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DYNAMICS OF SUGAR-METABOLIC ENZYMES AND SUGARS ACCUMULATION DURING WATERMELON (*CITRULLUS LANATUS*) FRUIT DEVELOPMENT

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Abstract

We analyzed sugar accumulation and the activities of sugar-metabolic enzymes in ripening fruits of three cultivars of watermelon; a high-sugar type "w2", a low-sugar type ("w1"), and their hybrid. In "w2", the glucose and fructose contents were higher than the sucrose content in the earlier stage of fruit development, and fruit growth was accompanied by increases in glucose, fructose, and sucrose contents. The sucrose content increased substantially after 20 days after anthesis (DAA) and it was the main soluble sugar in mature fruit (sucrose:hexoses ratio, 0.71). In "W1", the fructose and glucose contents were significantly higher than the sucrose content in mature fruit (sucrose:hexoses ratio, 0.25). Comparing the two parent cultivars, sucrose was the most important factor affecting the total sugar content in mature fruit, although glucose and fructose also contributed to total sugar contents. The fructose and glucose contents in the fruit of F_1 were mid-way between those of their parents, while the sucrose content was closer to that of "W1" (sucrose:hexoses ratio in F_1, 0.26). In the early stage of fruit development of "W2", the activities of acid invertase and neutral invertase were higher than those of sucrose synthase and sucrose phosphate synthase. After 20 DAA, the acid invertase and neutral invertase activities decreased and those of sucrose synthase and sucrose phosphate synthase increased, leading to increased sucrose content. In "W1", the activities of acid invertase and neutral invertase were higher than those of sucrose synthase and sucrose phosphate synthase at the early stage. The sucrose synthase and sucrose phosphate synthase activities were lower in "W1" than in "W2" at the later stages of fruit development. The patterns of sugar accumulation and sugar-metabolic enzyme activities during fruit development in F_1 were similar to those in "W1".

Key words: Watermelon; Fruit development; Sugar accumulation; Sugar-metabolic enzymes.

Introduction

Watermelon (*Citrullus lanatus*), a member of the gourd family, is an important fruit crop that is widely cultivated in warm and subtropical areas worldwide. It is known as "the king of fruits in summer". Watermelon produces large edible fruit that is an important component of the human diet, and its cultivation area accounts for approximately 7% of the world's total vegetable production area, according to FAO statistics (Erickson *et al*., 2005). Sugar content is one of the most important traits in watermelon. The amounts and types of sugars in the fresh fruit directly affect quality and flavor. Glucose, fructose, and sucrose function as sources of carbon and energy for non-photosynthetic tissues and are the most abundant free sugars in the flesh of watermelon fruit (Basson *et al*., 2010).

Sucrose phosphate synthase (SPS), sucrose synthase (SS), and invertases (acid invertase, AI, and neutral invertase, NI) are the key enzymes affecting sugar accumulation and composition (Ruan *et al*., 2010). Studies have shown that the activities of key sugar-metabolic enzymes are correlated with sugar accumulation in melon (Matsumoto *et al*., 2010), mango (Wongmetha *et al*., 2012), strawberry (Turhan, 2012), and sweet sorghum (Liu *et al*., 2013). An understanding of the activities of key sugar-metabolic enzymes involved in glucose, fructose, and sucrose accumulation in watermelon is important to control and improve fruit sweetness. At present, our knowledge of sugar metabolism during the growth and development of watermelon fruit is limited.

In the present study, three lines of watermelon were evaluated to study the changes in soluble sugar contents and the relationship with key sugar-metabolic enzymes in developing fruit. The three lines were a high-sugar type, a low-sugar type, and their first-filial generation.

Materials and Methods

Plant materials: The low-sugar line, "w-1" (P_1), accumulated 4%–5% soluble sugars and the high-sugar line, "w2" (P_2), accumulated 11% soluble sugars in mature fruit. Both were selfing lines. The F_1 population represented the hybrid obtained by crossing P_1 with P_2.

The experiments were conducted at the Melon Research Institute, School of Life Science, Huaibei Normal University, China. The three watermelon lines were cultivated in the same field with high ridges and mulch planting, with conventional field management. During the anthesis, the same female flower node was marked, and five fruits from every cultivar were collected on 15, 20, 25, 30, 35, 40, 45, 50, and 55 days after anthesis (DAA) as the fruit matured. The flesh was cut from the middle of each fruit, chopped into small pieces, and then immediately frozen in liquid nitrogen and stored at −80°C until analysis.

Extraction and determination of sugars: The soluble solids content was determined by a portable refractometer. Sucrose and fructose contents were assayed by the anthrone method, hexose content was assayed by the 3, 5-dinitrosalicylic acid method, and glucose content was calculated as the difference between hexose and fructose contents. The unit for all of these parameters was mg/g^{-1} FW.

Assays of sugar metabolic enzymes: The activities of the sugar metabolic enzymes SPS, SS, AI, and NI were assayed according to Gao (2000) with minor modifications, as follows:

(1) Extraction of enzymes: 2.0 g watermelon flesh was ground in a cold mortar with 5 mL extraction buffer (100 mmol/L Tris-HCl pH 7.0), and then centrifuged at 10000 r/min for 10 min at 2°C. Then, 3 mL supernatant was dialyzed against buffer (25 mmol/L Tris-HCl pH 7.0) at 4°C for 24 h and diluted to 5 mL with buffer before use.

(2) SPS activity assay: the reaction mixture consisted of 0.4 mL Tris-MES (100 mmol/L pH 7 .0), 0.1 mL uridine diphosphate glucose, 0.05 mL dialyzed enzyme solution, and distilled water to complete the volume to 1 mL. The mixture was incubated at 30° for 10 min before terminating the reaction by heating in boiling water for 3 min. Then, 0.1 mL NaOH (2 mol/L) was added and the mixture was cooled to room temperature before adding 3.5 mL HCl (30%) and 1 mL resorcinol (0.1%). The mixture was then heated at 80°C in a water bath for 10 min. After cooling, the absorbance of the mixture was measured at 480 nm. The unit of enzyme activity was μmol/(h·g).

(3) SS activity assay: this assay was conducted in the same way as that described for SPS, except that fructose-6-phosphate was replaced by 10 mol/L fructose in the reaction mixture.

(4) NI activity assay: the reaction mixture consisted of 0.95 mL acetic acid-K_3PO_4 (80 mmol/L pH 7.0) and 0.05 mL dialyzed enzyme solution. The mixture was incubated at 30°C in a water bath for 10 min and then the reaction was terminated by heating the mixture in boiling water for 3 min. Then, 1 mL 3, 5-dinitrosalicylic acid was added to the mixture before heating in boiling water for 5 min. After cooling, the absorbance of the mixture was measured at 540 nm. The unit of enzyme activity was μmol/(h·g).

(5) AI activity assay: This assay was conducted as described above for the NI activity assay, except that the reaction was conducted at pH 7.5.

Results

The soluble solids content in the three watermelon lines is shown in Fig. 1A. The soluble solids contents did not differ significantly among the three lines before 15 DAA. After 15 DAA, the soluble solids content increased to higher levels in P_2 than in P_1 and F_1 (from 4.83% at 15 DAA to 10.76% at 35 DAA in P_2). The soluble solids content did not increase substantially during fruit development of P_1 (maximum value, 4.6%). The was a 6.61% difference in soluble solids content between P_1 and P_2 fruit at maturity. The soluble solids content of F_1 was between those of its parents, higher than that of P_1 but lower than that of P_2. The highest soluble sugars content in mature F_1 fruit was 6.23%.

We monitored changes in the sucrose, fructose, and glucose contents in the ripening fruits of P_1, P_2, and F_1 (Fig. 1B–D). Fruit growth was accompanied by increases in sucrose, fructose, and glucose contents. In the earlier stage of fruit development, fructose and glucose contents were higher than the sucrose content, and the rates of increase differed among the three sugars. Among the three watermelon lines, the differences in fructose and glucose contents became significant after 15 DAA and the differences in sucrose contents became significant after 20 DAA. All of the sugars increased to higher concentrations in the fruit of P_2 than in the fruit of F_1 and P_1. In all three lines, the fructose and glucose contents increased until 30 DAA and then decreased as the fruit matured. In P_1, the fructose and glucose contents increased from 45 to 55 DAA. Sucrose accumulated to an approximately three-times higher concentration in P_2 than in F_1 and P_1 at 35 DAA. In F_1, the patterns of fructose and glucose accumulation were approximately mid-way between those of P_1 and P_2, while the pattern of sucrose accumulation was closer to that of P1.

The changes in the activities of the key sugar metabolic enzymes during the fruit development of P_1, P_2, and F_1 are shown in Fig. 2A–D. The activities of AI and NI increased during early fruit development in P_1 and P_2, and then decreased as the fruit matured. In F_1, the activities of AI and NI continuously decreased during fruit ripening. The activity of SS was higher in P_2 than in P_1 and F_1. In P_2, SS activity decreased until 20 DAA and then significantly increased to peak at 30 DAA before decreasing until the fruit reached maturity. The SS activity increased slowly in P_1 and F_1 until 35 DAA and 30 DAA, respectively, and then decreased until the fruit reached maturity. The SS activity in F_1 increased from 35 to 40 DAA. There were no significant differences in SPS activity among the three lines at 15 DAA. The SPS activity increased slowly from 20 to 30 DAA in P_1, P_2, and F_1, and then increased to a much higher level in P_2 than in the other two lines. The SPS activity decreased from 30 to 40 DAA in F_1, and decreased from 40 to 55 DAA in P_1.

Discussion

In the early fruit development stage of P_2 (high-sugar type), the glucose and fructose contents were higher than the sucrose content and fruit growth was accompanied by increases in glucose, fructose, and sucrose contents. After 30 DAA, the glucose and fructose contents decreased, but the sucrose content continued to increase until the fruit reached maturity. The results indicated that sucrose was the main component of total soluble sugars in the late stages of P_2 fruit development. Previous studies reported that fructose and glucose contents in watermelon fruit remained almost constant during fruit development, but sucrose content increased rapidly at 30 DAA and peaked at harvest (Elmstrom & Davis, 1981; Kano, 1991). Our results also showed that sucrose content was the most important factor affecting watermelon sweetness.

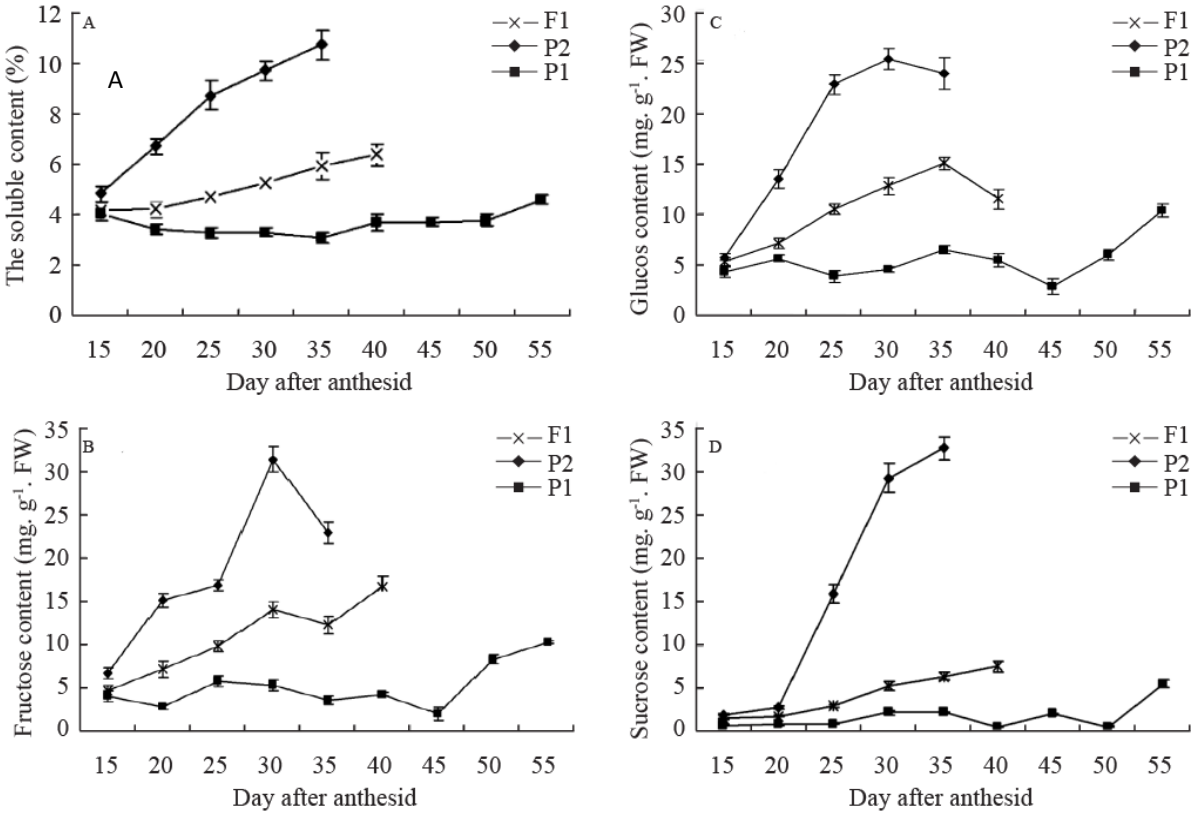

Fig. 1. Soluble solids content The developmental changes in sucrose.

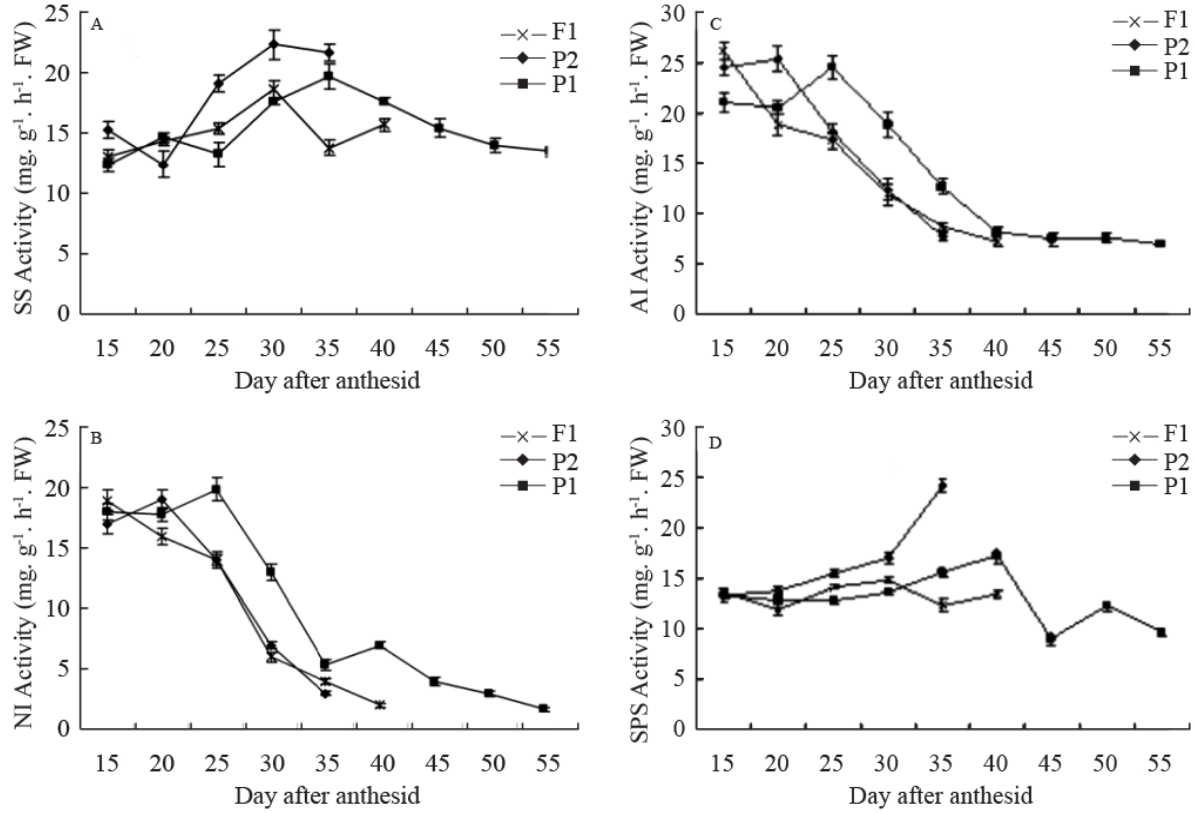

Fig. 2. Sugar-metabolic enzymes activities.

In the fruit development of P_1 (low-sugar type), the fructose and glucose contents were significantly higher than the sucrose content. The sucrose:hexoses ratio was 0.25 in mature fruit. Comparing the sugar accumulation and composition between P_1 and P_2, the increase in sucrose content was the key factor contributing to the high total sugars contents in the high-sugar type watermelon cultivar. Qazi *et al*. (2012) found that there was a high sucrose:hexoses ratio in mature fruits of high-sugar type cultivars. Liu *et al*. (2013) also found that most high-sugar type cultivars had high sucrose contents (mean 80.5 mg/g FW) and low hexoses (glucose + fructose) contents (mean, 5.7 mg/g FW). In this study, there were also significant increases in glucose and fructose contents in P_2 (hexose ratio of P_2 to P_1, 227%; sucrose ratio of P_2 to P_1, 651%). These results indicated that fructose, glucose, and sucrose accumulated to higher concentrations in the high-sugar watermelon type than in the low-sugar watermelon type.

We found that the AI and NI activities were higher than the SS and SPS activities in P_2 (high-sugar type) from 15 DAA to 20 DAA, but the sucrose content did not increase significantly during this period. Zhu *et al*. (1997) found that the activity of NI was not significantly correlated with sucrose content. After 20 DAA, there was a rapid increase in sucrose content accompanied by increasing activities of SS and SPS and decreasing activities of AI and NI. These results indicated that the activities of SPS and SS were important factors in the soluble sugars content in watermelon fruit, as reported for various other species (Hubbard *et al*., 1991; Jaleel *et al*., 2007). Kano *et al*. (2012) suggested that higher SPS activity in watermelon fruit resulted in the higher mean sucrose and fructose contents. Increased proportions of sucrose were found to be associated with increased SPS activity in melon fruit (Gao *et al*., 1999), butter squash (Irving *et al*., 1997), and rice (Yonekura *et al*., 2013). It has also been reported that a large increase in sugar content was associated with increased SPS and SS activities during walnut development (Li *et al*., 2012).

In P_1 (low-sugar type), the activities of AI and NI were higher than those of SPS and SS from 15 DAA to 25 DAA, but the AI and NI activities sharply declined after that. Moreover, the small increases in the activities of SPS and SS were not significant, so that the sucrose content increased only marginally in the maturing fruit. These results indicated that the differences in sugar content between high-sugar and low-sugar type watermelons are related to the activities of SPS and SS.

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