Genetic Control of Flowering Time in Rice, a Short-Day Plant[1]

Masahiro Yano*, Shoko Kojima[2], Yuji Takahashi, Hongxuan Lin[3], and Takuji Sasaki

Department of Molecular Genetics, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305–8602, Japan (M.Y., T.S.); Institute of the Society for Techno-innovation of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki 305-0854, Japan (S.K., Y.T.); and Bio-oriented Technology Research Advancement Institution, Omiya, Saitama 331-8537, Japan (H.L.)

Endogenous genetic factors and environmental signals control the time of flowering in plants. One of the environmental signals is photoperiod. Genetic control mechanisms for the photoperiodic response of flowering of long-day plants (LDPs) have been extensively analyzed through the use of Arabidopsis as a model plant (for review, see Coupland, 1998; Levy and Dean, 1998; Samach and Coupland, 2000). In contrast, mechanisms in short-day plants (SDPs) remain unclear, although many physiological studies have been performed on SDPs, such as *Pharbitis nil* (for review, see Lumsden, 1998). Recent progress in genome analysis has provided a new strategy for analyzing the genetic control of flowering in rice (*Oryza sativa*; SDP). Several studies have demonstrated that the structure of genes involved in the photoperiodic response of flowering in rice show remarkable similarity to those in Arabidopsis.

NATURAL VARIATIONS: A NEW RESOURCE FOR GENETIC ANALYSIS OF FLOWERING IN RICE

In rice, genetic analyses of flowering time (often called heading date) have been performed on mutants and natural variants. Several genes involved in the photoperiodic response (photoperiod sensitivity) have been identified (Yokoo et al., 1980; Yamagata et al., 1986; Yokoo and Okuno, 1993; Okumoto and Tanisaka, 1997). A series of nearly isogenic lines (NILs) for several photoperiod sensitivity genes have been developed to facilitate genetic analysis of flowering time in rice (Yamagata et al., 1986). However, the nature of the quantitative inheritance of flowering time has prevented us from performing more detailed analyses, including analysis of epistatic interactions and determination of chromosomal locations of genes. In the last decade, the progress in development of DNA markers made quantitative trait locus (QTL) analysis possible to clarify the number and nature of the genes controlling flowering time in rice (Yano and Sasaki, 1997).

We have performed a QTL analysis of heading date using several types of progeny derived from a single cross between rice cv Nipponbare (*japonica*) and rice cv Kasalath (*indica*) and have identified 14 QTLs controlling flowering time in rice (Fig. 1). Five QTLs, *Hd1* through *Hd5,* have been mapped based on analysis of the F_2 population (Yano et al., 1997), and an additional three QTLs, *Hd7*, *Hd8*, and *Hd11*, have been detected by using BC_1F_5 lines (Lin et al., 1998). In addition, other loci, *Hd6*, *Hd9*, *Hd10*, *Hd12*, *Hd13*, and *Hd14*, have been detected only when we used advanced backcross progeny, such as BC_3F_2 or BC_4F_2, but not F_2 or BC_1F_5 (Yamamoto et al., 2000; Lin et al., 2002; M. Yano, unpublished data).

The development of NILs by marker-assisted selection, in which a small chromosomal segment including the detected QTL of donor variety Kasalath was substituted into the Nipponbare genetic background, has provided many advantages for the genetic analysis of flowering time in rice (for review, see Yano and Sasaki, 1997). For example, the QTL-NILs can be used in the characterization of the photoperiodic response, epistatic interaction analysis, and fine genetic linkage mapping for target QTLs. The QTLs were classified into two groups based on the response of the QTL-NILs to photoperiod. Five QTLs, *Hd1*, *Hd2*, *Hd3*, *Hd5*, and *Hd6*, were found to confer the photoperiod sensitivity (Lin et al., 2000; Yamamoto et al., 2000; M. Yano, unpublished data). By the genetic analysis using QTL-NILs, the existence of an epistatic interaction between *Hd1* and *Hd3* was clarified. It was also suggested that the Kasalath allele of *Hd3* itself does not affect photoperiod sensitivity, but that it is involved in enhancement of expression of the Nipponbare alleles of photoperiod sensitivity QTLs, *Hd1* and *Hd2* (Lin et al., 2000). In addition, epistatic interaction between *Hd2* and *Hd6* was clearly detected in the analysis of the advanced progeny. The

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[2] Present address: College of Bioscience and Biotechnology, Chubu University, 1200 Matsumoto, Kasugai 487–8501, Japan.

[3] Present address: Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, People's Republic of China.

* Corresponding author; e-mail: myano@nias.affrc.go.jp; fax: 81–298–38–7468.

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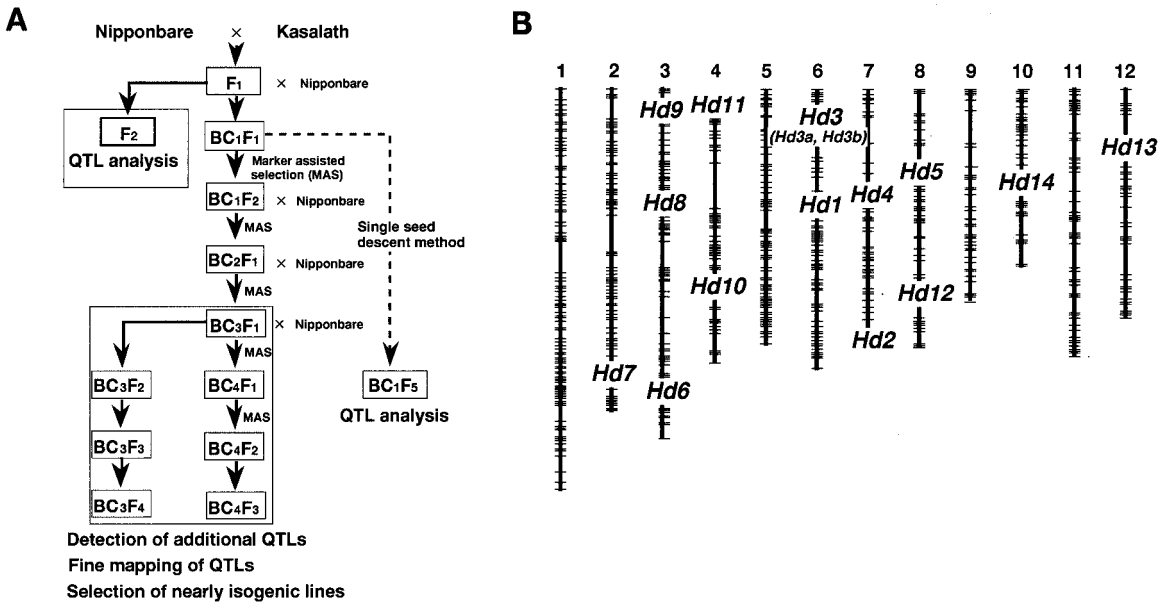

Figure 1. Plant materials used for the detection of QTLs and chromosomal locations of QTLs controlling flowering time. A, Mapping populations derived from a cross between Nipponbare and Kasalath. QTL mapping was performed by using F_2, BC_1F_5, BC_3F_2, and BC_4F_2 lines. Fine mapping and selection of NILs were conducted by using advanced backcross progeny. B, High-density RFLP linkage map showing chromosomal locations of QTLs (*Hd1–Hd14*) for flowering time.

effect of the Kasalath allele of *Hd6* could be observed only in the presence of the Nipponbare allele of *Hd2* (Yamamoto et al., 2000).

QTL-NILs could also be used for the fine mapping of target QTLs. Five QTLs, *Hd1*, *Hd2*, *Hd3*, *Hd6*, and *Hd9*, were mapped precisely on the genetic linkage map as single Mendelian factors (Yamamoto et al., 1998, 2000; Lin et al., 2002; H.X. Lin and M. Yano, unpublished data). Moreover, high-resolution mapping enabled us to dissect two tightly linked loci, *Hd3a* and *Hd3b*, in the *Hd3* region (Fig. 1; Monna et al., 2002). Analysis of the photoperiodic response in NILs of *Hd3a* and *Hd3b* revealed that the Kasalath allele of *Hd3a* promotes flowering under short-day (SD) conditions, and that the Kasalath allele of *Hd3b* delays late flowering under long-day (LD) and natural field conditions (Monna et al., 2002). Together, it is clearly demonstrated that genetic control mechanisms of flowering in rice could be dissected into each component by a series of genetic analyses of flowering date based on the QTL analysis.

MOLECULAR ANALYSIS OF GENES INVOLVED IN PHOTOPERIODIC RESPONSE

A major QTL, *Hd1*, which controls response to photoperiod, was cloned by means of a map-based cloning strategy (Yano et al., 2000). *Hd1* is an orthologue of *CO* (constans) in Arabidopsis (Putterill et al., 1995) and encodes a protein with the structure of a zinc finger domain and a nuclear localization signal. In addition, structural analysis demonstrated that the major gene controlling the response to photoperiod, photoperiod sensitivity 1 (*Se1*), is allelic to *Hd1*. The genetic study demonstrated that *Hd1* may function differently under SD and LD conditions to promote of flowering in the SD condition and inhibit it in the LD condition (Table I; Lin et al., 2000). Genetic linkage mapping and transgenic analysis clearly proved this bifunctional nature of *Hd1* expression (Yano et al., 2000). It is noteworthy that, under LD conditions, *Hd1* inhibits flowering of rice, whereas *CO* promotes flowering of Arabidopsis. This suggests that those genes may regulate the target genes in an opposite

Table I. *Genes identified that control flowering time in rice*

Gene	Effect on Flowering Time[a]	Putative Function	Arabidopsis Orthologue	Reference
Hd1	Early flowering in SD and late flowering in LD	Transcription factor	*CO*	Yano et al. (2000)
Hd6	Late flowering in LD	Protein kinase CK2α	*CK2*	Takahashi et al. (2001)
Hd3a	Early flowering in SD	Not clarified	*FT*	Kojima et al. (2001)
SE5	Late flowering in LD	Heme-oxygenase	*HY1*	Izawa et al. (2000)

[a] Effect of wild-type allele on the phenotype.

manner in LD. Because *CO* positively regulates the *FT* (flowering time T) and *SOC1* (suppressor of overexpression of *CO 1*) genes (Kobayashi et al., 1999; Onouchi et al., 2000; Samach et al., 2000) of Arabidopsis, *Hd1* may positively regulate the counterpart genes of rice in SD conditions and negatively in LD conditions. Recently, *PnCO*, with similarity to the Arabidopsis *CO* gene, was isolated by a differential display method in *P. nil* (Liu et al., 2001). The expression of *PnCO* was found to be photoperiodically regulated, and the Arabidopsis *co* mutant was complemented with *PnCO* cDNA. In addition to *Hd1* in rice, this result clearly supports the concept that a *CO*-like protein promotes flowering in different inductive photoperiods, SD and LD.

Another QTL, *Hd6*, located on the long arm of chromosome 3, is involved in rice photoperiod sensitivity (Yamamoto et al., 2000). The Kasalath allele inhibits flowering under natural and LD conditions but not under the SD condition. High-resolution and fine-scale genetic mapping of *Hd6* delimited the candidate for *Hd6* to a 26.4-kb genomic region; finally, it was proved by complementation analysis that *Hd6* encodes the α-subunit of protein kinase CK2 (CK2α; Takahashi et al., 2001; Table I). This result indicates that *CK2*α plays an important role in the photoperiodic response of flowering in rice. In Arabidopsis, CK2 interacts with and phosphorylates the Arabidopsis circadian clock-associated 1 protein (CCA1) in vitro (Sugano et al., 1998). Overexpression of the β-subunit of *CK2* shortened periods of rhythmic expression of *CCA1* and caused early flowering in both LD and SD conditions (Sugano et al., 1999). This suggests that *CK2* is involved in the control of flowering in Arabidopsis as well. These results demonstrate that a common mechanism may exist in the photoperiodic response of flowering in both SDPs and LDPs. It remains to be analyzed whether the alteration in circadian phenotypes, such as a daily rhythmic expression of a reporter gene, occurs in the NILs for *Hd6*. Recently, a good monitoring system of gene expression regulated by the circadian clock has been developed based on *cab1r::luc* transgenic plants (Sugiyama et al., 2001). It should be possible to analyze the alteration in circadian phenotypes using this system.

Hd3a, located on the short arm of chromosome 6, and involved in the photoperiodic response and in promoting flowering in the SD condition, was also identified by a map-based strategy (Kojima et al., 2001). *Hd3a* showed a high level of similarity with the *FT* gene (Kobayashi et al., 1999) that promotes flowering in LD conditions (Table I). Transgenic analysis revealed that the introduction of *Hd3a* resulted in early flowering in SD and LD conditions (Kojima et al., 2001). In addition, *Hd3a* mRNA is up-regulated in the SD conditions, which induces flowering in rice. These results suggest that *Hd3a* plays an important role for promotion of flowering in SD conditions.

Through the analysis of artificial mutants in rice, Izawa et al. (2000) demonstrated that phytochromes confer the photoperiodic control of flowering. They cloned the gene corresponding to the photoperiodic sensitivity 5 (*se5*) mutant (Yokoo and Okuno, 1993) that shows complete loss of the photoperiodic response of flowering. *SE5* encodes a putative heme oxygenase (*HY1* in Arabidopsis) involved in phytochrome chromophore biosynthesis. Light-stable phytochromes may play a major role in measuring the day length in rice, because *se5* mutants flowered early even under constant light conditions, in which the wild-type rice did not flower. It is noteworthy that rice *phyA* mutations did not affect flowering time in rice (Takano et al., 2001). It was also reported that a photoperiod sensitivity gene, Ma_3, encodes a phytochrome B in sorghum (*Sorghum bicolor* [L.] Moench; Childs et al., 1997). These results suggest that light-stable phytochromes play an important role in the photoperiodic induction of flowering in SDPs, although light-stable phytochromes generally inhibit flowering regardless of the photoperiodic responses in both LDPs and SDPs (Thomas, 1998; Lin, 2000). On the other hands, *PHY A* and *CRY2*, which are light-labile photoreceptors, are major players in the photoperiodic control of flowering in Arabidopsis (LDP; Guo et al., 1998; Johnson et al., 1994). Therefore, it would be interesting to examine the role of cryptochromes in the control of flowering time in rice.

In Arabidopsis, the signals from light/dark cycle received by phytochromes and cryptochromes are transmitted to the circadian clock (for review, see Levy and Dean, 1998). The circadian clock regulates the transcription factor gene *CO* (Suarez-Lopez et al., 2001). Then *CO* activates the *FT, SOC1*, and *LFY* (leafy) genes (Kobayashi et al., 1999; Samach et al., 2000), which in turn activate the ABC floral organ identity genes. In rice, expression profiles of *Hd1* and *Hd3a* remained to be analyzed with regards to photoperiods. However, based on the molecular structure of those genes and epistatic interactions, it is possible that *Hd1* acts like *CO* in Arabidopsis to mediate flowering signals from the environmental changes. Therefore, in rice, it can be speculated that *Hd1* mediates a signal from the circadian clock to the *Hd3a* gene.

FUTURE PROSPECTS

To understand the photoperiodic control of flowering in rice more comprehensively, other QTLs, such as *Hd2*, *Hd3b,* and *Hd5*, should be isolated. New genetic factors that control flowering must also be explored. To exploit a wide range of allelic variations in the genes controlling flowering in rice, wild relatives, which are adapted to specific environmental conditions, can be used as donor parents to develop

mapping populations. Chromosome segment substitution lines covering whole rice chromosomes have been developed through the use of wild relatives as donor parents and have been used for QTL analysis (for review, see Yano, 2001). In fact, new QTL for flowering time have been identified by using such wide cross combinations (Doi et al., 1998). There are also unique varieties within cultivated species. Rice varieties adapted to Hokkaido, the northernmost island in Japan, have a functional allele at the *Se1* (*Hd1*) locus (Ichitani et al., 1997, 1998). However, those varieties show complete loss of photoperiodic response of flowering, suggesting that some other genetic cofactor might be required to express photoperiodic response with *Hd1*. Those varieties should be used as parental lines in the QTL analysis of flowering time to detect such putative factors.

Arabidopsis flowering mutants often exhibit altered circadian clock phenotypes. Several genes involved in the circadian behavior of leaf movement of Arabidopsis have been identified through the QTL analysis of natural variation (Swarup et al., 1999). This approach detected some flowering-time genes, which have been reported in the previous studies, and new members of genes for the circadian system. The analysis of natural variation in circadian clock-related traits is an alternative strategy for finding new components of flowering time regulation in rice.

In addition to the phenotype-based approach mentioned above, the microarray system and differential display methods will contribute to identifying new components of the flowering time control system. In *P. nil*, the differential display method was used to isolate other candidate factors involved in the photoperiodic response of flowering (Sage-Ono et al., 1998). A cDNA, *PnC401*, which accumulated during the inductive dark period, was isolated. Fluctuations in *PnC401* mRNA abundance with regard to circadian rhythm and the day/night cycle suggested that *PnC401* might be involved in the photoperiodic response of flowering. *PnC401* showed no distinct similarity to known proteins but showed significant similarity to Arabidopsis expressed sequence tag. It will be interesting to learn the biological function of *PnC401* with regard to the photoperiodic response of flowering.

Several genes have been molecularly identified in rice. Although biochemical functions of Arabidopsis *CO* and *FT* seem to be conserved in rice *Hd1* and *Hd3a*, the inductive photoperiod for flowering is different between rice and Arabidopsis. This raises a simple question: What kind of gene(s) or mechanism(s) are involved in generating the completely opposite reaction to the photoperiod between SDPs and LDPs? Further comparative studies between Arabidopsis and rice will allow us to clarify conserved and/or diverse features in such an important and complex developmental system as flowering.

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