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Effect of green, yellow and purple radiation on biomass, photosynthesis, morphology and soluble sugar content of leafy lettuce via spectral wavebands "knock out"

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ABSTRACT

Spectral composition of artificial light is an important environmental factor for plants cultivated in controllable environment, eg., plant factory. In an attempt to explore the influence of different spectral wavebands on leaf lettuce growth and development, a new way of light treatment with turning off the specific narrow-wavebands from the wide spectrum was established. And leaf lettuce was cultivated under different light spectra with the spectral wavebands of green (LG), yellow (LY) and purple (LP) "knockout" respectively in controllable environment. Then changes in biomass, morphological indexes, photosynthetic gas exchange, chlorophyll content and soluble sugar content of lettuce were examined. Our results revealed that, compared with plants grown under wide-spectral LED light (CK), LG treatment showed significantly reduction of fresh shoot weight at 25 days after planting (DAP) comparing to CK. LY and LP obtained obviously higher fresh shoot weight and moisture content at 15 DAP, in contrast, significantly lower ratio of root to shoot than CK. And A/Ci in LG were significantly reduced by about 33.3% and 36.1% at 20 DAP and 25 DAP respectively compared with CK. In addition, LY showed significant increases in soluble sugar content compared to other treatments at three sampling stages, while the lowest values were obtained in CK. These results indicated that green, yellow and purple light had significant regulations on the biomass, photosynthesis and soluble sugar content in lettuce, depending on the different growth period.

1. Introduction

Vegetable production in controllable environment can defense the threats of outdoor severe climate and adverse environmental conditions. The yield and nutritional quality of vegetables can be improved by adjusting the cultivation environment conditions. Light is not only an essential energy source for plants, but also an important signal for plant growth and development (Chory and Li 1997; Clouse 2001; Kim et al. 2002). Apart from the effect of photon flux density (PFD) (Jeon et al. 2005; Ali et al. 2005), quality of light as well affects photosynthesis (Kim et al. 2004) and other developmental and biochemical processes such as germination, flowering (Taiz and Zeiger 2002; Liu et al. 2016) and stomatal regulation (Taylor and Assmann 2001). And it is also widely understood that light quality could positively affect phytochemical accumulation (Engelen-Eigles et al. 2006; Ohashi-Kaneko et al. 2007).

Light-emitting diode (LED) lighting systems have several unique advantages, including the ability to determine their spectral composition, small size, high photosynthetic efficiency, long lifespan, less thermal radiation as well as high safety performance (Morrow 2008). These lighting systems allow wavelengths to be matched to plant photoreceptors for providing better production and for influencing plant morphology and metabolic composition (Bourget 2008; Massa et al. 2008; Morrow 2008; Shao et al. 2015). Therefore, LED light sources have already been successfully used in facility agriculture and became the most promising artificial electronic light for plant cultivation in controllable environments, such as plant factory on earth and life support system in space (Morrow 2008; Fu et al. 2013). Also LED light source has been considered to be the most suitable light to study the effect of light quality on growth and development of plants. Previous studies reported that monochromatic and combined LED could modulate plant morphological specificity (Yanagi et al. 1996; Yanagi

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and Okamoto 1994; Yorio et al. 2001) and photosynthesis (Shin et al. 2003; Schuerger et al. 1997; Hogewoning et al. 2010), as well have significant influence on plant metabolic composition, such as vitamin C, flavonoid antioxidants and soluble sugar, soluble protein, nitrate (Li and Kubota 2009; Lin et al. 2013; Chen et al. 2014). Thus the photosynthetic efficiency, growth, morphology and metabolic composition could be improved by modulating the spectral composition of LED light sources.

Light sources used in current researches were always monochromatic LED lights with narrow waveband, to which plants had shown obvious responses. While in the natural environment, plants have adapted to the natural wide-spectrum by the long-term evolution. There might be coupling effects among different wavebands on plants, and could not be fully considered, and the specific functions on plant growth and development of specific wavebands in the wide spectrum were confused. In order to exactly examine the effects of green, purple and yellow light on plant growth, a new method was established, the designing of special spectrum was modeled after the principle of gene “knockout”. Using this method, the responses of plants to specific narrow wavebands could be invested from a different angle.

Lettuce (*Lactuca sativa* L. var. *youmaicai*) is one kind of the most popular leafy vegetable cultivated in controllable environment, and chosen as the plant material. Plant biomass, morphologic characteristics, photosynthetic pigments, gas exchange, soluble sugar have been analyzed. The results of this study show promise for improving plant growth and phytochemicals by regulating light environment, as well as can afford a new perspective for expanding and deepening research of the biological effects of light quality on plant.

2. Materials and methods

2.1. Design of a wide-spectrum LED light source

An LED panel (30 × 30 cm^2) was fabricated using 63 high-light LEDs of six types: violet (peak at 402 nm), blue (450 nm), green (520 nm), yellow (590 nm), red (660 nm) and far-red (850 nm). The chips for all the LEDs were bought from CREE Inc. (US) and the panel was custom-made from *E.* shine Systems Limited. The details for each type of LEDs are shown in Table S1. The same-type LEDs were located in one column, so there were six columns in total. Each type of LEDs could be controlled separately. Design drawings and the object of the LED light were shown in Fig. S1. Spectra of the LED light was measured with optical fiber spectrometer (Avaspec - 2048, USB2, UA, Avantes B. V., Netherlands), and shown in Fig. 1. Our LED light covering from 360 to 710 nm continuously could be considered as a wide-spectrum plant cultivation light source and also had the function of waveband “knock out”.

2.2. Evaluation for energy distribution of LED light in planting space

To control each individual narrow waveband, we assembled the same type of LEDs in the same column, but this caused uneven distribution of irradiation. In order to clarify the distribution of spectral radiation, we selected four ring-type surfaces (inner and outer radii were 7.5 and 15.5 cm respectively) in the vertical space under the LED panel. The distance from the lamp to each of the four ring-type surfaces was 34, 29, 20 and 9.5 cm, respectively. From each ring-type surface, 91 points were selected, of which one was the central point and the other 90 points were uniformly distributed within the surface. Radiation intensity of a specific waveband received by every point was measured with an Avaspec-2048-USB2 UA spectroradiometer (Avantes B. V., Netherlands). The radiation distribution of different spectral wavebands in the vertical space between the cultivation surface and the LED panel was calculated via interpolation-based spline integration (Fig. S2).

2.3. Design of waveband “knock-out” treatments

In order to comprehensively study the effects of each waveband on the growth of leaf lettuce, we set three “knock out” treatments at narrow wavebands of 360–420 nm (LP), 480–560 nm (LG), 560–610 nm (LY), respectively. An LED wide-spectrum light source was used as the normal control (CK). Spectra are shown in Fig. 1. According to the intensity distribution of the LED light source, the total light intensity received by the cultivation surface was kept constant at about 98–105 μ mol $m^{-2} s^{-1}$ by adjusting the distance from the LED lamp to the cultivation surface. The radiation of each waveband covered the whole cultivation space, but the distribution was uneven (Fig. S2). In order to keep the light radiation of various wavelengths received by every plant consistent during the whole growth period, we used rotary plates to make the plants slowly and circularly move at the speed of 1.5 RPM. Each treatment was repeated three times so as to avoid the possible error caused by environmental factors.

2.4. Plant cultivation and environment conditions

Seeds of leaf lettuce (*Lactuca sativa* L. Var. *youmaicai*) were germinated in a “Petri” plate (with moist filter paper) and hydroponically grown for 10 days in an environmentally controlled chamber. The temperature was at constant 20 °C under a light intensity of approximately 80 μ mol $m^{-2} s^{-1}$ photo flux density (PFD) for 24 h with cool white fluorescent lamps. The relative humidity was around 65%. Uniform-sized seedlings at one real-leaf stage were transplanted to a Styrofoam plate with eighteen holes (one plant per hole), and placed in a container (37 cm × 23.5 cm × 9 cm) filled with 1/2 Hoagland nutrient solution. The nutrient solution was renewed every three days and adjusted to pH 6.5 using 2 mol L^{-1} HNO_3. All treatments were performed in a greenhouse in which the temperature, relative humidity as well as the CO_2 concentration could be controlled. And the photoperiod was maintained at 24 h. The air temperature, relative humidity and [CO_2] for all treatments were 21 ± 2 °C, 35%～50%, and 350 ± 20 ppm throughout the whole experiment. Plants were harvested at 25 days after planting (DAP). In addition, all treatments were carried out at the same time to avoid the possible influence of growth conditions.

2.5. Methods of determination and analysis

2.5.1. Biomass and morphology

The whole growth time of leaf lettuce was divided into three periods: 15 DAP, 20 DAP and 25 DAP. Measurements including shoot fresh weight, shoot /root ratio dry weight (DW), height of plant, leaf width, leaf length and moisture content of shoot were performed, data and plant samples were collected at 15, 20, and 25 DAP for each light treatment. Plant samples were dried in a drying oven for 1 h at 120 °C, and further dried at 85 °C to the constant weight.

2.5.2. Leaf gas exchange measurements

We measured net photosynthesis (A, μmol CO_2 $m^{-2} s^{-1}$), internal CO_2 concentration (C_i, ppm). Data was periodically collected with the latest fully expanded leaves using a portable open gas exchange system (LI- 6400 XT OPEN 6.1, Ecoteck, USA), at a light intensity of 250 μmol $m^{-2} s^{-1}$ (supplied by in-built red/blue light-emitting diode). The temperature, [CO_2] and humidity were 21 ± 2 °C, 350 ± 20 ppm and 60% respectively. Then instantaneous carboxylation efficiency ($ICE = A/C_i$, μmol CO_2 $m^{-2} s^{-1} ppm^{-1}$) was calculated.

2.5.3. Chlorophylls measurements

Fresh plant samples were cleaned and cut into pieces after removing the midrib. Three repeats of the minced sample with 0.2 g each, were placed in a mortar respectively, and a little of quartz sand and calcium carbonate powder, and 2–3 ml of 95% ethanol were added, and then