NOTE

Wheat Mutant with Waxy Starch Showing Stable Hot Paste Viscosity

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The endosperm starch of cereal grains generally consists of amylose and amylopectin. When the starch is composed exclusively of amylopectin, it is referred to as "waxy" and exhibits low initial pasting temperature, clarity of paste, stickiness, resistance to retrogradation, and low syneresis. Although waxy starch is utilized widely in the food industry, the low viscosity stability due to the weakness of the starch granule constitutes a disadvantage for some applications. In such cases, starch is chemically cross-linked for stable viscosity (Shock 1967).

We previously produced hexaploid waxy wheats from a cross between cvs. Saikai168 and Tanikei A6099. When measured on the Rapid ViscoAnalyser (Newport Scientific, Narabeen, Australia), pasting profiles of the starch of waxy wheats and waxy maize were similar, though the peak viscosity of the waxy wheat was much higher than that of the waxy maize (Kiribuchi-Otobe et al 1997). Recently, we selected a waxy mutant of wheat showing more stable paste viscosity. This study dealt with the starch properties and the inheritance of this waxy mutant.

MATERIALS AND METHODS

Plant materials

Five common wheat lines (Norin 61, Kanto 107, Tanikei A6099, Tanikei H1881, Tanikei A6599-4) were used. Norin 61 is a normal amylose cultivar and Kanto 107 is a low-amylose line. Tanikei A6099 has lower amylose content than Kanto 107 due to ethyl methanesulphonate mutagenesis (Oda et al 1992). Tanikei H1881, previously designated as Waxy 1, is a waxy line (Kiribuchi-Otobe et al 1997). Tanikei A6599-4 is a mutant waxy line produced in this study. For genetic analysis, Tanikei A6599-4 was reciprocally crossed with Tanikei A6099 and Tanikei H1881.

Mutation Induction

About 1,400 seeds of the low-amylose mutant line Tanikei A6099 were treated with 4 m*M* sodium azide for 2 hr and sown in a field in November, 1991. In June 1992, all the spikes (≈1,500) of the M_1 plants were harvested and M_2 seeds (one seed from each spike) were sown in October in the same year. In June 1993, all the M_2 plants were harvested and threshed to give M_3 seeds. The M_3 seeds of 285 selected M_2 plants were ground and analyzed for apparent amylose content using an AutoAnalyser II (Bran+Luebbe, Norderstedt, Germany) as described by Oda et al (1992). Among them, five M_2 plants showed lower amylose content than Tanikei A6099, and their progenies (one M_3 line from each M_2 plants, five M_4 lines from each M_3 line) were produced by selfing. In June 1995, apparent amylose content of M_5 seeds was analyzed and one M_4 line showed homozygous waxy character: Tanikei A6599-4.

Amylose Content and Iodine Staining

Grains were milled to flour in a Brabender Quadramat Jr. mill and the endosperm starch was isolated by water washing and centrifugation. The starch was defatted using chloroform and methanol (3:1) and amylose content was analyzed by amperometric titration as described previously (Kiribuchi-Otobe et al 1997). Pollen grains isolated from anthers collected at flowering time and endosperm starch granules (nondefatted) were stained with a potassium iodide and iodine solution (0.2% KI, 0.04% I^2, w/v) and observed by a light microscope (40 or 100 magnification).

Starch Paste Viscosity

Starch paste viscosity was measured on the RVA. Starch (3 g, 13.5% mc basis, nondefatted) was mixed with 25 mL of distilled water. The suspension was heated from 34 to 94°C at a rate of 5°C/min and held at 94°C for 5 min, then cooled to 34°C at a rate of 5°C/min.

RESULTS AND DISCUSSION

The amylose contents of Norin 61, Kanto 107, and Tanikei A6099 were 31.8, 20.9, and 17.7%, respectively. Tanikei A6599-4 contained 1.6% amylose and was considered to be a waxy mutant. However it was not amylose-free like Tanikei H1881 (0.4% amylose). The difference between Tanikei A6599-4 and Tanikei H1881 was also shown in the color of the pollen grains and starch granules stained with potassium iodide and iodine solution (Fig. 1). The pollen grains and starch granules of Tanikei H1881 stained red-brown. On the other hand, Tanikei A6599-4 stained dark brown, though it was quite different from the purple color of Norin 61. The core of the starch granule stained darker than the periphery in both waxy starches.

In the reciprocal crosses between Tanikei A6099 and Tanikei A6599-4, the F_1 plants produced purple and dark brown pollen grains at a 1:1 ratio. Therefore, a single mutant gene induced in Tanikei A6099 must be responsible for the waxy character of Tanikei A6599-4, although it is obscure whether the mutation occurred at the *Wx* locus. For example in rice, the *du* gene that lowers endosperm amylose content is different from the *Wx* gene (Okuno et al. 1983). However, in the reciprocal crosses between Tanikei H1881 and Tanikei A6599-4, red-brown and dark brown pollen grains segregated at a 1:1 ratio, and no purple (nonwaxy) pollens were observed. Tanikei A6099 has null alleles at *Wx-A1* and *Wx-B1* loci, and only the *Wx-D1* gene produced Wx protein (Kiribuchi-Otobe et al 1997); Tanikei H1881 has null alleles at all three *Wx* loci (Kiribuchi-Otobe et al 1998). These data indicate that the mutant gene of Tanikei A6599-4 and the waxy gene of Tanikei H1881 were alleles and located at the *Wx-D1* locus.

As shown in Fig. 2, the starch pasting curve of Tanikei A6599-4 was unique. The initial pasting temperature, the peak viscosity and the final viscosity did not differ markedly between Tanikei A6599-4 and Tanikei H1881. However, the peak viscosity temperature was higher and the hot paste viscosity was more stable (higher) in Tanikei A6599-4 than Tanikei H1881. These traits were transmitted to the next generation. The cause of the stable hot paste viscosity of Tanikei A6599-4 is unknown. A simulated starch mixture (1.6% amylose) of Tanikei H1881 and Tanikei A6099 did not show the same result. In barley, a waxy cultivar containing ≈4% amylose and one that was amylose-free (0%) showed different pasting properties, however neither showed the stability like Tanikei A6599-4 (Bhatty and Rossnagel 1997). The starch of Tanikei A6599-4 might possibly contain no amylose, but rather its amylopectin molecule has long external branch chains that form complexes with iodine,

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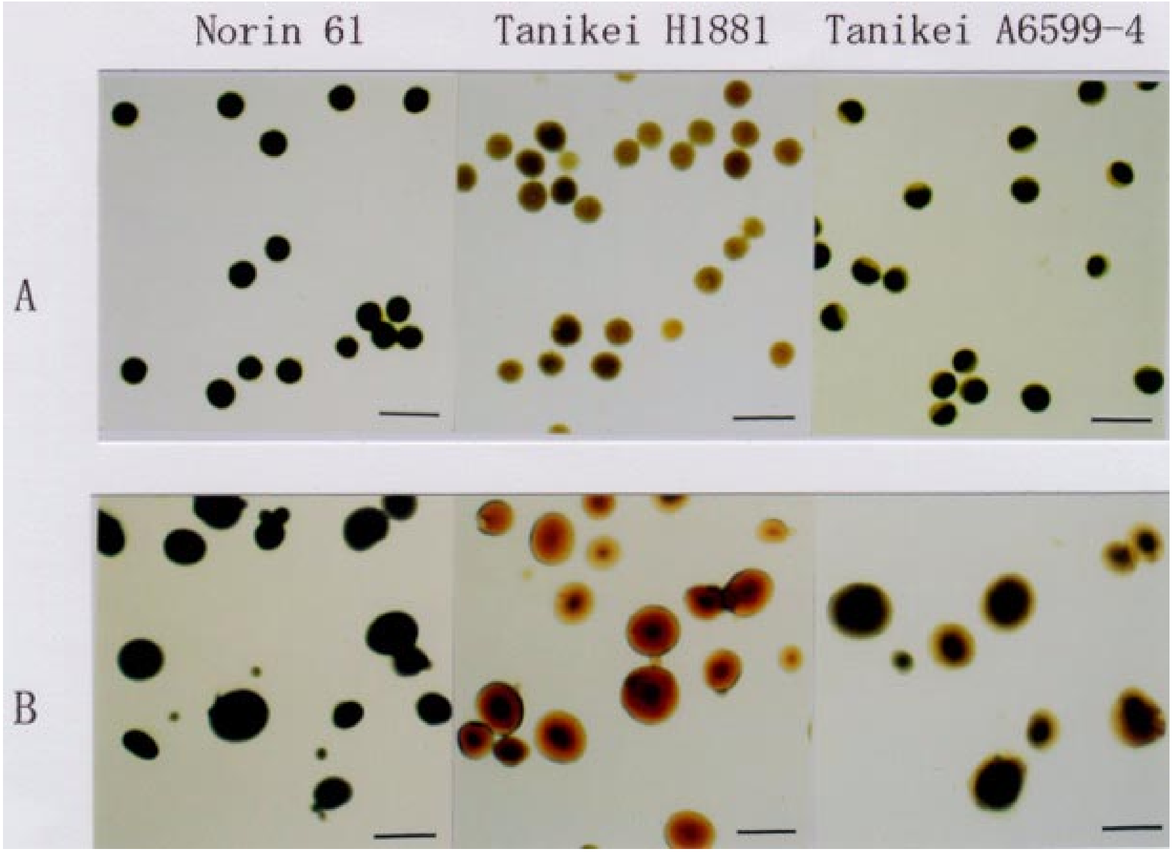

Fig. 1. Pollen grains (**A**) and endosperm starch granules (**B**) stained with potassium iodide and iodine solution. Bar = 120 μm in A, 30 μm in B.

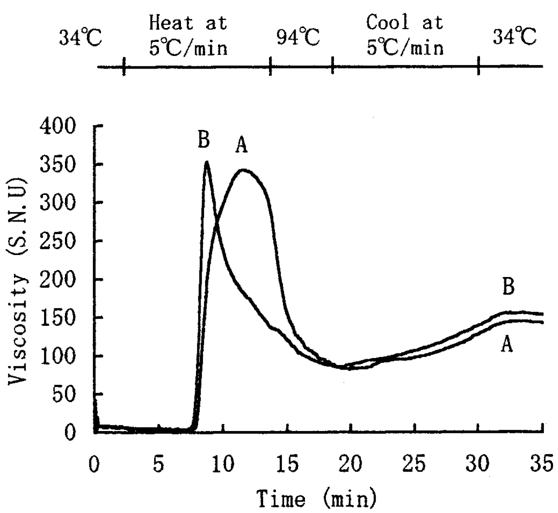

Fig. 2. Starch pasting curves. **A,** Tanikei A6599-4; **B,** Tanikei H1881.

because we determined the amylose content based on the iodine affinity. Jane and Chen (1992) demonstrated that the starch pasting properties were affected by amylose molecular size and amylopectin branch chain length. If the amylopectin chain profile of Tanikei A6599-4 is unique, it may be related to the stability, although the minor components of starch (lipid and phosphate) may have an effect (John 1992).

In the food industry, native or modified starch that meets specific product and processing conditions is valuable (Mauro 1997). Waxy starch, mainly from waxy maize, is used in baked foods, frozen foods, retort-packed foods, and so forth after modification by cross-linking to enhance the resistance to sheer and heat. Since the pasting profiles of the starch of Tanikei H1881 and the commercially available waxy maize starch were similar except for the peak viscosity (Kiribuchi-Otobe et al 1997), the starch of Tanikei A6599-4 with stable hot paste viscosity might be substituted for modified waxy maize starch.

CONCLUSIONS

A wheat mutant Tanikei A6599-4, which contained 1.6% amylose, exhibited a unique starch pasting curve. Hot paste viscosity was more stable than amylose-free wheat, although initial pasting temperature, peak viscosity and final viscosity did not differ markedly.

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