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# Variation for amylose content in wheat cultivars carrying different null alleles at the *Wx* loci

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**Abstract**

The wheat Wx proteins, controlling amylose synthesis in the endosperm, are the product of the *Wx* genes at a triplicate set of single-copy homoeoloci located on chromosomes 7A (*Wx-A1*), 4A (*Wx-B1*) and 7D (*Wx-D1*). Using a range of cultivars carrying the different null alleles at the *Wx* loci, variation for amylose content was investigated to determine the effects of each null allele on changing the content. Amylose content varied from 20% to 25% (100 mg starch base). A highly significant correlation was detected between the field-grown and growth chamber materials, indicating that amylose content is genotype-dependent. Genetic variation was mostly attributable to the differences between the five allele types at the *Wx* loci, indicating that the cultivar difference in amylose content is primarily under control of the *Wx* loci. It was confirmed that the effect of the null *Wx-B1b* on reducing amylose content is largest among the three null *Wx* alleles. Five cultivars carrying the *Wx-B1b* allele showed a significant variation by about 2% of amylose content, suggesting that a part of amylose synthesis is affected by genes located on background chromosomes. It appeared that a combined effect of the null *Wx-B1b* with *Wx-A1b* on reducing amylose content was not additive and led to lower content than the expected one.

**Key words :** amylose content, cultivar difference, *Triticum aestivum*, *Wx* gene, Wx protein

## INTRODUCTION

Starch is the major component of wheat (*Triticum aestivum* L.) grain, and its quality is extremely important in producing marketable wheat for end uses. The flour from soft wheat is often processed into the noodles in Asian countries such as Japan, China and Korea. Rapid gelatinization of starch is desirable for the processing and eating qualities of noodle (Moss, 1979 ; Oda *et al.*, 1980 ; Lee *et al.*, 1987 ; Toyokawa *et al.*, 1989). The storage starch in the endosperm usually consists of two carbohydrate polymers, amylose and amylopectin. The ratio of amylose

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to amylopectin, that is the apparent amylose content, varies from 21% to 30% (mg of amylose/100 mg of flour) in Japanese cultivars (Kuroda *et al.*, 1989). There is a strong relation between the amylose content and amount of the Wx proteins (Yamamori *et al.*, 1992; Miura and Tanii, 1994), providing a strong evidence that wheat Wx proteins are involved in amylose synthesis. Wheat has three distinct Wx proteins which are the products of the *Wx* genes at a triplicate set of single-copy homoeoloci located on chromosomes 7A (*Wx-A1*), 4A (*Wx-B1*) and 7D (*Wx-D1*) (Chao *et al.*, 1989). The ability of amylose synthesis is different among the *Wx* genes, being the largest ability of the *Wx-B1a*, followed by either *Wx-A1a* or *Wx-D1a* (Miura *et al.*, 1994; Miura and Sugawara, 1996).

For the noodle manufacture, low-amylose-content cultivars have been preferred (Yamamori *et al.*, 1992; Miura and Tanii, 1994). The Australian Standard White (ASW), a wheat brand composed of several cultivars and highly regarded in markets, has a reduced amylose content and suitable stickiness of noodles. ASW and Japanese cultivars which have been accepted into noodle classification are frequently lack the Wx-B1 protein (Miura and Tanii, 1994; Yamamori *et al.*, 1994; Zhao and Sharp, 1995). The fact supports the predominant effect of lacking the Wx-B1 protein due to the inactive *Wx-B1b* allele on reducing amylose content.

However, whether cultivars lacking the Wx-B1 protein vary for amylose content is not well documented. We also do not know how lacking either the Wx-A1 protein or Wx-D1 protein affect amylose content. In this experiment, using a range of spring wheats carrying the dfferent null *Wx* alleles, the effects of each allele on changing amylose content were investigated.

## MATERIALS AND METHODS

*Plant materials*

13 cultivars listed in Table 1 were used. They consist of three Japanese cultivars, Kanto 82, Kanto 79 and Kanto 107, five of the cultivars Cadoux, Eradu, Gamenya, Halberd and Kulin from Australia, and Bai Huo from China. Penawawa, Wadual and Wawawai were provided by Dr. C. Konzak, Wasington State University, USA. Chinese Spring (CS) was included as a check. They were grown under two environmental conditions, the experiment field of Obihiro Usiversity and the growth chamber, in the 1995 growing season. Wadual and Wawawai were raised only in the growth chamber.

Six plants of each cultivar were grown in the growth chamber with a mean temperature of 20°C (day/night; 24°C/16°C). In the field trial, each cultivar was represented by a single plot of 12 plants, spaced 10 cm between plants within a row and 30 cm between rows. The experiment plots were covered with transparent vinyl in order to protect from preharvest sprouting shortly after anthesis. Harvesting was carried out at maturity, and ears were hand-threshed in both experiments.

*Amylose content*

50-100 g grain samples conditioned to about 14% moisture content were milled on a Brabender Quadrant Junior Test Mill to produce flour of a 60% extraction. Starch granules were separated from the resultant flour in accordance with conventional methods. The amylose content was colorimetrically estimated using the Auto Analyzer System (Bran+Lubbe Co Ltd.) in Hokkaido Prefectural Kitami Agricultural Experiment Station and expressed as a percentage of apparent amylose content per 100 mg of starch granules. The assessment was carried out at least in triplicate.

*Identification of the* Wx *alleles*

Starch granule from mature grain was pre-

Table 1. The production of Wx proteins and identification of the alleles at the *Wx* loci of the cultivars used in the experiment

| Cultivars | Production of the Wx proteins | | | Alleles at the *Wx* loci |
|---|---|---|---|---|
| | Wx-A1 | Wx-B1 | Wx-D1 | |
| CS, Kulin, Wadual, Wawawai | + | + | + | *Wx-A1a*, *Wx-B1a*, *Wx-D1a* |
| Kanto 82 | − | + | + | *Wx-A1b*, *Wx-B1a*, *Wx-D1a* |
| Cadoux, Eradu, Gamenya Halberd, Penawawa | + | − | + | *Wx-A1a*, *Wx-B1b*, *Wx-D1a* |
| Bai Huo | + | + | − | *Wx-A1a*, *Wx-B1a*, *Wx-D1b* |
| Kanto 79, Kanto 107 | − | − | + | *Wx-A1b*, *Wx-B1b*, *Wx-D1a* |

pared with SDS extraction buffer which consisted of 0.55 M Tris-HCl pH 6.8, 2.3% SDS, 5% 2-mercaptoethanol and 10% glycerol (Echt and Schwartz, 1981). Starches were washed three times with the SDS buffer, and then twice with acetone.

An aliquot of 10 mg of each starch granule sample was mixed 400 $\mu$l of lysis buffer containing 8 M urea, 2% Nonidet-P 40, 2% ampholine pH 3.5-10, 5% 2-mercaptoethanol and 5% polyvinylpyrrolidone. The solutions were boiled for 1 min, and then centrifuged at 12000 rpm for 5 min at 4°C. Using the supernatant, the null alleles at the *Wx* loci of each cultivar were determined as lacking of particular Wx proteins encoded by the loci, employing the modified 2D-PAGE system (Nakamura *et al.*, 1993). 15% separating gels were in the ratio of 30 : 0.135 acrylamide/bis acrylamide. After electrophoresis, proteins were stained with silver-stain kits (Wako Pure Chemical Industry Ltd).

## RESULTS

The *Wx* alleles of cultivars examined were unambiguously identified by the 2D-PAGE pattern of Wx proteins and classified into five types of Table 1. CS, Kulin, Wadual and Wawawai produced all of the three Wx proteins. Kanto 82 was deficient for the Wx-A1 protein and Bai Huo lacked the Wx-D1 protein. Of the remaining seven cultivars, Kanto 79 and Kanto 107 produced only the Wx-D1 protein, and four cultivars from the ASW and Penawawa were the deficient of the Wx-B1 protein.

Amylose content varied from 20.7% to 24.9% in the field-grown materials, and from 19.3% to 25.2% in the growth chamber materials (Table 2). The correlation coefficient between the two growth conditions was highly significant ($r=0.94$, $P<0.01$). The amylose content of CS was 24.89% in field-grown matemials, and 25.18% in growth chamber materials. Kanto 107 and Kanto 79 lacking both the Wx-A1 and Wx-B1 proteins exhibited the lowest content in both growth conditions, being about a 5% lower content than CS. Cadoux, Eradu, Gamenya, Halberd and Penawawa lacked the Wx-B1 protein, and produced grains with 1-3% lower content than CS. While the amylose content of Kanto 82 with the *Wx-A1b* was not different from that of CS, Bai Huo with the *Wx-D1b* showed 1-2% lower content. Kulin had three Wx proteins, but exhibited a 1.2% lower amylose content than CS, over the two growth conditions.

The result of analysis of variance shown in Table 3 indicated that the difference in amylose content between cultivars was remarkable, compared to the variations caused by growth conditions and the cultivar x condition interaction. Partitioning of the genetic variation further revealed that the cultivar difference was largely attributable to the differences between the five allele types at the *Wx* loci, while variation

within the types was still significant.

Table 2. The deviations for amylose content of the cultivars from CS, grown in the field and growth chamber

| Cultivars | Deviations from CS (%) | |
|---|---|---|
| | Field | Growth chamber |
| Kulin | −1.22* | −1.20* |
| Wadual | — | −0.28 |
| Wawawai | — | −0.36 |
| Kanto 82 | −0.38 | −0.61 |
| Cadoux | −1.42* | −1.09* |
| Eradu | −2.84*** | −2.71*** |
| Gamenya | −1.20* | −1.17* |
| Halberd | −1.35* | −2.30*** |
| Penawawa | −2.76*** | −2.80*** |
| Bai Huo | −0.97 | −2.25*** |
| Kanto 79 | −4.23*** | −5.04*** |
| Kanto 107 | −4.15*** | −5.87*** |
| CS | 24.89 | 25.18 |

*, ***; Significantly different from CS at the 5% and 0.1% levels, respectively.

Table 3. Analysis of variance of amylose content of the 11 cultivars grown in the field and growth chamber

| Item | df | Mean squares |
|---|---|---|
| Cultivars, C. | 10 | 15.460*** |
| Between the *Wx* allele types | 4 | 33.373*** |
| Within the *Wx* allele types | 6 | 3.517** |
| Growth conditions, G. | 1 | 0.378 |
| C×G | 10 | 0.644 |
| Error | 44 | 0.709 |

**P<0.01, ***P<0.001.

## DISCUSSION

Variation for amylose content between the cultivars examined was from about 19 mg to 25 mg per 100 mg starch granules. This almost corresponded to the range of from 21% to 30% (100 mg flour base) detected over 133 cultivars (Kuroda *et al.*, 1989), and suggesting that around 20% amylose is the lowest limit on a cultivar level. A highly significant correlation of amylose content was detected between the field-grown and growth chamber materials, indicating that amylose content is genotype-dependent. A large part of genetic variation was explained by the differences between the five allele types at the *Wx* loci (Table 3). These indicate that the cultivar difference in amylose content is primarily under the control of major genes or the *Wx* loci. While only one cultivar with null *Wx-A1b* and that with null *Wx-D1b* were included in the experiment, it was clear that the effects of the three null alleles at the *Wx* loci on reducing amylose content are different. The null *Wx-B1b* reduced amylose content greatly compared to the *Wx-A1b* or the *Wx-D1b* alleles. These results agreed with our previous studies using aneuploid lines and single-chromosome substitution lines available in CS (Miura *et al.*, 1994; Miura and Sugawara, 1996).

The five cultivars with the *Wx-B1b* allele, however, had a significant variation by about 2% of amylose content (Tables 2, 3), suggesting that a part of amylose synthesis is affected by genetic background or by the genetic factors independent of the *Wx* loci. A small but practical difference appeared between Kulin and three cultivars, CS, Wadual and Wawawai, would also support this hypothesis, since the expression of the Wx proteins was normal and alike in these four cultivars. Furthermore, whereas Kanto 82 lacking the Wx-A1 protein had about 25% amylose and was not different from CS. Kanto 107 and Kanto 79 which lack both the Wx-A1 and Wx-B1 proteins exhibited the lowest amylose content of around 20%. This reduction by 5% was not explained by the sole effect of the *Wx-B1b*. Hence it appeared that a combined effect of null *Wx-B1b* with *Wx-A1b* on reducing amylose content was not additive.

Even if the amylose content is affected by genetic background, it is thought that the null *Wx-B1b* can produce the most reduced amylose content compared to other two null *Wx-A1b* and *Wx-D1b* or to the background genes. In addition, except for Kulin, all of the component cultivars of ASW examined carry the null *Wx-B1b* allele, and showed low amylose content as being suitable for making Japanese noodle. So it is confirmed that the low levels of amylose content in ASW are ascribable to the null *Wx-B1b* allele. This would lead to an idea that the breeding of low-amylose-content cultivars will success by introduction of the null *Wx-B1b* allele primarily. Although selection for low amylose in early generations could prove fruitful, screening in the segregating generations is impracticable due to difficulties in accurate measuring the amylose content on a single-grain basis. The Wx proteins are the product of the *Wx* genes. Using a modified SDS-PAGE system described by Kagawa *et al.* (1988), we have demonstrated that the Wx proteins of a half endosperm can separate readily (Miura and Tanii, 1994). This may permit screening of the grains with the null *Wx* alleles in large populations even in early segregating generations like $F_2$ and $B_1F_1$. The selected half grain including embryo will offer progenies in progressed generations to assess starch properties such as low amylose content and high paste viscosity.

Furthermore, if wheat breeder requires more reduced amylose content like Kanto 107 and Kanto 79, minor but significant modifications would be probable by means of combination of the *Wx-B1b* with other null alleles at the *Wx-A1* and *Wx-D1* loci. It is also desirable to manipulate genes located on background chromosomes which can produce a beneficial variation within the *Wx-B1b* type.

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## 異なる劣性 *Wx* 遺伝子をもつコムギ品種のアミロース含量の変異

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### 摘　　要

イネ科穀類の胚乳デンプンの特性は、構成成分であるアミロースとアミロペクチンの比率によって大きく変化する。6倍体コムギでは三つの *Wx* 座(*Wx-A1*、*Wx-B1*、*Wx-D1*)がコードするWxタンパク質がアミロース合成を司っている。*Wx* 遺伝子のアミロース合成能力には差異があり、*Wx-B1* 遺伝子の効果が最も大きい。

本試験では、それぞれの劣性対立遺伝子がアミロース含量をどの程度低下させるか調査した。劣性対立遺伝子 *Wx A1b* をもつ関東82号、劣性対立遺伝子 *Wx-B1b* をもつCadoux、Eradu、Gamenya、Halberd、Penawawa、劣性対立遺伝子 *Wx-D1b* をもつBai Huo、さらに *Wx-A1b* と *Wx-B1b* をともにもつ関東79号と関東107号を用いた(Table 1)。対照品種として3座とも優性対立遺伝子をもつChinese Spring(以下CS)とKulinを加えた。*Wx* 遺伝子型はWxタンパク質の二次元電気泳動パターンから同定した。圃場と人工気象室で栽培、登熟させた種子の胚乳デンプンをオートアナライザーで比色定量しアミロース含量を推定した。供試した11品種には圃場の材料で20.7～24.9%、人工気象室の材料で19.3%～25.2%の変異があった(Table 2)。環境間の高い相関($r=0.94$、$P<0.01$)から、アミロース含量は遺伝的支配を強く受ける形質であるといえる。関東82号とBai HuoのアミロースCS含量はCSより1～2%、*Wx-B1* 座が劣性な5品種は1～3%低く、系統間の遺伝変異は大部分が *Wx* 座の劣性対立遺伝子の違いで説明できた(Table 3)。*Wx-B1b* のアミロース含量を減少させる効果は、*Wx A1b*、*Wx-D1b* に比べ大きかった。しかし *Wx B1* 座が劣性である5品

種間の約２％の有意な変異や、CS と Kulin のアミロース含量の差異から、遺伝背景の影響や *Wx* 座以外の遺伝子効果が示唆された。さらに関東107号と関東79号のアミロース含量は20％程度で最も低く、二重劣性によるアミロース含量の低下が相加的でないことが示された。

**キーワード**：アミロース含量、品種間差異、コムギ、*Wx* 遺伝子、Wx タンパク質