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Inhibition of sucrose and galactosyl-sucrose oligosaccharide metabolism in leaves and fruits of melon (*Cucumis melo* L.) under low light stress

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ABSTRACT

Low light stress is a major limiting factor for melon to reach its full potential of fruit yield and quality in cold season production. This study investigated the effect of low light stress on sucrose synthesis and galactosyl-sucrose oligosaccharide loading in melon leaves, galactosyl-sucrose oligosaccharide unloading, and sugar accumulation in fruits under greenhouse conditions using the low light-tolerant cultivar Yujinxiang and the low light-sensitive cultivar Yuxue. The results showed that in melon leaves, sucrose content and activities of related synthase enzymes decreased significantly under low light stress. The reduction of sucrose content and the activity of sucrose phosphate synthase (SPS) and sucrose synthase in the synthesis direction (SS-s) in Yujinxiang (30.9%, 16.5%, 30.0%, respectively) were significantly less than those in Yuxue (60.6%, 31.6%, 40.5%, respectively). The raffinose and stachyose content as well as the activity of stachyose synthase also significantly decreased after shading. The reduction of stachyose synthase activity in Yujinxiang (23.8%) was significantly less than that in Yuxue (32.4%), indicating that sucrose synthesis and galactosyl-sucrose oligosaccharide loading in melon leaves were inhibited under low light stress and the low light-sensitive cultivar was more greatly influenced. In melon fruits, stachyose content and the activities of acid and alkaline α-galactosidases significantly decreased under low light conditions, but the raffinose content was higher from 10 to 20 days after anthesis, indicating that the imported assimilates reduced, and galactosyl-sucrose oligosaccharide unloading was inhibited after shading. During the earlier stage of fruit development, significant decreases in the activities of acid invertase (AI), neutral invertase (NI), and sucrose synthase in the cleavage direction (SS-c) were observed in the two cultivars after shading, sink strength reduced. In the later stage of fruit development, sucrose accumulation was inhibited by decreasing the activities of SPS and SS-s, but no significant difference was observed between the two cultivars suggesting that the difference of sugar content among the two cultivars might be due to the imported assimilates under low light conditions.

1. Introduction

In many plant species, photosynthetic product is exported exclusively in the form of sucrose; however, sugars translocated in phloem in Cucurbitaceae are mainly the galactosyl-sucrose oligosaccharides stachyose, raffinose, and sucrose (Mitchell et al., 1992; Hu et al., 2009; Ohkawa et al., 2010; Zhang et al., 2012). Hence, additional metabolic steps are required in melons during the source-sink translocation of carbohydrates. In leaves, the synthesis of galactosyl-sucrose oligosaccharides is the key step for carbohydrate loading from mesophyll to phloem cells and may be regulated by cell compartmentalization. This process takes place within the intermediary cells of the minor phloem veins (Turgeon et al., 1993, 2001;McCaskill and Turgron, 2007). Sucrose and galatinol, the two major precursors in synthesizing raffinose and stachyose, are thought to be delivered into the intermediary cells from the mesophyll via abundant plasmodesmata (Turgeon and Hepler, 1989; Rennie and Turgeon, 2009; Gil et al., 2011; Davidson et al., 2011; Zhang and Turgeon, 2018). Cell compartmentalization in leaves complicates carbohydrate metabolism, and the pathway of stachyose biosynthesis involves the following steps beyond sucrose biosynthesis: galactinol + sucrose → raffinose + myo-inositol, then galactinol + raffinose → stachyose + myo-inositol (Tanner and Kandler, 1968; Peterbauer et al., 2002; Hu et al., 2009). When galactosyl-sucrose oligosaccharides arrive at sink organs, they are thought to be initially hydrolyzed by α-galactosidase into sucrose and galactose: stachyose + H_2O → raffinose + galactose, then raffinose + H_2O → sucrose + galactose (Gross and Pharr, 1982; Chrost and Schmitz, 2000; Miao et al., 2007; Hu et al., 2017). Gao and Schaffer (1999a) discovered a

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novel alkaline α-galactosidase, termed form I, from melon fruit tissue that showed a high affinity for both raffinose and stachyose. This is distinguished from the previously described alkaline α-galactosidase form II (Gaudreault and Webb, 1982, 1983, 1986), which is specific to stachyose, suggesting that form I plays a physiologically significant role in unloading galactosyl-sucrose oligosaccharides in sink tissue (Gao et al., 1999b).

Although stachyose was the main sugar translocated in phloem sap, sucrose and its hydrolysis products, glucose and fructose, were found as the major sugars in fruit tissues; sucrose accumulation usually occurs in the final stage during fruit maturation (Burger and Schaffer, 2007; Kortstee et al., 2007; Yativ et al., 2010; Dai et al., 2011; Huang et al., 2017). In sugar-storing plant tissues, shifts in carbohydrate composition are usually accompanied by shifts in relative activities of sucrose metabolizing enzymes (Lingle and Dunlap, 1987; Burger and Schaffer, 2007; Datir and Joshi, 2016; Li et al., 2017). Previous studies have shown that the rapid accumulation of sucrose in melon fruit was usually characterized by a developmental loss of acid invertase (AI) activity (Lingle and Dunlap, 1987; Schaffer et al., 1987; McCollum et al., 1988; Hubbard et al., 1989; Chrost and Schmitz, 1997; Burger and Schaffer, 2007). This phenomenon was also found in tomato (*Solanum lycopersicun* L.) (Dali et al., 1992; Islam et al., 1996; Pressman et al., 2012), sugar cane (*Saccharum* L.) (Hatch and Glasziou, 1963; Gutiérrez-Miceli et al., 2002; Siswoyo and Oktavianawati, 2007; Datir and Joshi, 2016), and lychee (*Litchi chinensis* Sonn.) (Yang et al., 2013). It has been previously proposed that sucrose phosphate synthase (SPS) plays a key role in the activity of sucrose synthesis (Hubbard et al., 1989; Lester et al., 2001; Verma et al., 2011; Huang et al., 2017). Sucrose synthase in the synthesis direction (SS-s) was also thought to be directly involved in the synthesis of sucrose (Schaffer et al., 1987; Suzuki et al., 1996; Moscatello et al., 2011; Yang et al., 2013). The activities of SPS, SS-s, and neutral invertase (NI) were all found to be positively correlated with sucrose accumulation among eight melon genotypes (Burger and Schaffer, 2007).

Melon (*Cucumis melo* L.) originated from tropical regions of central Africa; hence, it is sensitive to low light conditions (Akashi et al., 2002). In the winter in northern China, melons must be cultivated in greenhouses, and stress caused by low light is one of the most important limiting factors affecting fruit production. Although the effect of low light on photosynthesis (Walcroft et al., 2002; Parlitz et al., 2011; Li et al., 2017; Dutta et al., 2017), antioxidant activities (Islam et al., 2003; Khandaker et al., 2008; Xu et al., 2008; Zhang et al., 2010; Somporn et al., 2012), sucrose metabolism (Chen et al., 2001; Geromel et al., 2008; Hu et al., 2016), and other physiological processes is well documented, limited information exists concerning the sucrose and galactosyl-sucrose oligosaccharide metabolism in leaves and fruits under low light stress in both melon and other Cucurbitaceae species. In this study, two melon cultivars (low light-tolerant Yujinxiang and low light-sensitive Yuxue) were shaded after anthesis, and carbohydrate levels as well as the activities of metabolic enzymes in source leaves and mesocarp tissues were studied over the course of melon fruit development. This study's aim was to gain a better understanding of the inhibition mechanism of source-sink carbohydrate translocation under low light stress.

2. Materials and methods

2.1. Plant materials and treatments

A low light-tolerant cultivar (Yujinxiang) and a low light-sensitive cultivar (Yuxue) of melon (*Cucumis melo* L.) were grown in the winter and spring, from December to March, in a greenhouse at the Gansu Academy of Agricultural Sciences in Lanzhou, China. Plants were watered regularly as needed and fertilized weekly with Hoagland nutrient solution. All plants were maintained at a single-stem condition by the manual removal of all lateral branches. Experimental treatments consisted of the following: (1) control under natural light in the greenhouse and (2) plants shaded with two layers of black nylon netting with a light intensity at the canopy less than 52% of the control. Treatments began after anthesis, and fruits at the tenth to fifteenth nodes were used. Mature leaves and fruits from the same node were sampled at 5, 10, 15, 20, 25, 30, and 35 days after anthesis. Samples were collected between 9:00 and 10:00 a.m. to avoid variability due to diurnal fluctuations in assimilate composition (Mitchell et al., 1992). For fruits, the mesocarp tissues were thinly sliced using a knife, and the epicarp, endocarp, and seeds were all removed. Leaf tissues were sampled by collecting 1.33 cm^2 discs with a cork borer. The leaf discs and mesocarp tissues were weighed, immediately frozen in liquid nitrogen, and stored at -80 °C.

2.2. Carbohydrate extraction and analysis

Leaf (2 g FW) and mesocarp (1 g FW) samples were ground extensively and extracted three times in 5 mL 80% (v/v) ethanol for 30 min. at 80 °C. The extracts were combined and evaporated to dryness in a vacuum using a Rotary Evaporator in a rotary flask in a 50 °C water bath. The residues were redissolved in 1 mL distilled water and passed through a 0.45 μm filter. Twenty-five microliters of sample were then injected into a high-performance liquid chromatograph (HPLC), containing an NH2P-50 4E column (Shodex Asahipak, 4.6 mm × 250 mm) at 35 °C using acetonitrile and water (70:30%) as eluent, with a flow rate of 1.0 mL per min. Stachyose, raffinose, sucrose, fructose, and glucose were identified by the comparison of retention times to known standards and quantified using a refractive index detector.

2.3. Enzyme extraction and assays

Stachyose synthase (STS) was extracted and assayed following the methods described by Huber et al. (1990). Tissues (0.5 g FW) were homogenized in a chilled mortar with four volumes of chilled extraction buffer containing 50 mM HEPES-NaOH (pH 7.0) and 20 mM 2-mercaptoethanol. The homogenate was centrifuged at 28,000 g for 20 min, the supernatant was dialyzed with 20 mM HEPES-NaOH buffer (pH 7.0) containing 20 mM 2-mercaptoethanol for 16 h, and this was subsequently used for the assay. One hundred milliliters of dialyzed extract was mixed with 100 mL of solution containing 25 mM HEPES-NaOH (pH 7.0), 20 mM 2-mercaptoethanol, 10 mM galactinol, and 40 mM raffinose for the reaction. A control was prepared similarly but without galactinol. The mixtures were incubated at 25 °C for 90 min and reactions were terminated by adding 100 mL 0.1 N NaOH. Then, the mixtures were boiled for 30 s and cooled to 25 °C. The formation of myo-inositol was detected using a total mixture of 1.0 mL containing 2 mM NAD, 0.1 U myo-inositol dehydrogenase [EC.1.1.1.18], and 50 mM Na_2CO_3 (pH 9.5), then incubated at 25 °C for 40 min. The reduction of NAD was measured spectrophotometrically at 340 nm.

For the assays of the α-galactosidases, tissue (1 g FW) was homogenized in a chilled mortar with four volumes of chilled extraction buffer containing 50 mM HEPES-NaOH (pH 7.5), 2 mM $MgCl_2$, 2 mM EDTA, and 5 mM DTT. The homogenate was centrifuged at 28,000*g* for 20 min and the supernatant was used for enzyme assays. Acid α-galactosidase activity was assayed in 200 μL of 100 mM pH 5.5 McIlvaine buffer with 10 mM stachyose. The activity of alkaline α-galactosidase form I was assayed in 200 μL of 100 mM pH 7.5 HEPES buffer with 10 mM raffinose and the activity of alkaline α-galactosidase form II was assayed in 200 μL of 100 mM pH 7.5 HEPES buffer with 10 mM stachyose (Gao and Schaffer, 1999). Reactions proceeded at 35 °C for 20 min and were terminated with 2 min of boiling. Enzyme activity was estimated by determining the amounts of galactose released, as described by Smart and Pharr (1980), using an enzyme coupled reaction with NAD and ß-galactose dehydrogenase [EC.1.1.1.48]. Enzyme activity was expressed as μmol of galactose released.

For assays of sucrose metabolism enzymes, samples (1 *g* FW) were