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QTL mapping of genes controlling ear emergence time and plant height on chromosome 5A of wheat

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Abstract Chromosome 5A of wheat carries major gene loci for agronomic traits including the vernalization requirement (*Vrn-A1*) and ear morphology (*Q*). To determine whether the genetic variation for ear emergence time and plant height is attributable to either of these major genes as pleiotropic effects or independent QTL, we combined a RFLP map constructed from 120 recombinant substitution lines derived from a cross between 'Chinese Spring' (Cappelle-Desprez 5A) and CS(*Triticum spelta* 5A) with data collected from field trials over 3 years. For ear emergence time the main effects on flowering time were by *Vrn-A1* and *QEet.ocs-5A.1*, the latter a QTL in the 28.6-cM *Xcdo584*/*Q* interval linked to *Q* by less than 10 cM. The CS(*T. spelta* 5A) allele at *QEet.ocs-5A.1* contributed to an earlier ear emergence time by 2.7–6.0 days, which was approximately equal to the effects of *Vrn-A1*. For plant height, three QTLs were identified on the long arm and linked in repulsion. The CS(*T. spelta* 5A) allele at *Vrn-A1* or closely linked to *Xfba068* contributed to a height reduction of 3.5–6.1 cm, whereas both the *Q* allele and *Qt.ocs-5A.1* allele within the *Xcdo1088*/*Xbcd9* interval from CS(Cappelle-Desprez 5A) produced a shorter plant. When plant height was partitioned into culm length and ear length, the *Vrn-A1* allele and CS(Cappelle-Desprez 5A) allele at *QCl.ocs-5A.1* within the *Xcd1088*/*Xbcd9* interval were found to contribute to a shorter culm. CS(*T. spelta* 5A) allele at *q* was a major determinant of a long ear, together with minor effects at *QEl.ocs-5A.1* within the *Xcdo1088*/*Xbcd9* interval.

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Introduction

Analyses of genes controlling ear emergence time in cereals are of practical importance because of the effects of these genes on plant adaptation and crop yield. Based on their interactions with environmental signals, the genes can be divided into three broad categories. These are photoperiod-response genes, vernalization response genes and earliness *per se* genes. For cereal production in cool climates, such as those of Asia and America, and northern Europe, the most important genes are those determining the spring/winter growth habit or vernalization response (Plaschke et al. 1993). In hexaploid wheat (*Triticum aestivum*), *Vrn-A1* on chromosome 5A appears to be the primary effect in controlling ear emergence time (Law 1966; Law et al. 1976).

Since in most situations later flowering plants tend to be taller simply due to their extended life cycle, allelic variation at *Vrn-A1* could regulate plant height. Chromosome 5A has been shown to carry multi-genes which affect plant height (Snape et al. 1985; Roberts 1990). One of these, *q*, which determines ear morphology would be expected to affect plant height (Snape et al. 1985). In addition to semi-dwarfing *Rht* genes, allelic variation at other loci associated with height reductions could have breeding potential for lodging resistance and yield increase. However, whether genetic variation for ear emergence time and plant height can be attributed to either of these major genes as pleiotropic effects or independent quantitative trait loci (QTLs) has not been defined in detail.

We had developed a homozygous population of 120 recombinant substitution lines for chromosome 5A and

mapped *Vrn-A1*, *q* and 10 restriction fragment length polymorphism (RFLP) markers (Kato et al. 1998). Using the linkage map constructed and the mapping population, manipulation and tagging of these major gene loci on chromosome 5A by RFLP markers could contribute to the breeding of high-yielding cultivars with a better plant form and adaptability to appropriate environments. The purpose of the work presented here was to identify QTL and QTL × environment interactions for ear emergence time and plant height by combining markers with data collected from field trials over 3 years.

Materials and methods

Population

Using the technique first described by Law (1966), we produced a population of 120 recombinant substitution lines from the F_1 between substitution lines for chromosome 5A from a spring accession of *T. spelta* and winter wheat cultivar 'Cappelle-Desprez' into a 'Chinese Spring' (CS) background (Kato et al. 1998). The parental substitution lines CS(Cappelle-Desprez 5A), which carries the alleles *vrn-A1*, *Q* and *B1*, and CS(*T. spelta* 5A), which carries alternative *Vrn-A1*, *q* and *b1* alleles, were developed and kindly provided by Dr. A.J. Worland, John Innes Centre, UK.

Field trials

Field trials were conducted at the experimental field of Obihiro University of Agriculture and Veterinary Medicine over the years 1993, 1994 and 1996. The mapping population and two parental lines were sown in the late April of each year. A randomized complete block design with five replicate blocks was used. Each line was represented by a single row plot of 11 plants, that were spaced 10 cm apart within rows. Rows were spaced 30 cm apart. Ear emergence time was scored as days to heading time from the July 1st. At maturity five random leading tillers were taken from each plot and used for the evaluation of plant height, which was subdivided into ear length and culm length.

RFLP assays

We earlier had constructed a genetic linkage map covering about 230 cM of chromosome 5A with the three major genes, *Vrn-A1*, *Q*, *B1*, and 10 RFLP markers (Kato et al. 1998). However, the map contained three gaps which did not meet a LOD threshold of 3.0 and recombination frequency of 0.40. In order to saturate these gaps, we analyzed BCD and CDO clone libraries obtained from Dr. M.E. Sorrells, Cornell University, USA and FBA clones from Dr. P. Leroy, INRA, France. RFLP assays were carried out as described by Kato et al. (1998).

Linkage and QTL analysis

Chi-square analyses were performed on each probe to detect deviations from the expected Mendelian segregation of 1:1. MAPMAKER/EXP (Lincoln et al. 1993) version 3.0 was used to map newly detected RFLP markers on our previous map (Kato et al. 1998). Linkage was declared when a LOD score threshold of 3.0 and recombination frequency of 0.40 were met.

QTL analysis was performed on the mean of the five replications. The phenotype data sets were analyzed by the simple interval mapping (SIM) and simplified composite interval mapping (sCIM) procedures using the software package MQTL (Tinker and Mather 1995). The linkage group was scanned at a 5-cM interval test statistic. Nine evenly 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.

Analyses of variance were also used within each trial to detect differences between the parental lines and differences between the mapping population.

Results

Linkage mapping

The reconstructed genetic linkage map is illustrated in Fig. 1. The map comprises 15 RFLP markers and three major gene loci, *Vrn-A1*, *Q* and *B1*. The total distance mapped covers about 230 cM of chromosome 5A. The 5 new RFLP markers showing non-distorted segregation from a 1:1 ratio could be mapped in the three gaps of our previous map in the intervals, *Xbcd926*/*Xcdo400*

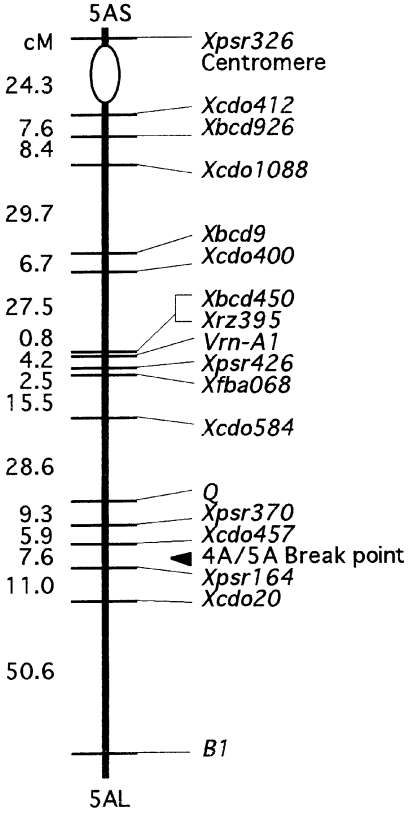

Fig. 1 Genetic linkage map of wheat chromosome 5A with 15 markers and three morphological genes

Table 1 Mean performance and range of four traits of the parental lines and the mapping population measured in the 3 years

Year	Lines		Ear emergence time (days[a])	Plant height (cm)	Culm length (cm)	Ear length (cm)
1993	CS(Cappelle-Desprez 5A)		19.4***	102.5**	96.3*	6.20***
	CS(*T. spelta* 5A)		10.6	113.9	104.1	9.83
	Mapping population	mean	14.6	107.7	99.8	7.95
		range	10.4–20.0	89.2–125.0	70.8–113.6	5.04–10.53
1994	CS(Cappelle-Desprez 5A)		11.6***	96.4*	90.1	6.26***
	CS(*T. spelta* 5A)		1.2	104.2	93.7	10.48
	Mapping population	mean	5.6	104.8	96.3	8.46
		range	1.0–12.8	86.0–120.5	81.7–109.5	5.94–11.22
1996	CS(Cappelle-Desprez 5A)		15.6**	88.7*	82.4	6.28***
	CS(*T. spelta* 5A)		11.8	96.3	86.8	9.46
	Mapping population	mean	13.6	95.9	87.7	8.19
		range	9.0–16.9	74.9–116.6	80.2–95.9	6.04–10.60

Significance levels: * $P \leq 0.05$–0.01, ** $P \leq 0.01$–0.001, *** $P < 0.001$
[a] Days from July 1st.

(45.0 cM), *Xpsr426/Q* (41.8 cM) and *Xpsr164/B1* (57.3 cM) (Kato et al. 1998).

Analysis of field data

In interpreting the data collected over the 3-year period we had to take the prevailing climatic conditions during these experiments into considertion. In the 1993 trial, cool and rainy weather throughout the growing season caused severe cold damage. While the conditions in the 1994 trial were fairly good, wheat plants in the 1996 trial were grown under cool and rainy weather from late June to July, and produced scabbed grains. Consequently, performance was very variable over the 3 years.

The mean performance and range of the four traits of the parental lines and the mapping population measured in each trial are listed in Table 1. While cool weather in 1993 and 1996 caused a large delay in ear emergence of CS(*T. spelta* 5A), this genotype was consistently earlier than CS(Cappelle-Desprez 5A). Over 3 years, there were significant differences between the parental lines for plant height and its component trait, ear length. *T. spelta* 5A confers a taller height and longer ear.

Analysis of variance detected highly significant differences between the recombinant lines for each trait in all 3 years. Line × year interaction was also significant for each trait. There were lines having phenotypic values greater than that of the higher parent and lower than that of the lower parent for plant height, culm length and ear length over the 3 years. For ear emergence time such transgressive segregants were not observed.

Ear emergence time

As shown in Fig. 2, two QTLs with large effects were identified at the *Vrn-A1* locus and the *Xcdo584/Q*

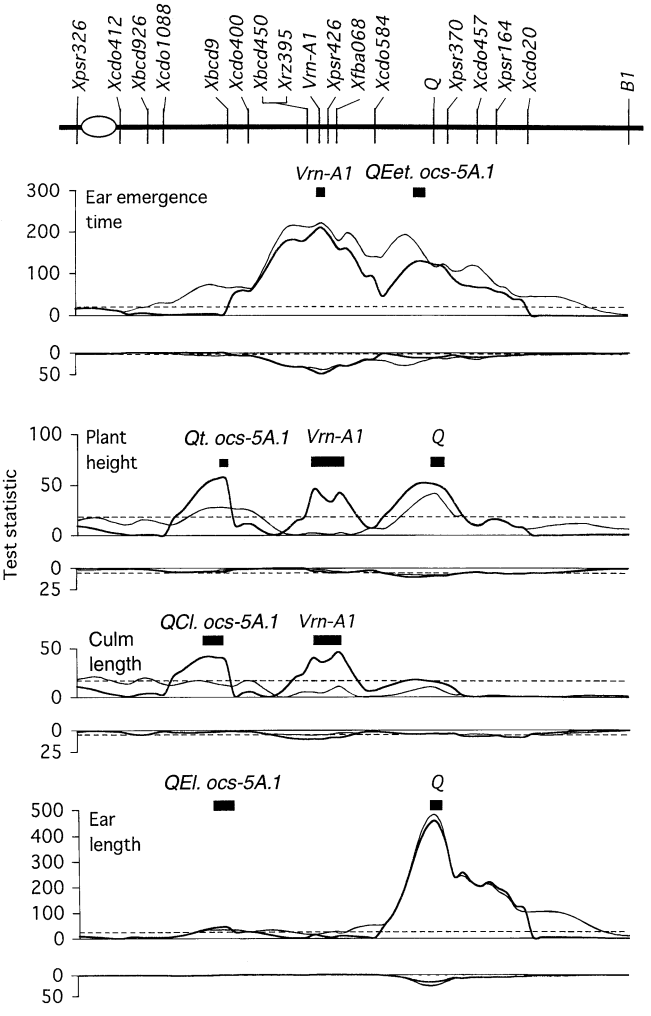

Fig. 2 Effect and position of QTLs for morphological traits. Both the QTL main effect (*upper*) and the QTL × years interaction (*lower*) were calculated by Simple Interval Mapping (*normal line*) and simplified Composite Interval Mapping (*bold line*). The *dotted line* indicates the 5% significant threshold level for Simple Interval Mapping

Table 2 Location of QTLs affecting for the four traits

Trait	Locus	Trial	Marker interval	Test statistic	r^2	Additive[a]
Ear emergence time	*Vrn-A1*	1993	*Vrn-A1*	61.0	0.40	3.4 c
		1994	*Vrn-A1*	111.3	0.60	5.5 c
		1996	*Xbcd450*	71.7	0.45	2.4 c
	QEet.ocs.5A.1	1993	*Xcdo584/Q*	55.8	0.37	3.9 c
		1994	*Xcdo584/Q*	85.2	0.51	6.0 c
		1996	*Xcdo584/Q*	67.0	0.43	2.7 c
Plant height	*Vrn-A1*	1994	*Xfba068*[b]	26.4[b]		6.1 c[b]
		1996	*Xfba068*[b]	19.8[b]		3.5 c[b]
	Q	1993	*Xcdo584/Q*	23.8	0.18	6.9 s
	Qt.ocs-5A.1	1993	*Xcdo1088/Xbcd9*	14.3	0.11	6.2 s
		1994	*Xbcd9*	9.3	0.07	3.9 s
Culm length	*Vrn-A1*	1994	*Xfba068*	10.8	0.09	3.8 c
		1996	*Xfba068*[b]	19.6[b]		3.2 c[b]
	QCl.ocs.-5A.1	1994	*Xcdo1088/Xbcd9*[b]	19.2[b]		5.3 s[b]
Ear length	*Q*	1993	*Q*	129.8	0.66	2.75 s
		1994	*Q*	234.6	0.86	3.16 s
		1996	*Q*	157.0	0.73	2.18 s
	QEl.ocs-5A.1	1994	*Xbcd9*	12.6	0.10	1.10 s

[a] Additive indicates additive SIM main effect of parent contributing higher value allele, where c = CS(Cappelle-Desprez 5A) and s = CS(*T. spelta* 5A)
[b] Indicates peak which can be detected only by sCIM, and these values were calculated by sCIM.

interval (*QEet.ocs-5A.1*) by sCIM, whereas SIM identified several QTLs located on the middle region of the long arm. As Tinker and Mather (1995) indicated, SIM is not able to resolve the combined effects of linked QTL if they are linked in coupling, while sCIM seems to give unbiased estimates. Thus, the two QTL detected by sCIM most likely represent the best estimation.

The CS(*T. spelta* 5A) allele, *Vrn-A1*, had additive effects of 3.4 days (1993), 5.5 days (1994) and 2.4 days (1996) in promoting ear emergence. Each of these explained more than 40% of the total phenotypic variance (Table 2). The second QTL, *QEet.ocs-5A.1*, was about 40 cM distant from *Vrn-A1* and linked to *Q* by less than 10 cM. The allele for earliness was from the earlier parent CS(*T. spelta* 5A) with additive effects of 3.9 days (1993), 6.0 days (1994) and 2.7 days (1996) which accounted for 37, 51 and 43% of the total phenotypic variance, respectively.

QTL × year interaction for the two QTLs was detected within the same significant marker intervals of the respective QTL main effects. Thus, the QTL × year interaction was due to changes in the magnitude of response at the two loci rather than changes in the alleles.

Plant height

We detected three QTLs linked in repulsion (Fig. 2) – a pleiotropic effect of *Vrn-A1* or the effect of a tightly linked locus to *Xfba068*. At this QTL the height reduction allele came from CS(*T. spelta* 5A) with additive effects of 6.1 cm (1994) and 3.5 cm (1996), which was the only height-reducing effect detected from the taller parent CS(*T. spelta* 5A). The second QTL, designated *Qt.ocs-5A.1*, was identified in the marker interval *Xcdo1088/Xbcd9*, proximal to *Xbcd9*, as a significant main effect by both SIM and sCIM. The allele from CS(Cappelle-Desprez 5A) at this QTL had additive effects of 6.2 cm (1993) and 3.9 cm (1994) on height reduction and explained 11% and 7% of the variability, respectively (Table 2). The effect associated with *Q* or tightly linked QTLs was detected as a significant main effect and QTL × year interaction by both SIM and sCIM. The gene effect was clear in the 1993 trial with an additive effect of 6.9 cm which accounted for 18% of the variability. Just as *Qt.ocs-5A.1*, the height reduction allele came from CS(Cappelle Desprez 5A). A significant QTL × year interaction found at this QTL was probably due to the disappearance or decrease of the effect in the 1994 and 1996 trials.

Culm length and ear length

To analyze the genetic factors affecting plant height in detail, we partitioned this trait into culm length and ear length. Two QTLs controlling culm length were mapped (Fig. 2). The effect of *Vrn-A1* or tightly linked QTL was identical to the *Vrn-A1* effect on plant height. The shorter-culm allele from CS(*T. spelta* 5A) at this QTL had an additive effect of 3.8 cm (1994) and 3.2 cm (1996). In the 1994 trial, a QTL corresponding to *Qt.ocs-5A.1* on plant height appeared within the

Xcdo1088/Xbcd9 interval and was designated *QCl.ocs-5A.1*.

For ear length both SIM and sCIM revealed the largest main effect of the *Q* locus. The *Q* × year interaction was significant but much less than the main effect (Fig. 2). The CS(*T. spelta* 5A) allele, *q*, had additive effects of a 2.75-cm (1993), a 3.16-cm (1994) and a 2.18-cm (1996) increase in length, which explained 66%, 86% and 73% of the total phenotypic variance, respectively (Table 2). These large effects of the *q* allele are an obvious result of the change in ear morphology. In the 1994 trial, a minor but significant QTL was detected in the *Xcdo1088/Xbcd9* interval. This QTL appeared to be closely linked to *Xbcd9*, and the longer-ear allele came from CS(*T. spelta* 5A), indicating its identity to *Qt.ocs-5A.1* or *QCl.ocs-5A.1*. The additive effect of this QTL was small, about 1 cm, and accounted for only 10% of the total phenotypic variance.

Discussion

As expected, the *Vrn-A1* gene from CS(*T. spelta* 5A) had a major effect on earlier ear emergence time over the 3 trial years. In addition, we have identified *QEet.ocs-5A.1* linked to *q* by less than 10 cM. This putative QTL, *QEet.ocs-5A.1*, was found to be comparable to *Vrn-A1* in the direction and magnitude of its additive effects and amount of variance accounted for. Since no transgressive segregants were found in our population (Table 1) and the test statistics of QTL × year interaction were much less than those of the QTL main effect (Fig. 2), *Vrn-A1* and *QEet.ocs-5A.1* must be major determinants of ear emergence time. The coupling of the alleles at these two loci would contribute the most potent effect on reduced days to ear emergence in *T. spelta* 5A, a phenomenon previously noted as whole chromosome effects (Law et al. 1976; Snape et al. 1976; Miura and Kuroshima 1996). Snape et al. (1985) demonstrated a pleiotropic effect or a closely linked locus of *q* on ear emergence time after sufficient vernalization to remove the effect of *Vrn-A1* allelic variation. We observed recombination between *q* and *QEet.ocs-5A.1* in our population. This confirms effects associated with the separate gene closely linked to *q*. *QEet.ocs-5A.1* may represent a major gene which was not previously identified or characterized. We now are determining whether this gene is for photoperiod response or for earliness *per se* using near isogenenic lines of *QEet.ocs-5A.1* selected by marker-assisted selection.

As described in Laurie's (1997) review on intra-chromosomal location of the major vernalization genes in Triticeae with respect to common RFLP markers, wheat *Vrn-A1*, *Vrn-D1* (5D) (Nelson et al. 1995; Galiba et al. 1995), rye *Sp1* (Plaschke et al. 1993) and barley *Sh2* (Laurie et al. 1995) have very similar map locations on the homoeologous group 5 chromosomes, suggesting that all three species share at least in part a common vernalization response mechanism. Although such syntenic relationships among Triticeae are also expected for *QEet.ocs-5A.1*, homologues of this gene have not been identified in the wheat 5B and 5D or in rye and barley. A simple explanation for this may be because allelic differences in QTLs between parental lines have been lacking, probably due to the limited number of cross-combinations studied.

For plant height we identified three QTL linked in repulsion. A pleiotropic effect of the *Vrn-A1* or the effect of a tightly linked locus to *Xfba068* was detected as the third QTL in which the shorter-plant allele came from the taller parent CS(*T. spelta* 5A). This pleiotropic effect of the *Vrn-A1* locus was anticipated because earlier-flowering plants conditioned by the *Vrn-A1* allele would be shorter simply due to a physical association between plant height and life cycle. When the genetic effects on plant height were partitioned into those for culm length and ear length, the three QTLs were found to consist of two QTLs for culm length and two QTLs for ear length. The transgression observed in these traits is associated with the inheritance of complementary QTL alleles from the two parents. However, it is doubtful that the three QTLs identified can perfectly explain the variation in the recombinant substitution lines where the observed range between the highest and lowest lines exceeded 35 cm in every year (Table 1). Such a wide range of the whole-chromosome effects of 5A has been reported by Miura and Kuroshima (1996). Generally, height seems to be affected by most genetic factors which regulate development, morphology and vigour. In addition to the three QTLs, therefore, the allelic variation at other undetected loci, including those on the short arm (Snape et al. 1985), could be responsible for the wide transgressive segregation in the recombinant substitution lines.

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