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Genetic variation at flowering time loci in wild and cultivated barley

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Abstract

The worldwide spread of barley cultivation required adaptation to agricultural environments far distant from those found in its centre of domestication. An important component of this adaptation is the timing of flowering, achieved predominantly in response to day length and temperature. Here, we use a collection of cultivars, landraces and wild barley accessions to investigate the origins and distribution of allelic diversity at four major flowering time loci, mutations at which have been under selection during the spread of barley cultivation into Europe. Our findings suggest that while mutant alleles at the *PPD-H1* and *PPD-H2* photoperiod loci occurred pre-domestication, the mutant vernalization non-responsive alleles utilized in landraces and cultivars at the *VRN-H1* and *VRN-H2* loci occurred post-domestication. The transition from wild to cultivated barley is associated with a doubling in the number of observed multi-locus flowering-time haplotypes, suggesting that the resulting phenotypic variation has aided adaptation to cultivation in the diverse ecogeographic locations encountered. Despite the importance of early-flowering alleles during the domestication of barley in Europe, we show that novel *VRN* alleles associated with early flowering in wild barley have been lost in domesticates, highlighting the potential of wild germplasm as a source of novel allelic variation for agronomic traits.

Keywords: barley; flowering time; germplasm; photoperiod; vernalization

Introduction

Cultivated barley (*Hordeum vulgare* ssp. *vulgare*) was domesticated from its wild progenitor (*H. vulgare* ssp. *spontaneum*) ~12,000 years ago within the Fertile Crescent (Zohary and Hopf, 2000), a region which spans Israel, Jordan and parts of Turkey. Barley is now one of the world's most important grain crops, and is cultivated in temperate environments throughout the world. One of the major genetic adaptations to the novel agricultural environments encountered during the post-domestication spread of cultivation was the modulation of flowering time, which is largely controlled in response to day length (photoperiod) and low temperature (vernalization). Four major genes controlling

barley flowering time have been identified (Laurie *et al.*, 1995). Wild-type alleles at the vernalization response loci (*VRN-H1* and *VRN-H2*) delay flowering in the absence of vernalization, thereby maintaining vegetative growth in wild barley and autumn-sown domesticates during the winter months, and preventing damage to sensitive floral organs (Cockram *et al.*, 2007a). Selection for vernalization-non-sensitive alleles at these loci resulted in spring-sown domesticates which lack a vernalization response, allowing avoidance of the comparatively cold winters encountered in northern Europe. Of the two major photoperiod response loci, *PPD-H1* modulates flowering in response to long-day (LD) photoperiods, while *PPD-H2* controls flowering in response to short-days (SDs). Mutations at *PPD-H1* resulted in photoperiod-non-sensitive alleles that remove the promotion of flowering in response to LDs (Turner *et al.*, 2005), extending the growing period and allowing domesticates to take advantage of the long cool and wet summers of

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north Europe. Deletions within the *PPD-H2* candidate gene *HvFT3* are associated with a delay in flowering under SDs (Faure *et al.*, 2007), which helps to maintain the vegetative stage in autumn-sown types until the onset of spring, even after the vernalization requirement has been met. Here, we use recently developed genetic markers diagnostic for allelic state at these major flowering time loci to explore diversity in wild and domesticated barley.

Methods

Genomic DNA was extracted as described by Jones *et al.* (2008). The PCR-based marker assaying for allelic variation at *VRN-H1* is described by Cockram *et al.* (2009). *VRN-H2* genotyping was performed using the assay described by Karsai *et al.* (2005). The *HvFT3* candidate gene at the *PPD-H2* locus was genotyped as described by Faure *et al.* (2007). *PPD-H1* genotypic data was sourced from Jones *et al.* (2008). Sequencing was performed as described by Cockram *et al.* (2008) and contig formation was conducted using the VectorNTI package (Eppendorf). Geodata were plotted using ArcGIS v.10 (ESRI). For clarity, we use the vernalization locus nomenclature described by Dubcovsky *et al.* (1998).

Results

We sourced a collection of 315 barley germplasm accessions with associated collection site geodata (longitude and latitude) from national genebanks (Fig. 1). Of these, 120 represent wild barley accessions, sampled from throughout their natural range. The remaining 195 accessions are landraces (cultivated, locally adapted barleys that pre-date systematic plant breeding), from across north-western Europe. Overlaying biome data illustrates that the western spread of barley domestication into the Mediterranean basin was into environments broadly similar to those encountered in the centre of domestication (Fig. 1). However, the spread into north and north-western Europe resulted in cultivation in regions with very different seasonal conditions and biomes. Genotypic analysis of *VRN-H1* intron 1 InDel variation finds that, while 98% of wild barley accessions are predicted to possess winter alleles, three accessions (all of which are located within Israel) carry spring alleles (Fig. 2). Of these, the amplicons obtained from two accessions suggest they possess that same allele, found at low frequency within landrace accessions from the western Mediterranean. Sequencing the *VRN-H1* amplicon obtained in the third accession finds that it possesses an intron 1 deletion not previously identified in cultivated barley (Cockram *et al.*, 2007b,c) (Supplementary Fig. S1,

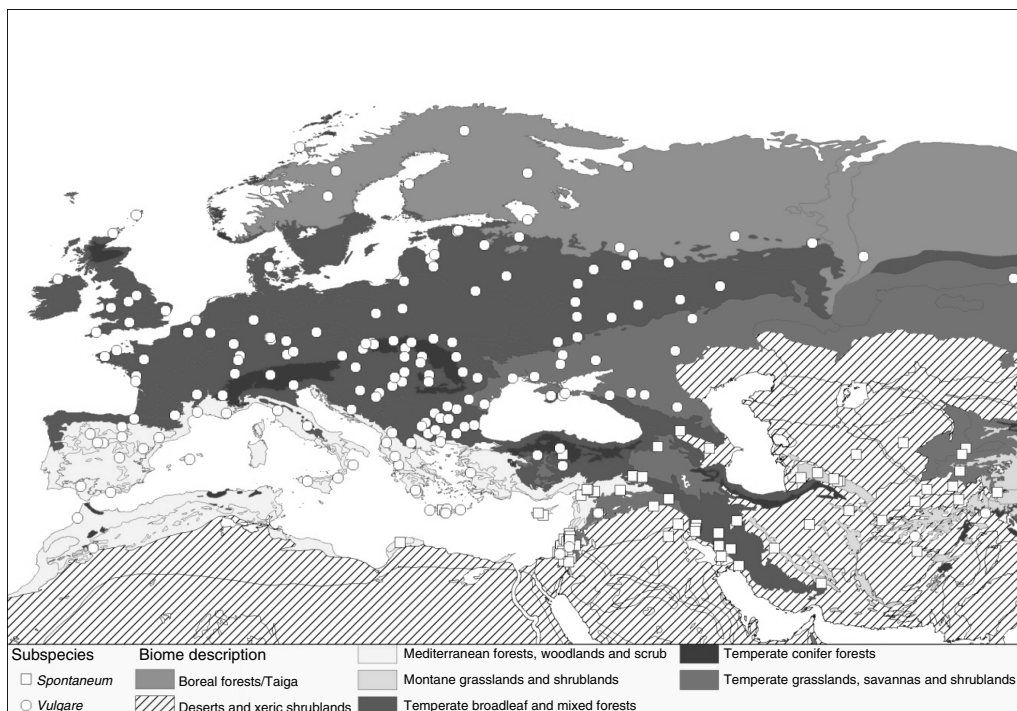


Fig. 1. Biome descriptions, overlaid with the geographic locations of germplasm collection sites for the landrace and wild barley accessions utilized in this study.

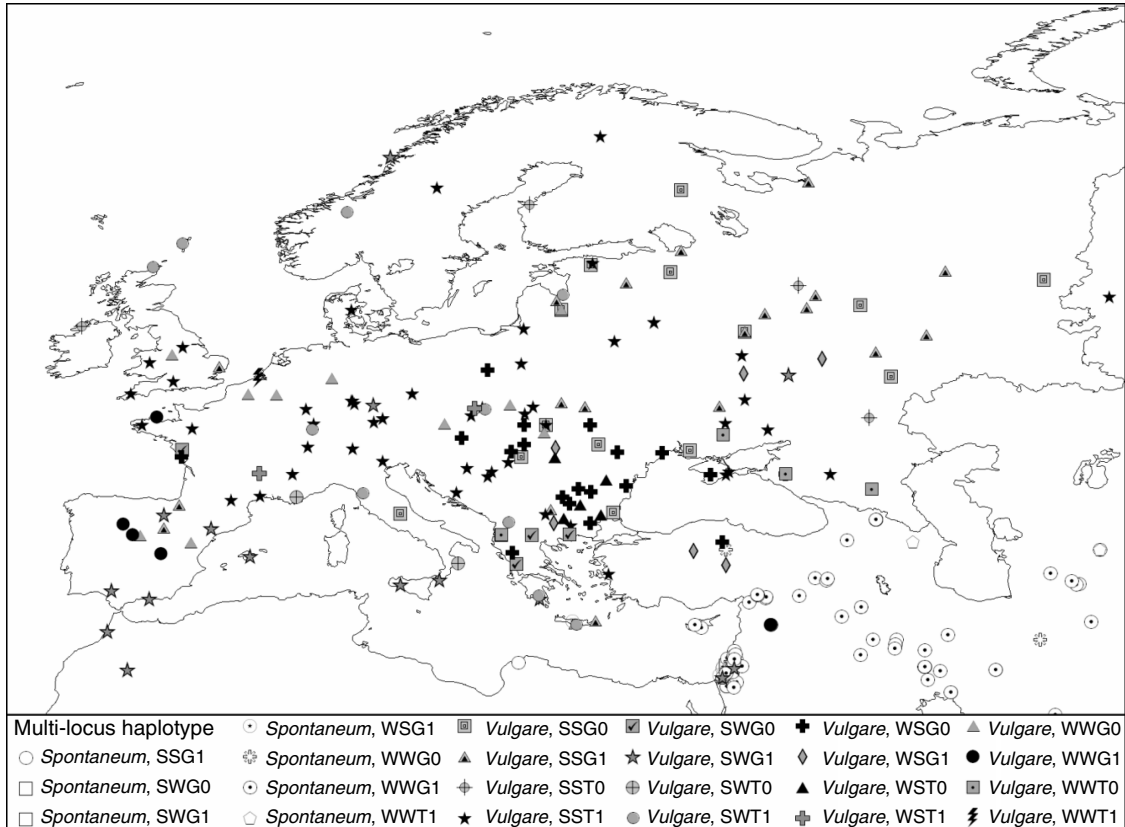


Fig. 2. Predicted allelic combinations at the major barley flowering time loci in landraces and wild barley. Allelic status predicted by *VRN-H1* and *VRN-H2* genotypes is indicated by S (spring) and W (winter). Allelic status at the diagnostic *PPD-H1* single-nucleotide polymorphism described by Turner *et al.* (2005): G, LD-photoperiod responsive (wild-type); T, LD-photoperiod non-responsive. *PPD-H2*: 1, *HvFT3* present (SD-photoperiod responsive, wild-type); 0, *HvFT3* absent (SD-photoperiod non-responsive).

available online only at <http://journals.cambridge.org>. PCR assays for the presence/absence of the three candidate genes at *VRN-H2* (*ZCCT-Ha*, *ZCCT-Hb* and *ZCCT-Hc*) shows that the deletion of all three genes associated with spring *vrn-H2* alleles in landrace and modern European cultivars (Cockram *et al.*, 2007b) is not found in wild barley. However, additional deletions are observed: individual deletions of *ZCCT-Ha* (ten accessions), *ZCCT-Hb* (one accession) and *ZCCT-Hc* (one accession), as well as a single example of a double deletion (*ZCCT-Ha* and *ZCCT-Hb*). Analysis of the *PPD-H2* candidate gene *HvFT3* shows that the mutated allele that delays flowering under SDs occurs at low frequency across the mid-to-western range of *H. vulgare* ssp. *spontaneum*. Overlaying genotypic data for allelic status at *PPD-H1* (sourced from Jones *et al.*, 2008) shows the presence of the mutant photoperiod non-responsive allele in wild barley from the east of its natural range. Simultaneous analysis of allelic status at all four flowering time loci finds an increase in haplotype number from 7 in wild barley, to 16 in landraces.

Conclusions

The modulation of flowering time during the post-domestication spread of barley cultivation allowed flexibility in the timing of sowing and harvesting, helping to maximise the yield. Our findings support the assumption that this flexibility was conferred both by the selection for mutations at major flowering time genes and the utilization of multiple allelic combinations at these loci. We find that the mutated photoperiod-non-responsive *ppd-H1* and photoperiod-responsive *ppd-H2* alleles originated pre-domestication. In contrast, the spring *Vrn-H1* and *vrn-H2* alleles found in modern European varieties (Cockram *et al.*, 2007b) are absent in wild barley. However, we identify rare instances of novel *VRN-H1* and *VRN-H2* deletions, predictive of spring alleles. Deletions within *VRN-H1* intron 1 result in the loss of vernalization requirement, and it is thought that the size and location of these deletions may have a quantitative effect on flowering time (Szűcs *et al.*, 2007). The identification of a novel deletion in ssp. *spontaneum* demonstrates that unexplored allelic

variation exists within wild germplasm. The presence of identical 6bp repeat motifs flanking this deletion suggests that it was formed by illegitimate recombination (IR) following double strand break (DSB) repair (Puchta, 2005), as previously observed in other *VRN1* deletions in barley and wheat (Cockram *et al.*, 2007c). As DSBs that occur anywhere within the intron have the potential to result in large deletions after IR, the creation of novel spring *Vrn-H1* alleles is predicted to be more frequent than the mutation of single DNA bases at a precise location within a gene. The predicted increased frequency of allele conversion supports our hypothesis that the spring *Vrn-H1* alleles observed in modern European cultivars arose post-domestication.

As was the case with *VRN-H1*, our data suggest the triple *ZCCT* deletion associated with spring *vrn-H2* alleles in all European germplasm surveyed to date (Cockram *et al.*, 2007b) occurred post-domestication. The prediction of single and double *ZCCT* deletions in wild barley indicates this genomic region may be prone to structural rearrangements. Genetic analysis of populations constructed from accessions differing for *ZCCT* copy number could help determine the relative function/phenotypic strength of each copy. Studies in human and animal genetics are increasingly finding copy number variation (CNV) to be an important component of functional genetic variation. Our findings suggest that mining germplasm for CNV could help resolve the contributions of duplicated genes in such cases. We hope to build on the preliminary analysis of barley germplasm presented here, with the aim of investigating the routes of cultivation into Europe, and the basis of genetic adaptation to local agricultural environments.

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