Heliyon 10 (2024) e26814

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

# Creating a new yellow and blue combination transparent film for *Panax ginseng* C.A. Meyer growth based on orthogonal designs

Zhenghai Zhang [a,*], Huixia Lei [a], Peihe Zheng [a], Yayu Zhang [b], Hai Sun [b], Cai Shao [b], Jingjing Zhao [c]

[a] *Jilin Agricultural Science and Technology University, Jilin, Jilin, 132109, China*
[b] *Institute of Special Animal and Plant Sciences of the Chinese Academy of Agricultural Sciences, Changchun, Jilin, 130112, China*
[c] *Changchun University, Changchun, Jilin, 130022, China*

A R T I C L E  I N F O

*Keywords:*
Chlorophyll fluorescence
Photosynthetic rate
Leaf area
Dry root weight
Ginsenoside content

A B S T R A C T

*Panax ginseng* C.A. Meyer originates from old-growth forest environments, where the light intensity and spectrum reaching the forest bed are influenced by the canopy and humidity. In farmlands, suitable light intensity for cultivation is achieved by controlling the light transmission rate using shading nets, while light quality is regulated by a cover of yellow or blue transparent film. Such films have a light quality distinct from that produced by old-growth forests. Herein, a large composite film was developed by alternating small pieces of yellow and blue transparent film. An orthogonal array was used to evaluate the influence of the small transparent film area (STFA), yellow transparent film (YTF) number, and blue transparent film (BTF) number on the associated changes in ginseng in a range of fluorescence-, photosynthesis-, morphology-, and crop quality-related factors. Our results showed that light intensity was influenced primarily by STFA, which caused an overall decrease, while the light quality ratio was affected primarily by YTF number, which increased the proportion of red light and decreased that of blue light, with corresponding influence on different growth parameters. Based on these observations, an improved yellow and blue combination transparent film (YBCTF) with the following characteristics was established: STFA: 15 × 15 cm, YTF: two pieces, and BTF: three pieces. The improved YBCTF facilitated efficient light energy use by the plants, and led to an increase in leaf area, the per leaf photosynthetic rate, dry root weight, and the per root single ginsenoside yield. The findings present a relatively low-cost approach for optimising the light environment of ginseng cultivated in farmland and other crops in large-scale agricultural settings.

## 1. Introduction

Wild ginseng (*Panax ginseng* C.A. Meyer) grows in old-growth forests, where tall trees, low shrubs, and herbage provide shade, and the high humidity refracts the sunlight; therefore, the light intensity and quality of sunlight are altered when they reach the ginseng leaf surface, and ginseng has adapted to such light environments for its growth [1,2].

A suitable light intensity for cultivated ginseng in farmland is achieved by controlling the light transmission rate through a black shading net in accordance with the latitude and season [3]. The light quality is regulated by a cover of yellow transparent film (YTF) or

* Corresponding author. No. 1. Xuefu Rd, Changyi Dist., Jilin City, Jilin Prov, China.
*E-mail address:* 258473227@qq.com (Z. Zhang).

https://doi.org/10.1016/j.heliyon.2024.e26814
Received 13 June 2023; Received in revised form 19 February 2024; Accepted 20 February 2024
Available online 25 February 2024

blue transparent film (BTF). However, such films produce a specific spectrum when sunlight passes through them: red light dominates the spectrum produced by YTF, and blue light dominates that produced by BTF, which are distinct to that in old-growth forests. BTF leads to decreased ginseng stem height and leaf area, but increased fresh root weight, while YTF induces the opposite effects [4]. Similar results have been obtained for ginseng treated with red and blue light-emitting diodes (LEDs); however, monochromatic red light causes abnormal leaf development, leaf senescence, and dysfunctional photosynthesis. These phenomena could be prevented by increasing the blue light proportion to 25%. The maximum leaf area, stem and root weight, and ginsenoside content of roots and leaves could be achieved using a mixed proportion of 50% red and 50% blue light [5]. Additionally, optimising of the light spectrum can facilitate a more resilient response to stress and partially mitigate the deleterious effects on plant photosynthesis [6].

Considering application of a suitable light spectrum can promote plant growth, improve product quality, and mitigate stress factors, a large composite film was developed in the present study by alternating combinations of small pieces of YTF and BTF. An orthogonal array was used to evaluate the influence of small transparent film area (STFA), yellow transparent film number (YTFN), and blue transparent film number (BTFN) on the associated fluorescence- [7], photosynthesis-, morphology-, and ginseng quality-related factors. Finally, an improved yellow and blue combination transparent film (YBCTF) that can provide optimal light conditions for cultivated ginseng was established. Considering plastic and glass-covered greenhouses that depend on sunlight as the primary light source are used extensively in vegetable, fruit, and flower production worldwide [8], our low-cost method was designed to also be utilised in such settings, to optimise the efficiency of light energy utilisation and plant growth, with potentially far-reaching benefits for agriculture.

## 2. Materials and methods

### 2.1. Experimental design

This experiment was conducted on open land of the Medicinal Botanical Garden of the Jilin Agricultural Science and Technology University, and the evaluation and validation experiments were conducted in 2020 and 2021, respectively. The evaluation experiment was designed as an $L_9(3^4)$ orthogonal array. The STFA was the first factor, with three levels: A1 (3 × 3 cm), A2 (7 × 7 cm), and A3 (15 × 15 cm). The levels of both the yellow (YTFN, factor 2) and blue transparent film number (BTFN, factor 3) were: Y1 and B1 (one piece), Y2 and B2 (two pieces), and Y3 and B3 (three pieces). These small pieces of YTF or BTF were arranged evenly according to the design number. In the validation experiment, the improved YBCTF was used as the test treatment, with the traditional YTF and BTF as controls. Each treatment included three randomly arranged plots, with samples obtained randomly from five points in each plot, with the average value of the samples taken as a replicate.

### 2.2. Plant materials and cultivation methods

In early May, the ungerminated roots of three-year-old ginseng (average root weight: 12.63 ± 0.43 g) were planted in a north-south orientation bed, 140 cm wide and 25 cm high, with a planting density of 10 × 20 cm. The roots were covered with 3 cm of soil and then with straw to preserve moisture. The bed was divided into 200 × 140 cm treatment plots and covered with a 150-cm high, arched frame, covered by YTF, BTF, or YBCTF. Finally, a black shading net was layered above the bed at a height of 200 cm, which blocked 70% of the incoming light.

### 2.3. Measurement indexes and methods

The photosynthetic photon flux density (PPFD) and spectrum were measured with a spectral analyser, HP330P (Hopoocoloryb, Hangzhou, China). The photosensitive measuring head was maintained in an upward position and at the same height as the leaf during measurement.

Maximum photochemical efficiency (Fv/Fm), actual photochemical efficiency [Y(II)], regulated thermal energy dissipation [Y(NPQ)], and the sum of non-regulated heat dissipation and fluorescence emission [Y(NO)] [9] were measured with IMAG-MIN/B (Heinz Walz GmbH, Effeltrich, Germany). The light source was a blue (450 nm) LED, the measuring light intensity was 0.05 μmol $m^{-2}$ $s^{-1}$, the actinic light intensity was 22 μmol $m^{-2}$ $s^{-1}$, and the dark-adaptation time was 15 min.

The photosynthetic rate (Pn) was measured using a GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany). The leaf chamber area was 3 $cm^2$, the gas flow rate was 750 μmol $s^{-1}$, and the gas mixer had its fan speed set at the seventh stage. In the evaluation experiment, the $CO_2$ concentration was set to 900 ppm, the temperature to 25 °C, and the relative humidity to 45%; these variables were unregulated in the validation experiment.

The measurement of chlorophyll fluorescence parameters and Pn was conducted from 9:00 a.m. to 12:00 p.m. on sunny days in late July (environmental temperature: 29.70 ± 1.55 °C, humidity: 45.90 ± 1.16%, light/dark photoperiod: 14:44/9:16 h), when the ginseng was in the green fruit stage. The relative chlorophyll content (Soil and Plant Analyser Development, SPAD) in the evaluation experiment was measured using the chlorophyll meter TYS-4N (Tuopuyunnong, Hangzhou, China), and the chlorophyll content in the validation experiment was determined using an assay kit (Cominbio, Suzhou, China). An image of a compound leaf's middle leaf was taken in mid-September, and leaf area was calculated using ImageJ 1.52v (National Institutes of Health, Bethesda, MA, USA).

In early October, when the leaves withered, the ginseng roots were dug out and washed, dried under natural processes, and weighed. They were subsequently ground and passed through a 60-mesh sieve. The total ginsenoside content was determined using ultraviolet spectrophotometry, according to the methodology in a previous report [10].

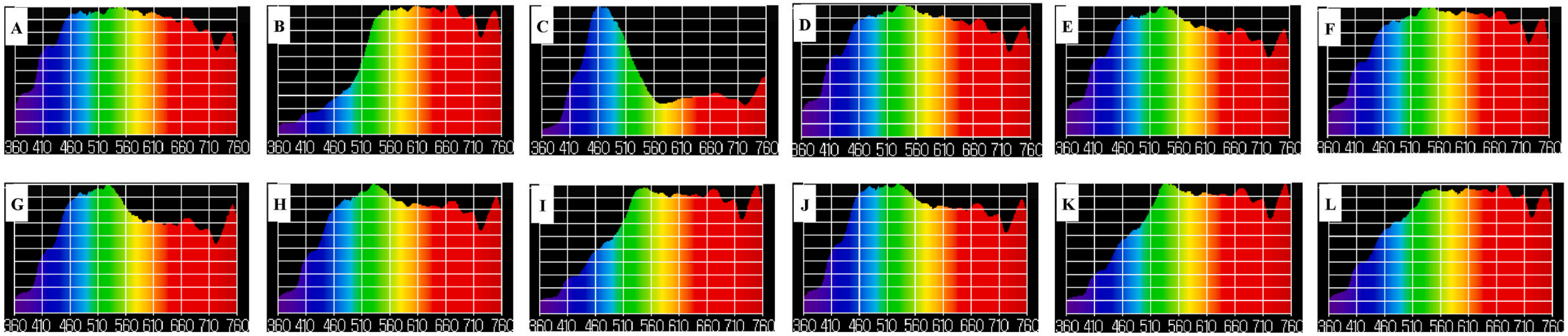


**Fig. 1.** Light characteristics of sunlight, yellow transparent film, blue transparent film, and yellow and blue combination transparent film. (A) Spectrum of solar light, (B) spectrum of light filtering through the yellow transparent film, (C) spectrum of light filtering through the blue transparent film, (D)–(L) spectrum of light filtering through combined transparent films. (D) 1 yellow and 1 blue (3 × 3 cm) piece, (E) 2 yellow and 2 blue (3 × 3 cm) pieces, (F) 3 yellow and 3 blue (3 × 3 cm) pieces, (G) 1 yellow and 2 blue (7 × 7 cm) pieces, (H) 2 yellow and 3 blue (7 × 7 cm) pieces, (I) 3 yellow and 1 blue (7 × 7 cm) piece, (J) 1 yellow and 3 blue (15 × 15 cm) pieces, (K) 2 yellow and 1 blue (15 × 15 cm) piece, and (L) 3 yellow and 2 blue (15 × 15 cm) pieces, respectively.

### 2.4. Statistical analysis

All data are presented as the mean ± standard deviation of three replicates. All analyses were conducted using SAS 8.01 (SAS Institute Inc., Cary, NC, USA). In the evaluation experiment, the contribution of each factor and its significance were estimated using analysis of variance (ANOVA). In the validation experiment, ANOVA was used to separate the influence of YBCTF, YTF, and BTF and estimate their significance. As all datasets were not normally distributed, and due to homogeneity of variance, the data were subjected to a non-parametric test and compared using Duncan's multiple range test ($P < 0.05$). The Pearson correlation coefficient was calculated to determine the relationships among the physiological, morphological, and quality parameters; a correlation plot was constructed with the Origin Pro software version 2022 (OrginLab Corporation, Northampton, MA, USA).

## 3. Results

### 3.1. Characteristics of sunlight, yellow transparent film, blue transparent film, and yellow and blue combination transparent film

There was a change in light intensity and spectrum composition after the sunlight filtered through the YTF, BTF, and YBCTF (Fig. 1). The sunlight intensity was 2166.45 ± 97.85 μmol $m^{-2}$ $s^{-1}$, with the proportions of blue and red light corresponding to 21.26 and 30.37%, respectively. The transmission light intensity of YTF was 203.41 ± 3.45 μmol $m^{-2}$ $s^{-1}$, and the proportions of blue and red light were 7.90 and 38.32%, respectively. Similarly, the transmission light intensity of BTF was 83.92 ± 1.91 μmol $m^{-2}$ $s^{-1}$, and the proportions of blue and red light were 37.51 and 19.00%, respectively.

STFA, followed by YTFN and then BTFN, significantly affected the light intensity of YBCTF. The transmission light intensity first decreased and then increased with an increase in STFA, but increased with YTFN and BTFN. The proportion of blue and red light was also markedly affected by YTFN, BTFN, and STFA, in this order of significance. The proportion of blue light decreased with an increase in STFA and YTFN and increased with BTFN. However, the proportion of red light showed the opposite trend (Tables 1 and 2).

### 3.2. Influence of different yellow and blue combination transparent film treatments on ginseng traits

Due to the difference in the light transmission rate and spectra between YTF and BTF, different combinations of STFA, YTFN, and BTFN had different effects on the light intensity and spectrum of YBCTF, with a corresponding influence on SPAD, chlorophyll fluorescence parameters, Pn, average dry root weight (ADRW), and total ginsenoside content (TGC; Table 3).

STFA significantly affected SPAD, Fv/Fm, Y(II), Y(NPQ), Pn, ADRW, and TGC without impacting Y(NO). The YTFN significantly affected Fv/Fm, Y(II), Y(NPQ), ADRW, and TGC, while it had no effect on SPAD or Y(NO). The BTFN had a significant influence on Y (II), Y(NPQ), ADRW, TGC, Y(NO), and Pn but did not impact SPAD or Fv/Fm. The order of influence of experimental factors on SPAD and Y(NPQ) was: STFA > BTFN > YTFN. Similarly, the order of influence on Fv/Fm, Y(II), Pn, ADRW, and TGC was: STFA > YTFN > BTFN. Interestingly, the order of the experimental factors' influence on Y(NO) displayed an opposite trend (Table 4).

A positive correlation was observed between an increase in STFA and increases in SPAD, Fv/Fm, Y(II), Pn, ADRW, and TGC, while Y (NPQ) and Y(NO) decreased. In a similar manner, Y(NPQ) and Y(NO) levels increased with an increase in the YTFN. Conversely, Y(II) and TGC appeared to decrease, while SPAD, Fv/Fm, Pn, and ADRW first increased before decreasing. An increase in the BTFN corresponded to an increase in SPAD, Y(NO), and TGC levels, with Fv/Fm and Y(II) also first increasing and then decreasing, while Y (NPQ), Pn, and ADRW levels first decreased before subsequently increasing. The optimal combinations of experimental factors for maximising the different growth parameters were: A3Y2B3 for SPAD, Pn, and ADRW, A3Y2B2 for Fv/Fm, A3Y1B2 for Y(II), A1Y3B1 for Y(NPQ), A3Y1B3 for TGC, and A3Y2B1for minimising the growth parameters of Y(NO) (Table 5).

For the eight inspection indexes, the levels which most frequently produced the best effects were: level A3 for STFA (seven times), level Y2 for YTFN (four times), and level B3 for BTFN (four times). Taken together, our results indicate that the treatment A3Y2B3 (STFA of 15 × 15 cm, two pieces of YTF, and three pieces of BTF) was the most conducive combination for optimal light energy utilisation, root dry weight increase, and ginsenoside accumulation in ginseng plants.

### 3.3. Validation of the improved yellow and blue combination transparent film

The light intensity of the improved YBCTF (123.32 ± 9.92 μmol $m^{-2}$ $s^{-1}$) was higher than that of BTF but lower than that of YTF (Fig. 2A). The proportion of blue- and red-light filtering through the improved YBCTF was 23.10 and 27.54%, respectively (Fig. 2B). By

**Table 1**
Order of influence and significance of STFA, YTFN, and BTFN on PPFD and the proportion of blue and red light under YBCTF.

| Factors | PPFD μ mol $m^{-2}$ $s^{-1}$ | | Blue light proportion % | | Red light proportion % | |
|---|---|---|---|---|---|---|
| | F value | *P* value | F value | *P* value | F value | *P* value |
| STFA | 145.49 | <0.0001 | 10.50 | 0.0010 | 7.15 | 0.0052 |
| YTFN | 30.61 | <0.0001 | 24.36 | <0.0001 | 38.45 | <0.0001 |
| BTFN | 4.09 | 0.0344 | 15.22 | 0.0001 | 13.59 | 0.0003 |
| *SE* | 3.48 | 0.0527 | 0.79 | 0.4703 | 1.63 | 0.2234 |

**Significance threshold**: $P < 0.05$.

**Table 2**
Trend of influence and significance of different levels of STFA, YTFN, and BTFN on light intensity (μmol $m^{-2}$ $s^{-1}$) and the proportion of blue and red light under YBCTF.

| Index | Level | Factor | | |
|---|---|---|---|---|
| | | STFA | YTFN | BTFN |
| PPFD | 1 | 356.50 a | 215.11 b | 226.12 b |
| | 2 | 162.62 c | 221.42 b | 249.40 ab |
| | 3 | 215.15 b | 297.75 a | 258.76 a |
| Blue light proportion | 1 | 18.99 a | 19.85 a | 15.13 b |
| | 2 | 16.83 b | 16.59 b | 18.03 a |
| | 3 | 15.96 b | 15.30 b | 18.57 a |
| Red light proportion | 1 | 31.36 b | 30.02 c | 33.15 a |
| | 2 | 31.66 b | 32.25 b | 31.54 b |
| | 3 | 32.82 a | 33.57 a | 31.14 b |

**Note**: Different lowercase letters indicate significant differences at $P < 0.05$.

**Table 3**
Influence of different treatments of YBCTF on ginseng PPFD, SPAD, chlorophyll fluorescence parameters, Pn, average dry root weight, and total ginsenoside content.

| Treatments | PPFD | SPAD | Fv/Fm | Y(II) | Y(NPQ) | Y(NO) | Pn (μmol $m^{-2}$ $s^{-1}$) | ADRW (g $root^{-1}$) | TGC (g $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 324.18 ± 11.99 | 30.56 ± 0.32 | 0.6783 ± 0.0216 | 0.359 ± 0.017 | 0.403 ± 0.025 | 0.237 ± 0.012 | 2.83 ± 0.60 | 3.45 ± 0.51 | 44.12 ± 1.90 |
| 2 | 322.79 ± 40.96 | 31.04 ± 0.82 | 0.7002 ± 0.0558 | 0.376 ± 0.032 | 0.371 ± 0.022 | 0.252 ± 0.029 | 2.85 ± 0.52 | 3.80 ± 0.65 | 45.51 ± 0.06 |
| 3 | 422.53 ± 34.49 | 30.27 ± 0.99 | 0.6052 ± 0.0180 | 0.311 ± 0.042 | 0.394 ± 0.046 | 0.294 ± 0.004 | 2.78 ± 0.66 | 2.49 ± 0.71 | 42.58 ± 1.47 |
| 4 | 136.65 ± 17.85 | 34.29 ± 0.80 | 0.7643 ± 0.0064 | 0.479 ± 0.014 | 0.269 ± 0.026 | 0.249 ± 0.019 | 3.97 ± 0.76 | 4.36 ± 0.60 | 57.60 ± 0.22 |
| 5 | 169.25 ± 29.16 | 35.90 ± 0.59 | 0.7603 ± 0.0050 | 0.415 ± 0.018 | 0.325 ± 0.013 | 0.259 ± 0.018 | 4.24 ± 0.55 | 5.59 ± 0.77 | 54.32 ± 0.26 |
| 6 | 181.96 ± 12.04 | 33.07 ± 0.60 | 0.7183 ± 0.0137 | 0.355 ± 0.012 | 0.388 ± 0.052 | 0.256 ± 0.041 | 3.47 ± 0.72 | 3.02 ± 0.72 | 48.08 ± 0.08 |
| 7 | 184.49 ± 23.32 | 37.00 ± 0.73 | 0.7575 ± 0.0033 | 0.462 ± 0.022 | 0.280 ± 0.014 | 0.257 ± 0.008 | 4.31 ± 0.58 | 6.44 ± 0.84 | 59.63 ± 2.92 |
| 8 | 172.21 ± 9.46 | 35.12 ± 0.74 | 0.7537 ± 0.0071 | 0.480 ± 0.006 | 0.278 ± 0.012 | 0.241 ± 0.010 | 4.43 ± 0.60 | 6.84 ± 0.66 | 51.28 ± 1.02 |
| 9 | 288.74 ± 37.10 | 35.19 ± 0.36 | 0.7569 ± 0.0026 | 0.463 ± 0.033 | 0.282 ± 0.011 | 0.253 ± 0.023 | 3.78 ± 0.62 | 4.54 ± 0.84 | 50.99 ± 0.49 |

comparison, the blue light proportion was higher than that measured for sunlight or passing through YTF but lower than that passing through BTF. The proportion of red light displayed an opposite trend.

The ginseng leaf area under the improved YBCTF was greater than that of ginseng grown under YTF or BTF—with no difference in leaf area between YTF and BTF—although chlorosis occurred earlier under YTF (Fig. 2C). The ginseng roots of plants grown under the improved YBCTF increased in length and diameter compared with the roots of plants grown under YTF or BTF. In addition, relative to BTF-grown ginseng, the roots of YTF-grown plants were shorter and thicker (Fig. 2D).

The physiological, morphological, and quality parameters of ginseng grown under the improved YBCTF, YTF, and BTF are shown in Table 6. Although the chlorophyll content of ginseng grown under the YBCTF was significantly higher than that of YTF- or BTF-grown plants, the chlorophyll fluorescence parameters were similar across the treatments. The Pn of ginseng grown under YTF was significantly higher than that of plants grown under the YBCTF or BTF. Furthermore, correlation analysis of physiological, morphological, and quality parameters revealed that the leaf area positively correlated with the per leaf photosynthetic rate (PLPR), ADRW, and the per root ginsenoside yield (PRGY; Fig. 3).

## 4. Discussion

The findings of our present work indicate that different combinations of STFA, YTFN, and BTFN altered the light intensity and quality of the YBCTF, with a varying impact on the ginseng traits assessed.

A high light intensity causes bleaching of leaves, and both an excessive and scarce amount of light led to a smaller leaf area in *Tetrastigma hemsleyanum* Diels et Gilg [11]. Blue light improves chlorophyll biosynthesis, while red light attenuates this process. *Brassica campestris* L. had a higher chlorophyll content under a 6:1 ratio of red to blue LEDs than under red or blue LEDs only [12]. In addition, due to the inhibition of cell expansion and division under blue light, a reduction in the proportion of blue light with a concomitant increase in red light may increase leaf surface area. Grafted tomato seedlings grown under mixed red and blue LEDs (R7:B3) had a significantly increased total leaf area than plants grown under red or blue LEDs, or under white fluorescent lamps only [13].

**Table 4**
Influence order and significance of STFA, YTFN, and BTFN on ginseng SPAD, chlorophyll fluorescence parameters, Pn, ADRW, and TGC.

| Factors | SPAD | | Fv/Fm | | Y(II) | | Y(NPQ) | | Y(NO) | | Pn (μmol $m^{-2}$ $s^{-1}$) | | ADRW (g $root^{-1}$) | | TGC (g $kg^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F value | *P* value | F value | *P* value | F value | *P* value | F value | *P* value | F value | *P* value | F value | *P* value | F value | *P* value | F value | *P* value |
| STFA | 35.20 | <0.0001 | 41.19 | <0.0001 | 50.97 | <0.0001 | 33.52 | <0.0001 | 0.64 | 0.0541 | 122.48 | <0.0001 | 176.62 | <0.0001 | 158.41 | <0.0001 |
| YTFN | 2.17 | 0.1431 | 12.96 | 0.0003 | 13.13 | 0.0004 | 4.35 | 0.0287 | 2.76 | 0.0932 | 16.22 | <0.0001 | 107.74 | <0.0001 | 55.62 | <0.0001 |
| BTFN | 2.73 | 0.0920 | 2.88 | 0.0822 | 8.52 | 0.0030 | 6.72 | 0.0066 | 3.95 | 0.0403 | 4.11 | 0.0340 | 8.75 | 0.0022 | 27.67 | <0.0001 |
| *SE* | 0.58 | 0.5717 | 1.67 | 0.2155 | 2.37 | 0.1257 | 3.14 | 0.0674 | 0.75 | 0.4891 | 2.16 | 0.143 | 0.49 | 0.6199 | 2.06 | 0.1565 |

**Significance threshold**: $P < 0.05$.

**Table 5**
Influence and significance of different combinations of STFA, YTFN, and BTFN on ginseng SPAD, chlorophyll fluorescence parameters, Pn, ADRW, and TGC.

| Index | Level | Factor | | |
|---|---|---|---|---|
| | | STFA | YTFN | BTFN |
| SPAD | 1 | 30.62 c | 33.95 a | 32.91 b |
| | 2 | 34.41 b | 34.02 a | 33.50 ab |
| | 3 | 35.77 a | 32.84 a | 34.39 a |
| Fv/Fm | 1 | 0.66 b | 0.73 a | 0.71 ab |
| | 2 | 0.74 a | 0.74 a | 0.73 a |
| | 3 | 0.75 a | 0.68 b | 0.70 b |
| Y(II) | 1 | 0.34 a | 0.43 a | 0.39 b |
| | 2 | 0.41 b | 0.42 a | 0.43 a |
| | 3 | 0.46 c | 0.37 b | 0.39 b |
| Y(NPQ) | 1 | 0.38 a | 0.31 b | 0.35 a |
| | 2 | 0.32 b | 0.32 b | 0.30 b |
| | 3 | 0.28 c | 0.35 a | 0.33 ab |
| Y(NO) | 1 | 0.26 a | 0.24 a | 0.24 b |
| | 2 | 0.25 a | 0.25 a | 0.25 ab |
| | 3 | 0.25 a | 0.26 a | 0.27 a |
| Pn | 1 | 2.82 c | 3.70 a | 3.57 b |
| | 2 | 3.88 b | 3.84 a | 3.53 b |
| | 3 | 4.18 a | 3.34 b | 3.77 a |
| ADRW | 1 | 3.25 c | 4.80 b | 4.43 b |
| | 2 | 4.35 b | 5.39 a | 4.26 b |
| | 3 | 5.93 a | 3.35 c | 4.84 a |
| TGC | 1 | 44.06 b | 53.78 a | 47.82 b |
| | 2 | 53.32 a | 50.36 b | 51.36 a |
| | 3 | 53.96 a | 47.21 c | 52.17 a |

**Note:** Different lowercase letters indicate a significant difference at $P < 0.05$. STFA levels: 1 (A1, 3 × 3 cm), 2 (A2, 7 × 7 cm), 3 (A3, 15 × 15 cm); YTFN levels: 1 (Y1, one piece), 2 (Y2, two pieces), 3 (Y3, three pieces); BTFN levels: 1 (B1, one piece), 2 (B2, two pieces), 3 (B3, three pieces).

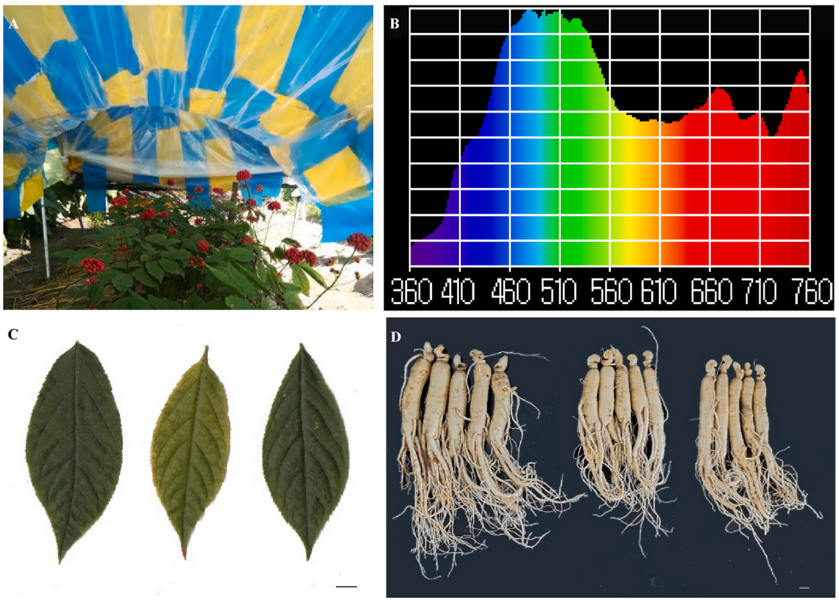


**Fig. 2.** Improved yellow and blue combination transparent film (YBCTF) and corresponding transmission light spectrum, and the growth condition of ginseng leaves and roots under the improved YBCTF, yellow transparent film (YTF), and blue transparent film (BTF). (A) Improved YBCTF, (B) spectrum of light filtering through the improved YBCTF, (C) ginseng leaves grown under the improved YBCTF, YTF, and BTF, respectively, (D) ginseng roots grown under the improved YBCTF, YTF, and BTF, respectively. Scale bar for panels C and D: 1 cm.

In the current study, the SPAD of ginseng grown under YBCTF was significantly influenced by STFA, which decreased the light intensity. Neither YTFN nor BTFN significantly affected SPAD, although they influenced the light quality ratio; the improved YBCTF had a higher proportion of blue light than YTF and a higher proportion of red light than BTF. This increased the chlorophyll content and leaf surface area of ginseng plants grown under the improved YBCTF.

**Table 6**
Influence of the improved YBCTF, YTF, and BTF, on ginseng chlorophyll content, chlorophyll fluorescence parameters, Pn, leaf area, per leaf photosynthetic rate, ADRW, and per root ginsenoside yield.

| Treatments | Chl content (mg $g^{1}$) | Fv/Fm | Y(II) | Y(NPQ) | Y(NO) | Pn (μmol $m^{-2}$ $s^{-1}$) | LFA ($cm^2$ $leaf^{1}$) | PLPR (μmol $s^{-1}$ $leaf^{-1}$) | ADRW (g $root^{-1}$) | TGC (g $kg^{-1}$) | PRGY (g $root^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| YBCTF | 1.87 ± 0.18 a | 0.7626 ± 0.0104 a | 0.341 ± 0.016 a | 0.408 ± 0.004 a | 0.250 ± 0.012 a | 1.58 ± 0.20 b | 29.70 ± 3.02 a | 0.0047 ± 0.0009 a | 4.43 ± 0.29 a | 53.76 ± 0.85 b | 0.23 ± 0.01 a |
| YTF | 1.15 ± 0.07 b | 0.7694 ± 0.0013 a | 0.357 ± 0.028 a | 0.406 ± 0.013 a | 0.236 ± 0.015 a | 1.90 ± 0.10 a | 20.90 ± 2.79 b | 0.0039 ± 0.0006 b | 3.04 ± 0.39 b | 47.13 ± 0.14 c | 0.14 ± 0.01 b |
| BTF | 1.20 ± 0.32 b | 0.7609 ± 0.0104 a | 0.355 ± 0.009 a | 0.394 ± 0.023 a | 0.249 ± 0.014 a | 1.37 ± 0.12 b | 20.82 ± 2.92 b | 0.0028 ± 0.0001 b | 2.63 ± 0.25 b | 58.42 ± 0.09 a | 0.15 ± 0.01 b |

**Note:** Different lowercase letters indicate significant differences at $P < 0.05$.

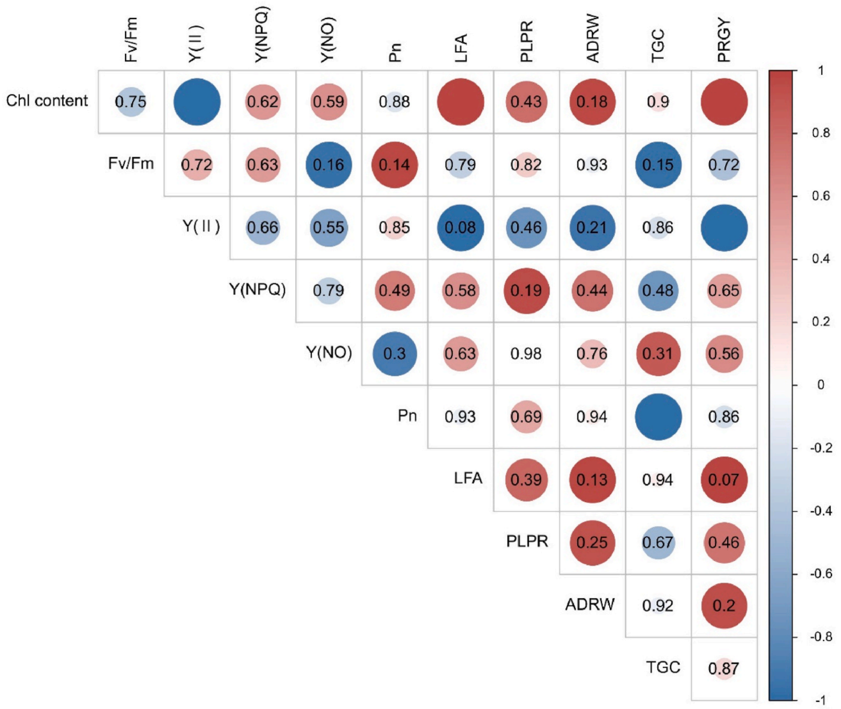


**Fig. 3.** Correlation plot of physiological, morphological, and quality parameters. The size and colour intensity of each circle are proportional to the Pearson correlation coefficient at $P < 0.05$. Red: positive correlations; blue: negative correlations. In the correlogram scale from −1 to +1, the Pearson correlation coefficients appear on the vertical axis. The numbers in the circles represent the *P*-value.

Our results indicate that Fv/Fm, Y(II), Y(NPQ), and Y(NO) were influenced by both the light intensity and the light quality ratio of the YBCTF. Fv/Fm was significantly influenced by STFA and YTFN, with a larger STFA leading to a reduced light intensity, while an increased YTFN increased the proportion of red light. Collectively, these factors caused ginseng leaves under the YBCTF to have an Fv/Fm that was between that of YTF- and BTF-grown plants. These results are similar to those of a previous study on lettuce, which reported that a relatively low light intensity leads to a higher Fv/Fm, while an excessively high or low light intensity decreases the Fv/Fm of lettuce [14]. Red light treatment of the red alga *Pyropia haitanensis* resulted in a significantly lower Fv/Fm than blue light treatment [15], while combined red and blue light treatment of *Oryza sativa* L. yielded a slightly lower Fv/Fm ratio than either red or blue light treatment alone [16]. This difference in Fv/Fm may be at least partially attributed to species differences or those between the continuous spectra of YTF, BTF, and YBCTF and the monochrome spectrum of LEDs.

Y(II), Y(NPQ), and Y(NO) reflect the distribution of excitation energy absorbed in photosystem-II (PSII). Y(II) corresponds to the fraction of energy that is photochemically converted in PSII; Y(NO) reflects the fraction of energy that is passively dissipated in the form of heat and fluorescence, mainly due to closed PSII reaction centres. In contrast, Y(NPQ) corresponds to the fraction of energy dissipated in the form of heat via the regulated photoprotective non-photochemical quenching mechanism. The successful regulation of energy distribution requires maximal values of Y(II), with a maximal ratio of Y(NPQ) to Y(NO) [17]. In our present study, Y(NO) was significantly influenced by BTFN, which increased the light intensity and the proportion of blue light. Y(NPQ) was also significantly affected by STFA and BTFN and, to a comparatively lesser yet significant extent, by YTFN. Light intensity overall exhibited an inverse correlation with STFA, with the proportion of blue light increasing with an increase in BTFN, and that of red light increasing with an increase in YTFN. A previous report argued that blue or red light alone compromised the antioxidant system of rice grown under a moderate light level, leading to decreased Y(II) and increased Y(NPQ) values [18]. This may explain why Y(NPQ) increased with YTFN, and Y(NO) increased with BTFN in our study. Furthermore, as the light intensities of YTF, BTF, and the improved YBCTF were all suitable for ginseng growth, there was no significant difference among their corresponding chlorophyll fluorescence parameters. However, the improved YBCTF had a more suitable proportion of blue and red light than YTF and BTF, resulting in a higher Y(NPQ)/Y(NO) ratio than that of BTF but a lower ratio than that of YTF. This suggests that ginseng grown under the improved YBCTF had a more efficient light energy utilisation than BTF-grown plants, which was, however, still lower than that in YTF-grown ginseng.

The Pn of ginseng grown under YBCTF was highly influenced by STFA and YTFN and slightly influenced by BTFN. Despite the fact that the proportion of red light increased with STFA and YTFN, the light intensity decreased with an increase in STFA. Ginseng grown under the improved YBCTF thus had lower Pn values than that grown under YTF, which in turn provided the highest red-light proportion and light intensity. However, ginseng grown under the improved YBCTF had a larger leaf surface area than that grown under YTF and BTF, which corresponded to a higher PLPR and ADRW. This is in accordance with previous studies, which showed that red light had higher photosynthetic efficiency than blue light, and that the combination of red and blue LEDs (1:1) was more conducive to efficient photosynthesis in potatoes than under monochromatic red or blue light [19]. Furthermore, the Pn of ginseng grown under a combination of R8G1B1 and R9G1B0 (based on chip number) had a higher Pn than that under other combinations of red, green, and blue LEDs. R8B2 and R8G1B1 also resulted in a larger leaf area than red, green, and blue LEDs, while R8B2 and R7G1B2 induced a higher fresh root weight [20].

Blue light receptor cryptochrome 1 regulates blue light and methyl jasmonate signalling pathways [21]. Methyl jasmonate has been shown to greatly increase the ginsenoside content in ginseng [22]. A suitable combination of red and blue LEDs (R9B1) resulted in a root ginsenoside content between that obtained from growth conditions using only blue or red light [20]. Our results also indicated that the root ginsenoside content increased with BTFN (which increased the blue light) and decreased with YTFN (which increased the red light). Ginseng grown under the improved YBCTF had a higher root ginsenoside content than that grown under YTF, but a lower ginsenoside content than that grown under BTF. However, the higher ADRW of ginseng grown under the improved YBCTF effectively offset the lower TGC, resulting in an overall higher yield of ginsenosides per single root than that induced by either YTF or BTF.

## 5. Conclusions

The results of this study suggest that light intensity was primarily influenced by STFA, which overall decreased light intensity, while the light quality ratio was primarily affected by YTFN, which increased the proportion of red light and decreased that of blue light. An STFA of 15 × 15 cm and a combination of two pieces of YTF and three pieces of BTF had the best effects on the light energy utilisation, root dry weight increase, and ginsenoside accumulation in ginseng plants. The improved YBCTF had a light intensity of $123.32 \pm 9.92$ μmol $m^{-2}$ $s^{-1}$, 23.10% blue light, and 27.54% red light, which lead to a larger leaf area, PLPR, ADRW, and PRGY in ginseng than those induced by YTF or BTF. The innovative YBCTF for optimising ginseng growth is cheap and suitable for mass production, making it potentially useful in large-scale crop applications. However, YBCTF had a large proportion of green light, which hampers plant growth. Further research should explore the effects of a combination of red and blue transparent films on ginseng growth to develop a more effective film.

## Funding

This work was supported by Jilin Agricultural Science and Technology University [grant number (2022)743].

## Data availability statement

All relevant data are included in the article.

## CRediT authorship contribution statement

**Zhenghai Zhang:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Huixia Lei:** Methodology, Investigation, Data curation. **Peihe Zheng:** Resources, Methodology, Investigation, Formal analysis, Data curation. **Yayu Zhang:** Resources, Conceptualization. **Hai Sun:** Software. **Cai Shao:** Resources. **Jingjing Zhao:** Data curation.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

We are grateful to Haifeng Zhang for his industrious field management and the relevant assistance provided.

## Abbreviation list

| | |
|---|---|
| ADRW | average dry root weight |
| BTF | blue transparent film |
| BTFN | blue transparent film number |
| Fv/Fm | maximum photochemical efficiency |
| LED | light-emitting diode |
| LFA | leaf area |
| Pn | photosynthetic rate |
| PLPR | per leaf photosynthetic rate |
| PPFD | photosynthetic photon flux density |
| PRGY | per root ginsenoside yield |
| PSII | photosystem-II |
| SPAD | Soil and plant analyser development |
| STFA | small transparent film area |
| TGC | total ginsenoside content |
| YBCTF | yellow and blue combination transparent film |
| Y(II) | actual photochemical efficiency |
| Y(NO) | sum of non-regulated heat dissipation and fluorescence emission |
| Y(NPQ) | regulated thermal energy dissipation |
| YTF | yellow transparent film |
| YTFN | yellow transparent film number |

## References

[1] H. Han, K.-m. Jang, J.-s. Chung, Selecting suitable sites for mountain ginseng (Panax ginseng) cultivation by using geographically weighted logistic regression, J. Mt. Sci. 14 (2017) 492–500, https://doi.org/10.1007/s11629-016-4118-9.

[2] N.H. Kim, M. Jayakodi, S.C. Lee, B.S. Choi, W. Jang, J. Lee, H.H. Kim, N.E. Waminal, M. Lakshmanan, B. van Nguyen, Y.S. Lee, H.S. Park, H.J. Koo, J.Y. Park, S. Perumal, H.J. Joh, H. Lee, J. Kim, I.S. Kim, K. Kim, L. Koduru, K.B. Kang, S.H. Sung, Y. Yu, D.S. Park, D. Choi, E. Seo, S. Kim, Y.C. Kim, D.Y. Hyun, Y.I. Park, C. Kim, T.H. Lee, H.U. Kim, M.S. Soh, Y. Lee, J.G. In, H.S. Kim, Y.M. Kim, D.C. Yang, R.A. Wing, D.Y. Lee, A.H. Paterson, T.J. Yang, Genome and evolution of the shade-requiring medicinal herb Panax ginseng, Plant Biotechnol. J. 16 (2018) 1904–1917, https://doi.org/10.1111/pbi.12926.

[3] T.S.C. Li, Asian and American ginseng-A review, HortTechnology 5 (1995) 27–34, https://doi.org/10.21273/HORTTECH.5.1.27.

[4] H. Park, M.-K. Lee, S.D. Ahan, Effect of light quality on the growth of panax ginseng in a phytotron with natural light, Korean J. Ginseng Sci. 13 (1989) 165–168.

[5] I. Jang, G. Do, S. Suh, J. Yu, I. Jang, J. Moon, C. Chun, Physiological responses and ginsenoside production of Panax ginseng seedlings grown under various ratios of red to blue light-emitting diodes, Hortic. Environ. Biotechnol. 61 (2020) 663–672, https://doi.org/10.1007/s13580-020-00255-5.

[6] M. Rahimi, M. Esmaeilizadeh, M.R. Malekzadeh Shamsabad, H.R. Roosta, P. Dąbrowski, M. Rapacz, A. Zieliński, J. Wróbel, H.M. Kalaji, Manipulation of light spectrum can improve the performance of photosynthetic apparatus of strawberry plants growing under salt and alkalinity stress, PLoS One 16 (2021) e0261585, https://doi.org/10.1371/journal.pone.0261585.

[7] J.M. Banks, Continuous excitation chlorophyll fluorescence parameters: a review for practitioners, Tree Physiol. 37 (2017) 1128–1136, https://doi.org/10.1093/treephys/tpx059.

[8] A. Yano, M. Cossu, Energy sustainable greenhouse crop cultivation using photovoltaic technologies, Renew. Sustain. Energy Rev. 109 (2019) 116–137, https://doi.org/10.1016/j.rser.2019.04.026.

[9] P.J. Ralph, R. Gademann, Rapid light curves: a powerful tool to assess photosynthetic activity, Aquat. Bot. 82 (2005) 222–237, https://doi.org/10.1016/j.aquabot.2005.02.006.

[10] M. Zhang, Y.X.J. Qin, D. Chen, P. Yang, Determination of the total ginsenosides in ginseng using the UV spectrophotometer and evaluation of the measurement uncertainty, Adv. Mater. Res. 490–495 (2012) 1290–1295, https://doi.org/10.4028/www.scientific.net/AMR.490-495.1290.

[11] Y. Dai, Z. Shen, Y. Liu, L. Wang, D. Hannaway, H. Lu, Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of Tetrastigma hemsleyanum Diels et Gilg, Environ. Exp. Bot. 65 (2009) 177–182, https://doi.org/10.1016/j.envexpbot.2008.12.008.

[12] X. Fan, J. Zang, Z. Xu, S. Guo, X. Jiao, X. Liu, Y. Gao, Effects of different light quality on growth, chlorophyll concentration and chlorophyll biosynthesis precursors of non-heading Chinese cabbage (Brassica campestris L.), Acta Physiol. Plant. 35 (2013) 2721–2726, https://doi.org/10.1007/s11738-013-1304-z.

[13] A.F. Yousef, M.M. Ali, H.M. Rizwan, M.A.A. Ahmed, W.M. Ali, H.M. Kalaji, N.E. Elsheery, J. Wro′bel, Y. Xu, F. Chen, Effects of light spectrum on morpho-physiological traits of grafted tomato seedlings, PLoS One 16 (5) (2021) e0250210, https://doi.org/10.1371/journal.pone.0250210.

[14] W. Fu, P. Li, Y. Wu, Effects of different light intensities on chlorophyll fluorescence characteristics and yield in lettuce, Sci. Hortic. 135 (2012) 45–51, https://doi.org/10.1016/j.scienta.2011.12.004.

[15] H. Wu, Effect of different light qualities on growth, pigment content, chlorophyll fluorescence, and antioxidant enzyme activity in the red alga pyropia haitanensis (bangiales, rhodophyta), BioMed Res. Int. 2016 (2016) 7383918, https://doi.org/10.1155/2016/7383918.

[16] C.C. Chen, M.Y. Huang, K.H. Lin, S.L. Wong, W.D. Huang, C.M. Yang, Effects of light quality on the growth, development and metabolism of rice seedlings (Oryza sativa L.), Res. J. Biotechnol. 9 (2014) 15–24.

[17] C. Klughammer, U. Schreiber, Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method, PAM Appl. Notes. 1 (2008) 27–35.

[18] S. Hamdani, N. Khan, S. Perveen, M. Qu, J. Jiang, Govindjee, X.G. Zhu, Changes in the photosynthesis properties and photoprotection capacity in rice (Oryza sativa) grown under red, blue, or white light, Photosynth. Res. 139 (2019) 107–121, https://doi.org/10.1007/s11120-018-0589-6.

[19] X. Yang, H. Xu, L. Shao, T. Li, Y. Wang, R. Wang, Response of photosynthetic capacity of tomato leaves to different LED light wavelength, Environ. Exp. Bot. 150 (2018) 161–171, https://doi.org/10.1016/j.envexpbot.2018.03.013.

[20] Y.J. Kim, T.K.L. Nguyen, M.M. Oh, Growth and ginsenosides content of ginseng sprouts according to LED-based light quality changes, Agronomy 10 (2020) 1979, https://doi.org/10.3390/agronomy10121979.
[21] H.J. Chen, T.Y. Fu, S.L. Yang, H.L. Hsieh, Correction: FIN219/JAR1 and cryptochrome1 antagonize each other to modulate photomorphogenesis under blue light in Arabidopsis, PLoS Genet. 14 (2018) e1007606, https://doi.org/10.1371/journal.pgen.1007606.
[22] M. Lu, H. Wong, W. Teng, Effects of elicitation on the production of saponin in cell culture of Panax ginseng, Plant Cell Rep. 20 (2001) 674–677, https://doi.org/10.1007/s002990100378.