

# Genetic control of flowering time in rice: integration of Mendelian genetics and genomics

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## Abstract

**Key message** Integration of previous Mendelian genetic analyses and recent molecular genomics approaches, such as linkage mapping and QTL cloning, dramatically strengthened our current understanding of genetic control of rice flowering time.

**Abstract** Flowering time is one of the most important agronomic traits for seed production in rice (*Oryza sativa* L.). It is controlled mainly by genes associated with photoperiod sensitivity, particularly in short-day plants such as rice. Since the early twentieth century, rice breeders and researchers have been interested in elucidating the genetic basis of flowering time because its modification is important for regional adaptation and yield optimization. Although flowering time is a complex trait controlled by many quantitative trait loci (QTLs), classical genetic studies have shown that many associated genes are inherited in accordance with Mendelian laws. Decoding the rice genome sequence opened a new era in understanding the genetic control of flowering time on the basis of genome-wide mapping and gene cloning. *Heading date 1 (Hd1)* was the first flowering time QTL to be isolated using natural variation in rice. Recent accumulation of information on rice genome has facilitated the cloning of other QTLs, including those with minor effects on flowering time. This information has allowed us to rediscover some of the flowering genes that were identified by classical Mendelian genetics. The genes characterized so far, including *Hd1*,

have been assigned to specific photoperiod pathways. In this review, we provide an overview of the studies that led to an in-depth understanding of the genetic control of flowering time in rice, and of the current state of improving and fine-tuning this trait for rice breeding.

## Introduction

After the rediscovery of the Mendelian concept of heredity in 1900, many genetic studies were performed to elucidate genes controlling plant phenotypes. Flowering is a profound transition from vegetative to reproductive development, and is determined largely by genetic pathways that integrate endogenous and exogenous signals. The ability of many plant species to initiate flowering at a time most favorable for their reproduction depends mainly on the accurate measurement of seasonal changes in daylength and temperature (Thomas and Vince-Pure 1997; Hayama et al. 2003).

In this review, we summarize the findings from many studies of rice flowering time based on classical Mendelian genetics and on molecular genetics and genomics. Studies performed over a century clearly showed that integration of these approaches dramatically improves our understanding of the genetic control of flowering time in rice.

## Mendelian genetics of flowering time in rice

Flowering time is important for regional adaptability and is easy to observe; its variability among rice varieties has been known for a long time. Studies of the inheritance of rice flowering time date back to the 1910s. On the basis of segregation patterns in progeny from experimental crosses between early- and late-flowering varieties, Hoshino (1915) suggested the involvement of at least three loci.

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Since the discovery of photoperiodism in plants (the response of an organism to the relative length of day and night) by Garner and Allard (1920, 1923), many researchers investigated flowering responses of rice varieties to day-length. These studies revealed that rice is a short-day (SD) plant: its flowering is promoted under SD conditions, and the difference in photoperiod sensitivity among varieties results in extensive variation in flowering time (reviewed in Chandraratna 1964; Vergara and Chang 1985).

To investigate the genetic control of flowering time in rice, early- and late-flowering varieties were crossed (Nomura and Yamazaki 1925; Jones 1928, 1933; Fuke 1955; Sampath and Seshu 1961), and the number of genes and their allelic effects were estimated from segregation patterns in  $F_2$  plants and their  $F_3$  progeny selected on the basis of flowering time (for example, early-, intermediate-, and late-flowering segregants). In  $F_2$  plants, the distribution of flowering time varied from continuous unimodal to clearly bimodal; the distribution depended on the photoperiod sensitivity of the parents used and on the environment (latitude and season) where the segregating population was grown. In several crosses, the ratio of late flowering to early flowering in  $F_2$  plants and their  $F_3$  progeny (3:1 or 15:1) was similar to the ratio expected from Mendel's law (Nomura and Yamazaki 1925; Jones 1928, 1933; Jones et al. 1935; Fuke 1955; Sampath and Seshu 1961). These results showed that one or two Mendelian genes likely play a major role, yet in some crosses, the presence of transgressive segregants among  $F_2$  plants suggested the contribution of additional gene(s) (Jones 1928; Jones et al. 1935). Moreover, segregation patterns suggesting a complementary effect of two genes (9 late-flowering: 7 early-flowering  $F_2$  plants) were also reported (Jones et al. 1935; Fuke 1955). These early studies eventually led to the identification of underlying genes, which are described in the following section.

### Classical Mendelian genes for flowering time in rice

From the 1920s, with the development of the chromosome theory of inheritance and the discovery of genetic linkage, the linkage relationship between flowering time genes and known loci (for other phenotypic traits) was investigated, and thereby the possibility of a connection between phenotype and genotype in rice was reinforced (Chao 1928; Jodon 1940).

Chandraratna (1953, 1955) found bimodal segregation in  $F_2$  plants from crosses between varieties insensitive and sensitive to photoperiod, suggesting control by a Mendelian gene. A dominant allele at the locus resulted in late flowering. The gene (called *Se*) was linked to a locus for apiculus color (Table 1). To explain variation in flowering time among Japanese varieties, Fuke (1955) distinguished

six genetic factors, *Z*, *M*, *K*, *G*, *O*, and *F*. Factor *K* had the largest effect on photoperiod sensitivity and was linked to a locus for apiculus color. Yokoo and Fujimaki (1971) reported that a late-flowering gene (designated *Lm*) is closely linked to a blast resistance gene in crosses between the *O. sativa* subspecies *japonica* and *indica* (Table 1). Yokoo and Kikuchi (1977) found that different alleles at the *Lm* locus determine photoperiod sensitivity. The gene for late flowering was also linked to a locus for apiculus color (called *C*) on chromosome 6. On the basis of linkage relationships among these flowering time loci (*Se*, *K*, and *Lm*) and the apiculus color locus, Yokoo et al. (1980) suggested that they are the same locus (Table 1), and that the *Se/K/Lm* gene contributes to flowering time variation among both *japonica* and *indica* varieties.

Syakudo and Kawase (1953) and Syakudo et al. (1954) proposed that the loci *E1* and *E2* are the main determinants of the segregation patterns of flowering time in the progeny of a cross between Japanese rice varieties (Table 1). Syakudo et al. (1954) suggested three additional loci (*E3*, *E4*, and *E5*), to interpret segregation of flowering time in crosses between other varieties, but *E4* and *E5* were later shown to be the same as *E1* and *E2*, respectively (Okumoto et al. 1991). Of the three genes, *E1* had the largest effect on flowering time and was investigated in detail; a recessive allele at the *E1* locus reduces photoperiod sensitivity (Yamagata et al. 1986). In addition, the allelic distribution of *E1*, *E2*, and *E3* among Japanese varieties was estimated from the results of crosses with tester lines, in which the genotypes for these genes had been defined. The dominant *E1* allele was distributed among varieties grown in southwestern Japan; the recessive *e1* allele was distributed among varieties grown in northern regions and was probably associated with early flowering (Okumoto et al. 1991, 1996). Linkage analyses of a trisomic line with phenotypic markers revealed that *E1* is located on chromosome 7 and is linked to the *rolled fine strip* and *slender glume* genes (Okumoto and Tanisaka 1997).

To delineate the effect of earliness genes carried by northern varieties, Tsai and Oka (1966) developed near-isogenic lines (NILs) for early flowering by introducing earliness genes into the background of a photoperiod-insensitive variety by successive backcrossing. Genetic analysis of these NILs revealed that the genes *E* and *M* are involved in early flowering; a dominant allele at the *E* locus and a recessive allele at the *M* locus (*m*) resulted in early flowering (Table 1). The effect of the *E* allele was enhanced in the presence of the *m* allele, implying epistasis between *E* and *M* (Tsai 1976). Recently, this epistasis was confirmed by Uwatoko et al. (2008). Independently of Tsai (1976), Sato et al. (1988) developed early-flowering NILs and identified the dominant earliness allele *Efl* (Table 1) on chromosome 10 by examining linkage to phenotypic markers and cytological

**Table 1** Classical Mendelian genes and isolated genes for natural variation in flowering time in rice

Gene symbol	Synonym	Effect on flowering	Chromosome	RAP ID <sup>a</sup>	Msu ID <sup>b</sup>	Description	References
<i>Se</i>	<i>Se1, K, Lm, Hd1</i>	SD promotion/LD repression	6	Os06g0275000	LOC_Os06g16370	Zinc-finger protein	Chandraratna (1953, 1955), Yokoo and Fujimaki (1971), Yano et al. (1997, 2000)
<i>E1</i>	<i>M, m-Ef1, Ghd7</i>	LD repression	7	Os07g0261200	LOC_Os07g15770	CCT (CONSTANS, CONSTANS-LIKE, and TIMING OF CHLORO-PHYLL A/B BINDING1) domain protein	Syakudo and Kawase (1953), Syakudo et al. (1954), Tsai and Oka (1966), Tsai (1976), Okumoto et al. (1992), Okumoto and Tanisaka (1997), Xue et al. (2008)
<i>E2</i>	<i>Hd17, E17, OsELF3-1, OsELF3, Hd-q</i>	SD/LD promotion	6	Os06g0142600	LOC_Os06g05060	Homolog of Arabidopsis EARLY FLOWERING 3 protein	Syakudo and Kawase (1953), Syakudo et al. (1954), Matsubara et al. (2008a), Monden et al. (2009), Yuan et al. (2009), Matsubara et al. (2012), Saito et al. (2012)
<i>E3</i>	<i>Hd6</i>	LD repression	3	Os03g0762000	LOC_Os03g55389	Similar to protein kinase CK2, alpha subunit	Syakudo and Kawase (1953), Syakudo et al. (1954), Takahashi et al. (2001)
<i>E</i>	<i>Ef1, Ehd1</i>	SD/LD promotion	10	Os10g0463400	LOC_Os10g32600	B-type response regulator	Tsai and Oka (1966), Tsai (1976), Sato et al. (1988), Doi et al. (2004), Saito et al. (2009)
<i>Hd3a</i>		SD promotion	6	Os06g0157700	LOC_Os06g06320	Florigen	Kojima et al. (2002)
<i>RFT1</i>		LD promotion	6	Os06g0157500	LOC_Os06g06300	Florigen	Kojima et al. (2002), Ogiso-Tanaka et al. (2013)
<i>DTH8</i>	<i>Ghd8, LHDI, Hd5, LH8</i>	SD promotion/LD repression	8	Os08g0174500	LOC_Os08g07740	Putative HAP3 subunit of CCAAT box-binding transcription factor	Wei et al. (2010), Dai et al. (2012), Fujino et al. (2013), Chen et al. (2014)
<i>DTH3</i>	<i>OsMADS50</i>	SD/LD promotion	3	Os03g0122600	LOC_Os03g03070; LOC_Os03g03100	MIKC-type MADS-box protein	Lee et al. (2004), Bian et al. (2011)
<i>DTH2</i>		LD promotion	2	Os02g0724000	LOC_Os02g49230	CONSTANS-like protein	Wu et al. (2013)
<i>Hd16</i>	<i>EL1</i>	LD repression	3	Os03g0793500	LOC_Os03g57940	Casein kinase I	Dai and Xue (2010), Hori et al. (2013), Kwon et al. (2014)
<i>OsPRR37</i>	<i>Hd2</i>	LD repression	7	Os07g0695100	LOC_Os07g49460	Pseudo-response regulator	Koo et al. (2013)
<i>Ehd4</i>		SD/LD promotion	3	Os03g0112700	LOC_Os03g02160	Zinc finger CCH domain-containing protein	Gao et al. (2013)
<i>Hd18</i>		SD/LD promotion	8	Os08g0143400	LOC_Os08g04780	Amine oxidase domain-containing protein	Shibaya et al. (2016)

<sup>a</sup> Locus ID of the Rice Annotation Project (<http://rapdb.dna.affrc.go.jp/>)<sup>b</sup> Locus ID of the Rice Genome Annotation Project, Michigan State University (<http://rice.plantbiology.msu.edu/>)

interchange breakpoints on the chromosome. An allelism test performed by crossing NILs for *Ef1* or *E*, which had the same background, placed these genes at the same locus (Sato et al. 1988). Okumoto et al. (1992) suggested that *M* is identical to *E1* by crossing NILs for the *m* allele (developed by Tsai 1976) with tester lines for *E1*, *E2*, or *E3* (Table 1).

Yokoo and Okuno (1993) found an early-flowering mutant among X-ray-irradiated plants. The mutant showed a significant reduction of photoperiod sensitivity. The mutated gene, designated *se5*, was located on chromosome 6 but was distinct from *Se/K/Lm*.

Thus, in the above studies, the chromosomal locations and allelic relationships of genes were evaluated from their linkage relationships to known phenotypic markers and using experimental crosses with tester lines or NILs for flowering time.

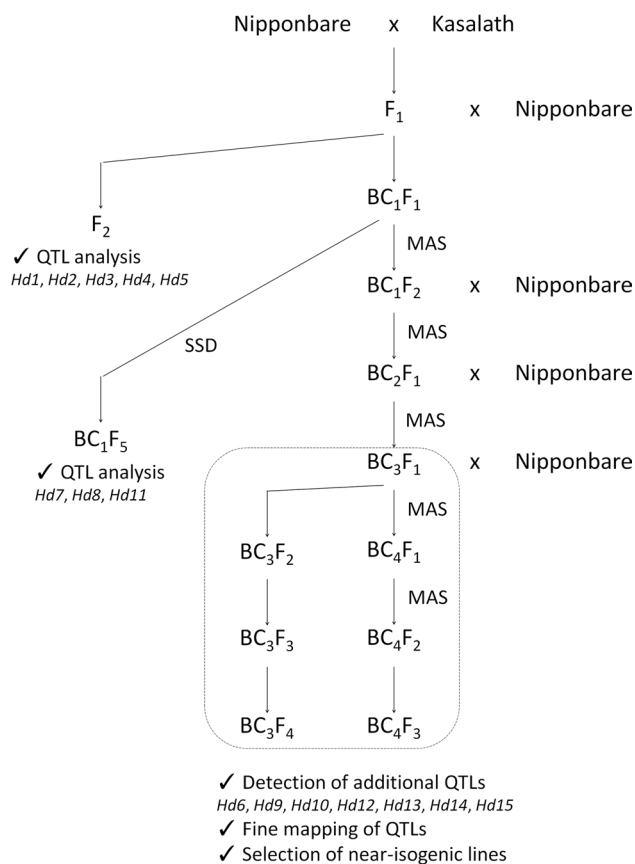
In addition to the six major genes described above (Table 1), to our knowledge, at least 15 genes have been reported so far (*Se2*, Yu and Yao 1968; *Se3*, Poonyarit et al. 1989; *Ef2*, *Ef3*, and *Ef4*, Tsai 1991; *Efx* and *Efy*, Sato et al. 1992; *Se4*, Ohshima et al. 1993; *Se6* and *Se7*, Tsai 1995; *Se9*, Ichitani et al. 1998a; *Se10* and *Se11*, Tsai 1999; *Ef5*, Khun et al. 2004; *Ef6*, Khun et al. 2006). However, little is known about the allelic relationships among these genes and their precise positions in the genome.

### Mendelizing flowering time in rice on the basis of QTL analysis

In the 1990s, the development of DNA markers allowed researchers to clarify the number and effects of the genes underlying flowering time by using quantitative trait locus (QTL) analysis (Li et al. 1995; Xiao et al. 1996; Yano et al. 1997).

Using several types of progeny derived from a single cross between the rice cultivars ‘Nipponbare’ (*japonica*) and ‘Kasalath’ (*indica*), our group identified 15 QTLs for flowering time (Fig. 1). Five of them (*Hd1–Hd5*) were mapped by QTL analysis of an  $F_2$  population (Yano et al. 1997), and *Hd7*, *Hd8*, and *Hd11* were detected with  $BC_1F_5$  lines (Lin et al. 1998). Other loci were detected only when we used advanced backcross progeny such as  $BC_3F_2$  or  $BC_4F_2$  (Yamamoto et al. 2000; Lin et al. 2002).

The development of NILs by marker-assisted selection (MAS) provided advantages for the genetic analysis of flowering time in rice. For example, QTL-NILs can be used to characterize epistatic interactions. This approach was used to clarify epistasis between *Hd1* and *Hd3* (Lin et al. 2000). Lin et al. (2000) suggested that the ‘Kasalath’ allele of *Hd3* does not affect photoperiod sensitivity but enhances the effect of the ‘Nipponbare’ alleles of the photoperiod sensitivity QTLs *Hd1* and *Hd2*. A significant epistatic interaction between *Hd2* and *Hd6* was detected in the analysis



**Fig. 1** Plant materials used for QTL mapping of flowering time in rice. Mapping populations were derived from a cross between the cultivars ‘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. QTLs are shown next to the mapping populations in which they were detected. MAS marker-assisted selection, SSD single seed descent. Modified from Fig. 1 in Yano et al. (2001)

of advanced progeny in which the effect of the ‘Kasalath’ allele of *Hd6* was observed only in the presence of the ‘Nipponbare’ allele of *Hd2* (Yamamoto et al. 2000).

QTL-NILs can also be used for fine mapping of target QTLs. In such advanced backcross progenies, *Hd1–Hd3*, *Hd6*, and *Hd9* were fine-mapped on a genetic linkage map as single Mendelian factors (Yamamoto et al. 1998, 2000; Lin et al. 2002). High-resolution mapping dissected two tightly linked loci, *Hd3a* and *Hd3b*, in the *Hd3* region (Monna et al. 2002). The ‘Kasalath’ allele of *Hd3a* promotes flowering under SD conditions, whereas that of *Hd3b* delays flowering under long-day (LD) and natural-field conditions (Monna et al. 2002). These efforts have led to map-based cloning of genes for flowering time and thereby improved our understanding of the function of these genes at the molecular level and of the gene regulatory network for flowering time in rice, as described in the following section.

## Molecular cloning of flowering time genes in rice

*Hdl* was the first rice flowering time gene cloned using natural variation (Yano et al. 2000). Using more than 9000 BC<sub>3</sub>F<sub>3</sub> plants, we defined the *Hdl* region within 12 kb on chromosome 6. This region contained one candidate gene highly similar to Arabidopsis *CONSTANS* (*CO*). Comparison of the candidate gene in ‘Nipponbare’ and ‘Kasalath’ revealed many sequence variations, including a 36-bp insertion and a 33-bp deletion (in exon 1) and a 2-bp deletion (in exon 2) in ‘Kasalath’. A 7.1-kb fragment of ‘Nipponbare’ containing the *Hdl* candidate gene was transferred into a NIL and was found to promote flowering under SD conditions. These results clearly indicated that the candidate gene homologous to Arabidopsis *CO* was *Hdl*.

More than 70 rice genes associated with flowering time have been isolated by using mutants, transgenic plants, and natural variation (Table 1; see also the OGRO database, Yamamoto et al. 2012). In segregating populations derived from crosses between rice varieties that differ in flowering time, at least 14 flowering time QTLs have been identified using natural variation and subsequently isolated by map-based cloning strategies (Table 1; Yano et al. 2000; Takahashi et al. 2001; Kojima et al. 2002; Doi et al. 2004; Xue et al. 2008; Wei et al. 2010; Bian et al. 2011; Matsubara et al. 2012; Gao et al. 2013; Hori et al. 2013; Koo et al. 2013; Ogiso-Tanaka et al. 2013; Wu et al. 2013; Shibaya et al. 2016). Rice mutants have also been used to isolate flowering time genes and investigate their functions (Izawa et al. 2000; Lee et al. 2004; Matsubara et al. 2008b; Kuromori et al. 2009; Saito et al. 2009, 2012; Dai and Xue 2010; Yang et al. 2013; Yokoo et al. 2014; Yoshitake et al. 2015). Other flowering time genes have been identified and their functions have been investigated by forward- and reverse-genetics approaches such as experiments using overexpression or knockdown transgenic plants. For example, the functions of *OsMADS34* and *OsTrx1* were revealed using knockdown transgenic plants, whereas the functions of *OsMADS50*, *OsMADS56*, and *OsMADS15* were identified using overexpressing transgenic plants (Ryu et al. 2009; Lu et al. 2012; Choi et al. 2014; Lin et al. 2014).

It should be noted that even in such a modern genomics era, classical Mendelian genetics is still in an important position in the analysis of the genetic control of flowering time in rice. For example, in map-based cloning, an association between phenotype and molecular marker is often examined based on the Mendel’s law. The law of dominance gives us a useful estimation about gene function; recessive phenotype may be caused by loss-of-function mutation of gene of interest.

## Relationships between genes identified by classical Mendelian genetics and molecular genomics

Unfortunately, in the almost studies on rice flowering time genes, different parental varieties or mutant lines have been used in classical Mendelian genetics and recent genomics approaches. Nevertheless, sequence comparison of isolated genes in rice varieties and mutant lines provided critical information about the connection between genes identified by the two approaches. For example, *Hdl* was isolated in the segregating populations of ‘Nipponbare’ and ‘Kasalath’, and sequence comparisons of *Hdl* in the two varieties revealed multiple sequence variations, such as insertions and deletions (Yano et al. 2000). In the same study, they analyzed sequences of the *Hdl* gene in the *Se1* mutants of HS66 and HS110 and their progenitor variety ‘Ginbozu’, in which the *Se1* gene had been considered as a different allele at the *Hdl* locus based on the chromosomal location on linkage maps (Tamura et al. 1998; Yamamoto et al. 1998). By sequence comparisons with the ‘Ginbozu’, HS66 had a 43-bp deletion and HS110 had a 433-bp insertion in their *Hdl* genes (Yano et al. 2000). Both polymorphisms in HS66 and HS110 were estimated to cause the nonfunctional protein of *Hdl*. These results could lead to the conclusion that *Hdl* is allelic to *Se1*. Other isolated genes have been also defined clear relationships with some classical Mendelian genes (Table 1). Tsai and Oka (1966) reported that a Taiwan variety ‘Taichung 65’ showed a long vegetative growth period and had a recessive allele of *Efl*. Sequencing analysis of *Ehd1* in various rice varieties revealed that the nonfunctional *Ehd1* allele had been identified only in Taiwan varieties including ‘Taichung 65’ (Saito et al. 2009), showing that *Ehd1* is allelic to *Efl*. In the same way, it could be estimated that *E1* (*M*), *E2* and *E3* are allelic to *Ghd7*, *Hd17* and *Hd6*, respectively (Ichitani et al. 1998b; Matsubara et al. 2008a; Monden et al. 2009; Saito et al. 2012). Thus, molecular genomics approaches including map-based cloning and sequencing analysis have unraveled correspondence between classical Mendelian genes and isolated genes.

## Gene regulatory network for flowering time in rice

Map-based cloning revealed a regulatory network of flowering time genes in rice. Rice photoperiodic flowering is controlled by two independent signaling pathways. The OsGI–Hd1–Hd3a pathway is evolutionarily related to the Arabidopsis GI–CO–FT pathway, whereas the other pathway, which includes *Ehd1*, *Ghd7*, *Ehd2*, *Ehd3*, and *Ehd4*, has no Arabidopsis counterpart.

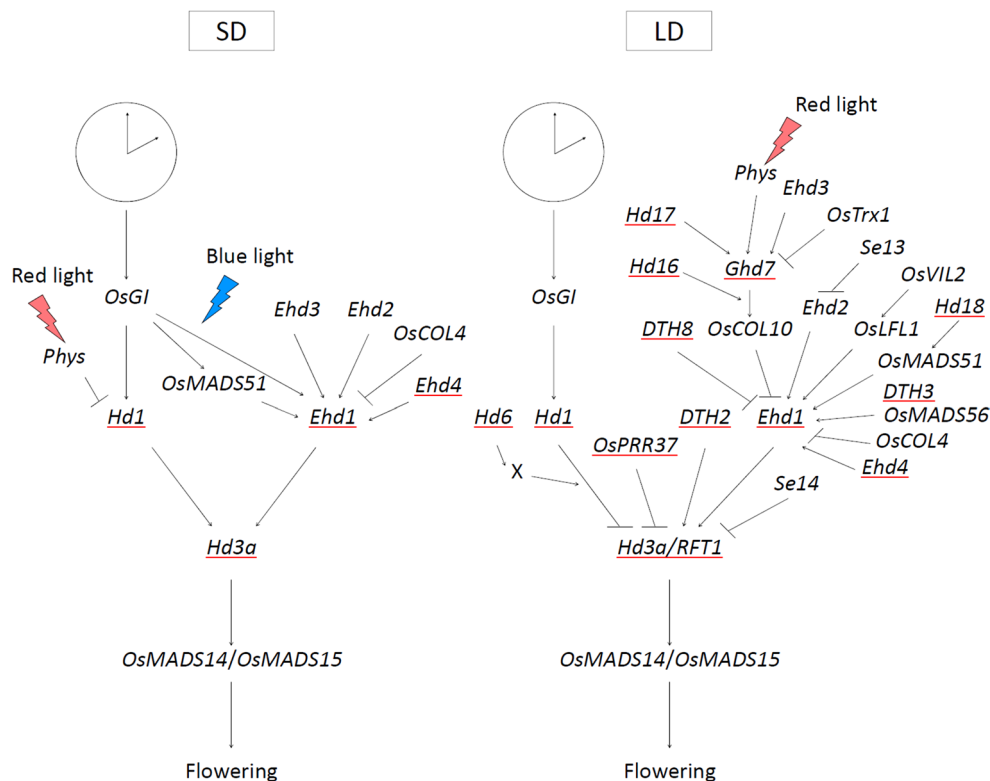
Under SD conditions, rice flowering is promoted by the transcription of *Hd3a*, which is activated independently



by Hd1 and Ehd1 (Yano et al. 2000; Kojima et al. 2002; Doi et al. 2004; Tamaki et al. 2007) (Fig. 2). Hd3a acts as a mobile flowering signal (florigen) (Tamaki et al. 2007). *OsGI* expression is regulated by the circadian clock and activates *Hd1* expression (Hayama et al. 2003). *Ehd1* expression is upregulated by the products of *Ehd2*, *Ehd3*, *Ehd4*, and *OsMADS51* and downregulated by the product of *OsCOL4*. *Ehd2* is a homolog of maize *indeterminate1* and is a strong promoter of flowering (Matsubara et al. 2008b). *Ehd3* encodes a putative transcriptional regulator with two plant homeodomain finger motifs (Matsubara et al. 2011). *Ehd4* encodes a zinc finger CCCH domain-containing protein, which is an *Oryza*-specific regulator of photoperiodic flowering (Gao et al. 2013). *OsGI* also strongly activates *Ehd1* expression, either directly or via *OsMADS51* (Itoh et al. 2010).

Under LD conditions, transcriptional activation of *Hd3a* is lower than under SD conditions; consequently, flowering is suppressed. Although Hd1 activates *Hd3a* expression under SD conditions, it represses *Hd3a* expression under LD conditions (Fig. 2); this functional conversion of Hd1 is caused by phytochrome-mediated signaling (Hayama and Coupland 2004; Izawa 2007). The Hd1 repressor function under LD conditions is enhanced by the activity of a kinase encoded by *Hd6* and is mediated by unknown

genes (Takahashi et al. 2001; Ogiso et al. 2010); it is also regulated by a complex of Hd1 and Ghd7 (Nemoto et al. 2016). *RFT1*, located within 11.5 kb of *Hd3a*, is an *Hd3a* paralog (Komiya et al. 2008). *RFT1* expression increases under LD conditions, and RFT1 moves from the leaves to the shoot apical meristem. These results strongly indicate that flowering time control in rice involves two florigen genes, *Hd3a* and *RFT1* (Komiya et al. 2008; 2009). *RFT1* expression is promoted by the product of *Ehd1* and *DTH2*, but is repressed by the product of *Se14* (Doi et al. 2004; Wu et al. 2013; Yokoo et al. 2014). *Ehd1* expression is induced by the products of *DTH3*, *OsMADS51*, *OsMADS56*, *Ehd2*, and *Ehd4*, but is repressed by the products of *DTH8*, *OsCOL4*, *OsCOL10*, and *OsLFL1*. DTH8 might form a complex with Hd1 to control flowering (Wei et al. 2010; Yan et al. 2011). DTH3 and *OsMADS56* form a complex that regulates *Ehd1* (Ryu et al. 2009). *OsMADS51* is upregulated by *Hd18* and induces *Ehd1* expression (Kim et al. 2007; Shibaya et al. 2016). *OsLFL1* is induced by the product of *OsVIL2*, and has been proposed to downregulate *Ehd1* expression (Peng et al. 2008; Yang et al. 2013). *Ehd2* is downregulated by *Se13*, and induces *Ehd1* expression (Matsubara et al. 2008b; Yoshitake et al. 2015). *OsCOL4* and *OsCOL10* are members of the *CONSTANS*-like family. *OsCOL4* is a constitutive repressor upstream of *Ehd1* (Lee



**Fig. 2** Gene regulatory network for flowering time in rice. The clocks at the top indicate the circadian clock. Genes with natural allelic variation are underlined. SD short-day conditions, LD long-day conditions. Arrows upregulation, bars downregulation

et al. 2010). *OsCOL10* downregulates the expression of *Ehd1* and is upregulated by the product of *Ghd7* (Tan et al. 2016). *Ghd7* expression is induced by the products of *Ehd3* and *Hd17* and is repressed by *OsTrx1*, which is a chromatin-remodeling factor having histone methyltransferase activity (Choi et al. 2014). *Ghd7* activity is increased by phosphorylation by the casein kinase I-like protein *Hd16* (Hori et al. 2013). *Hd16* also phosphorylates the product of *OsPRR37* (likely allelic to *Hd2*), which encodes a pseudo-response regulator protein (Koo et al. 2013). Regulation of flowering time genes downstream of *Hd3a* and *RFT1* is the same under both SD and LD conditions. *Hd3a* interacts with the 14-3-3 protein *OsFD* to initiate floral transition in the shoot apex (Purwestri et al. 2009). *OsFD* and *GF14c* induce the expression of *OsMADS14* and *OsMADS15* that control the phase transition of the SAM from vegetative to reproductive (Lim et al. 2000).

### Circadian clock genes control the expression of flowering time genes in rice

The expression of many rice flowering time genes is strictly controlled by a critical daylength (Izawa 2007; Itoh et al. 2010; Matsuzaki et al. 2015). For example, *Hd3a* and *Ehd1* are expressed in the morning under SD conditions, whereas *Ghd7* is expressed in the morning under LD conditions. *OsGI* expression shows daily circadian oscillation with a peak at the end of the light period (Hayama et al. 2002). Expressions of some flowering time genes are regulated by the circadian gating of light responses through phytochromes (red light receptors) and cryptochromes (blue light receptors) (Itoh et al. 2010). *Ghd7* expression is induced through phytochrome signaling, and sensitivity to red light is gated at the beginning of the light period under LD conditions. *Ehd1* expression confers clear gating responses of blue light pulses (Itoh et al. 2010). Recently, Matsuzaki et al. (2015) developed a statistical model for the expression of multiple genes with phase setting by sunlight and the circadian clock. The integration of the expression patterns of individual flowering time genes can accurately estimate both the internal biological time determined by the circadian clock and actual physical time of day (Matsuzaki et al. 2015). However, additional molecular genetics and physiological analyses are needed to understand the circadian clock and the gating mechanisms of flowering time pathways in rice.

### Genetic architecture of variation in flowering time among rice varieties

To date, more than 700 additional QTLs for flowering time have been detected on all 12 chromosomes by using segregating populations derived from crosses among rice

varieties (Yonemaru et al. 2010; Youens-Clark et al. 2011). To comprehensively characterize natural allelic variations in flowering time, we carried out QTL analyses in 12  $F_2$  populations derived from crosses of ‘Koshihikari’ (*japonica*), a widely grown elite Japanese cultivar commonly used as a parental line, with varieties originating from various regions in Asia (Ebana et al. 2011; Shibaya et al. 2011). A limited number of loci that coincided with *Hd1*, *Hd2*, *Hd6*, *RFT1*, *Ghd7*, *DTH8*, and *Hd16* explained some varietal differences, but additional QTLs are likely to be involved in the phenotypic variation in these populations.

To detect such “hidden” QTLs, we analyzed advanced backcross progeny ( $BC_4F_2$ ) derived from each cross combination of Ebana et al. (2011) and Shibaya et al. (2011) and detected a total of 255 QTLs distributed widely across the genome (Hori et al. 2015). We detected relatively large additive effects of 128 of these QTLs, which corresponded to the genomic positions of previously identified flowering time genes such as *Hd1*, *Hd2*, *Hd6*, *RFT1*, *Ghd7*, *DTH8*, and *Hd16*. Sequence analyses revealed that the chromosomal positions of the large-effect QTLs corresponded in the main to those of different alleles of the flowering time genes in 12 rice varieties. The other 127 QTLs were detected in chromosomal regions other than those of flowering time genes, and had relatively small additive effects. These results indicate that much of the phenotypic variation in flowering time can be explained by combinations of large- and small-effect QTLs.

Genome-wide association studies have also supported the hypothesis that allelic variations play an important role in the differences in flowering time among rice varieties (Zhao et al. 2011; Huang et al. 2012). Zhao et al. (2011) detected 10 genomic regions significantly associated with flowering time variation, although only *Hd1* was detected as a major QTL. These genomic regions explained fewer than 50 % of the phenotypic variations (Zhao et al. 2011). Huang et al. (2012) found 14 significant genomic regions: 5 around *Hd1*, *Ghd7*, *RCN1*, *OsGI*, and *Hd3a* and 9 newly discovered regions. The identified regions explained 36 % of the phenotypic variation in flowering time. The above studies suggest that multiple QTLs are associated with natural variation in flowering time in rice varieties.

### Regional adaptation conferred by allelic differences in flowering time genes

Early flowering conferred by deficient alleles of flowering time genes has been important for expanding the range of rice cultivation to high latitudes (Izawa 2007; Shrestha et al. 2014; Zheng et al. 2015), where early heading and maturity coincide with a period of optimal climatic conditions. Sequence analysis of the known flowering time genes, including *Hd1*, *Ghd7*, *DTH8*, *Hd16*, *OsPRR37*,

*DTH2*, and *Ehd4*, indicates that allelic differences contribute greatly to regional adaptation (Takahashi et al. 2009; Shrestha et al. 2014; Gómez-Ariza et al. 2015; Zheng et al. 2015). Functional alleles of *Hd1* are associated with late flowering and its nonfunctional alleles with early flowering under natural-daylength conditions; the geographic distribution of *Hd1* alleles suggests that favorable alleles have been selected by breeders to enhance rice productivity and adaptability for each region (Takahashi et al. 2009; Fujino et al. 2010; Ebana et al. 2011; Takahashi and Shimamoto 2011; Gómez-Ariza et al. 2015). Deficient or weak alleles of *Ghd7*, *DTH8*, *DTH2*, *Hd16*, and *OsPRR37* are distributed in cultivation areas at high latitudes (Xue et al. 2008; Wei et al. 2010; Fujino et al. 2013; Hori et al. 2013; Koo et al. 2013; Wu et al. 2013; Kwon et al. 2014), strongly suggesting that these alleles must be involved in the expansion of rice cultivation areas.

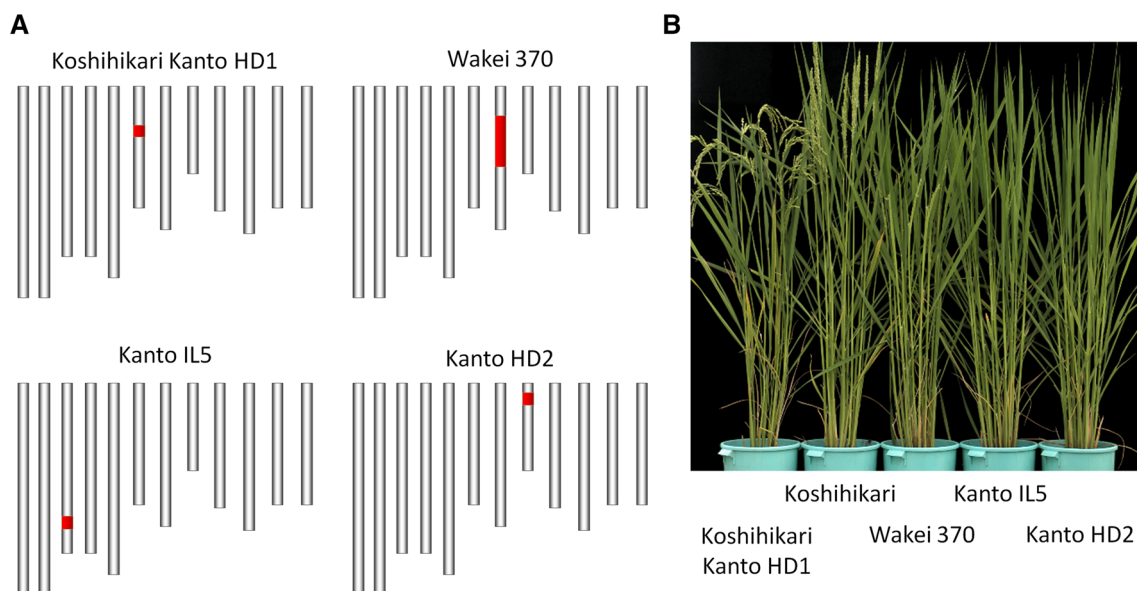
### Fine-tuning of flowering time by gene introgression into rice varieties

Information about additive effects of individual flowering time genes and their epistatic interactions allows the estimation of phenotypic effects, such as the degree of alteration in flowering time, when flowering time genes are introgressed into a particular rice variety. Rice breeders have used this information to develop novel rice varieties. Modulation of rice flowering time helps to avoid flowering and ripening during periods with high risk of drought, flooding, and extreme temperatures in each cultivation area (Bentley

et al. 2013). Plants can, thus, avoid severe stresses during the most sensitive developmental stages. Thus, the fine-tuning of flowering time is a major objective of rice breeding programs to adapt cultivars to regional cultivation systems and environmental conditions.

To facilitate modification of flowering time for use in commercial cultivars, we used MAS to develop a set of NILs of ‘Koshihikari’ with different alleles of flowering time genes derived from ‘Kasalath’ (Takeuchi et al. 2006; Takeuchi 2011). The set included NILs with a loss-of-function allele at *Hd1* (designated ‘Koshihikari Kanto HD1’) and gain-of-function alleles at *Hd4* (*Ghd7*) (‘Wakei 370’), *DTH8* (‘Kanto HD2’), and *Hd6* (‘Kanto IL5’) (Fig. 3). Each NIL contained less than 650 kb of the ‘Kasalath’ genome. ‘Koshihikari Kanto HD1’ flowered earlier than ‘Koshihikari’, whereas ‘Wakei 370’, ‘Kanto HD2’, and ‘Kanto IL5’ flowered later. Most agronomic traits of the NILs were similar to those of ‘Koshihikari’, although culm length and some other traits differed (Takeuchi et al. 2006; Takeuchi 2011). A recent large-scale QTL analysis of agronomic trait selected in rice breeding programs (Hori et al. 2012) demonstrated that flowering time genes can have pleiotropic effects on morphological traits (such as culm length and panicle length) and eating quality. Therefore, it is important to consider these pleiotropic effects to modify flowering time by introgression of corresponding genes.

Our results demonstrate that MAS for alteration of flowering time can be effective in rice. We started to develop novel NIL series in genetic backgrounds of elite rice varieties grown in many regions of Japan. These NILs could



**Fig. 3** Development of a series of isogenic lines of the Japanese elite cultivar ‘Koshihikari’ with different flowering times. **a** Graphical genotypes of the developed lines. Introgressed chromosome segments

with target flowering time genes are indicated in red. **b** Phenotypes of the same lines and ‘Koshihikari’ at flowering stage (color figure online)



have high agronomic performance in terms of high grain yield and optimum flowering time. Moreover, similar MAS strategies could be used to develop NILs of rice varieties grown in subtropical areas, where plants are at risk of both high-temperature stress and drought stress during flowering and ripening stages.

Because not only single genes but also interactions between genes affect flowering time (Lin et al. 2000; Yamamoto et al. 2000; Shibaya et al. 2011; Hori et al. 2013), one has to consider the genetic background of a rice variety when selecting the genes to introgress, combine, and pyramid for alteration of flowering time. Cultivation conditions also need to be considered, because the effects of flowering time genes often depend on daylength. For example, rice varieties with strong photoperiod sensitivity flower late in temperate areas (LD conditions during the growing season) and early in tropical areas (SD conditions). Accumulation of knowledge about flowering time genes and their regulatory network would facilitate the development of new rice varieties.

## Conclusions and perspectives

In the last two decades, tremendous progress in genome sequencing has improved our understanding of genetic and molecular mechanisms that control flowering time in rice. This progress was made possible not only by genomics approaches, such as QTL analysis and map-based cloning, but also the large number of rice accessions (including wild relatives) and genetic mapping populations derived from artificial crosses. Before the development of genomics, classical genetics based on the Mendelian concept had already shown that several genes and their interactions are involved in the control of flowering time in rice. A combination of expanding genome sequence information with appropriate tools and plant materials has dramatically enhanced our understanding of the genetic control mechanism. Eventually, this progress has contributed to rice breeding because it allows us to fine-tune flowering time by combining several QTLs.

Unlike flowering time, some other agriculturally and economically important rice traits, such as yield performance and stress tolerance, are difficult to measure and are strongly affected by environmental conditions. To improve them to satisfy the demand for food supply for still increasing world population under climate change, we have to understand the genetic complexity of these traits at a fundamental level. In this situation, survey of genetic resources and development of plant materials will also be inevitable to strengthen our understanding of these traits.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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