



Comparative genetic approaches to the identification of flowering time genes in temperate cereals

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Abstract

The timing of flowering during the year is an important adaptive character which impacts yield and quality in crop plants. The genetic basis of flowering time control is best understood in the model dicot arabidopsis (*Arabidopsis thaliana* [L.] Heynh.) and results from this system are briefly summarized. Recent results from rice are discussed in relation to arabidopsis, particularly in relation to conserved genes regulating flowering by photoperiod. How these results might benefit studies of temperate cereals is then considered. Genetic analysis of photoperiod and vernalization response in temperate cereals is summarized in relation to recent data pointing to greater evolutionary conservation of photoperiod response than vernalization response. The implications of these results for identifying additional genes in cereals are discussed. The aim of gene identification is to provide a better understanding of how plant development is controlled by environmental cues such as day length and temperature and to allow researchers to determine how many alleles of each gene are available. This information could be combined with physiologically-based whole plant growth models to provide an improved description of crop development. Improved understanding of the control of flowering would assist plant breeders in the selection of varieties with enhanced adaptation to existing environments or to new environments arising from climate change.

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1. Introduction

The timing of flowering is an important adaptive trait in wild plants. Genetic and physiological mechanisms have evolved to ensure that flowering occurs when conditions are most favorable to pollination,

seed development and seed dispersal. These constraints apply to the wild ancestors of crop species, but additional differences in the ways in which flowering are controlled are likely to have been selected during the development and spread of agriculture. This has allowed crop species to become adapted to environments outside the ecogeographical ranges of their wild ancestors.

Variation in flowering behavior can often be observed between ecotypes in wild species and

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between different adapted forms of crop species, particularly when plants from contrasting environments are compared. In some cases this has been shown to result from alteration in the way plants use environmental cues such as day length (photoperiod) and periods of low temperature (vernalization). Additional “earliness” factors not directly linked to environmental signals are also important. Major genes affecting the adaptation of model and crop plants have been defined by genetic analysis and in several cases, particularly in model species, the underlying genes have been cloned and identified.

Although genetic variation in photoperiod response, vernalization response and other earliness factors is well documented in crops, the identities of the majority of the underlying genes remain unknown. This poses several challenges. Firstly, for a given flowering time gene we have no clear idea of the extent of allelic variation that is available within cultivated germplasm or whether additional potentially beneficial alleles exist in wild ancestors or related species. Secondly, we have little information about how individual genes function, and even less about gene \times gene and gene \times environment interactions, which limits the degree to which flowering time can be designed to match a particular environment, either by the selection of allelic variation in conventional breeding programmes or by transgenic methods.

Lack of knowledge about allelic variation and gene function also inhibits the development of strategies for altering adaptation in response to projected changes in climate. This is exacerbated by the observation that different species respond differently to such changes (Fitter and Fitter, 2002), possibly because they use environmental cues in different ways. Such variation leads to changes in the structure of plant communities, and in agriculture can be expected to affect not only crop growth but also the relationships between crops, pests and competitor weed species. Therefore, there is a need to understand more about the ways in which crop development is affected by environment and how genetic variation within crop species can be deployed to enhance adaptation.

This paper discusses the prospects for understanding the genetic basis of one aspect of adaptation, namely the control of flowering time, and focuses on temperate cereals, particularly barley (*Hordeum*

vulgare L.) and bread wheat (*Triticum aestivum* L.). We briefly review what is known about the genetic control of flowering in model and crop species and discuss the ways in which comparative genetic methods, which integrate information from different species, might be used to advance understanding of temperate cereals.

2. Comparative methods

2.1. Gene homology

Before considering how comparative genetic methods are used, it is worth considering what they are and why they might be advantageous. Comparative genetics utilizes evolutionary conservation of individual genes, or conservation of gene order, in the chromosomes of related species. The attraction of comparative genetics is that discoveries in one species may be transferable to others which are experimentally less tractable. Many aspects of plant biology are best understood in the model dicot arabidopsis which has well documented experimental advantages including a small genome, now completely sequenced, and a large coordinated international effort devoted to developing resources for understanding gene function. In comparison, gene isolation has proven difficult in many crop species, including temperate cereals, and a major factor in this is the large sizes of their genomes [approximate haploid genome sizes in megabases ($\times 10^6$ base pairs) are 130 for arabidopsis, 450 for rice (*Oryza sativa* L.), 2500 for maize (*Zea mays* L.; about the size of the human genome), 5400 for barley and 17,000 for bread wheat]. This makes positional (also known as map-based) gene isolation difficult. In contrast to maize, temperate cereals lack endogenous transposable elements suitable for gene isolation by insertional mutagenesis (see Maes et al., 1999 for a review of this method).

In essence, comparative methods offer the potential for “fast-track” isolation of genes, reducing cost and effort, by virtue of evolutionary conservation of gene function. A good example comes from work on the control of plant growth by gibberellic acid (GA). Analysis of dwarf mutants of arabidopsis allowed the isolation and characterization of the *GIBBEREL-LIC ACID INSENSITIVE* (*GAI*) gene (Peng et al.,

1997). The *REDUCED HEIGHT 1 (Rht1)* gene of wheat was then isolated on the basis of sequence homology. This showed that the genes encoded highly conserved proteins. Furthermore, phenotypically similar GA insensitive dwarf phenotypes were caused by equivalent mutations that involved deletion of a specific region in the respective proteins (Peng et al., 1999). Further work has shown that *GAI* is a member of a small gene family termed the *DELLA* group that act as growth repressors and whose action is opposed by GA through a mechanism that leads to targeted destruction of *DELLA* proteins by the proteasome (Fu et al., 2002). Auxin signalling has also been shown to involve *DELLA* proteins, showing how the action of different hormone signals is integrated (Fu and Harberd, 2003). Clearly not all characters are conserved in this way. For example, several *Brassica* and temperate grass species have self-incompatibility systems, but the *S* locus system of the former is unrelated to the *S* and *Z* locus system of the latter (Hiscock and McInnis, 2003).

This highlights an obvious problem with comparative approaches which is that there is often no information on whether similar phenotypes are produced by variation in homologous genes or are the result of convergent evolution. However, with the increasing availability of DNA sequence information from many species it is now relatively easy to ask whether a gene cloned from *Arabidopsis*, for example, has a counterpart in cereals. Direct searching of wheat or barley cDNA or genomic libraries using *Arabidopsis* genes as hybridization probes is inefficient due to differences in nucleotide sequence of even the most highly conserved genes. Fortunately, the ability to search for wheat and barley homologues of genes from *Arabidopsis* and rice has been greatly enhanced by the availability of the complete genomic sequence of rice and the development of large numbers of expressed sequence tags (ESTs) which are partially sequenced cDNA clones (currently over 280,000 for rice, 350,000 for barley and 540,000 for wheat; <http://www.ncbi.nlm.nih.gov/dbEST/>). Thus, much of the work can now be done by database searching using bioinformatic tools. Mapping using selected cereal sequences can then determine if the candidate gene cosegregates with the phenotype of interest. This emphasizes the importance of high quality genetic analysis

of the target mutant or trait, even in cases where comparative approaches are used.

2.2. Comparative mapping

The section above deals with the conservation of individual gene function, but another important aspect is comparative mapping which aligns the genetic maps of different species using common markers, usually restriction fragment length polymorphism (RFLP) or polymerase chain reaction (PCR) markers based on the coding regions of evolutionarily conserved genes (see Devos and Gale, 2000; Paterson et al., 2000; Feuillet and Keller, 2002; Laurie and Devos, 2002; Bowers et al., 2003; for recent reviews). Comparative mapping has greatest benefit where one of the genomes is fully sequenced, and in the case of cereals this is rice. A high quality annotated sequence of rice is expected to be complete by the end of 2004, providing a great deal of information on the likely gene content of colinear regions in other species. Comparative mapping has to be approached with care, as changes in gene order can complicate the relationship between different species (for example, Sorrells et al., 2003). However, there is no doubt that this is an excellent way of generating new markers for particular regions and for identifying candidate genes.

The properties of temperate cereal genomes confer intrinsic disadvantages when it comes to gene isolation. They are also relatively disadvantaged in terms of the available model species. In dicots, the evolutionary separation of *Brassica* species from *Arabidopsis* is thought to have occurred 15–20 million years ago (mya; Bowers et al., 2003). The dicot (*Arabidopsis/Brassica*) lineage is far removed from the monocot lineage, which diverged 150–180 mya or perhaps as much as 235 mya (Wikström et al., 2001; Bowers et al., 2003, respectively) at one of the most basal branch points in the angiosperm phylogeny (Soltis et al., 1999). Temperate cereals are thought to have diverged from the “model” cereal rice about 60 million years ago. Thus, the power of model systems is proportionally reduced for the temperate cereals. Nevertheless, comparative approaches have great potential and the following sections discuss the experience of using *Arabidopsis* and rice in the study of flowering in temperate cereals.

3. The control of flowering in the model dicot arabidopsis

The genetic basis of flowering time control in arabidopsis is described in numerous excellent reviews (Simpson et al., 1999; Simpson and Dean, 2002; Mouradov et al., 2002; Yanovsky and Kay, 2003; Hayama and Coupland, 2003; Henderson et al., 2003; Sung and Amasino, 2004a). Briefly, flowering in arabidopsis is controlled by the interaction of the photoperiod, vernalization, autonomous and gibberellic acid pathways (Fig. 1). Photoperiod is perceived by light responsive elements including phytochromes (red and far-red light receptors) and cryptochromes (blue light receptors) and these are involved in setting the circadian clock. A key component of the photoperiod pathway is the *CONSTANS* (*CO*) gene whose expression is regulated by the circadian clock, giving a peak of expression late in

the day. Recent data from Valverde et al. (2004) show that the *CO* protein is stabilized by light late in the day through the actions of the *PHYTOCHROME A* (*PHYA*) and *CRYPTOCHROME 1* and *2* (*CRY1* and *CRY2*). *CO* protein is rapidly degraded in the dark, and at the start of the day any remaining *CO* protein is degraded by a mechanism involving *PHYTOCHROME B* (*PHYB*). This means that the functional form of the *CO* protein is only found in plants exposed to long days, where it promotes the expression of “pathway integrators” including *FLOWERING LOCUS T* (*FT*) and *AGAMOUS-LIKE20* (*AGL20*, also known as *SOCI*).

Changes in arabidopsis growth in response to shading, which includes an accelerated transition to flowering, are also mediated primarily by *PHYB*. This has recently been shown to be by regulation of *FT* expression. This shows that a light-quality pathway responding to changes in red/far red light ratio regulates

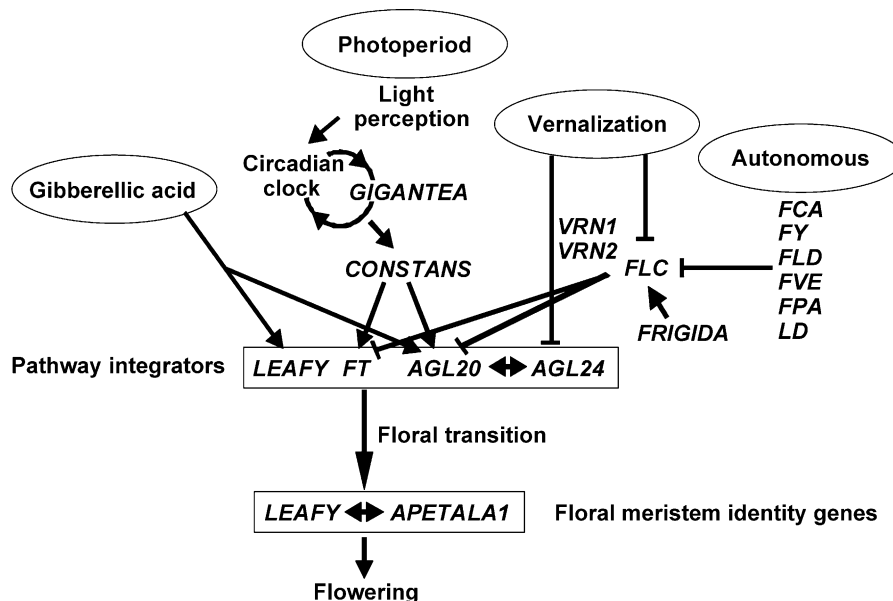


Fig. 1. Simplified model of pathways controlling flowering in *Arabidopsis thaliana*. Based on models from Simpson et al. (1999); Simpson and Dean (2002); Mouradov et al. (2002); Michaels et al. (2003a); Henderson et al. (2003). The transition from vegetative to reproductive growth (floral transition) requires the expression of *LEAFY* (*LFY*) and *APETALA1* (*API*; floral meristem identity genes). The expression of *LFY* and *API* is regulated by “pathway integrators”, particularly *FLOWERING LOCUS T* (*FT*) and *AGAMOUS-LIKE 20* (*AGL20*), so called because their expression level is determined by the balance of positive and negative signals derived from genes regulated by photoperiod, vernalization and gibberellic acid. The expression of the main repressor of flowering [*FLOWERING LOCUS C* (*FLC*)] is reduced by vernalization and by the action of genes in the autonomous pathway. The latter is so called because its constituent genes are not regulated by external cues such as photoperiod or vernalization. Arrows show promotion of gene expression, T-bars show inhibition of gene expression.

flowering time in plants and that this also acts by regulating one of the pathway integrators (Cerdan and Chory, 2003).

The expression of *FT* and *AGL20* is strongly repressed by *FLOWERING LOCUS C (FLC)*, a MADS-box transcription factor whose expression is promoted by *FRIGIDA (FRI)* and reduced by vernalization. Thus, flowering in unvernallized plants is strongly suppressed, even in long day conditions, by the action of *FLC*. In vernalized plants *FLC* levels are reduced and flowering can then be promoted by exposure to long day conditions through the action of CO on *FT* and *AGL20*. Vernalization also has an effect independent of *FLC* (Michaels and Amasino, 2001) and recent work suggests that this is through the regulation of another member of the MADS-box gene family, *AGL24* (Yu et al., 2002; Michaels et al., 2003a). The expression of pathway integrators *LFY* and *AGL20* is also promoted by GA, but it is unclear whether changes in GA level are important flowering time controls.

FLC expression is reduced in wild type arabidopsis plants during vernalization and remains suppressed after return to warm temperatures. Two *VERNALIZATION* genes (*VRN1* and *VRN2*) have recently been characterized that provide insights into how suppression of *FLC* is maintained. Mutants of *VRN1* or *VRN2* remain late flowering after vernalization because *FLC* levels return to pre-vernalization levels. *VRN1* encodes a general DNA-binding protein (Levy et al., 2002) while *VRN2* encodes a gene of the *polycomb* group believed to stably modify chromatin structure, analogous to the action of *polycomb* group genes in *Drosophila* (Gendall et al., 2001). Modification of chromatin structure is mitotically stable and provides a good explanation of the epigenetic nature of vernalization, of how stable repression of *FLC* is achieved, and how arabidopsis plants “remember” that they have been vernalized (Sung and Amasino, 2004b; Bastow et al., 2004). Interestingly, work on MADS-box genes related to *FLC* suggests that one of them (*MAF2*) has a role in preventing vernalization by short cold periods (Ratcliffe et al., 2003).

A wider role for chromatin modification in flowering is supported by recent work showing that the expression of the pathway integrator *FT* is influenced by *EARLY BOLTING IN SHORT DAYS* (a protein related to chromatin remodelling factors) and by

TERMINAL FLOWER 2 (a heterochromatin protein 1 homolog; Piñero et al., 2003 and Kotake et al., 2003, respectively).

In addition to vernalization, *FLC* transcription is also down regulated by the action of genes in the autonomous pathway (Fig. 1). These include *FCA* (Macknight et al., 1997; Quesada et al., 2003) and *FY* (Simpson et al., 2003) but exactly how these control *FLC* expression is currently unclear.

Although much of the work in arabidopsis has been based on induced mutations, there is also considerable interest in determining the genetic basis of flowering time variation between ecotypes. Comparison of winter and spring annual forms (analogous to winter and spring barley or wheat) shows that the major determinants are mutations at *FLC* or *FRI*. Several different mutations were found when spring types lacking *FRI* function were studied, suggesting that spring habit has evolved several times from a winter annual ancestor (Johanson et al., 2000). Similarly, mutations have been found that affect *FLC*. They reduce, rather than abolish, *FLC* activity by mutation of regulatory sequences (Michaels et al., 2003b). This attenuates *FLC* activity rather than removing it altogether, illustrating the subtlety of natural variation.

The results from work on *FRI* and *FLC* prompts the question of whether genes revealed by a mutation approach are the same genes that are revealed in comparisons of ecotypes. This is being actively studied in arabidopsis, but an interesting observation is that analysis of a population of recombinant inbred lines grown in controlled environment conditions and two field locations revealed several different quantitative trait loci (QTL) for flowering time (Weinig et al., 2002). This demonstrates that a wider range of genes is important than studies under laboratory conditions alone suggest.

4. Comparison of photoperiod response in arabidopsis and rice

Given the evolutionary separation of monocots and dicots, how likely is it that common genetic mechanisms will control flowering in arabidopsis and cereals? This can be understood through comparative analyses. In cereals, understanding the genes that control flowering time is best understood in rice (recently reviewed

by Yano, 2001; Yano et al., 2001; Izawa et al., 2003; Hayama and Coupland, 2003). Rice is very different to arabidopsis in flowering behavior, being a tropical species that shows promotion of flowering in response to short days and lacks a vernalization response. Therefore, it is particularly interesting to see if there is any conservation of flowering time control between these species.

Several flowering time genes have been isolated from rice, principally by positional cloning methods. Control of flowering in response to day length has been analyzed extensively, and interestingly the genes cloned to date relate directly to known genes in arabidopsis. *Hdl*, a major determinant of photoperiod response, was shown to be a homologue of *CO* (Yano et al., 2000). *Hd3a* was shown to be a homologue of *FT* (Kojima et al., 2002) and *Hd6* was shown to encode the alpha subunit of casein kinase II, a gene involved in control of the circadian clock in arabidopsis by phosphorylation of the CCA1 clock component (Takahashi et al., 2001). Rice *se5* was shown to be a homologue of *HY1*, encoding a heme oxygenase required for the synthesis of the chromophore component of phytochromes (Izawa et al., 2000). These results show that components of the photoperiod pathway, at least, are conserved between arabidopsis and rice. But if this is the case, why do these species show differences in response to photoperiod? Recent work in rice has shown that the circadian expression patterns of the rice homologues of *GIGANTEA* (*GI*) and *Hdl* (*CO*) are conserved, but that increased expression results in decreased levels of *FT* transcript. This indicates that the difference between long day and short day plants, or at least between arabidopsis and rice, is due to alteration in the way in which *CO* interacts with *FT* (Izawa et al., 2002; Hayama et al., 2003). In other words, the difference is due to “retuning” of a conserved photoperiod pathway rather than the evolution of completely novel control mechanisms.

5. Photoperiod, vernalization and earliness per se in barley and wheat

The winter annual habit of temperate cereals is superficially more similar to arabidopsis than to more closely related cereals such as rice. However, this

could be the result of convergent evolution as grasses have been suggested to have a tropical origin (Clayton and Renvoize, 1986). If this is correct, independent mechanisms may have evolved to regulate vernalization and long day photoperiod response in temperate cereals. To test this, we can ask whether temperate cereals have *CO*-like genes, whether these are the principal determinants of photoperiod response, and whether *FLC*-like genes control vernalization response.

5.1. Photoperiod response

In barley, two major loci regulating photoperiod response have been identified by genetic analyses. A cross between the winter variety ‘Igri’ and the spring variety ‘Triumph’ showed that in vernalized plants, flowering time under short days was largely controlled by the *Photoperiod-H2* (*Ppd-H2*) locus on the long arm of chromosome 1H while under long day conditions flowering was largely controlled by the *Ppd-H1* (also known as *Eam1*) locus of the short arm of chromosome 2H (Laurie et al., 1995). Major loci controlling photoperiod response have also been mapped to the short arms of the group 2 chromosomes of wheat and comparative mapping using common RFLP markers shows that the wheat and barley genes fall into equivalent intervals, suggesting that they are equivalent (orthologous) genes.

The question was then whether the major determinants of photoperiod response in barley and wheat were *CO* homologues, as in rice. In arabidopsis, *CO* is a member of a gene family comprising 17 members (Robson et al., 2001) that can conveniently be divided into three subgroups. The most *CO*-like (group I) genes are characterized by two B-boxes (regions that allow proteins to interact with each other; Borden, 1998) at the amino-terminus and a highly conserved 43 amino-acid region near the carboxy-terminus termed the CCT domain (Strayer et al., 2000; Robson et al., 2001). The CCT domain is found in all members of the family. Group II genes have a single B-box and group III genes have one B-box and an additional diverged zinc-finger domain. Analysis of cereal ESTs and rice genomic sequence revealed 16 genes that fell into the three subgroups defined in arabidopsis, showing that these subgroups predate monocot/dicot divergence (Griffiths et al., 2003).

CO-like genes were isolated from barley by screening genomic libraries with CCT domain probes from various rice genes and by subsequent rescreening with CCT domains from barley. This showed that rice and barley were similar and that the most CO-like subgroup comprised three genes in arabidopsis (*CO*, *COL1* and *COL2*), one gene in rice (*Hd1*) and two genes in barley (*HvCO1* and *HvCO2*; Griffiths et al., 2003). The genetic maps of wheat or barley and rice can be aligned from the positions of common RFLP markers and gene order is surprisingly well conserved (Kurata et al., 1994; Moore et al., 1995). This allows map location and sequence homology to be combined to assess the correspondence between barley and rice genes. *HvCO1* was located on barley chromosome 7H in a region previously shown to be collinear with the region of rice 6 containing *Hd1*. Sequence homology and comparative genetic mapping therefore confirmed that *HvCO1* and *Hd1* were orthologous genes. *HvCO2* had no counterpart in rice but is found in wheat (Nemoto et al., 2003). Interestingly, when rice plants with a mutated *Hd1* gene were transformed with the wheat gene, the normal rice photoperiod response was restored (Nemoto et al., 2003). Thus, the difference between wheat (promotion of flowering by long days) and rice (promotion of flowering by short days) is not determined by the CO-like genes themselves. The roles of CO-like genes in barley can also be tested using transgenic plants and this is currently under way.

Thus, while *CO/Hd1* homologues clearly exist in barley, and are likely to have roles in the control of flowering, their locations on chromosomes 6H and 7H show that they do not correspond to the major photoperiod loci defined by genetic analysis, which are located on 1H and 2H. There are two possible explanations for this. Temperate cereals may have evolved a new mechanism for controlling photoperiod response or the *Ppd* genes may correspond to other, perhaps as yet uncharacterized, genes in a conserved photoperiod pathway.

As *Ppd-H1* is a major determinant of flowering in barley we still wish to identify and characterize this gene. To do this, we are currently attempting a positional cloning approach in barley. This involves developing high resolution genetic maps to identify closely linked markers that can be used as hybridization probes to isolate clones from large insert libraries. These clones will then be sequenced to assess their

gene content and the individual genes examined in detail. This is a laborious process that can be expedited by use of rice genomic sequence, exploiting the conservation of gene order in the chromosomes of barley and rice. Briefly, cDNA clones mapped in rice were used for mapping in barley. This identified equivalent regions that could then be examined in detail. Additional markers can be made by amplifying sequences from rice but another highly effective method is to identify barley ESTs that match sequences in the corresponding region of rice. The barley sequences are then used as additional markers. This is effective as it preferentially generates markers for the target region.

In the case of *Ppd-H1*, mapping using rice cDNAs as probes showed that the relevant region of barley 2HS was collinear with a region on the end of the long arm of rice chromosome 7 (Dunford et al., 2002). However, a complication of this is that several markers also detected loci on rice chromosome 3. Detailed analysis of recently released rice genomic sequence confirms that these markers are part of an extensive duplication. Mapping in barley and maize showed consistent patterns of duplication, suggesting that the duplication occurred before the divergence of these species from a common ancestor (Fig. 2). This is supported by subsequent work on other duplicated regions in rice (Vandepoele et al., 2003). Because patterns of gene loss from duplicated regions are likely to be different in different lineages, the *Ppd-H1* region of barley 2HS may contain genes currently found on rice chromosomes 7, rice 3 or both. Such duplications are probably common in plant genomes and an understanding of their distribution is important for future uses of comparative mapping. It provides a more detailed understanding of genome evolution and assists in defining the gene content of key regions in other species.

No *Ppd* gene has been reported on wheat, barley or rye group 4 chromosomes or in corresponding regions of rice and maize (Dunford et al., 2002), suggesting that *Ppd-H1* and its wheat equivalents may have evolved after segment duplication and before divergence of the wheat and barley lineages (Fig. 2).

5.2. Vernalization response

Three major loci controlling vernalization response have been identified in barley by genetic analysis. For

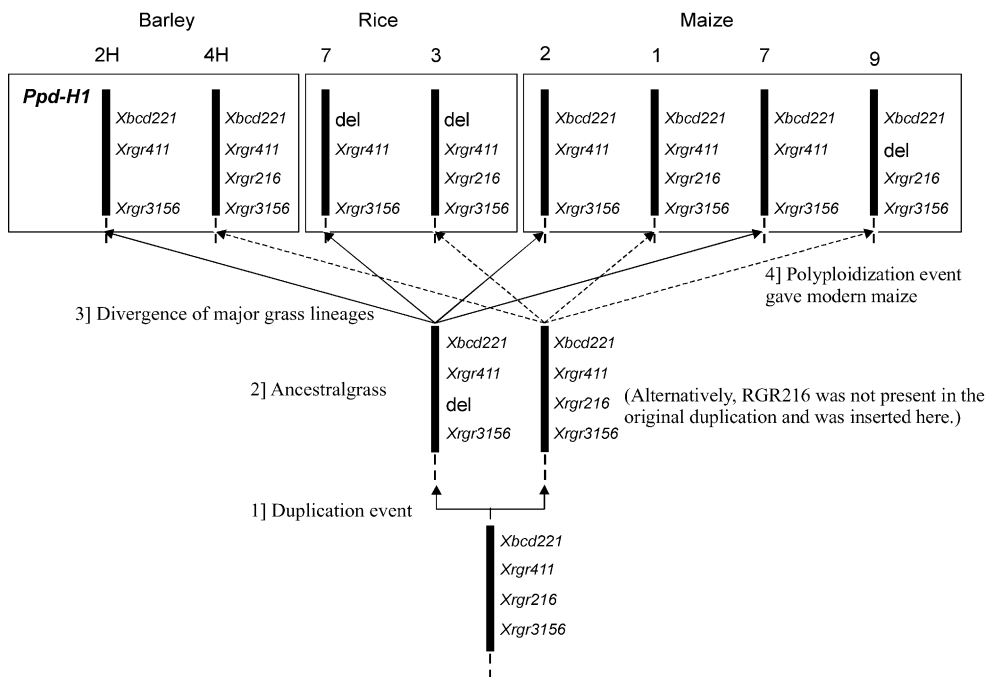


Fig. 2. Model for the evolution of duplicated markers shared by the *Ppd-H1* region of barley chromosome 2HS and barley chromosome 4H together with similar duplications in rice and maize. del indicates genes not detectable by Southern hybridization and probably deleted.

historical reasons, they are variously known as *Spring growth habit* (*Sgh*) or *Vernalization* (*Vrn*) genes. The *Vrn* nomenclature will be used here as this is also used in wheat (*N.B.* although they have the same name these are unrelated to the *Vrn* genes described in arabidopsis). *Vrn-H1* maps to the middle of the long arm of barley chromosome 5H and comparative mapping shows that major vernalization response genes map to equivalent positions in wheat (*Vrn-A1*, *-B1* and *-D1*) and rye (*Vrn-R1*; Laurie, 1997; Börner et al., 1998). For *Vrn-H1* and *Vrn-H3*, “spring” alleles (that allow flowering in the absence of a vernalization treatment) are semi-dominant. *Vrn1* loci in wheat and rye behave similarly. *Vrn3* has not been reported in wheat or rye. For *Vrn-H2* (located at the end of the long arm of barley chromosome 4H), the “spring” allele is recessive. No equivalent variation for *Vrn-H2* alleles is known in the tetraploid pasta wheats or the hexaploid bread wheats but variation is known in diploid einkorn wheat (*T. monococcum*; *Vrn-A^m2*; Dubcovsky et al., 1998). The genetic behaviour of *Vrn-H2* and *Vrn-A2* suggests that they encode a repressor of flowering analogous to *FLC* in arabidop-

sis. However, in contrast to conclusions from the analysis of photoperiod response, no cereal gene corresponding to *FLC* in sequence has been reported to date, nor have homologs of the *FLC* regulators *FRI* or *VRN2*.

Positional cloning in diploid wheat (*T. monococcum* L.) was used to identify the wheat *Vrn-A1* gene (Yan et al., 2003). Comparative mapping and high resolution mapping were used to define an interval that contained two genes, both members of the MADS-box family of transcription factors. One was most similar to the arabidopsis *AGAMOUS-LIKE 2* gene. The second was most closely related to *APETALA1* (*API*) and *FRUITFULL* (*FUL*), two related genes that are involved in the specification of floral meristem identity in arabidopsis. Based on differences in expression, the second gene was identified as *Vrn-A1*. Sequencing of alleles from the winter and spring genotypes used for the mapping population showed that the spring form had a 34 bp deletion in the promoter of the *API*-like gene.

API-like genes have previously been cloned from wheat (Murai et al., 1998) and barley (Schmitz et al.,

2000) but the direct connection to vernalization response had not been made. The same *API*-like gene was also identified by Danyluk et al. (2003) who used differential display to identify genes expressed in spring, but not in winter, wheat varieties and by Trevaskis et al. (2003) in an analysis of cereal MADS-box genes. Danyluk et al. (2003) also showed that the *API*-like gene was regulated by photoperiod. If the wheat gene is indeed a homolog of the arabidopsis *API/FUL* genes, this suggested that different mechanisms of vernalization response have evolved in dicot and monocot winter annuals, consistent with the fact that *FLC* and *FRI* were not found.

The existence of a different mechanism of vernalization response in cereals is strongly supported by recent work on the positional cloning of the *Vrn-A2* gene (Yan et al., 2004). This shows that *Vrn-A2* is distantly related to *CO* and is unrelated to the MADS-box family, to which *FLC* belongs. However, *Vrn-A2* and *FLC* behave similarly in showing reduced expression following extended periods of cold.

The model proposed by Yan et al. (2003, 2004) is that that *Vrn-A2* represses flowering by preventing expression of *Vrn-A1*. Spring forms can be generated either by mutating *Vrn-A2*, so that the repressor is absent or non-functional, or by mutating the *Vrn-A1* promoter so that it cannot be repressed by *Vrn-A2*.

5.3. Earliness per se genes

The vernalization genes discussed above distinguish winter and spring types of cereals. Variation in photoperiod response can be found between or within these classes. In addition, genetic studies commonly reveal the segregation of “earliness” alleles that do not appear to be specifically related to photoperiod or vernalization response. These “earliness” factors may be the major determinants of flowering time variation in crosses within spring or winter types. Various described as earliness per se (*eps*) or narrow sense earliness effects, they have considerable potential for modifying flowering time but are currently poorly understood and deserve increased study.

The effects of *eps* genes are generally less clear than those of the *Ppd* or *Vrn* genes described above, and *eps* loci have most often been detected as quantitative trait loci. They tend to occur in different loca-

tions in different crosses, suggesting that a relatively large number of genes can show *eps* effects, contrasting with the small number of *Ppd* and *Vrn* genes. An interesting question is whether *eps* genes are truly independent of environmental effects or whether they might regulate development in response to different cues such as variation in higher (non-vernalizing) temperatures. An example is a gene on chromosome 1HL of *T. monococcum* which was shown to have different effects on flowering time at 16 and 23 °C (Bullrich et al., 2002). Earliness factors can vary flowering time within spring or winter groups that have the same alleles at the major photoperiod and vernalization response loci and therefore have considerable potential for “tuning” plant development. Further studies of this class of genes will reveal the extent to which that can modulate rates of growth and how they achieve this control.

6. Discussion

6.1. Gene-based models

For technical reasons, genes that regulate development have hitherto been difficult to isolate from temperate cereals. This situation has greatly improved as a result of investment in genetic and genomic resources including large EST collections and genomic libraries. A major challenge now is to enhance methods for studying gene function and progress would be accelerated by the development of internationally coordinated programmes for the development of physical maps and populations of mutations. Once genes are identified, structured germplasm collections will assist searches for novel alleles which can be tested in breeding programmes.

Comparison of cDNA and genomic sequences suggests that between one-half and one-third of the genes in rice have no recognizable counterpart in arabidopsis (Kikuchi et al., 2003), reflecting their long evolutionary separation. Furthermore, temperate cereals have a relatively long separation from rice, perhaps 60 million years or more. Thus, we have to expect that a proportion of the genes in temperate cereals will have diverged either in sequence or function or both. For the many genes that are conserved, judicious use of comparative methods can expedite their analysis in

temperate cereals. Comparative approaches will become progressively easier as more genes are identified in model species and as more sequence information becomes available. The availability of rice genomic sequence also assists positional cloning in temperate cereals by predicting the gene content of collinear regions. Even for genes that are not conserved, rice provides a rich source of markers that assist high resolution mapping and positional cloning.

Studies of individual genes and the analysis of combinations of mutants have enabled detailed models of flowering time control to be developed in arabidopsis (Fig. 1). Such models give a qualitative description of gene interaction that establishes the order of gene action within pathways, the points at which pathways intersect and the molecular mechanisms by which genes act. There is no reason that similar models cannot be developed for cereals.

6.2. Genotype-based whole plant models

Whole plant models are predominantly based on crops and approach from a physiological perspective. They describe growth quantitatively, predicting the stage of development in relation to factors such as photothermal time. It now seems appropriate to consider whether gene and whole plant models can be combined. The potential benefits to crop physiologists would be the greater precision of models that incorporate genotype components. The benefits to geneticists include improved methods of quantifying development and identifying varietal differences that are of particular interest for genetic analysis. Models can also identify stages of development where studies of gene expression, using known genes or microarrays, might be particularly revealing. These ideas are explored in detail in several papers in a recent issue of the *Agronomy Journal* (Hunt et al., 2003; White and Hoogenboom, 2003; Yin et al., 2003).

There are two major challenges for the development of combined models. The first is to identify sufficient of the relevant genes to make the genotype of a given barley or wheat line accurate and complete, at least for the purposes of modelling development. Once genes are identified, the recognition of individual alleles is likely to be straightforward given the continuing innovations in novel high throughput genotyping methods (for example, Hardenbol et al., 2003).

The second problem is to incorporate the complexity of gene \times gene (photoperiod \times vernalization \times *eps*) and gene \times environment interactions. There are several ways in which this might be approached. One is to develop modeling principles using arabidopsis, where the information of the genes that regulate flowering is most extensive (Welch et al., 2003). For crops, the simplest situation would be to model near-isogenic lines that differ in the vernalization or photoperiod response genes that have been identified already, or are likely to be in the near future. This will require additional data on gene expression during the various growth phases of the plants and in different environmental conditions. Crosses between near-isogenic lines can be used to test simple gene \times gene and gene \times environment models. This incremental approach has the advantage of starting with a simple and experimentally tractable situation and it is realistic to think that such experiments could be done within the next 1–5 years. The models can then be expanded as additional genes are identified and as detailed phenotype data is acquired for wider ranges of genotypes and environments. Like the approaches described in Section 6.1, this aspect of flowering time research would benefit from international collaboration to provide high quality phenotype data from a range of genotypes grown in diverse environments.

A potential weakness of the above approach is that it focuses on known genes. Relatively few wheat or barley genotypes have been used for detailed genetic studies and it is likely that many potentially valuable flowering time genes remain to be defined, particularly in the *eps* category. To identify these, and to develop understanding to a level where they can be incorporated into models is a longer-term objective, perhaps in the 5–20 year range.

Incorporating gene effects into crop growth models is a worthwhile objective because it should enable more accurate prediction of plant behaviour and crop yield potential. In particular, it could provide valuable insights into which combinations of alleles favour adaptation to specific environments and how the introduction of new alleles for photoperiod response, vernalization response or earliness per se genes might allow varieties to be adapted to changing environments resulting from climate change.

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References

- Bastow, R., Mylne, J.S., Lister, C., Lippman, Z., Martienssen, R.A., Dean, C., 2004. Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature* 427, 164–167.
- Borden, K.L.B., 1998. RING fingers and B-boxes: zinc-binding protein–protein interaction domains. *Biochem. Cell Biol.* 76, 351–358.
- Börner, A., Korzun, V., Worland, A.J., 1998. Comparative genetic mapping of loci affecting plant height and development in cereals. *Euphytica* 100, 245–248.
- Bowers, J.E., Chapman, B.A., Rong, J.K., Paterson, A.H., 2003. Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. *Nature* 422, 433–438.
- Bullrich, L., Appendino, M.L., Tranquilli, G., Lewis, S., Dubcovsky, J., 2002. Mapping of a thermo-sensitive earliness per se gene on *Triticum monococcum* chromosome 1A^m. *Theor. Appl. Genet.* 105, 585–593.
- Cerdan, P.D., Chory, J., 2003. Regulation of flowering time by light quality. *Nature* 423, 881–885.
- Clayton, W.D., Renvoize, S.A., 1986. *Genera Graminum. Grasses of the world*. Kew Bulletin Additional Series XIII, Her Majesty's Stationary Office, London.
- Danyluk, J., Kane, N.A., Breton, G., Limin, A.E., Fowler, D.B., Sarhan, F., 2003. TaVRT-1, a putative transcription factor associated with vegetative to reproductive transition in cereals. *Plant Physiol.* 132, 1849–1860.
- Devos, K.M., Gale, M.D., 2000. Genome relationships: the grass model in current research. *Plant Cell.* 12, 637–646.
- Dubcovsky, J., Lijavetzky, D., Appendino, L., Tranquilli, G., 1998. Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement. *Theor. Appl. Genet.* 97, 968–975.
- Dunford, R.P., Yano, M., Kurata, N., Sasaki, T., Huestis, G., Rocheford, T., Laurie, D.A., 2002. Comparative mapping of the barley *Ppd-H1* photoperiod response gene region, which lies close to a junction between two rice linkage segments. *Genetics* 161, 825–834.
- Feuillet, C., Keller, B., 2002. Comparative genomics in the grass family: molecular characterization of grass genome structure and evolution. *Ann. Bot.* 89, 3–10.
- Fitter, A.H., Fitter, R.S.R., 2002. Rapid changes in flowering time in British plants. *Science* 296, 1689–1691.
- Fu, X.D., Harberd, N.P., 2003. Auxin promotes *Arabidopsis* root growth by modulating gibberellin response. *Nature* 421, 740–743.
- Fu, X.D., Richards, D.E., Ait-Ali, T., Hynes, L.W., Ougham, H., Peng, J.R., Harberd, N.P., 2002. Gibberellin-mediated proteasome-dependent degradation of the barley DELLA protein SLN1 repressor. *Plant Cell* 14, 3191–3200.
- Gendall, A.R., Levy, Y.Y., Wilson, A., Dean, C., 2001. The *VERNALIZATION 2* gene mediates the epigenetic regulation of vernalization in *Arabidopsis*. *Cell* 107, 525–535.
- Griffiths, S., Dunford, R.P., Coupland, G., Laurie, D.A., 2003. The evolution of *CONSTANS*-like gene families in barley (*Hordeum vulgare*), rice (*Oryza sativa*) and *Arabidopsis thaliana*. *Plant Physiol.* 131, 1855–1867.
- Hardenbol, P., Banér, J., Jain, M., Nilsson, M., Namsaraev, E.A., Karlin-Neumann, G.A., Fakhrai-Rad, H., Ronaghi, M., Willis, T.D., Landegren, U., Davis, R.W., 2003. Multiplexed genotyping with sequence-tagged molecular inversion probes. *Nat. Biotechnol.* 21, 673–678.
- Hayama, R., Coupland, G., 2003. Shedding light on the circadian clock and the photoperiodic control of flowering. *Curr. Opin. Plant Biol.* 6, 13–19.
- Hayama, R., Yokoi, S., Tamaki, S., Yano, M., Shimamoto, K., 2003. Adaptation of photoperiodic control pathways produces short-day flowering in rice. *Nature* 422, 719–722.
- Henderson, I.R., Shindo, C., Dean, C., 2003. The need for winter in the switch to flowering. *Annu. Rev. Genet.* 37, 371–392.
- Hiscock, S.J., McInnis, S.M., 2003. The diversity of self-incompatibility systems in flowering plants. *Plant Biol.* 5, 23–32.
- Hunt, L.A., Reynolds, M.P., Sayre, K.D., Rajaram, S., White, J.W., Yan, W., 2003. Crop modeling and the identification of stable coefficients that may reflect significant groups of genes. *Agron. J.* 95, 20–31.
- Izawa, T., Oikawa, T., Sugiyama, N., Tanisaka, T., Yano, M., Shimamoto, K., 2002. Phytochrome mediates the external light signal to repress *FT* orthologs in photoperiodic flowering of rice. *Genes Dev.* 16, 2006–2020.
- Izawa, T., Oikawa, T., Tokutomi, S., Okuno, K., Shimamoto, K., 2000. Phytochromes confer the photoperiodic control of flowering in rice (a short day plant). *Plant J.* 22, 391–399.
- Izawa, T., Takahashi, Y., Yano, M., 2003. Comparative biology comes into bloom: genomic and genetic comparison of flowering pathways in rice and *Arabidopsis*. *Curr. Opin. Plant Biol.* 6, 113–120.
- Johanson, U., West, J., Lister, C., Michaels, S., Amasino, R., Dean, C., 2000. Molecular analysis of *FRIGIDA*, a major determinant of natural variation in *Arabidopsis* flowering time. *Science* 290, 344–347.
- Kikuchi, S., Satoh, K., Nagata, T., Kawagashira, N., Doi, K., Kishimoto, N., Yazaki, J., Ishikawa, M., Yamada, H., Ooka, H., Hotta, I., Kojima, K., Namiki, T., Ohneda, E., Yahagi, W., Suzuki, K., Li, C.J., Ohtsuki, K., Shishiki, T., Otomo, Y., Murakami, K., Iida, Y., Sugano, S., Fujimura, T., Suzuki, Y., Tsunoda, Y., Kurosaki, T., Kodama, T., Masuda, H., Kobayashi, M., Xie, Q.H., Lu, M., Narikawa, R., Sugiyama, A., Mizuno, K., Yokomizo, S., Niikura, J., Ikeda, R., Ishibiki, J., Kawamata, M., Yoshimura, A., Miura, J., Kusumegi, T., Oka, M., Ryu, R., Ueda,

- M., Matsubara, K., Kawai, J., Carninci, P., Adachi, J., Aizawa, K., Arakawa, T., Fukuda, S., Hara, A., Hashidume, W., Hayatsu, N., Imotani, K., Ishii, Y., Itoh, M., Kagawa, I., Kondo, S., Konno, H., Miyazaki, A., Osato, N., Ota, Y., Saito, R., Sasaki, D., Sato, K., Shibata, K., Shinagawa, A., Shiraki, T., Yoshino, M., Hayashizaki, Y., 2003. Collection, mapping, and annotation of over 28,000 cDNA clones from japonica rice. *Science* 301, 376–379.
- Kojima, S., Takahashi, Y., Kobayashi, Y., Monna, L., Sasaki, T., Araki, T., Yano, M., 2002. *Hd3a*, a rice ortholog of the *Arabidopsis FT* gene, promotes transition to flowering downstream of *Hd1* under short-day conditions. *Plant Cell Physiol.* 43, 1096–1105.
- Kotake, T., Takada, S., Nakahigashi, K., Ohto, M., Goto, K., 2003. *Arabidopsis TERMINAL FLOWER 2* gene encodes a heterochromatin protein 1 homolog and represses both *FLOWERING LOCUS T* to regulate flowering time and several floral homeotic genes. *Plant Cell Physiol.* 44, 555–564.
- Kurata, N., Moore, G., Nagamura, Y., Foote, T., Yano, M., Minobe, Y., Gale, M.D., 1994. Conservation of genome structure between rice and wheat. *Biotechnology* 12, 276–278.
- Laurie, D.A., 1997. Comparative genetics of flowering time in cereals. *Plant Mol. Biol.* 35, 167–177.
- Laurie, D.A., Devos, K.M., 2002. Trends in comparative genetics and their potential impacts on wheat and barley research. *Plant Mol. Biol.* 48, 729–740.
- Laurie, D.A., Pratchett, N., Bezant, J.H., Snape, J.W., 1995. RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. *Genome* 38, 575–585.
- Levy, Y.Y., Mesnage, S., Mynne, J.S., Gendall, A.R., Dean, C., 2002. Multiple roles of *Arabidopsis VRN1* in vernalization and flowering time control. *Science* 297, 243–246.
- Macknight, R., Bancroft, I., Page, T., Lister, C., Schmidt, R., Love, K., Westphal, L., Murphy, G., Sherson, S., Cobbett, C., Dean, C., 1997. *FCA*, a gene controlling flowering time in *Arabidopsis*, encodes a protein containing RNA-binding domains. *Cell* 89, 737–745.
- Maes, T., De Keuleleire, P., Gerats, T., 1999. *Plant tagology*. *Trends Plant Sci.* 4, 90–96.
- Michaels, S.D., Amasino, R.M., 2001. Loss of *FLOWERING LOCUS C* activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell* 13, 935–941.
- Michaels, S.D., Ditta, G., Gustafson-Brown, C., Pelaz, S., Yanofsky, M., Amasino, R.M., 2003a. *AGL24* acts as a promoter of flowering in *Arabidopsis* and is positively regulated by vernalization. *Plant J.* 33, 867–874.
- Michaels, S.D., He, Y., Scortecci, K.C., Amasino, R.M., 2003b. Attenuation of *FLOWERING LOCUS C* activity as a mechanism for the evolution of summer-annual flowering behavior in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 100, 10102–10107.
- Moore, G., Devos, K.M., Wang, Z., Gale, M.D., 1995. Grasses, line up and form a circle. *Curr. Biol.* 5, 737–739.
- Mouradov, A., Cremer, F., Coupland, G., 2002. Control of flowering time: interacting pathways as a basis for diversity. *Plant Cell* S111–S130 (Suppl.).
- Murai, K., Murai, R., Takumi, S., Ogiwara, Y., 1998. Cloning and characterization of cDNAs corresponding to the wheat MADS box genes. In: Slinkard, A.E. (Ed.), *Proceedings of the Ninth International Wheat Genetic Symposium*. University Extension Press, University of Saskatchewan, pp. 89–94.
- Nemoto, Y., Kisaka, M., Fuse, T., Yano, M., Ogiwara, Y., 2003. Characterization and functional analysis of three wheat genes with homology to the *CONSTANS* flowering time gene in transgenic rice. *Plant J.* 36, 82–93.
- Paterson, A.H., Bowers, J.E., Burow, M.D., Draye, X., Elsik, C.G., Jiang, C.X., Katsar, C.S., Lan, T.H., Lin, Y.R., Ming, R.G., Wright, R.J., 2000. Comparative genomics of plant chromosomes. *Plant Cell* 12, 1523–1539.
- Peng, J., Carol, P., Richards, D.E., King, K.E., Cowling, R.J., Murphy, G.P., Harberd, N.P., 1997. The *Arabidopsis GAI* gene defines a signalling pathway that negatively regulates gibberellin responses. *Genes Dev.* 11, 3194–3205.
- Peng, J.R., Richards, D.E., Hartley, N.M., Murphy, G.P., Devos, K.M., Flintham, J.E., Beales, J., Fish, L.J., Worland, A.J., Pelica, F., Sudhakar, D., Christou, P., Snape, J.W., Gale, M.D., Harberd, N.P., 1999. ‘Green revolution’ genes encode mutant gibberellin response modulators. *Nature* 400, 256–261.
- Piñero, M., Gómez-Mena, C., Schaffer, R., Martínez-Zapater, J.M., Coupland, G., 2003. *EARLY BOLTING IN SHORT DAYS* is related to chromatin remodeling factors and regulates flowering in *Arabidopsis* by repressing *FT*. *Plant Cell* 15, 1552–1562.
- Quesada, V., Macknight, R., Dean, C., Simpson, G.G., 2003. Autoregulation of *FCA* pre-mRNA processing controls *Arabidopsis* flowering time. *EMBO J.* 22, 3142–3152.
- Ratcliffe, O.J., Kumimoto, R.W., Wong, B.J., Riechmann, J.L., 2003. Analysis of the *Arabidopsis MADS AFFECTING FLOWERING* gene family: *MAF2* prevents vernalization by short periods of cold. *Plant Cell* 15, 1159–1169.
- Robson, F., Costa, M.M.R., Hepworth, S.R., Vizir, I., Pineiro, M., Reeves, P.H., Putterill, J., Coupland, G., 2001. Functional importance of conserved domains in the flowering-time gene *CONSTANS* demonstrated by analysis of mutant alleles and transgenic plants. *Plant J.* 28, 619–631.
- Schmitz, J., Franzen, R., Ngyuen, T.H., Garcia-Maroto, F., Pozzi, C., Salamini, F., Rohde, W., 2000. Cloning, mapping and expression analysis of barley MADS-box genes. *Plant Mol. Biol.* 42, 899–913.
- Simpson, G.G., Dean, C., 2002. *Arabidopsis*, the Rosetta stone of flowering time? *Science* 296, 285–289.
- Simpson, G.G., Dijkwel, P.P., Quesada, V., Henderson, I., Dean, C., 2003. *FY* is an RNA 3′ end-processing factor that interacts with *FCA* to control the *Arabidopsis* floral transition. *Cell* 113, 777–787.
- Simpson, G.G., Gendall, A.R., Dean, C., 1999. When to switch to flowering. *Annu. Rev. Cell Dev. Biol.* 15, 519–550.
- Soltis, P.S., Soltis, D.E., Chase, M.W., 1999. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature* 402, 402–404.
- Sorrells, M.E., La Rota, M., Bermudez-Kandianis, C.E., Greene, R.A., Kantety, R., Munkvold, J.D., Miftahudin, M.A., Ma, X.F., Gustafson, P.J., Qi, L.L.L., Echallier, B., Gill, B.S., Matthews, D.E., Lazo, G.R., Chao, S.M., Anderson, O.D., Edwards, H.,

- Linkiewicz, A.M., Dubcovsky, J., Akhunov, E.D., Dvorak, J., Zhang, D.S., Nguyen, H.T., Peng, J.H., Lapitan, N.L.V., Gonzalez-Hernandez, J.L., Anderson, J.A., Hossain, K., Kalavacharla, V., Kianian, S.F., Choi, D.W., Close, T.J., Dilbirligi, M., Gill, K.S., Steber, C., Walker-Simmons, M.K., McGuire, P.E., Qualset, C.O., 2003. Comparative DNA sequence analysis of wheat and rice genomes. *Genome Res.* 13, 1818–1827.
- Strayer, C., Oyama, T., Schultz, T.F., Raman, R., Somers, D.E., Mas, P., Panda, S., Kreps, J.A., Kay, S.A., 2000. Cloning of the *Arabidopsis* clock gene *TOC1*, an autoregulatory response regulator homolog. *Science* 289, 768–771.
- Sung, S.B., Amasino, R.M., 2004a. Vernalization and epigenetics: how plants remember winter. *Curr. Opin. Plant Biol.* 7, 4–10.
- Sung, S.B., Amasino, R.M., 2004b. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature* 427, 159–164.
- Takahashi, Y., Shomura, A., Sasaki, T., Yano, M., 2001. *Hd6*, a rice quantitative trait locus involved in photoperiodic sensitivity, encodes the α subunit of protein kinase CK2. *Proc. Natl. Acad. Sci. U.S.A.* 98, 7922–7927.
- Trevaskis, B., Bagnall, D.J., Ellis, M.H., Peacock, W.J., Dennis, E.S., 2003. MADS box genes control vernalization-induced flowering in cereals. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13099–13104.
- Valverde, F., Mouradov, A., Soppe, W., Ravenscroft, D., Samach, A., Coupland, G., 2004. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Science* 303, 1003–1006.
- Vandepoele, K., Simillion, C., Van de Peer F Y., 2003. Evidence that rice and other cereals are ancient aneuploids. *Plant Cell* 15, 2192–2202.
- Welch, S.M., Roe, J.L., Dong, Z., 2003. A genetic neural network model of flowering time control in *Arabidopsis thaliana*. *Agron. J.* 95, 71–81.
- Weinig, C., Ungerer, M.C., Dorn, L.A., Kane, N.C., Toyonaga, Y., Halldorsdottir, S.S., Mackay, T.F.C., Purugganan, M.D., Schmitt, J., 2002. Novel loci control variation in reproductive timing in *Arabidopsis thaliana* in natural environments. *Genetics* 162, 1875–1884.
- White, J.W., Hoogenboom, G., 2003. Gene-based approaches to crop simulation: past experiences and future opportunities. *Agron. J.* 95, 52–64.
- Wikström, N., Savolainen, V., Chase, M.W., 2001. Evolution of the angiosperms: calibrating the family tree. *Proc. R. Soc. Lond. B* 268, 2211–2220.
- Yan, L., Loukoianov, A., Tranquilli, G., Helguera, M., Fahima, T., Dubcovsky, J., 2003. Positional cloning of the wheat vernalization gene *VRN1*. *Proc. Natl. Acad. Sci. U.S.A.* 100, 6263–6268.
- Yan, L., Loukoianov, A., Blechl, A., Tranquilli, G., Ramakrishna, W., SanMiguel, P., Bennetzen, J.L., Echenique, V., Dubcovsky, J., 2004. The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. *Science* 303, 1640–1644.
- Yano, M., 2001. Genetic and molecular dissection of naturally occurring variation. *Curr. Opin. Plant Biol.* 4, 130–135.
- Yano, M., Katayose, Y., Ashikara, M., Yamanouchi, U., Monna, L., Fuse, T., Baba, T., Yamamoto, K., Umehara, Y., Nagamura, Y., Sasaki, T., 2000. *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. *Plant Cell* 12, 2473–2483.
- Yano, M., Kojima, S., Takahashi, Y., Lin, H., Sasaki, T., 2001. Genetic control of flowering time in rice, a short-day plant. *Plant Physiol.* 127, 1425–1429.
- Yanovsky, M.J., Kay, S.A., 2003. Living by the calendar: how plants know when to flower. *Nat. Rev. Mol. Cell Biol.* 4, 265–275.
- Yin, X., Stam, P., Kropff, M.J., Schapendonk, H.C.M., 2003. Crop modeling, QTL mapping, and their complementary role in plant breeding. *Agron. J.* 95, 90–98.
- Yu, H., Xu, Y.F., Tan, E.L., Kumar, P.P., 2002. AGAMOUS-LIKE 24, a dosage-dependent mediator of the flowering signals. *Proc. Natl. Acad. Sci. U.S.A.* 99, 16336–16341.