Different light-quality colored films affect growth, photosynthesis, chloroplast ultrastructure, and triterpene acid accumulation in *Glechoma longituba* plants


Institute of Chinese Medicinal Materials, Nanjing Agricultural University, 210095 Nanjing, China
College of Agriculture, Henan University of Science and Technology, 471000 Luoyang, China
College of Horticulture and Plant Protection, Henan University of Science and Technology, 471000 Luoyang, China

Abstract

To elucidate the adaptive strategies of *Glechoma longituba* in response to different light-quality colored films, the growth, photosynthesis, chloroplast ultrastructure, and triterpene acid accumulation were analyzed. In this study, four colored films improved electron transport and maintained the function of PSII, and allocated more light energy absorbed for photochemical reactions, thus increasing the photosynthetic capacity and ultimately improving dry mass accumulation. Additionally, blue film (BF) and green film (GF) enhanced photosynthesis by increasing stomatal openness and chlorophyll contents and maintaining chloroplast structural integrity, thereby promoting dry mass and triterpene acid (TA) accumulation of *G. longituba*. Red film excessively increased starch grains, inhibited photosynthetic output and consequently reduced the concentration and yield of ursolic acid (UA). Yellow film decreased stomatal openness and chlorophyll concentrations, which was not conducive to chloroplast development, and also decreased the concentration and yield of UA. In conclusion, the application of BF and GF may represent an effective cultivation practice that can be used to achieve the highest TA yields in plantings of *G. longituba*.

Keywords: chlorophyll fluorescence; chloroplast structure; *Glechoma*; light-response curve; light spectrum; oleanolic acid; ursolic acid.

**Acknowledgments**: This study was funded by the project of the National Natural Science Foundation of China (41471243), the Expert Project of Variety Improvement of Traditional Chinese Medicine of Henan Province (21010439), and the Key Technologies Research and Development Program of Henan Province (212102110475, 222103810057).

**Conflict of interest**: The authors declare that they have no conflict of interest.

**Received 14 May 2022**
**Accepted 1 November 2022**
**Published online 24 November 2022**

**Corresponding author**
e-mail: hkdzlx@126.com (L.X. Zhang)
gqs@njau.edu.cn (Q.S. Guo)

**Abbreviations**: AQE – apparent quantum efficiency; BF – blue film; Car – carotenoid; Chl – chlorophyll; D – fraction of light absorbed in PSII antennae that is dissipated thermally; DM – dry mass; ETR – electron transfer rate; Ex – fraction of light absorbed in PSII antennae that is neither utilized in photosynthetic electron transport nor dissipated thermally; F₀ – minimal fluorescence yield of the dark-adapted state; F₀' – minimal fluorescence yield of the light-adapted state; FM – fresh mass; Fₘ – maximal fluorescence yield in the dark-adapted state; Fₘ' – maximal fluorescence yield in the light-adapted state; Fᵥ/Fₘ' – the efficiency of excitation capture of open PSII center; GF – green film; LCP – light-compensation point; LSP – light-saturation point; NPQ – nonphotochemical quenching coefficient; OA – oleanolic acid; P – fraction of light absorbed in PSII antennae that is utilized in PSII photochemistry; Pₘₙ₉₅ – light-saturated net photosynthetic rate; QA – photochemical quenching coefficient; R/B – ratio of red light and blue light; Rₒ – dark respiration rate; RF – red film; R/FR – ratio of red light to far-red light; UA – ursolic acid; WF – white film; YF – yellow film; Yield – quantum photochemical yield; Φₚₛₛ – effective quantum yield of PSII photochemistry.
Introduction

Light quality is an environmental factor that affects plant photomorphogenesis, photoperiodic response, and circadian rhythms. Light quality also has a major impact on plant growth and development, as well as primary and secondary metabolism (Arena et al. 2016, Miao et al. 2016, Esmaeilzadeh et al. 2021). Different families of photoreceptors in plants perceive and absorb light: phytochromes absorb red and far-red light (Smith 2000), phototropins, cryptochromes, and Zeitlupe family proteins all sense UV-A and blue wavelengths, and the photoreceptor UV RESISTANCE LOCUS 8 perceives UV-B wavelengths (Huché-Thélier et al. 2016). Light quality in different wavelengths can be perceived simultaneously and selectively by a range of different plant photoreceptors that induce complex crosstalk between photoreceptor-signaling pathways and overlapping sets of genes, implicating the participation of shared signaling components (Chen et al. 2004, Yan et al. 2019). For example, red light contributes to plant photomorphogenesis by inducing the conversion of phytochromes, a process that is crucial for photosynthetic apparatus development and starch accumulation, restricting the translocation of photosynthetic out of leaves. Red light also regulates the synthesis of phytochemicals, such as phenolics (Lee et al. 2014). Blue light regulates stomatal opening, chlorophyll and carotenoid biosynthesis, photomorphogenesis, as well as flavonol and anthocyanin production (Xu et al. 2014, Chen et al. 2017). Green light can also enhance carbon assimilation, promote photosynthesis and growth, increase antioxidant and aromatic compound accumulation in leaves, the latter of which has a positive effect on plant growth (Bian et al. 2018). Yellow light increases isoflavone content in soybean sprouts (Lee et al. 2007). These studies collectively indicate that different light-quality colored films can be used to manipulate plant growth, development, and metabolism.

Monochromatic light, however, cannot completely satisfy the needs of normal plant growth. For example, constant illumination with monochromatic red light may result in plants with abnormal leaf structure and anatomy, as well as lower photosynthetic rates, compared to plants provided by a combination of red and blue light or composite white light (Hogewoning et al. 2010). Plants exposed to monochromatic blue light for a long period may also be negatively affected, exhibiting impaired mesophyll conductance (Loreto et al. 2009), and a lower photosynthetic rate (Kim et al. 2004, Zhang et al. 2021). Previous studies have demonstrated that a combination of different wavelengths of monochromatic light in the visible spectrum is needed for normal plant growth, development, and photosynthesis (Wang et al. 2016). Different light-quality selective plastic films have been commonly used in field cultivation settings due to the ability to control the exposure of plants to sunlight, a strong and significant, non-monochromatic light source.

Photosynthesis involves two photosystems, which can only operate in coordination with each other for efficient photochemical reactions (Lunde et al. 2000). Chlorophyll fluorescence technology can accurately and nondestructively reflect the functional changes of the photosystems, and has been widely used in the physiological response and assessment of plant stress (Kalaji et al. 2014, 2018). Under LED-light source conditions in an artificial climate chamber, the apparent quantum efficiency (AQE) and the light-saturated net photosynthetic rate ($P_{\text{max}}$) of green onion leaves under blue light were significantly higher than those treated with other monochromatic light (Gao et al. 2020). Similarly, the leaves of sweet pepper seedlings in blue light were thicker, and the electron transport capacity, photosynthetic rate, and biomass were enhanced compared to red light (Li et al. 2020).

Glechoma longituba (Nakai) Kupr., a perennial herb in the Labiatae family (Menthaeae), can be used as a traditional herbal medicine (namely Glechomae herba) for the treatment of urolithiasis, cholelithiasis, diarrhea, as well as an antioxidant and antiseptic (Kim et al. 2011). G. longituba is a shade plant and suffers when growing under strong light, exhibiting reduced photosynthesis and growth, as well as reduced levels of TA (Zhang et al. 2015). Shading treatments can alleviate the degree of photoinhibition induced by strong light exposure and promote the accumulation of DM and bioactive metabolites (Stuefer and Huber 1998). The colored plastic films with different light-quality characteristics can not only affect photosynthesis, yield, and moisture retention but also affect the production of bioactive compounds (Miao et al. 2016). However, there are relatively few studies on the combination of growth, ultrastructure, photosynthetic and fluorescence parameters, and phytochemical production under different light-quality colored films.

In the present study, the main objective of this study was to determine the effects of different light-quality colored films on the growth, chloroplast ultrastructure, photosynthesis, yield, and health-promoting phytochemicals in G. longituba, namely ursolic acid (UA) and oleanolic acid (OA) in G. longituba. We hypothesized that (1) different light-quality colored films would improve stomatal openness, maintain chloroplast structure integrity, and enhance photosynthetic metabolism resulting in increased DM accumulation; and (2) the availability of the photosynthetic intermediates will enhance the biosynthesis and accumulation of health-promoting TA.

Materials and methods

Plant material and experimental conditions: A pot experiment was carried out at the experimental farm of the Institute of Chinese Medicinal Materials, Nanjing Agricultural University, Jiangsu Province, China. Clonal fragments of G. longituba obtained from healthy plants were planted in plastic pots filled with fertile sandy soil, and then cultivated for 20 d in an environmental chamber [1,000–1,200 μmol(photon) m$^{-2}$ s$^{-1}$, the daytime/night illumination time was 12/12 h (6:00–18:00 h), 25/18°C (day/night), and approximately 70% relative humidity]. After becoming established, healthy and homogenous plants that were derived from the cuttings were selected and enclosed in different light-quality colored films
RESPONSE OF GLECHOMA LONGITUBA TO LIGHT-QUALITY COLORED FILMS

(Weikang, Shanghai, China). Healthy, uniform plants were used in every treatment (n = 40).

The seedlings were grown in protected enclosures (L × W × H, 200 × 100 × 150 cm) that were each covered with one of five different light-quality colored plastic films composed of polyethylene: WF (white film, the control), RF (red film), YF (yellow film), BF (blue film), and GF (green film). Light radiation intensities above the plant canopies under the five different light-quality plastic films were determined at noon on a sunny day and adjusted to 36.6–38.4% of full sunlight using white nets and the films. The height between the lower edge of the different light-quality plastic films and the ground was approximately 15 cm for air circulation. Plants were irrigated daily to keep them well watered and full-strength Hoagland's solution was administered at seven-day intervals. The position of each of the plastic pots was constantly changed during the experiment to ensure that each plant received a similar level of light. These plants were kept under different light-quality colored plastic films in June until they were harvested in September. The daily photoperiod was consistent with natural sunlight during the experiment. Plant growth, chloroplast ultrastructure, pigment content, photosynthesis, chlorophyll (Chl) fluorescence, and secondary metabolism (production of TA) were analyzed after four months of growth under different light-quality treatments.

The spectral characteristics of the different light-quality colored films: The spectral composition of the radiation inside the enclosures in each light-quality treatment was determined with a Lambda 25 spectroradiometer in the wavelength range of 300–800 nm at a 2-nm interval (Perkin Elmer, Waltham, Massachusetts, US). The transmittance spectra of different light-quality colored films were indicated in Fig. 1A and the transmittance ratio of different light-quality spectra was calculated and indicated in a radar chart (Fig. 1B). The WF control transmitted the maximum UV light (300–400 nm), accounting for 14.9% of its spectral profile, which was significantly higher than the other colored films. BF and GF significantly reduced UV light, relative to the WF control, RF and YF transmitted very little UV light. The highest transmittance of blue light (400–500 nm wavelengths) occurred with the BF, accounting for 29.0% of its spectral profile, followed by WF at 20.4%, while blue light transmittance in GF, YF, and RF were lower, especially in the RF which contained almost no blue light in its spectral profile. Significant differences in the level of transmittance of green light (500–600 nm wavelengths) were also observed among the different colored films. The highest level of green light transmittance occurred with the YF, followed by WF and GF, while the spectral profile of RF contained almost no green light. Significant differences in the level of transmitted green light (500–600 nm wavelengths) were also observed among the different colored films. The ratio of red light to far-red light (R/B) transmitted by the different colored films was also significantly different (Fig. 1B). RF transmitted the highest level of far-red light (700–800 nm wavelengths), accounting for 55.6% of its spectral profile, a level of transmittance that was significantly higher than the transmittance of far-red light of the other colored films. BF and GF both had a high level of far-red light transmittance, while the lowest level was observed in RF. Notably, RF transmitted the reddest light and the least blue light. Thus RF had the highest ratio of red light to blue light (R/B) transmittance, which was significantly higher than observed in the other colored films. The R/B values under WF, RF, YF, BF, and GF were 1.06, 17.56.60, 29.73, 0.24, and 3.31, respectively. No significant difference in the R/B ratio was observed between WF, YF, BF, and GF. The ratio of red light to far-red light (R/FR) transmitted by the different colored films was also significantly different (Fig. 1B). WF had the highest R/FR ratio as it had a high transmittance of red light and the lowest transmittance of far-red light. The R/FR transmitted by YF, RF, and GF was considerably lower than WF, and BF had the lowest R/FR. In summary, WF had relatively small differences in the transmittance of different light spectrum wavelengths. The spectral profile of RF was mainly red and far-red.
light, with almost no ultraviolet, blue, or green light. The spectral profile YF was mainly far-red, red, and green light with very little blue light and almost no UV light. The spectral profile of BF was mainly far-red light, followed by blue and UV light, with a low proportion of red light and green light. The spectral profile of GF was primarily composed of far-red light, accounting for 45.8% of its spectral profile, followed by green and red light, and a low proportion of UV light and blue light.

Growth analysis: When the experiment was terminated, the aerial portion of the plants in the five different light quality treatments was harvested and weighed to determine the fresh mass (FM) of the aboveground part per plant, and then oven-dried to a constant mass to record DM. The dried samples were then ground and sieved for subsequent analysis of metabolites.

Observation of stomatal traits and chloroplast ultrastructure: The nail polish imprint method was used to visualize the morphology of stomata located on the abaxial epidermis (Zhang et al. 2015). Digital images were obtained using a light microscope (Zeiss, Axio Imager A1m, Jena, Germany). Dry, nail-polish imprints of five leaves from different plants in each treatment were prepared and the number of abaxial stomata in a specific area was recorded (n = 5). Four areas, 0.156 mm² in size containing a minimum of 30 stomata in each leaf sample were selected and analyzed. The analysis was carried out three times. Stomatal density was calculated as previously described (Ceulemans et al. 1995).

Leaf samples for transmission electron microscopy were prepared according to the method of Zhang et al. (2015). In brief, samples of leaf lamina (2 × 2 mm) were fixed in 2.5% glutaraldehyde, rinsed with 0.1 M phosphate buffer solution (pH 7.4), and post-fixed in 1% osmium tetroxide. Samples were then dehydrated in a graded series of ethanol, and embedded in Epon-812 resin. Ultrathin sections were cut on a Reichert ultramicrotome (Leica, Germany), mounted on grids, and then stained with 2% uranyl acetate and lead citrate. The leaf sections were observed and photographed on a transmission electron microscope (Hitachi, H-7650, Tokyo, Japan). Leaf cross-sections were analyzed on an Axio Imager optical microscope (Zeiss, Jena, Germany).

The number of chloroplast per cell, the number of grana per chloroplast, and the number of lamella per grumna were counted in 50 selected areas of view. The length and width of chloroplasts, the proportion of starch per area of chloroplast, and the thickness of grana were also estimated in the selected views. Fifteen leaf cross-section samples from different plants were analyzed for each treatment.

Photosynthetic pigments: The concentration of total chlorophyll (Chl) and carotenoid (Car) were determined following the method of Lichtenhaller (1987). The fresh leaves were immersed in 80% acetone solution for 48 h in the dark, then the absorbance values of the supernatants were measured at 470, 646, and 663 nm using a spectrophotometer (Lambda 25, Perkin Elmer, USA).

Light-response curves were measured from 09:00 to 11:00 h on sunny days. Three intact, healthy leaves of the different plants in each treatment were randomly selected and light-response curves were measured with a portable photosynthesis meter (Li-6400, LI-COR, USA). The auto-program function was used to obtain the light-response curves, which included light settings of 1,800; 1,500; 1,200; 900, 400, 200, 160, 120, 90, 60, 30, and 0 μmol(photon) m⁻² s⁻¹, a chamber temperature of 25°C, a reference CO₂ concentration of 380 μmol mol⁻¹, a flow rate of 500 μmol s⁻¹, a minimum waiting time of 120 s, and a maximum waiting time of 150 s. All the procedures were repeated three times for each treatment but three different leaves, giving a total of nine light-response curve data for each treatment. The light-saturated net photosynthetic rate (Fm' – Fo), light-saturation point (LSP), light-compensation point (LCP), dark respiration rate (Rd), and apparent quantum efficiency (AqE) were calculated as described in previous studies (Ye 2007). The plants were watered to saturation at 18:00 h before the measurement of the light-response curves.

Chlorophyll fluorescence was determined using a pulse modulation fluorometer (FMS-2, Hansatech, UK) after the measurement of the photosynthetic parameters was completed. After dark adaptation for 2 h, the minimal fluorescence yield of the dark-adapted state (Fo) was measured by applying a low-intensity (<0.1 μmol(photon) m⁻² s⁻¹, 600 Hz) red-measuring light source, and the maximal fluorescence yield of the dark-adapted state (Fm) was determined using a saturating light pulse of 6,000 μmol(photon) m⁻² s⁻¹. Steady-state Chl fluorescence (F0) of the continuously illuminated marked leaves was measured with sunlight from 9:30 to 11:00 h on sunny days. The maximum fluorescence yield in the light-adapted state (Fm') and the minimum fluorescence yield in the light-adapted state (F0') were obtained using saturating pulses (0.8 s) of white light [8,000 μmol(photon) m⁻² s⁻¹] and far-red light [3 s, 5 μmol(photon) m⁻² s⁻¹], respectively. The efficiency of excitation capture of open PSII center (Fv'/Fm'), effective quantum yield of PSII photochemistry (ΦPSII), photochemical quenching coefficient (qP), nonphotochemical quenching coefficient (NPQ), electron transfer rate (ETR), and quantum photochemical yield (Yield) were calculated following to the formulas (van Kooten and Snel 1990): Fv'/Fm' = (F0' – Fo)/Fm', ΦPSII = (Fm' – F0')/Fm, NPQ = (Fm – F0)/Fm', ETR = ΦPSII × PPFD × 0.5 × 0.84 (PAR is photosynthetic active radiation), Yield = (Fm’ – F0’)/Fm’. The fraction of light absorbed in PSII antennae was calculated according to the equations proposed by Demmig-Adams et al. (1996): the fraction of light absorbed in PSII antennae that is utilized in PSII photochemistry (P) = Fv'/Fm' × qP, the fraction of light absorbed that is utilized in thermal dissipation (D) = 1 – Fv'/Fm', and the fraction of light absorbed that is neither utilized in photochemistry nor dissipated thermally (Ex) = Fv'/Fm' × (1 – qP).

The parameters of Chl fluorescence of 15 leaf samples from different plants were measured for each treatment, and three values were recorded for each leaf sample.
**Measurement of UA and OA:** For the analysis of TA, 0.5 g of previously dried and ground powder of leaves were immersed in 20 mL of a solution of 75% ethanol and 1% formic acid and extracted by sonication for 30 min. Supernatants obtained after centrifugation at 10,000 rpm for 10 min were filtered through a 0.45-µm millipore filter (Waters, Massachusetts, USA). Analysis of UA and OA was conducted using a reversed-phase HPLC LC-20AT (Shimadzu) equipped with an Agilent ZORBAX SB-Aq C18 column (250 × 4.6 mm, 5 µm). Sample separation was performed using a mobile phase of methanol–0.5% ammonium acetate (88:12, v/v) at a flow rate of 0.6 mL min⁻¹ and a detection wavelength of 210 nm. The concentrations of UA and OA were quantified based on standard curves generated using chromatographic grade UA and OA (Wang et al. 2008).

**Statistical analysis:** One-way analysis of variance (ANOVA) was performed on the data using SPSS 16.0 (SPSS, Chicago, USA), and the least significant difference (LSD) test was used to perform multiple comparison analyses between groups (P<0.05). The presented data represent means ± standard error (SE). A correlation analysis was also conducted using SPSS software to analyze the relationship between the accumulation of TA in *G. longituba* and other measured parameters.

**Results**

**FM and DM accumulation:** RF, YF, BF, and GF all promoted an increase in the FM and DM of the aboveground portion of *G. longituba* plants, relative to WF (Fig. 2). Only BF and GF had the greatest promoting effect on FM and DM, which was significantly higher than that in RF, YF, and WF.

**Stomatal characteristics and chloroplast ultrastructure:** As shown in Table 1, the highest level of stomatal density was observed under WF and was significantly higher than the other treatments. No significant difference in stomatal density was observed among the RF, BF, and GF treatments, however, stomatal density in the YF was significantly lower than that in the other treatments. Additionally, BF significantly increased stomatal length, stomatal width, and stomatal opening, relative to WF. RF and GF also increased stomatal length, stomatal width, and the stomatal opening, while YF decreased stomatal length and stomatal width.

Chloroplast grana lamellae in leaves of *G. longituba* under WF were neatly arranged with many thylakoids, enlarged starch grains, and a small number of osmophilic grains (Fig. 3). The chloroplast grana lamellae were also arranged neatly under RF, however, the grana thylakoids were more stacked and thicker, relative to chloroplast structure under WF. In addition, the structure of stroma lamellae was visible in the RF treatment and chloroplasts contained a huge volume of starch, relative to the WF treatment. The arrangement of chloroplast grana and stroma lamellae under YF was loose and disorderly with poorly defined grana lamellae and few starch grains. Grana lamellae were arranged neatly with a large number of stacked thylakoids in the BF treatment, and chloroplasts contained a large volume of starch grains. Chloroplast grana lamellae were neatly arranged in the GF treatment with stacked thylakoids and large starch grains along with a few osmophilic grains.

The BF treatment had the highest number of chloroplasts per cell, followed by the RF, GF, and WF treatments (Table 2), while the YF treatment exhibited the lowest number of chloroplasts per cell, which was significantly lower than that in the BF treatment. There was no significant difference in chloroplast length among all treatments. The largest chloroplast width was observed in the BF treatment, followed by the GF, RF, and YF treatments. The chloroplast length-to-width ratio was not significantly different between the different colored film treatments. The proportion of starch grains in the cross-section of chloroplasts was the highest in the RF treatment, however, the value was not significantly different from the BF treatment except WF, GF, and YF treatments. The maximum number of grana per chloroplast was observed in the BF treatment but was not significantly different from the number in the WF, RF, and GF treatments except YF treatment. BF significantly increased the number of grana lamellae in chloroplasts, relative to WF, while GF and RF had no significant effect, and the lowest number of grana lamellae in chloroplasts was observed in the YF treatment. Maximum grana thickness was observed in the BF, however, the differences between the BF, GF, RF, and WF were not significant, while the lowest value was observed in YF.

**Chl and Car contents:** The content of Chl *a*, Chl *b*, and total Chl were higher in the different colored film treatments compared with the WF treatment (Table 1).
This was especially true for the BF treatment in which the pigment contents were significantly higher than it was in other film treatments. The pigment contents were also significantly higher in RF treatment and were not significantly different in YF and GF treatments compared with WF treatment. Car content was the highest in BF treatment but not significantly different from RF treatment. The promoting effect of BF and RF on Car content was significantly higher than it was under WF, YF, and GF.

**Photosynthesis:** A differential effect on the net photosynthetic rate, based on the light-response curve, was observed in different colored plastic film treatments, ranging from high to low as follows: BF > GF > RF > YF > WF (Fig. 4). As indicated in Table 1, BF significantly increased the $P_{\text{max}}$ in $G.\ longituba$ leaves, which was significantly higher under BF and GF than that under WF. RF and YF also increased $P_{\text{max}}$, but the effect was not significant. No significant difference in the AQE was observed between the different colored film treatments. The BF treatment had the highest $R_D$, and no significant difference in $R_D$ was observed between the WF, BF, and GF treatments. The $R_D$ of RF was significantly lower than that of other treatments. The highest value of LSP was
RESPONSE OF GLECHOMA LONGITUBA TO LIGHT-QUALITY COLORED FILMS

observed in the BF treatment, and LSP in the BF and GF treatments was significantly higher than that in the WF, RF, and YF treatments. The maximum value for LCP was observed in the GF treatment. YF, BF, and GF all promoted a significant increase in LCP, while RF significantly reduced LCP in *G. longituba* leaves, relative to WF.

**Chl fluorescence parameters and energy allocation:** The maximum value of Fv'/Fm' was observed in the BF treatment, and the lowest Fv'/Fm' value was in the WF treatment (Fig. 5A). No significant differences in Fv'/Fm' values were found between the BF, GF, RF, and YF treatments, however, all four of these treatments had significantly higher Fv'/Fm' values than that of the WF treatment. The maximum value of ΦPSII was also observed under BF but was not significantly different from that under GF and RF. ΦPSII under YF was significantly lower than it was under BF, however, ΦPSII values under YF, GF, and RF were not significantly different from each other. The qP value in the RF, YF, BF, and GF treatments was significantly higher than that in the WF treatment, but the highest increase was observed under GF, although the increase among the BF, GF, and RF treatments was not significant. The qP value under YF was significantly lower than that under GF treatment, but not significantly different than under BF and RF, relative to WF. The parameter NPQ in the RF, YF, BF, and GF treatments was significantly lower than that in the WF treatment. Relative to the WF treatment, the maximum values of ETR and Yield were observed in BF, and their values in colored light-quality film treatments were significantly higher than that in WF. The P value of the BF treatment was the highest, followed by the GF, RF, YF, and WF treatments (Fig. 5B). And WF treatment exhibited the maximum value of D and Ex, followed by the YF, RF, GF, and BF treatments.

**The concentration and the yield of UA and OA:** Both GF and BF treatments enhanced the concentration of UA in *G. longituba*, while YF and RF treatments slightly decreased the concentration of UA compared with WF (Fig. 6). The highest yield of UA per plant was observed in the BF treatment and was significantly higher than that in WF, RF, YF, and GF treatments. The enhancement of UA yield in the RF and YF treatments was not statistically significant. The concentration of OA was the highest in the BF treatment, but there was no significant difference between BF, GF, and RF treatments. YF treatment had no significant effect on the concentration of OA, relative to WF treatment. The highest yield of OA per plant was observed in the BF treatment, which was significantly higher than the yield in the WF, RF, YF, and GF treatments. BF, GF, and RF treatments had significant effects on OA yield relative to WF, but no significant effect was observed for YF.

---

Table 2. Effects of light-quality colored films on chloroplast ultrastructure in leaves of *Glechoma longituba*. BF – blue film; GF – green film; RF – red film; WF – white film; YF – yellow film. Different lowercase letters indicate significant differences at *P* < 0.05 by the least significant difference (LSD). Data are represented as the means ± SE (*n* = 15).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of chloroplasts per cell</th>
<th>Chloroplast size</th>
<th>Proportion of starch per area of chloroplast section [%]</th>
<th>Number of grana per chloroplast</th>
<th>Number of lamellae per granum</th>
<th>Thickness of grana [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Length [µm]</td>
<td>Width [µm]</td>
<td>Length/width</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WF</td>
<td>5.75 ± 0.48&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.75 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.02 ± 0.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.73 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.14 ± 4.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.40 ± 1.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>RF</td>
<td>7.00 ± 0.82&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.71 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.34 ± 0.14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.55 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.29 ± 2.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.67 ± 1.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>YF</td>
<td>5.25 ± 0.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.75 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.19 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.81 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.20 ± 1.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.50 ± 1.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BF</td>
<td>7.75 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.23 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.78 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.55 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.07 ± 2.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.11 ± 0.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GF</td>
<td>6.20 ± 0.58&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.61 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.44 ± 0.66&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.59 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.33 ± 2.69&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>21.83 ± 1.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Fig. 4. Photosynthesis (*P*<sub>N</sub>)–photosynthetic photon flux density (PPFD) response curves of *Glechoma longituba* leaves under different light-quality colored films (*n* = 9). BF – blue film; GF – green film; RF – red film; WF – white film; YF – yellow film.
Discussion

In this study, a highly significant negative correlation ($r = -0.992, P = 0.001$) was observed between DM accumulation and the R/FR ratio in the different spectral profiles provided by the different colored films, indicating that the DM accumulation was greatly affected by far-red light in *G. longituba*. Far-red light was also shown to stimulate the growth of marigold and salvia bedding plants (Heo *et al.* 2002). Supplemental far-red light also significantly increased plant height, leaf area, FM, and DM of the aboveground portions of *Mesembryanthemum crystallinum* (Meng *et al.* 2022). The enhanced growth may be induced due to the level of far-red light transmitted by the different colored films because plants utilize far-red light as a major signal of shading and the proximity of potential competitors (Tegelberg *et al.* 2004). Low R/FR ratios promote the elongation of plant stems and thus provide more opportunities to obtain light energy, which would enhance plant development (Tegelberg *et al.* 2004). The effect of different red/far-red ratios on the growth and photosynthetic parameters was demonstrated in *M. crystallinum* (Meng *et al.* 2022).

In our study, BF, RF, and GF enhanced the synthesis of Chl $a$, Chl $b$, and Car, indicating that the spectral profile provided by BF, RF, and GF could enhance the ability of *G. longituba* to utilize short-wavelength (blue) and long-wavelength (red) lights, and improve its ability...
to capture light energy. In contrast, YF had no obvious promoting effect on photosynthetic pigments. Our results were consistent with a study in white birch indicating that the highest content of Chl was observed under blue light (Sæbo et al. 1995). In *Diosyma versipellis*, BF treatment promotes the content of Chl a, Chl b, and total Chl, while YF inhibits the accumulation of Chl, which is consistent with our study (He et al. 2018). However, the highest concentration of Chl in *Ginkgo biloba* leaves was observed under GF, followed by BF, while the lowest occurred under WF (Leng et al. 2002). In *Arabidopsis thaliana*, there was no significant difference in the content of Chl a and Chl b between Col-0 and C24 under blue light (BL; 450 nm), red light (RL; 650 nm) and amber light (AL; 595 nm) treatments, while Chl a content significantly increased in *A. thaliana* accession Est-1 under red light (Yavari et al. 2021); the different results in different plant materials indicated that the accumulation of photosynthetic pigments was also related to the spectral components of the treatments and the genotype of the plant materials. The Chl a/b value of plants often decreases when the proportion of red light decreases and the proportion of blue light increases (Xie et al. 2017). In this study, the proportion of red light was lower and the proportion of blue light was higher under BF and GF, which may be the reason for the lower Chl a/b under these two treatments. Generally, plants with higher LSP and LCP can perform photosynthesis well under strong light (Xu et al. 2021). In this study, the contents of Chl and Car under BF and GF with the higher LSP and LCP were higher than those under WF, which showed that G. longituba had a better acclimation to a higher light under BF and GF, thereby showing higher *P* _{Nmax}.

Previous studies have demonstrated that the size and distribution of stomata on plant leaves are closely related to the growth of plants, with vigorously growing plants having larger but fewer stomata (Paek and Hahn 2000). In the present study, the length and width of stomata in the lower epidermis of *G. longituba* leaves were significantly greater in BF treatment, relative to the other treatments. Stomatal density, however, was lower under BF than that under WF, GF, and RF. These results are consistent with the results of Li et al. (2010). Our results indicate that blue light significantly enhances the size of stomata, as well as the size of the stomatal openness, suggesting that stomatal morphology may be one of the factors contributing to the higher photosynthetic rate observed under BF.

Chloroplast ultrastructure is closely associated with photosynthetic function, and light quality affects both the structure and function of chloroplasts. The ultrastructure of chloroplasts and the photosynthetic capacity of leaves were differentially affected by the different light-quality colored films. BF enhanced the formation of the chloroplast stroma layer, the number of chloroplast grana, as well as the number of grana lamella to a certain extent. These results are in agreement with the reported effect of that blue light on chloroplasts of leaves in potato plantlets *in vitro* (Sæbo et al. 1995), cucumber seedlings (Miao et al. 2019), and apple seedlings (Li et al. 2021), which exhibited a greater number of grana and a fewer number of starch grains compared to chloroplasts in plants exposed to red light, suggesting that blue light is essential for normal chloroplast development. The persistence of starch accumulation in chloroplasts reflects that transport is inhibited and will damage the structure and function of chloroplasts (Paul and Foyer 2001). More starch grains in chloroplast squeezed severely the spatial distribution of grana and stroma lamella under RF, so the number of grana and lamella decreased, which led to its reduced photosynthetic function compared to that under BF. These results are similar to the effects of blue light and red light treatments on the development of chloroplasts in cucumber (Wang et al. 2015). Similar to BF, the proportion and volume of starch grains under GF were also distinctly reduced compared to RF, indicating that the functional area (active in photosynthesis) of chloroplasts was larger, which would be conducive to the production and export of assimilates (Sæbo et al. 1995). The physiological and structural characteristics described above explain the lower *P* _{Nmax} in plants under RF, and the higher *P* _{Nmax} under BF and GF.

PSII plays an important role in maintaining light efficiency (Dąbrowski et al. 2016), and Chl fluorescence parameters can characterize the function of PSII in leaves (Kalaji et al. 2014, 2018). In our study, the light quality under BF, GF, RF, and YF was beneficial to electron transfer in PSII reaction centers, increased the ETR in the PSII reaction center, and could significantly enhance the efficiency of excitation capture of the open PSII center. These results are similar to previous studies on green onion and sweet pepper (Gao et al. 2020, Li et al. 2020). In particular, BF treatment with the highest *F* _{v}/*F* _{m} and photochemical reaction allocation ratio *P* and the lowest NPQ, D, and Ex, contributed to protecting and maintaining the photosynthetic apparatus in *G. longituba* leaves, providing the highest potential activity of PSII and utilization efficiency of light energy. In ginger, it was also found that blue and green film treatments could improve the fluorescence parameters, such as *F* _{v}/*F* _{m}, *F* _{v'}/*F* _{m'}, and Φ _{PSII} (Zhang et al. 2008). *G. longituba* leaves under GF also had a strong photosynthetic capacity, indicating that the spectral light distribution under GF had a significant positive regulatory effect on the growth of *G. longituba*, while RF also promoted to lesser extent photosynthesis in *G. longituba* leaves. In contrast, the proportion of light energy absorbed by *G. longituba* leaves under WF and YF was low, and most of the absorbed light energy was dissipated thermally but not utilized in PSII photochemistry, which may be the reason for the lower *P* _{Nmax} and DM accumulation under WF.

Light quality can also affect the secondary metabolism of plants, and the production of metabolites directly linked to photosynthesis such as terpenoids, which are synthesized in the mevalonic acid (MVA) pathway in chloroplasts (Morfopoulos et al. 2014). The dependence of terpene production on light is because light facilitates the biosynthesis of the immediate precursor, dimethylallyl diphosphate, which is produced in the methylerthritol phosphate (MEP) pathway, a part of photosynthetic metabolism. Reduction steps are involved in this process.
(Morfopoulos et al. 2014). Isoprenoid production is co-driven by the activity of isoprene synthase (Isps) and the availability of NADPH and/or ATP. The central intermediate precursor farnesyl pyrophosphate (FPP) is subsequently produced, the common precursor of triterpenoids, and the electron transport system in photosynthesis may control this process (Lichtenhaler 1999, Morfopoulos et al. 2014). The increased production of TA under BF and GF observed in our study could be attributed to the enhanced photosynthetic metabolism and availability of the photosynthetic intermediate, dimethylallyl diphosphate, which is required to initiate the mevalonic acid pathway (Pallozzi et al. 2013). The lower NPQ and the balance of photons available to PSI and PSII would also establish a more efficient electron transport between PSI and PSII, maintain the electron transport chain, and improve the electron balance between NADPH and ATP, the latter of which is closely correlated with terpenoid production (Pollastri et al. 2014). These factors could potentially explain why triterpene biosynthesis was enhanced under BF and GF, relative to the other colored film treatments.

Conclusions: This present study demonstrated that different light-quality colored films, especially blue and green plastic films could increase the photosynthetic capacity of _G. longituba_ leaves by enhancing stomatal openness, enhancing photosynthetic metabolism and availability of the photosynthetic intermediate, finally promoting DM and TA accumulation of _G. longituba_. The use of blue and green plastic films in the production process would provide a spectral profile that could enhance the growth and development of the medicinal and aromatic plant _G. longituba_ and improve the yield of UA and OA.

References


Miao L., Zhang Y. et al.: Colored light-quality selective plastic films affect anthocyanin content, enzyme activities, and the expression of flavonoid genes in strawberry (Fragaria × ananassa) fruit. – Food Chem. 207: 93-100, 2016.