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Vrn-H1 and *Vrn-H2* allelic diversity in barley may explain specific adaptation to the Mediterranean environments

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Introduction

Some barley genotypes may require prior exposure to low temperature before floral initiation proceeds under inductive photoperiod. Takahashi and Yasuda (1970) reported that three pairs of genes *Sh*, Sh_2 and Sh_3 (on chromosomes 4HL, 5HL and 1HL, respectively), were responsible for the spring/winter growth class of barley. Their position was verified by Laurie *et al*. (1995) in a spring x winter cross. The genes *Sh*, Sh_2 and Sh_3 are all for the spring habit and only a single genotype ($Sh\_sh_2sh_2sh_3\ sh_3$), is capable of exhibiting winter-type growth. A multiple allelic series at the Sh_2 locus conditions variation in the requirement for vernalization (Takahashi and Yasuda, 1970).

The use of molecular techniques led to the identification and positional cloning of vernalization genes in several cereals: *Vrn-Am1* (Yan *et al.,* 2003) and *Vrn-Am2* (Yan *et al.,* 2004a) in diploid wheat; *VRN-1* (*TaVRT1*, Danyluk *et al.,* 2003; *WAP1*, Trevaskis *et al.,* 2003) in hexaploid wheat and *VRN-H1* and *VRN-H2* in barley (von Zitzewitz *et al*., 2005). *VRN1* encodes an *AP1*-like MADS box transcription factor, and *VRN2* was mapped to a chromosome region containing ZCCT zinc finger transcription factor genes. In barley, *VRN-H1* corresponds to the gene *HvBM5A* (Yan *et al*., 2005) and *VRN-H2* to the candidate *ZCCT-H* genes (Dubcovsky *et al*., 2005; Karsai *et al*., 2005).

Mutations in the promoter or first intron of the *VRN1* gene sequence are associated with spring alleles of *VRN1* in wheat and barley (Yan *et al.,* 2004b, Fu *et al*., 2005, von Zitzewitz *et al*., 2005). Loss of function mutations in *ZCCT1* are associated with the early flowering *VRN2* spring habit phenotype in wheat (Yan *et al*., 2004a) and barley (Dubcovsky *et al*., 2005; von Zitzewitz *et al.,* 2005).

Yan *et al.* (2004a) proposed a model to explain the *VRN1*/*VRN2* interaction in diploid wheat, in which *VRN-Am2* acts as a repressor of *VRN-Am1*, controlled by vernalization. This was consistent with genetic data that revealed an epistatic interaction between *VRN1* and *VRN2* in diploid wheat (Tranquilli and Dubcovsky, 2000). Recently, Trevaskis *et al.* (2006) examined the regulation of *VRN-H1* and *VRN-H2* and found evidence suggesting that *VRN1-H1* is regulated primarily by vernalization and developmental cues, whereas day-length would be the major determinant of the *ZCCT* loci in *VRN-H2*.

In this study, allelic diversity for those genes was evaluated in a wide sample of genotypes, winter and spring, 2 and 6-row barleys, mainly from the Western Mediterranean region. It included 159 landraces from the Spanish Barley Core Collection (SBCC, Igartua *et al*., 1998) and a set of 80 accessions (reference cultivars) mainly from other European countries. Specific alleles for *VRN-H1* were found in the Iberian Peninsula, in a higher frequency than that seen in other materials. Allelic diversity matches with flowering time phenotypic responses across several series of field trials and controlled conditions experiments. The phenotypic differences apparently may entail adaptive responses. These results led us to initiate a marker-assisted selection program to introduce the vernalization genes of a Spanish accession into the French winter cultivar Plaisant.

Results and discussion

Germplasm screening for VRN-H1 and VRN-H2

PCR amplifications were carried out with specific primers to discriminate different forms of the *VRN-H1* intron 1 and the *VRN-H2* loci, as reported in a previous work (von Zitzewitz *et al*.,2005). Different size products were found in the first intron of *VRN-H1* (Fig. 1), ranging from 5250 to 150 bp, or even a complete deletion in some spring cultivars. A total of 9 different alleles were detected in this set of genotypes.

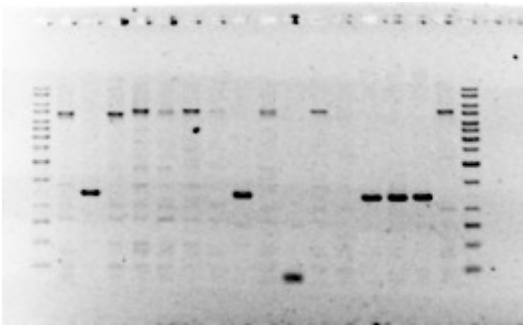


Fig. 1. Amplificacion of different alleles of the *HvBM5A* intron 1 using FideliTaq (USB), and primer set HvBM5.55F/56R. From left to right: Ager, Albacete, Almunia, Barberousse, Dobla, Hatif de Grignon, Monlon, Pané, Alpha, Beka, Igri, Triumph, three SBCC accessions and Plaisant. Marker: 1 kb ladder (10kb-0.25 kb).

As reported, only two alleles were found for *VRN-H2*: presence or absence of the *HvZCCT* loci (von Zitzewitz *et al*.,2005). Combining the observed variation at *VRN-H1* and *VRN-H2*, a total of 17 haplotypes were found. These haplotypes are unevenly distributed across the germplasm examined (Table 1).

Table 1. Survey of barley cultivars and SBCC landraces according to *VRN-H1* and *VRN-H2*

Hap.	VRN-H1 HvBM5	VRN-H2 HvZCCT	Reference cultivars	SBCC landraces	Phenotype
I	5250	1400	11	0	winter
II	5150	1400	16	0	winter
III	5250	1400	2	0	winter
IV	4850	1400	5	93	winter
V	1200	1400	5	47	intermediate
VI	1200	1400	2	0	intermediate
VII	150	1400	4	2	spring
VIII	n	1400	2	5	spring
IX	5250	null	2	0	facultative
X	5200	null	1	0	spring
XI	5150	null	2	0	facultative
XII	4850	null	1	1	facultative
XIII	1200	null	1	4	spring
XIV	1200	null	1	0	spring
XV	150	null	10	5	spring
XVI	n	null	11	2	spring
XVII	n	null	4	0	spring

Haplotypes I to III correspond to typical winter varieties, characterized by a large intron in *VRN-H1* and presence of the repressor *VRN-H2*. They were found in cultivars from Germany, UK or France. Haplotype IV is present in a few winter varieties such as Ager from France, Almunia from Spain, or Athene from Germany. However, this haplotype was found in a majority of landraces from the SBCC that, according to this information, are supposed to be winter types. Haplotype V is specific from the Mediterranean region. It is found in landrace-derived cultivars from Spain (Albacete or Pané), Greece (Athenais) or Syria (S-36), and in 47 SBCC landraces. Haplotype VI (Orria) was previously classified as a winter cultivar but it actually behaves in the field as a spring one (Igartua *et al.,* 1999). Haplotypes VII (Cameo, Gaelic or S-45) and VIII (Hassan) were found in a few varieties that were previously classified as both either winter and or spring by Igartua *et al.* (1999). Haplotypes IX, XI and XII correspond to facultative types, i.e. presence of a large intron in *VRN-H1* and absence of *VRN-H2*, as reported for the cultivar Dicktoo (Karsai *et al*., 2005; von Zitzewitz *et al.,* 2005). Haplotypes XV and XVI, both containing different deletions in the first intron of *VRN-H1*, are typical of spring varieties such as Morex or Triumph, respectively.

Phenotypic characterization

Evaluation of the SBCC under field conditions allowed the comparison of haplotypes under normal (autumn sowing), and late (spring sowing) conditions. In the autumn-sown trials, both winter and spring types flowered in a narrow range of time (13 days). A much larger range of variation between haplotypes was seen in the late-sown trial (37 days). Plants having haplotypes with the larger introns were the latest (186 days); haplotype IV (4850 bp intron) was intermediate (170 days) whereas plants carrying haplotype V flowered as early as the spring types (157 days), (data not shown).

A subset of accessions was evaluated under controlled conditions, with long photoperiod (16 h daylight), varying the duration of the vernalization treatment (0, 15, 30 or 45 days), as reported (Karsai *et al*., 2005). Although based on a limited sample of genotypes, the results of this experiment define a gradation in their vernalization requirement (Fig. 2). In general, large introns in *VRN-H1* are associated with winter growth habit and different size deletions related to medium vernalization requirement or no vernalization response, in spring types (Table 1; Figs 1 and 2).

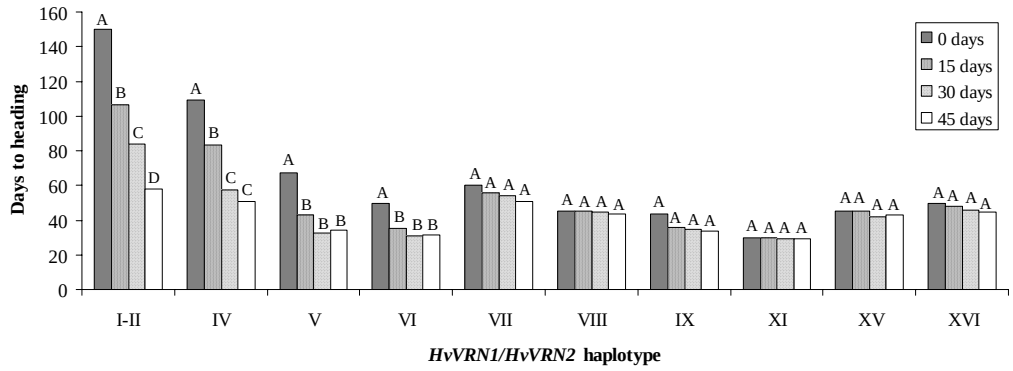
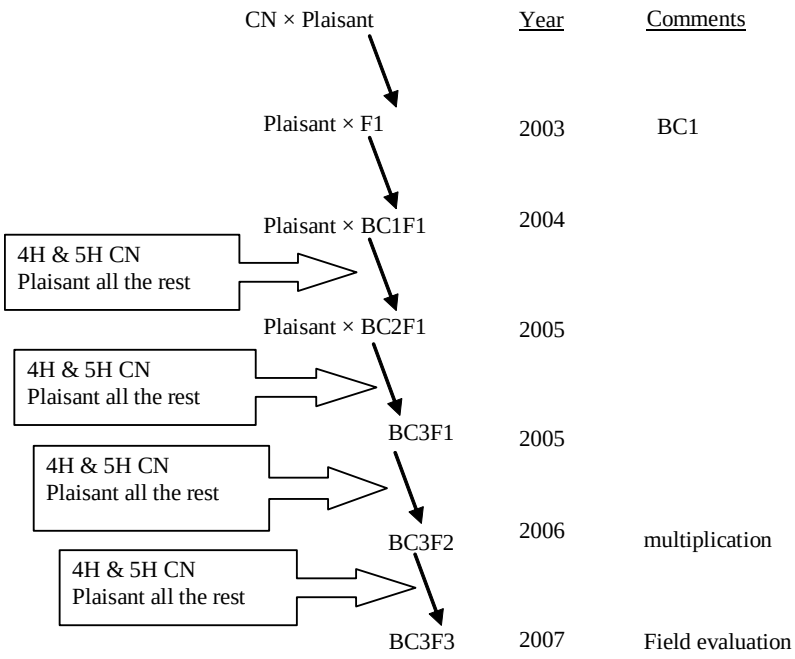


Fig. 2. Average days to heading of different *VRN-H1*/*VRN-H2* haplotypes grown under controlled conditions, after 0, 15, 30 or 45 days of vernalization. Tested genotypes are: Kompolti korai (I), Plaisant (II), 3 SBCC landraces (IV), 3 SBCC landraces (V), Cierzo & Orria (VI), Gaelic (VII), Hassan (VIII), Hatif de Grignon & Dicktoo (IX), Monlon (XI), Kym (XV) and Alexis (XVI). Letters indicate means separation within each haplotype.

Marker-assisted backcrossing

In parallel to this study, in order to use the observed natural variation in practical breeding, we started a marker-assisted backcrossing program to reduce the vernalization requirement of an

improved winter cultivar (Plaisant, haplotype II) by introgressing the vernalization genes from a local Spanish accession (CNE58, haplotype V).



We used a three-stage selection strategy as proposed by Frisch *et al*. (1999). This involved using flanking markers for the target genes in chromosomes 4H (*VRN-H2*) and 5H (*VRN-H1*) and background selection trying to recover the genotype of the recurrent parent with another 36 markers, first in the same chromosomes and then in the rest of the genome. Future evaluation under field conditions will reveal the result of this process.

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References

Danyluk, J., Kane, N.A., Breton, G., Limin, A.E., Fowler, D.B. and Sarhan, F. (2003). *Plant Physiol.,* 132: 1849-1860.

Dubcovsky, J., Chen, C. and Yan, L. (2005). *Mol. Breed.,* 15: 395-407.

Frisch, M., Bohn, M. and Melchinger, A.E. (1999). *Crop Sci.,* 39: 1295-1301.

Fu, D., Szücs, P., Yan, L., Helguera, M., Skinner, J.S., von Zitzewitz, J., Hayes, P.M. and Dubcovsky, J. (2005). *Mol. Gen. Genomics,* 273: 54-65.

Igartua, E., Gracia, M.P., Lasa, J.M., Medina, B., Molina-Cano, J.L., Montoya, J.L. and Romagosa, I. (1998). *Genet. Resour. Crop Ev.,* 45: 475-481.

Igartua, E., Casas, A.M., Ciudad, F., Montoya, J.L. and Romagosa, I. (1999). *Heredity,* 83: 551-559.

Karsai I., Szücks, P., Mészáros, K., Filichkina, T., Hayes, P.M., Skinner, J.S., Láng, L. and Bedő, Z. (2005). *Theor. Appl. Genet.,* 110: 1458-1466.

Laurie, D.A., Pratchett, N., Bezant, J.H. and Snape, J.W. (1995). *Genome,* 38: 575-585.

Takahashi, R. and Yasuda, S. (1970). *Barley Genetics II*, Nilan, R.A. (ed.). Washington State University Press, Washington, pp. 388-408.

Tranquilli, G. and Dubcovsky, J. (2000). *J. Hered.,* 91: 304-306.

Trevaskis, B., Bagnall, D.J., Ellis, M.H., Peacock, W.J. and Dennis, E.S. (2003). *Proc. Natl Acad. Sci.*, USA 100: 13099-13104.

Trevaskis, B., Hemming, M.N., Peacock, W.J. and Dennis, E.S. (2006). *Plant Physiol.*, 140: 1397-1405.

von Zitzewitz,J., Szücs, P., Dubcovsky, J., Yan, L., Francia, E., Pecchioni, N., Casas, A., Chen,

T.H.H., Hayes, P.M. and Skinner, J.S. (2005). *Plant Mol. Biol,* 59: 447-465.
Yan, L., Loukoianov, A., Tranquilli, G., Helguera, M., Fahima, T. and Dubcovsky, J. (2003). *Proc. Natl Acad. Sci.,* USA 100: 6263-6268.
Yan, L., Loukoianov, A., Blechl, A., Tranquilli, G., Ramakrishna, W., San Miguel, P., Bennetzen, J.L., Echenique, V. and Dubcovsky, J. (2004a). *Science,* 303: 1640-1644.
Yan, L., Helguera, M., Kato, K., Fukuyama, S., Sherman, J. and Dubcovsky, J. (2004b). *Theor. Appl. Genet.,* 109: 1677-1686.
Yan, L., von Zitzewitz, J., Skinner, J.S., Hayes, P.M. and Dubcovsky, J. (2005). *Genome,* 48: 905-912.