Changes in Sugar Metabolism Enzyme Activities in Cultivars of Two Pumpkin Species (*Cucurbita maxima* and *C. moschata*) during Fruit Development

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Summary

Sugar accumulation and sugar metabolism enzyme activities were measured in two distinct pumpkin cultivars, 'Miyako' (*C. maxima*) and 'Hayato' (*C. moschata*) during fruit development. Sugar accumulation levels in the fruits were different between these cultivars. At the harvesting stage, more glucose and fructose were accumulated in 'Hayato' than in 'Miyako'. Sucrose content was low in young fruit when its catabolic enzyme activities were highest. The accumulation of sucrose with fruit development in both cultivars coincided with increasing sucrose phosphate synthase (SPS) activity and decreasing catabolic enzyme activities. The amount of sucrose content was indistinguishable in both cultivars, whereas, higher SPS activity was observed in 'Hayato'. SPS contributes to sucrose accumulation in the late stage of pumpkin fruit development, but the amount of accumulated sucrose could not be explained solely on SPS activity.

Key Words: pumpkin, starch, sucrose phosphate synthase, sugar.

Introduction

Because of differences in fruit texture, mealiness and sweetness, between *C. maxima* and *C. moschata*, Japanese customers tend to prefer to *C. maxima* fruit rather than *C. moschata*; thus, there is a demand for pumpkin fruit containing high amounts of starch and sugar. Recently, Terazawa et al. (2001) reported the existence in differences in sugar accumulation levels in the fruits of cultivars 'Miyako' (*C. maxima*) and 'Hayato' (*C. moschata*) during development. At harvest, total sugar content in 'Hayato' fruit was higher than that of 'Miyako', which was attributed to the residual glucose and fructose accumulated in 'Hayato' fruit. Thus, it may be that differences in carbohydrate metabolism mechanism exist between these two species. Apparently, photo-assimilates from leaves are accumulated in fruit as different carbohydrates that contribute to fruit quality. However, little information of related enzymes in sugar metabolism exist for pumpkin. In this study, we analyzed two different pumpkin cultivars for sugar metabolism enzymes during fruit development to compare their means of carbohydrates accumulation.

Materials and Methods

Plant material

Pumpkins, 'Miyako' and 'Hayato', grown in Kanagawa Agricultural Institute Research field, Miura, Kanagawa, were harvested 0, 10, 20, 30 and 40 days after pollination (DAP). Fruit mesocarp were sliced, immediately frozen in liquid nitrogen and stored at –85 °C.

Carbohydrate analysis

The mesocarp samples were extracted with 80 % (v/v) ethanol, and the levels of sucrose, glucose, fructose and mannose in the extract determined by HPLC analysis, equipped with Asahipak NH₂P–50 column (4.6 mm I.D. × 250 mm, acetonitril : water = 70 : 30) and a refractive index detector.

Enzyme extraction

Tissue samples were ground using mortar and pestle in the presence of liquid N₂. The homogenates were desalted according to the method described by Hubbard et al. (1989). Enzyme extraction was carried out under 4 °C condition.

Assay of enzymatic activities

For assay of acid invertase (AI), 0.1 ml reaction mixture consisted of 100 mM Na–citrate, pH 5.0, 120 mM sucrose and the enzyme solution, whereas for

Received; February 20, 2003. Accepted; May 15, 2003.

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neutral invertase (NI), 50 mM Hepes-NaOH, pH 7.5 substituted for Na-citrate. For sucrose synthase (SS) cleavage direction (SS-c), the reaction mixture consisted of 30 mM Hepes-NaOH, pH 7.5, 200 mM sucrose, 5 mM uridine diphosphate (UDP) and the enzyme solution. The mixture was incubated at 30 °C for 30 min and stopped by dipping in boiling water for 10 min. Hexose sugar concentration was determined by measuring NADP reduction (Hubbard et al., 1989; Tanase and Yamaki, 2000). For SPS assay, the reaction mixture (0.07 ml) consisted of 50 mM Hepes-NaOH, pH 7.5, 15 mM $MgCl_2$, 10 mM UDP-glucose, 5 mM fructose-6-phosphate, 15 mM glucose-6-phosphate and enzyme solution; the mixture of SS synthesis (SS-s) assay consisting of 15 mM Hepes-NaOH, pH 7.5, 5 mM $MgCl_2$, 2 mM UDP-glucose, 50 mM fructose and enzyme solution. They were incubated at 30 °C for 30 min and the resulting synthesized sucrose was measured by the anthron- H_2SO_4 method (Van Handel, 1968).

Results and Discussion

Changes in sugar composition and starch accumulation during fruit development

During the initial stage of development, glucose and fructose were the major hexoses in both cultivars (Fig. 1). In 'Miyako', these sugars decreased 20 DAP, whereas little change occurred in 'Hayato'. The rapid loss of glucose and fructose found in 'Miyako' was also observed in buttercup squash 'Delica' (*C. maxima*) (Irving et al., 1997). In our cultivars, sucrose increased gradually with fruit development.

Changes in the activities of sugar metabolism enzymes

Sucrose cleaving activities, such as invertase or SS-c, were highest at anthesis 0 DAP fruit. SS-c activities rapidly decreased 10 DAP in 'Miyako' or 20 DAP in 'Hayato' (Fig. 2A). Both AI and NI activities declined with fruit development in both cultivars (Fig. 2B). During early development, the contribution of AI or SS-

Fig. 1. Changes in sugar composition in the developing fruit of pumpkin cultivars, 'Miyako' and 'Hayato'.

Fig. 2. Changes in sugar metabolism enzyme activities during fruit development of two pumpkin cultivars. A, sucrose synthase both cleaving and synthesis direction (SS-c and SS-s); B, acid (AI) and neutral (NI) invertase; C, sucrose phosphate synthase (SPS). Vertical bars indicate SE.

c to sucrose hydrolysis occurs in many kinds of sink tissues (Dali et al., 1992; Hubbard and Pharr, 1992; Hubbard et al., 1991; Miron and Schaffer, 1991; Ross et al., 1994; Sung et al., 1994). In both pumpkin cultivars, these enzymatic activities seem to contribute to lower the content of sucrose in young fruit.

The activity of SS-s changed little, remaining relatively low through the development in both cultivars (Fig. 2A). One of the SS isoforms isolated from Japanese pear was prove to sucrose accumulation (Tanase and Yamaki, 2000), but not in the case of pumpkin.

That SPS contributes in sucrose accumulation at the latter stage of development in *Cucurbita* fruit was reaffirmed in this study. SPS activity increased with fruit development in both cultivars (Fig. 2C), furthermore, the sucrose content increased concurrently (Fig. 1), indicating that SPS contributes sucrose accumulation. However, the sucrose content was not distinguishable in

both cultivars, whereas SPS activity was lower in 'Miyako' than in 'Hayato' (Fig. 2C). A decrease in glucose content in 'Miyako' fruit that coincided with starch accumulation (data not shown) did not occur in 'Hayato'. Thus, in pumpkin, sucrose accumulation with fruit development is attributed to SPS activity, but the amount of sucrose is regulated by its conversion to starch. In 'Miyako', most of the photoassimilates were converted to starch. In source tissue, regulation of the interconversion of starch and sucrose is explained diurnal cycling model. However, the mechanism in sink tissue has not been explored; we are currently measuring starch metabolism enzyme activities. Further studies related to starch accumulating enzyme(s) are required to elucidate the carbohydrate metabolic mechanism in pumpkin fruit.

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異なるカボチャ品種の果実生長にともなう 糖代謝関連酵素活性の変動

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摘 要

カボチャ品種‘みやこ’(*Cucurbita maxima*)および‘はやと’(*C. moschata*)の果実生長における糖の蓄積と糖代謝関連酵素活性の変動を測定した。糖の蓄積パターンは品種間で異なっており、収穫時では‘はやと’は‘みやこ’よりグルコースやフルクトースの蓄積量が多かった。未熟果ではスクロース含量が低く、また、スクロース分解酵素類の活性が高かった。両品種とも生長にともなって、スクロースリン酸合成酵素 (SPS) 活性が増大するとともにスクロース分解酵素類の活性が減少し、スクロースが蓄積した。SPS活性は‘はやと’のほうが高かったが、両品種のスクロースの蓄積量に大きな差は認められなかった。SPSは果実生長後期のスクロースの蓄積に関与するが、カボチャの場合その蓄積量についてはSPS活性だけでは説明できなかった。