Relationship between Acid Invertase Activity and Hexose Content in Sweet Potato Storage Roots

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To elucidate the factors affecting the varietal differences in the hexose content in raw storage roots of sweet potato, relationships between the free sugar contents and the activities of acid and neutral invertases were investigated. There was a highly significant relationship between hexose contents and acid invertase activities when the cultivar Georgia Jet was excluded. The cultivar Georgia Jet showed a peculiar pattern of free sugar composition and invertase activities and was considered to have a different biochemical basis from the others. Although not all cultivars showed a strong relationship between hexose content and acid invertase activity, it was concluded that the acid invertase activity in sweet potato roots played an important role in the varietal differences in their hexose content.

Keywords: *Ipomoea batatas; glucose; fructose; sucrose; free sugar*

INTRODUCTION

Free sugars are one of the most important constituents for determining the quality of fruits and vegetables. Numerous workers have studied the free sugar composition, metabolism, and physiology of many horticultural crops. The relationships between free sugar composition and saccharide-metabolizing enzymes have been extensively studied on some fruits and vegetables (Hubbard et al., 1991; Stommel, 1992; Yelle et al., 1991).

Storage root of sweet potato contains free sugars that are important in determining root quality, although the largest storage compound is starch. Many researchers extensively studied the varietal differences in free sugar composition of heated roots, specifically in the formation of maltose and/or degradation of starch by endogenous amylolytic activity (Walter et al., 1975; Shen and Sterling, 1981; Takahata et al., 1994). Some researchers reported that free sugars in raw sweet potato roots consisted of sucrose, glucose, and fructose (Picha, 1986; Truong et al., 1986). Picha (1985) showed the diversity in the hexose content among six U.S. cultivars. We also reported that several cultivars showed distinct differences in free sugar composition in raw storage root (Takahata et al., 1992). However, the enzymatic system is not well understood, and its relationship with free sugar composition has not been studied until the present. To improve the quality of sweet potato storage roots, biochemical factors controlling free sugar composition in raw storage roots should be clarified.

In this paper, to elucidate the biochemical differences associated with varietal differences in the hexose content of sweet potato storage roots, relationships between free sugar contents and enzyme activities were investigated by using representative sweet potato cultivars.

MATERIALS AND METHODS

Plant Materials and Tissue Sampling. The seedlings of six sweet potato cultivars were planted in mid-May in an experimental field at Kyushu National Agricultural Experiment Station, and the storage roots were harvested and sampled in mid-October 1994. Developmental studies were performed on some cultivars, which were sampled at four different times before harvest. The sampling dates were 69 (stage 1), 89 (stage 2), 112 (stage 3), 133 (stage 4), and 154 (stage 5, at harvest) days after planting the seedlings. Immediately after sampling, the storage roots were washed and cut. The central portion of each root was then frozen in liquid nitrogen and stored at −80 °C for analysis of free sugar content and invertase activity. Three roots per cultivar were provided at every sampling period.

Extraction and Assay of Invertases. Frozen sweet potato tissue was ground into a powder with a mortar and pestle in liquid nitrogen. The ground powder was homogenized with 50 mM Hepes−NaOH buffer (pH 7.4) containing 4 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, 12.5% (v/v) glycerol, 0.5 mg/mL bovine serum albumin, 10% (w/w) polyvinylpolypyrrolidone, and 0.05% (v/v) Triton X-100 at a 1/2 (w/v) tissue-to-buffer ratio. Homogenates were centrifuged at 27200*g* for 10 min, and aliquots of the supernatant were desalted with 50 mM Hepes−NaOH buffer (pH 7.4) containing 4 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, and 12.5% (v/v) glycerol using a Sephadex G-25 column (Pharmacia).

The reaction mixture (500 μL) for determining the acid invertase activity contained 100 mM sodium acetate (pH 4.8), 100 mM sucrose, and 100 μL of desalted extract. Identical conditions were used to assay neutral invertase, except that the reaction mixture contained 100 mM Hepes−NaOH (pH 7.4). The reaction mixture was incubated at 30 °C, and the reaction was terminated by adding 500 μL of Somogyi copper reagent at 0 and 60 min after initiation with enzyme extract (Copeland, 1990). The amount of reducing sugar produced was measured by the Somogyi−Nelson method with glucose as a standard. No residual activity was detected in the insoluble pellets when they were resuspended and assayed after washing three times with 10 mM Hepes−NaOH (pH 7.4) containing 1 mM 2-mercaptoethanol.

Free Sugar Determinations. Extraction and determination of free sugars were performed according to the method in Takahata et al. (1992).

RESULTS

Varietal Differences in Free Sugar Contents and Enzyme Activities. The cultivars used in the current study showed various patterns of free sugar composition in the raw storage roots (Table 1). The fructose content was slightly higher than or almost the same as the glucose content. Shiroyutaka (SY) and Naeshirazu (NS) contained a negligible amount of hexose, and their

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Table 1. Free Sugar Contents and Enzyme Activities in Six Sweet Potato Cultivars at Harvest[a]

	free sugar content (mg/g, fresh weight basis)				enzyme activities[c]	
	fructose	glucose	sucrose	Suc/Hex[b]	acid invertase	neutral invertase
SY	0.420 ± 0.180	0.090 ± 0.016	11.7 ± 1.97	22.9	0.51 ± 0.18	10.5 ± 2.93
NS	0.447 ± 0.350	0.273 ± 0.177	21.1 ± 0.387	29.3	0.63 ± 0.06	11.0 ± 3.43
T5	10.8 ± 1.12	9.02 ± 1.16	16.2 ± 1.52	0.82	6.12 ± 1.14	9.47 ± 2.97
M2	9.14 ± 2.06	9.25 ± 2.84	14.5 ± 2.77	0.79	6.26 ± 1.16	9.75 ± 4.58
GJ	12.2 ± 2.58	11.6 ± 4.09	37.2 ± 0.787	1.56	2.53 ± 0.18	4.01 ± 2.60
SS	4.10 ± 1.77	3.76 ± 1.31	24.9 ± 2.40	3.17	3.66 ± 1.06	7.87 ± 1.78

[a] Free sugar contents and enzyme activities are expressed as means of three roots ± SD. [b] Sucrose/(fructose + glucose). [c] μmol glc/g·h, fresh weight basis.

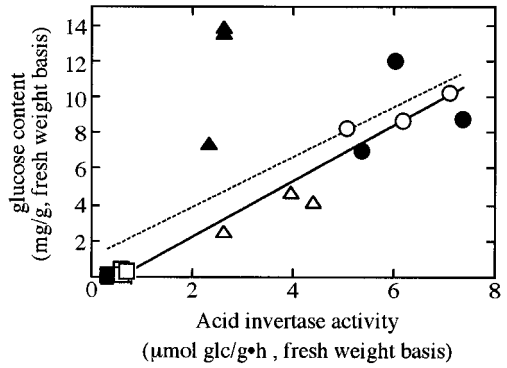

Figure 1. Scattergram of acid invertase activities and glucose contents in six sweet potato cultivars: M2 (●), T5 (○), SY (■), NS (□), GJ (▲),SS (△) The dotted line represents the linear regression for all samples, $y = 1.196 + 1.360x$ ($r = 0.679$, $n = 18$). The solid line represents the linear regression excluding GJ, $y = -0.812 + 1.539$ ($r = 0.958$, $n = 15$).

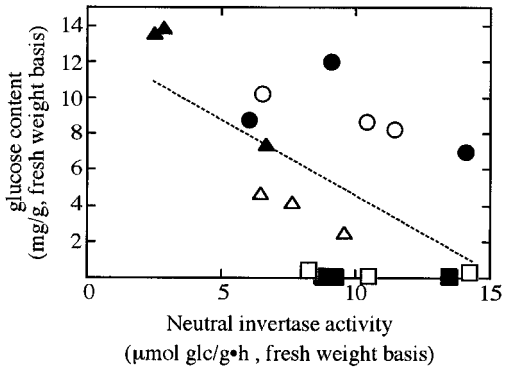

Figure 2. Scattergram of neutral invertase activities and glucose contents in six sweet potato cultivars. Symbols are the same as in Figure 1. The dotted line represents the linear regression for all samples, $y = 13.05 - 0.841x$ ($r = 0.574$, $n = 18$).

sucrose/hexose ratios (Suc/Hex) were far greater than those of the other cultivars. These two cultivars also had similar traits in acid and neutral invertase activities. The activities of acid invertase were the lowest and those of neutral invertase were the highest among the six cultivars. On the contrary, Tokai 5 (T5) and Murasakibaru 2 (M2) contained significantly greater amounts of hexose than SY and NS and their Suc/Hex ratios were less than 1.0. The acid invertase activities of these two cultivars were also significantly higher than those of SY and NS, whereas the neutral invertase activities were at a similar level. Georgia Jet (GJ) and Shirosengan (SS) showed different patterns of free sugar composition compared to the other cultivars. GJ had a larger amount of hexose than SY and NS, but its sucrose content was also higher than those of the other cultivars. The acid invertase activity of GJ was lower than those of T5 and M2, but higher than those of SY and NS. The activity of neutral invertase in GJ was the lowest in this experiment. SS had a trait intermediate between those of SY/NS and T5/M2 with respect to the hexose content and acid invertase activity and between those of GJ and the others with respect to the sucrose content and neutral invertase activity.

The relationships between the acid invertase activities and the glucose contents in the raw roots are plotted in Figure 1. An increasing activity of acid invertase correlated with an increase in the glucose content. However, GJ had a relatively high glucose content compared with its acid invertase activity. Slightly significant relationships were observed between glucose contents and neutral invertase activities, but the relationships were negatively correlated (Figure 2).

Changes in Free Sugar, Invertase Activities, and Dry Matter Contents during Storage Root Development. Figure 3A–C shows changes in the free sugar and dry matter contents during storage root development in three representative cultivars. The activities of acid and neutral invertases were also assayed in SY and M2 (Figure 3D). Glucose and fructose were present at approximately equal concentrations. SY showed a negligible amount of glucose, whereas M2 and GJ had significantly higher contents of glucose than SY throughout development. Sucrose content levels were similar among the cultivars at the initial stage, but GJ increased its sucrose content during development. Sucrose contents of SY and M2 were similar and tended to decrease throughout development. Dry matter contents of SY and M2 increased during development, while that of GJ remained constant. Changes in neutral invertase activities of SY and M2 were similar during development. On the other hand, changes in acid invertase activities were different between SY and M2: the acid invertase activity of SY was lower than that of M2 and continued to decrease throughout development. The activity of M2 was high at the initial stage, declined in stage 3, and increased again at the later stage of development.

DISCUSSION

Accumulation of hexoses during the storage of potato tubers has been studied extensively on a biochemical basis. In the study of cold-stored potatoes, Pressey (1969) reported that the acid invertase had a significant role in reducing sugar formation, although the reducing sugar content was not proportional to the invertase activity. Richardson et al. (1990) also reported that a distinct genotypic variation in the extent of hexose accumulation was not always reflected by that in acid invertase activity. Recently, the same research group reported that the differences between genotypes in the rate of hexose accumulation were related to acid inver-

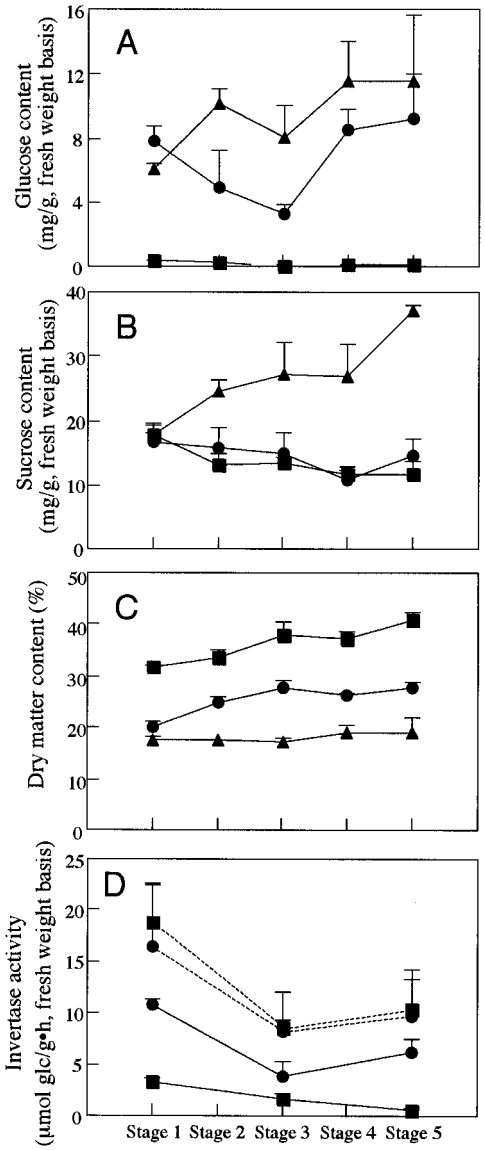

Figure 3. Changes in glucose (A), sucrose (B), and dry matter contents (C) and in acid and neutral invertase activities (D) during storage root development: M2 (●), SY (■), GJ (▲). In panel D, the dotted and solid lines represent the activities of neutral and acid invertase, respectively. Vertical bars represent SD of three replicates.

tase (Ross and Davies, 1992). However, in the case of potato tubers immediately after harvest, no biochemical knowledge concerning varietal differences in hexose content was obtained because varietal differences in hexose content were not significant.

In this paper, the influence of invertase activities on the varietal differences in free sugar contents of sweet potato storage roots was studied immediately after harvest using six cultivars with significantly different hexose contents. A highly significant correlation was observed between acid invertase activities and glucose contents among the roots, except for GJ (Figure 1). The correlation coefficient was 0.679 among all roots (n = 18), but it rose to 0.958 when GJ was excluded (n = 15). Between neutral invertase activities and glucose contents, a slightly significant but negative correlation was observed (Figure 2). The correlation coefficient was −0.574 among all roots (n = 18). When GJ was excluded, there was no significant relationship (r = −0.272, n = 15). Thus, acid invertase might have a significant role in hexose content but neutral invertase might not. Developmental studies support the significant role of acid invertase relative to hexose content (Figure 3). SY that contained a negligible amount of glucose throughout development showed a declining acid invertase activity during development. The hexose content in M2 changed concomitantly with the change in its acid invertase activity. The changes in neutral invertase activities in both cultivars were almost the same and had no relationship with hexose content. These results showed that the acid invertase activities were the primary determinant of the hexose content in raw sweet potato storage roots, except for GJ.

Neutral invertase was reported to be a cytosolic enzyme, while acid invertase was reported in the vacuole (apRees, 1988; Preiss, 1982). Free sugars in sweet potato are considered to be compartmented in the vacuole; thus, only acid invertase is deduced to affect the free sugar composition. For the same reason, neutral invertase does not affect free sugar content, but it is presumably involved in the metabolic flow from sugars to starch during root development.

In our previous paper (Takahata et al., 1992), the sweet potato cultivars were divided into three groups on the basis of free sugar composition in steamed roots. M2,T5, and GJ were classified into group 3, which was characterized by the high hexose content, whereas SS was assigned to group 3 or group 1, which was characterized by low hexose content, depending on the year. In this study, the differences among the cultivars with high hexose content were studied in more detail. GJ is considered to be different from M2 and T5 with respect to free sugar metabolism in raw storage roots, because Suc/Hex was higher and the sucrose content was far larger than those of the other two cultivars (Table 1). This difference agrees with the results of developmental studies, which indicated a large accumulation of sucrose and a constantly low level of dry matter during the development of GJ (Figure 3). The steady increase in sucrose content during the development of GJ indicates the decrease in the starch content. GJ might accumulate sucrose instead of starch and is considered to have a different biochemical basis compared to the others. However, the patterns of sugar−starch−enzyme relationships in sweet potato appear to be very complex.

GJ had a greater amount of hexose compared to its low acid invertase activity (Figure 1). The reason for this comparatively high content of hexose in GJ may depend on its high content of sucrose, a substrate for the hydrolysis of invertase. Because the glucose and fructose contents were approximately equal in GJ (Table 1), this implies an important role of acid invertase activity in regulating hexose concentration in GJ. Although the free sugar metabolism in GJ is still unknown, acid invertase activity in GJ affects the hexose content in the raw storage roots. On the other hand, the levels of hexose content and acid invertase activity in SS were intermediate and tended to be unstable. This variability might be the reason why SS was assigned to different groups depending on the year (Takahata et al., 1992).

In conclusion, although not all cultivars showed a strong relationship between hexose content and acid invertase activity, the acid invertase activity in sweet potato roots played an important role in the varietal differences in their hexose contents.

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LITERATURE CITED

apRees, T. Hexose phosphate metabolism by nonphotosynthetic tissues of higher plants. In *The Biochemistry of Plants. Vol. 14 Carbohydrates*; Preiss, J., Ed.; Academic Press: London, 1988; pp 1−33.

Copeland, L. Enzymes of sucrose metabolism. In *Methods in Plant Biochemistry. Vol. 3 Enzymes of Primary Metabolism*; Lea, P. J., Ed.; Academic Press: London, 1990; pp 73−85.

Hubbard, N. L.; Pharr, D. M.; Huber, S. C. Sucrose phosphate synthase and other sucrose metabolizing enzymes in fruits of various species. *Physiol. Plant.* **1991**, *82*, 191−196.

Picha, D. H. HPLC determination of sugars in raw and baked sweet potatoes. *J. Food Sci.* **1985**, *50*, 1189−1190, 1210.

Picha, D. H. Sugar content of baked sweet potatoes from different cultivars and lengths of storage. *J. Food Sci.* **1986**, *51*, 845−846, 848.

Preiss, J. Regulation of the biosynthesis and degradation of starch. *Annu. Rev. Plant Physiol.* **1982**, *33*, 431−454.

Pressey, R. Role of invertase in the accumulation of sugars in cold-stored potatoes. *Am. Potato J.* **1969**, *46*, 291−297.

Richardson, D. L.; Davies, H. V.; Ross, H. A.; Mackay, G. R. Invertase activity and its relation to hexose accumulation in potato tubers. *J. Exp. Bot.* **1990**, *41*, 95−99.

Ross, H. A.; Davies, H. V. Sucrose metabolism in tubers of potato (*Solanaum tuberosum* L.). *Plant Physiol.* **1992**, *98*, 287−293.

Shen, M. C.; Sterling, C. Changes in starch and other carbohydrates in baking *Ipomoea batatas*. *Starch/Staerke* **1981**, *33*, 261−268.

Stommel, J. R. Enzymic components of sucrose accumulation in the wild tomato species *Lycopersicon peruvianum*. *Plant Physiol.* **1992**, *99*, 324−328.

Takahata, Y.; Noda, T.; Nagata, T. Varietal diversity of free sugar composition in storage root of sweet potato. *Jpn. J. Breed.* **1992**, *42*, 515−521.

Takahata, Y.; Noda, T.; Nagata, T. Effect of β-amylase stability and starch gelatinization during heating on varietal differences in maltose content in sweet potatoes. *J. Agric. Food Chem.* **1994**, *42*, 2564−2569.

Truong, V. D.; Biermann, C. J.; Marlett, J. A. Simple sugars, oligosaccarides, and starch concentration in raw and cooked sweet potato. *J. Agric. Food Chem.* **1986**, *34*, 421−425.

Walter, W. M.; Purcell, A. E.; Nelson, A. M. Effects of amylolytic enzymes on "moistness" and carbohydrate changes of baked sweet potato cultivars. *J. Food Sci.* **1975**, *40*, 793−796.

Yelle, S.; Chetelat, R. T.; Dorais, M.; DeVerna, J. W.; Bennett, A. B. Sink metabolism in tomato fruit. IV. Genetic and biochemical analysis of sucrose accumulation. *Plant Physiol.* **1991**, *95*, 1026−1035.

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