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Metabolic process of the ^{14}C-sugars on the translocation pathways of cucumber plants

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ABSTRACT

Studies on the metabolic process of photoassimilates and enhancement of sugar accumulation into fruit are important in fruit crop production. The metabolic process of the ^{14}C-photoassimilates in cucumber plants was analyzed with respect to the vascular system. At 4 h after the start of $^{14}CO_2$ feeding, the ^{14}C-photoassimilates synthesized in a selected leaf on the main shoot were translocated to the vascular bundles of the internode just below the $^{14}CO_2$-fed leaf. The radioactivity of ^{14}C-stachyose was as high as that of ^{14}C-sucrose in the vascular bundles of petiole and internode just below the $^{14}CO_2$-fed leaf as well as in the midrib, while the radioactivity of ^{14}C-stachyose was lower than that of ^{14}C-sucrose in the mesophyll. The ^{14}C-photoassimilates appeared to have been translocated without any metabolic change in the translocation pathways between the petiole and internode just below the $^{14}CO_2$-fed leaf, because the ratio of ^{14}C-stachyose radioactivity in the two parts was similar. At 8 h after the start of $^{14}CO_2$ feeding, the ^{14}C-photoassimilates were translocated to the fruit. In the vascular bundles of the peduncle, the ratios of the radioactivity of ^{14}C-stachyose and ^{14}C-raffinose were lower, and the ratio of the radioactivity of ^{14}C-sucrose was higher, than that at the petiole and internode just below the $^{14}CO_2$-fed leaf at 8 h after the start of $^{14}CO_2$ feeding. Therefore, it seemed that ^{14}C-stachyose and ^{14}C-raffinose were hydrolyzed to ^{14}C-sucrose in the peduncle.

1. Introduction

Sugar is necessary for energy supply and the synthesis of various molecules during fruit development. Sugar also generates cell turgor to enlarge fruit during the cell enlargement stage of fruit. For example, the suppression of a proton pump gene, which is important in sugar accumulation into fruits cells, inhibits fruit development (Amemiya et al., 2006). In addition, the translocation and accumulation of sugar into fruit contribute to maintaining high photosynthetic activity by withdrawing photosynthates from leaf cells, because the accumulation of photosynthates in leaf cells decreases the expression of photosynthesis-related genes (Rolland et al., 2002). This finding indicates the importance in sink strength. Therefore, studies on the metabolic process of photoassimilates and enhancement of sugar accumulation into fruit are important in fruit crop production.

In fruity vegetables such as tomato and cucumber, the photoassimilates synthesized in the leaves (source) are translocated to the fruits (sink) via the vascular bundles of the petiole and stem. The photoassimilates are translocated as sugars, and sucrose is the typical translocation sugar in many plants. The raffinose family oligosaccharides (RFOs) such as raffinose and stachyose are translocated in cucurbit plants (Mitchell et al., 1992). Raffinose is synthesized from sucrose and galactinol by raffinose synthase (EC 2.4.1.82), and stachyose is synthesized from raffinose and galactinol by stachyose synthase (EC 2.4.1.67), in mature melon leaves (Schmitz and Holthaus, 1986) (Fig. 1). Galactinol, the galactosyl donor of the synthesis of RFOs, is synthesized by galactinol synthase (EC 2.4.1.123). Stachyose synthase and galactinol synthase localized in the intermediary cells of the leaf miner vine (Holthaus and Schmitz, 1991; Beebe and Turgeon, 1992; Volk et al., 2003). However, in cucurbit fruits, monosaccharides such as fructose and glucose, and disaccharides such as sucrose are mainly detected, and raffinose and stachyose are rarely detected (Hubbard et al., 1989).

There have been many reports about the sugar translocation and sink strength in fruit of tomato, which translocates sucrose from leaves to fruit. For example, the relationship between the vascular system and distribution pattern of the photoassimilates has been reported by Shishido et al. (1988) and Li et al. (2000). Ho (1996), in tomato, suggested that the sink strength is related to the

Abbreviation: RFOs, raffinose family oligosaccharides.

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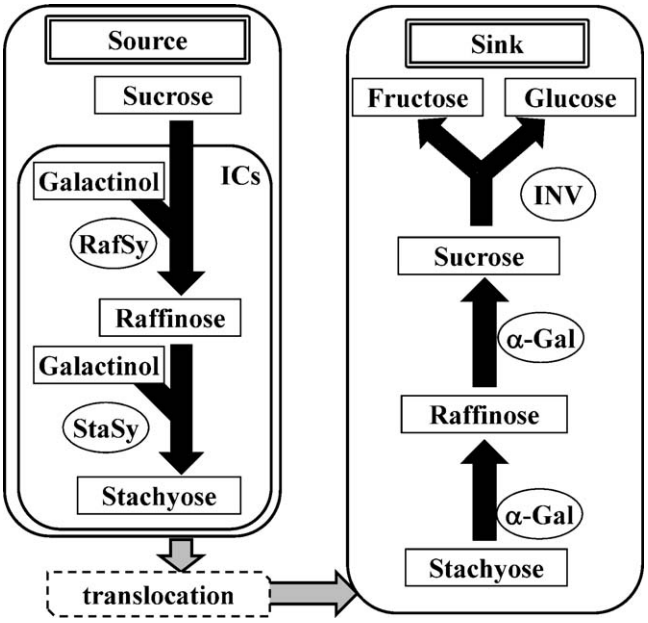

Fig. 1. Raffinose family oligosaccharides metabolic pathway in cucurbit plants. ICs: intermediary cells. α-Gal: α-galactosidase. INV: invertase. RafSy: raffinose synthase. StaSy: stachyose synthase.

routes of sugar transport into fruit and sucrose synthase and ADP-glucose pyrophosphorylase determine the sink strength in young fruit. In addition, Fridman et al. (2000) reported that one of the quantitative trait loci for tomato sugar contents is the cell wall invertase gene. This cell wall invertase functions in the placenta (Fridman et al., 2004). Therefore, in the unloading of sucrose, the hydrolysis of sucrose in the fruit placenta is important for accumulating high soluble solids in the fruit.

In cucumber plants, a relationship between the vascular system and distribution pattern of the photoassimilates has been reported (Kanahama and Saito, 1986, 1988). It is known that RFOs are metabolized by α-galactosidase (EC 3.2.1.22). Raffinose is hydrolyzed to galactose and sucrose, while stachyose is hydrolyzed to galactose and raffinose (Fig. 1). Because of this, α-galactosidase activity has been investigated mainly in fruit flesh through the fruit development of melon and squash (Gao et al., 1999; Gao and Schaffer, 1999; Irving et al., 1997). As the cell wall invertase in tomato plants, the first step of metabolism of translocated sugar could be important for the sink strength in cucumber plants. However, in contrast to tomato plants in which sucrose is hydrolyzed in the fruit placenta, information is lacking for tissues in which raffinose and stachyose are metabolized first. Therefore, in this experiment, we examined, by $^{14}CO_2$ treatment, the metabolic process of RFOs in the translocation pathways according to the vascular system in cucumber plants.

2. Materials and methods

Cucumber plants (*Cucumis sativus* L. 'Sharp 1') (Saitama Gensyu Ikuseikai Co., Ltd.) were used for the experiment. The seeds were sown in March 2001, and the seedlings were transplanted to 15-cm-diameter clay pots in a glasshouse.

To examine the metabolic process of ^{14}C-photoassimilates in the main stem of cucumber plants, plants with the 10th leaf expanded were moved to a natural light type phytotrons controlled at 24 °C, and 0.37 MBq of $^{14}CO_2$ was fed to the 7th leaf for 1 h. Four, eight or twenty-four hours after the start of $^{14}CO_2$ feeding, the $^{14}CO_2$-fed leaf was divided into three parts: the mesophyll, midrib and petiole. The petiole was divided into two parts: the vascular bundles and parenchyma. The internodes just below (the 7th internode) and above (the 8th internode) the $^{14}CO_2$-fed leaf were collected and divided into the vascular bundles and the parenchyma.

To examine the metabolic process of ^{14}C-photoassimilates in the cucumber fruit, a fruit was set on the 10th or nearest to the 10th node. Five days after anthesis, the plants were moved to a natural light type phytotrons controlled at 24 °C, and 0.37 MBq of $^{14}CO_2$ was fed for 1 h to the leaf on the same node as the fruit set. Eight, 24 or 48 h after the start of $^{14}CO_2$ feeding, the peduncle and receptacle were collected and divided into the vascular bundles and parenchyma.

Alcohol-soluble sugars were extracted from the divided sample with 80% ethanol at 80 °C. Maltose of 0.1 mg was dissolved in the extracts as an internal standard. The extracts were purified by reverse-phase chromatography using Sep-Pak C_{18} (Waters).

The concentration of the alcohol-soluble sugars and the radioactivity of ^{14}C-sugars were analyzed by radio high-performance liquid chromatography (radio-HPLC) using a Gilson 305 pump (Gilson), a Gilson 132 refractive index monitor (Gilson), and a Raytest Ramona radioactive flow-through monitor (Raytest) as shown in Fig. 2. The Asahipak NH2P-50 4E column (Showa Denko) was used for separation, with 75% acetonitrile (1 ml/min) as the mobile phase. The chromatograms were analyzed by WINNIE ver.1.1 software (Raytest). The sugars (fructose, glucose, sucrose, raffinose and stachyose) were identified by the retention time. The concentration of sugars and the radioactivity of ^{14}C-sugars were calculated from the peak area of each chromatogram. The compositions of alcohol-soluble sugars were expressed as the percentage of total sugars. The radioactivities of ^{14}C-sugars were expressed in amounts of disintegration per minute per gram of fresh weight.

Data were analyzed by Tukey–Kramer's HSD test at the 5% level significance.

3. Results and discussion

3.1. ^{14}C-sugar metabolism on the translocation pathway in the leaves and main stems of cucumber plants

At 4 h after the start of $^{14}CO_2$ feeding to the 7th leaf, hexose (fructose and glucose) and sucrose were detected as the alcohol-soluble main sugars in the vascular bundles of the petiole and internode just below (the 7th internode) the $^{14}CO_2$-fed leaf, and raffinose and stachyose were barely detected (Fig. 2A and B). Among the ^{14}C-sugars, high radioactivities of ^{14}C-hexose and ^{14}C-sucrose were detected, and radioactivities of ^{14}C-raffinose and ^{14}C-stachyose were also detected (Fig. 2C and D). At 4 h after the start of $^{14}CO_2$ feeding, the radioactivity of ^{14}C-sugars in the vascular bundles of the 7th internode just below the $^{14}CO_2$-fed leaf was higher than in the parenchyma of the 7th internode (Fig. 3B). The radioactivity of ^{14}C-sugars in the vascular bundles of the 8th internode just above the $^{14}CO_2$-fed leaf was lower than that in the vascular bundles of the 7th internode (Fig. 3B). These results indicated that the ^{14}C-photoassmilates are first translocated to the internode just below the $^{14}CO_2$-fed leaf. This finding is supported by the previous studies in cucumber and bean plants (Kanahama and Saito, 1988; Biddulph and Cory, 1965). They reported that metabolites from mature leaves first move downward by one node though the vascular bundle connected to the petiole, and then divide into upward- and downward-moving components via anastomosing of the bundles. At 8 h after the start of $^{14}CO_2$ feeding, the radioactivity of ^{14}C-sugars in the vascular bundles of the 7th internode was similar to that in the parenchyma of the 7th internodes (Fig. 3C). Thus, most of the ^{14}C-photoassmilates were