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Segregation analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines

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The purpose of this study was to analyze the genetic segregation of heading traits in wheat using recombinant inbred lines (RILs) of hexaploid wheat, derived from *Triticum aestivum* cv. Chinese Spring and *T. spelta* var. *duhameliamum*. The population was examined under controlled environmental conditions as well as in the field. This strategy differentiated the effect of three genetic factors (vernalization requirement, photoperiod sensitivity and narrow-sense earliness) and identified their interactions. Correlation analysis showed that photoperiod sensitivity and narrow-sense earliness are critical for heading time in the field. Single-marker analysis using 322 molecular markers segregating among RIL detected a total of 38 linked markers for each genetic factor and heading in the field. In interval analysis, two *Vrn* genes (*Vrn-B1* and *Vrn-D1*) and *Ppd-B1* were mapped on chromosomes 5B, 5D and 2B, respectively. It was noticed that *Vrn-B1* on 5B from the spelt wheat conferred a strong-spring habit equivalent to the homoeologous *Vrn-A1*. Quantitative trait locus analysis also showed that *Ppd-B1* was not detected under the short-day condition without vernalization treatment, and that there were two types of genes for photoperiod sensitivity, dependent on and independent of vernalization treatment.
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Introduction

Heading time in wheat is governed by three major genetic factors: vernalization requirement, photoperiod sensitivity and narrow-sense earliness (Kato and Yamagata, 1988). The first two factors are environment dependent, while the latter is environment independent. Generally, wheat cultivars are divided into two types (winter and spring types), depending on their requirement for cold temperature to initiate heading. This is known as vernalization requirement, which determines growth habits; winter and spring types. Wheat is essentially a long-day plant, and most varieties require a certain period of long day for heading. This feature is known as photoperiod sensitivity. On the other hand, narrow-sense earliness is an environment-independent factor defined as earliness in completely vernalized plants under the 24-h daylength regime (Takahashi and Yasuda, 1958).

Segregation studies and aneuploid analyses have indicated that each of the above genetic factors is controlled by multiple homoeoallelic genes. For the vernalization requirement, three major genes, *Vrn-A1*, *-B1* and *-D1*, have been well characterized and are reported to be located on the long arms of chromosomes 5A, 5B and 5D, respectively (Law *et al*, 1976; Maystrenko, 1980; Scarth and Law, 1983; Hoogendoorn, 1985a; Worland and Law, 1986; Galiba *et al*, 1995; Nelson *et al*, 1995; Korzun *et al*, 1997; McIntosh *et al*, 1998). For photoperiod sensitivity, three major genes, *Ppd-A1*, *Ppd-B1* and *Ppd-D1* (initially *Ppd3*, *Ppd2* and *Ppd1*, respectively), also thought to be homoeoallelic, are located on chromosomes 2A, 2B and 2D, respectively (Welsh *et al*, 1973, Law *et al*, 1978, Scarth and Law, 1983). Semidominant alleles of the *Vrn* and *Ppd* genes promote heading without requirement for cold treatment or long daylength, respectively. In addition to these genes, several other genes have been reported to be involved in the control of heading time, including late heading (Islam-Faridi *et al*, 1996; Law *et al*, 1998). Contrary to vernalization requirement and photoperiod sensitivity, it is known that narrow-sense earliness is polygenic.

Genetic analysis of heading has been conducted mainly by using F_2 population and/or aneuploid or chromosome substitution lines. Recent development of molecular markers for linkage maps has allowed the development of quantitative trait locus (QTL) analysis, which could identify the exact numbers and positions of the responsible genes as well as their interactions. In the present study, segregation analysis of heading was applied to a series of recombinant inbred lines (RILs) of hexaploid wheat. RILs are appropriate for QTL analysis because of their homozygosity. In addition, RILs are useful for detecting gene interactions because they can be repeatedly examined for their phenotypes under different environmental conditions. Linkage analysis was conducted to identify QTLs for genetic factors of heading, and to examine the interactions and contribution of such factors to heading earliness of

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hexaploid wheat. Using these analyses, *Ppd-B1* and *Vrn-B1* were located on chromosomes 2B and 5B, respectively.

Materials and methods

Plant materials

The RILs used in this study were established at F_8 generation by the single-seed descent method from an F_2 family between *Triticum aestivum* cv. Chinese Spring (CS) and *Triticum spelta* var. *duhamelianum* KT19-1 (SP) (Ahmed *et al*, 2000). These two parental lines were selected from the genetic resource bank of KIBR because of the relatively large genetic diversity and lack of any genetic distortion of segregation among them (Liu and Tsunewaki, 1991). CS and SP are moderate- and strong-spring types, respectively, and the former has been reported to harbor the *Vrn-D1* and *Ppd-B1*, respectively (Pugsley, 1972; Scarth and Law, 1983). Nulli-tetrasomic lines of CS derived by Sears (1954) were used to determine the homoeology of the RFLP marker locations.

Growth condition

The winter-sown trial was conducted at the experimental field of KIBR during the period from 1999 to 2000. The days to heading from the 1st of April were recorded for each plant with five observations for two replications. The same RILs were also grown under four controlled conditions, combining the presence or absence of vernalization treatment with long or short daylength as follows: VL: vernalization/long day; VS: vernalization/short day; NL: nonvernalization/long day; NS: nonvernalization/short day. In all conditions, seeds were imbibed at 4°C for 2 days to synchronize germination, and then incubated for 3 days at 20°C and thereafter transplanted into 5 cm × 5 cm pots. Six 5-day-old seedlings of each genotype were vernalized in a phytotron (Koito Co.) controlled at 5/10°C for 14-h dark/10-h light cycles for 6 weeks. Seeds for treatments without vernalization were germinated 5 weeks later to allow the seedlings to enter into the different daylength regime at the same growing stage as the vernalized seedlings. When the vernalization period was completed, one-half of the plants were grown under the long-day condition where plants were exposed to illumination by using fluorescence lamps (National FCL30BR) throughout the entire night time. The rest of the plants were subjected to the short-day condition with 16-h darkness by covering the plants with a sheet in a greenhouse. Under both conditions, the temperature was maintained at 25 ± 3°C throughout the day. The days to unfolding of the flag leaf from the start of the photoperiod treatment were recorded as days to heading. Vernalization requirement and photoperiod sensitivity for each RIL were calculated as the deviation of the mean of heading days under VL from those under NL and VS, respectively.

Genetic mapping and QTL analysis

A skeleton RFLP map of this RIL population has been reported by Ahmed *et al* (2000). Additional RFLP markers were analyzed for polymorphisms to construct detailed maps of chromosomes 2B, 5A, 5B and 5D. DNA probes, including PSR clone libraries, which were kindly provided by Dr MD Gale, John Innes Centre, Norwich, UK, BCD, WG and CDO clone libraries, which were generously donated by Dr ME Sorrells, Cornell University, NY, USA, and KSU clone libraries, which were obtained from Dr BS Gill, Kansas State University, KS, USA, were used. WEC clones were the original cDNA that were expressed differentially by vernalization treatment of developing embryos (Shindo and Sasakuma, 2002). The *Xgwm* and *Xgdm* loci were microsatellite markers, which were reported by Röder *et al* (1998) and Pestsova *et al* (2000), respectively. Methods of Southern hybridization and microsatellite examination were described in our previous report (Ahmed *et al*, 2000). In total, 66 RILs were genotyped for 322 polymorphic markers. The linkage maps were constructed using MAPMAKER 2.0 (Lander *et al*, 1987), and map distances were calculated using the Kosambi mapping function.

The association between individual marker loci and QTLs for heading under the controlled environments and in the field was evaluated by single-marker analysis using QGene software (Nelson, 1997). The simple interval analysis via flanking-marker regression method (Haley and Knott, 1992) was also performed under this program to identify QTLs on the genetic maps of 2B and group 5 chromosomes. The interval size was set at 1 cM, and the threshold value of the LOD score was considered as 2.0 for detecting the presence of a QTL. The significant level of the LOD threshold was examined by permuting the data (Churchill and Doerge, 1994), as implemented by the permutation analysis module.

Results

Heading time of RIL under different conditions

The heading time of parental lines, CS and SP, was significantly different in all growth conditions (Table 1). Under field condition, there was no significant difference between two replications ($r = 0.840$). The averages of

Table 1 Days to heading of RILs in field and under four controlled conditions

	Field	*Vernalization treatment*		*Nonvernalization treatment*	
		Long day (VL)	*Short day (VS)*	*Long day (NL)*	*Short day (NS)*
CS	35.7 (0.58)	26.0 (0.00)	42.0 (0.00)	45.5 (4.90)	94.0 (2.80)
SP	45.7 (0.58)	33.5 (0.71)	91.0 (1.40)	38.5 (0.70)	121.0 (0.00)
RILs average	37.3	29.9	67.0	50.3	101.1
Range	28.0–46.0	24.5–39.0	36.0–117.5	33.0–120.0	47.0–160.0
Variance	16.1	9.8	627.3	455.1	746.0

Standard deviations of parental lines are in parentheses.

days to heading from the 1st of April of these lines were 35.7 and 45.7 days, respectively. Under VL, SP headed later than CS by 7.5 days, while CS headed earlier than SP by 49.0 and 27.0 days under the two short-day conditions (VS and NS), respectively. SP headed in 38.5 days under NL, which was the only condition where SP headed earlier than CS.

Under the four conditions, all RILs headed during the experimental periods (Table 1). The smallest variance was observed under VL where the deviation between CS and SP was small, indicating that this condition was the most effective to promote heading. Under NL, where the variance was 455.1, 13 lines headed later than two parental lines, suggesting transgressive segregation. The largest variance was recorded under NS, where the widest range was observed.

Frequency distribution of genetic factors of heading

The degree of vernalization requirement and photoperiod sensitivity were calculated by subtracting the days to heading under VL from those under NL and VS, respectively, because heading under VL corresponded to the degree of narrow-sense earliness. The gene segregations in the RIL population of heading for these genetic factors, heading in field and heading under NS, were estimated from the frequency distributions (Figure 1a–e).

Since the distribution of narrow-sense earliness was continuous with a small range (Figure 1a), genetic variation for this character was relatively small in this population. The distribution of vernalization requirement was divided into two groups: the first group (ranging from 4 to 24 days) contained 53 lines including both parental lines, and the second (28–88 days) contained 13 lines that segregated transgressively (Figure 1b). The ratio between the two fitted a two-gene system, indicating that the genotypes of vernalization requirement are different between the two parental lines ($\chi^2 = 0.31$). This finding suggests that four genotypes of these genes would segregate in a 1:1:1:1 ratio among RILs, and that the large degree of vernalization requirement in transgressive segregants is the result of the double recessive genotype. The distribution of photoperiod sensitivity was divided on 32 days into two groups, which contained 34 and 32 lines, respectively (Figure 1c). As CS, involved in the early group, has been reported to harbor *Ppd-B1* (Pugsley, 1972), our result suggests that SP has the recessive *ppd-B1* allele, and that *Ppd-B1* and *ppd-B1* segregated among RILs in a 1:1 ratio ($\chi^2 = 0.061$).

Distributions of heading in field and heading under NS were continuous (Figure 1d and e). These results suggest the involvement of several genes and their mutual interaction under these conditions.

Correlation among genetic factors of heading

Table 2 shows the correlation coefficients among the genetic factors of heading and heading under NS and in the field. There were significant correlations between heading in the field and narrow-sense earliness ($r = 0.686$, $P < 0.01$) as well as photoperiod sensitivity ($r = 0.796$, $P < 0.01$). These findings suggest that autumn sowing in Yokohama (35°N) could fulfill vernalization requirement, while photoperiod sensitivity would remain and determine the timing of heading in the field together with narrow-sense earliness. In contrast to heading in the field, heading under NS correlated significantly with all three genetic factors, indicating that these factors interact mutually under the no-vernalization and short-day condition. There was no significant relationship between vernalization requirement and photoperiod sensitivity, although narrow-sense earliness tended to correlate significantly with these factors ($r = 0.305$, $P < 0.05$ and $r = 0.557$, $P < 0.01$, respectively).

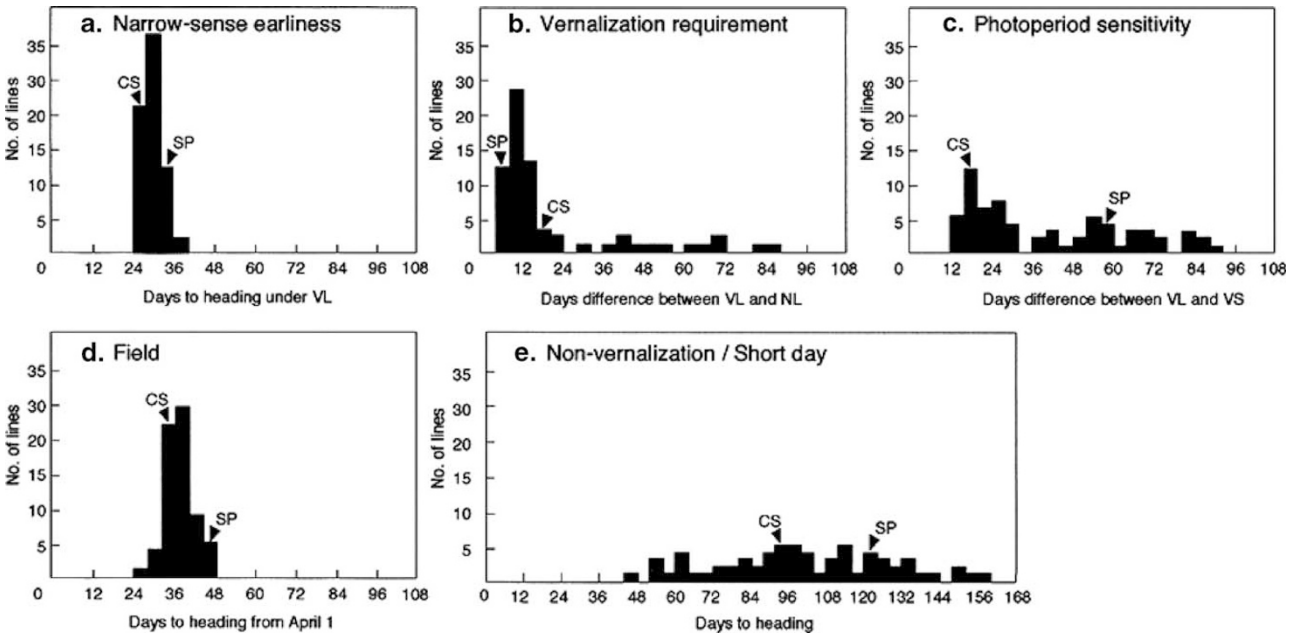

Figure 1 Frequency distribution of RILs for three genetic factors of heading (a–c), heading in field (d) and heading under nonvernalization/short-day condition (e).

Table 2 Correlation coefficients between heading factors and heading in field

	Correlation coefficient (*r*)			
	Narrow-sense earliness	*Photoperiod sensitivity*	*Vernalization requirement*	*Heading under NS*
Narrow-sense earliness				
Photoperiod sensitivity	0.557**			
Vernalization requirement	0.305*	−0.008		
Heading under NS	0.618**	0.692**	0.499**	
Heading in field	0.686**	0.796**	0.053	0.612**

*$P<0.05$, **$P<0.01$.

Table 3 Single-marker analysis using ANOVA; markers with significant linkage to the genetic factors of heading and heading in field and under NS ($P<0.01$)

Heading factors	*Linked markers*	*Location*	*Early allele*	F-*value*	*Contribution* ($r \times 100$)	*Probability*	*Additive effect*
Narrow-sense earliness	*Xpsr135b*	2B	CS	14.8	19.2	0.0003	−1.4
	Xabc451	2B	CS	13.9	18.1	0.0004	−1.3
	Xcdo202	6B	SP	9.6	13.8	0.0030	1.2
	X1Bamyl	1A	SP	7.5	11.5	0.0081	1.0
Vernalization requirement	*Xcdo1326b*	5B	SP	9.7	13.8	0.0028	7.5
	XksuAB17	—[a]	SP	9.2	13.3	0.0035	7.5
	Xpsr901	2A	SP	7.8	11.0	0.0070	7.4
	Xbcd1823b	3A	SP	7.6	10.8	0.0075	6.7
	Xwec70	5D	CS	7.2	10.6	0.0092	−6.7
Photoperiod sensitivity	*Xpsr135b*	2B	CS	23.6	27.5	0.0000	−12.4
	Xpsr126b	2B	CS	18.6	22.5	0.0001	−10.9
	Xpsr131	2B	CS	15.3	20.0	0.0002	−10.5
	Xpsr574a	5A	SP	14.4	19.1	0.0003	9.7
	Xpsr103	7A	CS	11.6	16.7	0.0012	−9.5
	Xrz630b	5B	SP	10.9	14.9	0.0016	8.9
	Xbcd1030b	5B	SP	9.9	13.8	0.0025	8.7
	Xpsr584	4B	CS	8.0	11.5	0.0064	−8.0
	Xcdo475	—	SP	7.8	11.3	0.0071	7.9
	Xcdo412a	5A	SP	7.6	11.0	0.0078	7.7
	Xgwm57	7B	CS	7.5	10.5	0.0079	−7.5
Heading in field	*Xpsr135b*	2B	CS	20.3	24.6	0.0000	−2.0
	Xwec78	5B	SP	14.7	19.2	0.0003	1.7
	Xgdm132	6D	SP	12.5	16.3	0.0008	1.6
	Xgwm469	6D	SP	11.4	15.8	0.0013	1.6
	Xpsr131	2B	CS	11.1	15.4	0.0015	−1.6
	Xrz630b	5B	SP	9.8	13.6	0.0027	1.5
	Xgwm428	7D	CS	9.3	12.8	0.0034	−1.4
	Xgwm234	5B	SP	8.9	12.7	0.0040	1.4
	Xpsr103	7A	CS	8.4	12.7	0.0053	−1.4
	XksuF11b	2A	SP	7.6	10.9	0.0078	1.3
Heading under NS	*Xrz630b*	5B	SP	18.9	23.4	0.0001	13.0
	Xbcd1030b	5B	SP	17.9	22.4	0.0001	13.0
	Xpsr103	7A	CS	16.2	21.8	0.0002	−12.9
	Xrz166a	1	CS	11.9	16.3	0.0010	−10.7
	Xcdo1160	1A	SP	10.3	14.7	0.0021	10.7
	Xcdo202	6B	SP	10.2	14.5	0.0022	10.4
	Xrz166b	1	CS	8.5	12.0	0.0050	−9.2
	Xcdo1160b	7A	SP	7.3	10.7	0.0088	8.5

[a]The chromosome location was not determined.

QTL analysis for heading factors

Based on the genotypes of each RIL with 322 marker loci, single-marker analysis was conducted to identify QTLs for each genetic factor, heading under NS, and heading in the field. The results listed in Table 3 showed that 38 markers were significantly linked to those five phenotypes ($P<0.01$, in each case).

Four markers on 2A, 2B and 6B were found to link to narrow-sense earliness. For vernalization requirement, five markers, which included *Xcdo1326* and *Xwec70*

located on chromosomes 5B and 5D, respectively, were significant. The allele of SP in four markers except *Xwec70* resulted in a small degree of vernalization requirement. For photoperiod sensitivity, 11 markers were linked significantly; among them three markers *Xpsr135b*, *Xpsr126b* and *Xpsr131* on chromosome 2B showed high linkage scores. The SP alleles of four markers, *Xpsr574a*, *Xcdo412a* on 5A, *Xrz630b* and *Xbcd1030b* on 5B, respectively, caused early heading under the VS condition. Out of 10 markers linking to heading in the field experiment, six were significant only in this field experiment, while the rest (*Xpsr135b*, *Xpsr131*, *Xrz630b* and *Xpsr103*) were simultaneously linked significantly to photoperiod sensitivity.

Although the results of correlation analysis shown in Table 2 suggested that heading under NS correlated with three genetic factors, only four out of eight markers were significant under NS conditions. *Xpsr135b*, *Xpsr126b* and *Xpsr131* on 2B, which were linked to photoperiod sensitivity with high degree, were not significantly related to heading under NS. In contrast to these markers, *Xrz630b*, *Xbcd1030b* and *Xpsr103* were linked to both photoperiod sensitivity and heading under NS. No common markers were found for NS and vernalization requirement.

Map locations of genes for vernalization requirement and photoperiod sensitivity

A total of 46 markers were mapped on homoeologous group 5, and eight were on 2B, on which chromosomes the major genes for vernalization requirement and photoperiod sensitivity were located. The interval analysis was conducted to determine the exact positions for these major genes (Figure 2a–d). On 5A, there was no LOD peak for vernalization requirement in this popula-

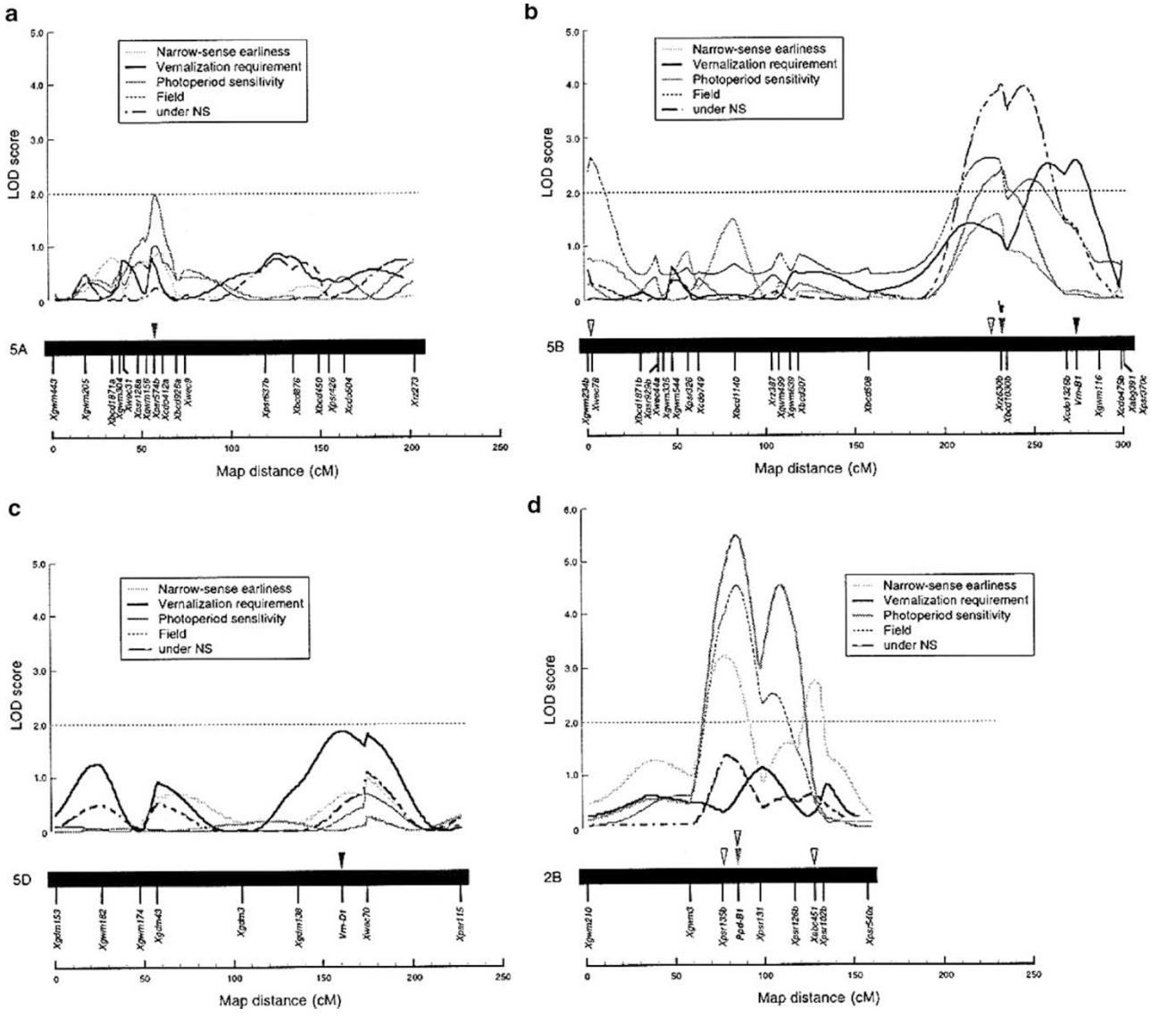

Figure 2 (a–d). LOD scores by interval analysis by QGene for narrow-sense earliness, vernalization requirement, photoperiod sensitivity, heading in field and heading under nonvernalization/short-day condition. Arrowheads show the map position of peak LOD scores >2.0 for each genetic factor; (▽) for narrow-sense earliness, (▼) for vernalization requirement, (▼) for photoperiod sensitivity, (▽) for heading in field and (▼) for heading under NS.

tion (Figure 2a), although three RFLP markers, BCD450, PSR426 and CDO504, had been reported as the tight-linked markers to *Vrn-A1* (Galiba *et al*, 1995; Kato *et al*, 1998). On the other hand, a large LOD peak ($P<0.01$) for vernalization requirement was detected in the 15.8 cM *Xcdo1326b–Xgwm116* interval on 5B (Figure 2b). The results of single-marker analysis showed that vernalization requirement was most strongly linked to *Xcdo1326b*, where the SP allele was early heading under NL. This result suggests that a strong spring-type gene should locate in this interval. On this chromosome, four other QTLs were also detected for photoperiod sensitivity, heading in the field and heading under NS, respectively ($P<0.05$, <0.05 and <0.01, respectively). Among them, a locus in the 3.5 cM interval between *Xrz630b* and *Xcdo1030b* was involved in both photoperiod sensitivity and heading under NS.

On 5D, a peak (LOD = 1.9, $P<0.05$) of vernalization requirement was observed in the *Xgdm138–Xwec70* interval, although it was lower than 2.0 (Figure 2c). Since the CS allele resulted in early heading, it was suggested that *Vrn-D1* would be located on this interval. On group 5 chromosomes, there was no homoeology among mapped loci except *Xbcd1871a* and *Xbcd1871b* on 5A and 5B.

Interval analysis of chromosome 2B showed that the largest LOD score peak ($P<0.01$) for photoperiod sensitivity was at 17.9 cM in the at *Xpsr135b–Xpsr131* interval (Figure 2d). Since the results in Table 3 showed that the early alleles of these markers were derived from CS, *Ppd-B1* gene was mapped in this interval. At the same point, another LOD peak ($P<0.01$) was also recorded for heading in the field. On this chromosome, two QTLs for narrow-sense earliness were also detected at the $P<0.01$ level in the *Xpsr135b–Xpsr131* and *Xabc451–Xpsr102b* intervals, respectively.

Discussion

The present study is the first report of the linkage mapping of a strong-spring habit gene, *Vrn-B1* on chromosome 5B of hexaploid wheat. The existence of a homoeologous gene on 5B to *Vrn-A1* and *Vrn-D1* for vernalization requirement has been suggested by Hoogendoorn (1985a). Law and Worland (1997) concluded that *Vrn-B1* should be located on 5B at a homoeologous position to *Vrn-A1* and *Vrn-D1* by utilizing the substitution lines of CS with 5B from the Italian variety Mara. However, there were conflicting reports as to whether the *Vrn* gene on chromosome 5B corresponded to *Vrn2* or *Vrn4*, which had been identified by segregation tests with the marker lines (Hoogendoorn, 1985b; Maystrenko, 1980). McIntosh *et al* (1998) summarized that the previous designations of *Vrn2* and *Vrn4* were probably the same, or allelic, and then were designated as *Vrn-B1* in the new nomenclature system. Recently, Iwaki *et al* (2002) reported that *Vrn2* should be equivalent to *Vrn-B1* by analysis using monosomic lines of DwarfA, and identified a molecular marker closely linked to this gene on the long arm of chromosome 5B.

Preliminary results based on test crosses with near-isogenic lines of *Vrn* genes showed that no segregated F_2 was observed only in the cross with Triple Dirk(B) harboring *Vrn2*, indicating that the spring habit of SP is conferred by *Vrn2* (Shindo and Sasakuma, unpublished data). Thus, it was concluded that the LOD peak in the *Xcdo1326b–Xgwm116* interval on 5B should correspond to the location of *Vrn-B1* of *T. spelta* var. *duhamelianum* KT19-1. In this interval, the clone CDO1326 was mapped, which was previously reported to link *Vrn-A1* on 5A in hexaploid wheat (Nelson *et al*, 1995) or *Vrn-A^{m}1* in diploid wheat (Dubcovsky *et al*, 1998). These facts also supported the homoeologous location of *Vrn-B1*. It has been reported that insensitivity to vernalization treatment of *Vrn2* (equivalent to *Vrn-B1*) was weaker than that of *Vrn-A1*, *Vrn-D1* or *Vrn4* in the near-isogenic lines series of Triple Dirk (Gotoh, 1976). However, our preliminary and present results indicate that the strong-spring habit of *Vrn-B1*, which is allelic to *Vrn2*, is equivalent to *Vrn-A1*.

On chromosome 5D, a LOD peak of slightly less than 2.0 was detected for *Vrn-D1* in the *Xgdm138–Xwec70* interval. Among RILs, four genotypes of *Vrn* genes, *Vrn-B1 Vrn-D1*, *Vrn-B1 vrn-D1*, *vrn-B1 Vrn-D1* and *vrn-B1 vrn-D1*, would segregate in a 1:1:1:1 ratio. It was hypothesized that the relatively weak effect of *Vrn-D1* would be covered by that of *Vrn-B1*, so that there would be no difference in the degree of vernalization requirement between *Vrn-B1 Vrn-D1* and *Vrn-B1 vrn-D1*. As a result of this epistatic effect of *Vrn-B1*, the effect of the dominant *Vrn-D1* for spring habit should be evaluated only from segregants lacking *Vrn-B1*, such as *vrn-B1 Vrn-D1* and *vrn-B1 vrn-D1*.

Our study demonstrated that heading in the field is influenced by narrow-sense earliness and photoperiod sensitivity. In particular, *Ppd-B1* on chromosome 2B played a major role. On the other hand, QTLs specific to heading in the field were also detected on 2A, 5B, 6D and 7D. In the field, the three genetic factors should interact mutually. In the case of winter sowing in Yokohama, seedlings sometimes encounter temperatures below freezing point, and temperature and daylength vary simultaneously and continuously.

The single-marker analysis indicated that several QTLs were involved for photoperiod sensitivity in hexaploid wheat, apart from *Ppd-B1* on 2B. QTLs on 5B and 7A are involved in both photoperiod sensitivity and heading under NS. On the other hand, *Ppd-B1* or a QTL on 5A is not involved in heading under the NS condition. This result indicated that genes involved in photoperiod sensitivity are categorized by at least two groups: one is a group of genes responding to a photoperiod independent of vernalization requirement, and the other group of genes, including *Ppd-B1*, cannot exert their effect until vernalization requirement is completely fulfilled. Previous studies of wheat have shown that vernalization treatment is a prerequisite for response to a photoperiod (Vince-Prue, 1975).

We also detected two QTLs for narrow-sense earliness on 2B. One is located close to the *Ppd-B1* locus while the other is in the *Xabc451–Xpsr102b* interval. The latter is possibly the same as the one reported by Worland (1996), owing to the position near the centromere on RFLP maps. In barley, *eps2S*, located near the centromere region of the 2H chromosome, has been reported to be a QTL for narrow-sense earliness (Laurie *et al*, 1995). Thus, this locus could represent the orthologous gene to *eps2S*. Although Kato *et al* (1999) reported a locus for narrow-sense earliness on 5A of hexaploid wheat, we

could not find any loci for this character on group 5 chromosomes in the present study.

The QTL analysis used in the present study demonstrated that photoperiod sensitivity and narrow-sense earliness critically determine heading in the field. In particular, chromosome 2B is important for heading as it contains many critical genes for heading. Our results are important with regard to the breeding of early-heading varieties. Several new findings of our study were possible following the use of QTL analysis and construction of the skeleton map based on RIL. Segregation data are available on the KOMUGI web site (http://www.shigen.nig.ac.jp/wheat/wheat.html), and the material can be provided on request.

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