Theor Appl Genet (2004) 110: 106–115
DOI 10.1007/s00122-004-1799-1

ORIGINAL PAPER

E. Hanocq · M. Niarquin · E. Heumez · M. Rousset
J. Le Gouis

Detection and mapping of QTL for earliness components in a bread wheat recombinant inbred lines population

Received: 19 December 2003 / Accepted: 12 August 2004 / Published online: 12 November 2004
© Springer-Verlag 2004

Abstract Earliness, an adaptative trait and factor of variation for agronomic characters, is a major trait in plant breeding. Its constituent traits, photoperiod sensitivity (PS), vernalization requirement (VR) and intrinsic earliness (IE), are largely under independent genetic controls. Mapping of major genes and quantitative trait loci (QTL) controlling these components is in progress. Most of the studies focusing on earliness considered it as a whole or through one (or two) of its components. The purpose of this study was to detect and map QTL for the three traits together through an experimental design combining field trials and controlled growth conditions. QTL were mapped in a population of F_7 recombinant inbred lines derived by single-seed descent from a cross between two French varieties, 'Renan' and 'Récital'. A map was previously constructed, based on 194 lines and 254 markers, covering about 77% of the genome. Globally, 13 QTL with a LOD > 2.5 were detected, of which four control PS, five control VR and four control IE. Two major photoperiod sensitive QTL, together explaining more than 31% of the phenotypic variation, were mapped on chromosomes 2B and 2D, at the same position as the two major genes *Ppd-B1* and *Ppd-D1*. One major VR QTL explaining (depending on the year) 21.8–39.6% of the phenotypic variation was mapped on 5A. Among the other QTL, two QTL of PS and VR not referenced so far were detected on 5A and 6D, respectively. A VR QTL already detected on 2B in a connected population was confirmed.

Communicated by H. H. Geiger

E. Hanocq (✉) · M. Niarquin · E. Heumez
M. Rousset · J. Le Gouis
Institut National de la Recherche Agronomique (INRA),
Unité de Génétique et Amélioration des Plantes,
B.P. 50136, 80203 Péronne Cedex, France
E-mail: hanocq@mons.inra.fr
Tel.: +33-3-22857510
Fax: +33-3-22856996

Introduction

Earliness can be considered as an adaptative trait (Worland 1996) and it is one of the main factors of variation when studying agronomic characters. Consequently, it is a major trait to consider in plant breeding. Progress in its understanding could have great consequence in the latter area.

Earliness is a complex trait that is often characterized with only a synthetic measure: heading time or flowering time for classical sowing dates. For a more efficient control, it is necessary for breeders to more finely characterize earliness. Earliness may be partitioned into its three components: photoperiod sensitivity (PS) (Garner and Allard 1923), vernalization requirement (VR) (T.D. Lysenko in 1928) and IE (Yasuda and Shimoyama 1965). Intrinsic earliness (IE) may be defined as the earliness inducing differences between varieties in developmental rate, independent of day length and vernalization response.

During the 1970s and 1980s, using Mendelian and cytological approaches, geneticists located most of the main genes controlling earliness components in wheat (Law 1987). Then, using aneuploidy in wheat, two homeologous major genes sets have been studied: the *Ppd* series, located on the group 2 chromosomes, controlling sensitivity to photoperiod (Welsh et al. 1973; Scarth and Law 1983; Sharp and Soltes-Rak 1988) and the *Vrn* series, located on the group 5 chromosomes, controlling VR (Pugsley 1971, 1972; Law et al. 1978; Maystrenko 1980). Sprayed over the genome, other genes controlling one of these two traits or IE, with minor effect, have been identified (Scarth and Law 1983; Hoogendoorn 1985).

In the 1980s, simultaneous development of molecular biology aspects and theory of QTL detection allowed large progress in this area. Markers were then used to describe DNA variability. RFLP markers (Soller and Beckman 1982; Lander and Bostein 1989) were progressively supplanted by highly numerous and

polymorph markers such as RAPD, AFLP and microsatellites. After marker mapping, association of genome region with quantitative traits can be tested to detect QTL. Simple marker analysis, interval mapping (Lander and Bostein 1989) using flanking-marker regression method (Haley and Knott 1992), composite interval mapping [(CIM) Jansen and Stam 1994; Zeng 1994] and multi-trait analysis (Jiang and Zeng 1995) were methods successively developed to detect QTL. Such methods are now largely used in revealing regions of chromosome including genes involved in the control of a trait.

As a direct issue of the cytogenetical works, in wheat, QTL and gene mapping were first essentially focused on specific chromosomes, often using single-chromosome recombinant lines. They concerned mainly the group 5 chromosomes and VR. Galiba et al. (1995) and Kato et al. (1998, 1999) on 5A, Miura et al. (1992); Barrett et al. (2002); Toth et al. (2003); Leonova et al. (2003) on 5B and Kato et al. (2001) on 5D are examples of important papers on the topic. Only Worland (1996) was interested in developing such analysis of PS on 2D. Few authors used a global approach on the whole genome. In barley, Laurie et al. (1995) considered PS, spring habit and IE together, whereas Börner et al. (2000) focused on IE. In wheat, Sourdille et al. (2000) studied PS and IE. Shindo et al. (2003) investigated the three earliness components together by using a *Triticum aestivum* × *Triticum spelta* cross. Other authors worked on heading or flowering time as an easily measurable trait when analyzing other agronomic traits (Börner et al. 2002; Gervais et al. 2003; Kulwal et al. 2003). They studied plants grown in one condition, and then they detected QTL for only one of the three earliness components or for a mixture of those components.

The purpose of this study was to detect and map QTL for earliness components in an F_7 recombinant inbred line (RIL) bread wheat population, using a design mainly developed in field. The three earliness components were then analyzed together on wheat agronomic material. Thus, several specific QTL have been found for each of the three components. Existence of some segregating major genes is confirmed and some not referenced so far QTL are described.

Materials and methods

Plant material and growth conditions

A population of 194 F_7 RILs obtained by single-seed descent (SSD) lines was produced from the 'Renan' × 'Récital' cross. For classical field sowing, 'Recital' is an early variety, while 'Renan' is a fairly late one. The SSD population together with the parents were characterized in field experiments at Estrées-Mons (latitude: 49°52′44″), INRA, northern France. Heading Date (HD) was registered over 3 years (1999/2000–2001/2002), when half of the ears totally emerged from the leaf sheath. In October 1999, the 194 RILs were sown in 6.5-m^2 plots with two replicates. During the two following years, the design consisted of four field sowing or planting dates: 1 October sowing, 1 March sowing and 2 May plantings. For October and March sowings, two rows of 3 m, 18 cm apart, were sown per genotype. In May, unvernalized and previously artificially vernalized plants were planted. For the vernalized treatment, seeds were imbibed during 3 days at 19°C and then transplanted in 38-cm diameter lumps of peat. After 1 day, they were settled at 5.5°C under 8 h of light, using fluorescence lamps for 8 weeks. Unvernalized plants, instead of being placed at 5.5°C, were grown in a greenhouse at a mean daily temperature of 14.2°C. In May, when planting in field, vernalized and unvernalized plants had cumulated roughly the same mean daily temperature.

Earliness components evaluation

Standard screen air temperature was registered 2 m above the ground.

PS was estimated by considering HD for the October sowings; October is a classical date for winter bread wheat in the north of France. Mean temperature in Estrées-Mons at this period was 9.5°C, allowing a completely efficient vernalization. At sowing, day length was about 10 h. Decreasing day length was highly limiting. HD for such a sowing reflects mainly PS (Masle et al. 1989).

VR was characterized by analyzing the results of the March and May plantings. In March, the rapid increasing day length was about 12.5 h at sowing, and the mean daily temperature was 8°C. These values were 15 h and 13°C for the May planting. For such a sowing, vernalization is the main factor controlling development (Masle et al. 1989). The May planting with unvernalized plants is a useful tool to perform an all-or-none screening of the genotypes: genotypes that headed, despite a lack of vernalizing temperature, and genotypes that always stayed in a vegetative state. In order to have a continuous ranking of the genotypes for VR, HD values for the March sowing, which provide only a partial natural vernalization, were compared to HD values for fully vernalized plants. For this purpose, as proposed by Kato and Yamashita (1991), a ratio was estimated as HD from the first of June, obtained after artificial vernalization divided by HD from the first of June for the March sowing. The lower the ratio was, the more delayed was the HD due to incomplete field vernalization. A zero value was assigned for genotypes that never headed for the March sowing.

Masle et al. (1989) suggested using the vernalized May planting to estimate IE. Indeed, under such a condition, the rather long day length was considered as not limiting, and vernalization was artificially fully ensured. By definition, IE is independent of photoperiod and vernalization aspects. Nevertheless IE and PS

measures were correlated; thus, we were led to transform them.

Statistical analysis

The Pearson correlation coefficients between years or traits were estimated using the PROC CORR procedure of the SAS Institute (1991) software. Replicate and/or year and genotype effects were studied by analysis of variance (ANOVA) with the PROC GLM procedure of the SAS Institute. Heritability was estimated: $h_2 = \sigma_G{}^2/[\sigma_{G_2} + (\sigma_E{}^2/r)]$, with $\sigma_G{}^2$ being the genetic variance, $\sigma_E{}^2$ the residual variance and r the number of year and within year replicates. Year and within-year replicates were considered both as classical replicates, which led likely to underestimate heritability.

The correlated IE and PS measures were transformed by rotation. The two coordinate axes x (photoperiod measure) and y (IE measure) were transformed into two new orthogonal axes, x' and y', through a rotation of angle $\hbar$ as follows:

$$\begin{vmatrix} x' \\ y' \end{vmatrix} = \begin{vmatrix} \cos\hbar & \sin\hbar \\ -\sin\hbar & \cos\hbar \end{vmatrix} \begin{vmatrix} x \\ y \end{vmatrix}.$$

By choosing an adequate angle $\hbar$,x' and y' are not correlated. $\hbar$, was estimated by using the PROC PRINCOMP procedure of the SAS Institute.

QTL detection and mapping

The QTL analyses were based on a map constructed through a Genoplante project (Groos et al. 2002). For the present analysis, 254 markers, essentially microsatellite, were used, covering 2,639 cM (77% of the genome), with an average of one marker each 12 cM.

One QTL analysis was performed on each trait measured every year. For the October 1999 sowing, mean HD over the two replicates was used. Interactions between QTL have been tested. The QTL detection was performed with the PlabQTL software (Utz and Melchinger 2000). Results of the CIM method (Jansen and Stam 1994) are presented in this paper. The step used for interval mapping was 2 cM. A LOD score threshold of 2.5 was considered to register a QTL as significant. This threshold was determined with 1,000 permutations for a α value of 10%. An F-to-enter value of 9.79 was used, resulting in selecting as cofactors, depending on the trait and the year, three to eight markers. Confidence intervals were set as the map interval corresponding to a 1-LOD decline either side of the LOD peak.

Results

Phenotypic measure of the three earliness components

Distribution of the mean values are represented on Figs. 1, 2 and 3 for PS, IE and VR, respectively. Means were calculated over all the years and/or replicates.

PS

Results on HD reflecting PS showed a clear contrasted behaviour between 'Renan' and 'Récital' (Fig. 1). 'Renan', more sensitive to photoperiod, headed 12 days later than 'Récital'. In the SSD population, HD had a clear, continuous normal distribution, with a slight long

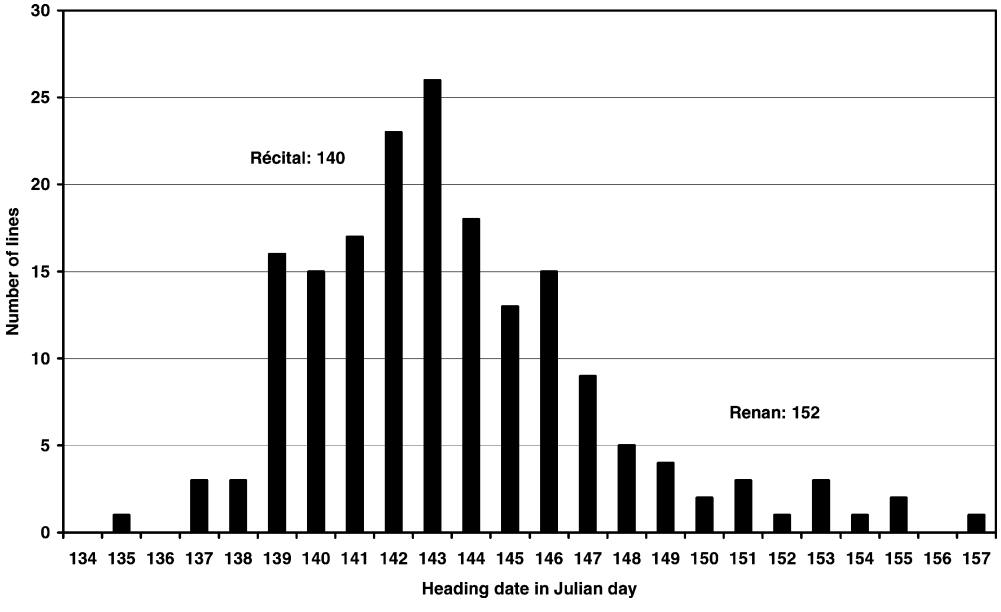


Fig. 1 Distribution of the mean heading date (HD) values for the October sowings in the F_7 recombinant inbred lines (RILs) derived from the cross 'Renan' × 'Récital' [measure of the photoperiod sensitivity (PS)]

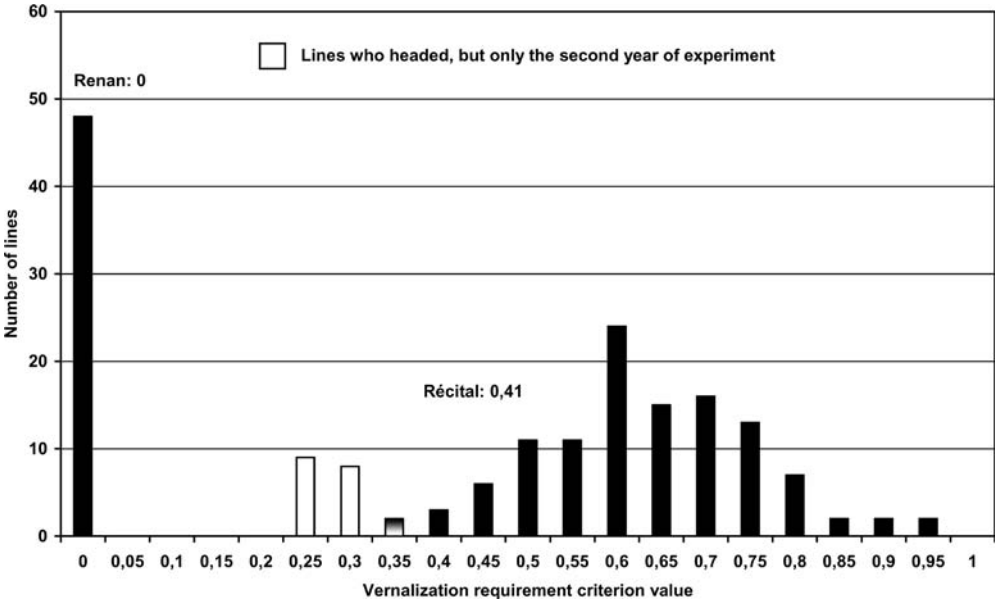


Fig. 2 Distribution of the mean vernalization requirement (VR) criterion values (HD values from the first of June under vernalized treatment divided by HD values from the first of June for the March sowing) in the F_7 RILs derived from the cross 'Renan' × 'Récital'. *White bars* are lines that headed only the second year of experiment

tail for high values as for 'Renan'. The range of HD was 22 days. Heritability was 0.86, and pairwise phenotypic correlations between years and/or replicates for PS were between 0.83 and 0.97.

IE

A normal distribution was observed in the whole population, with a slight relative surplus of early genotypes (Fig. 2). The range of HD was 16 days. Heritability was only 0.54, and the correlation between years was only 0.58.

VR

Without artificial vernalization, no genotypes headed when planted in May. They all required some vernalization. All lines with 8 weeks of artificial vernalization reached heading stage. By comparison, when vernalization is partial (March sowing), only 113 SSD lines headed in the 2 years of experiment (Fig. 3), and 18 headed only in the second one characterized by a cold spring. 'Récital' headed late and with an unusual very low number of spikes. The rest of the population, like 'Renan', stayed at a vegetative status. Heritability was 0.74 and correlation between years was 0.77.

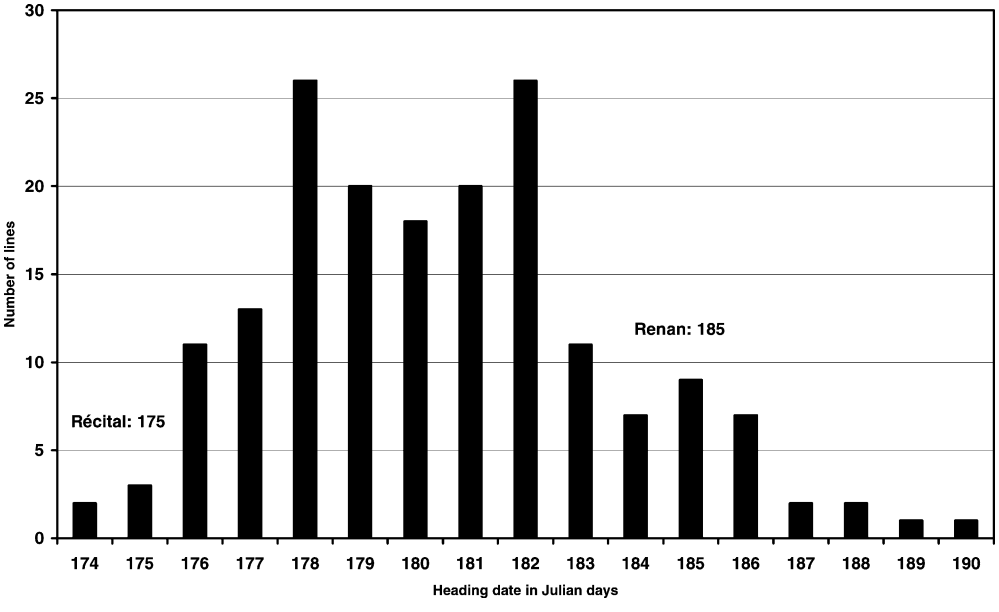


Fig. 3 Distribution of the mean HD values for the May planting with 8 weeks of artificial vernalization in the F_7 RILs derived from the cross 'Renan' × 'Récital' (measure of the IE)

New variables derived from PS and IE criteria

As a whole, VR was not correlated to the two other traits (−0.01 and −0.09 with PS and IE measures, respectively). On the contrary, correlation between PS and IE was surprisingly very high (0.79). To obtain independent coordinate axes from these two variables, we used an angle $\hbar$, of 0.67 rad and 0.81 rad the first and second years of the experiment, respectively. For these 2 years, correlation between x (photoperiod measurement) and x' (new variable derived from photoperiod measurement) coordinates were 0.95 and 0.90. x' represented largely the original photoperiod measurement. Correlation between years for x' was 0.82. For IE, correlations between y and y' were 0.48 and 0.39 for the 2 years of experiment, whereas correlation between years for y' was only 0.15.

QTL detection and mapping

Several QTL were found for the various traits studied. Among all the pairwise QTL interactions tested, none of them had a significant effect.

PS

Four QTL with a LOD higher than 2.5 have been detected on four chromosomes (Table 1; Fig. 4). Two of them, located on 2B (PSQTL_2B) and 2D (PSQTL_2D), were very great QTL, each explaining 13.1–20.5% of the variation of HD, depending of the year. These two QTL have been identified for the 3 years. The two other QTL, located on 5A (PSQTL_5A) and 7D (PSQTL_7D), have been detected for only 1 year. Jointly, the four PS QTL detected in the SSD 'Renan' × 'Récital' population explained between 31.9% and 45.8% of the whole phenotypic variation for PS, depending of the year.

VR

Five QTL were detected on chromosomes 2B, 5A, 5B, 5D and 6D when analyzing VR criteria (Table 1; Fig. 4). Three of them were identified for the 2 years of experiment; nevertheless, the two others had LOD scores higher than 2 for the 2 years (Table 1). Among these QTL, one QTL on the 5A chromosome (VRQTL_5A) had a strong effect on the control of VR; it explained between 21.2% and 39.6% of the phenotypic variation. Jointly, between 31.0% and 55.0% of the variation in the population was explained by the five detected QTL.

IE

Four QTL, on chromosomes 2B, 2D, 5B and 7A, have been detected as controlling IE. Compared to some PS or VR QTL values, none of the IE QTL had a strong effect. The two QTL on 2B (IEQTL_2B) and 2D (IEQTL_2D) have been identified for the 2 years of experiments (Table 1; Fig. 4). They were detected at the same position as the two major PS QTL (PSQTL_2B and PSQTL_2D). The QTL together described between 27.2% and 28.6% of the HD variation observed under the vernalized treatment, with a maximum individual R^2 of 12.4%.

New variables derived from PS and IE criteria

QTL detections were performed on the two new variables obtained from PS and IE by rotation. With

Table 1 Earliness QTL detected in the F7 RILs population derived from 'Renan' × 'Récital'. **QTL possibly corresponding to the known major genes Ppd and Vrn**

Chromosome	Closest marker	Confidence interval (cM)	LOD (1999–2001)	R^2 for loci with a LOD > 2.5 (1999–2001)	Allele providing earlier heading dates[a]
Heading date QTL for October sowings (sensitivity to photoperiod)					
2B	***Xgwm148***	**14**	**7.9-7.6-6.0**	**20.5-18.2-13.1**	**Rc**
2D	***Xgwm484***	**18**	**6.2-7.7-6.4**	**16.1-19.8-18.8**	**Rc**
5A	*Xgwm264c*	24	2.8-1.3-0.9	5.9-.-.	Rc
7D	*pch1*	8	2.1-2.6-1.1	.-7.8-.	Rn
QTL for vernalization requirement criterion					
2B	*Xgwm374*	24	2.8-1.9	4.1-.	Rc
5A	***Xgwm271b***	**10**	**18.8-7.1**	**39.6-21.8**	**Rc**
5B	*Xgwm639a*	34	3.4-2.3	6.8-.	Rn
5D	*Xbcd1421*	14	2.2-3.0	.-4.7	Rn
6D	*Xcfd42*	18	2.6-2.7	4.5-4.5	Rn
Heading date QTL for fully vernalized plants (intrinsic earliness)					
2B	*Xgwm148*	24	3.5-2.5	12.4-7.2	Rc
2D	*Xgwm261*	28	3.2-4.5	8.2-11.4	Rc
5B	*Xgwm371*	26	1.9-3.9	.-8.6	Rn
7A	*Xrz995*	20	2.9-1.3	8.0-.	Rn

[a]Alleles are 'Renan' (*Rn*) or 'Récital' (*Rc*)

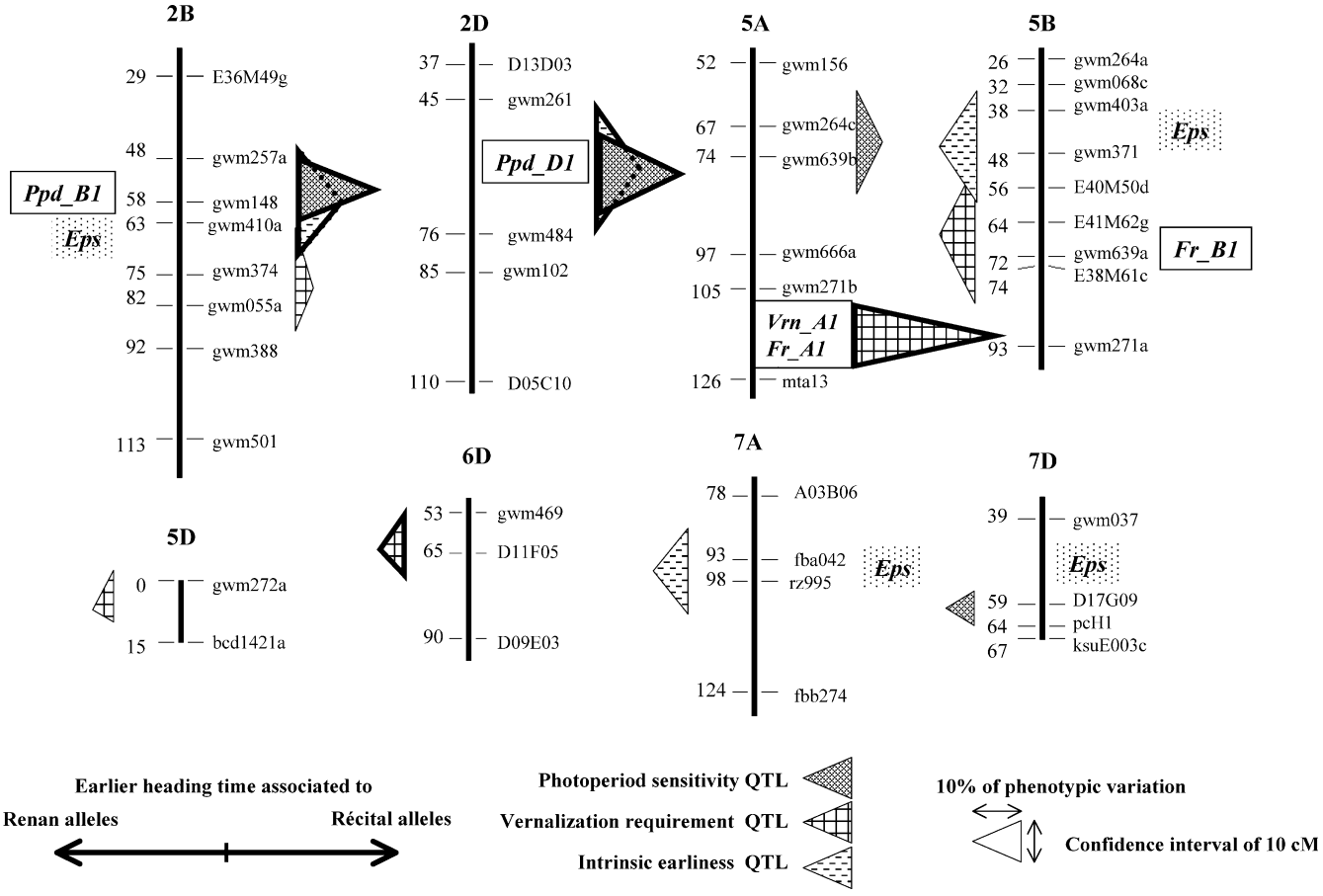


Fig. 4 Genetic map of chromosome segments containing QTL for PS, VR and IE detected in the F_7 RILs derived from the cross 'Renan' × 'Récital'. *Bold triangles* are QTL detected whatever the year of experiment

the transformed photoperiod data set, the two major PS QTL on 2B and 2D were detected on the same position, with absolutely similar LOD and R^2 values, whereas the two other PS QTL having previously a lower effect were not detected any more. Concerning IE, none of the IE QTL was detected with the transformed variable.

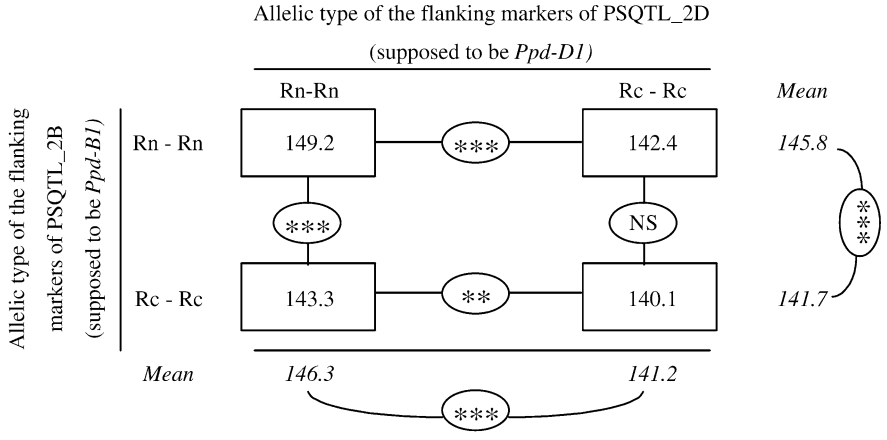
Globally, QTL were mapped on chromosomes 2B, 2D, 5A, 5B, 5D, 6D, 7A and 7D. For every QTL mapped on 2B, 2D and 5A, earlier HD was associated with the 'Récital' allele. Conversely, for every QTL mapped on 5B, 5D, 6D, 7A and 7D, earlier HD were associated with the 'Renan' allele.

Effect of the major QTL for PS and VR

QTL detection can provide valuable markers for assisted selection. In such a way, it can be interesting to evaluate the effect of the alleles of the three major QTL presented above (PSQTL_2B, PSQTL_2D and VRQTL_5A). Alleles were supposed to be known through the information at flanking markers.

PS effect

The flanking markers of PSQTL_2D were *Xgwm484* and *Xgwm261*. Those of PSQTL_2B *were Xgwm148* and *Xgwm157a*. Lines having 'Renan' (Rn) and 'Récital' (Rc) alleles at both flanking markers of PSQTL_2D had a mean HD averaged over the 3 years of experiment of 146.3 and 141.2, respectively (Fig. 5). For the PSQTL_2B, these values were 145.8 and 141.7, respectively. Thus, the mean phenotypic effect of PSQTL_2D and PSQTL_2B were 5.1 days and 4.1 days, respectively; in both cases, the Rc allele provided earlier HD. In addition, Fig. 5 shows HD results when crossing genotyping information for these two couples of markers. A highly significant difference of 9.1 days between extreme groups was observed. These values can be compared to the 22 days' difference registered for individual HD between extreme lines of the whole population (Fig. 1). The overall effect of interaction between PSQTL_2D and PSQTL_2B was not significant. Nevertheless, some particular interactions were highly significant. Indeed, when considering the subpopulation having the Rc allele for PSQTL_2D, the allele PSQTL_2B effect was not



Rn = Renan allele; Rc = Récital allele

Fig. 5 Epistatic interactions between PSQTL_2B and PSQTL_2D detected in the F_7 RILs derived from the cross 'Renan' (*Rn*) × 'Récital' (*Rc*) in the same position as *Ppd-B1* and *Ppd-D1* genes. Mean HD used to estimate PS for each allelic type combinations. *** $P < 0.0001$, ** $P < 0.001$, *NS* non-significant difference

significant. On the contrary, for the subpopulation having the Rn allele for PSQTL_2D, the 5.9-days allele PSQTL_2B effect was highly significant ($P < 0.0001$). Now, in the case of the Rc allele for PSQTL_2B, the allele PSQTL_2D effect on HD was 3.2 days ($P = 0.0008$). With the Rn allele for the PSQTL_2B, the allele PSQTL_2D effect was highly significant (6.8 days).

VR effect

The flanking markers of VRQTL_5A were *Xmta13* and *Xgwm271b*. Forty-nine lines had the Rn allele at both loci. Twenty-four of them (49%) never headed, eight headed only the second year and 17 (35%) headed for the 2 years. Seventy-seven lines had the Rc alleles. Among them, 62 (93%) headed for the 2 years, whereas the remaining 7% never headed. The mean values of the VR criterion were 0.21 and 0.61 for the 49 and 67 lines, respectively. If comparing to HD under the vernalization treatment, HD was delayed for March sowing due to incomplete vernalization. The mean delay of these 49 lines was 52.9 days, whereas it was 17.5 for the 62 lines. The phenotypic effect of the VRQTL_5D was thus estimated at 35.4 days of additional delay.

Discussion

Phenotypic HD results

'Renan' and 'Récital' are much contrasted relative to the three components of earliness. 'Récital' is commonly an early variety, having a very low PS, a medium VR and a high IE. The low PS of 'Récital' could come from its Mexican parent 'R-267' (Zeller et al. 1993). Mexican wheat cultivars are known to carry genes providing unsensitivity to photoperiod (Law and Worland 1997). 'Renan' has a quite high PS, a very high VR and a low IE. Its very high VR could come from its Russian parent, 'Mironovskaya 808' (INRA 1997). 'Mironovskaya 808' has been largely studied as winter genotype by Stelmakh and Avsenin (1983) cited by Stelmakh (1998) with a high VR (Kosner and Pankova 1998).

PS and IE were continuous traits in the population. Conversely, the VR trait showed all or none characteristics. Indeed, under partial vernalization, only a part of the population headed, whereas the other part stayed at a vegetative status. For lines that headed, VR had a continuous normal pattern.

QTL detection

Overall considerations

Between four and five QTL were detected depending on the trait studied. These values are similar to the mean values computed on 47 published studies almost exclusively on diploid plant species by Kearsey and Farquhar (1998).

Groups 2, 5, and to a lesser extent, group 7 chromosomes are known to have a major role in the developmental control in wheat (Law and Worland 1997). In the 'Renan' × 'Récital' population, QTL were mapped on 8 chromosomes, of which seven are of these three groups.

PS

The study of HD from October sowings resulted in the detection of four QTL. The two QTL located on 2B and 2D detected for the three experimental years can be considered as major QTL. Together they explained between 32% to 38% of the phenotypic variation. From cytogenetical works, the important effect on PS of chromosomes 2B (Welsh et al. 1973) and 2D (Law 1966) has been well established. Worland (1996) and Worland et al. (1998) mapped the major genes *Ppd_ D1* (formerly *Ppd1*) on 2D and *Ppd_ B1* (formerly *Ppd2*) on 2B, with RFLP markers, using single-chromosome recombinant lines for chromosomes 2B of 'Chinese Spring' and 2D of 'Mara' in a common 'Cappelle'–'Desprez' background.

Microsatellite markers are now the most widely used markers, and links between RFLP and microsatellite maps can be established through maps like that developed by Röder et al. (1998). Using such a map, it appears that the two main PS QTL (PSQTL_2D and PSQTL_2B) were located in the same region as the Ppd genes. Alleles of 'Récital' conferred a higher earliness.

Probably, 'Récital' has a *Ppd_ D1* allele, as suspected by Worland et al. (1994), and possibly a *Ppd_ B1* allele, too. The mean effect of PS QTL_2D in the 'Renan' × 'Récital' population (5.1 days) was completely in accordance with values estimated by Worland (1996). Depending on genetic background and trials, the effect of *Ppd_ D1* was between 4.6 days and 7.8 days. Worland (1996) estimated the effect of the *Ppd_ B1* gene, too. Thus, in a 'Cappelle'–'Desprez' background, while the effect of *Ppd_ D1* was 7.8 days, the effect of *Ppd_ B1* was 6.2 days. In the same way (in this paper) the mean PSQTL_2B effect (4.1 days) was inferior to the PSQTL_2D one. Worland (1996) mentioned that the effect of accumulating two or more *Ppd* genes has never been tested. Nevertheless, through cytogenetical works, Welsh et al. (1973) found that *Ppd_ D1* was epistatic to the other alleles, and Law et al. (1978) found that *Ppd* genes were dominant, fully or partially inhibiting the PS. Results obtained in the present study suggest that there is an epistatic relationship between *Ppd_D1* and *Ppd_ B1*. *Ppd_ D1* is highly epistatic toward *Ppd_B1* (no significant effect of PSQTL_2B with the 'Récital' allele at PSQTL_2D) but in an incomplete way: in this study there was a cumulative effect of these two genes (joint effect of 9.2 days compared to marginal effect of 6.8 days and 5.9 days for PSQTL_2D and PSQTL_2B, respectively).

The 7D PS QTL detected in this study was also detected by Gervais et al. (2003). Until now, the last PS QTL detected here on 5A had never been reported. Moreover, given the respective position of this PS QTL and *Vrn_ A1*, there is no possible confusion between them.

VR

The most potent genes determining VR pertain to the homoeologous group 5 series of *Vrn* genes (Pugsley 1971, 1972; Law et al. 1976).

In this study, a major VR QTL (VRQTL_5A), explaining 22% to 40% of the phenotypic variation, has been detected on 5A. Nelson et al. (1995) and Galiba et al. (1995) mapped *Vrn1* (renamed *Vrn_ A1* by McIntosh et al. 1998) on 5A. The major QTL detected in our study likely would correspond to the *Vrn1* gene. Snape et al. (1976) showed that the *Vrn_ A1* gene is predominant in reducing the VR in European wheat varieties. Kosner and Pankova (1998) mentioned that the *Vrn_ A1* gene is epistatic and inhibits completely VR. This is not the case in the 'Récital' × 'Renan' population, since none of the lines headed under the unvernalized treatment, and there was always a delay due to incomplete vernalization. Nevertheless, Stelmakh (1993) stated that the effect of the *Vrn* genes on VR was dependent on the PS of the genetic background. Indeed, a *Vrn* gene in a photosensitive background gives an alternative phenotype; if the background is photoinsensitive, cultivars have a spring phenotype but late. In the present study, no epistatic relationship between *Ppd* and *Vrn* genes was detected at a significant level. These results could also suggest that there would be various alleles at the *Vrn_ A1* locus or a second gene as identified on the A genome by Shindo et al. (2002).

On 5B, the *Vrn-B1* gene (formerly *Vrn2*) was recently finely mapped by Barrett et al. (2002), Leonova et al. (2003) and Toth et al. (2003). The frost resistance gene (*Fr-B1*) was mapped by Toth et al. (2003). The VR QTL detected on 5B in the 'Renan' × 'Récital' population is not located in the same position as the *Vrn-B1* gene but rather in the same position as the *Fr-B1* gene. On 5B, these two genes are quite distant one from the other (more than 40 cM).

When using common microsatellite markers from Röder et al. (1998) and Snape et al. (2001) maps, in the 'Renan' × 'Récital' population, the VR QTL detected on 5D is distant from more than 100 cM of frost resistance or VR genes. Then, on 5D, the VR QTL detected in this study does correspond neither to *Vrn-D1* nor to *Fr-D1* mapped by Snape et al. (2001).

The two last VR QTL were detected on 2B and 6D. Using cytogenetic studies, chromosome 6D (Law and Worland 1997), as well as group 2 chromosomes (Kuspira and Unrau 1957; Halloran and Boydell 1967), were found to have an effect on VR control. Nevertheless, up to now, no VR QTL or genes have been mapped on these two groups of chromosomes, except recently one QTL on 2B in a connected population by Hanocq et al. (2003). This QTL was located in a similar position as the VR QTL detected on the 'Renan' × 'Récital' population.

IE

The two main IE QTL have been located in the same position as PSQTL_2B and PSQTL_2D, probably corresponding to *Ppd-B1* and *Ppd-D1* genes. Shindo et al. (2003) also found an IE QTL close to *Ppd-B1*. Three hypotheses can be put forth. Firstly, IE genes do exist closed to the *Ppd* genes, and they have a quite large effect. Shindo et al. (2003) concluded that. Consequently, they also concluded that there was an effect of IE for autumn sowings. Nevertheless, in their study, IE was significantly correlated to PS and VR (all traits registered in completely controlled conditions), which is not in agreement with the definition of IE. Secondly, the location of IE QTL can be due to a pleiotropic effect of *Ppd* genes as mentioned by Worland (1996). Thirdly, confusion between PS and IE effects can be suspected. Likely, HD for fully artificially vernalized plants, placed

in field in May, would be partially controlled by photoperiod aspects. Indeed, day length in May in northern France is not optimal for cereal growth. To study this last hypothesis, a new variable was derived from HD measured for the May planting originally considered as an IE measurement. This new variable can not be considered either as a measure of HD for the May planting, but it is derived from it and it is not statistically correlated to PS and VR aspects according to the narrow-sense definition of IE. No more QTL were then detected on 2B and 2D. These results would suggest that there was a real mixing between PS and IE measurements. Eventually, would this new variable be a better measure of IE? The poor correlation between years for this new variable does not strengthen this idea.

The last two IE QTL, on 5B and 7A, were located in the same position of QTL already known in wheat (Toth et al. 2003) and/or barley (Laurie et al., 1995). The 5B QTL is in the same region as both an IE QTL and the *eps5L* QTL detected in wheat and in Barley, respectively. On 7A, the IE QTL is syntenic to the *eps7S* QTL of Laurie et al. (1995). These two QTL were not detected when analyzing the transformed HD registered in May.

Acknowledgements We thank J.-P. Noclercq and O. Jaminon for their excellent technical assistance in growing plants in controlled conditions as well as D. Bouthors and D. Brasseur for their help in planting plants in field. We thank G. Doussinault for his help in planning the experimental design. We also thank L. Moreau and V. Fontaine for their valuable comments and suggestions on the manuscript.

References

Barrett B, Bayram M, Kidwell K (2002) Identifying AFLP and microsatellite markers for vernalization response gene *Vrn-B1* in hexaploid wheat using reciprocal mapping populations. Plant Breed 121:400–406

Börner A, Buck-Sorlin GH, Hayes PM, Korzun V, Malyshev S, Stracke S (2000) Genetics and molecular mapping of genes determining flowering time in barley. In: Proceedings of the 8th International barley genetics symposium, Adelaide, Australia, pp 55–57

Börner A, Schumann E, Fürste A, Cöster H, Leithold B, Röder MS, Weber WE (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L). Theor Appl Genet 105:921–936

Galiba G, Quarrie SA, Sutka J, Morgunov A, Snape JW (1995) RFLP mapping of the vernalization (*Vrn1*) and frost resistance (*Fr1*) genes on chromosome 5A of wheat. Theor Appl Genet 90:1174–1179

Garner W, Allard AH (1923) Further studies in photoperiodism, the response of the plant to relative length of day and night. J Agric Res 23:871–920

Gervais L, Dedryver F, Morlais JY, Bodusseau V, Negre S, Bilous M, Groos C, Trottet M (2003) Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat. Theor Appl Genet 106:961–970

Groos C, Gay G, Perretant M-R, Gervais L, Bernard M, Dedryver F, Charmet G (2002) Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a white × red grain bread-wheat cross. Theor Appl Genet 104:39–47

Haley CS, Knott SA (1992) A simple regression model for interval mapping in line crosses. Heredity 69:315–324

Halloran GM, Boydell CW (1967) Wheat chromosomes with genes for vernalization response. Can J Genet Cytol 9:632–639

Hanocq E, Sayers EJ, Niarquin M, Le Gouis J, Charmet G, Gervais L, Dedryver F, Duranton N, Marty N, Dufour P, Rousset M, Worland AJ (2003) A QTL analysis for earliness under field and controlled conditions in a bread wheat doubled-haploid population In: Börner A, Snape JW, Law CN (eds) Proceedings of the 12th EWAC conference, John Innes Centre, Norwich, 1–6 July 2002, pp 57–59

Hoogendoorn J (1985) A reciprocal F1 monosomic analysis of the genetic control of the time of ear emergence, number of leaves and number of spikelets in wheat (*Triticum aestivum* L.). Euphytica 34:545–558

INRA (1997) Catalogue of genetic resources bread wheat and barley. Small Grain Cereal Network 1997, p 91

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447–1455

Jiang C, Zeng ZD (1995) Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics 140:1111–1127

Kato K, Yamashita S (1991) Varietal variation in photoperiodic response, chilling requirement and narrow-sense earliness and their relation to heading time in wheat (*Triticum aestivum* L.). Jpn J Breed 41:475–484

Kato K, Miura H, Akiyama M, Kuroshima M, Sawada S (1998) RFLP mapping of the three major genes, Vrn1, Q and B1, on the long arm of chromosome 5A of wheat. Euphytica 101:91–95

Kato K, Miura H, Sawada S (1999) Detection of an earliness per se quantitative trait locus in the proximal region of wheat chromosome 5AL. Plant Breed 118:391–394

Kato H, Taketa S, Ban T, Iriki N, Murai K (2001) The influence of a spring habit gene, Vrn-D1, on heading time in wheat. Plant Breed 120:115–120

Kearsey MJ, Farquhar AG (1998) QTL analysis in plants; where are we now? Heredity 80:137–142

Kosner J, Pankova K (1998) The detection of allelic variants at the recessive *vrn* loci of winter wheat. Euphytica 101:9–16

Kulwal PL, Roy JK, Balyan HS, Gupta PK (2003) QTL mapping for growth and leaf characters in bread wheat. Plant Sci 164:267–277

Kuspira J, Unrau J (1957) Genetic analyses of certain characters in common wheat using whole chromosome substitution lines. Can J Plant Sci 37:300–326

Lander ES, Bostein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Laurie DA, Pratchett N, Benzant JH, Snape JW (1995) RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. Genome 38:575–585

Law CN (1966) The locations of genetic factors affecting a quantitative character in wheat. Genetics 53:487–498

Law CN (1987) The genetic control of day length response in wheat. In: Averton JG (ed) Manipulation of flowering. Butterworth, London, pp 225–240

Law CN, Worland AJ (1997) Genetic analyses of some flowering time and adaptative traits in wheat. New Phytol 137:19–28

Law CN, Worland AJ, Giorgi B (1976) The genetic control of ear-emergence time by chromosome 5A and 5D of wheat. Heredity 36:49–58

Law CN, Sutka J, Worland AJ (1978) A genetic study of day-length response in wheat. Heredity 41:185–191

Leonova I, Pestsova E, Salina E, Efremova T, Röder M, Börner A (2003) Mapping of the Vrn-B1 gene in *Triticum aestivum* using microsatellite markers. Plant Breed 122:209–212

Masle J, Doussinault G, Sun B (1989) Response of wheat genotypes to temperature and photoperiod in natural conditions. Crop Sci 29:712–721

Maystrenko OI (1980) Cytogenetic study of the growth habit and ear emergence time on chromosome 2B of wheat. In: Well-being of mankind and genetics. Proceedings of the fourteenth international congress of genetics, Moscow, vol 1. MIR, Moscow, pp 267–282

Mc Intosh RA, Hart GE, Devos KM, Rogers J, Gale MD (1998) Catalogue of gene symbols for wheat: 1998 supplement. Wheat Infor Serv 86:54–91

Miura H, Parker BB, Snape JW (1992) The location of major genes and associated quantitative trait loci on chromosome arm 5BL of wheat. Theor Appl Genet 85:197–204

Nelson JC, Sorrells ME, Van Deynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD, Anderson JA (1995) Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141:721–731

Pugsley AT (1971) A genetic analysis of the spring-winter habit of growth in wheat. Aust J Agric Res 22:21–31

Pugsley AT (1972) Additional genes inhibiting winter habit in wheat. Euphytica 21:547–552

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

SAS Institute Inc (1991) SAS user's guide: release 6.03 eds. SAS Institute, Cary

Scarth R, Law CN (1983) The location of the photoperiod gene Ppd2 and an additional genetic factor for ear-emergence time on chromosome 2B of wheat. Heredity 51:607–619

Sharp PJ, Soltes-Rak E (1988) Homoeologous relationships between wheat group 2 chromosome arms as determined by RFLP analysis. In: Proceedings of 7th international wheat genetics symposium, Cambridge, pp 635–637

Shindo C, Sasakuma T, Watanabe N, Noda K (2002) Two-gene systems of vernalization requirement and narrow-sense earliness in einkorn wheat. Genome 45:563–569

Shindo C, Tsujimoto H, Sasakuma T (2003) Segregation analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines. Heredity 90:56–63

Snape JW, Law CN, Worland AJ (1976) Chromosome variation for loci controlling ear-emergence time on chromosome 2B of wheat. Heredity 37:335–340

Snape JW, Sarma R, Quarrie SA, Fish L, Galiba G, Sutka J (2001) Mapping genes for flowering time and frost tolerance in cereals using precise genetic stocks. Euphytica 120:309–315

Soller M, Beckman JS (1982) Restriction fragment length polymorphisms and genetic improvement. In: Second world congress on genetics applied to livestock production, 6. Round tables, 4–8 October 1982, pp 396–404

Sourdille P, Snape JW, Cadalen T, Charmet G, Nakata N, Bernard S, Bernard M (2000) Detection of QTLs for heading time and photoperiod response in wheat using a doubled-haploid population. Genome 43:487–494

Stelmakh AF (1993) Genetic effect of Vrn genes on heading date and agronomic traits in bread wheat. Euphytica 65:53–60

Stelmakh AF (1998) Genetic systems regulating flowering response in wheat. Euphytica 100:359–369

Stelmakh AF, Avsenin VI (1983) Development of congenic lines on *Vrn1-Vrn3* loci (in Russian). Naucz Tech Bull VSGI 2:24–28

Toth B, Galiba G, Feher E, Sutka J, Snape JW (2003) Mapping genes affecting flowering time and frost resistance on chromosome 5B of wheat. Theor Appl Genet 107:509–514

Utz HF, Melchinger AE (2000) PLABQTL A computer program to map QTL

Welsh JR, Keim DL, Pirasteh B, Richards RD (1973) Genetic control of photoperiod response in wheat. In: Sears ER, Sears LMS (eds) Proceedings of the 4th international wheat genetics symposium, Columbia, pp 879–884

Worland AJ (1996) The influence of flowering time genes on environmental adaptability in European wheats. Euphytica 89:49–57

Worland AJ, Appendino ML, Sayers EJ (1994) The distribution, in European winter wheats, of genes that influence ecoclimatic adaptability whilst determining photoperiodic insensitivity and plant height. Euphytica 80:219–228

Worland AJ, Börner A, Korzun V, Li WM, Petrovic S, Sayers EJ (1998) The influence of photoperiod genes on the adaptability of European winter wheats. Euphytica 100:385–394

Yasuda S, Shimoyama H (1965) Analysis of internal factors influencing the heading time of wheat varieties. Ber Ohara Inst Landw Biol Okayama Univ 13:23–28

Zeller FJ, Lutz J, Reimlein EI, Limpert E, Koenig J (1993) Identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L.). II. French cultivars. Agronomie 13:201–207

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468