Varietal Diversity of Free Sugar Composition in Storage Root of Sweet Potato

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Free sugar composition of the storage root of sweet potato cultivars and breeding lines was investigated by high performance liquid chromatography (HPLC) in 1989 and 1990. Based on the cluster analysis of the free sugar composition, the genotypes were classified into three groups. Among the 54 entries, 48 lines belonged to group 1 which was characterized by a relatively small amount of fructose and glucose, moderate amount of sucrose and large amount of maltose in steamed roots. In two breeding lines belonging to the group 2, Kyushu89 and Chugoku37, the maltose content was very low in the steamed roots, suggesting the lack of β -amylase activity in these cultivars. The other 3 lines in group 3 showed relatively high fructose and glucose contents. The genotypes in group 2 and group 3 are considered to be valuable materials for the breeding of sweet potato. The value of the correlation coefficient of the maltose content between the two years which was 0.835 (except for the low maltose genotypes), was close to that of the dry matter content. On the other hand, the correlation coefficient of the sucrose content was 0.649. It is concluded that the maltose content is as phenotypically stable as the dry matter content. However, the sucrose content is more easily affected by environmental conditions.

KEY WORDS: *Ipomoea batatas*, free sugar composition, HPLC analysis, quality, varietal diversity

Introduction

In Japan, sweet potatoes are mainly used for table consumption and starch production, and recently the amount for food processing has gradually increased. Eating quality of sweet potato is largely influenced by the sweetness associated with the presence of free sugars. Free sugar composition is also important in food processing. PICHÁ (1985) who developed isocratic HPLC techniques for the quantitative analysis of free sugars in raw and baked sweet potato studied a few cultivars (PICHÁ 1986). TRUONG *et al.* (1986) also analysed the free sugar composition of some cultivars by gas-chromatography (GC) and HPLC. BABA *et al.* (1987) reported that the reducing sugar contents of steamed sweet potatoes of 37 cultivars ranged widely from 0.6% to 13.7%. However, the above reports would not provide satisfactory information about the free sugar composition for the breeding of sweet potato to meet the increasing demand for table consumption and the new requirement for food processing.

In this paper, we report on the varietal diversity of the free sugar composition in the storage root of sweet potato cultivars and breeding lines. We also analysed the phenotypic stability of the sucrose and maltose content during a two year period.

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Materials and Methods

Samples

Fifty four sweet potato cultivars and breeding lines were grown without mulching in 1989 and 1990 in the same experimental field in Nishigoshi, Kumamoto (thick high-humic andosols), with different plot allocations. These materials consisted of 19 Japanese Norin cultivars, 19 breeding lines, 7 indigenous varieties, and 9 introduced varieties. The seed tubers were provided as virus free from the Laboratory of Sweetpotato Breeding, Kyushu National Agricultural Experiment Station. In 1989 the materials were transplanted on May 18 and harvested on October 18 and in 1990 they were transplanted on May 15 and harvested on October 16. They were blocked in 1 row $\times$ 10 hills with two replications in 1989, and without replication in 1990. Six medium-sized roots per plot were sampled, and then stored at room temperature until use. Three of them each were used for the raw and steamed samples, respectively.

In 1989, the following preparation procedures were carried out 10~14 days after harvest for the raw samples, and 19~23 days for the steamed ones, while in 1990, 2~4 days after harvest for the raw samples, and 6~10 days for the steamed ones.

Preparation of Raw Samples

Three roots each per plot were washed and longitudinally cut in half or quarter, and each piece was further cut laterally. Two symmetrical pieces were peeled and cut into small pieces for freeze-drying (Fig. 1). The freeze-dried materials were milled until they passed through a 500 μ m mesh sieve. The flour samples were stored at -30°C until use for analysis. Five hundred mg of the flour was extracted with 20 ml of an ethanol-water solution (80:20) by shaking at 40°C overnight. The supernatant was passed through Sep-Pak C₁₈ (Japan Millipore Ltd.) before HPLC analysis.

Fig. 1. Illustration of sampling method
Left: lateral view Right: top view
Areas with shading were sampled.

Fig. 2. Average free sugar composition of genotypes in each group. The groups were classified based on the cluster analysis of the free sugar composition in 1990.

Preparation of Steamed Samples

Each root was washed and steamed for 40~60 minutes depending on its size and cooled at room temperature. Three roots each per plot were longitudinally cut in half or quarter, and subsequently each piece was cut laterally. Two symmetrical pieces were peeled and cut into lateral slices (Fig. 1). The slices were pressed and rolled in a nylon bag to obtain a uniform paste. The paste samples were stored at -30°C until use for analysis. The subsequent procedures were the same as those for the 'raw samples' except for the sampling of the 1.5g paste.

HPLC Analysis

We modified the PICA's method to use a polyamine (pentaethylene-hexamine)-bonded hydrophilic polymar gel. A JASCO (Japanese Spectroscopic Co., Ltd.) 800 PU series equipment was used with a 250 mm $\times$ 4.6 mm i.d. column packed with NH2P-50 (Asahi Chemical Industry Co., Ltd., Japan) at 40°C . The mobile phase consisted of a degassed acetonitrile-water solution (70:30) at a flow rate of 0.75 ml/min. Samples were injected via a 20 μl sample loop. Identification of each sugar was based on HPLC retention times using authentic standards. Recovery test was carried out using the standard addition method with sucrose for the raw samples and with maltose for the steamed ones. The value was approximately 97% and the test was considered to be quantitative.

Results and Discussion

In cluster analysis by four free sugars contents of steamed roots of 54 entries in 1990, the first principal component contributed to the glucose and fructose content, and the second principal component to the maltose content. Based on the cluster analysis using first and second principal component, genotypes were classified into three groups. Analysis of the data in 1989 gave similar results, although Shiroseengan was found to belong to group 1 in 1990, and to group 3 in 1989. The average free sugar composition in each of the three groups is shown in Fig. 2, which illustrates the characteristics of the groups. Table 1 and 2 show the free sugar composition of the representative genotypes in each group. Raw roots contained a negligible amount of maltose, because raw starch which is not gelatinized is resistant to the hydrolyzation of β -amylase.

In the present study, 48 genotypes including all of the leading cultivars in Japan like Beniazuma, Kokei 14 and Koganeseengan were classified to group 1. The free sugar composition of this group was characterized by a relatively small amount of fructose and glucose, a moderate amount of sucrose and a large amount of maltose. It is interesting to note that both cultivars for table consumption (Beniazuma, Kokei 14, etc.) and cultivars for starch production (Koganeseengan, Shiroyutaka, etc.) exhibited a similar free sugar composition. The reason of the above would be: 1. the parental lines for starch production are preliminary same as those for table use which showed relatively small genetic diversity, and 2, cultivars bred in Japan have not been selected for free sugar contents.

The genotypes in group 2 were characterized by a very small amount of maltose which may be due to the lower or lack of β -amylase activity. The low content of reducing sugars is considered to be a suitable trait for cooking and/or processing associated with frying

Table 1. Dry matter content and free sugar composition of steamed and raw roots of sweetpotato genotypes in 1990

Genotype	Steamed root					Raw root			
	D.M ¹⁾	Fru ²⁾	Glc ²⁾	Suc ²⁾	Mal ²⁾	D.M ¹⁾	Fru ²⁾	Glc ²⁾	Suc ²⁾
Koganesengan	37.9	3.45	3.46	24.9	117.8	38.0	0.69	0.40	16.5
Beniazuma	38.4	1.57	1.70	24.0	119.6	38.6	0.96	0.42	17.3
Shiroyutaka	37.2	0.32	0.99	28.8	110.4	37.5	1.24	0.23	18.7
Benitome	39.5	1.16	2.61	30.1	131.4	38.3	1.02	0.16	18.2
Kokei14	39.8	3.21	3.97	19.1	107.4	38.9	0.84	0.64	15.0
Beniaka	38.0	1.16	0.60	29.9	106.2	35.6	0.32	0.19	14.7
Satsumahikari	38.6	4.19	3.48	21.0	1.8	35.8	2.57	1.74	17.0
Kyushu89	41.3	2.49	2.78	25.1	2.1	38.9	1.40	0.23	13.9
Chugoku37	41.3	3.35	3.91	16.8	2.3	35.2	2.86	2.15	13.7
Murasakibaru2	29.4	17.56	18.05	9.3	83.7	28.3	6.38	6.30	9.6
Tokai5	28.9	13.04	12.16	8.9	65.7	26.5	10.57	18.88	9.1
Georgia Jet	19.2	14.42	15.04	30.9	41.5	17.8	6.09	8.50	31.2

¹⁾ Dry matter content (%)

²⁾ Free sugar content (mg/g, Fresh weight basis)

Note: Each datum is a mean of three roots. HPLC injection was performed on three roots each.

Table 2. Dry matter content and free sugar composition of steamed and raw roots of sweetpotato genotypes in 1989

Genotype	Steamed root					Raw root			
	D.M ¹⁾	Fru ²⁾	Glc ²⁾	Suc ²⁾	Mal ²⁾	D.M ¹⁾	Fru ²⁾	Glc ²⁾	Suc ²⁾
Koganesengan	41.7	3.24	3.36	34.2	131.9	39.9	2.82	3.02	30.9
Beniazuma	43.7	2.47	2.66	38.7	149.1	41.6	1.87	2.13	26.3
Shiroyutaka	40.1	1.20	1.67	35.3	142.0	38.1	0.89	0.70	29.4
Benitome	43.5	2.55	3.82	38.8	156.6	42.0	2.21	2.81	36.0
Kokei14	43.5	6.56	6.46	24.4	145.8	39.9	6.18	6.99	17.0
Beniaka	37.5	1.97	2.90	33.1	98.4	34.4	0.77	0.83	26.6
Satsumahikari	36.5	8.56	8.89	26.0	4.9	36.9	7.54	8.35	22.7
Kyushu89	43.7	3.61	4.27	20.8	4.2	42.4	3.16	3.67	22.1
Chugoku37	41.8	7.55	7.39	18.9	3.2	39.6	8.80	9.12	17.8
Murasakibaru2	31.3	17.98	16.53	12.5	113.2	30.2	13.30	12.56	11.0
Tokai5	35.9	14.92	14.13	10.3	87.2	28.8	14.13	12.38	8.7
Georgia Jet	22.2	12.88	17.43	43.9	55.2	20.1	12.23	14.91	27.1

¹⁾ Dry matter content (%)

²⁾ Free sugar content (mg/g, Fresh weight basis)

Note: Each datum is a mean of two replications. HPLC injection was performed on two samples which were prepared from two replications each.

Fig. 3. Scatter diagram of maltose content in steamed roots between 1989 and 1990.

Fig. 4. Scatter diagram of dry matter content in steamed roots between 1989 and 1990

Fig. 5. Scatter diagram of sucrose content in steamed roots between 1989 and 1990.

because a larger amount of reducing sugars may give rise to more browning in the frying process. Satsumahikari registered in 1987 as a cultivar for food processing was characterized by a lower β -amylase activity (KUKIMURA *et al.* 1988). In the present study, two breeding lines with the same characters as those of Satsumahikari were developed. Especially, Kyushu89 with a white skin was characterized by a higher dry matter content and lower reducing sugar (fructose, glucose) content compared with Satsumahikari. Kyushu89 is considered to be a breeding material of sweet potato for food processing.

The genotypes in group 3 were characterized by relatively larger amounts of fructose and glucose and a lower dry matter content. Fructose is 1.15~1.75 times as sweet as sucrose, and so fructose is the sweetest sugar (SHALLENBERGER 1963). It is also reported that fructose becomes sweeter at low temperature (TSUZUKI and YAMAZAKI 1953). For the processing of sweet potato for frozen or chilled products, these genotypes are likely to be useful for the breeding of sweet potato varieties with a new taste.

Figure 3 shows the scatter diagram of the maltose content in steamed roots between 1989 and 1990. The free sugar content in 1989 was higher than that in 1990, presumably due to the longer duration of root storage period in 1989 compared to 1990. The correlation coefficient between 1989 and 1990 of the maltose content was 0.922, and even after low maltose genotypes belonging to group 2 were omitted, the value of the coefficient was 0.835. Fig. 4 and 5 show the scatter diagrams of the dry matter and sucrose contents. The correlation coefficient of the dry matter content between the two years was 0.895, and even after omitting cv. Georgia Jet which contains a very small

amount of dry matter among all the genotypes examined, the value of the coefficient was 0.844. On the other hand, the value of the correlation coefficient of the sucrose content was 0.649.

Sucrose is a major component in the carbohydrate metabolism of sweet potato and of most plants, and its metabolism is very complicated (PREISS 1982, Ho 1988). Accordingly, the sucrose content is easily affected by various environmental conditions. Maltose in the sweet potato root is produced only when the roots have been heated, and it is not an essential component for sweet potato growth. The formation of maltose in the sweet potato root is related to the activity of β -amylase and starch gelatinization (KIRIBUCHI *et al.* 1976) and the mechanism is apparently simpler than that of the formation of sucrose. This seems to make maltose contents phenotypically stable.

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カンショ 塊根中遊離糖組成の品種間変異

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カンショの遊離糖組成は、その食味に影響を与えるばかりでなく、油加工時に還元糖が製品褐変の原因になることなどから、加工時においても重要視されている。現在までに、遊離糖を還元糖あるいは全糖当量として調査した報告は数多くみられるが、各個の遊離糖を分別定量した報告は少なく、また、多くの品種・系統を対象とした分別定量の報告はなされていない。

本報では、遊離糖組成の品種(系統を含む)間変異と年次変動を明らかにするため、1989年、1990年の2か年にわたって同一の圃場で栽培した54品種・系統のカンショを供試し、生塊根・蒸煮塊根それぞれの遊離糖(フルクトース、グルコース、スクロース、蒸煮塊根の場合はさらにマルトース)を高速液体クロマトグラフィー(HPLC)を用いて調査した。

1990年の遊離糖データをもとにクラスター分析を行ったところ供試品種を3つのグループに分けることができた(Fig. 2, Table 1, 2)。グループ1の品種は一般的な糖組成すなわち少量のフルクトース・グルコース、中間量のスクロース、大量のマルトース(蒸煮塊根のみ)を含んでおり、48品種がこのグループに属した。コガネセンガン、ベニアズマなどの国内主要品種はすべてこのグループに含まれた。グループ2の品種は蒸煮塊根中のマルトース含量が極度に少なく、低糖系統として育成されたサツマヒカリ以外に新たに2系統(九州89号、中国37号)がこのグループに含まれた。特に、九州89号は既存低糖品種であるサツマヒカリに比べて乾物率が高く、還元糖含量が少なく、また皮色も白のため、今後の加工用低糖系統の改良のための育種素材として有用である。グループ3に属する3品種はフルクトース・グルコースの含量が他に比べて高く、乾物率は低かった。フルクトースはスクロースの1.15~1.75倍甘く、また低温になるほど甘味が増すことが知られているため、グループ3は、新しいタイプの食用あるいは加工用品種の作出など、今後の育種素材として興味ある特性を有するものと考えられた。

年次変動については、蒸煮塊根でみた場合、低糖品種を除いた両年次間のマルトース含量の相関係数が0.835、極端に乾物率の低かったGeorgia Jetを除いた乾物率のそれでは0.844となり、マルトース含量は乾物率と同程度の安定性を有する形質であった。一方、スクロースの相関係数は0.649で、上記の2形質ほど安定しておらず、環境条件の影響を受け易いことがわかった(Fig. 3, 4, 5)。