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Epistatic Interaction Between Vernalization Genes *Vrn-A^{m}1* and *Vrn-A^{m}2* in Diploid Wheat

G. Tranquilli and J. Dubcovsky

Genes *Vrn-A^{m}1* and *Vrn-A^{m}2* control the vernalization requirement in diploid wheat (*Triticum monococcum*). The epistatic interaction between these two genes on flowering date was studied here using a factorial analysis of variance. One hundred and two F_2 plants were classified according to their genotypes for molecular markers tightly linked to *Vrn-A^{m}1* and *Vrn-A^{m}2.* Mean comparisons showed that the *Vrn-A^{m}2* allele for winter growth habit was dominant to the *vrn-A^{m}2* allele for spring growth habit and that the *Vrn-A^{m}1* allele for spring growth habit was dominant to the *vrn-A^{m}1* allele for winter growth habit. A significant interaction was found between these two genes, suggesting that they work in the same developmental pathway. Plants homozygous for the recessive *vrn-A^{m}2* allele for spring growth habit flowered earlier than plants from the *Vrn-A^{m}2* class independently of the alleles present at *Vrn-A^{m}1.* However, differences in heading date between plants with the *Vrn-A^{m}1* allele and those with the *vrn-A^{m}1* allele were significant only when the dominant *Vrn-A^{m}2* allele was present. A genetic model for the action of these two vernalization genes is proposed in which the role of *Vrn-A^{m}1* is to counteract the *Vrn-A^{m}2*-mediated delay of flowering.

Wheat is produced worldwide in a large range of environments. This wide adaptability is partially based on the exploitation of genetic variation in sensitivity to daylength and the vernalization requirement to adjust flowering time. Of particular importance for adaptation to autumn sowings are the genes controlling the vernalization requirement that prevents flower development during winter, providing protection to the environmentally sensitive floral organs.

The response to vernalization in wheat is mainly controlled by the *Vrn-1* genes located on the long arms of chromosomes 5A, 5B, and 5D (McIntosh et al. 1998). Cultivars carrying the dominant allele *Vrn-1* at any of these three loci exhibit a reduced vernalization requirement and a spring growth habit; cultivars homozygous for the recessive alleles at all *Vrn-1* loci behave as winter genotypes.

Genes orthologous to *Vrn-1* have been reported in other cereals, including rye (*Vrn-R1*) (Plaschke et al. 1993), barley (*Vrn-H1*) (Laurie et al. 1995), and diploid wheat (*Triticum monococcum*) (*Vrn-A^{m}1*) (Dubcovsky et al. 1998). Recently, a new wheat vernalization gene designated *Vrn-A^{m}2* was mapped in the distal region of chromosome 5A^{m}L more than 50 cM distal to *Vrn-A^{m}1.* It was suggested that this gene is orthologous to *Vrn-H2* (originally *Sh*) from barley (Dubcovsky et al. 1998).

Analyses of the epistatic interactions between *Vrn-H1* and *Vrn-H2* in barley have shown that the *vrn-H2* allele for spring growth habit is epistatic to *vrn-H1,* and that the *Vrn-H1* allele for spring growth habit is epistatic to *Vrn-H2* (Takahashi and Yasuda 1971). Based on the proposed orthology between barley and *T. monococcum* vernalization genes, we hypothesized that similar interactions would be observed in wheat. To test this hypothesis the epistatic interaction between *Vrn-A^{m}1* and *Vrn-A^{m}2* was determined in an F_2 population of *T. monococcum* segregating for both loci. Based on the results of this experiment, a genetic model for the action of these two genes in the regulation of wheat heading time is proposed.

Materials and Methods

Two spring accessions of *T. monococcum, T. monococcum* ssp. *monococcum* DV92 and *T. monococcum* ssp. *aegilopoides* G2528, previously characterized as having different combinations of vernalization genes (Dubcovsky et al. 1998), were used in the present study. The allelic constitu-

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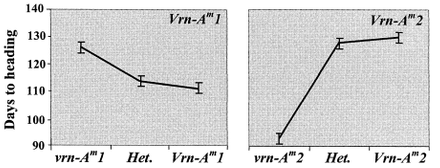

Figure 1. Main effects of *Vrn-A^{m}1* and *Vrn-A^{m}2* on flowering time. Bars represent one standard error of the mean. Genotypes at the *Vrn-A^{m}1* and *Vrn-A^{m}2* loci were inferred from RFLP loci *Xwg644* (completely linked to *Vrn-A^{m}1*) and *Xbcd402* (completely linked to *Vrn-A^{m}2*).

Table 1. Factorial (2 × 2) ANOVA for flowering time

Source of variation	Degrees of freedom	Mean squares	*F*	*P*
Vrn-A^{m}1	1	1831	8.6	.004
Vrn-A^{m}2	1	19,624	92.1	.000
*Vrn-A^{m}1*Vrn-A^{m}2*	1	877	4.1	.045
Error	98	213		

Genotypes at the *Vrn-A^{m}1* and *Vrn-A^{m}2* loci were inferred from RFLP loci *Xwg644* (completely linked to *Vrn-A^{m}1*) and *Xbcd402* (completely linked to *Vrn-A^{m}2*).

tion for *T. monococcum* ssp. *monococcum* DV92 is *vrn-A^{m}1/vrn-A^{m}2* and for *T. monococcum* ssp. *aegilopoides* G2528, *Vrn-A^{m}1/Vrn-A^{m}2* (Dubcovsky et al. 1998).

These two accessions were crossed using accession G2528 as the female parent. Three F_1 plants were self-pollinated and 102 F_2 plants were planted on July 31, 1997, in a greenhouse at 24°C. The number of days between planting and complete emergence of the first spike was scored for each F_2 plant. Genotypes at RFLP loci *Xwg644* (completely linked to *Vrn-A^{m}1*) and *Xbcd402* (completely linked to *Vrn-A^{m}2*) (Dubcovsky et al. 1998) were used to infer the *Vrn* genotype of each F_2 plant. Based on the complete linkage of the *Vrn* genes with these RFLP markers in a population of 74 F_2 plants (Dubcovsky et al. 1998) it is possible to calculate a maximum genetic distance of 2 cM for a 95% confidence interval (Hanson 1959).

The requirement for vernalization in diploid wheat is not absolute, and even plants with winter growth habit generally flower within 6 months. Therefore a quantitative analysis was necessary to show the interaction between these two genes. The data was first evaluated in a 3 × 3 factorial experiment considering each locus as a factor with three levels: homozygous DV92, heterozygous, and homozygous G2528. Tukey comparisons were performed among least-squares means. The nonsignificantly different classes were merged for a second statistical analysis using a 2 × 2 factorial analysis. All statistical analyses were performed using SAS (SAS Institute 1994).

Results

Dominance Relationships

The 3 × 3 factorial analysis of variance (ANOVA) showed significant differences in flowering time among genotypes for both *Vrn-A^{m}1* ($P = .0026$) and *Vrn-A^{m}2* ($P = .0001$). Comparisons of least-squares means for the *Vrn-A^{m}1* locus, showed no significant differences ($P = .83$) between the heterozygous class and the class homozygous for the G2528 *Vrn-A^{m}1* allele (Figure 1). Plants from these two classes were significantly earlier than plants from the class homozygous for the DV92 *vrn-A^{m}1* allele ($P < .02$; Figure 1). These results indicated that the *Vrn-A^{m}1* allele was dominant for reduced vernalization requirement and spring growth habit.

For the *Vrn-A^{m}2* locus (Figure 1), no significant differences ($P = .87$) were found between the heterozygous class and the homozygous G2528 *Vrn-A^{m}2* allele. Plants from these two classes flowered significantly later than plants homozygous for the DV92 *vrn-A^{m}2* allele ($P < .0001$; Figure 1). These results indicated that the allele for winter growth habit *Vrn-A^{m}2* was dominant for increased vernalization requirement and winter growth habit (Figure 1).

Epistatic Interaction

Interaction was not significant in the 3 × 3 factorial ANOVA ($P > .05$). However, this interaction was an average of lineal and quadratic interaction components. To increase the power to detect the lineal by lineal interaction, heterozygous and dominant homozygous classes were merged. The merged classes did not show significant differences for heading date in the 3 × 3 factorial ANOVA.

The 2 × 2 factorial ANOVA showed a significant interaction between *Vrn-A^{m}1* and *Vrn-A^{m}2* (Table 1; $P < .05$). To determine the origin of this interaction, the effect of each locus was analyzed within each class of the other locus by four one-way ANOVAs (Figure 2).

The heading date of the plants carrying different allelic variants for *Vrn-A^{m}1* was dependent on the *Vrn-A^{m}2* allele present. When plants carrying *Vrn-A^{m}1* were compared with plants carrying *vrn-A^{m}1* within the recessive *vrn-A^{m}2* class, no significant differences were detected ($P = .52$; Figure 2A). When the same comparison was made within the dominant *Vrn-A^{m}2* class differences in heading date were highly significant ($P < .0001$; Figure 2B). Within the *Vrn-A^{m}2* class, plants homozygous for the recessive *vrn-A^{m}1* flowered 20 days later than the other plants (Figure 2B).

Differences in flowering date between the allelic classes at the *Vrn-A^{m}2* locus were significant, regardless of the *Vrn-A^{m}1* class analyzed ($P < .0001$; Figure 2 C,D). Within the *Vrn-A^{m}1* class, plants with the *Vrn-A^{m}2* allele flowered 31 days later than plants with the *vrn-A^{m}2* allele (Figure 2D). Within the *vrn-A^{m}1* class the delay in flowering time for plants with the *Vrn-A^{m}2* allele relative to the plants with the *vrn-A^{m}2* allele was even longer (47 days) due to the presence of true winter types (*vrn-A^{m}1/Vrn-A^{m}2*; Figure 2C).

Discussion

Results from this experiment are a good example of the power of comparative genetics. Information generated almost 30 years ago for barley (Takahashi and Yasuda 1971) was used here to predict the

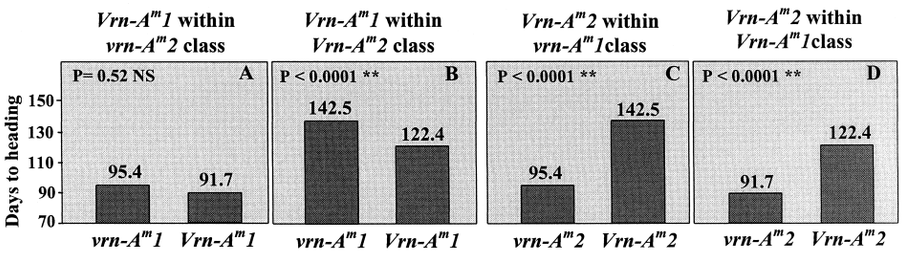

Figure 2. Effect of *Vrn-A^{m}1* and *Vrn-A^{m}2* on flowering time when analyzed within each class of the other locus. Heterozygous and homozygous dominant classes were merged. *P* indicates the probability of significant differences in flowering time between the allelic classes determined by one-way ANOVAs.

epistatic interaction between wheat vernalization genes. The experimental data confirmed the original hypothesis and demonstrated that wheat and barley vernalization genes are similar not only in their map location but also in their intralocus and interloci relationships. Wheat *Vrn-A^{m}1* and barley *Vrn-H1* are both dominant for spring growth habit, and *Vrn-A^{m}2* and *Vrn-H2* are both dominant for winter growth habit. In both species the alleles for spring growth habit are epistatic to the alleles for winter growth habit at the other locus.

If these epistatic interactions were similar in polyploid wheat, they could provide an explanation for the absence of reports on *Vrn-2* in hexaploid bread wheats. Under this hypothesis, the epistatic effects of the *vrn-2* alleles to the *vrn-1* alleles would be observed only when recessive alleles are present simultaneously at *vrn-A2, vrn-B2,* and *vrn-D2* in a plant that also has recessive alleles at all *Vrn-1* loci. If the frequencies of recessive *vrn-2* alleles at the different genomes were low, the simultaneous presence of recessive *vrn-2* alleles at the three homologous loci would be an infrequent event.

If dominance is assumed to be an indication of an active allele, then *Vrn-A2* can be considered to actively increase the vernalization requirement, while *Vrn-A1* can be considered to actively reduce the vernalization requirement. Halloran (1967) tested the latter hypothesis in hexaploid wheat using nullisomic and tetrasomic lines for chromosomes of homologous group 5. When the dosage of one of the homoalleles was decreased in nullisomic plants, the vernalization requirement was increased. Conversely, when dosage of one of these genes was increased in tetrasomic plants, the vernalization requirement was reduced and the unvernalized plants flowered early. Therefore it was concluded that the *Vrn-1* gene actively promotes early flowering or reduces the vernalization requirement.

In *T. monococcum,* the epistatic effect of the recessive allele *vrn-A^{m}2* for spring growth habit on *Vrn-A^{m}1* suggests that though *Vrn-A^{m}1* and *Vrn-A^{m}2* genes are both involved in the regulation of floral initiation, they act at different points of the regulatory pathway. Based on the results of this experiment the following model is suggested. The active *Vrn-A^{m}2* allele is associated with a mechanism of repression of the constitutive floral initiation pathway in diploid wheat. In turn, this inhibitor gene is inhibited or suppressed by vernalization treatment or by the action of the dominant *Vrn-A^{m}1* allele. The postulated action of the *Vrn-A^{m}1* gene product on *Vrn-A^{m}2* explains the absence of the effect of the *Vrn-A^{m}1* locus when the recessive *vrn-A^{m}2* allele was present.

This model parallels the MCDK flowering model for *Arabidopsis* proposed by Martinez-Zapater et al. (Weigel 1995). The MCDK model postulates the existence of a constitutive central flowering pathway regulated by genes that produce inhibitors, which can either suppress the activity of the genes within the pathway or inactivate their products. In turn, these inhibitor genes can be subjected to other inhibitor or suppressor genes and/or to environmental controls such as daylength or temperature (Law et al. 1994).

The detailed analyses available in *Arabidopsis* have shown a complex circuitry of epistatic interactions in the regulation of flowering time (Koornneef et al. 1998; Lee and Amasino 1995; Michaels and Amasino 1999). A similar complexity is expected in the control of heading date in wheat, including interactions between the environment, different vernalization genes, daylength-sensitive genes, and earliness per se genes (Laurie 1997). The model proposed here for the *Vrn-A^{m}1* and *Vrn-A^{m}2* genes is just one piece of the complex puzzle of interactions involved in the regulation of flowering time in wheat.

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