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 autotetraploid (2n = 6x = 90) (Shiotani and Kawase 1989) and an outcrossing vegetative propagation crop. The highly heterozygous, outcrossing polyploidy in...
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 variants and 15 normal clones, 26 families were generated. The gametic ratios expected for each of these

**Table 1. Segregation of variants with low pasting temperature in F1 progenies and expected genotypes of their parents**

| No. | Female                | Male       | Pasting temperature of parents (°C) | No. of F1 plants | Expected ratio | χ² | P | Genotype of parents |
|-----|-----------------------|------------|-------------------------------------|------------------|----------------|----|----|----------------------|
|     | Female | Male | Female | Male | Normal | Variant |     |     |                      |
| 1   | 99L03-1 | Self | 60.9 | 60.9 | 9 | 0 | 0:1 | sssss | sssss |
| 2   | Quick Sweet | 99L03-1 | 56.8 | 60.9 | 20 | 0 | 0:1 | sssss | sssss |
| 3   | Quick Sweet | 99L04-6 | 56.8 | 57.7 | 15 | 0 | 0:1 | sssss | sssss |
| 4   | 99L04-13 | Quick Sweet | 59.5 | 56.8 | 27 | 0 | 0:1 | sssss | sssss |
| 5   | 99L04-13 | Quick Sweet | 59.5 | 56.5 | 17 | 0 | 0:1 | sssss | sssss |
| 6   | 99L04-3 | 99L04-13 | 56.5 | 59.5 | 22 | 0 | 0:1 | sssss | sssss |
| 7   | 99L04-3 | Kan98122-14 | 56.5 | 55.8 | 20 | 0 | 0:1 | sssss | sssss |
| 8   | Kyukei97230-5 | Quick Sweet | 68.9 | 67.7 | 24 | 1 | 1:1 | 0.019 | 0.891 | SSSSS | sssss |
| 9   | Quick Sweet | Kyukei00214-3 | 56.8 | 67.7 | 23 | 1 | 1:1 | 0.021 | 0.884 | SSSSS | sssss |
| 10  | Kyukei97230-5 | kyukei00214-1 | 68.9 | 58.7 | 8 | 1 | 1:1 | 1.190 | 0.275 | SSSSS | sssss |
| 11  | Norin No. 5 | Quick Sweet | 72.8 | 56.8 | 41 | 9 | 4:1 | 1.047 | 0.819 | SSSSS | sssss |
| 12  | Quick Sweet | Miyano No. 36 | 56.8 | 70.5 | 39 | 9 | 4:1 | 0.125 | 0.724 | SSSSS | sssss |
| 13  | Tamayutaka | Quick Sweet | 72.5 | 56.8 | 157 | 3 | 19:1 | 3.289 | 0.070 | SSSSS | SSSSS |
| 14  | Quick Sweet | Daichinoyume | 56.8 | 73.4 | 60 | 4 | 19:1 | 0.125 | 0.346 | SSSSS | SSSSS |
| 15  | Satsuma Starch | Quick Sweet | 73.5 | 56.8 | 48 | 2 | 19:1 | 0.105 | 0.746 | SSSSS | SSSSS |
| 16  | Kyukei236 | Kyukei02250-209 | 72.0 | 57.1 | 39 | 5 | 19:1 | 3.753 | 0.053 | SSSSS | SSSSS |
| 17  | Kyukei02250-209 | Kyukei236 | 57.1 | 72.0 | 51 | 2 | 19:1 | 0.168 | 0.682 | SSSSS | SSSSS |
| 18  | Kyukei00142-6 | Kyukei02250-209 | 71.0 | 57.1 | 50 | 4 | 19:1 | 0.659 | 0.417 | SSSSS | SSSSS |
| 19  | Beniaizuma | 99L03-1 | 75.6 | 60.9 | 46 | 3 | 19:1 | 0.130 | 0.718 | SSSSS | SSSSS |
| 20  | Quick Sweet | Kyushu No. 30 | 56.8 | 76.4 | 49 | 1 | 19:1 | 0.947 | 0.330 | SSSSS | SSSSS |
| 21  | Kyushu No. 127 | Kyukei97230-5 | 66.4 | 68.9 | 40 | 13 | 3:1 | 0.006 | 0.937 | SSSSS | SSSSS |
| 22  | Kyushu No. 127 | Kyukei00214-3 | 66.4 | 67.7 | 42 | 11 | 3:1 | 0.509 | 0.475 | SSSSS | SSSSS |
| 23  | Kyushu No. 127 | Norin No. 5 | 66.4 | 72.8 | 42 | 8 | 9:1 | 2.000 | 0.157 | SSSSS | SSSSS |
| 24  | Siroyutaka | Quick Sweet | 75.5 | 56.8 | 191 | 0 | 1:0 | ≥SSSS | sSSS | sSSS | ≥SSSS |
| 25  | Quick Sweet | Koganesengan | 56.8 | 75.0 | 65 | 0 | 1:0 | SS | SSSS | sSSS | ≥SSSS |
| 26  | Kyukei02250-209 | Konasenri | 57.1 | 74.8 | 53 | 0 | 1:0 | sSSS | sSSS | sSSS | ≥SSSS |

\( ^a \) The mean of pasting temperatures of each parent examined in multi-year.

\( ^b \) 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 Ss: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<sub>1</sub> progenies had low pasting temperature (Fig. 1A). The other crosses between ‘Quick Sweet’ and normal clones had two groups of progenies, or all normal progenies (Fig. 1B–1E). The progenies from the cross between ‘Kyukei97230-5’ and ‘Quick Sweet’ segregated at 26:27 for the normal and variant groups (Fig. 1B). The ratio of segregants between ‘Quick Sweet’ and ‘Miyano No. 36’ was 39:9 (Fig. 1C), between ‘Quick Sweet’ and ‘Daichinoyume’ it was 60:4 (Fig. 1D), and that between ‘Quick Sweet’ and ‘Koganesengan’ was 65:0 (Fig. 1E), 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
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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, 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. ‘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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