Physiological Responses of the *Tetrastigma hemsleyanum* Plant under Different Color Films

Yan Bai and Wen Chen

Zhejiang Provincial Key Laboratory of Resources Protection and Innovation of Traditional Chinese Medicine, Hangzhou, Zhejiang 311300, P.R. China; School of Forestry and Biotechnology, Zhejiang Agriculture and Forestry University, Hangzhou, Zhejiang 311300, P.R. China; State Key Laboratory of Subtropical Silviculture, Zhejiang Agriculture and Forestry University, Hangzhou, Zhejiang 311300, P.R. China

Shou-Zan Liu

Zhejiang Provincial Key Laboratory of Resources Protection and Innovation of Traditional Chinese Medicine, Hangzhou, Zhejiang 311300, P.R. China; Botanical Garden, Zhejiang Agricultural and Forestry University, Lin'an, Zhejiang 311300, P.R. China

Lin-Yu Xu and Zhe Li

Zhejiang Provincial Key Laboratory of Resources Protection and Innovation of Traditional Chinese Medicine, Hangzhou, Zhejiang 311300, P.R. China; School of Forestry and Biotechnology, Zhejiang Agriculture and Forestry University, Hangzhou, Zhejiang 311300, P.R. China; State Key Laboratory of Subtropical Silviculture, Zhejiang Agriculture and Forestry University, Hangzhou, Zhejiang 311300, P.R. China

Bin Liu

School of Agriculture and Biology, Shanghai Jiao Tong University, Key Laboratory of Urban Agriculture, Ministry of Agriculture, Shanghai 200240, P.R. China

Additional index words. flavonoid, color film, flavonoid enzyme activity

Abstract. *Tetrastigma hemsleyanum* is a traditional Chinese medicine herb, commonly used for its anti-inflammatory and antitumor properties. Flavonoids are the main functional constituents of *T. hemsleyanum*, and their production in the herb is affected by light quality. *T. hemsleyanum* is a shade-loving plant and is usually covered by black shade nets during cultivation. However, there are only a few studies on the effects of using color films on growth and flavonoid synthesis in *T. hemsleyanum*. In this study, we measured the influence of five different color films on growth indexes—sugar, soluble amino acid, soluble protein, and flavonoid content—and flavonoid-synthesizing enzyme activities in *T. hemsleyanum*. The films used were colorless plastic film as the control group (CK-W), red film (RF), yellow film (YF), green film (GF), and blue film (BF). BF promoted plant growth and increased yield, as evidenced by the highest growth indexes, soluble amino acid content, and chalcone isomerase (CHI) enzyme activity. RF increased the content of secondary metabolites, thereby enhancing herb quality, as evidenced by the highest phenylalanine ammonia lyase (PAL) activity and increased flavonoid content.

Tetrastigma hemsleyanum is a precious plant in China (Wang et al., 2018a). It is known for its anti-inflammatory, antioxidant, and antitumor activities (Chen et al., 2017; Wang et al., 2017; Zhu et al., 2020) in Chinese herbology. Wu et al. (2019) reported that the flavonoids present in *T. hemsleyanum* have a noticeable antitumor effect in leukemia and colon cancer. Wang et al. (2019a) reported that n-butanol and ethyl acetate extractions of *T. hemsleyanum* have superior antiviral activity against the respiratory syncytial virus than ribavirin. Ding et al. (2019)

and Hu et al. (2021) reported that the chemical constituents of *T. hemsleyanum* have antiviral activity against influenza viruses.

Light is one of the most important environmental factors that regulate growth, development, and metabolism in plants (Cheng et al., 2018; Hu et al., 2019; Xu et al., 2018). Light quality during seedling development influences the morphology of the plant (Li et al., 2021), and regulates gene expression and metabolism in plants (Dong et al., 2018). Wang et al. (2018b) indicated that light has a significant effect on enzyme activities in the

medicinal plant *Gynostemma pentaphyllum*. Being a sciophilous plant, *T. hemsleyanum* is commonly cultivated as undergrowth vegetation. Our previous study showed that the total flavonoid content in the leaves and roots of *T. hemsleyanum* is affected by light (Xu et al., 2018). However, the types of flavonoids and their related enzymes that are specifically influenced by light are unclear.

In this study, *T. hemsleyanum* was grown under color films to simulate different light conditions. Color films are widely used in *T. hemsleyanum* cultivation to decrease light intensity (Gao and Fang, 2014). In addition, the use of color films is much cheaper than direct treatment with lamps. Therefore, the use of color films is a practical approach for large-scale cultivation.

Many studies on the use of color films and their effects in agriculture have been conducted. The use of films can enhance the conversion of organic carbon and stimulate the emergence of brace roots in maize (Jin et al., 2020; Zhou et al., 2020). In addition, films with different colors have different physiological effects on plants. Black film increases dry matter accumulation and water use efficiency in maize (Zhang et al., 2018), whereas red films increase the expression of flavonoid genes and the enzyme activities in strawberries (*Fragaria × ananassa*) (Miao et al., 2016). However, there are few studies on the effects of color films in promoting flavonoid synthesis in *T. hemsleyanum* (Cheng et al., 2018). We studied the growth and flavonoid synthesis in *T. hemsleyanum* under four different color films.

This study focused on the effects of different light qualities, by using colored plastic films, on the growth characteristics, flavonoid-related enzyme activities, and flavonoid monomer contents of *T. hemsleyanum*. The aim of the study was to find the most suitable light conditions, by using color films, for plant growth and flavonoid accumulation to benefit production and further research.

Materials and Methods

Plants and their growing conditions. *T. hemsleyanum* plants were under cultivation from Mar. 2016 to Feb. 2019, and were maintained in a greenhouse located at the State Key Laboratory of Subtropical Silviculture, Zhejiang Agriculture and Forestry University, Hangzhou, China (lat. 30°15'N, long. 119°43'E). Three-year-old *T. hemsleyanum* Diels et Gilg plants were identified by Zhou Aicun, Doctor of Traditional Chinese Medicine Identification (specimen code PEY0063158, Chinese Virtual Herbarium; <http://www.cvh.ac.cn>). These plants were grown under five different light conditions—CK-W, RF, YF, GF, and BF—by using polyvinylchloride transparent films (Qihang Plastic Co., Ltd., Dongguan, China) for a complete growth circle of 9 months. Light intensities between different treatments were kept consistent by adjusting the number of holes in the shading net. The transmittance of different films is shown in Fig. 1.

Light intensities were adjusted to 70% in all treatment groups by using shading nets

Fig. 1. Wavelength range of light film.

(measured by a luxmeter; Victor Electronic Instrument Co., Ltd., Hangzhou, China) at 12:00 noon every sunny day. All plants were conserved with regular watering, weeding, and spraying of insecticides. To get a more accurate data, each treatment consisted of five replicates, among which three of good and similar growth status were chosen for analysis.

Physiological index. The physiological index included growth target, reducing sugar content, soluble amino acid content, and soluble protein content. Three plants were

selected from each treatment, and growth indexes such as stem length, maximum blade length, maximum blade width, specific leaf weight, and fresh root weight were measured. Reducing sugar content in the leaves of *T. hemsleyanum* was determined using the Anthrone method (Li, 2000). Soluble amino acid content was determined using the Ninhydrin coloring method (Liu et al., 2018a). Soluble protein content was determined using the Bradford method (Chen et al., 2003).

Flavonoid-synthesizing metabolic enzymes activities index. For each treatment, 0.5 g of leaf and fresh root samples was accurately weighed and ground into a homogenate with 10 mL liquid nitrogen (pH 8.8) and 0.1 mol/L boric acid–borax buffer [1 mmol/L ethylenediaminetetraacetic acid, 5 mmol/L β -mercaptoethanol, 0.5 mmol/L ascorbic acid, and 1% (w/v) polyvinylpyrrolidone]. Activities of CHI and chalcone synthase (CHS) were determined using the CHI/CHS Kit (Wanxiang Hengyuan Co., Ltd., Tianjin, China). PAL activity was measured using the method of Li (2000).

Flavonoid monomer content determination. An ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) method (Zhang et al., 2017) was used to quantify flavonoid monomers using the chromatogram of the standard mixture of naringin, isorhamnetin-3-O-glucoside, orientin, isoorientin, astragalin, luteoloside, isovitexin, hesperidin, calycosin-7-O- β -D-glucoside, isquercitrin, eriodictyol, hispidulin, and lonicerin (Yuanye Biotechnology Co., Ltd., Shanghai, China). Standard curves for the calculation are shown in Table 1.

Statistical analysis. For the statistical analysis, data were subjected to one-way analysis of variance ($P < 0.01$ or $P < 0.05$) using SPSS Statistics 22.0, Origin 2018 Software (SPSS, Inc., Chicago, IL).

Results

Blue light promotes growth of *T. hemsleyanum*. The morphological traits of *T. hemsleyanum* were affected significantly by different treatments, especially in the BF and RF groups (Table 2). Each characteristic of the growth index showed the maximum value with the BF treatment among all treatments (stem length, 48.53 cm; maximum blade length, 5.8 cm; maximum blade width, 2.77 cm; specific leaf weight, 3.51 g; and fresh root weight, 12.78 g), followed by the RF group with respect to specific leaf weight (3.47 g). However, but the RF group showed the lowest fresh root weight (3.66 g) among all groups. The results indicated that blue light promoted the growth of *T. hemsleyanum*.

Red light promotes accumulation of five flavonoid compounds in leaves. UPLC-MS/MS detected 11 flavonoid compounds in the leaves of *T. hemsleyanum*, which are shown in Fig. 2. In all replicates studied, isovitexin was found at the greatest concentration among all flavonoid compounds detected (Fig. 2A).

Replicates under RF treatment contained a significantly higher concentration of some flavonoid compounds than those under other treatments; those compounds were isovitexin (2523.60 $\mu\text{g/mL}$), orientin (Fig. 2B, 25.11 $\mu\text{g/mL}$), isoorientin (Fig. 2C, 14.81 $\mu\text{g/mL}$), lonicerin (Fig. 2F, 2.17 $\mu\text{g/mL}$), and Astragalin (Fig. 2H, 0.22 $\mu\text{g/mL}$). Luteoloside content (Fig. 2D) was the greatest with the CK-W treatment (4.54 $\mu\text{g/mL}$), followed by the BF treatment (4.52 $\mu\text{g/mL}$). Isovitexin, isoorientin, and lonicerin levels were lowest with the GF treatment among all treatments. These results show that color films affect the accumulation of flavonoids in *T. hemsleyanum* considerably.

Red light benefits activity of PAL and blue light benefits activity of CHI. The effects of different light treatments on the three major flavonoid-synthesizing enzymes (PAL, CHS, and CHI) were determined independently in three growth periods. *T. hemsleyanum* grows rapidly in the spring, and accumulates nutrients and flavonoids in the summer and autumn. Thus, we chose these three growth periods—spring rapid growth period (SRP), summer dormancy period (SDP) and autumn rapid growth period (ARP)—to determine enzymes activity.

PAL, CHS, and CHI enzyme activity differed among treatments and growth periods (Fig. 3). The greatest PAL activity was observed in the RF group, whereas PAL targets were significantly low in both the YF and the GF groups during all three growth periods (Fig. 3A). The lowest CHS activity was observed in the YF group; mostly lower CHS activity was observed during the SDP than during SRP and ARP (Fig. 3B). The greatest CHI activity was observed in the CK-W group (280.73 U/mL) during the SRP period among all treatment groups (Fig. 3C), whereas the greatest CHI activity was found in the BF group during SDP and ARP (285.40 U/mL).

In summary, red light, blue light, and white light showed significant upregulation

Received for publication 15 Jan. 2021. Accepted for publication 23 Mar. 2021.
Published online 27 May 2021.

This research was supported by the Forestry Science and Technology Extension Foundation of Central Government Financial Project (grant number 2019TS08); Key Research and Development Program of Zhejiang Province (grant number 2021C02043); the Forestry special fund of Zhejiang Provincial Forestry Bureau (grant number lqly2020-03); Forestry Scientific Research Extension Foundation of Zhejiang Province (grant number 2019B04); and the Public Beneficial Program of Zhejiang Province (grant number 2015C32096).

Jian Yang, China Academy of Chinese Medical Sciences, and Xiao-hui Fan, Institute of Genetics and Developmental Biology, helped to determine the content of flavonoid compounds. Yi-qing Xu, Zhejiang A&F University, helped in transmittance determination. Xue-Qian Wu and Bing-Song Zheng provided insight into the experimental design and chemical analysis.

Y.B. and W.C. contributed equally to this work. Y.B. and B.L. designed the research; W.C., L.X. Z.L., and S.L. performed the experiments and analyzed the data; W.C. wrote the manuscript; Y.B., B.L., and S.L. revised the manuscript.

Y.B. is the corresponding author. E-mail: hzbaiyan@zafu.edu.cn.

This is an open access article distributed under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

Table 1. High-performance liquid chromatography chromatogram of *Tetrastigma hemsleyanum* flavonoids in leaf and root.

Chemical compound	Regression equation	r value	Linear range (μg/mL)
Naringin	$y = 3,972,039.5x + 64,183.6$	0.9976	0.010–0.500
Isorhamnetin 3-O-glucoside	$y = 8,988,517.2x + 5,992,231.1$	0.9982	0.020–1.000
Orientin	$y = 9,071,337.1x + 33,093.6$	0.9996	1.00–100.0
Isoorientin	$y = 4,845,698.3x + 4,222,807.8$	0.9987	1.00–100.0
Astragalin	$y = 2,210,508.5x + 2,507,321.4$	0.9986	0.010–0.500
Luteoloside	$y = 3,168,802.6x + 166,068.3$	0.9994	0.10–5.00
Isovitexin	$y = 8,542,235.9x + 18,454,664.6$	0.9987	20.0–2000.0
Hesperidin	$y = 12,553,808.9x + 77,366.1$	0.9986	0.020–1.000
Calycosin 7-O-glucoside	$y = 22,042.7x + 2,463.8$	0.9986	0.020–1.000
Isoquercitrin	$y = 3,356,305.1x + 593,847.8$	0.9989	0.020–1.000
Eriodictyol	$y = 691,932.1x + 84,659.5$	0.9991	0.010–0.500
Hispidulin	$y = 207,676.0x + 105,248.9$	0.9993	0.020–1.000
Lonicerin	$y = 17,697.4x + 3,389.0$	0.9998	0.10–5.00

of PAL and CHS activity, but yellow and green lights showed negative effects on these activities. Yellow light had a facilitating effect on CHI activity, similar to that of blue and white lights. On the contrary, CHI activity under green light was lower than that for all other lights except red light.

Three soluble substances are affected under RF and BF. The greatest reducing sugar content was found in the CK-W group (12.82 mg/g) and the lowest content was found in the GF group (7.23 mg/g) among all films (Fig. 4A).

Light quality also affected amino acid content (Fig. 4B), with the greatest content observed in the BF treatment group (0.89 μg/g), which was significantly greater than that in the other groups. The lowest content was found in the GF treatment group (0.79 μg/g), whereas in the RF and CK-W treatment groups, it was found to be 0.81 μg/g and 0.82 μg/g, respectively. These results indicate that blue light promotes the accumulation of amino acids, whereas green and red lights show the opposite effects.

Soluble protein content was the greatest in the CK-W treatment group among all five groups (Fig. 4C), followed by the GF treatment group (1.96 μg/g). The lowest soluble protein content was found in the BF treatment group (1.55 μg/g), followed by the RF treatment group (1.66 μg/g). Overall, BF treatment had a marked influence on *T. hemsleyanum*.

Discussion

Light is an important factor that influences growth and development in plants; lights of short wavelengths affect morphological structure and metabolic synthesis in plants. In

particular, blue light improves growth in shade-tolerant herbs (Lv et al., 2016; Zhang et al., 2020). Wang et al. (2019b) and Liu et al. (2013) reported that blue light improves growth and biomass accumulation in *Bletilla striata* and *Anoectochilus roxburghii*, and blade area in leaves of *A. roxburghii*. Kim et al. (2014) reported that blue light increases expansin gene expression (genes reported to control expansin proteins), which leads to a significant increase in stem length and plant height in cherry tomato (*Solanum lycopersicum* L. 'Cuty'). Our study indicated similar effects; blue light had a positive effect on maximum blade width, maximum blade length, and fresh root weight.

Light also affects the accumulation of flavonoid compounds (Brunetti et al., 2018), such as stimulating enzyme activity involved in metabolic pathways of flavonoid synthesis. In particular, blue light enhances the production of many flavonoid compounds (Taulavuori et al., 2016). A study by Cheng et al. (2018) showed that red light increases total flavonoid content in *T. hemsleyanum*. PAL and CHS are two major enzymes associated with flavonoid synthesis pathways (Liu et al., 2006). In *Cyclocarya paliurus*, PAL and CHS enzyme activity correlates significantly and positively with flavonoid content, which can be increased by blue and red lights (Liu et al., 2018b). Similar results have been reported for *A. roxburghii* and *Polygala tenuifolia* Willd (Peng et al., 2018; Wang et al., 2018c).

Red and blue lights have been reported to increase PAL enzyme activity (Liang et al., 2017; Wang et al., 2007). Our results were similar to these findings: red light showed the maximum positive effect on PAL enzyme activity in *T. hemsleyanum*, followed by blue

light, thereby increasing the accumulation of flavonoid compounds.

CHS enzyme activity also showed a similar trend, except for CHS ARP and CHS SRP activity. CHS gene expression is controlled by different light treatments and plays an important role in regulating the metabolic pathways in flavonoid synthesis (Zhao et al., 2017), and the overall expression of CHS family genes regulates the production of flavonoids (Wang et al., 2018d). In *Arabidopsis thaliana*, blue light receptor cryptochromes (CRY1 and CRY2) respond to blue light; thus, blue light upregulates CHS gene expression, which promotes flavonoid synthesis and accumulation in the plant (Lin, 2009; Wade et al., 2001). Blue light could trigger CRY1/CRY2-COP1 interaction to enable regulatory factors SmHY5 and SmMYB1 to combine with CHS genes (Jiang et al., 2016). Our results further confirmed that red and blue lights could promote CHS activity.

CHI is the first enzyme in flavonoid isomerization and usually controls flavonoid isomerization in metabolic pathways (Jiang et al., 2015; Lim and Li, 2017). However, there is not enough information about the light-regulated mechanism of CHI genes in flavonoid biosynthesis in *T. hemsleyanum* and other plants. Total flavonoid content is increased significantly by blue light in *Erigeron breviscapus* (Su et al., 2006). The flavonoids rutin and quercetin accumulate more under blue light in *Fagopyrum esculentum* Moench, whereas the two flavonoids accumulate more under red light in *Fagopyrum tataricum* (L.) Gaertn (Lee et al., 2014) than under other lights. In our research findings, RF and GF showed inhibitory effects on CHI activity, but BF showed promoting effects. Orientin, isoorientin, lonicerin, and luteoloside contents under RF and BF were greater than the other treatments. By the comprehensive consideration of the enzymes (PAL, CHS, and CHI), red and blue lights might promote the accumulation of flavonoid compounds by inducing promoters of flavonoid synthesis genes that regulate these enzymes.

Furthermore, soluble substance (reducing sugar, soluble amino acid, and soluble protein) contents were affected dramatically by using color films. These contents changed to reduce stress damage, and flavonoid biosynthesis is related to environmental stress. Thus, soluble substances reflected the degree of plant stress and predicted the synthesis of flavonoids. Replicates cultivated under white light had a greater reducing sugar content, but green light had the opposite trend. Zheng

Table 2. The effects of different color filters on increments of *Tetrastigma hemsleyanum*.

Treatment group	Stem length (cm)	Maximum leaf length (cm)	Maximum leaf width (cm)	Specific leaf wt (g)	Fresh root wt (g)
CK-W	37.60 ± 6.09 Aab	4.97 ± 0.21 Bb	2.53 ± 0.15 ABa	2.41 ± 0.48 Ab	10.74 ± 1.30 Bb
RF	35.47 ± 2.02 Aab	4.33 ± 0.25 Cc	2.03 ± 0.12 Db	3.47 ± 1.19 Aab	3.66 ± 0.75 Dc
YF	31.93 ± 2.50 Ab	4.67 ± 0.12 BCb	2.30 ± 0.10 Cc	2.80 ± 0.31 Aab	7.55 ± 0.81 Cb
GF	36.93 ± 1.71 Aab	4.73 ± 0.15 BCb	2.46 ± 0.06 BCc	3.31 ± 1.10 Aab	9.86 ± 1.21 Bb
BF	48.53 ± 9.84 Aa	5.80 ± 0.10 Aa	2.77 ± 0.06 Aa	3.51 ± 0.42 Aa	12.78 ± 1.06 Aa

Different capital letters indicate a significant difference at the 0.01 level; different lowercase letters indicate a significant difference at the 0.05 level.

CK-W = colorless plastic film, control group; RF = red film; YF = yellow film; GF = green film; BF = blue film.

Fig. 2. Flavonoid contents responded to color filters in leaves of *Tetrastigma hemsleyanum*. (A) Isovitexin. (B) Orientin. (C) Isoorientin. (D) Luteoloside. (E) Isoquercitrin. (F) Lonicerin. (G) Hesperidin. (H) Astragalin. (I) Naringin. (J) Hispidulin. (K) Isorhamnetin-3-*O*-glucoside. Different lowercase letters indicate a significant difference at the 0.05 level ($P < 0.05$). CK-W, colorless plastic film, control group; RF, red film; YF, yellow film; GF, green film; BF, blue film.

Fig. 3. (A) Phenylalanine ammonia lyase (PAL), (B) chalcone synthase (CHS), and (C) chalcone isomerase (CHI) activity in leaves of *Tetrastigma hemsleyanum*. Different lowercase letters indicate a significant difference at the 0.05 level ($P < 0.05$). CK-W, colorless plastic film, control group; RF, red film; YF, yellow film; GF, green film; BF, blue film; SRP, spring rapid growth period; SDP, summer dormancy period; ARP, autumn rapid growth period.

Fig. 4. The effects of different color filters on soluble substances in *Tetrastigma hemsleyanum*. (A) Reducing sugar content. (B) Soluble amino acid content. (C) Soluble protein content. Different lowercase letters indicate a significant difference at the 0.05 level ($P < 0.05$). CK-W, colorless plastic film, control group; RF, red film; YF, yellow film; GF, green film; BF, blue film.

et al. (2016) and Liu et al. (2014) reported similar results with green light with regard to the accumulation of reducing sugar in *Lycopersicon esculentum* Mill. and *Curcuma longa*. Our results showed that BF could increase soluble amino acid content significantly, but reduce soluble protein content, similar to many previous studies. The studies of Ban et al. (2019) and Lafuente et al. (2021) showed that blue light can increase soluble amino acid content significantly in *Pisum sativum* Linn. sprouts and *Toona sinensis* (A. Juss.) Roem sprouts, respectively. Xie et al. (2018) reported that blue light decreased soluble protein content in *Heuchera micrantha* Douglas ex Lindl. 'Palace Purple'. A study by Li et al. (2017) showed that blue light, which is one of the environmental triggers, can decrease soluble protein content by the upregulation of genes involved in microtubules, chlorophyll synthesis, and sugar degradation, and by the downregulation of auxin-repressed protein. Some studies confirmed that plants can increase or decrease the soluble substance content to adjust osmosis pressure to prevent damage caused by environmental triggers (Wang et al., 2019c; Zhao et al., 2015). A certain degree of plant damage may induce flavonoid synthesis (Wang et al., 2019d), along with soluble substances.

Color film enhances the utilization ratio of light and increases fruit quality in agricultural production (Wang et al., 2018d), including Chinese traditional herb production. In addition, color film is made of plastic, which is cheap and easily available, and can be used on large scale to improve quality in the developing herb industry. In our study, the use of RF increased flavonoid content, and BF improved growth and enzyme activities, thereby improving the medicinal value of *T. hemsleyanum*.

Conclusion

Conclusively, this study shows that BF promotes plant growth and increases yield, whereas RF enhances some secondary metabolites and increases the content of five flavonoid compounds (isovitexin, orientin, isoorientin, lonicerin, and astragaline). These findings provide a future perspective for *T. hemsleyanum* cultivation at a large scale.

Literature Cited

- Ban, T.T., X.H. Li, and C. Ma. 2019. Effect of light quality on the growth and quality of *Pisum sativum* Linn. Sprouts North Hort. 43(13):77–82, doi: 10.11937/bfy.20183820.
- Brunetti, C., A. Fini, F. Sebastiani, A. Gori, and M. Tattini. 2018. Modulation of phytohormone signaling: A primary function of flavonoids in plant-environment interactions. Front. Plant Sci. 9(20):1042, doi: 10.3389/fpls.2018.01042.
- Chen, H., S.B. Liao, D. Chen, P. Xie, and J. Huang. 2017. Acute toxicity tests of anti-inflammatory extracts of *Tetrastigma hemsleyanum*'s aerial part. J. Fujian Med. Univ. 51(5):287–290, doi: 10.3969/j.issn.1672-4194.2017.05.004.
- Chen, J.H., L. Tao, and J. Li. 2003. Guide for the experiments of biochemistry. Science Press, Beijing, China. (in Chinese).
- Cheng, X.Y., Z.L. Yang, X. Yang, and M. Tan. 2018. Effects of light quality on the growth and content of active ingredients of *Tetrastigma hemsleyanum*. For. Res. 31(5):98–103, doi: 10.13275/j.cnki.lykxyj.2018.05.013.
- Ding, F.J., J.T. Liu, R.K. Du, Q.H. Yu, L.L. Gong, H.Q. Jiang, R. Rong, and D.F. Vincenzo. 2019. Qualitative and quantitative analysis for the chemical constituents of *Tetrastigma hemsleyanum* Diels et Gilg using ultra-high performance liquid chromatography/hybrid quadrupole-orbitrap mass spectrometry and preliminary screening for anti-influenza virus components. Evid. Based Complement. Alternat. Med. 2019: 9414926, doi: 10.1155/2019/9414926.
- Dong, F., C.Z. Wang, Y.Q. Ren, X.Z. Zhang, L.X. Wang, and S.Q. Liu. 2018. Effects of light qualities on sugar contents, activities and gene expression of related enzymes involved in sugar metabolism of tomato fruit. Plant Physiol. J. 54(9):1507–1515, doi: 10.13592/j.cnki.ppj.2017.0534.
- Gao, Z.W. and W.N. Fang. 2014. *Radix tetrastigma* facility and cultivation method. China Patent CN103988699A, filed 30 May 2014, and issued 20 Aug. 2014.
- Hu, X.T., S.Z. Liu, Y. Bai, L.Y. Xu, H. Ding, X.Q. Wu, H.S. Xu, and B.S. Zheng. 2019. Effects of different shading treatments on physiology, biochemistry and total flavonoids of *Tetrastigma hemsleyanum* in Zhejiang Province. Guihaia 39(7):925–932, doi: 10.11931/guihaia.gxzw201803056. (in Chinese, with English abstract).
- Hu, W.Y., Y.J. Zheng, P.G. Xia, and Z.S. Liang. 2021. The research progresses and future prospects of *Tetrastigma hemsleyanum* Diels et Gilg: A valuable Chinese herbal medicine. J. Ethnopharmacol. 271:113836, doi: 10.1016/j.jep.2021.113836.
- Jiang, M.M., L. Ren, H.L. Lian, Y. Liu, and H. Chen. 2016. Novel insight into the mechanism underlying light-controlled anthocyanin accumulation in eggplant (*Solanum melongena* L.). Plant Sci. 249:46–58, doi: 10.1016/j.plantsci.2016.04.001.
- Jiang, W.B., Q.G. Yin, R.R. Wu, G.S. Zheng, J.Y. Liu, A.D. Richard, and Y.Z. Pang. 2015. Role of a chalcone isomerase-like protein in flavonoid biosynthesis in *Arabidopsis thaliana*. J. Expt. Bot. 66(22):7165–7179, doi: 10.1093/jxb/erv413.
- Jin, X.X., R.G. Aaron, F.S. Muhammad, S.Y. Li, F. Timothy, and J.K. Wang. 2020. Plastic film mulching and nitrogen fertilization enhance the conversion of newly-added maize straw to water-soluble organic carbon. Soil Tillage Res. 197:104527, doi: 10.1016/j.still.2019.104527.
- Kim, E.Y., S.A. Park, B.J. Park, Y. Lee, and M.M. Oh. 2014. Growth and antioxidant phenolic compounds in cherry tomato seedlings grown under monochromatic light-emitting diodes. Hort. Environ. Biotechnol. 55(6):506–513, doi: 10.1007/s13580-014-0121-7.
- Lafuente, M.T., P. Romero, and A.R. Ballester. 2021. Coordinated activation of the metabolic pathways induced by LED blue light in citrus fruit. Food Chem. 341(1):128050, doi: 10.1016/j.foodchem.2020.128050.
- Lee, S.W., J.M. Seo, M.K. Lee, J.H. Chun, A. Paulrayer, V.A. Mariadhas, S. Tatsuro, A.A. Naif, and S.J. Kim. 2014. Influence of different LED lamps on the production of phenolic compounds in common and Tartary buckwheat sprouts. Ind. Crops Prod. 54:320–326, doi: 10.1016/j.indcrop.2014.01.024.
- Li, C.X., Z.G. Xu, R.Q. Dong, S.X. Chang, L.Z. Wang, M. Khalil-Ur-Rehman, and J.M. Tao. 2017. An RNA-Seq analysis of grape plantlets grown in vitro reveals different responses to blue, green, red LED light, and white fluorescent light. Front. Plant Sci. 8:78, doi: 10.3389/fpls.2017.00078.
- Li, H.S. 2000. Principles and experimental techniques of plant physiology and biochemistry. Higher Education Press, Beijing, China. (in Chinese).
- Li, J.F., C.Y. Yi, C.R. Zhang, F. Pan, C. Xie, W.Z. Zhou, and C.F. Zhou. 2021. Effects of light quality on leaf growth and photosynthetic fluorescence of *Brasenia schreberi* seedlings. Heliyon 7(1): E06082, doi: 10.1016/j.heliyon.2021.e06082.
- Liang, D., H. Xu, H.C. Qin, G. Fan, X. Wang, and D.Y. Zhang. 2017. Cloning and transformation of the *PAL2-3* gene in soybean (*Glycine max* L.). Soybean Sci. 36(4):508–513, doi: 10.11861/j.issn.1000-9841.2017.04.0508.

- Lim, W.S. and J.R. Li. 2017. Synergetic effect of the onion CHI gene on the PAP1 regulatory gene for enhancing the flavonoid profile of tomato skin. *Sci. Rep.* 7(1):316–326, doi: 10.1038/s41598-017-12355-x.
- Lin, J.Z. 2009. Functional analysis of *Arabidopsis* 4CL3 gene in flavonoid biosynthesis, p. 99–102. Hunan University, Hunan, China, doi: 10.7666/d.y1725482.
- Liu, C.J., Y.Y. Yang, N. Wang, and P. Yu. 2018a. Optimization of detection conditions of amino acids in okra by ninhydrin colorimetric method. *China Food Addit.* 1:187–193, doi: 10.3969/j.issn.1006-2513.2018.01.020. (in Chinese, with English abstract).
- Liu, J.F., M.Y. Wang, Y.J. Tang, Y.P. Fan, S.C. Zhong, and Q. Chen. 2014. Effects of light qualities on physiological characteristics and accumulation of secondary metabolites in rhizomes of *Curcuma longa* L. *Plant Physiol.* 50(12):1871–1879, doi: 10.13592/j.cnki.pppj.2014.0446. (in Chinese, with English abstract).
- Liu, M.L., M.H. Su, D.M. Pan, and W. Wang. 2013. The effect of different LED light on the growth of *Anoectochilus roxburghii*. *Subtrop. Plant Sci.* 42(1):46–48, doi: 10.3969/j.issn.1009-7791.2013.01.011.
- Liu, R.R., S.H. Xu, J.L. Li, Y.L. Hu, and Z.P. Lin. 2006. Expression profile of a PAL gene from *Astragalus membranaceus* var. *Mongolicus* and its crucial role in flux into flavonoid biosynthesis. *Plant Cell Rep.* 25(7):705–710, doi: 10.1007/s00299-005-0072-7.
- Liu, Y., S. Fang, W. Yang, X. Shang, and X. Fu. 2018b. Light quality affects flavonoid production and related gene expression in *Cyclocarya paliurus*. *J. Photobiol.* 179:66–73, doi: 10.1016/j.jphotobiol.2018.01.002.
- Lv, Z.C., L.L. Ye, and Y.H. Peng. 2016. Effects of blue LEDs with different wavelength on the growth of tissue culture seedlings of *Anoectochilus formosanus* Hayata. *J. Zhongkai Univ. Agr. Technol.* 29(4):1–6, doi: 0.3969/j.issn.1674-5663.2016.04.001.
- Miao, L.X., Y.C. Zhang, X.F. Yang, J.P. Xiao, H.Q. Zhang, Z.F. Zhang, Y.Z. Wang, and G.H. Jiang. 2016. 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, doi: 10.1016/j.foodchem.2016.02.077.
- Peng, L., T. Zhao, B.Y. Yang, Y.R. An, T. Huang, T. Sun, A.P. Liu, M. Wang, and B.X. Hu. 2018. Effects of light quality and intensity on growth, related enzymes activity, and components of *Polygala tenuifolia*. *Chin. Tradit. Herbal Drugs* 49(21):5004–5009, doi: 10.7501/j.issn.0253-2670.2018.21.008. (in Chinese, with English abstract).
- Su, W.H., G.F. Zhang, X.H. Li, F.X. Gu, and B.L. Shi. 2006. Effect of light intensity and light quality on growth and total flavonoid accumulation of *Erigeron breviscapus*. *Chin. Tradit. Herbal Drugs* 37(8):1244–1247, doi: 10.3321/j.issn:0253-2670.2006.08.051. (in Chinese, with English abstract).
- Tautilavuori, K., V. Hyöky, J. Oksanen, E. Tautilavuori, and J. Julkunen-Tiitto. 2016. Species-specific differences in synthesis of flavonoids and phenolic acids under increasing periods of enhanced blue light. *Environ. Exp. Bot.* 121:145–150, doi: 10.1016/j.envexpbot.2015.04.002.
- Wade, H.K., T.N. Bibikova, W.J. Valentine, and G.I. Jenkins. 2001. Interactions within a network of phytochrome, cryptochrome and UV-B phototransduction pathways regulate chalcone synthase gene expression in *Arabidopsis* leaf tissue. *Plant J.* 25(6):675–685, doi: 10.1046/j.1365-313x.2001.01001.x.
- Wang, D.D., R. Gao, and B. Yan. 2019a. Experimental study on the effects of Radix *Tetrastigma* on respiratory syncytial virus. *Nat. Prod. Res. Dev.* 31(6):1070–1074, doi: 10.16333/j.1001-6880.2019.6.022. (in Chinese, with English abstract).
- Wang, F., Q. Wang, and X. Zhao. 2019c. Research progress of phenotype and physiological response mechanism of plants under low temperature stress. *Mol. Plant Breed.* 17(15):5144–5153, doi: 10.13271/j.mpb.017.005144. (in Chinese, with English abstract).
- Wang, J.R., Q. Yi, Q.Q. Tie, L.X. Peng, and G. Zhao. 2019d. Effects of different light quality on flavonoids and antioxidant activity of Tartary buckwheat sprout. *Food Sci. Technol.* 44(5):213–218, doi: 10.13684/j.cnki.spkj.2019.05.039. (in Chinese, with English abstract).
- Wang, L., L.L. Shi, and Y.J. Liu. 2007. Effects of different light treatments on growth and PAL activity of the suspension-cultured cells of *Rhodiola fastigiata*. *Sci. Silvae Sin.* 43(6):52–56, doi: 10.3321/j.issn:1001-7488.2007.06.009. (in Chinese, with English abstract).
- Wang, T., X.R. Tian, X.Y. Wu, Z. Luo, G. Li, X.L. Peng, and S.B. Liu. 2018b. Effect of light quality on total gypenosides accumulation and related key enzyme gene expression in *Gynostemma pentaphyllum*. *Chin. Herb. Med.* 10(1):34–39, doi: 10.1016/j.chmed.2017.12.004.
- Wang, T.T., Z. Zhan, J. Ma, Y.Q. Chen, and Y. Li. 2019b. Effects of light quality on growth and photosynthetic characteristics of *Bletilla striata* in vitro. *Guihaia* 1–8. 8 Apr. 2021. <http://kns.cnki.net/kcms/detail/45.1134.Q.20190729.1529.012.html>. (in Chinese, with English abstract).
- Wang, W., M.H. Su, H.H. Li, B.Y. Zeng, Q. Chang, and Z.X. Lai. 2018c. Effects of supplemental lighting with different light qualities on growth and secondary metabolite content of *Anoectochilus roxburghii*. *PeerJ* 6:e5274, doi: 10.7717/peerj.5274.
- Wang, Y., W. Jiang, W. Ye, C. Fu, A.G. Matthew, S.S. Pamela, E.S. Douglas, and Y. Qiu. 2018a. Evolutionary insights from comparative transcriptome and transcriptome-wide coalescence analyses in *Tetrastigma hemsleyanum*. *BMC Plant Biol.* 18(1):208, doi: 10.1186/s12870-018-1429-8.
- Wang, Z.B., Q.B. Yu, W.X. Shen, C.A. El Mohtar, X.C. Zhao, and F.G. Gmitter. 2018d. Functional study of CHS gene family members in citrus revealed a novel CHS gene affecting the production of flavonoids. *BMC Plant Biol.* 18(1):189, doi: 10.1186/s12870-018-1418-y.
- Wang, Z.F., Z.R. Yang, Y.W. Zhang, Q.L. Li, and Y.F. Zhao. 2017. Study on effect of total flavonoids from Radix *Tetrastigma* on human hepatocellular carcinoma HepG-2 cells and xenograft tumor in nude mice. *Chin. Arch. Tradit. Chin. Med.* 35(10):2615–2620, doi: 10.13193/j.issn.1673-7717.2017.10.038. (in Chinese, with English abstract).
- Wu, H.Y., Y.T. Wu, Y.Y. Chen, H.L. Luo, and H.F. Jiang. 2019. Effect of Radix *Tetrastigma hemsleyanum* flavone on apoptosis and MAPK signaling pathway in myeloid leukemia NB-4 cells. *Chin. J. Pathophysiol.* 35(8):1451–1456, doi: 10.3969/j.issn.1000-4718.2019.08.016. (in Chinese, with English abstract).
- Xie, M.M., Y. Sun, L.Q. Yin, J. Wu, X.Q. Tuo, Q. Shen, and Y.C. Zhang. 2018. Effects of different LED light qualities on growth and physiological characteristics of *Heuchera* spp. plantlets. *Mol. Plant Breed.* 16(6):2001–2008, doi: 10.13271/j.mpb.016.002001. (in Chinese, with English abstract).
- Xu, L.Y., S.Z. Liu, Y. Bai, H. Ding, X.T. Hu, X.Q. Wu, H.S. Xu, and B.S. Zheng. 2018. Effects of light intensity treatments on photosynthetic characteristics in *Tetrastigma hemsleyanum*. *J. Zhejiang A&F Univ.* 35(3):467–475, doi: 10.11833/j.issn.2095-0756.2018.03.010. (in Chinese, with English abstract).
- Zhang, L.L., S.J. Sun, Z.J. Chen, H. Jiang, X.D. Zhang, and C.C. Dao. 2018. Effects of different colored plastic film mulching and planting density on dry matter accumulation and yield of spring maize. *J. Appl. Ecol.* 29(1):113–124, doi: 10.13287/j.1001-9332.201801.019. (in Chinese, with English abstract).
- Zhang, S.C., J.Q. Ma, H.Y. Zou, L. Zhang, S.H. Li, and Y.P. Wang. 2020. The combination of blue and red LED light improves growth and phenolic acid contents in *Salvia miltiorrhiza* Bunge. *Ind. Crops Prod.* 158:112959, doi: 10.1016/j.indcrop.2020.112959.
- Zhang, Y., J. Yang, Y.Y. Zhao, G. Yang, Y. Yuan, and Y.P. Liu. 2017. Contents determination of nine vitamin in four kinds of suspension cells of Xuelian by UPLC-MS/MS. *Mod. Chin. Med.* 19(12):1697–1701, doi: 10.13313/j.issn.1673-4890.2017.12.008. (in Chinese, with English abstract).
- Zhao, X., Y. Shi, Y. Liu, R.L. Jia, and X.R. Li. 2015. Osmotic adjustment of soil biocrust mosses in response to desiccation stress. *Pedosphere* 25(3):459–467, doi: 10.1016/S1002-0160(15)30013-8.
- Zhao, X.R., Y. Yang, X.P. Luo, P.F. Yao, A.H. Wang, H.X. Zhao, and Q. Wu. 2017. Cloning and analysis of chalcone synthase gene FtCHS1 promoter in *Fagopyrum tataricum* Gaertn. *Plant Sci. J.* 35(4):543–550, doi: 10.11913/PSJ.2095-0837.2017.40543. (in Chinese, with English abstract).
- Zheng, D.M., Z.B. Lin, Y.Q. Chen, and B.Y. Lin. 2016. Effect of different light quality on the yield and quality of cherry tomato. *J. Shanxi Agr. Univ. Nat. Sci. Educ.* 36(8):567–571, doi: 10.13842/j.cnki.issn1671-8151.2016.08.007. (in Chinese, with English abstract).
- Zhou, B.Y., J.Q. Wang, H.B. Zhang, H.H. Shi, Y.F. Fei, S.Y. Huang, Y.Z. Tong, D.S. Wen, Y.M. Luo, and B. Damià. 2020. Microplastics in agricultural soils on the coastal plain of Hangzhou Bay, East China: Multiple sources other than plastic mulching film. *J. Hazard. Mater.* 388:121814, doi: 10.1016/j.jhazmat.2019.121814.
- Zhu, B.Q., C.D. Qian, F.M. Zhou, J.J. Guo, N.P. Chen, C.X. Gao, B. Jin, and Z.S. Ding. 2020. Antipyretic and antitumor effects of a purified polysaccharide from aerial parts of *Tetrastigma hemsleyanum*. *J. Ethnopharmacol.* 253, doi: 10.1016/j.jep.2020.112663.