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Sucrose accumulation in watermelon fruits: Genetic variation and biochemical analysis

Merav Yativ [a,b], Idan Harary [a,b], Shmuel Wolf [a,b,*]

[a] The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, The Robert H. Smith Faculty of Agriculture, Food and Environment, PO Box 12, Rehovot 76100, Israel

[b] The Otto Warburg Minerva Center for Agricultural Biotechnology, The Hebrew University of Jerusalem, The Robert H. Smith Faculty of Agriculture, Food and Environment, PO Box 12, Rehovot 76100, Israel

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ABSTRACT

Sugar accumulation, the key process determining fruit quality, is controlled by both the translocation of sugars and their metabolism in developing fruits. Sugar composition in watermelon, as in all cucurbit fruits, includes sucrose, fructose and glucose. The proportions of these three sugars are determined primarily by three enzyme families: invertases, sucrose synthases (SuSys) and sucrose phosphate synthases (SPSs). The goal of the present research was to explore the process of sugar metabolism in watermelon fruits. Crosses between the domestic watermelon (*Citrullus lanatus*) and three wild species provided a wide germplasm to explore genetic variability in sugar composition and metabolism. This survey demonstrated great genetic variability in sugar content and in the proportions of sucrose, glucose and fructose in mature fruits. Genotypes accumulating high and low percentage of sucrose provided an experimental system to study sugar metabolism in developing fruits. Insoluble invertase activity was high and constant throughout fruit development in control lines and in genotypes accumulating low levels of sucrose, while in genotypes accumulating high levels of sucrose, activity declined sharply 4 weeks after pollination. Soluble acid invertase activity was significantly lower in genotypes accumulating high levels of sucrose than in low-sucrose-accumulating genotypes. Conversely, activities of SuSy and SPS were higher in the high-sucrose-accumulating genotypes. The present results establish that, within the genus *Citrullus*, there are genotypes that accumulate a high percentage of sucrose in the fruit, while others accumulate high percentages of glucose and fructose. The significant negative correlation between insoluble invertase activity and fruit sucrose level suggests that sucrose accumulation is affected by both phloem unloading and sugar metabolism.

Introduction

The sweetness of Cucurbitaceae fruits, such as melons and watermelons, is the central characteristic in determining fruit quality. Watermelon sweetness is determined by the total sugar content and by the ratios among the main accumulated sugars – glucose, fructose and sucrose (Brown and Summers, 1985). Accumulation of glucose and fructose in watermelon fruits is evident at early stages of fruit development, while sucrose accumulation is detected only 3 or 4 weeks post-anthesis (Elmstrom and Davis, 1981; Brown and Summers, 1985). In mature commercial watermelon fruits, proportions of sucrose and glucose are in the range of 20–40% of total sugars, while the proportion of fructose is in the range of 30–50%.

Wide genetic variation in sugar content of melon fruits has been observed. Interestingly, genotypes that accumulate high levels of total sugars are those that accumulate high levels of sucrose, while genotypes having fruits with low sugar content do not accumulate sucrose (Stepansky et al., 1999). In some genotypes, sucrose accumulation is accompanied by a reduction in the concentration of glucose and fructose, suggesting that the accumulated sucrose is synthesized from available glucose and fructose (Chrost and Schmitz, 1996). In other studies, sucrose accumulation was observed, while the levels of glucose and fructose remained constant (McCollum et al., 1988; Hubbard et al., 1989). Collectively, these results suggest that genetic variation in total sugar concentrations, and in the ratios among the various sugars, are due to variation in the process of sugar metabolism during fruit development.

Stachyose and raffinose are the main sugars translocated in the phloem of cucurbit plants. As concentrations of these

Abbreviations: BC, backcross; DW, dry weight; FW, fresh weight; NI, neutral invertase; SPS, sucrose phosphate synthase; SuSy, sucrose synthase

* Corresponding author at: The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, and the Otto Warburg Minerva Center for Agricultural Biotechnology, The Hebrew University of Jerusalem, The Robert H. Smith Faculty of Agriculture, Food and Environment, PO Box 12, Rehovot 76100, Israel.
Tel.: +972 8 9489428; fax: 972 8 9462385.
E-mail address: swolf@agri.huji.ac.il (S. Wolf).

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oligosaccharides in the fruit flesh are negligible, it is assumed that they are hydrolyzed (by α-galactosidase) while being unloaded from the sieve tube and entering the sink tissue. The three major enzyme families involved in sugar metabolism in fruits are the invertases, sucrose synthase (SuSy) and sucrose phosphate synthase (SPS). The plant invertases (EC 3.2.1.26) belong to an enzyme family that hydrolyzes sucrose to glucose and fructose in a one-way reaction. Three kinds of invertases have been characterized based on their optimal pH activity and their localization in the cell (Sturm, 1999; Roitsch and Gonzalez, 2004). Soluble acid invertase is localized in the vacuole, with optimal activity at pH 4.5–5.5 (Leigh et al., 1979), cell wall invertase, also called insoluble acid invertase, is an extracellular enzyme, with optimal activity at pH 3.5–5.5 (Karuppiah et al., 1989; Iwatsubo et al., 1992), and neutral invertase (NI), also called soluble NI, shows optimal activity at pH 7–7.8. A significant negative correlation has been observed between soluble acid invertase activity and sucrose accumulation in tomato fruits (Miron and Schaffer, 1991; Yelle et al., 1991), sugar cane (Zhu et al., 1997) and melon fruits (Schaffer et al., 1987; Hubbard et al., 1989; Lester et al., 2001). On the other hand, cell wall (insoluble) invertase activity has been found to be constant during melon fruit development, although sucrose concentration gradually increases (Schaffer et al., 1987).

Susy is a key enzyme catalyzing sucrose synthesis and hydrolysis. Activity of this enzyme in the direction of sucrose synthesis is optimal at pH 7–9, and for sucrose hydrolysis, at pH 6.2–7.3, while its K_m values for fructose are in the range of 30–150 and 1–5 mM for sucrose hydrolysis and synthesis, respectively (Sun et al., 1992). Substantial activity of this enzyme has been associated with rapid accumulation of hexoses in tomato fruits (Sun et al., 1992; Shahidul Islam et al., 1996). These authors suggested that SuSy activity may be involved in determining tomato fruit sink activity. In melon fruits, however, SuSy activity has not been found to play an important role in sucrose accumulation (Hubbard et al., 1989).

SPS is a key enzyme in sucrose synthesis. This enzyme is localized and active in the cytosol of photosynthetically active tissues, as well as in sink organs such as seeds and fruit (Huber and Huber, 1996). Positive relationships have been found between SPS activity and sucrose accumulation in tomato (Dali et al., 1992) and melon (Hubbard et al., 1989) fruits. An increase in SPS activity at late stages of melon fruit development, together with a decrease in invertase activity, results in accumulation of sucrose (Hubbard et al., 1989).

In the present study, we characterized high genetic variation in the proportions of sugars in watermelon fruits. Analyses of the activity of enzymes involved in sugar metabolism were carried out to explore the factors contributing to this genetic variation and the mechanism of sucrose accumulation in the fruit.

Materials and methods

Plant material and growth conditions

Genetic material included accessions from four *Citrullus* species: *C. lanatus*, *C. colocynthis*, *C. ecirrhosus* and *C. rhemii*. Two cultivars ('Sugar Baby' and 'Mallali') of *C. lanatus* were crossed with accessions of the three species. Altogether, the first set of observations included more than 150 lines including F2 crosses and backcrosses (BCs) between the various F1's and either parent. About 500 F7–F9 lines (stemming from a cross between 'Sugar Baby' of 'Malali' and *C. colocynthis*) were analyzed for sugar content in the mature fruit and seven were selected for more detailed analyses. An additional three commercial varieties served as controls in the various experiments: hybeid 313, Odem and Crimson Sweet.

Plants were grown in the field under a 40-mesh net to prevent insect access, particularly that of bees. Flowers were hand-pollinated and tagged. Developing fruits were collected at 12, 19, 27, 35 and 42 days after pollination for sugar analyses and determination of invertase activity. For the determination of SuSy and SPS activities, fruits were collected at four stages of fruit development: 20, 30, 40 and 50 days after pollination.

Sugar determination

Fruits samples (5–10 g) were collected from the center flesh of the fruit into test tubes and kept on ice. Samples were stored at −80 °C for further analyses of sugar content and enzyme activity.

Squeezed fruit juice (1 mL) was centrifuged (9000*g*) for 10 min at 4 °C. The juice was diluted with ddH_2O by a constant factor and filtered through a 0.45-μm HPLC nylon filter (Gelman, Ann Arbor, MI, USA). Sugars were separated in an analytical HPLC system (Pump System 320, Kontron, Switzerland) fitted with a Sugar-Pak I column (6.5 × 300 mm; Waters) using a refractive-index detector (model 475, Kontron).

Invertase extraction and assay

Protein extractions for invertase activity analyses were performed according to Miron and Schaffer (1991), with some

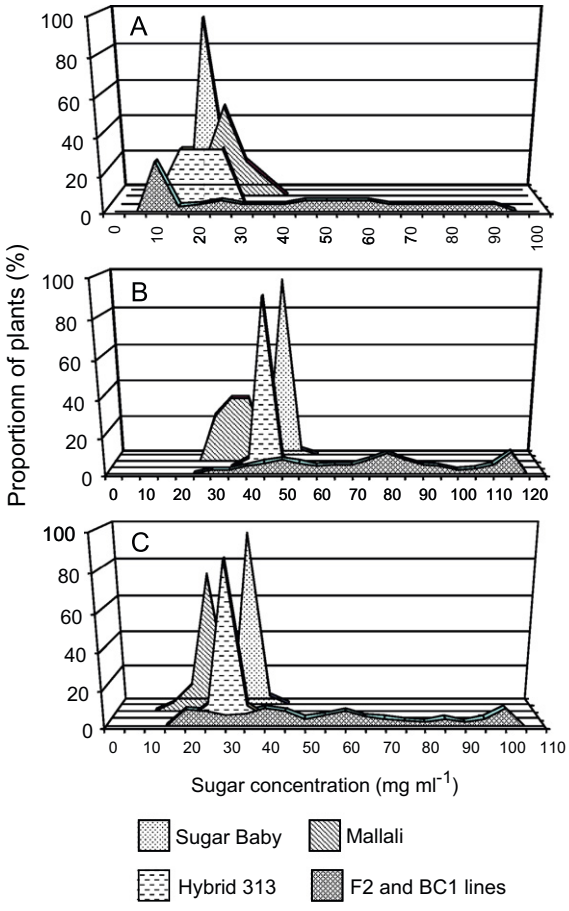

Fig. 1. Sucrose (A), fructose (B) and glucose (C) concentrations in mature watermelon fruits of the commercial varieties Mallali, Sugar Baby and hybrid 313 and over 150 F2 and backcrosses among the wild species *Citrullus colocynthis*, *C. ecirrhosus* and *C. rhemii* and the commercial *C. lanatus* cultivars (Sugar Baby and Mallali).