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Growth, leaf anatomy, and photosynthesis of cotton (*Gossypium hirsutum* L.) seedlings in response to four light-emitting diodes and high pressure sodium lamp

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Abstract

Background Light is a critical factor in plant growth and development, particularly in controlled environments. Light-emitting diodes (LEDs) have become a reliable alternative to conventional high pressure sodium (HSP) lamps because they are more efficient and versatile in light sources. In contrast to well-known specialized LED light spectra for vegetables, the appropriate LED lights for crops such as cotton remain unknown.

Results In this growth chamber study, we selected and compared four LED lights with varying percentages (26.44%–68.68%) of red light (R, 600–700 nm), combined with other lights, for their effects on growth, leaf anatomy, and photosynthesis of cotton seedlings, using HSP lamp as a control. The total photosynthetic photon flux density (PPFD) was (215 ± 2) μmol·m^{-2}·s^{-1} for all LEDs and HSP lamp. The results showed significant differences in all tested parameters among lights, and the percentage of far red (FR, 701–780 nm) within the range of 3.03%–11.86% was positively correlated with plant growth (characterized by leaf number and area, plant height, stem diameter, and total biomass), palisade layer thickness, photosynthesis rate (P_n), and stomatal conductance (G_s). The ratio of R/FR (4.445–11.497) negatively influenced the growth of cotton seedlings, and blue light (B) suppressed stem elongation but increased palisade cell length, chlorophyll content, and P_n.

Conclusion The LED 2 was superior to other LED lights and HSP lamp. It had the highest ratio of FR within the total PPFD (11.86%) and the lowest ratio of R/FR (4.445). LED 2 may therefore be used to replace HPS lamp under controlled environments for the study of cotton at the seedling stage.

Keywords Cotton seedling, Light-emitting diodes, Biomass, Palisade cell, Photosynthesis

Introduction

Plants require photosynthetically active radiation (PAR) with wavelengths ranging from 400 to 700 nm for photosynthesis (Carruthers et al., 2001). Changes in light quality can alter both plant shape and photosynthetic responses, although the consequences vary widely among species (Brown et al., 1995; Rahman et al., 2021). Red wavelengths are most effective for triggering photosynthetic activity (Hogewoning et al., 2012). However, when grown with red light as the only source of irradiation, some dicotyledonous plants (*Brassica alboglabra*, cucumber, *Arabidopsis*

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thaliana) showed enhanced hypocotyl and stem elongation, leaf extension, and reduced chlorophyll (Chl) levels (He et al., 2015; Ooi et al., 2016; Hernández et al., 2016; Wang et al., 2016). Such morphological deviation can be recovered when red-LED is supplemented with blue light (Ouzounis et al., 2016). In addition, the blue light increases stomatal opening due to activating H^+-ATPase on the plasma membrane and driving K^+ flux into the guard cells (Doi et al., 2015), whereas too much blue light induces photoinhibition (Zeiger et al., 1977; Muneer et al., 2014; Tokuno et al., 2012). Green light has the potential to enter the leaf deeper than blue or red light, enhancing carbon fixation and consequently promoting plant production (Al Murad et al., 2021; Sun et al., 1998). Furthermore, green light can counteract blue-light-induced stomatal opening (Smith et al., 2017). Far-red light (700–800 nm) mediates plant growth and developmental processes, especially in shaded environments (Park et al., 2017), and moderate-intensity UV light has been shown to promote photosynthesis (Verdaguer et al., 2017). However, the sensitivity of plants to light quality always varied with species and growth stages (Massa et al., 2008; Terfa et al., 2013).

During the traditional cultivation of crops, the sun light is excellent in a natural environment. However, with the development of science and technology, controlled-environment agriculture (CEA) systems are designed to provide optimal growing conditions for crops and to prevent disease and pest damage (Parrish et al., 2021). Moreover, biological and agricultural experiments were usually performed in CEA systems (Tan et al., 2022a, b). Artificial illumination is one of the major methods in CEA. High-pressure sodium (HPS) lamps are common artificial lighting sources. They provide broad-spectrum light, mostly in the yellow (565 to 590 nm), orange (590 to 625 nm), and red (625 to 700 nm) spectra, with less light in the blue region (400 to 500 nm) (Islam et al., 2012). However, HPS lamps have a low efficiency of only 25%–30%, which not only requires considerable energy but also induces light stress in plants (Randall et al., 2014). Light-emitting diodes (LEDs) are solid-state, semiconducting diodes that can generate light in the 250 to 1000 nm wavelength range (Piovene et al., 2015). Compared with traditional HPS lamps lighting, LEDs offer a longer life period, greater spectrum specificity, and consume less energy to produce light (D'Souza et al., 2015; Dayani et al., 2016). This type of light source was initially used in plant growth in the 2000s and is now commonly utilized in industrial-scale plant cultivation (Singh et al., 2015).

Cotton (*Gossypium hirsutum* L.) is an important cash crop that is typically grown in field conditions. However, numerous cotton physiological and molecular studies have been conducted in controlled environments. For instance, our previous study (Wang et al., 2012) was carried out in a growth chamber to elucidate the phytohormone basis of the feedback regulation of leaf senescence induced by potassium deficiency in cotton. The study conducted by Arias-Gaguancela et al. (2023) in a controlled environment revealed the mechanistic understanding of cotton growth modulation by NAE (9-hydroxy linoleoylethanolamide) oxylipins. As mentioned above, there is also a tendency for LEDs to replace HSP lamps in cotton-grown chambers. However, there is limited research on the optimal LED lights for cotton growth (Li et al., 2017). To address this issue, we selected several LED lights with varying ratios of red wavelengths and compared their effects on the growth of cotton seedlings with HPS lamp as a control. The goal was to determine whether one of the LED lights could be efficiently used to replace HSP lamps when an indoor study is carried out during the seedling stage of cotton, which will enhance cotton physiological and molecular study in the future.

Materials and method

Plant material and growth conditions

The experiment was carried out in a growth chamber at China Agricultural University, Beijing. The cotton variety, SCRC 22, developed by the Cotton Research Center, Shandong Academy of Agricultural Sciences, was used in this study. The seeds were surface-sterilized in 9% H_2O_2 for 30 min, washed in tap water, and germinated in sand medium for four days. Then, the seedlings were transplanted into 6.0 L plastic pots (24 × 17 × 15 cm) with modified Hoagland's solution (Wang et al., 2012). There were six plants per pot and the nutrient solutions were changed twice a week. The light/dark cycle, temperature, and humidity were maintained at 14/10 h, (28 ± 1)/(20 ± 1) ℃, and 50%–60%, respectively. An air pump was used for continuous aeration to provide O_2.

Light treatments

Red light is one of the primary bands absorbed by chlorophyll (McCree, 1971). We selected four LED light sources with varied red to total photosynthetic photon flux density (PPFD) ratios (LED 1, 68.68%; LED 2, 52.73%; LED 3, 41.09%; and LED 4, 26.44%) from products of Jiupo Biotechnology Co., Ltd. (Fuzhou, China). The high pressure sodium lamp (HPS lamp, 400 w, Townlite Electric Lighting Co., Ltd, Changzhou, China) served as a control.

The spectrum of tested light sources was monitored at the top of pots using a spectrometer (ALP-01, Asensetek Lighting Passport Pro, Taiwan, China). Its spectral range lies within 380–780 nm, and the far red

(FR), red (R), green (G), blue (B), and ultraviolet-A (UV-A) wavelengths are within 701–780, 600–700, 500–599, 400–499, and 380–399 nm, respectively. Figure 1 shows the spectrum of the tested lights, and Table 1 presents the photosynthetic photon flux density (PPFD) of each wavelength and their ratios (%) relative to the total PPFD [(215 ± 2) μmol·m^{-2}·s^{-1}] which was the same among each light source. The ratios of any two lights for each light source are listed in Table 2.

The light sources were randomly positioned in the growth chamber and separated by polyvinyl chloride (PVC) boards. Five pots (five replicates) were set under each kind of light. The experiment was repeated independently twice and produced the same results. The data of the second repeat were analyzed in this study.

Considering the possible impact of heat generated by the HPS lamp on plant growth, the growth chamber was equipped with a central air conditioner, and the wind speed remained constant throughout the day to keep room temperature even. In addition, the thermohygrographs were placed under each light source to monitor real-time temperature and humidity, which guided us to modulate the wind speed of central air conditioner.

Measurement of seedling growth and development

Plant height (from the junction of root and hypocotyl to apical meristem), stem diameter (at the junction of root and hypocotyl), and the number of unfolded true leaves were counted every two days after the plants

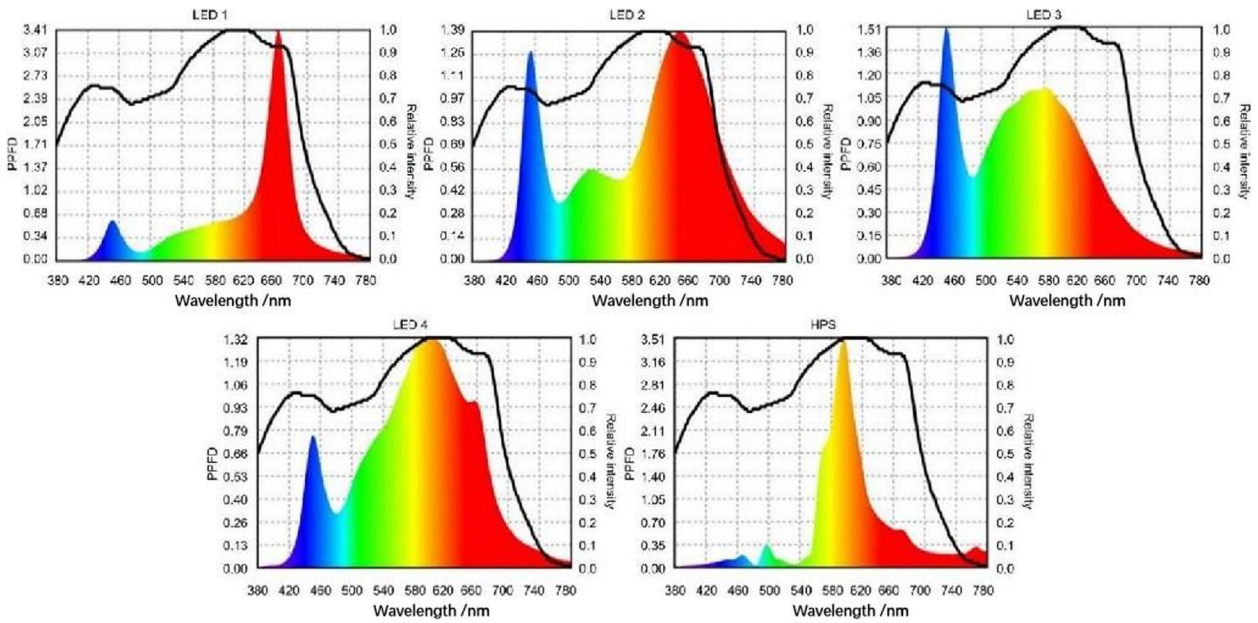

Fig. 1 Color spectrum of light-emitting diodes (LEDs) and high pressure sodium (HPS) lamp. PPFD (μmol·m^{-2}·s^{-1}): photosynthetic photon flux density. Black line represents the visible spectra of solar radiation in the 380–780 nm range

Table 1 Photosynthetic photon flux density (PPFD) of far red (FR, 701–780 nm), red (R, 600–700 nm), green (G, 500–599 nm), blue (B, 400–499 nm), and ultraviolet-A (UV-A, 380–399 nm) for LEDs (light-emitting diodes) and HPS (high pressure sodium) lamp, and ratio of FR (PFR), R (PR), G (PG), B (PB) and UV-A (PUV-A) to total photosynthetically active radiation (PAR) of each light source

Light	PPFD / (μmol·m^{-2}·s^{-1})						Ratio of light to PAR /%				
	PAR	FR	R	G	B	UV-A	PFR /%	PR /%	PG /%	PB /%	PUV-A /%
LED 1	213.45	12.75	146.59	44.71	22.16	0.09	5.97	68.68	20.95	10.38	0.04
LED 2	214.64	25.46	113.17	53.64	47.83	0.12	11.86	52.73	24.99	22.29	0.05
LED 3	215.87	8.62	88.70	92.96	34.21	0.25	3.99	41.09	43.06	15.85	0.12
LED 4	213.98	6.48	56.53	98.21	59.19	0.13	3.03	26.44	45.89	27.66	0.06
HPS lamp	216.34	19.38	100.40	104.15	11.86	0.52	8.96	46.41	48.14	5.48	0.24

Table 2 Ratios of any two colors relative to the photosynthetic photon flux density (PPFD) of each light source. Far red (FR, 701–780 nm), red (R, 600–700 nm), green (G, 500–599 nm), blue (B, 400–499 nm), and ultraviolet-A (UV-A, 380–399 nm)

Light	R/FR	G/FR	B/FR	UV-A/FR	R/G	R/B	R/UV-A	G/B	G/UV-A	B/UV-A
LED 1	11.50	3.51	1.74	0.01	3.28	6.62	1628.78	2.02	496.78	246.22
LED 2	4.45	2.11	1.88	0.01	2.11	2.37	943.08	1.12	447.00	398.58
LED 3	10.29	10.78	3.97	0.03	0.95	2.59	354.80	2.72	371.84	136.84
LED 4	8.72	15.16	9.13	0.02	0.58	0.96	434.85	1.66	755.46	455.31
HPS	5.18	5.37	0.61	0.03	0.96	8.47	193.08	8.78	200.29	22.81

were transplanted into the nutrient solution. Plants were harvested at 28 days after transplanting (DAT), and separated into roots, stem (including petiole), and each true leaf. The leaf area was measured using a portable area meter (LI-3050C, Li-Cor Inc, Lincoln, NE, USA), and all samples were dried at 80 ℃ for five days.

Leaf anatomy

Before harvesting, the leaf segments (1 cm^2) without the main vein from the third or fourth leaf were sampled using a hole puncher. They were kept for three days in the 10 mL formaldehyde-based fixative solution (70% alcohol: formalin: acetic acid = 90: 5: 5, v/v/v). After dehydrating via ethyl-alcohol series (75%, 85%, 95%, and 100%), the leaf samples were embedded in paraffin at a melting temperature of 58–60 ℃. Then they were microtome sectioned at a thickness of 10 μm (Leika RM2265, Leika, Wetzlar, Germany), mounted on glass slides, and stained with safranine and quick green stain (Ruzin, 1999). The stained segment of leaf tissues was examined using an OLYMPUS BX-51 microscope (Olympus, Tokyo, Japan). Images were analyzed using an OLYMPUS CCD camera (Olympus, Tokyo, Japan) to measure leaf thickness, stratum corneum, epidermis, palisade, and spongy tissues. Each treatment had five replicates, and the results of each replicate were averaged across three leaf sections.

Measurement of photosynthetic pigments and leaf gas exchange traits

The soil and plant analyzer development (SPAD)-502 (Minolta Corporation, Ltd., Osaka, Japan) was used to estimate the SPAD value between the midrib and the leaf edge. Three SPAD measurements were collected per leaf and averaged. About 0.2 g fresh leaf tissues without main vein were soaked in 10 mL of extraction solution (anhydrous ethanol: acetone = 1:2, v/v) for 48 h in the dark. A spectrophotometer (UV-2550, Shimadzu, Tokyo, Japan) was used to measure absorbance at 440, 645, and 663 nm. The contents of Chl a, Chl b, and carotenoids (Car) were calculated according to Wellburn (1994). At 6 and 28 DAT, the net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) of cotyledons and all true leaves were determined with a portable photosynthesis system (LI-6400XT, Li-Cor Inc, Lincoln, NE, USA). Photosynthetic parameters were measured under (55 ± 5)% relative humidity, 6 cm^2 leaf area, (23 ± 2) ℃ in the sample cell, (21 ± 2) ℃ leaf thermocouple, (450 ± 5) μmol(CO_2)·mol^{-1} (CO_2), 200 μmol·s^{-1} flow rate, and 200 μmol·m^{-2} ·s^{-1} photosynthetically active radiation (PAR).

Statistical analyses

The data were subjected to a one-way analysis of variance (ANOVA) in SPSS 25.0 (IBM Inc. Chicago, USA), and significant differences between treatment means were identified at a 95% confidence level using Tukey's multiple comparisons test. When the variances were uneven, we used Games-Howell under the unequal variance assumption. Correlations between characteristics of light sources and growth traits of seedlings were analyzed using R 4.1.3. Before this, the percentage data such as the percentage of each light and the ratio between two lights were normalized using arcsine transformation. The heatmap of the correlation coefficient matrix was also generated by R 4.1.3.

Results

Plant growth and development

As shown in Fig. 2A, seedlings grown under LED 2 and LED 4 produced true leaves earlier than those under other lights. Also, LED 2 produced more leaves than other LED lights and HPS lamp at 16, 18, and 24 DAT (Fig. 2B). However, LED 3 had the lowest number of leaves after 18 DAT.

The plants under HPS lamp were the tallest from 4 DAT, while those under LED 4 were the shortest. Plant height under LED 2 was significantly shorter than that under HPS lamp from 6 to 14 DAT, but similar to the latter from 14 DAT (Fig. 2C). The stem diameter of plants

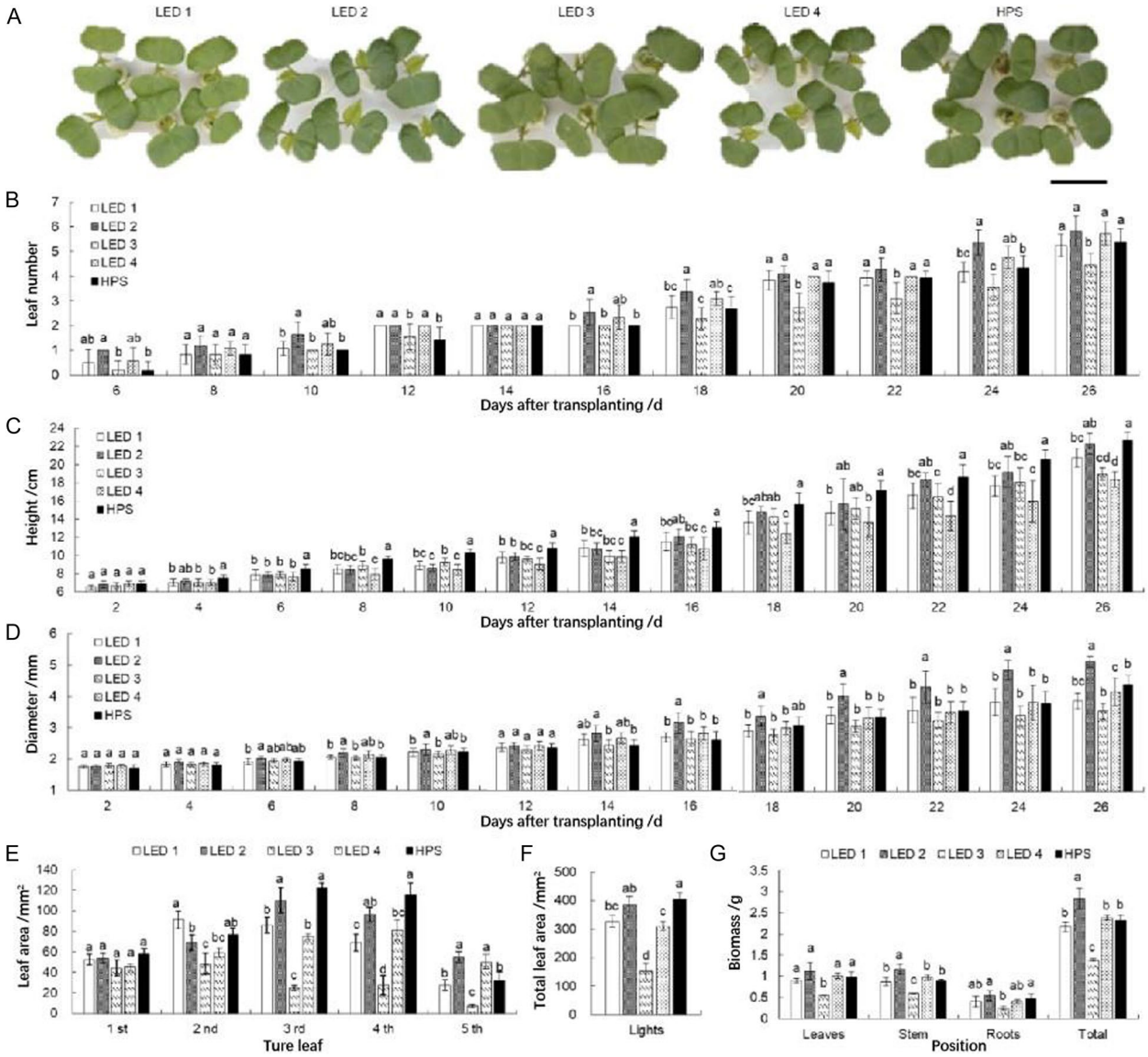

Fig. 2 Cotton seedlings at 7 DAT (days after transplanting from sand medium at emergence) under LEDs (light-emitting diodes) and HPS (high pressure sodium) lamp (**A**). The bar is 5 cm. Effect of light sources on the number of true leaves over time (**B**), plant height (**C**) measured from the junction of root and hypocotyl to the apex, stem diameter (**D**) measured at the junction of root and hypocotyls, area of each true leaf (**E**) measured at 28 DAT, total leaf area (**F**) and biomass (**G**) determined at 28 DAT. Each value is a mean of five replicates ± standard deviation (SD). Different letters indicate significant differences at $p < 0.05$

under LED 2 was the largest from 16 to 26 DAT, whereas that of plants under LED 3 was the smallest post 16 DAT (Fig. 2D).

LED 2 and HPS lamp produced leaves with larger total leaf area than other LED lights (Fig. 2F), owing mostly to their bigger third and fourth leaves (Fig. 2E). Conversely, LED 3 had the lowest total leaf area (60.3% less than that of LED 2) because of its small third, fourth, and fifth leaves (Fig. 2E).

LED 2 produced the highest biomass among lights, and LED 3 produced the lowest (Fig. 2G). There were no significant differences in biomass among LED 1, LED 4, and HPS lamp (Fig. 2G).

Leaf anatomy

There were obvious differences in anatomy of the true leaf (the third expanded leaf from the apex) among light sources (Fig. 3). Notably, the palisade and spongy cells of leaves under LED 4 were tightly structured,

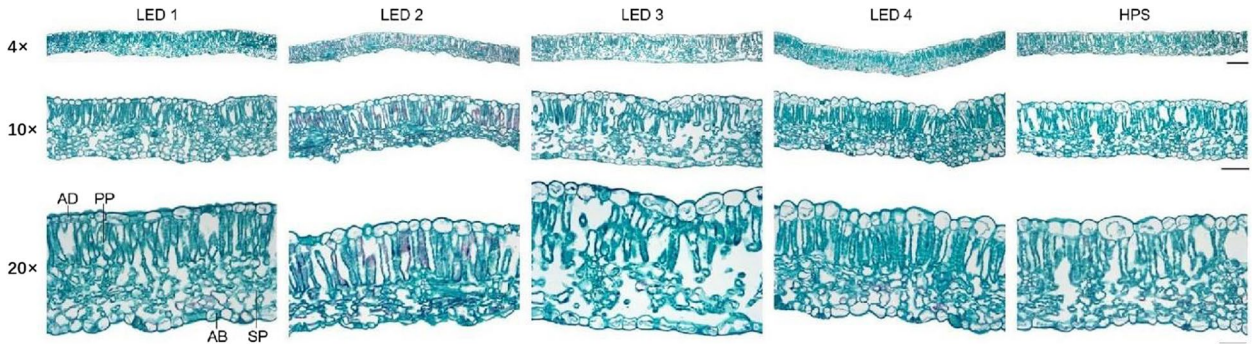

Fig. 3 Longitudinal section of the third expanded leaf from the apex under LEDs (light-emitting diodes) and HPS (high pressure sodium) lamp. The leaf tissue was stained with Safranine O-fast green and was examined with OLYMPUS CCD camera at × 4, × 10, and × 20 magnitudes. AD: adaxial epidermis; AB: abaxial epidermis; PP: palisade parenchyma; SP: spongy parenchyma. The bar is 200, 100, and 50 μm for × 4, × 10, and × 20 magnitudes

whereas those under LED 3 had loose spongy and palisade layers. In addition, leaves under LED 2 showed the longest palisade parenchyma cells (29.9% and 29.5% longer than that of LED 3 and HPS lamp, respectively) and a much higher palisade-to-spongy ratio than other lights (Fig. 3, Table 3). Also, the total thickness of the leaf and length of spongy layer under LED 1 and HPS lamp was significantly greater than those under other lights (Table 3).

SPAD and photosynthetic pigments

The SPAD value of cotyledons treated with LED 2 was much higher than that of other treatments. For the fourth true leaves, LED 4 always had higher SPAD value, then followed by LED 2 (Fig. 4A). In comparison to other lights, LED 3 and HPS lamp showed lower contents of Chl a, Chl b, the sum of Chl a and Chl b, and carotenoid in the first and second leaves, while LED 2 had higher contents of Chl a, Chl b, the sum of Chl a and Chl b, and carotenoid in the third, fourth, and fifth leaves (Fig. 4B ~ D&F). Each true leaf under LED 3 exhibited slightly greater contents of Chl a to Chl b than other light sources (Fig. 4E), indicating a limited capacity to utilize blue light.

Leaf photosynthesis

Among light sources, LED 2 had the greatest P_n, G_s, C_i, and E in cotyledons, albeit the differences in P_n and E between LED 2 and LED 4 were negligible (Fig. 5A ~ D). Also, the P_n, G_s, and E of all true leaves (except the third leaf) under LED 2 were greater than those under other light sources (Fig. 5E ~ H). Specifically, the photosynthetic rate of the 2nd, 4th, and 5th true leaves of LED 2 was 52.1%, 75.9%, and 52.1% higher than those of HPS lamp, respectively. However, LED 2 showed greater C_i only in the first and second leaves (Fig. 5F).

Correlation analysis

The correlation analysis between various light quality metrics and plant parameters is shown in Fig. 6. Although we studied LEDs with varied percentages of red light (PR), this characteristic of light quality significantly correlated with only Chl a to Chl b and C_i. Conversely, the percentage of FR in total PPFD (PFR) showed a significantly positive correlation with all five traits of

Table 3 Effects of light sources on total leaf thickness, length of palisade parenchyma cells, length of spongy layer as well as thickness of adaxial and abaxial epidermis, and ratio of palisade to spongy tissue. Values are means of 5 replicates ± standard deviation (SD). Means in the same column with different letters differ significantly ($p < 0.05$)

Light	Total thickness /μm	Length of palisade parenchyma cells /μm	Length of spongy layer /μm	Thickness of adaxial epidermis /μm	Thickness of abaxial epidermis /μm	Ratio of palisade to spongy tissue
LED 1	211.43 ± 8.19 a	67.43 ± 2.22 bc	102.77 ± 4.65 a	22.18 ± 0.68 bc	19.48 ± 1.05 a	0.66 ± 0.03 c
LED 2	171.60 ± 10.25 c	90.44 ± 3.61 a	51.12 ± 1.03 c	20.27 ± 2.07 c	8.78 ± 0.95 c	1.78 ± 0.15 a
LED 3	189.20 ± 1.71 b	63.37 ± 3.15 c	88.39 ± 4.14 b	20.32 ± 1.04 c	15.56 ± 1.78 b	0.72 ± 0.04 c
LED 4	191.38 ± 4.81 b	72.56 ± 3.15 b	81.02 ± 1.40 b	29.22 ± 2.95 a	17.14 ± 0.82 b	0.91 ± 0.06 b
HPS	206.54 ± 6.08 a	63.76 ± 2.13 c	102.54 ± 6.45 a	24.91 ± 2.05 b	16.60 ± 1.22 b	0.62 ± 0.04 c

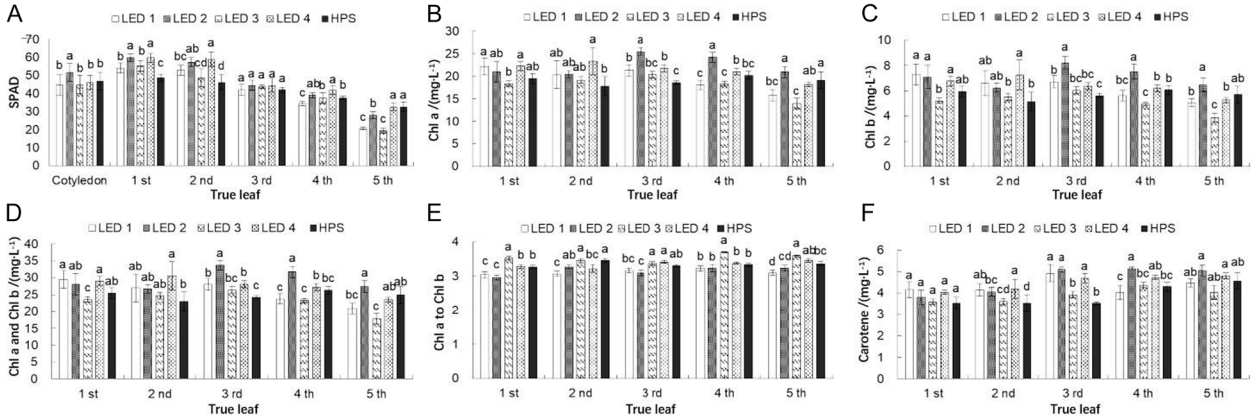

Fig. 4 Effects of light sources on the SPAD value (**A**), chlorophyll a (Chl a) (**B**), chlorophyll b (Chl b) (**C**), the sum of Chl a and Chl b (**D**), ratio of Chl a to Chl b (**E**), and carotene content (**F**) of each true leaf. Each value is a mean of five replicates ± standard deviation (SD). Different letters indicate significant differences at $P < 0.05$

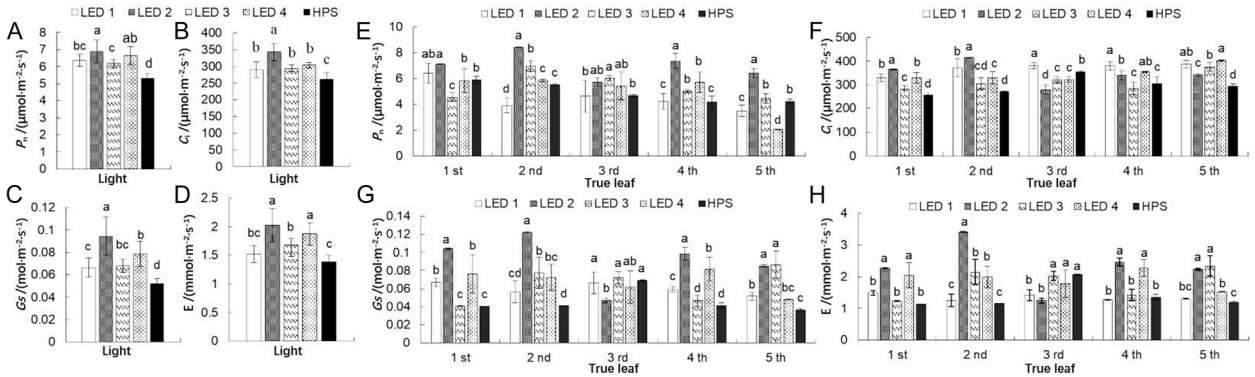

Fig. 5 Effects of light sources on gas exchange parameters of cotton cotyledons (**A** ~ **D**) and true leaves (**E** ~ **H**), including net photosynthetic rate (P_n) (**A**, **E**), intercellular CO_2 concentration (C_i) (**B**, **F**), stomatal conductance (G_s) (**C**, **G**), and transpiration rate (E) (**D**, **H**). Each value is a mean of five replicates ± standard deviation (SD). Different letters indicate significant differences at $P < 0.05$

plant growth and development (leaf count, leaf area, plant height, stem diameter, and total biomass), which could be possibly explained by the positive relationship between PFR and the length of palisade parenchyma cells, P_n, and G_s, respectively. Also, the ratio of R, G, and UV-A to FR significantly correlated with most traits of plant growth, the length of palisade parenchyma cells, the sum of Chl a and Chl b, P_n, and G_s.

There was a negative correlation between the percentage of B in PPFD (PB) and plant height, but positive relationships between PB and the length of palisade parenchyma cells, the sum of Chl a and Chl b, P_n, and G_s, respectively. Also, there were significantly positive correlations between B to UV-A and leaf count, total biomass, the length of palisade parenchyma cells, the sum of Chl a and Chl b, P_n, G_s, and E, respectively. In addition, we found that plant height, the length of spongy layer, and total leaf thickness increased, but the length of palisade layer, P_n, and G_s decreased with increased R/B (from 0.96 to 8.47).

Discussion

In this study, we found considerable differences in plant growth, leaf anatomy, photosynthetic pigments, and gas exchange parameters among four LEDs lights and HSP lamp. Overall, the LED 2 was superior to other lights (including HSP lamp), especially in terms of leaf number, stem diameter, biomass, length of palisade cell, the sum of Chl a and Chl b, and photosynthetic rate. This LED light was characterized by the highest ratio of FR to total PPFD (11.86%), and the lowest ratios of R/FR (4.45), G/FR (2.11), and UV-A/FR (0.01).

A higher percentage of FR and lower R/FR ratio enhanced growth of cotton seedlings

The inverse linear relationship between the stem elongation and the R/FR ratio has been reported in many species (Smith, 1994). A low R/FR can alter the auxin (indole-3-acetic acid, IAA) and gibberellin (GA) levels of plants via expression of related genes to increase plant height (Shibuya et al., 2013; Tao et al.,

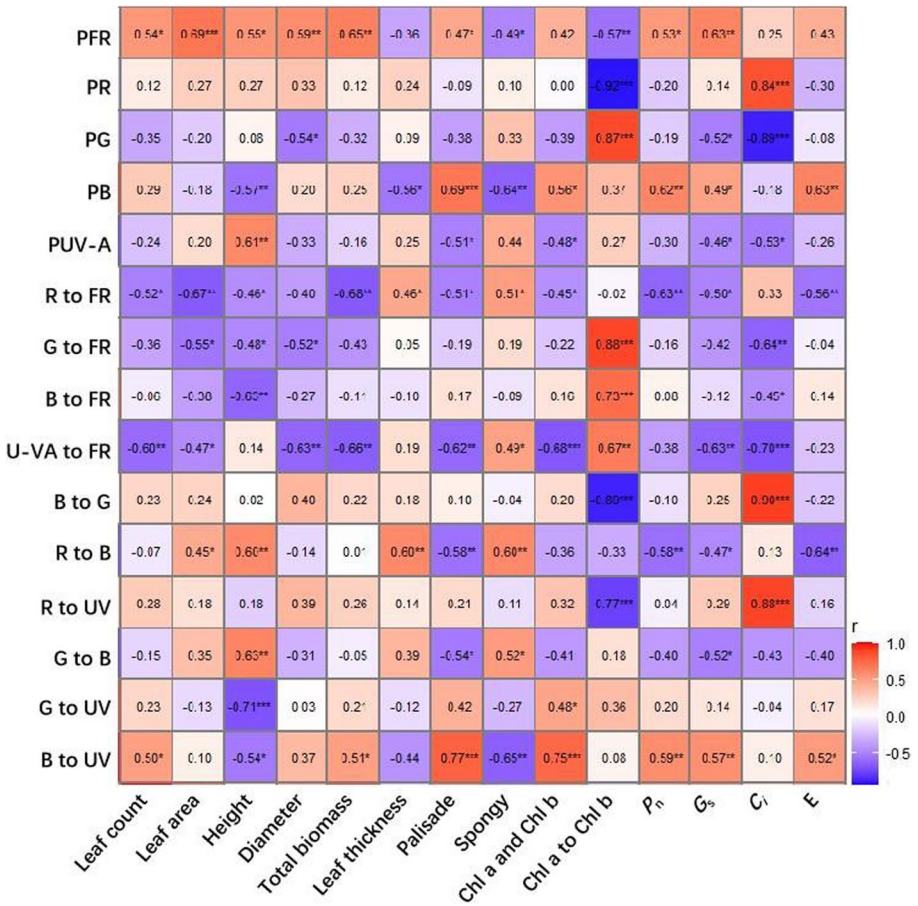

Fig. 6 Heat map of linear correlation coefficients matrix between light quality metrics and plant traits. FR: far red; R: red; G: green; B: blue; UV-A: ultraviolet-A. PFR, PR, PG, PB, PUV-A: the percentage of FR, R, G, B, and UV-A in total PPFD (photosynthetic photon flux density). Palisade: the length of palisade parenchyma cells; Spongy: the length of spongy layer; Chl: chlorophyll; P_n: net photosynthetic rate; G_s: stomatal conductance; C_i: intercellular CO_2 concentration; E: transpiration rate. The percentage of each light and the ratio between two lights were normalized with arcsine transformation. The normality of other data used for this correlation analysis was verified

2008; Morelli et al., 2000). However, the leaf expansion response to R/FR varies, ranging from inhibition to promotion, depending on growth conditions and species (Demotes-Mainard et al., 2016). For instance, leaf expansion is promoted under low R/FR when the PPFD is sufficient for growth while it is inhibited under low R/FR when the PPFD is excessively low (thus the carbon supply for leaf growth is limited). Also, the content of chlorophyll and carotenoids varies among species when far-red light increases or R/FR decreases (Tan et al., 2022a, b). A decrease in R/FR generally reduces leaf thickness (Gommers et al., 2013), and thinner shade leaves showed lower light compensation points and lower maintenance respiration rates than thicker leaves, maintaining a higher net photosynthesis under lower light (Weraduwage et al., 2015). Park et al. (2017) showed that a small increase in quantum yield with FR photons can have large direct effects on whole-plant net assimilation and considered that the inclusion of FR promoted plant growth indirectly through leaf expansion and directly through an increase in whole-plant net assimilation.

In this study, we found that the component of FR in total PPFD across 380–780 nm (within the range of 3.03%–11.86%) had a positive correlation with the growth of cotton seedlings, and the R/FR (within the range of 4.45–11.50) had a negative correlation with the growth of cotton seedlings. For example, in this study, the LED 2 had the highest FR percent and the lowest ratio of R/FR; it showed the thinnest leaves, the highest chlorophyll content and net P_n (in most leaves) as well as the strongest plant and the greatest biomass, with the second-most plant height and leaf area. These results are overall consistent with previous reports (Park et al., 2017; Tan et al.,

2022a, b), suggesting that a relatively higher FR is beneficial for the growth of cotton seedlings in many aspects.

Blue light increased palisade cell length, chlorophyll content, and photosynthetic activity but suppressed stem elongation

Blue light is less efficient for photosynthetic reactions compared with green or red photons due to its high energy not being fully utilized. Hence, a low intensity of blue light is sufficient for complete photosynthesis (Izzo et al., 2020). It has been shown that in reaction to blue light, a portion of phototropin 2 (PHOT2, a blue light photoreceptor) is translocated from the plasma membrane to the Golgi apparatus (Kong et al., 2006), which is essential for vesicle trafficking (Hawes, 2005). As a result, PHOT2 may enhance palisade cell growth by modifying auxin distribution via its interaction with ADP ribosylation factor proteins. Therefore, plants grown under blue light usually have longer leaf palisade cells than those grown under red or green light (Chang et al., 2016). In addition, blue light has been found to stimulate the production of chlorophyll and carotenoids in plants to counteract lower photosynthetic efficiency (Christie et al., 2015; Bian et al., 2015; Snowden et al., 2016). Lastly, blue light plays a crucial role in regulating the opening of stomata, the plants grown in a blue-light-rich environment usually exhibit greater G_s. This is because guard cells use photophosphorylation to produce ATP in response to blue light, which activates the plasma membrane's H^+-ATPase and causes K^+ flow into the guard cells (Doi et al., 2015).

Taken together, blue light enhances the net photosynthetic rate (Chang et al., 2016; Wang et al., 2016). In contrast to the higher photosynthetic activity, blue light generally suppresses extension growth, which is regulated both by cryptochromes and phytochrome since they affect endogenous levels of gibberellins (Zhao et al., 2007; Fantini et al., 2019). Similarly, we found positive correlations between the percentage of blue light (within the range of 5.48%–27.66%) and the length of palisade cells, the content of chlorophyll, the G_s and P_n, respectively, but a negative correlation with plant height. Thus, the HPS lamp with the lowest blue light percentage produced the tallest plants, and the LED 4 with the highest blue light percentage produced the shortest plants (19.05% shorter than that of HPS lamp).

The optimal R/B for cotton growth may vary with the participation of other lights

Blue and red light are the main contributors to photosynthesis and growth (Yeh et al., 2009). Plants develop more productively and healthily when exposed to a mixture of blue and red light as compared with just one of them (Naznin et al., 2019). In studies of artificial lighting for plant development, the ratio of R/B is frequently taken into consideration (Sobczak et al., 2021; Zheng et al., 2017; Naznin et al., 2019). Li et al. (2017) reported that cotton seedlings prefer 8.00 of R/B rather than 0.33, 1.00, and 3.00 for growth and development when only red and blue light was provided. In the present study, the HSP lamp had an R/B of 8.47 which was closer to 8.00. However, the LED 2, producing 21.8% more biomass than HSP lamp, showed an R/B of only 2.37. This discrepancy in optimum R/B for cotton seedlings growth between literature and our results may be due to the inclusion of other lights besides red and blue in this study. Whatever, there were still linear relationships observed between R/B (varying from 0.96 to 8.47) and some morphological and physiological traits in current study. For instance, when the R/B increased, the plant height, leaf area, and thickness as well as spongy layer were enhanced to some extent, but the length of palisade cell and P_n were inhibited. We suggest that the ideal R/B ratio for cotton growth and development may change when additional lights are included.

Conclusion

In the present study, the light sources [four light-emitting diodes (LEDs) and high pressure sodium (HSP) lamp] greatly affected the growth, leaf anatomy, and photosynthetic capacity of cotton seedlings. The percentage of far red (FR, 701–780 nm) in total PPFD (3.03%–11.86%) showed a significantly positive correlation with growth and photosynthesis. However, the ratio of R/FR (4.45–11.50) had a significantly negative correlation with them. Also, the blue light inhibited plant height but enhanced photosynthesis. The LED 2 with the highest percentage of FR (11.86%) and the lowest ratio of R/FR (4.45) produced the most leaves and highest biomass, which may be due to the relatively elongated palisade cell, superior chlorophyll contents, and photosynthesis rate. This LED light can be used in the growth chamber to replace HSP lamp during the study of cotton seedlings. Considering that optimal illumination is not only species-specific but also stage-specific (Rahman et al., 2021), we suggest that LED 2 may not necessarily be suitable for cotton yield formation and fiber development.

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Authors' contributions

Tian XL conceived and designed the experiments, Zhang YC and Liao BP performed the experiments, Zhang YC analyzed the data and wrote the manuscript, Tian XL and Zhang YC revised the manuscript, Li FJ and Du MW

designed the methodology and organized the manuscript. Eneji AE has done the phonetic polishing of the manuscript. All of the authors read and approved the final manuscript.

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Availability of data and materials
The datasets used during this study can be provided on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All the authors and co-authors have given their consent for publication.

Competing interests
All authors declare no competing interests.

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