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Research article

Secondary and sucrose metabolism regulated by different light quality combinations involved in melon tolerance to powdery mildew

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ABSTRACT

We evaluated the effect of different light combinations on powdery mildew resistance and growth of melon seedlings. Light-emitting diodes were used as the light source and there were five light combinations: white light (420–680 nm); blue light (460 nm); red light (635 nm); RB31 (ratio of red and blue light, 3: 1); and RB71 (ratio of red and blue light, 7: 1). Compared with other treatments, blue light significantly decreased the incidence of powdery mildew in leaves of melon seedlings. Under blue light, H_2O_2 showed higher accumulation, and the content of phenolics, flavonoid and tannins, as well as expression of the genes involved in synthesis of these substances, significantly increased compared with other treatments before and after infection. Lignin content and expression of the genes related to its synthesis were also induced by blue light before infection. Melon irradiated with RB31 light showed the best growth parameters. Compared with white light, red light and RB71, RB31 showed higher accumulation of lignin and lower incidence of powdery mildew. We conclude that blue light increases melon resistance to powdery mildew, which is dependent on the induction of secondary metabolism that may be related to H_2O_2 accumulation before infection. Induction of tolerance of melon seeds to powdery mildew by RB31 is due to higher levels of sucrose metabolism and accumulation of lignin.

1. Introduction

Melon is a widely cultivated horticultural crop worldwide. Healthy seedlings are beneficial to melon plant growth and development because of higher tolerance to biotic and abiotic stress. In the growing season, melon is often infected with powdery mildew, and necrotic lesions in the leaves are induced, which results in lower net photosynthetic rate, inhibiting plant growth and decreasing both fruit yield and quality (Spotts and Cervantes, 1986; Li et al., 2015).

Light-emitting diodes (LEDs) have been used widely as a controlled light source for plant growth and development (Ahmad and Cashmore, 1996; Sellaro et al., 2009). A number of scholars carried out extensive research in light-quality-mediated plant tolerance to pathogens. It has been found that blue light obviously reduce the proportion of wilted plants in solanaceae mosaic virus (Schuerger and Brown, 1997), and also Kook et al. (2013) found that lettuce resistance to Botrytis disease was induced by blue light. Previous study showed that blue light can obvious induce flavonoid metabolism pathway, promote the synthesis of anthocyanins in lettuce (Li and Kubota, 2009). Besides, the metabolism of phenylalanine can also be induced by blue light, then promote downstream mediators, aliphatic glucosinolate in broccoli (Kopsell et al., 2014) and chlorogenic acid accumulation in tomato (Taulavuori et al., 2013), and Wang et al. (2010) also found blue light could induce the expression of *PAL* and *PPO* during the *Sphaerotheca fuliginea* infection in cucumber. Therefore, it was proposed that the pathogen resistance induced by blue light was dependent on its function of regulating secondary metabolism.

In general, higher plants will produce a variety of disease-resistance reactions following recognition of a variety of bacterial and fungal pathogens, such as reactive oxygen burst, SA or JA signal transduction, and synthesis of phytoalexins (Janda and Ruelland, 2015). Among these, as the earliest cellular response, the reactive oxygen species (ROS) signaling pathway is regarded as an important disease-resistant reaction (Delledonne et al., 2002; Czajkowski et al., 2015) and H_2O_2 is considered as the center of attention in plant pathology. After pathogenic attack, plants can rapidly accumulate H_2O_2, and Apostol et al. (1989) found that oligogalacturonide from fungi induces H_2O_2 production in soybean cells. In the process of plant against infection, ROS

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have a dual role: on the one hand, high concentration of ROS may kill both plant and invading pathogen and, on the other hand, low concentration of ROS can act as the signal for the induction of antioxidant and pathogenesis-related genes in plant tissues adjacent to infection sites (Torres et al., 2006; Hafez et al., 2012).

It is known that plant disease resistance includes physical and chemical resistance. One of the physical resistance characteristics is cell wall thickening, which is dependent on lignin synthesis. Miedes et al. (2014) reported that phenolic compounds involved in lignin synthesis are important substances in plant disease resistance. Therefore, the ability of lignin synthesis is considered to be an important indicator of plant disease resistance. Phytoalexins, including the phenolics, flavonoids and tannins, is considered to be the key material of plant chemical resistance (Yusuf et al., 2016). Phytoalexins are important in the production of phenylalanine metabolism, in which phenylalanine ammonia-lyase (PAL), cinnamic acid 4-hydroxylase (C4H), 4-coumarate:CoA ligase (4CL) are three key enzymes and have attracted much attention with regards plant resistance to pathogens. And also, *CHS*, *FLS*, *LDOX* are the important genes of secondary metabolism pathway whose products are flavonoid and condensed tannin, which are closed associated with plant disease-resistance.

The synthesis of sucrose is an important biological process and it plays an important role in plant growth. Besides providing a carbon source, as a signal, sucrose is also important in plant defense reactions to biotic and abiotic stresses (Bolouri-Moghaddam et al., 2010; Tsutsui et al., 2015). Within hours of being infected with a pathogen, a series of defensive responses are quickly induced, which includes synthesis of phytoalexins and NADPH, which are essential for production of ROS; these processes require sugar metabolism to provide energy (Pugin et al., 1997). Moreover, in the interaction between microbes and plants, it is important to accumulate sucrose in plants (Morkunas et al., 2005). Kwon et al. (2011) indicated that sucrose is related to hormone production in plant defense mechanisms. Thus it plays an important role in the response to disease.

In this study we found that composition of different light spectra had significantly different effects on muskmelon seedling growth and the occurrence of powdery mildew. To elucidate the mechanism, ROS metabolism, accumulation of primary and secondary metabolic substances and expression of related enzymes were analyzed.

2. Materials and methods

2.1. Plant seedling preparation

Silver melon, a powdery-mildew-sensitive cultivar, was used as experimental material. After germinating at 28 °C, seeds were sown in plug trays (20 × 50 × 8 cm, 50 spans) containing peat, vermiculite and perlite (2: 1: 1). Melon plants were cultivated with half-strength Hoagland's solution (Vangessel et al., 2009), and the containers were kept in a greenhouse maintained with a 14-h photoperiod and temperature controlled at 18/28 °C (night/day). The plants were used for treatment when the second leaves were fully expanded.

2.2. Light conditions for melon seedling cultivation

The light treatment was conducted in each enclosed chamber, which could exclude interference each other. The LED environment were programmed to provide a 12-h light/12-h dark photoperiod and the photosynthetic photon flux (PPF) was set as 300 ± 5 μmol/m^2·s. The LED spectrum is shown in Fig. 1; there were five light treatments: white light (420–680 nm); blue light (460 nm); red light (635 nm); RB31 (ratio of red and blue light, 3: 1); and RB71 (ratio of red and blue light, 7: 1).

2.3. Plant seedling treatment

Muskmelon seedlings with two real leaves were set in the light treatment. To explore the effect of different light combinations on plant growth, growth indexes were assayed after 15 days of light treatment without powdery mildew inoculation. To explore the effect of different light combinations on the accumulation of disease-resistant substances in melon, after 5 days of cultivation with different light treatments, leaf samples were taken, and immediately frozen in liquid nitrogen and stored at −80 °C until analysis. After sampling, powdery mildew was inoculated. Seven days later, leaves were sampled and stored at −80 °C for analysis of physiological parameters.

2.4. Powdery mildew (Podosphaera xanthii) inoculation

Conidia of powdery mildew (*P. xanthii*) were gathered from naturally infected melon leaves in Shandong Agricultural University greenhouses. Twenty-four hours before gathering, old conidia were shaken off to keep the pathogen fresh. Conidial suspension was prepared by brushing the source leaves with distilled water. The spore concentration was kept to approximately 10^6 conidia/ml using a hemocytometer. The pathogens were sprayed on the muskmelon leaves. The inoculated leaves were covered with polyethylene film for 24 h in a dark environment. After 12 days, incidence rate and disease index were measured, and the results of staining were observed. The disease index was investigated 12 days after infection following the guidelines provided by the Institute of Inspection and Testing for Pesticides, Ministry of Agriculture China (IITP, 1993). Disease index was calculated according to the equation: Disease index (%) = $[\sum (rn_r)/9N_t] \times 100$ (r = rate value, n_r = number of disease leave with a rating of r, and N_t = total number of leaves tested).

2.5. Analysis for secondary metabolites

Total phenolic and flavonoid contents were measured using the Folin–Ciocalteu method (Toor and Savage, 2005). Leaf samples (3 g) were ground quickly in 18 ml 40% aqueous methanol. The grinding fluid was put into a bain marie (60 °C) for 1 h, and then centrifuged at 10 000 *g* for 15 min at 4 °C. The dilute Folin–Ciocalteu's reagent (1 ml) was added to the supernatant, and the mixture was allowed to stand for 3 min after full shaking. Then, the mixture was shaken well and reset to static for 2 h after placing in 3 ml 7.5% Na_2CO_3. The supernatant was assayed by measuring the absorbance at 765 nm. The flavonoid was also used the same extract (Toor and Savage, 2005). Samples (5 ml) were reacted with 5% NaNO (300 μl) and allowed to stand for 5 min after shaking. Then, the mixture was successively reacted with 10% $Al_2(NO_3)_3$ (300 μl) and 4% NaOH (4 ml) and allowed to stand for 6 and 10 min, respectively. The supernatant was assayed by measuring the absorbance at 510 nm. The amount of condensed tannins was measured using Lin's method (Lin et al., 2006). The leaf samples (0.1 g) were ground in 70% acetone/methanol (5 ml) and allowed to stand for 30 min. Then, the samples were centrifuged at 4000 *g* for 15 min. Reaction mixtures contained 1 ml supernatant and 6 ml alcohol/hydrochloric acid. The mixtures were placed in boiling water for 75 min. The supernatant was assayed by measuring the absorbance at 550 nm. Total lignin content was determined by the Klason method with modifications (Sewalt et al., 1997). The reaction mixtures contained dry leaf sample (0.2 g) and 25% acetyl bromide in glacial acetic acid (0.5 ml) and were put in the bain marie at 70 °C for 30 min. The supernatant was assayed by measuring the absorbance at 280 nm after adding and blending with glacial acetic acid (5 ml) and 7.5 M hydroxylamine hydrochloride (0.1 ml). The amount of hydroxyproline-rich glycoproteins (HRGP) was correlated positively with Hyp (hydroxyproline) in the cell wall, so we determined the content of Hyp as the content of HRGP in the cell wall. The amount of Hyp was measured using Risteli's method (Risteli et al., 2004) and the supernatant was assayed by measuring the