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Effects of light quality on CO_2 assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*

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ABSTRACT

Light quality is thought to affect many plant physiological processes during growth and development, particularly photosynthesis. We examined how light quality influences plant photosynthesis by analyzing changes in photosynthetic parameters and expression levels of some photosynthesis related genes of cucumber (*Cucumis sativus* L. cv. Jinyou No. 1) plants. The plants were grown under different light qualities: purple (*P*), blue (*B*), green (*G*), yellow (*Y*), red (*R*) and white light (*W*) of the same photosynthetic photon flux density (PFD) about 350 μmol m^{-2} s^{-1} for 5 days. The results show that all plants grown under monochromatic light had reduced growth, CO_2 assimilation rate (Pn) and quantum yield of PSII electron transport (Φ_{PSII}) as compared with plants grown under *W*, and these reductions were more significant in the plants under *G*, Y and *R*. The decrease in Φ_{PSII} is mostly due to the reduction in photochemical quenching (qP). Interestingly, *P*- and *B*-grown plants had higher stomatal conductance (Gs), total and initial Rubisco activities and higher transcriptional levels of 10 genes which encode key enzymes in the Calvin cycle together with higher total soluble sugars, sucrose and starch contents as compared with *W*-grown plants, whereas in *G*-, *Y*-, and *R*-grown plants these parameters declined. Therefore, the reduction in Pn under *P* and *B* is likely the result of inactivation of photosystems, whilst under *Y*, G and *R* it is caused by, in addition to photosystem inactivation, the closure of stomata and the transcriptional down-regulation of genes for the Calvin cycle enzymes such as *rbc* L and *rca*. In conclusion, light quality alters plant photosynthesis by the effects on the activity of photosynthetic apparatus in leaves and the effects on the expression and/or activity of the Calvin cycle enzymes.

1. Introduction

Light is a predominant source of energy for plant photosynthesis and also an important signal for plant growth and development. It is known that not only are plants able to respond to the intensity of light but also to its quality or colour [1]. The spectral irradiance of solar radiation is a mixture of UV radiation, photosynthetically active radiation (PAR), far red and near infrared, ranging from 280 nm to 1100 nm [2]. However, little is known about how light quality influences plant growth and development, especially photosynthesis. Strong UV-*B* radiation can cause the damage to plant photosynthesis, such as losses of both activity and content of Rubisco [3] and sedoheptulose 1,7-biphosphatase [4], inactivation of photosynthetic electron transport chain [4,5] and induction of stomatal closure [6]. A few studies have been done to investigate the effects of light quality on plants by using only two colours, blue and red [7,8]. They showed that under blue light plants had greater stomatal opening, higher Chl a/b ratios, smaller amounts of light harvesting Chl a/b-binding protein in photosystem II (PSII), higher photosynthetic electron-transport activity per unit of Chl content and higher Rubisco activity than plants grown under red light [9,10]. Recently, Matsuda et al. [11] found that rice plants grown under red light in combination with blue light had higher photosynthetic rates and higher total nitrogen (N) content in leaves than those grown under red light alone. The effects of different light qualities on gene expression have been investigated mostly under white light conditions [12]. Thus how light quality affects both photosynthesis and gene expression needs to be further elucidated by using more light colours or a serious of defined light qualities.

There have been difficulties to generate different light qualities. Until now, the most commonly used light sources are fluorescent lamps, high intensity discharge lamps, high-pressure mercury bulbs, high-pressure metal-halide bulbs or sodium lamps. These broad spectrum lights have various limitations in application and are consequently not an optimal radiation source for plants. Recently, light-emitting diodes (LEDs) have been developed as an alternative light source for plants because of their wavelength

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specificity and narrow bandwidth, small mass and solid state construction, long life as well as minimum heating [13]. Furthermore, LEDs can provide a better combination of the visible spectrum for optimizing photosynthesis and growth. Thus, the application of LEDs provides with a new way for studying how different light qualities affect photosynthesis and a better source of supplemental light to natural sunlight even for agricultural production.

The objective of this study is to examine how light quality affects plant photosynthesis and growth. CO_2 assimilation, chlorophyll-fluorescence quenching, stomatal characteristics, Rubisco activity, the transcriptional levels of genes which encode some key enzymes in the Calvin cycle and carbohydrate metabolites have been determined in leaves of cucumber plants after exposure to different light colours.

2. Materials and methods

2.1. Plant material and light treatments

Cucumber (*Cucumis sativus* L.cv.Jinyou No.1) seeds were germinated in trays filled with a mixture of peat, vermiculite and perlite (6:3:1, V:V:V) in a greenhouse. When the first true leaf fully expanded, seedlings were transplanted into plastic pots (15 cm diameter and 15 cm depth, one seedling per pot) containing the same medium. The seedlings were watered daily with half-strength Enshi nutrient solution [14] and grown under natural light in a greenhouse till the 4-leaf stage.

As a multiwavelength white light control (*W*), some plants were kept under natural light and supplemented with incandescent reflector lamps (100 W, Nanjing Special Lamp Co., China). The other plants were moved into closed cabinets and given 5 d exposure to purple light (*P*) with a maximum intensity at 394.6 nm, blue light (*B*) with a maximum intensity at 452.5 nm, green light (*G*) with a maximum intensity at 522.5 nm, yellow light (*Y*) with a maximum intensity at 594.5 nm and red light (*R*) with a maximum intensity at 628.6 nm. *P*, *B*, *G*, Y and *R* were created by light-emitting photodiodes (ZDL-100 W, Nichia, Japan). All treatments were kept with a 12 h dark/light photoperiod and the same light intensity expressed as photosynthetic photon flux density (PFD) about 350 μmol m^{-2} s^{-1} which was measured daily above of the plant canopies and maintained by adjusting the distance of the LED lamps to the plant canopies. All plants were then transferred to *W* conditions for another 3 d and were then ready for analysis. The light intensity and spectral distributions of the LEDs were measured with a quantum sensor (Li-Cor, USA) and a STC 4000 spectrometer (Everfine Photo-E-Info Co., China) (Fig. 1), respectively.

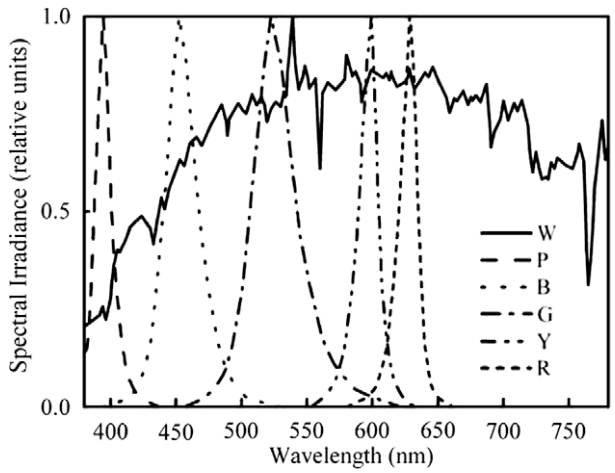

Fig. 1. Relative spectral distribution of the LEDs and white light used in this study: *W* = white light; *P* = purple light; *B* = blue light; *G* = green light; *Y* = yellow light; *R* = red light.

2.2. Gas exchange and chlorophyll fluorescence measurements

The 4th leaf was used for the gas exchange analysis with an infrared gas analyzer (LI-6400; Li-COR Lincoln NE, USA) under the conditions at 25 °C, with a CO_2 concentration of 450 μmol mol^{-1} and 60% relative humidity. Artificial irradiation was supplied to the leaf from different LED sources but their respective intensities were the same about PFD 350 μmol m^{-2} s^{-1}.

Chlorophyll fluorescence was measured using an Imaging-PAM Chlorophyll Fluorometer attached a computer-operated PAM-control unit (Walz, Effeltrich, Germany). The seedlings were kept in dark for about 20 min before measurement. The intensities of actinic light and saturating light setting were 280 μmol mol^{-2} s^{-1} and 2500 μmol mol^{-2} s^{-1} PAR, respectively. The maximal photochemical efficiency of PSII (Fv/Fm), relative quantum efficiency of PSII photochemistry (Φ_{PSII}), photochemical quenching (qP), and the efficiency of excitation energy capture by open PSII reaction centres (Fv'/Fm') were measured and calculated according to the method described previously [15].

2.3. Measurements of stomatal index and stomatal density

The number of stomata and epidermal cells were counted on the 3rd leaf at the 4-leaf stage. The imprints of abaxial (lower) epidermis were taken by using colourless adhesive tapes and then measured with a microscope (DMIRB, Leica, Germany) and calculated by Image-Pro Plus software. In each treatment, the imprints of five individual leaves were used to count stomata and epidermal cells. Five areas of 0.04 mm^2 were randomly selected and analyzed on each leaf. Stomatal density and stomatal index were determined according to the method described by Ceulemans et al. [16].

2.4. Measurements of total chlorophyll, soluble protein more blank and Rubisco activity

Chlorophyll was extracted by 80% cold acetone (v/v) and determined as described [17]. The frozen leaf sample was extracted immediately in ice-cold buffer including 50 mM Tris–HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM EDTA, 10% PVP, 12.5% (v/v) glycerin and 10 mM β-mercaptoethanol, using a chilled pestle and mortar. The soluble protein was measured by the method of Bradford [18]. Rubisco activity was assayed spectrophotometrically at 25 °C as described previously [19] with some modification [20]. The extraction buffer for total Rubisco activity measurement contained 33 mM Tris–HCl (pH 7.5), 0.67 mM EDTA, 33 mM $MgCl_2$ and 10 mM $NaHCO_3$. Initial Rubisco activity was measured in a 0.1 ml reaction mixture containing 5 mM HEPES–NaOH (pH 8.0), 2 mM $MgCl_2$, 1 mM $NaHCO_3$, 0.1 mM EDTA, 0.25 mM DTT, 1 U 3-phosphoglyceric phosphokinase, 1 U creatine phosphokinase, 1 U glyceraldehyde, 3-phosphate dehydrogenase, 0.5 mM ATP, 0.015 mM NADH, 0.5 mM phosphocreatine, 0.06 mM RuBP and 10 μl enzyme solution. The reaction was monitored for 90 s with A340.

2.5. RNA extraction and RT-PCR for gene expression analysis

Total RNA was extracted from cucumber leaves after exposure to different light quality regimes for 3 d using TRIZOL reagent (Sangon, China). Total RNA was dissolved in DEPC-treated water after extraction. First-strand cDNA was synthesized using a RevertAid™ cDNA Synthesis Kit (Fermentas, USA) according to the manufacturer's instruction. Specific gene primers of 10 Calvin cycle genes as shown in Table 1 were used for RT-PCR.