to low mobility of
261 the nutrient in the cotton phloem [19], owing to high transpiration rate, the main driving
262 force of B transport within the plant [55]. The amount of B accumulated in the plant body
263 increased exponentially with 2.6, 5.52, 7.78 and 10.04 mg B Kg⁻¹ of soil (Table 3). Lower
264 concentration of B in seeds as compared to other plants parts (Table 3) might be due to B
265 movement through xylem, which has no direct connection to seed [56]. Moreover, flowers
266 and seeds may not be able to take up B directly from the soil [57]; [58] and [45]. The
267 partitioning of B in various plant tissues showed significant variations with increasing B
268 level and it was assimilated in the order of leaf > shoot > root (Table 3) [59].

269 Different fiber quality traits of both cultivars were improved with soil applied B in
270 calcareous soil (Table 4). Maximum GOT, staple length, fiber fineness and strength were
271 recorded with 2.6 mg B (Table 4). The results were confirmed by the findings of [14] who
272 reported that application of B enhanced the GOT, staple length and fiber fineness and
273 strength of cotton genotypes but the degrees of effects were varied among genotypes. In
274 present study, from the two cultivars, CIM-600 produced better quality fiber at different
275 levels of B. Moreover, higher concentration (above 2.6 mg) of B deteriorates the fiber
276 quality (Table 4). Our results are quite in line with the findings of [60], who reported that
277 fiber quality was positively affected by application of B. Although fiber quality was
278 improved with different treatments, however, it was the best with 2.6 mg B. Our results
279 supported the findings of [61] who reported that soil applied B affected staple length, fiber
280 fineness and strength and uniformity ratio.

281 **Conclusion**

282 Boron application into the medium of cotton growing considerably improved the growth,
283 different yield and quality parameters. Different gas exchange parameters were also improved,
284 which ultimately improved the performance of cotton in saline soil. From the tow cotton
285 genotypes, CIM-600 showed better results as compared to CIM-616. Among different B
286 applications, 2.6 mg B Kg⁻¹ of soil was remain superior to others.

287 **Acknowledgments**

288 We acknowledge the financial support for this study from Directorate of Research, Bahauddin
289 Zakariya University, Multan, Pakistan.

290 **References**

- 291 1. Ahmed N, Abid M, Ahmad F, Ullah MA, Javaid Q, Ali MA. Impact of boron
292 fertilization on dry matter production and mineral constitution of irrigated cotton.
293 Pak J Bot. 2011; 43: 2903-2910.
- 294 2. Govt. of Pakistan. Pakistan Economic Survey. 2017-18, Economic Adviser's Wing,
295 Finance Division, Government of Pakistan. Islamabad; 2017-2018. pp. 121.
- 296 3. Yeates SJ, Constable GA, McCumstie T. Irrigated cotton in the tropical dry season. III:
297 Impact of temperature, cultivar and sowing date on fiber quality. Field Crops
298 Res. 2010; 116: 300-307.
- 299 4. Rashid A, Rafique E. Boron deficiency in cotton grown in calcareous soils of Pakistan,
300 II: Correction and criteria for foliar diagnosis. In Boron in Plant and Animal
301 Nutrition, eds. Goldbach HE, Brown PH, Rerkasem B, Thellier T, Wimmer MA,
302 Bell RW, New York: Academic Kluwer/Plenum. 2002. Pp. 357-362.

303 5. Rashid A, Rafique E, Ali N. Micronutrient deficiencies in rainfed calcareous soils of
304 Pakistan. II. Boron Nutrition of the peanut plant. Commun Soil Sci Pl Analysis.
305 1997; 28: 149-59.

306 6. Zhao D, Oosterhuis DM. Cotton growth and physiological responses to boron deficiency.
307 J Plant Nutr. 2003; 26: 855-867.

308 7. Ahmed N, Abid M, Rashid A, Ali MA, Ammanullah M. Boron requirement of irrigated
309 cotton in a typic haplocambid for optimum productivity and seed composition.
310 Commun Soil Sci Plant Anal. 2013; 44: 1293-309.

311 8. Rosolem CA, Quaggio JA, Silva NM. Algodoao, Amendoim e Soja. In: (Eds.): Ferreira,
312 M.E., Cruz. M.C.P., Raij, van. B., Abreu C.A. Mironutrients e element
313 ostoxicosna agricultura. Jaboticabal. 2001. pp. 321-354.

314 9. Communar G, Keren R. Boron adsorption by soils as affected by dissolved organic
315 matter from treated sewage effluent. Soil Sci Soc Am J. 2008; 72: 492-499.

316 10. Rosolem CA, Biscaro T. Boron adsorption and leaching in a Brazilian Oxisol Pesq.
317 Agrop Brasil. 2007; 42: 1473-1478.

318 11. Kekeç G. Effect of Boron pollution on some crops germination, determination of
319 modifications in genetic structure by using RAPD method and reducing this
320 effect by using growth hormones. Fatih University, Istanbul. 2008.

321 12. Ayvaz M. Bazi Arpa Çeşitlerinde Borun Büyüme ve Gelişime Üzerine Etkileri,
322 Yüksek Lisans Tezi, Biyoloji Anabilim Dalı. 2002. pp. 21-32.

323 13. Satya S, Pitchai JG, Indiran R. Boron nutrition of crops in relation to yield and quality-
324 A review. Agri Rev. 2009; 30: 139-144.

325 14. Ahmad S, Akhtar LH, Iqbal N, Nasim M. Cotton (*Gossypium hirsutum* L.) varieties
326 responded differently to foliar applied boron in terms of quality and yield. *Soil*
327 *Environ.* 2009; 28: 88-92.

328 15. Zhao D, Oosterhuis DM. Cotton carbon exchange, nonstructural carbohydrates and
329 boron distribution in tissues during development of boron deficiency. *Field*
330 *Crops Res.* 2002; 78: 75-87.

331 16. Sankaranarayanan K, Praharaj CS, Nalayini P, Bandyopadhyay KK, Gopalakrishnan N.
332 Effect of magnesium, zinc, iron and boron application on yield and quality of
333 cotton (*Gossypium hirsutum*). *Ind J Agri Sci.* 2010; 80: 699-703.

334 17. Rosolem CA, Deus ACF, Martins PO, Léles EP. Acúmulo e distribuição de boro em
335 cultivares de algodão. *Rev Bras Ciênc Solo.* 2012; 20: 265-266.

336 18. Rosolem CA, Costa A. Cotton growth and boron distribution in the plants as affected
337 by a temporary deficiency of boron. *J Plant Nutr.* 2000; 23: 815-825.

338 19. Oliveira RH, Milaneze RSD, Moraes-Dallaqua MA, Rosolem CA. Boron deficiency
339 inhibits petiole and peduncle cell development and reduces growth of cotton. *J*
340 *Plant Nutr.* 2006; 29: 2035-2048.

341 20. Zhou GF, Liu YZ, Sheng O, Wei QJ, Yang CQ, Peng SA. Transcription profiles of
342 boron-deficiency-responsive genes in citrus rootstock root by suppression
343 subtractive hybridization and cDNA microarray. *Front Plant Sci.* 2015; 5: 795.

344 21. Beato VM, Rexach J, Navarro-Gochicoa MT, Camacho-Cristóbal JJ, Herrera-
345 Rodríguez MB, Maldonado JM, et al. A tobacco asparagine synthetase gene
346 responds to carbon and nitrogen status and its root expression is affected under
347 boron stress. *Plant Science.* 2010; 178: 289-298.

348 22. Chen LS, Han S, Qi YP, Yang LT. Boron stresses and tolerance in citrus. Afr J
349 Biotechnol. 2012; 11: 5961-5969.

350 23. Hajiboland R, Farhanghi F, Aliasgharpour M. Morphological and anatomical
351 modifications in leaf, stem and roots of four plant species under boron deficiency
352 conditions. Folia Hort. 2012; 24: 41-51.

353 24. Lu YB, Yang LT, Li Y, Xu J, Liao TT, Chen YB, et al. Effects of boron deficiency on
354 major metabolites, key enzymes and gas exchange in leaves and roots of Citrus
355 sinensis seedlings. Tree physiology. 2014; 34: 608-618.

356 25. Durbak AR, Phillips KA, Pike S, O'Neill MA, Mares J, Gallavotti A, et al. Transport of
357 boron by the tassel-less1 aquaporin is critical for vegetative and reproductive
358 development in maize. The Plant Cell. 2014; 26: 2978-2995.

359 26. Leonard A, Holloway B, Guo M, Rupe M, Yu G, Beatty M, et al. Tassel-less1 encodes
360 a boron channel protein required for inflorescence development in maize. Plant
361 Cell Physiol. 2014; 55: 1044-1054.

362 27. Camacho-Cristobal JJ, Herrera-Rodriguez MB, Beato VM, Rexach J, Navarro-
363 Gochicoa MT, Maldonado JM, et al. The expression of several cell wall-related
364 genes in Arabidopsis roots in down-regulate under boron deficiency. Environ
365 Exp Bot. 2008; 63: 351-58.

366 28. Koshiba T, Kobayashi M, Ishihara A, Matoh T. Boron nutrition of cultured tobacco
367 BY-2 cells. VI. Calcium is involved in early responses to boron deprivation.
368 Plant Cell Physiol. 2010; 51: 323-327.

369 29. Mengel K, Kirkby EA. Boron. In: *Principles of plant nutrition*. 621-638, Kluwer
370 Academic Publishers (5th ed.) Dordrecht/ Boston/ London, Netherlands. 2001.

371 30. Reid R. Update on boron toxicity and tolerance in plants. *In: Xu F, Goldbach HE,*
372 *Brown PH, Bell RW, Fujiwara T, Hunt CD, Goldberg S, Shi L. (eds.). Advances*
373 *in Plant and Animal Boron Nutrition.* Springer, Dordrecht, The Netherlands.
374 2007. pp. 83-90.

375 31. Fontes RLF, Medeiros JF, Neves JCL, Carvalho OS, Medeiros JC. Growth of Brazilian
376 cotton cultivars in response to soil applied boron. *J Plant Nutr.* 2008; 31: 902-
377 918.

378 32. Rosolem CA, Esteves JAF, Ferelli L. Responses of cotton cultivars to boron in nutrient
379 solution. *Sci Agric.* 1999; 56: 705-711.

380 33. Rochester I. Nutrient uptake and export from an Australian cotton field. *Nutr Cycle*
381 *Agroecosyst.* 2007; 77: 213-223.

382 34. Rashid A. Boron deficiency in soils and crops of Pakistan: Diagnosis and Management.
383 PARC, Islamabad, Pakistan. 2006.

384 35. Paull JG, Cartwright B, Rathjen AJ. Responses of wheat and barley genotypes to toxic
385 concentrations of soil boron. *Euphytica.* 1998; 39: 137-144.

386 36. Aitkin RL, McCallum LE. Boron toxicity in soil solution. *Aust J Soil Res.* 1988; 26:
387 605-610.

388 37. Bingham FT. Boron. In *Methods of soil analysis, part 2ed.* A.L. Page. et al., 2nd ed.,
389 431-447. Madison, WI: ASA and SSSA. 1982.

390 38. Zellweger. Advance fibre information system for neps: Zellweger, Uster Ltd., Uster
391 Switzerland. 1992. Pp. 12

392 39. Standard ASTM. Standard Test Method for Flexural Properties of Unreinforced and
393 Reinforced Plastics and Electrical Insulation Materials. In American Society for
394 Testing and Materials. 1997.

395 40. Steel RGD, Torrie JH, Deekey DA. Principles and procedures of statistics: A
396 biometrical approach. 3rd ed. McGraw Hill Book. Int. Co. New York. 1997. pp.
397 400-428.

398 41. Hall D, Boron Fact Sheet. <http://www.soilquality.org.au/factsheets/boron> (Retrieved on
399 13th October, 2012 at 14:45pm) 2008.

400 42. Gupta U, Hitesh S. Impact of boron deficiency on plant growth. Int J Bioassays. 2013;
401 1048-1050.

402 43. Zafar M, Abbasi MK, Khan MA, Khaliq A, Sultan T, Aslam M. Effect of plant growth-
403 promoting rhizobacteria on growth, nodulation and nutrient accumulation of
404 lentil under controlled conditions. Pedosphere. 2012; 22: 848-859.

405 44. Brown PH, Bellaloui N, Wimmer MA, Bassil ES, Ruiz J, Hu H, et al. Boron in plant
406 biology. Plant Biol. 2002; 4: 205-223.

407 45. Shah JA, Zia-ul-Hassan S, Rajpar I, Sial MA. Response of cotton genotypes to boron
408 under B-deficient and B-adequate conditions. Pak J Bot. 2015; 47: 1657-1663.

409 46. Dordas C. Foliar boron application affects lint and seed yield and improves seed quality
410 of cotton grown on calcareous soils. Nutr Cycl Agroecosyst. 2006; 76: 19-28.

411 47. Mohsen S, Rashidi M, Yarmohammadi-Samani P. Influence of different application
412 rates of boron on biological growth and fiber quality of cotton. American-
413 Eurasian J Agri Environ Sci. 2013; 13: 548-552.

414 48. Sotiropoulos TE, Therios NI, Dimassi NK, Bosbalidis A, Kofilidis G. Nutritional status,
415 growth, CO₂ assimilation, and leaf anatomical responses in two kiwi fruit species
416 under boron toxicity. *J Plant Nutr.* 2002; 5: 1244-1261.

417 49. Nadim M, Awan I, Baloch M, Khan E, Naveed K, Khan M. Response of wheat
418 (*Triticum aestivum* L.) to different micronutrients and their application methods.
419 *J Anim Plant Sci.* 2012; 22: 113-119.

420 50. Wojcik P, Wojcik M, Klamkowski K. Response of apple trees to boron fertilization
421 under conditions of low soil boron availability. *Sci Hortic-Amsterdam.* 2008;
422 116: 58-64.

423 51. Han S, Chen LS, Jiang HX, Smith BR, Yang LT, Xie CY. Boron deficiency decreases
424 growth and photosynthesis, and increases starch and hexoses in leaves of citrus
425 seedlings. *J Plant Physiol.* 2008; 165: 1331-1341.

426 52. Li Y, Hou L, Song B, Yang L, Li L. Effects of increased nitrogen and phosphorus
427 deposition on offspring performance of two dominant species in a temperate
428 steppe ecosystem. *Sci Rep.* 2017; 7: 40951-40961.

429 53. Camacho-Cristobal JJ, Lunar L, Lafont F, Baumert A, Gonzalez-Fontes A. Boron
430 deficiency causes accumulation of chlorogenic acid and caffeoyl polyamine
431 conjugates in tobacco Leaves. *J Plant Physiol.* 2004; 161: 879-881.

432 54. Rosolem CA, Leite VM. Coffee leaf and stem anatomy under boron deficiency. *Rev*
433 *Bras Ciênc do Solo.* 2007; 31: 477-483.

434 55. Ahmed N, Abid M, Ahmad F. Boron toxicity in irrigated cotton (*Gossypium hirsutum*
435 L.). *Pak J Bot.* 2008; 40: 2443-2452.

436 56. Dell B, Huang L, Bell RW. Boron in plant reproduction. In: (Eds.): Goldbach *et al.*,
437 Boron in plant and animal nutrition. Kluwer Academic/Pleum Publishers, N Y,
438 USA. 2002. pp: 103-117.

439 57. Perica S, Brown PH, Connell JH, Nyomora AMS, Dordas C, Hu H, et al. Foliar boron
440 application improves flower fertility and fruit set of olive. Hort Sci. 2001; 36:
441 714-716.

442 58. Asad A, Blamey FPC, Edwards DG. Effects of boron foliar applications on vegetative
443 and reproductive growth of sunflower. Anal Bot. 2003; 92: 565-570.

444 59. Reid RJ, Hayes JE, Post A, Stangoulis JCR, Graham RD. A critical analysis of the
445 causes of boron toxicity in plants. Plant Cell and Environ. 2004; 25: 1405-1414.

446 60. Görmüs O. Interactive effect of nitrogen and boron on cotton yield and fiber quality.
447 Turk J Agri For. 2005; 29: 51-59.

448 61. Abid M, Ahmed N, Ali A, Chaudhry MA, Hussain J. Influence of soil-applied boron on
449 yield, fiber quality and leaf boron contents of cotton (*Gossypium hirsutum* L.). J
450 Agri Soc Sci. 2007; 3: 7-10.

451