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L. Monna · H.X.Lin · S. Kojima · T. Sasaki
M. Yano

Genetic dissection of a genomic region for a quantitative trait locus, *Hd3*, into two loci, *Hd3a* and *Hd3b*, controlling heading date in rice

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Abstract The rice photoperiod sensitivity gene *Hd3* was originally detected as a heading date-related quantitative trait locus localized on chromosome 6 of rice. High-resolution linkage mapping of *Hd3* was performed using a large segregating population derived from advanced backcross progeny between a *japonica* variety, Nipponbare, and an *indica* variety, Kasalath. To determine the genotype of *Hd3*, we employed progeny testing under natural field and short-day conditions. As a result, two tightly linked loci, *Hd3a* and *Hd3b*, were identified in the *Hd3* region. Nearly-isogenic lines for *Hd3a* and *Hd3b* were selected from progeny using marker-assisted selection. The inheritance mode of both *Hd3a* and *Hd3b* was found to be additive. Analysis of daylength response in nearly-isogenic lines of *Hd3a* and *Hd3b* showed that the Kasalath allele at *Hd3a* promotes heading under short-day conditions while that at *Hd3b* causes late heading under long-day and natural field conditions.

Keywords *Oryza sativa* L. · QTL · Photoperiod sensitivity · Nearly-isogenic line · Linkage analysis

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L. Monna · S. Kojima
Institute of the Society for Techno-innovation of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki 305-0854, Japan

H.X. Lin
Bio-oriented Technology Research Advancement Institution, Omiya, Saitama 331-8537, Japan

T. Sasaki · M. Yano (✉)
Department of Molecular Genetics,
National Institute of Agrobiological Sciences,
2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan
e-mail: myano@nias.affrc.go.jp
Fax: +81-298-387468

Present address:
L. Monna, Plant Genome Center, Tsukuba, Ibaraki 305-0856, Japan

Introduction

Quantitative trait locus (QTL) analysis is a powerful method for identifying genes controlling various phenomena or functions exhibiting complex inheritance. The recent development and availability of molecular markers have made it feasible to detect various QTLs. Heading time of rice under natural field (NF) conditions is one trait under the control of these QTLs. Several putative QTLs controlling heading date have been analyzed using molecular markers (Li et al. 1995; Xiao et al. 1995; Lin et al. 1996, 1998; Yano et al. 1997). We identified five QTLs, *Hd1* to *Hd5*, based on a high-density linkage map using an F_2 population derived from a cross between a *japonica* variety, Nipponbare, and an *indica* variety, Kasalath (Yano et al. 1997). Using nearly-isogenic lines (NILs) of QTLs, Lin et al. (2000) revealed that *Hd1*, *Hd2*, and *Hd3* are involved in photoperiod sensitivity.

The Kasalath allele of *Hd3* causes late heading under natural photoperiodic conditions from April to August in Tsukuba, Japan (Yamamoto et al. 1998). *Hd3* as a QTL has been detected on the distal part of the short arm of chromosome 6, close to restriction fragment length polymorphism (RFLP) marker C226A (Yano et al. 1997). Subsequently, it was accurately mapped by using 93 segregants derived from advanced backcross progeny in which the *Hd3* region was heterozygous and most other chromosomal regions were homozygous for the Nipponbare allele (Yamamoto et al. 1998). In addition, analysis with an NIL for *Hd3* revealed that the Kasalath allele reduces days-to-heading under short-day (SD) conditions and increases it under long-day (LD) conditions (Lin et al. 2000). Because *Hd3* has been mapped as a single Mendelian locus in RFLP linkage mapping (Yamamoto et al. 1998), the Kasalath allele was considered to have two functions: inhibition of heading under NF conditions and promotion of heading under SD conditions.

To map *Hd3* more precisely for the goal of map-based cloning, we performed a high-resolution linkage analysis using 595 plants. Progeny testing under SD and NF con-

ditions was used to determine the genotype of *Hd3*. We detected two tightly linked genes controlling heading date in the *Hd3* region. Using NILs, we also clarified that promotion and inhibition of heading under SD and LD conditions are differentially regulated by both genes.

Materials and methods

Plant materials

The population used for high-resolution linkage mapping of *Hd3* was generated by self-pollinating two individuals (designated $95BC_4\ F_2$ -18-17 and -79) selected from advanced backcross progeny derived from a cross between Nipponbare and Kasalath. The resultant 595 plants were grown in an experimental paddy field at the National Institute of Agrobiological Resources, Tsukuba, Ibaraki, Japan. Total DNA of these plants was extracted from leaves by the CTAB method (Murray and Thompson 1980). Self-pollinated seeds were also collected for progeny testing.

Linkage analysis

Twelve RFLP markers flanking *Hd3* were used for high-resolution linkage mapping (Harushima et al. 1998; Yamamoto et al. 1998). A end clone was generated from a yeast artificial chromosome (YAC) clone, Y6828, by the method described by Umehara et al. (1995) and used as an additional RFLP marker. RFLP analysis was performed according to the procedure of Kurata et al. (1994).

Progeny testing

Plants in which recombination occurred in the regions flanking *Hd3* were selected for progeny testing to determine the genotype of *Hd3*. Twenty self-pollinated progeny of each plant were grown in a growth chamber under a 10/14-h (light/dark) photoperiod (light intensity: 700 µmol photon/m^2 /s), at 28 °C for 12 h, 24 °C for 12 h. Progeny were also tested under NF conditions in an experimental paddy field in Tsukuba. The duration from seeding to heading lasted from April to August. The mean daylengths and temperatures during the rice cultivation season were as follows: 12:56 h and 14.3 °C in April; 14:04 h and 18.7 °C in May; 14:37 h and 19.8 °C in June; 14:17 h and 23.5 °C in July; 13:26 h and 25.2 °C in August. Twenty self-pollinated progeny of each plant were tested. "Heading day" was defined as the day when the top of the first panicle appeared. "Days-to-heading" was defined as the number of days from seeding to heading.

Cleaved amplified polymorphic sequence (CAPS) analysis

For CAPS analysis (Konieczny and Ausubel 1993), a small piece of rice leaf (approximately 0.5 cm^2) was ground in a 1.5-ml tube containing 300 µl of 100 m*M* Tris-HCl, 1 *M* KCl, and 10 m*M* EDTA. Crude DNA in the centrifuged supernatant was precipitated by isopropanol, and the pellet was redissolved in 50 µl H_2 O. One microliter of this DNA extract was used as a template for polymerase chain reaction (PCR) amplification.

For the genotyping of *Hd3a* and *Hd3b*, two CAPS markers – CP15 and R1952, respectively – were used. DNA of each plant was amplified with a pair of primers: CP15 U (5′-ACCGCAGGTCTCCTTGTCATT-3′) and CP15 L (5′-GCTATTGCCATCGCCTTGTGT-3′). The amplified product was digested with *Msp*I and separated by agarose gel electrophoresis. This CAPS marker, CP15, was located between B174 and C1032 (S. Kojima, L. Monna and M. Yano, unpublished data) and used to determine genotype of *Hd3a*. For *Hd3b*, primers R1952U (5′-CCTTGGCCCTTGAATAGTTG-3′) and R1952L (5′-TGCTCGATTCTGACAAAGTT-3′)

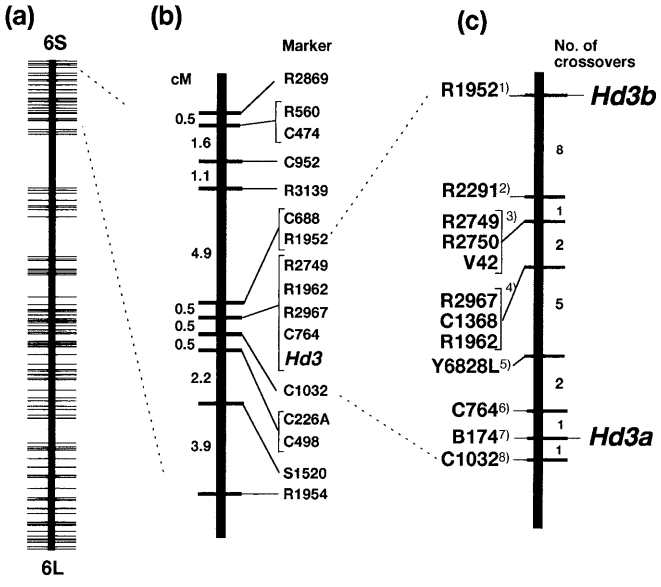

Fig. 1a–c Genetic linkage maps of the *Hd3* region on the short arm of chromosome 6. **a** Framework of high-density RFLP linkage map of Chromosome 6 (Harushima et al. 1998). **b** Linkage map of *Hd3* region previously reported (Yamamoto et al. 1998). **c** High-resolution linkage map constructed in this study using 595 segregants. Numbers of plants in which recombination occurred between adjacent markers are indicated on the *right* of the map. The marker loci are *numbered*, and the genotypes of each recombinant at each marker locus are shown in Tables 1 and 2

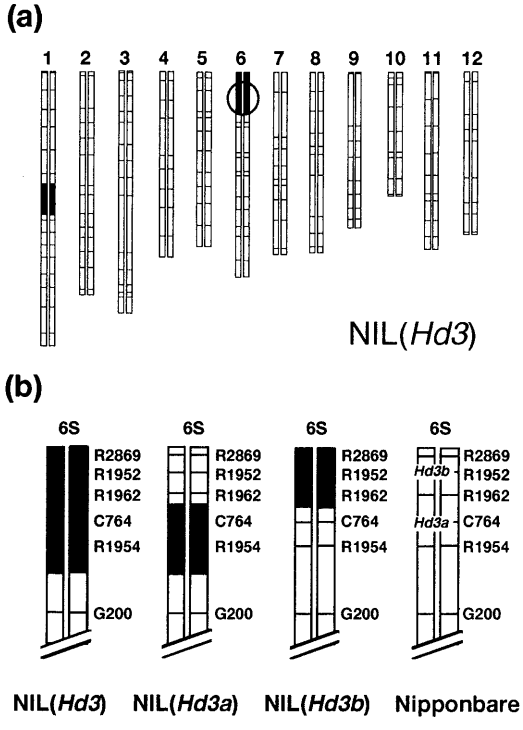

Fig. 2a,b Graphical representation of genotypes of NIL(*Hd3*), NIL(*Hd3a*), and NIL(*Hd3b*). **a** Graphical representation of genotype of NIL(*Hd3*). A chromosomal segment of Kasalath containing both *Hd3a* and *Hd3b* is substituted into the genetic background of Nipponbare (*circle*). **b** Graphical representation of genotypes of the distal end of chromosome 6 in NIL(*Hd3*), NIL(*Hd3a*), NIL(*Hd3b*), and Nipponbare. Genotypes of other chromosomal regions are the same as in NIL(*Hd3*) (**a**)

Table 1 Frequency distribution for days to heading in the F_3 progenies under a short daylength condition

Plant no.	Marker loci[a] and genotypes[b]								Days to heading								*Hd3* genotype[b]
	1	2	3	4	5	6	7	8	37	39	41	43	45	47	49	51	
14-049	K	K	K	H	H	H	H	H		1	1	8	5	3	2		H
14-077	H	H	H	H	K	K	K	K			7	12	1				K
14-079	K	K	K	K	H	H	H	H			1	4	8	4	1		H
14-276	H	H	H	H	H	H	H	N			4	10	4	1	1		H
14-294	H	H	H	H	H	K	K	K		1	9	9		1			K
16-019	N	N	N	N	H	H	H	H				2	11	2	4	1	H
16-068	N	N	N	N	H	H	H	H				3	7	2	7	1	H
16-116	H	H	H	H	H	K	K	K				10	10				K
16-128	N	N	N	H	H	H	H	H		2	4	6	5	4	2		H
16-182	N	N	N	N	H	H	H	H				7	3	3	6	1	H
16-220	K	K	K	K	K	K	H	H		1	5	3	2	6	2		H
16-290	H	H	N	N	N	N	N	N					1	3	13	3	N
Nipponbare	N	N	N	N	N	N	N	N					2	6	2		N

[a] The eight marker loci numbered 1–8 are shown in Fig. 1c
[b] Genotypes: N, homozygous for Nipponbare allele; K, homozygous for Kasalath allele; H, heterozygous.

Table 2 Frequency distribution for days to heading in the F_3 progenies under a natural field condition

Plant no.	Marker loci[a] and genotypes[b]								Days to heading									*Hd3* genotype[b]
	1	2	3	4	5	6	7	8	117	118	119	120	121	122	123	124	125	
14-049	K	K	K	H	H	H	H	H					1	2	12	3	1	K
14-077	H	H	H	H	K	K	K	K	4	2	3	6	3	2	1			H
14-079	K	K	K	K	H	H	H	H				2		5	6	4	5	K
14-151	H	N	N	N	N	N	N	N			4	3	6	5		1		H
14-206	H	N	N	N	N	N	N	N				1	3	2	9	2	3	H
14-227	N	H	H	H	H	H	H	H		4	11	4	3					N
14-276	H	H	H	H	H	H	H	N	1		4	6	7	3	1			H
14-294	H	H	H	H	H	K	K	K		2		6	8	4	2			H
16-019	N	N	N	N	H	H	H	H		1	6	8	5	1				N
16-068	N	N	N	N	H	H	H	H			5	6	7	2				N
16-070	K	H	H	H	H	H	H	H							4	6	9	K
16-116	H	H	H	H	H	K	K	K		3	1	8	5	3	1	1		H
16-128	N	N	N	H	H	H	H	H		6	5	8	3					N
16-139	H	K	K	K	K	K	K	K		2	3	3	6	2	4	1	1	H
16-158	H	N	N	N	N	N	N	N				1	2	4	9	2	3	H
16-166	H	N	N	N	N	N	N	N				2	3	6	5	1	3	H
16-182	N	N	N	N	H	H	H	H		1	4	10	7					N
16-205	N	H	H	H	H	H	H	H		1		6	4	5	2			N
16-220	K	K	K	K	K	K	H	H				1	1	3	9	4	4	K
16-290	H	H	N	N	N	N	N	N				1	4	4	6	1	5	H
Nipponbare	N	N	N	N	N	N	N	N			3	14	3					N

[a] The eight marker loci numbered 1–8 are shown in Fig. 1c
[b] Genotypes: N, homozygous for Nipponbare allele; K, homozygous for Kasalath allele; H, heterozygous. The *Hd3* genotype of 16-205 (shown in *italics*) was certified by repeated progeny testing in the next year (data not shown)

and restriction enzyme *Sac*I were used. These primers were designed from expressed sequence tags (ESTs) released by the Rice Genome Research Program (Yamamoto and Sasaki 1997).

Selection of NILs for *Hd3a* and *Hd3b*

From the individuals used for fine mapping, a plant was selected in which the *Hd3a* region (C1032–R1962) was homozygous for the Nipponbare allele and the *Hd3b* region (distal side of R1962) was heterozygous (Fig. 1). From its self-pollinated progeny, one plant was selected in which the *Hd3b* region was homozygous for the Kasalath allele. The progeny of this plant were designated as NIL(*Hd3b*) (Fig. 2). Another NIL homozygous for the Kasalath allele at *Hd3a* in the genetic background of Nipponbare, called NIL(*Hd3a*), was also selected (Fig. 2). The selection was made with the two CAPS markers described above. NIL(*Hd3*), which is homozygous for the Kasalath allele at both *Hd3a* and *Hd3b*, was developed by Lin et al. (2000).

Analysis of response of NILs under different daylength conditions

Three NILs for *Hd3a*, *Hd3b*, and *Hd3* and their recurrent variety Nipponbare were grown under different daylength conditions. Three growth conditions were used: SD (10 h of light) and LD (14 h of light) in a growth chamber, and NF. The materials were planted with two replications (five plants per replication). Days-to-heading of ten plants in each line was scored, and the least significant difference (LSD) test was used to detect differences among lines.

Results

High-resolution linkage mapping by RFLP markers

Linkage mapping revealed that 20 plants were recombinant between RFLP markers R1952 and C1032 (Fig. 1).

This interval was previously defined as the *Hd3* candidate region (Yamamoto et al. 1998). One recombination was detected between R2291 and R2749 (which co-segregates with R2750 and V42; hereafter identified simply as R2749), and two were detected between R2749 and R2967 (co-segregating with C1368 and R1962), although these markers had been mapped at the same locus in our Nipponbare–Kasalath F_2 population (Harushima et al. 1998). A new RFLP marker, Y6828L, generated by cloning of the end sequence of the YAC clone Y6828, was mapped between C764 and R2967. The overall resolution of this map was better than that of the map reported previously (Yamamoto et al. 1998).

Progeny testing under SD and NF conditions

Progeny were tested under SD conditions for genotyping *Hd3*. We first selected three individuals with a recombination between R2291 and R2967, then seven with crossovers between R2967 and C764, and finally one with recombination between C764 and B174 and one with a recombination between B174 and C1032. The first progeny testing mapped *Hd3* on the centromere side of R2291; eight individuals with crossovers between R1952 and R2291 were not tested further. Self-pollinated progeny of selected plants grown under SD conditions showed early heading (approximately 42 days), late heading (approximately 48 days), and segregation from early to late (39–50 days), representing plants homozygous for the Kasalath allele, homozygous for the Nipponbare allele, and heterozygous, respectively (Table 1). This result and the RFLP data localized *Hd3* within the interval of C764 and C1032; no recombination was observed between *Hd3* and B174 (Fig. 1).

Progeny of all 20 plants with recombination between R1952 and C1032 were grown in an experimental paddy field, and days-to-heading was scored. Based on the variation in days-to-heading, the genotype of each selected plant was determined (Table 2). As a result, *Hd3* co-segregated with R1952.

The results of linkage mapping under both SD and NF conditions suggested that there were two heading date-related genes, designated *Hd3a* and *Hd3b*, in the *Hd3* region identified by Yamamoto et al. (1998). The Kasalath allele at *Hd3a* promoted heading under SD conditions, and the Kasalath allele at *Hd3b* delayed heading under NF conditions. Nineteen recombinations among 595 individuals were detected between these two genes, giving the genetic distance between them as approximately 1.6 cM.

Inheritance mode of *Hd3a* and *Hd3b*

To clarify the mode of inheritance of *Hd3a* and *Hd3b*, we monitored days-to-heading of each plant in the segregating populations under SD and NF conditions. The genotypes were estimated by CAPS analysis.

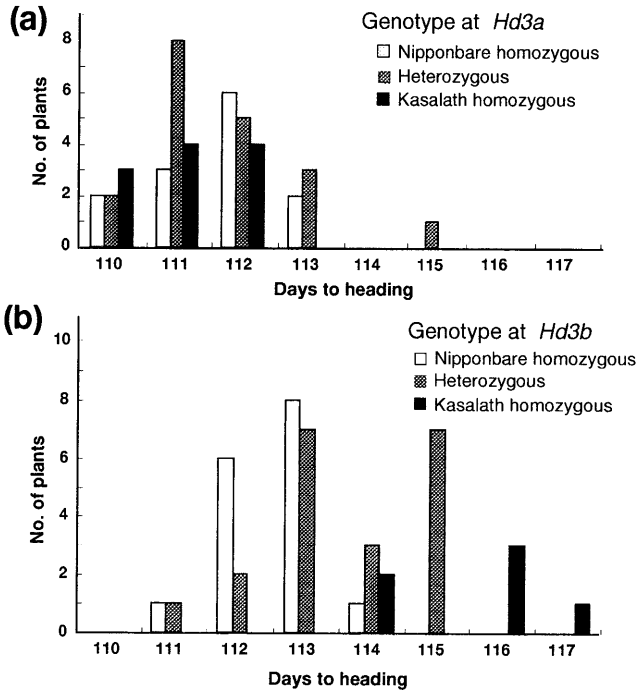

Fig. 3 Frequency distribution of days-to-heading in segregating populations of *Hd3a* (**a**) and *Hd3b* (**b**) under natural field conditions. Genotypes of *Hd3a* and *Hd3b* are represented as a *white bar* (homozygous for Nipponbare allele), *shaded bar* (heterozygous), and *black bar* (homozygous for Kasalath allele)

Under NF conditions, a wide variation in days-to-heading occurred in the *Hd3b* segregating population, but not in the *Hd3a* population (Fig. 3). The Kasalath allele at *Hd3b* delays heading; plants heterozygous for *Hd3b* showed intermediate scores for days-to-heading (Fig. 3b). In the population with Nipponbare *Hd3b* and segregating *Hd3a* grown under NF conditions, no shift in days-to-heading occurred based on the genotypes of *Hd3a* (Fig. 3a). Therefore, *Hd3b* may affect days-to-heading under NF conditions, but the effect of *Hd3a* was not obvious under NF conditions. The intermediate distribution of days-to-heading of plants heterozygous for *Hd3b* suggests that the genetics of *Hd3b* is additive.

On the other hand, under SD conditions, there was an obvious relationship between days-to-heading and the genotypes of *Hd3a* when a population with Nipponbare *Hd3b* and segregating *Hd3a* was tested: Kasalath *Hd3a* promotes heading and Nipponbare *Hd3a* delays heading (Fig. 4a). However, no shift in days-to-heading occurred according to the genotypes of *Hd3b* (Fig. 4b). Therefore, it can be concluded that *Hd3a* caused the difference in heading date under SD conditions and that the effect of *Hd3b* can be ignored under SD conditions. The intermediate distribution of days-to-heading of plants heterozygous for *Hd3a* also suggests that the genetics of *Hd3a* is additive.

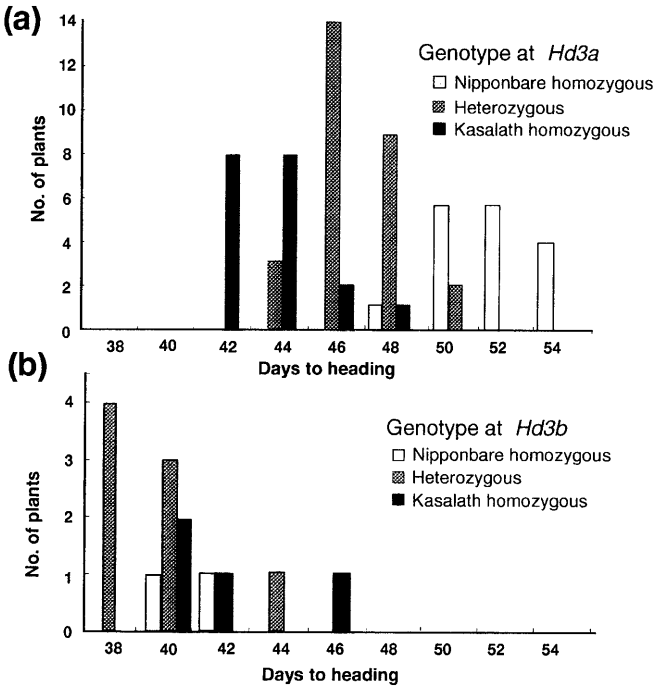

Fig. 4 Frequency distribution of days-to-heading in segregating populations of *Hd3a* (**a**) and *Hd3b* (**b**) under short-day conditions. Genotypes of *Hd3a* and *Hd3b* are represented as a *white bar* (homozygous for Nipponbare allele), *shaded bar* (heterozygous), and *black bar* (homozygous for Kasalath allele)

Table 3 Days to heading (mean[a] ± SD) of nearly-isogenic lines and their recurrent variety, Nipponbare, under two photoperiod treatments and natural field conditions (SD standard deviation)

Lines	Short daylength (10 h)	Long daylength (14 h)	Natural field
NIL(*Hd3a*)	41.7±0.9c	106.8±2.4c	114.4±1.1bc
NIL(*Hd3b*)	49.2±1.1a	117.7±3.2a	118.8±1.8a
NIL(*Hd3*)	40.6±1.2d	108.9±2.5bc	120.4±2.5a
Nipponbare	47.7±0.9b	110.2±2.1b	113.2±0.8c

[a] Means within a column followed by different letters (a, b, c, and d) are significantly different at $P \leqq 0.05$ by the least significant difference test

Photoperiodic response of NILs for *Hd3a* and *Hd3b*

NILs were selected from self-pollinated progeny of plants chosen from the segregating population for linkage analysis. Graphical genotypes of these NILs are shown in Fig. 2.

We developed an NIL homozygous for Nipponbare allele at *Hd3a* and for Kasalath at *Hd3b* [NIL(*Hd3b*)] and an NIL homozygous for Kasalath at *Hd3a* and for Nipponbare at *Hd3b* [NIL(*Hd3a*)] by marker-assisted selection. Lin et al. (2000) have already developed an NIL homozygous for Kasalath at both *Hd3a* and *Hd3b* [NIL(*Hd3*)]. These NILs are isogenic to Nipponbare except for one segment in the proximal region of chromosome 1 (Fig. 2). No heading date-related QTLs have previously been detected on this region (Yano et al. 1997; Lin et al. 1998; Yamamoto et al. 2000).

These NILs and Nipponbare (control) were grown under SD, LD, and NF conditions, and days-to-heading of each line was scored (Table 3). NIL(*Hd3a*) showed earlier heading than Nipponbare under SD conditions, but no difference under NF conditions. It also showed earlier heading than Nipponbare under LD conditions, but the difference was small. On the other hand, NIL(*Hd3b*) showed later heading under LD and NF conditions, but only a small difference under SD conditions. These results confirmed the existence of two loci, *Hd3a* and *Hd3b*, which are differentially regulated by two photoperiodic conditions, SD and LD.

Discussion

One of the questions about QTLs is whether a QTL consists of a single gene (or factor) or of a cluster of genes with relatively weak effects. Previous studies suggested that QTLs for heading date – *Hd1*, *Hd2*, *Hd3*, and *Hd6* – represent single Mendelian factors (Yamamoto et al. 1998, 2000). However, the present study provides evidence for the existence of two tightly linked genes with related functions in a small chromosomal region originally considered a single QTL. In linkage analysis using 93 segregants, Yamamoto et al. (1998) considered *Hd3* to be a single gene controlling heading date. Lin et al. (2000), using an NIL, considered it a typical photoperiod-sensitivity gene that both decreases days-to-heading under SD conditions and increases it under LD conditions. However, in our study, higher-resolution mapping with 595 segregants and progeny testing under both SD and NF conditions revealed two tightly linked genes. Because we previously detected *Hd3* under NF conditions, we could detect only *Hd3b* in that study (Yano et al. 1997). Using SD conditions in this study enabled us to detect the new locus, *Hd3a*.

Epistatic interactions have been observed between *Hd3* and *Hd1* under SD conditions, and between *Hd3* and *Hd2* under NF conditions (Lin et al. 2000). However, because we have revealed two heading date-related genes at the *Hd3* region, interactions between *Hd3a* and *Hd3b*, and between *Hd1* and *Hd2*, should be analyzed under both NF and SD conditions. Our results suggest that an epistatic interaction between *Hd3b* and *Hd2* might appear under NF but not SD conditions because the phenotypic effect of *Hd3b* is evident only under NF conditions. On the other hand, *Hd3a* might interact with *Hd1* and *Hd2* under SD conditions.

Hd1 is allelic to *Se1*, a major photoperiod sensitivity gene (Yano et al. 2000). Another photoperiod-sensitivity gene, *En-Se1*, has been mapped on the short arm of chromosome 6; it enhances the photoperiod sensitivity of *Se1*, resulting in late heading under NF conditions (Sano 1992). Yano et al. (1997) and Yamamoto et al. (1998) suggested that *En-Se1* might be allelic to *Hd3*, based on

a comparison of their map positions. Considering how *En-Se1* increases days-to-heading under NF conditions, *Hd3b* rather than *Hd3a* seems to be functionally analogous to *En-Se1*. A recent study, however, mapped *En-Se1* between C764 and R845 and it co-segregated with B174 and C1032 (Monna et al. 2000); thus, *Hd3b* cannot be allelic to *En-Se1*. Based on the comparison of map positions, *Hd3a* and *En-Se1* are likely to be allelic. Further analysis will be necessary to clarify the relationship between *Hd3a* and *En-Se1*.

Allelic differences between Nipponbare and Kasalath at both *Hd3a* and *Hd3b* occurred spontaneously. Therefore, we cannot presume which allele functions in the photoperiod response. In appearance, the Kasalath allele at *Hd3a* might promote heading under SD conditions and can thus be called a 'flowering promoter' under SD, but the Nipponbare allele at *Hd3a* could act as a 'flowering inhibitor'. The same question is raised for *Hd3b*. The answer remains uncertain until both genes are cloned.

In this study, by means of high-resolution linkage analysis, we genetically dissected two tightly linked heading-date-related genes in a region previously considered to be one locus. In primary QTL analysis, it is difficult to identify genes with related functions when they lie close to each other. This study clearly demonstrated that high-resolution, fine-scale genetic mapping can overcome this situation.

In *Arabidopsis*, many genes involved in flowering time have been isolated (reviewed by Levy and Dean 1998; Fowler et al. 1999; Kobayashi et al. 1999; Sheldon et al. 1999). One of them, *CONSTANS* (*CO*), encodes a putative transcription factor that has a zinc finger domain (Putterill et al. 1995). Homologs of *CO* function in *Brassica napus* (Robert et al. 1998) and rice (Yano et al. 2000). Song et al. (1998) compared the structures of homologs of *CO* derived from random EST sequencing analysis in rice and *Arabidopsis CO*. They suggested one homolog, R2931 (same clone as R2967), to be a probable candidate gene for *Hd3*. This hypothesis was refuted based on the high-resolution mapping in this study. Hence, neither *Hd3a* nor *Hd3b* co-segregated with R2967. We are currently trying to identify both *Hd3a* and *Hd3b* genes using a map-based strategy. It should also be necessary to compare the chromosomal locations of other classes of genes controlling flowering time in *Arabidopsis* to facilitate the identification of probable candidate genes for *Hd3a* and *Hd3b*.

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References

Fowler S, Lee K, Onouchi H, Samach A, Richardson K, Morris B, Coupland G, Putterill J (1999) *GIGANTEA*: a circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. EMBO J 18:4679–4688

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS, Sasaki T (1998) A high density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148:479–494

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. Science 286:1960–1962

Konieczny A, Ausubel FM (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403–410

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin S-Y, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang Z-X, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nat Genet 8:365–372

Levy YY, Dean C (1998) The transition to flowering. Plant Cell 10:1973–1989

Li ZK, Pinson SRM, Park WD (1995) Identification of quantitative trait loci (QTLs) for heading date and plant height in cultivated rice (*Oryza sativa* L.). Theor Appl Genet 91:374–381

Lin HX, Qian HR, Xiong ZM, Min SK, Zhen KL (1996) Mapping of major genes and minor genes for heading date in several rice varieties (*Oryza sativa* L.). Chin J Genet 23:107–114

Lin HX, Yamamoto T, Sasaki T, Yano M (2000) Characterization and detection of epistatic interactions of three QTLs, *Hd1*, *Hd2*, and *Hd3*, controlling heading date in rice using nearly isogenic lines. Theor Appl Genet 101:1021–1028

Lin SY, Sasaki T, Yano M (1998) Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa* L. using backcross inbred lines. Theor Appl Genet 96:997–1003

Monna L, Sano Y, Yano M (2000) Genetic mapping of rice photoperiod sensitivity gene *Enhancer-Se1*. Rice Genet Newsl 17:40–42

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res 8:4321–4325

Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell 80:847–857

Robert LS, Robson F, Sharpe A, Lydiate D, Coupland G (1998) Conserved structure and function of the *Arabidopsis* flowering time gene *CONSTANS* in *Brassica napus*. Plant Mol Biol 37:763–772

Sano Y (1992) Genetic comparisons of chromosome 6 between wild and cultivated rice. Jpn J Breed 42:561–572

Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards A, Peacock WJ, Dennis E (1999) The *FLF* MADS box gene: a repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. Plant Cell 11:445–458

Song J, Yamamoto K, Shomura A, Itadani H, Zhong HS, Yano M, Sasaki T (1998) Isolation and mapping of a family of putative zinc-finger protein cDNAs from rice. DNA Res 30:95–101

Umehara Y, Inagaki A, Tanoue H, Yasukochi Y, Nagamura Y, Saji S, Otsuki Y, Fujimura T, Kurata N, Minobe Y (1995) Construction and characterization of a rice YAC library for physical mapping. Mol Breed 1:79–89

Xiao J, Li J, Yuan L, Tanksley SD (1995) Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics 140:745–754

Yamamoto K, Sasaki T (1997) Large-scale EST sequencing in rice. Plant Mol Biol 35:135–144

Yamamoto T, Kuboki Y, Lin SY, Sasaki T, Yano M (1998) Fine scale mapping of quantitative trait loci, *Hd-1*, *Hd-2* and *Hd-3*, controlling heading date of rice, as single Mendelian factors. Theor Appl Genet 97:37–44

Yamamoto T, Lin HX, Sasaki T, Yano M (2000) Identification of heading date quantitative trait locus *Hd6*, and characterization of its epistatic interaction with *Hd2* in rice using advanced backcross progeny. Genetics 154:885–891

Yano M, Harushima Y, Nagamura Y, Kurata N, Minobe Y, Sasaki T (1997) Identification of quantitative trait loci controlling heading date in rice using a high-density linkage map. Theor Appl Genet 95:1025–1032

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. Plant Cell 12:2473–2483