

Increase in Apparent Amylose Content and Change in Starch Pasting Properties at Cool Growth Temperatures in Mutant Wheat

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ABSTRACT

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Some mutant wheat lines with low-amylose content were grown in a field and greenhouse (15 or 20°C) to compare apparent amylose content and starch pasting properties. The apparent amylose content of flour and starch increased and starch pasting parameters as measured by a Rapid Visco Analyser (RVA) changed in the greenhouse (at cool temperatures) during seed maturation. Densitometric analysis of the protein band separated by electrophoresis suggested that the increase in amylose content by cool temperature was related to the amount of Wx-D1 protein. This data suggests that the *Wx-D1* gene was responsible for these changes.

In wheat starch from Tanikei A6099 and Tanikei A6598 at 15°C, the value of final viscosity and total setback was higher than that from the field. In wheat starch from Tanikei A6599-4 (waxy mutant with stable hot paste viscosity), the peak viscosity temperature was higher and time maintained >80% of the peak was shorter at 15°C than that from the field. Genetic analysis using doubled-haploid (DH) lines from a combination of Tanikei A6599-4 and Kanto 118 (low-amylose line) showed that apparent amylose content increased and the starch pasting curve and properties changed in waxy progenies similar to Tanikei A6599-4.

Cereal grain endosperm starch generally consists of amylose and amylopectin. Amylose content is used as one of the parameters characterizing starch, and plays an important role in wheat quality because it affects starch properties. For Japanese white salted noodles (udon), lower amylose tends to increase glutinosity, a condition most Japanese prefer. In our breeding program, amylose content of flour was measured to select and characterize breeding lines because of an important breeding objective. Many measurements of apparent amylose content based on colorimetric method can be rapidly performed.

Elevated growth temperatures decrease the amylose content of rice (Asaoka et al 1984) and maize (Ferguson and Zuber 1962) starch. Although the effect of environmental temperature on barley starch amylose content was ambiguous (Tester and Karkalas 2001), in wheat, amylose contents increase slightly as a function of temperature (Tester et al 1995). Potato tuber and microtuber are similar to wheat (Cottrell et al 1995; Debon et al 1998) although authors report little effect (Hizukuri 1969; Tester et al 1999). The amylose content of soybean seedling hypocotyls and cotyledon starch shows little response to environmental temperature (Hizukuri 1969).

Amylose synthesis is controlled by the amount of waxy (Wx) protein, *waxy* (Wx) gene product, which is granule-bound starch synthase I (GBSSI) (Nelson and Rines 1962; Echt and Schwarz 1981). Hexaploid wheat has three Wx genes (*Wx-A1*, *B1*, and *D1*) coding for Wx protein isoforms (Wx-A1, B1, and D1) (Chao et al 1989; Nakamura et al 1993). A normal amylose content line has three active Wx proteins coded by *Wx-A1a*, *Wx-B1a*, and *Wx-D1a*. Variation in amylose content is related to deficiencies in one or two of the three Wx proteins (Yamamori et al 2000). A low-amylose content line having null alleles in the *Wx-A1* and *B1* gene (*Wx-A1b*, *Wx-B1b*) was bred in Japan, whose lines have only Wx-D1 protein.

Amylose content in rice with a *Wx^b* allele endosperm is affected by genetic regulation and by temperature during seed development. Wx protein increased at cool temperatures, resulting in higher content of amylose in mature seeds (Asaoka et al 1984; Sano et al 1985; Umemoto et al 1995). Insoluble and soluble starch synthase activity in potato microtuber is especially sensitive to growth temperature (Debon et al 1998).

During wheat seed maturation in the field, the highest temperature generally reaches 25°C or more in Tsukuba and in most of Japan. Many low-amylose wheat lines, including mutants, have been bred in Japan, but the effect of temperature during seed maturation on amylose content in these lines remains to be explained.

We analyzed apparent amylose content and the amount of Wx protein at cool temperatures (15 or 20°C) during seed development in low-amylose wheat with the *Wx-D1* gene to compare with those grown in the field. RVA parameters correlated to amylose content and RVA findings correlated to noodle quality were also used (Toyokawa et al 1989).

Tanikei A6599-4, assumed to be a waxy mutant wheat, exhibited a unique starch pasting curve with stable hot paste viscosity by RVA (Kiribuchi-Otobe et al 1998). We used doubled-haploid (DH) lines from a combination of Tanikei A6599-4 and another low-amylose line to study the origin of the change in apparent amylose content and starch pasting analyzed by RVA.

MATERIALS AND METHODS

Plant Materials

The plant materials used were Kanto 107, Tanikei A6099, Tanikei A6598, and Tanikei A6599-4. Kanto 107 is a low-amylose line with a null allele at *Wx-A1* and *Wx-B1* locus (*Wx-A1b*, *Wx-B1b*, *Wx-D1a*), and only Wx-D1 protein is active. Tanikei A6099 was induced from Kanto 107 by ethyl methane sulfonate treatment (Oda et al 1992). Previously, Yanagisawa et al (2001) showed that the amount of Wx-D1 protein in Tanikei A6099 is lower than that in Kanto 107. Tanikei A6598 and Tanikei A6599-4 were induced from Tanikei A6099 by sodium azide treatment (Kiribuchi-Otobe et al 1998, 2001a). None of these lines have Wx-A1 and B1 protein (*Wx-A1b*, *Wx-B1b*: null allele in *Wx-A1* and *B1* gene). Amylose content is Kanto 107 > Tanikei A6099 > Tanikei A6598 > Tanikei A6599-4. Point mutation in the *Wx-D1* gene results in amino acid substitution (A→T) at position 258 in the mature Wx-D1 protein in Tanikei A6599-4 (Yanagisawa et al 2001), and this gene was assigned the allele designation *Wx-D1e*.

DH lines from Kanto 118 (*Wx-D1a*) × Tanikei A6599-4 (*Wx-D1e*) were produced by a maize method in which F₁ plants of Kanto 118 × Tanikei A6599-4 were pollinated with maize pollen followed by immature embryo culture (Inagaki et al 1997). Kanto 118, an offspring of Kanto 107 is a low-amylose line in which only the Wx-D1 protein is produced. All DH lines used in this research have *Wx-D1e* alleles (Yanagisawa et al 2003), so we used them to survey the origin of changes in amylose and the starch pasting curve.

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In field conditions, seeds were sown from the end of October to the beginning of November. The flowering stage is from the end of April to the beginning of May. The ripening stage starts at the beginning of June. During seed maturation, the highest temperature was over 25°C (May to June) in Tsukuba, Japan. In greenhouse conditions, the light/dark period was 8/16 hr and the temperature was constant at 15 or 20°C during seed maturation.

Apparent Amylose Content and Iodine Staining

For flour preparation, crushed grains were filtered with a 100-µm filter. The apparent amylose content of the flour was determined using a Technicon Auto analyzer based on iodine staining. Regression curve was derived using potato amylose (Sigma) as the standard. When the value of 100 mg of wheat flour measured colorimetrically corresponded to that of A mg of potato amylose, the apparent amylose content of the flour was defined as A% (Oda et al 1992). Starch was isolated by washing in water and centrifugation. Apparent amylose content of starch was determined based on colorimetric measurement of the iodine-starch complex (absorbance at 620 nm). A potato amylose (Sigma Type III) (100% amylose) and waxy wheat (0% amylose) starch mixture was used as the standard. Apparent means that the contribution of amylopectin, protein, lipid, and water content to absorbance was not considered. The experiment was run five times. Endosperm starch granules were stained with potassium iodide and iodine solution (0.2% KI, 0.04% I₂, w/v) and observed by light microscopy.

SDS-PAGE

We suspended 10 mg of starch granules in the extraction buffer (140 µL) containing 10% (v/v) glycerol, 0.0625M 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride (Tris-HCl) (pH 6.8), 2.5% (w/v) SDS, and 5% (v/v) 2-mercaptoethanol and heated in boiling water for 10 min. Gelatinized starch solutions were cooled on ice immediately and left overnight before centrifuging at 10,000 rpm for 10 min at 4°C. We applied 20 µL of supernatants to SDS-PAGE (10% separation gel). Electrophoresis was done at 20 mA for 2 hr. Proteins were detected by a silver staining kit (Wako Pure Chemical Industries, Japan). To determine the amount of Wx-D1 protein, densitometric analysis was performed using a public-domain Scion image Beta 4.02 program for Windows (available at <http://www.scioncorp.com>).

Starch Paste Viscosity

Starch paste viscosity was measured by an RVA. We mixed 3 g of starch in 25 mL (12%) of distilled water (Kiribuchi-Otobe et al 1998). The suspension was heated from 35 to 95°C at 5°C/min and held at 95°C for 5 min, then cooled to 35°C at 5°C/min. Peak viscosity (PV), minimum viscosity (MV), and final viscosity (FV) were recorded, and breakdown (BD [PV – MV]), total setback (TSV [FV – MV]), and setback (SV [PV – FV]) were calculated. In Tanikei A6599-4 and DH lines, the peak viscosity temperature and time maintained at >80% of PV (Kiribuchi-Otobe et al 2001b) was also selected to determine hot paste viscosity stability.

RESULTS AND DISCUSSION

Apparent Amylose Content and Amount of Wx-D1 Protein

The apparent amylose content of endosperm flour and starch from the field and greenhouse (15°C) are shown in Table I. Except for Kanto 107, the apparent amylose content of flour and starch differed significantly between the field and greenhouse (*t*-test). For Tanikei A6099, Tanikei A6598, and Tanikei A6599-4, the apparent amylose content of starch also differed significantly between the field and greenhouse (*t*-test) (Table I). Tanikei A6599-4 was grown at 20°C in addition to 15°C. The amylose content at 20°C differed significantly in both the field and greenhouse at 15°C (Table I).

In some cases, large differences in apparent amylose content were found between flour and starch, perhaps because the contribution of amylopectin, protein, lipid, and water content to absorbance was not considered, and also because the measurement between flour and starch differed.

During wheat seed maturation in the field, the highest temperature reached >25°C in Tsukuba and in most of Japan. The apparent amylose content of flour of these low-amylose content lines, analyzed by autoanalyzer, was not severely affected by year-to-year fluctuations (Yamaguchi et al 1998; Kiribuchi-Otobe et al 2001a). The apparent amylose content of starch from the greenhouse was higher than that in the field in all DH lines with a waxy phenotype (Table I). Densitometric analysis of the Wx-D1 protein in these mutant lines between the field and greenhouse (15°C) showed that the amount of Wx-D1 protein grown in the greenhouse (15°C) produced more than that grown in the field (Table II). The fact that the increase apparent amylose content of flour and starch (Table I) and the amount of Wx-D1 protein in Tanikei A6099, A6598, and A6599-4 grown in the greenhouse

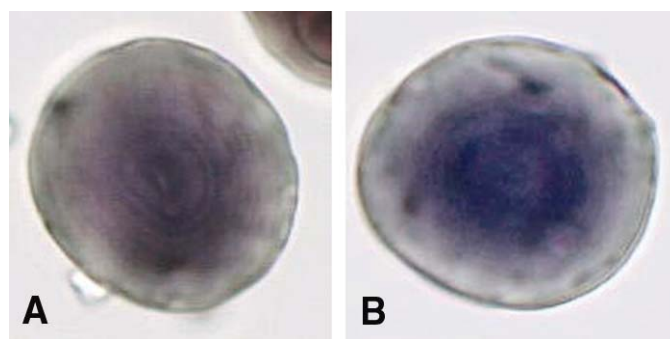


Fig. 1. Starch granule stained with potassium iodide and iodine solution grown in field conditions (A) and grown in greenhouse (15°C) (B).

TABLE I
Apparent Amylose Content (%) of Flour and Starch
(average ± standard deviation)

	Field	Greenhouse	
		15°C	20°C
Flour			
Kanto 107	23.22 ± 1.09	22.62 ± 2.32 ^{ns}	nd
Tanikei A6099	17.61 ± 1.16	18.61 ± 1.08**	nd
Tanikei A6598	14.68 ± 0.94	17.86 ± 2.16**	nd
Tanikei A6599-4	5.88 ± 0.74	8.85 ± 0.99**	6.71 ± 0.27**
Starch			
Tanikei A6099	24.47 ± 0.88	26.67 ± 0.52**	nd
Tanikei A6598	21.31 ± 1.41	24.45 ± 0.99**	nd
Tanikei A6599-4	5.56 ± 0.13	9.34 ± 0.30**	6.95 ± 0.16**
DH-975	4.53 ± 0.35	9.20 ± 0.39**	nd
DH-977	5.83 ± 0.34	9.24 ± 0.15**	nd
DH-980	5.18 ± 0.55	7.99 ± 0.11**	nd
DH-982	5.82 ± 0.38	8.33 ± 0.26**	nd
DH-985	6.25 ± 0.58	10.65 ± 0.05**	nd
DH-987	5.79 ± 0.11	7.84 ± 0.14**	nd
DH-988	5.20 ± 0.26	6.60 ± 0.58**	nd
DH-989	6.18 ± 0.27	9.91 ± 0.27**	nd
DH-990	5.03 ± 0.32	7.45 ± 0.20**	nd
DH-991	6.27 ± 0.12	8.67 ± 0.15**	nd

^a ns, not significant; *, **, significant at *P* < 0.01 and 0.05.

TABLE II
Relative Amount of Wx-D1 Protein for Field and Greenhouse^a

Line	Field	Greenhouse (15°C)
Tanikei A6099	100	119
Tanikei A6598	100	130
Tanikei A6599-4	100	107

^a Value of each line grown in field is 100 (average of three times).

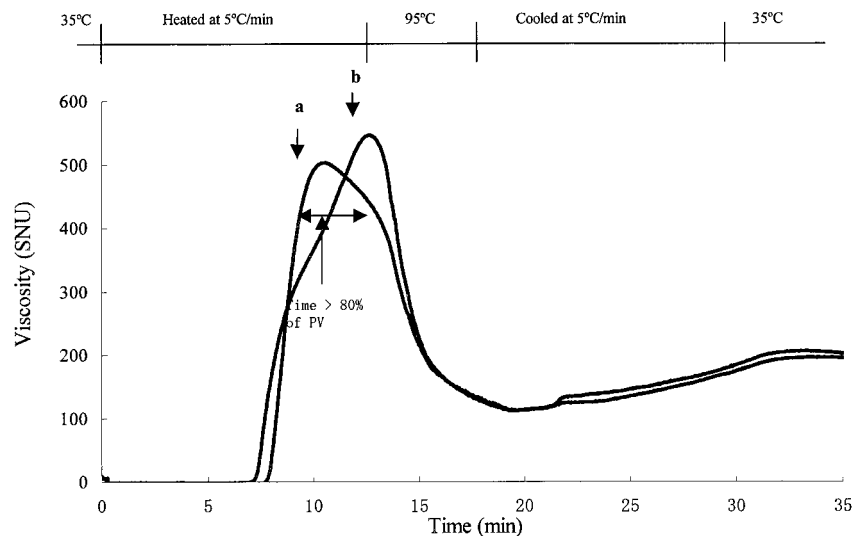


Fig. 2. Starch pasting curves in RVA measurement of Tanikei A6599-4 grown in field conditions (a) and grown in greenhouse (15°C) (b).

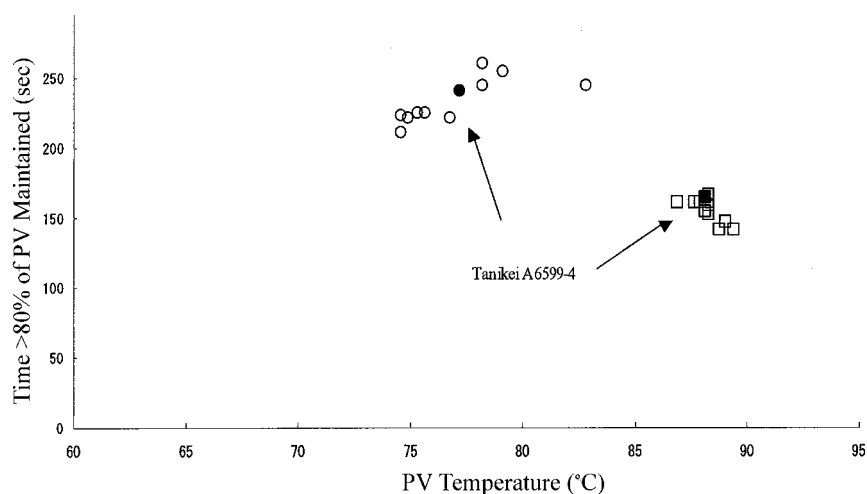


Fig. 3. Change in hot paste viscosity stability in Tanikei A6599-4 and DH lines with waxy phenotype grown in field conditions (○, ●) and grown in greenhouse (15°C) (□, ■).

compared to that grown in the field (Table II) suggested that the *Wx-D1* gene was related to the response to temperature. The apparent amylose content of Tanikei H1881 (no waxy protein) changed only negligibly (data not shown), supporting this hypothesis.

Observing starch stained with an iodide-iodine solution, that of Tanikei A6099 and A6598 stained purple, the color difference between the field and greenhouse was not clear (data not shown), and in Tanikei A6599-4, starch stained dark-brown in the field and starch from the greenhouse stained more bluish (Fig. 1).

Tanikei A6099, A6598, and A6599-4 were mutant lines originating in Kanto 107. The promoter region of the *Wx-D1* gene of Tanikei A6099 may have mutated to become responsive to temperature changes and this was transmitted to Tanikei A6598 and Tanikei A6599-4. *Wx-D1* genes in these mutants were also used to monitor responses to slight changes in temperature. Further study is needed to determine the molecular mechanism of the *Wx-D1* gene in mutant wheat.

Tester et al (1995) found that contents of total amylose and lipid-free amylose slightly increased with temperature in nonmutant wheat. Apparent amylose content of flour in Kanto 107 (*Wx-Alb*, *Wx-B1b*, *Wx-D1a*) grown in the field was little higher than that grown in the greenhouse (15°C), but there was no statistically significant difference (Table I). In these cases, other factors like enzyme activity may be related to the alteration of amylose content by growth temperature. Rice with the *Wx^a* allele, in which

Wx protein level is ≈ 10 -fold higher than that in rice with the *Wx^b* allele, produces seeds containing more amylose (Sano 1984; Isshiki et al 1998). The longer rice plants are exposed to cool temperatures, the higher the levels of the *Wx* protein and the greater the accumulation of amylose (Hirano and Sano 1998), therefore they suggested that the *Wx* gene promoter is responsive to cool (18°C) temperatures. A mutation at the 5' splicing site in the first intron of the *Wx* gene is associated with different sensitivity to temperature during seed development (Larkin and Park 1999).

A point mutation in the *Wx-D1* gene resulting in amino acid substitution (A→T) occurred at position 258 in the mature *Wx-D1* protein in Tanikei A6599-4. This gene was assigned to *Wx-D1e* allele to *Wx-D1* locus (Yanagisawa et al 2001). This mutated *Wx-D1* protein coded by *Wx-D1e* was responsible for the waxy character of Tanikei A6599-4 (Yanagisawa et al 2003), and the *Wx-D1e* gene is responsible for GBSSI enzyme activity. The fact that apparent amylose content was increased by cool temperatures suggests that the mutated *Wx-D1* protein produced little amylose. Apparent amylose content is not controlled by the *Wx* gene alone because the amylopectin structure influences the apparent amylose level.

We cannot rule out the possibility that the increase in apparent amylose content actually reflects the long chain of amylopectin because apparent amylose content was determined colorimetrically and the contribution of amylopectin to absorbance was not

TABLE III
Comparison of RVA Parameters for DH Lines Between Field and Greenhouse (15°C) Growth Conditions (no repeat)

	PV		MV		FV		BD		TSV		SV	
	Field	15°C	Field	15°C	Field	15°C	Field	15°C	Field	15°C	Field	15°C
Tanikei A6099	336.7	365.2	140.9	150.8	273.4	355.0	195.8	214.3	132.5	204.2	63.3	10.2
Tanikei A6598	353.0	376.5	131.9	148.7	280.8	340.4	221.1	227.8	148.9	191.8	72.2	36.1
Tanikei A6599-4	502.5	545.6	111.4	111.2	195.8	205.4	391.1	434.4	84.3	94.3	306.8	340.2
DH-975	543.0	484.6	115.5	108.2	198.3	198.6	427.5	376.4	82.8	90.4	344.8	286.0
DH-977	526.8	566.2	118.8	124.7	194.9	207.0	407.9	441.5	76.1	82.3	331.8	359.2
DH-980	543.0	547.0	115.5	104.8	198.3	201.3	427.5	442.2	82.8	96.4	344.8	345.8
DH-982	497.0	544.8	114.9	114.8	198.0	203.5	382.1	430.1	83.1	88.8	299.0	341.3
DH-985	573.6	546.0	117.3	122.3	198.0	217.8	456.3	423.8	80.7	95.6	375.6	328.2
DH-987	569.9	568.3	118.8	112.2	191.3	201.6	451.1	456.1	72.5	89.4	378.6	366.7
DH-988	494.3	503.0	108.3	101.0	188.1	191.0	385.9	402.0	79.8	90.0	306.2	312.0
DH-989	532.1	593.3	131.4	137.3	196.4	218.9	400.7	456.0	65.0	81.7	335.7	374.3
DH-990	551.1	570.6	131.9	125.3	197.8	209.0	419.2	445.3	65.9	83.7	353.3	361.6
DH-991	504.8	548.1	124.6	119.3	193.4	201.2	380.2	428.8	68.8	81.8	311.3	346.9

considered. Because KI-I₂ cannot be incorporated into lipid-complexed amylose, amylose content of the lipid-free starch determined colorimetrically was higher than that of the lipid-bound starch. Tester et al (1995) found that the contents of lipid-complexed amylose varied independently of temperature in wheat. In barley and wheat starch, elevated growth temperatures increased the amount of starch lipids (Tester and Karkalas 2001). To summarize, there is little possibility that the content of lipid-complexed amylose grown at 15°C increased.

Denyar et al (2001) indicated that the availability of malto-oligosaccharides (MOS) and the concentration of adenosine-5' diphospho-glucose (ADPG) influenced the amylose synthesis. Further studies of these factors in amylose content will be required.

Starch Pasting Properties

Starch pasting viscosity as analyzed by RVA in Tanikei A6599-4 from the field and greenhouse (15°C) are shown in Fig. 2. Compared with starch matured at 15°C, the temperature at the peak viscosity was higher and time maintained at >80% of peak was shorter than that of starch from the field. Using DH lines with a waxy phenotype, these characteristics of starch pasting were similar to Tanikei A6599-4 (Fig. 3). The waxy character and stable hot paste viscosity of Tanikei A6599-4 are controlled by the same *Wx-D1* gene. The finding that the temperature attained for the peak viscosity was higher and time maintained at >80% of peak was shorter than that of starch from the field in Tanikei A6599-4 and all DH lines with *Wx-D1e* by cool temperatures analyzed by RVA (Figs. 2 and 3) suggests that this mutated *Wx-D1* gene is related to changing starch properties. Stable hot paste viscosity was affected by the concentration of starch (Kiribuchi-Otobe et al 2001b). In an experiment with 9% concentration, PV temperature and starch pasting patterns were similar for both the field and greenhouse (15°C) (data not shown).

FV and TSV were also higher at 15°C than in the field (Table III) and other characteristics did not conspicuously differ between the field and 15°C in Tanikei A6599-4 and all DH lines. Tanikei A6099 and A6598 pasting curves did not differ between the field and greenhouse, but FV and TSV also increased in the greenhouse (Table III). Zeng et al (1997) reported significant correlations between apparent amylose content and PV, MV, FV, BD, TSV, and SV in RVA. Yamamori et al (2000) also pointed out that these RVA parameters were correlated to amylose content. RVA parameters such as FV and TSV, which increased according to the increase of amylose content, were used to monitor slight changes in temperature during seed maturation.

Physicochemical properties of starch in solution are likely direct functions of the molecular constitution of the polymer including molecular size, unit chain length distribution, branching pattern, degree of phosphate substitution, and granule size and distribution (Blennow et al 2001). Phosphate-monoester derivatives in starch

increase the paste viscosity. Potato starch, which contains a large amount of phosphate-monoesters, is more resistant to heat and shearing than cereal starches, but hot paste stability is lost when potassium bound to phosphate-monoester is displaced by other cations (Hofstee and de Willigen 1956). Kiribuchi-Otobe et al (2001) showed that the peak viscosity of potato starch decreased markedly by the addition of NaCl, whereas the pasting curve of Tanikei A6599-4 starch did not change. Starch pasting properties of Tanikei A6599-4 grown at 15°C also did not change by the addition of NaCl (data not shown). Therefore the pasting curve change of Tanikei A6599-4 grown at 15°C is unrelated to the content of phosphate-monoesters.

Elevation of growth temperature tends to decrease the number or size of starch granules, and increases the gelatinization temperature of wheat starch due primarily to the enhanced registration of amylopectin double helices and probably enhanced rigidity of amorphous region (Tester et al 1995). There is the possibility that the difference of the size of starch granules and amylopectin crystallinity between those grown in the field and those grown in the greenhouse (15°C) reflected the change of starch pasting curve in Tanikei A6599-4.

Myllarinen et al (1998) found that the gelatinization peak temperature measured by differential scanning calorimetry (DSC) was lower for barley cultivars grown at low temperatures. This is in agreement with our study because the DSC gelatinization peak temperature was similar to the RVA gelatinization temperature.

CONCLUSIONS

The apparent amylose content of flour and starch in low-amylose content lines (Tanikei A6099, A6598, and A6599-4) with only *Wx-D1* protein increased at cool temperatures during seed maturation. The *Wx-D1* gene was related to the increase of the apparent amylose content.

The starch pasting curve measured by RVA changed in starch of Tanikei A6599-4 (waxy mutant with stable hot paste viscosity) at cool temperature. Stable hot paste viscosity was affected by temperature during seed maturation. This changing was also related to the *Wx-D1* gene by using doubled-haploid (DH) lines.

LITERATURE CITED

- Asaoka, M., Okuno, K., Sugimoto, Y., Kawakami, J., and Fuwa, H. 1984. Effect of environmental temperature during development of rice plants on some properties of endosperm starch. *Starch* 36:189-193.
- Blennow, A., Bay-Smidt, A. M., and Bauer, R. 2001. Amylopectin aggregation as a function of starch phosphate content studied by size exclusion chromatography and on-line refractive index and light scattering. *Int. J. Biol. Macromol.* 28:409-420.
- Chao, S., Sharp, P. J., Worland, E. J., Warham, A. J., Koebner, R. M. D., and Gale, M. D. 1989. RFLP-based genetic maps of wheat homoeolo-

- gous group 7 chromosomes. *Theor. Appl. Genet.* 78:495-504.
- Cottrell, J. E., Duffus, C. M., Paterson, L., and MacKay, G. R. 1995. Properties of potato starch: Effects of genotype and growing conditions. *Phytochemistry* 40:1057-1064.
- Debon, S. J. J., Tester, R. F., Millam, S., and Davies, H. V. 1998. Effects of temperature on the synthesis, composition and physical properties of potato microtuber starch. *J. Sci. Food Agric.* 76:599-607.
- Denyer, K., Johnson, P., Zeeman, S., and Smith, A. M. 2001. The control of amylose synthesis. *J. Plant. Physiol.* 158:479-487.
- Echt, C. S., and Schwartz, D. 1981. Evidence for the inclusion of controlling elements within the structural gene at the waxy locus in maize. *Genetics* 99:275-284.
- Ferguson, V. L., and Zuber, M. S. 1962. Influence of environment on amylose content of maize endosperm. *Crop Sci.* 2:209-211.
- Hirano, H. Y., and Sano, Y. 1998. Enhancement of Wx gene expression and the accumulation of amylose in response to cool temperatures during seed development in rice. *Plant Cell Physiol.* 39:807-812.
- Hizukuri, S. 1969. The effect of environment temperature of plants on the physicochemical properties of their starches. *J. Jpn. Soc. Starch Sci.* 17:73-88.
- Hofstee, J., and de Willigen, A. H. A. 1956. Starch. Pages 1-33 in: *Foodstuffs, Their Plasticity, Fluidity and Consistency.* G. W. S. Bair, ed. J. Nikuni and T. Isemura, trans. Asakura Shoten: Tokyo.
- Inagaki, M., Nagamine, N., and Mujeeb-Kazi, A. 1997. Use of pollen storage and detached-tiller culture in wheat polyploid production through wide crosses. *Cereal Res. Commun.* 25:7-13.
- Isshiki, M., Morino, K., Nakajima, M., Okagaki, R. J., Wessler, S. R., Izawa, T., and Shimamoto, K. 1998. A naturally occurring functional allele of the rice waxy locus has a GT to TT mutation at the 5' splice site of the first intron. *Plant J.* 15:133-138.
- Kiribuchi-Otobe, C., Yanagisawa, T., and Yoshida, H. 1998. Wheat mutant with waxy starch showing stable hot paste viscosity. *Cereal Chem.* 75:671-672.
- Kiribuchi-Otobe, C., Yanagisawa, T., Yoshida, H., Yamaguchi, I., Seko, H., Ushiyama, T., and Amano, Y. 2001a. Breeding of "Wheat Norin-PL 8 (Tanikei A6599-4)", a waxy wheat line with starch showing a stable hot paste viscosity. *Bull. NICS* 2:63-74.
- Kiribuchi-Otobe, C., Yanagisawa, T., and Yoshida, H. 2001b. Genetic analysis and some properties of starch in waxy mutant wheat Tanikei A6599-4. *Breed. Sci.* 51:241-245.
- Larkin, P. D., and Park, W. D. 1999. Transcript accumulation and utilization of alternate and non-consensus splice sites in rice granule-bound starch synthase are temperature-sensitive and controlled by a single-nucleotide polymorphism. *Plant Molec. Biol.* 40:719-727.
- Myllarinen, P., Schulman, A. H., Salovaara, H., and Poutanen, K. 1998. The effect of growth temperature on gelatinization properties of barley starch. *Acta Agric. Scand. Sect. B—Soil and Plant Sci.* 48:85-90.
- Nakamura, T., Yamamori, M., Hirano, H., and Hidaka, S. 1993. Identification of three Wx proteins in wheat (*Triticum aestivum* L.). *Biochem. Genet.* 31:75-86.
- Nelson, O. E., and Rines, H. W. 1962. The enzymatic deficiency in the waxy mutant of maize *Biochem. Biophys. Res. Comm.* 9:297-300.
- Oda, S., Kiribuchi, C., and Seko, H. 1992. A bread wheat mutant with low amylose content induced by ethyl methanesulphonate. *Jap. J. Breed.* 42:151-154.
- Sano, Y. 1984. Differential regulation of waxy gene expression in rice endosperm. *Theor. Appl. Genet.* 68:467-473.
- Sano, Y., Maekawa, M., and Kikuchi, H. 1985. Temperature effects on the Wx protein level and amylose content in the endosperm of rice. *J. Hered.* 6:221-222.
- Tester, R. F., Morrison, W. R., Ellis, R. H., Piggott, J. R., Batts, G. R., Wheeler, T. R., Morison, J. I. L., Hadley, P., and Ledward, D. A. 1995. Effects of elevated growth temperature and carbon-dioxide levels on some physicochemical properties of wheat starch. *J. Cereal Sci.* 22:63-71.
- Tester, R. F., Debon, S. J. J., Davies, H. V., and Gidley, M. J. 1999. Effect of temperature on the synthesis, composition and physical properties of potato starch. *J. Sci. Food Agric.* 79:2045-2051.
- Tester, R., and Karkalas, J. 2001. The effects of environmental conditions on the structural features and physico-chemical properties of starches. *Starch* 53:513-519.
- Toyokawa, H., Rubenthaler, G. L., Powers, J. R., and Schanus, E. G. 1989. Japanese noodle qualities. II. Starch components. *Cereal Chem.* 66:215-219.
- Umemoto, T., Nakamura, Y., and Ishikura, N. 1995. Activity of starch synthase and the amylose content in rice endosperm. *Phytochemistry* 40:1613-1616.
- Yamaguchi, I., Kiribuchi-Otobe, C., Yanagisawa, T., Ushiyama, T., Seko, H., Amano, Y., Miyagawa, S., Kuroda, A., and Oda, S. 1998. Breeding of wheat Norin-PL 7 (Tanikei A6099): New germplasm accession with low amylose content. *Bull. Natl. Agric. Res. Cent.* 29:1-11.
- Yamamori, M., and 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.
- Yanagisawa, T., Kiribuchi-Otobe, C., and Yoshida, H. 2001. An alanine to threonine change in the Wx-D1 protein reduces GBSS I activity in waxy mutant wheat. *Euphytica* 121:209-214.
- Yanagisawa, T., Kiribuchi-Otobe, C., Hirano, H.-Y., Suzuki, Y., and Fujita, M. 2003. Detection of single nucleotide polymorphism (SNP) controlling the waxy character in wheat by using a derived cleaved amplified polymorphic sequence (dCAPS) marker. *Theor. Appl. Genet.* 107:84-88.
- Zeng, M., Morris, C. F., Batey, I. L., and Wrigley, C. W. 1997. Sources of variation for starch gelatinization, pasting, and gelation properties in wheat. *Cereal Chem.* 74:63-71.

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