



The Pseudo-Response Regulator Ppd-H1 Provides Adaptation to Photoperiod in Barley

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with species richness. If complementarity, facilitation, and sampling effects do contribute to positive effects of diversity on carbon storage on BCI, as often has been observed in simpler communities (1–5), then actual carbon storage in species-poor communities may be lower than our models predict. Indeed, high diversity within the BCI plot may reduce losses of carbon to density-dependent effects of herbivores and pathogens (33, 34).

Species extinctions are rarely random but rather are driven by the interaction between species traits and environmental change. Our results show that tropical forest carbon storage depends on species composition and on the mode and manner in which species are lost. By extension, carbon storage in reforested landscapes depends especially on the functional diversity of the available species pool. Because variability decreases with species richness, and because extinction scenarios differ widely in magnitude and direction, management options that favor high diversity will maximize predictability for tropical forest carbon storage and sequestration.

We have examined only one of many ecosystem services provided by tropical forests. Extinction scenarios that maximize carbon storage may minimize other services such as flood protection, nutrient retention, cultural services, pollination, biological control, and provisioning of fruits, nuts, and bush meat (10). Human

domination of terrestrial and aquatic landscapes has made us increasingly dependent on a reduced number of species to provide critical ecosystem services. Given uncertainty in both the nature of extinction and the variety of ecosystem services required for human well-being, we may best be able to meet these demands by maximizing the pool of species on which we depend.

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Table S1
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The Pseudo-Response Regulator *Ppd-H1* Provides Adaptation to Photoperiod in Barley

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Plants commonly use photoperiod (day length) to control the timing of flowering during the year, and variation in photoperiod response has been selected in many crops to provide adaptation to different environments and farming practices. Positional cloning identified *Ppd-H1*, the major determinant of barley photoperiod response, as a pseudo-response regulator, a class of genes involved in circadian clock function. Reduced photoperiod responsiveness of the *ppd-H1* mutant, which is highly advantageous in spring-sown varieties, is explained by altered circadian expression of the photoperiod pathway gene *CONSTANS* and reduced expression of its downstream target, *FT*, a key regulator of flowering.

Plants have evolved sophisticated controls to ensure that flowering occurs when there is the greatest chance of pollination, seed de-

velopment, and seed dispersal. Usually this involves restricting flowering to a specific time of year. To achieve this, many plants use photoperiod as an environmental cue to regulate development. The timing of flowering has important impacts on crop yield, and the modification of responses to environmental cues by human selection has been central to the success and spread of agriculture.

The control of flowering by photoperiod is understood best in the long-day (LD) dicot *Arabidopsis* and the short-day (SD) monocot cereal rice. In *Arabidopsis*, expression of *GIGANTEA* (*GI*) and *CONSTANS* (*CO*) is regulated by the circadian clock such that coincidence of the *CO* expression peak with light only occurs in LD conditions. Light-stabilized *CO* protein is a transcription factor inducing downstream genes, including *FLOWERING LOCUS T* (*FT*) (1, 2).

In rice, analyses of natural variation showed that *Heading date1* (*Hd1*), a major determinant of photoperiod response, is an ortholog of *CO* (3), that *Hd3a* is an ortholog of *FT* (4), and that *GI* is also conserved (5). However, the interaction of *Hd1* with *FT* is altered such that *FT* expression is inhibited in LDs (2, 5). The rice *Ehd1* gene also controls photoperiod response but has no direct counterpart in *Arabidopsis* and regulates *FT* independently of *Hd1* (6). Photoperiod response in rice therefore has conserved and novel aspects compared with *Arabidopsis*, but in both species increased *FT* expression is crucial to the induction of flowering. Genes controlling photoperiod response in temperate cereals such as barley (*Hordeum vulgare*) have not been identified previously.

Barley varieties can be broadly classified as winter or spring types. Winter (fall-sown) bar-

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leys require vernalization and usually show strong promotion of flowering in response to LDs. This is typical of *H. spontaneum*, the wild progenitor of barley, suggesting that this is the ancestral condition. Spring (spring-sown) barleys lack vernalization requirement and show weak or strong response to LDs depending on whether they have been selected for long or short growing seasons, respectively. In long growing seasons, as in Western Europe and much of North America, reduced response to photoperiod allows spring-sown plants to extend the period of vegetative growth and accumulate additional biomass that supports higher yields.

The major determinant of LD response in barley is the *Photoperiod-H1* (*Ppd-H1*) locus (7, 8). The late-flowering *ppd-H1* allele is recessive (Fig. 1A), suggesting that reduced response results from a mutation that impairs gene function. *Ppd-H1* does not correspond to either of the barley *CO*-like genes (*HvCO1* and *HvCO2*) (9), showing that different major determinants of photoperiod adaptation have been selected in barley and rice.

We identified *Ppd-H1* by positional cloning, using colinearity of the barley *Ppd-H1* region with rice and *Brachypodium* (10). Fine-scale mapping using lines derived from an Igr1 (*Ppd-H1*) and Triumph (*ppd-H1*) cross (Fig. 1, B and C) enabled a physical map of the *Ppd-H1* region to be developed (Fig. 1D). Recombinants defined a region containing a single gene that was a pseudo-response regulator (*PRR*) most similar overall to *Arabidopsis* *PRR7* (fig. S1). *PRR* proteins are characterized by two conserved regions, a pseudoreceiver domain with similarities to bacterial two-component signaling systems and a CO, CO-like, and TOC1 (CCT) domain that is also found in the CO family (11). The barley *PRR* gene was amplified by polymerase chain reaction (PCR) from Igr1 and two *H. spontaneum* accessions (JIC-1894 and JIC-1947) crossed with Igr1 and shown to have the *Ppd-H1* allele. Morex, which provided the bacteria artificial chromosome (BAC) sequence, was crossed with Igr1 and shown to have the *ppd-H1* allele. Other *ppd-H1* lines sequenced were Triumph, Golden Promise, and Optic. This revealed 23 polymorphisms, of which 7 were single nucleotide polymorphisms (SNPs) that produced amino acid changes distinguishing *Ppd-H1* and *ppd-H1* alleles (1, 12, 15, 20, 21, 22, and 23 in Fig. 1E). Regions containing these SNPs were sequenced from a further eight *H. spontaneum* accessions known to be early flowering in LDs and nine barley varieties previously classified as early or late flowering in LDs (table S3). In the extended set, four SNPs (1, 15, 22, and 23) remained completely associated with *Ppd-H1* or *ppd-H1* alleles (Fig. 2). Three were in regions of low conservation with rice and *Arabidopsis* (fig.

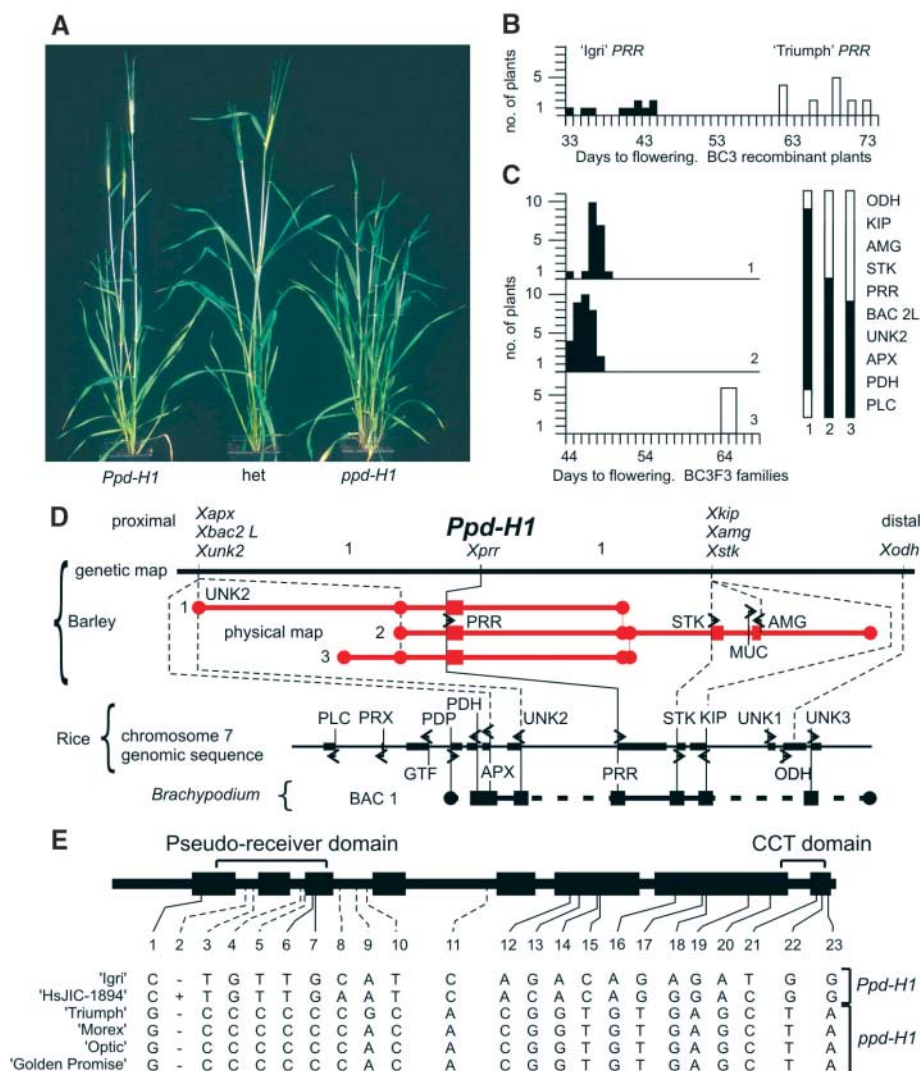


Fig. 1. Flowering phenotypes, genetic and physical mapping of the *Ppd-H1* locus, and sequence variation between alleles. (A) Phenotypes of homozygous *Ppd-H1* (left), heterozygous *Ppd-H1/ppd-H1* (middle), and homozygous *ppd-H1* (right) plants. (B) Flowering time (days to awn emergence) of BC₃ (backcross 3) recombinant plants. (C) Flowering time of selected families with their respective homozygous recombinant chromosomes (right) where black segments have Igr1 alleles and white segments have Triumph alleles. (D) Genetic and physical maps of the *Ppd-H1* region in barley and colinear regions in rice and *Brachypodium*. The barley genetic map has its basis in 2336 Igr1 × Triumph BC₃ plants and shows the numbers of recombinants in the intervals flanking the *Ppd-H1* locus. Key barley BAC clones are drawn to the same scale as the rice genomic sequence. Circles are BAC end sequences. BAC 2 was completely sequenced (AY943294). Rice chromosome 7 genomic sequence (AP005199) has annotated genes (listed in table S1) as black rectangles. The *Brachypodium* BAC shows gene content, with the solid line indicating genes with confirmed order and orientation. (E) Structure of *Ppd-H1* (the eight exons are shown as black rectangles) and positions of the 23 polymorphisms identified in fully sequenced *Ppd-H1* and *ppd-H1* alleles. 1 and 3 to 23 were SNPs, whereas 2 was a 5-base pair (bp) insertion/deletion polymorphism (indel). Polymorphisms in exons are indicated by solid lines.

S1), but the fourth produced a Gly-to-Trp change in the CCT domain affecting a residue that is conserved in all CCT domain genes identified to date (fig. S2) and that is the most likely causal basis of the *ppd-H1* mutation. The CCT domain mutation was a G-to-T change, which removed a *Bst*UI restriction site, providing a simple PCR-based assay for the *ppd-H1* allele (fig. S3).

Arabidopsis *prp7* mutants showed delayed flowering in LDs but showed no significant

effect in SDs (12, 13), similar to the effect of *ppd-H1* (7, 8). *prp7* mutants also lengthen the period of clock-mediated leaf movement (14) and affect the expression of clock components *CCA1* and *LHY*, implicating the gene in the phasing of the clock in relation to light (15, 16). These results suggested that *ppd-H1* might affect flowering by altering the expression of photoperiod pathway genes that have circadian control. To test this, we compared gene expression in Triumph (*ppd-H1*) with a Tri-

Fig. 2. Genotypes of seven barley varieties and 10 *H. spontaneum* accessions carrying the *Ppd-H1* allele and seven barley varieties carrying the *ppd-H1* allele at the seven SNPs that produce amino acid changes in the predicted protein. Polymorphism positions are shown in Fig. 1E. HsJIC-164 has a 9-bp deletion spanning SNP20. Amino acids that distinguish the alleles are shown above and below in bold: A, Ala; G, Gly; H, His; P, Pro; Q, Gln; S, Ser; T, Thr; and W, Trp.

	SNP 1	SNP 12	SNP 15	SNP 19	SNP 20	SNP 22	SNP 23	
	H		P			G	A	<i>Ppd-H1</i>
'Igri'	C	A	C	G	A	G	G	
'Dairokkaku'	C	C	C	G	G	G	G	
'Funza'	C	C	C	G	G	G	G	
'Hayakiso'	C	C	C	A	G	G	G	
'Haruna Nijo'	C	C	C	G	G	G	G	
'Nigrinudum'	C	C	C	G	G	G	G	
'Steptoe'	C	C	C	G	G	G	G	
HsJIC-1894	C	A	C	G	A	G	G	
HsJIC-1947	C	C	C	G	G	G	G	
HsJIC-16	C	C	C	G	G	G	G	
HsJIC-52	C	C	C	G	G	G	G	
HsJIC-144	C	C	C	G	G	G	G	
HsJIC-164	C	C	C	G	-	G	G	
HsJIC-209	C	C	C	G	G	G	G	
HsJIC-1284	C	C	C	G	G	G	G	
HsJIC-1377	C	C	C	G	G	G	G	
HsJIC-2602	C	C	C	G	G	G	G	
'Triumph'	G	C	T	A	G	T	A	<i>ppd-H1</i>
'Morex'	G	C	T	A	G	T	A	
'Barke'	G	C	T	A	G	T	A	
'Blenheim'	G	C	T	A	G	T	A	
'Kym'	G	C	T	G	G	T	A	
'Golden Promise'	G	C	T	A	G	T	A	
'Optic'	G	C	T	A	G	T	A	
	Q		S			W	T	

umph line into which the *Ppd-H1* allele from Igri had been introgressed.

In LDs *Ppd-H1* was expressed predominantly in the early part of the day (Fig. 3A), similar to the expression patterns of *Arabidopsis PRR7* and related genes in rice. An entrainment experiment confirmed that the barley gene was under circadian control, as previously shown for *Arabidopsis* and rice *PRR* genes (17, 18). Although *PRR* genes are implicated in clock function we detected no significant difference between *Ppd-H1* and *ppd-H1* plants in the expression of *Ppd-H1* itself or the barley homolog of *GI* (*HvGI*) (Fig. 3B). However, two barley *CO*-like genes (*HvCO1* and *HvCO2*) were affected. *ppd-H1* plants showed reduced expression of *HvCO1* at 8 and 12 hours (Fig. 3C), and *HvCO2* was more significantly affected with reduced expression throughout the light period and a delay in the expression peak of about 4 hours (Fig. 3D). By analogy with *Arabidopsis*, the reduced expression of *HvCO1* and *HvCO2* during the latter part of the light period in *ppd-H1* plants should reduce *FT* expression. We first tested whether barley *CO* genes behaved like *CO* in *Arabidopsis* by analyzing their expression under SDs [8 hours of light (fig. S4)]. *HvCO2* expression was lower at the start of the day but peaked at a similar time in SD and LD, whereas *HvCO1* peaked at 20 hours in SDs. The later peak of *HvCO1* expression in SDs and the higher expression of both genes at dawn in LDs were similar to *CO* in *Arabidopsis* (19). We then isolated

a barley *FT* (*HvFT*) gene that is orthologous to rice *Hd3a* (10). Expression of *HvFT* was consistently very low in SDs (figs. S4 and S5) and was markedly lower in *ppd-H1* in LDs (Fig. 3E). The late-flowering phenotype of *ppd-H1* can therefore be explained through known photoperiod mechanisms by a reduction in *FT* expression resulting from altered circadian timing of *CO* expression. The lack of effect on *HvGI* expression suggests that the *ppd-H1* mutation does not have a strong disruptive effect on clock function or that the barley mutation affects an output linking the circadian clock to the *HvCO* genes. However, additional effects such as a direct role in *HvFT* expression cannot be ruled out.

Previous work (14) surveying 150 *Arabidopsis* accessions identified *PRR* genes as candidates for quantitative trait loci that provide adaptive variation by modulating circadian timing. Clock period length was correlated with latitude of origin, suggesting that these genes provide adaptive variation in photoperiod response. The identification of *Ppd-H1* as a *PRR* gene shows that the *PRR* family is of general importance for adaptation to natural and agricultural settings. Notably, comparative mapping shows that the major wheat photoperiod response genes are in colinear regions on the group 2 chromosomes (20) and that *Hd2* is in the colinear region of rice chromosome 7 (21), making these attractive targets for further analysis. The availability of *Ppd-H1* will provide greater understanding of the ways

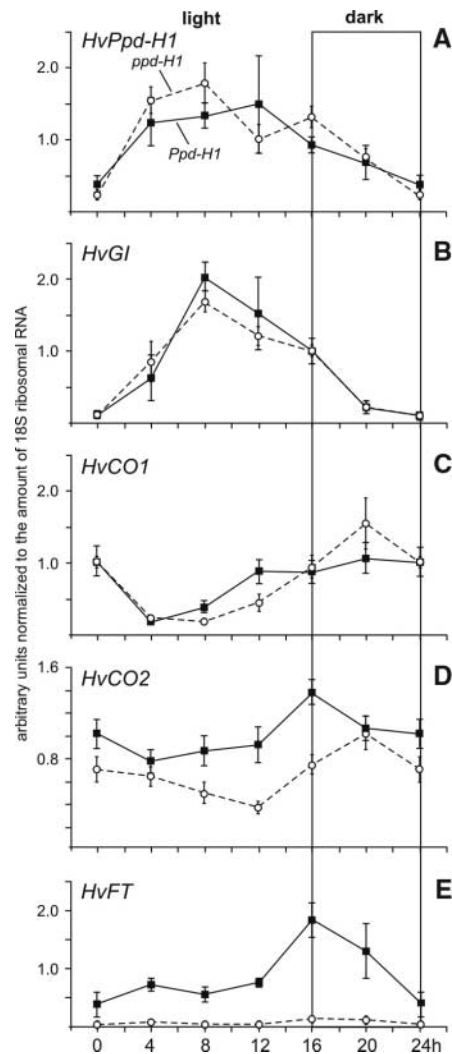


Fig. 3. Gene expression patterns in *Ppd-H1* (■, solid line) and *ppd-H1* (○, dashed line) plants grown in LD (16 hours of light) conditions and sampled at 4-hour intervals over a 24-hour period: (A) *HvPpd-H1*, (B) *HvGI*, (C) *HvCO1*, (D) *HvCO2*, and (E) *HvFT*. Means and standard deviations from three independent experiments are shown expressed in arbitrary units normalized against the amount of 18S rRNA (10). Primers and primer positions are given in table S4. Error bars indicate SEM.

in which cereal development is regulated by environmental cues, allowing plant breeders to tailor crops to specific environments and to adjust varieties to new conditions arising from climate change.

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