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Using genetics to advance breeding: The winter barley example

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SUMMARY – The winter hardiness of cereal crops is associated with low temperature tolerance, vernalization (the requirement of exposure to an extended period of low temperature), and photoperiod sensitivity. Understanding the genetics of these traits, using agronomically relevant germplasm, will provide new opportunities for sustainable and productive barley production. Using a new generation of germplasm and massively parallel data sets we are anticipating new insights into the ancestral and derived conditions of barley.

The winter hardiness of cereal crops is associated with the requirement of exposure to an extended period of low temperature (vernalization) in order to achieve a timely transition from vegetative to reproductive growth. Understanding the genetics of vernalization is a prerequisite to improving winterhardiness and improved winterhardiness will allow for more sustainable and productive cereal crop production in the face of global warming.

The classification of growth habit in barley (*Hordeum vulgare* subsp. *vulgare*) is based on vernalization requirement and can be described as winter, facultative, or spring. Vernalization requirement in barley is not absolute – an unvernalized winter genotype will eventually flower under favorable growth conditions. However, the flowering is so delayed as to be agronomically unacceptable (Karsai *et al*., 2001). Technically speaking, winter barley genotypes do not "require" vernalization − since they will eventually flower. It may be more appropriate to say they are very "responsive", or "sensitive" to vernalization in that the number of days elapsed from planting to flowering will be substantially reduced if plants receive a vernalization treatment.

The genetic basis of vernalization response/sensitivity in barley was first described, based on phenotypic data, in terms of a three-locus epistatic model (Takahashi and Yasuda 1971). Using current nomenclature and chromosome designations, the loci are *VRN-H1* (5H), *VRN-H2* (4H) and *VRN-H3* (1H). Winter genotypes have the allelic architecture *Vrn-H2_/vrn-H1vrn-H1/vrn-H3vrn-H3*. All other allelic configurations lead to a lack of significant vernalization response, e.g. spring and facultative growth habit. There is no allelic variation at *VRN-H3* in most cultivated genotypes, reducing the genetic model to two factors (Yasuda *et al*., 1993).

HvBM5A, a MADS-box floral meristem identity gene, is considered to be the determinant of *VRN-H1* (Danyluk *et al*., 2003; Trevaskis *et al*., 2003; von Zitzewitz *et al*., 2005; Yan *et al*., 2003). In wheat and barley, allelic variation at *VRN-1* is ascribed to mutations in the promoter and/or first intron (Yan *et al*., 2003; Yan *et al*., 2004; a von Zitzewitz *et al*., 2005). The key regulatory region in the intron has been narrowed down to a highly conserved 0.44-kb segment "critical region" (Fu *et al*., 2005; von Zitzewitz *et al*., 2005).

A zinc finger CCT domain transcription factor (*ZCCT*), which is a flowering repressor down-regulated by vernalization, is the candidate for wheat and barley *VRN-2* (Yan *et al*., 2004b). Allelic variation at this locus is ascribed to loss-of-function mutations or complete deletion leading to recessive spring growth habit (Dubcovsky *et al*., 2005; Karsai *et al*., 2005; von Zitzewitz *et al*., 2005; Yan *et al*., 2004b). There are three tightly linked *ZCCT* genes in barley (*ZCCT-Ha*, *ZCCT-Hb* and *ZCCT-Hc*) and two in diploid wheat (*ZCCT-1* and *ZCCT-2*) (Dubcovsky *et al*., 2005; Karsai *et al*., 2005; Yan *et al*., 2004b). In wheat and barley, *ZCCT-1* and *ZCCT-Ha* (respectively) were reported to be are the most likely candidates for *VRN-2* (Dubcovsky *et al*., 2005; Yan *et al*., 2004b). However, Trevaskis *et al*. (2006) and Yan *et al*. (2004b) have shown that during vernalization under long-day conditions, the expression of *ZCCT-Hb* and the two wheat *ZCCT* genes are down-regulated.

There is evidence that the photoperiod and vernalization sensitivity pathways that culminate in flowering are connected. High levels of barley and wheat *ZCCT* expression were detected only when photoperiod-responsive plants were grown under long-day conditions (Dubcovsky *et al*., 2006; Trevaskis *et al*., 2006). These results provide a molecular basis for explaining quantitative trait locus (QTL) associations of *VRN-H2* with photoperiod sensitivity (Karsai *et al*., 2005; Karsai *et al*., 2006; Laurie *et al*., 1995; Szűcs *et al*., 2006). In photoperiod-responsive wheat and barley plants grown under short-day conditions (at either cold or room temperature) *ZCCT* expression was repressed, but there was no increase in *VRN-1* expression. This suggests the presence of at least one other *VRN-1* repressor (Dubcovsky *et al*., 2006; Trevaskis *et al*., 2006). A candidate gene is *VRT-2*, a putative flowering repressor MADS-box gene regulated by both vernalization and day length; this gene shows higher expression under short-day (*vs* long-day) in a photoperiod-sensitive barley (Kane *et al*., 2005). *HvVRT-2* is on the short arm of chromosome 7H and is associated with photoperiod sensitivity QTL (Szűcs *et al*., 2006).

The two-locus epistatic model for *VRN* gene interaction is supported by extensive phenotypic data in wheat and barley populations segregating for growth habit (Dubcovsky *et al*., 2005; Dubcovsky *et al*., 2006; Fu *et al.,* 2005; Karsai *et al*., 2005; Kóti *et al*., 2006; Laurie *et al*., 1995; Takahashi and Yasuda 1971; Tranquilli and Dubcovsky 2000; Yan *et al.,* 2003; Yan *et al.,* 2004a; Yan *et al.,* 2004b). A molecular model explaining the *VRN-2/VRN-1* epistatic interaction in winter cereals was proposed by Yan *et al*. (2003; 2004a; 2004b). According to this model, *VRN-2* encodes a dominant repressor which inhibits the expression of *VRN-1*. Vernalization down-regulates *Vrn-2* expression, allowing expression of *vrn-1* alleles in winter habit genotypes. No or little vernalization sensitivity is observed in genotypes with recessive *vrn-2* alleles (loss-of-function mutations or complete deletions of *ZCCT*), regardless of the allelic state at *VRN-1*. Likewise, genotypes with a dominant *Vrn-2* allele but with a dominant *Vrn-1* allele (lacking a repressor binding site in the promoter and/or in the intron 1) will show minimal vernalization sensitivity.

We have genetically validated the *VRN-H2*/*VRN-H1* epistatic model under long-day conditions, using allele sequence and flowering time phenotypic data from segregating barley populations. The uniqueness of this approach is that the three parental accessions are all spring habit but based on allele sequencing we determined that each genotype has a winter type allele at either the *VRN-H1* or the *VRN-H2* locus. We predicted that we would observe vernalization sensitivity (delayed flowering in the absence of vernalization) in a subset of segregating progeny of specific crosses. Dicktoo has a winter (*vrn-H1*) *HvBM5A* allele but has a deletion of all three *ZCCT-H* genes and is therefore homozygous *vrn-H2*. This particular allele configuration defines the facultative growth habit *(sensu* von Zitzewitz *et al*., 2005). The Oregon Wolfe Barley Dominant genetic stock has a spring (*Vrn-H1*) *HvBM5A* allele due to a large deletion of the intron 1. All three *ZCCT-H* genes are present in this accession, making it *Vrn-H2*. (Fu *et al*., 2005). In an extensive screen of barley germplasm with *VRN-H1*- and *VRN-H2*-specific primers, we found that the *ZCCT-H* genes and the 436 bp vernalization critical region of *HvBM5A* intron 1 are present in the spring habit accession Calicuchima-sib (I. Karsai, personal communication). We therefore made three crosses: Dicktoo × Oregon Wolfe Barley Dominant; Dicktoo × Calicuchima-sib, and Calicuchima-sib × Oregon Wolfe Barley Dominant. We hypothesized that we would observe vernalization sensitivity in the progeny of Dicktoo (*vrn-H2vrn-H2*/*vrn-H1vrn-H1*) × Oregon Wolfe Barley Dominant (*Vrn-H2Vrn-H2*/*Vrn-H1Vrn-H1*) cross. The crosses with Calicuchima-sib were designed to determine if this accession is an exception to the two-locus model or if it has novel "spring" alleles at *VRN-H1* and/or *VRN-H2*.

The results of this research validate the two-locus epistatic model for vernalization and provide a predictive tool for germplasm screening and selection. We have discovered winter alleles in exotic and adapted spring barley varieties of interest for their human nutritional properties. Targeted crosses among such lines, followed by marker assisted selection in their progeny, should expedite the development of winter habit barley varieties with unique quality profiles.

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