JARQ 40 (4), 327 – 331 (2006) http://www.jircas.affrc.go.jp

REVIEW

Waxy and Low-Amylose Mutants of Bread Wheat (*Triticum aestivum* L.) and their Starch, Flour and Grain Properties

Takeshi YASUI*

Crop Breeding Division, National Agricultural Research Center for Western Region
(Fukuyama, Hiroshima 721–8514, Japan)

Abstract

Bread wheat has three waxy proteins (= granule-bound starch synthase I, GBSSI), which are genetically controlled by three homoeologous loci, *Wx-A1*, *Wx-B1* and *Wx-D1*. Several mutant lines with waxy and low-amylose endosperm were induced from cv. Kanto 107, which has two null *Wx-A1b* and *Wx-B1b* alleles and a functional *Wx-D1a* allele. Starch was isolated from their grain and its amylose content, chain-length distribution profiles and thermal properties were determined. Waxy mutant lines, K107Wx1 and K107Wx2, had a new null allele named *Wx-D1d* on the *Wx-D1* locus; a low-amylose mutant line, K107Afpp4, had a new less functional allele named *Wx-D1g* on the locus. Paste viscosity is markedly suppressed in waxy flour when pasting properties are measured in water using a Rapid Visco Analyzer because waxy starch swells greatly and would disintegrate at a lower temperature where α-amylase is still active. Flour yield of the waxy mutant lines was lower than that of Kanto 107 because the waxy lines had decreased starch content and increased fat and β-glucan contents more than the wild type. Possible uses of the mutant lines were described from the standpoint of starch science and genetic resource.

Discipline: Food / Genetic resources
Additional key words: amylopectin, chain-length distribution profile, endosperm

Introduction

Starch is the major component of bread wheat (*Triticum aestivum* L.) grain and its properties affect the quality of final products. The starch granule consists of two types of glucose polymers, i.e., the highly branched amylopectin and essentially linear amylose, and other minor components, including lipids and proteins. Amylose content and amylopectin structure are principal factors influencing starch properties. Granule-bound starch synthase I (GBSSI, EC 2.4.1.21) is a key enzyme involved in amylose biosynthesis[2]. Since it is allohexaploid, bread wheat has three waxy (Wx) proteins, Wx-A1, Wx-B1, and Wx-D1, which are considered GBSSI and genetically controlled by three homoeologous loci, *Wx-A1*, *Wx-B1*, and *Wx-D1*[5] on chromosome arms 7AS, 4AL, and 7DS[1]. The lower amylose content of endosperm starch was correlated with the lack of Wx protein(s)[6,10]. Several waxy bread wheat lines were bred by crossing cv. Bai Huo carrying a null allele on the *Wx-D1* locus with cultivars, including Kanto 107, carrying null alleles on *Wx-A1* and *Wx-B1* loci[7,11]. Since the dosage effect of the functional Wx protein genes is present, starches with decreased amylose content at levels unavailable in present cultivars/lines were produced by crossing between waxy and non-waxy lines lacking two Wx proteins[8,12]. Although this is a reliable approach for obtaining starch samples with controlled amylose content, it is laborious and yields only a small amount of material. Therefore, genetically altered materials are further required to clarify the effects of amylose content on starch, flour, and grain properties. Since Kanto 107 is known to be deficient in both Wx-A1 and Wx-B1 proteins, a mutation at the *Wx-D1* locus can produce a waxy and low-amylose genotype without a substantial change of the genetic background of the parent. The present review summarizes the production of wheat mutants with decreased amylose content from Kanto 107 and their starch, flour, and grain properties.

*Corresponding author: fax +81–84–924–7893; e-mail yas@affrc.go.jp
Received 7 December 2005; accepted 3 February 2006.

Selection of waxy and low-amylose endosperm mutants

About 2,000 mature seeds of cv. Kanto 107 were treated with 0.5% ethyl methanesulphonate (EMS) solution. Of the 2,000 EMS-treated M_1 seeds, 1,928 plants were able to grow and 10,634 M_2 seeds were obtained; 56 M_1 plants did not produce seeds. Cross sections from M_2 seeds were stained with a 0.08% iodine-0.8% potassium iodide (I_2-KI) solution, and five seeds became red-brown. This observation suggested that these seeds had a waxy endosperm. Their progenies also had a waxy endosperm and were named K107Wx1, K107Wx2[18], K107Wx3, K107Wx4, and K107Wx5.

After waxy endosperm variants were screened from M_2 seeds derived from EMS-treated M_1 seeds of cv. Kanto 107, 3,880 non-waxy M_2 seeds were sown in an experimental field at National Agriculture Research Center (Tsukuba). Because no major morphological changes were observed in the growth and maturation stages of M_2 plants, 1,267 were randomly selected and an emerged spike of each plant was bagged to prevent outcrossing and to obtain self-pollinated seeds. Selected plants were harvested at maturity. Whole meal was prepared from the bulked grain of a single plant. Pasting properties of whole meal were measured using a Rapid Visco Analyzer (RVA), and six variants with altered pasting profile were selected. The progenies of four variants had similar pasting profiles to their M_2 parents and they were named K107Afpp1, K107Afpp2, K107Afpp3, and K107Afpp4[16]. The M_3 generation of the other two variants showed segregated RVA profiles including waxy and Kanto 107 type profiles, and so it was considered that they were heterozygotes at M_2 generation. Their waxy descendants were named K107Wx6 and K107Wx7, respectively.

In the following sections the properties of K107Wx1, K107Wx2 and K107Afpp4 are described.

Starch properties

The amylose content of the endosperm starch isolated from the waxy mutant lines, K107Wx1 and K107Wx2, was 0.9% by the concanavalin A (ConA) method[18]. The amylose content of K107Afpp4 starch by the ConA method was 12.7%, lower than its wild type, 21.9%. Apparent amylose content measured by amperometric titration of K107Afpp4 starch was 15.9% while that of Kanto 107 was 25.3%. The results suggested that K107Afpp4 has endosperm starch with decreased amylose content at about 60% of its wild type, Kanto 107[16]. Gel permeation chromatography on Sepharose CL-2B (Fig. 1) showed that the mutant starch has fewer amounts of the lower molecular weight fraction than wild type starch, i.e., K107Wx2 contained 3.8% of the lower molecular weight fraction, and K107Afpp4 contained 12.1% of the lower molecular weight fraction, while cv. Kanto 107 had 18.9% of the fraction[16]. Differential scanning calorimetry (DSC) showed that the waxy mutant lines lacked an endothermic peak at *ca.* 95ºC for the melting of the amylose-lipid complex[18]. DSC indicated that K107Afpp4 starch has a higher onset, peak, and final gelatinization temperature than the wild type. The gelatinization enthalpy of the K107Afpp4 starch was, however, not significantly different from that of the parent[16].

The chain-length distribution profiles of waxy mutant starches have an increased ratio of relatively long side-chains and decreased ratio of relatively short side-chains compared with Kanto 107. The difference in the distribution profile, however, was within the variation observed in starches from the closely related species of the *Triticum-Aegilops* group[17]. The difference in the chain-length distribution profiles between K107Afpp4 and Kanto 107 was negligible (Yasui, unpublished).

The endosperm starch from two waxy mutant lines did not contain any detectable amount of Wx-D1 protein by SDS-PAGE. EMS treatment induced the mutation at the *Wx-D1* locus of Kanto 107, which resulted in the lack of deposition of Wx-D1 protein in the endosperm starch

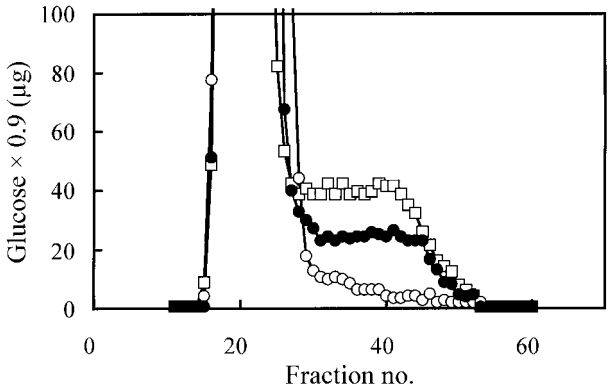

Fig. 1. Elution profiles of starches from K107Afpp4, K107Wx2 and Kanto 107 on a Sepharose CL-2B column

Starch (5 mg) was gelatinized with 50 μL of 1 mol/L NaOH and diluted with water to 500 μL. An aliquot of the solution (400 μL) was applied to the column (1 cm i.d. × 33 cm) and eluted with 0.02 mol/L NaOH at a flow rate of 0.2 mL/min. Fractions (0.5 mL) were collected and assayed for total carbohydrates using the phenol-sulfuric acid method.

●: K107Afpp4, ○: K107Wx2, □: Kanto 107.

of the mutant lines[18]. A Wx-D1 protein band was detected, however, in K107Afpp4 starch. The band intensity was similar to that of Kanto 107 starch, and so Wx-D1 proteins were presumably expressed at the same extent in both materials suggesting that the GBSSI of K107Afpp4 has a structure different from that of Kanto 107 and is less functional than that of the wild type[13].

Flour properties

In RVA measurement, whole meal paste of K107Wx1 showed a viscosity peak at a lower temperature (Fig. 2).

In another RVA measurement condition, pastes from K107Wx1 and K107Wx2 flour showed a lower peak temperature and a lower peak, holding and final viscosity than that from Kanto 107 flour when water was used as the pasting medium. Breakdown, setback, and setback from peak of waxy flour paste were also lower than for non-waxy paste. In these flours, α-amylase (EC 3.2.1.1) activity was slightly higher in Kanto 107 than in the two waxy mutant lines. Pasting properties were measured in 0.1% silver nitrate solution to eliminate the effects of enzyme activity affecting paste viscosity, and marked changes were observed in viscosity, but not temperature parameters: the peak viscosity of mutant lines, for example, increased from about 150 RVU to above 320 RVU, and of Kanto 107 from about 230 RVU to above 310 RVU when silver nitrate was added to the pasting medium. Silver nitrate solution brought the most noticeable difference in breakdown, i.e., breakdown of waxy flour was less than that of non-waxy flour in water, but greater in the silver nitrate solution. Since waxy starch swells greatly and would disintegrate at a lower temperature where α-amylase is still active, amylopectin molecules would be hydrolyzed by active α-amylase in swelled and disintegrated waxy starch granules when pasting properties are measured in water. The increase in paste viscosity is thus markedly suppressed in waxy flour. In non-waxy flour, however, non-waxy starch does not swell so extensively as waxy starch, so less hydrolysis would occur at lower temperature. At higher temperature, non-waxy starch would swell and disintegrate enough for enzymatic hydrolysis, α-amylase would be heat-denatured and lose activity, and, as a result, less amylopectin hydrolysis would occur and pasting viscosity is not suppressed remarkably even in the pasting medium of water. The activity of α-amylase in flour affects the paste viscosity of flour, especially waxy flour[15].

Whole meal paste of K107Afpp4 showed markedly increased viscosity at 6 min and decreased peak time (Fig. 3), while pasting temperature, peak temperature, and peak viscosity remained unchanged[16].

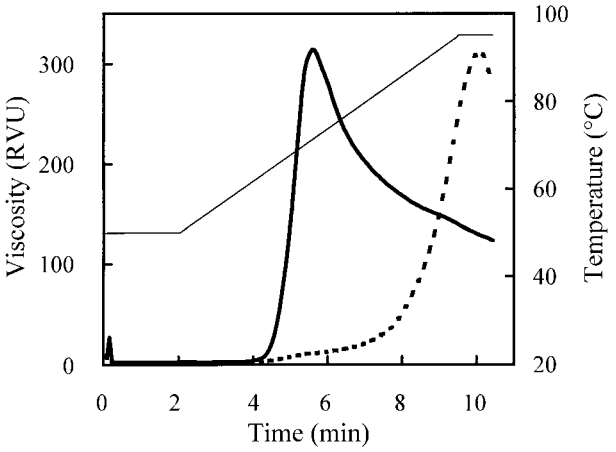

Fig. 2. RVA profiles of flours from K107Wx1 and Kanto 107
Pasting properties of flour were measured using a Rapid Visco Analyser 3D+ (RVA) (Newport Scientific, Warriewood, Australia); 3.5 g of flour dispersed in 25.0 ± 0.1 mL of 0.1% silver nitrate solution was held 2 min at 50ºC, heated to 95ºC at 6ºC/min, and held 1 min at 95ºC. The paddle was rotated at 960 rpm for the first 10 s, then at 160 rpm for the remainder of the test.
—— : K107Wx2, - - - : Kanto 107, — : Temperature.

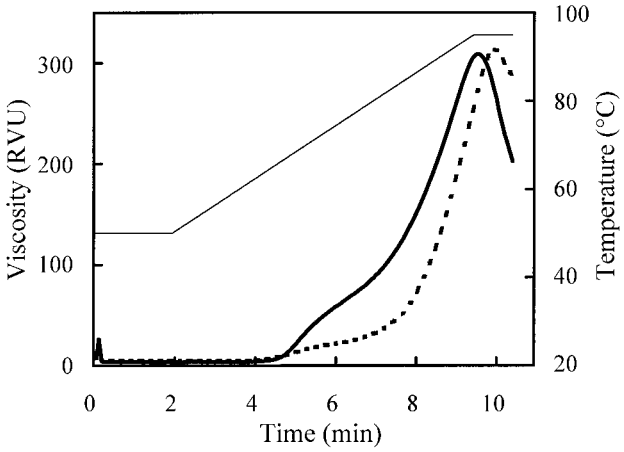

Fig. 3. RVA profiles of whole meals from K107Afpp4 and Kanto 107
Pasting properties are described in Fig. 2;
4.0 g of whole meal was used.
—— : K107Afpp4, - - - : Kanto 107, — : Temperature.

Grain properties

Thousand-grain weights of K107Wx1 and K107Afpp4 were not significantly different from that of the wild type, but that of K107Wx2 was decreased from Kanto 107[16,18]. The starch content of the grain from waxy mutant lines was slightly but significantly lower than that of Kanto 107[15], while the starch content of K107Afpp4 grain was not decreased from Kanto 107[16].

The averaged flour yield of waxy mutant lines was about 40%, and that of the non-waxy parent was about 50% at a feed rate of 40–80 g/min using a Quadrumat Junior test mill: waxy mutant lines had about 10 percentage point lower flour yield than Kanto 107[15]. The fat and β-glucan content of waxy mutants was significantly higher than that of the parent, while protein content did not differ markedly between them[15]. The starch content of waxy mutant lines was about 95% of the non-waxy parent, but reduced starch content alone could not explain the lower flour yield of waxy mutants[15]. It is probable that higher fat content in the mutants' grain reduced flour flowability and yielded less flour. The increased β-glucan content in waxy mutants implied an increased thickness of the endosperm cell wall, which could alter milling properties and lower flour yield[15].

The flour yield of K107Afpp4 was about 63% while that of Kanto 107 was 65% using a Buhler test mill but their statistical significance was obscure (Yasui, unpublished).

Genetic analyses of alleles responsible for waxy and low-amylose endosperm

K107Wx1 and K107Wx2 have a null allele at the *Wx-D1* locus, but its intragenic structure was found to be different from *Wx-D1b*. The allelic symbol for the null allele of the waxy mutants is *Wx-D1d*[14], because *Wx-D1a* was found in many cultivars including Chinese Spring and Kanto 107, *Wx-D1b* in cv. Bai Huo, and *Wx-D1c* in cv. Scoutland[10]. The RVA profile of K107Afpp4 was governed by a single major gene, which was partially dominant to the *Wx-D1a* allele of its wild type, Kanto 107, and also to the null *Wx-D1d* allele of the backcrossed waxy line with the genetic background of Kanto 107. No other type of RVA profile including Kanto 107 type was observed in the F_2 plants from the cross between backcrossed waxy line/K107Afpp4. These results indicated that the gene responsible for the characteristic RVA profile of K107Afpp4 is located on the *Wx-D1* locus and other loci are not concerned with the trait, suggesting a new allele on the locus, which is designated *Wx-D1g*[13], because nonfunctional *Wx-D1e* was found in NP150 and less functional *Wx-D1f* in Tanikei A 6599-4[4].

Conclusions

Two other mutant lines with low-amylose endosperm were reported to be induced from Kanto 107[3,9]. Mutant induction from cultivars/lines carrying two non-functional Wx protein alleles would be useful and practical in obtaining materials with negligible and decreased amylose content without substantially altering their genetic background and to develop new genetic resources with mutated Wx protein alleles.

K107Wx1, K107Wx2 and K107Afpp4, and other stocks having the genetic background of Kanto 107 (Table 1) are surely useful for the comparative studies on the relationship between the structure and activity of GBSSI and on the relationship between amylose content and starch properties. The studies using near-isogenic waxy and non-waxy lines would clarify the cause of the low flour yield of the waxy mutants. The use of waxy and low-amylose lines in food and non-food industries are negligible so far because these industries have established processes for the final products and are conservative to the use of novel raw materials when their

Table 1. Properties of genetic stocks carrying known *Wx-D1* alleles with the genetic background of Kanto 107

Material	Allele	Amylose (%)	RVA profile	
Kanto 107	*Wx-D1a*	21.9	Kanto 107 type	<Kanto 107>
Backcrossed waxy line	*Wx-D1b*: null	(2.0 >)	Waxy type	<Bai Huo>
–	*Wx-D1c*	–	–	<Scoutland>
K107Wx1, K107Wx2	*Wx-D1d*: null	0.9	Waxy type	<K107Wx1>
–	*Wx-D1e*: null	(2.0 >)	(Waxy type)	<NP150>
Tanikei A6599-4	*Wx-D1f*	4.4	Tanikei A6599-4 type	<Tanikei A6599-4>
K107Afpp4	*Wx-D1g*	12.7	K107Afpp4 type	<K107Afpp4>

Amylose content was determined by concanavalin A method.
( ): Expected, –: Not available/unknown, < >: Representative cultivar or line carrying the allele.

properties are obscure.

The amylose content decreased the most by a lack of the Wx-B1 protein, followed by a lack of Wx-D1 protein, and the least by the Wx-A1 protein deficiency, which indicated the differential effects of the three null alleles for the Wx protein to amylose biosynthesis[12]. Bread wheat cultivars which constitute an Australian wheat blend, AWB noodle, are known to lack Wx-B1 protein. It is generally said that AWB noodle has a superior quality for white salted noodles and Japanese udon-style noodles. Although the amylose level of AWB noodle is low compared with ordinary Japanese cultivars with three wild-type Wx protein genes, it is considered that the amylose level of AWB noodle is not always suitable for udon-noodle making. In different genetic backgrounds and/or growing conditions of bread wheat cultivars/lines, the best amylose level for noodle-making might be a somewhat lower or higher amylose content than AWB noodle. For this kind of fine genetic control of amylose content of a cultivar/line, a series of altered but functional Wx protein alleles such as *Wx-D1f* and *Wx-D1g* are the prerequisites as well as non-functional null alleles. We would be able to adjust the amylose content of a bread wheat cultivar/line to the 0–30% level without any technical difficulties using conventional breeding techniques when we further discover novel genes and/or artificially induced mutant genes for altered GBSSI activity on the three Wx protein loci.

Acknowledgments

National Agricultural Research Center for Western Region is supported by grant-in-aid from the Ministry of Agriculture, Forestry and Fisheries. The author would like to thank all those involved in our research.

References

1. Chao, S. et al. (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. *Theor. Appl. Genet.*, **78**, 495–504.
2. Echt, C. S. & Schwarz, D. (1981) Evidence for the inclusion of controlling elements within the structural gene at the waxy locus in maize. *Genetics*, **99**, 275–284.
3. Kiribuchi-Otobe, C. et al. (1998) Wheat mutant with waxy starch showing stable hot paste viscosity. *Cereal Chem.*, **75**, 671–672.
4. McIntosh, R. A. et al. (2003) Catalogue of gene symbols for wheat. *In* Proceedings, vol. 4, 10th international wheat genetics symposium, eds. Pogna, N. E. et al., Paestum, Italy. Available in MacGene CD, and on line at http://wheat.pw.usda.gov/ggpages/wgc/2003/.
5. Nakamura, T. et al. (1993a) Identification of three Wx proteins in wheat (*Triticum aestivum* L.). *Biochem. Genet.*, **31**, 75–86.
6. Nakamura, T. et al. (1993b) Decrease of waxy (Wx) protein in two common wheat cultivars with low amylose content. *Plant Breed*., **111**, 99–105.
7. Nakamura, T. et al. (1995) Production of waxy (amylose-free) wheats. *Mol. Gen. Genet.*, **248**, 253–259.
8. Sasaki, T., Yasui, T. & Matsuki, J. (2000) Effect of amylose content on gelatinization, retrogradation, and pasting properties of starches from waxy and nonwaxy wheat and their F1 seeds. *Cereal Chem.*, **77**, 58–63.
9. Yamaguchi, I. et al. (1998) Breeding of "Wheat Norin-PL 7 (Tanikei A6099)" a new germplasm accession with low amylose content. *Bull. Natl. Agric. Res. Cent.*, **29**, 1–11 [In Japanese with English summary].
10. Yamamori, M. et al. (1994) Waxy protein deficiency and chromosomal location of coding genes in common wheat. *Theor. Appl. Genet.*, **89**, 179–184.
11. Yamamori, M., Nakamura, T. & Nagamine, T. (1995) Inheritance of waxy endosperm character in a common wheat lacking three Wx proteins. *Breed. Sci.*, **45**, 377–379.
12. Yamamori, M. & Quynh, N. T. (2000) Differential effects of Wx-A1, -B1 and -D1 protein deficiencies on apparent amylose content and starch pasting properties in common wheat. *Theor. Appl. Genet.*, **100**, 32–38.
13. Yasui, T. (2004) A new allele on *Wx-D1* locus causes an altered flour-pasting profile of the low-amylose bread wheat (*Triticum aestivum* L.) mutant, K107Afpp4. *Breed. Sci.*, **54**, 281–286.
14. Yasui, T., Sasaki, T. & Matsuki, J. (1998) Waxy bread wheat mutants, K107Wx1 and K107Wx2, have a new null allele on *Wx-D1* locus. *Breed. Sci.*, **48**, 405–407.
15. Yasui, T., Sasaki, T. & Matsuki, J. (1999) Milling and flour pasting properties of waxy endosperm mutant lines of bread wheat (*Triticum aestivum* L). *J. Sci. Food Agric.*, **79**, 687–692.
16. Yasui, T., Sasaki, T. & Matsuki, J. (2002) Starch properties of a bread wheat (*Triticum aestivum* L.) mutant with an altered flour-pasting profile. *J. Cereal Sci.*, **35**, 11–16.
17. Yasui, T., Sasaki, T. & Matsuki, J. (2005) Variation in the chain-length distribution profiles of endosperm starch from *Triticum* and *Aegilops* species. *Starch/Stärke*, **57**, 521–530.
18. Yasui, T. et al. (1997) Waxy endosperm mutants of bread wheat (*Triticum aestivum* L.) and their starch properties. *Breed. Sci.*, **47**, 161–163.