

EFFECT OF LIGHT QUALITY ON PHOTOSYNTHESIS AND CONTENTS OF ACTIVE INGREDIENTS *SAXIFRAGA STOLONIFERA* CURT

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Abstract

To provide a theoretical basis for artificial cultivation of *Saxifraga stolonifera*, we cultured the plants under different types of agriculture films and compared their effect on photosynthesis, leaf microstructure and active ingredient contents using Li 6400 portable photosynthesis instrument, paraffin method and high performance liquid chromatography (HPLC). The results showed that the quality of transmitted light differed significantly under different film sheds, showing the highest UV transmittance under the white film shed as well as the highest red/blue ratio and lowest green/red ratio under the red film shed. Leaf gas exchange of *S. stolonifera* showed a “bimodal” diurnal variation curve under all sheds. Among them, *S. stolonifera* under red film shed showed the highest daily average net photosynthetic rate (P_N) of $7.62 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the greatest P_N from the fitted photosynthesis-light response curve. Chlorophyll fluorescence parameter displayed that *S. stolonifera* under the red film shed had the highest electron transfer rate of $58.4 \mu\text{mol m}^{-2} \text{s}^{-1}$. *Saxifraga stolonifera* under red shed had the thickest mesophyll tissue and the highest active ingredient content and biological yield. In conclusion, shedding with red film is most conducive for photosynthesis of *S. stolonifera*.

Key words: Light qualities, Photosynthesis, *Saxifraga stolonifera*.

Abbreviations: AQY – apparent quantum efficiency; Car – carotenoids; Chl *a(b)* – chlorophyll *a(b)*; C_i – intercellular CO_2 concentration; DM – dry mass; E – transpiration rate; ETR – electron transport rate; FM – fresh mass; F_v/F_0 – potential activity of PSII photochemistry; F_v/F_m – maximal quantum yield of PSII photochemistry; HPLC – high performance liquid chromatography; LCP – light compensation point; LE – lower epidermis; LSP – leaf light saturation point; PAR – Photosynthetically active radiation; P_N – net photosynthetic rate; $P_{N \text{ max}}$ – light-saturated net photosynthetic rate; PTT – palisade tissue thickness; q_N – nonphotochemical quenching coefficient; q_P – photochemical quenching coefficient; SLM – specific leaf mass; STT – spongy tissue thickness; UE – upper epidermis; Φ_{PSII} – effective quantum yield of PSII photochemistry.

Introduction

Light is the key factor for plant photosynthesis. Its intensity and quality have significant impacts on plant photosynthesis. Plants often change their photosynthetic pigments and leaf structures to adapt to different environments (Macintyre *et al.*, 2002). In recent years, many scholars utilize light with different qualities to adjust crop growth. Different light qualities have been found to play different important regulatory roles on plant growth and development (Hans *et al.*, 2014; Gong *et al.*, 2013), leaf gas exchange (Zhang *et al.*, 2008; Liu *et al.*, 2010), secondary metabolites formation (Su *et al.*, 2012; Chen *et al.*, 2013) among others.

Saxifraga stolonifera is an evergreen perennial plant in family Saxifragaceae. Its whole plant can be used as antitussive and anti-inflammatory medicines (Qin *et al.*, 2013). In addition, it contains high contents of bergenin and gallic acid. The former is an isocoumarin compound and has been recorded in “Pharmacopoeia of People's Republic of China” as an antitussive and expectorant drug and a potent drug for treatment of chronic bronchitis, emphysema and other respiratory diseases (Chinese Pharmacopoeia Commission, 2010). Recent studies have shown that gallic acid and other organic

acids in *S. stolonifera* have certain anti-androgen effects and can regulate prostate cells growth and apoptosis and inhibit prostate cancer (Zhou *et al.*, 2013). Because *S. stolonifera* is easy to breed and manage and has fewer diseases and pests, it can be used as an ideal plant source to extract bergenin and gallic acid. However, *S. stolonifera* cultivation was rarely reported. We have studied its photosynthesis in recent years (He *et al.*, 2012). *S. stolonifera* is a shade requiring plant. Shading conditions with light transmittance of 40% is most suitable for its growth while strong sunlight is adverse to its growth (He *et al.*, 2010), which significantly limits its large-scale cultivation. Agricultural films covering blocks part of sunlight and light intensity and quality under shades have a direct relationship with permeation performance of agricultural films, therefore it has been widely used in *S. stolonifera* cultivation (Pearson *et al.* 1995; Ding & Zhou, 2008; Wei *et al.*, 2009). In this paper, we compared the effects of shading of commonly used agricultural films with different colors on photosynthesis characteristics and key ingredients contents of *S. stolonifera* and identified the best colored film suitable for planting *S. stolonifera* with the hope to provide a theoretical basis for artificial cultivation of *S. stolonifera*.

Materials and Methods

Plant materials and culture condition: *S. stolonifera* was collected from Biological Park in Huaihua, Hunan Province, China. The experiments were conducted in the Biological Park in Huaihua, Hunan Province, China at 17–25°C and CO₂ concentration of about 400 μmol (CO₂) mol⁻¹. Based on the previous study (He *et al.*, 2012), the photoperiod was controlled at 400 μmol (photon) m⁻² s⁻¹ at noon by shade net. A total of 15 healthy *S. stolonifera* plants at three-leaves stage were evenly planted in each 80 x 100 cm² plot with black humus soils on March 2014. These plots were then covered with 80 cm wide, 100 cm long and 100 cm high shed prepared using white, red and blue Polyethylene films with thickness of 0.1 mm. The light intensity were adjusted to a similar level with transparent light intensity of 400 μmol (photon) m⁻² s⁻¹ at noon.

The experiments included three blocks, representing three replications and each block contained three plots covered by white, red and blue films, respectively. These three treatments within each block were randomly placed. *S. stolonifera* was planted in colored film sheds for 30 days and four newborn and fully developed leaves in each plot were randomly selected for experiments.

Spectral characteristics: The spectral characteristics of the three films were obtained using *Li 1800* spectroradiometer (*Li-Cor*, USA) and used to calculate the ratios of ultraviolet (300–400 nm), blue (450–500 nm), green (500–570 nm) and red (620–700 nm) light penetrating the films.

Measurement of gas exchange: The diurnal variations of P_N (net photosynthetic rate) and E (transpiration rate) of healthy leaves were measured every 1.5h from 08:30 am to 17:30 pm in sunny days using a *Li 6400* portable photosynthesis system (*Li-Cor*, USA). Their averages were used for data analysis.

Light response curve was measured from 09:00–11:00 am in sunny days at natural temperature and CO₂ concentration. PAR (Photosynthetically active radiation) of 1,800, 1,500, 1,200, 1,000, 700, 500, 300, 200, 150, 100, 50, 20 and 0 μmol m⁻² s⁻¹ was generated from the *Li-Cor* LED light source equipped with the instrument. The AQY (apparent quantum efficiency) was calculated based on the initial slope of the P_N -PAR curve (PAR < 150 μmol m⁻² s⁻¹). The light saturation point, light compensation point and maximum photosynthetic rate were calculated as described previously (Bassman & Zwier, 1991).

Photosynthetic pigment contents: Photosynthetic pigments were extracted using 80% acetone and measured using a *Beckman DU 800 UV-V*(USA) spectrophotometer as described previously by Lichtenthaler (Zou 1995).

Determination of chlorophyll fluorescence parameters: After wrapped with aluminum foil for at least 30 min to adapt to darkness at room temperature,

leaf blades were first subjected to weak modulated measuring light to measure minimal fluorescence yield of the dark-adapted state (F_0), then in turn to saturated light pulse to measure maximal fluorescence yield of the dark-adapted state (F_m), to activation light to measure steady-state fluorescence yield (F_s), and to saturated light pulse in the presence of actinic light to measure maximal fluorescence yield of the light-adapted state (F_m'). At the moment of turning off the activation light, leaf blades were subjected to far-infrared light for a few seconds to rapidly activate PSII reaction center for measurement of minimal fluorescence yield of the light-adapted state (F_0'). All these parameters were measured using *Li 6400* at the measurement light and strong flash light set following the manuals provided by the manufacturer. The following fluorescence parameters were calculated: $q_P = (F_m' - F_s)/(F_m' - F_0')$; $q_N = (F_m - F_m')/(F_m - F_0)$; $\Phi_{PSII} = (F_m' - F_s)/F_m'$; $ETR = PPFD \times \Phi_{PSII} \times 0.84 \times 0.5$ (Zhang 1999).

Observation of leaf cross – section structural features:

Healthy leaves under different films treatment were collected and prepared as 5 mm x 10 mm blocks with midrib at the center. After fixed with FAA fixative solution and dehydrated with ethanol, the blocks were cleared with xylene, embedded in paraffin and cut into 10 μm sections using *Leica RM 2145 slicer* (Germany). The sections were stained using saffron and examined under an *Olympus microscope* (Japan). One field per section of 10 sections per sample was photographed and analyzed using *Motic Images Plus 2.0* software to obtain the thickness of upper and lower epidermis, palisade tissues and spongy tissues.

Specific leaf mass and water content: Healthy leaves were randomly selected, rinsed with deionized water and surface dried with filter papers. A fixed area of leaves blades was collected using a puncher. After weighed to obtain fresh mass, they were placed at 105°C for 10 min and at 80°C to constant dry mass. The specific leaf mass (SLM) and water content were calculated based on their fresh mass and dry mass.

Determination of gallic acid and bergenin contents:

Whole *S. stolonifera* plants treated with different films were collected, washed and incubated at 105°C for 10 min and at 80°C to constant weight. After weighed, they were crushed and filtered through a 120-mesh sieve. About 1.0 g of *S. Stolonifera* powder was accurately taken, mixed with methanol at ratio of 1:25 (g:mL) in a 50 mL Erlenmeyer flask, sonicated for 35 min and filtered. The remaining powder was extracted one more times. The filtrates were combined, concentrated to about 5 mL using a rotary evaporator, mixed with methanol to 25 mL in a volumetric flask and filtered through a 0.22 μm membrane. Gallic acid and bergenin were separated using *LC 20AT liquid chromatograph* (Japan) with a *Agilent Eclipse XDB-C18* (150 mm × 4.6 mm, 5 μm) column at the following conditions: the mobile phase was methanol: 0.1% phosphoric acid = 73:29, flow rate was 1.02 ml min⁻¹, detection wavelength was 272 nm, column temperature was 25°C and injection volume was 20 μL and their contents were calculated based on the peak area using external standard method.

Statistical analysis: The each experimental data set were calculated the mean and standard deviation. And Duncan multiple comparisons were conducted among treatments using *SPSS 13.0* software, and the significance level were set at $\alpha=0.05$.

Results

Components of irradiance spectrum: The optical properties of different films varied significantly. The white film had the highest ultraviolet transmission rate of 0.86%, followed by the red film (Table 1). In addition, because the red film could transmit more red light, it had the highest red/blue ratio of 1.63. Similarly, blue film could transmit more blue light, thus had the lowest red/blue ratio.

Comparison of photosynthesis of *S. stolonifera* in different film sheds

Diurnal variations of P_N and E : The diurnal variations of P_N of *S. stolonifera* under different film sheds all showed “bimodal” curves with the highest value at 10:00 am and break phenomenon at 13:00 – 14:00 pm (Fig. 1). The daily average P_N was the highest of 7.62 $\mu\text{mol}(\text{CO}_2)\text{m}^{-2}\text{s}^{-1}$ under red film. The diurnal variations of E showed the monodomal curves with peaks all around 4.10 $\text{mmol}(\text{H}_2\text{O})\text{m}^{-2}\text{s}^{-1}$ at 13:00 pm in *S. stolonifera*.

Response curves of leaf photosynthesis to light intensity: The response curves of leaf photosynthesis to light intensity showed that P_N increased rapidly with photon density increasing to up to 150 $\mu\text{mol}(\text{CO}_2)\text{m}^{-2}\text{s}^{-1}$, then slowly declined with photon density further increasing (Fig. 2). Apparent quantum efficiency (AQY) obtained using fitting calculation showed that blue shed had the highest AQY of 0.058 $\mu\text{mol}(\text{CO}_2)\text{m}^{-2}\text{s}^{-1}$ and lowest LCP (light compensation point) of 19.6 $\mu\text{mol}(\text{photon})\text{m}^{-2}\text{s}^{-1}$ (Table 2), indicating that *S. stolonifera* under blue film shed had the strongest ability to use the weak light. The leaf light saturation point (LSP) was the highest of 1,308 $\mu\text{mol}(\text{photon})\text{m}^{-2}\text{s}^{-1}$ under white film shed and the fitted maximum P_N was the highest of 8.93 $\mu\text{mol}(\text{CO}_2)\text{m}^{-2}\text{s}^{-1}$ under red film shed, indicating that *S. stolonifera* under white film shed and red film shed had better ability to use strong light.

Photosynthetic pigment contents: Photosynthetic pigment contents were significantly different in *S. stolonifera* under different film sheds. In detail, total chlorophyll and carotenoid contents were the highest of 1.64 mg g^{-1} (FM) and 0.30 mg g^{-1} (FM), respectively, under blue film shed and the total chlorophyll was the

lowest of only 1.12 mg g^{-1} (FM) under white film shed (Table 3). Because *S. stolonifera* under blue film shed contained more chlorophyll, it also had the highest chlorophyll a/b ratio of 2.96.

Chlorophyll fluorescence parameters: Maximal quantum yield of PSII photochemistry (F_v/F_m) as the maximum photochemical efficiency of PSII reflects the primary efficiency of PSII photochemical reaction center. Table 4 showed that chlorophyll fluorescence parameters F_v/F_m was not significantly different among *S. stolonifera* under different film sheds, while potential activity of PSII photochemistry (F_v/F_0) was significantly higher under red film sheds than under blue and white film sheds, indicating that PSII reaction center of *S. stolonifera* under red film sheds had the highest energy capture efficiency and potential activity. The data of q_P (photochemical quenching coefficient) and q_N (nonphotochemical quenching coefficient) showed that the openness of PSII reaction center was the lowest in *S. stolonifera* under white film shed. Effective quantum yield of PSII photochemistry (Φ_{PSII}) showed that photochemical reaction efficiency was the highest under red film shed and the lowest under white film shed, indicating that *S. stolonifera* under red film shed had the highest electronic transfer rate (ETR).

Micro-structures: Leaf micro-structures of *S. stolonifera* under different film sheds mainly varied in the thickness of palisade tissues and spongy tissues. The palisade and spongy tissues were the thickest of 45.1 μm and 149 μm , respectively, under red film shed, followed by those under white films shed (Table 5, Fig. 3). But the vascular bundle size showed no significant difference among *S. stolonifera* in the three film sheds.

Leaf water content and specific leaf mass: The leaf water contents were higher than 90% and not significantly different among *S. stolonifera* under different film sheds. However, the specific leaf masses were significantly different among *S. stolonifera* under different film sheds, showing the highest of 4.12 mg cm^{-2} under red film shed and the lowest of 2.57 mg cm^{-2} under blue shed (Table 5).

Active ingredients contents: Table 6 showed that the biomass was significantly different among *S. stolonifera* under different film sheds. In detail, the dry weight of *S. stolonifera* was the highest under red film shed and the lowest of 23.7 g m^{-2} under blue film shed. The content of gallic acid and bergenin levels were the highest under red film shed, reaching to 0.81 g mg^{-2} and 3.53 g mg^{-2} , respectively. The yields of gallic acid and bergenin under red film shed were significantly higher than the other two.

Table 1. The components of representative irradiance spectrum in different films.

Item	Ratio of transmission spectrum (%)				Red/Blue	Green/Red
	Ultraviolet	Blue	Green	Red		
White film shed	0.86 ± 0.07 a	15.5 ± 1.32 b	26.8 ± 2.33 b	19.4 ± 1.89 b	1.25 ± 0.11 b	1.38 ± 0.15 b
Red film shed	0.75 ± 0.06 b	13.6 ± 1.27 c	22.2 ± 2.18 c	22.3 ± 1.66 a	1.63 ± 0.14 a	0.99 ± 0.08 c
Blue film shed	0.64 ± 0.04 c	22.1 ± 1.75 a	30.1 ± 2.47 a	10.2 ± 0.78 c	0.46 ± 0.06 c	2.94 ± 0.18 a

Values in table are presented as mean ± standard deviation (n=9)

Means followed by the same letter do not differ based on the Duncan's New Multiple Range Test at $p \leq 0.05$

Table 2. Photosynthetic parameters of *S. stolonifera* in film sheds of different colors.

Item	AQY	LCP	LSP	$P_{N \max}$
	$[\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}]$	$[\mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}]$	$[\mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}]$	$[\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}]$
White film shed	0.049 b	29.5 a	1,308 a	8.85 a
Red film shed	0.051 b	22.4 b	1,221 b	8.93 a
Blue film shed	0.058 a	19.6 c	1,140 c	5.60 b

Values in table are obtained by Nonlinear Fitting by SPSS 13.0

Means followed by the same letter do not differ based on the Duncan's New Multiple Range Test at $p \leq 0.05$

AQY – apparent quantum efficiency; LCP – light compensation point; LSP – leaf light saturation point; $P_{N \max}$ – light-saturated net photosynthetic rate

Table 3. Comparison of chlorophyll content [$\text{mg g}^{-1}(\text{FM})$] of *S. stolonifera* in film sheds of different colors.

Item	Chl a	Chl b	Total Chl	Chl a/b	Car
White film shed	0.82 ± 0.02 c	0.31 ± 0.01 b	1.12 ± 0.02 c	2.73 ± 0.01 b	0.25 ± 0.01 b
Red film shed	0.90 ± 0.02 b	0.32 ± 0.02 b	1.21 ± 0.01 b	2.89 ± 0.23 a	0.26 ± 0.02 b
Blue film shed	1.22 ± 0.01 a	0.41 ± 0.03 a	1.64 ± 0.07 a	2.96 ± 0.20 a	0.30 ± 0.02 a

Values in table are presented as mean \pm standard deviation (n=9)

Means followed by the same letter do not differ based on the Duncan's New Multiple Range Test at $p \leq 0.05$

Chl – chlorophyll; Car – carotenoids; FM – fresh mass

Table 4. Comparison of chlorophyll fluorescence parameters of *S. stolonifera* in film sheds of different colors.

Item	F_v/F_m	F_v/F_0	q_p	q_N	Φ_{PSII}	ETR ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)
White film shed	0.778 ± 0.021 a ^y	3.53 ± 0.399 b	0.477 ± 0.090 b	0.784 ± 0.037 a	0.239 ± 0.047 c	30.1 ± 2.51 c
Red film shed	0.800 ± 0.002 a	3.89 ± 0.196 a	0.792 ± 0.011 a	0.604 ± 0.046 c	0.509 ± 0.013 a	64.1 ± 5.98 a
Blue film shed	0.779 ± 0.016 a	3.54 ± 0.313 b	0.771 ± 0.017 a	0.655 ± 0.075 b	0.463 ± 0.036 b	58.3 ± 4.37 b

The photon flux density for actinic light (PPFD) is $300 \mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$

Values in table are presented as mean \pm standard deviation (n=9)

Means followed by the same letter do not differ based on the Duncan's New Multiple Range Test at $p \leq 0.05$

ETR – electron transport rate; F_v/F_0 – potential activity of PSII photochemistry; F_v/F_m – maximal quantum yield of PSII photochemistry; q_N – nonphotochemical quenching coefficient; q_p – photochemical quenching coefficient; Φ_{PSII} – effective quantum yield of PSII photochemistry

Table 5. Comparison of tissue structure of *S. stolonifera* in film sheds of different colors.

Item	Water content (%)	SLM (mg cm^{-2})	UE (μm)	LE (μm)	PTT (μm)	STT (μm)	Diameter of vascular bundles (μm)	
							Transverse	Vertical
White film shed	92.0 ± 0.02 a	3.69 ± 0.22 b	24.7 ± 2.52 a	14.7 ± 1.53 b	41.7 ± 4.72 b	140 ± 10.5 b	73.8 ± 5.11 a	123 ± 11.3 a
Red film shed	91.7 ± 0.01 a	4.12 ± 0.27 a	25.8 ± 3.55 a	17.7 ± 1.91 a	45.1 ± 2.17 a	149 ± 13.7 a	74.7 ± 7.07 a	123 ± 4.24 a
Blue film shed	93.2 ± 0.00 a	2.57 ± 0.19 c	24.7 ± 2.30 a	14.1 ± 1.17 b	36.2 ± 1.04 c	116 ± 5.57 c	74.1 ± 5.61 a	122 ± 15.5 a

Values in table are presented as mean \pm standard deviation (n=9)

Means followed by the same letter do not differ based on the Duncan's New Multiple Range Test at $p \leq 0.05$

Specific leaf mass are measured by dry weight. SLM – specific leaf mass; UE – upper epidermis; LE – lower epidermis; PTT – palisade tissue thickness; STT – spongy tissue thickness

Table 6. Comparison of bergenin and gallic acid contents of *S. stolonifera* in film sheds of different colors.

Item	DW (g m^{-2})	Content [$\text{mg g}^{-1}(\text{DW})$]		Yield [$\text{mg m}^{-2}(\text{DW})$]	
		Gallic acid	Bergenin	Gallic acid	Bergenin
White film shed	26.3 ± 2.14 ab	0.73 ± 0.06 ab	3.46 ± 0.36 a	19.2 ± 1.65 b	91.0 ± 6.59 a
Red film shed	28.4 ± 1.78 a	0.81 ± 0.07 a	3.53 ± 0.41 a	23.0 ± 1.72 a	100 ± 8.25 a
Blue film shed	23.7 ± 2.31 b	0.67 ± 0.05 b	3.34 ± 0.35 a	15.9 ± 1.19 c	79.2 ± 6.91 b

Values in table are presented as mean \pm standard deviation (n=9)

Means followed by the same letter do not differ based on the Duncan's New Multiple Range Test at $p \leq 0.05$

DW – dry weight

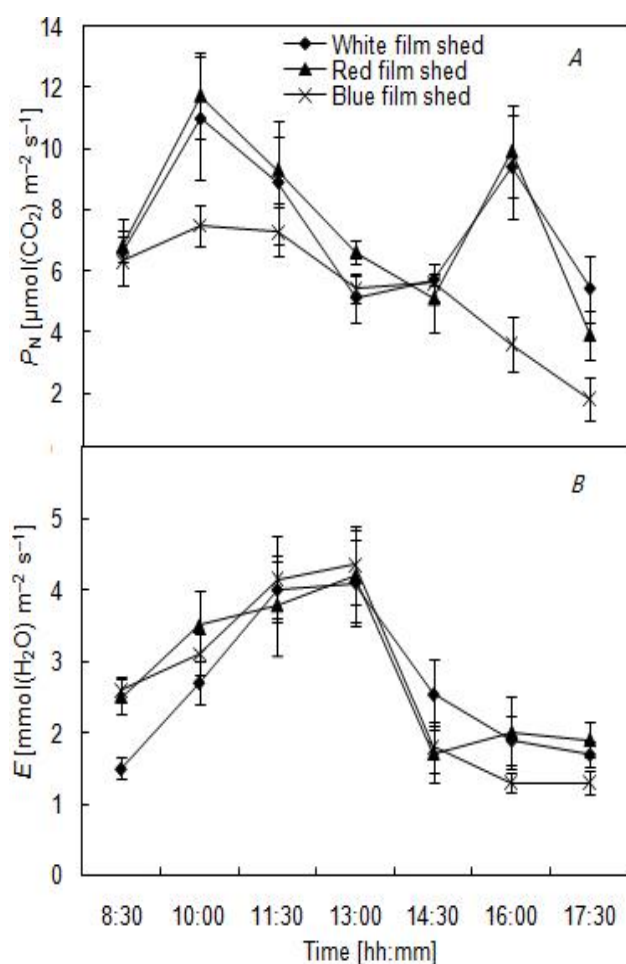


Fig. 1. The daily variation of (A) net photosynthetic rate (P_N) and (B) transpiration rate (E) of *S. stolonifera* in film sheds of different colors.

Error bars reflect the standard deviation of biological duplicates (n=9).

Discussions

Colored film shedding could produce dual effects on light intensity and quality qualities within the sheds (Tian *et al.*, 2013). In this study, the light intensity under different film sheds was maintained to the same. Thus, the calculated red/blue ratio and green/red ratio were significantly different among different colored film sheds. Light qualities have been shown to play an important role in regulating plant photosynthesis (Leong *et al.*, 1985). The absorption wavelength of plants is mainly in the red-orange and blue-violet light areas (Andrew *et al.*, 1997) and plants with different photosynthetic rates respond differently to light quality (Xu *et al.*, 2005). Previous studies have shown that red light could increase plant P_N and photosynthetic product accumulation by regulating development of photosynthetic organs (Ni *et al.*, 2009). *S. stolonifera* under red film shed had the highest daily average P_N , and the light response curve and its fitting formula showed that *S. stolonifera* under red film shed had the highest leaf P_N . This phenomenon should be related to the light quality under different sheds. Red film shed had more red light and significantly higher red/blue ratio than two other film sheds, indicating that red light is in favor of improving P_N of *S. stolonifera*.

Chlorophyll is an important pigment involved in light energy absorption, transfer and conversion in photosynthetic process. Its content and composition could significantly influence photosynthetic rate. Plants with higher chlorophyll b content could better absorb red and far-red light (Han & Chen, 2013). Treatment with light of different qualities can also influence chlorophyll a/b ratio in plants (Baig *et al.*, 2005; Cao *et al.*, 2013). Chlorophyll a/b ratio was the highest in *S. stolonifera* under blue film shed, which had minimum red/blue ratio. The results verified that blue light could increase chlorophyll a/b ratio (Jiang & Pan, 2006). Chlorophyll content was the highest under blue film shed possibly due to difference in starch accumulation of leaf mesophyll cells among different plants. Carotenoids as accessory pigments of chloroplast photosynthetic antenna can dissipate excessive energy of PSII in a non-irradiated mode, protecting chlorophyll from light damage. Our results showed that carotenoid content was the highest in *S. stolonifera* under blue film shed, which had the lowest red/blue ratio, indicating that appropriate blue light could increase leaf carotenoid content in *S. stolonifera*, which was consistent with a previous report (Wen *et al.*, 2011).

Photochemical quenching reflects the ratio of energy absorbed by PSII antenna used for photochemical electron transfer. To maintain a high level of photochemical quenching, it is necessary to maintain PSII reaction center in the "open" state. Thus, photochemical quenching also reflects to some extent the openness of PSII reaction center (Van Kooten & Snel, 1990). *S. stolonifera* under different film sheds had similar leaf F_v/F_m values, indicating that *S. stolonifera* under different film sheds all had good PSII function. The q_N of *S. stolonifera* leaves under white film shed was the highest, which meant that the majority of light energy absorbed by *S. stolonifera* leaves cannot be used in photosynthetic electron transport and was dissipated in the form of heat, which was one kind of self-protection mechanisms in plants (Jin *et al.*, 2003). Previous studies had reported that *S. stolonifera* was intolerant to strong light, but could still maintain higher photosynthesis rate under artificial red/blue light, possibly due to different light qualities (He *et al.*, 2012). This study showed that *S. stolonifera* under the white film shed had the lowest q_p and highest q_N , indicating that *S. stolonifera* had suffered from adverse effects, possibly caused by the highest UV transmittance under white film shed.

Light qualities have significant effects on the structure of leaves. Increased red/blue ratio could lead to thicker spongy and palisade tissues of *S. stolonifera*. Because active ingredients mainly presented in the mesophyll tissues, thickening leaf mesophyll tissues could increase not only biological production but also active ingredients biomass. The effect of light on plant secondary metabolism is very complicated. Different plants and different secondary metabolites responded differently to light qualities (Gao *et al.*, 2012), which were related to the differences in plant active ingredient

synthesis pathways. Photosynthesis is the only way to produce primary metabolites, which is not only necessary for plant life activity, but also for the precursors of plant secondary metabolites. The synthesis and transformation of secondary metabolites actively and continuously occurred in all actively growing cells (Mc-Mullen *et al.*, 1998). Our study showed that red light was conducive to the bergenin and gallic acid accumulation, which was consistent with the effect of red light on photosynthetic rate.

In summary, red light was in favor of photosynthesis, as well as accumulation of active ingredients and biomass in *S. stolonifera*. Therefore, *S. stolonifera* should be best planted in red film shed to maintain a high photosynthesis level, high PSII electron transfer rate, and appropriately increased leaf thickness, as well as ensure high biomass yield and high contents of important active ingredients. Overall, red film shedding could be used as a means to regulate light environment of *S. stolonifera* cultivation.

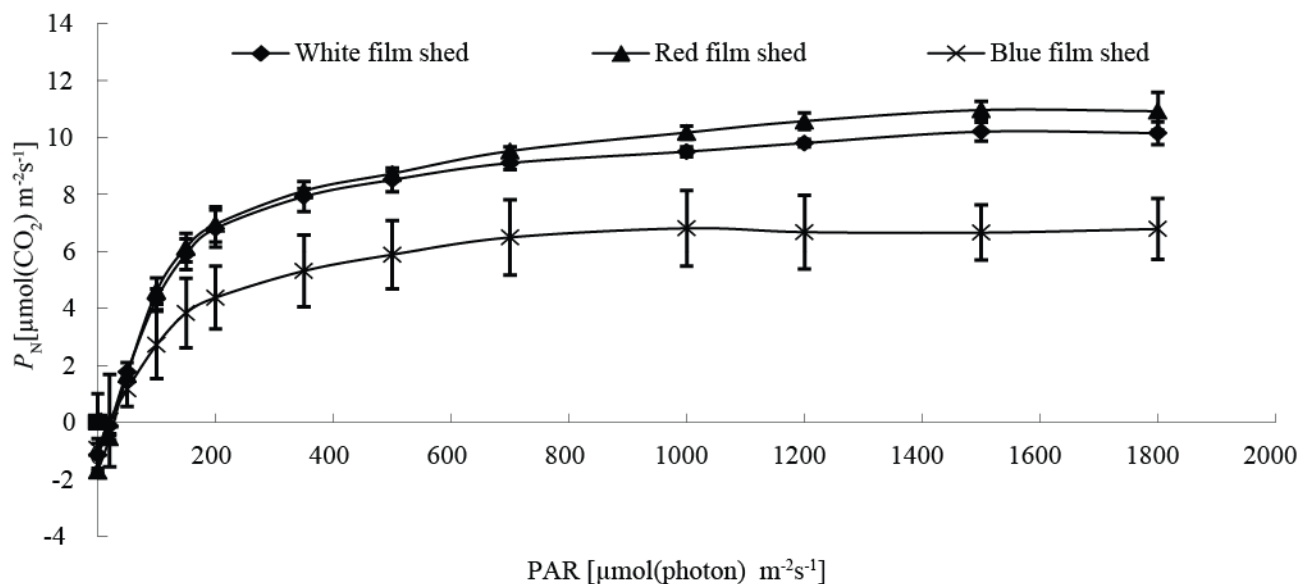


Fig. 2. Net photosynthetic rate (P_N) of *S. Stolonifera* under different irradiances. Error bars reflect the standard deviation of biological duplicates ($n=9$). PAR – Photosynthetically active radiation

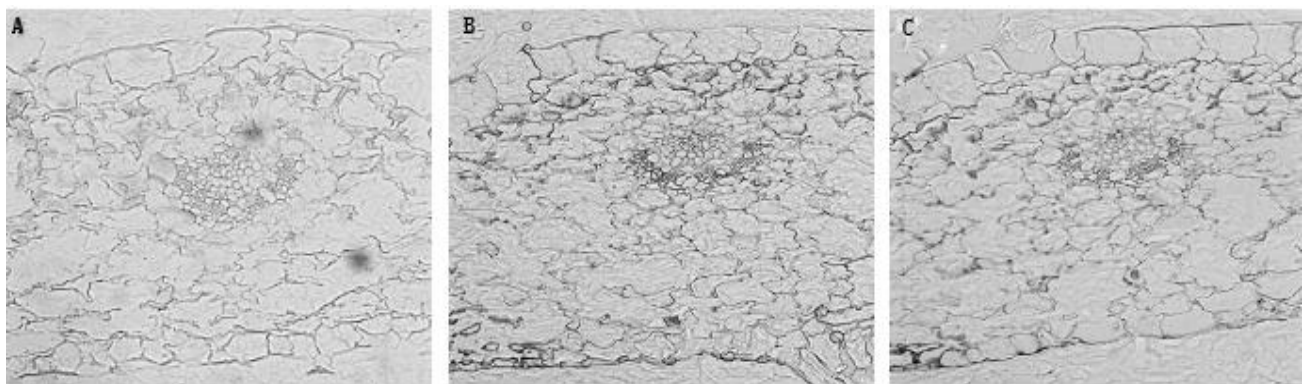


Fig. 3. The Microstructure of *S. Stolonifera* under different irradiances. A, B, C were the leaf cross-sections under white film, red film and blue film treatment (X100)

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