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Note

Inheritance of low pasting temperature in sweetpotato starch and the dosage effect of wild-type alleles

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Sweetpotato (*Ipomoea batatas* (L.) Lam.), which is an outcrossing hexaploid, is one of the most important starch-producing crops in the world. During the last decade, new sweetpotato cultivars, e.g. 'Quick Sweet', which have approximately 20°C lower pasting temperature, slower retrogradation and higher digestibility of raw starch than ordinary cultivars, have been developed in Japan. Genetic analysis of these variants with low pasting temperature starch was conducted in this study. Using 8 variants and 15 normal clones, 26 families were generated. The results from analyzing these progenies suggested that this trait is a qualitative character controlled by one recessive allele (designated *spt*), which is inherited in a hexasomic manner. A dosage effect of the wild-type *Spt* allele was found for starch pasting temperature, although the effect was not linear. These results will aid breeders to develop sweetpotato cultivars with a range of starch pasting temperatures.

Key Words: sweetpotato, inheritance, starch, pasting temperature, polyploid, gene-dosage effect.

Introduction

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is one of the most important starch-producing crops in the tropics and warm-temperate regions of the world. This crop is used as a raw material for alcohol fermentation and the production of sugar syrup and glucose in East Asia (Woolf 1992). Recently, research has focused on the potential of sweetpotato as both a food material and as a renewable resource for bioethanol production. The physicochemical properties of starch strongly influence the quality of food processing materials and industrial products. In particular, the gelatinization temperature of starch influences the conversion rate of starch to sugar in the first step of ethanol production (Srichuwong *et al.* 2012).

During the last decade, new sweetpotato cultivars with novel starch properties, e.g. 'Quick Sweet' and 'Konamizuki', have been developed in Japan (Katayama *et al.* 2003, 2012). The starches of these cultivars have cracked granule shapes, approximately 20°C lower pasting temperature, slower retrogradation and higher digestibility of raw starch compared with ordinary cultivars (Katayama *et al.* 2004, 2011, Kitahara *et al.* 2005). In addition, these starches were qualitatively different from those of ordinary cultivars in terms of gelatinization temperature and chain-length distribution of amylopectin molecules (Katayama *et al.* 2002, Kitahara *et al.* 2005). Storage roots of 'Quick Sweet' can be cooked quickly and taste good even when cooked rapidly in a microwave oven, because of its low pasting temperature. These cultivars and their starches are expected to be suitable for foodstuffs such as gelatinized cakes and pastries. In addition, low temperature-gelatinizing starches give a definite advantage over ordinary cultivars in terms of energy-saving during liquefaction for bioethanol production (Srichuwong *et al.* 2012).

Changes in the structure of amylopectin molecules that lead to low pasting temperature of starch have been reported in other plants such as pea (Craig *et al.* 1998), potato (Edwards *et al.* 1999, Lloyd *et al.* 1999), rice (Umemoto *et al.* 2004) and maize (Zhang *et al.* 2004). These results and previous studies of transgenic sweetpotato (Kitahara *et al.* 2011, Takahata *et al.* 2010) suggested that the lack of or inhibition of starch synthase II was responsible for changes in the structure of amylopectin and the low pasting temperature of starch.

Sweetpotato is an autohexaploid (2n = 6x = 90) (Shiotani and Kawase 1989) and an outcrossing vegetative propagation crop. The highly heterozygous, outcrossing polyploidy in

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sweetpotato complicates its genetic analysis. Most varieties of sweetpotato show self- and cross-incompatibility, low natural flowering ability and low seed fertility (Fujise 1964). Therefore, sweetpotato breeders have to utilize painstaking breeding processes such as a grafting method for flower induction and identification of incompatibility groups before crossing. In addition, the accumulation of genome sequence information for sweetpotato has been slower than in selfing diploid crops (Hirakawa *et al.* 2015, Monden *et al.* 2015). This study was conducted to elucidate the inheritance mode of variants with low pasting temperature in order to improve starch properties in sweetpotato. Genetic analysis of the variants with low pasting temperature was conducted from 26 test crosses using variants and normal clones. We also investigated the relationship between starch pasting temperature and the number of wild type alleles in crossing parents.

Materials and Methods

We used 'Quick Sweet' and breeding lines with low pasting temperature and cultivars or lines with normal pasting temperature for crossing (**Supplemental Table 1**). Using 8 variants and 15 normal clones, 26 families were generated (**Table 1**). The upper 7 families (No. 1–7) represented crosses between the variant parents ('Quick Sweet' and its progeny) or were generated by selfing the variant. The next 16 families (No. 8–20, 24–26) were produced by crosses between variants and normal clones. The remaining three families (No. 21–23) were generated by crossing between normal clones. The crosses were made by hand pollination in the greenhouse at NARO Kyushu Okinawa Agricultural Research Center, during 1998, 2002, 2003, 2005, 2006 and 2012. In total, 3,439 seeds from 26 families were collected and sown in a seedbed, and seedlings were transplanted to the fields of NARO Institute of Crop Science in 1999 and 2014 or NARO Kyushu Okinawa Agricultural Research Center between 2003 and 2008. Two to four storage roots per plant were harvested for starch extraction. Starch extraction from storage roots was conducted in accordance with a previously reported method (Katayama *et al.* 2004). Starch pasting temperature was investigated using a Rapid Visco-Analyzer (RVA-3D) with 7% starch suspension (Katayama *et al.* 2004).

Previous cytological and genetic studies indicated that sweetpotato is an autohexaploid (Monden *et al.* 2015, Shiotani and Kawase 1989), and its inheritance mode is hexasomic (Kumagai *et al.* 1990). From results of the crossing test, we assumed that low pasting temperature of starch is controlled by a single recessive mutant allele, *spt*. The genotypes of low pasting temperature variants were *spt spt spt spt spt spt* (*ssssss* for short) and the genotypes of normal clones were *Ssssss*, *SSssss*, *SSSsss*, *SSSSss*, *SSSSSs* or *SSSSSS*. The gametic ratios expected for each of these

Table 1. Segregation of variants with low pasting temperature in F_1 progenies and expected genotypes of their parents

Cross combination			Pasting temperature of parents (°C)[a]		No. of F_1 plants		Expected ratio	χ^2	P	Genotype of parents	
No.	Female	Male	Female	Male	Normal	Variant[b]				Female	Male
1	99L03-1	Self	60.9	60.9	0	9	0:1			*ssssss*	*ssssss*
2	Quick Sweet	99L03-1	56.8	60.9	0	20	0:1			*ssssss*	*ssssss*
3	Quick Sweet	99L04-6	56.8	57.7	0	15	0:1			*ssssss*	*ssssss*
4	99L04-13	Quick Sweet	59.5	56.8	0	27	0:1			*ssssss*	*ssssss*
5	99L04-13	99L04-3	59.5	56.5	0	17	0:1			*ssssss*	*ssssss*
6	99L04-3	99L04-13	56.5	59.5	0	22	0:1			*ssssss*	*ssssss*
7	99L04-3	Kan98122-14	56.5	55.8	0	20	0:1			*ssssss*	*ssssss*
8	Kyukei97230-5	Quick Sweet	68.9	56.8	26	27	1:1	0.019	0.891	*Ssssss*	*ssssss*
9	Quick Sweet	Kyukei00214-3	56.8	67.7	24	23	1:1	0.021	0.884	*ssssss*	*Ssssss*
10	Kyukei97230-5	kyukei00214-1	68.9	58.7	8	13	1:1	1.190	0.275	*Ssssss*	*ssssss*
11	Norin No. 5	Quick Sweet	72.8	56.8	41	9	4:1	0.125	0.724	*SSssss*	*ssssss*
12	Quick Sweet	Miyano No. 36	56.8	70.5	39	9	4:1	0.047	0.829	*ssssss*	*SSssss*
13	Tamayutaka	Quick Sweet	72.5	56.8	157	3	19:1	3.289	0.070	*SSSsss*	*ssssss*
14	Quick Sweet	Daichinoyume	56.8	73.4	60	4	19:1	0.211	0.646	*ssssss*	*SSSsss*
15	Satsuma Starch	Quick Sweet	73.5	56.8	48	2	19:1	0.105	0.746	*SSSsss*	*ssssss*
16	Kyukei236	Kyukei00214-1	72.0	58.7	39	5	19:1	3.751	0.053	*SSSsss*	*ssssss*
17	Kyukei02250-209	Kyukei236	57.1	72.0	51	2	19:1	0.168	0.682	*ssssss*	*SSSsss*
18	Kyukei00142-6	Kyukei02250-209	71.0	57.1	50	4	19:1	0.659	0.417	*SSSsss*	*ssssss*
19	Beniazuma	99L03-1	75.6	60.9	46	3	19:1	0.130	0.718	*SSSsss*	*ssssss*
20	Quick Sweet	Kyushu No. 30	56.8	76.4	49	1	19:1	0.947	0.330	*ssssss*	*SSSsss*
21	Kyushu No. 127	Kyukei97230-5	66.4	68.9	40	13	3:1	0.006	0.937	*Ssssss*	*Ssssss*
22	Kyushu No. 127	Kyukei00214-3	66.4	67.7	42	11	3:1	0.509	0.475	*Ssssss*	*Ssssss*
23	Kyushu No. 127	Norin No. 5	66.4	72.8	42	8	9:1	2.000	0.157	*Ssssss*	*SSssss*
24	Siroyutaka	Quick Sweet	75.5	56.8	191	0	1:0			≥*SSSS*	*ssssss*
25	Quick Sweet	Koganesengan	56.8	75.0	65	0	1:0			*ssssss*	≥*SSSS*
26	Kyukei02250-209	Konasenri	57.1	74.8	53	0	1:0			*ssssss*	≥*SSSS*

[a] The mean of pasting temperatures of each parent examined in multi-year.
[b] Variants with low pasting temperature.

Table 2. Genetic hypothesis for the pasting temperature in sweetpotato starch

Genotype	Phenotype	Gametic output	Expected ratio from test cross (normal:variant)
ssssss	Variant[a]	*sss*	0:1
Ssssss	Normal	1*Sss*:1*sss*	1:1
SSssss	Normal	1*SSs*:3*Sss*:1*sss*	4:1
SSSsss	Normal	1*SSS*:9*SSs*:9*Sss*:1*sss*	19:1
SSSSss	Normal	1*SSS*:3*SSs*:1*Sss*	1:0
SSSSSs	Normal	1*SSS*:1*SSs*	1:0
SSSSSS	Normal	*SSS*	1:0

[a] Variants with low pasting temperature.

genotypes are presented in **Table 2**. All gametes of low pasting temperature variants were *sss*, and the ratio of normal to variant gametes of normal clones with a *Ssssss* genotype was 1 *Sss*:1 *sss*, and so on. The segregation ratio from crosses between this genotype and the variant was 1 *Ssssss*:1 *ssssss*, equating to 1 normal:1 variant in terms of phenotype. The possible segregation ratios of normal versus variant phenotypes for test crosses involving the various genotypes of normal clones using this hypothesis would be 1:1, 4:1, 19:1, and 1:0 (**Table 2**). Chi-square tests were used to evaluate the goodness of fit between the observed and expected ratios of progenies.

Results

Fig. 1 shows the frequency distributions of starch pasting temperatures in 257 seedlings from five families analyzed using the RVA. The pasting temperatures of progenies ranged from 53.0 to 77.0°C. These progenies could be broadly classified into two groups. The first group had low pasting temperature ranging from 53.0 to 64.0°C, and the second group had normal pasting temperature ranging from 63.0 to 77.0°C. We decided that the low pasting temperature variants and normal clones of the progenies were classified by a break in the distribution of pasting temperature between 63.0 and 64.0°C. These five families differed in the ratios of segregation. Both 'Quick Sweet' and '99L04-13' had low pasting temperature, and all of their F_1 progenies had low pasting temperature (**Fig. 1**A). The other crosses between 'Quick Sweet' and normal clones had two groups of progenies, or all normal progenies (**Fig. 1**B–1E). The progenies from the cross between 'Kyukei97230-5' and 'Quick Sweet' segregated at 26:27 for the normal and variant groups (**Fig. 1**B). The ratio of segregants between 'Quick Sweet' and 'Miyano No. 36' was 39:9 (**Fig. 1**C), between 'Quick Sweet' and 'Daichinoyume' it was 60:4 (**Fig. 1**D), and that between 'Quick Sweet' and 'Koganesengan' was 65:0 (**Fig. 1**E), for normal and variants groups, respectively.

Results of 26 testcrosses are shown in **Table 1**. The upper 7 families (No. 1–7) from crosses between variant parents generated all variant progenies. In the next 13

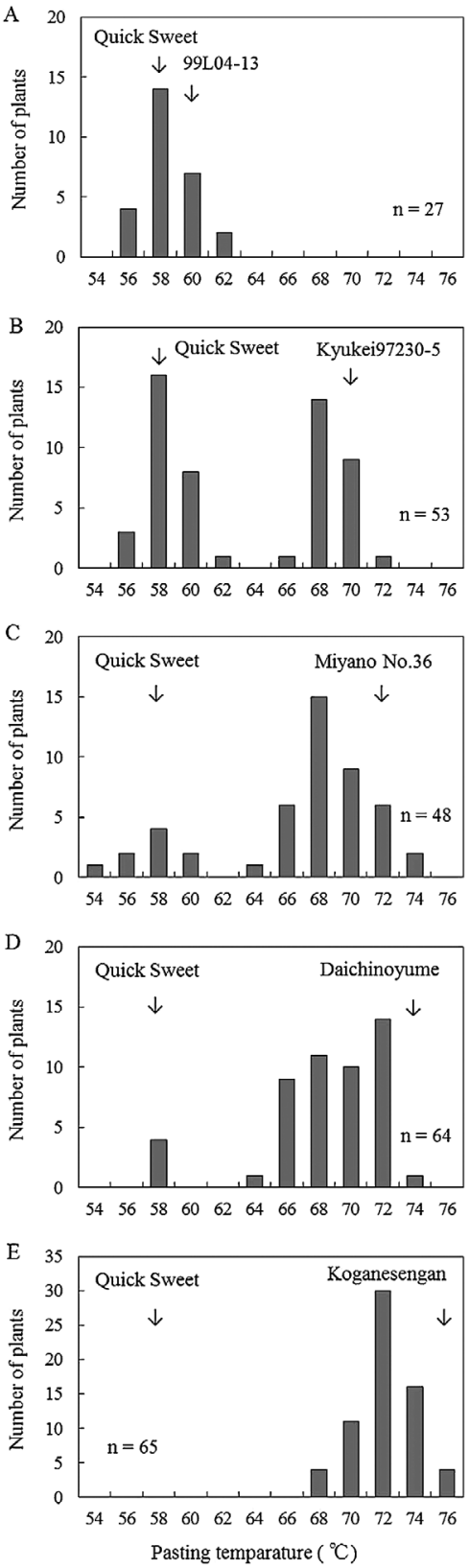

Fig. 1. The frequency distributions of starch pasting temperatures in the F_1 progenies of five families. Arrows show the pasting temperatures of the parental lines. A: 99L04-13 × Quick Sweet, B: Kyukei97230-5 × Quick Sweet, C: Quick Sweet × Miyano No. 36, D: Quick Sweet × Daichinoyume, E: Quick Sweet × Koganesengan.

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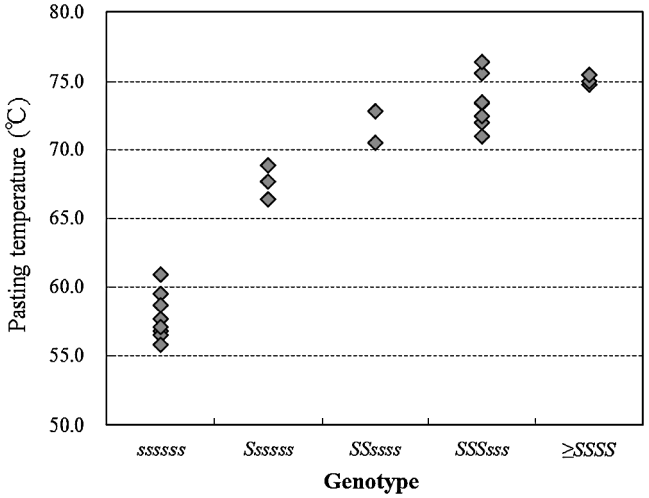

Fig. 2. Relationship between starch pasting temperature and the number of wild-type *Spt* alleles. Each box shows the mean pasting temperature of each parent examined in multi-year.

families (No. 8–20) from crosses between normal and variant parents, all progenies had various segregation ratios (1:1, 4:1, or 19:1) for normal and variant phenotypes. The lower three families (No. 24–26) from crosses between normal and variant parents had no variant segregants. The remaining three families (No. 21–23) were crosses between normal parents ('Kyushu No. 127' and other lines), and segregated variant progenies. 'Kyushu No. 127' shows approximately 10°C lower pasting temperature than ordinary cultivars. We assumed that its genotype is *Ssssss*, and the expected ratios for normal and variant phenotypes in these three families were 3:1 or 9:1.

Fig. 2 shows the relationship between pasting temperature and the number of wild-type *Spt* alleles in the crossing parents. The variants of nulliplex genotypes (*ssssss*) had approximately 15–20°C lower pasting temperature than the quadruplex (*SSSSss*) and pentaplex (*SSSSSs*)/hexaplex (*SSSSSS*) genotypes, which showed normal phenotypes. The simplex genotypes (*Sssssss*) had approximately 10°C lower pasting temperature than the normal phenotypes (≥*SSSS*). The duplex (*SSssss*) and triplex (*SSSsss*) genotypes had similar pasting temperatures for the normal phenotypes (≥*SSSS*), or slightly lower pasting temperatures than the normal phenotypes (≥*SSSS*). A dosage effect of wild-type *Spt* alleles was found for starch pasting temperature, although the effect was not linear.

Discussion

Theoretical prediction of segregation is difficult but important in sweetpotato breeding because of the complexity of hexaploid genetics. Frequently, a trait controlled by a single gene can be quantitatively scored by phenotype owing to the specificity of its segregation in autopolyploids (Watanabe 2015). From these results of crossing tests, we assumed that a pasting temperature lower than approximately 63°C is a qualitative character controlled by a single recessive gene. The results of 26 test crosses agreed well with this hypothesis. The segregation of all variant progenies in 7 families (No. 1–7) between variant parents, strongly supported the hypothesis that this trait is recessive (**Table 1**).

The starch in these variants exhibited not only low pasting temperature, but also cracked granule shape, slow retrogradation, high digestibility of raw starch and a high proportion in short outer chains of amylopectin molecules (Katayama *et al.* 2002, 2004, 2011, Kitahara *et al.* 2005). In other plants such as pea (Craig *et al.* 1998), potato (Edwards *et al.* 1999, Lloyd *et al.* 1999), rice (Umemoto *et al.* 2004) and maize (Zhang *et al.* 2004), it has been reported that an increase in short chains in amylopectin molecules leads to low starch pasting temperature, which is caused by a lack of or inhibition of starch synthase II. Previous studies in sweetpotato also reported that inhibition of expression of the starch synthase II gene led to low pasting temperature of sweetpotato starch, and the expression of starch synthase II gene was decreased in cultivars with low pasting temperature (Kitahara *et al.* 2011, Takahata *et al.* 2010). These results suggested that *Spt* allele encodes starch synthase II, and the variant phenotype of the *spt* allele resulted from a mutation(s) in the gene encoding starch synthase II.

Most sweetpotato starch is used to make sugar syrup or glucose, with the reminder employed to make foodstuffs such as starch noodles and gelatinized cakes, so-called in Japanese "*Warabi-mochi*" and "*Kuzu-mochi*", in Japan. Sweetpotato starches with low pasting temperature and slow retrogradation are suitable for foodstuffs such as gelatinized cakes and pasties. In 2012, 'Konamizuki', which has low pasting temperature, slow retrogradation and high digestibility of raw starch, was registered as a new cultivar for starch production from a cross between '99L04-3' (genotype is *ssssss*) and 'Kyukei236' (genotype is *SSSsss*) (Katayama *et al.* 2012). Starch yield of 'Konamizuki' is higher than that of 'Quick Sweet' and is close to that of a leading Japanese variety for starch production. 'Konamizuki' is expected to be used in foodstuffs such as gelatinized cakes and has the potential to be used as a renewable resource for bioethanol production.

The present study showed that sweetpotato can be subject to improvement of starch pasting temperature. Variants with nulliplex genotypes can be used as a tester to check the genotype of any breeding materials, and simplex or duplex genotypes are available as parents to develop breeding materials with low pasting temperature. In addition, this study demonstrated that the wild-type *Spt* allele has a gene-dosage effect on starch pasting temperature in sweetpotato. In a previous study, a dosage effect of the wild-type granule-bound starch synthase (GBSS) allele was found for GBSS activity and for amylose content in tetraploid potato tubers (Flipse *et al.* 1996). There was an almost linear relationship between the number of wild-type alleles and GBSS activity,

but this linear relationship was not observed for amylose content. It was expected that such polyploid crops could show a gene-dosage effect on various characters. Recently, a rapid screening method for low pasting temperature starches in sweetpotato was reported (Kobayashi *et al.* 2014). Sweetpotato breeders will be able to develop a series of sweetpotato cultivars with various pasting temperatures of starches by using these findings.

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