

# Blue LED light promoting the growth, accumulation of high-value isoflavonoids and astragalosides, antioxidant response, and biosynthesis gene expression in *Astragalus membranaceus* (Fisch.) Bunge hairy root cultures

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## Research Article

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# Abstract

The root of *Astragalus membranaceus* (Fisch.) Bunge is one of the most frequently used herbs in traditional Chinese medicine (TCM) formulae for fighting COVID-19 infections, due to the presence of isoflavonoids and astragalosides associated with antiviral and immune-enhancing activities. For the first time, the exposure of *A. membranaceus* hairy root cultures (AMHRCs) to different colors of LED lights *i.e.*, red, green, blue, red/green/blue (1/1/1, RGB), and white, was conducted to promote the root growth and accumulation of isoflavonoids and astragalosides. LED light treatment regardless of colors was found beneficial for root growth, which might be a result of the formation of more root hairs upon light stimulation. Blue LED light was found most effective for enhancing phytochemical accumulation. Results showed that the productivity of root biomass in blue-light grown AMHRCs with an initial inoculum size of 0.6% for 55 days was 1.40-fold higher than that in dark (control), and yields of high-value isoflavonoids and astragalosides including calycosin, formononetin, astragaloside IV, and astragaloside I increased by 3.17-fold, 2.66-fold, 1.78-fold, and 1.52-fold relative to control, respectively. Moreover, the photooxidative stress together with transcriptional activation of biosynthesis genes might contribute to the enhanced accumulation of isoflavonoids and astragalosides in blue-light grown AMHRCs. Overall, this work offered a feasible approach for obtaining higher yields of root biomass and medicinally important compounds in AMHRCs *via* the simple supplementation of blue LED light, which made blue-light grown AMHRCs industrially attractive as plant factory in controlled growing systems.

## 1. Introduction

*Astragalus membranaceus* (Fisch.) Bunge, a perennial species of Leguminosae family, is widely distributed in Eastern Asia, Europe, and North America (Sheik et al. 2021). *A. membranaceus* root is a famous herb in traditional Chinese medicine (TCM) and also recorded in pharmacopoeias of Japan, Korea, the United States, and Europe, which is highly appreciated for the multiple pharmacological functions such as immunomodulatory, antitumor, antiviral, antifatigue, anti-inflammatory, antioxidant, cardioprotective, antihyperglycemic, neuroprotective, and hepatoprotective activities (Fu et al. 2014; Li et al. 2020; Sheik et al. 2021). Notably, *A. membranaceus* root is one of the most frequently used herbs in TCM formulae for fighting COVID-19 infections (Lee et al. 2021).

Isoflavonoids and astragalosides are well-known as the primary bioactive ingredients in *A. membranaceus* root (Fu et al. 2014). Nevertheless, the levels of these bioactive phytochemicals in field-grown *A. membranaceus* root always fluctuate owing to the geographical, ecological, and seasonal variations (Fu et al. 2014; Ionkova et al. 2014). In this regard, our research team has established an *in vitro* platform *i.e.*, *A. membranaceus* hairy root cultures (AMHRCs), for the stable production of isoflavonoids and astragalosides (Jiao et al. 2014, 2015a). However, there is still a need to search safe, simple, and effective strategies that can both improve the productivity of root biomass and enhance the accumulation of medicinally important compounds in AMHRCs, which will contribute to promoting the industrial application process of this *in vitro* platform as plant factory in pharmaceutical fields.

LED lights have been extensively used in controlled growing systems for amending crop yield, nutritional value, and phytochemical level, because they are more environmentally friendly and economically favorable as compared with conventional light sources (Bantis et al. 2018). Moreover, LED lights are able to affect the proliferation, morphogenesis, and differentiation of cells/tissues/organs as well as the biosynthesis of secondary metabolites in plant *in vitro* cultures (Hashim et al. 2021). Recent studies have showed that the supplementation of different colors of LED lights could lead to the increased biomass productivity together with higher yields of pharmacologically important phytochemicals in *in vitro* cultures of some medicinally important plants, such as *Salvia miltiorrhiza*, *Cnidium officinale* Makino, and *Rhodiola imbricata* Edgew (Adil et al. 2019; Kapoor et al. 2018; Zhang et al. 2020). In view of this, it is highly expected to manipulate LED lights to promote the root growth and enhance the production of isoflavonoids and astragalosides in AMHRCs.

In the present study, the effects of different colors of LED lights *i.e.*, red, green, blue, red/green/blue (1/1/1, RGB), and white on the root growth, morphogenesis, and phytochemical accumulation in AMHRCs were initially investigated. Subsequently, AMHRCs with different initial inoculum sizes were exposed to the selected blue light to determine the appropriate conditions required for achieving the optimal biomass productivity and phytochemical yields. Afterwards, contents of typical primary metabolites, antioxidant response, expression of genes involved in isoflavonoid and astragaloside biosynthesis pathways were evaluated to reveal the molecular events associated with phytochemical enhancement in blue-light grown AMHRCs. To our knowledge, this is the first study regarding the conscious manipulation of LED lights for promoting the root growth and accumulation of high-value isoflavonoids and astragalosides in AMHRCs.

## 2. Materials And Methods

### 2.1. Preparation of AMHRCs

A leading hairy root line (AMHRL II) with the fast growth rate and high phytochemical productivity was obtained by *Agrobacterium rhizogenes*-mediated transformation of *A. membranaceus* leaf explants as previously reported (Jiao et al. 2014). In this work, AMHRCs were prepared by inoculating a certain amount of AMHRL II into 100 mL Erlenmeyer flasks containing 50 mL MS solid medium supplemented with 30 g/L sucrose, and then used for the following LED illumination experiments.

### 2.2. Exposure of AMHRCs to LED lights

A combination of shelves installed with different colors of LED lamps emitting red light (660 nm), green light (510 nm), blue light (460 nm), RGB light (red/green/blue = 1/1/1), and white light (400–700 nm), was applied for the illumination treatment of AMHRCs. A shelf without lamps was used for the culture of AMHRCs under dark. All shelves were covered by blackout fabric to avoid the contamination of lights from neighboring shelves and environments. The combined shelves were placed in an air-conditioned room at 28°C. The light intensity between LED lamps and culture flasks was in the range of 10 to 15 W/m<sup>2</sup> as measured by a 36701 solar radiation sensor coupled with a 3415FX photometer (Spectrum

Technologies, Inc., USA). The illumination experiments were conducted as follows. Initially, a series of AMHRCs with an initial inoculum size of 0.4% were exposed to different LED lights (red, green, blue, RGB, and white) and dark for 62 days, and sampled every 7 days after 20 days of proliferation culture. Subsequently, a series of AMHRCs with different initial inoculum sizes (0.2%, 0.4%, 0.6%, and 0.8%) were cultured under the selected blue light and dark for 76 days, and samples were also harvested at 7-day intervals after 20 days of proliferation culture.

### ***2.3. Determination of root growth***

After removing the residual medium carefully, the obtained hairy roots were weighed for determination of fresh weight (FW). Subsequently, the hairy roots were dried in a vacuum oven at 50°C and then weighed for measurement of dry weight (DW). The fresh weight and dry weight (biomass productivity) of hairy roots were reported as grams per liter of medium (g/L).

### ***2.4. Microscopic observation***

Microscopic observation was conducted to study the influence of LED lights on micromorphology of hairy roots. Samples were prepared using the method reported previously (Gai et al. 2020). A DM 4000B fluorescence microscope (Leica, Germany) equipped with a DS-Ri1 digital camera (Nikon, Japan) was used to acquire images under the magnification of  $\times 10$ .

### ***2.5. UPLC-MS/MS analysis***

The phytochemical extraction from dried hairy roots was carried out using the ultrasonic-assisted method reported previously (Jiao et al. 2014). The extracting solution was evaporated to dryness, redissolved in acetonitrile (UPLC-MS grade), and filtered through a PVDF membrane (0.22  $\mu\text{m}$ ) for UPLC-MS/MS analysis. The determination of four high-value isoflavonoids and astragalosides including calycosin (CA), formononetin (FO), astragaloside IV (AG IV), and astragaloside I (AG I) was performed using a developed UPLC-MS/MS method adopting multiple reaction monitoring (MRM) mode on a 1290 Infinity UPLC coupled to a 6460 ESI QqQ-MS/MS system (Agilent Technologies, USA). The injection volume was 1  $\mu\text{L}$ , and analyte separation was conducted on an Agilent ZORBAX Eclipse Plus C18 column (50 mm  $\times$  2.1 mm I.D., 1.8  $\mu\text{m}$ ) with a gradient elution using binary mobile phases of acetonitrile and water (0–2 min, 25–35% acetonitrile; 2–3 min, 35–40% acetonitrile; 3–5 min, 40–70% acetonitrile; 5–5.1 min, 70–25% acetonitrile; and 5.1–5.5 min, 25% acetonitrile) at a flow rate of 0.4 mL/min. By the aid of an automated MS method development software (Agilent MassHunter Optimizer), the key MRM parameters of MS/MS (ionization mode, precursor/product ion transition, fragmentor voltage, and collision energy) of each analyte were optimized and summarized in Table S1. The quantitative analysis of CA, FO, AG IV, and AG I in extracts of AMHRCs was achieved *via* screening of their specific precursor ion-to-product ion transitions. The yield of each analyte was calculated through the corresponding calibration curve, and expressed as microgram per gram of root samples (DW).

### ***2.6. Determination of typical primary metabolites***

Amounts of two typical primary metabolites *i.e.*, free amino acids (FAA) and reducing sugars (RS) in fresh hairy roots were determined using AA-2-W kit and HYT-2-Y kit (Suzhou Comin Biotechnology Co., Ltd., China), respectively. Contents of FAA and RS were expressed as microgram per gram of hairy roots (FW).

## 2.7. Evaluation of photooxidative stress

Levels of hydrogen peroxide ( $H_2O_2$ ) and malondialdehyde (MDA) together with activities of catalase (CAT) and peroxidase (POD) in fresh hairy roots were evaluated using  $H_2O_2$ -2-Y kit, MDA-2-Y kit, CAT-2-W kit, and POD-2-Y kit (Suzhou Comin Biotechnology Co., Ltd., China), respectively.  $H_2O_2$  content was expressed as micromole per gram of hairy roots (FW), while MDA reported as nanomole per gram of hairy roots (FW). CAT activity was expressed as micromole of  $H_2O_2$  degraded in one gram of hairy roots (FW) per minute, while POD reported as enzyme active unit per gram of hairy roots (FW).

## 2.8. qRT-PCR analysis

Specific primers of enzyme genes involved in biosynthesis pathways of isoflavonoids (*PAL*, *C4H*, *4CL*, *CHS*, *CHR*, *CHI*, *IFS*, and *I3'H*) and astragalosides (*AACT*, *HMGR*, *MK*, *PMK*, *MVD*, *IDI*, *FPS*, *SS*, *SE*, and *CAS*) were designed as reported previously (Jiao et al. 2015b, 2016). Total RNA extraction, cDNA synthesis, and the preparation of qRT-PCR reaction solutions were conducted using MiniBEST Plant RNA Extraction kit, PrimeScript™ RT reagent kit, and SYBR Premix Ex Taq™ II kit (Takara Biotechnology Co. Ltd., Dalian, China), respectively. The transcription of all target genes was determined on a LightCycler 96 instrument (Roche Diagnostics GmbH, Germany) using the amplification procedure reported previously (Jiao et al. 2015b, 2016). The relative expression level of each gene relative to *ubiquitin* was calculated using the  $\Delta\Delta CT$  method (Livak and Schmittgen 2001).

## 2.9. Statistical analysis

All experiments were carried out in triplicate, and results were expressed as averages  $\pm$  standard deviations. Analyses of significant differences between different groups of data ( $P < 0.05$ ) were achieved by Duncan's multiple comparison test using SPSS statistical software 17.0 (SPSS Inc, Chicago, USA).

# 3. Results And Discussion

## 3.1. Effects of different LED lights on root growth and morphology in AMHRCs

Generally, the long-term exposure of plant in *vitro* cultures to LED lights with different spectral qualities may cause diverse influences on growth and morphology of cells/tissues/organs (Landi et al. 2020). Therefore, the root growth and morphology in AMHRCs under different colors of LED lights (red, green, blue, RGB, and white) and dark were investigated in the present study. In contrast to the typical characteristics of root growth (lack of geotropism) in dark cultures, LED light treatment regardless of colors resulted in the light-avoidance growth of hairy roots, *i.e.*, root growth toward the medium being evident (Fig. 1). As shown in Fig. 2A and B, all light treatment significantly promoted root growth in terms

of fresh/dry weights. However, the effects of different colors of LED lights on root growth varied significantly, with the magnitude of biomass productivity during the plateau period (48 to 62 days) being in the order of red > RGB > green > blue > white > dark (Fig. 2B). The observation of micromorphology of hairy roots was beneficial for understanding the effects of LED lights on their growth. Interestingly, all light treatment was found to induce the formation of more root hairs in comparison with dark (Fig. 3), which was similar to the previous report that the root hair formation in *Arabidopsis thaliana* was strongly stimulated by light illumination (Simone et al. 2000). Accordingly, the enhanced density of root hairs upon LED light treatment might contribute to the uptake of nutrients in the medium thus promoting the root growth in AMHRCs.

### ***3.2. Effects of different LED lights on phytochemical accumulation in AMHRCs***

Apart from the influence on plant growth and morphology, the long-term light exposure can trigger plant defense response to cope with light stress by enhancing the synthesis of photoprotective compounds that are generally possess biological/pharmacological activities, such as flavonoids, carotenoids, and terpenoids (Alrifai et al. 2019; Landi et al. 2020). It is well documented that the supplementation of different colors of LED lights could induce the production of bioactive secondary metabolites in various plant *in vitro* cultures (Hashim et al. 2021). Nevertheless, the effects of LED lights on phytochemical production are species-specific (Landi et al. 2020). Accordingly, the effects of different colors of LED lights (red, green, blue, RGB, and white) on the production of high-value isoflavonoids (CA and FO) and astragalosides (AG IV and AG I) in AMHRCs need to study in the present study.

It is clearly observed from Fig. 4 that blue and red LED lights exhibited superiority in promoting the accumulation of four target compounds in comparison with dark, which was consistent with many findings that blue and red lights were effective in stimulating the production of secondary metabolites in plants (Alrifai et al. 2019; Landi et al. 2020; Zhang et al. 2020). Moreover, blue LED light was found most effective in enhancing the accumulation of isoflavonoids and astragalosides, which might be ascribed to that blue light could cause more severe light stress in plant cells due to its higher photon energy (Alrifai et al. 2019). Additionally, contents of all target compounds in blue-grown AMHRCs were noticed to increase significantly after 41 days (Fig. 4). This supported that light, acting as a unique environmental factor that can interfere with plant metabolism, required the long-term exposure to stimulate the biosynthesis of secondary metabolites (Landi et al. 2020). Overall, the results obtained here, together with the findings of “3.1” section, demonstrated the promising potential of blue light that could achieve the purpose of both improving the productivity of root biomass and enhancing the accumulation of medicinally important compounds in AMHRCs.

### ***3.3. Effects of different initial inoculum sizes on root growth and phytochemical accumulation in AMHRCs under blue LED light***

The initial inoculum size of cells/tissues/organs is a crucial factor that determines the productivity of biomass and secondary metabolites in plant *in vitro* cultures due to the limited nutrients in culture medium (Isah et al. 2018). In this regard, it is necessary to study the effects of different initial inoculum sizes (0.2%, 0.4%, 0.6%, and 0.8%) on root growth and phytochemical accumulation in AMHRCs under the selected blue LED light. As exhibited in Fig. 5A and B, different inoculum sizes indeed had a significant effect on fresh/dry weights of hairy roots. The magnitude of biomass productivity during the plateau period (55 to 76 days) was in the order of 0.6% > 0.8% > 0.4% > 0.2% (Fig. 5B). As shown in Fig. 6, the accumulation profiles of four target compounds were also significantly influenced by different inoculum sizes. For the two isoflavonoids (CA and FO), both yields were highest during the period of 48 to 62 days for inoculum size of 0.6% (Fig. 6). For the two astragalosides (AG IV and AG I), inoculum size of 0.8% was found more favorable for their accumulation, but both yields were also at higher levels from 48 to 55 days for inoculum size of 0.6% (Fig. 6). Overall, the initial inoculum size of 0.6% with exposure time of 55 days under blue LED light was determined as the appropriate conditions for obtaining the optimal productivity of root biomass and high-value phytochemicals in AMHRCs, which provided a valuable reference for the possible industrial application in the future.

Generally, hairy roots are cultured under dark conditions for growth and phytochemical production (Chandra and Chandra 2018). In this study, AMHRCs cultured in darkness under the aforementioned conditions (inoculum size of 0.6% and 55 days) were used as control to know the augmentation in phytochemical yields and biomass productivity in blue-light grown AMHRCs. Four target compounds in extracts of control and blue-light grown AMHRCs were determined by a developed UPLC-MS/MS method *via* screening of the specific precursor ion-to-product ion transitions, including  $[M-H]^- 283.1 \rightarrow 268.1$  for CA (Fig. 7A),  $[M-H]^- 267.1 \rightarrow 252.1$  for FO (Fig. 7B),  $[M + Na]^+ 807.5 \rightarrow 627.4$  for AG IV (Fig. 7C), and  $[M + Na]^+ 891.5 \rightarrow 711.5$  for AG I (Fig. 7D). After calculation, yields of CA ( $153.97 \pm 8.72 \mu\text{g/g DW}$ ), FO ( $106.59 \pm 4.61 \mu\text{g/g DW}$ ), AG IV ( $206.49 \pm 16.24 \mu\text{g/g DW}$ ), and AG I ( $1431.26 \pm 76.83 \mu\text{g/g DW}$ ) increased by 3.17-fold, 2.66-fold, 1.78-fold, and 1.52-fold in blue-light grown AMHRCs as compared with control ( $48.52 \pm 3.86 \mu\text{g/g DW}$  of CA,  $40.11 \pm 3.10 \mu\text{g/g DW}$  of FO,  $115.96 \pm 7.21 \mu\text{g/g DW}$  of AG IV, and  $940.47 \pm 55.56 \mu\text{g/g DW}$  of AG I), respectively. Moreover, the productivity of root biomass ( $12.68 \pm 1.57 \text{ g/L}$ ) in blue-light grown AMHRCs was 1.40-fold higher than control ( $9.03 \pm 0.49 \text{ g/L}$ ). The obtained findings here indicated that the simple supplementation of blue LED light could make AMHRCs industrially attractive as plant factory in controlled growing systems for obtaining higher productivity of root biomass and valuable isoflavonoids and astragalosides.

### 3.4. Contents of FAA and RS in AMHRCs under blue LED light

It is known that secondary metabolites such as flavonoids and terpenoids are derived from glycolysis, TCA cycle, pentose phosphate pathway, aliphatic amino acids, and aromatic amino acids (Aharoni and Galili 2011). Additionally, RS can provide energy after glycolysis, which is favorable for biosynthesis of secondary metabolites to counteract environmental stresses (Saddhe et al. 2020). As shown in Fig. 8A

and B, contents of both FAA and RS in blue-light grown AMHRCs decreased significantly after 34 days. Meanwhile, yields of four target compounds in blue-light grown AMHRCs were found to increase obviously after 34 days (Fig. 6), which suggested that the enhanced accumulation of isoflavonoids and astragalosides might require more consumption of primary metabolites such as FAA and RS. In other words, the light stress in AMHRCs caused by long-term exposure of blue LED light could direct the primary metabolic flow toward the biosynthesis of photoprotective secondary metabolites such as isoflavonoids and astragalosides.

### 3.5. Antioxidant response in AMHRCs under blue LED light

Blue LED light was reported to have higher photon energy that likely induced the photooxidative stress in plant cells (Alrifai et al. 2019). It is known that plant can cope with oxidative stress mainly by modulating antioxidant enzyme system (Gill and Tuteja 2010).  $H_2O_2$  is considered as the most stable reactive oxygen species (ROS), and CAT is able to catalyze the conversion of  $H_2O_2$  to oxygen and water (Gill and Tuteja 2010). As shown in Fig. 8C and E, the change in  $H_2O_2$  content was noticed to be negatively correlated with that in CAT activity, which indicated that antioxidant enzyme system was indeed activated as a positive-feedback response to scavenge the excessive ROS in AMHRCs caused by blue LED light. In addition, there was no significant change in MDA content along with POD activity (except 62 days) during the entire period (Fig. 8D and E), which suggested that the accumulation of intracellular ROS in blue-light grown AMHRCs did not reach the level that could trigger the lipid oxidation on cell/organelle membranes. Moreover, it is worth mentioning that  $H_2O_2$  is an important signal molecule in plants that can be involved in the acclimatory signal transduction responsive to environmental stresses (Gill and Tuteja 2010). It was reasonable to speculate that the high level of  $H_2O_2$  in blue-light grown AMHRCs at 48 days could act as the secondary messenger that possibly activated light signal transduction.

### 3.6. Expression of biosynthesis genes in AMHRCs under blue LED light

Upon the illumination of blue light, the photoreceptors *i.e.*, cryptochromes in plants that can transduce light information to downstream signaling, thereby activating the expression of genes involved in regulating plant growth, development, and secondary metabolism (Alrifai et al. 2019; Landi et al. 2020; Xu 2020). Thus, the investigation of transcriptional profiles of enzyme genes involved in biosynthesis pathways of isoflavonoids (*PAL*, *C4H*, *4CL*, *CHS*, *CHR*, *CHI*, *IFS*, and *I3'H*) and astragalosides (*AACT*, *HMGR*, *MK*, *PMK*, *MVD*, *IDI*, *FPS*, *SS*, *SE*, and *CAS*) would contribute to shedding light on molecular events associated with the enhanced accumulation of isoflavonoids and astragalosides in blue-light grown AMHRCs.

As shown in Fig. 9A, the expression of all tested genes in isoflavonoid biosynthesis pathway was gradually activated from 20 to 48 days in AMHRCs upon blue LED light treatment, which should be responsible for the increasing accumulation of CA and FO during this period (Fig. 6). Factually, the photoactivated cryptochromes can enhance the production of a master regulator of light signaling *i.e.*,

HY5 (ELONGATED HYPOCOTYL5), which is able to bind to cis-acting elements such as G-box and ACE-box in promoters of structural genes in flavonoid biosynthesis pathway thus promoting their transcription (Xu 2020; Zhao et al. 2022). The superior expression of *CHI* (10.40-fold increase) and *CHR* (9.17-fold increase) at 48 days might be attributed to the mechanism stated above. Notably, *HMGR* exhibited the tremendous transcriptional abundance (96.44-fold increase at 48 days) among all investigated genes in astragaloside biosynthesis pathway (Fig. 9B), which suggested that this gene might play a key role in the induction of astragaloside biosynthesis in AMHRCs under blue LED light. It was reported that phytochrome-interacting factor 3 (PIF3, a basic helix-loop-helix transcription factor) could bind to the G-box in the promoter of *HMGR* (Kim et al. 2013). As inferred, blue LED light might activate the light-responsive PIF3 thus remarkably up-regulating *HMGR* expression for promoting AG IV and AG I biosynthesis in AMHRCs. Overall, the results obtained here demonstrated that blue LED light could significantly activated the transcription of biosynthesis genes (such as *CHI*, *CHR*, and *HMGR*) via cryptochrome-mediated light signal transduction for the enhanced accumulation of isoflavonoids and astragalosides in AMHRCs.

## 4. Conclusions

In the present study, the supplementation of blue LED light could simultaneously promote the root growth and accumulation of medicinally important compounds in AMHRCs. In comparison with control, the enhanced productivity of root biomass (1.40-fold increase) together with higher yields of CA (3.17-fold increase), FO (2.66-fold increase), AG IV (1.78-fold increase), and AG I (1.52-fold increase), were achieved in blue-light grown AMHRCs with an initial inoculum size of 0.6% for 55 days. The enhanced density of root hairs in blue-light grown AMHRCs might account for the increased biomass productivity. Moreover, the photooxidative stress and transcriptional activation of biosynthesis genes might contribute to the enhanced accumulation of photoprotective phytochemicals (such as isoflavonoids and astragalosides) in AMHRCs upon blue LED light treatment. Overall, AMHRCs culture under blue LED light could serve as plant factory in controlled growing systems for the large-scale production of high-value isoflavonoids and astragalosides, which would exhibit great prospects in pharmaceutical industries dealing with coronaviruses viruses such as COVID-19.

## Declarations

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### Author contributions

**Qing-Yan Gai:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing-original draft, Funding acquisition. **Xue Feng:** Methodology, Validation, Investigation, Data

Curation. **Jiao Jiao**: Conceptualization, Methodology, Formal analysis, Data Curation, Writing-review & editing, Supervision, Project administration, Funding acquisition. **Xiao-Jie Xu**: Methodology, Validation, Investigation, Data Curation. **Jin-Xian Fu**: Formal analysis, Investigation, Validation. **Xiao-Jia He**: Investigation, Validation. **Yu-Jie Fu**: Resources, Funding acquisition.

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## Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

## Data availability statements

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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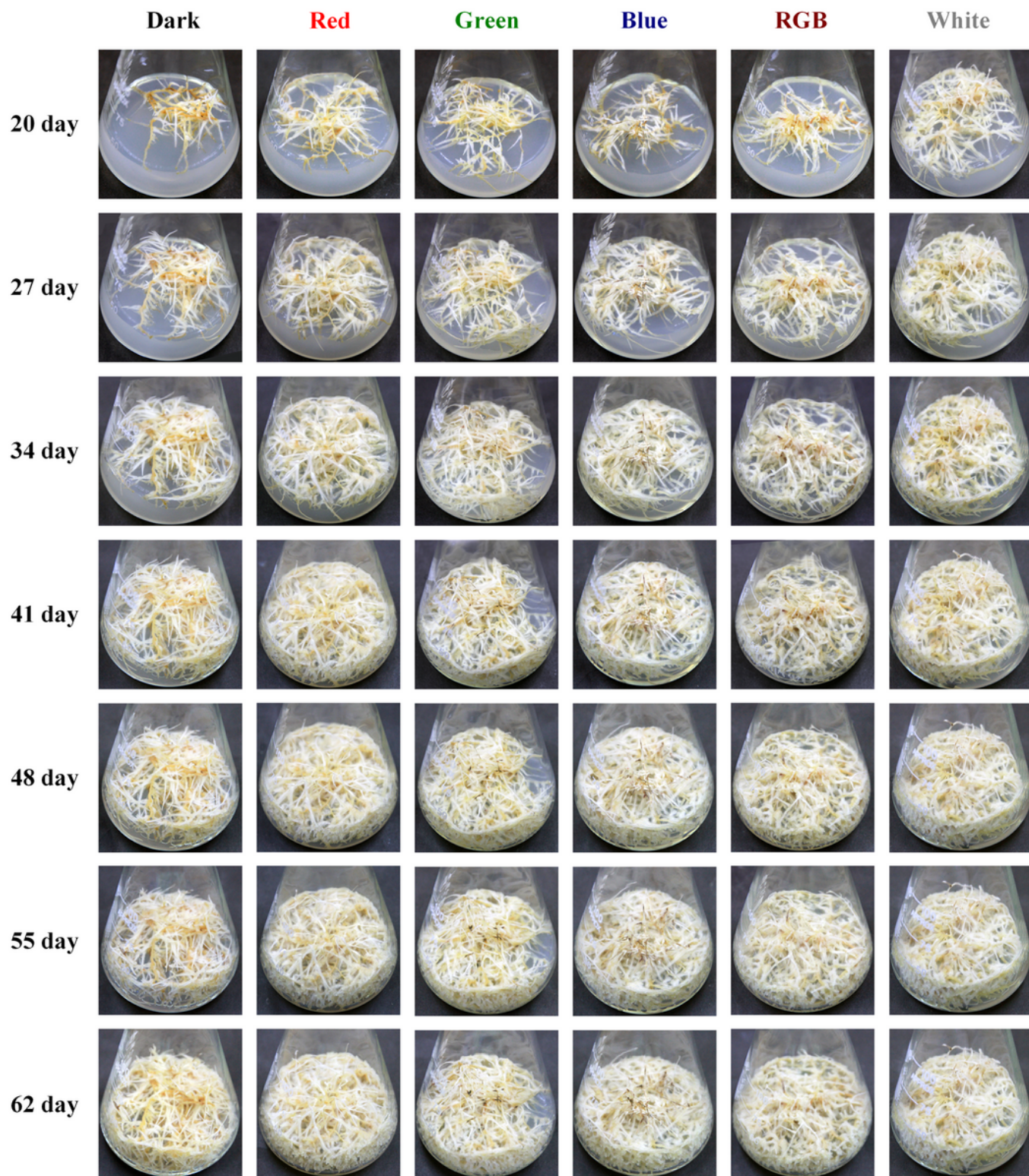
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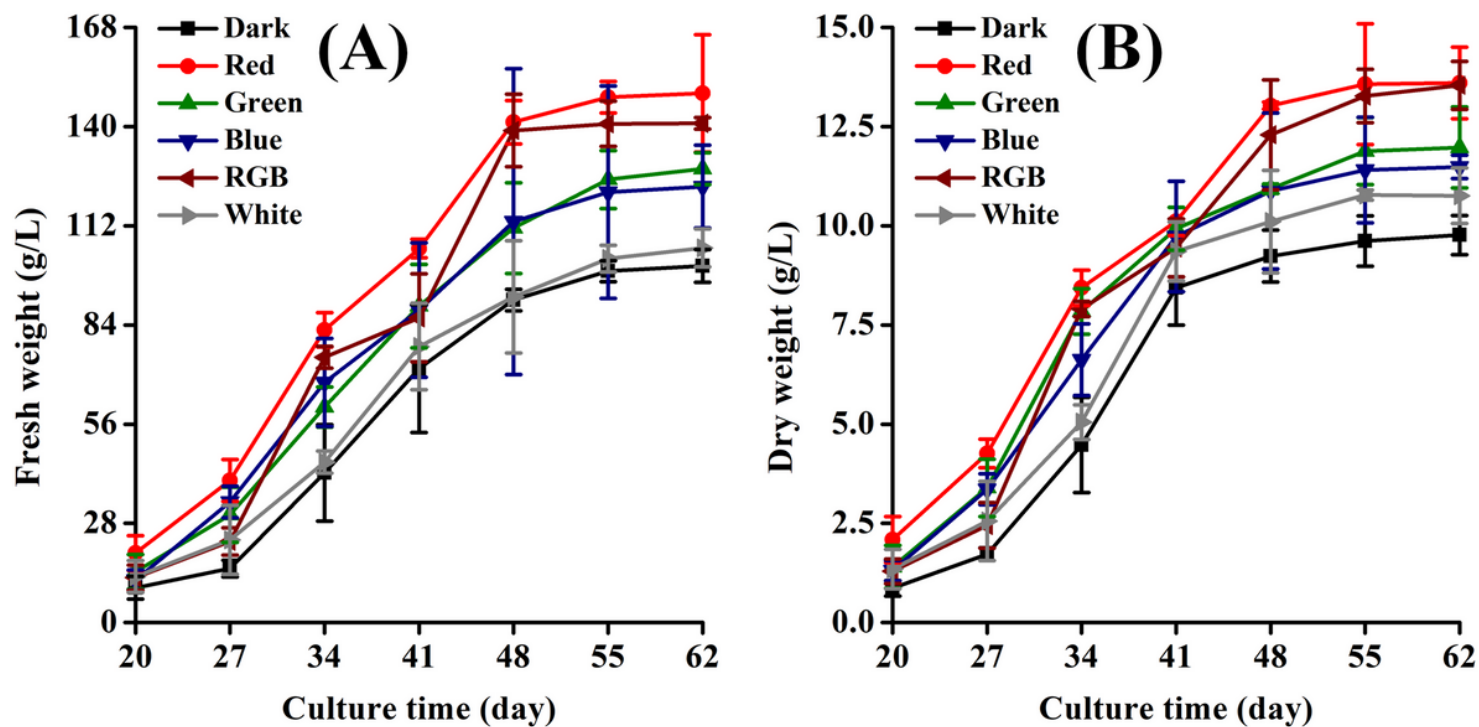
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## Figures



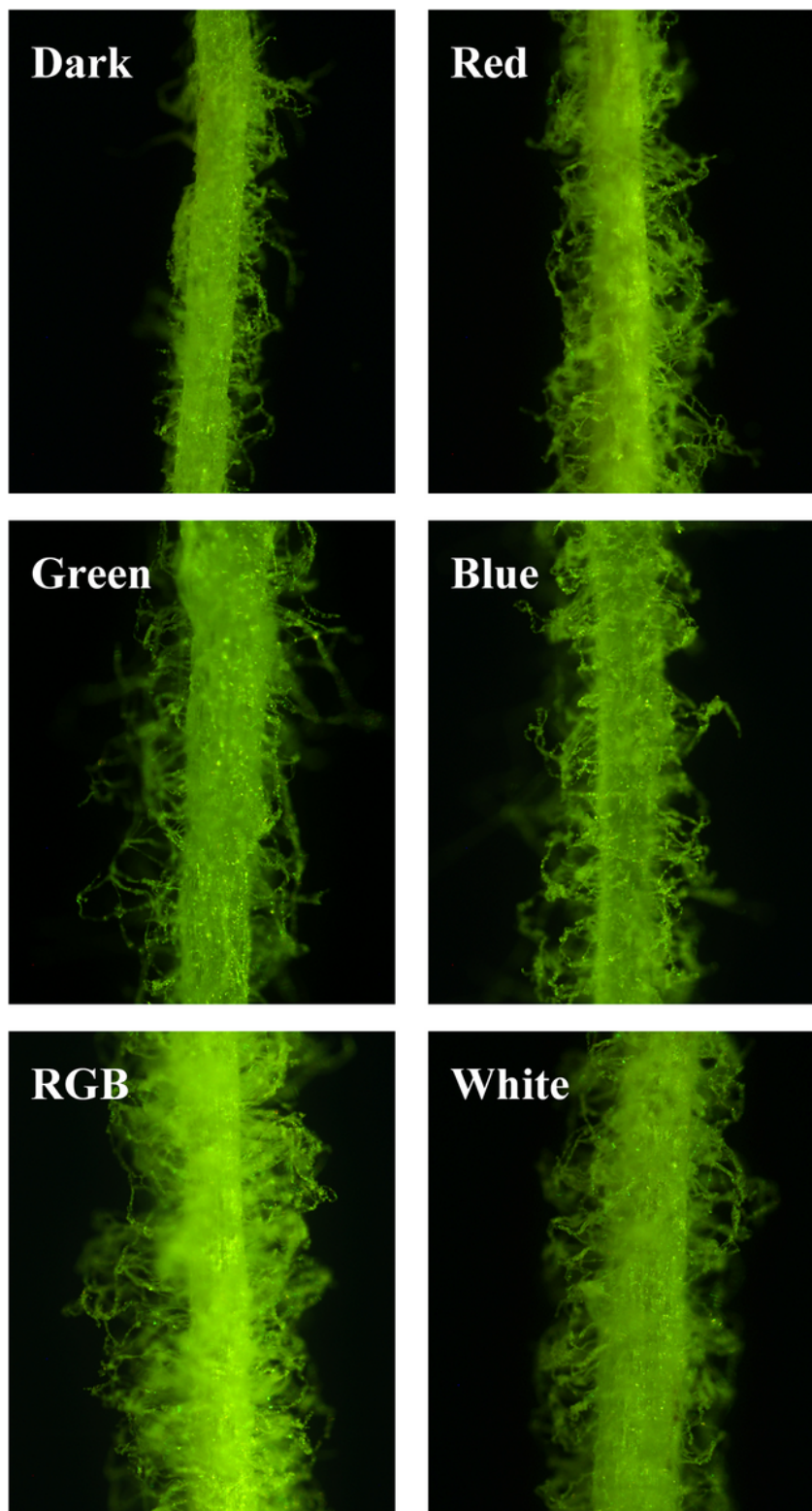
**Figure 1**

Effects of different colors of LED lights (red, green, blue, RGB, and white) and dark on morphology in AMHRCs over the period of 62 days.



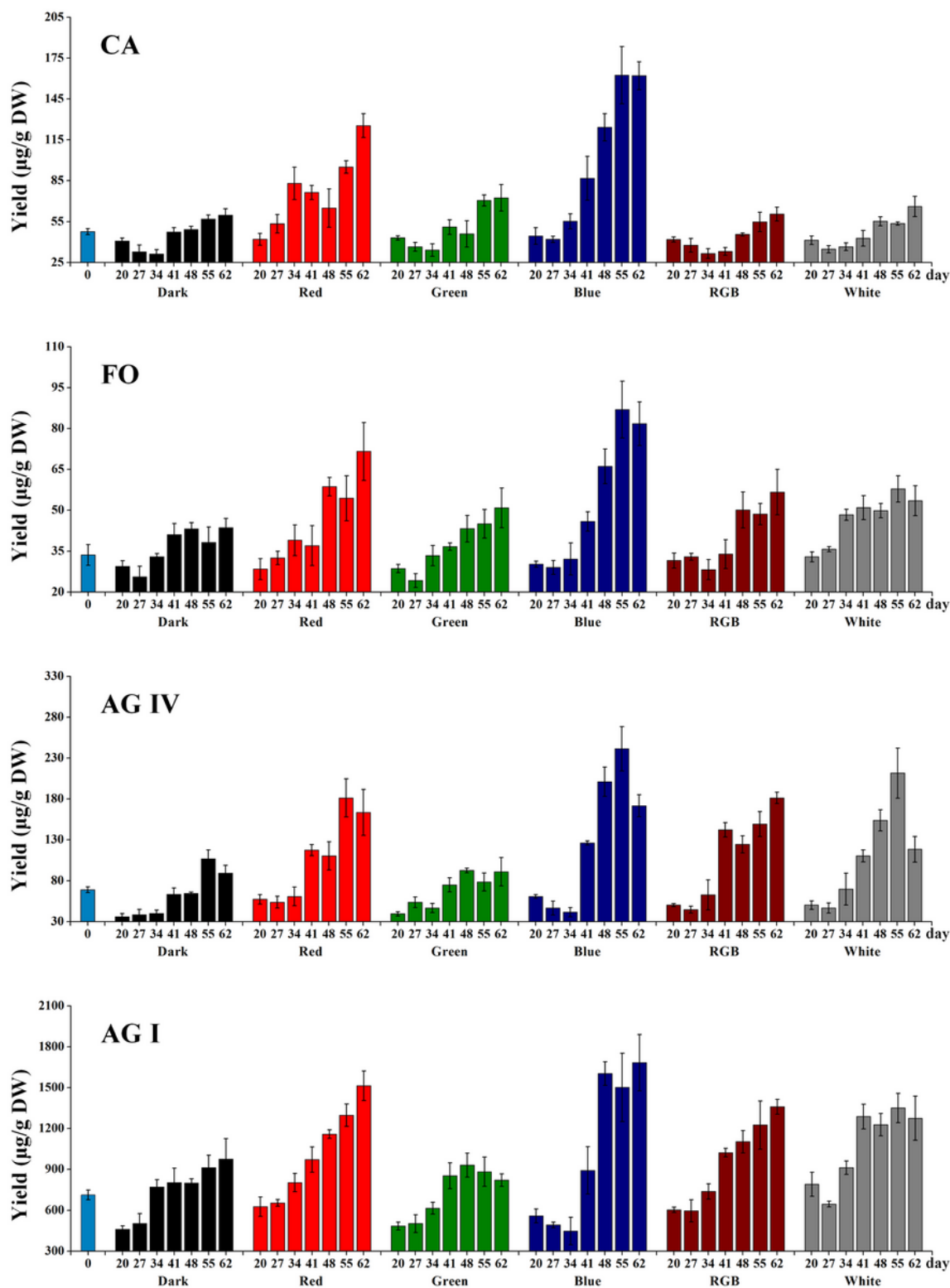
**Figure 2**

Effects of different colors of LED lights (red, green, blue, RGB, and white) and dark on fresh weight (A) and dry weight (B) in AMHRCs over the period of 62 days.



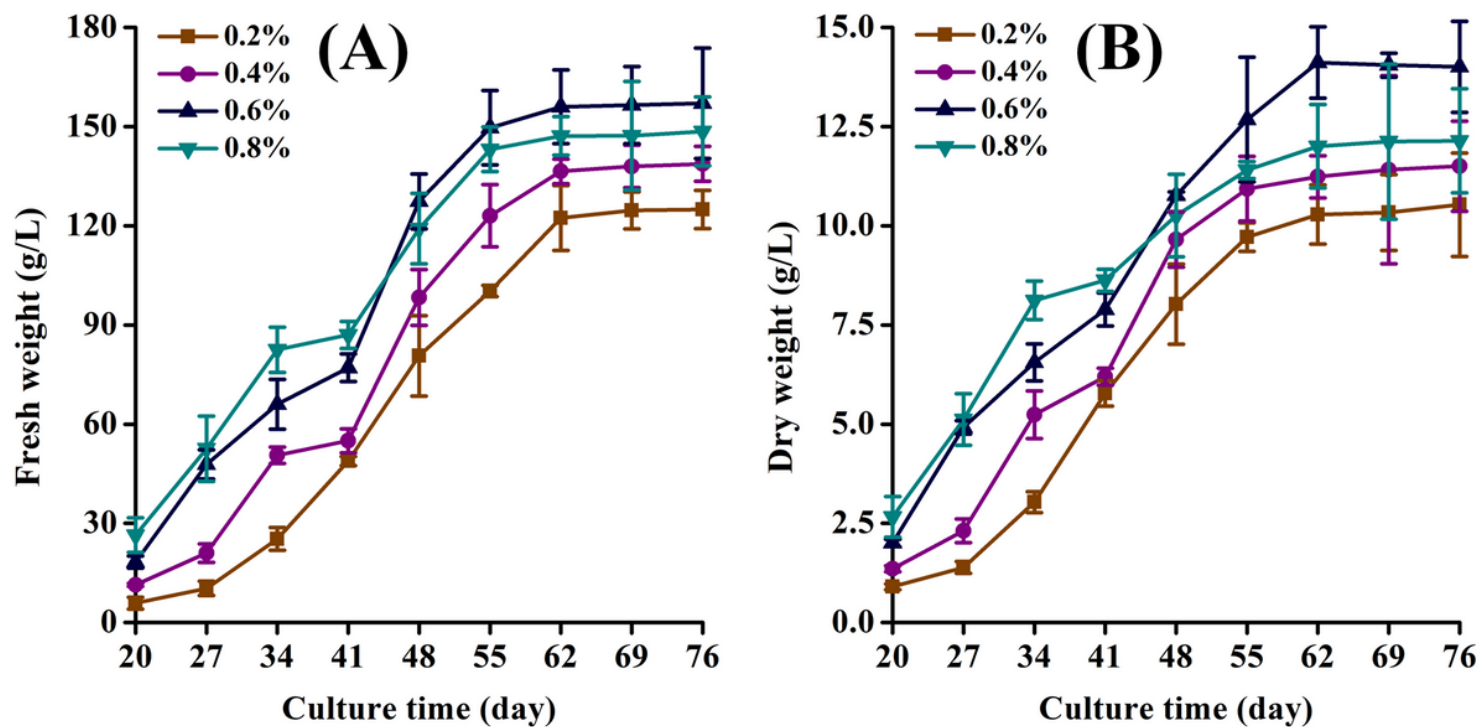
**Figure 3**

Micromorphology of hairy roots treated by different colors of LED lights (red, green, blue, RGB, and white) and dark for 55 days under fluorescence source (magnification of  $\times 10$ ).



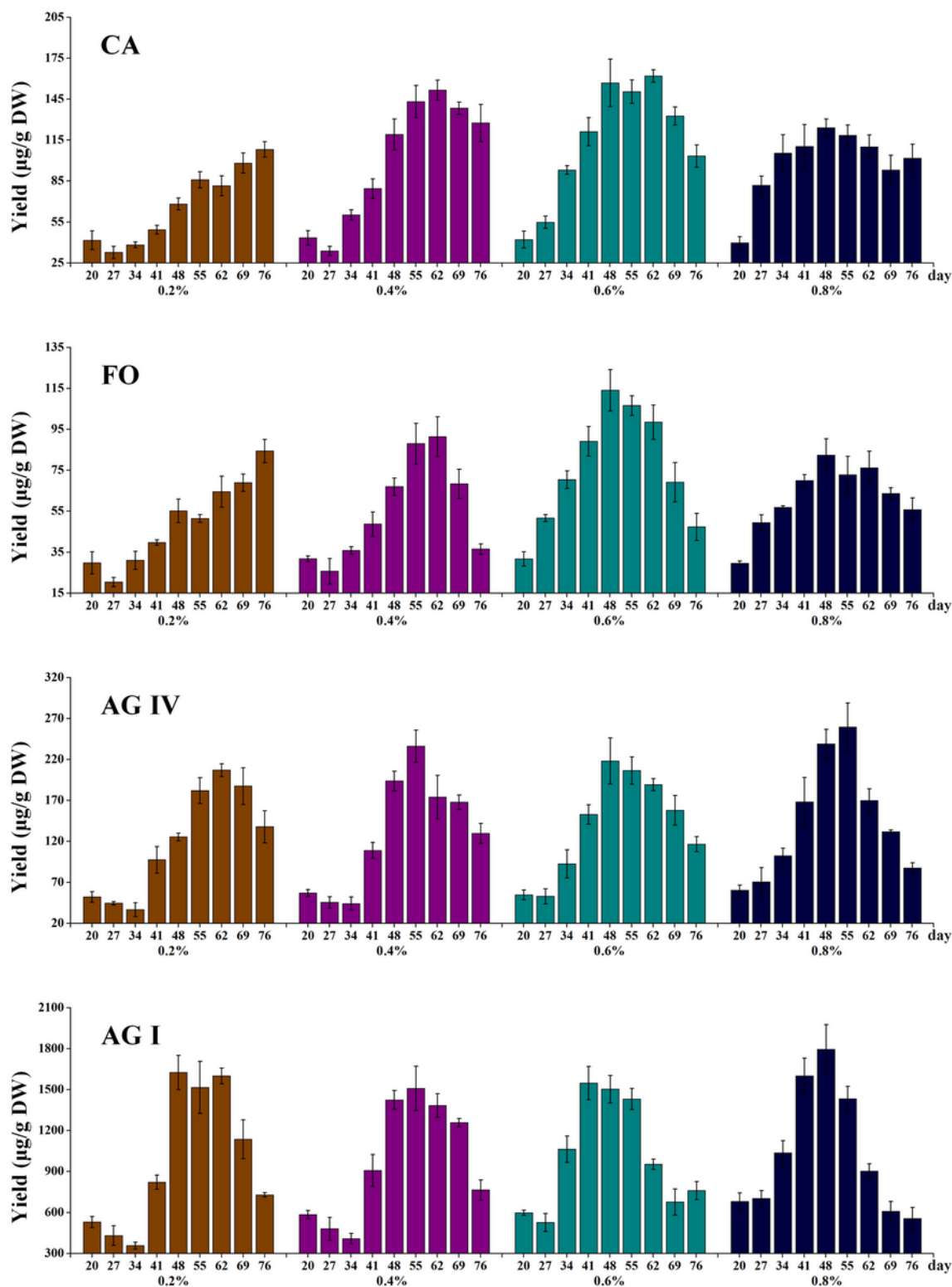
**Figure 4**

Effects of different colors of LED lights (red, green, blue, RGB, and white) and dark on the accumulation of isoflavonoids (CA and FO) and astragalosides (AG IV and AG I) in AMHRCs over the period of 62 days.



**Figure 5**

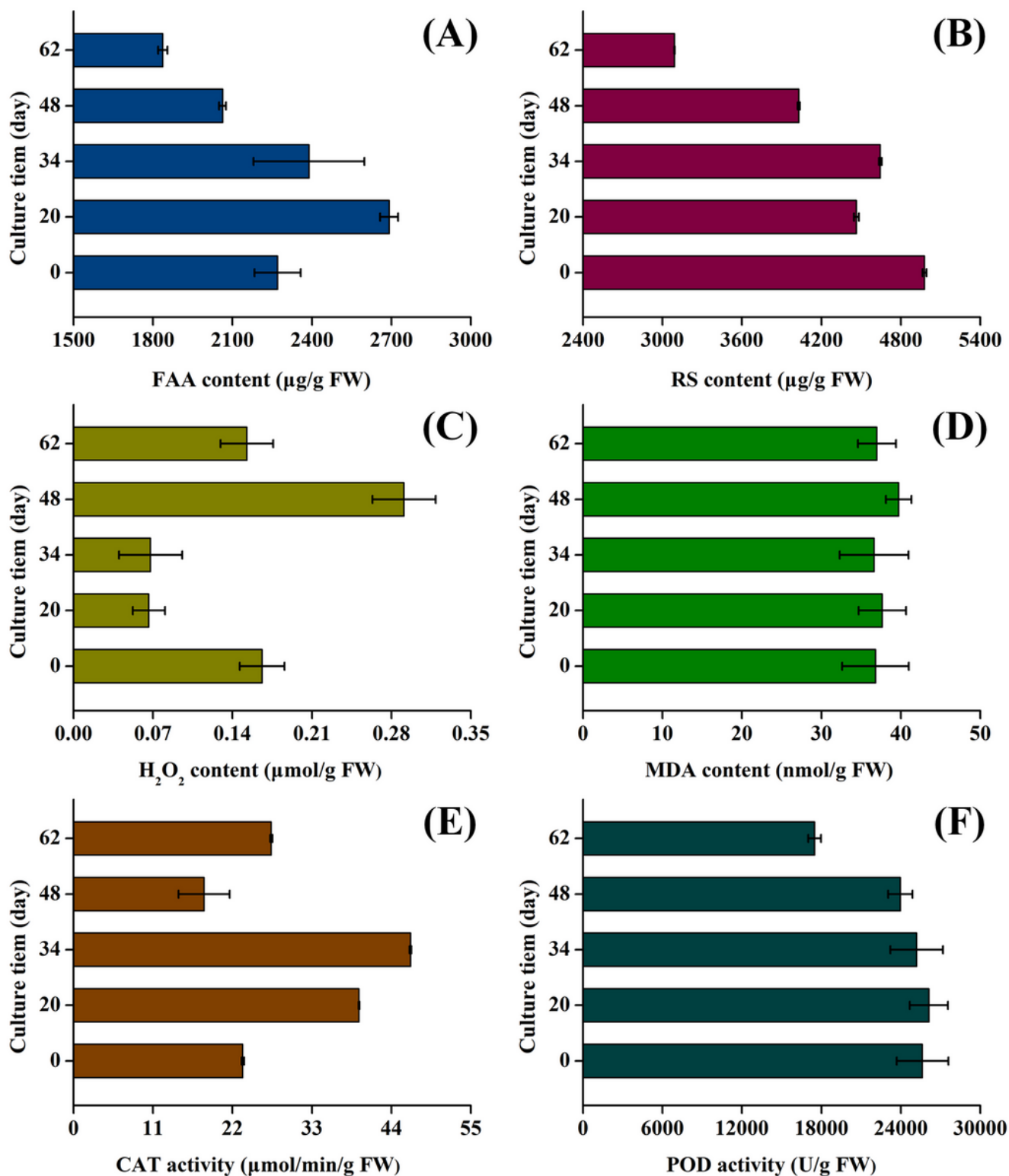
Effects of different initial inoculum sizes (0.2%, 0.4%, 0.6%, and 0.8%) on fresh weight (A) and dry weight (B) in AMHRCs under blue LED light over the period of 76 days.



**Figure 6**

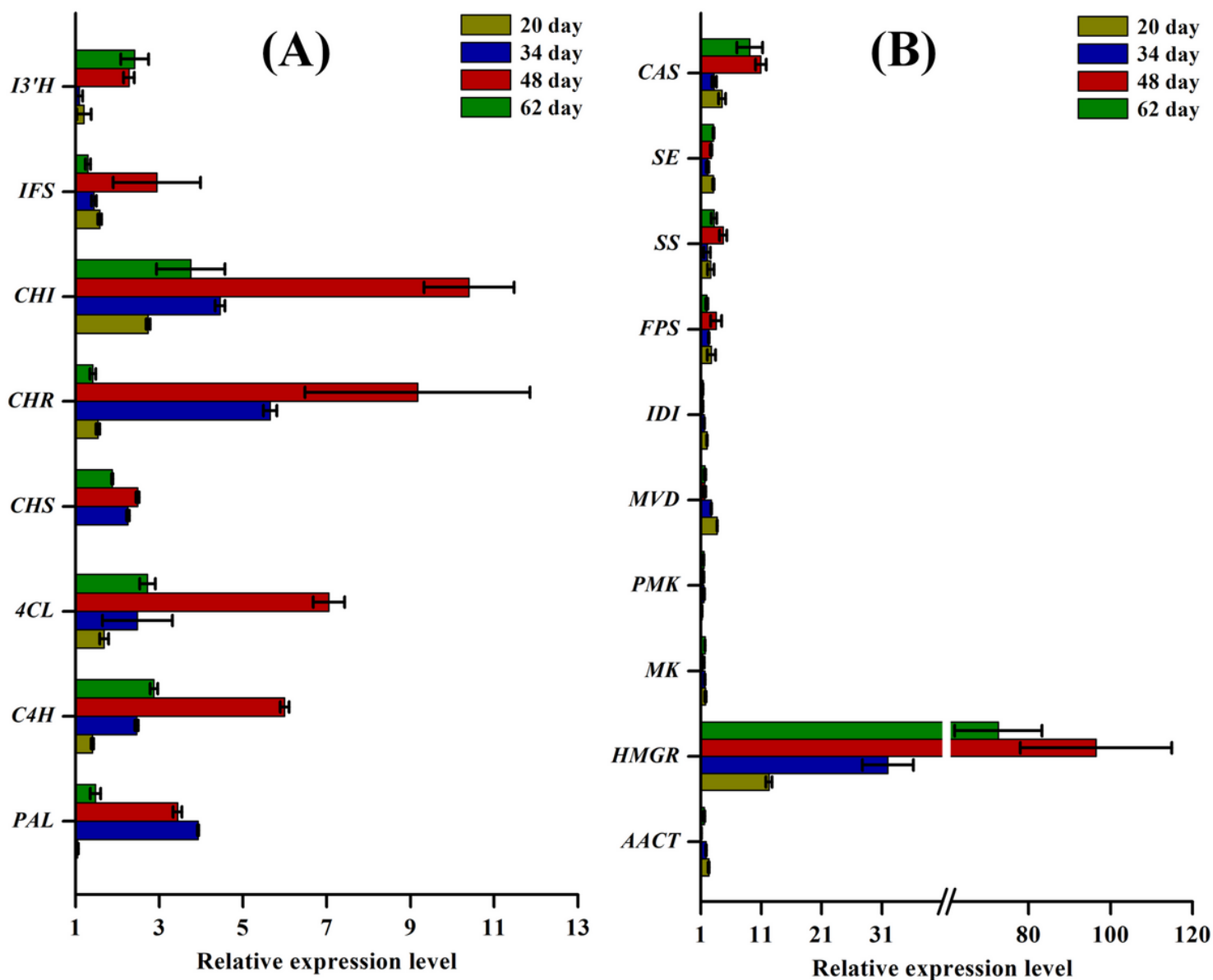
Effects of different initial inoculum sizes (0.2%, 0.4%, 0.6%, and 0.8%) on the accumulation of isoflavonoids (CA and FO) and astragalosides (AG IV and AG I) in AMHRCs over the period of 76 days.





**Figure 8**

Contents of FAA (A), RS (B),  $\text{H}_2\text{O}_2$  (C), MDA (D) together with activities of CAT (E) and POD (F) in blue-light grown AMHRCs (initial inoculum size of 0.6%) within 62 days.



**Figure 9**

Expression profiles of enzyme genes involved in biosynthesis pathways of isoflavonoids (*PAL*, *C4H*, *4CL*, *CHS*, *CHR*, *CHI*, *IFS*, and *I3'H*) and astragalosides (*AACT*, *HMGR*, *MK*, *PMK*, *MVD*, *IDI*, *FPS*, *SS*, *SE*, and *CAS*) in blue-light grown AMHRCs (initial inoculum size of 0.2%) within 62 days.

## Supplementary Files

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