

## Review

# Carbohydrate components in sweetpotato storage roots: their diversities and genetic improvement

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Carbohydrates are important components in sweetpotatoes in terms of both their industrial use and eating quality. Although there has been a narrow range of diversity in the properties of sweetpotato starch, unique varieties and experimental lines with different starch traits have been produced recently both by conventional breeding and genetic engineering. The diversity in maltose content, free sugar composition and textural properties in sweetpotato cultivars is also important for their eating quality and processing of storage roots. In this review, we summarize the current status of research on and breeding for these important traits and discuss the future prospects for research in this area.

**Key Words:** sweetpotato, *Ipomoea batatas*, carbohydrate, starch, maltose, free sugar, texture.

## 1. Introduction

The sweetpotato (*Ipomoea batatas* (L.) Lam) is cultivated on about 8 million ha and about 110 million tons are harvested annually throughout the world (FAOSTAT 2015). Among ordinary crops, it ranked tenth in the world in 2013 in production in terms of both quantity and value. It is cultivated mainly in Asian and African countries, China in particular, where about 70% of worldwide production occurs (FAOSTAT 2015). In Japan, sweetpotato breeding has been carried out for more than 100 years, starting in 1914 when the method of cross-breeding for practical purposes was initiated (Kukimura *et al.* 1990). The storage root is used not only as a whole root for human and livestock consumption but also in manufactured products such as starch, spirits, and regional domestic products. Starch is a fundamental component in the storage roots of the sweetpotato and plays a significant role in various aspects of its utilization. The eating quality of the sweetpotato depends on its texture and sweetness, and the both of these qualities are largely based on the quantity and quality of carbohydrates (Walter *et al.* 1975, Walter 1987). In this review, we summarize the recent

progress and new findings in the research, including genetic engineering research, on the carbohydrate components in sweetpotato storage roots.

## 2. Physicochemical properties of recent sweetpotato starches in Japan

Starch is the most important carbohydrate component in the storage root of the sweetpotato. Of the many botanical starches, cereal starches such as those found in corn seeds generally have a smaller granular size, higher pasting temperature, and lower pasting viscosity. On the other hand, tuber starches such as those found in potato tubers grown underground generally have a larger granular size, lower pasting temperature, and higher pasting viscosity. The general properties of sweetpotato starch appear to take a middle position among the many starches (Suganuma *et al.* 1996). It appears that sweetpotatoes of the conventional varieties and early breeding varieties, as well as currently popular varieties in Japan, which were selected primarily based on their desirable agronomic characteristics, such as disease resistance and high and stable yield, have relatively similar starches in terms of their pasting properties, amylose content, phosphate content, and the chain-length distribution of the debranched starch (Kitahara *et al.* 1996a). However, during the course of exploring the properties of sweetpotato starch, a few unique starches exhibiting a low amylose

Communicated by Kenji Katayama

Received August 17, 2016. Accepted October 31, 2016.

First Published Online in J-STAGE on February 16, 2017.

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content (Kitahara *et al.* 1996b), a moderately low gelatinization temperature (Kitahara *et al.* 1999), a very low gelatinization temperature (Katayama *et al.* 2002, 2003, 2004), and a somewhat high amylose content (Katayama *et al.* 2011) were discovered in Japan. Subsequently, sweetpotato breeding that is focused on the properties of starch has developed new superior varieties and diverse lines as genetic resources. One result of these efforts is that a ground-breaking variety for the manufacture of starch, ‘Konamizuki’ (KM), has been bred at the Kyushu Okinawa Agricultural Research Center, National Agriculture and Food Research Organization of Japan (KARC/NARO, Katayama *et al.* 2012). This variety is expected to provide a new starch material for foods, as the gelatinized starch shows slow retrogradation and desirable gel-forming properties.

Because a comprehensive review of the physicochemical properties of sweetpotato starches has recently been published (Zhu and Wang 2014), the present chapter in this review will focus on the starch properties of recently bred sweetpotatoes in Japan, including the KM variety. Moreover, we introduce a variety of technical books and reviews in order to elucidate some of the fundamental background to the structures and physical properties of starch (*e.g.* Ai and Jane 2015, BeMiller 2007, Bertoft 2013, Hizukuri *et al.* 2006, Pérez and Bertoft 2010, Vamadevan and Bertoft 2015).

### 2-1. General properties of sweetpotato starches

**Table 1** summarizes the general properties of starches derived from recently developed sweetpotato varieties in Japan, including those of the leading cultivars, ‘Koganesengan’ and ‘Kokei 14’ (Kitahara *et al.* 2014). The pasting temperature determined using the Rapid Visco Analyzer (RVA) ranged from 58.4°C to 76.2°C, and the gelatinization peak temperature determined using a differential scanning

calorimeter ranged from 50.1°C to 74.2°C. The gelatinization enthalpy ranged from 12.9 J/g to 16.1 J/g; two starches with low gelatinization temperatures, which were derived from the ‘Quick Sweet’ and KM varieties, also showed low gelatinization enthalpy values. Arguably, the use of these starches for industrial applications would save energy, as they gelatinize at low temperatures with low enthalpy. Some structural properties of the starches are also shown in **Table 1**. The apparent amylose contents of the starches ranged from 18.6% to 20.7% for the ‘Koganesengan’ and ‘Okikogane’ varieties, respectively, showing a narrow range of variation and values typical of as standard sweetpotato starches (Zhu and Wang 2014). The diversity of the amylose contents of Japanese sweetpotato starches appears to be low (11.3–25.3%, Katayama *et al.* 2004, 2011, Kitahara *et al.* 1996b, Tokimura *et al.* 2002), although waxy starches have been found in root crops, such as potato (Hovenkamp-Hermelink *et al.* 1987), cassava (Ceballos *et al.* 2007), and yam (Pérez *et al.* 2011). Such waxy starches are expected to offer great potential for the food industry. In addition, the sweetpotato starches of the ‘Quick Sweet’ and KM varieties showed the common properties of a low phosphate content and B-type crystalline structure, as well as similar pasting and gelatinization properties. Thus, according to the pasting and gelatinization behaviors and structural properties, the sweetpotato starches in Japan were roughly divided into two groups: (1) starches with a low gelatinization temperature; and (2) the so-called standard sweetpotato starches. Recently, it was reported that the trait of having a low gelatinization temperature exhibited by sweetpotato starch is a qualitative characteristic controlled by a *spt* recessive allele and inherited in a hexasomic manner (Katayama *et al.* 2015). The sweetpotato starches with a low gelatinization temperature are unique to Japan at present.

**Table 1.** General properties of starches from recently bred sweetpotato cultivars in Japan

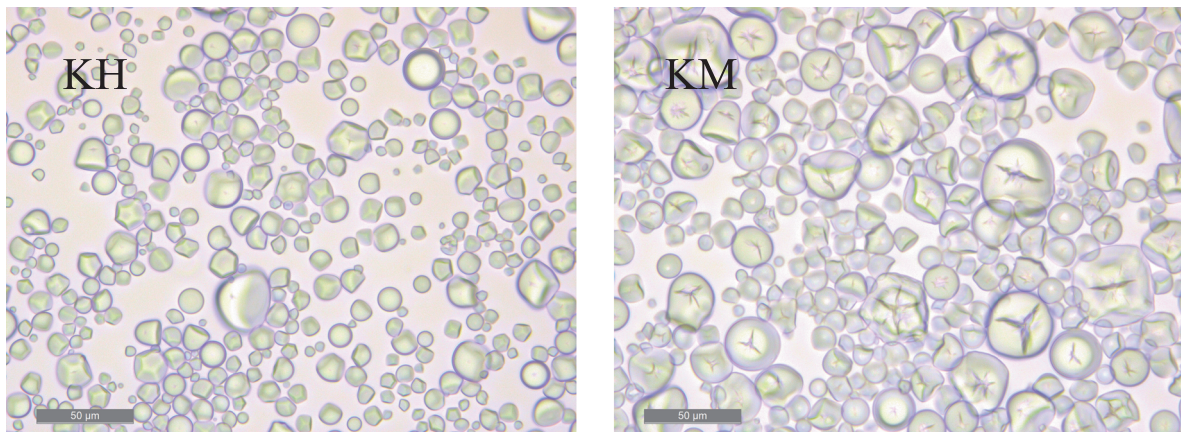
Main use (Trait)	RVA pasting temperature (°C)	DSC peak gelatinization temperature (°C)	DSC gelatinization enthalpy (J/g)	Apparent amylose content (%)	Attached phosphate content (μmol/g)	Median granular size (μm)	Crystalline type
Recently bred cultivars							
Konahomare	Starch production (High yield, High starch)	75.6	74.2	15.2	20.4	6.43	Ca
Daichinoyume	Starch production (High yield, High starch)	74.7	70.1	15.1	19.3	3.51	Ca
Okikogane	Food processing, Cooking (Low starch, Low sweetness)	74.9	73.2	14.8	20.7	5.59	Ca
Konamizuki	Starch production (High yield, High starch, Low gelatinization temperature)	58.4	52.2	13.1	19.9	1.27	B
Quick Sweet	Vegetable use (Low gelatinization temperature)	58.8	50.1	12.9	20	1.17	B
Traditional bred cultivars							
Koganesengan	Leading cultivar for shochu production (High yield, High starch, Good flavor)	76.2	71.7	16.1	18.6	5.63	Ca
Kokei 14	Leading cultivar for vegetable use (Good taste)	75.6	71.9	14.9	19.3	4.61	Ca

All measurements are conducted in accordance with the previous methods (Kitahara *et al.* 2014).

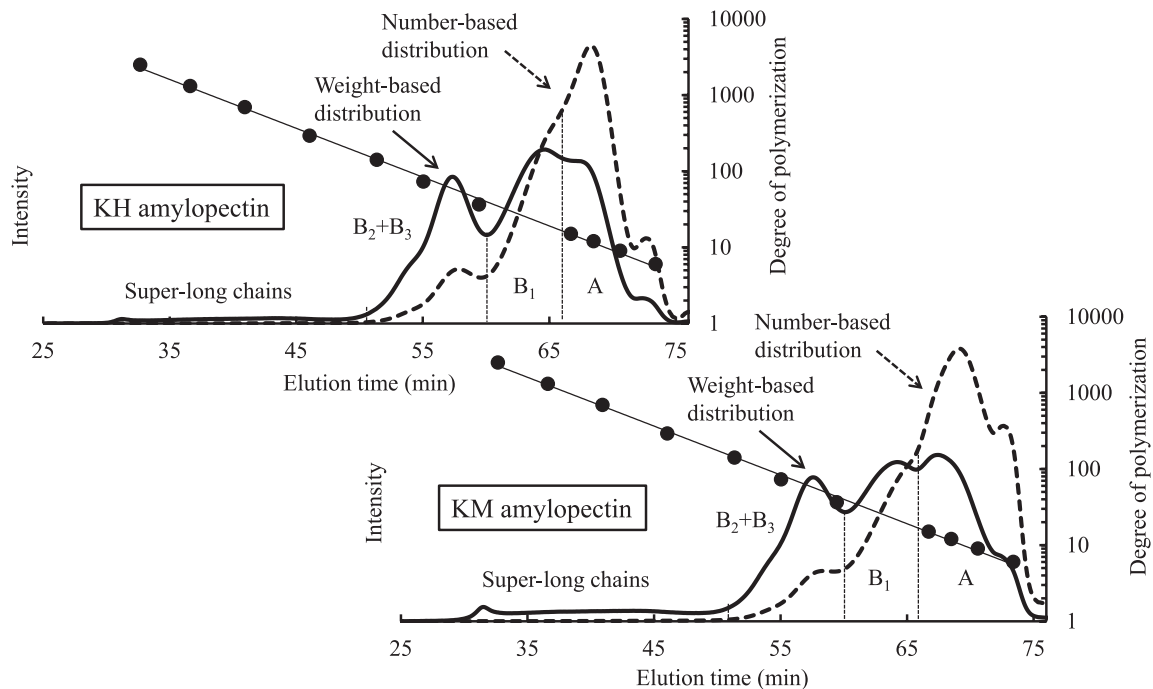
**2-2. Distinctive molecular structures of starch exhibiting a low gelatinization temperature**

In general, sweetpotato starch has a diameter of 2–50 μm and is bell-shaped, round, or polygonal shaped. **Fig. 1** shows the morphological differences between the two types of sweetpotato starches, namely the standard sweetpotato starch and the unique sweetpotato starch exhibiting a low gelatinization temperature. Many larger granules with cracks at the center were observed in the KM starch than in the ‘Konahomare’ (KH) starch. The median granular sizes were 17.6 μm and 13.6 μm for the unique and standard

starches, respectively (**Table 1**). **Fig. 2** shows the chain-length distributions of the isolated amylopectins from KH and KM starches on two linked columns of Superose 6 10/300 GL and Superdex Peptide 10/300 GL (GE Healthcare Japan). We devised a convenient and useful method to compare number-based chain-length distributions between starches (Kitahara *et al.* 2014). In simple terms, each output of the refractive index detector through the weight-based chain length distribution was divided by each degree of polymerization (DP) value obtained from a calibration curve with commercial Pullulan standards (Shodex Standard P-82



**Fig. 1.** Optical microphotographs of Konahomare (KH) and Konamizuki (KM) starch granules (Kitahara *et al.* 2014). Bars in the photographs represent 50 μm.



**Fig. 2.** Weight-based chain-length distributions determined by gel permeation chromatography with refractive index detection and their calculated number-based chain-length distributions of Konahomare (KH) and Konamizuki (KM) amylopectins (Kitahara *et al.* 2014). The solid line indicates the distribution of weight-based chain lengths, and the dotted line indicates the distribution of number-based chain lengths. Vertical dotted lines indicate the division of the distribution into unit-chain fractions of the cluster structure of amylopectin. Commercial pullulan standards (closed circle) with known DPs were used for the column system.

and P2) at the retention time, providing the number-based chain-length distribution. This calculation was performed using the Microsoft Excel software. Following Hanashiro *et al.* (2002), the calculated number-based chain length distribution was divided into three fractions, A, B<sub>1</sub>, and B<sub>2</sub> + B<sub>3</sub>, at inflection points. The molar amounts of the A, B<sub>1</sub>, and B<sub>2</sub> + B<sub>3</sub> fractions were estimated to be 61.7 %, 28.4 % and 9.7% for the KH amylopectin, and 69.4 %, 22.5 %, and 7.9% for the KM amylopectin, respectively. The periodic distribution of the KM amylopectin showed a clearer difference from that of the KH amylopectin than did their weight-based chain length distributions. Compared with KH amylopectin, KM amylopectin had more A chains, in particular, as well as twice the number of short chains with DP 6–10 and fewer B<sub>1</sub> chains; however, the numbers of B<sub>2</sub> + B<sub>3</sub> chains were comparable between the KH and KM amylopectins. Because maltooligosaccharides with DP 6–10 do not readily crystallize into a double-helical configuration (Gidley and Bulpin 1987, Pfannmüller 1987), the short chains with DP 6–10 in KM amylopectin are scarcely involved in the crystalline structure of starch granules, and their form can be imagined as many downy hairs in a cluster of amylopectin molecules. Such short chains with DP 6–10 act as an unstable factor for maintaining the starch granules, resulting in the ease of gelatinization of starch granules. On the other hand, the hydrated short chains after gelatinization act to resist crystallization, resulting in a high water-holding ability and slow retrogradation. The abundance of short chains with DP 6–10 in amylopectin molecules accounts for the reduction in starch synthase II activity observed in major crops (Edwards *et al.* 1999, Lloyd *et al.* 1999, Morell *et al.* 2003, Nakamura *et al.* 2005, Umemoto *et al.* 2002, Yamamori *et al.* 2000, Zhang *et al.* 2004) including the sweetpotato (Takahata *et al.* 2010). Furthermore, amylose-like super-long chains eluted at 30–50 min were observed in both amylopectins. It was clear that KM amylopectin had a larger amount of the super-long chains than KH amylopectin. The super-long chains of KM amylopectin are considered to play the role of crosslinking between each molecule in the starch paste, and are probably responsible for their strong gel-forming ability.

In this chapter, the structural properties of two types of Japanese sweetpotato starches were outlined. In particular, the KM variety has desirable agronomic characteristics and is expected to offer a highly functional starch that can offer energy-saving gelatinization, slow retrogradation, and high enzyme-digestibility, for use in food materials (Katayama *et al.* 2012, Kitahara *et al.* 2014) or as a feedstock for bioethanol production (Srichuwong *et al.* 2012).

### 3. Development of starch metabolic pathway engineering in sweetpotato

Genetic engineering of the amylose content has great potential to improve the quality of sweetpotato starch for the development of new dietary and industrial applications.

Metabolic engineering of the starch pathway by genetic transformation has progressed in crops such as rice, potatoes, and maize. In the sweetpotato, starch metabolic engineering has been advanced by a number of Japanese scientists (Kimura *et al.* 2001, Otani *et al.* 2007, Shimada *et al.* 2006, Takahata *et al.* 2010).

The starch biosynthesis pathways from adenosine diphosphoglucose (ADP-glucose) are catalyzed by multiple enzymes. The enzyme granule-bound starch synthase I (GBSSI) is the key enzyme in the biosynthesis of amylose, whereas the soluble starch synthases (SS), the starch branching enzymes (SBEs) I and II (SBEI and SBEII), and the debranching enzymes (DBE) act in concert to produce amylopectin. In the potato, antisense inhibition of *GBSSI* and *SBEII* resulted in amylose-free starch and a moderate increase in the apparent amylose level up to 38% (Jobling *et al.* 1999, Visser *et al.* 1991), respectively. We had been succeeded in producing an amylose-free sweetpotato with co-suppression of the *GBSSI* gene via the introduction of *GBSSI* cDNA into the sweetpotato genome (Kimura *et al.* 2001). However, the production frequency of the expected amylose-free transformed plants obtained using the co-suppression method was very low. Furthermore, the amylose-free character obtained using antisense-RNA or co-suppression was not stable in the vegetative propagated progenies of the potato and sweetpotato. Therefore, the production of starches that were amylose-free and exhibited other starch characteristics by new metabolic engineering had been expected in sweetpotato.

The double-stranded RNA (dsRNA)-mediated interference (RNAi) method has been found to be effective for the genetic improvement of crops (Waterhouse *et al.* 1998). The genetic manipulation of key enzymes related to the starch biosynthesis pathway using the RNAi method might produce altered starches such as high-amylose, very-high-amylose, and amylose-free starches in the sweetpotato. In the present chapter, we describe the modification of sweetpotato starch by using dsRNA of the sweetpotato *GBSSI*, *SBEI*, *SBEII*, *SSII*, *AGPa1*, and *AGPβ1A* genes.

#### 3-1. Transformation methods of sweetpotato

To produce knock-down mutants by RNAi it is essential to establish a genetic transformation system in the sweetpotato. To date, several works on establishing genetic transformation in the sweetpotato have been reported. An *Agrobacterium tumefaciens*-mediated transformation method targeting the embryogenic callus (EC) might be reasonable and reliable in the sweetpotato. The most important point in establishing a transformation system of the sweetpotato must be the induction of competent cells such as ECs which possess high regeneration potential and the trait of fast growth. Our routine methods for the induction of ECs and for transformation have already been reported (Otani and Shimada 1996, Otani *et al.* 1998). We currently use binary vector pZH2B (Hajdukiewicz *et al.* 1994, Kuroda *et al.* 2010) for the *Agrobacterium*-mediated transformation of

**Table 2.** Characteristics of transgenic plants transformed with dsRNA of starch biosynthesis genes of the sweetpotato

Plant lines	Genes	Growth of aerial part	Total fresh weight of storage root per plant <sup>a</sup>	Sprouting trait of storage roots	Starch yield <sup>a</sup>	Amylose content (%) <sup>b</sup>	Reference
Kokei 14	–	Normal	100.0	–	100.0	10.25 ± 0.4	–
Kokei 14/GBSSI	<i>wx</i>	Normal	96.8	late	88.3	0	Otani <i>et al.</i> 2007
Kokei 14/SBEII	<i>ae</i>	Normal	92.1	late	89.7	24.85 ± 0.6	Shimada <i>et al.</i> 2006
White Star	–	Normal	100.0	–	100.0	nd	–
White Star/SSII	<i>dul</i>	Normal	87.1	nd	98.0	nd	Takahata <i>et al.</i> 2010

<sup>a</sup> Data are expressed as relative ratios compared to non-transgenic Kokei 14 or White Star as 100%.

<sup>b</sup> Average ± S.D. (n = 3–5).

nd: not determined.

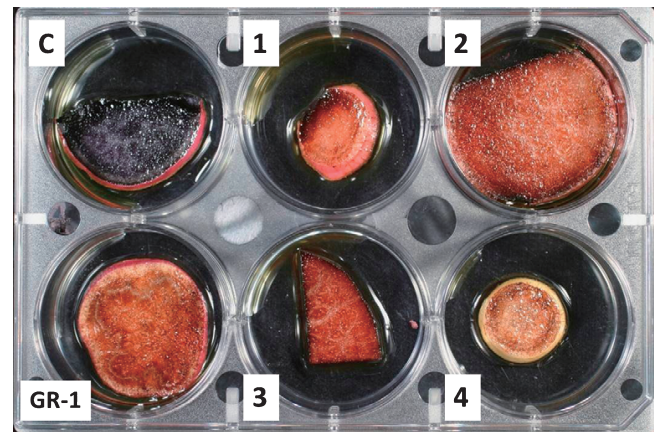
the sweetpotato because of the ease of cloning and because of its higher transformation efficiency (unpublished data).

### 3-2. Genetic manipulation of starch properties in the sweetpotato

**3-2-1. GBSSI.** The GBSSI, which catalyzes the formation of amylose, is one of the key enzymes in the biosynthesis of starch. Amylose-free or low-amylose starch was produced by the antisense GBSSI RNA method in potatoes (Kuipers *et al.* 1994a, 1994b, Visser *et al.* 1991) and rice (Shimada *et al.* 1993). However, the amylose-free character of potatoes achieved by antisense RNA-mediated inhibition was not completely stable, and the efficiency of the production of amylose-free plants was low (Kuipers *et al.* 1994a, 1994b). On the other hand, several transgenic plants with the dsRNA of *IbGBSSI* were obtained by *Agrobacterium*-mediated transformation at a high frequency. No morphological differences in whole plants were observed between non-transgenic ‘Kokei 14’ and transgenic plants with the dsRNA of *IbGBSSI* (Table 2). When storage root slices of the transgenic plants with the dsRNA of *IbGBSSI* and non-transgenic ‘Kokei 14’ plants were stained by an iodine solution, 28 of 38 transgenic lines showed a red-brown stain, while the other 10 lines showed a dark blue color similar to non-transgenic roots (Fig. 3). Consequently, 73.7% of these transgenic plants contained amylose-free starch. The amylose-free character of transgenic plants with the dsRNA of *IbGBSSI* was stable in transgenic sweetpotato plants through both vegetative propagation and sexual crossing (Fig. 3).

**3-2-2. SBE.** The SBE, 1,4- $\alpha$ -D-glucan-6- $\alpha$ -[1,4-glucan]-transferase, is a key enzyme in starch biosynthesis. It acts on glucose polymers and catalyzes the excision and transfer of glucan chains to either similar or different glucan molecules (Hussain *et al.* 2003, Pan and Nelson 1984). The SBEs can be divided into two classes, SBEI and SBEII classes, with distinct structures and enzymatic properties (Martin and Smith 1995). SBEs of SBEI class prefer amylose as a substrate and transfers relatively longer glucan chains (up to DP 30, with the majority being DP 10–13) (Kuriki *et al.* 1997, Takeda *et al.* 1993). On the other hand, SBEs of the SBEII class prefer amylopectin as a substrate and transfer relatively shorter chains (DP 6–14) (Guan and Preiss 1993).

High amylose starches are more hard to be decomposed by  $\alpha$ -amylase (EC 3.2.1.1) than normal starches, resulting in



**Fig. 3.** Inheritance of the amylose-free character of storage roots of transgenic sweetpotatoes by sexual crossing (Otani *et al.* 2007). C: non-transgenic Kokei 14’ plant, GR-1: transgenic amylose-free plant line with dsRNA of *IbGBSSI*. 1–4: progenies of GR-1 crossed with cv. ‘Chikei 682-11’.

low digestibility (Tetlow 2011). High amylose starches showed several functional effects for health benefits such as lower postprandial glucose response (Nestel *et al.* 1996) and reduced energy due to its low caloric value (Brown 2004). Therefore, foods containing high amylose starches are beneficial for the human health. Transgenic potato and wheat with high-amylose levels were produced by antisense RNAs and RNAi for *SBE* genes, respectively (Regina *et al.* 2006, Schwall *et al.* 2000). To produce high-amylose sweetpotatoes, dsRNAs of *IbSBEII* was expressed in transgenic sweetpotato plants (Shimada *et al.* 2006). The gene expression levels of endogenous *IbSBEII* were decreased in the storage roots of the transgenic plants. In the *IbSBEII* dsRNA transgenic plants, the amylose content in storage roots increased to 242.4%, when compared with the control plant (Table 2). On the other hand, starch yield in the storage root of *IbSBEII* dsRNA transgenic plant was decreased slightly (89.7%) when compared with the control plant (Table 2). Morphological differences in the aerial part and storage roots of the *IbSBEII* dsRNA transgenic plants were small (Table 2). Harvested storage roots showed very slow sprouting compared to those of non-transgenic ‘Kokei 14’, which was similar to the storage roots of transgenic plants with the dsRNA of *IbGBSSI*. Production and characterization of

transgenic sweetpotatoes in which dsRNAs of *IbSBEI* and *IbSBEs* (*IbSBEI* + *II*) are expressed is now in progress.

**3-2-3. SSII.** The activity of isoform II of SS (*SSII*) was considered to be a critical factor in the changes to the structure of starch granules and the low-temperature pasting property. Thus, the production of transgenic sweetpotato plants having low-pasting-temperature starch was believed to be caused by the dsRNA of *IbSSII*-mediated gene silencing.

Several transgenic plants with the dsRNA of *IbSSII* were obtained by *Agrobacterium*-mediated transformation (Takahata *et al.* 2010). No morphological differences in the aerial part or the storage roots were observed between non-transgenic ‘White Star’ and transgenic plants, although total fresh weight of the storage roots had been decreased slightly (Table 2). All of the starches from transgenic plants with the dsRNA of *IbSSII* showed lower pasting temperatures and breakdown. The pasting temperatures in transgenic plants were approximately 10–15°C lower than in non-transgenic ‘White Star’ plants. Further physicochemical properties of transgenic plants were examined and it was found that the starches of transgenic plants showed slower retrogradation and higher digestibility (Kitahara *et al.* 2011). Those properties of starches from transgenic plants with the dsRNA of *IbSSII* should lead to benefits not only in the table use of these plants but also in food industry applications and bio-fuel production. Thus, the knock-down trait of *IbSSII* should be combined with other starch biosynthesis-related genes to produce unique and useful starches.

**3-2-4. AGPase.** ADP-glucose pyrophosphorylase, EC 2.7.7.27 (AGPase), is an enzyme that catalyzes the reaction in which ADP-glucose and pyrophosphate are made from glucose-1-phosphate and ATP. ADP-glucose is the only or most important precursor for plant starch synthesis, and AGPase is thought to be the key enzyme in this biosynthetic pathway (Martin and Smith 1995, Preiss 1996). Most plant AGPases are tetramers consisting of two  $\alpha$ -subunits and two  $\beta$ -subunits (Preiss 1996). The genes of these subunits are referred to as *Brittle2* and *Shrunken2*, respectively, and recessive mutants of these genes contain more sugars than wild *Zea mays* strains (Hannah and Nelson 1975, 1976). Production and characterization of transgenic sweetpotatoes in which dsRNAs of *IbAGPa1* (*IbAGPS*) and *IbAGP $\beta$ 1A* (*IbAGPL*) are expressed are now in progress

**3-2-5. ISA.** Isoamylases (ISAs) are DBEs that cut the  $\alpha$ -1,6-glycosidic bond in starch (Nakamura 1996). These enzymes are thought to contribute to the trimming of starch. Three isoforms are known to occur in plants. The gene of ISAI, one of these isoforms, is called *Sugary1*, and the recessive mutant of this gene contains more sugars than wild *Z. mays* strains. Several transgenic plants with the dsRNA of *IbISAI*, *IbISAI* or *IbISAI* were regenerated, and the investigation of the morphological and physicochemical properties of these transgenic plants is in progress.

#### 4. Starch, free sugars and other carbohydrates associated with sweetness and texture of cooked sweetpotato cultivars

Sweetpotatoes are used as food, forage, and ingredient in processed food and industrial products, in which they are often included in the form of starch. In Japan, approximately half of all sweetpotatoes produced, about 0.45 million tons, are sold in fresh markets; the eating quality of sweetpotato as well as its physiological functions such as antioxidant activity have attracted a great deal of attention. The eating quality of sweetpotato practically depends on its texture and sweetness, and both of these qualities are largely dependent on the quantity and quality of carbohydrates contained in the sweetpotato (Walter *et al.* 1975, Walter 1987). In this chapter, the starch and free sugar characteristics associated with the sweetness and texture of steamed storage roots of sweetpotato cultivars are described.

##### 4-1. Diversity in maltose content is associated with the sweetness of cooked sweetpotato cultivars

The free sugars that predominantly existed in the storage roots of sweetpotatoes are fructose, glucose, sucrose, and maltose (Picha 1986a). Among these, maltose is hardly detected in raw fresh storage roots, and the other three sugars are presented in both raw and cooked storage roots. Takahata *et al.* (1992) classified the genotypes of sweetpotato cultivars and breeding lines into three groups based on the free sugar composition of their steamed storage roots. Group 1 consisted of cultivars and lines that contain a large amount of maltose in their steamed roots. Group 2 consisted of species that generate a small amount of maltose via steaming. The breeding lines that contain relatively large amounts of fructose and glucose were classified as group 3. The cultivars and lines belonging to group 1 show high  $\beta$ -amylase activity, while those belonging to group 2 are completely or mostly lacking this enzyme activity.

The storage roots of current Japanese sweetpotato cultivars suitable for table use contain 10 to 25% (w/w) starch, which is the largest constituent of the solid component of sweetpotato storage roots. In the cooking process, starch is gelatinized and then partially dissolved by two kinds of amylolytic enzymes, *i.e.*  $\alpha$ - and  $\beta$ -amylase, to generate a large amount of maltose (Morrison *et al.* 1993). Maltose is the sugar highest in content among the four free sugars, namely fructose, glucose, sucrose, and maltose, that are mainly detected in cooked storage roots of ordinary sweetpotato cultivars. Although maltose has less than half of the sweetness of the other three sugars, it is mainly responsible for the sweetness of the cooked storage root of ordinary sweetpotato cultivars, which in this context refers to cultivars having  $\beta$ -amylase activity, because of its 10-times-higher concentration of maltose in comparison to the other sugars. The maltose concentration in steamed storage roots increased to a peak of approximately 10% (w/w), with the enzymic activity increasing up to about 0.2 mmol maltose/mg protein

of enzyme solution/min, and however, the maltose concentration did not increase with increasing activity if it increased to a level above 0.2 mmol maltose/mg/min (Nakamura *et al.* 2014b). This result suggests that the activity of  $\beta$ -amylase in storage root is not the only definitive factor in the generation of maltose by cooking with heat in the sweetpotato.

One of the other limiting factors in maltose generation would be starch gelatinization. Starch gelatinization is a prerequisite for maltose generation by  $\beta$ -amylase because the enzyme of the sweetpotato is not able to attack raw (not gelatinized) starch granules (Ito *et al.* 1968). Starch gelatinization properties are characterized by various physicochemical phenomena such as thermal reaction, structural transition, and temperature-dependent changes in the viscosity of the starch suspension (Biliaderis 2009). The starch pasting temperature, the temperature at which the increase in viscosity of starch suspension is recognized for the first time during a temperature increase, is an index of the facility of starch gelatinization. The pasting or gelatinization temperature of isolated starch from sweetpotato storage roots is closely correlated with the distribution ratio of the chain-length of branch glucose in amylopectin molecules (Noda *et al.* 1998). The increase in the distribution ratio of the shorter chain (DP 6–10) in sweetpotato amylopectin caused a distinct reduction of the gelatinization and pasting temperature of starch determined by differential scanning calorimetry (DSC) and RVA, respectively. It is also well known that the temperature during the growth period of the sweetpotato greatly affects the amylopectin structure and the resulting gelatinization or pasting properties of sweetpotato starch (Noda *et al.* 2001). The pasting temperature measured by RVA using a 7% (w/w) suspension of isolated starch ranges from 50°C to 75°C for sweetpotato cultivars and breeding lines (Katayama *et al.* 2015). The cultivars and breeding lines with lower pasting temperatures are able to generate a larger amount of maltose than those with higher pasting temperatures if the cultivars and lines are similar in terms of  $\beta$ -amylase activity. The maltose concentration in the steamed storage roots of sweetpotato cultivars with  $\beta$ -amylase activity was negatively correlated with pasting temperature as measured by RVA using isolated starch (Nakamura *et al.* 2014b). ‘Quick Sweet’, developed at the beginning of 21<sup>st</sup> century, was the first sweetpotato cultivar having unique starch gelatinization characteristics: its pasting temperature was around 55°C, which was approximately 20°C lower than those of other ordinary cultivars (Katayama *et al.* 2002, 2003, 2004). Consequently, this cultivar was able to begin maltose generation at about a 20°C lower temperature than ‘Beniazuma’, which contains normal starch and whose pasting temperature is ca. 75°C. Also, the maltose concentration in steamed storage roots of ‘Quick Sweet’ was about 40% higher than that in ‘Beniazuma’ (Nakamura *et al.* 2014a). The deterioration of the starch pasting temperature is associated with the inhibition of the expression of the *starch synthase II* gene (Takahata *et al.* 2010) and is qualitatively

regulated by the dosage of recessive alleles (Katayama *et al.* 2015). The maltose generation in ‘Quick Sweet’ would continue at temperatures over 90°C because its  $\beta$ -amylase activity maintains a sufficient level despite the serious inhibition of this enzyme activity in ‘Beniazuma’. Takahata *et al.* (1994) reported that the heat stability of  $\beta$ -amylase and the gelatinization of starch granules play an important role in high maltose generation. The development of new cultivars having low starch pasting temperatures such as ‘Quick Sweet’ continues to progress because they are effective for increasing the processability and functionality of the sweetpotato or its starch (Katayama *et al.* 2012, Kuranouchi *et al.* 2012).

As for the group 2, two cultivars, ‘Satumahikari’ and ‘Okikogane’ were developed in the past 30 years (Baba *et al.* 1987). They do not produce practical amount of maltose as a result of heating due to a complete lack of or to having only traces of  $\beta$ -amylase activity in their storage roots. The trait of an absence of or an extremely low level of  $\beta$ -amylase activity is believed to be inherited easily because this genetic mutation is brought on by only a single recessive allele in their germplasm (Kumagai *et al.* 1990). Such types of genetic variation will raise the potential of the sweetpotato as a crop for food and other uses.

#### 4-2. Other free sugars

The other three sugars, namely fructose, glucose, and sucrose, are contained in both raw and cooked storage roots. The contents of these sugars do not show any significant changes after cooking; however, they show remarkable changes in raw storage roots during storage. Generally speaking, the sucrose content in raw storage roots of sweetpotatoes gradually increases together with a decrease in the starch content during storage (Picha 1986b), and lowering the storage temperature stimulates these changes (Picha 1987). The changes in the activity of sucrose-metabolizing enzymes such as sucrose synthase and sucrose phosphate synthase during storage have been extensively reported for potatoes, but few reports have been published on this topic in sweetpotato (Masuda *et al.* 2007, Takahata *et al.* 1995). The contents of fructose and glucose are highly correlated with the activity of acid invertase, which catalyze the conversion of sucrose into fructose and glucose (Takahata *et al.* 1996). Although the two sugars are known to have a close relationship to the quality of potatoes after storage (Matsuura-Endo *et al.* 2004, 2006), their role in the eating quality of sweetpotatoes remains to be investigated.

#### 4-3. Textural characteristics

Another important factor in determining the eating quality of baked or steamed sweetpotato storage roots is their textural character, which consists of two major types, *i.e.* mealy and soggy. In the current breeding system in Japan, the textural character of a cultivar or breeding line is sensorially determined after cooking by comparison with the textural character of the standard cultivar (‘Kokei-14’) and

the character is sorted into five categories: mealy, slightly mealy, intermediate, slightly soggy, and soggy. “Intermediate” means that the texture is almost equal to the texture of the standard cultivar. This sensory evaluation system has some problems; for example, the texture of the standard cultivar often differs between harvest locations or from year to year, and the tasting peculiarity of the panel members is liable to influence the evaluation results. Therefore, the relationships between the textural character of the sweetpotato and the physicochemical properties of its constituents need to be determined to establish an invariant evaluation system of the texture of the sweetpotato. The most primary constituent associated with the texture of sweetpotato storage roots is starch. The concentration of starch presented in raw fresh storage roots is generally well correlated to the texture of the cooked root; a higher starch concentration tends to result in a mealy texture (Nara 1957). It can be assumed that the concentration of starch remaining in the root after cooking must be very closely related to the texture. Since the maltose generation during heat cooking with heat results from starch degradation by amylolytic enzymes, it will significantly decrease the starch content and consequently induce the textural changes in the cooked storage root. However, there is little information about starch degradation regarding the texture of sweetpotatoes (Nara 1951, Walter *et al.* 1975). The starch degradation that occurs in storage roots during the storage period presumably induces textural changes in the cooked roots; however, there is also little information about the enzymic mechanism of starch degradation in the sweetpotato during the storage period (Takahata *et al.* 1995). Further investigation of the relationships between starch metabolism and the texture of steamed or baked sweetpotatoes in relation to storability is needed.

The amylose content will also influence the texture, although this content in the sweetpotato is lower than those of other roots and tuberous crops and has less variability compared with rice and wheat. The alteration of amylose content by conventional breeding techniques (Kitahara *et al.* 1996b) or by genetic transformation (Kimura *et al.* 2001) induced variations into the eating quality and the processability of sweetpotato storage roots.

The organoleptic characteristics such as the texture of cooked sweetpotatoes are also affected by other physical properties of the storage root tissues and cells. Relationships between the degree of disintegration of steamed tissues and the starch content in fresh raw tissues are recognized in sweetpotato storage roots (Nakamura *et al.* 2010, 2015) as well as in potatoes (Matsuura-Endo *et al.* 2002). The contents of pectic substances and concomitant Ca ions are also correlating factors of the degree of storage root tissue disintegration. Pectic substances, particularly chelating agent-soluble ones, play important roles together with divalent cations in cell adhesion in plant tissue (Grant *et al.* 1973). The treatment of storage root tissues of sweetpotatoes with chelating agents decreases their firmness in association with histological changes in the microstructure of the cell wall

(Fuchigami *et al.* 2002), and divalent cations are reported to have a preventive effect against the decrease in the firmness of potatoes (Ross *et al.* 2011). As for sweetpotatoes, the disintegration rate of steamed storage root tissues is correlated negatively with the contents per fresh weight of the chelating agent-soluble pectic fraction and calcium ions, which are divalent cations (Nakamura *et al.* 2015). Although other cell wall polysaccharides such as cellulose and hemicellulose in sweetpotato storage roots have been analyzed in terms of their chemical composition (Noda *et al.* 1994), little information about their functions in the texture of cooked sweetpotato roots is available (Shen and Sterling 1981) and further investigations are still needed.

## 5. Conclusion and future

There is no doubt that the sweetpotato is an important crop not only as a staple food, but also as a supplier of starch raw materials. However, the lack of variations in its starch traits has prevented the demand for sweetpotatoes from expanding. Because of its high ploidy level, self-sterility, and cross-incompatibility, it has been difficult to find useful mutants of sweetpotatoes. However, a ground-breaking variety for use in the manufacture of starch, Konamizuki (KM), was bred recently, and this variety has begun to spur new demand among food processing manufacturers. Moreover, progress in tissue culture and in the genetic transformation of the sweetpotato enables the production of various mutants by RNAi-mediated gene silencing. To date, several mutants related to starch pathways in the sweetpotato were produced artificially by RNAi methods as described in this review. Now, it has become possible to provide a wide range of amylose contents (0% (GBSSI-RNAi), 10–20% (non-transgenic), 26–29% (SBEII-RNAi)) in sweetpotato starch.

Recently the importance of sweetness has risen to meet the needs of consumers in Japan. The varietal diversity in maltose production during the heating of sweetpotato storage roots has been well clarified over the past several decades. However, information on other free sugars, such as sucrose, fructose, and glucose, and on textural properties is still insufficient, as is our understanding of the changes in these components, including maltose, during the storage of sweetpotatoes. Further studies involving food-processing chemists and post-harvest physiologists are also needed.

The sweetpotato has not lent itself well to molecular breeding; however, the establishment of genetic transformation and the RNAi-mediated gene silencing method in this plant species has led to the creation of several useful mutants, and has made it comparable to other crops such as potatoes, rice, and maize. In addition, the draft genome sequence of *I. trifida*, the closest wild relative of the sweetpotato, has been reported (Hirakawa *et al.* 2015). We hope that several novel unique starches will be produced through marker-assisted and/or transgenic techniques in sweetpotato plants in the near future. Also, our research continues and we expect to further develop and discover novel sweetpotatoes



with starches exhibiting distinct properties or sugars associated with attractive traits, thereby contributing to the promotion of sweetpotato utilization.

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