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Amylose content and starch properties generated by five variant *Wx* alleles for granule-bound starch synthase in common wheat (*Triticum aestivum* L.)

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Abstract In common and durum wheats (*Triticum aestivum* L. and *T. durum* Desf.), variant *waxy* (*Wx*) alleles have been reported for three Wx proteins (Wx-A1, -B1 and -D1), responsible for amylose synthesis in flour starch. Five variant alleles, *Wx-A1c*, *-A1e*, *-B1c*, *-B1d* and *-D1c*, were examined to elucidate their effects on amylose content in flour starch. Common wheat lines carrying a Wx protein produced by one variant (e.g., *Wx-A1c*) and one control (e.g., *Wx-A1a*) allele were bred and their starches were compared. Results showed that *Wx-A1e* did not produce amylose (waxy phenotype), whereas three alleles (*Wx-A1c*, *-B1c* and *-B1d)* reduced amylose, and *-D1c* might have increased it slightly. Most data on blue value, swelling power and starch paste clarity in water and dimethyl sulphoxide also suggested the variant *Wx* alleles either reduced or increased amylose content.

Keywords *Triticum aestivum* · Starch · Amylose · Starch synthase · *Wx* allele

Introduction

Flour starch of hexaploid wheat (*Triticum aestivum* L.) usually consists of about 25% amylose (essentially a linear glucose polymer) and about 75% amylopectin (branched glucose polymer). The amylose percentage in starch of wheat flour affects the physicochemical properties of flour or its end-products. For example, compared to normal amylose level, low amylose starch may have higher swelling power (SP), lower set back and final viscosity in a Rapid Visco Analyzer (Sasaki and Matsuki 1998; Yamamori and Quynh 2000; Wickramasinghe et al. 2003), produce a softer gel (Ishida et al. 2003), and/or affect the eating quality of noodles (Oda et al. 1980). Thus, amylose content in wheat flour has been of interest for researchers of flour quality, food products, and breeding (Seib 2000).

Cereal starch is produced by the coordinated activities of starch synthase, starch branching enzyme, debranching enzyme, and other enzymes (Rahman et al. 2000). Waxy (Wx) protein is a (starch) granule-bound starch synthase I responsible for amylose synthesis. Because hexaploid common wheat consists of AABBDD genomes, it has three homoeologous *Wx* genes, i.e. *Wx-A1*, *Wx-B1* and *Wx-D1*. The gene *Wx-A1* belongs to the A genome, *-B1* to the B genome, and *-D1* to the D genome (Chao et al. 1989).

Analyses of the wheat Wx protein by two-dimensional gel electrophoresis showed protein polymorphism on isoelectric points or molecular weight; that is, the Wx protein was separated into the Wx-A1, -B1 and -D1 proteins (Nakamura et al. 1993). The *Wx-A1a*, *-B1a* and *-D1a* alleles are wild types present in the cultivar Chinese Spring, whereas *b* alleles are null types producing no Wx protein (Nakamura et al. 1993).

Gel electrophoresis using world wheat cultivars has found further variant Wx proteins or alleles; for example, *Wx-A1c*, *-B1c* and *-D1c* are in hexaploid common wheat (Yamamori et al. 1994), and *Wx-A1d*,

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-A1e and *-B1d* occur in tetraploid durum wheat (Yamamori et al. 1995). Other variant *Wx* alleles have been reported (Kiribuchi-Otobe et al. 1998; Rodríguez-Quijano et al. 1998; Nieto-Taladriz and Rodrígues-Quijano 2000; Marcoz-Ragot et al. 2000; Yasui 2004).

Variant Wx proteins may yield different amounts of amylose if they alter the ability to produce amylose. In fact, mutant alleles *Wx-D1f*(*e*) and *g*, which were generated by chemical mutagenesis, produced mutant Wx-D1 protein and lower amylose content than the wild allele *Wx-D1a* (Kiribuchi-Otobe et al. 1998; Yanagisawa et al. 2001; Yasui 2004). In this report, different types of wheat, with different amylose contents, were used to examine starch properties in order to determine which traits are usable for distinguishing them. In addition, the main purpose of the study was to describe the effects of the variant alleles *Wx-A1c*, *-A1e*, *-B1c*, *-B1d*, and *-D1c* (Yamamori et al. 1994, 1995) on amylose and starch.

Materials and methods

Wheat materials

For flour starch characterization, five types of wheat were used (Yamamori and Quynh 2000; Yamamori et al. 2006), viz. waxy (amylose-free) wheat (abbreviation wx) with no Wx protein, wheat with Wx-A1 protein (WxA), wheat with Wx-A1 and -D1 (WxAD) and wheat with Wx-A1, -B1 and -D1 (wild type, WxABD). High amylose wheat (HA) has Wx-A1, -B1 and -D1 but lacks another starch synthase (starch synthase IIa or SGP-1). Owing to the loss of SGP-1, starch of HA wheat appears to have a high content of amylose (Yamamori et al. 2006).

The five variant *Wx* alleles were *Wx-A1c*, *-A1e*, *-B1c* *-B1d* and *-D1c* (Yamamori et al. 1994, 1995) and seeds used were F_7 or F_8 generations. These were harvested in 2000 or 2001. *Wx-A1c* was derived from five common wheat cultivars (Pakistan Zairaishu WB 357, Pakistan Zairaishu WB 6, Pakistan Zairaishu (49P 70–27), Pakistan Zairaishu QT 105, Pakistan Zairaishu WB 27), *Wx-B1c* from four cultivars (Chousen 40, Junbuk 12, Cikotaba, AF 24), *Wx-D1c* from one cultivar (Scoutland), *Wx-A1e* from *Triticum durum* (KU 3659) and *Wx-B1d* was derived from *T. durum* (KU 4213D).

After crossing between the above wheats and a waxy wheat (which had no Wx proteins), lines with only one Wx protein produced by the variant *Wx* allele were selected. The presence or absence of Wx proteins was determined by one- or two- dimensional SDS-gel electrophoresis (Yamamori and Quynh 2000). Because *Wx-A1e* and *-B1d* were derived from durums ($2n = 28$), hexaploid ($2n = 42$) progenies were selected.

For control samples of variant *Wx* alleles, wheats with one Wx protein produced by either of *Wx-A1a*, *Wx-B1e* and *Wx-D1a* were used (Yamamori and Quynh 2000). The alleles *Wx-A1a* and *Wx-B1e* were derived from the cultivar Bai Huo, whereas *Wx-D1a* was derived from Kanto 107.

Starch isolation

Hammer-crushed grains were homogenized in a solution consisting of 2% sodium dodecyl sulfate (SDS) and 10% glycerol. Homogenates were passed through a 100 µm nylon mesh and centrifuged. A yellowish layer was removed with a spatula, and then mixed with the SDS solution. This was repeated twice, then the pellet was washed with distilled water twice, and twice with acetone. The resulting starch was used for measuring amylose, blue value (BV) and maximum absorbance (λ_{max}).

Water-washed starches (Yamamori et al. 2006) were used for the other analyses, viz. swelling power (SP), glucoamylase digestibility, starch paste clarity, and freeze-thaw stability.

Amylose content

Amylose was measured using an amylose/amylopectin assay kit (Megazyme Int. Ireland, Ireland) by the concanavalin A (Con A) method according to the manufacturer's protocol.

Swelling power (SP)

SP of water-washed starches was examined as per Yamamori et al. (2006). Starches (160 mg) were weighed in a 10 ml test tube, then 5 or 6.5 ml of 0.1% $AgNO_3$ was added. Capped test tubes were incubated at 70°C in a water bath for 10 min with shaking, then incubated in boiling water for 10 min. After cooling, tubes were centrifuged at 2,100×*g* for 10 min and the

supernatant was removed. SP was measured as sediment weight divided by dry sample weight (g/g).

SP using 40 mg starch followed Konik-Rose et al. (2001) with a slight modification: Instead of 1.0 ml of distilled water, 1.5 ml of 0.1% $AgNO_3$ was used.

Glucoamylase digestibility

The procedure followed the methods of Wickramasinghe et al. (2003) with slight modifications. Starch (20 mg) was suspended in 500 µl of distilled water with 250 µl of 0.1 M acetate buffer (pH 5.0) and 250 µl of glucoamylase (5 units). After shaking at 40°C for 4 h, 2 ml of ethanol and 10 ml of distilled water were added and centrifuged. Sugars of the supernatant (0.5 ml) were measured; 1 ml of 3–5 dinitrosalicylic acid reagent was added, and the mixture was soaked in a boiling water bath for 5 min, then 4.5 ml of distilled water was added. Absorbance at 535 nm was then measured. The value was expressed as mg of maltose released per gram of starch.

Starch paste clarity

The paste clarity of starch in water was determined according to the method of Craig et al. (1989). Starch (50 mg, db) was suspended in 5 ml of distilled water in a capped glass tube, and the tube was soaked in a 95°C water bath for 30 min with shaking every 5 min. After cooling to room temperature, paste clarity (percent transmittance (%T) at 650 nm) was measured by spectrophotometer (U-200A, Hitachi, Japan) against a water blank. It was also measured after being stored at 4°C for 1, 3 and 7 days.

The paste clarity of starch in dimethyl sulphoxide (dehydrated DMSO containing max. 0.005% water, Wako Co., Japan) was determined according to the method of Singh et al. (2006). Starch (50 mg, db) was suspended in 10 ml of dehydrated DMSO, and the tube was shaken at 23°C. Paste clarity (%T at 640 nm) was measured against a DMSO blank after 4, 8, 16, 24, 36, 48 and 60 h.

Freeze-thaw stability of starch gel

The freeze-thaw stability of starch was examined according to the method of Zheng and Sosulski (1998) with slight modifications. Starch paste was prepared using a Rapid Visco Analyzer (RVA) (Newport Scientific, Australia); 1 g (dry basis) of water-washed starch in 12.5 ml of distilled water was subjected to the RVA (Yamamori et al. 2006). Using about 2.0 g of cooled fresh gel, the amount of water (free water) separating from the gel after centrifugation for 10 min at 4,500×g was measured. Using cooked gel in a plastic 50 ml test tube, the amounts of expelled and absorbed water were determined through three freeze-thaw cycles (freezing at −20°C for 16 h, thawing at 40°C for 2 h) over 76 h. The amount of expelled water was measured as separated water by decanting the thawed gel. After removing the expelled water, the absorbed water was measured as separated water by centrifuging about 2.0 g of gel at 4,500×g. Net syneresis (%) was calculated using the formula: expelled water + absorbed water − free water (%).

Apparent blue value (BV) and maximum absorbance (λ_{max})

The BV and λ_{max} were measured as described by Yamamori et al. (2006). Absorption curves of gelatinized starch-iodine complexes were measured at 500–700 nm to determine the absorbance at 680 nm (apparent BV) and λ_{max} of the iodine-starch complex.

Results and discussion

Starch characterization of wheats with five different amylose levels

Amylose content in starches of the five wheats (wx, WxA, WxAD, WxABD and HA) are shown in Table 1. Statistical analyses showed they produced different levels of amylose. Starch SP was distinguishable among the five wheats though SP of wx was not determined because the wx starch did not form a rigid gel (Table 1); amylose content was negatively correlated with the value of SP.

Glucoamylase treatment produced more maltose in the order of WxABD, WxAD < WxA < HA < wx (Table 1). Except for HA, amylose content tended to be negatively correlated with maltose release. These results corroborated those of previous studies (Sasaki and Matsuki 1998; Yamamori and Quynh 2000; Wickramasinghe et al. 2003). In the following analyses

Table 1 Amylose content, swelling power (SP) and glucoamylase digestibility of five wheat groups

Wheat	Amylose (%)	SP of 160 mg starch (g/g)	Glucoamylase digestibility (maltose mg/g starch)
Wx	1.9 ± 0.6a	–	881 ± 52d
WxA	23.8 ± 0.6b	29.7 ± 0.3d	387 ± 46b
WxAD	28.9 ± 1.1c	24.0 ± 1.0c	249 ± 41a
WxABD	29.7 ± 0.4c	19.2 ± 1.7b	240 ± 41a
HA	36.0 ± 1.1d	10.2 ± 0.3a	613 ± 35c

For each group, four lines were used and values were derived from four measurements of amylose and glucoamylase digestibility, and eight measurements of SP (two measurements per sample)

Values followed by the same letter in a column are not significantly different ($P < 0.05$, Tukey's studentized range test)

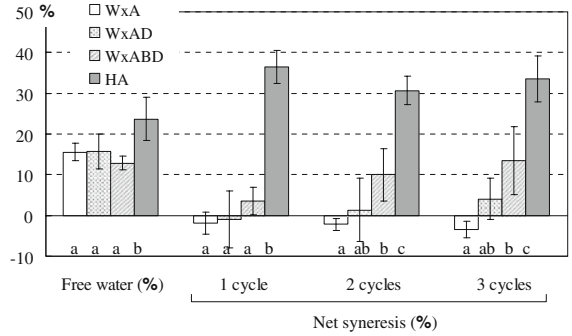

Fig. 2 Free water and net syneresis in freeze-thaw stability of starch gels. For each wheat, four lines were used and values were derived from four measurements (one measurement per sample). *Columns* with the same subscript letter in the cycle are not significantly different ($P < 0.05$, Tukey's studentized range test)

of wheats having variant *Wx* alleles, amylose and SP were examined.

Paste clarity in water varied among starches of the five wheats; wheat with higher amylose content showed a lower %T at 650 nm (Fig. 1). After boiling, the %T of WxA, WxAD, WxABD and HA decreased as days passed while that of wx was comparatively stable and high. The %T of five wheat starches differed from each other within 1 day. On the other hand, paste clarity in DMSO increased over time (Fig. 1). After 36–60 h, WxABD, WxAD, WxA and wx were distinguishable while the HA starch showed a unique trend. In the following analyses of the variant *Wx* alleles, paste clarities in both water and DMSO were examined.

The absorbed water of wx wheat starch was not measured because the starch gel fell to the bottom of the tube after centrifugation. Thus, net syneresis of wx was not estimated. Net syneresis of starches of the other four wheats rose as their amylose content increased (Fig. 2). After two or three freeze-thaw cycles, the differences among the starches became more distinct.

The values of SP, starch paste clarity in water and net syneresis of the five wheats seemed to be either negatively or positively related to amylose content. However, regarding glucoamylase digestibility and paste clarity in DMSO, the values for HA starch were closer to those of the wx starch than to those for WxABD. This uniqueness of the HA starch might be attributed to its altered structure of amylopectin which was caused not by the Wx protein but the starch synthase IIa (Yamamori et al. 2000; Hanashiro et al. 2004).

Wx-A1c and *-A1e* (variant alleles of *Wx-A1*)

On a two-dimensional electrophoretic gel, the variant Wx-A1 protein coded by *Wx-A1c* showed slightly

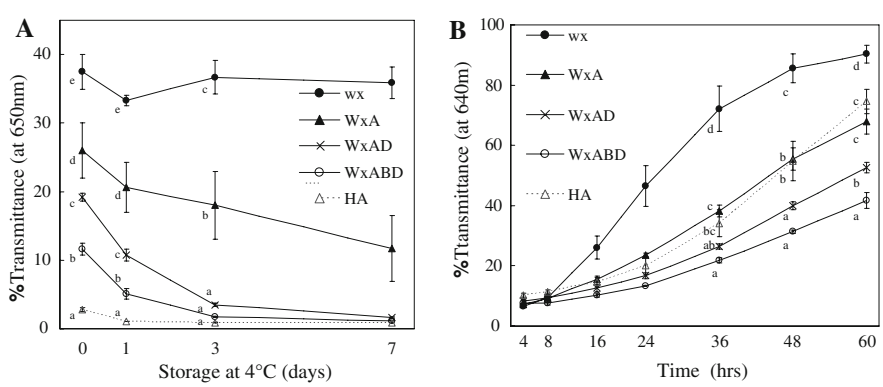

Fig. 1 Starch paste clarity in water (**a**) and DMSO (**b**). For each allelic group, four lines were used and values were derived from eight measurements (two measurements per sample). *Marks* with the same letter on the storage or time are not significantly different ($P < 0.05$, Tukey's studentized range test)

Table 2 Amylose content, blue value, maximum absorbance (λ_{max}) and swelling power of wheat starches having variant *Wx* alleles

Wx allele		Origin	Amylose (%)		Blue value	λ max(nm)	Swelling power (g/g)	
			2000	2001			160 mg	40 mg
Wx-A1	*c*	WB 357	13.8 ± 0.1**	9.6 ± 1.0**	0.219 ± 0.011**	555 ± 1**	30.4 ± 0.7**	–
		WB 6	13.5 ± 0.6**	9.5 ± 1.9**	0.216 ± 0.014**	556 ± 2**	30.3 ± 1.6**	20.4 ± 1.2^{ns}
		49 P70-27	12.3 ± 1.8**	11.7 ± 0.7**	0.214 ± 0.016**	555 ± 3**	32.6 ± 1.2**	22.1 ± 0.5**
		QT 105	14.8 ± 0.9**	11.1 ± 0.4**	0.228 ± 0.009**	557 ± 2**	31.3 ± 0.8**	21.3 ± 0.1*
		WB 27	13.1 ± 1.6**	12.4 ± 0.3**	0.214 ± 0.008**	557 ± 2**	30.5 ± 0.9**	21.7 ± 0.9**
		(Average)	13.5 ± 0.9**	10.9 ± 1.3**	0.218 ± 0.006**	556 ± 1**	31.0 ± 1.0**	21.4 ± 0.7**
	a	Bai Huo	23.2 ± 0.3	17.6 ± 1.8	0.286 ± 0.024	569 ± 4	28.0 ± 0.8	18.2 ± 1.2
	e	*T. durum*	–	0.5 ± 0.0^{ns}	0.084 ± 0.008^{ns}	534 ± 0**	–	–
	b (null)	Kanto 107	–	0.5 ± 0.1	0.086 ± 0.007	529 ± 1	–	–
Wx-B1	*c*	Chousen 40	19.4 ± 0.9**	16.4 ± 1.2**	0.243 ± 0.020**	566 ± 2**	27.0 ± 0.5**	–
		Junbuk 12	20.0 ± 1.3**	20.8 ± 1.4**	0.255 ± 0.006**	573 ± 1**	26.5 ± 0.6**	16.4 ± 0.9**
		Cikotaba	22.3 ± 1.0**	21.3 ± 1.2*	0.279 ± 0.010^{ns}	575 ± 2^{ns}	24.0 ± 1.4**	14.9 ± 1.0**
		AF 24	23.6 ± 1.2^{ns}	21.9 ± 0.6*	0.278 ± 0.016^{ns}	577 ± 5^{ns}	22.6 ± 1.3^{ns}	15.1 ± 0.9**
		(Average)	21.3 ± 2.0*	20.1 ± 2.5*	0.264 ± 0.018^{ns}	573 ± 5^{ns}	25.0 ± 2.1**	15.4 ± 0.8**
	d	*T. durum*	–	21.0 ± 0.7**	0.279 ± 0.012^{ns}	578 ± 4^{ns}	25.2 ± 1.2 **	–
	e	Bai Huo	25.0 ± 0.4	24.3 ± 1.2	0.288 ± 0.011	578 ± 2	22.1 ± 0.7	12.8 ± 0.5
Wx-D1	*c*	Scoutland	24.8 ± 1.0^{ns}	25.3 ± 1.2^{ns}	0.327 ± 0.015^{ns}	579 ± 2**	22.6 ± 1.0**	14.1 ± 0.9**
	a	Kanto 107	24.2 ± 0.8	23.1 ± 1.5	0.292 ± 0.026	570 ± 4	25.9 ± 0.3	17.5 ± 2.1

For each allele, one to four lines were used and values were derived from four measurements of amylose, BV and λ_{max}, from four to eight measurements of 160 mg SP and four measurements of 40 mg SP. For amylose measurement, starches from the 2000 and 2001 harvests were used, and starches from the 2001 harvest were used for the other measurements

**, * Significant at 0.01 and 0.05 (Student's *t*-test), respectively

ns, not significant

lower molecular weight (higher mobility in SDS-gel electrophoresis) and a slightly more basic isoelectric point than that coded by the wild allele *Wx-A1a* (Yamamori et al. 1994). Amylose percentages in starch produced by *Wx-A1c* which was derived from five Pakistani cultivars ranged from 12.3 to 14.8% in 2000 and 9.5 to 12.4% in 2001 whereas *Wx-A1a* was 23.2% in 2000 and 17.6% in 2001 (Table 2). The observation that amylose in 2001 was lower than in 2000 might be attributable to experimental error. However, the results suggest that *Wx-A1c* yielded about 60% of the amylose level generated by *Wx-A1a*.

Both the BV (0.214–0.228) and λ_{max} (555–557 nm) of the starch-iodine complex from *Wx-A1c* were significantly lower than those of *Wx-A1a* (0.286 and 569 nm), suggesting there was less amylose in starch from the *Wx-A1c* genotype compared with the *Wx-A1a* genotype. The SP of 160 mg starch produced by *Wx-A1c* ranged from 30.3 to 32.6 g/g, statistically greater than that of *Wx-A1a* starch (28.0 g/g). The SP of 40 mg starch produced by *Wx-A1c* (20.4–22.1 g/g) was also higher than that of the *Wx-A1a* starch (18.2 g/g). Because wheat starches with lower amylose tend to increase SP (Sasaki and Matsuki 1998; Yamamori and Quynh 2000), these results mean that *Wx-A1c* generates less amylose in starch than the wild *Wx-A1a*.

Paste clarities of the *Wx-A1c* starch in water were significantly higher than those of *Wx-A1a* at 0, 1, 3 and 7 days after treatment (Table 3). Furthermore, paste clarities of the *Wx-A1c* starch in DMSO at 36, 48 and 60 h after treatment were also significantly higher than those of *Wx-A1a* starch. These results provide further evidence that less amylose is produced by *Wx-A1c* (40%) than by *Wx-A1a*.

The Wx-A1 protein from the variant *Wx-A1e* of *T. durum* showed a more basic isoelectric point on the electrophoretic gel (Yamamori et al. 1995). Starch from *Wx-A1e* showed 0.5% amylose which was the same as waxy starch from the null allele *Wx-A1b*, and the BV of the *Wx-A1e* starch (0.084) was almost the

Table 3 Starch paste clarity in water (%T at 650 nm) and DMSO (%T at 640 nm)

Wx allele		Days after boiling water treatment				Hours after DMSO treatment		
		0	1	3	7	36	48	60
Wx-A1	*c*	28.9 ± 2.8*	24.6 ± 2.4**	23.9 ± 2.5**	23.5 ± 2.6**	54.6 ± 6.1**	66.0 ± 5.3**	77.4 ± 4.3**
	a	23.2 ± 1.6	14.1 ± 1.1	12.0 ± 0.3	9.5 ± 1.5	40.8 ± 1.5	54.6 ± 2.5	70.3 ± 2.2
Wx-B1	*c*	21.3 ± 2.7^{ns}	14.0 ± 3.0*	8.0 ± 4.2^{ns}	4.9 ± 3.9^{ns}	23.8 ± 4.4^{ns}	32.8 ± 6.7*	45.4 ± 9.3^{ns}
	d	24.3 ± 1.3**	15.0 ± 0.5**	6.5 ± 0.7**	2.8 ± 0.4**	29.8 ± 1.0^{ns}	42.9 ± 2.5^{ns}	59.1 ± 3.5^{ns}
	e	19.2 ± 1.1	10.0 ± 1.2	3.6 ± 0.7	1.8 ± 0.4	28.4 ± 2.9	40.7 ± 5.1	55.2 ± 6.2
Wx-D1	*c*	20.0 ± 4.1^{ns}	9.0 ± 2.9^{ns}	2.8 ± 1.0*	1.5 ± 0.4*	24.8 ± 3.1**	34.3 ± 6.0**	47.5 ± 8.6**
	a	16.8 ± 2.9	8.1 ± 1.9	5.3 ± 0.9	3.0 ± 1.0	36.1 ± 5.7	49.7 ± 7.0	64.3 ± 6.7

Values in water are derived from 13 measurements of *Wx-A1c*, four of *-A1a*, 14 of *Wx-B1c*, three of *-B1d*, four of *-B1e*, *Wx-D1c* and three for *-D1a*. Values in DMSO are from 14 measurements of *Wx-A1c*, four of *-A1a*, eight of *Wx-B1c*, four of *-B1d*, and five measurements of *-B1e*, *Wx-D1c* and *-D1a*

**, * Significant at 0.01 and 0.05 (Student's *t*-test), respectively

ns, not significant

same as that of *Wx-A1b* (0.086) (Table 2). Although λ_{max} of *Wx-A1e* starch (534 nm) was greater than that of *Wx-A1b* (529 nm), it was lower than *Wx-A1a* starch (569 nm). The flour starches and pollen grains of *Wx-A1e* stained by a KI-I_2 solution turned red-brown like those of waxy wheat, showing a waxy (amylose-free) phenotype. These results mean that *Wx-A1e* produces the Wx-A1 protein but it does not produce amylose, presumably due to the inability to synthesize amylose.

This kind of alteration was reported for the allele *Wx-D1f*(*e*); in the mutant Tanikei A6599-4, *Wx-D1f* produced the Wx-D1 protein, but showed much less amylose or waxy phenotype (1.6% amylose of *Wx-D1f* was slightly more than typical waxy (0.4%) (Kiribuchi-Otobe et al. 1998). The point mutation in *Wx-D1f* generated an alanine-to-threonine change and decreased enzymatic activity in the Wx-D1 protein (Yanagisawa et al. 2001, 2003).

Wx-B1c and *-B1d* (variant alleles of *Wx-B1*)

The Wx-B1 protein from the variant *Wx-B1c* showed a slightly more basic isoelectric point than the alleles *Wx-B1a* of Chinese Spring and *Wx-B1e* of Bai Huo (Yamamori et al. 1994; Yamamori and Quynh 2000). The amylose content of *Wx-B1c* from four cultivars was less than that of *Wx-B1e* in both years, except for AF 24 in 2000 (Table 2). On average, *Wx-B1c* produced about 85% of the amylose level of *Wx-B1e*.

The BV and λ_{max} of Chousen 40 and Junbuku 12 were lower than those of *Wx-B1e*, but those of Cikotaba and AF 24 were not significantly different from *Wx-B1e*. In SP of 160 mg starch, *Wx-B1c* from AF 24 was not significantly different from *Wx-B1e*, whereas other starches from *Wx-B1c* genotypes were different. In 40 mg starch SP, Junbuku 12, Cikotaba, AF 24 and their mean were higher than *Wx-B1e*. These results suggest the amylose level in starch produced by *Wx-B1c* genotypes was somewhat less (15%) than those with *Wx-B1e*.

Though the starch paste clarity of *Wx-B1c* in water was greater than that of *Wx-B1e*, it was statistically different from *Wx-B1e* only at 1 day after treatment (Table 3). On the other hand, the starch paste clarity of *Wx-B1c* in DMSO was lower than that of *Wx-B1e*. Paste clarity in water seemed to provide supporting evidence that *Wx-B1c* produces less amylose than *Wx-B1e*, but paste clarity in DMSO did not.

The Wx-B1 protein from the variant *Wx-B1d* of *T. durum* showed a slightly higher basic isoelectric point and a lower molecular weight compared to wild *Wx-B1a* (Yamamori et al. 1995). *Wx-B1d* produced 21.0% amylose which was less than *Wx-B1e* (24.3%) (Table 2). Although the BV and λ_{max} of *Wx-B1d* genotypes did not differ from those with *Wx-B1e*, the SP of 160 mg starch was significantly higher than that of *Wx-B1e*. The starch paste clarity of *Wx-B1d* in water was statistically higher than that of *Wx-B1e* on the 4 days examined, but its paste clarity in DMSO did not differ from that of *Wx-B1e* (Table 3). These results suggest that, like *Wx-B1c*, *Wx-B1d* also produced somewhat less amylose than *Wx-B1e*.

In this study, the allele *Wx-B1e* was used as a control for the variants *Wx-B1c* and *-B1d*, because the

only available wheat possessing only the Wx-B1 protein was bred using Bai Huo carrying *Wx-B1e* (not *-B1a*) as a parent (Yamamori and Quynh 2000). The Wx protein from *Wx-B1e* showed a slightly higher molecular weight on an electrophoretic gel than that from *Wx-B1a* and the allele was reported to occur in 12–16% of common wheat cultivars (Marcoz-Ragot et al. 2000; Yamamori and Quynh 2000).

In durum wheat, amylose from the genotype *Wx-A1a Wx-B1e*(*c'*) appeared to be higher (by ~2.9%) than that from *Wx-A1a Wx-B1a* (Nieto-Taladriz and Rodrígues-Quijano 2000; Rodríguez-Quijano et al. 2003), suggesting that *Wx-B1e* produces slightly more amylose than *Wx-B1a*. However, *Wx-B1e* in Bai Huo has not been compared to *Wx-B1a* in a common wheat.

Wx-D1c (variant allele of *Wx-D1*)

The Wx-D1 protein from *Wx-D1c* showed a slightly more basic isoelectric point than that from *Wx-D1a* (Yamamori et al. 1994). The average amylose values of *Wx-D1c* from Scoutland in the two harvest years were a little higher than those of *Wx-D1a*, but statistical differences were not detected (Table 2). Compared to *Wx-D1a*, the BV of *Wx-D1c* starch showed a higher value, but it was not statistically significant. The λ_{max} of *Wx-D1c* was greater than *Wx-D1a*, and the SP's of 160 and 40 mg starches were smaller than *Wx-D1a*. The starch paste clarity of *Wx-D1c* in water was significantly lower than that of *Wx-D1a* at 3 and 7 days after treatment, but it was not lower on the other 2 days (days zero and one) (Table 3). The starch paste clarity of *Wx-D1c* in DMSO was lower than that of *Wx-D1a* at 36, 48 and 60 h after treatment. These results suggest that *Wx-D1c* may produce a little more amylose (by 1–2%) than *Wx-D1a*.

For *Wx-D1*, seven alleles (Wx-*D1a* to *-D1 g*) have been reported (Yasui 2006); *Wx-D1a* is the wild standard, *-D1b*, *d*, and *e* are null alleles whereas *f* and *g* produce less amylose than *a*.

The present analyses suggest that the amylose synthesizing ability of *Wx* alleles is *Wx-A1b*(null) = *Wx-A1e* < *Wx-A1c* < *Wx-A1a* < *Wx-B1c* ≒ *Wx-B1d* ≦ *Wx-D1a* < *Wx-D1c* ≦ *Wx-B1e*. In our previous study (Yamamori and Quynh 2000), amylose content in flour starch was related to the amount of Wx protein; the amounts of Wx protein and amylose increased in the order of *Wx-A1a* < *-D1a* < *-B1e*. The present study indicates that Wx proteins with altered pIs or molecular weights also influence amylose content. The findings of *Wx* allelic variation for amylose production will contribute to the genetic and precision control of amylose percentage in flour. In addition, this study has shown that wheat lines carrying one of three Wx proteins are useful for examining the effect of the Wx protein because the influences of the other two Wx proteins are excluded.

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