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FLOWERING NEWSLETTER REVIEW

Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity

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Abstract

The control of flowering is central to reproductive success in plants, and has a major impact on grain yield in crop species. The global importance of temperate cereal crops such as wheat and barley has meant emphasis has long been placed on understanding the genetics of flowering in order to enhance yield. Leads gained from the dissection of the molecular genetics of model species have combined with comparative genetic approaches, recently resulting in the isolation of the first flowering time genes in wheat and barley. This paper reviews the genetics and genes involved in cereal flowering pathways and the current understanding of how two of the principal genes, *Vrn* and *Ppd*, have been involved in domestication and adaptation to local environments, and the implications for future breeding programmes are discussed.

Key words: Barley, diversity, domestication, flowering, gene, wheat.

Introduction

Globally, cereals (including wheat, barley, and rice) are significant sources of food and animal feed, constituting over 50% of worldwide crop production (http://www.fao.org/). To maximize yield, it is essential to tailor the life cycle of cereals to the agro-environments in which they are grown. The transition from vegetative to reproductive growth is a critical developmental switch and a key adaptive trait in both crop and wild cereal species that ensures that plants set their flowers at an optimum time for pollination, seed development, and dispersal. Temperate environments with a long growing season allow cereal crops to flower late in the year and thus exploit an extended vegetative period for resource storage. Conversely, early flowering has evolved as an adaptation to short growing seasons. Knowingly, or unknowingly, farmers throughout history and, latterly, plant breeders have selected differences in flowering time to increase yield and extend the agricultural flexibility and eco-geographical range of crops. This is illustrated by our ability to cultivate modern wheat (*Triticum aestivum* L.) in environments far removed from the origins of cultivation in the Fertile Crescent.

Flowering time is a complex trait that shows almost continuous variation in cereals. Unravelling its molecular intricacies in species such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), with large, complex genomes and few genomic resources, has resulted in the comparative use of floral pathways from model plant species. In this context, data from *Arabidopsis thaliana* (L.) Heynh. (reviewed by Henderson and Dean, 2004; Bäurle and Dean, 2006) have been particularly useful. Likewise, recent research in rice (*Oryza sativa* L.) has extended our knowledge of flowering processes in a model grass species, which is of considerable relevance to other cultivated cereal species. However, different growth strategies compromise the relevance of rice as a model for the temperate cereals: rice is a short-day (SD) plant with no vernalization requirement, while wheat and barley (as well as *Arabidopsis*) are long-day (LD) plants which use vernalization as a control. Despite these differing responses to environmental signals, and the ancient divergence of the monocots and their magnoliid relatives from the eudicot lineage, examples of orthologous genes have been found to be involved in identical flowering

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pathways in *Arabidopsis*, rice, maize (*Zea mays* L.), wheat, and barley. However, either the exact position in the pathway or the way in which some of these respond to the same environmental signals has been modulated (Yano *et al*., 2001; Kojima *et al*., 2002; Hayama *et al*., 2003). The identification of orthologous genes with analogous function illustrates that the molecular dissection of flowering pathways in both rice and *Arabidopsis* assists the identification of genes within the corresponding pathway in temperate cereals and provides a conceptual framework within which to analyse variation in flowering time.

As increasing numbers of genes controlling key agronomic traits in cereals are identified and the development of molecular markers is facilitated by the availability of genome sequences, plant breeding will move into an increasingly technology-driven era where crops are fine-tuned by pyramiding of quantitative trait loci (QTLs) in conjunction with allele-specific markers. A clear example of this approach has recently been demonstrated in rice where combining loci for grain number and plant height provides the beneficial properties of both traits (Ashikari *et al*., 2006). With this comes the promise of future breeding efforts that will precisely tailor the adaptation of temperate cereals to existing environments, and will enhance performance in new conditions predicted by climate change.

This paper reviews the genetics and genes involved in the control of flowering in barley and wheat, and their roles in domestication, and will discuss how natural and induced genetic variation can be exploited to maintain continued varietal improvement for current and future agro-environments.

Genetic control of flowering time in wheat and barley

All plants undergo several developmental transitions during their life cycle and, like many of these, the transition from vegetative to reproductive phase is stimulated by environmental and developmental signals. Physiologically, most temperate cereals can be categorized according to their response to prolonged periods of cold (vernalization) and daylength (photoperiod). Autumn-sown varieties require vernalization to promote subsequent flowering, and commonly display a strong promotion of flowering in response to growth under LDs. Spring-sown varieties lack a vernalization requirement and can display a weak or strong response to LDs. The use of substitution lines and crosses between winter and spring types has identified a number of discrete loci that mediate the response to such environmental signals. Two important pathways within this network include the vernalization (*Vrn*) and photoperiod (*Ppd*) genes (Fig. 1; Table 1).

Three major loci controlling the vernalization response in both wheat and barley map to collinear locations in their respective genomes, suggesting that they represent orthologous genes (Laurie *et al*., 1995; Dubcovsky *et al*., 1998; Karsai *et al*., 2005; Yan *et al*., 2005, 2006). The most extensively studied group map to the long arms of the group 5 chromosomes in barley (*VRN-H1*), the diploid einkorn wheat *Triticum monococcum* L. ssp. *monococcum* (*VRN-A^{m}1*), wheat (*VRN-A1*, *VRN-B1*, *VRN-D1*), and rye (*Secale cereale* L.) (*VRN-R1*), with dominant or semi-dominant alleles in all species conferring vernalization-insensitive, spring-type lines (Law, 1966; Law *et al*., 1976; Snape *et al*., 1985; Plaschke *et al*., 1993; Laurie *et al*., 1995). A second series of *Vrn* genes have been mapped to collinear locations in barley (*VRN-H2*) and *T. monococcum* (*VRN-A^{m}2*), with recessive alleles conferring insensitivity to vernalization (Takahashi and Yasuda, 1971; Hacket *et al*., 1992; Dubcovsky *et al*., 1998). Analysis of dominance and interaction between the two major *Vrn* loci in barley and wheat shows that they display similar interactions, with spring alleles epistatic to winter alleles in both species. Barley also possesses a third locus, *VRN-H3*, originally mapped to chromosome 1H by linkage to morphological markers (Takahashi and Yasuda, 1971). Renewed investigation has found *VRN-H3* to map to chromosome 7HS, collinear to *VRN-B3* located on chromosome 7BS in *T. aestivum* (Law, 1966; Law and Wolfe, 1966; Law and Worland, 1997; Chao *et al*., 1989; Yan *et al*., 2006).

Similarly, major loci affecting the photoperiod response have been mapped to collinear positions on the short arm of the group 2 chromosomes in wheat (Welsh *et al*., 1973; Law *et al*., 1978; Scarth and Law, 1983) and barley (Laurie *et al*., 1995), although the effect of these loci differs between the species. In barley, dominant alleles at *Ppd-H1* confer early flowering under LDs, but have no effect under SDs. A homoeologous series of *Ppd* loci has been mapped to the short arms of group 2 chromosomes in wheat, and are ranked *Ppd-D1>Ppd-B1>Ppd-A1* in terms of their potency (Worland *et al*., 1998), although *Ppd-A1* remains poorly characterized. In wheat, dominant *Ppd* alleles greatly reduce sensitivity to photoperiod and confer an early flowering phenotype in SD and LD conditions, resulting in yield benefits under certain agro-environments (Worland *et al*., 1998). The contrast between the barley and wheat mutations can be interpreted as a loss of function in barley (failure to activate the photoperiod pathway correctly under LDs) and a gain of function in wheat (constitutive activation of the photoperiod pathway, irrespective of daylength). A second major photoperiod response locus, *Ppd-H2*, has been mapped to chromosome 1H in a winter× spring barley cross (Laurie *et al*., 1995), with the allele from the winter parent delaying flowering under SD. This locus provides a delay in flowering under unfavourable conditions, complementary to that conferred by the vernalization requirement. No equivalent loci have been identified in wheat, although flowering time effects are known for the group 1 chromosomes (Law *et al*., 1998).

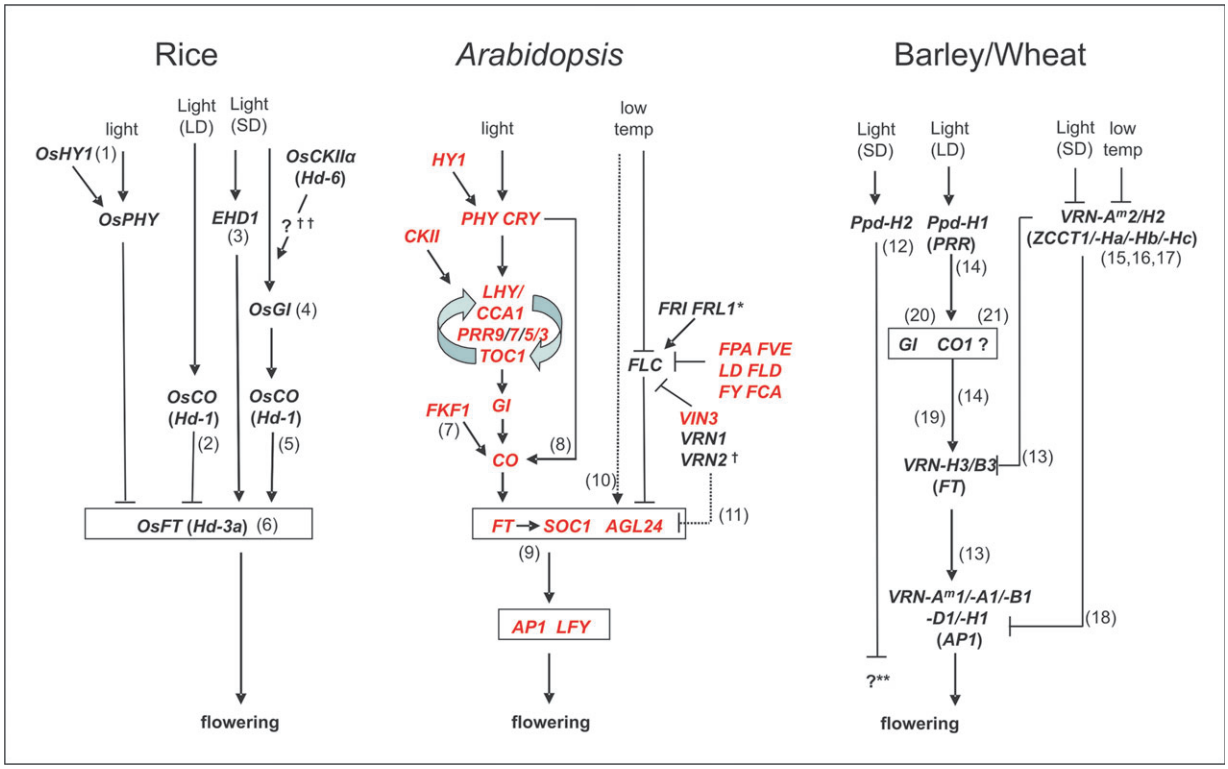

Fig. 1. Major flowering pathway genes of *Arabidopsis*, rice, and barley/wheat. Red denotes *Arabidopsis* genes for which putative wheat/barley orthologues have been identified in public sequence databases. (1) Izawa *et al*., 2000; (2) Hayama *et al*., 2003; (3) Doi *et al*., 2004; (4) Izawa *et al*., 2002; (5) Yano *et al*., 2000; (6) Kojima *et al*., 2002; (7) Nelson *et al*., 2000; (8) stabilization of CO protein, Valverde *et al*., 2004; (9) Yoo *et al*., 2005; (10) Yu *et al*., 2002; (11) Levy *et al*., 2002; (12) Laurie *et al*., 1995; (13) Yan *et al*., 2006; (14) Turner *et al*., 2005; (15) Yan *et al*., 2004*a*; (16) Dubcovsky *et al*., 2006; (17) Trevaskis *et al*., 2006; (18) Yan *et al*., 2003; (19) Christodoulou (2002), vernalization affects genes downstream of *HvGI* and *HvCO1* in barley; (20, 21) *GI* and *CO* orthologues in barley are described by Dunford *et al*. (2005) and Griffiths *et al*. (2003), respectively. In addition *Arabidopsis* photoperiod and autonomous pathway references can be found in recent reviews by Thomas (2006) and Henderson and Dean (2003), respectively. (*) The identification of *FRIL1*, which shows dispersed amino acid conservation with *FRI*, allows *FRI*/*FRI*-like genes to be tentatively identified in rice. (**) *Ppd-H2* affects flowering under SD but its interaction with downstream genes is unknown. (†) *VRN2* is a member of the VEFS-box group of genes that include *EMBRYONIC FLOWER2* (*EMF2*) and *FERTILIZATION INDEPENDENT SEED2* (*FIS2*). Cereal ESTs most closely resemble *EMF2* and no convincing equivalent of *VRN2* can be found. (††) In *Arabidopsis*, CKII acts to phosphorylate CCA1. The precise positioning within the rice photoperiod pathway has not been determined.

As illustrated above, a detailed knowledge of genetics permitted comparative approaches within temperate cereals to identify several series of homoeologous flowering time loci. At the same time, advances in the dissection of floral pathways in model species unravelled a framework with which the application of comparative genetics could help our understanding of wheat and barley flowering time loci.

Leads from model species

The observation that *Arabidopsis* has similar floral responses to vernalization and photoperiod suggests that genes identified within its flowering pathways could play orthologous roles in temperate cereals (Fig. 1). As in the case of winter cereal varieties, the vernalization response in *Arabidopsis* prevents development of the floral meristem during harmful temperatures, while allowing the plant to gain biomass during the winter months. The molecular genetics of the vernalization requirement in *Arabidopsis* and its interaction with other flowering pathways is well defined (recently reviewed by Sung and Amasino, 2005), with natural variation at two major genes, *FLOWERING LOCUS C* (*FLC*) and *FRIGIDA* (*FRI*), determining the classification of vernalization-responsive and non-responsive ecotypes. FRI promotes up-regulation of *FLC*, a MADS-box transcription factor that acts as a central repressor of flowering (Michaels and Amasino, 1999; Sheldon *et al*., 1999; Johanson *et al*., 2000). During vernalization, *FLC* is down-regulated in proportion to the duration of cold treatment, and remains low on subsequent transfer to warm temperatures, permitting subsequent competence to flower. Screening vernalization-responsive lines for mutants that remain late flowering after vernalization resulted in the identification of two genes, *VERNALIZATION 1* (*VRN1*) and *VERNALIZATION 2* (*VRN2*), required to maintain *FLC* repression (Gendall *et al*., 2001; Levy *et al*., 2002). However, despite the analogous vernalization-response phenotypes of *Arabidopsis* and temperate grasses, no clear cereal orthologues of these genes have been identified,

Table 1. *Major floral pathway loci and genes from* Arabidopsis*, barley,* T. monococcum, T. aestivum*, and rye*

References as main text. NB. Vernalization and photoperiod loci in wheat and barley have been inconsistently referred to in different publications. For example, some use *VRN-H1* and *Vrn-H1* to denote the locus and dominant allele, respectively; in others *Vrn-H1* has been used to denote the locus, with no reference to allelic state. Therefore, care should be taken to interpret nomenclature in the correct context. In this paper, to highlight collinear relationships and avoid confusion with similarly named genes in *Arabidopsis*, the vernalization locus nomenclature previously described by Dubcovsky *et al.* (1998) is used. Photoperiod locus nomenclature follows that used by Turner *et al.* (2005).

Locus (synonyms)	Gene name	Predicted protein	Pathway	Function
Arabidopsis				
AGL24	*AGL24*	MADS-box	Pathway integrator	Activate floral organ identity genes
AP1	*AP1*	MADS-box	Meristem identity, Floral organ identity	Activate floral organ identity genes Control floral development
CCA1	*CCA1*	Myb-related transcription factor	Photoperiod	Components of central oscillator
CO	*CO*	B-box, CCT-domain	Photoperiod	Output of central oscillator
CRY1-2	*CRY1-2*	FAD-binding domain	Light quality	Blue light perception
FCA	*FCA*	RNA-binding	Autonomous	*FLC* repression
FKF1	*FKF1*	Flavin-binding, kelch repeat	Photoperiod	Promote peak *CO* transcription
FLC	*FLC*	MADS-box	Vernalization	Central repressor of flowering
FLD	*FLD*	HDAC-associated protein	Autonomous	*FLC* repression
FPA	*FPA*	RNA-binding protein	Autonomous	*FLC* repression
FRI	*FRI*	Coiled-coil	Vernalization	Up-regulate *FLC*
FRL1	*FRL1*	Related to *FRI*	Vernalization	Up-regulate *FLC*
FT	*FT*	Putative kinase inhibitor	Pathway integrator	Activate floral organ identity genes
FVE	*FVE*	MSI4	Autonomous	*FLC* repression
FY	*FY*	Polyadenylation factor	Autonomous	*FLC* repression
GI	*GI*	Nuclear protein	Photoperiod	Output of central oscillator
HY1	*HY1*	Haemoxygenase	Photoperiod	Chromophore synthesis
LD	*LD*	Homeodomain protein	Autonomous	*FLC* repression
LFY	*LFY*	Plant-specific transcription factor	Pathway integrator	Activate floral organ identity genes
LHY	*LHY*	Myb-related transcription factor	Photoperiod	Components of central oscillator
PHYA-E	*PHYA-E*	Phytochrome	Light quality	Light sensors
PRR9/7/3/5	*PRR9/7/3/5*	CCT-domain	Photoperiod	Components of central oscillator
SOC1	*SOC1*[a]	MADS-box	Pathway integrator	Activate floral organ identity genes
TOC1 (*PRR1*)	*TOC1*	CCT-domain	Photoperiod	Initiate cold-mediated *FLC* repression
VIN3	*VIN3*	PHD, VID-domain	Vernalization	Cold-mediated *FLC* repression
VRN1	*VRN1*	B3-domain, DNA-binding	Vernalization	*FLC* repression post-vernalization
VRN2	*VRN2*	Su(z)12-like polycomb protein	Vernalization	*FLC* repression post-vernalization
Rice				
EHD1	*EHD1*	B-type response regulator	Photoperiod	Promote flowering under SDs
Hd1 (*Se1*)	*CO*	B-box, CCT-domain	Photoperiod	Promote flowering under SDs
Hd3a	*FT*	Putative kinase inhibitor	Photoperiod	Promote flowering under SDs
Hd6	*CKX2α*	Protein kinase	Photoperiod	Promote flowering under SDs
Se5	*HY1*	Haem oxygenase	Photoperiod	Chromophore synthesis
Barley				
VRN-H1 (*Sh2*, *Sgh2*)	*BM5A*[b]	MADS-box, AP1-like	Vernalization	Recessive alleles promote flowering after vernalization
VRN-H2 (*Sh*, *Sgh*)	*ZCCT-Ha/-Hb/-Hc*	B-box, CCT-domain	Vernalization/ photoperiod	Dominant alleles promote flowering after vernalization
VRN-H3 (*Sh3*, *Sgh3*)	*HvFT*	Putative kinase inhibitor	Vernalization/ photoperiod	Recessive alleles promote flowering after vernalization, and are up-regulated in LDs
Ppd-H1 (*Eam1*)	*PRR*	Pseudo-receiver and CCT-domain	Photoperiod	Light-sensitive allele promotes flowering under LDs
Ppd-H2	Not cloned	Not cloned	Photoperiod	Light-sensitive allele delays flowering under SDs

Table 1. (*Continued*)

Locus (synonyms)	Gene name	Predicted protein	Pathway	Function
T. monococcum				
VRN-A^{m}1 (*VRN-1*)	*VRN1*	MADS-box, AP1-like	Vernalization	Promote flowering after vernalization
VRN-A^{m}2 (*VRN-2*)	*ZCCT1*	B-box, CCT-domain	Vernalization	Promote flowering after vernalization and are up-regulated under SDs
T. aestivum				
VRN-A1 (*Vrn1*)	–[c]	MADS-box, AP1-like	Vernalization	Promote flowering after vernalization
VRN-B1 (*Vrn2*)	–[c]	MADS-box, AP1-like	Vernalization	Promote flowering after vernalization
VRN-D1 (*Vrn3*)	–[c]	MADS-box, AP1-like	Vernalization	Promote flowering after vernalization
VRN-B3 (*Vrn-B4*)	*TaFT*	Putative kinase inhibitor	Vernalization	Recessive alleles promote flowering after vernalization and are up-regulated in LDs
Ppd-A1 (*Ppd1*)	Not cloned	Not cloned	Photoperiod	Promote flowering in SDs and LDs
Ppd-B1 (*Ppd2*)	Not cloned	Not cloned	Photoperiod	Promote flowering in SDs and LDs
Ppd-D1 (*Ppd3*)	Not cloned	Not cloned	Photoperiod	Promote flowering in SDs and LDs
Rye				
Vrn-R1 (*Sp1*)			Vernalization	Recessive alleles promote flowering after vernalization

[a] Also known as *AGL20* (Lee *et al*., 2000)
[b] also known as *HvAP1a* (Yan *et al*., 2005)
[c] *AP1*-like genes from hexaploid wheat have been variously named *TaMADS#11* (Murai *et al*., 1998), *WAP1* (Murai *et al*., 2003, who reported cloning 5A, 5B, and 5D copies, but whose sequences and genome-specific names remain unpublished), *TaVRT-1* (Danyluk *et al*., 2003), and *AP1-5A* (Beales *et al*., 2005)

suggesting the two lineages have evolved vernalization pathways independently. This observation is supported by current phylogeny (Kellogg, 1998; Soltis *et al*., 2002; http://www.mobot.org/ MOBOT/research/APweb/), with parsimonious interpretation suggesting that the ancestral cereal was an SD, vernalization-unresponsive plant (Fig. 2). Recently, *VERNALIZATION INSENSITIVE 3* (*VIN3*) has been found to mediate the cold-induced repression of *FLC* in *Arabidopsis* (Sung and Amasino, 2004). In contrast to other *Arabidopsis* vernalization pathway genes, sequence database searches show that *VIN3*-like genes are conserved in monocots including rice, maize, sorghum (*Sorghum bicolor* L.), barley, and wheat. This raises the question of why these are genes conserved in the absence of convincing orthologues of *FLC*, *VRN1*, and *VRN2*. An answer may lie in the relatively uncharacterized *FLC*-independent vernalization-response pathway in *Arabidopsis* (Fig. 1), mediated in part by the floral integrator, *AGAMOUS-LIKE 24* (Yu *et al*., 2002; Michaels *et al*., 2003), a MADS-box transcription factor for which cereal orthologues have been identified (Zhao *et al*., 2006). The widely held view that monocots and dicots have evolved independent vernalization pathways is further confounded following the isolation of *FRIGIDA-LIKE 1* (*FRL1*), a homologue of *FRI* that functions to maintain the regulation of *FLC* (Michaels *et al*., 2004). *FRL1* shows an unusual dispersed pattern of amino acid conservation with *FRI*, permitting the identification of a larger gene family in *Arabidopsis*, as well as putative orthologues in grass species. Clearly, differences in the vernalization pathways of monocots and dicots have evolved; the extent to which conserved components of this pathway are functionally equivalent is yet to be determined.

In contrast to the unproven relevance of model species in the identification of cereal *Vrn* genes, comparative genetics of photoperiod pathways has been more promising. Detailed understanding of the photoperiod pathway in *Arabidopsis* (recently reviewed by Imaizumi and Kay, 2006) provides a source of candidate genes for *Ppd* loci in the Triticeae (Table 1). Photoperiodic timekeeping involves the circadian clock, with the central oscillator generating rhythms of approximately 24 h, entrained to the environmental conditions the plant experiences by the transmission of light and temperature signals to the oscillator via the input pathway. This integration determines the regulation of output pathways, one of which is the control of flowering by daylength. It is the genes in this pathway, principally *GIGANTEA* (*GI*), *CONSTANS* (*CO*), and *FLOWERING LOCUS T* (*FT*), that constitute candidates for grass photoperiod pathway loci. Dunford *et al*. (2005) demonstrated that, although expression of the

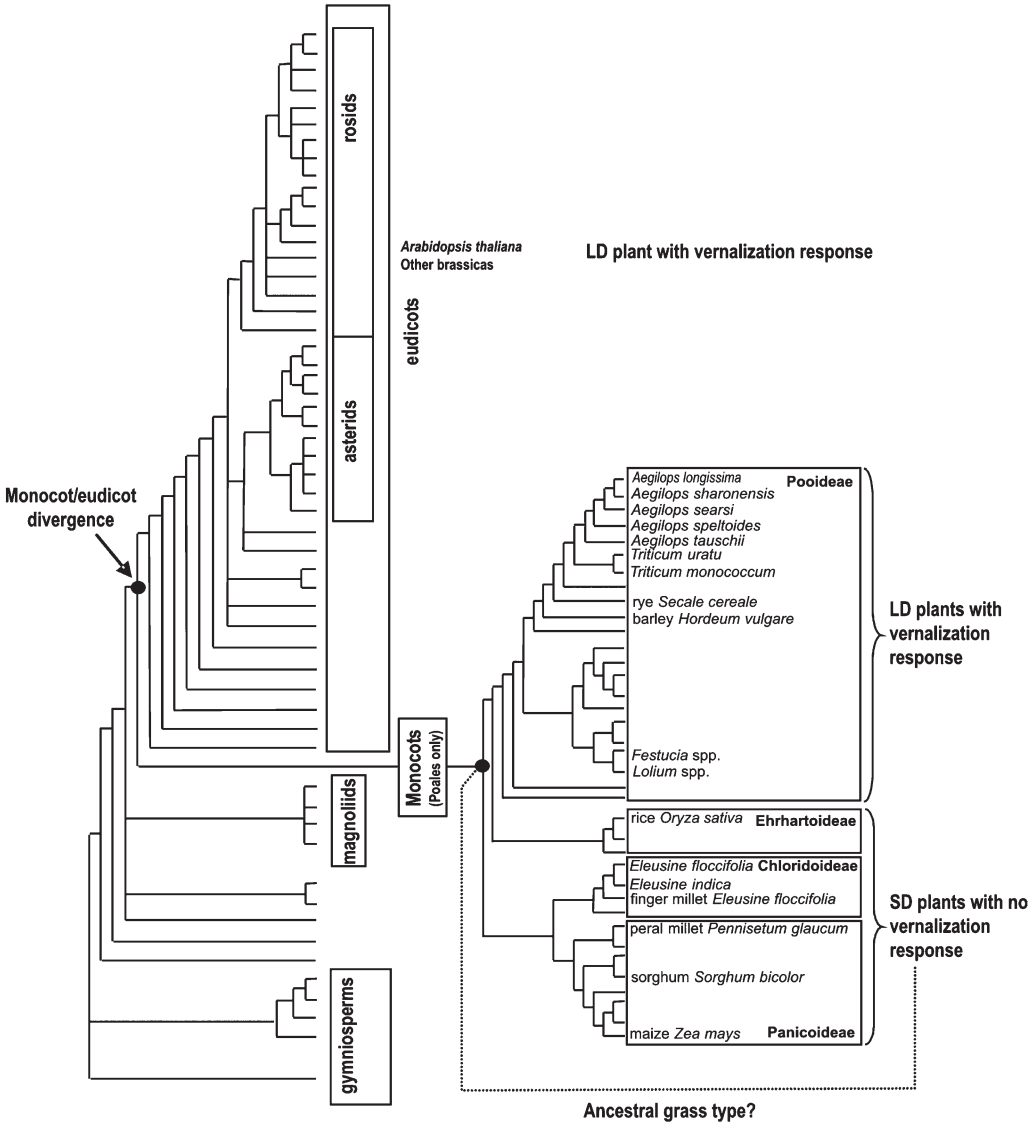

Fig. 2. Seed plant phylogeny, indicating photoperiod and vernalization responses. Adapted from the Missouri Botanical garden Angiosperm Phylogeny Website, Version 7 (http://www.mobot.org/MOBOT/research/APweb/), Kellogg (1998) and Soltis *et al*. (2002).

barley orthologue of *GI* (*HvGI*) shows similar diurnal rhythms to *AtGI*, its map position does not correspond to known cereal flowering time QTLs. Similarly, mapping of *HvFT* (Yan *et al*., 2006), and barley and wheat *CO*-like genes (Griffiths *et al*., 2003; Nemoto *et al*., 2003) shows that none is a convincing candidate for *Ppd-H1* or *Ppd-H2*, although *HvCO1*, *HvCO2*, and *LpCO* from perennial ryegrass (*Lolium perenne* L.) show circadian oscillation of expression as observed in *Arabidopsis* (Martin *et al*., 2004; Turner *et al*., 2005).

Studies of natural variation in rice have shown that orthologues of *Arabidopsis* photoperiod pathway genes underlie several flowering time QTLs (Fig. 1; Table 1). The first, *Heading date 1* (*Hd1*), a major determinant of daylength sensitivity, was found to encode an orthologue of *AtCO* (Yano *et al*., 2000). Soon after, Kojima *et al*. (2002) found that a second rice flowering time QTL, *Hd3a*, represents an orthologue of the *Arabidopsis* floral pathway integrator *FT*, and has been shown to promote flowering under SDs (Monna *et al*., 2002). Overexpression of *OsGI* leads to up-regulation of *OsCO* and down-regulation of *OsFT* expression, resulting in late flowering under SDs and LDs (Hayama *et al*., 2003), providing further evidence of conserved photoperiod pathway components. In addition, *Hd6*, a rice QTL implicated in the control of flowering by photosensitivity (Yamamoto *et al*., 2000), has also been cloned, and encodes the alpha subunit of *CASEIN KINASE 2 alpha* (*CK2*α) (Takahashi *et al*., 2001), which, in *Arabidopsis*, acts by phosphorylation of the CIRCADIAN CLOCK ASSOCIATED1 (CCA1) protein (Suagano *et al*., 1998). The contrasting photoperiod responses of *Arabidopsis* and rice are due to differences

in the relationship between *CO* and *FT* (Hayama and Coupland, 2004). In rice, *Hd1* (*CO*) promotes *Hd3a* (*FT*) expression in SDs and represses it in LDs, while, in *Arabidopsis*, *CO* promotes *FT* expression in LDs. In this case, dramatic phenotypic variation results from modification of an evolutionarily conserved pathway.

Positional cloning of flowering time genes in temperate cereals

Based on a foundation of detailed understanding of the genetics of cereal flowering time loci and the comparative approaches outlined here, several temperate grass flowering time genes have now been cloned (Table 1).

Vrn *genes*

VRN-A^{m}1, a major determinant of flowering in *T. monococcum*, was the first cereal vernalization locus isolated (Yan *et al*., 2003). To compensate for the large physical distances expected in a cereal genome, gene content of the region was revealed by a multi-species bridging strategy exploiting collinearity with rice and sorghum. Two MADS-box transcription factors were defined by the mapping interval. The gene showing sequence similarity to the *Arabidopsis* meristem identity gene, *APETALA 1* (*AP1*), was considered as the best candidate, based on polymorphisms in its promoter and transcriptional up-regulation in vernalization-responsive lines during cold treatment.

Soon after, a combination of fine mapping, expression analysis, and RNAi down-regulation showed that *VRN-A^{m}2* is encoded by *ZCCT1*, whose protein is predicted to contain a zinc finger and CCT domain, found in *CO*, *CO*-like, and *TOC1* genes (Yan *et al*., 2004*a*). *ZCCT1* is down-regulated in both vernalization-sensitive and -insensitive lines during cold treatment. However, insensitive alleles at *VRN-A^{m}2* contain a mutation that results in an R/W amino acid substitution at a highly conserved residue within the CCT domain. Mutation of this residue in the *Arabidopsis co-7* mutant results in a severe effect on flowering time (Robson *et al*., 2001), suggesting that this point mutation is the likely cause of spring growth habit in *T. monococcum*. The decrease in expression of functional *ZCCT1* alleles during vernalization is concomitant with the up-regulation of *AP1* and the subsequent competence of the apical meristem to flower under inductive photoperiods. The previously established epistatic relationships between the two loci suggest a model in which ZCCT1 acts by repressing expression of *AP1* thus maintaining vegetative growth in vernalization-sensitive varieties. During cold treatment, the down-regulaton of *ZCCT1* permits stable up-regulation of *AP1* and competence to flower (Fig. 1).

The vernalization-responsive phenotype of cereal wild progenitors, and the interactions between the two major genes involved suggest that a dominant mutation at *VRN-A^{m}1* would confer insensitivity to ZCCT1-mediated repression. Indeed, vernalization-insensitive alleles of the *VRN-A^{m}1* candidate gene, *AP1*, contain a series of small deletions in the promoter spanning a region with a CArG-box motif. Such motifs have previously been shown to be *cis*-acting sites for the interaction with MADS-box genes in *Arabidopsis* (Tilly *et al*., 1998). The deletion of this binding site is thought to make the gene under its control 'blind' to the repression mediated directly, or indirectly, by ZCCT1. A combination of comparative mapping and expression analysis has identified orthologous *ZCCT1* and *AP1* genes in a variety of other cereals (Danyluk *et al*., 2003; Murai *et al*., 2003; Petersen *et al*., 2004; Dubcovsky *et al*., 2005; Andersen *et al*., 2006). In barley, the *VRN-H2* locus contains three *ZCCT* genes, with a deletion of all three loci resulting in the creation of recessive spring alleles in almost all lines studied (von Zitzewitz *et al*., 2005).

Although multiple spring *T. monococcum* lines show disruption of the CArG-box in the *AP1* promoter, the independent selection of orthologous *Vrn* loci since the divergence of temperate crop species has resulted in the utilization of a range of alternative deletions within putative regulatory regions. Comparative sequence analysis of the orthologous *AP1*-like MADS-box gene in barley (*BM5A*), as well as *AP1* alleles from A, B, and D genomes of hexaploid wheat has identified a range of deletions within the first intron that define a 2.8 kb conserved region thought to contain critical regulatory elements (Yan *et al*., 2004*b*; Fu *et al*., 2005; von Zitzewitz *et al*., 2005). Interestingly, recent studies of epistatic models between barley and *T. monococcum Vrn* loci have shown similar, but slightly different interactions (Dubcovsky *et al*., 2005). The selection of alternative mutations at orthologous cereal *AP1*-like genes indicates that intron 1 deletions are more effective at removing the repression mediated by *ZCCT* genes than disruptions of the CArG-box (Dubcovsky *et al*., 2005). This suggests that comparative sequence analysis may inform the selection or creation of novel alleles with specific *Vrn* response phenotypes within Triticeae crop species. The detailed molecular analysis of cereal *ZCCT* and *AP1*-like genes undertaken to date will allow characterization of large wheat and barley germplasm collections, and allow the dissection of the adaptive value of the observed alleles and allele combinations in response to vernalization (Fu *et al*., 2005).

Recently, the barley *VRN-H3* vernalization locus, and the collinear *VRN-B3* locus from *T. aestivum*, have been shown to encode an orthologue of the *Arabidopsis* floral pathway integrator, *FT*, and are collinear with *OsFT* in rice, which underlies the photoperiod QTL, *Hd3a* (Yan *et al*., 2006). *VRN3* transcripts from both species are up-regulated in response to vernalization and growth under

SDs, indicating that the vernalization and photoperiod pathways interact in temperate cereals, and highlighting functional similarity with *OsFT*. The dominant (early flowering) *TaFT* allele is associated with the insertion of a retroelement in the promoter; in barley, polymorphisms within the first intron are associated with the early allele. The limited germplasm screens completed to date suggest that the early *Vrn-B3* allele has not been extensively used in commercial varieties, and represents a novel source with which to modulate wheat flowering time (Yan *et al*., 2006).

Ppd *genes*

Unlike the situation in vernalization pathways, QTL analysis in rice show that functional grass orthologues of *Arabidopsis* photoperiod genes do exist. However, mapping the Triticeae orthologues of *Arabidopsis* photoperiod genes *CO*, *FT*, and *GI* shows that different major determinants of photoperiod have been selected in Triticeae relative to rice. This view is validated by the recent cloning of the *Ppd-H1* locus in barley (Turner *et al*., 2005). Fine mapping using a cross between the photosensitive variety 'Igri' (*Ppd-H1*) and the late flowering, non-sensitive variety 'Triumph' (*ppd-H1*) defined an interval containing a single gene encoding a pseudo-response regulator (*PRR*), most similar to *Arabidopsis PRR7*. In *Arabidopsis*, *PRR* genes form a small family of five circadian clock-associated genes that include *TIMING OF CAB EXPRESSION 1* (*TOC1*), a component of the central oscillator. *PRR7* is thought to act close to the central oscillator or in temperature and light input pathways (Salome and McClung, 2005). A single point mutation within the CCT domain of *PRR* results in an amino acid substitution at a conserved position, and is thought to result in insensitivity to growth under LD, analogous to the phenotype of several *Arabidopsis prr7* mutants (Yamamoto *et al*., 2003; Nakamichi *et al*., 2005). Orthologues of *HvPRR7* from the A, B, and D genomes of wheat have been characterized (D Laurie *et al*., unpublished data), and represent strong candidates for the genes underlying the syntenous *Ppd* series in wheat. However, due to the differences in phenotype associated with the wheat and barley *Ppd1* loci, alternative mutations are expected.

Comparative markers show the *Ppd-H1* region is syntenous to a section of rice chromosome 7 that contains the heading date QTL, *Hd*2 (Dunford *et al*., 2002). The hypothesis that these loci may be controlled by orthologous genes has recently been supported by the mapping of a member of the rice *PRR* gene family to the *Hd2* interval, with one of the mapping parents containing a severe lesion in the CCT domain (Murakami *et al*., 2005). This raises the possibility that characterization of temperate cereal flowering time genes could soon inform corresponding pathways in rice.

Additional temperature and photoperiod sensitivity loci

As well as the major *Vrn* and *Ppd* response loci in wheat and barley, studies have identified additional loci responsive to light and temperature that represent additional targets for use in breeding. Photoperiod response loci have been reported on chromosomes 3D (Miura and Worland, 1994) and 6D (Islam-Faridi *et al*., 1996), while loci controlling the vernalization response have been found on 3B (Zemetra and Morris, 1984; Miura and Worland, 1994), the group 6 chromosomes (Islam-Faridi *et al*., 1996), and 7A (Law and Worland, 1997). Recently, Kane *et al*. (2005) mapped orthologous MADS-box genes in barley (*HvVRT*-2) and hexaploid wheat (*TaVRT*-2) to the short arms of the Triticeae group 7 chromosomes. These belong to the MADS11-like clade from *Solanum tuberosum* L., whose members in *Arabidopsis* and *Antirrhinum majus* L. have been shown to affect flowering (Hartmann *et al*., 2000; Yu *et al*., 2002; Masiero *et al*., 2004). In both wheat and barley, *VRT-2* is down-regulated during vernalization, analogous to the expression of cereal *ZCCT* genes, supporting the *TaVRT-2* homeologues as candidates for the 7A vernalization loci discussed above.

In barley, *HvVRT-2* also marks the peak QTL for a photoperiod response locus mapped to 7HS (Szücs *et al*., 2006). Interestingly, interaction between temperature and photoperiod has previously been identified in the cereal cold tolerance pathways (Crosatti *et al*., 1999; Fowler *et al*., 2001), and increasing evidence is emerging that similar interactions may exist between the major loci controlling flowering time. This was noted by Danyluk *et al*. (2003), who reported that the wheat *AP1*-like genes showed differential expression in response to photoperiod. Subsequently, *VRN-H1* and *VRN-H2* (or tightly linked) loci have been associated with photoperiod response in three barley crosses (Karsai *et al*., 2005; Szücs *et al*., 2006; Trevaskis *et al*., 2006).

'Earliness per se*' genes*

Additional cereal loci that promote flowering independently of environmental cues have been identified, and are variously termed '*earliness per se*' (*eps*) or 'narrow sense' earliness genes. Numerous *eps* and flowering time QTLs have been mapped in both barley (Hackett *et al*., 1992; Laurie *et al*., 1995; Kato *et al*., 2002) and wheat (Scarth and Law, 1983; Hoogendoorn, 1985; Snape *et al*., 1985; Zemetra *et al*., 1986; Miura and Worland, 1994; Kato *et al*., 2002), although almost all remain ill-defined to date. Recently, Bullrich *et al*. (2002) reported mapping the thermo-sensitive *eps* gene, *Eps-A^{m}1*, to chromosome 1A^{m}L in *T. monococcum*, thus questioning whether *eps* genes are truly independent of environmental cues. The characterization of *Eps-A^{m}1* suggests that once *eps* loci are resolved in backgrounds in which the effects of additional

flowering time loci have been removed, many may not truly be independent of environmental signals. The relatively large numbers of cereal *eps* loci suggest that variation in genes controlling flowering time is common compared with the major *Vrn* and *Ppd* genes. Progress in defining and utilizing *eps* loci will rely on the development of backcross lines to isolate individual QTLs as Mendelian characters to allow accurate mapping, an approach that has proven highly successful in rice (Yano *et al*., 2001; Ebitani *et al*., 2005). As variation at *eps* loci is found both within and between spring and winter varieties, *eps* genes represent an, as yet, untapped source of variation for targeted breeding that should allow 'fine-tuning' of flowering time within these two agri-types.

Domestication of temperate cereals: role of the *Vrn* and *Ppd* genes in adaptation to local environments

Wild einkorn (*Triticum monococcum* L. ssp. *aegilopoides* (Link) Thell.) and emmer (*Triticum turgidum* L. (Thell.) ssp. *dicoccoides* (Körn. Ex Asch. & Graebn.) Thell.) wheats, and *Hordeum spontaneum* C. Koch, the extant progenitors of present day wheat and barley varieties, were first harvested from natural stands in and around the Fertile Crescent 13 000–10 300 years before present (BP) (Feldman, 2001). They are adapted to the prevailing climatic patterns of that region, where the bulk of the annual rainfall falls in the autumn and spring, followed by hot and dry summer months. Under these conditions, cereals maximize their fitness by using the autumn rains to establish vegetative structures before winter, and use vernalization as a mechanism to delay flowering until winter and the danger of frost damage has passed. In addition, the ability to detect increasing photoperiod allows these species to flower and to complete grain filling using spring moisture ahead of the hot summer.

The first cereals to be domesticated in the Fertile Crescent presumably shared the vernalization and photoperiod response phenotypes and biennial growth habit of their wild relatives. However, the spread of agriculture into Europe 8500 to 5000 years BP required the selection of novel adaptive traits suited to the new environments encountered. There are two reasons why the early farmers that domesticated cereals might have selected against the ancestral biennial growth habit. Firstly, spring forms which could be sown and harvested in a short season fitted the predominantly nomadic lifestyle of preMesopotamian culture. Secondly, as farmers settled in the most fertile locations, the use of rapid-cycling spring lines allowed sowing two successive crops each year. Thus, the evolution of spring types from a predominantly winter ancestral state is a key event in the post-domestication spread of temperate cereals.

Successful spring and winter sowing requires divergent agronomic characters. For example, winter varieties require cold hardiness, and resistance to distinct disease pressures including root diseases. For this reason, the division of the species gene-pool by seasonal type has persisted to the present day, and distinct clustering of winter and spring types is notable in all surveys of cultivated genomic diversity (Backes *et al*., 2003; Koebner *et al*., 2003). Although loci across the entire genome may have significantly different frequencies in spring versus winter populations, strong bias in haplotype distribution may be observed at loci closely linked to major flowering loci. For instance, a single haplotype of the endosperm gene, *Beta-amylase I* (*Bmy1*) has been observed in 95% of all European winter barley varieties (Chiapparino *et al*., 2006). This is attributable to the virtual invariance in haplotype constitution of the same European cultivated winter barleys at the closely linked *VRN-H2* locus (J Cockram, F Leigh, E Chiapparino, IR Mackay, DA Laurie, D O'Sullivan, unpublished data). As the identities of the two major cereal *Vrn* loci are now known, the patterns of diversity and effects of selection on these genes can be studied in detail. However, few data have, as yet, been published, and are mostly limited to a small number of modern cultivars. Extended analysis should prove invaluable, as illustrated by the comparative sequence analysis of a limited number of deletions identified in wheat and barley *AP1*-like genes (discussed previously).

Following the cloning of *Ppd-H1*, the molecular diversity of responsive and non-responsive alleles within an extended collection of diverse barley germplasm has been investigated (H Jones, F Leigh, IR Mackay, L Smith, T Brown, W Powell, unpublished data). In landrace material, polymorphism was identified at the 'causative' single nucleotide polymorphism (SNP) identified by Turner *et al*. (2005), confirming that responsive and non-responsive alleles were present. Mapping landrace *Ppd-H1* alleles according to accession origin reveals a geographic structure to the observed diversity (Fig. 3a). Genotypes predicting a non-responsive phenotype were prevalent in landraces from central and northern Europe, where long growing seasons with moisture available for grain filling over the summer favour late flowering conferred by such alleles. Photoperiod-responsive alleles were predominant in landraces from south-west Asia, southern Europe, and the Mediterranean basin, and were found in all *H. spontaneum* lines studied, consistent with the view that wild barley initiates flowering in response to LDs. The non-responsive allele was not found in *H. spontaneum*, suggesting the mutation occurred post-domestication during the radiating spread of cereals throughout Europe by human cultivation. The latitudinal cline in photoperiod response found in barley landrace material indicates that the non-responsive *ppd-H1* mutation created a phenotype that early farmers

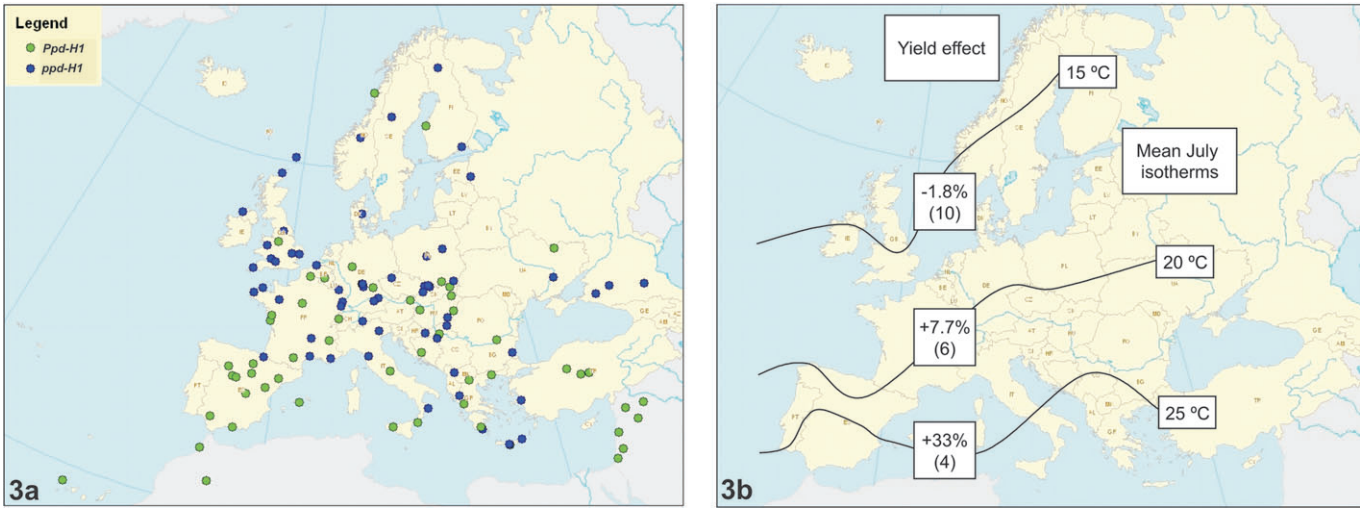

Fig. 3. (A) Distribution of responsive (*Ppd-H1*) and non-responsive (*ppd-H1*) alleles in a collection of European barley landraces (H Jones, DA Leigh, IR Mackay, L Smith, T Brown, W Powell, unpublished data). (B) Percentage yield effect of substituting the photoperiod-insensitive *Ppd-D1* allele into 'Capelle-Desprez' (years of trialling in parentheses) adapted from Worland *et al.* (1998). Increasing benefit is seen with decreasing latitude and this correlates with growth conditions such as mean summer temperatures. Early flowering wheats perform better in southern locations because grain filling is completed before the onset of high temperatures and associated water deficit. (Fig. 3B adapted from: Laurie, 2004).

selected and maintained because it conferred an ecological advantage in the environmental conditions of northern Europe. This, along with the selection of crops with no vernalization requirement, represents important milestones in post-domestication adaptation.

A relationship between geographical position and genotype is also observed in wheat, where photoperiod-insensitive forms (which flower early in short and long days) predominate in regions with hot dry summers. Photoperiod insensitivity is conferred by semi-dominant alleles, primarily at the *Ppd-D1* and *Ppd-B1* loci (Welsh *et al.*, 1973; Worland and Sayers, 1996; Law and Worland, 1997). The benefit of early flowering is illustrated by the studies of Worland *et al.* (1998), in which 'Cappelle-Desprez' (*ppd-D1*) was compared with a near-isogenic line carrying the insensitive *Ppd-D1* allele. Over several years of testing, the early flowering line conferred a yield penalty in England, a modest yield benefit in Germany, and a strong yield benefit in the former Yugoslavia (Fig. 3b).

Future prospects

Biotechnology and breeding in wheat and barley is on the cusp of a revolution. Starved for so long of the rich resources that the model plant species boast, significant genetic, EST sequence, and molecular marker resources are now available (www.gramene.org; www.tigr.org) and credible plans to sequence gene-dense regions of the wheat genome are developing (www.wheatgenome.org). In addition, for both wheat and barley, effective transformation procedures (Patel *et al.*, 2000; Bhalla, 2006) and virus-induced gene-silencing systems (VIGS) (Hein *et al.*, 2005; Scofield *et al.*, 2005) have been developed that add the capability for rapid functional testing of genes. As these developments are exploited, the ease with which the genes underlying QTLs for key agronomic traits can be isolated and functionally validated will dramatically increase. Many of these studies will focus on QTLs that impact on yield potential, particularly in relation to sustainable production under changing climatic conditions. When fully characterized, it is predicted that many of the genes controlling these traits will be shown to be involved in the control of flowering.

The cloning of *Ppd-H1* (Turner *et al.*, 2005) provides an exemplar of how such studies might proceed, as it allows the collinear wheat genes (*Ppd-A1*/*-B1*/*-D1*) to be isolated. From this starting point, the full extent of allelic diversity within wheat can be described. By careful preparation of near-isogenic lines in an appropriate genotype using allele-specific and flanking markers, the precise relationship between flowering time and sequence variation can be quantified, and pleitrophic effects, such as fewer tillers and spikelets in the ear that are generally associated with photoperiod insensitive genotypes (Worland and Snape, 2001), investigated.

The interaction of the flowering control genes in wheat and barley with the circadian clock mechanism would also appear to be a fertile area for study in relation to crop improvement. In *Arabidopsis*, *PRR* genes have been implicated in providing adaptive responses to photoperiod in growth at different latitudes by modulating circadian timing (Michael *et al.*, 2003). Plants in which the clock period is correctly matched to the day/night cycle are more photosynthetically efficient and productive than those grown in mismatched environments (Dodd *et al.*, 2005).

The extent to which this latter observation extends to wheat and barley and how it might be exploited to increase productivity remains to be determined. Nevertheless, it does, yet again, highlight the immense value of research in model species. A challenge for crop scientists in the 21st century is to integrate knowledge from model systems with burgeoning genomic and genetic resources and systems for functional testing in crop species. Once genes are identified and allelic variation characterized, it is extremely important that this research is effectively exploited by ensuring that appropriate transfer mechanisms for commercial exploitation, including pre-breeding, are in place.

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