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Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement

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Abstract The adaptability of *Triticum aestivum* to a large range of environments is partially due to genetic differences in sensitivity to vernalization. The most potent gene reducing the vernalization requirement in hexaploid wheat is *Vrn-A1*. An orthologous vernalization gene, designated *Vrn-A^{m}1*, was mapped in the diploid wheat *Triticum monococcum* between RFLP markers *Xwg908* and *Xabg702* on the long arm of chromosome 5A^{m}L. The orthology of *VrnA^{m}1* with *Vrn-A1* (5A wheat, originally *Vrn1*), *Vrn-D1* (5D wheat, originally *Vrn3*), *Vrn-R1* (5R rye, originally *Sp1*) and *Vrn-H1* (5H barley, originally *Sh2*) was shown by mapping RFLP markers linked to these vernalization genes on the *T. monococcum* linkage map. A second vernalization gene, designated *Vrn-A^{m}2*, was found in the distal region of chromosome 5A^{m}L within a segment translocated from homoeologous group 4. This gene is completely linked to RFLP marker *Xbcd402* and located between the same RFLP markers (*Xβ-Amy-1* and *Xmwg616*) as the *Vrn-H2* (originally *Sh*) locus in *Hordeum vulgare*.

Key words *Triticum monococcum* · Vernalization genes · *Vrn-1* · *Vrn-2* · RFLP · Comparative maps

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Introduction

Genetic variation in vernalization response and day-length sensitivity plays an important role in the adaptability of wheat (*Triticum aestivum* L.) to a large range of environments. Genes controlling vernalization requirement (*Vrn* genes) divide bread-wheat varieties into two main categories distinguished by their adaptation to different sowing dates and referred to as winter or spring wheats. Autumn sown or winter wheats require a period of low temperature (vernalization) for floral initiation. This mechanism prevents the onset of floral development during winter.

Genes controlling vernalization requirement in hexaploid wheat and other temperate cereals have been extensively characterized. Based on the evidence for orthology among some of these genes obtained by other workers (Law et al. 1976; Plaschke et al. 1993; Galiba et al. 1995; Laurie et al. 1995; Nelson et al. 1995) and in this paper, the classical nomenclature of cereal vernalization genes has been replaced by one reflecting orthologous relationships (McIntosh et al. 1998). This new nomenclature of vernalization genes will be used throughout this paper.

The most potent gene in reducing the vernalization requirement in hexaploid wheat is *Vrn-A1* (originally *Vrn1*) (Pugsley 1971). This gene was mapped in the long arm of chromosome 5A (Unrau 1950; Morrison 1960; Maystrenko 1974; Halloran 1975; Law et al. 1976; Maystrenko 1980; Galiba et al. 1995; Nelson et al. 1995; Korzun et al. 1997). *Vrn-A1* is orthologous to *Vrn-D1* (originally *Vrn3*) located in chromosome arm 5DL (Law et al. 1976; Galiba et al. 1995; Nelson et al. 1995) and to *Vrn-B1* (originally *Vrn4* and *Vrn2*) located in chromosome 5B (McIntosh et al. 1998). It has been also suggested that wheat *Vrn-1* genes are orthologous to *Vrn-H1* (originally *Sh2*) located in barley chromosome arm 5HL (Laurie et al. 1995), and to *Vrn-R1* (originally

Sp1) located in rye chromosome arm 5RL (Plaschke et al. 1993).

Genetic studies of a large set of spring barley varieties identified two additional vernalization genes located in chromosomes 4H and 1H (Takahashi and Yasuda 1971). These genes are re-designated *Vrn-H2* (originally *Sh*) and *Vrn-H3* (originally *Sh3*) respectively. *Vrn-H3* variation was found predominantly in barley varieties from extreme high or low latitudes, and crosses between temperate varieties are therefore expected to segregate only for *Vrn-H1* and *Vrn-H2*. No wheat gene orthologous to *Vrn-H2* or *Vrn-H3* has been identified.

Other genes affecting the transition to flowering that are sensitive to vernalization have been reported in chromosome arm 7BS (Law 1966; Law and Wolfe 1966; Law 1967; Chao et al. 1989), chromosome 3B (Zemetra and Morris 1984; Miura and Worland 1994), and chromosomes 6A, 6B, and 6D (Islam-Faridi et al. 1996). New names will be assigned to these additional vernalization genes as their orthology is established.

Flowering time is determined not only by genes that control the vernalization response but also by genes that control the photoperiod response or affect flowering time independently of photoperiod and vernalization (earliness *per se*). The complex interactions among genes of these three classes often result in continuous variation in flowering time that is usually analyzed by *Q*uantitative *T*rait *L*oci (QTL) techniques (Laurie et al. 1995; Li et al. 1995; Siripoonwiwat et al. 1996; Holland et al. 1997). However, crosses between spring and winter lines grown without vernalization in greenhouses under long days and continuous warm conditions, may segregate into two clear cut classes, those not inhibited to flower (spring) and those which remain vegetative (winter) (Plaschke et al. 1993). This characteristic clear-cut segregation for growth habit was detected in two *Triticum monococcum* segregating populations.

The objective of the present paper is to map vernalization genes in *T. monococcum* and to establish their relationship with genes affecting flowering time in other cereals.

Materials and methods

Mapping populations

Two F_2 populations of *T. monococcum* were used for mapping chromosome $5A^m$. The first one included 76 F_2 plants from a cross between wild *T. monococcum* subsp. *aegilopoides* (Link.) Thell. accessions from Turkey (G1777, winter) and Iran (G2528, spring). Partial maps of chromosome $1A^m$ and the short arm of chromosome $5A^m$ from this population have been published (Dubcovsky and Dvorak 1995; Dubcovsky et al. 1995). The second mapping population included 74 F_2 individuals from a cross between a cultivated *T. monococcum* (DV92, spring) and a wild *T. monococcum* ssp. *aegilopoides* from Lebanon (G3116, winter). Thirteen new loci were added to the published map of chromosome $5A^m$ (Dubcovsky et al. 1996). Loci were assigned to chromosome arms using di-telocentric stocks of Chinese Spring (*T. aestivum*) (Sears and Sears 1979).

Mapping technique

Nuclear DNAs were isolated from leaves of single F_2 plants or 10–20 pooled F_3 plants following the procedure of Dvorak et al. (1988). Southern hybridization was performed as described earlier (Dubcovsky et al. 1994). Maps were constructed with the computer program Mapmaker/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992) using the Kosambi function (Kosambi 1943). Multipoint analysis was used on individual linkage groups, using a LOD threshold of 2.0. Preferred orders were checked by the "RIPPLE" command with a window size of 5 and a LOD threshold of 2. Markers ordered at a LOD < 2.0 were placed in the preferred locations and are indicated by parentheses on the maps. The significance of the differences between recombination fractions in the same intervals in different maps was determined by the Z-test. Variances of the recombination-fraction estimates were calculated according to Allard (1956).

To facilitate map comparisons among genera, RFLP markers from chromosome regions associated with flowering time were selected from genetic maps of different cereals. RFLP markers from rice (Causse et al. 1994; Li et al. 1995, Saghai Maroof et al. 1996), oat (O'Donoughue et al. 1995; Van Deynze et al. 1995 b; Siripoonwiwat et al. 1996), barley (Laurie et al. 1995), rye (Plaschke et al. 1993), and *T. aestivum* (Galiba et al. 1995; Nelson et al. 1995) were included in the *T. monococcum* maps. Raw data for the new loci included in previously published *T. monococcum* maps are available at the GrainGenes database (http://probe.nalusda.gov:8300/cgi-bin/browse/graingenes).

Vernalization requirement

Flowering time was studied in the parents of the *T. monococcum* mapping populations (G1777, G2528, G3116, and DV92) and in *T. aestivum* varieties Sonora 64 (spring) and Klein Rendidor (winter). The two bread-wheat varieties, used as spring and winter controls, have a minimum photoperiodic response. Plants from the six lines were randomly assigned to four different treatments resulting from the factorial combination of two vernalization and two photoperiodic treatments. One-half of the plants were vernalized for 6 weeks in a chamber at 10°C (8-h day length), the other half were germinated 5 weeks later and grown in a chamber at 25°C. When vernalized and unvernalized plants were at the same developmental stage, they were transferred to a greenhouse and randomly assigned to two photoperiodic treatments. Half of the plants within each vernalization treatment were grown under continuous light and the other half under short 8-h day length conditions. Four-to-ten plants of each genotype (depending on survival) were analyzed for heading-date for each treatment combination.

Progeny tests for vernalization requirement were performed in a greenhouse at 20°C using 10–15 F_3 plants per F_2 plant. The number of F_3 plants tested was increased to 20 for those F_2 plants showing recombination between the RFLP markers flanking the vernalization gene. In this way, the possibility of finding no F_3 individual with the recessive genotype from a heterozygous F_2 plant just by chance was reduced to less than 0.001.

Results and discussion

Mapping of *Vrn-A^{m}1* (cross G1777 × G2528)

Vernalized G1777 and G2528 plants grown under long-day conditions showed identical ear-emergence times. Large differences in heading-date were observed between vernalized plants grown under short- and long-day conditions indicating that genes controlling photoperiod response are present in both parents. Unvernalized plants of G1777, grown under long-day conditions, failed to flower even 2 months after the heading-date of G2528.

The F_2 population grown under long-day conditions showed 60 spring and 16 winter plants, a ratio conforming to the expected 3:1 ratio for a single-gene model (χ^2, $P = 0.56$). This ratio confirmed the F_1 results, indicating that this vernalization gene is dominant for the spring habit. Thirty four out of the 60 F_3 families derived from spring F_2 plants, showed segregation for vernalization requirement and were recorded as H (heterozygous) for mapping purposes. Nineteen families were recorded as B (homozygous spring), and seven were recorded as C (either homozygous spring or heterozygous) because of insufficient F_3 individuals for the progeny tests.

A gene controlling vernalization response in this population, henceforth designated *Vrn-A^{m}1*, was mapped on the long arm of chromosome 5A^m approximately 60 cM from the centromere and 30 cM proximal to the 5A^{m}L/4A^{m}L-translocation break point (Fig. 1). The location of *Vrn-A^{m}1* within the *Xabg702–Xwg908* interval (Fig. 1) is supported by a high LOD score (Fig. 1, LOD = 8.92). *Vrn-A^{m}1* and the completely linked *Xwg644* locus are one-crossover distal to *Xcdo465* (Fig. 1, LOD > 3.0). The progeny test for F_3 family 55 showing the crossover between *Xcdo465* and *Vrn-A^{m}1* was repeated two times. In each case RFLP analysis of the F_3 plants confirmed the presence of the crossover. The low LOD score (LOD = 1.83) of locus *Xpsr426* in the G1777 × G2528 populations was due to the impossibility to score the G2528 restriction fragment.

Mapping of *Vrn-A^{m}2* (Cross DV92 × G3116)

Vernalized plants of G3116 (winter type) grown under long-day conditions flowered 30 days earlier than plants of DV92 (spring type) grown under the same conditions. Both lines showed a strong response to photoperiod. Unvernalized plants of G3116 grown under long-day conditions failed to flower even 2 months after the heading-date of unvernalized DV92.

The F_2 population grown in the greenhouse under long-day conditions yielded 55 winter and 19 spring plants, a ratio conforming to the expected 3:1 ratio in a single-gene model (χ^2, $P = 0.56$). The observed segregation ratio indicated that this vernalization gene, henceforth designated *Vrn-A^{m}2*, is dominant for the winter habit under the conditions of this study.

The *Vrn-A^{m}2* locus was mapped on the long arm of chromosome 5A^m, approximately 125 cM from the centromere and 25 cM distal to the 5A^{m}L/4A^{m}L- translocation break point. Based on F_2 data alone, *Vrn-A^{m}2* was completely linked to RFLP markers *Xwg199* and *Xbcd402.* Progeny tests of 12 winter F_2 plants showing crossovers between *Xwg199* and *Xβ-Amy-1* provided additional information on the location of *Vrn-A^{m}2.* These F_3 data indicated that *Vrn-A^{m}2* is completely linked to *Xbcd402* and two crossovers distal to the completely linked markers *Xwg199* and *Xmwg616* (LOD 4.3, Fig. 1).

Comparative mapping with other *Triticeae* species

Comparative mapping of chromosome 5A^m of *T. monococcum* and homoeologous group-5 chromosomes from other *Triticeae* genomes needs to take into account the presence of chromosome structural differences (Devos et al. 1995; Nelson et al. 1995; Dubcovsky et al. 1996). Of these, relevant to this paper is a reciprocal translocation involving the distal 40–50 cM of chromosome arms 4AL and 5AL. This translocation is present in the A genome of diploid, tetraploid and hexaploid wheats (Devos et al. 1995; Dubcovsky et al. 1996). Because of this translocation, *Vrn-A^{m}1* and *Vrn-A^{m}2* are located on the same chromosome arm but belong to different homoeologous groups. *Vrn-A^{m}1* is proximal to the 5A^{m}L/4A^{m}L translocation break point in the region that is homoeologous to group-5 chromosomes, whereas *Vrn-A^{m}2* is distal to this translocation break point in a region that is homoeologous to group-4 chromosomes in other wheat and *Triticeae* genomes.

Comparative mapping in the *Vrn-1* region

Vrn-A^{m}1 was mapped in the cross between G1777 and G2528. The proportion of polymorphic loci detected between these two wild *T. monococcum* lines is lower than the proportion of polymorphic loci detected between cultivated DV92 and wild G3116 in the other mapping population. Because of the lower level of polymorphism it was not always possible to include all the important RFLP markers required for comparative mapping of *Vrn-A^{m}1* in the G1777 × G2528 cross. Collinearity between the maps of both crosses helped to extrapolate linkage information from one *T. monococcum* map to the other. Collinearity of the long arms of chromosome 5A^{m}L from the G1777 × G2528 and DV92 × G3116 mapping populations is based on the presence of 14 collinear markers. Collinearity within

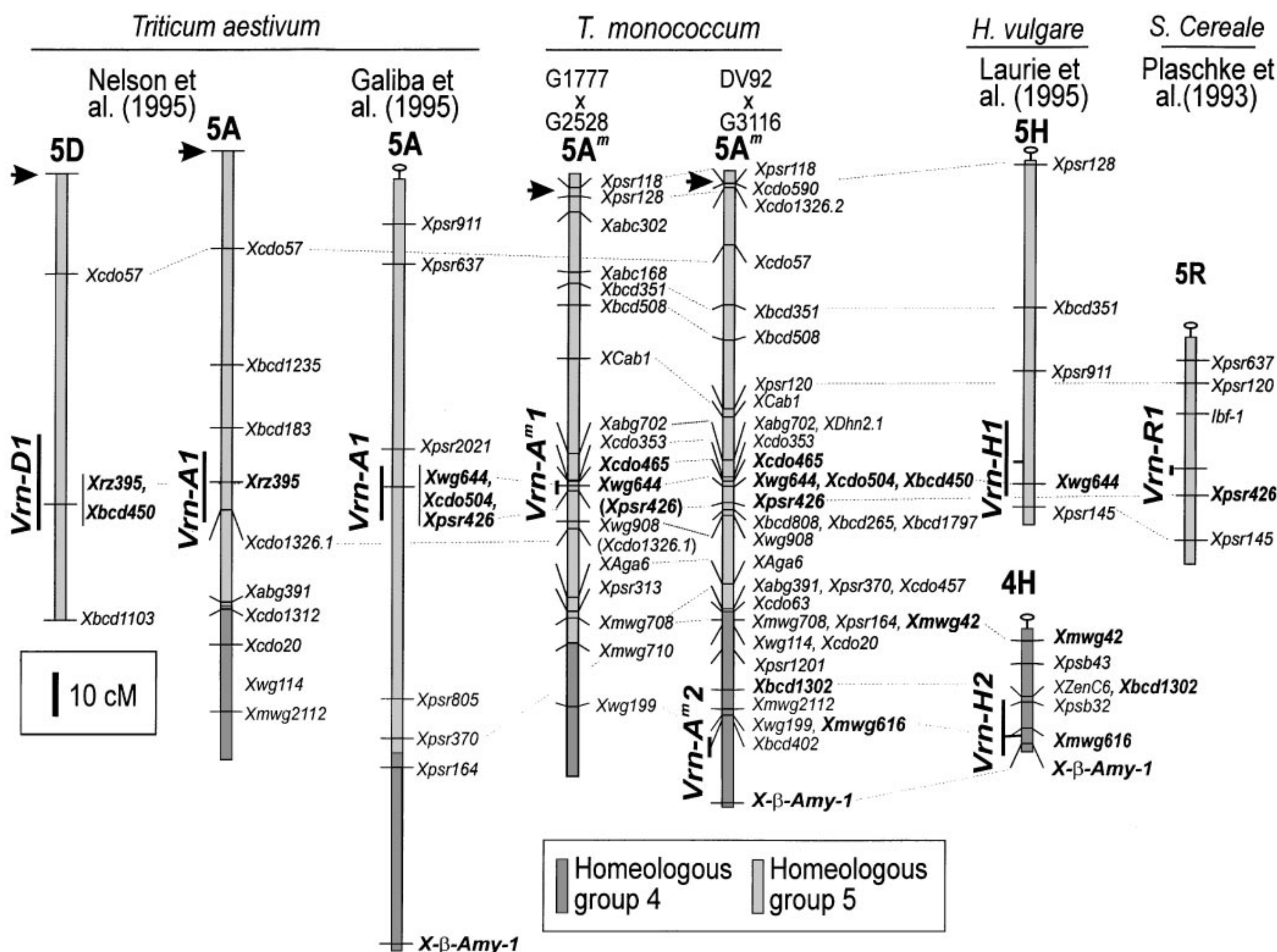

Fig. 1 Comparative RFLP maps of *T. monococcum* chromosome arms 5A^{m}L and homoeologous chromosome regions in other *Triticeae* species. Distances between markers in all chromosomes are proportional to the scale on the left side of the figure. *Solid arrows* indicate the position of the centromere and ⚲ indicates the direction of the centromere for chromosome segments not including the centromere. Markers ordered at LOD < 2.0 are indicated by *parentheses*. Putative orthologous loci are connected with *dotted lines*. Critical loci used to establish the orthology of vernalization genes are indicated in *bold face*. Some markers present on the published maps have been omitted for the sake of clarity. Complete linkage between *XZenC6* and *Xbcd1302* in barley is based on a personal communication from D. A. Laurie, and on our own unpublished mapping results in a population of recombinant substitution lines of chromosomes 4D and 4B

the *Vrn-A^{m}1* region is further indicated by similar genetic distances between flanking markers *Xabg702* and *Xwg908* (14.4 cM and 12.4 cM respectively), and by the presence of RFLP markers *Xcdo353*, *Xcdo465*, *Xwg644* and *Xpsr426* within this interval in both maps (Fig. 1). Therefore, it will be assumed in the following discussion that loci located between *Xabg702* and *Xwg908* on the map based on the DV92 × G3116 cross will be also closely linked to *Vrn-A^{m}1* on the map based on the G1777 × G2528 cross.

Vernalization genes have been located on the same map regions as *Vrn-A^{m}1* in other *Triticum* genomes. In hexaploid wheat, the *Vrn-A1* locus is closely linked to *Xwg644*, *Xcdo504*, *Xpsr426* (Galiba et al. 1995; Korzun et al. 1997) and *Xcdo1326* (Nelson et al. 1995) on the long arm of the map of chromosome 5A (Fig. 1). These four markers are also closely linked to *Vrn-A^{m}1* on both *T. monococcum* 5A^{m}L maps. RFLP marker *Xbcd450* is closely linked to the *Vrn-D1* locus on the map of the long arm of chromosome 5D and is also included in the *Xabg702–Xwg908* interval on the map based on the DV92 × G3116 cross (Fig. 1). Moreover, in the latter cross *Xbcd450* is completely linked to *Xwg644*, which in turn is completely linked to *Vrn-A^{m}1* on the map based on the G1777 × G2528 cross (Fig. 1). The simplest explanation is that *Vrn-A^{m}1*, *Vrn-A1*, and *Vrn-D1* are derived from a common ancestral gene (orthologous genes).

Collinearity between *Vrn-1* and RFLP loci from homoeologous group 5 can be extended to other genera of

the tribe Triticeae. In rye, a gene for vernalization requirement, designated *Vrn-R1* (originally *Sp1*), was mapped 6 cM proximal to the *Xpsr426* locus (Plaschke et al. 1993). *Vrn-A^{m}1* was also proximal to *Xpsr426* on the map based on the G1777 × G2528 cross (Fig. 1). In barley, a more distant relative of wheat, RFLP markers *Xbcd265* and *XDhn2.1* were found to flank a QTL for heading-date and a QTL for winter survival probably related to the presence of a vernalization gene (Pan et al. 1994). The same markers flanked *Xwg644* and *Vrn-A^{m}1* in *T. monococcum* (Fig. 1). A recent study using 69 double-haploid barley lines mapped the vernalization gene *Vrn-H1* (originally *Sh2*) approximately 5 cM proximal to *Xwg644* (Laurie et al. 1995). This genetic distance is not significantly different (*Z*-test, $P = 0.06$) from the absence of recombination observed between *Xwg644* and *Vrn-A^{m}1* in *T. monococcum* (Fig. 1). The 5-cM distance between *Vrn-H1* and *Xwg644* should be considered as an approximation because of the large gap proximal to *Xwg644* on the map of chromosome arm 5HL (22.6 cM) and the segregation of other genes affecting flowering time in the same mapping population (Laurie et al. 1995).

Van Deynze et al. (1995b) suggested that the QTL for heading-date on oat *L*inkage *G*roup (LG) 24 corresponds to vernalization genes *Vrn-A1* and *Vrn-D1* on the maps of wheat chromosomes 5 A and 5D respectively. Holland et al. (1997) confirmed the presence of a QTL on LG 24 associated with a vernalization response and showed that the correlation between this response and the RFLP markers peaked at *Xbcd1968*, decreasing for markers located on both sides of this locus (*Xcdo1523*, *Xcdo484*, *Xbcd1797*). No significant effect was detected on oat LG 24 in the region proximal to *Xbcd1797*, including *Xbcd808* and *Xcdo353* (Holland et al. 1997). The last two RFLP markers are closely linked to *Vrn-A^{m}1* in *T. monococcum* (Fig. 2), casting some doubt on the orthology between the oat vernalization gene and wheat *Vrn-1*.

The distal and proximal regions of wheat chromosome arm 5L are homoeologous to rice chromosomes 3 and 9, respectively (Van Deynze et al. 1995 a) (Fig. 2). The *Vrn-A^{m}1* locus on the map of *T. monococcum* 5A^{m}L was very close to the point where homoeology between wheat and rice chromosomes changes from rice chromosome 3 to rice chromosome 9. The location of *Vrn-A^{m}1* one-crossover distal to *Xcdo353* suggests that the wheat chromosome region encompassing *Vrn-A^{m}1* is homoeologous to a region on rice chromosome 3 and not to a region on rice chromosome 9. No QTL for heading-date has been reported for this region in the rice genome (Li et al. 1995; Lin et al. 1995). However, a QTL for heading-date was identified on the map of rice chromosome 9 (Li et al. 1995), in a region homoeologous to the centromeric region of the long arm of group-5 chromosomes in the *Triticeae* (Moore et al. 1995; Van Deynze et al. 1995 a) (Fig. 2). This region, located between RFLP markers *Xbcd926* and *Xcdo590*, is more than 60 cM proximal to *Vrn-A^{m}1* in *T. monococcum*, and overlaps with a QTL for earliness *per se* reported in the centromeric region of barley chromosome 5HL (Laurie et al. 1995).

Comparative mapping in the *Vrn-2* region

The *Vrn-A^{m}2* locus is the first vernalization-response gene mapped in wheat homoeologous group 4. In barley, *Vrn-H2* (originally *Sh*) was assigned to chromosome 4H more than 25 years ago based on linkage with morphological markers (Takahashi and Yasuda 1971). Linkage studies using isozyme markers showed that *Vrn-H2* is closely linked (4.3 cM) to *β-Amy-1* isozyme polymorphisms in chromosome 4H (Chojecki et al. 1989; Hackett et al. 1992). More recently, studies using RFLP markers established that a QTL for vernalization response is located between RFLP markers *Xmwg616* and *Xβ-Amy-1* (Laurie et al. 1995). Model fitting suggested that *Vrn-H2* was 1-cM distal to *Xmwg616* and 2.6-cM proximal to *Xβ-Amy-1* (Laurie et al. 1995).

In *T. monococcum Vrn-A^{m}2* mapped 1.4-cM distal to *Xmwg616* and 18.4-cM proximal to *Xβ-Amy-1*. It should be noted that the *Xβ-Amy-1–Vrn-H2* interval on the barley map (Hackett et al. 1992; Laurie et al. 1995) is 4–7 fold shorter than the same interval on the *T. monococcum* map (Fig. 1). This difference most likely reflects shorter terminal regions on the barley maps based on double-haploid mapping populations compared to the terminal regions on this *T. monococcum* map based on an F_2 mapping population (Dubcovsky et al. 1996). Close linkage of both *Vrn-H2* and *Vrn-A^{m}2* with *Xmwg616* and their proximal position relative to *Xβ-Amy-1* (Fig. 1) suggest that *Vrn-A^{m}2* and *Vrn-H2* are orthologous. Orthology between these loci is further supported by the fact that segregating populations with a dominant winter habit were observed for both loci.

The chromosome region encompassing *Vrn-A^{m}2* is homoeologous to a region of diploid oat chromosome F and hexaploid oat LG 5 (Van Deynze et al. 1995 b). A QTL for heading-date, not associated with vernalization response, was detected in LG 5 in hexaploid oat (Siripoonwiwat et al. 1996; Holland et al. 1997). Van Deynze et al. (1995 b) pointed out that this heading-date locus lies in the same chromosome region [common marker *Xcdo20* (Causse et al. 1994)] as a heading-date locus in rice homoeologous chromosome 3 (Li et al. 1995; Lin et al. 1995) (Fig. 2). They also suggested (see Van Deynze et al. 1995 b, Fig. 4) a correspondence of this rice QTL for heading-date and the *Vrn-H2* gene controlling the vernalization response in barley. Although this may be the case, the presence of a QTL for earliness *per se* proximal to *Vrn-H2* in barley chromosome arm 4HL (Laurie et al. 1995) offers an alternative

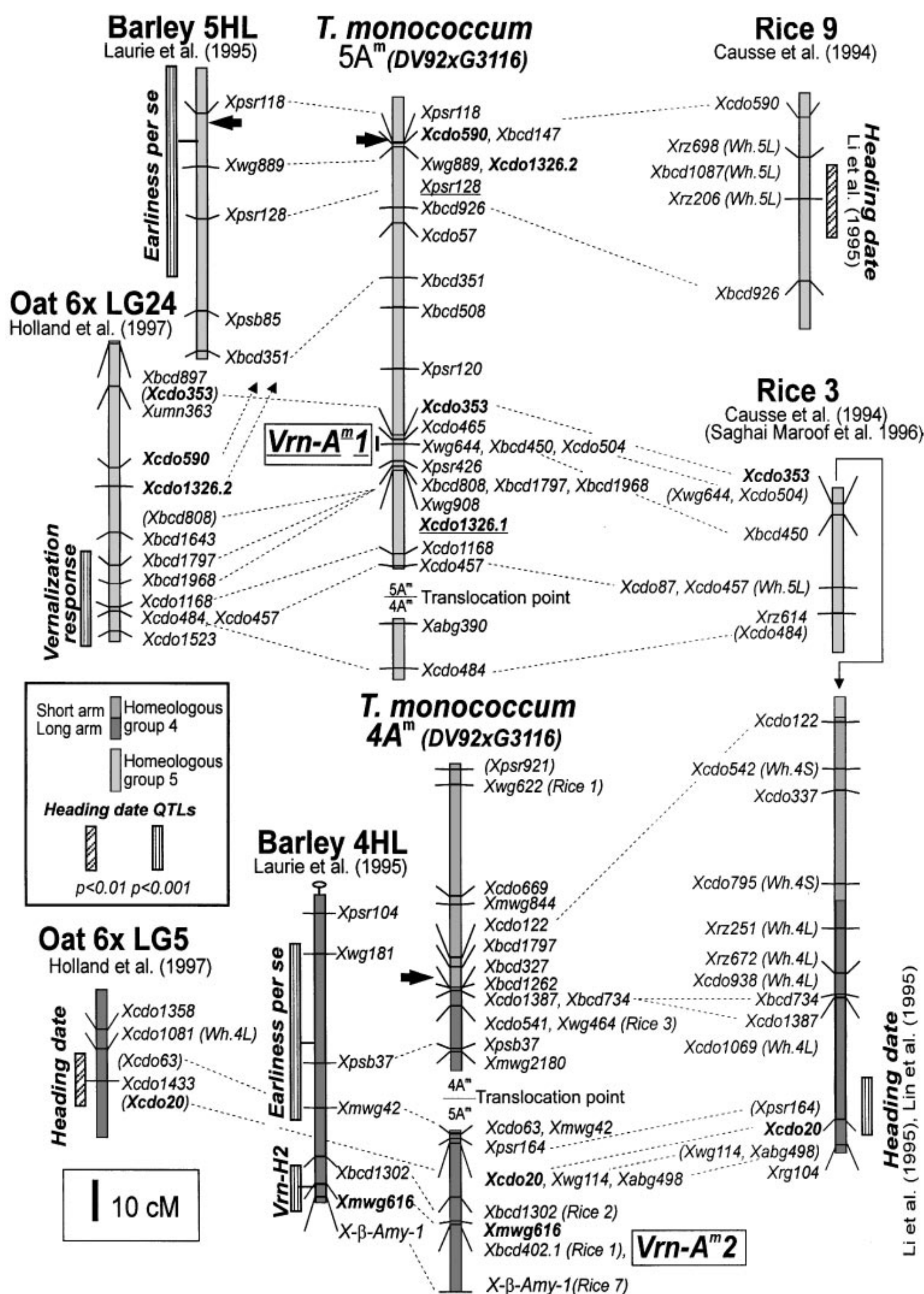

Fig. 2 Comparative RFLP maps of *T. monococcum* chromosomes $4A^m$ and $5A^m$ and homoeologous chromosome regions in *Hordeum vulgare* (2x), *Avena sativa* (6x) and *Oryza sativa* (2x). To facilitate the comparison, *T. monococcum* chromosomes have been reorganized as before the $4A^mL/5A^mL$ translocation event; and chromosome 3 of rice has been divided and the region homoeologous to wheat chromosome 5 has been inverted. Putative orthologous markers are connected with *dotted lines.* Information about the orthology of loci not present in the compared maps but available at the GrainGenes database are indicated in parentheses after the markers name (*Wh.* = wheat). The *T. monococcum* maps are based on the DV92 × G3116 cross, but three additional loci (*underlined*) from the cross G1777 × G2528 have been intercalated in the $5A^m$ map. Distances between markers in all chromosomes are proportional to the scale on the left side of the figure. *Solid arrows* indicate the position of the centromere and ⚲ indicates the direction of the centromere for chromosome segments not including the centromere. Markers with LOD < 2 are indicated by *parentheses.* The most likely location of barley QTLs are indicated by *horizontal lines* connecting the QTLs and the chromosome (Laurie et al. 1995). Some markers present on the published maps have been omitted for the sake of clarity

possibility (Fig. 2). QTLs for heading-date in oat LG 5 and rice chromosome 3 both peak at marker *Xcdo20*, which is more than 20 cM proximal to *Vrn-2* in *Triticeae* homoeologous group 4 (Fig. 2). This suggests that QTLs for heading-date in rice chromosome 3, and oat LG 5 may be orthologous to the QTL for earliness *per se* in barley chromosome 4H rather than to *Vrn-2* (Fig. 2). Additional markers flanking the different QTLs need to be mapped in these species to discriminate between these alternative hypotheses.

Dominance and epistatic interactions between *Vrn-A^m1* and *Vrn A^m2*

Dominance relationships between alleles for spring and winter habit at the *Vrn-A^m1* and *Vrn-A^m2* loci detected in these experiments are similar to those observed at the orthologous genes in barley (Takahashi and Yasuda 1971). In *T. aestivum* (6x) spring habit is generally dominant to winter habit. *Vrn-A1* and all other vernalization loci, except those recently reported from

group 6, promote early flowering or a reduced vernalization requirement. Absence of chromosomes of homoeologous group 5 increases the vernalization requirement whereas their increased dosage reduces this requirement (Law et al. 1994). Aneuploids of homoeologous group 6 show opposite effects to those observed for homoeologous group 5. The absence of these chromosomes accelerates flowering, whereas increased dosage delays flowering and increases the vernalization requirement (Law et al. 1994; Islam-Faridi et al. 1996). It was suggested that group-6 genes produce an inhibitor of flowering that is neutralized either by vernalization or by a suppressor produced by group-5 and -7 *Vrn* genes (Miura and Worland 1994; Islam-Faridi et al. 1996). This may also be true for the *T. monococcum* $Vrn\text{-}A^{m}2$ locus since the dominance of the winter habit suggests that the gene product of the $Vrn\text{-}A^{m}2$ gene is for 'lateness'.

An interesting question is why the *Vrn-2* locus has not been observed in hexaploid wheat. It is possible that epistatic interactions between *Vrn-1* and *Vrn-2* alleles are similar to those in barley and that a series of orthologous *Vrn-2* loci showing similar degrees of expressivity exists in the three *T. aestivum* genomes. Under those circumstances, *Vrn-2* will determine a spring habit in *T. aestivum* only if the three *Vrn-2* loci are simultaneously homozygous for the alleles determining spring habit. Lack of allelic variation at any of these *Vrn-2* loci would preclude its detection in polyploid wheat. Under these assumptions the detection of *Vrn-2* is an unlikely event, and may explain the absence of previous reports for *Vrn-2* in hexaploid wheat.

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