Theor Appl Genet (2005) 110: 1458–1466
DOI 10.1007/s00122-005-1979-7

ORIGINAL PAPER

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The *Vrn-H2* locus is a major determinant of flowering time in a facultative × winter growth habit barley (*Hordeum vulgare* L.) mapping population

Received: 15 December 2004 / Accepted: 22 February 2005 / Published online: 16 April 2005

Abstract With the aim of dissecting the genetic determinants of flowering time, vernalization response, and photoperiod sensitivity, we mapped the candidate genes for *Vrn-H2* and *Vrn-H1* in a facultative × winter barley mapping population and determined their relationships with flowering time and vernalization via QTL analysis. The *Vrn-H2* candidate *ZCCT-H* genes were completely missing from the facultative parent and present in the winter barley parent. This gene was the major determinant of flowering time under long photoperiods in controlled environment experiments, irrespective of vernalization, and under spring-sown field experiments. It was the sole determinant of vernalization response, but the effect of the deletion was modulated by photoperiods when the vernalization requirement was fulfilled. There was no effect under short photoperiods. The *Vrn-H1* candidate gene (*HvBM5A*) was mapped based on a microsatellite polymorphism we identified in the promoter of this gene. Otherwise, the *HvBM5A* alleles for the two parents were identical. Therefore, the significant flowering time QTL effect associated with this locus suggests tight linkage rather than pleiotropy. This QTL effect was smaller in magnitude than those associated with the *Vrn-H2* locus and was significant in two-way interactions with *Vrn-H2*. The *Vrn-H1* locus had no effect on vernalization response. Our results support the *Vrn-H2*/*Vrn-H1* repressor/structural gene model for vernalization response in barley and suggest that photoperiod may also affect the *Vrn* genes or tightly linked loci.

Communicated by J.W. Snape

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Introduction

Vernalization—the requirement of a period of low temperature for a plant to transition from a vegetative to a reproductive state—is an important trait in many plants, including winter annual forms of the Triticeae. The term "winter habit" is commonly used to describe autumn-sown cereals, and a vernalization requirement is often found in the winter annual forms of the Triticeae. Most winter barley varieties are better described as vernalization-responsive than vernalization-requiring, as they will eventually flower without vernalization (Karsai et al. 2004b). The vernalization-responsive phenotype is often found in conjunction with photoperiod sensitivity, a delay in flowering when plants are grown under short-day conditions. However, in a survey of winter habit forms of barley, the two traits were found to be highly correlated but not always associated (Karsai et al. 2001). Genetic evidence supports the hypothesis that multiple genes control each phenotype, with crosstalk between the genes in the two pathways (Danyluk et al. 2003). Vernalization and photoperiod sensitivity may contribute to low temperature tolerance by maintaining plants in a vegetative state, but the fact that genotypes with comparable vernalization and photoperiod phenotypes may differ significantly in their level of cold tolerance (Karsai et al. 2001) suggests yet a third low-temperature tolerance pathway, with the potential for connections between the vernalization and photoperiod pathways. For example, genes controlling low-temperature tolerance remain upregulated as long as the vernalization requirement and/or photoperiod duration requirements are not met (Danyluk et al. 2003).

Vernalization in barley is determined by three major loci: *Vrn-H2* (formerly *Sh*) on chromosome 4H, *Vrn-H1*

(*Sh2*) on chromosome 5H, and *Vrn-H3* (*Sh3*) on chromosome 1H (Takahashi and Yasuda 1971; Hayes et al. 1993; Laurie et al. 1995). The vernalization-responsive haplotype is *vrn-H1Vrn-H2vrn-H3*; all other allelic combinations at the three loci confer spring habit (Takahashi and Yasuda 1971). Allelic variation at the *Vrn-H3* locus is found predominantly in barleys from extreme high or low latitudes (Yasuda et al. 1993), reducing the vernalization phenotype to a two-locus model in most cultivated germplasm (Yasuda et al. 1993; Laurie et al. 1995). Among the *vrn* loci, *Vrn1* on the group 5 chromosomes of the Triticeae (A, B, D, and H genomes) is the most extensively characterized in terms of its effects and inheritance (Law et al. 1976; Galiba et al. 1995; Kato et al. 1999). Yan et al. (2003) identified the candidate gene for this locus, a MADS-box gene family member similar to the *AP1* floral meristem identification gene of *Arabidopsis*. In spring habit genotypes, *Vrn1* is expressed from the beginning of the life cycle; whereas in winter habit genotypes, expression of *Vrn1* is repressed until the vernalization requirement is met (Yan et al. 2003; Danyluk et al. 2003). *Vrn-H2* was first mapped to 4HL by its linkage with morphological (*yh)* and biochemical (*Bmy-1*) markers (Takahashi and Yasuda 1971; Hackett et al. 1992). In *Triticum monococcum*, the *Vrn-A*^{m} *2* locus is on the translocated segment of 5A^{m} L derived from 4A^{m} L (Dubcovsky et al. 1998; Tranquilli and Dubcovsky 2000). The candidate gene for the *Vrn-A2* is *ZCCT1*, which is implicated as a repressor of *Vrn-A1* that is downregulated by vernalization (Yan et al. 2004). In barley, there are three *ZCCT* genes, designated *Ha*, *Hb*, and *Hc*. At this time, it is not known which one/ones is/are the repressor/s of *Vrn-H1* (Dubcovsky et al. 2005).

We have described two patterns of photoperiod sensitivity in barley: (1) under short-photoperiod regimes ($<$ 12 h light/24 h), the flowering of some genotypes was extremely delayed, or even prevented, and (2) under long-photoperiod regimes ($>$ 14 h light/24 h), the flowering of some genotypes was significantly accelerated (Karsai et al. 2004b). The candidate genes for these two patterns are the *Ppd-H1* and *Ppd-H2* loci. The *Ppd-H1* photoperiod sensitivity locus was first described by Laurie et al. (1994) on the short arm of chromosome 2H. In barley, *Ppd-H1* is a principal determinant of flowering time under long photoperiods (Karsai et al. 1997), whereas *Ppd-H2* on chromosome 1H is a principal determinant under short photoperiods (Laurie et al. 1995). Candidate genes for the *Ppd* loci have not been reported.

In order to understand the genetic basis of vernalization response, photoperiod sensitivity, and low-temperature tolerance, we are conducting an ongoing series of comparative experiments involving the genotype 'Dicktoo' and its progeny from crosses with multiple genotypes representing different combinations of growth habit and low-temperature tolerance phenotypes. 'Dicktoo' is a ''facultative'' genotype, meaning it is photoperiod-sensitive but has no vernalization response. In the 'Dicktoo' × 'Morex' (facultative × spring growth habit) mapping population, QTL coincident with the expected positions of *Ppd-H1* and *Vrn-H1* were significant determinants of flowering time, and their epistatic interactions resulted in transgressive segregants (Pan et al. 1994). In this report, we describe results from crossing 'Dicktoo' with 'Kompolti korai', a Hungarian winter barley that contrasts with 'Dicktoo' for key adaptation traits (Karsai et al. 2001). Preliminary experiments with this population showed that neither of the regions around *Vrn-H1* nor *Ppd-H1* were significant main-effect determinants of flowering time in the progeny of this cross (Karsai et al. 2004a). Accordingly, our objectives were to identify the determinants of flowering time, vernalization response, and photoperiod sensitivity in the 'Dicktoo' × 'Kompolti korai' population.

Materials and methods

Plant materials

A doubled haploid (DH) population of 95 lines was developed from the F_1 of 'Dicktoo' × 'Kompolti korai' at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, using anther culture. The US variety 'Dicktoo' (released in 1952) has good frost tolerance, no vernalization response, and is photoperiod-sensitive (Pan et al. 1994; Karsai et al. 1997). The Hungarian variety 'Kompolti korai' ('Ager' × 'Ledeci Beta', released in 1973) has moderate frost tolerance, a strong vernalization requirement, and is less sensitive to photoperiod duration than 'Dicktoo' (Karsai et al. 2001).

Phenotypic characterization

The 95 DH lines and the two parents were phenotyped in a series of controlled environment tests involving combinations of vernalization and different photoperiods in the Martonvásár phytotron, following the procedures described by Karsai et al. (1997, 2004b). The two vernalization treatments were (1) no vernalization and (2) vernalization of seedlings for 42 days at 3°C, with a 9 h-light/16 h-dark photoperiod regime and low light intensity (12–13 μmol m^{-2} s^{-1}). Seedlings for the unvernalized treatment were established one week prior to the end of the vernalization treatment so that seedlings from both treatments were transplanted at the same time and approximately at the same growth stage (seedlings with one to two leaves). The photoperiod treatments consisted of 8-, 16-, and 24-h light regimes per 24-h period. The intensity (345 ± 22 μmol m^{-2} s^{-1}) and type of the daily light (metal halide lamps) were kept constant within each photoperiod regime and remained the same for all three photoperiods, the only difference being in the length of the light period. The temperature was also kept constant at 18°C day and night in all the

treatments. Two replications were used, due to space limitations in the growth chambers. The number of days elapsed from the start of the photoperiod treatments to heading was recorded for each experimental unit (developmental phase 49 on the Zadock's scale, Tottman and Makepeace 1979). The vernalization response of each genotype was calculated as the difference in flowering time between unvernalized (uv), and vernalized (v) plants within each photoperiod regime (8, 16, and 24 h). The parents and the mapping population of 95 DH lines were also grown under field conditions, using fall and spring plantings. The experimental field is located at latitude of 47°21′N and at longitude of 18°49′E, with an altitude of 150 m. Each genotype was planted as a single head row together with head rows of each parent assigned evenly to the field plan. The fall-sown experiment was planted on 19 October 2003, the spring-sown experiment on 19 March 2004. The average monthly temperatures were as follows: −2.3°C in January, 1.5°C in February, 4.4°C in March, and 11.2°C in April. The daily photoperiod was 12 h light/24 h and increasing by the time of plantlet emergence in the spring-sown experiment. The flowering time of each plot was determined as the number of days elapsed between 1 January and the day when 50% of the inflorescences in the plot were at the developmental stage of 49 on Zadock's scale (Tottman and Makepeace 1979).

Genotyping

Linkage maps were created for chromosomes reported to have photoperiod and vernalization loci (1H, 2H, 4H, and 5H), using an array of marker types assayed on the 95 DH lines. The RFLP loci are named using standard North American Barley Genome Project (NABGP) nomenclature. The RFLP probes converted to STS primers were also utilized; the primer sequences were kindly provided by Dr. T. Blake (Montana State University, Bozeman, Mon., USA). To differentiate them from the RFLP loci, the standard NABGP nomenclature was used, but lowercase letters were employed. The RAPD loci were mapped using OPERON primer sets (the first two digits following the letter stand for the number of the primer within the given set, whereas the next digits show the size of the polymorphic band in base pairs). The SSR loci are named per the nomenclature employed by Ramsay et al. (2000). Special markers characterizing the region around *Vrn-H2* (4H) were the following:

1. The *Bmy1* SSR version of the biochemical marker *Bmy1* was assayed using the information reported by Pillen et al. (2000).
2. Primers HvSnf2.02F (5′-cctggccacaaaaacaatcagc-3′) and HvSnf2.04R (5′-cgttgttgtttctctataatgcagc-3′) were designed and used for amplifying 'Dicktoo' and 'Kompolti korai' alleles (382 bp and 214 bp, respectively) at the *HvSnf2* locus (GenBank accession no. AF459085). This gene codes for a transcriptional regulation protein and found to be physically tightly linked to *Vrn-A2* (Yan et al. 2004).
3. Primers for identifying the three forms of *ZCCT-H* the candidate gene for *Vrn-H2*; HvZCCT.06F (5′-cctagttaaaacatatatccatagagc-3′) and HvZCCT.07R (5′-gatcgttgcgttgctaatagtg-3′) were designed to co-amplify a 307-bp long fragment of *ZCCT-Ha* gene (AY485977) and a 273-bp fragment of *ZCCT-Hb* gene (GenBank accession no. AY485978). Primers HvZCCT.HcF (5′-caccatcgcatgatgcac-3′) and HvZCCT.HcR (5′-tcatatggcgaagctggag-3′) were used to amplify a 194-bp long fragment of the *ZCCT-Hc* gene (AY687931).

We are conducting an ongoing series of experiments characterizing the *HvBM5A* gene (the candidate gene for the *Vrn-H1* locus on chromosome 5H) at the structural and functional levels in an array of barley germplasm. Based on unpublished data, we designed and used SSR primers HvBM5.82F (5′-atatctactccagcctagggtac-3′) and HvBM5.83R (5′-cgcgaatctcccccatattgc-3′) for mapping the *HvBM5A* gene (AY750995) in the 'Dicktoo' × 'Kompolti korai' DH population. Linkage maps were constructed using MAPMAKER, version 3.0 (Lander et al. 1987), and QTL analyses were performed using simple interval mapping (SIM) of MAPMAKER/QTL (Lander and Botstein 1989), with a significance level of LOD3 and the composite interval mapping (CIM) of WinQTL Cartographer, version 2.0 (Statistical Genetics, North Carolina State University, Raleigh, N.C., USA), with a significance level of LR 12. Two-gene interactions between major genes were analyzed through comparing the values of the four allele classes.

Results

Mapping genes involved in vernalization and photoperiod response

Locus orders and distances in linkage maps of chromosomes 1H, 2H, 4H, and 5H were in agreement with previous reports (Pan et al. 1994; Ramsay et al. 2000, the Steptoe × 'Morex' BIN map: http://barleygenomics.wsu.edu). No significant main QTL effect or interaction was detected on chromosome 1H in the predicted position of *Ppd-H2*, whereas main QTL effects and/or interactions were identified on chromosomes 2H, 4H, and 5H (Fig. 1). The linkage maps of the latter three chromosomes are shown in Fig. 2. The probable position of *Ppd-H1* gene (2H) is in vicinity of *ABG358* and *ABG459*. On 4H, all the three *ZCCT-H* genes, *ZCCT-Ha, ZCCT-Hb*, and *ZCCT-Hc* cosegregate and are deleted in 'Dicktoo' (*Vrn-H2*$^{-}$) and present in 'Kompolti korai' (*Vrn-H2*$^{+}$). To confirm the dominant marker segregation of the *ZCCT-H* loci, the *Snf2* locus, which is physically linked to *ZCCT1* in *T. monococcum* (Yan et al. 2004), was also analyzed. The 168-bp long insertion/deletion between the DNA sequences of

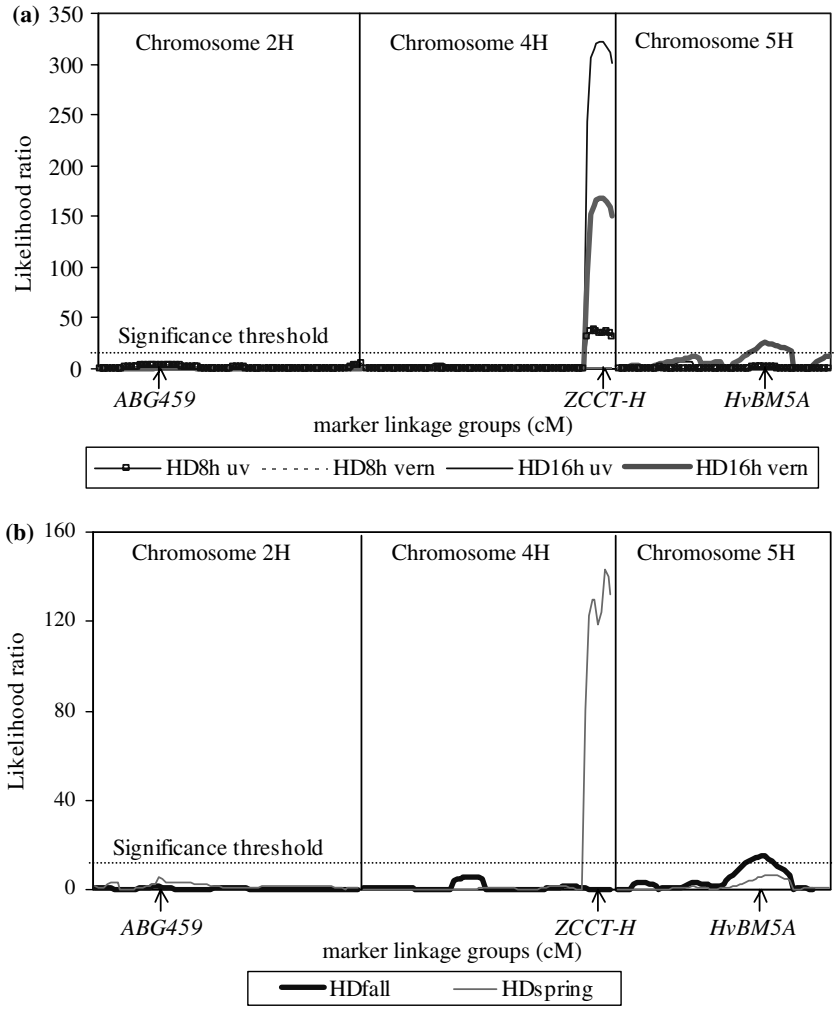

Fig. 1 Heading date QTL main effects in the composite interval mapping analysis in 'Dicktoo' × 'Kompolti korai' barley population measured in (**a**) controlled environments and (**b**) under field-sown conditions

'Dicktoo' and 'Kompolti korai' made it possible to use *HvSnf2* as a codominant marker. *HvSnf2* alleles showed complete cosegregation with the presence/absence of *ZCCT-Ha, ZCCT-Hb*, *ZZCT-Hc*, and this gene cluster mapped 8.0 cM proximal to *Bmy1*. Using the 20-bp differences in the promoter microsatellite, the *HvBM5A* gene (Danyluk et al. 2003; Yan et al. 2003) was mapped to the long arm of chromosome 5H.

Vrn-H2 is the major determinant of flowering time

The *ZCCT-H* gene cluster is completely deleted in 'Dicktoo', making this genotype a natural "knock-out" for the *Vrn-H2* locus. In the DH population, the presence/absence of the *Vrn-H2* gene was the most significant determinant of flowering time under long photoperiods (16 h and 24 h) in controlled environment tests, producing highly significant QTL peaks both in SIM and CIM analyses. Under the 16-h photoperiod regime, it explained 96% of the phenotypic variance for flowering time in the unvernalized treatment, with a LOD score of 68 (LR 321). The flowering time phenotype showed a bimodal distribution; the average flowering times of *Vrn-H2*$^{-}$ and *Vrn-H2*$^{+}$ lines were 40 days and 138 days, respectively (Fig. 3a). Vernalization treatment did not significantly influence the flowering time of the *Vrn-H2*$^{-}$ lines, but it dramatically reduced the number of days to flowering for *Vrn-H2*$^{+}$ DH lines (an average reduction of 72 days). Even with vernalization, the *Vrn-H2*$^{+}$ and *Vrn-H2*$^{-}$ groups remained distinctly separated, with a significant difference of 27 days between their average flowering times (Fig. 3b). After vernalization, the allele phase at *Vrn-H2* accounted for 80% of the phenotypic variation, with a LOD score of 31 (LR 168). A similar pattern was observed under the 24-h photoperiod regime, where the average flowering time values for the *Vrn-H2*$^{-}$ and *Vrn-H2*$^{+}$ classes were 28 days and 109 days (uv), and 29 days and 55 days (v). The r^2 values were 0.9 and 0.8 and the LOD scores 64 and 44, respectively (LR 309 and 220). The *Vrn-H2* locus was the only determinant of vernalization response in this population [$r^2 = 0.9$ and LOD = 39 (LR 200) at 16 h, and $r^2 = 0.9$ and LOD = 44 (LR 221) at 24 h photoperiod].

Under a short photoperiod (8 h), the effect of *Vrn-H2* allele phase was much smaller, and its significance depended on the vernalization treatment. Without vernalization, the presence or absence of *Vrn-H2*

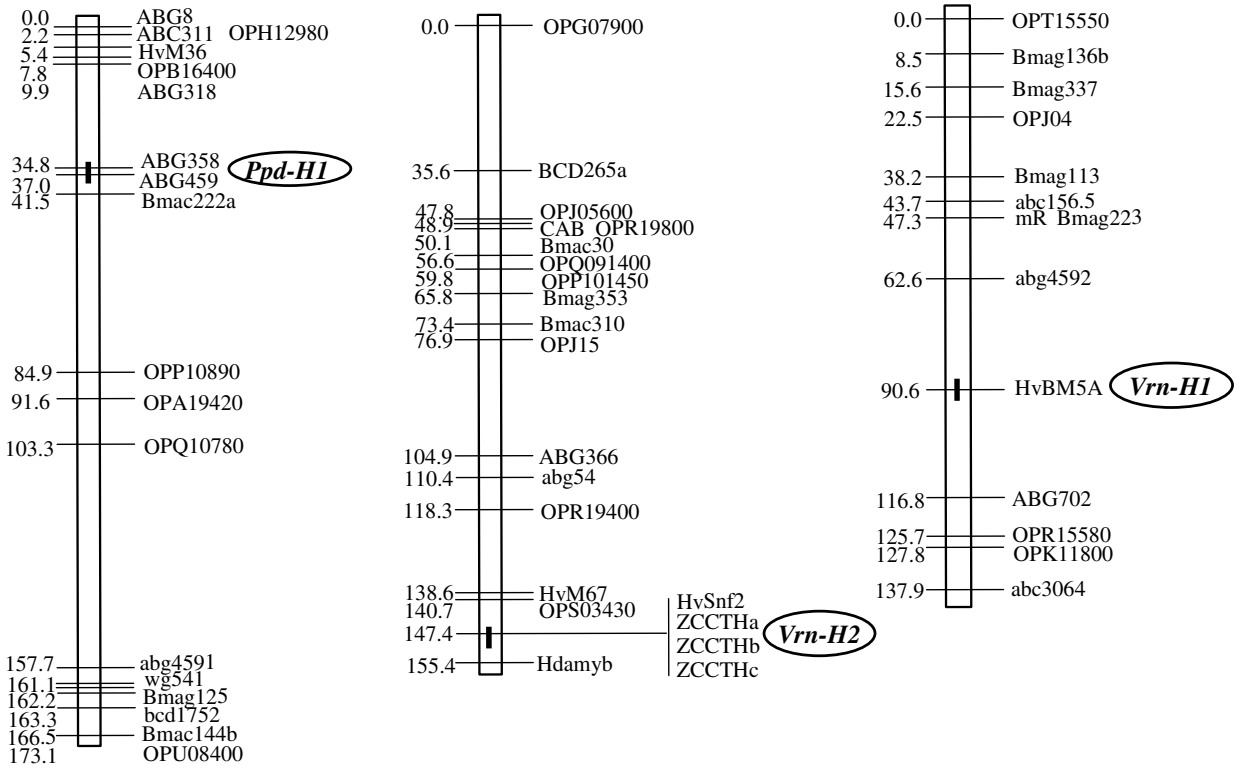

Fig. 2 Linkage maps of chromosomes 2H, 4H, and 5H in the 'Dicktoo' × 'Kompolti korai' population, sharing inferred positions of *Ppd-H1* (based on Pan et al. 1994 and barley BIN map: http://barleygenomics.wsu.edu), *Vrn-H2* (based on mapping *ZCCT-H*), and *Vrn-H1* (based on mapping *HvBM5A*)

explained 32% of the phenotypic variation with a LOD score of 6 (LR 36, Fig. 3c). On average, the *Vrn-H2*$^{-}$ lines flowered earlier than those with the gene (198 vs 236 days). With vernalization, there was no significant difference between the two allele classes, and the presence/absence of *Vrn-H2* gene had no effect on flowering time (Fig. 3d). Under an 8-h photoperiod, and when vernalized, 'Kompolti korai' was significantly (42 days) earlier than 'Dicktoo'.

Under field conditions, the effects of the *Vrn-H2* gene on flowering time depended on the sowing time. It had no effect on flowering time under autumn-sown conditions; the distribution pattern of the two allele classes was very similar (Fig. 3e). 'Kompolti korai' flowered significantly earlier (125 days) than the population mean (128 days) and 'Dicktoo' significantly later (132 days). Under spring-sown conditions, the presence/absence of *Vrn-H2* explained 80% of the phenotypic variance with a LOD score of 29 (LR 143), and the population was again separated into two distinct groups (Fig. 3f). The average flowering time of *Vrn-H2*$^{-}$ lines was 152 days, whereas that of the *Vrn-H2*$^{+}$ lines was 168 days. 'Dicktoo' headed significantly later (156 days) and 'Kompolti korai' significantly earlier (166 days) than their respective group means.

The significant QTL main effects detected on chromosome 5H coincided with *HvBM5A*. In SIM analysis, only one QTL was identified at this location, which was significantly associated with flowering time under autumn-sown field conditions (LOD = 4.7 and LR 15). This QTL explained 33% of the phenotypic variance, with the 'Dicktoo' contributing the later-flowering allele. In addition to this QTL, in CIM analysis, significant heading date QTL were also detected in vernalized treatment under long photoperiods (LR 26 at 16 h vernalization and LR 21 at 24 h vernalization), both explaining a low portion of the phenotypic variation (5 and 3%, respectively). No major QTL effect was detected on chromosome 2H either with SIM or CIM.

Interactions of *Vrn-H2* with other loci

Although there were two distinct flowering time groups when the population was sorted by *Vrn-H2* allele classes under long photoperiods and spring-sown conditions, there was also variation within each of the allele classes. Earlier- and later-flowering phenotypic transgressive segregants were observed under long photoperiods irrespective of the vernalization treatment, at short photoperiods when vernalized, and in the spring-sown field test. The transgressive segregants suggest that *Vrn-H2* "alleles" interact with alleles at other loci. Based on the previous results from the 'Dicktoo' × 'Morex' population, two logical targets for interaction were *Vrn-H1* (5H), and *Ppd-H1* (2H) (Karsai et al. 1997). In the case of *Vrn-H1*, the effect of allele phase in the gene (*HvBM5A*) itself could be assessed. There were highly

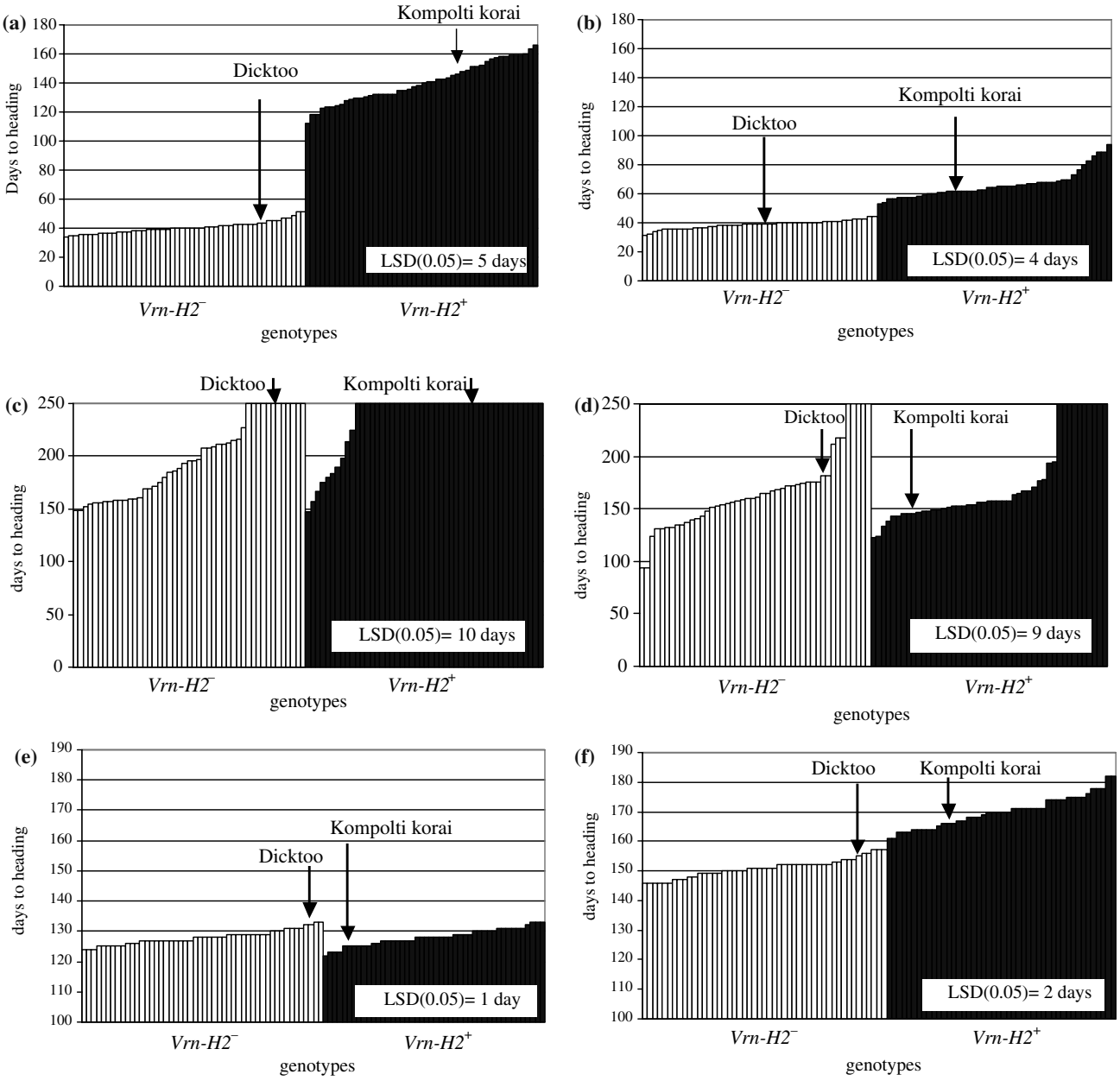

Fig. 3 Flowering time of *Vrn-H2*$^{-}$ and *Vrn-H2*$^{+}$ DH lines in the 'Dicktoo' × 'Kompolti korai' mapping populations under six growing conditions: 16 h light/24 h unvernalized (**a**) and vernalized (**b**), 8 h light/24 h unvernalized (**c**) and vernalized (**d**) under controlled environment, and field autumn-sown (**e**) and spring-sown (**f**). The *Vrn-H2* allele structure was inferred by the codominant *HvSnf2* locus, which cosegregated with the *ZCCT-H* presence/deletion

significant *Vrn-H2* and *Vrn-H1* two-locus interactions (Table 1). In *Vrn-H2*$^{-}$ lines, the allele phase at *Vrn-H1* was a significant determinant of flowering time under long photoperiods, irrespective of the vernalization treatment. The same effect was also observed under both autumn and spring-sown field tests. Under all these conditions, lines having the Kompolti allele at *Vrn-H1* flowered significantly earlier. The allele phase at *Vrn-H1* had some effect on the flowering time of *Vrn-H2*$^{+}$ lines, but this was only apparent when their vernalization requirement was satisfied. In these cases (16-h, 24-h vernalized, autumn-sown), the Kompolti allele at *Vrn-H1* resulted in earlier flowering. This interaction pattern may explain the differences found between the SIM and CIM QTL analyses. The interaction between these two genes had a very small effect on vernalization response and no effect on heading under short photoperiods.

In the case of *Ppd-H1*, the candidate gene has not been identified, and interaction with *Vrn-H2* could only be inferred based on linked markers. Considering the three markers (*ABG358*, *ABG459*, and *Bmac222a*) flanking the *Ppd-H1* region, the interactions with *ABG459* were consistently the highest, and are therefore shown in Table 1. No significant interactions were found

Table 1 Two-locus interaction between the *Vrn-H2* and *Vrn-H1* loci and between *Vrn-H2* and the region around *Ppd-H1* locus (inferred by allele type at *ABG459*) for flowering time (*FT*) and vernalization response (*VR*) under various growing conditions

Traits	Parental values			Allele classes[c] for *Vrn-H2* and *Vrn-H1*				Allele classes[c] for *Vrn-H2* and *ABG459*			
	'Dicktoo'	'Kompolti korai'	LSD (0.05)	DD[b] ($n=23$)	DK ($n=25$)	KD ($n=29$)	KK ($n=18$)	DD ($n=29$)	DK ($n=18$)	KD ($n=27$)	KK ($n=21$)
FT8 huv[a]	250	250	10	206a	190a	241b	234b	208b	180a	233c	240c
FT8 hv	188	146	9	165a	163a	171a	169a	165a	162a	171a	173a
FT16 huv	42	145	5	44b	37a	140c	139c	41a	40a	136b	139b
FT16 hv	39	61	4	41b	37a	69c	62d	39a	39a	66b	65b
FT24 huv	29	111	4	29b	26a	110c	112c	28a	27a	110b	111b
FT24 hv	27	52	2	30b	28a	57c	51d	30a	29a	56b	54b
VR8 h	62	104	-	41ab	25a	73c	61bc	40b	15a	60bc	67c
VR16 h	3	84	-	3b	0a	71c	78c	2a	1a	70b	74b
VR24 h	1	58	-	–1a	–1a	53b	61b	-1a	-2a	54b	57b
FT autumn-sown	132	125	1	129b	127a	129b	127a	128a	128a	128a	127a
FT spring-sown	156	166	2	153b	149a	169c	167c	151a	151a	170b	167b

[a]The numeral refers to the photoperiod duration (h light/24 h), *uv* unvernalized, *v* vernalized
[b]*D* 'Dicktoo' allele, *K* 'Kompolti korai' allele
[c]Allele class means with the same letter within each of the two-locus interactions are not significantly different from each other

for the long photoperiod regimes or for the field-sown experiments. Significant interactions were observed for flowering time under short photoperiods in the unvernalized treatment, and for vernalization response under short photoperiods. Under these conditions, the *Vrn-H2*$^-$ DH lines with the Kompolti allele at the inferred position of the *Ppd-H1* locus were earlier flowering and showed a smaller difference in flowering time between the unvernalized versus vernalized treatments.

Discussion

Identification of the *Vrn-H2* (4H) gene and its effect on flowering

We found that the three members of the *ZCCT-H* gene family, candidates for the *Vrn-H2* vernalization response gene, are completely deleted in 'Dicktoo' and present in 'Kompolti korai'. In the progeny of 'Dicktoo' × 'Kompolti korai', no recombinants were found between the three genes. We cannot therefore, determine which gene (or genes) within the family encodes the repressor of *Vrn-H1*, as hypothesized by Yan et al. (2004). Dubcovsky et al. (2005) recently presented evidence that *ZCCT-Ha* is the most likely candidate. Considering the cosegregating members of the *ZCCT-H* family as a single locus—*Vrn-H2*—we determined that it is the major determinant of vernalization response in the 'Dicktoo' × 'Kompolti korai' population.

Using QTL analysis tools, we observed other phenotypes—photoperiod-dependent vernalization response and regulation of flowering time—that could be pleiotropic effects of the *Vrn-H2* locus, or they could be due to a tightly linked gene (or genes). The vernalization response effects of *Vrn-H2* are photoperiod-dependent in that the presence/absence of *Vrn-H2*, had an effect on vernalization response only under long (18-h and 24-h) photoperiods; there was no effect on vernalization response at short (8-h) photoperiods. This suggests that *Vrn-H2* (or a tightly linked gene) may be regulated by photoperiod duration such that *Vrn-H2* ceases to be a repressor of *Vrn-H1* only after completion of the vernalization requirement and achievement of a photoperiod of sufficient duration. We also observed significant QTL effects for flowering time in conjunction with the presence of the *ZCCT-H* genes at long photoperiods and after satisfaction of the vernalization requirement, which suggests a continued role in regulation of growth and development, or the effects of a tightly linked gene. Additional work is needed to explain this finding, because the available data suggest that *Vrn-H2* ceases to transcribe the encoded repressor of *Vrn-H1* upon completion of the vernalization requirement (Yan et al. 2004) and no candidate genes for photoperiod regulation (e.g. phytochromes or cryptochromes) are reported for this region of chromosome 4H.

Identification of the *Vrn-H1* (5H) gene and its effect on flowering

We found that the 'Dicktoo' and Kompolti *HvBM5A* (*Vrn-H1*) alleles are identical throughout their coding regions (AY785828, AY785835) and their promoters (AY785817, AY785824), except for the number of repeats in a promoter-localized microsatellite. Fu et al. (2005) demonstrated that a 436-bp segment of the first intron of *HvBM5A* may have conserved regulatory elements important for the determination of vernalization response. There are no differences between the 'Dicktoo' and 'Kompolti korai' intron 1 sequences (AY750994, AY866487) in this putative regulatory region. The promoter-localized microsatellite polymorphism was used

for mapping the locus in the 'Dicktoo' × Kompolti population, and it maps to the expected position of *Vrn-H1* on chromosome 5H. This microsatellite should be a useful tool for mapping the *Vrn-H1* locus in barley mapping populations. No vernalization response QTL were found in conjunction with the *Vrn-H1* locus, which we attribute to the identical parental alleles, except for the aforementioned microsatellite. Most importantly, this finding supports the two-locus epistatic model for vernalization response proposed by Yan et al. (2004). In the 'Dicktoo' × 'Kompolti korai' mapping population, the presence of the *ZCCT-H* cluster is the dominant *Vrn-H2* allele, whereas the deletion in 'Dicktoo' is the recessive allele. 'Dicktoo' and 'Kompolti korai' both have identical recessive (*sensu* Yan et al. 2003) alleles at *Vrn-H1* and as a result, the only genetic factor that is segregating for vernalization response is *Vrn-H2* on chromosome 4H.

We detected a significant QTL for flowering time under autumn-sown field conditions coincident with the *Vrn-H1* locus, which may be a pleiotropic effect or the effect of a tightly linked gene. In *T. monococcum*, a *PhyC* gene was identified approximately 0.1 cM proximal to *Vrn-1* (Yan et al. 2003). The phytochrome polypeptide family is known to be red- and far-red-light-absorbing photoreceptors, light-regulated Ser/Thr-specific protein kinases that regulate diverse photomorphogenic processes in plants and as such, they are key signalling components in the photoperiodic control of flowering (Fankhauser and Staiger 2002). We have mapped the *PhyC* locus 1 cM from *HvBM5A* in the 'Dicktoo' × 'Morex' mapping population (unpublished data).

Interaction between *Vrn-H2* and *Vrn-H1*

The size of the 'Dicktoo' × 'Kompolti korai' mapping population is sufficient for mapping Mendelian genes and estimating QTL main-effects, but it is insufficient for extensive analysis of the multi-gene interactions that are likely to be important in such complex phenotypes as vernalization response, photoperiod sensitivity, and flowering time. No main effect QTL or interactions were found in the case of *Ppd-H2*. In the case of *Vrn-H2* × *Ppd-H1*, there were significant interactions, but only under an 8-h photoperiod. We also observed a two-locus interaction of the *Ppd-H1* locus in the 'Dicktoo' × 'Morex' population (Karsai et al. 1997), but with *Vrn-H1* rather than with *Vrn-H2*.

We detected intriguing significant interactions between the *Vrn-H2* and *Vrn-H1* loci. Under 16-h and 24-h photoperiods, the non-parental configuration of *Vrn-H2*$^{-}$ (e.g., 'Dicktoo') alleles with 'Kompolti korai' alleles at *Vrn-H1* led to very early flowering transgressive segregants. In other words, substitution of the 'Kompolti korai' *Vrn-H2* allele with the 'Dicktoo' deletion of the gene revealed a new pattern of flowering time effects for the 'Kompolti korai' *Vrn-H1* allele. Two possible explanations for this effect are (1) functional polymorphisms between the 'Dicktoo' and 'Kompolti korai' alleles at some other region of *Vrn-H1* besides the coding region and sequenced portion of the promoter and intron 1 or (2) the effects of a linked gene with a significant effect on growth and development rates. The interaction analysis also demonstrated that 'Dicktoo' carries the recessive (e.g., ''vernalization competent'') alleles at *Vrn-H1*, because all DH lines with 'Dicktoo' alleles at *Vrn-H1* and 'Kompolti korai' alleles at *Vrn-H2* (e.g., *vrn-H1vrn-H1Vrn-H2Vrn-H2* genotypes) showed a vernalization response.

In conclusion, we have shown that the 'Dicktoo' × 'Kompolti korai' mapping population supports the two-locus epistatic model for vernalization proposed by Yan et al. (2004) based on phenotypic segregation, mapping data, and allele sequence at candidate loci. We have also shown that, in barley, photoperiod has an important main effect and interactive role with vernalization in determining flowering time, although it remains to be determined to what extent this is due to regulation of *Vrn* genes by *Ppd*-sensitivity genes and how much is due to the effects of *Ppd*-sensitivity genes tightly linked to *Vrn* genes, forming ''winter hardiness co-adaptive gene clusters.''

Acknowledgements This project was funded by the Hungarian National Research and Innovation Fund (NKFP) research grant no. 4/0020/2002 and the United States National Science Foundation project no. DBIO 110124.

References

Danyluk J, Kane ND, Breton G, Limin AE, Fowler DB, Sarhan F (2003) *TaVRT-1*, a putative transcription factor associated with vegetative to reproductive transition in cereals. Plant Physiol 132:1849–1860

Dubcovsky J, Lijavetzky D, Appendino L, Tranquilli G (1998) Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement. Theor Appl Genet 97:968–975

Dubcovsky J, Chen C, Yan L (2005) Molecular characterization of the allelic variation at the *VRN-H2* vernalization locus in barley. Mol Breed (in press)

Fankhauser C, Staiger D (2002) Photoreceptors in *Arabidopsis thaliana*: light perception, signal transduction and entrainment of the endogenous clock. Planta 216:1–16

Fu D, Szűcs P, Yan L, Helguera M, Skinner JS, von Zitzewitz J, Hayes PM, Dubcovsky J (2005) Large deletions within the *VRN-1* first intron are associated with spring growth habit in barley and wheat. Mol Genet Genomics (in press) DOI: 10.1007/s00438-004-1095-4.

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

Hackett CA, Ellis RP, Forster BP, McNicol JW, Macaulay M (1992) Statistical analysis of a linkage experiment in barley involving quantitative trait loci for height and ear-emergence time and two genetic markers on chromosome 4. Theor Appl Genet 85:120–126

Hayes PM, Liu BH, Knapp SJ, Chen F, Jones B, Blake T, Frankowiak JD, Rasmusson D, Sorrells ME, Ullrich SE, Wesenberg D, Kleinhofs A (1993) Quantitative trait locus effects and environmental interaction in a sample of North American barley germplasm. Theor Appl Genet 87:392–401

Karsai I, Mészáros K, Hayes PM, Bedő Z (1997) Effects of loci on chromosomes 2 (2H) and 7 (5H) on developmental patterns in barley (*Hordeum vulgare* L.) under different photoperiod regimes. Theor Appl Genet 94:612–618

Karsai I, Mészáros K, Láng L, Hayes PM, Bedő Z (2001) Multivariate analysis of traits determining adaptation in cultivated barley. Plant Breed 120:217–222

Karsai I, Mészáros K, Szűcs P, Filichkina T, Hayes PM, Láng L, Bedő Z (2004a) Effect of *Vrn-H2* vernalization response locus (4H) on plant development in a winter barley mapping population. In: Proceedings of the 9th barley genetic conference, June 20–26, Brno, pp 289–294

Karsai I, Hayes PM, Kling J, Matus IA, Mészáros K, Láng L, Bedő Z, Sato K (2004b) Genetic variation in component traits of flowering time in *Hordeum vulgare* subsp. *spontaneum* accessions characterized in controlled environments. Crop Sci 44:1622–1632

Kato K, Miura H, Sawada S (1999) QTL mapping of genes controlling ear emergence time and plant height on chromosome 5A of wheat. Theor Appl Genet 98:472–477

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

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interaction computer package for constructing primary genetic linkage maps for experimental and natural populations. Genomics 1:174–181

Laurie DA, Pratchett N, Bezant JH, Snape JW (1994) Genetic analysis of a photoperiod response gene on the short arm of chromosome 2(2H) of *Hordeum vulgare* (barley). Heredity 72:619–627

Laurie DA, Pratchett N, Bezant 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, Worland AJ, Giorgi B (1976) The genetic control of ear-emergence time by chromosomes 5A and 5D of wheat. Heredity 36:49–58

Pan A, Hayes PM, Chen F, Chen THH, Blake T, Wright S, Karsai I, Bedő Z (1994) Genetic analysis of the components of winterhardiness in barley (*Hordeum vulgare* L.). Theor Appl Genet 89:900–910

Pillen K, Binder A, Kreuzkam B, Ramsay L, Waugh R, Förster J, Léon J (2000) Mapping of EMBL-derived barley microsatellites and their use to differentiate German barley cultivars. Theor Appl Genet 101:652–660

Ramsay L, Macaulay M, degli Ivanissevich S, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005

Takahashi R, Yasuda S (1971) Genetics of earliness and growth habit in barley. In: Nilan RA (ed) Barley genetics II. Washington State University Press, Pullman, pp 388–408

Tottman DR, Makepeace RJ (1979) An explanation of the decimal code for the growth stages of cereals, with illustrations. Ann Appl Biol 93:221–234

Tranquilli G, Dubcovsky J (2000) Epistatic interaction between vernalization genes *Vrn-A*^{m} *1* and *Vrn-A*^{m} 2 in diploid wheat. J Hered 91:304–306

Yan L, Echenique V, Busso C, SanMiguel P, Ramakrishna W, Bennetzen JL, Harrington S, Dubcovsky J (2002) Cereal genes similar to *Snf2* define a new subfamily that includes human and mouse genes. Mol Genet Genomics 268:488–499

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. PNAS 100:6263–6268

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004) The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. Science 303:1640–1644

Yasuda S, Hayashi J, Moriya I (1993) Genetic constitution for spring growth habit and some other characters in barley cultivars in the Mediterranean coastal regions. Euphytica 70:77–83