Genetic Analysis and Some Properties of Starch in Waxy Mutant Wheat Tanikei A6599-4

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Waxy mutant wheat Tanikei A6599-4, which was induced from Tanikei A6099 (low-amylose line), exhibited a unique pasting curve with stable hot paste viscosity in the Rapid ViscoAnalyser (RVA) measurement. Analysis of the amylase content of reciprocal $F_1$ seeds revealed the incomplete dominance and gene dosage effect in hexaploid wheat. In the dominance among multiple Wx-D1 alleles, the Wx-D1 allele of Tanikei A6099 partially dominated that of Tanikei A6599-4, and the allele of Tanikei A6599-4 partially dominated that of Tanikei H1881 (waxy line of amylase-free type). Genetic analysis using DH$_2$ lines suggested that both the waxy character and stable hot paste viscosity of Tanikei A6599-4 are controlled by the same mutated Wx-D1 gene. We studied the pasting properties of Tanikei A6599-4 starch and compared them to those of other starches. At a low suspension concentration (6%), the peak viscosity of Tanikei A6599-4 was closer to that of Tanikei H1881 but the peak viscosity temperature was the same as that of Norin 61 (nonwaxy line). Addition of NaCl did not affect the starch pasting properties of Tanikei A6599-4, while potato starch which shows a more stable hot paste viscosity than cereal starches, was significantly affected by the NaCl treatment.

Key Words: *Triticum aestivum*, waxy, amylase content, Rapid ViscoAnalyser, pasting property.

Introduction

Amylose synthesis in the cereal grain endosperm is mainly controlled by the granule-bound starch synthase known as the Wx protein (Echt and Schwartz 1981, Sano 1984, Rohde et al. 1988). Endosperm starch generally consists of amyllose and amylpectin, but in the mutant lacking the Wx protein, endosperm starch consists exclusively of amylpectin and is referred to as “waxy starch.” Although the waxy endosperm has been identified in several crops (Eriksson 1969), it was not found in hexaploid wheats, possibly because of the triplication of Wx genes (Wx-A1, Wx-B1, Wx-D1) coding for Wx protein isoforms (Wx-A1, Wx-B1, Wx-D1) (Chao et al. 1989, Nakamura et al. 1993). The first waxy hexaploid wheats were obtained in Japan from 3 different sources: (1) cross combination between Kanto 107 and Bai Huo (Nakamura et al. 1995), (2) cross combination between Saikai 168 and Tanikei A6099 (Kiribuchi-Otobe et al. 1997), and (3) mutation from Kanto 107 (Yasui et al. 1997). These waxy lines showed similar characteristics; amylase content was almost zero (amylase-free), pollen grains and endosperm starch granules stained red-brown with a potassium iodide and iodine solution, and no Wx proteins were observed in the endosperm (Nakamura et al. 1995, Kiribuchi-Otobe et al. 1997, Yasui et al. 1997). They were referred to as “amylase-free”, based on the interpretation of Nakamura et al. (1995). Allelism test conducted by pollen analysis showed that they had null alleles at 3 Wx loci (Kiribuchi-Otobe et al. 1998a).

We obtained a different type of waxy wheat, Tanikei A6599-4, by sodium azide treatment (Kiribuchi-Otobe et al. 1998b). Analysis by amperometric titration showed that the line contained 1.6% amylase and its pollen grains and endosperm starch granules stained dark brown with a potassium iodide and iodine solution. The line contained the same amount of Wx protein as its parental line Tanikei A6099 (Yanagisawa et al. 2001). Rapid ViscoAnalyser (RVA) measurement showed that the peak viscosity temperature was higher and the hot paste viscosity was more stable in Tanikei A6599-4 than in amylase-free wheat (Kiribuchi-Otobe et al. 1998b). Although pollen analysis suggested that the waxy character of Tanikei A6599-4 was due to a mutation occurring at the Wx-D1 locus (Kiribuchi-Otobe et al. 1998b), certain aspects remained to be analyzed, e.g., dominance among multiple Wx-D1 alleles and whether the stable hot paste viscosity in Tanikei A6599-4 was controlled by the same mutated Wx-D1 gene responsible for its waxy character. We studied the mode of inheritance of the waxy character in detail and provide information on the unique pasting properties of Tanikei A6599-4 and their genesis.

Materials and Methods

Determination of apparent amylase content of F1 seeds

Materials studied included Norin 61 (normal amylase), Tanikei A6099 (low-amylose), Tanikei H1881 (waxy: amylose-free), and Tanikei A6599-4 (waxy: a small amount of amylase). Norin 61 is a standard cultivar with 3 Wx protein isoforms. Tanikei A6099 is a low-amylose mutant line induced from Kanto 107 by EMS treatment (Oda et al.
Both Tanikei A6099 and Kanto 107 lack Wx-A1 and Wx-B1 proteins, and the amount of the Wx-D1 protein in Tanikei A6099 is lower than that in Kanto 107 (Yanagisawa et al. 1996). Tanikei H1881 is a waxy wheat of amyllose-free type derived from a cross combination between Saikai 168 and Tanikei A6099 (Kiribuchi-Otobe et al. 1997). Tanikei A6599-4 is a waxy mutant line induced from Tanikei A6099 by sodium azide treatment (Kiribuchi-Otobe et al.). In 1995, Tanikei H1881 was reciprocally crossed with Norin 61 and Tanikei A6099 to yield F1 seeds. In 1996, Tanikei A6599-4 was reciprocally crossed with Tanikei A6099 and Tanikei H1881 to yield F1 seeds. Self-pollinated seeds were also prepared. In all the cases, central florets were removed and the number of florets per spike was adjusted to 20 and their awns were cut off to equalize the grain filling conditions. Using a motor drill, flour was obtained from the endosperm of each seed. The apparent amyllose content of the flour was colorimetrically determined using a Technicon Autoanalyser as described elsewhere (Oda et al. 1992).

Inheritance of stable hot paste viscosity

Kanto 118 and Tanikei A6599-4 were used as parents. Kanto 118, an offspring of Kanto 107, is a low-amyllose line in which only the Wx-D1 gene produces the Wx protein. We obtained 22 doubled haploid (DH) lines by haploid breeding using the maize technique (Suenaga and Nakajima 1989, Ushiyama et al. 1991) from F1 hybrids between Kanto 118 and Tanikei A6599-4. Grains of all the DH2 lines and their parents were milled into flour using a Brabender Quadrumat Jr. mill. Grains of Tankei H1881 were also milled into flour. Four gram of flour was mixed with 25 ml of 0.01 M silver nitrate (AgNO3) solution and the paste viscosity was measured using the RVA. AgNO3 was used as an inhibitor of α-amylase in flour. The suspension was heated from 34 to 94°C at 5°C/min and held at 94°C for 5 min, then cooled to 34°C at 5°C/min.

Starch pasting properties

Starches of Norin 61, Tanikei H1881, and Tanikei A6599-4 were isolated by washing with water and centrifugation as described elsewhere (Kiribuchi-Otobe et al. 1998b). Potato starch was purchased from Wako Pure Chemical Industries, Ltd. To evaluate the concentration effect, 1.5, 2.25, and 3 g of wheat starch were mixed with 25 ml of distilled water to prepare 6%, 9% and 12% suspensions. To evaluate the effect of sodium chloride (NaCl), 3 g of wheat starch and 2 g of potato starch were suspended in 25 ml of distilled water or 0.08% NaCl solution. The pasting viscosity of the suspension was measured using the RVA as described above.

Results

Determination of apparent amyllose content of F1 seeds.

The amyllose content of the endosperm flour of F1 seeds and their parents is shown in Table 1. Since the flour amyllose content was determined colorimetrically and the contribution of amyllopectin to the absorbance was not considered, the waxy lines showed a higher apparent amyllose content than their actual content. In our previous paper where the same Technicon Autoanalyser was used, we reported that the amyllose content of Norin 61 and Tankei A6099 (previously designated as M3-84) was about 30 and 15% (Oda et al. 1992). Compared to these values, the present values were lower by 2-4%. The removal of central

<table>
<thead>
<tr>
<th>Year</th>
<th>Cross combination</th>
<th>Wx gene dosage</th>
<th>Apparent amyllose content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wx-A1 Wx-B1 Wx-D1</td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td>Norin 61 (self)</td>
<td>3 3 3</td>
<td>25.0 ± 0.9 a</td>
</tr>
<tr>
<td></td>
<td>Tanikei A6099 (self)</td>
<td>0 0 3</td>
<td>13.1 ± 1.5 d</td>
</tr>
<tr>
<td></td>
<td>Tanikei H1881 (self)</td>
<td>0 0 0</td>
<td>2.2 ± 0.6 g</td>
</tr>
<tr>
<td></td>
<td>Norin61/Tanikei H1881</td>
<td>2 2 2</td>
<td>22.1 ± 1.5 b</td>
</tr>
<tr>
<td></td>
<td>Tanikei H1881/Norin 61</td>
<td>1 1 1</td>
<td>17.5 ± 2.3 c</td>
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<td></td>
<td>Tanikei A6099/Tanikei H1881</td>
<td>0 0 2</td>
<td>8.5 ± 0.6 e</td>
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<tr>
<td></td>
<td>Tanikei H1881/Tanikei A6099</td>
<td>0 0 1</td>
<td>5.0 ± 0.4 f</td>
</tr>
<tr>
<td>1996</td>
<td>Tanikei A6099 (self)</td>
<td>0 0 3</td>
<td>12.4 ± 0.9 a</td>
</tr>
<tr>
<td></td>
<td>Tanikei H1881 (self)</td>
<td>0 0 0</td>
<td>2.1 ± 0.1 g</td>
</tr>
<tr>
<td></td>
<td>Tanikei A6599-4 (self)</td>
<td>0 0 3</td>
<td>3.7 ± 0.4 d</td>
</tr>
<tr>
<td></td>
<td>Tanikei A6099/Tanikei A6599-4</td>
<td>0 0 2+1</td>
<td>8.7 ± 0.6 b</td>
</tr>
<tr>
<td></td>
<td>Tanikei A6599-4/Tanikei A6099</td>
<td>0 0 1+2</td>
<td>6.3 ± 0.4 c</td>
</tr>
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<td>2.7 ± 0.1 f</td>
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<tr>
<td></td>
<td>Tanikei A6599-4/Tanikei H1881</td>
<td>0 0 2</td>
<td>3.1 ± 0.2 c</td>
</tr>
</tbody>
</table>

Values followed by different letters in the same year differ significantly based on the LSD method at P = 0.05. Underlined parts in the Wx-D1 row indicate mutated genes (— Tankei A6099, — Tankei A6599-4).
florets and cutting of awns probably modified the ripening conditions and affected the amylase content but not the order relation. F1 seeds showed values intermediate between those of the parents in all the cross combinations (Table 1), indicating that the gene of the line with a higher amylase content was partially dominant over that of the line with a lower content. The amylase content differed between reciprocal F1 seeds. When the line with a lower amylase content was used as a maternal parent, F1 seeds showed a lower amylase content than when it was used as a pollen parent. These results suggest the presence of a gene dosage effect, because the endosperm had 1 dose of the paternal and 2 doses of maternal alelle due to double fertilization.

Inheritance of stable hot paste viscosity

Twenty-two DH2 lines segregated into waxy 11 : nonwaxy 11, indicating a good fit to the expected 1 : 1 ratio. To determine the stability of hot paste viscosity, we selected the peak viscosity temperature and time maintained above 80% of peak viscosity (Fig. 1). All the waxy lines showed a pasting curve similar to that of Tanikei A6599-4, and none of the nonwaxy lines exhibited a stable hot paste viscosity (Fig. 2). Tanikei H1881 was plotted in a separate area. These results suggest that both the waxy character and stable hot paste viscosity of Tanikei A6599-4 were controlled by the same gene, which must be Wx-D1, since the waxy character of Tanikei A6599-4 was ascribed to the mutation at the Wx-D1 locus (Kiribuchi-Otobe et al. 1998b).

Starch pasting properties

In the pasting curves of 3 cultivars with different starch concentrations (Fig. 3), the peak viscosity dropped as the concentration decreased in the all cases. The behavior of the peak viscosity temperature was noteworthy. The peak viscosity temperature of Norin 61 starch was constant at 94°C (maximum temperature), while waxy wheat starch (Tanikei H1881 and Tanikei A6599-4) showed a higher value for the peak viscosity temperature as the concentration decreased. When the 2 types of waxy wheat were compared, the increase in Tanikei A6599-4 (72.5°C → 90.5°C → 94°C) was larger than that in Tanikei H1881 (68.5°C → 70.5°C → 75.5°C).

The effect of NaCl on the starch pasting properties
(Fig. 4) showed that Tanikei A6599-4 starch pasting was only negligibly affected and its stability was maintained, while potato starch pasting, known to be more stable than that of cereal starches, was significantly affected by NaCl, showing a markedly reduced peak viscosity.

**Discussion**

The Wx gene coding for Wx protein (= granule-bound starch synthase) is the principal gene that modifies the amylose content of cereal endosperm, although other genes such as du, ae, or amol were identified in maize, rice, and barley (Creech 1968, Satoh and Omura 1981, Okuno et al. 1983, Yano et al. 1985, Ulrich and Eslick 1978). In diploid cereals, the Wx gene dosage in the endosperm of the ordinary type is 3, and incomplete dominance and dosage effect of Wx gene have been reported in maize and rice (Sager 1950, Okuno et al. 1983, Sano 1984). Since hexaploid wheat has 3 homoeologous Wx genes (Wx-A1, Wx-B1, Wx-D1) on group 7 chromosomes (Chao et al. 1989, Nakamura et al. 1993), the Wx gene dosage in the endosperm of the ordinary type is 9 in total. Although it had already been reported that the line having 3 null alleles at 1 or 2 of the 3 homologous Wx loci, e.g., [null null null, null null null, Wx-D1 Wx-D1 Wx-D1] contains less amylose than the ordinary one (Yamamori et al. 1994, Graybosch et al.1998), the amylose content in a line having 1 or 2 null alleles at each Wx locus, e.g., [Wx-A1 null null, Wx-B1 null null, Wx-D1 null null] remained to be determined. We analyzed the amylose content of reciprocal F1 seeds in 4 cross combinations (Table 1). In 3 cross combinations, excluding that between Norin 61 and Tanikei H1881, only the Wx-D1 gene was involved, as in the case of the diploid plants. In the dominance among multiple Wx-D1 alleles, the Wx-D1 allele of Tanikei A6099 partially dominated those of Tanikei A6599-4 and Tanikei H1881, and the allele of Tanikei A6599-4 partially dominated that of Tanikei H1881. In the cross combination between Norin 61 and Tanikei H1881, 3 Wx genes were involved, and F1 seeds of Norin 61/Tanikei H1881 showed a lower and those of Tanikei H1881/Norin 61 a much lower amylose content than Norin 61, indicating that incomplete dominance and dosage effect of Wx genes are evident in wheat regardless of polyploidy.

Compared to the amylose-free waxy lines, Tanikei A6599-4 is unique, as it contains a small amount of amylose and its starch and pollen grains stained dark brown with a KI-I2 solution (Kiribuchi-Otobe et al. 1998b). The present study showed that at a 6% concentration, the peak viscosity of Tanikei A6599-4 starch was closer to that of Tanikei H1881, but the peak viscosity temperature was the same, 94°C, as that for Norin 61 (Fig. 3). This phenomenon is compatible with the microscopic observation of the swelling process of individual starch granules heated in excess water (Otobe and Kiribuchi-Otobe 1999). They reported that Tanikei A6599-4 starch exhibited morphological changes similar to those of amylose-free wheat, but that its swelling ratio was close to that of nonwaxy wheat.

Yanagisawa et al. (2001) observed that Tanikei A6599-4 contained the same amount of Wx-D1 protein as Tanikei A6099 but with 1 base change from G to A leading a substitution of 1 amino acid from alanine to threonine. Assuming that the Wx-D1 protein mutation reduced the starch synthase activity, the reduction of the amylose content is understandable. Such waxy mutants that produce inactive Wx proteins were reported in maize and rice (Echt and Schwartz 1981, Yano et al. 1988). Furthermore, our study showed that the stable hot paste viscosity was also controlled by the mutated Wx-D1 gene. If the endosperm starch of Tanikei A6599-4 is a chimera of waxy and nonwaxy starch, such a unique pasting property may be explainable, because swollen nonwaxy starch granules with a higher peak viscosity temperature may maintain viscosity
after waxy starch granules collapsed. Nevertheless, such an assumption can be ruled out because the starch granules of Taniki A6599-4 uniformly stained dark brown with a potassium iodine and iodide solution (Kiribuchi-Otobe et al. 1998b) and a simulated 1.6% amylase mixture of Taniki A1881 and Taniki A6099 did not show hot paste stability (Kiribuchi-Otobe et al. 1998b).

It is generally assumed that phosphate-monoester derivatives in starch increase the paste viscosity. Potato starch, which contains a large amount of phosphatemonoesters, is more resistant to heat and shearing than cereal starches, but its hot paste stability is lost when potassium iodide is added to phosphate-monoester is displaced by other cations (Hofstee and de Willigen 1956). Our present study also showed that the peak viscosity of potato starch decreased markedly by the addition of NaCl, whereas the pasting curve of Taniki A6599-4 starch did not change (Fig. 4). Therefore, we consider that the stability of the hot paste viscosity of Taniki A6599-4 is unrelated to the content of phosphate-monoesters, although we have not measured it. It remains to be determined why the hot paste viscosity of Taniki A6599-4 is stable. Although the effect of the presence of 1.6% amylase cannot be ruled out, it is likely that the starch property mostly depends on the amylpectin structure in Taniki A6599-4. If the mutated Wx-D1 gene affects both amylase and amylpectin synthesis, it is of deep interest and deserves further exploration.

Literature Cited


