Effects of different light qualities and plant growth regulators on the growth and secondary metabolites contents of L. macranthoides seedlings

Peiyin Wu
Yangtze University
Guiyuan Wang
Yangtze University
Zhengyan Cao
Yangtze University
Yanni Liu
Chongqing University of Arts and Sciences
Ning Xia
Chongqing University of Arts and Sciences
Qiyao Wang
Chongqing University of Arts and Sciences
Shuo Si
Chongqing University of Arts and Sciences
Xinlong Shen
Chongqing University of Arts and Sciences
Yingge Yao
Chongqing University of Arts and Sciences
Ning Tang
Chongqing University of Arts and Sciences
Feng Xu
Yangtze University
Zexiong Chen (chenzexiong1979@163.com)
Chongqing University of Arts and Sciences

Research Article

Keywords:

Posted Date: October 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2049205/v1

Abstract

Lonicera macranthoides, a medicinal herb mainly distributed in South China, is widely utilized in Chinese traditional medicines for its high yield and strong pharmacological properties rich in phenolic acids and flavonoids. However, the factors regulating plant growth and secondary metabolism in *L. macranthoides* are still largely unknown. In this study, the effects of different light qualities and hormone combinations on seedlings growth performance and metabolites production were investigated. The results showed that plants under red light produced greatest biomass of the shoots and roots, and also promoted plant photosynthesis as indicated by significantly greater values for Pn, Gs, Ci, and Tr, followed by white and blue LED. The tissue culture seedlings exhibited maximum plant height and stem diameter on the medium with 1.0mg/L 6-benzyladenine (BA) + 1.0mg/L Indole 3-butyric acid (IBA), while produced the highest amounts of phenolic acids and flavonoids in medium containing 2.0mg/L BA + 0.2mg/L IBA. Red light dramatically enhanced the accumulation of chlorogenic acid (CGA), ferulic acid, luteoloside, and rutin than those under other light spectra conditions on the medium with low level of auxin. However, higher levels of auxin dramatically inhibited the CGA production under red light. Moreover, blue light can promote the accumulation of chlorogenic acid and luteoloside on these two media. The results indicate that the plant growth and secondary metabolism were dramatically influenced by light quality and auxin/cytokinin combinations. Light and auxin signaling crosstalk programmed secondary metabolites accumulation. Our findings provide effective strategies aiming to enhance biomass yield and bioactive compounds production in *L. macranthoides*.

Introduction

Lonicera macranthoides Hand. Mazz, an evergreen shrub, is belonging to the Caprifoliaceae family and mainly distributed in South China. *L. macranthoides* is one of the most important resources for the Chinese herbal drug Flos Lonicerae and widely used and highly appreciated in Chinese traditional medicine (Tang et al. 2021). A variety of secondary metabolites, including phenolic acids, flavonoids, volatile oils and saponins were detected in the floral buds or flowers of *L. macranthoides*. Among them, the compounds, including chlorogenic acid, luteoloside, caffeic acid, and rutin are the key secondary metabolites in *L. macranthoides*, which have important biological and pharmacological activities, such as antibacterial, antipyretic, antioxidant, and hepatoprotective ability effects. Due to its high yield and excellent properties of thirst-quenching, heat-clearing, and detoxifying, *L. macranthoides* is incorporated within approximately one-third of traditional Chinese medicine preparations, and besides, also widely utilized in health care products, cosmetics, shampoos, beverages, flower tea, and baked goods (Li et al. 2020). Moreover, *L. macranthoides* is referred to as a “plant antibiotic”, which played a critical role in the prevention and treatment of severe infectious diseases, such as SARS coronavirus and H1N1 flu virus and had a potential effect inhibiting against the COVID-19 outbreak (Yu et al. 2020). In view of its pharmacological effects, the current demand for *L. macranthoides* is growing in the medical field. It’s an effective way to solve the problem of insufficient resources by elevation of biomass yield and secondary

metabolites accumulation. However, the factors that regulate plant growth and secondary metabolism in *L. macranthoides* are still largely unknown.

Light, including light intensity, photoperiod, light quality, is one of the important external factors regulating plant growth and the accumulation of secondary metabolites. Plants have different growth responses when exposed to different light qualities in an in vitro culture. Specific responses are triggered by light in plants depending on the wavelength applied. The blue light (400–500 nm) controls phototropism, leaf growth, stem growth and anthocyanin accumulation. In *Achillea millefolium L*, blue spectrum provided the highest dry matter accumulation, number of roots, percentage of rooting and survival (Alvarenga et al. 2015). The red light (660 nm) controls germination, chloroplast function and stem and petiole growth. Rehman et al. (2020) observed that red light significantly increased shoot and leaf biomass, plant height, number of leaves per plant, and stem diameter by increasing the Chl content and therefore promoting the highest photosynthetic capacity. Both red and far-red light influence flowering and gene expression (Carvalho and Folta 2014). Many reports have suggested that light sources directly stimulated the production of important secondary metabolites including anthocyanins, artemisinin, caffeic acid derivatives and flavonoids. Previous study showed that red LED light promotes biomass, flowering and secondary metabolites accumulation in hydroponically grown *Hypericum perforatum L* (Karimi et al. 2022). Red light treatment showed significantly higher content of chlorogenic acid and was optimum for maximum biomass accumulation and antioxidant activity in calli of *W. somnifera* (Adil et al. 2019). Blue light promotes the accumulation of phenolic acids such as chlorogenic acid in *Schisandra chinensis* callus (Szopa and Ekiert 2016). Szopa et al. (2018) also reported that phenolic acids and flavonoids in three aronia in vitro cultures, reaching maximum values under blue light. In *Rhodiola imbricata*, compared to other light conditions, the callus cultures grown under red light accumulated maximum amount of biomass and was observed maximum specific growth rate, but the callus cultures exposed to blue light accumulated maximum amount of total phenolics and flavonoids (Kapoor et al. 2018). To date, there is no available information concerning the effects of different light spectra on plant growth performance and secondary metabolism in *L. macranthoides*.

In general, plant growth regulators (PGRs) strongly regulate plant growth, cell differentiation and metabolites formation in vitro cultures. The optimal medium with appropriate concentration of auxin and cytokinin is the critical determinant in controlling plantlet growth and metabolite production. Monney et al. (2016) shown that cultures maintained on MS medium supplemented with 3 mg/L BA, in combination with 0.1 mg/L IBA recorded the highest shoot induction, mean shoot length and mean number of nodes per explant. In *Allium hookeri*, application of BA (1.5 mg/L) with IBA (0.5mg/L) promoted the root initiation and growth, while no root initiation was observed in MS and MS supplemented with BA (Chauhan et al. 2015). BA and IBA also can promote shoot proliferation of Blue Honeysuckle (*Lonicera caerulea L.*) (Mihaljević et al. 2019). The effects of different PGRs on secondary metabolite production have previously been confirmed in various plants. Previous study shown that highest flavonoid of *Gynura procumbens* production was obtained in combination treatment of MS medium supplemented with BA 8 mg/L and IAA 2 mg/L (Pramita et al. 2018). Shoots of *Lamium album* cultivated on MS medium supplemented with BA from 0.6 up to 0.8 mg/L and IBA in concentration of 0.9 mg/L showed enhanced

content of total phenols and flavonoids (Dimitrova et al. 2011). It indicates the greatest plant growth rate and secondary metabolites production involves a balance between auxin and cytokinin contents. However, the optimal concentration and ratio of auxin and cytokinin in culture medium for the maximum production of seedling biomass and metabolites in *L. macranthoides* are still a mystery.

In this study, we determine investigate the significance of light with different wavelengths, including red, blue, green, yellow, white, purple and darkness, as well as different hormone compose and densities on plant growth, photosynthesis, and secondary metabolites accumulation, especially the key medicinal components in *L. macranthoides*. The ultimate objective was to propose lighting conditions and hormone combinations for increased biomass yield and accumulation of these bioactive metabolites in *L. macranthoides*.

Materials And Methods

Plant materials and treatments

The tissue culture (TC) seedlings of *Lonicera macranthoides* Hand.-Mazz (cv Yulei No.1) were used in this study, grown in a phytotron in Chongqing University of Arts and Sciences (Chongqing, China). For the light quality treatments, rooted TC seedlings were transplanted into holed plates and cultured in a climate chamber under controlled conditions (12/12h light/dark photoperiod at 25/20 ^{o}C). After 5 days, the seedlings were transferred to the culture shelves with different light conditions. The seedlings were irradiated using LED light source with different spectral structures, including white (W, 390-760nm), yellow (Y, 590 ± 5nm), green (G, 525 ± 5nm), red (R, 660 ± 5nm), blue (B, 450 ± 5nm), black (D), purple (P, 380 ± 5nm). Each light treatment employed 3 replicates. After 30 days of treatment, seedlings growth parameters were determined.

For the plant hormones treatments, uniform tissue culture plantlets (3 to 5cm) were used as materials and inoculated on 8 types of culture medium with different hormone combinations, which includes T1(0.2mg/L 6-BA + 0.2 mg/L IBA), T2(1.0mg/L 6-BA + 0.2 mg/L IBA), T3(2.0mg/L 6-BA + 0.2 mg/L IBA), T4 (4.0mg/L 6-BA + 0.2 mg/L IBA), T5(1.0 mg/L 6-BA + 1.0 mg/L IBA), T6(2.0mg/L 6-BA + 2.0 mg/L IBA), T7(4.0mg/L 6-BA + 4.0mg/L IBA), and T8(2.0mg/L 6-BA + 6.0 mg/L IBA). Three replicates were performed in each hormone treatment. Five bottles of TC plantlet with 10 ~ 12 plants were taken as one replicate. After three consecutive subcultures (30 days for one subculture), plant growth parameters and the contents of secondary metabolites were determined. Then, the optimal hormone combinations for plant growth and the accumulation of secondary metabolites were screened respectively.

Finally, in order to evaluate the effects of the crosstalk between hormone and light on the secondary metabolism, TC plantlets on different medium were subjected to culture conditions under seven different light qualities. Seedlings were harvested and used for experimental measurement after 30 days of cultivation.

Determination of plant growth and photosynthetic parameters

For the light quality treatments, the seedling growth parameters, including the fresh weight of root, stem, leaf and the whole plant, the dry weight of the whole plant, the stem length and diameter, and the seedling vigor index was determined by using the formula, that is stem diameter/stem length*DW of whole plant. For the hormone treatments, the TC plantlet growth parameters, including seedling height, stem diameter, the dry weight of the whole seedling and callus, were determined.

Photosynthetic parameters of treated seedlings, including the net photosynthetic rate (Pn), intercellular CO2 concentration (Ci), stomatal conductance (Gs), and transpiration rate (Tr) were measured on a clear and cloudless day using an LI-6400 XT portable CO2/H2O analysis system (Li-COR Inc., Lincoln, NE). The middle portions of the uppermost fully expanded leaves were analyzed using the following conditions: molar flow of air per unit leaf area: 500 mmol l-1 m-2 s-1; effective radiation of the light source: 1000 mol m-2 s-1; and CO2 concentration: 380.0 mol mol-1. Each measurement was performed on 3 positions of the youngest fully expanded leaf and the average value was used for the analysis.

Analysis of variance (ANOVA) was used to evaluate the data for each parameter. The means were compared with Duncan s multiple range tests using the Sigmaplot 12.5 software and $P < 0.05$ was set as the threshold for significance.

Determination of the contents of secondary metabolites

To evaluate the effects of hormone and light quality on secondary metabolism, the important secondary metabolites in *L. macranthoides*, such as caffeic acid, chlorogenic acid, cinnamic acid, ferulic acid, *p*-coumaric acid, luteoloside and rutin in treated TC seedlings were determined by HPLC. Briefly, tissues were subjected to lyophilization and homogenization with a grinding miller. A total of 0.25 g powder was extracted with 20 mL of 70% (v/v) ethanol by ultrasonication for 30 min. Then the extracts were cooled to room temperature and centrifuged at 4,000 rpm for 10 min, afterward, the supernatant was filtered through a 0.22 μM microfiltration membrane for secondary metabolites analysis by HPLC.

HPLC was carried out on a Shimadzu LC-20A HPLC analyzer (Shimadzu Corporation, Kyoto, Japan), equipped with a LC-20AT pump, a SIL-20A auto sampler, a CBM-20A system controller, an SPD-M20A diode array detector (DAD) and a CTO-20A column oven. Separation was performed on a Shimadzu Shim-Pack VP-ODS C18 column (250 × 4.6 mm, 5 μm) using gradient elution. The mobile phase was as follows. A was 2% (v/v) formic acid in deionized water, while B was 2% formic acid in 80% (v/v) methanol. A linear gradient was programmed from 15% B tilled 8.00 min, followed by a 15 ~ 50% B linear gradient from 8.01 min to 25.00 min, and finally a 50% B linear gradient from 25.01 min to 40.00 min. The flow rate was 0.7 mL/min. The column and the detector were set at 35°C. A volume of 20 μL supernatant was injected and the detection wavelength was monitored at 280nm, 320nm and 360 nm. All standards (> 98%) were purchased from SIGMA (Sigma-Aldrich, St. Louis, MO, USA). The contents of secondary metabolites were analyzed in triplicate and calculated based on peak area measurements.

Statistical significance was performed with Sigmaplot 12.5 software using Duncan's new multiple range test.

Results

Growth characteristics in response to light quality

Thirty days after light treatment, the seedling growth status of *L. macranthoides* was significantly affected (Fig. 1). Red light treatment is most beneficial to the growth of seedlings. Under red light, seedlings were more vigorous than those under other types of light spectrum, while seedlings also grew well under blue and white light. When the seedlings were treated with yellow and green light, the root growth was inhibited. Under purple light, seedlings showed weak appearance. The seedlings died after 30 days in the dark (Fig. 1).

The result showed that the biomass yield, including the fresh weight (FW) of root, leaf, stem and the whole plant, and the dry weight (DW) of the whole plant, was highest under red light, followed by those under yellow, while, blue and green light in proper order. Seedlings under purple light and dark conditions possessed the lowest biomass accumulation (Fig. 2C, D, E, G, and H). With regards to stem growth, the stem lengths of the seedlings treated with red and yellow light were longer than others, while the stem diameter of seedlings under red light was largest among the seedlings treated by various light qualities (Fig. 2A and B). We observed that the seedling vigor index (VI) was dramatically influenced in response to varied light qualities. The maximum VI was recorded in seedlings treated by red light, followed by those in white, yellow and blue light, at the same time minimum was noticed in those under purple light and in dark (Fig. 2F).

Effects of light qualities on photosynthetic parameters

Since the extremely low aboveground biomass in seedlings under purple light and in dark, these samples were discarded in the following experiments. The effect of various light treatments on the photosynthetic parameters, including Pn, Gs, Ci, and Tr, in the leaves of *L. macranthoides* were observed in Fig. 3. The result showed that light qualities remarkably affected the Pn, Gs, Ci, and Tr of the seedlings. Under red light, the seedlings displayed the greatest value of Pn [5.5567 μmol(CO_2) m^{-2} s^{-1}], Tr [0.1407 μmol(CO_2) m^{-2} s^{-1}], Gs [3.2227e^{-3} μmol(CO2) m^{-2} s^{-1}] and Ci [320.6337 μmol(CO2) m^{-2} s^{-1}], followed by those traits of seedlings under blue, yellow and green light treatments. While, minimums for Pn [2.7133μmol (CO_2) m^{-2} s^{-1}], Tr [0.0353 μmol(CO_2) m^{-2} s^{-1}], Gs [6.7603e^{-4} μmol(CO_2) m^{-2} s^{-1}] and Ci [223.8772 μmol (CO_2) m^{-2} s^{-1}] were noted in seedlings under the while light treatment (Fig. 3). Red light promoted plant photosynthesis as indicated by the maximum values for leaves Pn, Gs, Ci, and Tr.

Effects of different culture media on the growth index of L. macranthoides

The effects of plant hormone, including the concentrations of cytokinin and auxin and the ratio of cytokinin to auxin, on the growth rate of TC plantlets were shown in Fig. 4. When the plantlets were cultured in the medium with low level of auxin (0.2 mg/L IBA), the growth rate displayed the tendency rising up at the beginning and declining in late following with the increase of the cytokinin content in the medium (from T1 to T4). And the plantlets cultured in the medium with 1.0 mg/L 6BA (T2) showed the maximum biomass production including plant height, stem diameter, the dry weight of the whole plantlet and callus (Fig. 4A, B and C). When the ratio of cytokinin to auxin was 1 in the medium, the plantlets growth in the medium with 1.0mg/L 6BA and 1.0mg/L IBA (T5) were greater than those cultured in media with higher levels of 6BA and IBA (Fig. 4A, B and C). In addition, the callus growth presented the tendency rising up with the increase of the auxin content in the medium (from T5 to T8). On the whole, No. 5 medium is considered the optimal medium for TC seedling growth.

Effects of different culture media on secondary metabolites accumulation of L. macranthoides

The results showed that the substrates with different concentrations of cytokinin and auxin can dramatically influence the accumulation of key secondary metabolites in TC seedlings of *L.macranthoides* (Fig. 5). When the plantlets were cultured in the substrates with low level of auxin (0.2 mg/L IBA), the contents of four compounds, such as caffeic acid, ferulic acid, luteoloside and rutin, displayed an upward tendency with the increasing of the cytokinin content in the medium (from T1 to T4) (Fig. 5A, D, E and F). When the concentration of 6BA in the medium at a certain level, the contents of these compounds, except rutin, in seedings cultured in the medium with low level of IBA were significantly higher than those in the medium with high level of IBA, such as T2 vs T5, T3 vs T6 and T4 vs T7 (Fig. 5A, D and E). For cinnamic acid, no obvious was found in its content among the eight treatments (Fig. 5C). With regard to chlorogenic acid (CGA), the key metabolite and active ingredient in *L.macranthoides*, showed the greatest accumulation in the plantlets grown in the medium supplemented with 2.0mg/L 6-BA and 0.2 mg/L IBA (T3) (Fig. 5B). Accordingly, the total amount of phenolic acids presented the same trend as CGA contents. In addition, the production of *p*-coumaric acid, the precursor of CGA, was remarkably larger in seedlings cultured in No.3 medium (T3) than those in other seven media (Fig. 5G). Above all, No. 3 medium is considered the optimal medium for the accumulation of secondary metabolites in TC seedlings.

Effects of different hormone levels and light qualities on the accumulation of secondary metabolites

To characterize the effects of cross talk between hormone and light on secondary metabolites accumulation, seedlings cultured in T3 and T5 media under five types of light qualities were examined. T3 culture medium has lower level of auxin and higher ratio of cytokinin to auxin, while there was higher level of auxin and lower ratio of cytokinin to auxin in T5 culture medium. For the seedlings in T3 medium, three compounds, such as CGA, ferulic acid, and rutin, were found to accumulate at the highest level

under red light, that was 44.5 mg/g FW, 161.29 μg/g FW, and 606.25 μg/g FW respectively, followed by those under yellow and green light, while the lowest levels of these compounds were observed in plantlets under white light, that was 25.52 mg/g FW, 56 μg/g FW, and 296.22 μg/g FW respectively (Fig. 6A). Under green light, the seedlings accumulated the greatest amount of caffeic acid, cinnamic acid, and luteoloside, that was 252.36 μg/g FW, 161.78 μg/g FW, and 57.37 μg/g FW respectively, while lowest level of these three compounds were presented in the plantlets under white light, that was 123.12 μg/g FW, 45.53 μg/g FW, and 12.61 μg/g FW respectively, (Fig. 6A). The accumulation of *p*-coumaric acid exhibited the greatest (311.56 μg/g FW) and lowest levels (0 μg/g FW) in the seedlings under red and green light, respectively (Fig. 6A).

With regard to the seedlings in T5 medium, yellow light can promote the production of caffeic acid (174.1 μg/g FW), ferulic acid (150.31 μg/g FW), luteoloside (55.29 μg/g FW) and *p*-coumaric acid (369.53 μg/g FW), while white and blue light facilitate the accumulation of CGA, that was 57.33 mg/g FW and 56.64 mg/g FW, respectively. For cinnamic acid, no remarkable difference in its content was observed among different light qualities treatments. Green light can accelerate the production of rutin, but dramatically inhibit the accumulation of caffeic acid, CGA, ferulic acid, luteoloside, and *p*-coumaric acid. Under blue light, the yield of caffeic acid and rutin was markedly suppressed, whereas luteoloside and *p*-coumaric acid exhibited the lowest level in seedlings under blue light (Fig. 6B).

According to the integration of data, we can see that under white light, higher auxin level facilitated the production of all detected compounds expect *p*-coumaric acid. Under blue light, higher auxin can promote the yield of CGA, ferulic acid, and *p*-coumaric acid. However, under red and green light, higher auxin significantly suppressed the production of CGA, while under yellow light, there was no obvious effects of auxin concentration on the secondary metabolites' accumulation (Fig. 6). To sum up, the crosstalk between light and auxin dramatically affected the accumulation of CGA and other secondary metabolites.

Discussion

Regulation of light quality on plant growth and photosynthesis

Light, quantity and particularly quality, is a key regulator for plant growth and development processes. Among the varied light spectra, red and blue lights are the main lights absorbed by plant photo-receptors, phytochromes and cryptochromes respectively (Batista et al. 2018). The LEDs application to the plant cultivation was first reported by Bula et al. (1991), who demonstrated that the leaf shape, color, and texture of *Lactuca sativa* under red LED, supplemented with blue fluorescent lamps, were no difference from those in plants cultivated under cool white fluorescent and incandescent lamps. However, the potential application of LED light for sustainable agriculture production needs further research with more plant species. Several studies observed that red, blue light and their combination contribute to root formation and shoot elongation in *Stevia rebaudiana* (Ramírez-Mosqueda et al. 2017), shoot growth and

improved plant regeneration capacity in *Jatropha curcas* (Daud et al. 2013), shoot and root biomass in *Vaccinium corymbosum* (Hung et al. 2016). In *Arabidopsis*, red light significantly increased leaf area growth and biomass compared with those in other light qualities, including blue light, amber light and fluorescent light (Yavari et al. 2021). Kong et al. (2021) also observed that the plant height, stem diameter and shoot dry weight under blue light were less than those under red light. Similarly, in the present study, the *L.macranthoides* seedling morphogenesis and growth were significantly influenced under different types of light qualities (Figs. 1 and 2), of which, red light produced the largest plantlets and the greatest shoot and root biomass, indicating that red light favored leaf expansion, stem elongation, root formation and biomass accumulation, while yellow light significantly increased leaf growth and seedling height. This is partly consistent with previous study in *Camptotheca acuminata* seedlings (Yu et al. 2017). They found that compared to seedlings grown under white light, red light promotes plant growth as reflected by higher total biomass, shoot mass, seedling height and larger leaf area, whereas seedlings growth and yield were significantly reduced under blue and yellow light treatments. However, higher plant height was observed in seedlings cultivated in yellow and blue light compared with those in white light (Fig. 2A). These results show that the effects of spectral quality on growth traits varied according to the plant species.

Photosynthesis utilizes light energy to convert CO2 into organic compounds, and therefore is strongly modulated by light quality, especially red and blue light, the major energy sources for photosynthetic CO2 assimilation in plants (Wang et al. 2016). Previous studies demonstrated that red light promotes photosynthetic capacity in *Arabidopisis* (Yavari et al. 2021) and *C. acuminata* (Yu et al. 2017). Herein, changes in plant growth performance under varied light quality were closely correlated with photosynthesis. The Pn, Gs, Ci, and Tr of red-light treatment were the greatest but the white-light treatment was least (Fig. 3), suggesting an activation in the photosynthetic rate of plants under red light, which may due to that red wavelengths are efficiently absorbed by photosynthetic pigments. However, the effects of blue light on photosynthesis were controversial depending different crops. We found that the photosynthetic parameters, such as Pn and Ci, were dramatically higher in plantlets under red light than those under blue light, while no remarkable difference was exhibited in Gs and Tr in seedlings under red light and blue light (Fig. 3). However, in cherry tomato, with respect to those in red light, seedlings grown in blue light exhibited higher Pn and Gs (Kong et al. 2021). In contrast to red, yellow and white light, blue light reduced Pn, Gs, Ci, and Tr notably in *C. acuminata* (Yu et al. 2017). In our opinion, red light produced larger leaf area, resulted in higher light harvesting and net photosynthetic rate (Shafiq et al. 2021), whereas blue light promoted greater stomatal opening (Hogewoning et al. 2007), thereby increasing CO2 supply and consequently elevating transpiration rate (Tr). In brief, this result displayed that as an efficient light source, red light facilitates leaf energy assimilation, triggering the promotion of plant growth and photosynthesis.

Regulation of auxin and cytokinin on plantlets growth and secondary metabolites accumulation

Among the main plant hormones, auxin play fairly important roles in *in vitro* root meristem and development (Su et al. 2011). Cytokinin is one of the most important promoters for shoot proliferation growth (Pramita et al. 2018), of which, BA and IBA were proved to be more efficient in shoot multiplication and root induction, respectively in *Rumex cyprius* Murb (Al Khateeb et al. 2017). Kadhim et al. (2019) showed that when the plantlets of *Lonicera caerulea* cultivated in the medium with low level of IBA and BA, the high concentrations of BA and IBA can induce shoot branching and root formation and elongation, respectively. In this study, we observed that higher levels of cytokinin and auxin strongly inhibit plant stem elongation to decrease the dry weight, while the high concentration of auxin promote callus formation in tissue culture seedlings of *L. macranthoides* (Fig. 4). This is in accordance with the result in *Chlorophytum borivilianum* that higher concentrations of BA inhibited the shoot length (Ashraf et al. 2014). Overall, we considered that a spatio-temporal balance between auxin and cytokinin appears to be crucial for proper plantlets development in vitro.

Phytohormones are critical factors regulating plant secondary metabolism, but there is little information available on the relationship between phenolics accumulation and the levels of cytokinin and auxin (Jogawat et al. 2021). Roussos et al. (2021) noted that total phenolic compounds as well as individual phenolic ones of the apricot fruit were not influenced by cytokinin and auxin application. Similar results have been obtained after exogenous auxin application in tomato, where the phenolic compound concentration was not significantly influenced (Li et al. 2017) and in cucumber, after either auxin or cytokinin application (Qian et al. 2018). Skała et al. (2015) also reported that there was no marked difference in chlorogenic acid levels in plants of *R. carthamoides* regenerated in vitro supplemented with cytokinin and auxin and in the seed-derived plants growing in greenhouse. However, this study presented that higher concentration of cytokinin in the medium with low level of auxin enhanced the levels of caffeic acid, ferulic acid, luteoloside, rutin and *p*-coumaric acid (Fig. 5), main functional phenolic acids and biological activities in *L. macranthoides* (Liu et al. 2020). This was in line with the previous study that transgenic ipt tobacco overproducing cytokinins overaccumulates five times higher content of phenolic acids, including caffeic, chlorogenic, and p-coumaric acids during in vitro growth. They also pointed out that the higher phenolics might cause the decline in auxin content and thus break the balance of phytohormones (Schnablová et al. 2006). In addition, the ectopic expression of CKX in barley has led to the activation of the biosynthesis of flavonoids (Vojta et al. 2016). Moreover, Çakmakçı et al. (2020) reviewed that external auxin application increased several volatile organic compounds, such as nerol and geraniol. Three AUX/IAA suppressor proteins, IAA5, 6, and 19, have been shown to regulate aliphatic glucosinolate biosynthesis (Jogawat et al. 2021). The above findings indicate the crucial role of cytokinin and auxin in regulating secondary metabolism. Appropriate ratio of auxin and cytokinin was needed to obtain the largest biomass yield and secondary metabolites accumulation for in vitro plant growth.

The interaction between hormone and light on secondary metabolism

To date, numerous studies have concentrated on evaluating the effects of light quality on the concentration of phenolic compounds in plants, particularly phenolic acids and flavonoids (Bian et al.

2015). According to the previous reports, red and blue light displayed significant effects on phenolics accumulation. (Samuolienė et al. 2012) demonstrated that when red LED light was applied for 16 h before harvest, the phenolic content in red and light-green leaf lettuce was increased by 52.7 and 14.5%. In *Morinda citrifolia*, red light produced higher amounts of phenolics and flavonoids, compared with blue light (Baque et al. 2010). However, in pea sprouts, red and far-red light inhibits the phenolic accumulation (Liu et al. 2016). Zhang et al. (2019) documented that total phenolic and flavonoid content were not sensitive to red and far-red light in soybean microgreens, whereas blue light promoting effects on the accumulation of these compounds. In this study, we observed that when the plantlets grown in medium with low level of auxin (T3), red light dramatically enhanced the accumulation of several phenolics, including CGA, ferulic acid, luteoloside, rutin and *p*-coumaric acid than those under other light spectra conditions, but CGA content was strongly inhibited under red light in seedlings cultivated in medium with higher level of auxin (T5) (Fig. 6). This is consistent with the previous study on presenting that the highest overall amounts of phenolic acids were obtained under blue light in plantlets cultivated on the MS medium supplemented with 3.0 mg/L of cytokinin (BA) and 1.0 mg/L of auxin (NAA), while red light and far-red light proved to be less favorable for the accumulation of these metabolites (Szopa and Ekiert 2016). Similarly, several studies also displayed that blue light treatment enhanced the production of phenolic acids and flavonoids in various plant species, such as arugula (Taulavuori et al. 2018), *Rhodiola imbricata* (Kapoor et al. 2018) and aronias (Szopa et al. 2018). Besides, white and green light also significantly regulate the accumulation of phenolics and flavonoids. In the present study, when the plants grown in the T3 medium, white and green light significantly promoted and inhibited the production of these metabolites, respectively (Fig. 6). This was inconsistent with the previous studies that green light decreased the accumulation of phenolic compounds in soybean microgreens and lettuce (Zhang et al. 2019). It has been also reported that white lights are beneficial to the total production of phenolic acids in in vitro cultures of *Ruta graveolens* and *R. divaricate* (Gazolla et al. 2017). Therefore, the effect of light spectra on phenolic metabolite might vary among different plant species.

Based on the results, we noticed that the effect of each monochromatic light on the level of different phenolic compounds is various, and it is difficult to maximize the content of each compound under one light condition (Zhang et al. 2019).Nevertheless, monochromatic light can be used to elevate the production of targeted metabolites, for example red light for the enrichments of ferulic acid, luteoloside, rutin and p-coumaric acid, blue light for CGA production, green light for caffeic acid, cinnamic acid and rutin accumulation (Fig. 6). Moreover, light and auxin signaling cross-talk programmed secondary metabolism. Higher levels of auxin promoted the accumulation of phenolics and flavonoids under white light, while enhanced and inhibited the production of CGA, the key bioactive compound in *L. macranthoides*, under blue and red light, respectively (Fig. 6). Though light and hormone interact with each other in regulating seedling deetiolation, chlorophyll biosynthesis and lateral root development in plants, there are limited reports concerning secondary metabolism regulated by light-hormone crosstalk (Biswal and Panigrahi 2021; Khan et al. 2018). We hypothesized that light signaling components such as HY5, COP1 and PIF integrate light and hormone signaling in regulating phenolics production in *L. macranthoides*.

Conclusions

In summary, the *L.macranthoides* seedling growth was significantly influenced under different light qualities. Red light produced the highest plant height, largest plantlets and greatest shoot and root biomass. The photosynthetic parameters, including Pn, Tr, Gs and Ci were strongly elevated in seedlings of *L. macranthoides* grown under red light than those under other light qualities, indicating that red light was beneficial to the plant growth and photosynthesis. Meanwhile, a balance between auxin and cytokinin appears to be crucial for proper plantlets development and metabolites production in vitro. The tissue culture seedlings exhibited maximum yield biomass on the medium with higher level of auxin, while produced the highest amounts of bioactive compounds in medium with lower level of auxin. Blue light plays an important role in secondary metabolites biosynthesis, which can promote the accumulation of CGA and luteoloside on these two media. However, when lower auxin was applied, red light dramatically enhanced the accumulation of chlorogenic acid (CGA), ferulic acid, luteoloside, and rutin, whereas higher levels of auxin remarkably reduced the CGA production under red light. It indicates that light and auxin cross-talk regulates secondary metabolism. The underlying mechanisms remain to be further explored.

Abbreviations

PGRs
plant growth regulators
BA
6-benzyladenine
IBA
Indole 3-butyric acid
FW
fresh weight
DW
dry weight
VI
vigor index
Pn
photosynthetic rate
Ci
intercellular CO2 concentration
Gs
stomatal conductance
Tr
transpiration rate
TC

tissue culture
CGA
chlorogenic acid
ANOVA
Analysis of variance
DAD
diode array detector.

Declarations

Acknowledgements

This research was funded by the National Natural Science Foundation of China (no. 31770338, 32270401), the Projects for Innovative Research Groups of Chongqing Universities (no. CXQT21028) and the Chongqing Natural Science Foundation (no. cstc2017jcyjBX0026). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Author contributions

Conceptualization, NT, GW and ZC; Methodology, PW and ZC; Software, YL, NX and QW; Validation, NT and ZC; Formal analysis, PW and SS; Investigation, YL and NX; Resources, PW and ZC; Data curation, XS and YY; Writing-original draft, PW; Writing-review and editing, NT, GW, FX and ZC; Visualization, ZC; Supervision, ZC and FX; Funding acquisition, ZC. All authors have read and agreed to the published version of the manuscript.

References

1. Adil M, Abbasi BH, ul Haq I (2019) Red light controlled callus morphogenetic patterns and secondary metabolites production in Withania somnifera L. Biotechnology Reports 24:e00380
2. Al Khateeb W, Alu'datt M, Al Zghoul H, Kanaan R, El-Oqlah A, Lahham J (2017) Enhancement of phenolic compounds production in in vitro grown Rumex cyprius Murb. Acta physiologiae plantarum 39 (1):1–13
3. Alvarenga ICA, Pacheco FV, Silva ST, Bertolucci SKV, Pinto JEBP (2015) In vitro culture of Achillea millefolium L.: quality and intensity of light on growth and production of volatiles. Plant Cell, Tissue Organ Culture 122 (2):299–308
4. Ashraf MF, Aziz MA, Kemat N, Ismail I (2014) Effect of cytokinin types, concentrations and their interactions on in vitro shoot regeneration of Chlorophytum borivilianum Sant. & Fernandez. Electronic Journal of Biotechnology 17 (6):275–279
5. Baque M, Hahn E-J, Paek K-Y (2010) Growth, secondary metabolite production and antioxidant enzyme response of Morinda citrifolia adventitious root as affected by auxin and cytokinin. Plant Biotechnology Reports 4 (2):109–116
6. Batista DS, Felipe SHS, Silva TD, de Castro KM, Mamedes-Rodrigues TC, Miranda NA, Ríos-Ríos AM, Faria DV, Fortini EA, Chagas K (2018) Light quality in plant tissue culture: does it matter? In Vitro Cellular Developmental Biology-Plant 54 (3):195–215
7. Bian ZH, Yang QC, Liu WK (2015) Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: a review. Journal of the Science of Food Agriculture 95 (5):869–877
8. Biswal DP, Panigrahi KCS (2021) Light-and hormone-mediated development in non-flowering plants: An overview. Planta 253 (1):1–24
9. Bula RJ, Morrow RC, Tibbitts T, Barta D, Ignatius R, Martin T (1991) Light-emitting diodes as a radiation source for plants. HortScience 26 (2):203–205
10. Çakmakçı R, Mosber G, Milton AH, Alatürk F, Ali B (2020) The effect of auxin and auxin-producing bacteria on the growth, essential oil yield, and composition in medicinal and aromatic plants. Current Microbiology 77 (4):564–577
11. Carvalho SD, Folta KM (2014) Environmentally modified organisms–expanding genetic potential with light. Critical Reviews in Plant Sciences 33 (6):486–508
12. Chauhan HK, Bisht AK, Singh L (2015) Effects of phytohormones BA (6-benzylaminopurine) and IBA (Indole-3-butyric acid) on shoot and root multiplication in Allium hookeri Thw. Int J Environ Sci Technol 10:87–93
13. Daud N, Faizal A, Geelen D (2013) Adventitious rooting of Jatropha curcas L. is stimulated by phloroglucinol and by red LED light. In Vitro Cellular Developmental Biology-Plant 49 (2):183–190
14. Dimitrova M, Yordanova Z, Kapchina-Toteva V Influence of 6-benzylaminopurine and indole-3-butyric acid on in vitro propagation and secondary metabolites accumulation in Lamium album from Lozen mountain. In: I International Symposium on Medicinal, Aromatic and Nutraceutical Plants from Mountainous Areas (MAP-Mountain 2011) 955, 2011. pp 315–320

15. Gazolla AP, Curado FMLMJ, Pedroso RCN, Filho LCK, de Fátima Sales J, Rosa M, Costa AC, Pauletti PM, Januário AH, Silva FG (2017) The influence of light quality on phenolic acid and biflavonoid production in'Anacardium othonianum'Rizz. seedlings grown'in vitro'. Australian Journal of Crop Science 11 (5):528–534
16. Hogewoning S, Maljaars H, Harbinson J (2007) The acclimation of photosynthesis in cucumber leaves to different ratios of red and blue light. Photosynthesis Research 91 (2–3):287–288
17. Hung CD, Hong C-H, Kim S-K, Lee K-H, Park J-Y, Nam M-W, Choi D-H, Lee H-I (2016) LED light for in vitro and ex vitro efficient growth of economically important highbush blueberry (Vaccinium corymbosum L.). Acta Physiologiae Plantarum 38 (6):1–9
18. Jogawat A, Yadav B, Lakra N, Singh AK, Narayan OP (2021) Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: A review. Physiologia Plantarum 172 (2):1106–1132
19. Kadhim ZK, Al-Shareefi MJ, Lateef SM (2019) Effect of growth regulators on in vitro micropropagation of blue honeysuckle (Lonicera caerulea L.). Research on Crops 20 (3)
20. Kapoor S, Raghuvanshi R, Bhardwaj P, Sood H, Saxena S, Chaurasia OP (2018) Influence of light quality on growth, secondary metabolites production and antioxidant activity in callus culture of Rhodiola imbricata Edgew. Journal of Photochemistry Photobiology B: Biology 183:258–265
21. Karimi M, Ahmadi N, Ebrahimi M (2022) Red LED light promotes biomass, flowering and secondary metabolites accumulation in hydroponically grown Hypericum perforatum L.(cv. Topas). Industrial Crops Products 175:114239
22. Khan T, Abbasi BH, Khan MA (2018) The interplay between light, plant growth regulators and elicitors on growth and secondary metabolism in cell cultures of Fagonia indica. Journal of Photochemistry Photobiology B: Biology 185:153–160
23. Kong L, Wen Y, Jiao X, Liu X, Xu Z (2021) Interactive regulation of light quality and temperature on cherry tomato growth and photosynthesis. Environmental Experimental Botany 182:104326
24. Li J, Khan ZU, Tao X, Mao L, Luo Z, Ying T (2017) Effects of exogenous auxin on pigments and primary metabolite profile of postharvest tomato fruit during ripening. Scientia Horticulturae 219:90–97
25. Li RJ, Kuang XP, Wang WJ, Wan CP, Li Wx (2020) Comparison of chemical constitution and bioactivity among different parts of Lonicera japonica Thunb. Journal of the Science of Food Agriculture 100 (2):614–622
26. Liu H, Chen Y, Hu T, Zhang S, Zhang Y, Zhao T, Yu H, Kang Y (2016) The influence of light-emitting diodes on the phenolic compounds and antioxidant activities in pea sprouts. Journal of Functional Foods 25:459–465
27. Liu R-y, Deng J, Lin X-L, Li Y-m, Lin Y, Xia B-h, Lin L-m (2020) Metabolomics Reveals Distinct Metabolites between Lonicera japonica and Lonicera macranthoides Based on GC-MS. Journal of Chemistry 2020

28. Mihaljević I, Tomaš V, Vuković D, Dugalić K (2019) Propagation of three Blue Honeysuckle (Lonicera caerulea L.) cultivars in in vitro culture. Pomologia Croatica: Glasilo Hrvatskog agronomskog društva 23 (1–2):41–48
29. Monney MAD, Amissah N, Blay E (2016) Influence of BA and IBA or NAA Combinations on Micropropagation of Cryptolepis sanguinolenta. American Journal of Plant Sciences 7 (3):572–580
30. Pramita AD, Kristanti AN, Utami ESW, Manuhara YSW (2018) Production of biomass and flavonoid of Gynura procumbens (Lour.) Merr shoots culture in temporary immersion system. Journal of Genetic Engineering Biotechnology 16 (2):639–643
31. Qian C, Ren N, Wang J, Xu Q, Chen X, Qi X (2018) Effects of exogenous application of CPPU, NAA and GA4 + 7 on parthenocarpy and fruit quality in cucumber (Cucumis sativus L.). Food chemistry 243:410–413
32. Ramírez-Mosqueda MA, Iglesias-Andreu LG, Bautista-Aguilar JR (2017) The effect of light quality on growth and development of in vitro plantlet of Stevia rebaudiana Bertoni. Sugar Tech 19 (3):331–336
33. Rehman M, Fahad S, Saleem MH, Hafeez M, Rahman M, Liu F, Deng G (2020) Red light optimized physiological traits and enhanced the growth of ramie (Boehmeria nivea L.). Photosynthetica 58 (4):922–931
34. Roussos P, Ntanos E, Denaxa N, Tsafouros A, Bouali I, Nikolakakos V, Assimakopoulou A (2021) Auxin (triclopyr) and cytokinin (forchlorfenuron) differentially affect fruit physiological, organoleptic and phytochemical properties of two apricot cultivars. Acta Physiologiae Plantarum 43 (2):1–12
35. Samuolienė G, Sirtautas R, Brazaitytė A, Duchovskis P (2012) LED lighting and seasonality effects antioxidant properties of baby leaf lettuce. Food chemistry 134 (3):1494–1499
36. Schnablová R, Synková H, Vičánková A, Burketová L, Eder J, Cvikrová M (2006) Transgenic ipt tobacco overproducing cytokinins overaccumulates phenolic compounds during in vitro growth. Plant Physiology Biochemistry 44 (10):526–534
37. Shafiq I, Hussain S, Raza MA, Iqbal N, ASGHAR MA, Ali R, FAN Y-f, Mumtaz M, Shoaib M, Ansar M (2021) Crop photosynthetic response to light quality and light intensity. Journal of Integrative Agriculture 20 (1):4–23
38. Skała E, Grąbkowska R, Sitarek P, Kuźma Ł, Błauż A, Wysokińska H (2015) Rhaponticum carthamoides regeneration through direct and indirect organogenesis, molecular profiles and secondary metabolite production. Plant Cell, Tissue Organ Culture 123 (1):83–98
39. Su Y-H, Liu Y-B, Zhang X-S (2011) Auxin–cytokinin interaction regulates meristem development. Molecular plant 4 (4):616–625
40. Szopa A, Ekiert H (2016) The importance of applied light quality on the production of lignans and phenolic acids in Schisandra chinensis (Turcz.) Baill. cultures in vitro. Plant Cell, Tissue Organ Culture 127 (1):115–121
41. Szopa A, Starzec A, Ekiert H (2018) The importance of monochromatic lights in the production of phenolic acids and flavonoids in shoot cultures of Aronia melanocarpa, Aronia arbutifolia and

Aronia× prunifolia. Journal of Photochemistry Photobiology B: Biology 179:91–97

42. Tang N, Cao Z, Yang C, Ran D, Wu P, Gao H, He N, Liu G, Chen Z (2021) A R2R3-MYB transcriptional activator LmMYB15 regulates chlorogenic acid biosynthesis and phenylpropanoid metabolism in Lonicera macranthoides. Plant Science 308:110924
43. Taulavuori K, Pyysalo A, Taulavuori E, Julkunen-Tiitto R (2018) Responses of phenolic acid and flavonoid synthesis to blue and blue-violet light depends on plant species. Environmental Experimental Botany 150:183–187
44. Vojta P, Kokáš F, Husičková A, Grúz J, Bergougnoux V, Marchetti CF, Jiskrova E, Ježilová E, Mik V, Ikeda Y (2016) Whole transcriptome analysis of transgenic barley with altered cytokinin homeostasis and increased tolerance to drought stress. New Biotechnology 33 (5):676–691
45. Wang J, Lu W, Tong Y, Yang Q (2016) Leaf morphology, photosynthetic performance, chlorophyll fluorescence, stomatal development of lettuce (Lactuca sativa L.) exposed to different ratios of red light to blue light. Frontiers in plant science 7:250
46. Yavari N, Tripathi R, Wu B-S, MacPherson S, Singh J, Lefsrud M (2021) The effect of light quality on plant physiology, photosynthetic, and stress response in Arabidopsis thaliana leaves. PloS one 16 (3):e0247380
47. Yu J-w, Wang L, Bao L-d (2020) Exploring the active compounds of traditional Mongolian medicine in intervention of novel coronavirus (COVID-19) based on molecular docking method. Journal of functional foods 71:104016
48. Yu W, Liu Y, Song L, Jacobs DF, Du X, Ying Y, Shao Q, Wu J (2017) Effect of differential light quality on morphology, photosynthesis, and antioxidant enzyme activity in Camptotheca acuminata seedlings. Journal of Plant Growth Regulation 36 (1):148–160
49. Zhang X, Bian Z, Li S, Chen X, Lu C (2019) Comparative analysis of phenolic compound profiles, antioxidant capacities, and expressions of phenolic biosynthesis-related genes in soybean microgreens grown under different light spectra. Journal of agricultural food chemistry 67 (49):13577–13588

Figures

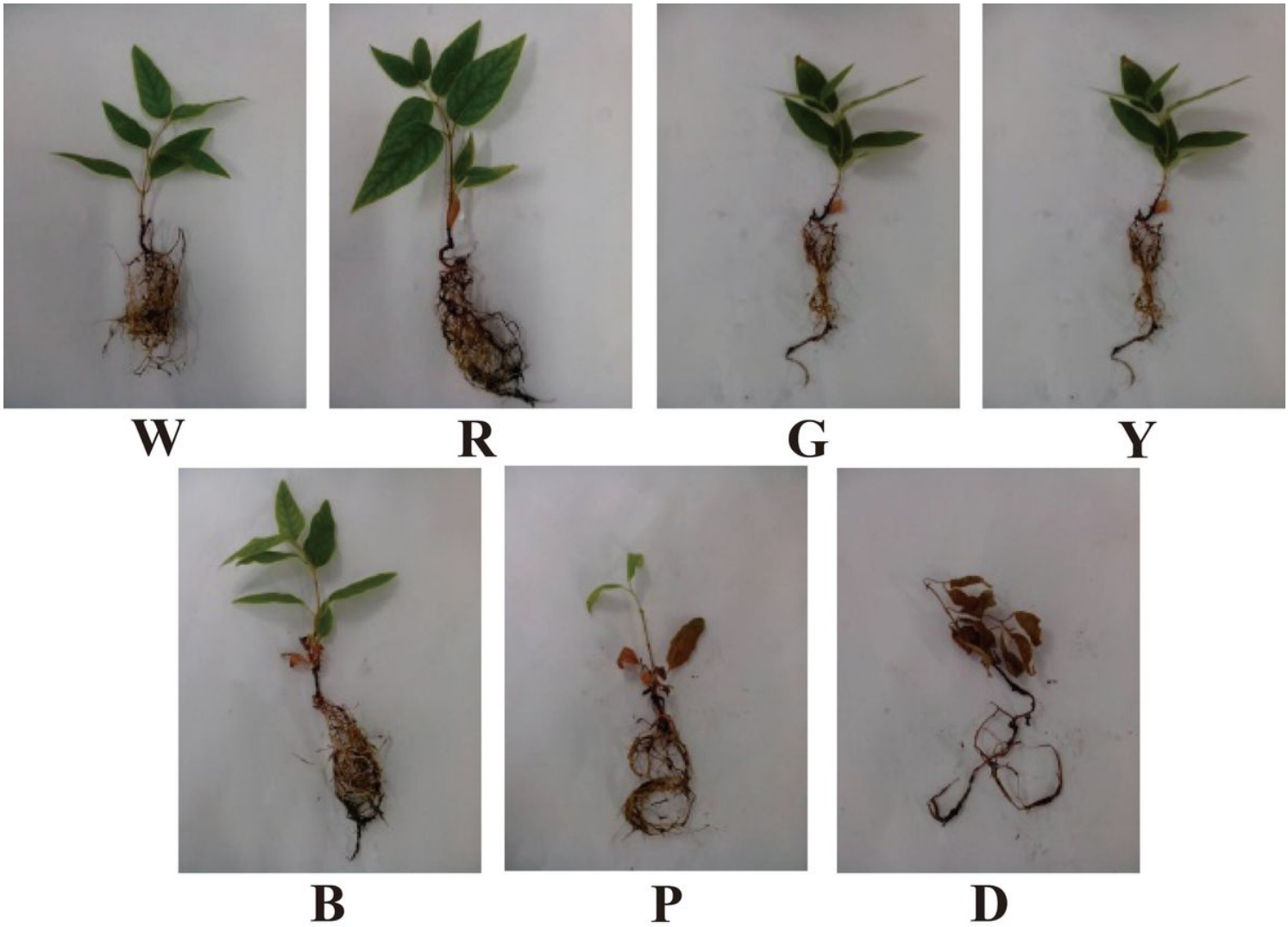

Figure 1

Effects of different light quality on plant growth performance of *L. macranthoides* seedlings.

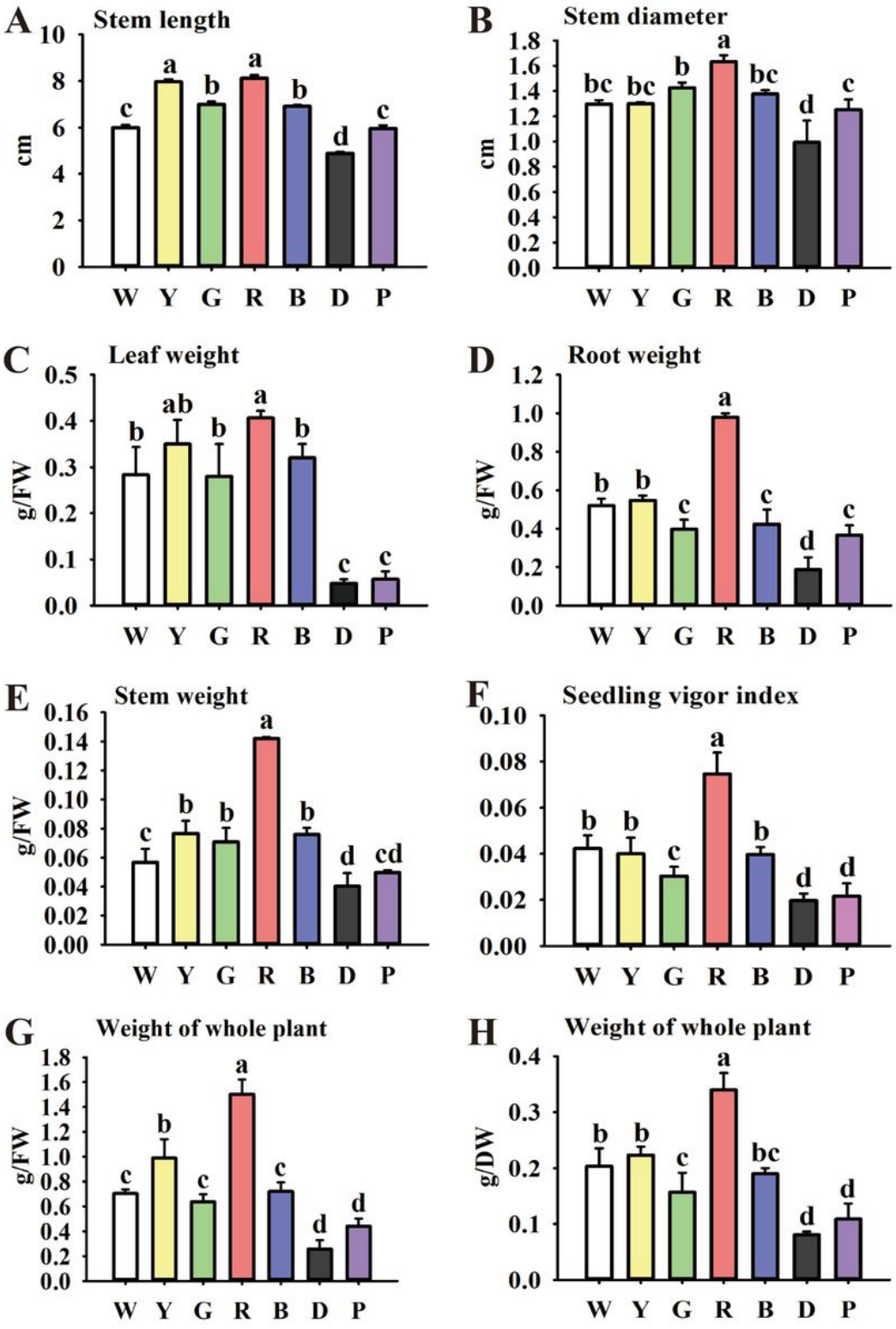

Figure 2

Effects of different light quality on plant growth index in *L. macranthoides*. (A) stem length, (B) stem diameter, (C) leaf fresh weight, (D) root fresh weight, (F) stem fresh weight, (G) seedling vigor index (H) fresh weight of whole plant, (I) dry weight of whole plant. Yellow (Y), purple (P), red (R), blue (B), green (G), and white (W) light. Error bars represent SE of three biological repetitions. Duncan's multiple range test

was used to analyze the significance, and significant differences (P < 0.05) are marked by different letters.

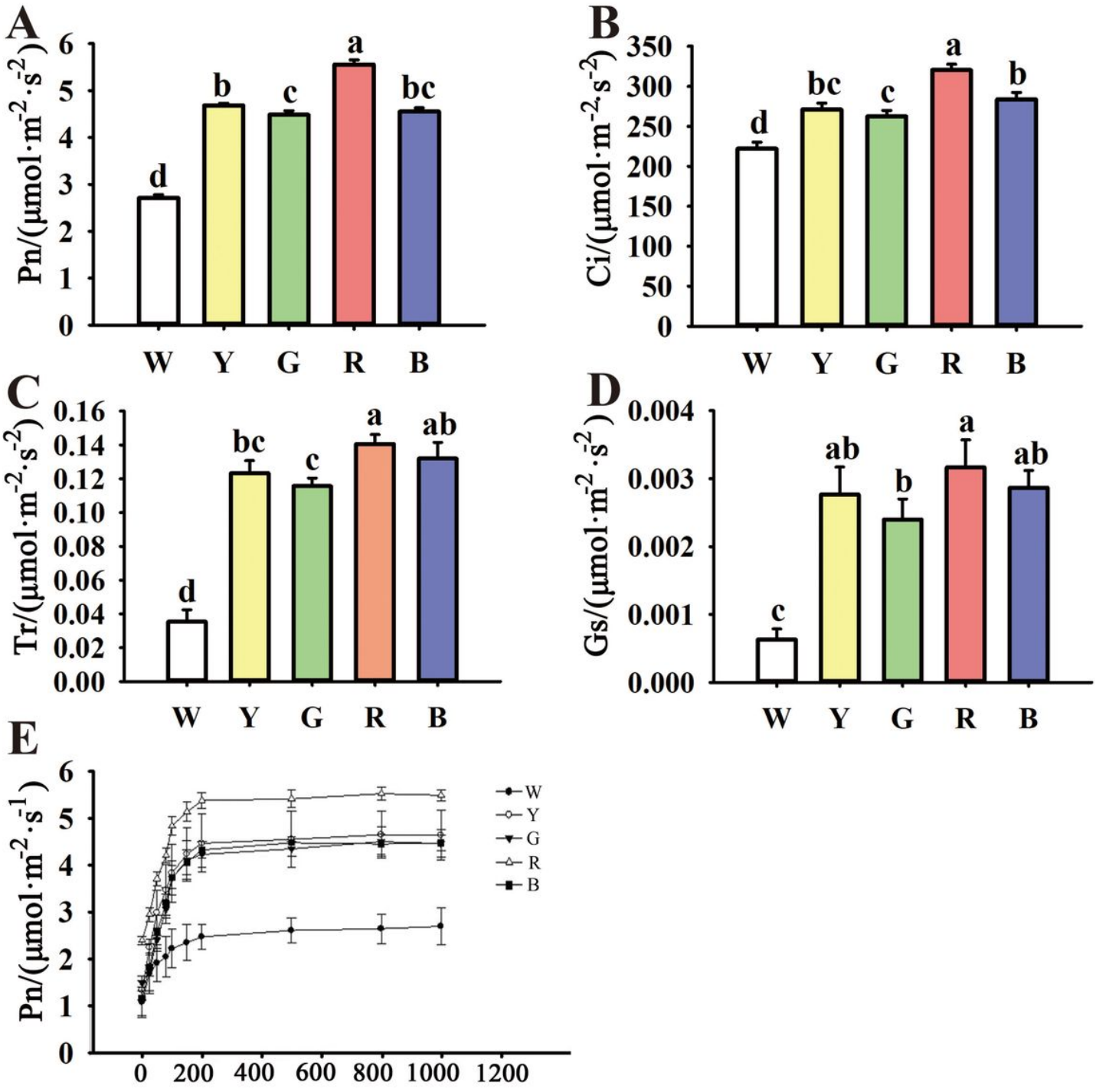

Figure 3

Effects of different light qualities on the photosynthesis of *L. macranthoides* leaves. (A) net photosynthetic rate (Pn), (B) intercellular CO2 contentration (Ci), (C) transpiration rate (Tr), (D) stomatal conductance (Gs), (E) Pn-PAR curves. Error bars represent SE of three biological repetitions. Duncan's multiple range test was used to analyze the significance, and significant differences (P < 0.05) are marked by different letters.

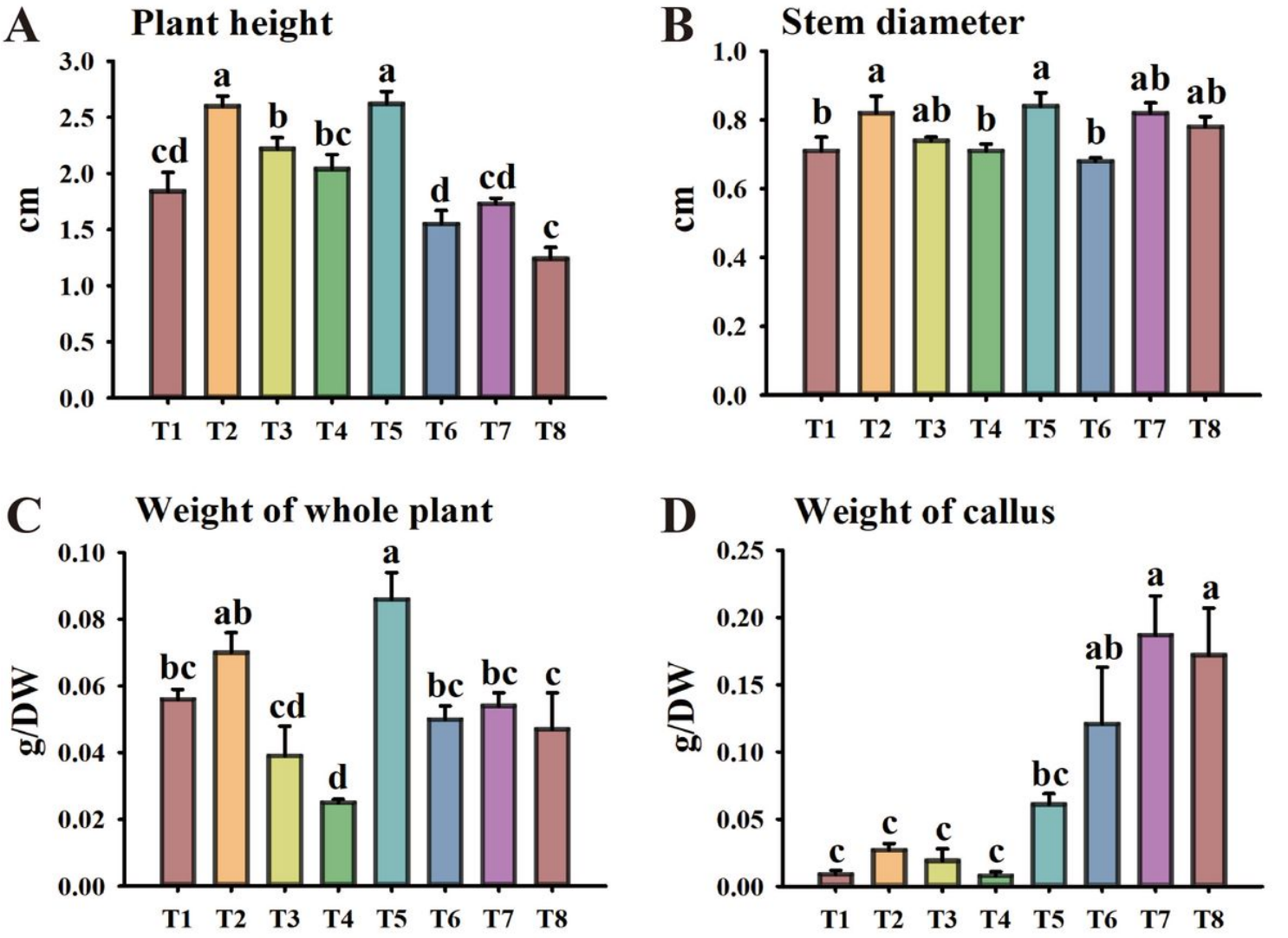

Figure 4

Effects of different culture media on the growth index of *L. macranthoides*. (A) plant height, (B) stem diameter, (C) dry matter weight, (D) dry weight of callus. 0.2 mg/L BA and 0.2 mg/L IBA (T1), 1.0 mg/L BA and 0.2 mg/L IBA (T2), 2.0 mg/L BA and 0.2 mg/L IBA (T3), 4.0 mg/L BA and 0.2 mg/L IBA (T4), 1.0 mg/L BA and 1.0 IBA mg/L (T5), 2.0 mg/L BA and 2.0 mg/L IBA (T6), 4.0 mg/L BA and 4.0 mg/L IBA (T7), 2.0 mg/L BA and 6.0 mg/L IBA (T8). Error bars represent SE of three biological repetitions. Duncan's multiple range test was used to analyze the significance, and significant differences ($P < 0.05$) are marked by different letters.

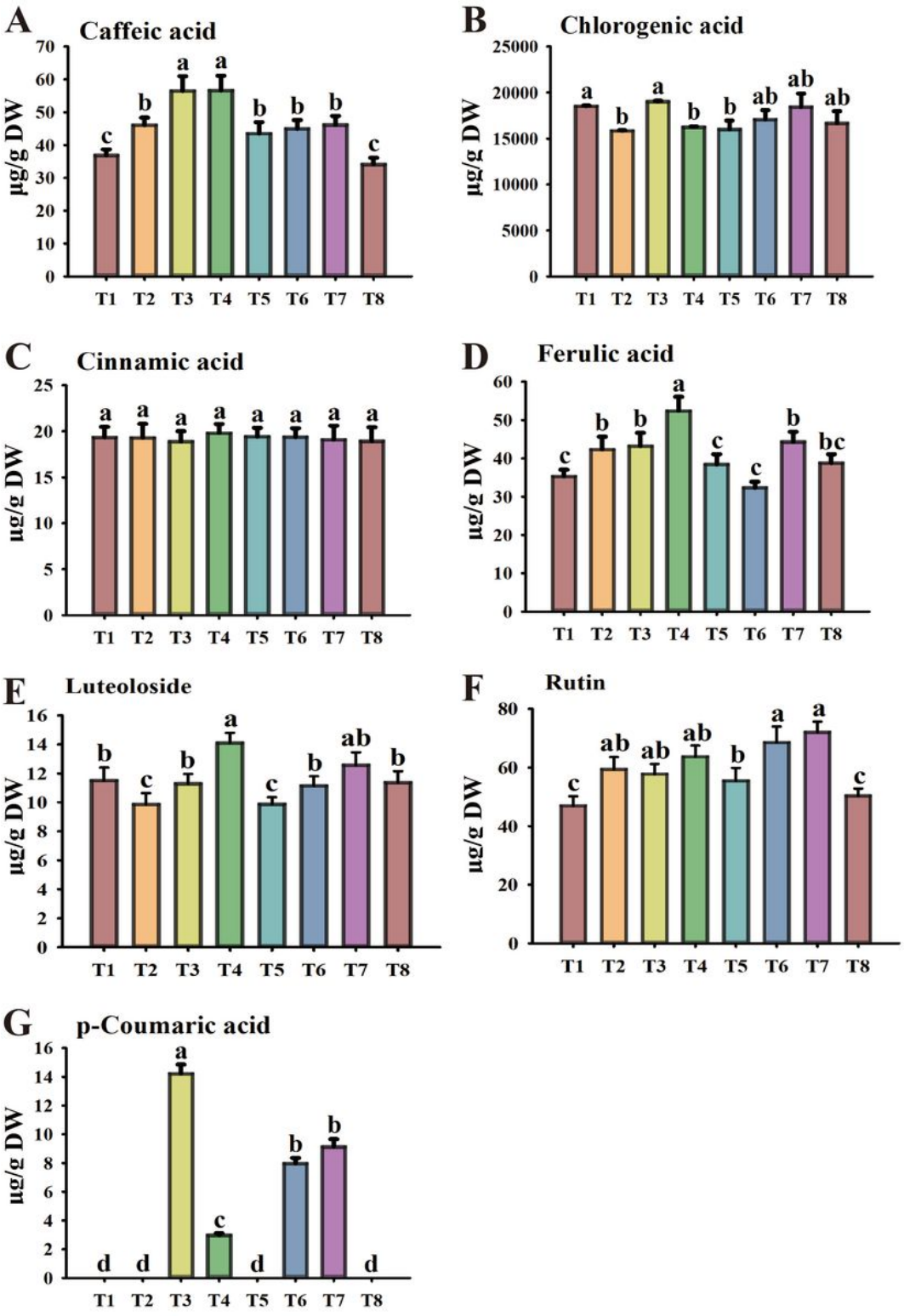

Figure 5

Effect of auxin and cytokinin concentration on secondary metabolites accumulation in *L. macranthoides*. (A) caffeic acid, (B) chlorogenic acid, (C) cinnamic acid, (D) ferulic acid, (E) luteoloside, (F) rutin, (G) *p*-coumaric acid. Error bars represent SE of three biological repetitions. Duncan's multiple range test was used to analyze the significance, and significant differences ($P < 0.05$) are marked by different letters.

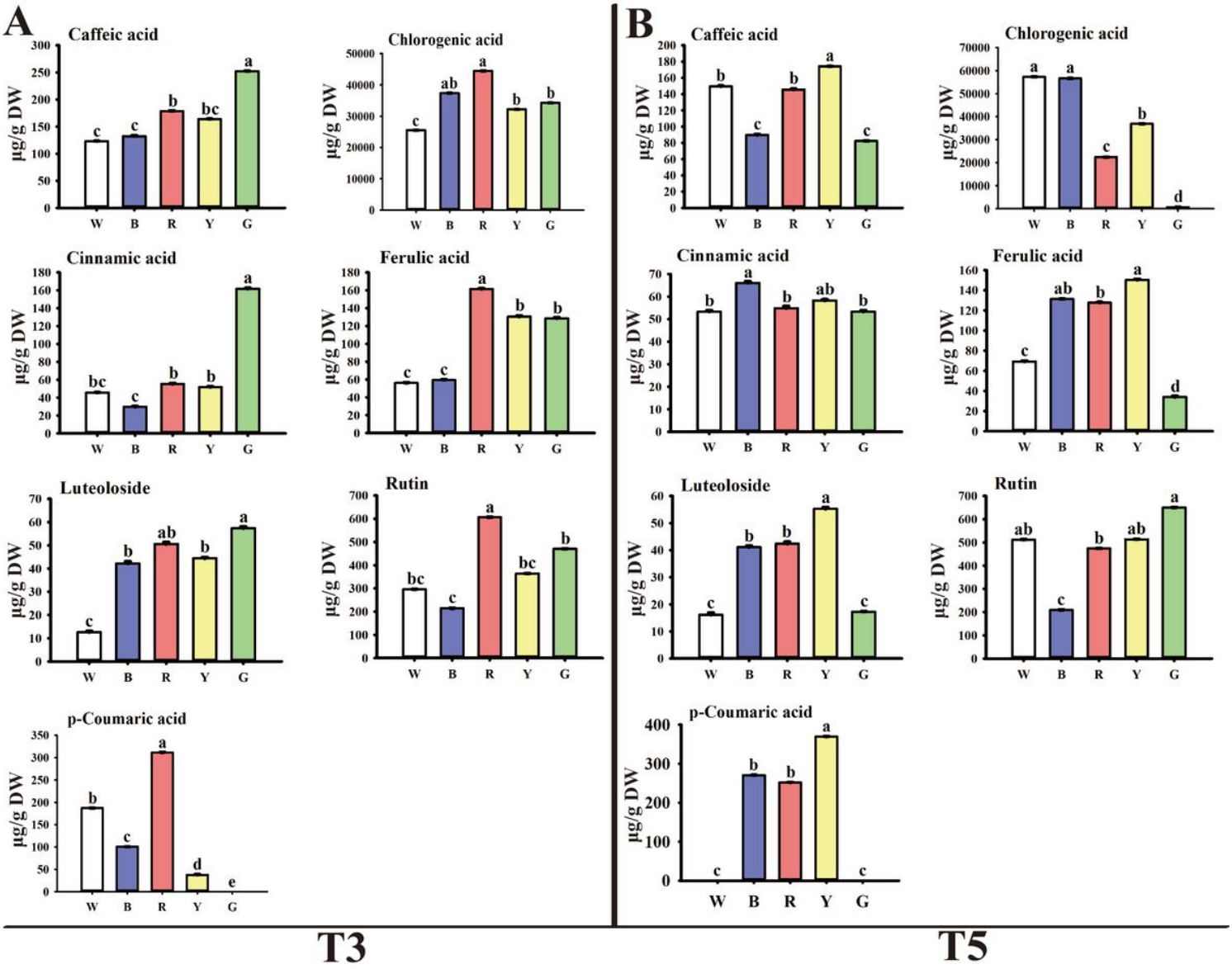

Figure 6

Effects of different hormone levels and light qualities on the accumulation of secondary metabolites. (A) caffeic acid, (B) chlorogenic acid, (C) cinnamic acid, (D) ferulic acid, (E) luteoloside, (F) rutin, (G) *p*-coumaric acid. Error bars represent SE of three biological repetitions. Duncan's multiple range test was used to analyze the significance, and significant differences ($P < 0.05$) are marked by different letters.