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Carbohydrate accumulation and sucrose metabolism responses in tomato seedling leaves when subjected to different light qualities

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ABSTRACT

Light qualities are thought to affect many plant physiological processes during growth and development. To investigate how light qualities influence tomato (*Solanum lycopersicum*) seedlings, the present study evaluated the effects of different light qualities generated by light emitting diodes (LEDs) with the same photosynthetic photon flux density (PPFD), including monochromic light red (657 nm, R), blue (457 nm, B), purple (417 nm, P) or white (W), combination of R and B lights (R:B = 1:1, 1R1B and R:B = 3:1, 3R1B) on the net photosynthetic rate (Pn), growth rate, carbohydrate content and sucrose-metabolizing enzyme activities. The results showed that relative to W, the seedling plant height and stem diameter were significantly promoted by combination of R and B lights and monochromic R light. However, the level of root growth was lower under R and P light, and the seedling growth and Pn were significantly suppressed under the latter. Additionally, R light significantly increased the contents of fructose and glucose, and combination of R and B lights significantly enhanced total carbohydrate, starch and sucrose accumulation, especially for 3R1B treatment. Activity of sucrose synthase (SS) was promoted under the different treatments and reached its highest value under 3R1B, which appeared to be a major contributor to the significantly higher content of starch under this treatment. Furthermore, R, B and P light reduced activity of sucrose phosphate synthase (SPS). Activities of acid invertase (AI) and neutral invertase (NI) were significantly increased by R light, but were markedly reduced under P. The results presented here indicated that monochromic R and combination lights 3R1B, could regulate the plant morphology and photosynthesis by the effects on the metabolism of carbohydrate into fructose, glucose, sucrose and starch, mainly through the enhanced activities of AI and NI under the former treatment, while SS and SPS in the latter treatment, respectively. They also improved the end-product output in tomato leaves, and may ultimately improve the yield and quality of tomato fruit.

1. Introduction

Light is the most important source of energy for plant photosynthesis and is also an important signal for plant growth and development (Hudák et al., 2005; Jiao et al., 2007). It is known that plants can respond to the quantity of light and photoperiod length, and to its quality (Neff et al., 2000; Yamazaki, 2010). Light qualities are known to control morphogenesis, growth and differentiation of plant cells, tissues and organ cultures (Moshe and Dalia, 2007; Gupta and Jatothu, 2013). For instance, the plant height of tomato (*Solanum lycopersicum*) was reduced under B light, but expression of some photosynthesis-related genes was up-regulated, and the electron transport rate values were enhanced. Whereas, the Pn and expression of other photosynthesis-related genes were promoted under R light (Wu et al., 2014). Our previous studies found that the plant height, specific leaf area (SLA) and intercellular CO_2 concentration of tomato seedlings was significantly promoted by R light, the root growth was inhibited and the seedling index was reduced. The seedling growth was significantly suppressed under B light and the chlorophyll content was decreased (Wang et al., 2017a). Moreover, combination of R and B lights could improve transcription levels of nitrogen metabolism-related enzymes and activities of some key enzymes in this pathway (Wang et al., 2017b).

Light emitting diodes (LEDs) are regarded as a new light source that can be used to enhance the production of horticultural crops under closed conditions (Gómez and Mitchell, 2015; Hernández and Kubota, 2016). Therefore, it is more accurate, practical and relevant to use LEDs to investigate the effects of light qualities on plant growth and development (Morrow, 2008). Carbohydrate accumulation plays an

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important role in plant growth and development. Starch and sucrose are the major storage carbohydrate in many plants and the principal form in which carbon is transported through the plants (Duffus and Duffus, 1984; Smith, 1999). However, previous reports have scarcely described the effects of LED light source on the biosynthesis of carbohydrate metabolites in plants. In this paper, the carbohydrate content and activities of sucrose metabolism associated enzymes, including acid invertase (AI), neutral invertase (NI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) in tomato seedlings cultured under different LED light treatments was investigated to examine how light qualities affect plant photosynthesis and growth.

2. Materials and methods

2.1. Light treatments

All the LEDs were produced by the Unihero Co., Shenzhen, China. The light treatments were designated as monochromic R light with a maximum intensity at 657 nm, B with a maximum intensity at 457 nm, P with a maximum intensity at 417 nm and a multiwavelength W LED (CK), combination of R and B LEDs (R:B = 1:1, 1R1B: 50% R light at a wavelength of 657 nm and 50% B light at a wavelength of 457 nm and R:B = 3:1, 3R1B: 75% R light at a wavelength of 657 nm and 25% B light at a wavelength of 457 nm). All the plants tested were subjected to a 12 h dark/light photoperiod and the same light intensity, which was a photosynthetic photon flux density (PPFD) of 300 ± 3 μmol m^{-2} s^{-1}. This was measured using a quantum sensor (LI-250, LI-COR Inc., Lincoln, NE, USA) and adjusted at the top of plants. The distance between the LEDs and the top of plants was approximately 10 cm. The spectral photon flux density distributions (SPDs) of the LED light were measured using a spectroradiometer (Unispec-SC Spectral Analysis System, PP Systems Inc., Haverhill, MA, USA).

2.2. Plant material and sampling procedure

The experiment was conducted from April to June 2015 in a greenhouse and a closed artificial climate chamber (ACC, Zhejiang Qiushi Environment Co., Zhejiang, China) at the Horticultural Research Center, Shandong Agricultural University, P. R. China. Tomato (*Solanum lycopersicum* L. cv. SV0313TG) seeds (Seminis Vegetable Seeds, Inc., CA, USA) were immersed in water for 15 min at 55 °C and then soaked in cold water for 8 h. The seeds were sown into plug trays filled with a mixture of peat (Floragard Seed 2, Floragard Co., Oldenburg, Germany) and vermiculite (2:1, V/V), and then, grown in the greenhouse. When the first true leaf had fully expanded, the seedlings were transplanted into plastic pots (6.5 cm diameter and 6.5 cm depth, one seedling per pot) containing the same medium. Then, 180 seedlings were selected and moved into ACC, randomized into six groups, and cultured under the six light treatments for 30 days (d). The seedlings were watered daily with half-strength Yamazaki's nutrient solution. Five random plants from each light treatment were sampled after periods of 1 (on the day of transplant), 6, 12, 18, 24 and 30 d of LEDs treatment for morphological and biochemical analysis (Fig. 1). The controlled environment was ventilated, which meant that the CO_2 level was the same as the atmospheric CO_2 level. The relative humidity (RH) was maintained at 70 ± 10% and the growth temperature was 28/19 °C (day/night).

2.3. Plant net photosynthetic rate (Pn), growth parameters and biomass analysis

The second fully expanded leaf that formed under each treatment was used to determine the Pn values using an infrared gas analyzer (LI-6400, Li-COR, USA) at 25 °C, under saturated CO_2 conditions, and at 70% relative humidity. The light source was inbuilt Li-6400 RB lamps with an intensity of 350 μmol m^{-2} s^{-1}. Artificial irradiation was supplied to the leaf from different LED sources, but their respective intensities were about PPFD of 100 μmol m^{-2} s^{-1}. Plant height was measured from the main stem base to the top of the plant by a ruler, and the stem diameter was measured with a vernier caliper. The dry weight (DW) of the seedlings, including shoot and root, was determined according to the method of Lin et al. (2013) by drying the seedlings in a drying oven for 48 h at 70 °C until a constant weight was reached.

2.4. Carbohydrate analysis

Freeze-dried leaf samples (200 mg) were used to determine the carbohydrate content. The carbohydrate was extracted in 25 mL 80% ethanol (v/v) overnight and the supernatants were analyzed for glucose, fructose, sucrose and total carbohydrate contents. The pellets were boiled for 3 h in 10 mL 2% HCl (v/v) and then the supernatants were collected for starch content analysis. The total carbohydrate, glucose, fructose, sucrose and starch contents were determined according to Buysse and Merckx (1993).

2.5. Enzyme assays

Enzyme extraction was determined as previously described (Nielsen et al., 1991) with some modifications. Fresh plant material (2 g) was sampled and then powdered in a mortar at 4 °C with four volumes of ice-cold buffer containing 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)/NaOH (pH 7.5), 10 mM $MgCl_2$, 1 mM ethylene diamine tetraacetic acid (EDTA), 0.1% (w/v) bovine serum albumin (BSA), 0.5% (w/v) of polyvinylpyrrolidone (PVP), 0.05% (v/v) TritonX-100, and 2.5 mM dithiothreitol (DTT). The homogenate was centrifuged at 12,000*g* for 20 min at 4 °C. The supernatant was recovered as the crude enzyme and used for the AI, NI, SS and SPS enzyme activity assays.

The AI and NI activities were assayed according to Jiang et al. (2014). The soluble activity was assayed by adding 50 μL reaction buffer (0.1 M acetic acid buffer and 1% sucrose, pH 5.5) to 50 μL crude enzyme, and incubating the mixture at 34 °C for 1 h. The control was 50 μL crude enzymes incubated at 100 °C for 10 min. The reaction was stopped by boiling the mixture for 5 min and adding 1.5 mL of 3, 5-dinitrosalicylic acid, after the mixture had been incubated in a water bath at 100 °C for 5 min. The mixture was made up to 25 mL with distilled water. The AI activity was determined from the absorbance at 540 nm. The assay for NI activity was similar to the AI assay except that the reaction was performed in phosphate buffer (pH 7.5).

The SS assays followed Tsai et al. (1985) and Douglas et al. (1988). The SS activity was determined by adding a sample of the crude enzyme mixture to 50 μL of 50 mM HEPES-NaOH (pH 7.5), 20 μL of 50 mM $MgCl_2$, 15 μL of 100 mM uridine diphosphate glucose (UDPG), 10 μL of distilled water, and 15 μL of 100 mM fructose. The mixture was incubated in a water bath at 30 °C for 30 min. The reaction was stopped by adding 200 μL of 2 M NaOH and 1.5 mL concentrated hydrochloric acid. Exactly 0.5 mL 0.1% hydroquinone was added after the mixture had been incubated in a water bath at 30 °C for 10 min. The SS activity was determined from the absorbance at 480 nm.

The measurement SPS activity followed Yu (1985) and Wardlaw and Willenbrink (1994). The assay for SPS activity was similar to that of SS except that the fructose was changed to 100 mM fructose 6-phosphate (Fru6P).

2.6. Statistical analysis

The experiments had a completely randomized design. There were five replicates with five plants in each treatment. The data were analyzed by one-way analysis of variance (ANOVA), by the SPSS 11.0 statistics program (SPSS Inc., Chicago, IL, USA), and the differences between the means were tested using Duncan's multiple range test ($P < 0.05$). The figures were created using Origin (version 7.5,