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Differential effects of the null alleles at the three *Wx* loci on the starch-pasting properties of wheat

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Abstract The amylose/amylopectin ratio and the pasting properties of wheat starch are important in producing marketable flour products, especially Japanese noodles. To determine if null mutations at the three *Wx* loci confer differences in starch-pasting viscosity, we analyzed the variation associated with the null mutations in three separate sets of recombinant substitution lines of chromosomes 7A, 4A and 7D produced from crosses between Chinese Spring and three single-chromosome substitution lines carrying the null *Wx* alleles. Differential effects of null alleles at the three *Wx* loci on starch-pasting properties were revealed. With respect to chromosome 4A, the effect of the *Wx-B1b* allele, giving a higher peak and breakdown viscosity, was unambiguous. In addition, a QTL of minor effect was identified near the centromere on the short arm. The presence or absence of the Wx-A1 protein gave some variation in peak and breakdown viscosity, but the effects of *Wx-Alb* were much smaller than those of the *Wx-Blb* allele. Associated effects of the *Wx-D1* locus were detected for the breakdown viscosity as the null *Wx-D1b* allele produced a higher viscosity than the wild-type *Wx-D1a*. While negative correlations between amylose content and breakdown viscosity were common in the three populations, the null mutations at the *Wx* loci produced some variation independent of amylose content. The genetic variation detected for breakdown viscosity was more evident than that for peak viscosity in all three recombinant populations.

Key words *Triticum aestivum* · Amylose content · Recombinant substitution line · Starch-pasting property · *Wx* gene

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Introduction

Wheat (*Triticum aestivum* L.) endosperm starch normally contains about 20–30% amylose, the remainder being amylopectin. Amylose consists of essentially linear molecules of α-(1–4)-linked α-D-glucopyranosyl units, whereas amylopectin is made up of highly branched molecules of α-D-glucopyranosyl units linked, primarily by α-(1–4) bonds, with branches resulting from α-(1–6) linkages. Two distinct types of starch synthases, isoforms of granule-bound starch synthase (GBSS) and soluble starch synthases, are involved in the conversion of ADP-glucose to the starch polymers. The major GBSS I with a molecular weight of about 60 kDa, the so called Waxy (Wx) protein, is responsible for amylose production. The soluble starch synthases act together with branching enzymes to synthesize amylopectin. Three isoforms of the wheat Wx proteins, Wx-A1, Wx-B1 and Wx-D1, have been identified by 2D-PAGE (Nakamura et al. 1993a). They are encoded by three homoeologous *Wx* loci, *Wx-A1*, *Wx-B1* and *Wx-D1*, located on 7AS, 4AL and 7DS, respectively (Chao et al. 1989; Nakamura et al. 1993a). Yamamori et al. (1994) have found various cultivars with null alleles for each of the three controlling loci. There is a correlation between the presence of *Wx* null alleles and a lower amylose content across cultivars (Yamamori et al. 1992; Miura and Tanii 1994). The three *Wx* genes have different effects on modifying amylose content, with the *Wx-B1b* allele providing the largest reduction in amylose through the lack of the Wx-B1 protein in comparison with the other null alleles, *Wx-A1b* and *Wx-D1b* (Miura et al. 1994; Miura and Sugawara 1996). The wild-type *Wx-B1a* therefore predominates for amylose synthesis capacity, followed by *Wx-D1a* and *Wx-A1a* (Miura et al. 1999).

The amylose/amylopectin ratio of wheat starch is extremely important in producing marketable flour products. The flour from cultivars with a lower amylose content due to the null *Wx-B1b* allele have a higher Japanese noodle quality (Yamamori et al. 1992; Nakamura et al. 1993b; Miura and Tanii 1994; Zhao et al. 1998). It is

also agreed that the quality of Japanese noodles is related to their starch-pasting properties, which are involved in starch gelatinization, pasting, and gelation processes (Nagao et al. 1977; Moss 1980; Oda et al. 1980; Toyokawa et al. 1989). Wheat flour with a high swelling volume and swelling power (Crosbie 1991; McCormick et al. 1991), high peak viscosity (Moss 1980; Lee et al. 1987; Crosbie et al. 1990), low gelatinization temperature (Oh et al. 1985; Endo et al. 1988) and a high rate of breakdown during viscoamylography (Oda et al. 1980; Konik et al. 1992) is desirable for high-quality white salted noodles. Therefore, in addition to a low amylose content, starch properties for processing and eating quality should include higher starch-pasting characteristics.

The starch-pasting properties of wheat flour show genetic variation (McCormick et al. 1995; Miura and Tanii 1995; Zeng et al. 1997). Generally, a lower amylose content corresponds to a higher peak paste viscosity. Zeng et al. (1997) have demonstrated that reduced starch amylose due to decreased GBSS profoundly affects starch gelatinization, pasting, and gelation properties. On the other hand, there is a near continuous spectrum of variation in flour viscosity between wild-type and mutant genotypes (Udall et al. 1999), and there are cultivars showing high scores in both amylose content and peak viscosity (Miura and Tanii 1994). These facts argue against a model in which a lower amylose content produced by the null mutation is always responsible for a higher peak viscosity. Zhao et al. (1998) provided clear evidence for the genetic association of the null *Wx-B1b* allele with a high peak viscosity, and suggested that the molecular basis for this association is not simply due to a decrease in amylose content. Further, although associated effects of chromosomes 7A and 7D on peak viscosity were found (Miura and Tanii 1995; Watanabe and Miura 1997), the effects of the null alleles at the *Wx-A1* and *Wx-D1* loci have not yet been studied in detail.

To determine if the null *Wx* mutations at the three *Wx* loci confer categorical differences in starch-pasting viscosity, we developed three separate sets of recombinant substitution lines for chromosomes 7A, 4A, and 7D, and analyzed the effect of the null mutations on starch properties. In the present paper, we report differential effects on the null alleles at the three *Wx* loci on starch-pasting properties, especially on peak viscosity and breakdown viscosity. Further, we mapped quantitative genetic variation for these characters on chromosome 4A.

Materials and methods

Plant materials

Previously we developed single-chromosome substitution lines for chromosomes 7A, 4A and 7D with a Chinese Spring (CS) genetic background (Miura and Sugawara 1996). CS carries the *Wx-A1a*, *Wx-B1a* and *Wx-D1a* alleles and thus produces all three Wx proteins. The chromosome 7A substitution line, CS*11/Kanto107 7A, lacks Wx-A1 protein as it has the *Wx-A1b* allele. Similarly, CS*11/Kanto107 4A carrying *Wx-B1b* does not produce Wx-B1 protein, and CS*8/Bai Huo 7D with the *Wx-D1b* allele has no GBSS I activity to produce the Wx-D1 protein.

Using the procedures described by Law (1966), three sets of homozygous recombinant substitution lines (RSLs) for chromosomes 7A, 4A and 7D were developed separately from F_1s between CS and each of the single-chromosome substitution lines. The development of RSLs for chromosome 4A has already been described by Araki et al. (1999). In total 101 different RSLs for chromosome 7A, 95 RSLs for 4A and 105 RSLs for 7D were included in the present experiment. Since chromosome 5D of CS carries the dominant *Vrn-D1* allele promoting the spring growth habit (Pugsley 1972), all RSLs and the parental substitution lines are spring wheats.

Identification of the *Wx* allele types

To classify the RSLs for the *Wx* allele types, electrophoretic analysis of starch granule-bound protein was performed. Starch granule preparation and SDS-PAGE were conducted as described by Nakamura et al. (1993a), with the modification that a 15% SDS polyacrylamide gel with an acrylamide/BIS concentration of 30:0.135 was used for electrophoresis.

Starch preparation

Spring-sown trials were conducted in the experimental field of Obihiro University of Agriculture and Veterinary Medicine. The RSL-4A population and the parental genotypes were grown in 1997, and the RSL-7A and RSL-7D populations were examined in 1998. In the 2nd year, CS and the three single-chromosome substitution lines were included. Each genotype was represented by a single plot of 15 plants, spaced 10 cm between plants within a row and 30 cm between rows. After anthesis, the experimental plots were covered to prevent pre-harvest sprouting. Grain samples were milled on a Brabender Quadrant Junior Test Mill to a final extraction rate of 60%. Starch granules were separated using conventional methods (Miura et al. 1994).

Amylose content and starch-pasting properties

The amylose content of 100 mg of starch granules was colorimetrically determined using a Auto Analyzer System II (Bran+Lubbe Co.) as described by Miura et al. (1994). The assessment was carried out at least twice.

Starch peak viscosity and breakdown viscosity were measured on a Rapid Visco Analyzer (RVA, Newport Scientific Pty. Ltd.). Three grams of starch were mixed with 25 ml of distilled water. The suspension was heated from 55°C to 95°C at the rate of 5°C/min and held at 95°C for 5 min, then cooled to 55°C at the rate of 5°C/min. The primary starch-pasting viscosity parameters derived from the RVA curve included peak viscosity and minimum viscosity (the lowest viscosity after the hold at 95°C). Using these parameters breakdown viscosity was calculated as the peak minus the minimum viscosity. The analysis was made at least twice. Peak viscosity and breakdown viscosity were measured in Rapid Visco Units (RVU).

Data analysis

Analyses of variance (ANOVA) were performed for starch-property data to detect differences between parental genotypes and to partition the variation between RSLs in each chromosome population. A one-way ANOVA was also employed for each *Wx* locus to detect significant differences between the allele class-means by comparing them with the variation between lines within classes.

Detection of QTLs on chromosome 4A

Previously, we constructed a linkage map of chromosome 4A for *Wx-B1* and nine other markers from an initial population of

Table 1 Mean performance of parental genotypes, together with the mean and range in the *Wx* allele classes of the three recombinant subsitution line (RSL) populations for amylose content and starch-pasting properties

Item	No. of RSLs	Amylose content (%)		Peak viscosity (RVU)		Breakdown viscosity (RVU)	
		Mean	Range	Mean	Range	Mean	Range
Parental genotypes (1997)							
CS		24.9		240		118	
CS*11/Kanto107 4A		22.9		265		152	
RSLs for chromosome 4A	95						
Wx-B1a	44	24.1	23.3–25.0	221	193–254	111	98–129
Wx-B1b	51	22.8***	22.2–23.5	247***	191–302	144***	100–184
Parental genotypes (1998)							
CS		25.1		226		111	
CS*11/Kanto107 4A		23.1		242		142	
CS*11/Kanto107 7A		24.0		195		99	
CS*8/Bai Huo 7D		23.9		318		174	
RSLs for chromosome 7A	101						
Wx-A1a	49	24.7	23.6–25.5	225	178–248	106	72–128
Wx-A1b	52	23.9***	22.7–25.2	228 ns	191–248	117**	96–135
RSLs for chromosome 7D	105						
Wx-D1a	48	25.0	24.1–25.9	267	212–316	131	96–157
Wx-D1b	57	24.0***	23.0–25.2	296***	236–330	166***	129–190

, *: Significant differences between the allele class means at the 0.01 and 0.001 probability levels, respectively
ns: non-significant

98 RSLs (Araki et al. 1999). This map was utilized for the determination and localization of the QTLs for starch-pasting properties. QTL analysis was performed using the software package MQTL (Tinker and Mather 1995). The data sets of peak viscosity and breakdown viscosity were analysed by the simple interval mapping (SIM) and simplified composite interval mapping (sCIM) procedures. The linkage group was scanned at a 5-cM interval for the test statistic. Seven evenly spaced background markers were specified for sCIM, and type-I 5% significance thresholds were estimated with 1000 permutations.

Results

Classification of the *Wx* allele types

Of the 95 RSLs for chromosome 4A, 44 lines were found to produce the Wx-B1 protein by the SDS-PAGE system, thus classified as the CS type with *Wx-B1a*. The remaining 51 lines were deficient in the Wx-B1 protein and were identified as the CS*11/Kanto107 4A type with *Wx-B1b*. Of the 101 RSLs for chromosome 7A, 49 lines were identified as the CS type with *Wx-A1a*, and 52 lines identified as the CS*11/Kanto107 7A type with *Wx-A1b*. Of the 105 recombinant lines for chromosome 7D, 48 lines were classified as the CS type with *Wx-D1a*, and 57 lines identified as the CS*8/Bai Huo 7D type with *Wx-D1b*. All of the RSL popopulations showed the expected 1:1 ratio for normal segregation. This indicated that there was no genetic or genotypic selection in favor of RSLs carrying the particular allele at each *Wx* locus.

Amylose content

The mean performance of parental genotypes, together with the mean and range in the three sets of RSLs for amylose content and starch-pasting properties is given in Table 1. Starch granules from CS produced about 25.0% amylose over 2 years. CS*11/Kanto107 4A showed a significantly lower content, by about 2.0%, than CS. In the RSLs for chromosome 4A, the amylose content of lines with *Wx-B1a* ranged from 23.3% to 25.0%, while the lines with *Wx-B1b* ranged from 22.2% to 23.5%. The null *Wx-B1b* RSLs as a group exhibited a significantly lower amylose content by more than 1% compared to those that produced the Wx-B1 protein. The deficiency of the Wx-B1 protein due to the null *Wx-B1b* allele caused a clear reduction in amylose content.

CS*11/Kanto107 7A and CS*8/Bai Huo 7D showed a significantly lower content by about 1.0% than CS. The ranges of amylose content were almost similar in the RSLs for chromosomes 7A and 7D, ranging from 22.7 to 25.5% in RSL-7A and from 23.0 to 25.9% in RSL-7D. About a 1% amylose difference was detected between the allele class-means in each chromosome population. ANOVA revealed highly significant differences between the RSLs in each population and the majority of variation between lines was explained by the allelic differences at *Wx-A1* and *Wx-D1*, respectively. Thus, as with the *Wx-B1* locus, null alleles at the *Wx-A1* and *Wx-D1* loci were found to have large effects on the reduction in amylose content.

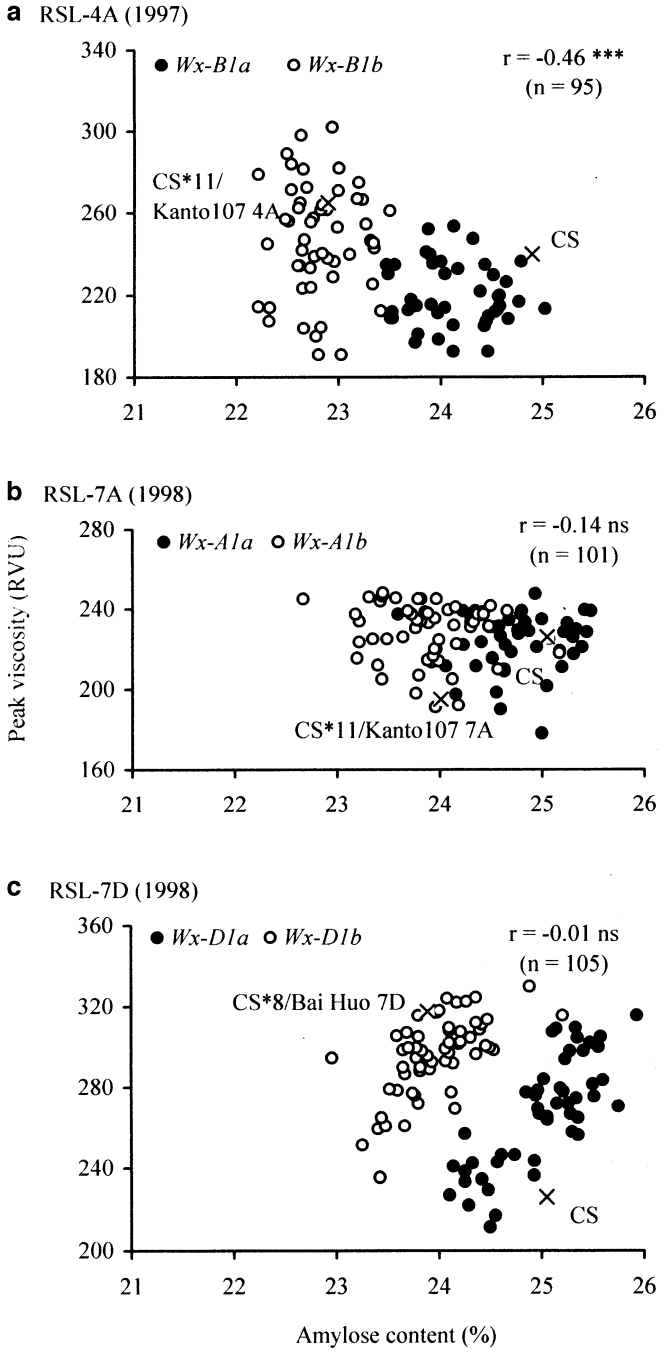

Fig. 1 Relationship between amylose content and peak viscosity of starch in the recombinant substitution lines (RSLs) of chromosomes 4A (**a**), 7A (**b**) and 7D (**c**). *** $P<0.001$, ns: non-significant

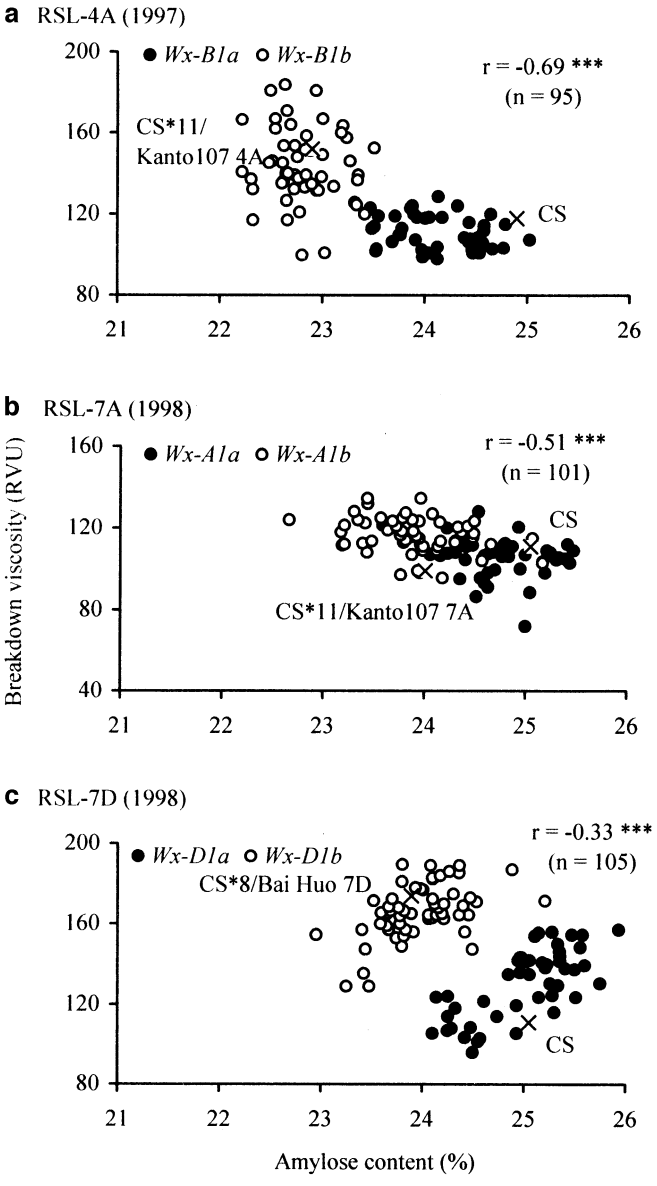

Fig. 2 Relationship between amylose content and breakdown viscosity of starch in the recombinant substitution lines (RSLs) of chromosomes 4A (**a**), 7A (**b**) and 7D (**c**). *** $P<0.001$

Peak viscosity

The peak viscosity of CS*11/Kanto107 4A was about 15 RVU higher than that of CS. The 95 RSL-4A obtained from the CS×CS*11/Kanto107 4A cross showed a large variation ranging from 191 to 302 RVU. When the RSLs were separated into the *Wx-B1a* and *Wx-B1b* allele classes, the *Wx-B1b* class had a larger variation while no RSLs in the *Wx-B1a* class produced more than 260 RVU in the 1997 trial (Fig. 1a). ANOVA indicated a significant difference between the allele class-means at the *Wx-B1* locus, and peak viscosity was negatively correlated with amylose content (r=0.46, $P<0.001$), suggesting an associated effect of the *Wx-B1* locus on peak viscosity.

Among the parental substitution lines in the 1998 experiment, CS*11/Kanto107 7A showed a low peak viscosity of 195 RVU, which is similar to CS. The 178–248 RVU range in the 101 RSLs for chromosome 7A was much smaller than the detected in the RSL-4A and RSL-7D populations (Fig. 1b). The RSLs in the *Wx-A1a* and *Wx-A1b* allele classes showed similar ranges to each other and there was no significant difference between the class-means for peak viscosity. The lower

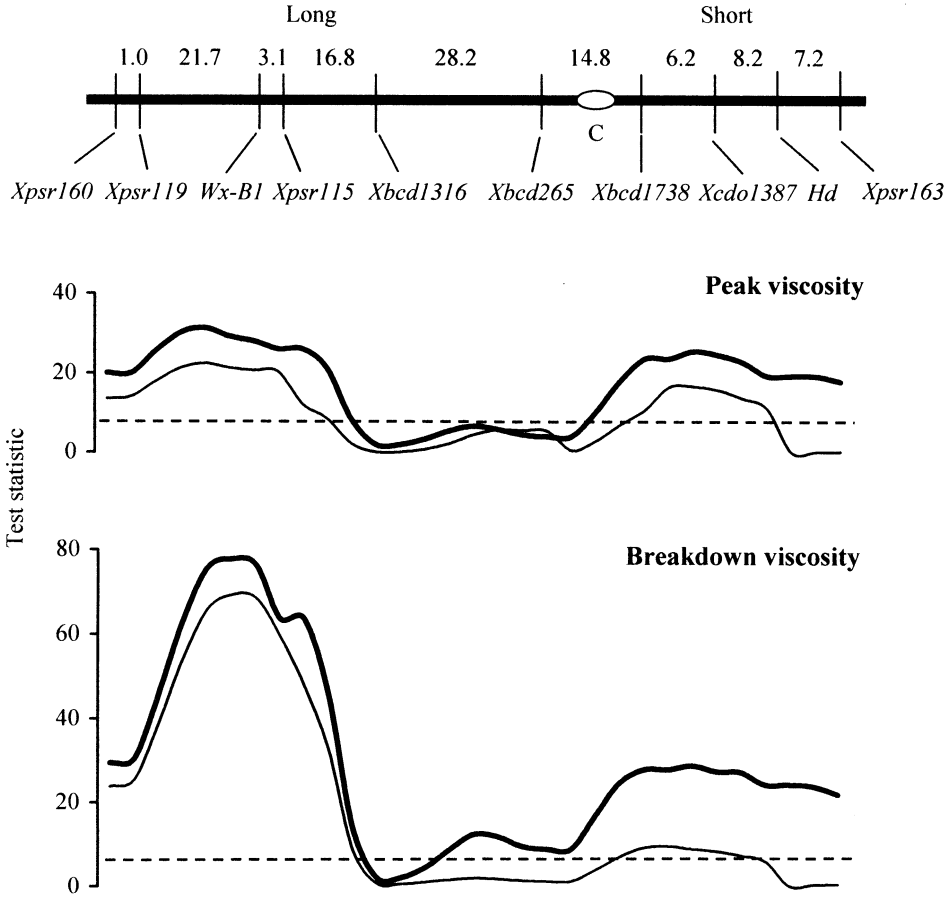

Fig. 3 Effects and positions of QTLs for starch peak viscosity (top) and breakdown viscosity (bottom) detected by MQTL analysis of chromosome 4A. The QTL main effect calculated by both Simple Interval Mapping (*bold line*) and simplified Composite Interval Mapping (*normal line*) is presented. The *dotted line* indicates the 5% significance threshold level for SIM. The *horizontal black bar* at the top represents chromosome 4A with the markers, map distances (cM), and centromere (*C*) indicated

amylose content in the *Wx-A1b* allele class was not correlated with a higher peak viscosity (r=–0.14).

CS*8/Bai Huo 7D exhibited a high peak viscosity of 318 RVU, very different from that of CS. Of the 105 RSLs derived from the CS×CS*8/Bai Huo 7D cross, as a group the 57 RSLs in the *Wx-D1b* allele class produced a significantly higher peak viscosity (296 RVU) than the *Wx-D1a* allele class of 48 RSLs (267 RVU). However, the distributions of the individual RSL values in these two classes almost completely overlapped and thus the reduction in amylose content in the RSLs with *Wx-D1b* did not result in a high peak viscosity (r=0.01, Fig. 1c).

Breakdown viscosity

Associated effects of the *Wx-B1* locus on breakdown viscosity were larger than those on peak viscosity. Over 2 years, starch from CS*11/Kanto107 4A had about a 150 RVU breakdown viscosity, which was consistently higher than those from CS. In the RSL-4A population, a significant difference between the allele class-means was revealed. The mean value of around 150 RVU in the *Wx-B1b* class was almost equal to the breakdown viscosity of CS*11/Kanto107 4A, and the *Wx-B1a* class breakdown viscosity corresponded to the about 110 RVU breakdown viscosity of CS. The 95 RSLs showed a clear bimodal distribution of breakdown viscosity. The variation in this population was related directly to the allele types at the *Wx-B1* locus (Fig. 2a), since most of the high-breakdown viscosity RSLs have the null allele while the lower RSLs carry the wild-type allele, resulting in a highly significant negative correlation between breakdown viscosity and amylose content (r=–0.69, P<0.001).

CS*11/Kanto107 7A and CS*8/Bai Huo 7D showed a breakdown viscosity of 99 and 174 RVU, respectively. The RSLs-7A population showed a small variation, ranging from 72 to 135 RVU. On the other hand, the range of 96–190 RVU in breakdown viscosity in the RSL-7D population was relatively large and comparable to the range in the RSL-4A population. As shown in Fig. 2b, a significant negative correlation between breakdown viscosity and amylose content was detected in RSL-7A (r=–0.51, P<0.001). The RSLs carrying *Wx-A1b* tended to have a low amylose content and a high breakdown viscosity.

As in the RSL-4A population, associated effects of the *Wx-D1* locus on breakdown viscosity were detected. The 105 RSLs for chromosome 7D showed a clear bimodal distribution (Fig. 2c). Most of the RSLs with a high breakdown viscosity have the null *Wx-D1b* allele while the lower RSLs carry *Wx-D1a*, providing a significant difference of about 35 RVU between the allele classes. In contrast to peak viscosity, a negative corre-

Table 2 Location of QTLs on chromosome 4A for starch-pasting properties

Trait	Marker interval	r^2	Additive[a]
Peak viscosity	*Xpsr119/Wx-B1*	0.28	30
	Xbcd1738/Xcdo1387	0.24	26
Breakdown viscosity	*Xpsr119/Wx-B1*	0.56	34
	Xbcd1738/Xcdo1387	0.26	22

[a] Additive: indicates an additive SIM main effect, all from Kanto107

lation between breakdown viscosity and amylose content was found (r=–0.33, P<0.001), indicating the effects of the null *Wx-D1b* allele both on the reduction in amylose content and on the increase in breakdown viscosity.

QTLs for starch-pasting properties on chromosome 4A

For peak viscosity, two QTLs linked in coupling were identified by both SIM and sCIM (Fig. 3). The major QTL effect was detected in the 21.7-cM *Xpsr 119/Wx-B1* interval, close to the latter locus. The allelic difference at this locus accounted for 28% of the RSLs variation, and the higher peak viscosity allele came from CS*11/Kanto107 4A with an additive effect of 30 RVU (Table 2). The second QTL of minor effect was detected in the *Xbcd1738/Xcdo1387* interval on the short arm, which explained 24% of the line variation. Again the CS*11/Kanto107 4A allele at this QTL contributed to a high peak viscosity with an additive effect of 26 RVU.

As expected, two QTLs for breakdown viscosity were identified and mapped to very similar positions, the *Xpsr119/Wx-B1* and *Xbcd1738/Xcdo1387* intervals, to the QTLs for peak viscosity (Fig. 3). At these two QTLs, the high-breakdown viscosity alleles came from CS*11/Kanto107 4A with additive effects of 34 RVU in the *Xpsr119/Wx-B1* QTL and 22 RVU in the *Xbcd1738/Xcdo1387* QTL, which explained 56% and 26% of the variation, respectively (Table 2).

The two QTLs for peak viscosity were presumed to be identical with the breakdown-viscosity QTLs. In terms of the two test statistics, the percentage of the variation accounted for and the additive effects, the QTL effects determined for breakdown viscosity were higher than those for peak viscosity.

Discussion

With respect to chromosome 4A, the null *Wx-B1b* allele or the allele from CS*11/Kanto107 4A at the *Xpsr119/Wx-B1* QTL both contributed to a lower amylose content, and a higher peak and breakdown viscosity, properties which are desirable for noodle quality. Such trait correlations may result either from pleiotropic effects of a single locus or from tight linkage of a locus (loci) controlling the traits. If the effects observed in this research are not due to *Wx-B1* but rather to a tightly linked gene located in the 21.7-cM *Xpsr119/Wx-B1* interval, recombination between *Wx-B1* and another locus (loci) would be expected. However, this possibility is cancelled out by the fact that there were no such recombinant lines having a combination of the *Wx-B1a* allele with a high peak or breakdown viscosity (Figs. 1a, 2a). In addition, as far as is known, the gene encoding other proteins for starch metabolism such as soluble starch synthases and starch branching enzymes are not located on chromosome 4A (Denyer et al. 1995; Rahman et al. 1995; Yamamori and Endo 1996; Morell et al. 1997; Nagamine et al. 1997). Hence, it is possible to conclude that the higher peak and breakdown viscosity associated with the *Xpsr119/Wx-B1* interval is due to effects of the *Wx-B1b* allele.

MQTL analysis indicated that the null *Wx-B1b* allele has an additive effect with an increase of 30 and 34 RVU for peak and breakdown viscosity, respectively. These effects may be associated with the difference in amylose content between the allele classes, since the allelic difference at this locus is a primary factor accounting for more than 70% of the variation in amylose content (Araki et al. 1999). However, the RSLs in the *Wx-B1b* allele class with only small differences in amylose content differed appreciably in starch-pasting viscosity, indicating some variation in this that is not accounted for by variation in amylose content. These results are in agreement with the research carried out by Zhao et al. (1998) who pointed out that the molecular basis for the effect of the *Wx-B1* null mutation is not simply due to a decrease in amylose content, but rather that the null mutation most likely causes a subtle change in starch structure. However, within the limits of the assays of Zhao et al. (1998) and the present study, the structural basis for this association is difficult to determine. Instead, our result implies the control of a previously unidentified secondary genetic factor. The analysis shown in Fig. 3 revealed a possible QTL effect as a candidate for such a secondary factor. The same map position, the *Xbcd1738/Xcdo1387* interval, on the short arm of chromosome 4A was shared by *QAmc.ocs-4A.1* for amylose content (Araki et al. 1999), and a QTL for starch-pasting viscosity. This putative QTL of minor effect might involve variation within the allele classes, but this issue will require further investigation.

For chromosome 7A carrying *Wx-A1*, a negative and significant correlation between breakdown viscosity and amylose content was detected in the RSL-7A population (r=–0.51, P<0.001). In addition, Miura and Tanii (1995) found that removal of chromosome 7A produced a reduction in peak viscosity. These results might suggest an associated effect of the absence of the Wx-A1 protein on a higher pasting viscosity. Certainly, the RSLs carrying *Wx-A1b* tended to have low amylose contents and high breakdown viscosity; however, the effects of *Wx-A1b* on elevated viscosity seem unremarkable, as the breakdown-viscosity variation in the two allele classes of

Wx-A1 was condensed and not substantial enough to confer categorical differences in the viscosity between classes. Therefore, it is concluded that, even if the presence or absence of the Wx-A1 protein gives some variation on the starch-pasting viscosity, the effects of the null *Wx-A1b* are much smaller than those of the *Wx-B1b* allele.

Associated effects of *Wx-D1* on breakdown viscosity were clearly detected, while there was little effect on peak viscosity. As in the RSL-4A population, a bimodal distribution for breakdown viscosity was found in the RSL-7D population. As a group the RSLs with the null *Wx-D1b* allele produced about a 35 RVU higher viscosity than the RSLs with *Wx-D1a*. This may demonstrate the contribution of the *Wx-D1* null mutation to a higher breakdown viscosity. Consequently, as far as this experiment is concerned, we conclude that there are differential effects on the null mutations at the three *Wx* loci on starch-pasting viscosity.

However, when we discuss the effects of chromosomes 7A and 7D on starch properties, it seems important to consider the co-location on the group-7 chromosomes of genes for enzymes related to starch synthesis. For example, the starch branching enzyme (SBE) isolated from wheat endosperm is fractionated into two distinct isoforms, SBE I and SBE II. Of these, SBE I with an approximately 85 kDa molecular mass further consists of several isoforms. The genes controlling some of the SBE I isoforms have been assigned to the short arms of the homoeologous group-7 chromosomes (Morell et al. 1997; Nagamine et al. 1997). The genes for ADP-glucose pyrophosphorylase have been mapped on chromosome 7 (Devos and Gale 1997). In addition, the minor starch granule protein of 100–115 kDa molecular weight are also encoded by homoeologous loci on the short arms of the group-7 chromosomes (Denyer et al. 1995; Yamamori and Endo 1996), and have been suggested to be soluble starch synthases (Takaoka et al. 1997). The respective roles of these two classes of proteins in wheat starch synthesis have not yet been unambiguously defined (Morell et al. 1997). Therefore, prior to drawing a more precise conclusion about the associated effects of the null *Wx-A1* and *Wx-D1* mutations on starch-pasting properties, it remains to be seen if these enzymes, other than GBSS I, affect starch-pasting properties and if there are polymorphisms at these loci in our RSL populations for chromosomes 7A and 7D. At present, the lack of a sufficient number of polymorphic marker loci hampers the analysis of these chromosomes. In further work, the three sets of recombinant substitution-line populations will be useful as tools for defining the genetic variation associated with the other loci mentioned above, and for identifying QTL effects under the uniform genetic background of CS by growing them in different environments.

Any information increasing our understanding of the genetic mechanisms of the starch-pasting properties has the potential to improve the breeding process. The results derived from the current experiment have general consequences for the strategies of breeding wheat cultivars with a preferable quality for white noodle production. At the very least, the associated effects of *Wx-B1* were common for amylose content and starch-pasting properties, indicating that it should be possible to modify several aspects of starch properties simultaneously. This supports the idea that the null *Wx-B1b* allele is worth selecting not only for a lower amylose content (Araki et al. 1999; Miura et al. 1999), but also for a higher starch-pasting viscosity in breeding programs (Miura and Tanii 1994; Zhao et al. 1998). Furthermore, Udall et al. (1999) have mapped several quantitative trait loci (QTLs) for peak viscosity and these QTL effects are independent of variation due to the *Wx* null mutations because the mapping population used was not segregating for the *Wx* loci. Such QTLs, including the *Xbcd1738/Xcdo1387* QTL on 4A identified here, may have breeding potential after introduction of the null mutation at the *Wx* loci.

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