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Changes in carbohydrate levels and their metabolic enzymes in leaves, phloem sap and mesocarp during cucumber (*Cucumis sativus* L.) fruit development

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ABSTRACT

Levels of carbohydrates and activities of metabolic enzymes were examined in leaves (source), phloem sap (flow) and mesocarp tissues (sink) in the course of cucumber (*Cucumis sativus* L.) fruit development, from 2 days before anthesis to 20 days after anthesis. While total sugar levels increased in all the three sampling organs, starch levels declined in leaves and mesocarp tissues as fruit development progressed. Glucose and fructose were the primary contributors to the soluble sugar pools in mature leaves. Stachyose was found as the most important component of the phloem sap extracts, followed by sucrose and raffinose. However, the primary sugars accumulated in mesocarp tissues were glucose and fructose, not stachyose or sucrose. Activities of sucrose synthesizing enzymes (sucrose phosphate synthase plus sucrose synthase in the synthesizing direction) exceeded that of sucrose degrading enzymes (acid invertase, neutral invertase plus sucrose synthase in the degrading direction) in leaves, which might cause a sucrose pool utilized in raffinose and stachyose biosynthesis. While alkaline a-galactosidase form I activity declined, stachyose synthase activity showed a rapid increase until 12 days after anthesis and only subsequently decreased in leaves. Activities of sucrose degrading enzymes were always much higher than that of sucrose synthesizing enzymes in mesocarp tissues. Thus, sucrose accumulation could not occur in mesocarp tissues. While stachyose synthase activity steadily decreased, alkaline a-galactosidase form I activity showed a moderate increase before decrease in mesocarp tissues. The relationship between levels of soluble sugars and activities of relative enzymes was also discussed.

1. Introduction

In many plant species, such as in Arabidopsis, tomato, soybean, maize, sugar beet or tobacco, assimilated CO_2 is exported exclusively in the form of sucrose. However, in addition to sucrose, raffinose and stachyose are also used for long-distance transport in Cucurbitaceae (Hendrix, 1982; Mitchell et al., 1992). Stachyose biosynthesis involves the following three steps beyond sucrose biosynthetic pathway (Fig. 1).

Stachyose synthase (STS, EC 2.4.1.67) which is the terminal transferase in stachyose formation (Taji et al., 2002) may control carbon partitioning between sucrose and stachyose. The initial step in stachyose and raffinose catabolism is thought to be hydrolyzed by α-galactosidase (α-Gal, EC 3.2.1.22) (Smart and Pharr, 1980), resulting in sucrose and galactose. Different forms of α-Gal in plants can be classified as acid or alkaline, based on the optimal pH for their activities. Furthermore, alkaline α-Gal can be further classified into form I and form II. Acid α-Gal and alkaline α-Gal form II prefer raffinose and stachyose as substrate, respectively. However, alkaline α-Gal form I shows high affinity for both raffinose and stachyose (Gao and Schaffer, 1999). It is known that alkaline α-Gal form I is the most important α-Gal in regulating the hydrolysis of stachyose and raffinose in melon fruit (Gao and Schaffer, 1999). Thus, it is undoubtedly that STS and alkaline α-Gal form I are two important enzymes of stachyose metabolism.

Great efforts have been made to study the carbohydrate metabolism in leaves (Pharr et al., 1985; Robbins and Pharr, 1987, 1988; Janoudi et al., 1993) and developing fruits (Handley et al., 1983; Hubbard et al., 1989; Chrost and Schmitz, 1997; Gao et al., 1999) of cucurbit species. However, these studies focused on carbohydrate metabolism in leaves or fruits alone. Little information

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Abbreviations: a-Gal, a-galactosidase; AI, acid invertase; DAA, days after anthesis; DDT, dithiothreitol; EDTA, ethylenediamine tetra-acetic acid; FW, fresh weight; HEPES, 4-(2-hydroxyethyl-1-piperazine) ethanesulfonic acid; HPLC, high-performance liquid chromatography; NI, neutral invertase; SPS, sucrose phosphate synthase; SS, sucrose synthase; STS, stachyose synthesis.

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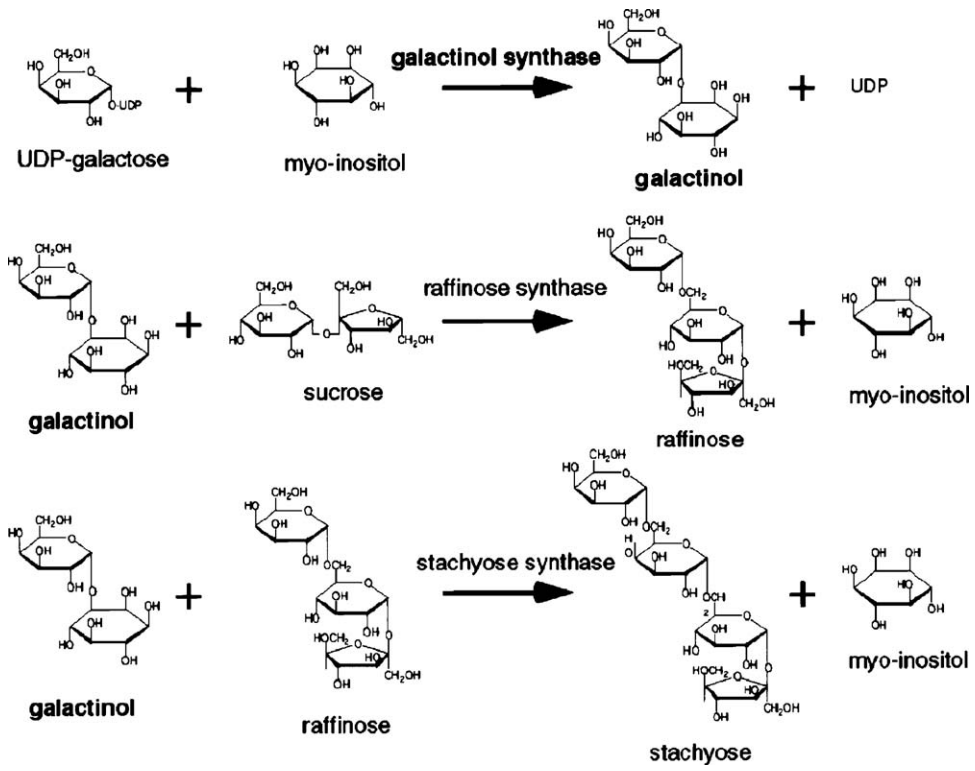

Fig. 1. Stachyose biosynthetic pathway in plants (Taji et al., 2002).

exists simultaneously concerning the sugar metabolism in source leaves, phloem sap and fruits, not only in cucumber but also in other species.

In the present study, which aims to gain a better insight into the cucumber carbohydrate metabolism, carbohydrate levels in source leaves, phloem sap and mesocarp tissues, as well as the activities of metabolic enzymes in source leaves and mesocarp tissues, were studied during cucumber fruit development.

2. Materials and methods

2.1. Plant material

Cucumbers (*Cucumis sativus* L cv. Guonong 25) were grown in autumn–winter season under natural conditions in a greenhouse at the China Agriculture University in Beijing. Plants were watered regularly as needed and fertilized weekly with Hoagland nutrient solution. All plants were maintained in a single-stem condition by mechanical removal of all lateral branches. Fruits at the fifth to eighth node were used in this experiment. Fruit age, measured as days after anthesis (DAA), was determined by tagging each flower at anthesis.

Mature leaves, phloem sap and fruits from the same node were sampled 2 days before anthesis and at 0, 4, 8, 12, 16, and 20 DAA. Samples were selected only between 09:00 a.m. and 10:00 a.m. to avoid variability due to diurnal fluctuations in assimilate composition (Mitchell et al., 1992). Leaf tissues were sampled by removing 1.33 cm^2 discs with a cork borer. For the fruits collected at 4 DAA or older, the mesocarp tissues were sliced, with epicarp, endocarp and seeds all removed. Endocarp tissues refer to the gelatinous tissues surrounding the seeds and include the associated placental tissues. Epicarp tissues were just removed for younger fruits. The leaf discs and mesocarp tissues were weighed and immediately frozen in liquid nitrogen and stored at −80 °C.

2.2. Collection of phloem sap

Phloem sap samples were obtained by making two widely spaced incisions into the stem internodes directly above and below the node of the sampled fruit according to Mitchell et al. (1992). After excision, 50 μL of phloem exudates was collected in a capillary tube and immediately transferred to a microfuge tube containing 450 μL of 80% ethanol in order to stop enzymatic activities. The tube was placed on ice and stored at −20 °C.

2.3. Carbohydrate extraction and analysis

Leaf and mesocarp samples (both 1 g FW) 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 *vacuo* in a rotary flask in a 40 °C waterbath. The residues were re-dissolved in 1 mL distilled water and passed through 0.45 μm filter. Ten microliters of sample was then injected into high performance liquid chromatography (HPLC), which consists of a Sugar-Park column (Waters, 6.5 mm × 300 mm) that operated isocratically at 75 °C with water as eluant at 0.5 mL min^{-1} and a refractive index detector. Eluted sugars were identified and quantified from retention time and peak heights of sugar standards. Phloem sap samples were centrifuged for 5 min at 3000 × *g* to pellet the precipitated protein and the resulting supernatant was treated as leaf and mesocarp samples to analyze the soluble sugars.

Starch was extracted by incubating the residue left after ethanolic extraction with 30% $HClO_4$ over night according to Wang et al. (1993). The glucose liberated was analyzed with anthrone-H_2SO_4 reagent.

2.4. Enzyme extraction and assays

STS was extracted and assayed according to Huber et al. (1990). Tissues (0.5 g FW) were homogenized in a chilled mortar with 4