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Identification of genetic loci affecting amylose content and agronomic traits on chromosome 4A of wheat

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Abstract Chromosome 4A of wheat carries the *Wx-B1* gene encoding the granule-bound starch synthase involved in amylose synthesis in the endosperm. To determine the pleiotropic effects of this locus and effects of independent QTLs on agronomic traits, genetical analysis of chromosome 4A was conducted using 98 single-chromosome recombinant substitution lines derived from a cross of Chinese Spring and Chinese Spring (Kanto107 4A) with a low amylose content due to the null *Wx-B1b* allele. For amylose content, most of the genetic variation was explained by the allelic difference at the *Wx-B1* locus. An additional QTL of minor effect was mapped in the 6.2-cM *Xbcd1738/Xcdo1387* interval on the short arm, where the allele from Kanto107 led to an increase in amylose content. Field trials over two seasons revealed a pleiotropic effect of *Wx-B1*, or else the effect of a closely linked QTL, on ear emergence time. A QTL linked to *Wx-B1* was detected for plant height. For plant yield and its components, there was no evidence for significant main effects associated with *Wx-B1* or adjacent regions. One plant-yield QTL was identified by RFLP markers on the short arm and this was identical to QTLs controlling spikelet number/ear and grain weight/ear. At these QTLs for agronomic traits, alleles from Kanto107 contributed to an earlier emergence time, a height reduction and an yield increase.

Key words *Triticum aestivum* L. · Amylose content · QTLs · *Wx-B1* · Yield traits

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Introduction

Starch is the major component of the wheat grain, making up 65–70% of the dry weight of mature grain (Rahman et al. 1995). Starch consists essentially of two different forms of polymers of glucose: linear amylose and branched amylopectin. The amylose/amylopectin ratio of starch is extremely important in producing marketable flour products, because it affects the quality of end-uses (Moss 1979; Oda et al. 1980; Toyokawa et al. 1989). Low-amylose-content cultivars are preferred for the manufacture of certain noodle types (Yamamori et al. 1992; Miura and Tanii 1994), and the potential use of starch with a reduced amylose content is a current topic of discussion among wheat breeders and geneticists. Therefore, a better understanding of the factors modifying amylose content is required for the development of effective selection- or screening-procedures for use in breeding.

The endosperm contains two distinct types of starch synthases, granule-bound starch synthase (GBSS) and soluble starch synthases, which act together with the starch-branching enzyme to synthesize amylopectin. The major GBSS I of 60 kDa, so called the Wx protein, is the key enzyme involved in amylose synthesis, and is encoded by the *Wx* loci (Tsai 1974; Echt and Schwartz 1981). In hexaploid wheat (*Triticum aestivum* L.), three Wx proteins, Wx-A1, Wx-B1 and Wx-D1, are coded by the three homoeologous *Wx* loci, *Wx-A1*(7AS), *Wx-B1*(4AL) and *Wx-D1*(7DS), respectively (Chao et al. 1989; Nakamura et al. 1993 a). The three *Wx* genes have different effects on amylose content, in particular the null *Wx-B1b* allele is associated with the largest reduction through the lack of the Wx-B1 protein (Miura et al. 1994; Miura and Sugawara 1996). Cultivars that are accepted into white-salted noodle market-classifications lack the Wx-B1 protein (Miura and Tanii 1994; Yamamori et al. 1994; Zhao et al. 1998). In breeding programs aimed to improve noodle quality,

the genetic manipulation of *Wx-B1* and chromosome 4A seems to be a prerequisite. However, whether the *Wx-B1* locus and the adjacent region of 4AL affect agronomically and economically important traits, such as flowering time and plant yield, has not been reported.

A low-amylose content of the Japanese spring wheat 'Kanto107' has received much attention in breeding programs. This cultivar lacks both of the Wx-A1 and Wx-B1 proteins and only the Wx-D1 protein, produced by *Wx-D1a*, synthesizes amylose (Nakamura et al. 1993 b). Genetic variation associated with chromosome 4A for important agronomic traits was noted when the null allele at the *Wx-B1* locus of 'Kanto107' was introduced into a 'Chinese Spring' (CS) genetic background by producing the single-chromosome substitution line (Miura and Sugawara 1996). In the present paper, we report the effects of allelic differences at the *Wx-B1* locus on amylose content and quantitative agronomic traits, together with the effects of independent quantitative trait loci (QTLs).

Materials and methods

Plant material

The CS (Kanto107 4A) substitution line had previously been developed by 11 backcrosses of the monosomic substitution to CS monosomic 4A prior to the extraction of the disomic substitution. CS(Kanto107 4A), with a null *Wx-B1b* allele, has been shown to lack the Wx-B1 protein and has a 2–3% lower amylose content than CS (Miura and Sugawara 1996).

Using the procedures described by Law (1966), homozygous recombinant substitution lines were developed for chromosome 4A from the F_1 between CS and CS(Kanto107 4A). In total, 98 different homozygous disomic plants were extracted, then grown to maturity and allowed to self-pollinate.

Identification of the *Wx-B1* allele type and measurement of amylose content

To classify the recombinant substitution lines for the *Wx-B1* allele, electrophoretic analysis of starch-granule bound protein was performed. Starch-granule preparation and SDS-PAGE were conducted as described by Nakamura et al. (1993 a), with the modification that a 15% SDS polyacrylamide gel with an acrylamide/BIS concentration of 30 : 0.135 was used for electrophoresis.

For the evaluation of amylose content in the endosperm, all recombinant lines and the parents were grown in the field in 1996 and 1997. After anthesis, all lines were covered to prevent preharvest sprouting. Grain samples were milled on a Brabender Quadrant Junior Test Mill to a final extraction rate of a 60%. Starch granules were separated using conventional methods. The amylose content per 100 mg of starch granules was colorimetrically determined using the Auto Analyzer System II (Bran and Lubbe Co.) as described by Miura et al. (1994).

RFLP analysis

DNA of each recombinant line and parent was extracted from young leaves by a modified CTAB method (Murray and Thompson 1980). Restriction-enzyme digestion, Southern blotting, and hybridization were performed as described in Kato et al. (1998). PSR clone libraries, wheat cDNA or genomic DNA, were obtained from Dr. M. D. Gale, John Innes Centre, UK. BCD clone libraries, barley cDNAs, and CDO clone libraries, as well as oat cDNAs, were obtained from Dr. M. E. Sorrells, Cornell University, USA. Of these, the probes which were known from previous reports (Gale et al. 1995; Nelson et al. 1995) to be located on chromosome 4A were utilized. Using a "Gene Images" kit (Amersham LIFE SCIENCE), a non-isotope labelling and detecting system, the 98 recombinant lines were genotyped for RFLP markers polymorphic between the parents.

Chi-square analysis was performed between the observed segregation ratio of each RFLP marker and the theoretical 1 : 1 ratio. The linkage map was constructed using MAPMAKER/EXP3 (Lander et al. 1987), and the recombination frequencies were converted to centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944). The marker ordering, distance and degree of chromosome coverage were assessed via comparisons with the consensus maps of Gale et al. (1995) and Nelson et al. (1995).

Field trials

Field trials were conducted at the experimental field of Obihiro University of Agriculture and Veterinary Medicine over two seasons, 1996 and 1997. The parental lines and the recombinant lines were grown as a single-row plot of 12 plants, spaced 10-cm within and 30-cm between rows. A randomized block design with five replicates was employed. Ear emergence time was scored as days to heading date from the 1st July. At maturity five random leading tillers were taken from each plot and used for the evaluation of plant height and yield components, including spikelet number/ear, grain weight/ear and 50-grain weight. The remainder of each plot was harvested to score plant yield and tiller number/plant.

Detection of QTLs

Analyses of variance (ANOVA) were used to detect differences between parents and to partition the variation between recombinant lines, between seasons, and for line × season interaction. Estimates of variance components, including genetic variance and genotype by environment interaction variance, were calculated by equating the mean squares to their expected values. They were then used to estimate heritability values. A one-way ANOVA was employed for each marker to detect significant differences between allele class means by comparing them with the variation between lines within classes.

QTL analysis was also performed using the software package MQTL (Tinker and Mather 1995) which can detect both QTL main effects and QTL × season interaction. The phenotype data sets were analyzed by the simple interval mapping (SIM) and simplified composite interval mapping (sCIM) procedures. The linkage group was scanned at a 5-cM interval test statistic. Six even-spaced background markers were specified for sCIM. Except for multiple-environment sCIM analysis, where precise test statistics are not computable, type-I 5% significance thresholds were established with 1000 permutations. QTL inference were made as QTLs were declared at positions where SIM peaks were significant for either QTL main effects or QTL × season interaction with strong sCIM peaks.

Results

Linkage map

Of the 98 recombinant lines, 47 were found by the SDS-PAGE system to produce the Wx-B1 protein, and

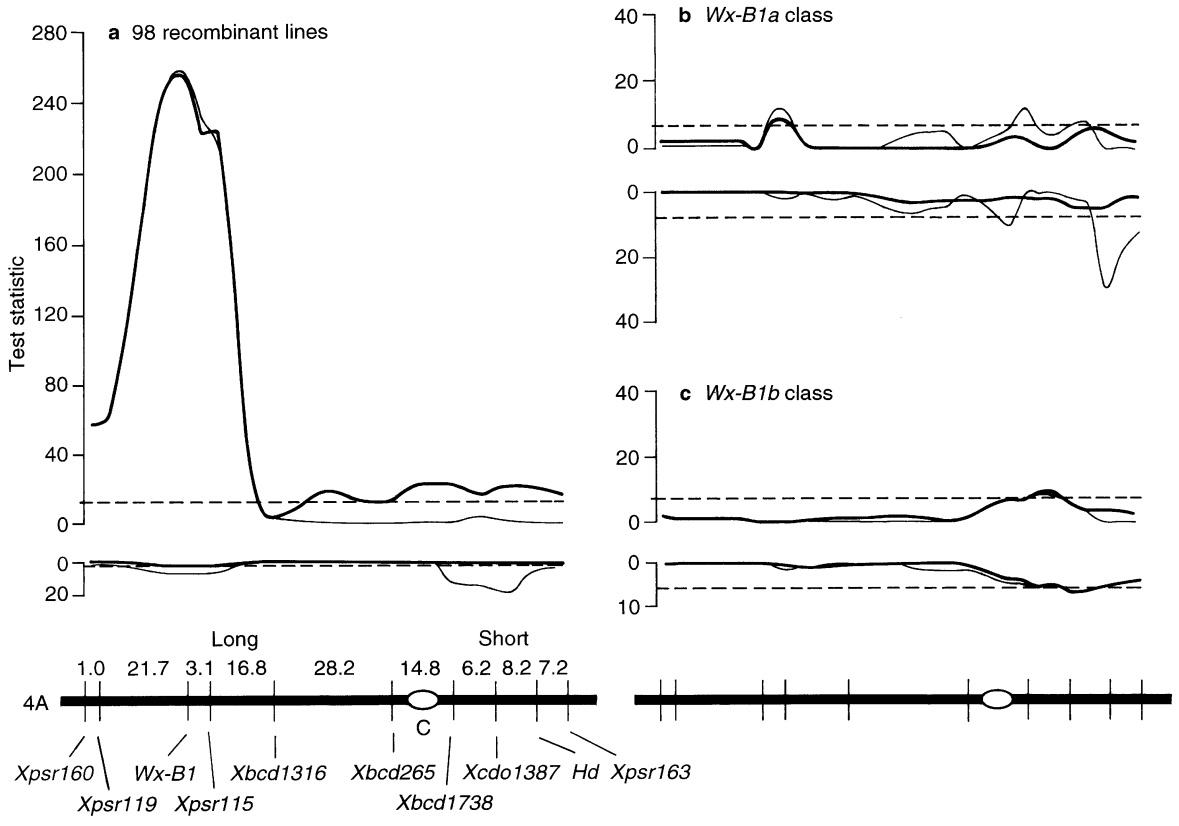

Fig. 1 Effects and positions of QTLs for amylose content detected by MQTL analysis using all of 98 recombinant lines (**a**), 47 lines of the *Wx-B1a* class (**b**) and 51 lines of the *Wx-B1b* class (**c**). The QTL main effect (upper) and the QTL × season interaction (lower) calculated by both of Simple Interval Mapping (*bold line*) and simplified Composite Interval Mapping (*normal line*) are presented. The *dotted line* indicates the 5% significance threshold level for SIM. The *horizontal black bar* at the bottom represents chromosome 4A with both markers and map distances in cM. *C* centromere

were thus identified as the CS-type with *Wx-B1a*. The remaining 51 lines were deficient in the Wx-B1 protein and identified as the CS(Kanto107 4A)-type with *Wx-B1b*. CS chromosome 4A carries a dominant allele at the awn inhibitor *Hd* locus (Sears 1954) and suppresses awn development, whereas CS(Kanto107 4A) confers an awned phenotype by the recessive *hd* allele. The recombinant substitution lines were classified by eye into unawned (51 lines) with *Hd*, and awned (47 lines) with *hd*.

Of the RFLP markers detecting polymorphism between the parents, eight showing non-distorted segregation from the expected 1 : 1 ratio were used for map construction. The resulting genetic linkage map is illustrated in Fig. 1. Compared to previously reported maps by Gale et al. (1995) and Nelson et al. (1995), the map covered approximately 107 cM, from the terminal region of the long arm defined by *Xpsr160* to the central part of the short arm as marked by *Xpsr163*. *Wx-B1* mapped to the long arm at about a 60-cM distance from the centromere, and was closely linked to *Xpsr115*, a proximal marker to the 7BS/5AL break point.

Amylose content

Starch granules from CS produced about a 25.0% amylose content over two seasons. CS(Kanto107 4A) showed a consistently, and significantly, lower content by about 2.0% than CS. The amylose content in the recombinant lines is given in Fig. 2. The 22.1–25.1% range in the recombinant lines was consistent between seasons and a highly significant correlation was detected ($r = 0.75$, $P < 0.001$) with a heritability value of 0.696. As expected, the deficiency of the Wx-B1 protein due to the null *Wx-B1b* allele caused a clear reduction in the amylose content. MQTL analysis showed that the allelic difference at *Wx-B1* was a major factor accounting for more than 70% of the variation in each season (Fig. 1a).

However, the 51 lines with the null *Wx-B1b* allele had a distribution from 22.1 to 23.9% amylose with a significant correlation between seasons ($r = 0.37$, $P < 0.01$) while the remaining 47 lines with *Wx-B1a* varied from 23.1 to 25.1%, suggesting the involvement of an additional genetic factor(s). To determine if the variation of

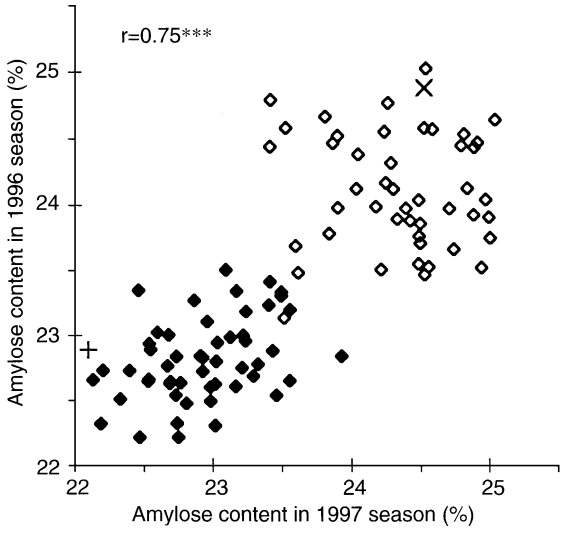

Fig. 2 Variation for amylose content in 98 recombinant lines for chromosome 4A in the 1996 and 1997 seasons. × CS, + CS(Kanto107 4A), ◇ 47 lines with the *Wx-B1a* allele, ◆ 51 lines with the *Wx-B1b* allele. Significance level ***$P < 0.001$

about 2.0% observed between lines within the allele classes included genetic variation, QTL analysis was carried out separately for the *Wx-B1a* and *Wx-B1b* classes (Fig. 1b,c). In the *Wx-B1b* allele class, both ANOVA and MQTL revealed a significant effect associated with the *Xbcd1738/Xcdo1387* interval on the short arm. This QTL, designated *QAmc.ocs-4A.1*, accounted for about 17% of the variation in the lines within the *Wx-B1b* class. The Kanto107 allele at *QAmc.ocs.1* produced a higher amylose content with an additive effect of 0.3%. In the *Wx-A1a* allele class, ANOVA confirmed the minor effect associated with *Xcdo1387*, whilst MQTL analysis failed to detect this effect.

Analysis of agronomic traits

The mean phenotypes of the parents, as well as the mean and range in the recombinant lines for the agronomic traits, are presented in Table 1. ANOVA detected highly significant differences ($P < 0.001$) between the recombinant lines for each trait. The estimated heritability values ranged from 0.216 for tiller number/plant to 0.698 for spikelet number/ear. These values suggest that chromosome 4A has effects on the traits examined and that these genetic factors segregate among the recombinant lines. Line × season interactions were also significant for all traits except spikelet number/ear. However, when the expected variance components were extracted from the mean squares of ANOVA tables, it was found that line × season interactions contributed around 6% or less of the total variation (2.80% for grain weight/ear; 6.50% for tiller number/plant).

Ear emergence time and plant height

Over two seasons of trials, CS(Kanto107 4A) flowered 2.6–3.0 days earlier than CS (Table 1). One QTL with a large effect was identified at, or closely linked, with the *Wx-B1* locus (Fig. 3). ANOVA showed that this effect was most strongly associated with *Wx-B1*. The allele from CS(Kanto107 4A) contributed to accelerated flowering, having additive effects of 1.2 days (1996) and 1.6 days (1997). This QTL, designated *QEet.ocs-4A.1*, accounted for approximately 40% of the variation in the lines in each season (Table 2).

The mean height of CS was 109.2 cm (1996) and 114.4 cm (1997), approximately 8–9 cm higher than CS(Kanto107 4A). The recombinant lines ranged from 91.5 cm to 110.7 cm in the 1996 trial, and from 95.9 cm

Table 1 Mean performance of parents, together with the mean and range in the recombinant lines for agronomic traits

Item	Ear emergence time (days)[a]	Plant height (cm)	Plant yield (g)	Spikelet no./ear	Grain wt./ear (g)	Tiller no./plant	50-grain weight (g)
1996 season							
Parents							
CS	13.4***	109.2**	10.4	13.1	1.16	17.8*	1.66
CS(Kanto107 4A)	10.8	101.1	9.2	14.6	1.19	13.7	1.59
Recombinant lines							
Mean	12.6	101.9	8.4	12.3	0.99	14.6	1.62
Range	10.8–14.8	91.5–110.7	4.7–12.8	9.8–15.7	0.64–1.34	11.7–19.8	1.45–1.77
1997 season							
Parents							
CS	12.6***	114.4**	15.2	15.1*	1.42	14.1	1.79
CS(Kanto107 4A)	9.6	105.4	13.9	16.3	1.49	14.8	1.71
Recombinant lines							
Mean	12.8	106.5	12.5	15.1	1.32	12.9	1.71
Range	10.0–15.2	95.9–120.1	7.4–17.6	12.9–17.5	0.94–1.65	9.7–18.1	1.54–1.89

Significance levels: * = 0.05–0.01, ** = 0.01–0.001, *** = 0.001
[a] Days from the 1st July

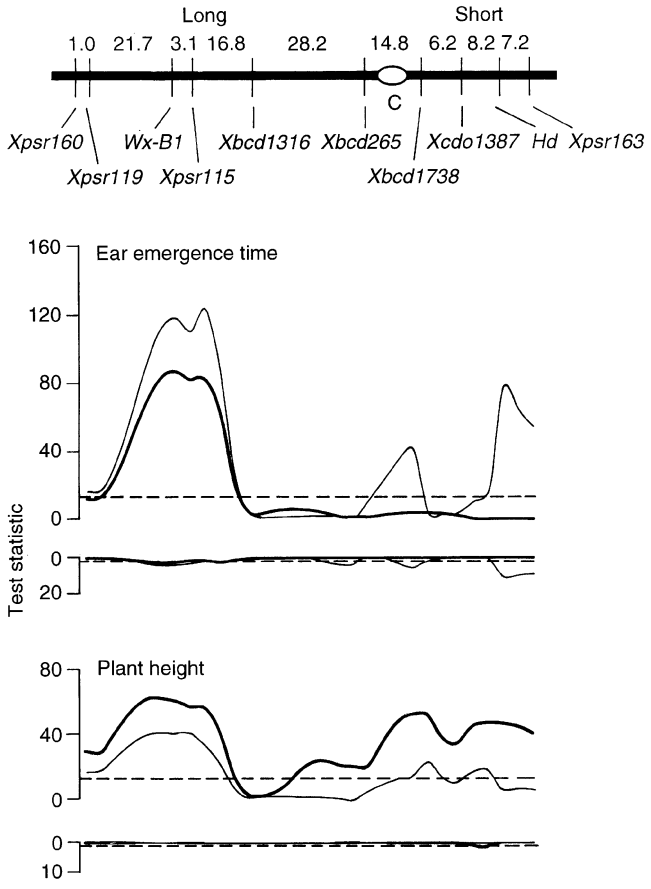

Fig. 3 Effects and positions of QTLs for ear emergence time (*top*) and plant height (*bottom*) detected by MQTL analysis on chromosome 4A. The definition of *lines* is the same as in Fig. 1

to 120.1 cm in the 1997 trial. However, the lines having heights significantly greater than CS and shorter than CS(Kanto107 4A) were not observed, showing the absence of transgressive segregation. A QTL, *QHt.ocs-4A.1*, was detected in the 21.7-cM *Xpsr119/Wx-B1* interval (Fig. 3), close to the latter locus where the height-reducing allele came from CS(Kanto107 4A) with additive effects of 4.8 cm (1996) and 5.7 cm (1997). This primary QTL accounted for about 30% of the line variation. The second QTL, *QHt.ocs-4A.2*, of lesser effect was identified in the *Xbcd1738/Hd* interval on the short arm, which explained 20% (1996) and 26% (1997) of the line variation. Like *QHt.ocs-4A.1*, the Kanto107 allele at this QTL produced a reduced height.

Plant yield and its components

While the two parents did not differ widely in plant yield, the recombinant progeny showed a large variation, ranging from 4.7 g to 12.8 g (1996) and from 7.4 g to 17.6 g (1997). Plant yield in the 1996 trial was about two-thirds of that in the 1997 trial, being partly due to head scab (*Microdochium nivale* Samuels et Hallett) infection. Compared to the parents, the recombinant lines contained several lower-yielding lines. Over the two seasons, 14 lines showed a significantly lower yield performance than the parents, indicating the occurrence of transgressive segregation.

As shown in Fig. 4, there was no evidence for significant main effects of the *Wx-B1* locus, or its adjacent regions, on plant yield. One QTL, designed *QYld.ocs-4A.1*, was detected by *Xbcd1738* on the short arm. At *QYld.ocs-4A.1*, the yield-increasing allele came from CS(Kanto107 4A) with additive effects of 1.4 g (1996) and 1.7 g (1997) which explained 27% and 20% of the variability, respectively (Table 2).

Of the yield components, a QTL for spikelet number/ear, *QSpn.ocs-4A.1*, and that for grain weight/ear, *QGwe.ocs.-4A.1*, were highly genetically correlated with each other and mapped to very similar positions to *QYld.ocs-4A.1* (Fig. 4). *QSpn.ocs-4A.1* explained

Table 2 Location of QTLs affecting agronomic traits

Trait	Locus	Trial	Marker interval	Test statistic	r^2	Additive[a]
Ear emergence time	*QEet.ocs-4A.1*	1996	*Wx-B1*	46.0	0.37	1.2 c
		1997	*Wx-B1*	44.0	0.36	1.6 c
Plant height	*QHt.ocs-4A.1*	1996	*Xpsr119/Wx-B1*	33.5	0.29	4.8 c
		1997	*Xpsr119/Wx-B1*	29.6	0.27	5.7 c
	QHt.ocs-4A.2	1996	*Xbcd1738/Hd*	30.8	0.20	4.5 c
		1997	*Xbcd1738/Hd*	22.6	0.26	4.6 c
Plant yield	*QYld.ocs-4A.1*	1996	*Xbcd1738*	31.1	0.27	1.4 k
		1997	*Xbcd1738*	18.5	0.17	1.7 k
Spikelet no./ear	*QSpn.ocs-4A.1*	1996	*Xbcd1738*	71.1	0.52	1.8 k
		1997	*Xbcd1738*	60.9	0.46	1.4 k
Grain wt./ear	*QGwe.ocs-4A.1*	1996	*Xbcd1738*	31.4	0.27	0.14 k
		1997	*Xbcd1738/Xcdo1387*	12.9	0.12	0.09 k
Tiller no./plant	*QTn.ocs-4A.1*	1996	*Xpsr163*	9.9	0.10	1.0 k
		1997	*Hd/Xpsr163*	17.1	0.16	1.2 k
50-grain weight	*QFgw.ocs-4A.1*	1996	*Xbcd265/Xbcd1738*	18.3	0.17	0.06 k

[a] Additive: indicates an additive SIM main effect of the parent contributing to a higher-value allele, where c = CS and k = CS (Kanto107 4A)

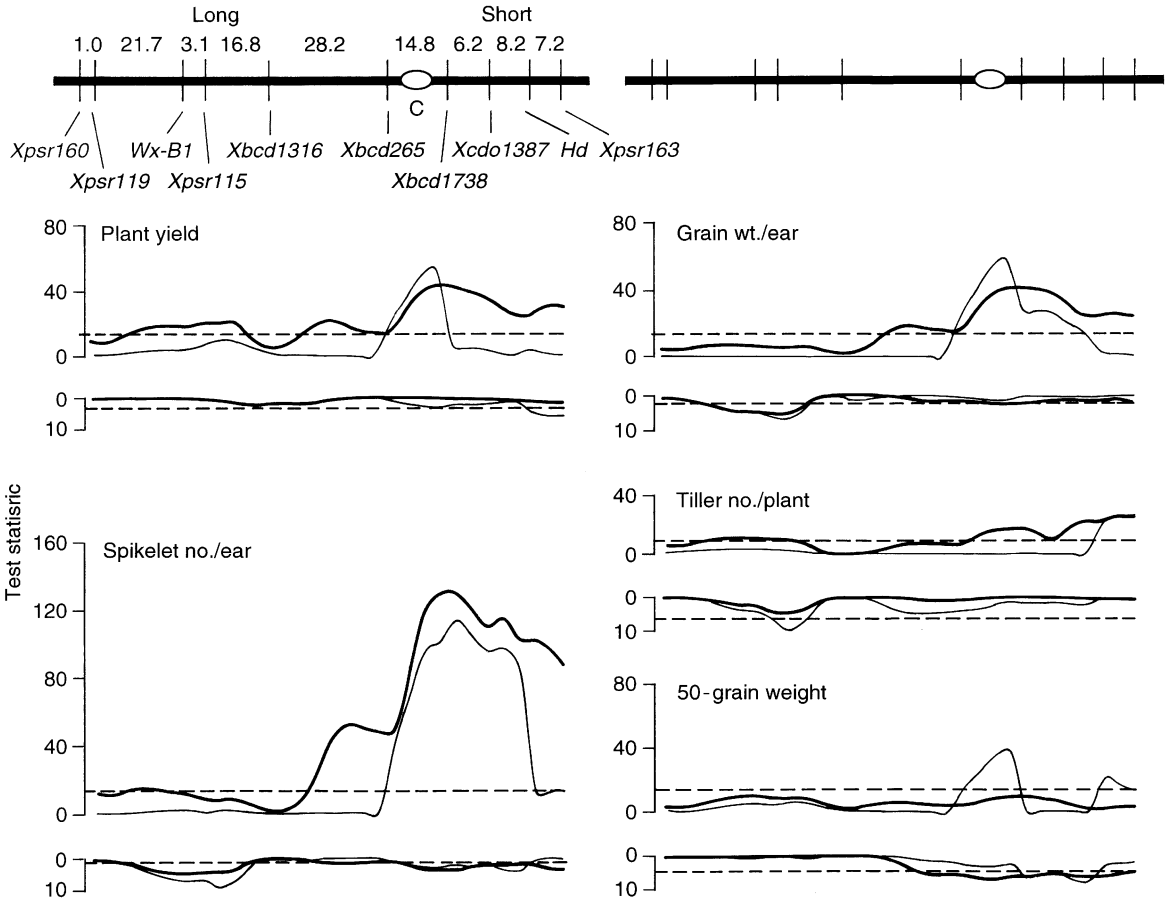

Fig. 4 Effects and positions of QTLs for plant yield and its four component traits detected by MQTL analysis on chromosome 4A. The definition of *lines* is the same as in Fig. 1

approximately 50% of the total phenotypic variance in the recombinant lines, and the Kanto107 allele with additive effects of 1.8 (1996) and 1.4 (1997) contributed to increased spikelet number. The high grain weight/ear allele at *QGwe.ocs-4A.1* again came from CS(Kanto107 4A), and had additive effects of 0.14 g (1996) and 0.09 g (1997) accounting for 27% and 12% of the phenotypic variation, respectively (Table 2). MQTL detected QTL × season interactions on the long arm for spikelet number/ear and grain weight/ear suggesting minor effects, but these were much smaller than the main effects of *QSpn.ocs-4A.1* and *QGwe.ocs-4A.1*. Most of the lower-yielding segregants also produced a lower spikelet number and grain weight/ear than the lower parent.

On the other hand, one QTL associated with tiller number/plant, *QTn.ocs-4A.1*, was found at the most distal marker *Xpsr163* of the short arm (Fig. 4). The additive effects of this QTL were small and accounted for only 10% (1996) and 16% (1997) of the phenotypic variance (Table 2). In the 1996 trial, a QTL for 50-grain weight, *QFgw.ocs-4A.1*, was detected in the centromere region flanked by *Xbcd265* and *Xbcd1738*.

Discussion

The recombinant substitution analysis of this study confirms a close association of a reduction in amylose content with lack of the Wx-B1 protein encoded by the null *Wx-B1b* allele. Reduced-amylose wheats have been shown to confer superior performance in some noodle applications and may confer enhanced water absorption to baked goods. The null *Wx-B1b* allele is also suggested to contribute to the improvement of starch-paste properties such as a high peak viscosity (Zhao et al. 1998; Araki et al. unpublished) which is an important factor for noodle (and bread) manufacture. Compared to single null lines, like CS(Kanto107 4A), or double-null lines with *Wx-A1b* and *Wx-D1b*, double-null lines with *Wx-B1b* and *Wx-D1b* or with *Wx-B1b* and *Wx-A1b* are more likely to provide consistent sources of low-amylose starch and high paste viscosity (Miura et al. 1998). Therefore, it is preferred that double-null cultivars and advanced breeding lines should be used to carry *Wx-B1b* into the development of locally adapted wheats carrying desirable starch properties.

Even though amylose content is mostly influenced by the *Wx* genes, we show that they do not explain all the variation in this trait. A modifying factor, *QAmc.ocs-4A.1*, was detected in the present study and

mapped in the 6.2-cM *Xbcd1738/Xcdo1387* interval on the short arm. This QTL might account for variation within the *Wx-B1* allele classes. Since the effect of *QAmc.ocs-4A.1* was masked in lines with *Wx-B1a*, an epistatic interaction with *Wx-B1* appeared to decrease the action of *QAmc.ocs-4A.1* in the presence of the Wx-B1 protein. The availability of genetic loci which alter the amylose content around 0.5–1.0% make it feasible to "fine-tune" genotypes to meet specific requirements of flours. Molecular markers will allow the *QAmc.ocs-4A.1* locus with minor effect to be used more precisely to manipulate amylose content than would be possible on the basis of phenotypic selection.

For changing the amylose content, at least two kinds of genes should be considered; one associated with starch synthesis and another with starch hydrolysis. As far as is known, chromosome 4A carries no loci encoding other proteins, except Wx-B1, mediating soluble-starch synthases and the starch-branching enzyme. The short arms of the group-7 chromosomes have homoeologous genes encoding starch granule-bound proteins, with molecular weights of 100–105 kDa, which have starch-synthase activity (Denyer et al. 1995). The possibility that the translocated segment on 4A from 7BS contains these genes is refuted by the fact that 7BS produces the starch granule-bound protein of 100 kDa (Denyer et al. 1995). The starch hydrolyzing enzymes, α-amylase and β-amylase, are of significance in starch quality and can modify the amylose/amylopectin ratio. The production of α-amylase-1 is controlled by genes on the long arms of group-6 chromosomes, and that of α-amylase-2 by genes on the group-7 long arms (Gale et al. 1995). While the homoeologous *β-amy-1* loci are located on group-4 chromosomes, *β-amy-A1* is in the translocated segment on 5A from 4A. Furthermore, two sucrose synthase loci, *Sus1* and *Ss1*, have been mapped on the short arms of homoeologous group 7 (Devos et al. 1995). Since the *Wx* locus is flanked with *Sus1* and *Ss1*, the long arm of 4A can localize the sucrose synthase loci on the translocated segment from 7BS. This evidence indicates that *QAmc.ocs-4A.1* on the short arm is independent of known genes affecting starch quality or sucrose synthesis. Whether *QAmc.ocs-4A.1* encodes an enzyme(s) in the starch-synthesis pathway or acts as a regulator gene should be investigated.

In our population, *QEet.ocs-4A.1*, closely linked to *Wx-B1*, was identified via its effect on flowering time. Hoogendoorn (1985) reported an earliness *per se* factor on chromosome 4A but any linkage with *Wx-B1* is unclear. In barley, a significant linkage of an earliness *per se* gene (*eps7S*) with the *Wx* locus has been implied (Backes et al. 1995; Laurie et al. 1995). Comparative mapping of wheat, barley and rye using molecular markers shows that, even if translocations are taken into account, the order of genes including QTLs is conserved on homoeologous chromosome segments (e.g. Laurie 1997). This predicts a possible relationship between *QEet.ocs-4A.1* and barley *eps7S*, but few common markers exist between the QTL studies and thus it is at present difficult to assess the synteny of these genes. The region adjacent to the *Wx-B1* locus was also associated with plant height. The position of this putative *QHt.ocs-4A.1* is contained within the cluster of *Wx-B1* and *QEet.ocs-4A.1*. While Bezant et al. (1997) located a barley plant-height QTL (*QHt.psb-7H*) on the distal part of the *Wx* locus, no QTLs for plant height have been reported in the vicinity of the *Wx* genes in other Triticeae species.

Although phenotypic differences conditioned by *QEet.ocs-4A.1* and *QHt.ocs-4A.1* are small, compared to the effects of the major *Vrn* or *Ppd* genes on flowering time and those of the *Rht* genes on plant height respectively, an allelic difference at *QEet.ocs-4A.1* can lead to about a 3.0-day variation for flowering time, while alleles at *QHt.ocs-4A.1* produce a 10-cm change in plant height (Table 2). Thus, these QTLs located in the adjacent regions of *Wx-B1* could have breeding potential. If this is the case, the expression of the Wx-B1 protein might be used to select for these traits. Conversely, because of its effects on starch properties, selection for the *Wx-B1b* allele may also lead to inadvertent selection for heading date and plant height. In this regard, Miura and Tanii (1994) demonstrated that lack of the Wx proteins can be identified readily using a half-endosperm in an SDS-PAGE system. This may permit the screening of grains with the null *Wx-B1b* in large populations, even in early segregating generations like F_2 and B_1F_1. The selected half-grain with the embryo will allow an assessment of starch properties and morphological traits, including flowering time and plant height.

For plant yield and its components, there was no evidence for significant main effects associated with *Wx-B1* or adjacent regions. One QTL affecting plant yield was detected on the short arm and a QTL for spikelet number (grain weight/ear) was mapped in a similar position to the plant-yield QTL, so it was not enough to explain both the transgressive segregation in the recombinant substitution lines and the lack of significant differences between the parents. Additional QTLs which would have been able to indicate a correlation with plant yield, such as the tiller number QTLs, might be segregating on chromosome 4A, but were not detected due to the lack of appropriate markers. It is also possible that, like the QTL for 50-grain weight, the effects of genes could be too weak to be clearly detected within our population. Since all of the transgressive segregants detected were the lower-yielding lines compared to the parents, the residual variation on chromosomes other than 4A should also be considered. Hence the possibility of linkage of *Wx-B1* and yield QTLs would not be cancelled out, though, even if they occur, such QTL effects are expected to be smaller than the effects of the plant-yield QTL, *QYld.ocs-4A.1*, on the short arm. At least in

barley, the multiple map compilation by Hayes et al. (1997) shows that no close linkage of yield QTLs with the *Wx* locus on 7HS have been found in five different doubled-haploid populations. From this information and our present results, we propose that wheat breeders can manipulate the *Wx-B1* locus while giving less attention to yield QTLs.

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