Journal of Plant Biology, June 2007, 50(3) : 241-248

REVIEW

Diversified Mechanisms for Regulating Flowering Time in a Short-Day Plant Rice

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Flowering in rice is influenced by not only endogenous factors that comprise an autonomous pathway, but also environmental effects, such as photoperiod, water availability, and temperature just before floral initiation. Recent molecular genetics studies have elucidated the functional roles of genes involved in the photoperiod pathway, e.g., photoreceptors, circadian clock components, and short-day (SD) promotion factors. Although these molecular players are well conserved between rice and *Arabidopsis*, their actual genetic functions are distinct. This is exemplified by *Hd1* (a *CO* counterpart) and phytochromes, in particular, rice *PHYA*. *Hd1* has a dual role in regulating flowering time and the expression of *Hd3a* (an *FT* counterpart) repression under long-day (LD) conditions while promotion under SDs. Models have been proposed to explain these photoperiod-dependent antagonistic activities. Some regulatory factors are present in only one of the model systems, e.g., *FLC* in *Arabidopsis* or *Ehd1* in rice. Furthermore, epistatic relationships vary among such flowering regulators as *Hd3a* (*FT*), *OsMADS50* (*SOC1*), and *OsMADS14* (*AP1*). Further experiments to probe these differences will be essential to enlarging our understanding of the diversified flowering regulation mechanisms in rice.

Keywords: Arabidopsis, diversification, flowering time, rice, short-day plant

FACTORS AFFECTING FLOWERING TIME IN RICE

To survive and evolve, plants must transmit their genetic traits to their progeny. Even though asexual reproduction is employed in some species, all land plants have sexual mechanisms that can adapt to changing environments. Because flowering is the key developmental process in achieving successful reproduction, elaborate mechanisms for regulating flowering time are essential to survival. Depending on habitat, each plant has a particular mechanism that integrates numerous internal and external signals. Among the 2867 Korean angiosperm species, flowering patterns range in diversity according to seasonal changes (Kang and Jang, 2004). Their internal signals may include growth stage and levels of phytohormones, e.g., gibberellin (GA), while abiotic (photoperiod, temperature, nutrients) and biotic (competition, pollinators, herbivores) conditions could be the external signals. Although flowering has been extensively studied in angiosperms (Ausin et al., 2005; Corbesier and Coupland, 2006; Imaizumi and Kay, 2006; Roux et al., 2006), most of that research has focused on *Arabidopsis*, a long-day (LD) plant that blooms in spring. However, here we will review the flowering signal pathways in rice, a short-day crop that blooms in late summer.

The ancestor of cultivated rice (*Oryza sativa*) is believed to have originated in the southeastern part of the Asian continent (Hoshikawa, 1989). Over the course of cultivation and migration, the ancestral type differentiated into three groups: javanica, indica, and japonica. To survive at different latitudes and in regions ranging from tropical to temperate, rice developed photoperiod-sensing mechanisms. Although it is an obligate short-day (SD) plant, it has now diverged to obligate, facultative, and neutral SD genotypes. Of the hundreds of varieties studied at the International Rice Research Institute, about 30% are obligate, with the remaining ones displaying either a facultative or neutral response to controlled LD conditions (Vergara and Chang, 1985). Whereas more than 90% of the obligate varieties are cultivated in tropical areas, most others in the temperate or subtropical areas are facultative, exhibiting a quantitative response to short days and even flowering under continuous light (Yu and Yao, 1967). Those genotypes that either are cultivated in high-latitude temperate regions (Okumoto et al., 1996; Fujino, 2003) or which are early-season cultivars in low-latitude temperate areas are considered day-neutral (Shen et al., 1965).

Through crossings between 'Nipponbare' and 'Kasalath', 15 flowering time QTLs (*Hd1* through *Hd14*, and including *Hd3a* and *Hd3b*) have been identified (Yano et al., 2001). NILs have been developed for these QTLs, and used to fully characterize the responses to photoperiod (10 and 14 h). Three types of photoperiod-sensitive genes have been elucidated: the first group (*Hd1* and *Hd2*) responds to LDs and SDs in opposite manners while those in the second group (*Hd3b*, *Hd4*, *Hd5*, *Hd6*, *Hd9*, etc.) are stimulated only by LDs (Lin et al., 2000, 2002, 2003; Yamamoto et al., 2000; Monna et al., 2002), and *Hd3a* (in the third group) is induced only under SDs (Monna et al., 2002).

In rice, the temperature just before floral initiation may strongly affect flowering time (Vergara and Lilis, 1968; Shibata et al., 1973; Yin et al., 1997). This influence is stronger in photoperiod-sensitive cultivars, suggesting that temperature has an effect on photoinduction in addition to its role as a general modifier of developmental rate (Yin et al., 1997). Certain photoperiod-sensitive QTLs, i.e., *Hd1* and *Hd2*, are associated with this thermal response, consistent with the fact that the photoinduction process is sensitive to temperature (Nakagawa et al., 2005). *Hd9* is also related to this response, even though it is not correlated with photoperiod sensitivity (Lin et al., 2002; Nakagawa et al., 2005).

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Applying molecular genetics approaches with these temperature-sensitive genes will be valuable in elucidating the role of temperature in the regulation of flowering time. Finally, flowering is also associated with the availability of water, especially at latitudes where monsoons provide predictable rainfall at certain times of the year (Maheswaran et al., 2000).

Considerable genetics evaluations in *Arabidopsis* have enabled researchers to identify many of the genes that respond to photoperiod, light quality, vernalization, ambient temperature, and GA. Likewise, the rice genome contains almost all of the homologous genes, except *FLC* and *FRI*, that are involved in this vernalization in *Arabidopsis*. Although most function in a similar manner in rice, some, especially those active in photoperiod sensitivity act differently.

GENERAL MODELS EXPLAINING DAY-LENGTH RECOGNITION IN PLANTS

To flower at the appropriate time, plants must perceive changes in day length. An external coincidence model, first proposed by Erwin Bünning, is currently the most consistent with the available genetic evidence (Bünning, 1936; Pittendrigh and Minis, 1964). In that model, light has dual functions: 1) resetting of the circadian clock to establish the photo-inducible phase and 2) an external signal. The clock entrained by light generates a rhythm that is approximately 24 h long and sensitive to light at a certain phase. Consequently, if a plant is grown under a specific day length that causes it to be exposed to light at this particular phase, then flowering is either induced if the plant shows an LD response (such as with *Arabidopsis*), or repressed if the plant exhibits an SD response (such as with rice). In contrast, an internal coincidence model proposes that the floral response occurs under conditions in which two distinct circadian rhythms produce a harmony that recognizes day length. Two differentially entrained rhythms are brought into the same phase under a day length that promotes flowering but, when under any other day length, these two rhythms will be out of phase. Even though studies of photoperiodism in insects support this model (Nunes and Saunders, 1999), detailed analyses have not yet been carried out with plants.

Therefore, the photoperiodic flowering pathway can be separated into two functional domains: a circadian clock and a circadian-regulated day length measurement mechanism. Plants defective in this pathway do not demonstrate a day length-specific acceleration of flowering. These mutants are said to be photoperiod-insensitive, flowering at the same time whether under LDs or SDs. Izawa et al. (2002) have applied this external coincidence model to rice, based on results that indicate Hd1 protein levels correspond to the photoinducible phase, whereas the active Pfr form of phytochromes corresponds to the external signal.

PHOTORECEPTORS

To sense the diversity of light wavelengths and intensities, and to entrain circadian rhythm, plants use small families of specialized photoreceptors, the major ones being the phytochromes and cryptochromes. Molecular phylogenetics analyses indicate that the angiosperm phytochrome gene family is composed of four subfamilies -- *PHYTOCHROME A* (*PHYA*), *PHYB*, *PHYC/F*, and *PHYE* (Alba et al., 2000). Rice has only three genes, *PHYA*, *PHYB*, and *PHYC* (Takano et al., 2005), whereas *Arabidopsis* has all five, because *PHYD* has been further derived from an ancestral *PHYB* gene by a recent gene duplication event (Clack et al., 1994).

A general effect of phytochromes in flowering can be inferred from mutations in *PHOTOPERIODSENSITIVITY5* (Se5). This gene encodes a protein similar to *Arabidopsis* HY1, a heme oxygenase, which participates in biosynthesis of phytochrome chromophore. The *se5* mutants exhibit severe early flowering, both in LD and SD (Izawa et al., 2000). Similarly, *hy1* mutants manifest a severe early flowering phenotype independent of photoperiod (Goto et al., 1991). These results demonstrate that phytochromes are an essential photoreceptor for the regulation of day-length responses, and that those receptors generally function to repress flowering in both rice and *Arabidopsis*.

Roles for rice phytochromes have been analyzed using single and double mutants (Takano et al., 2005). The rice *phyB* mutants flower earlier than the wild type under both LDs and SDs, which is expected based on data published for the *Arabidopsis phyB* mutant (Childs et al., 1997). However, under extended dark periods (8-h L/16-h D) or end-of-day FR treatments in SD (10-h L/14-h D), both the wild type and the *phyB* mutants flower simultaneously. These treatments may remove the functional PHYB and make Hd1 fully active to promote flowering. Analyses of *phyC* monogenic and *phyB phyC* double mutants have demonstrated that *PHYC* along with *PHYB* is required for delaying floral initiation in response to LD, but has no effect on flowering time in SD. In *Arabidopsis*, a monogenic mutation in *PHYB* or *PHYC* also causes early flowering under inadequate photoperiods (Monte et al., 2003). Nevertheless, *PHYB* functioning is dominant over *PHYC* in *Arabidopsis* while the contributions of *PHYB* and *PHYC* are equal in rice.

As long as both *PHYB* and *PHYC* are induced normally, *PHYA* has little effect on the determination of flowering time, but in the absence of *PHYB* or *PHYC*, this contribution of *PHYA* to delayed flowering is greatly increased in LD. This suggests that *PHYA* acts at different points along the LD pathway than do *PHYB* and *PHYC* in order to suppress floral induction in response to long days. Under SDs, the *phyA* mutant shows slightly later flowering compared with the wild type, regardless of the presence of *PHYC*. The *phyA phyB* double mutants, which almost entirely lack all three phytochromes, flower significantly later than the wild type or any of the other *phy* mutants under SD conditions. These results show that *PHYA* functions quite differently between rice and *Arabidopsis*, especially in combination with other phytochromes. In *Arabidopsis*, the flowering time of *phyA phyB* double mutants is intermediate between those of single mutants, demonstrating additivity or antagonism between these phytochromes (Neff and Chory, 1998). The *phyA phyC* double mutation causes late flowering, suggesting that *PHYA* and *PHYC* play redundant roles in promoting flowering under LDs (Monte et al., 2003).

Whereas the *Arabidopsis* genome contains two copies of the blue-light receptors *CRY1* and *CRY2*, the rice genome has three, named *OsCRY1a*, *OsCRY1b*, and *OsCRY2* (Hirose et al., 2006). Among them, OsCRY2 protein is negatively regulated by both blue- and red-light treatments; lines with suppressed levels of *OsCRY2* mRNA flower later than the WT in both SD and LD (Hirose et al., 2006). In *Arabidopsis*, the effects of blue light on flowering time acceleration are mainly mediated by *CRY2*, but also partially by *CRY1* (Lin, 2002; Mockler et al., 2003).

CIRCADIAN CLOCK

The circadian clock system is often divided into three general parts (Dunlap, 1999). A central oscillator is the core of the system, responsible for driving 24-h rhythms. This oscillator is entrained to day-night or temperature cycles through a mechanism involving input pathways that transmit light or temperature signals to the core oscillator. Output pathways are controlled by the oscillator and represent a wide range of biochemical and developmental pathways. The control of flowering by day length presumably is regulated by one or more of these output branches. In this way, the oscillator can determine the activity of diurnal rhythms in output genes, and these genes can set the light-sensitive phase for triggering the floral transition.

To respond to environmental cues, a circadian clock should adjust its endogenous cycle to precisely 24 h. Fluence-rate dependence of free-running periods (FRPs) and phase response by a light pulse are involved in these entrainment mechanisms (Johnson, 1999; Roenneberg and Merrow, 2000). Physiological studies using the CAB1R luciferase reporter system have shown that both factors are implicated in such entrainment mechanisms in rice (Sugiyama et al., 2001). Based on the fact that the FRP in continuous darkness after light/dark entrainment is nearly equal to that in constant illumination of a middle fluence-rate, phase re-setting upon light/dark transitions in daily cycles may be sufficient to explain the environment mechanisms in rice (Sugiyama et al., 2001).

At the molecular level, comparison of the genome sequences between rice and *Arabidopsis* suggests that molecules comprising the circadian clock are widely conserved. For example, *CCA1/LHY*- and *TOC1/ELF4*-like genes, putative components of the core oscillator that composes the negative feedback loop, are found in the rice genome (Izawa et al., 2003). Actually, *OsLHY* exhibits circadian rhythms with a phase similar to that of *LHY* from *Arabidopsis* (Izawa et al., 2002). Furthermore, genes similar to *Arabidopsis ZTL* and *ELF3*, involved in light input to the clock, and a gene homologous to *Arabidopsis GI* that mediates clock output to the flowering promotion pathway, also occur in the rice genome (Izawa et al., 2003).

In *Arabidopsis*, numerous reports have been made of the role for a circadian clock in flowering time regulation. Two basic observations support the existence of circadian rhythms. First, most mutants affected in their clock show flowering time alterations in response to photoperiod (Schaffer et al., 1998; Wang and Tobin, 1998; Strayer et al., 2000; Doyle et al., 2002). Second, most *Arabidopsis* genes involved in the photoperiod flowering pathway exhibit circadian regulation of their expression (Fowler et al., 1999; Kardailsky et al., 1999; Suarez-Lopez et al., 2001). In rice, Takahashi et al. (2001) have used map-based cloning for *Heading date6* (*Hd6*) to identify a gene with close homology to *CK2*, a kinase-regulating circadian clock functioning in *Arabidopsis*. *OsGI*, the rice *GI* homolog, regulates flowering time in response to photoperiodic conditions; lines with reduced levels of *OsGI* mRNA show delayed flowering under inductive SDs whereas in LD they flower only slightly later than the wild type (Hayama et al., 2003). Furthermore, not only *OsGI* but also key photoperiodic flowering regulators, e.g., *Heading date1* (*Hd1*), *Heading date3a* (*Hd3a*), and *Early heading date 1* (*Ehd1*), have expression patterns that are circadian clock-dependent. Thus, the clock components in rice share similar functional roles with *Arabidopsis* to control flowering in response to photoperiod.

The action of photoreceptors in photoperiodic flowering can be mediated by either a circadian clock-dependent or -independent pathway. Detailed analyses using an *se5* mutant have shown that phytochromes mediate an external light signal to repress mRNA expression of major floral-inducer genes, but may not be necessary for the entrainment of circadian clocks under LD and SD conditions (Izawa et al., 2002). Other photoreceptors also will redundantly work to entrain the circadian clock. This phenomenon is commonly observed in *Arabidopsis* (Millar et al., 1995; Yanovsky et al., 2000). Therefore, considering the fact that *se5* plants are completely insensitive to photoperiod (Izawa et al., 2000), an alternative phototransduction pathway, not mediated by circadian clocks, must exist in rice.

PHOTOPERIOD-DEPENDENT ANTAGONISTIC ROLES OF *HD1*

Map-based cloning of major QTLs responsible for heading date in rice have identified *Hd1* and *Hd3a*, which are respective homologs of the *Arabidopsis CONSTANS* (*CO*) (Yano et al., 2000) and *FLOWERING LOCUS T* (*FT*) (Kojima et al., 2002). Recent studies in *Arabidopsis* suggest that the interaction between circadian rhythms and light signaling may occur at the level of *CO* transcript and CO protein stability. While large amounts of *CO* mRNA can be measured only during the night under SDs, high *CO* levels occur at the end of the day in LD when plants can be exposed to light. In contrast, the CO protein level is strongly detectable only in LD at the end of the day. This is probably due to its instability under darkness. Consequently, *CO* functions to induce the expression of *FT* only in LD. The *FT* gene was originally identified using a late-flowering mutant; its transcript is induced specifically in LD (Samach et al., 2000).

Rice *Hd1* also shows a circadian rhythm, peaking at dusk in LD and in the middle of the night in SD (Izawa et al., 2002; Kojima et al., 2002; Hayama et al., 2003). Furthermore, as with *FT* in *Arabidopsis*, *Hd3a* acts as a flowering activator under SD-inductive conditions (Kojima et al., 2002). However, in contrast to *Arabidopsis CO*, *Hd1* has a dual role in regulating *Hd3a* expression and flowering time -- repression under LDs and promotion in SD (Yano et al., 2000). Thus, the high *Hd1* expression noted at dusk in LD results in a signal that inhibits *Hd3a* expression and represses flowering. Alternatively, high *Hd1* expression in the middle of the night in SD generates a signal that enhances *Hd3a* expression and activates flowering.

Although the photoperiod-dependence of Hd1 protein levels has not been reported, like CO, Hd1 may also be regulated post-transcriptionally. However, it is speculated that Hd1 may not be degraded through the proteasome-dependent degradation pathway because the protein works under both photoperiod conditions while CO only operates in LD. One possible explanation for this antagonistic role is that other light-regulated components participate in this process (Fig. 1). At least 16 *CONSTANS-Like* (*COL*) genes exist in the rice genome; some show circadian rhythms similar to *Hd1* while others have contrasting rhythmic expression (Shin et al., 2004). In winter wheat, *VRN2* encoding a COL protein acts as a repressor of flowering in plants that have not been vernalized (Yan et al., 2004). Hd1 may perform opposite roles through its interaction with different COL proteins. In LD, Hd1 may cooperate with VRN2-like protein to inhibit flowering while forming a protein complex with other COL proteins, thereby acting as a flowering activator in SD (Fig. 1A).

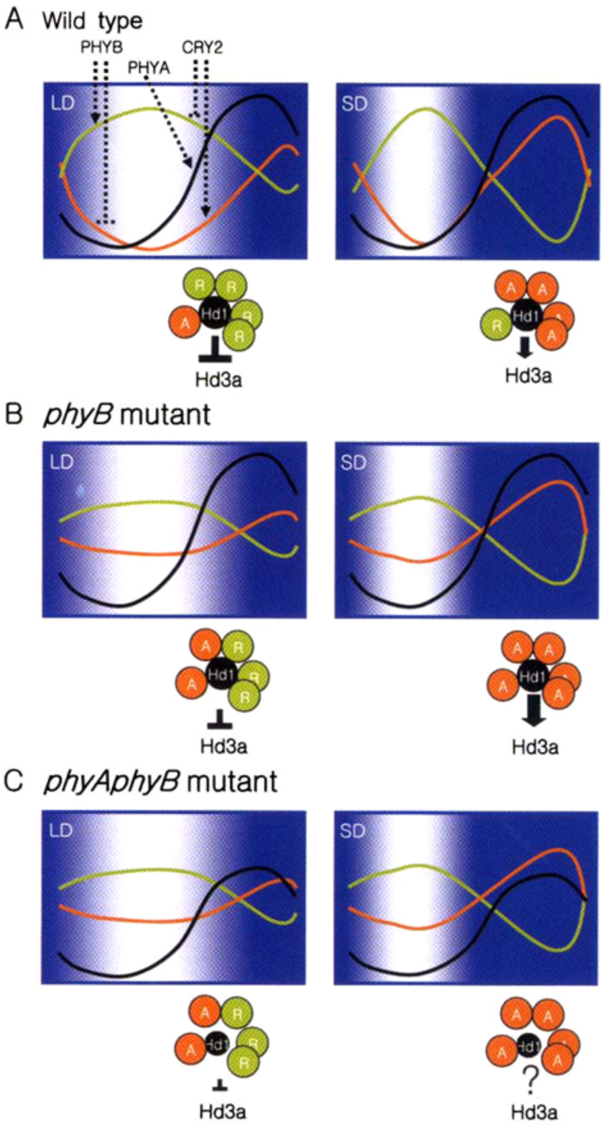

Figure 1. Model explaining antagonistic roles of *Hd1*. We proposed a model (**A**) in which Hd1 forms a preferential complex with a repressor in LD or with an activator in SD. In *osphyB* mutants (**B**), the repressor level is decreased, thereby inducing early flowering. We suggest that *PHYA* stabilizes Hd1 as in *Arabidopsis*. If so, we can then explain why the *phyA phyB* double mutant shows more significant early flowering than the single *phyB* mutant under LDs (**C**, left). However, in following this assumption, we cannot explain the later-flowering phenotype of the double mutant over the single *phyA* mutant under SDs (**C**, right). Black, Hd1; blue, repressor; red; activator.

Furthermore, rice Hd1 protein may be regulated by photoreceptors in a manner different from that for *Arabidopsis*. For example, *phyB* mutations in the latter cause high accumulations of CO protein during the daytime, indicating that PHYB promotes the reduction of CO protein especially in the early morning (Valverde et al., 2004). However, rice PHYB seems not to decrease levels of Hd1 protein because elevated amounts during the day induce late flowering in LD. Actually, the rice *phyB* mutant promotes early flowering under both SD and LD conditions (Takano et al., 2005). *OsPHYB* might activate a flowering repressor or inhibit a flowering activator, perhaps cooperating with *Hd1* (Fig. 1B). If so, in an *osphyB* mutant background, *Hd1* should activate flowering regardless of photoperiod. In *Arabidopsis*, both PHYA and cryptochromes appear to stabilize CO protein at the end of the light period because mutations in these photoreceptors cause reductions in CO protein abundance during the day time in LD (Valverde et al., 2004). Likewise, OsPHYA may stabilize Hd1 protein (Fig. 1A). If true, this explains why *osphyA* mutants develop early flowering in LD under an *osphyB* mutant background (Fig. 1C, decreased *Hd1* level in LD induces early flowering) but are late-flowering in SD (decreased *Hd1* level in SD induces late flowering). However, it is still confusing why the *phyA phyB* double mutant flowers later than the single *phyA* mutant in SD.

Taking another perspective, the opposing roles of *Hd1* may not arise from differences in the composition or stability of the protein complex (Fig. 1B). The post-transcriptional modification status of Hd1, e.g., phosphorylation, may be switched by different qualities of external signals. In *Caenorhabditis elegans*, the same transcriptional complex can induce or inhibit the transcription of genes directly, depending on external signals (Eastburn and Han, 2004).

SD-PROMOTION PATHWAY

In *Arabidopsis*, the LD-promotion pathway is mainly mediated by *CO*, *FT*, and *SOC1*. There, *SOC1* acts as a flowering inducer via converging photoperiod, autonomous, vernalization, and GA pathways (Lee et al., 2006). *FT* also functions as a flowering activator, not only in the photoperiod pathway but also in the autonomous and vernalization pathways (Samach et al., 2000; Blazquez et al., 2003). *FT* and *SOC1*, identified as downstream targets of *CO*, may have a parallel relationship (Onouchi et al., 2000; Samach

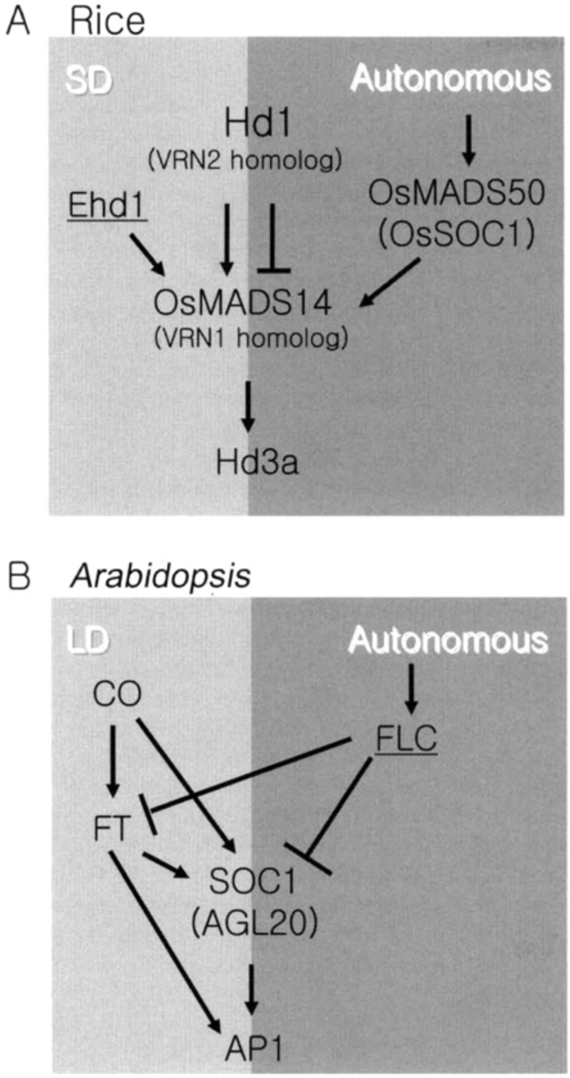

Figure 2. Different epistatic relationships among flowering regulators have been suggested between rice and *Arabidopsis*. (**A**) In rice, the AP1 homolog *OsMADS14* appears to mediate the SD-promotion signal from *Hd1* to *Hd3a*, while it integrates LD signals from *Hd1* and *OsMADS50*. A similar epistatic relationship between *OsMADS14* and *Hd1* has been elucidated in winter wheat, where *VRN1* promotes flowering that is under the control of *VRN2*. We propose that the autonomous signal mediated by *OsMADS50* may compete with the repressive signal transferred through *Hd1*. (**B**) In *Arabidopsis*, the LD-promotion pathway is mainly mediated by *CO*, *FT*, and SOC1, while the autonomous pathway is integrated into *FLC*. Although *FT* and *SOC1* were first identified as downstream targets of *CO*, and a parallel relationship has been suggested, other lines of evidence show that a genetic hierarchy exists between *FT* and *SOC1*.

et al., 2000). However, other lines of evidence raise the possibility that some sort of genetic hierarchy exists between *FT* and *SOC1* (Fig. 2B). Expression of the latter is affected by the loss of *FT* functioning, while activation-tagged *FT* mutants induce *SOC1* upregulation (Schmid et al., 2003; Michaels et al., 2005; Moon et al., 2005). In comparison, *FT* expression remains unaffected by either losses or gains in *SOC1* functioning. All of these results suggest that, although *FT* appears to be a primary target of *CO*, these two integrators are not simply parallel, at least within the photoperiod pathway.

As mentioned, molecules corresponding to *CO* and *FT* are well conserved in rice, and they act on the SD-promotion pathway. However, the role of the rice *SOC1* homolog *OsMADS50* (*OsSOC1*) seems to be diversified. Regarding flowering time, *OsMADS50* acts as a flowering activator similar to *SOC1* because ectopic expression causes dramatic early flowering at the regenerating callus stage, whereas T-DNA insertional mutants show considerable late flowering, being delayed about a month in the paddy field (Lee et al., 2004). However, a reversed genetic hierarchy between *FT* and *SOC1* appears to exist in rice because mutations in *OsMADS50* cause reduced levels of *Hd3a* transcripts (Lee et al., 2004) (Fig. 2A). Compared with *Hd3a*, higher expression levels of *OsMADS50* are measured at the seedling stage, thereby supporting this assumption.

The epistatic relationship between the AP1-group MADS-box gene and the FT-like gene seems to be different in rice than in *Arabidopsis*, in which *AP1* determines floral meristem identity in the downstream of *FT*. Rice has four members homologous to *AP1*. One, *OsMADS14*, appears to function as a major flowering activator in the upstream of *Hd3a*. Ectopic expression of *OsMADS14* causes significant early flowering at the callus stage (Jeon et al., 2000), and *Hd3a* is markedly upregulated in transgenic plants (unpublished data). *OsMADS14* transcript is more abundant in mature leaves than in young leaves (Jang et al., 2002). The wheat MADS-box gene *VRN1*, most homologous to *OsMADS14*, promotes flowering under the control of *VRN2*, the *Hd1* homolog in wheat (Danyluk et al., 2003; Trevaskis et al., 2003). These reports demonstrate that *OsMADS14* mediates the SD-promotion signal from *Hd1* to *Hd3a* (Fig. 2A).

Interestingly, an alternative rice-specific SD-promotion pathway exists that functions independently of *Hd1*. *Ehd1*, which encodes a B-type response regulator, promotes flowering by inducing the expression of *Hd3a*, *FTL1*, and *OsMADS14* (Doi et al., 2004). *FTL1* is a close *Hd3a* homolog that performs a role similar to *Hd3a* (Doi et al., 2004). This indicates that a novel two-component signaling cascade may be intrinsically involved in the photoperiodic flowering pathway in rice (Fig. 2A).

AUTONOMOUS PATHWAY

Because rice flowers even under continuous LD conditions (14 h light/10 h dark), there should exist autonomous promotion pathways that overcome the repression mediated by photoreceptors and some innate negative regulators. In rice, *OsMADS50* seems to work as a major flowering activator on the autonomous pathway, as demonstrated by *osmads50* mutants that develop considerable late flowering (Lee et al., 2004). Compared with the transgenic controls, RNAi transgenic plants also flower 1~2 months later when grown in the paddy under long days. Therefore, we propose that the flower-promoting signal mediated by *OsMADS50* may compete with the flowering-repression signal generated by *OsGI* and *Hd1* (Fig. 2A). In the absence of *Hd1*, *OsMADS50* induces the expression of *Hd3a*, *OsMADS14*, and *OsMADS1* and, consequently, early flowering occurs (An and An, 2000; Lee et al., 2004). However, *OsMADS50* is not a solitary flowering inducer in LD because *osmads50* knockout mutant plants do eventually flower.

In *Arabidopsis*, *FLC* integrates autonomous and vernalization pathways, and the converged signal is delivered to *SOC1*. However, the rice genome does not contain *FLC* homologs, and the autonomous pathway appears to directly integrate into *OsMADS50*. However, we do not rule out the possibility of a flowering repressor that functionally corresponds to *FLC* in rice. This is because ectopic expression of *Arabidopsis FLC* results in late flowering by suppressing *OsMADS50* expression at the onset of floral initiation (Tadege et al., 2003). Based on the fact that *FLC* and *SOC1* are regulated by chromatin remodeling factors (Noh and Noh, 2006), *OsMADS50* and some unknown inducers or repressors are also likely regulated by chromatin modifiers. For example, rice genes that might be putative targets are those that encode proteins similar to the VIN3 complex repressing *FLC* expression, the PAF1 complex activating *FLC* expression, *MSI1* activating *SOC1* expression, and an SWI/SNF ATPase Homolog *AtBRM* repressing the levels of *CO*, *FT*, and *SOC1* transcripts.

Most of the known heading-time loci in rice control photoperiod sensitivity; only a few, such as *Ef1*, are responsible for the vegetative growth period (Yokoo and Kikuchi, 1982; Sato et al., 1988). Characterization of those vegetative-stage loci will further elucidate the autonomous pathway in rice.

NIGHT BREAK EFFECTS

The night break (NB) effect on flowering has long been known, being first described by Hamner and Bonner (1938). Its inhibition of short-day-plant development is best evidenced by very short exposure to illumination during the night (Thomas and Vince-Prue, 1997). In contrast, the promotional effect of NB in LD plants has been reported in a limited number of species, and longer exposure to light is generally required (Thomas and Vince-Prue, 1997). For instance, in *Arabidopsis*, clear stimulation of floral initiation is observed when at least 1 h of light is applied every day, in the middle of the dark period, until the onset of flowering (Goto et al., 1991). However, for rice, 10 min of light exposure in the middle of a 14-h night causes an obvious delay in flowering (Ishikawa et al., 2005). Compared with the numerous NB investigations conducted at the physiological level, very little is known about this phenomenon at the molecular level. Recent, intensive NB experiments in rice have provided valuable clues at the molecular level (Ishikawa et al., 2005). A single NB event can strongly suppress the mRNA of *Hd3a* without affecting the mRNAs of *OsGI* and *Hd1*. The *osphyB* mutation abolishes this NB effect on flowering, indicating mediation by *OsPHYB*. It is speculated that *Hd3a* suppression by NB is coordinated via two pathways -- *Hd1*-dependent and-independent -- but it is unclear whether the suppression mechanism of *PHYB* in LD can be applied to NB effects as well.

CONCLUDING REMARKS

Comparison of the flowering signals between short-day rice and long-day *Arabidopsis* have revealed that, although these two plant systems have several identical steps, significant functional divergences have occurred. In particular, the absence of *FLC* and *FRI* homologs in the rice genome indicates that these genes were introduced to the *Arabidopsis* genome during its adaptation to cold temperatures. However, this does not insure the absence of a vernalization pathway in rice because that genome carries a set of genes homologous to the wheat genes responsible for vernalization. Rice QTL analyses have dissected several loci that respond to temperature just before floral initiation, suggesting that this species has a temperature-sensing mechanism integrated into its floral induction pathway. In *Arabidopsis*, ambient temperature affects flowering time by regulating the expression of *FT* mRNA mediated by *FVE* and *FCA* (Blazquez et al., 2003). Therefore, applying the *Arabidopsis* model to rice will be interesting.

Genes encoding photoreceptors, circadian clock components, and photoperiod promotion/ repression pathways are well conserved between rice and *Arabidopsis*. However, the actual genetic functions differ between the two, as exemplified by phytochromes and *Hd1*. Furthermore, the epistatic relationships of flowering regulators are probably different. Although significant progress has been made toward determining the regulatory elements that control flowering time, a large number of genes have not yet been identified in rice. Finding those missing links and elucidating the relationships among regulatory elements will provide further understanding of the molecular mechanisms that dictate flowering in rice, the best model system for a short-day crop.

ACKNOWLEDGEMENTS

We apologize to colleagues for not citing all relevant papers due to space constraints. This work was supported, in part, by grants from the Crop Functional Genomic Center, the 21st Century Frontier Program (Grant CG1111), Korea; from the Biogreen 21 Program, Rural Development Administration, Korea; and from the Korea Science and Engineering Foundation (KOSEF) through the National Research Laboratory Program funded by the Ministry of Science and Technology (M10600000270-06J0000-27010).

Received March 6, 2007; accepted March 21, 2007.

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