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Full Length Article

Influence of Continuous Illumination on Growth and Secondary Metabolism of Pakchoi (*Brassica campestris*)

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Abstract

Changes in the light quality alter the growth and metabolic responses of plants, although studies are limited on these lines. Using white light (CK and CLW), red (R), blue (B), combined far-red (RB+FR) and red combined blue (RB) light-emitting diodes (LEDs), we investigated the effect of different light qualities on growth and phytochemicals of pakchoi (*Brassica campestris* L.). After 24 h, the flavonoid and phenolic compounds content under CLW, RB and RB+FR treatment significantly increased compared to white light. With added FR light, the dry mass and leaf area increased by 67 and 94% as compared to white light. The study demonstrated that continuous light combines different light quality treatment could be used to improve growth and nutrition of pakchoi. © 2019 Friends Science Publishers

Keywords: Continuous light; Growth; Light quality; Pakchoi; Phenolics

Introduction

Light is a key important environmental factor affecting plant growth and phytochemical metabolism. Artificial light is currently applied in horticulture to improve the yield, quality and phytochemical composition of cultivated plants. Red and blue light are the primary focus of current research, owing to the reason that they are used in leaves plant for photosynthesis. In addition, far-red light also has effects on plant growth and development (Folta and Maruhnich, 2007; Tsormpatsidis *et al*., 2008; Li and Kubota, 2009).

In controlled environment, cultivating plants under continuous light (CL) is a way of enhancing the growth of vegetables efficiently. Exposure to continuous light affects some growth parameters, such as the quantum yield, net photosynthetic rate and metabolism and accumulation of metabolites, etc. (Pettersen *et al*., 2010; Lachinee *et al*., 2017; Abdullah *et al*., 2018). The mechanism of using continuous light in vegetables production eventually depends on the cost-to-benefit ratio (Ikkonen *et al*., 2015).

Light quality is one of the most important environmental factors affecting secondary metabolites biosynthesis in plants and influence secondary metabolites biosynthesis in many ways (Zoratti *et al*., 2014). For example, blue light could increase total anthocyanin content in strawberry fruit. Likewise, blue light increased the activation of its catalyzed enzymes, such as phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate: CoA ligase (4CL) and chalcone synthase (CHS) (Xu *et al*., 2014). Also, blue light and red light may regulate anthocyanin biosynthesis via activation of FaMYB10 expression, which is a positive regulator of anthocyanin accumulation (Zhang *et al*., 2018).

A number of examples suggest that the flavonoid and phenolic acid biosynthesis is influenced by light quality. Blue light could induce flavonoids and phenolic acids in a variety of plants, such as lettuce and pea seedlings. However, these responses are species-specific (Liu *et al*., 2016; Taulavuori *et al*., 2018).

The research on the effects of CL on plant growth has mainly focused on the induced injury and circadian clock (Velez-Ramirez *et al*., 2011). The effect of continuously applied light of different qualities on the content of phytochemicals remains largely unclear. The main objective of this study was to investigate whether CL light and the combination of far-red with RB LED light have positive effects on promote the synthesis of secondary metabolites. The results will provide theoretical basis for studying the light quality affecting plant secondary metabolism. Furthermore, this study will be beneficial in designing suitable light sources for the production of vegetables with enhanced phytochemical content in the greenhouses.

Materials and Methods

Experimental Material and Growth Conditions

The varieties of pakchoi was Suzhouqing (*Brassica campestris* L. ssp. *Chinensis Makino*), which was supplied by the Jiangsu Academy of Agricultural Sciences of

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Vegetables. Seed of pakchoi were kept in the wet sponge seedling block, and grown under halide lamp for 12 h light period inside a growth chamber maintained at 20~22℃. At four leaf stage, the uniform selected seedlings were cultured under LED light schedules for 24, 48 and 72 h. We set the seedlings into hydroponic box filled with total Hoagland nutrient solution (pH 6.0 and EC 1.2 dS/m) containing 5 mmol L^{-1} NO^{-}_{3}-N, 1 mmol L^{-1} P and 5 mmol L^{-1} K, while the nutrient solution changed once in a day. There were two fanners in the controlled environment, which to maintain the uniform CO_2 level in the chamber.

Light Treatments

The LED light used in the test were white light (peak wavelength 400–700 nm), red light (peak wavelength 660 nm), blue light (peak wavelength 445 nm) and far red light (peak wavelength 735 nm). Seedings were allocated to three groups and maintained under four schedules: continuous white light, the light quality ratio of red light: blue light was 1:1 and red light: blue light: far red light was 13:13:1. Seedlings grown under white LED light with a 12 h photoperiod was set as the control (CK). The photosynthetic photon flux density (PPFD) was maintained at 150 μmol m^{-2} s^{-1} by adjusting the distance between LED light sources and the plant. The parameters of the light in each treatment are shown in Table 1.

Plant Sampling

When the treatments were initiated, 12 plants were sampled from each light treatment (four plants per replicate, three replicates per light treatment). We sampled at 24, 48 and 72 h. The leaves of plants were immediately treated with liquid nitrogen after collection and then stored at -80℃ for secondary metabolites measurements. The growth room was equipped with an air exchange system which provided sufficient outdoor air inside the room.

Fresh and Dry Mass

The fresh mass of each plant was weighed right after harvested. After weighed the fresh mass, the plants were dried in an oven at 100ºC for 20 h and dry masses were weighed then.

Determination of Anthocyanin, Total phenolic compounds and Flavonoids

Anthocyanin: The method of the pH differential shift method was used to determine the total anthocyanin (Giusti and Wrolstad, 2001; Li and Kubota, 2009). A 0.1 g freeze-dried sample was put into 5 mL 2% of hydrochloric acid-methanol solutions for 48 h and then separated the liquid extract by centrifugation at 8000 rpm for 8 min. Two sample diluents were prepared: sodium acetate buffer (0.4 M, pH 4.5) and potassium chloride buffer (0.025 M, pH 1.0). Then, 800 μL of extract was diluted to 2.0 mL with two different buffers. After 20 min reaction, the reaction fluids was filtered through the 0.2 μm pore size filter paper and then measured the absorbance at 515 nm and 700 nm, respectively, using a spectrophotometer (OPT-2000, ABDPE CO., Beijing, China). Total anthocyanins concentrations were expressed as cyaniding-3-glucoside equivalent values.

Total phenolic compounds: The modified method to Singleton and Rossi (1965) was used for this measurement. Freeze-dried samples (0.1 g) was extracted with 5 mL of 50% methanol in 30℃ water bath and shaken at 200 rpm for 5 h. Then, mixed 1 mL reactive fluid with 0.2 mL Folin-Phenol reagent. The mixture was vortexed and added to 2.0 mL of 7% Na_2CO_3 at 25℃ for 3 h and then measured the absorbance at 735 nm using a spectrophotometer.

Total flavonoids: The total flavonoids were determined by using a method of Chen *et al.* (2015) which we improved. Freeze-dried samples (0.1 g) were reflux extracted with methanol in 60 for 40 min. Then 0.15 mL of 5% $NaNO_2$ and 0.5 mL methanolic extract were added in a 5 mL tube and let the reaction solution stand for 10 min at 20℃. Then added 0.15 mL of 10% $AlCl_3 \cdot 6H_2O$ to the mixture, kept for 5 min and then added 1 mL of 1 M NaOH. The chromogenic reaction was stayed for 20 min and measured the absorbance at 510 nm using a spectrophotometer. Rutin was used to prepare the standard curve, and the amount of flavonoid was express as rutin equivalent.

Statistical Analysis

Statistical analyses were conducted using Statistical Product and Service Solutions for Windows, version 16.0 (SPSS Inc., Japan). The data were analyzed using one-way ANOVA and the differences between the means were tested using Tukey Test ($P<0.05$).

Results

Effect of CL and different LED Qualities Lights on Biomass of Pakchoi

Growth of pakchoi was significantly affected by applying 72 h of CL and different quality lights (Table 1). After 72 h of CL treatment, the dry mass increased significantly by 68% under the FRB treatment compared to the CK (Table 2). Plant fresh mass was not significantly affected with four treatments.

Effect of CL and different LED Light Qualities on Phytochemical Accumulation of Pakchoi

Continuous light and different quality lights significantly affected phytochemicals content of pakchoi leaf (Table 3). After 72 h, the anthocyanin content under CLW and CK

Table 1: Major technique parameters of different light spectral energy distribution

Light treatment	Peak wavelength λp (nm)	PPFD (μmol m^{-2} s^{-1})	Ratios Red/far-red
CK	6000k	150	10.1
CLW	6000k	150	10.1
RB	460+660	150	>100
RB+F	460+660+735	150	4.5

Table 2: Fresh weight (g), dry weight (g) and leaf area (cm^2) of pakchoi under the different light treatments

Treatments	FW (g)		DW (g)		Leaf area (cm^2)
	0 h	72 h	0h	72 h	72h
CK	6.59a	9.41a	0.67a	0.73b	42.1c
CLW	6.19a	9.64a	0.62a	1.08ab	54.3c
RB	6.46a	9.40a	0.67a	1.08ab	67.5b
FRB	6.81a	10.80a	0.71a	1.23a	82.1a

Means within the same column followed by different lower case letters are significantly different between treatments at P =0.05 according to Duncan's multiple range test

Table 3: Phytochemical components of pakchoi leaf in response to light treatments

Time (h)	Treatments	Anthocyanins (μg g^{-1} fresh weight)	Flavonoid (μg g^{-1} fresh weight)	Phenolic compounds (mg g^{-1} fresh weight)
24	CK	31.90b	29.98c	17.88c
	CLW	68.04a	39.84b	23.96b
	RB	46.62b	40.75b	26.49b
	RBF	60.66ab	55.28a	32.88a
48	CK	39.60c	29.97b	17.14b
	CLW	113.16a	31.53b	19.87b
	RB	67.74c	45.42ab	23.67a
	RBF	78.78b	49.47a	27.83a
72	CK	89.76b	33.87a	16.41b
	CLW	128.28a	37.60a	17.28b
	RB	46.44c	35.91a	18.99a
	RBF	49.80c	33.93a	21.69a

Means within the same column followed by different lower case letters are significantly different between treatments at P =0.05 according to Duncan's multiple range test

displayed an upward trend, while the anthocyanin content under RB and FRB treatment first increased and then showed a marked decrease. At 72 h, the CLW and RB treatments resulted in the high anthocyanin content and CLW being significantly higher than those three treatments. The flavonoid content under continuous light treatment had a similar trend, which decrease as the light duration increased. From hour 24 to 48 h, the flavonoid content under BR treatment increased by 30% in 48 h and then decreased in 72 h. The total phenolic compounds in pakchoi leaves under four treatments were decreased at 72 h significantly. Compared with CK, the total phenolic content increased with CL, RB and FRB by 34, 48 and 83%, respectively, at 24 h time duration.

Discussion

On growth of lettuce plants, a similar effect of FR was reported (Li and Kubota, 2009). One of the factors influencing biomass is leaf area, the treatment with supplemental FR would create lowest R/FR ratio of the light environment, and this light environment can enlarged leaf area. Larger increase in leaf area was for more light interception, which may led to the more photosynthesis and a significantly increase on biomass.

Previously studies have demonstrated that anthocyanin biosynthesis in plants was regulated by light. For example, phytochrome was involved in anthocyanin synthesis (Ramalho *et al*., 2002). Zhang *et al*. (2018) have reported that red light combined with blue light induced anthocyanin accumulation in strawberry fruit. However, Miao *et al*. (2016) reported that using blue plastic films led to the decrease of anthocyanin content in strawberry. This may be relevant to species, growth environment and other factors. Previous experiments were all 12 h of photoperiod, which was quite different from our experimental treatment. Another important factor for anthocyanin biosynthesis is the R/FR ratio. An increase in this ratio from 0.5 to 3.07 has been shown to increase anthocyanin content with increasing R/FR ratio, when the R/FR ratios is within a range of 0.5–3.07 (Li and Kubota, 2009). In our study, compared to the control, the supplemental FR treatment significantly reduced anthocyanin content. This finding agrees with a previous research reported by Li and Kubota (2009).

Different light qualities could regulate the formation of flavonoid methyl derivatives and flavonoid glucoside derivatives. However, a recent study showed that the effects of light quality on the synthesis of flavonoids are plant species dependent (Cope and Bugbee, 2013). In this study,

we found that providing far-red light facilitates flavonoid accumulation under 24 to 48 h continuous illumination. In general, shorter wavelengths lead to the greater accumulation of flavonoids. We found different experimental results, which might be due to the continuous illumination and specific spectral wavelengths, while flavonoid compounds accumulated might be a defense of plant when under the specific light wavelengths (Liu *et al*., 2018).

About the effect of light on total phenolics, a previous study found blue light significantly increased the content of gallic acid which is an important phenolic acid. On the other hand, an increase in phenolics may be due to the higher activity of PAL enzyme under blue light (Engelsma, 1974). Although part of the mechanism is still not clear, it is speculated that red light modulates the synthesis of phenolics due to the increase in cytokinin level (Qamaruddin and Tillberg, 1989; Galuszka *et al*., 2005). We need to do more studies to clarify the mechanisms.

Conclusion

The CL and different light wavelengths could be used as a supplemental cultivation practice for promotion of growth and nutritional value of pakchoi. Providing continuous illumination could enhance phytochemicals accumulation. Under the condition of continuous light, exposure to far-red light increased biomass, flavonoid and phenolic concentration, but resulted in lower anthocyanin concentrations. Further studies are needed to explore the regulation of light-environment by selected light qualities in both growth chamber and greenhouse.

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