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Full Length Article

Physiological Responses of Tung Tree (*Vernicia fordii*) Saplings to Different Red, White and Blue Light-Emitting Diodes

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Abstract

Tung tree (*Vernicia fordii* Hemsley) is a fast-growing heliophile with high photosynthetic efficiency. The objective of this study was to investigate the effects of different light qualities on the growth, photosynthesis, chlorophyll fluorescence parameters, leaf thickness and stomata status of tung tree saplings grown in an artificial chamber. The saplings were exposed to four light qualities with photosynthetic active irradiance at 100 μmol·m^{-2}·s^{-1}: white light-emitting diodes (LEDs) as a control (CK), red and blue (R: B=8: 2) LEDs (RB), red LEDs (R) and blue LEDs (B). The growth, chlorophyll content (Chl.), photosynthesis and physiological activity were quantified at 20 and 40 d after treatment (DAT). Compared to the control, at 40 DAT, stem length, Chl., net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), photochemical quenching coefficient (qP) and electron transfer rate (ETR) of the saplings with blue light were significantly increased by approximately 7.32, 60.17, 59.06, 194.78, 129.37, 35.42 and 56.39%, respectively (P<0.05). Red light significantly decreased leaf number and seedling height by 12 and 15%, respectively (P<0.05), and caused leaf obvious sagging. Furthermore, leaf thickness, palisade cell length, SOD activity and POD activity with red light compared to CK were significantly increased by 27.49, 18.96, 337.40 and 203.11%, respectively. The promotion and inhibition of red and blue light were relatively slow in various aspects. We conclude that tung tree adapts to growing under blue light and suggest that blue light can ensure tung tree proper growth in greenhouse environments.

Keywords: *Vernicia fordii*; LED sources; Photosynthesis; Chlorophyll fluorescence

Introduction

Tung tree (*Vernicia fordii* Hemsley) is a deciduous tree of the genus *Vernicia* (Euphorbiaceae). It is one of four major woody oil seed tree species including *Camellia oleifera*, *Juglans regia* and *Sapium sebiferum* in China (He, 2001; Hu *et al*., 2006). Tung oil from its seeds exhibits traits that are highly valued in many industrial applications including rapid drying, chemical resistance, adhesiveness and sleekness (Park *et al*., 2007; Pfister *et al*., 2008; Cao and Shockey, 2012). Additionally, tung oil is the preferred material for manufacturing environmentally friendly paint. With the decrease of crude oil and the increasing pressure of environmental protection, tung tree has become an excellent bio-energy tree and makes enormous contribution to the Chinese economy. However, genetic transformation system of tung tree has not been established yet, although tung tree regeneration by *in vitro* hypocotyl via indirect organogenesis has been reported (Lin *et al*., 2016). Light is one of the important elements for the growth of tissue culture saplings. The rapid growth of tissue culture saplings is desirable for efficient conducting tissue culture saplings experiments. To our knowledge, the saplings of tung tree are slowly growing under white light-emitting diodes, which results in poor quality of transplanted saplings in greenhouse and lower regeneration activity.

The light energy is indispensable for plant growth and photosynthesis. The use of light-emitting diode (LED) sources in greenhouse seedling cultivation has been greatly improved. The LED light source is the result of the fourth innovation in the field of lighting. It is the most advanced and practical light source in modern times. The LED light source converts electric energy into light energy and replaces sunlight to participate in photosynthesis (Bula *et al*., 1991; 1994; Wang *et al*., 2006). Many studies have indicated that different plants have their own optimum light and different lights have different effects. For example, the photosynthetic rate and water use efficiency of *C. lanceolatat* saplings under red light were significantly higher than those of other light quality when the saplings are

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growing under the low light condition (≤200 μmol·m^{-2}·s^{-1}; Wang *et al*., 2017). In addition, RB light source was better than other LED light sources for the growth of *Arabidopsis thaliana*, chrysanthemum, radish, rapeseed and cucumber (Kim, 2004; Samuolienė *et al*., 2011; Cao *et al*., 2013; Hernández, 2016; Li *et al*., 2017). Red light can promote growth and nutrient absorption of lettuce leaf buds, whereas blue light can increase content of anthocyanins and carotenoids in lettuce seedlings (Li, 2009; Shin *et al*., 2012; Wei *et al*., 2018). Therefore, studying the effects of different light qualities can be beneficial to screen the LED light source that is most suitable for the growth and can provide useful references for the cultivation and management of the cultivated plantlets, especially cultivating the light-loving plant tung tree.

Light is a major environmental factor which significantly affects leaf photosynthesis and traits limiting plant growth and survivorship, and then determines the geographical distribution of plants (Kim *et al*., 2011; Wang *et al*., 2018). In this study, we investigated the changes in growth, photosynthesis, chlorophyll fluorescence parameters, leaf thickness and stomata status of tung tree saplings, under white LEDs (CK), red and blue (R: B=8: 2) LEDs (RB), red LEDs (R) and blue LEDs (B). The aims were to understand acclimation under different light conditions and to determine the optimal light quality for cultivating tung tree saplings.

Materials and Methods

Plant Materials and Light Quality Treatments

In November 2017, ripe fruits of the tung tree were collected from the same mother tree at national conservation bank of the tung tree germplasm resources in Xiangxi Region, Hunan Province. Seeds were removed from the fruits and disinfected with 0.5% potassium permanganate solution. In the natural environment, all seeds were dried to the point that they can be stored by the air in the shade. The stored seeds were mixed with wet sand in December 2017. In early April 2018, newly germinated seeds were selected and sown into plastic containers (18 cm×18 cm×14 cm) containing a substrate containing the substrate was vermiculite, perlite, peat and loess uniformly mixed with a ratio of 1:1:1:1. A total of 72 saplings were used in the experiment. Seedling cultivation was carried out under the natural conditions at the Central South University of Forestry and Technology (28°10′N; 113°23′E) for one month, and the saplings were under the normal management. In May 5, 2018, after the second true leaf of the seedling grew, aplings were all transferred to the artificial chamber with 28.0℃ of constant temperature, 70.0% of relative humidity and 450 μmol·mol^{-1} of average CO_2 concentration. The saplings were cultured with a 12-photoperiod of light and exposed to an photosynthetic active radiation of 100 μmol·m^{-2}·s^{-1} under four LED lights: white LEDs (CK), red and blue (R: B=8: 2) LEDs (RB), red LEDs (R) and blue LEDs (B), which were named CK, RB, R and B, respectively. The value of the photosynthetic active radiation was measured by Li-6400xt (LI-COR Biosciences, Lincoln, NE, USA). All 72 saplings were randomly divided into four groups and placed in each light environment. All the experimental saplings were randomly assigned to each light treatment. The papers were used to prevent other light sources from affecting the test results. The trial lasted for 40 days in two phases. The samples were harvested and the data were collected after the seedling was transferred every 20 days.

Growth and Biomass Measurement

At 20 and 40 DAT nine saplings were randomly selected from each treatment for analysis of the growth and biomass. Nine replicates were set up for each treatment. The sapling height was measured from the main stem base to the top of the seedling by the tape and the ground diameter was measured by the Vernier Caliper at the base of the stem.

Chlorophyll Analysis

At 20 and 40 DAT the leaves of nine saplings at a similar position were respectively used to extract the chlorophyll within each treatment. The samples were respectively soaked in 10 mL ethanol-acetone mixed solution (the volume ratio is 1:1) for 24 h in 4°C darkness until the leaf turned white (Zhang, 1986). The absorbance was measured with a spectrophotometer (UV-1100 MAPADA, China) at 663 nm (OD_{663}) for chlorophyll a content (Chl-a) and at 645 nm (OD_{645}) for chlorophyll b content (Chl-b). The chlorophyll content was calculated from the following equations:

$$\text{Chl-a (mg/dm}^2\text{)} = 12.72\times OD_{663} - 2.59\times OD_{645}$$

$$\text{Chl-b (mg/dm}^2\text{)} = 22.88\times OD_{645} - 4.68\times OD_{663}$$

$$\text{Chl (a + b) (mg/dm}^2\text{)} = \text{Chl-a} + \text{Chl-b}$$

Gas Exchange Measurement

At 20 and 40 DAT, the photosynthetic parameters of the sapling leaf were measured with Li-6400xt (LI-COR Biosciences, Lincoln, NE, USA), a portable photosynthesis system between 9:00 am and 11:00 am during each measurement period (one leaf per plant; nine plants per replicate) (Xu *et al*., 2019). A transparent basal chamber with natural light was used for determination and the CO_2 concentration was 400 μmol·mol^{-1} that was supplied by the small cylinder with a flow rate of 500 μmol·s^{-1}. The light response curve was measured with the 6400-LED red-blue light source automatic light-curve system, and the photosynthetic effective radiation gradient was set as: 1800, 1500, 1200, 900, 600, 300, 200, 150, 100, 75, 50, 25 and 0 μmol·m^{-2}·s^{-1}. Nine saplings were selected from each

treatment, and leaves with the same growth and similar growth positions were selected for measurement on each sapling. There were nine replicates for each treatment that nine saplings were selected from each treatment.

Chlorophyll Fluorescence Analysis

At 20 and 40 DAT, the chlorophyll fluorescence parameters were measured with Li-6400xt (LI-COR Biosciences, Lincoln, NE, USA). Nine replicates were set up for each treatment and nine saplings were selected from each treatment for the chlorophyll fluorescence analysis. Referring to the operating manual of Li-6400xt, the leaves of saplings after a dark adaptation were given detection light and then the initial fluorescence (F_o) was determined. Then the leaves were applied a saturation pulse for 0.8 s at the photosynthetic active radiation of 7200 μmol·m^{-2}·s^{-1} to determine the maximal fluorescence (F_m) under the dark adaptation. The parameters such as photochemical quenching coefficient (*qN*), photochemical quenching coefficient (*qP*) and electron transfer rate (ETR) were measured after the activation of light in the leaf chamber for more than 30 min.

Anatomical Features of Leaves

At 20 and 40 DAT after the end of photosynthesis measurement, the test samples were excised from the leaves at a similar position for each treatment. Nine saplings were selected for each treatment. Leaf sections (0.5 cm^2: 1 cm× 0.5 cm) were taken from fully expanded leaves between the two side veins. These saplings sections were dipped in 20 mL of FAA fixative containing 70% ethanol, glacial acetic acid and formaldehyde (95:5:5, v/v/v) for half a month before the experiment. Leaf samples were dehydrated in a graded ethanol series (70, 80, 90, 95 and 100%), embedded in paraffin, sectioned to slices, mounted on glass slides and treated with a safranin and acid fast green staining procedure (Zeng *et al*., 2008). Stained sections of leaf tissues were analyzed with a Leica DMi8 inverted microscope (Leica Inc. Germany). Images were concomitantly viewed with Leica Application Suite (Version 4.12.0) on the computer and analyzed. The cross-sections of the leaves were analyzed to measure the leaf thickness and palisade mesophyll thickness.

Leaf Protein Concentration Determination

At 40 DAT, after photosynthesis measurement, fresh leaves (0.3 g) were collected at a similar position of nine in each treatment. The samples were stored in -80°C freezer before measurement. The UV Absorption Method was used to measure the protein concentration before measuring the enzyme activity. Plant leaves (0.3 g fresh weight) were ground in 8 mL phosphate buffer (0.1 mol/L, pH 7.4) at room temperature. The homogenate was centrifuged at 4000 rpm for 10 min under 20°C. The supernatant was transferred into a volumetric flask supplemented with the phosphate buffer for a constant volume of 10 mL. The supernatant was measured at 280 nm and 260 nm wavelength on a UV-spectrophotometer with phosphate buffer as the blank control.

Leaf Antioxidant Enzymes Determination

At 40 DAT, after photosynthesis measurement, fresh leaves (0.3 g) were collected at a similar position of nine in each treatment. The samples were stored in -80°C freezer before measurement.

The leaves were ground with 2.7 mL phosphate buffer (0.1 mol/L, pH 7.4) in an ice bath. First, the homogenate was centrifuged at 4°C, 3500 rpm for 10 min. The resulting supernatant was collected for determination of the activities of superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7) and catalase (CAT, EC1.11.1.6) using commercial assay kits which were purchased from Nanjing Jian Cheng Bioengineering Institute (Nanjing, China). All enzymes were detected using a spectrophotometer (UV-1100 MAPADA, China) and nine saplings were used to provide leaf tissues in each experimental treatment.

The activity of SOD was determined by measuring the inhibition rate of enzyme to $O_2^-\cdot$ produced by the xanthine morpholine with xanthine oxidase using the SOD assay kit. Each endpoint assay detected the red substances of the reaction system by absorbance at 550 nm after 40 min of reaction time at 37°C. One unit SOD activity (U) was defined as the quantity of SOD required to produce 50% inhibition of reduction of nitrite in 1 mL reaction solution by measuring the change of absorbance at 550 nm.

The POD activity was measured based on the change of absorbance at 420 nm by catalyzing H_2O_2. One unit was defined as the amount of enzyme which was used to catalyze 1 μg substrate by 1 mg substrate by 1.0 g fresh tissues in the reaction system at 37°C. POD activity was calculated as the formula according to POD assay kit.

The CAT activity was measured based on the hydrolysis reaction of hydrogen peroxide (H_2O_2) with CAT, which could be terminated by molybdenum acid (MA) to produce yellow MA-H_2O_2 complex. CAT activity was calculated by the decrease in absorbance at 405 nm due to degradation of H_2O_2. One unit was defined as the amount of enzyme that caused the decompose of 1 μmol hydrogen peroxide (H_2O_2) per second at 37°C in 1.0 g fresh tissue according to CAT assay kit (Li *et al*., 2013).

Stomatal Study

At 40 DAT after the end of photosynthesis measurement, one leaf was selected from the same position of nine saplings in each treatment for stomatal analysis. Absorbent cotton fiber was wet with water and used to wipe the lower

epidermis of the leaves. Then when the leaves were dried, a thin layer was smeared between the two side veins with transparent nail polish. After the nail polish air-dried and formed a film, the thin film was gently peeled off with a pair of tweezers. It was placed on the glass slide with a small amount of sterile water covered it surface. With a cover slip covered on, then it was made into a temporary observation slide. The slides made by the leaf epidermal fingerprint of cotton with the transparent nail polish method were observed using an optical microscope (Zeng *et al*., 2008). Slides were analyzed with a Leica DMi8 inverted microscope (Leica Inc. Germany) and images were concomitantly viewed with Leica Application Suite (Version 4.12.0) on the computer.

Statistical Analysis

Experiments were conducted as a complete randomized design with nine replications. The data were analyzed by Analysis of variance (ANOVA) followed by the Duncan's Multiple Range Test (DMRT) using the IBM SPSS Statistic software. The significance was set at the $P<0.05$ level.

Results

Plant Growth and Biomass Yield

Different LED light qualities had variable effects on the growth and biomass parameter of tung saplings (Fig. 1). There was no significant difference among sapling ground diameter at 20 and 40 DAT ($P<0.05$). However, the seedling height was decreased by 4.35 and 5.20% at 20 DAT and by 5.09 and 15.21% at 40 DAT with RB light and R light, respectively. Compared to the control, at 40 DAT, the leaf number and the sapling height increased by 2.49 and 7.32%, respectively, under the B light (Table 1). In addition, it can be found from the Fig. 1 that the sapling leaves exhibited an obvious sagging phenomenon under R light (Fig. 1Bc).

Chlorophyll Contents

Among four different LED light treatments at 20 DAT, the value of the Chl-a, the Chl-b and the total chlorophyll contents in RB light were the highest, and in the B light were lowest. Compared with the control, at 40 DAT, the Chl-a content in leaves treated with RB and B lights significantly increased 47.37 and 68.42%, respectively ($P<0.05$). The Chl-b content of RB, R and B lights were higher in contents, which were 56.67, 28.83 and 28.83% higher, than that of the control and no difference was detected among these three treatments. The total chlorophyll contents in plants treated with RB, R and B lights increased by 49.78, 18.18 and 60.17%, respectively than that of the control at 40 DAT (Table 2). Clearly, blue light was the most favorable to increase chlorophyll content of tung tree saplings.

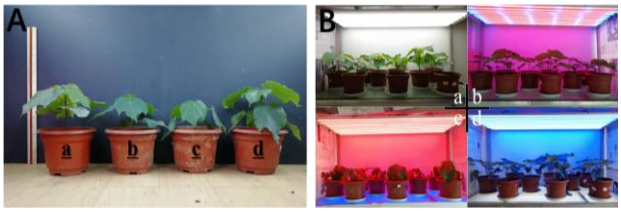

Fig. 1: The growth situation of tung tree saplings under four LED light qualities
A: Comparison of growth characteristics of tung tree saplings under four LED light qualities
B: Four processing environments
a: white LED (CK); b: red and blue (R: B=8: 2) LED; c: red LED (R); d: blue LED (B)

Gas Exchange Parameters

Different LED light qualities have variable effects on the photosynthetic parameters of tung tree saplings. At 20 DAT, the net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E) of the saplings under the B light were significantly higher by 51.08, 108.77 and 81.98% than CK, respectively ($P<0.05$). But these values with RB light and R light were significantly lower than that of control. Especially under red light, P_n, g_s and E decreased by 81.98, 86.00 and 74.30% than that of control, respectively. At 40 DAT, the values of P_n, g_s and E with the B light was also the highest than that of other light treatments. In addition, the values of P_n, g_s, C_i and E with RB light were higher than that of control group and among g_s and E were significantly increased by 71.78 and 51.38% (Fig. 2).

The Light Response Curve

The net photosynthetic rate (P_n) on the light response curves of the four treatments showed significant differences from the photosynthetic active radiation at 200 μmol·m^{-2}·s^{-1}, and P_n reached the maximum at around 1800 μmol·m^{-2}·s^{-1}. At 20 DAT, under B light, P_n was the maximum and it was significantly higher than that of the other three treatments ($P<0.05$). The value of P_n was the lowest in the R light condition, and there was no significant difference between the CK and RB light. At 40 DAT, the value of the RB light was higher than that of the CK, and there was a significant difference ($P<0.05$). Though the P_n of the B light decreased a little, it still maintained the maximum value and the R light was the lowest (Fig. 3).

Chlorophyll Fluorescence analysis

The chlorophyll fluorescence parameters were significantly different under different LED lights ($P<0.05$). With R light, initial fluorescence (F_o) in both two periods was the highest, being 16.68 and 15.59% higher than the CK, respectively. At 20 DAT, under the RB and the B light, the values of

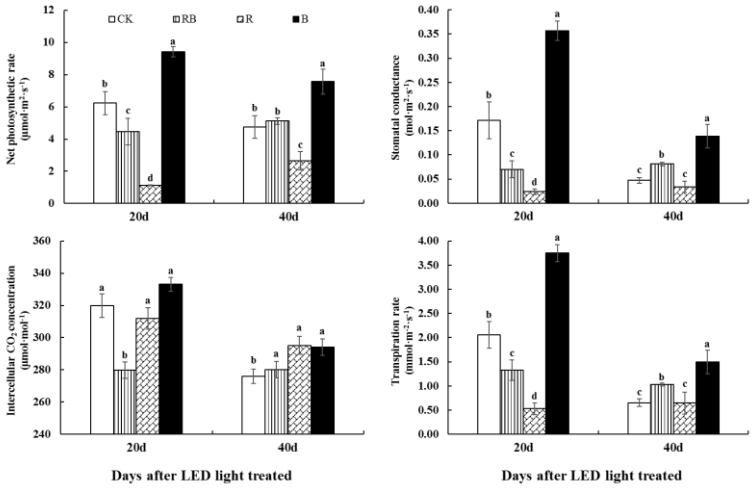

Fig. 2: Effects of different LED light qualities on net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (E) in tung tree saplings. Different lowercase letters within the column indicate significant differences at $P<0.05$ according to Duncan's test (n=9). The bars represent the standard error

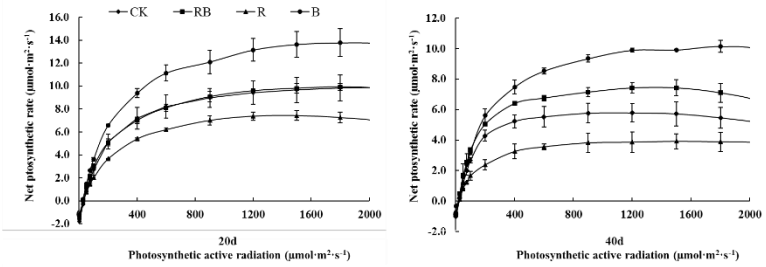

Fig. 3: Effects of different LED light qualities on the light response curve in tung tree saplings. The bars represent the standard error (n=9)

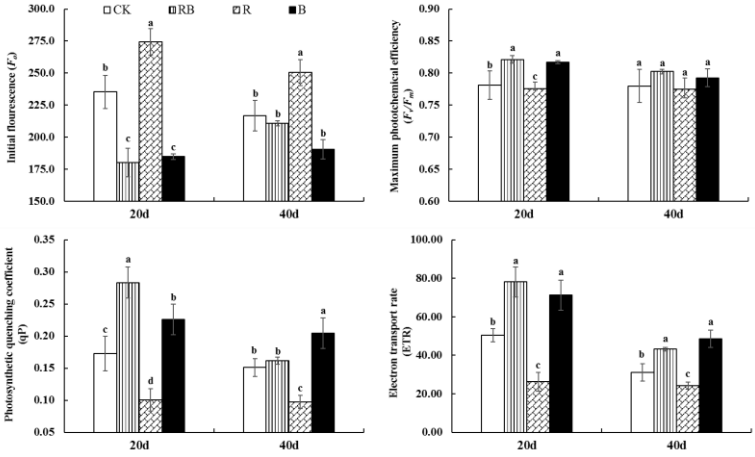

Fig. 4: Effects of different LED light qualities on initial fluorescence (F_o), maximum photochemical efficiency (F_v/F_m), photochemical quenching coefficient (qP) and electron transfer rate (ETR) in tung tree saplings. Different lowercase letters within the column indicate significant differences at $P<0.05$ according to Duncan's test (n=9). The bars represent the standard error

maximum photochemical efficiency (F_v/F_m) were significantly higher than that of the control, being 5.13 and 4.57% higher. At 20 DAT, photochemical quenching coefficient (qP) and electron transfer rate (ETR) with the B light were the highest, and increased by 64.21 and 55.04%, respectively than the CK. At 40 DAT, qP and ETR with the B light were higher than the CK by 35.42 and 56.39%, respectively. However, qP and ETR under the R light were

zTable 1: Effects of different LED light qualities on tung tree saplings growth and biomass parameter

Light treatment	Days after LED light treatment					
	20 d			40 d		
	Ground diameter (mm)	Seedling height (cm)	The leaf number	Ground diameter (mm)	Seedling height (cm)	The leaf number
CK	6.49±1.04a	15.18±3.01a	6.17±0.41b	6.61±1.00a	17.49±2.70ab	6.83±0.41a
RB (8:2)	5.35±1.01a	14.52±1.92a	6.00±0.00b	5.92±1.25a	16.60±3.30ab	6.50±0.55b
R	6.01±0.77a	14.39±1.85a	6.00±0.00b	6.09±0.78a	14.83±1.90b	6.00±0.00c
B	6.05±1.12a	15.50±3.64a	6.67±0.52a	6.38±1.21a	18.77±2.43a	7.00±0.00a

Different lowercase letters within the column indicate significant differences at $P<0.05$ according to Duncan's test (n=9)

Table 2: Effects of different LED light qualities on chlorophyll contents in tung tree saplings

Light treatment	Days after LED light treatment					
	20 d			40 d		
	Chl-a (mg/dm^{-2})	Chl-b (mg/dm^{-2})	Chl. (mg/dm^{-2})	Chl-a (mg/dm^{-2})	Chl-b (mg/dm^{-2})	Chl. (mg/dm^{-2})
CK	3.06±0.61ab	0.76±0.08b	3.82±0.69ab	1.71±0.41b	0.60±0.25b	2.31±0.47c
RB (8:2)	3.42±0.05a	1.05±0.18a	4.46±0.23a	2.52±0.33a	0.94±0.13a	3.46±0.45a
R	2.98±0.08ab	0.94±0.13ab	3.92±0.20ab	1.91±0.38b	0.82±0.13a	2.73±0.50b
B	2.62±0.22b	0.71±0.15b	3.33±0.37b	2.88±0.63a	0.82±0.27a	3.70±0.52a

Different lowercase letters within the column indicate significant differences at P<0.05 according to Duncan's test (n=9)

Table 3: Effects of different LED light qualities on leaf thickness and palisade cell length in tung tree saplings

Light treatment	Days after LED light treatment			
	20 d		40 d	
	Palisade mesophyll thickness (μm)	Leaf thickness (μm)	Palisade mesophyll thickness (μm)	Leaf thickness (μm)
CK	49.44±3.73a	105.74±2.96c	54.19±3.10a	123.70±2.65b
RB (8:2)	29.81±1.07b	134.75±4.67b	42.50±0.66c	131.43±1.87b
R	46.74±0.90a	153.36±2.75a	40.91±0.20c	134.02±1.63b
B	47.24±0.88a	141.99±8.03b	49.95±0.49b	159.36±6.35a

Different lowercase letters within the column indicate significant differences at $P<0.05$ according to Duncan's test (n=9)

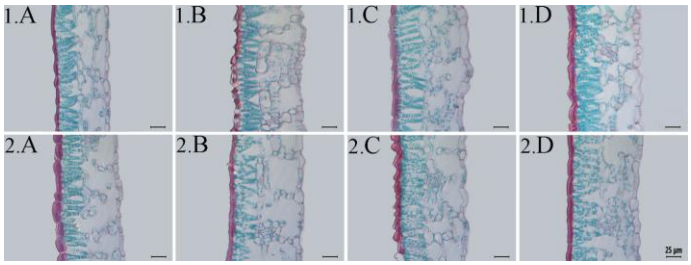

Fig. 5: Anatomical structure of tung tree seedling leaves under different light qualities at 20 and 40 DAT (40×)
1: 20 DAT; 2: 40 DAT
A: white LED (CK); B: red and blue (R: B=8: 2) LED; C: red LED (R); D: blue LED (B)

the lowest, showing a decreased by 35.67 and 21.80%, respectively than the CK (Fig. 4).

Anatomical Features of Leaves

Compared to the control, at 20 DAT, the leaf thickness under the R light was increased by 45.04%. The palisade mesophyll thickness was the smallest with the RB light. At 40 DAT, the leaf thickness with the B light was increased by 28.83%, higher than that of the control (Table 3). The palisade mesophyll thickness of the RB, R and B light condition was significantly lower than that of the CK. The leaf thickness of the B light was significantly higher than other three treatments. And the palisade mesophyll with R light at 20 DAT and with RB light at 40 DAT were loosely arranged (Fig. 5).

Antioxidant Enzyme Activity

At 40 DAT the protein concentration of CK treatment was the highest. Compared to the control, protein concentrations of RB, R and B conditions were significantly decreased by 58.87, 65.96 and 38.30%, respectively (Fig. 6A). The R light increased the activity of both SOD and POD enzymes, and this effect became more pronounced for SOD enzymes (Fig. 6B). Compared to the control, POD activity in the RB, the R and the B light were increased by 142.18, 203.11 and 64.92%, respectively (Fig. 6C). The activity of CAT under the CK treatment was the highest, and the CAT activity in RB, R and B lights were reduced by 59.17, 50.00 and 40.00% ($P<0.05$), compared to the control, respectively (Fig. 6D).

Table 4: Effects of different LED light qualities on stomata status in tung tree saplings

Light treatment	Stomatal density /per mm^2	Open stomata /per mm^2	Stomatal opening rate
CK	156.62±8.10a	93.50±5.36c	0.60±0.011d
RB (8:2)	155.45±5.36a	128.56±8.82a	0.83±0.031b
R	148.43±4.05a	105.19±6.07bc	0.71±0.022c
B	127.40±2.02b	113.37±5.36b	0.89±0.029a

Different lowercase letters within the column indicate significant differences at $P<0.05$ according to Duncan's test (n=5)

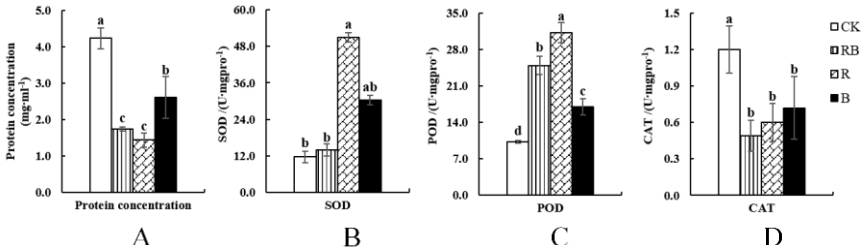

Fig. 6: Effects of different LED light qualities on the protein concentration, SOD, POD and CAT activity in tung tree saplings at 40 DAT. Different lowercase letters within the column indicate significant differences at $P<0.05$ according to Duncan's test (n=9). The bars represent the standard error

Stomata Status of Leaves

At 40 DAT the stomatal density of the leaves' lower epidermis in the CK treatment was the highest, and that of saplings under the B light was 18.66% significantly lower than that of the CK. However, the number of the opening stoma per unit area of the B light was 21.25% higher than that of the control group. The stomatal opening rate of the CK was the lowest, and the B light got the highest number, 49.0% higher than that of the CK (Table 4 and Fig. 7).

Discussion

Tung tree grows fast, blossoms and yields fruits in three years, due to the high efficiency of photosynthesis. In this experiment, we found that different LED light sources have different effects on the growth and photosynthetic indexes of tung tree saplings. Among them, photosynthesis was stimulated by the light energy and the accumulation of photosynthetic products makes plants grew fast. In our study, the B light promoted growth of saplings the most significantly at 40 DAT of cultivation. As it shows, the stem length and the leaf number of tung tree saplings with B light were higher than that of other treatments, while the R light significantly reduced the growth of saplings and appeared obvious sagging phenomenon (Table 1 and Fig. 1). These results corresponded to those of previous studies that the B light can improve the stem length and biomass accumulation in eggplant, *Rehmannia glutinosa* and red leaf lettuce (Hirai *et al*., 2005; Johkan *et al*., 2010; Manivannan *et al*., 2015). The cherry tomato seedlings under RB light are significantly stronger and shorter than those under R light condition (Liu *et al*., 2009). In a greenhouse high-wire tomato, adding some blue light is advantageous for growth and yield (Kaiser *et al*., 2018). Therefore, from the perspective of the growth, the B light can promoted growth of tung tree saplings.

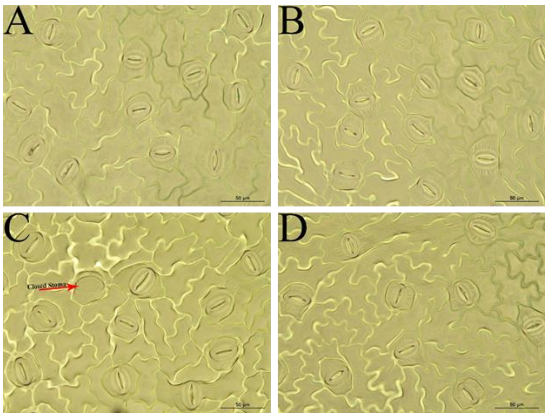

Fig. 7: The stomata status under four LED light qualities in tung tree saplings at 40 DAT
A: white LED (CK); **B**: red and blue (R: B=8: 2) LED; **C**: red LED (R); **D**: blue LED (B)

Chlorophylls are the primary light harvester pigments for photosynthesis. Chl-a and Chl-b increase light capture efficiency and enhance the net photosynthesis rate (Melkozernov and Blankenship, 2006; Kowitcharoen *et al*., 2015). In this experiment, at 20 DAT, the value of the Chl-a, the Chl-b and the Chl. with the RB light were the highest, and the B light were lowest. However, there was a significant difference in the chlorophyll content at 40 DAT. The B light significantly increased the chlorophyll content which is consistent with the previous reports on plantlets under the B light (Table 2; Poudel *et al*., 2008; Mizuno *et al*., 2009). At the same time, studies have shown that chlorophyll content and P_n were generally positively correlated. Experimental results showed that P_n under the B light was the highest at 40 DAT, and it was significantly higher than that of the control group, followed by the value under the RB (8:2) light (Fig. 2). This is consistent with the

chlorophyll content. However, P_n in R light condition was lower than that of the control group, while the chlorophyll content was opposite. From anatomical attributes, it was clear that the leaves under R light are the thickest. The main reason for leaf thickness was due to the increased number of palisade cells per unit area. An increase in the number of cells causes an increase in the number of chloroplasts and the chlorophyll contents. This means that the chlorophyll content cannot completely replace the photosynthetic rate to explain the problem.

Photosynthetic response curve reflects the changes of sapling photosynthetic rate with the increase and decrease of photosynthetically active radiation. Therefore, the measurement of photosynthetic response curve is the important means to study plants under adverse conditions (Xu, 2002; Fu *et al*., 2006). The result showed that the net photosynthetic rate in the B light condition was always higher than that of the other three treatments, while the R light was significantly lower than that of the control group (Fig. 3). The results showed that the saplings of tung tree were more efficient harvester of the blue light under different photosynthetic active radiations.

The limiting factors of photosynthesis can be classified into stomatal and non-stomatal limitations under the external environmental stress. When the C_i and g_s decrease simultaneously, the decrease of P_n is mainly caused by stomatal restriction. If the decrease of P_n is accompanied by the increase of C_i, the major limiting factor of photosynthesis is a non-stomatal factor (Farquhar and Sharkey, 1982). The P_n and g_s of the saplings under the B light were higher than that of the CK treatment whilst the values of the saplings under the R light were lower than that of the CK treatment. This indicated that photosynthetic effect on tung tree saplings was caused by stomatal limitation under different light qualities. Under the B light, the number of stomatal opening per unit area was significantly higher than that of the CK treatment. The stomatal density of the saplings under the B light was the lowest, while the value of P_n was the highest at 7.560 μmol·m^{2}·s^{-1}, and significantly higher than other three treatments (P<0.05). Therefore, stomatal restriction in different light treatments had important effects on photosynthesis of tung tree saplings.

Under the R light, an increase in F_o was mainly caused by the inactivation of the PSII partial reflection center (Maxwell and Johnson, 2000). F_o was significantly decreased and qP and ETR were significantly increased under the RB and B lights. The result with the B light and the study of rice seedlings were similar (Chen *et al*., 2014). F_o significantly increased and ETR significantly decreased under the R light, indicating that the reflection center entered a closed state under the R light stress. This appears to result in blocked electron transfer and increasing heat dissipation, thus reducing the photosynthetic rate of tung tree saplings.

The SOD and CAT are considered as the first line defense antioxidant enzymes that detoxify the reactive oxygen species and thus reduce damage to plant cells (Naz *et al*., 2019). SOD is the primary free radical scavenger in cells. The increase of SOD enzyme activity has a special effect of anti-aging. The decreased level of SOD in organism means the visual index of senescence and death. The SOD activity in the R and B light was significantly higher than that of the control group. The POD activity was generally higher in aged tissues and lower in young tissues. The POD activity in the R light was the highest, indicating that the saplings in the R light have endured a certain level of light stress. CAT is involved in photorespiration. The CAT activity of the CK was significantly higher than that of other treatments, but net photosynthetic rate was not at its maximum. A previous research on *R. glutinosa* showed that the B light significantly increased the antioxidant enzyme activities in both leaves and roots (Manivannan *et al*., 2015) which are inconsistent with the result of this present study. This means that the analysis of a single factor should be combined with all the results of a comprehensive analysis. All three enzymes were related to plant photosynthesis, and the difference of enzyme activity under different treatments further indicated that different light qualities have different effects on saplings.

Conclusion

Based on the growth, physiological and biochemical responses to different light quality treatments, we conclude that tung tree adapts to grow under blue light, promoting the growth of tung tree saplings and increasing rate of photosynthesis. Red light treatment demonstrates an initial increase in fluorescence, influence growth and photosynthesis of tung tree saplings, which was caused probably by stomatal factors. Thus, blue light condition is recommended to ensure the proper growth of tung tree in greenhouse environments.

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