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Waxy Endosperm Mutants of Bread Wheat (*Triticum aestivum* L.) and Their Starch Properties

Takeshi Yasui1, Tomoko Sasaki1, Junko Matsuki1 and Makoto Yamamori2

1) National Agriculture Research Center (NARC), Ministry of Agriculture, Forestry and Fisheries (MAFF), Tsukuba, Ibaraki 305, Japan
2) National Institute of Agrobiological Resources (NIAR), MAFF, Tsukuba, Ibaraki 305, Japan

Summary
Two waxy bread wheat lines, K107Wx1 and K107Wx2, have been developed by treating cv. Kanto 107 seeds with ethyl methanesulphonate (EMS). About 2,000 seeds of the cultivar were soaked for 4 hours in 0.5% EMS dissolved in 7% ethanol, and then 2,000 seeds were sown in a greenhouse and 10,654 M2 seeds were obtained from 1,872 M1 plants. Cross sections of the distal endosperms of 4,000 M2 seeds were stained with an iodine-potassium iodide solution, and two seeds stained red-brown. Since the M2 and M4 seeds derived from the remaining proximal halves of these two seeds showed the characteristic feature of waxy endosperm, the waxy endosperm character was considered to be genetically fixed in these two mutant lines, which were designated as K107Wx1 and K107Wx2. The amylose content of endosperm starch from both mutant lines was 0.9%, and no waxy proteins were detected in the starch of either line. The agronomic characters of K107Wx1 and K107Wx2 were very similar to those of cv. Kanto 107 grown under field conditions, except for the heading dates of the mutant lines and the 1,000-grain weight of K107Wx2. These results indicate that the mutagen treatment of seeds is effective in inducing of waxy, i.e. amylose-free, mutants when a bread wheat cultivar has a single waxy protein in their endosperm starch.

Key Words: amylose-free, bread wheat, starch, *Triticum aestivum* L., waxy mutant.

Introduction
Starch is the major component of the bread wheat (*Triticum aestivum* L.) kernel, and its properties affect the quality of the products made from wheat flour. The eating quality of white Japanese noodles (Udon), for example, correlates negatively with the amylose content of flour (Oda et al. 1980). Bread wheat cultivars and breeding lines with a low amylose content have therefore been released in Japan to improve the noodle quality (Yamada 1994). Since bread wheat is allohexaploid, it has three types of waxy (Wx) proteins, i.e., Wx-A1, Wx-B1, and Wx-D1 (Nakamura et al. 1993 a). Deficiency in one or two type(s) of Wx proteins explains the low amylose content of low-amylose cultivars and lines (Nakamura et al. 1993b, Yamamori et al. 1994). Several waxy wheat lines have been developed by cross-breeding using Wx-A1 and Wx-B1 protein-deficient cultivars and a Wx-D1 protein-deficient cultivar (Nakamura et al. 1995, Yamamori et al. 1995, Hoshino et al. 1996). Since cv. 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 genotype without a substantial change of the genetic background of the parent. The present paper reports the production of two waxy endosperm mutant lines from cv. Kanto 107 and the properties of starch isolated from their endosperm.

Materials and Methods

Waxy endosperm mutants
About 2,000 mature seeds of cv. Kanto 107 were soaked for 4 hours in 0.5% ethyl methanesulphonate (EMS) dissolved in 7% ethanol. Seeds were then washed five times with 200 ml of water and sown in seedbeds filled with vermiculite, and grown in a greenhouse. Mature M2 seeds were harvested and cross sections of the distal endosperm were stained with an iodine-potassium iodide (I2-KI) solution to detect a waxy (amylose-free) mutant. The color of the stained endosperm cross sections was observed by the unaided eye and using a microscope. The remaining proximal halves of M2 seeds that stained red-brown were planted in pots and grown in a greenhouse to obtain M3 seeds. M3 plants were also grown in a greenhouse to harvest M4 seeds. The seeds of cv. Kanto 107 were treated with EMS in August, 1993, and waxy M4 seeds were harvested in April 1996. To compare their agronomic characters with those of cv. Kanto 107, waxy M4 seedlings were transplanted into an experimental field at the National Agriculture Research Center (NARC, Tsukuba) in November, 1995, and cultivated under ordinary conditions.

Starch properties
Starch was isolated below 4 °C from degermed 29 and 47 M3 seeds of two waxy mutant lines using a modification of the method of Sulaiman and Morrison (1990). The amylose content of starch was determined using a

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modification of the method of Yun and Matheson (1990) with an amylopectin/amyllose assay kit (Megazyme, Sydney). Thermal properties were measured according to the method of Kohyama and Nishinari (1991) and using a differential scanning calorimeter, SSC 5300, with a DSC120U module (Seiko Instruments, Tokyo) calibrated with indium.

Wx proteins extracted from the endosperm starch isolated from M₄ grains were separated by SDS-PAGE using acrylamide/BIS (30:0.135) gels (Nakamura et al. 1992).

Results and Discussion

Waxy endosperm mutants

Of the 2,000 EMS-treated M₁ seeds, 1,928 plants were able to grow and 10,634 M₂ seeds were obtained; 56 M₁ plants did not produce seeds. Cross sections from randomly selected 4,000 M₂ seeds were stained with an I₂-KI solution, and two seeds became red-brown. This observation suggested that these seeds had a waxy endosperm. The remaining proximal parts of these seeds were grown, and 178 and 107 M₃ seeds were obtained. All the cross sections from the distal endosperms of all of the M₃ seeds stained red-brown with the I₂-KI solution. The remaining proximal parts of five M₃ seeds each from each M₂ plant were grown and M₄ grains were obtained. Twelve M₄ seeds each from M₃ plants stained red-brown with the I₂-KI solution. These results indicated that the waxy character was genetically fixed in these lines, which were designated as K107Wx1 and K107Wx2.

Starch properties

Since the endosperm of both mutant lines stained red-brown with the I₂-KI solution, it was assumed that the two lines contained a very small amount of amyllose in the endosperm starch. The amyllose content of the endosperm starch isolated from the mutant lines was 0.9%. Differential scanning calorimetric profiles of the mutant lines failed to reveal an endothermic peak at ca. 95 °C for the melting of the amyllose-lipid complex (Fig. 1). These results strongly indicate that the endosperm starch of the mutant lines was essentially amyllose-free and that these mutants were waxy (glutinous or amyllose-free).

Since the peak gelatinization temperature and gelatinization enthalpy of the starches from K107Wx1 were 60.3 °C and 13.1 J/g, and those of K107Wx2 were 60.4 °C and 13.6 J/g, the thermal properties of starches from the mutant lines were essentially identical.

Wx proteins

Fig. 2 shows the SDS-PAGE results for the proteins extracted from the endosperm starch of the mutant lines and cvs. Kanto 107 and Norin 61. Cv. Norin 61 showed two prominent Wx protein bands for Wx-A1, Wx-B1, and Wx-D1 proteins, and cv. Kanto 107 a single Wx-D1 protein band. The two mutant lines, however, did not contain any detectable amount of Wx protein. EMS treatment thus induced the mutation at the Wx-D1 gene in cv. Kanto 107, which resulted in the lack of deposition of Wx protein in the endosperm starch of the mutant lines.

Agronomic characters

Table 1 shows the agronomic variation in the two mutant lines and cv. Kanto 107. The average culm length and spike length of the two mutant lines did not differ significantly from those of its parent. The 1,000-grain weight of K107Wx1 was similar to that of cv. Kanto 107, but the K107Wx2 grain was lighter than that of the parent. The heading dates of the mutant lines were delayed by 2-5 days compared with cv. Kanto 107. The glume and grain color and culm angle of the waxy mutant lines were indistinguishable from those of cv. Kanto 107.
In diploid wheat species, Kanzaki and Noda (1988) reported the production of the waxy endosperm mutant of *Triticum monococcum* L. after EMS treatment. By using EMS, Oda et al. (1992) obtained a mutant line with a reduced amylose content from cv. Kanto 107.

K107Wx1 and K107Wx2 are, to the authors' knowledge, the first waxy lines of hexaploid wheat induced by mutagen treatment. The induction of a waxy mutant in bread wheat was thus found to be possible when a single Wx protein is present. These mutant lines would be useful for the structural elucidation of *Wx-D1* gene, and for clarifying the effects of the amylose-amylopectin ratio on physicochemical properties of starch paste and flour dough and on the quality of final products, especially white Japanese noodle. Breeding of the near-isogenic waxy lines of cv. Kanto 107 will be considerably easier when these materials are used as non-recurrent parent. The allelic status in *Wx-D1* locus will be revealed by the allelism test between these two mutant lines and cross-bred waxy lines. The genetic analysis of waxy character and protein profiles in the endosperm of these waxy mutant lines will be published separately.

**Literature Cited**


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**Table 1. Agroscopic characters of waxy endosperm mutant lines, K107Wx1 and K107Wx2**

<table>
<thead>
<tr>
<th></th>
<th>Culm length (cm)</th>
<th>Spike length (cm)</th>
<th>1000-grain weight (g)</th>
<th>Heading date</th>
</tr>
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<tbody>
<tr>
<td>K107Wx1</td>
<td>84.9±4.43</td>
<td>10.07±0.879</td>
<td>46.4±3.25</td>
<td>May 3</td>
</tr>
<tr>
<td>K107Wx2</td>
<td>86.0±3.38</td>
<td>10.04±0.551</td>
<td>44.9±1.75</td>
<td>May 6</td>
</tr>
<tr>
<td>Kanto 107</td>
<td>86.8±3.75</td>
<td>10.40±0.629</td>
<td>47.7±2.18</td>
<td>May 1</td>
</tr>
<tr>
<td>Difference between K107Wx1 and Kanto 107</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Difference between K107Wx2 and Kanto 107</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Difference between K107Wx1 and K107Wx2</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
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1) mean ± standard deviation.

**,**, significant at the 1% level; ns, not significant.