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A comparison of sugar-accumulating patterns and relative compositions in developing fruits of two oriental melon varieties as determined by HPLC

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Abstract

Sugar-accumulating patterns and compositions were compared between two oriental melon varieties, "Huangjingua" (*Cucumis melo* var. *makuwa* Makino) and "Yuegua" (*Cucumis melo* var. *conomon* Makino). Sucrose and reducing sugars were measured in different mesocarp tissues of developing fruits. They were all characterized by enhanced accumulation of glucose and fructose during early fruit development with almost no sucrose detectable. However, a transition of sucrose enhancement was accompanied by fruit maturing in the variety "Huangjingua", while no such transition was observed in the variety "Yuegua" that merely had a sucrose content throughout development. In "Huangjingua", both sucrose and total sugar gradients were observed, ascending from mesocarp adjacent to pedicle, middle part of mesocarp, and up to mesocarp adjacent to umbilicus. However, no obvious gradient in sucrose accumulation was seen among three mesocarp tissues examined. In terms of sweetness index, fructose is the chief contributor to sugar accumulation in both varieties. Also, the melon variety "Huangjingua" could be comparatively considered as a high-sucrose accumulator and "Yuegua" a minor-sucrose accumulator.

Keywords: Oriental melons; Sugar accumulation; High-sucrose accumulator; Minor-sucrose accumulator

1. Introduction

Melon (*Cucumis melo* L.) crops are very diversified and are comprised of six subvarieties (Robinson & Decker-Walters, 1997), among which the oriental melons are important. They are widely grown in China and eastern Asian countries and favoured by consumers, largely due to their high qualities and special flavours. Generally, soluble solids content is one criterion applied to judge fruit quality of melons. This is primarily determined by sugar content which is principally a phenomenon of sucrose accumulation (Yamaguchi, Hughes, Yabumoto, & Jennings, 1977). In muskmelon fruit, >97% of the total soluble solids are soluble sugars with sucrose accounting for 50% of the total (Bianco & Pratt, 1977). This is true of most netted melons (Chrost & Schmitz, 1997; Hayata, Li, & Osajima, 2001; Hubbard, Huber, & Pharr, 1989; Lester, Arias, & Lim, 2001; McCollum, Huber, & Cantliffe, 1988; Schaffer, Aloni, & Fogelman, 1987; Zhang, Li, Chen, Qian, & Zhang, 2003). As sweetness is one of the primary criteria for grading melon fruits, the acceptability of fresh fruits is mainly influenced by sugar level and composition, that ultimately affect fruit quality and flavour. Although it is widely believed that the *Cucurbitaceae* crops transport galactosyl-sucrose (raffinose and stachyose) rather than sucrose, the sugar that finally enters the fruit is obviously sucrose (Gross & Pharr, 1982). Therefore, the metabolic fate of the sucrose synthesized in the leaf source,

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that finally enters the fruit sink, might determine sugar-accumulating patterns in developing fruits and final sugar composition at maturity. However, in melon crops, most of the workers on sugar accumulation have concentrated on netted melons. So far, less information is available about sugar-accumulating patterns and compositions in developing fruits of oriental melons.

In the present paper, two oriental melon varieties were used for comparative analysis of sugar-accumulating patterns and their compositions in an attempt to determine the developmental changes in sucrose metabolism. A sharp difference in sugar-accumulating patterns was observed between these two oriental melons. The results obtained herewith might be helpful for clarifing whether the oriental varieties undergo similar developmental changes in sucrose metabolism to those observed in netted melons, and might contribute to a better understanding of sugar composition affecting the distinct flavour of oriental melons.

2. Materials and methods

2.1. Plant material

The oriental melons used in the investigation are of two varieties, "Huangjingua" (*C. melo* var. *makuwa* Makino) and "Yuegua" (*C. melo* var. *conomon* Makino). The former is yellow in colour, oblong in shape, and small-sized with ca. 0.5 kg per fruit (Fig. 1(a)), while the latter is green in colour with chimeric stripes, oblong in shape, and large-sized with ca. 2.0 kg per fruit (Fig. 1(b)). The seeds were sown in the greenhouse and cultivated from June through September in 2001. All plants were vertically trained to a single main vine and fruits were allowed to set on similar node positions to guarantee their uniformities in fruit size and similar growing conditions. Female flowers were hand-pollinated and tagged at anthesis, and one fruit per plant was allowed to set. Fruits were harvested at 5 day intervals starting from 0 until 30 days after pollination (DAP).

Each sampled fruit was divided into three edible tissues, namely mesocarp adjacent to pedicel, mesocarp (middle part), and mesocarp adjacent to umbilicus. As it could not be subdivided at 0 DAP, the intact fruit was sampled and blended. Plugs were removed from the equatorial section of each fruit with a cork borer. The mesocarps were sliced into sections, approximately 1–2 cm in width. After being sliced, they were quickly frozen in liquid nitrogen and stored at −74 °C. There were three replicates for each treatment and each replicate was performed twice.

2.2. Analysis of soluble sugars

Sugars were determined as described by Lowell, Tomlinson, and Koch (1989) and Komatsu, Takanokura, and Moriguchi (1999). Samples of mesocarp tissues (Fig. 2) (ca. 1 g fresh weight [FW]) were homogenized for 5 min in 5 volumes of extracted solution (ethanol:chloroform:water=12:5:3, L/L). Water and chloroform were then added to bring the final E:C:W ratio to 10:6:5. Subsequent separation of the chloroform layer allowed removal of lipids and pigments. The remaining aqueous-alcohol phase was evaporated to dryness in a vacuum at 50 °C and re-dissolved in 1 ml distilled water. Analysis of soluble sugars was performed by high performance liquid chromatography (Shimadzu Co., Kyoto, Japan) with an NH_2 column at 30 °C using 65% acetonitrile solution (1 ml min^{-1}) as a mobile phase and a refractive index detector (Shimadzu RID-10A). Peaks were quantified by using corresponding analytical software. Sweetness index was calculated on the basis of relative values (sucrose being rated as 145, glucose as 100, and fructose as 200).

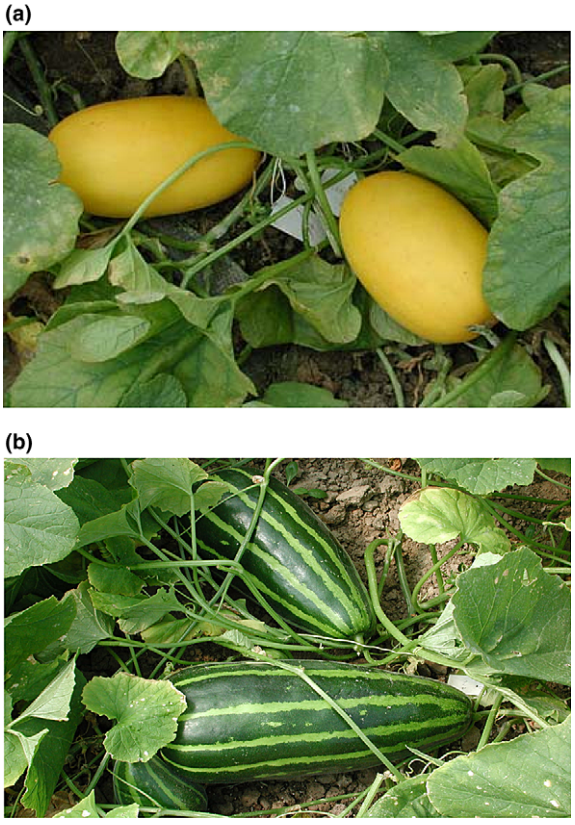

Fig. 1. Oriental melon varieties "Huangjingua" (a) and "Yuegua" (b).

3. Results

3.1. Sugar accumulation in pedicel parts of mesocarp

Sucrose and reducing sugar contents in developing fruits were measured at 5 day intervals starting from the instant day after pollination (0 DAP) until 30

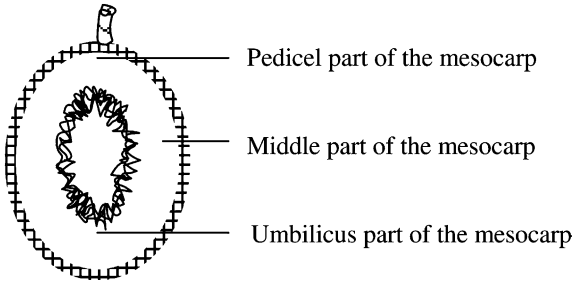

Fig. 2. Sampled portions of fruit tissues.

DAP (Fig. 3). In "Huangjingua", reducing sugars, both glucose and fructose, began to rise sharply in mesocarp tissue adjacent to pedicel within 10 DAP after anthesis (Fig. 3(a)). They decreased rapidly from 15 until 20 DAP and subsequently increased until maturity, at which the fructose level was highest, and glucose and sucrose contents were similar at fruit maturity. Meanwhile, almost no sucrose was detectable in developing fruits within 20 DAP, and the onset of sucrose occurred at 20 DAP and thereafter a sharp increase was observed, reaching its highest value at fruit maturity. In "Yuegua", reducing sugar contents increased markedly from 0 to 5 DAP, and then remained at a high level with fruit development (Fig. 3(b)). At 30 DAP, glucose and fructose contents were similar in mesocarp tissues adjacent to pedicel. However, sucrose level retained at a very low level and no sucrose accumulation was observed throughout development.

3.2. Sugar accumulation in middle parts of mesocarp

Upon anthesis in "Huangjingua", reducing sugars started to accumulate steadily until maturity, at which the fructose level was highest (Fig. 4(a)). There were no sharp fluctuations of reducing sugar contents in middle parts of mesocarp before 20 DAP, in contrast to pedicel parts of mesocarp. The reducing sugars increased steadily within 20 DAP and a marked enhancement was also observed thereafter, concomitantly with the rapid transition of sucrose accumulation. Meanwhile, almost no sucrose accumulation was found before 20 DAP. A sharp increase in sucrose accumulation occurred from 20 until 30 DAP, at which the fruits were fully mature. At maturity, fructose was relatively predominant in the middle parts of mesocarp tissues. Similarly, in "Yuegua" developing fruits, reducing sugar accumulation was enhanced quickly within 5 DAP and thereafter changed smoothly until 30 DAP (Fig. 4(b)). At 30 DAP, the glucose level was highest, followed by fructose. The accumulating pattern for sucrose in the middle part of mesocarp was quite similar to that in the pedicel part of mesocarps showing almost no sucrose synthesis.

3.3. Sugar accumulation in umbilicus parts of mesocarp

In umbilicus parts of mesocarp from "Huangjingua", both glucose and fructose levels rose rapidly within 5 DAP, after which they showed a steady increase with fruit maturing (Fig. 5(a)). In mature fruits harvested at 30 DAP, the fructose level was highest, followed by glucose and sucrose. However, in umbilicus parts of mesocarp from "Yuegua", reducing sugars, both glucose and fructose displayed similar sharp enhancement within 5 DAP, as observed in its counterparts of "Huangjingua". However, the onset of sucrose accumulation in mesocarps adjacent to umbilicus was 15 DAP, 5 days earlier than that in either the mesocarps adjacent to pedicle or middle part of the mesocarps. Likewise, in "Yuegua" variety, the accumulation of reducing sugars showed a quick increase within 5 DAP, followed by a gradual elevation, in which it was not so fluctuant as detected in "Huangjingua" (Fig. 5(a)). Moreover, the glucose became predominant in the reducing sugars, with fructose being the second, starting from 15 until 30 DAP. Likewise, sucrose was barely detectable in umbilicus parts of mesocarps from developing fruits of "Yuegua".

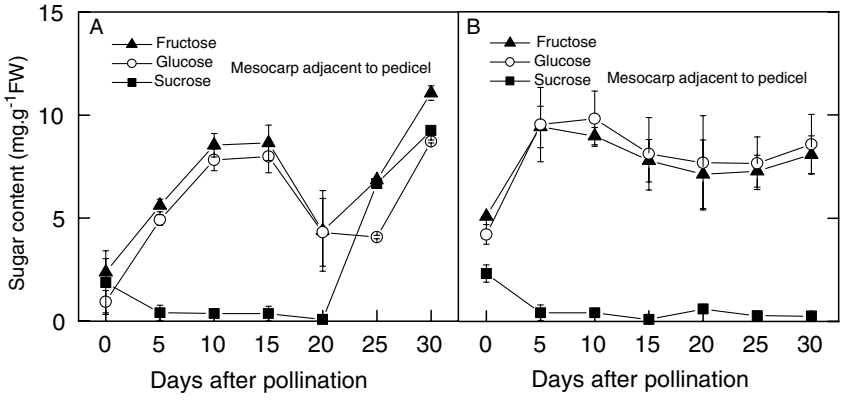

Fig. 3. Sugar contents of mesocarp tissue adjacent to pedicel from developing fruits of "Huangjingua": (a) and "Yuegua" (b).

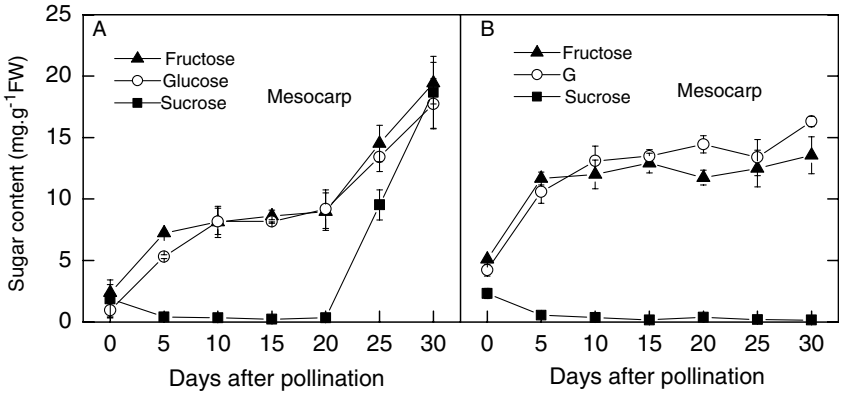

Fig. 4. Sugar contents of middle parts of mesocarp from developing fruits of "Huangjingua" (a) and "Yuegua" (b).

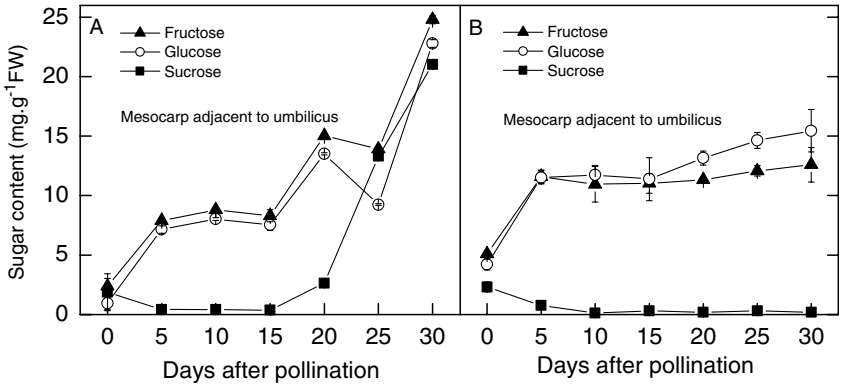

Fig. 5. Sugar contents of mesocarp adjacent to umbilicus tissue from developing fruits of "Huangjingua" (a) and "Yuegua" (b).

In addition, by conversion of sugar content into sweetness index, in three portions of "Huangjingua" mature fruits, the sweetness index reached a maximum value of 10289.8 in umbilicus, followed by 8339.1 in middle part and 4428.70 in pedicel, respectively (Figs. 3(a), 4(a) and 5(a)). In "Huangjingua", the contribution of sucrose to sweetness index was 29.7% in pedicel, 32.5% in middle part and 30.3% in umbilicus. However, in "Yuegua", the corresponding value was merely 0.7%, 0.5% and 1.5% (Figs. 3(b), 4(b), and 5(b)).

There was a dynamic change in sucrose accumulation observed in "Huangjingua" developing fruits. An obvious gradient of sucrose accumulation was also detected, ascending from pedicel to middle and umbilicus parts of mesocarps (Fig. 6(a)). The sucrose accumulation was enhanced markedly after approximately 15 DAP in the umbilicus part of mesocarp that caused unparalleled sucrose accumulation in different portions of mesocarps. This phenomenon became quite remarkable among three portions after 20 DAP. In comparison, no sucrose-accumulating gradient was found in three portions of "Yuegua" developing fruits (Fig. 6(b)).

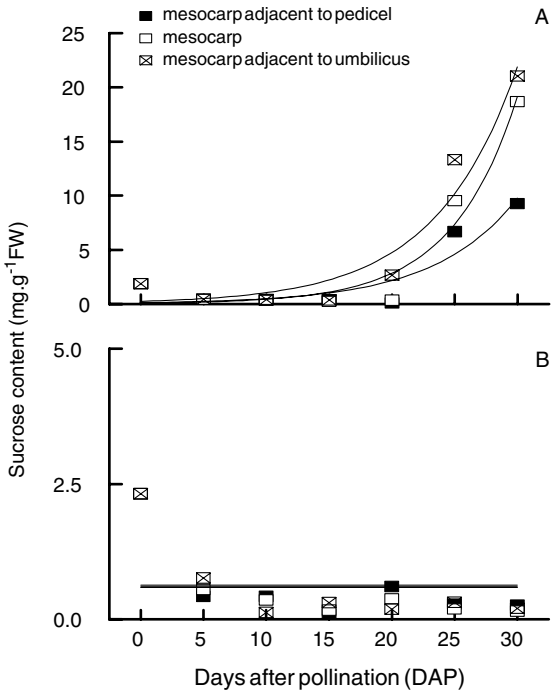

Fig. 6. Curves for sucrose levels in three portions of mesocarp tissues from developing fruits of "Huangjingua" (a) and "Yuegua" (b).

4. Discussion

Fruit flavour is a function of both taste (e.g. sugars and acids), and aroma components (Malundo, Shewfelt, & Scott, 1995). However, the main component dictating muskmelon fruit eating quality is sugar concentration or sweetness (Yamaguchi et al., 1977). In "Huangjingua", there is a sharp difference in sugar accumulation observed among three portions with the highest in umbilicus and lowest in pedicel (Figs. 3(a), 4(a) and 5(a)). This may explain why people actually feel that the umbilicus portion of mesocarp tissues taste sweetest and the pedicel portion least sweet. Based on the comparative results obtained, it is quite reasonable to consider the "Huangjingua" genotype as a high-sucrose accumulator and "Yuegua" a minor sucrose accumulator.

In netted melons, as reported by many researchers, sucrose was the chief contributor to sweetness, approximately accounting for 50% or more among total soluble sugars (Chrost & Schmitz, 1997; Hayata et al., 2001; Hubbard et al., 1989; Lester et al., 2001; Zhang et al., 2003). Nevertheless, in the oriental sucrose-accumulator "Huangjingua", sucrose only shared approximately 30% of total soluble sugar contents (Figs. 3(a), 4(a) and 5(a)). In contrast to most netted melon, in which sucrose was predominant, fructose was the predominant sugar in three portions of mesocarps in the sucrose-accumulator "Huangjingua", either in terms of sweetness index contribution or percentage relative to total sugars. By comparison, glucose was the prevailing sugar on the basis of percentage relative to total sugars in the minor sucrose accumulator "Yuegua". However, fructose was still the chief contributor to sweetness index. It was implied from these facts that oriental melons possessed different dominant sugar components and ratios in comparison to netted melons and even between these two oriental melons. As fruit flavour is also influenced by sugar components and ratios, in addition to acid and aroma, the quantitative differences between sugar compositions might be more or less responsible for flavour differences between netted and oriental melons. So far, it has been found, only in tomato fruits, that the sugars are primarily the reducing sugars, fructose and glucose, with trace amounts of sucrose (Davies & Hobson, 1981). Interestingly, in "Yuegua" developing fruits, the sugar-accumulating patterns and compositions were quite similar to cultivated tomato that had only minor-sucrose accumulation in mature fruits. In the minor-sucrose accumulator, "Yuegua", the low sucrose content was maintained throughout development, which also conformed with an observation in a species of cucumber (Schaffer et al., 1987). Generally, there is a very strict date of fruit harvest in sucrose-accumulators, in case fruit quality is severely influenced, (if harvested prior to adequate maturity). Conversely, as there is no sucrose accumulation transition in the minor-sucrose accumulator, "Yuegua", the date of fruit harvest is relatively quite flexible without adversely affecting fruit eating quality.

Many studies have shown that sucrose synthase (EC 2.4.1.13), acting toward sucrose synthesis (SS-s), plays an important role in sucrose-accumulation in sucrose-accumulating fruits, such as peach (Kobashi, Gemma, & Iwahori, 1999; Moriguchi, Sanada, & Yamaki, 1990; Moriguchi, Ishizawa, Sanda, Teramoto, & Yamaki, 1991), whereas sucrose phosphate synthase (EC 2.4.1.14) is the key enzyme involved in sucrose-accumulation in strawberry (Hubbard, Pharr, & Huber, 1991), wild tomato (Dali, Michaud, & Yelle, 1992; Miron & Schaffer, 1991) and banana (Cordenunsi & Lajolo, 1995). McCollum et al. (1988) and Schaffer et al. (1987) suggested that sucrose accumulation in muskmelon is associated with an increase in SS-s activity, accompanied by a decrease in acid invertase activity. In contrast Lingle and Dunlap (1987) reported that SPS activity increased with a decrease in AI activity during fruit maturation. Moreover, Hubbard et al. (1989) found that SPS rather than SS, was the key enzyme responsible for sucrose-accumulation in muskmelon. The observations aforementioned indicate that there are probably different predominating sucrose-metabolizing enzymes involved in sugar-accumulation of melons.

However, it is unclear, as yet, what are the major enzymatic factors responsible for distinctive sugar-accumulating patterns in oriental melons. These two varieties potentially serve as a good model for comparatively investigating whether differences in sugar accumulation between high sucrose-accumulators and minor-sucrose-accumulators could be related to sucrose-metabolizing enzymes, and how intrinsic and extrinsic elements play roles in sugar accumulation. This seems to be extremely interesting and is worthy of further investigation. The comparative analysis of dynamic changes and expressions of corresponding sucrose-metabolizing enzymes is in progress in the laboratory.

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