



Article

# Changes in Leaf Structural and Functional Characteristics when Changing Planting Density at Different Growth Stages Alters Cotton Lint Yield under a New Planting Model

Aziz Khan <sup>1</sup>, Jie Zheng <sup>1</sup>, Daniel Kean Yuen Tan <sup>2</sup>, Ahmad Khan <sup>3</sup>, Kashif Akhtar <sup>4</sup>, Xiangjun Kong <sup>1,3</sup>, Fazal Munsif <sup>1,3</sup>, Anas Iqbal <sup>1</sup>, Muhammad Zahir Afridi <sup>3</sup>, Abid Ullah <sup>5</sup>, Shah Fahad <sup>6</sup> and Ruiyang Zhou <sup>1,\*</sup>

<sup>1</sup> Key Laboratory of Plant Genetics and Breeding, College of Agriculture, Guangxi University, Nanning 530005, China; azizkhanurlandi@gmail.com (A.K.); summerjackk@gmail.com (J.Z.); kongxiangjun201010@163.com (X.K.); munsiffazal@yahoo.com (F.M.); anasiqbalagr@gmail.com (A.I.)

<sup>2</sup> Plant Breeding Institute, Sydney Institute of Agriculture, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Sydney, NSW 2006, Australia; daniel.tan@sydney.edu.au

<sup>3</sup> Department of Agronomy, University of Agriculture, Peshawar 25000, Khyber Pakhtunkhwa, Pakistan; ahmad0936@yahoo.com (A.K.); drmzahir@aup.edu.pk (M.Z.A.)

<sup>4</sup> Institute of Nuclear Agricultural Sciences, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, China; kashif@zju.edu.cn

<sup>5</sup> Department of Botany, University of Malakand, Chakdara Dir Lower, Malakand, Khyber Pakhtunkhwa 18800, Pakistan; abid.ullah@uom.edu.pk

<sup>6</sup> Department of Agriculture, University of Swabi, Swabi 23561, Khyber Pakhtunkhwa, Pakistan; shah\_fahad80@yahoo.com

\* Correspondence: ruiyangzhou@aliyun.com or ruiyangzh@gmail.com

Received: 22 October 2019; Accepted: 3 December 2019; Published: 7 December 2019



**Abstract:** Manipulation of planting density and choice of variety are effective management components in any cropping system that aims to enhance the balance between environmental resource availability and crop requirements. One-time fertilization at first flower with a medium plant stand under late sowing has not yet been attempted. To fill this knowledge gap, changes in leaf structural (stomatal density, stomatal length, stomata width, stomatal pore perimeter, and leaf thickness), leaf gas exchange, and chlorophyll fluorescence attributes of different cotton varieties were made in order to change the planting densities to improve lint yield under a new planting model. A two-year field evaluation was carried out on cotton varieties—V<sub>1</sub> (Zhongmian-16) and V<sub>2</sub> (J-4B)—to examine the effect of changing the planting density (D<sub>1</sub>, low, 3 × 10<sup>4</sup>; D<sub>2</sub>, moderate, 6 × 10<sup>4</sup>; and D<sub>3</sub>, dense, 9 × 10<sup>4</sup>) on cotton lint yield, leaf structure, chlorophyll fluorescence, and leaf gas exchange attribute responses. Across these varieties, J-4B had higher lint yield compared with Zhongmian-16 in both years. Plants at high density had depressed leaf structural traits, net photosynthetic rate, stomatal conductance, intercellular CO<sub>2</sub> uptake, quenching (qP), actual quantum yield of photosystem II (ΦPSII), and maximum quantum yield of PSII (F<sub>v</sub>/F<sub>m</sub>) in both years. Crops at moderate density had improved leaf gas exchange traits, stomatal density, number of stomata, pore perimeter, length, and width, as well as increased qP, ΦPSII, and F<sub>v</sub>/F<sub>m</sub> compared with low- and high-density plants. Improvement in leaf structural and functional traits contributed to 15.9%–10.7% and 12.3%–10.5% more boll m<sup>-2</sup>, with 20.6%–13.4% and 28.9%–24.1% higher lint yield averaged across both years, respectively, under moderate planting density compared with low and high density. In conclusion, the data underscore the importance of proper agronomic methods for cotton production, and that J-4B and Zhongmian-16 varieties, grown under moderate and lower densities, could be a promising option based on improved lint yield in subtropical regions.

**Keywords:** leaf chlorophyll fluorescence; fiber yield; leaf gas exchange; leaf structure

---

## 1. Introduction

Cotton (*Gossypium hirsutum* L.) is a natural white fiber and cash crop that is grown globally [1]. The cotton plant is characterized by indeterminate growth habits and shows morphological and physiological adaptation to a wide range of environmental and management practices, including planting density and cultivar. An expanding population necessitates global efforts to increase crop production, especially those fulfilling food and fiber needs. Currently, numerous management practices have been introduced for cotton production systems, but lint production per unit area has remained stagnant [2]. High input costs combined with multiple management and material inputs have threatened cotton productivity. [2]. An efficient agricultural production system characterized by moderate planting density with one-time fertilization under a short growing season can reduce inputs without yield loss [3,4].

Planting density and choice of cultivar are important agronomic practices that have the potential to optimize the canopy photosynthetic rate and crop productivity of any cropping system [5]. Changes in plant architecture and canopy dynamics in response to planting density can have impacts on disease incidence, water use, canopy temperature, and enzymatic activity of assimilate metabolism [6]. Manipulations of planting density in cotton have remarkable impacts on biomass partitioning, nutrient uptake, boll distribution, changes in the light spectrum, and crop production [3,5,7,8], which can influence yield and profits for producers [9]. Plants at high density can minimize evaporation and irrigation frequency, as well as increase the utilization of irrigation water [10,11]. In contrast, high-density planting can slow down leaf appearance and reduce open boll density [12], boll weight, and boll number [7]. It also delays leaf senescence [13] and decreases nitrogen use efficiency and nitrogen recovery efficiency [14]. A planting density of up to nine plants  $m^{-2}$  has been reported to sustain leaf photosynthetic rate and reproductive organ biomass formation by increasing plant potassium uptake at various developmental stages. However, a sowing density of  $>10$  plants  $m^{-2}$  and subsequent shading can result in disease infestation, small boll size, fruit shedding, delayed maturity, and decreased individual plant development [4]. Dense planting can also delay crop maturity by promoting vegetative growth and can substantially depress net photosynthetic rate [4] due to decreased RuBP carboxylase activity and chlorophyll content [15]. High planting density can increase the auxin (IAA) content and enhance auxin polar transport by increasing the expression of the auxin biosynthesis gene (*GhYUC5*) and the auxin polar transport gene (*GhPIN1*). It can also inhibit vegetative branching by decreasing IAA, cytokinin, gibberellic acid, and brassinosteroid contents, followed by increased strigolactone content due to differential expression of hormone-associated genes in the tips of vegetative branches [15]. Optimal plant density can ensure healthy plant development by maintaining a core population of plants synchronizing boll number and fiber quality to achieve optimal yield [16].

Leaf morphological and physiological attributes are important players in photosynthetic regulation [4] and can provide a structural framework for gas exchange as well as optimize the photosynthetic function [17]. Cotton leaf surface characteristics, including cuticular thickening, wax layer, and trichomes, play critical roles in the variability of optical properties [18]. Generally, leaves developed under high sunlight can have thicker and smaller leaves with well-developed plastid tissues, greater stomatal density, and smaller granal stacks than shade leaves [19]. Plants under low density planting have a lower chlorophyll content and a higher electron transfer rate and ribulose-1,5-bi-phosphate carboxylase/oxygenase compared with high-density planting [20,21]. Leaves developed under lower density (sun leaves) are tolerant to strong light; conversely, shade leaves have weak photoprotection potential and are more sensitive to high light [17,21].

Studies regarding cotton growth and lint yield in response to diverse populations are common [22–24]. However, we are the first to report the effects of changing the planting density

on cotton lint yield, leaf structure, chlorophyll fluorescence, and leaf gas exchange characteristics in subtropical regions. The objectives of this study were to investigate leaf structural and functional characteristics in response to different planting densities and varieties. It also explored optimal plant density and variety for improved lint yield in subtropical regions.

## 2. Materials and Methods

### 2.1. Plant Material and Experimental Site

Seeds of two cotton cultivars— $V_1$  (Zhongmian-16) and  $V_2$  (J-4B)—were procured from the Cotton Research Institute, Chinese Academy of Agricultural Sciences and were grown under field conditions for two years. A replicated two-year (2017 and 2018 growing seasons) field experiment was conducted at Guangxi University, Nanning, China. The soil properties of the experimental field were sandy loam and yellowish, having a pH of 6.5; organic matter of 23.37 mg kg<sup>-1</sup>; and available nitrogen, phosphorus, and potassium content of 53.24, 77.58, and 6.30 mg kg<sup>-1</sup>, respectively. The experimental design layout was a balanced split plot with three replications.

### 2.2. Crop Management and Experimental Design

Before sowing, the experimental field was ploughed, laser leveled approximately three weeks prior, and covered with plastic film to conserve moisture and suppress weed germination. The experiments were designed in a split plot arrangement with three replications of each of the six treatment combinations. Two cotton varieties ( $V_1$ , Zhongmian-16;  $V_2$ , J-4B), were randomly allocated to the main plots and three plant population levels ( $D_1$ , low,  $3 \times 10^4$ ;  $D_2$ , medium,  $6 \times 10^4$ ; and  $D_3$ , dense,  $9 \times 10^4$  ha<sup>-1</sup>) were randomized in subplots. By increasing the precision of comparisons, split plot arrangements were adopted. Seeds were sown on 5 June in double rows on each raised plot (3.0 m wide and 11 m long), with a total plot size of 33.0 m<sup>2</sup>. Each subplot was 11 m long and 1.5 m wide, consisting of four rows with narrow (10 cm) and wide (66 cm) row spaces for a total of eight rows on each main plot. Plant-to-plant spacing was controlled according to the corresponding population level. Crops were irrigated one day after sowing to ensure uniform germination. Cotton seedlings were hand-thinned at the third leaf stage to the target population level for each plot. A basal application of phosphorus (P<sub>2</sub>O<sub>5</sub>) at 66 kg ha<sup>-1</sup>, nitrogen (N) at 170 N kg ha<sup>-1</sup>, and potash (K<sub>2</sub>O) at 190 kg ha<sup>-1</sup> was applied using superphosphate (12% P<sub>2</sub>O<sub>5</sub>), urea (46% N), and potassium chloride (59% K<sub>2</sub>O), respectively, during the pinhead stage. A plant growth regulator (i.e., mepiquat chloride) at the rate of 0.057 active ingredient ha<sup>-1</sup> was sprayed to control vegetative growth. All the necessary field management practices were performed according to crop requirements during the whole crop cycle.

### 2.3. Data Collection

Data on leaf structure, chlorophyll fluorescence, leaf gas exchange attributes, cotton yield, and yield contributors were recorded for each treatment in three replications. The details of each measurement are given below.

### 2.4. Yield and Yield Components

To assess cotton yield, fully opened bolls were hand-picked at three times in each treatment. The harvested seed cotton was sun-dried to  $\leq 11\%$  moisture content [16]. The seed cotton was ginned to calculate seed cotton and lint yield. During the second picking, 100 mature bolls were manually picked to calculate single boll mass and lint percentage. Seed cotton yield of 100 bolls was divided by the number of bolls to assess individual boll weight. Lint % was determined using the lint yield of 100 bolls and divided by seed cotton mass.

### 2.5. Cotton Leaf Structure Attributes

Ten plants in each plot were randomly tagged to measure leaf structure and plant growth characteristics at the boll setting stage. Leaf thickness was determined on 10 fully expanded leaves from the upper part of three plants (functional leaves, i.e., upper fourth leaf). A hand-held micrometer (Mitutoyo Digital Micrometer Model 293-185, Kawasaki, Japan) with a digital display and a clutch that ensured uniform pressure [25] was used for leaf thickness assessment. A  $5 \times 8$  mm leaf section was removed for each treatment. Samples were then added into 10 mL tubes containing 50%, 5%, and 5% alcohol solution, formaldehyde, and glacial acetic acid, respectively. Scanning electron microscopy was performed at Guanxi Medical University using a SUPRA 55VP (Carl Zeiss AG, Oberkochen, Germany). Image software was used to assess cotton leaf stomatal length, width, density, and pore perimeter according to the method reported in [26].

### 2.6. Chlorophyll Fluorescence Traits

Cotton leaf chlorophyll fluorescence attributes were measured on a fully expanded functional leaf (upper fourth leaf on the main stem) on a sunny day (between 1000 and 1200 h) via a portable mini PAM-2100 fluorometer coupled with a 2030-B leaf (Walz, Germany). Maximum ( $F_m$ ) and minimum ( $F_o$ ) fluorescence values of dark-adapted leaves (photosystem II (PSII) centers open) were measured using leaf clips. The maximum and minimum fluorescence values were assessed at  $0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$  with a frequency of 0.6 kHz and a 0.8 saturating pulse at  $>8000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. Maximum quantum yield of PSII photochemistry ( $F_v/F_m$ ) was calculated as  $F_v/F_m = 1 - (F_o/F_m)$  [27]. The effective quantum yield of PSII photochemistry of light-adapted leaves was determined by  $\Phi_{\text{PSII}} (F_m' - F)/F_m'$  [28]. Coefficient of photochemical quenching (qP) was assessed using the formula  $qP = (F_m' - F_s)/(F_m' - F_o')$  [29]. Minimal fluorescence of light-adapted leaves ( $F_o'$ ) was calculated according to the equation  $F_o' = F_o/(F_v/F_m + F_o/F_m')$  done by [28]. Nonphotochemical quenching (NPQ) was recorded according to [6] as  $NPQ = (F_m - F_m')/F_m'$ , where  $F_m$  represents the value of the predawn observations. The electron transport rate (ETR) was assessed using a leaf absorptance of 0.85 and half of the absorbed light was partitioned to each photosystem:  $ETR = \text{PSII} \times \text{PPFD} \times 0.85 \times 0.5$  [30].

### 2.7. Leaf Gas Exchange Attributes

At squaring, flowering, peak bloom, and boll setting stages, fully expanded leaves from the upper part of three plants (functional leaves, i.e., upper fourth leaf) were chosen to assess net photosynthetic rate (Pn), stomatal conductance ( $g_s$ ), intercellular  $\text{CO}_2$  concentration ( $C_i$ ), and transpiration rate (E). Net rate of photosynthesis was measured from the six functional leaves of three plants in each treatment using a portable infrared gas exchange analyzer (Li-6400, Li-Cor, Lincoln, NE, USA). These observations were made on a clear day between 10:00 a.m. and 12:00 p.m. Beijing time in each experimental unit of four replications. Leaves in each plot followed the following adjustments: PAR,  $1800 \mu\text{mol m}^{-2} \text{s}^{-2}$ ; air flow,  $389.42 \text{ mmol}^{-1} \text{ m}^{-2} \text{s}^{-1}$ ; water vapor pressure into leaf chamber, 3.13 mbar; leaf temperature,  $30 \text{ }^\circ\text{C}$ ; ambient temperature,  $33.69 \text{ }^\circ\text{C}$ ; and ambient carbon dioxide concentration,  $330\text{--}350 \text{ mol mol}^{-1}$ .

### 2.8. Statistical Analysis

All the data were processed using Microsoft Excel 2016. Figures were plotted using Sigma Plot 14.00 software. Analysis of variance was implemented using SAS software (version 8.1, SAS Institute, Cary, NC, USA). The initial combined data showed no interactions with years. Therefore, the data were pooled and presented across the two years. Means of planting density were separated using the least significant difference (LSD) test at the 5% probability level. Both planting densities and cultivars were taken as main factors and fixed effects with cropping season as the repetitive measured factor with a fixed effect. Similarly, the interaction was taken as fixed effects and treatment  $\times$  replication interaction, which was taken as a random effect. Differences among treatments imply statistical difference ( $p = 0.05$ ).

### 3. Results

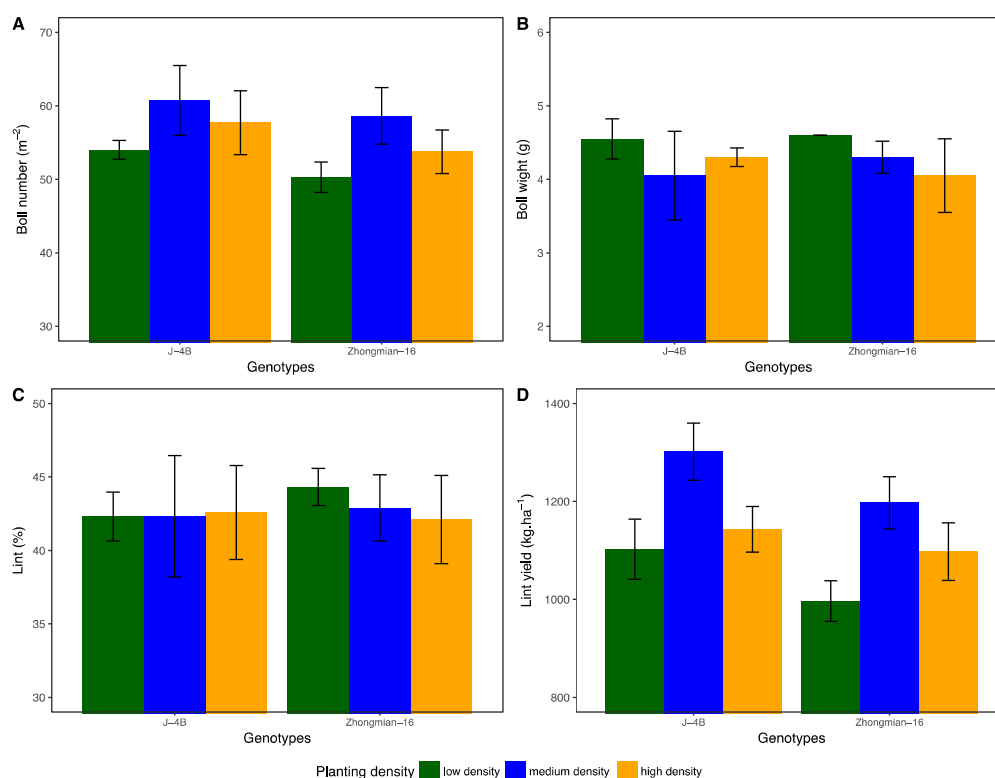
#### 3.1. Yield and Yield Components

The analysis of variance (Table 1) showed that effects of year, planting density, variety, and their interaction on cotton yield and yield contributors. The year effect was statistically significant, but the differences were not large. Planting density and variety did not affect lint percentage and boll weight. There were 14.5% and 7.1% more bolls  $m^{-2}$  with a 19% and 11.5% higher lint yield in moderate-density crops compared with low- and high-density crops, respectively. Under high-density conditions, a reduction of 9.6% and 2.3% was noted in boll weight and lint percentage, respectively, compared with low- and moderate-density crops. Across the varieties, J-4B produced 6% and 7.8% greater bolls  $m^{-2}$  and lint yield, respectively, compared with the Zhongmian-16 variety (Figure 1A–D). Interaction was significant for density  $\times$  variety across two years. Cotton plant individual boll weight, boll density  $m^{-2}$ , and lint yield were highest under moderate-to-high planting density for J-4B, while under low-density conditions, Zhongmian-16 had a higher boll weight.

**Table 1.** Summary of mean square (MS) values from analysis of variance (ANOVA) for cotton yield and yield contributors.

Source of Variance	Year	Density	Variety	Density $\times$ Variety
Bolls number ( $m^{-2}$ )	20.59 *	170.7 **	96.13 **	2.995 **
Boll weight (g)	2.402 **	0.640 **	0.003 ns	0.190 **
Lint (%)	121.5 **	3.001 ns	4.448 ns	4.749 ns
Lint yield ( $kg\ ha^{-1}$ )	50,400 **	123,003 **	65,451 **	3561 **

Different values obtained from ANOVA represent \* significant at  $p < 0.05$ , \*\* significant at  $p < 0.01$  and ns: nonsignificant.



**Figure 1.** Cotton (in response to different planting densities and cultivars: (a) boll number ( $m^{-2}$ ); (b) boll weight (g); (c) lint (%); (d) lint yield ( $kg\ ha^{-1}$ )). Values are the sum of three independent replicates. Error bars correspond to confidence interval at  $p = 0.05$ .

### 3.2. Leaf Structure Attributes

Cotton leaf structural characteristics (e.g., stomatal density, length, width, pore perimeters, and leaf thickness) significantly influenced by planting density and cultivar (Table 2). Under dense crops, leaf stomatal density, length, width, and pores were reduced by 7.1% and 11.7%; 3.3% and 9.3%; and 11.2%, 2.2%, and 7.9% compared with lower- and medium-density crops, respectively. Likewise, J-4B had improved stomatal density, length, width, pores, and leaf thickness by 10.3%, 13.7%, 1.1%, 9.9%, and 10.7%, respectively, compared with the Zhongmian-16 variety. Significant density  $\times$  variety interaction revealed that, unlike J-4B, increasing planting density reduced stomatal density, length, width, and pore perimeters in Zhongmian-16 during both growing seasons.

**Table 2.** Cotton leaf structural attributes as influenced by planting density and cultivars.

Treatment	Plant Height (cm)	Stomatal Density (mm <sup>-2</sup> )	Stomata Length (μm)	Stomata Width (μm)	Stomatal Pore Perimeter (μm)	Leaf Thickness (μm)
Year (Y)						
Year 2017	66.9a	28.8a	146.3a	20.8a	28.3a	143.0a
Year 2018	45.6b	20.1b	125.3b	14.3b	20.9b	106.6b
Density (D)						
D <sub>1</sub> (low)	57.0a	25.3a	144.9a	18.3a	25.6a	128.9a
D <sub>2</sub> (moderate)	56.1ab	24.7a	134.6b	18.1a	24.8b	124.5b
D <sub>3</sub> (high)	57.0a	23.3b	127.9c	16.3a	23.4c	121.0c
Variety (V)						
V <sub>1</sub> (Zhongmian-16)	59.1a	23.3b	129.2b	17.7a	23.1b	117.9b
V <sub>2</sub> (J-4B)	53.5b	25.6a	142.4a	17.5a	26.2a	131.7a
Source of variance						
Y	4091 **	689.8 **	4001 **	381.1 *	485.47 **	11916.8 *
D	5.53 *	12.83 **	878.3 **	15.55 ns	0.422 **	3.10 **
V	276.39 **	48.22 *	1579 **	0.358 *	89.30 *	1717.6 **
D $\times$ V	744.18 **	256.9 **	8971 **	208.6 **	201.59 *	6219.4 **

Values within columns followed by the same letter are statistically insignificant at the 0.05 level. \*\* significant at  $p < 0.01$  and \* significant at  $p < 0.05$ . ns: nonsignificant.

### 3.3. Chlorophyll Fluorescence Traits

During both years, planting densities, varieties, and their interaction had significant impacts on chlorophyll fluorescence traits in different growth stages (Tables 3–5). Except the squaring stage,  $\Phi$ PSII at first bloom, peak bloom, and boll setting stages were increased by moderate-density compared with low- and high-density crops, while the  $F_v/F_m$  yield was greater at all growth stages (Table 3). Across the varieties, J-4B had higher  $\Phi$ PSII and  $F_v/F_m$  at peak bloom and boll setting stages than Zhongmian-16, respectively. The interaction between density  $\times$  variety remained significant for  $\Phi$ PSII and  $F_v/F_m$  at different growth stages. The J-4B variety with moderate crops had greater  $\Phi$ PSII and  $F_v/F_m$  across the years.

Significant variation between planting densities, varieties, and years was found for photochemical quenching (qP) and nonphotochemical quenching (NPQ) of cotton at all growth stages (Table 4). Across densities, medium competitive plants yielded higher qP and NPQ rates. The variety J-4B resulted in higher qP at squaring and boll setting stages, while Zhongmian-16 had higher NPQ rates at first and full bloom stages. The interaction showed that J-4B had a higher qP under moderate density at different growth stages. J-4B had higher values for NPQ at low density compared with Zhongmian-16, followed by moderate density for the same variety at the peak bloom stage.

Significant differences existed between years, densities, and varieties for the ETR at four growth stages (Table 5). Interaction between density  $\times$  variety revealed substantial variation between varieties to planting density at all growth stages. Increased planting density substantially reduced ETR at all growth stages in both years (Table 5). The low-density plants improved ETR at squaring, first, peak bloom, and boll setting stages, followed by moderate density, while there was a lower ETR in high-density crops. A higher ETR was noted for the variety Zhongmian-16 at squaring, first, and peak bloom stages compared with J-4B; however, J-4B had a higher ETR at the boll setting stage than



Zhongmian-16. ETR values were substantially reduced under high density for both varieties. Lower planting density had higher ETR values for Zhongmian-16 or J-4B during both years.

**Table 3.** Quantum and maximum quantum yield of photosystem II (PSII) of cotton cultivars under varied planting densities.

Treatment	Squaring	First Bloom	Peak Bloom	Boll Set
Quantum yield ( $\Phi$ PSII)				
Year				
Year 2017	0.37b	0.57a	0.57a	0.55a
Year 2018	0.43a	0.51b	0.48b	0.38b
Density (D)				
D <sub>1</sub> (low)	0.40a	0.53b	0.49b	0.46b
D <sub>2</sub> (moderate)	0.40a	0.57a	0.58a	0.52a
D <sub>3</sub> (high)	0.40a	0.52b	0.50b	0.43c
Variety (V)				
V <sub>1</sub> (Zhongmian-16)	0.38a	0.52a	0.48b	0.52a
V <sub>2</sub> (J-4B)	0.41a	0.56a	0.56a	0.56a
Source of variance				
Y	0.034 **	0.029 **	0.07 **	0.260 **
D	0.008 ns	0.010 *	0.025 **	0.038 **
V	0.007 ns	0.009 ns	0.053 **	0.072 **
D × V	0.010 ns	0.007 ns	0.038 **	0.017 *
Maximal quantum yield ( $F_v/F_m$ )				
Year				
Year 2017	0.79a	0.78a	0.78a	0.73a
Year 2018	0.44b	0.70b	0.76b	0.59b
Density (D)				
D <sub>1</sub> (low)	0.62b	0.76a	0.78a	0.67b
D <sub>2</sub> (moderate)	0.63a	0.76a	0.78a	0.70a
D <sub>3</sub> (high)	0.59c	0.71b	0.75b	0.62c
Variety (V)				
V <sub>1</sub> (Zhongmian-16)	0.59a	0.74a	0.77a	0.63b
V <sub>2</sub> (J-4B)	0.62a	0.74a	0.77a	0.69a
Source of variance				
Y	1.123 **	0.057 **	0.004 **	0.161 **
D	0.006 **	0.008 **	0.004 **	0.023 **
V	0.008 ns	0.001 ns	0.003 ns	0.036 *
D × V	0.008 ns	0.017 *	0.004 ns	0.016 ns

Values within columns followed by the same letter are statistically insignificant at the 0.05 level. \* indicate significant at  $p < 0.05$ , \*\* significant at  $p < 0.01$  and ns: nonsignificant.

**Table 4.** Photochemical and nonphotochemical quenching of cotton cultivars under varied planting densities.

Treatment	Squaring	First Bloom	Peak Bloom	Boll Set
Photochemical quenching (qP)				
Year				
Year 2017	0.63a	0.78a	0.75a	0.7a
Year 2018	0.64a	0.69b	0.61b	0.6b
Density (D)				
D <sub>1</sub> (low)	0.64b	0.74b	0.65b	0.63b
D <sub>2</sub> (moderate)	0.60c	0.83a	0.73a	0.79a
D <sub>3</sub> (high)	0.66a	0.64c	0.65b	0.58c

Table 4. Cont.

Treatment	Squaring	First Bloom	Peak Bloom	Boll Set
Photochemical quenching (qP)				
Variety (V)				
V <sub>1</sub> (Zhongmian-16)	0.62a	0.70b	0.68a	0.63b
V <sub>2</sub> (J-4B)	0.65a	0.77a	0.67a	0.70a
Variance				
Y	0.002 ns	0.078 **	0.156 **	0.137 **
D	0.009 **	0.109 **	0.023 **	0.148 **
V	0.008 ns	0.036 **	0.002 ns	0.048 **
D × V	0.001 ns	0.029 **	0.016 *	0.034 **
Nonphotochemical quenching (NPQ)				
Year				
Year 2017	1.07a	1.78a	1.86a	1.33a
Year 2018	0.82b	0.64b	0.97b	0.95b
Density (D)				
D <sub>1</sub> (low)	1.06a	1.39a	1.91a	1.24a
D <sub>2</sub> (moderate)	0.98b	1.21b	1.26b	1.15b
D <sub>3</sub> (high)	0.77c	1.04c	1.08c	1.04c
Variety (V)				
V <sub>1</sub> (Zhongmian-16)	0.94a	1.25a	1.55a	1.18a
V <sub>2</sub> (J-4B)	0.94a	1.17b	1.28b	1.10a
Source of variance				
Y	0.555 **	10.856 **	7.124 **	1.355 **
D	0.257 **	0.374 **	2.277 **	0.128 **
V	0.001 ns	0.051 **	0.699 **	0.049 ns
D × V	0.007 ns	0.002 ns	0.613 **	0.004 ns

Values within columns followed by the same letter are statistically insignificant at the 0.05 level. \*\* significant at  $p < 0.01$  and \* significant at  $p < 0.05$ . ns: nonsignificant.

Table 5. Electron transport rate (ETR) of cotton cultivars at different planting densities.

Treatment	Squaring	First Bloom	Peak Bloom	Boll Set
Year				
Year 2017	118.7b	168.0a	167.6a	166.3a
Year 2018	136.3a	162.2b	130.9b	109.9b
Density (D)				
D <sub>1</sub> (low)	140.7a	172.8a	156.2a	156.0a
D <sub>2</sub> (moderate)	125.0b	162.3b	147.7b	131.4b
D <sub>3</sub> (high)	116.8c	160.3c	143.8c	126.9c
Variety (V)				
V <sub>1</sub> (Zhongmian-16)	129.9a	167.1a	151.9a	133.9b
V <sub>2</sub> (J-4B)	125.0b	163.2b	146.6b	142.3a
Source of variance				
Y	2770 **	301.6 **	12115 **	28685 **
D	1769 **	538.1 **	478.7 **	2940
V	216.1 **	126.2 **	261.4 **	641.8 **
D × V	34.08 **	113.9 **	50.60 **	930.1 **

Values within columns followed by the same letter are statistically insignificant at the 0.05 level. \*\* significant at  $p < 0.01$  and ns: nonsignificant.



### 3.4. Leaf Gas Exchange Attributes

Cotton leaf gas attributes were significantly influenced by plant density, variety, and growing year (Tables 6 and 7). Under moderate-density conditions, net photosynthetic rate (Pn) was increased at all growth stages except squaring, while stomatal conductance ( $g_s$ ) was higher at the first bloom and boll setting stages. Plants under high density had significantly lower Pn and  $g_s$  compared with low and moderate density (Table 6). J-4B had higher Pn and  $g_s$  compared with Zhongmian-16 under moderate density. Interaction between density  $\times$  variety was significant only at full bloom and boll setting for Pn and at the peak bloom stage for  $g_s$ . J-4B under low-to-moderate planting density had a higher Pn at squaring and first bloom stages, while it was higher in Zhongmian-16 at the peak bloom and boll set stages. A higher  $g_s$  under moderate planting density was noted in J-4B at the peak stage than Zhongmian-16 at low or high density.

**Table 6.** Net photosynthetic rate (Pn) and stomatal conductance ( $g_s$ ) of cotton cultivars at varied planting densities.

Treatment	Squaring	First Bloom	Peak Bloom	Boll Set
Photosynthesis (Pn ( $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ))				
Year				
Year 2017	25.5a	27.0a	32.3b	35.9a
Year 2018	26.0a	26.8a	32.5a	35.7b
Density (D)				
D <sub>1</sub> (low)	25.8a	26.4b	31.9c	34.8c
D <sub>2</sub> (moderate)	25.5b	27.7a	33.3a	36.8a
D <sub>3</sub> (high)	25.9a	26.2b	32.1b	35.8b
Variety (V)				
V <sub>1</sub> (Zhongmian-16)	25.6a	26.6b	32.1b	35.6b
V <sub>2</sub> (J-4B)	25.9a	27.2a	32.7a	36.1a
Source of variance				
Y	2.576 ns	0.276 ns	0.681 **	0.123 **
D	0.735 **	5.623 **	6.544 **	11.56 **
V	0.664 ns	3.453 *	3.901 **	1.823 **
D $\times$ V	0.323 ns	0.948 ns	7.696 **	3.399 **
Stomatal conductance ( $g_s$ ( $\text{mol}(\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$ ))				
Year				
Year 2017	0.49a	0.58a	0.45a	0.33a
Year 2018	0.49a	0.55a	0.44a	0.32a
Density (D)				
D <sub>1</sub> (low)	0.48a	0.54b	0.46a	0.31b
D <sub>2</sub> (moderate)	0.49a	0.61a	0.46a	0.35a
D <sub>3</sub> (high)	0.49a	0.55b	0.42a	0.33ab
Variety (V)				
V <sub>1</sub> (Zhongmian-16)	0.45a	0.55b	0.43b	0.32b
V <sub>2</sub> (J-4B)	0.48a	0.58a	0.46a	0.34a
Source of variance				
Y	0.001 ns	0.005 ns	0.003 ns	0.006 ns
D	0.003 ns	0.027 **	0.006 ns	0.044 *
V	0.004 ns	0.017 **	0.019 ns	0.064 **
D $\times$ V	0.004 ns	0.016 ns	0.008 **	0.006 ns

Values within columns followed by the same letter are statistically insignificant at the 0.05 level. \*\* significant at  $p < 0.01$  and \* significant at  $p < 0.05$ . ns: nonsignificant.

**Table 7.** Intercellular CO<sub>2</sub> concentration (C<sub>i</sub>) and transpiration rate (E) of cotton cultivars under different planting densities.

Treatment	Squaring	First Bloom	Peak Bloom	Boll Setting
Intercellular CO <sub>2</sub> concentration (C <sub>i</sub> (μmol (CO <sub>2</sub> ) m <sup>-2</sup> s <sup>-1</sup> ))				
Year				
Year 2017	246.0a	274.6a	167.6a	243.2a
Year 2018	243.8b	271.6b	164.9b	239.3b
Density (D)				
D <sub>1</sub> (low)	248.7a	272.2c	164.8b	242.2a
D <sub>2</sub> (moderate)	244.0b	274.7a	167.9a	242.1a
D <sub>3</sub> (high)	241.9c	272.4b	165.9b	239.4b
Variety (V)				
V <sub>1</sub> (J-4B)	245.9a	274.6a	166.9a	241.5a
V <sub>2</sub> (Zhongmian-16)	243.9b	271.6b	165.5b	240.9b
Source of variance				
Y	42.25 **	81.00 **	64.00 **	132.25 **
D	145.63 **	21.948 **	28.89 **	29.84 **
V	37.21 **	0.0004 **	17.64 **	2.89 **
D × V	16.74 **	8.703 **	25.40 **	56.12 **
Transpiration rate (E (mmol (H <sub>2</sub> O) m <sup>-2</sup> s <sup>-1</sup> ))				
Year				
Year 2017	6.8a	9.1a	6.6a	4.6a
Year 2018	6.7b	9.1b	6.5b	4.4b
Density (D)				
D <sub>1</sub> (low)	6.6c	9.3a	6.7a	4.4a
D <sub>2</sub> (moderate)	6.7b	9.1b	6.5b	4.4b
D <sub>3</sub> (high)	6.8a	9.0c	6.1c	4.4b
Variety (V)				
V <sub>1</sub> (J-4B)	6.68b	9.21a	6.48b	4.52a
V <sub>2</sub> (Zhongmian-16)	6.82a	9.05b	6.68a	4.29b
Source of variance				
Y	2770 **	0.007 **	301.6 **	28685 **
D	1769 **	0.203 **	538.1 **	2940 **
V	216.0 **	0.226 **	126.2 **	641.8 **
D × V	34.10 **	0.139 **	113.9 **	930.1 **

Values within columns followed by the same letter are statistically insignificant at the 0.05 level. \*\* significant at  $p < 0.01$  and ns: nonsignificant.

Increasing planting density significantly reduced C<sub>i</sub> in cotton leaves for both varieties. Plants with moderate density had higher C<sub>i</sub> uptake at first bloom and peak bloom stages compared with low- and high-density crops, respectively (Table 7). Plants under low density resulted in a higher rate of E during first bloom, peak bloom, and boll set stages compared with moderate- and high-density crops, respectively (Table 7). Across the varieties, Zhongmian-16 yielded higher for both C<sub>i</sub> uptake and E rates compared with J-4B. Interaction between density × variety remained significant at all growth stages for C<sub>i</sub>. The transpiration rate was decreased in both varieties when the planting density increased.

#### 4. Discussion

The current study has provided new data on the common perception that high planting density significantly decreases leaf structural characteristics, such as stomatal density, length, width, pore perimeter, and leaf thickness, as well as functional traits (leaf gas exchange and chlorophyll fluorescence traits), which leads to lint yield loss. However, we found that improved leaf functional and structural traits for J-4B under moderate density had a higher lint yield. Under high-density treatment, reductions

in lint yield for Zhongmian-16 were associated with repression in leaf structural and functional attributes, which in turn caused depression in leaf photosynthetic capacity due to nutrient competition. The difference between varieties from changing planting density might be associated with canopy architecture and genetic variation. Therefore, these changes in varieties might have significant impacts on leaf structural and functional attributes and, ultimately, on yield formation.

High planting density responses to cotton lint yield, growth, biomass production, nutrient uptake, and fiber quality have been extensively investigated [3,4,13,22]. The mechanisms of interplant competitiveness under low-to-high planting density on leaf structure, chlorophyll fluorescence, and leaf gas exchange attributes for optimal cotton lint yield have not yet been reported. Across densities, the moderate population had a higher boll number  $m^{-2}$  with improved lint yield for J-4B compared with Zhongmian-16 across two years. High-density plants substantially reduced yield and yield components in both years, probably due to competition for nutrients. The phenomenon of increased lint yield under moderate density can be associated with improved leaf structural and chlorophyll fluorescence traits and higher leaf photosynthetic capacity, which resulted in higher boll density  $m^{-2}$  compared with other densities.

Moderate density favors dry matter partitioning to the reproductive structures rather than vegetative organs [31] and less fruit shedding compared with denser plants. The reductions in lint yield under high density can be attributed to decreased leaf structural and physiological traits, which were observed in this study. The differences that existed between varieties for yield when changing planting density might be attributable to canopy architecture. Differences in plant canopy architectural traits among varieties have an impact on growth characteristics and lint yield. These data further confirmed that an appropriate selection of variety and optimal density can contribute to successful cotton production. Reducing population density may also have other implications, such as decreased frequency and insecticide inputs per season without any yield loss to increase profit. Moreover, high plant density can substantially depress leaf structural and physiological attributes, which in turn cause a severe yield penalty.

Plants respond to ambient and management interventions via architectural and structural changes. Plant growth and leaf morphological attributes, including stomatal density, size, number of pores, width, length, and leaf thickness features, are pivotal windows regulating leaf photosynthetic capacity [10,25] and offer a structural framework for  $CO_2$  exchange and optimization of photosynthetic activities, which in turn can improve crop yield [17]. In this study, high planting density substantially decreased leaf thickness, stomatal density, leaf length, width, and number of stomatal pores. Limitations in these attributes disrupted the photosynthetic capacity of plants by restricting entry of  $CO_2$  to the mesophyll through the stomata of leaves, which is extremely responsive to light environments. Thus, the exchange of  $CO_2$  by means of stomata might be restricted [32]. Higher stomatal density, thicker leaves, and rapid metabolite transfer between the mesophyll and bundle sheath cells can favor higher leaf photosynthetic capacity [33]. Increasing planting density has been proposed to decrease the stomatal density of wheat leaves [34]. A greater stomatal size can facilitate  $CO_2$  distribution into the leaf due to its conductance being proportional to the square of the effective radius of the stomatal pore, resulting in increased stomatal conductance [35]. However, the responses of leaf structural attributes vary under different abiotic stresses in different plant species or varieties [36]. These data suggest that plants under high-density conditions have significantly decreased leaf morphological characteristics, which might be particularly responsible for depressing leaf photosynthetic capacity.

Chlorophyll fluorescence is a nondestructive evaluation of PSII activity. In plant physiology, this technique is commonly used and has become a classical method for crop improvement, screening of beneficial traits, and linking genomic knowledge to phenological response. Due to the sensitivity of PSII to undesirable ambient conditions, this is a useful method for understanding photosynthetic mechanisms and a good indicator of how plants respond to ambient change [37,38].

$\Phi PSII$  is a measure of light energy capture efficiency, which reflects the actual primary sunlight energy conversion efficiency of the PSII reaction center [15]. In this study,  $\Phi PSII$  substantially declined

under high-density conditions. Probably, a lower  $\Phi_{PSII}$  value under high-density conditions did not efficiently convert photon energy to chemical energy; however, this phenomenon needs further exploration. Under shading conditions, a low  $\Phi_{PSII}$  may be responsible for depressing Pn due to the adjustment in photochemical reaction centers [39], which was observed in our study. The efficient use of limited light energy and the degree of the PSII reaction center openness can increase, resulting in improved energy conversion efficiency. This is associated with the increase of  $F_v/F_m$ ,  $\Phi_{PSII}$ , and qP at early shading [40]. The maximal photochemical efficiency of PSII ( $F_v/F_m$ ) determines the potential quantum efficiency of PSII [41]. In this study,  $F_v/F_m$  had higher values under low rather than high planting density, which is consistent with [32], and reductions in  $F_v/F_m$  values might be due to the lower values of  $F_m$  and increased values of  $F_o$ . The ETR is an important chlorophyll fluorescence attribute affected by the external light environment. The rate of ETR declined from low to high density in this study, which corresponds with [40], and shading can significantly decrease ETR values by affecting PSII photochemical reaction centers and consequently diminish the primary stable quinone acceptor of PSII, leading to a decrease in the activity of photosynthetic electron transport efficiency via PSII [27,33]. NPQ can have critical roles in the nonradiative dissipation of surplus light energy [42]. A low-light environment can cause a reduction in NPQ, possibly associated with reduced light energy [32,43]. In this study, a severe decline in NPQ values was noted under high-density compared with low-density crops. This can be explained as the decreased NPQ being associated with the decreased efficiency of photochemical reactions through the reduced fraction of incident light in photochemical energy utilization, which resulted in lower thermal dissipation in PSII [44]. The rate of photochemical quenching (qP) under dense crops showed a substantial reduction compared with low and moderate densities. Probably, a low-light environment can cause reductions in the amount of pigment and the efficiency of photochemical energy conversion, resulting in the depressed quantum yield of PSII and decreased qP. The qP reflects the efficiency of light quantum harvested by PSII to chemical energy and represents the openness degree of the PSII reaction center, and a greater qP results in greater activity of electron transfer in PSII.

Leaf gas exchange traits can play a central role in biomass formation and the prime determination of cotton lint yield [45]. High planting density results in rapid canopy closure and an increase in radiation interception, which reduces weed competition [46], but this impedes leaf gas exchange traits, leading to yield loss [47]. In the current study, cotton leaf gas exchange parameters were substantially depressed under close planting at different growth stages. Accordingly, high-density conditions resulted in reductions in leaf stomatal density, length, width, pores, and leaf thickness, probably due to mutual shading, which may be responsible for depressing stomatal conductance ( $g_s$ ) and  $CO_2$  uptake through the stomata, which in turn suppressed the photosynthetic capacity. Plants under high-density conditions can significantly decrease  $g_s$  and  $C_i$ , which can negatively influence the photosynthetic system [38]. The  $CO_2$  concentration plays a central role in net photosynthetic rate (Pn), but this varies across species and ambient conditions [48,49]. The  $g_s$  might respond to alterations in Pn and thus prevents  $C_i$  near saturation. The primary function of stomata is to avoid desiccation and enable the passage of  $CO_2$ . Stomata induce a substantial disruption in the  $CO_2$  assimilation rate, which reduces more in  $C_4$  than  $C_3$  plants. The stomatal limitation of Pn is the role of stomatal resistance to contribute to "resistance" to  $CO_2$  uptake and stomatal limitation in spite of a decline in  $C_i$  [50]. The higher transpiration (E) rates in low-density conditions may have been due to low mutual shading, which allowed rapid stomata opening. Our data showed that high plant density substantially decreased leaf thickness, stomatal density, width, length, and stomatal pores and resulted in lower  $C_i$  and  $g_s$ , which in turn depressed leaf photosynthetic capacity.

## 5. Conclusions

In the present study, planting densities and varieties significantly influenced lint yield by affecting leaf stomatal density, thickness, width, length, pore perimeter, leaf gas exchange, and chlorophyll fluorescence characteristics. The J-4B variety in the moderate-density condition produced a higher

lint yield due to improved leaf structure, leaf gas exchange, and chlorophyll fluorescence attributes compared with low or high planting densities. Plants at high density substantially depressed leaf stomatal density, thickness, width, length, and pore perimeter, probably due to more competition for nutrients compared with low and moderate planting densities in both varieties. The offset in these attributes further disrupted  $\Phi$ PSII,  $F_v/F_m$ , ETR, and NPQ, which in turn reduced leaf photosynthetic capacity and consequently, lint yield loss. Conclusively, J-4B and Zhongmian-16 grown under medium- and lower-density conditions may be a promising option based on improved leaf structural and functional traits in subtropical regions. Our data will substantially contribute to cotton breeding programs in subtropical environments in the future.

**Author Contributions:** Conceptualization, A.K. (Aziz Khan) and R.Z.; methodology, A.K. (Aziz Khan); investigation, J.Z., Z.Z., X.K. and A.I.; review and editing, D.K.Y.T. and A.U.; formal analysis, A.K. (Ahmad Khan), K.A. and F.M.; software, M.Z.A., A.B. and S.F.

**Funding:** We are thankful for the financial supported by National Natural Science Foundation of China (Grant No. 31360348). The supporters did not play any role in the design, analysis, or interpretation of this work and the relevant data.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

PSII, photosystem II;  $\Phi$ PSII, actual quantum yield of PSII;  $F_v/F_m$ , maximal photochemical efficiency of PSII; ETR, electron transport rate; NPQ, nonphotochemical quenching; qP, photochemical quenching; Pn, net photosynthetic rate;  $g_s$ , stomatal conductance;  $C_i$ , intercellular CO<sub>2</sub> concentration; E, transpiration rate; HNR, height-to-node ratio; D<sub>1</sub>, low; D<sub>2</sub>, moderate; D<sub>3</sub>, high density; V<sub>1</sub>, Zhongmian-16; V<sub>2</sub>, J-4B.

## References

1. Constable, G.A.; Bange, M.P. The yield potential of cotton (*Gossypium hirsutum* L.). *Field Crops Res.* **2015**, *182*, 98–106. [[CrossRef](#)]
2. Dai, J.; Dong, H. Intensive cotton farming technologies in China: Achievements, challenges and countermeasures. *Field Crops Res.* **2014**, *155*, 99–110. [[CrossRef](#)]
3. Khan, A.; Najeeb, U.; Wang, L. Planting density and sowing date strongly influence growth and lint yield of cotton crops. *Field Crops Res.* **2019**, *209*, 129–135. [[CrossRef](#)]
4. Khan, A.; Wang, L.; Ali, S. Optimal planting density and sowing date can improve cotton yield by maintaining reproductive organ biomass and enhancing potassium uptake. *Field Crops Res.* **2017**, *214*, 164–174. [[CrossRef](#)]
5. Yao, H.S.; Zhang, Y.L.; Yi, X.P.; Zhang, X.J.; Zhang, W.F. Cotton responds to different plant population densities by adjusting specific leaf area to optimize canopy photosynthetic use efficiency of light and nitrogen. *Field Crops Res.* **2016**, *188*, 10–16. [[CrossRef](#)]
6. Kalaji, H.M.; Schansker, G.; Brestic, M.; Bussotti, F.; Calatayud, A.; Ferroni, L.; Losciale, P. Frequently asked questions about chlorophyll fluorescence, the sequel. *Photosynth. Res.* **2017**, *132*, 13–66. [[CrossRef](#)] [[PubMed](#)]
7. Dong, H.; Li, W.; Tang, W.; Li, Z.; Zhang, D.; Niu, Y. Yield, quality and leaf senescence of cotton grown at varying planting dates and plant densities in the Yellow River Valley of China. *Field Crops Res.* **2006**, *98*, 106–115. [[CrossRef](#)]
8. Wherley, B.G.; Gardner, D.S.; Metzger, J.D. Tall fescue photomorphogenesis as influenced by changes in the spectral composition and light intensity. *Crop Sci.* **2005**, *45*, 562–568. [[CrossRef](#)]
9. Adams, C.; Thapa, S.; Kimura, E. Determination of a plant population density threshold for optimizing cotton lint yield: A synthesis. *Field Crops Res.* **2019**, *230*, 11–16. [[CrossRef](#)]
10. Yao, H.; Zhang, Y.; Yi, X. Plant density alters nitrogen partitioning among photosynthetic components, leaf photosynthetic capacity and photosynthetic nitrogen use efficiency in field-grown cotton. *Field Crop Res.* **2015**, *184*, 39–49. [[CrossRef](#)]
11. Antonietta, M.; Fanello, D.D.; Acciaresi, H.A.; Guiamet, J.J. Senescence and yield responses to plant density in stay green and earlier-senescing maize hybrids from Argentina. *Field Crop Res.* **2014**, *155*, 111–119. [[CrossRef](#)]
12. Sawan, Z.M. Plant density; plant growth retardants: Its direct and residual effects on cotton yield and fiber properties. *Cogent Biol.* **2016**, *2*, 1234959. [[CrossRef](#)]



13. Luo, Z.; Liu, H.; Li, W.; Zhao, Q.; Dai, J.; Tian, L.; Dong, H. Effects of reduced nitrogen rate on cotton yield and nitrogen use efficiency as mediated by application mode or plant density. *Field Crops Res.* **2018**, *218*, 150–157. [[CrossRef](#)]
14. Li, P.; Dong, H.; Zheng, C.; Sun, M.; Liu, A.; Wang, G.; Pang, C. Optimizing nitrogen application rate and plant density for improving cotton yield and nitrogen use efficiency in the North China Plain. *PLoS ONE* **2017**, *12*, e0185550. [[CrossRef](#)] [[PubMed](#)]
15. Li, T.; Zhang, Y.; Dai, J.; Dong, H.; Kong, X. High plant density inhibits vegetative branching in cotton by altering hormone contents and photosynthetic production. *Field Crops Res* **2019**, *230*, 121–131. [[CrossRef](#)]
16. Dong, H.Z.; Kong, X.Q.; Li, W.J.; Tang, W.; Zhang, D.M. Effects of plant density and nitrogen and potassium fertilization on cotton yield and uptake of major nutrients in two fields with varying fertility. *Field Crops Res.* **2010**, *119*, 106–113. [[CrossRef](#)]
17. Jiang, C.D.; Wang, X.; Gao, H.Y.; Shi, L.; Chow, W.S. Systemic regulation of leaf anatomical structure, photosynthetic performance, and high-light tolerance in sorghum. *Plant Physiol.* **2011**, *155*, 1416–1424. [[CrossRef](#)]
18. Bondada, B.R.; Oosterhuis, D.M. Comparative Epidermal Ultrastructure of Cotton (*Gossypium hirsutum* L.) Leaf, Bract and Capsule Wall. *Ann. Bot.* **2000**, *86*, 1143–1152. [[CrossRef](#)]
19. Anderson, J.M. Photo regulation of the composition, function, and structure of thylakoid membranes. *Ann. Rev. Plant Physiol.* **1986**, *37*, 93–136. [[CrossRef](#)]
20. Marchiori, P.E.R.; Machado, E.C.; Ribeiro, R.V. Photosynthetic limitations imposed by self-shading in field-grown sugarcane varieties. *Field Crops Res.* **2014**, *155*, 30–37. [[CrossRef](#)]
21. Naramoto, M.; Katahata, S.I.; Mukai, Y.; Kakubari, Y. Photosynthetic acclimation and photoinhibition on exposure to high light in shade-developed leaves of *Fagus crenata* seedlings. *Flora* **2006**, *201*, 120–126. [[CrossRef](#)]
22. Tung, S.A.; Huang, Y.; Hafeez, A.; Ali, S.; Khan, A.; Souliyanonh, B.; Yang, G. Mepiquat chloride effects on cotton yield and biomass accumulation under late sowing and high density. *Field Crops Res.* **2018**, *215*, 59–65. [[CrossRef](#)]
23. Mao, L.; Zhang, L.; Zhao, X.; Liu, S.; Zhang, S.; Li, Z. Crop growth, light utilization and yield of relay intercropped cotton as affected by plant density and a plant growth regulator. *Field Crop Res.* **2014**, *155*, 67–76. [[CrossRef](#)]
24. Liao, J.; Ma, F.Y.; Fan, H. Effects of sowing rate on population growth, canopy light distribution and yield of drip irrigated spring wheat. *J. Triticeae Crops* **2012**, *32*, 739–742.
25. Pauli, D.; White, J.W.; Andrade-Sanchez, P.; Conley, M.M.; Heun, J.; Thorp, K.R.; Gore, M.A. Investigation of the influence of leaf thickness on canopy reflectance and physiological traits in upland and Pima cotton populations. *Front. Plant Sci.* **2017**, *8*, 1405. [[CrossRef](#)] [[PubMed](#)]
26. Zhou, C.B.; Xie, C. A simple method to quantify the size and shape of stomatal pore. In Proceedings of the 17th International Congress on Photosynthesis Research, Maastricht, The Netherlands, 7–12 August 2016.
27. Genty, B.; Briantais, J.M.; Baker, N.R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* **1989**, *1*, 87–92. [[CrossRef](#)]
28. Oxborough, K.; Baker, N.R. Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components—calculation of qP and Fv/Fm without measuring Fo. *Photosynth. Res.* **1997**, *54*, 135–142. [[CrossRef](#)]
29. Krause, G.H.; Weis, E. Chlorophyll fluorescence and photosynthesis: The basics. *Annu. Rev. Plant Biol.* **1991**, *42*, 313–349. [[CrossRef](#)]
30. Kromkamp, J.; Barranguet, C.; Penne, J. Determination of microphytobenthos quantum efficiency and photosynthetic activity by means of variable chlorophyll fluorescence. *Mar. Ecol. Prog. Ser.* **1998**, *162*, 45–55. [[CrossRef](#)]
31. Pettigrew, W.T.; Gerik, T.J. Cotton leaf photosynthesis and carbon metabolism. *Adv. Agron.* **2007**, *94*, 209–236.
32. Li, T.; Liu, L.N.; Jiang, C.D.; Liu, Y.J.; Shi, L. Effects of mutual shading on the regulation of photosynthesis in field-grown sorghum. *J. Photochem. Photobiol. B Biol.* **2014**, *137*, 31–38. [[CrossRef](#)] [[PubMed](#)]
33. Szczepanik, J.; Minchin, P.E.H.; Sowiński, P. On the mechanism of C4 photosynthesis intermediate exchange between Kranz mesophyll and bundle sheath cells in grasses. *J. Exp. Bot.* **2008**, *59*, 1137–1147.

34. Xiao, Y.; Tholen, D.; Zhu, X.G. The influence of leaf anatomy on the internal light environment and photosynthetic electron transport rate: Exploration with a new leaf ray tracing model. *J. Exp. Bot.* **2016**, *67*, 6021–6035. [[CrossRef](#)] [[PubMed](#)]
35. Xu, Z.; Zhou, G. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J. Exp. Bot.* **2008**, *59*, 3317–3325. [[CrossRef](#)]
36. Liu, S.; Liu, J.; Cao, J.; Bai, C.; Shi, R. Stomatal distribution and character analysis of leaf epidermis of jujube under drought stress. *J. Anhui Agric. Sci.* **2006**, *34*, 1315–1318.
37. Stirbet, A.; Lazár, D.; Kromdijk, J. Chlorophyll a fluorescence induction: Can just a one-second measurement be used to quantify abiotic stress responses. *Photosynthetica* **2018**, *56*, 86–104. [[CrossRef](#)]
38. Murchie, E.H.; Lawson, T. Chlorophyll fluorescence analysis: A guide to good practice and understanding some new applications. *J. Exp. Bot.* **2013**, *64*, 3983–3998. [[CrossRef](#)]
39. Chen, B.L.; Yang, H.K.; Ma, Y.N.; Liu, J.R.; Lv, F.J.; Chen, J.; Zhou, Z.G. Effect of shading on yield, fiber quality and physiological characteristics of cotton subtending leaves on different fruiting positions. *Photosynthetica* **2017**, *55*, 240–250. [[CrossRef](#)]
40. Zhong, X.M.; Shi, Z.S.; Li, F.H.; Huang, H.J. Photosynthesis and chlorophyll fluorescence of infertile and fertile stalks of paired near-isogenic lines in maize (*Zea mays* L.) under shade conditions. *Photosynthetica* **2014**, *52*, 597–603. [[CrossRef](#)]
41. Singh, S.K.; Badgular, G.; Reddy, V.R.; Fleisher, D.H.; Bunce, J.A. Carbon dioxide diffusion across stomata and mesophyll and photo-biochemical processes as affected by growth CO<sub>2</sub> and phosphorus nutrition in cotton. *J. Plant Physiol.* **2013**, *170*, 801–813. [[CrossRef](#)]
42. Zhao, W.Q.; Meng, Y.L.; Chen, B.L.; Wang, Y.H.; Li, W.F.; Zhou, Z.G. Effects of fruiting-branch position, temperature-light factors and nitrogen rates on cotton (*Gossypium hirsutum* L.) fiber strength formation. *Sci. Agric. Sin.* **2011**, *48*, 3721–3732.
43. Dai, Y.; Shen, Z.; Liu, Y.; Wang, L.; Hannaway, D.; Lu, H. Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels Gilg. *Environ and Exp. Bot.* **2009**, *65*, 177–182. [[CrossRef](#)]
44. Guo, C.J.; Qi, W.M.; Yi, Z.; Long, W.Y. Effects of shading on photosynthetic characteristics and chlorophyll fluorescence parameters in leaves of *Hydrangea macrophylla*. *Chin. J. Plant Ecol.* **2017**, *41*, 570–576.
45. Zahoor, R.; Dong, H.; Abid, M.; Zhao, W.; Wang Zhou, Y.Z. Potassium fertilizer improves drought stress alleviation potential in cotton by enhancing photosynthesis and carbohydrate metabolism. *Environ. Exp. Bot.* **2017**, *137*, 73–83. [[CrossRef](#)]
46. Yao, H.; Zhang, Y.; Yi, X.; Zuo, W.; Lei, Z.; Sui, L.; Zhang, W. Characters in light-response curves of canopy photosynthetic use efficiency of light and N in responses to plant density in field-grown cotton. *Field Crops Res.* **2016**, *203*, 192–200. [[CrossRef](#)]
47. Riar, R.; Wells, R.; Edmisten, K.; Jordan, D.; Bachelier, J. Cotton yield and canopy closure in North Carolina as influenced by row width, plant population, and leaf morphology. *Crop Sci.* **2013**, *53*, 1704–1711. [[CrossRef](#)]
48. Wang, J.; Chen, Y.; Wang, P.; Li, Y.S.; Wang, G.; Liu, P.; Khan, A. Leaf gas exchange, phosphorus uptake, growth and yield responses of cotton cultivars to different phosphorus rates. *Photosynthetica* **2018**, *56*, 1414–1421. [[CrossRef](#)]
49. Ku, S.; Edwards, G. Oxygen inhibition of photosynthesis. II. Kinetic characteristics as affected by temperature. *Plant Physiol.* **1977**, *59*, 991–999. [[CrossRef](#)]
50. Farquhar, G.D.; Sharkey, T.D. Stomatal conductance and photo-synthesis. *Annu. Rev. Plant Physiol.* **1982**, *33*, 317–345. [[CrossRef](#)]

