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Differential effects of Wx-A1, -B1 and -D1 protein deficiencies on apparent amylose content and starch pasting properties in common wheat

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Abstract Waxy (Wx) protein is a granule-bound starch synthase (GBSS) responsible for amylose production in cereal endosperm. Eight isolines of wheat (*Triticum aestivum* L.) having different combinations of presence and absence of three Wx proteins, Wx-A1, -B1, and -D1, were produced in order to elucidate the effect of Wx protein deficiencies on the apparent amylose content and starch-pasting properties. An improved SDS gel electrophoresis showed that 'Bai Huo' (a parental wheat) carried a variant Wx-B1 protein from an allele, *Wx-B1e*. Thus, wheat lines of types 1, 2, 4, and 6 examined in this study contained a variant *Wx-B1* allele and not the standard allele, *Wx-B1a*. The results from 3 years of experiments using 176 lines derived from two cross-combinations showed that apparent amylose content increased the least in type 8 (waxy) having no Wx proteins and, in ascending order, increased in type 5 (only the Wx-A1 protein is present) <type 7 (Wx-D1) <type 6 (Wx-B1) <type 3 (Wx-A1 and -D1) <type 4 (Wx-A1 and -B1) <type 2 (Wx-B1 and -D1) <type 1 (three Wx proteins). However, Tukey' s studentized range test did not detect significant differences in some cases. Densitometric analysis suggested that the amylose content was related to the amount of the Wx protein in the eight types. Parameters in the Rapid Visco-Analyzer test and swelling power were correlated to amylose content. Consequently, amylose content and pasting properties of starch were determined to be influenced the most by the lack of the Wx-B1 protein, followed by a lack of Wx-D1, and leastly by the Wx-A1 deficiency, which indicated the presence of differential effects of the three null alleles for the Wx protein.

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Introduction

The major component of wheat flour is starch, which accounts for about three-quarters of the composition of flour. There are two types of starch, amylose and amylopectin. Amylose is less branched, or essentially a linear glucose polymer, while amylopectin is highly branched. Owing to the presence of two kinds of glucose polymer, amylose content (%) is used as one of the parameters characterizing the starch of flour. In common wheat (*Triticum aestivum* L.), the range of amylose content is restricted compared to that of rice whose amylose content ranges from 0 to about 30% (Nakagahra et al. 1986). In Japanese wheat cultivars, the apparent amylose content of flour ranges from 22% to 30% (Kuroda et al. 1989). Waxy (amylose-free) wheats have been produced in Japan (Nakamura et al. 1995; Kiribuchi-Otobe et al. 1997; Yasui et al. 1997).

Amylose content plays an important role in the quality of wheat since it affects starch properties. For the Japanese white salted noodles, 'udon', lower amylose tends to increase the glutinosity of the noodles, a condition which most Japanese prefer. Comparatively low amylose content is one of the reasons why an Australian wheat brand, Australian Standard White (ASW), is considered suitable for 'udon' noodles (Oda et al. 1980; Taira et al. 1989; Toyokawa et al. 1989). Thus, elucidation of the genetic basis for amylose variation will be helpful for breeding wheat with good noodle-making quality.

Waxy (Wx) protein, which is produced by the *waxy* genes, is a granule-bound starch synthase (GBSS) responsible for amylose synthesis. Because common wheat is allohexaploid (AABBDD genomes), it has three different Wx proteins, Wx-A1, -B1, and -D1. Yamamori et al. (1994) reported the world-wide distribution of Wx protein deficiencies in wheats. This result together with

Table 1 Classification of wheat into eight types based on presence (+) and absence (–) of Wx-A1, -B1, and -D1 proteins

Type	Presence (+) and absence (-) of Wx protein		
	-A1	-B1	-D1
1	+	+	+
2	–	+	+
3	+	–	+
4	+	+	–
5	+	–	–
6	–	+	–
7	–	–	+
8(waxy)	–	–	–

amylose contents in Japanese wheat (Kuroda et al. 1989) suggested that variation in amylose content is related to deficiencies in one or two of three Wx proteins. Based on the presence and absence of three Wx proteins, common wheat can be classified into eight types (Table 1). Although not all types (types 1, 2, 3 and 7) exist in Japanese wheat, the effects of Wx protein deficiencies on amylose content seem to differ between the Wx-A1 and Wx-B1 proteins; lack of the Wx-B1 protein reduced amylose content relative to lack of the Wx-A1 protein (Yamamori et al. 1994). For the effects of the three Wx proteins on amylose content to be understood, eight types should be produced. Discovery of the null allele for the Wx-D1 protein in a Chinese wheat enabled the eight types to be obtained (Yamamori et al. 1994).

In the study reported here, the eight possible types of wheat were produced by crossing Japanese wheats lacking both the Wx-A1 and -B1 proteins to a Chinese wheat lacking the Wx-D1 protein. Apparent amylose content in the eight types obtained from 3-year experiments and the relationship between amylose content and amount of the Wx protein are described. Starch-pasting properties analyzed by the Rapid Visco-Analyzer and swelling power were also examined because these characters are related to noodle quality (Toyokawa et al. 1989; McCormick et al. 1991; Konik et al. 1993).

Materials and methods

Plant materials

In order to produce the eight types of wheats, which were classified on the basis of presence and absence of three Wx proteins (Table 1), we crossed Japanese cultivar 'Kanto 107' and/or 'Saikai 173' (type 7) to a Chinese cultivar, 'Bai Huo' (type 4). By the single-seed descent (SSD) method, 176 F_6 lines in total (85 lines from 'Bai Huo'/'Kanto 107' and 91 lines from 'Bai Huo'/'Saikai 173') were bred. In the 1995/1996 experiment (1996 harvesting year), F_7 seeds were harvested from 1 F_6 plant of each line at Ishigaki in Okinawa prefecture (southern part of Japan). In the 1996/1997 (1997 harvesting year) and 1997/1998 (1998 harvesting year) experiments, F_8 and F_9 seeds were harvested at Tsukuba in Ibaraki prefecture (middle part of Japan), respectively.

The effect of one dosage of three *Wx* alleles was analyzed by pollinating type-8 (waxy) line by types-5, -6, and -7 lines to obtain endosperm starches from F_1 seeds in 1997. The endosperm genotype of F_1 (type 8/type 5) seed is *Wx-A1a -A1b -A1b, Wx-B1b -B1b -B1b, Wx-D1b -D1b -D1b*, which was abbreviated to *Aaa, bbb, ddd*. Alleles *Wx-A1a*, *-B1a*, and *-D1a* are standard alleles (dominant) producing the Wx proteins present in 'Chinese Spring', and *Wx-A1b*, *-B1b*, and *-D1b* are null alleles (recessive) which can not produce the Wx protein (Nakamura et al. 1993a; Yamamori et al. 1994).

Electrophoresis

For starch preparation, crushed grains were steeped in 2% sodium dodecyl sulfate (SDS) solution and homogenized. The solution was passed through a nylon mesh, centrifuged at 15,000 rpm for 1 min, and the yellowish upper layer of the starch pellet was removed using a spatula. Washing of starch, centrifugation, and removal of a yellowish layer were repeated by 2% SDS, and a protein extraction buffer (55 m*M* Tris/HCl, pH 6.8, 2.3% SDS, 5% 2-mercaptoethanol, 10% glycerol) (Echt and Schwartz 1981). The starch was then washed twice by water, twice by acetone, and air-dried. The Wx protein was extracted by heating the starch in the protein extraction buffer in boiling water (Echt and Schwartz 1981); the supernatant was subsequently used. Eight types were identified by two-dimensional gel electrophoresis (2D-PAGE), in which isoelectric focusing was used for the first dimension and SDS-polyacrylamide gel electrophoresis was used for the second dimension (2D) (Nakamura et al. 1993a).

A new variant of the Wx-B1 protein was examined by applying a modified SDS-PAGE by Zhao and Sharp (1996). The gel size was $9 \times 6 \times 0.1$ cm, and the acrylamide concentration of the separation gel was 12.5% or 15%. Proteins were detected by a silver staining kit (Wako Pure Chemical Industries, Japan). We examined 331 wheat cultivars from six countries and regions. The previous study by 2D-PAGE (Yamamori et al. 1994) revealed that all these cultivars carried three Wx proteins (type 1).

To determine the amount of the Wx protein, we subjected the protein band of the Wx protein on Laemmli's SDS-PAGE gel to densitometry (Molecular Dynamics). This gel system could not separate the three Wx proteins into bands but produced one band of the Wx protein (Yamamori et al. 1992), which was suitable for densitometric analysis. For an internal standard, the 80-kDa band that is SGP-3 (Yamamori and Endo 1996) was used because its amount seemed to be constant. Three measurements for three lines of types 1–7, 'Bai Huo', 'Kanto 107', and 'Saikai 173', were made. The amount of the Wx protein relative to that of 'Norin 61' (100) was calculated.

Apparent amylose content

Apparent amylose content was measured by an Autoanalyzer (Bran+Luebbe) using 50 mg starch; the technique is based on colorimetric measurement of the iodine-starch complex (absorbance at 600 nm). For controls, the amylose content of waxy wheat starch was considered to be 0.6% and wheat starch purchased from Wako Pure Chemical Industries to contain 31.2% (Yamamori et al. 1995b). The amylose content (31.2%) was determined using potato amylose and amylopectin as controls. For each line of types 1–8, amylose was measured once, 'Norin 61', 'Bai Huo', 'Kanto 107', and 'Saikai 173' were analyzed in duplicate; and starch from F_1 endosperm was measured in triplicate.

Starch-pasting properties measured by the Rapid Visco-Analyzer and swelling power

To examine the starch-pasting properties of the eight types, we prepared 3 g of water-washed starch from wholemeal flour in 25 ml distilled water and subjected this suspension to the Rapid Visco-Analyzer (RVA) (Newport Scientific, Australia). 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 (Kiribuchi-Otobe et al. 1997). Peak viscosity (PV), minimum viscosity (MV) (or holding strength), and final viscosity (FV) were

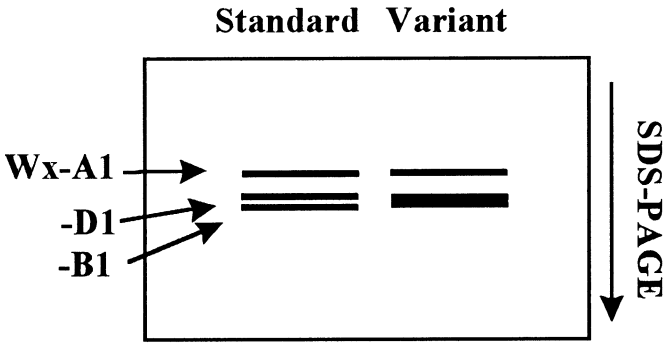

Fig. 1 One-dimensional electrophoretic profile of standard Wx-B1 protein from allele *Wx-B1a* and a variant from *Wx-B1e*

Table 2 Distribution of wheat cultivars carrying variant Wx-B1 protein revealed by an improved SDS-PAGE

Origin	Number of cultivars	
	Examined	Variant Wx-B1 produced by *Wx-B1e* (%)
Japan	64	2 (3.1)
China	52	3 (5.8)
USA	51	9 (17.6)
Australia	47	0 (0.0)
Russia	64	30 (46.9)
Western Europe	53	8 (15.1)
Total	331	52 (15.7%)

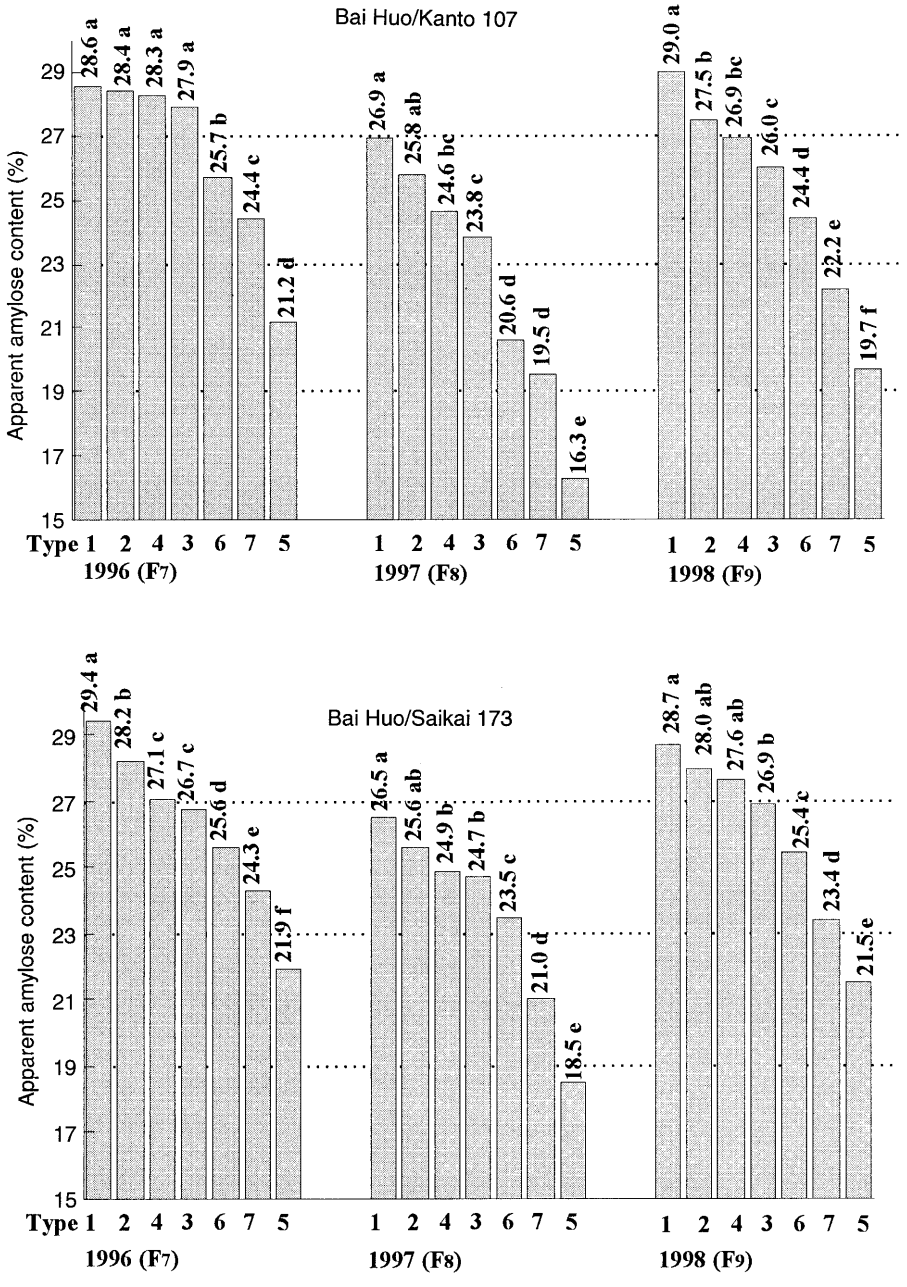

Fig. 2 Apparent amylose contents of wheat lines of types 1–7 in 3-year experiments. Amylose contents are shown on the *top* of the *column*. Values *followed* by the same *letter* in the same year and cross-combination are not significantly different ($P<0.05$, Tukey's studentized range test)

Table 3 Amylose contents in endosperm starch of F_1 seeds

Genotypes of F_1 endosperm	Apparent amylose content (%)	
	Cross A[a]	Cross B
Aaa, bbb, ddd[b]	6.7 a[c]	7.1 a
aaa, bbb, Ddd	8.3 b	7.7 b
aaa, Bbb, ddd	13.0 c	12.6 c

[a] Parents (F_7) of cross A were bred from 'Bai Huo'/'Kanto 107'; those of cross B were bred from 'Bai Huo'/'Saikai 173'
[b] Genotype *Aaa, bbb, ddd* indicates one dosage of *Wx-A1a* and null for both *Wx-B1* and *-D1*, which was bred by a cross, type 8(waxy)/type 5. Genotype *aaa, bbb, Ddd* was from type 8/type 7, and *aaa, Bbb, ddd* was from type 8/type 6
[c] Values followed by the same letter in the same column are not significantly different (P<0.05, Tukey's studentized range test)

recorded, and break-down (PV minus MV), total set back (FV minus MV), and set back (PV minus FV) were calculated. Five lines were used for types 1–7, and three lines for type 8, and there were all analyzed once. 'Norin 61', 'Bai Huo', 'Kanto 107', and 'Saikai 173' were analyzed in duplicate.

Swelling power was examined according to a modified method of McCormick et al. (1991). Water-washed starch (0.16 g) was weighed into a test tube, and 5 ml of 0.1% $AgNO_3$ was added (Yasui et al. 1996a). Capped test tubes were incubated at 70°C in a water bath for 10 min, then in boiling water for 10 min. After cooling in water, tubes were centrifuged at 1,700 *g* for 4 min, and the supernatant was removed. Swelling power was measured as sediment weight (g/g). The water content of the starch was determined by drying the starch at 135°C for 1 h. Five lines of types 1–7, which were used in the RVA test, were examined in quadruplicate. 'Norin 61', 'Bai Huo', 'Kanto 107', and 'Saikai 173' were analyzed seven or eight times. Because type 8 (waxy) can not form a gel (Yasui et al. 1996b), it was not examined.

Results

A new variant for the Wx-B1 protein

The SDS-PAGE method of Zhao and Sharp (1996) can separate three wheat Wx proteins as three bands on a gel. This method was used to identify the presence and absence of three Wx proteins in the lines bred by the SSD method. Three Wx proteins of wheat cultivars 'Norin 61' and 'Chinese Spring' (control cultivars) yielded three bands as reported by Zhao and Sharp (1996). However, type-1 wheat from 'Kanto 107'/'Bai Huo' and 'Saikai 173'/'Bai Huo', which were identified by 2D-PAGE, did not produce three bands but two on SDS-PAGE (Fig. 1). The band with the higher molecular weight was the Wx-A1 protein and the lower band should consist of the Wx-B1 and -D1 proteins. The electrophoregram showed that the migration rate of the Wx-B1 protein in type-1 wheat was almost the same as that of Wx-D1 or slightly slower than that of Wx-B1 of 'Norin 61'. This result shows that 'Bai Huo' carries a new variant of the Wx-B1 protein. The frequency of the variant Wx-B1 protein varied among 331 cultivars from six countries or regions (0–47%), and the average frequency was 15.7% (Table 2). Cultivars carrying the variant Wx-B1 were, for example, 'Norin 44', 'Canthatch', 'Blue Boy II', 'Turkey Red', 'Eureka' and 'Götz'.

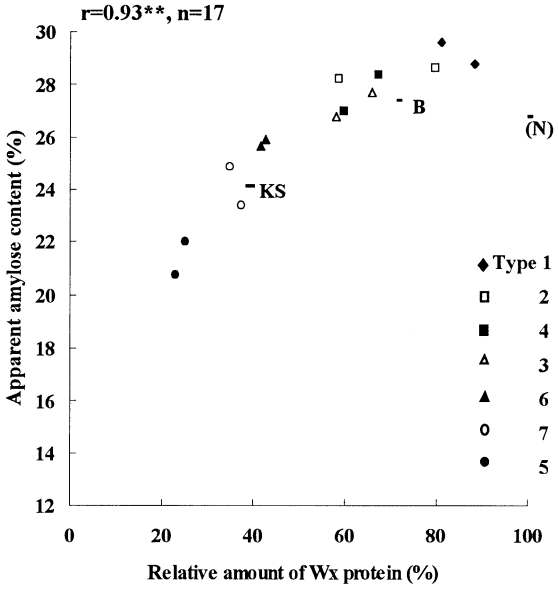

Fig. 3 Relationship between apparent amylose content and amount of the Wx protein measured by densitometry. *K* shows 'Kanto 107', *S* 'Saikai 173', *B* 'Bai Huo', *N* 'Norin 61'. 'Norin 61' is a control, and its value is 100. Though 'Norin 61' has three Wx proteins, it contains less amylose (26.8%) than type 1(28.6%, 29.4%). ** P<0.01

Effect of Wx protein deficiency on amylose content

By means of 2D-PAGE, F_7 seeds were classified into eight types, for each of which 5 (type 8)–18 (type 6) lines were analyzed. In both cross-combinations and during the 3 years of experiments, average apparent amylose content increased in the order of types 5 (lowest), 7, 6, 3, 4, 2, and 1 (highest) (Fig. 2). However, in some cases, Tukey's range test did not detect a significant difference (P<0.05); for example, among types 1–4 from 'Bai Huo'/'Kanto 107' (1996). Amylose percentage in the 1997 harvesting year was lower than those in 1996 and 1998 years, which might be due to year-to-year fluctuations. Types lacking two Wx proteins (types 5, 6, and 7) produced less amylose than types lacking one Wx protein (types 2, 3, and 4), and showed significant differences among the three types (Tukey's range test, P<0.05). The result that amylose increased in the order of types 5, 7, 6 means that, among the three Wx proteins, the Wx-A1 protein is the least responsible for amylose production, then the Wx-D1 protein, with the Wx-B1 protein generating the most amylose. In order to confirm this, we measured amylose percentages in F_1 seeds (1997 harvesting year). Amylose levels increased in the order of endosperms carrying one dose of *Wx-A1* (*Aaa, bbb, ddd*), endosperms carrying one *Wx-D1* (*aaa, bbb, Ddd*), then one *Wx-B1* (*aaa, Bbb, ddd*) (Table 3).

Densitometric analysis of the Wx protein in seven types of wheat (Fig. 3) showed a significant correlation between amounts of the Wx protein and amylose con-

Table 4 Starch-pasting properties by RVA and swelling power analyses in eight types and cultivars of wheat

Types and cultivars	Peak viscosity (SNU[a])	Minimum viscosity (SNU)	Final viscosity (SNU)	Break down (SNU)	Total set back (SNU)	Set back (SNU)	Swelling power (g/g)
Bai Huo/Kanto 107							
Type 1	232±14 a[c]	128±6 a	414±15 a	104±11 a	286±11 a	182±15 a	18.1±0.9 a
2	262±17 ab	128±8 a	371±13 b	134±18 b	243±8 b	109±24 b	19.6±0.8 ab
4	278±10 b	127±6 ab	372±18 b	151±10 bc	245±14 b	94±20 b	20.1±0.5 bc
3	298±23 b	129±15 a	353±22 bc	169±8 cd	224±9 b	55±6 c	21.4±0.7 cd
6	288±20 b	121±8 abc	322±14 c	167±16 cd	201±8 c	34±18 c	22.8±1.1 d
7	281±21 b	102±10 c	249±18 d	179±12 de	147±11 d	–32±9 d	25.0±0.7 e
5	283±15 b	106±13 bc	229±23 d	177±12 cde	123±13 e	–54±16 d	26.0±0.5 e
8 (waxy)	272±16 b	75±9 d	139±12 e	197±10 e	64±6 f	–133±4 e	–
Bai Huo/Saikai 173							
Type 1	241±20 a	130±9 a	410±17 a	111±15 a	280±20 a	169±21 a	17.5±0.8 a
2	263±16 ab	126±4 a	404±17 a	136±13 ab	277±14 a	141±6 a	18.7±0.3 a
4	291±17 bc	132±10 a	378±26 a	159±11 bc	246±20 b	87±27 b	20.9±1.2 b
3	303±13 c	120±8 ab	336±13 b	183±7 cde	216±7 c	33±11 c	22.6±0.4 c
6	277±21 bc	108±6 b	300±19 bc	168±20 cd	192±13 c	23±26 c	23.3±0.9 c
7	295±12 bc	107±8 b	265±18 c	189±16 de	158±12 d	–31±20 d	25.3±0.7 d
5	303±9 c	108±8 b	226±6 d	196±11 de	119±6 e	–77±8 e	26.4±1.0 d
8 (waxy)	290±8 bc	83±8 c	149±4 e	206±4 e	65±5 f	–141±4 f	–
Bai Huo (type 4)	250[d]	117	341	133	224	92	21.6±0.5
Kanto 107 (type 7)	296	112	275	185	163	–21	24.0±1.2
Saikai 173 (type 7)	302	113	275	190	162	–27.5	24.7±0.8
Norin 61 (type 1)	200	105	382	95	277	182	16.6±0.6
Coefficient of correlation[b]	–0.58* (–0.23)	0.85** (0.89**)	0.96** (0.88**)	–0.78** (–0.61**)	0.96** (0.85**)	0.93** (0.79**)	–0.94** (–0.88**)

[a] Stirring number
[b] Coefficient of correlations between amylose contents (Fig. 2) and peak viscosity – swelling power were calculated from the data of types 1—7 (n=14). Values in parentheses were calculated from data of types 1—8, 'Bai Huo', 'Kanto 107', 'Saikai 173' and 'Norin 61' (n=18 in SP or n=20 in the rest). ** P<0.01, * P<0.05
[c] Average ± standard deviation. Values followed by the same letter in the same column are not significantly different (P<0.05, Tukey's studentized range test)
[d] Analyzed in duplicate. Standard deviations are not shown

tents (1996 harvesting year) (r=0.93**, n=17, types 1–7 from two combinations, 'Bai Huo', 'Kanto 107', 'Saikai 173'). It also showed that the amount of the Wx protein tended to increase as Wx-A1 (type 5) <Wx-D1 (type 7) <Wx-B1 (type 6). However, Tukey's range test did not classify them into three groups. Due to this probable quantitative difference between the three Wx proteins, the effect of each Wx protein on amylose content would differ in wheat (Fig. 2). The quantitative differences between type 7 and types 1, 2, or ratio (%) of type 7/type 1 and type 7/type 2, were 43% and 54%, respectively, which were consistent with the following studies: Graybosch et al. (1998) reported the Wx protein content of US cultivar 'Ike' (type 7) was about 50% that of the wild type (type 1); the amount of the Wx protein in 'Kanto 107' (type 7) was 40 or 50% that of 'Norin 98' (type 2) (Yamamori et al. 1992; Nakamura et al. 1993b).

Starch-pasting properties by RVA and swelling power

In RVA and swelling power analyses, starches from the 1998 harvesting year were used. All parameters except for PV in RVA (Table 4) were significantly (P<0.01) correlated to amylose contents (Fig. 2) of types 1–7 derived from two cross combinations and/or all lines and cultivars used. The breakdown showed a negative correlation, and others were positive. Zeng et a l. (1997) reported a significant negative correlation between amylose content and PV in RVA which was not detected in this study. However, except for type 2, types 3–8 showed significantly (P<0.05, Tukey's range test) higher PV than type 1 which is the most frequent type of wheat germplasm (Yamamori et al. 1994). PV of type 8 (waxy) was similar to or a little lower than that of 'Kanto 107'. This result was different from that of both Kiribuchi-Otobe et al. (1997) and Hayakawa et al. (1997). Compared to 'Tanikei A6099' (a mutant of 'Kanto 107' with less amylose than 'Kanto 107'), a higher PV of waxy wheat was shown by Kiribuchi-Otobe et al. (1997), whereas another waxy wheat generated a lower PV than 'Kanto 107' (Hayakawa et al. 1997). Both groups reported that waxy wheats showed a low minimum and final viscosity and a low pasting temperature in the RVA test, all of which were detected in this study. PV is known to be influenced by amylase or sprouting damage. In this study sprouted grains were removed by visual inspection, but amylase activity was not measured. PV might be reduced by some factor in this experiment. Thus, it

was probable that the negative correlation between PV and amylose content was not high (r=-0.58*, P<0.05) or undetectable (r=-0.23) (Table 4). Though no parameters in the RVA test could distinguish all of the eight types, total set back and set back appeared to be the best parameters for classifying the eight types.

Swelling power (SP) was negatively correlated to amylose content; the average of SP in seven types increased the least in type 1, increasing in the order 2, 4, 3, 6, 7, and 5(the highest), which was the reverse order of their average amylose contents (Table 4; Fig. 2). Tukey's range test (P<0.05) grouped types 1–7 into five or six types. Types 5 and 6 from both cross-combinations were not significantly different though they were different in apparent amylose content.

Discussion

This study showed differential effects of deficiencies of the three Wx proteins on amylose content. The results from 3 years showed amylose increased the least in type 5 and increased in the order 7<6<3<4<2<1. The difference among the seven types is explained by the different amount of Wx proteins (Wx-A1<-D1<-B1). Amylose content was not linearly proportional to the dose of *Wx* allele in maize (Tsai 1974), which suggested that as the level of the wild type is approached (three doses of *Wx* allele), amylose content tends to plateau. A similar trend was observed in rice. As the rice Wx protein increased, amylose content became saturated (Hirano 1993). Figure 3 also suggested the presence of a plateau for amylose in wheat. The molecular mechanism causing the quantitative differences in the three Wx proteins is unknown. The amount of Wx-A1 protein was about 57% of that of the Wx-B1 in this study (Fig. 3). This percentage was 45% in 'Bai Huo', and it ranged from 65% to 74% in three tetraploid wheats (Yamamori et al. 1995b). In rice, the amount of the Wx protein derived from allele Wx^b is about 10% of that from Wx^a (Sano 1984). Molecular studies (Cai et el. 1998; Isshiki et al. 1998) suggest that this difference results from abnormal splicing of the first intron of the Wx^b gene, which accumulates less normal mRNA of the *waxy* gene in Wx^b rice.

The Wx-B1 protein in 'Bai Huo' is a new variant which is likely to be the same Wx-B1 as that reported by Rodríguez-Quijano et al. (1998). They showed a novel Wx-B1 protein that migrated slightly slower than normal Wx-B1 (*Wx-B1a* product) on one-dimensional SDS-PAGE. They provisionally named the allele *Wx-B1c'* because there was a possibility it was the *Wx-B1c* product. However, 2D-PAGE showed that 'Bai Huo' does not have *Wx-B1c* (Yamamori et al. 1994). Therefore, we propose to designate the *Wx-B1* allele in 'Bai Huo' as *Wx-B1e*, which will be a synonym for *Wx-B1c'*. Gene symbols of *Wx-B1a* to *-B1d* have been used in common and tetraploid wheats (Yamamori et al. 1994; Yamamori et al. 1995a). The electrophoretic gel (9 × 6 × 0.1 cm) used was smaller than that of Zhao and Sharp (1996), which may be the reason why the Wx-B1 protein from *Wx-B1e* could not be separated from the *Wx-D1a* product in our gel. It must be noted that types 1, 2, 4 and 6 in this study do not have the *Wx-B1a* product but *Wx-B1e*. The results of 2D-PAGE (Nakamura 1993a; Yamamori et al. 1994) suggest that the Wx-B1 protein from *Wx-B1a* forms the largest portion among the three Wx proteins. Therefore, results using the standard allele *Wx-B1a* will probably not differ from the present results.

Correlations between amylose contents and starch-pasting properties in the eight possible types of wheat (Table 4) are consistent with the following results reported by three groups. Zeng et al. (1997) examined the relationship between amylose content and parameters of the RVA test using wheat cultivars including types 1, 3, and 7 ('Kanto 107'). They reported significant correlations between apparent amylose content and PV, MV, FV, break down, total set back, and set back in RVA. Zhao et al. (1998) reported that breeding lines with the GBSS-4A null genotype (which corresponds to type 3) showed higher PV and FV in RVA, higher flour swelling volume (FSV), and lower blue value than the wild type (type 1). FSV is a parameter used to characterize starch-pasting property and is closely related to SP (McCormick et al. 1991; Crosbie 1991). A blue value (absorbance of iodine-starch complex) corresponds to amylose content in this study. According to Tukey's range test, type 1 and type 3 in this study were significantly different in PV and FV by the RVA test, SP, and amylose content (Table 4; Fig. 2). Sasaki and Matsuki (1998) reported a significant negative correlation (r=-0.924) between amylose content and swelling power in six wheat cultivars of types 1 ('Norin 31', 'Chinese Spring', 'Norin 61'), 3 ('Haruhikari', 'Chihokukomugi'), and 7 ('Kanto 107').

Amylose content, parameters shown by RVA, and SP are known to be related to the eating quality (softness, elasticity) of Japanese white salted noodles, 'udon' (Oda et al. 1980; Toyokawa et al. 1989; McCormick et al. 1991; Crosbie 1991; Konik et al. 1993; Panozzo and McCormick 1993). The results demonstrated that flour or starch with lower amylose, higher peak viscosity in RVA, and higher SP received higher scores of eating quality in noodle sensory tests. Because types 1 and 5 were clearly distinguishable in amylose content and pasting properties (Fig. 2; Table 4), it is anticipated that they will produce noodles with different eating quality or texture. Though type-7 cultivars have been released to Japanese farmers, type 5 has not.

Apparent amylose content is not controlled by the *waxy* gene alone because the amylopectin structure influences the apparent amylose level or blue value. Enzymes different from the Wx protein (branching enzyme and starch synthase) are involved in amylopectin synthesis. However, genotypes or alleles affecting the amylopectin structure of wheat have not been studied in detail. This study showed a close correlation of types 1–7 to amylose content and/or starch-pasting properties. Classifying breeding lines based on the presence and absence of three Wx proteins will be helpful for estimating the starch-past-

ing properties or pre-assessment of noodle quality. Further, the eight types may become materials for studying which level of amylose or which type is optimum for eating quality (softness and elasticity) in ‘udon’ noodles. Consequently, combinations of three null alleles for the Wx proteins will influence noodle-making quality through differential control of amylose content in wheat.

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