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Effects of intermittent light exposure with red and blue light emitting diodes on growth and carbohydrate accumulation of lettuce

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ABSTRACT

In this study, effects of continuous and intermittent irradiation of mixed red (R) and blue (B) light (RB) provided by LEDs on the growth and carbohydrate accumulations of lettuce were examined. Combined RB was provided 16 h per day (24 h) in all the six treatments, among which, continuous RB with only one light/dark (L/D) cycle of 16 h/8 h in treatment L/D(1) was regarded as control. Five intermittent RB treatments that were respectively L/D(2) with two L/D cycles of 8 h/4 h over a 24-h period, L/D(3) with three L/D cycles of 6 h/3 h or 4 h/2 h, L/D(4) with four L/D cycles of 4 h/2 h, L/D(6) with six L/D cycles of 3 h/1.5 h or 1 h/0.5 h, and L/D(8) with eight L/D cycles of 2 h/1 h were set up. Results indicated that shoot biomass of lettuce was increased by intermittent RB treatment L/D(2), L/D(4), L/D(6) and L/D(8) to different extents, while decreased significantly by L/D(3) compared with the control. Significantly higher hexose/sucrose ratio (in comparison with any other treatment) accompanied with the strongest combined activities of sucrose degrading enzymes as well as the weakest combined activities of sucrose synthesizing enzymes was observed in plants treated with L/D(2) treatment. Significantly higher content of soluble sugar especially fructose as well as lower contents of crude fiber and starch were observed in lettuce exposed to L/D(2) and L/D(3) compared with any other treatment. All intermittent treatments significantly enhanced the glucose content in lettuce compared with the control. Both L/D(2) and L/D(3) treatments promoted lettuce taste via improving the sweetness and crispness, whereas only L/D(2) simultaneously increased the shoot biomass (in terms of dry weight) compared with the control.

1. Introduction

Red (R) and blue (B) lights are predominantly absorbed by photosynthetic pigments in plants and have a strong influence on plant architecture, photosynthetic apparatus development and phytochemical synthesis (Shao et al., 2015; Giliberto et al., 2005). Combined R and B has been regarded as optimal light quality to support the normal growth of most plants. So far, lots of studies have been performed focusing on the effects of combined RB on plant cultivations from the aspects of light intensity, R/B ratio and photoperiod of artificial lights (Li et al., 2013; Fu et al., 2012; Wang et al., 2016). However, only a few studies have dealt with the influence of irradiation modes on the electric energy utilization efficiency by plants. Yamada et al. (2000) found that dry weight of sweet potato plant was 1.1 times higher in stepwise PPF (photosynthetic photon flux) condition than that in constant PPF condition based on the same integrated PPF. Thus, stepwise PPF might be a useful method to increase the electric energy utilization efficiency. Hoffmann et al. (2016) reported that alternating illumination with high/low blue light enhanced the potential of pepper leaves to accumulate epidermal flavonols and triggered the synthesis of anthocyanins and carotenoids. Chen et al. (2017) reported that alternating R/B light with the alternating intervals of 8h and 1h resulted in higher yield of lettuce than the cocurrent RB light based on equal energy consumption. Kurata et al. (2000) proposed that anthocyanin production by strawberry cells depended not only on light intensity but also the light/dark cycle operations with hour-scale or second-scale periods. Sivakumar et al. (2006) compared the influence of intermittent light on sweet potato plantlets in vitro with that of continuous light, and found that intermittent RB resulted in greater dry weight and carbohydrate content than the continuous RB light. As reported by Jao and Fang (2004), continuous/pulse light and simultaneous/alternating light can be generated by an LED light source with a proper driver design. Therefore, it is worthy to investigate the optimized irradiation patterns taking both plant growth and electricity cost into account. It can be expected that optimized RB irradiation modes will achieve ideal electric energy utilization efficiency.

The composition and content of carbohydrate determine not only the quality but also the flavor of vegetables (Fillion and Kilcast, 2002;

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Chadwick et al., 2016; Stokkom et al., 2016). Lin et al. (2013) reported that soluble sugar content in lettuce was increased by RBW (RB plus white light) treatment compared with FL (fluorescent) or RB treatment. Li et al. (2013) demonstrated that soluble sugar and starch contents in rapeseed plantlets exposed to monochromic R were significantly greater than those exposed to FL. Li et al. (2010) stated that R might be the best light for the accumulation of sucrose, starch and soluble sugar in upland cotton plantlets compared with B, RB, or FL. Kong et al. (2008) observed higher concentrations of starch and sucrose in *Doritaenopsis* plants exposed to RB than those grown under monorchromic R or B; Choi et al. (2015) reported that the formation of sucrose in strawberry was inhibited under monochromic B compared with R or RB. However, knowledge on the effects of intermittent RB on the composition and accumulation of carbohydrate in lettuce is limited.

Soluble sugar, mainly consisting of fructose, glucose and sucrose, is directly responsible for the sweetness of lettuce (Chadwick et al., 2016). Higher proportion of soluble sugar in total carbohydrate resulted in better taste for lettuce (Lin et al., 2013). The total sweetness index (TSI) used to indicate sweetness of horticultural produce is based on the content and sweetness coefficient of each sugar (Magwaza and Opara, 2015). The key enzymes responsible for sucrose metabolism are respectively invertase (EC 3.2.1.26), sucrose synthase (SS, EC 2.4.1.13), and sucrose phosphate synthase (SPS, EC 2.4.1.14). The regulation of carbohydrate metabolism have been investigated extensively based upon the functions of those enzymes (Qi et al., 2005; Choudhury et al., 2009).

The aim of this study was to determine the effects of different irradiation modes of combined RB on the growth and carbohydrate accumulations of lettuce. Plant morphology, biomass, the contents of soluble sugar, starch and crude fiber, as well as the activities of sucrose metabolism enzymes in lettuce were investigated. Results from this study are expected to improve the application modes of RB light and optimize lighting strategies without adding additional energy consumption in a horticultural production system.

2. Materials and methods

2.1. Experimental set-up and growth conditions

After incubation at 4 °C on moistened gauze for 5 d, germinated lettuce seeds (*Lactuca sativa* var. *crispa* 'Green Oak Leaf') were sown in sponge cubes and hydroponically grown in an environmentally controlled growth room. Environmental conditions in the experiment were set at 23 °C, 70% relative humidity (RH) and 700 μmol mol^{-1} CO_2 level. There were 3 hydroponic boxes in each treatment and 20 plants were planted in each box. The Hoagland's solution was used and renewed per week, the electrical conductivity (EC) and pH were adjusted to 0.11–0.12 S m^{-1} and 5.8–6.0 respectively. The plants were irradiated with six light treatments with different radiation modes described below and harvested at 60 days after sowing (DAS).

2.2. LED panels and the light treatments

Irradiation treatments were performed using two-color LED panels which provided R with peak wavelength of 660 nm and B with peak wavelength of 450 nm determined by a spectrophotometer (Ocean Optics, model-SD 650, USA). Irradiation intensity of R and B could be individually controlled by regulating electric current of power DC supply, and intermittent period of R and B could be adjusted via the built-in timing switches. R and B were concurrently provided and the photosynthetic photon flux density (PPFD) of R and B was respectively set at 180 μmol m^{-2} s^{-1} and 20 μmol m^{-2} s^{-1} in all treatments as measured with a light quantum meter at plant canopy level (LI-250A, LI-COR, USA).

As shown in Fig. 1, combined R and B (RB) were provided 16 h per day (24 h) in all the six treatments. Continuous RB with only one light/dark (L/D) cycle of 16 h/8 h in treatment L/D(1) was regarded as control, while five intermittent RB treatments that were respectively L/D(2) with two L/D cycles of 8 h/4 h over a 24-h period, L/D(3) with three L/D cycles of 6 h/3 h or 4 h/2 h, L/D(4) with four L/D cycles of 4 h/2 h, L/D(6) with six L/D cycles of 3 h/1.5 h or 1 h/0.5 h, and L/D(8) with eight L/D cycles of 2 h/1 h were set up. The daily light integral as well as the total energy consumption were completely the same among all treatments.

2.3. Sampling and biometric measurements

At 60 DAS, the growth light in all treatments was turned off, only leaving interior lighting provided by white LED tubes. Samples for all measurements were harvested at 60 DAS, and six plants randomly taken from each hydroponic box were regarded as a repetition (three repetitions in per treatment). Plant height was measured from the stem base to the top of lettuce plant, while stem diameter was measured at the upper apex of stem using a vernier calliper. Fresh weight (FW) and the contents of chlorophyll (Chl) and carotenoid (Car) were determined using fresh lettuce samples. Dry weight (DW) and carbohydrate content were determined using the oven-dried lettuce samples (70 °C for 48 h). The materials for enzyme activity measurement were sampled after the growth light was turned off for approximately 3 h, thereafter the samples were instantly frozen with liquid nitrogen and stored at −80 °C in a super cold refrigerator. The ratio of shoot and root (S/R) was determined from shoot/root DW. Moreover, plant morphology description was conducted based on six representative plants chosen from each treatment at harvest.

2.4. Determination of chlorophyll and carotenoid

A total of 0.2 g fresh samples from the mature leaves of lettuce (the fifth fully expanded leaf, counted from inside outwards) were ground in a mortar, and then washed using 80% acetone and subsequently filtered (repeated until the leaf turned white). The filtrates were diluted to a total volume of 100 ml with distilled water. The absorbance of the extraction at 470 nm, 645 nm, and 663 nm was respectively measured by a TU-1810s spectrophotometer (PERSEE, Beijing, China). Concentrations of Chl and Car were determined using the following equations (Lichtenthaler and Wellburn, 1983):

$$\text{Chl a (mg/g)} = \frac{(12.72 \times OD_{663} - 2.59 \times OD_{645})V}{1000\,W}$$

$$\text{Ch1 b (mg/g)} = \frac{(22.88 \times OD_{645} - 4.67 \times OD_{663})V}{1000\,W}$$

$$\text{Car (mg/g)} = \frac{((1000 \times OD_{470} - 3.27 \times \text{Chl.a} - 104 \times \text{Chl.b}) / 229)V}{1000\,W}$$

V is the total volume of acetone extract (ml) and W is the fresh weight (g) of the sample.

2.5. Determination of carbohydrate

1.0 g (DW) lettuce shoot sample was extracted in 5 ml 80% (v/v) ethanol for 30 min in a 80 °C water bath and subsequently centrifuged at 12,000 × *g* for 10 min (repeated twice). The supernatant were combined for measuring sugar (fructose, glucose, and sucrose) contents, while the precipitate was collected for measuring starch content. (1) For sugar content determination: the supernatant was evaporated in a 85 °C waterbath, then the residues were re-dissolved in 20 ml distilled water and subsequently passed through 0.45 μm microporous membrane. Fructose, glucose, and sucrose contents were carried out via the HPLC system (Waters, model-e2695, USA) equipped with 3.5 μm Waters XBridge Amide column (4.6 × 150 mm) at 30 °C. The mobile phase was acetonitrile/water (75/25, v/v) at a flow rate of 1.0 ml min^{-1}, and the concentrations of the separated sugars were determined according to the corresponding standards (Standard substance