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Characterization and detection of epistatic interactions of 3 QTLs, *Hd1*, *Hd2*, and *Hd3*, controlling heading date in rice using nearly isogenic lines

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Abstract To characterize quantitative trait loci (QTLs), we used marker-assisted selection (MAS) to develop three nearly isogenic lines (NILs) differing only for the presence of a single, specific QTL (QTL-NILs) – *Hd1*, *Hd2*, and *Hd3* – for heading date in rice. The three lines contained the chromosomal region of the target QTL from donor variety Kasalath (*indica*) in the genetic background of var. Nipponbare (*japonica*). To analyze epistatic interactions in pairs of these QTLs, we also used MAS to develop four combined QTL-NILs with 2 of the 3 QTLs or with all 3. Different daylength treatment testing of the QTL-NILs revealed that the three QTLs control photoperiod sensitivity. Genetic analysis of F_2 populations derived from crosses between the three QTL-NILs with a single QTL using molecular markers revealed the existence of epistatic interactions between *Hd1* and *Hd2*, and *Hd2* and *Hd3*. These interactions were also confirmed by the analysis of combined QTL-NILs under different daylength conditions. The existence of an epistatic interaction between *Hd1* and *Hd3* was also clarified. Based on these results, we suggest that the Kasalath allele of *Hd3* does not affect photoperiod sensitivity by itself but that it is involved in enhancement of the expression of the Nipponbare alleles of *Hd1* and *Hd2*.

Key words *Oryza sativa* L. · Quantitative trait loci · Photoperiod sensitivity · Digenic interaction · Nearly isogenic lines

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Introduction

Rice is a major crop, cultivated extensively in a wide range of latitudes from 53°N to 35°S. Heading date is a critical trait for adaptation to different cultivation areas and cropping seasons, and in rice photoperiod sensitivity (PS) and basic vegetative growth (BVG) are the main determinants of heading date. Several major genes controlling PS in rice have been identified, including *Se1* (photoperiod sensitivity 1), *Se3–Se7*, and *E1–E3* (heading date 1–3) (Yokoo et al. 1980; Yamagata et al. 1986; Poonyarit et al. 1989; Yokoo and Okuno 1993; Tsai 1995; Kinoshita 1998). Among the known PS genes, *Se1*, *Se3*, and *Se5* have been located on chromosome 6, and *E1* and *E3* on chromosomes 7 and 3, respectively (Yokoo et al. 1980; Yokoo and Okuno 1993; Causse et al. 1994; Okumoto et al. 1996; Okumoto and Tanisaka 1997; Kinoshita 1998). Many quantitative trait loci (QTLs) for heading date in rice have been recently mapped using DNA markers (Mackill et al. 1993; Li et al. 1995; Xiao et al. 1995, 1996; Lin et al. 1996; Yano et al. 1997; Lin et al. 1998; Tamura et al. 1998). Li et al. (1995) detected 3 QTLs for heading date; *QHd3a*, *QHd8a* and *QHd9a* were mapped on chromosome 3, 8 and 9, respectively. Xiao et al. (1995) identified 5 QTLs, *dth3–1*, *dth3–2*, *dth4*, *dth7*, and *dth8*. In an other study, 6 QTLs, *hd1*, *hd3*, *hd6a*, *hd6b*, *hd8*, and *hd12* were identified using an F_2 population from a cross of two *indica* varieties (Lin et al. 1996). The nomenclature *hd* was different from that reported by Yano et al. (1997). However, it was difficult to compare the chromosomal position precisely among these QTLs, and the allelic relationships between these QTLs and known major genes remain unknown.

Yano et al. (1997) identified 5 QTLs (*Hd1–Hd5*) conferring heading date in rice 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. Three new QTLs for heading date were detected by using a backcross inbred line (BIL) population (BC_1F_5) derived from the same cross combination (Lin et al. 1998). Further, fine mapping was carried out on 3 putative QTLs (*Hd1–Hd3*) as a single Mendelian factor, using advanced backcross progeny (Yamamoto et al. 1998). These 3 QTLs have been precisely mapped on chromosomes *6* (*Hd1* with the closest markers, C235 and S2539 and *Hd3* with the closest marker, C764) and 7 (*Hd2* with the closest marker, C728). However, their function – whether or not they are involved in the response to photoperiod or basic vegetative growth – has not been clarified.

Based on classical quantitative genetic theory (Cockerham 1954), the phenotypic variance for quantitative traits can be partitioned into an additive portion resulting from the average effects of genes, a portion resulting from dominant effects (allelic interactions of genes), and a portion resulting from epistatic effects (non-allelic interactions). Studies on epistasis have been carried out by classical quantitative genetic methods (Spickett and Thoday 1966; Mather and Jinks 1982; Pooni et al. 1987; Allar 1988). However, these studies, not based on QTL analysis, took into consideration the whole genetic system controlling the quantitative traits and therefore could not provided information on the epistatic interactions between individual QTLs.

Recent genetic analyses of quantitative traits using molecular markers have revealed that they have a potential value in evaluating epistatic interactions between individual loci (Paterson et al. 1991; Stuber et al. 1992; DeVincente and Tanksley 1993; Doebley et al. 1995; Lark et al. 1995; Eshed and Zamir 1996). A few examples have been reported so far in rice (Xiao et al. 1995; Li et al. 1997; Yano et al. 1997). However, these studies did not clearly support the existence of specific interactions among QTLs because the analyses of primary populations (for example, F_2 populations and recombinant inbred line populations) could not provide much information on epistatic interactions among QTLs due to several reasons, such as interference by the variation derived from other QTLs and small population size (Tanksley 1993; Yano and Sasaki 1997). Tanksley (1993) also suggested that novel plant materials, such as nearly isogenic lines, would be required to clarify the nature of epistatic interaction between QTLs.

In the study reported here, we characterized *Hd1*, *Hd2*, and *Hd3* and analyzed interactions among them using nearly isogenic lines with target QTLs (QTL-NILs) developed by marker-assisted selection (MAS), under different daylength conditions and natural conditions.

Materials and methods

Plant materials

The parental line, Nipponbare, a *japonica* variety exhibits a photoperiod sensitivity, and the other parental line, Kasalath, an *indica* variety shows less photoperiod sensitivity. Days to heading of Nipponbare and Kasalath are 118 and 110 days, respectively, under natural field conditions at Tsukuba, Japan. Three QTL-NILs with a single target QTL, called NIL(*Hd1*), NIL(*Hd2*), and NIL(*Hd3*), were selected from backcross progenies derived from a cross between Nipponbare as the recurrent parent and Kasalath as the donor parent by MAS. NIL(*Hd1*) was selected from the BC_4F_2 generation, and NIL(*Hd2*) and NIL(*Hd3*) were selected from the BC_3F_2 generation. Three combined QTL-NILs with three pairs of QTLs – NIL(*Hd1*/*Hd2*), NIL(*Hd1*/*Hd3*), and NIL(*Hd2*/*Hd3*) – and 1 combined QTL-NIL with all three QTLs – NIL (*Hd1*/*Hd2*/ *Hd3*) – were developed. These combined lines were also selected from the BC_3F_2 generation or from F_2 populations derived from crosses between QTL-NILs with a single target QTL.

Three F_2 populations derived from cross combinations between three pairs of NILs with a single QTL were constructed. These F_2 populations (96 plants each), seven NILs and Nipponbare as a control were cultivated in a paddy field at the National Institute of Agrobiological Resources, Tsukuba, Japan. The duration from seeding to heading was from April to August. The mean daylengths and mean temperatures under natural conditions were as following, 12h 56 min and 14.3°C for April, 14 h 4 min and 18.7°C for May, 14 h 37 min and 19.8°C for June, 14 h 17 min and 23.5°C for July, and 13 h 26 min and 25.2°C for August. Days to heading (days from seeding to heading) were recorded for each individual plant. The genotypes for each plant were determined by restriction fragment length polymorphism (RFLP) analysis of each segregating chromosomal region.

RFLP analysis

RFLP analysis followed the procedures described by Kurata et al. (1994). Eight restriction enzymes – *Apa*I, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Kpn*I – were used to digest genomic DNA. Digested DNA was blotted onto a nylon membrane (Boehringer Mannheim, Mannheim, Germany). Southern hybridization and detection were done with an ECL direct labeling and detection system (Amersham Pharmacia Biotech, Buckinghamshire, UK). A total of 127 RFLP markers covering the whole rice genome were used to estimate the status of substitution of chromosome segments in all processes of selection (Harushima et al. 1998). In the F_2 analysis, tightly linked RFLP markers (S2539 for *Hd1*, C728 for *Hd2*, and C764 for *Hd3*) were used to determine the genotype of the target QTLs.

Daylength treatment test

To clarify the function of QTLs and the existence of epistatic interactions among them, we grew Nipponbare (control), three QTL-NILs with a single QTL, three combined NILs with a pair of QTLs, and one combined NIL with three QTLs in growth chambers with different daylength conditions at 26°C for 12 h and 22°C for 12 h. Two daylength conditions, a short daylength (10.0 h) (SD) and a long daylength (13.5 h) (LD), were used. The materials were planted in the growth chambers with two replications (5 plants per replication). Days to heading were recorded for each plant.

Statistical analysis

The least significant difference (LSD) test was used to compare the differences between means of days to heading recorded for each line. The mean values of days to heading recorded for each NIL were compared with that recorded for the isogenic control,

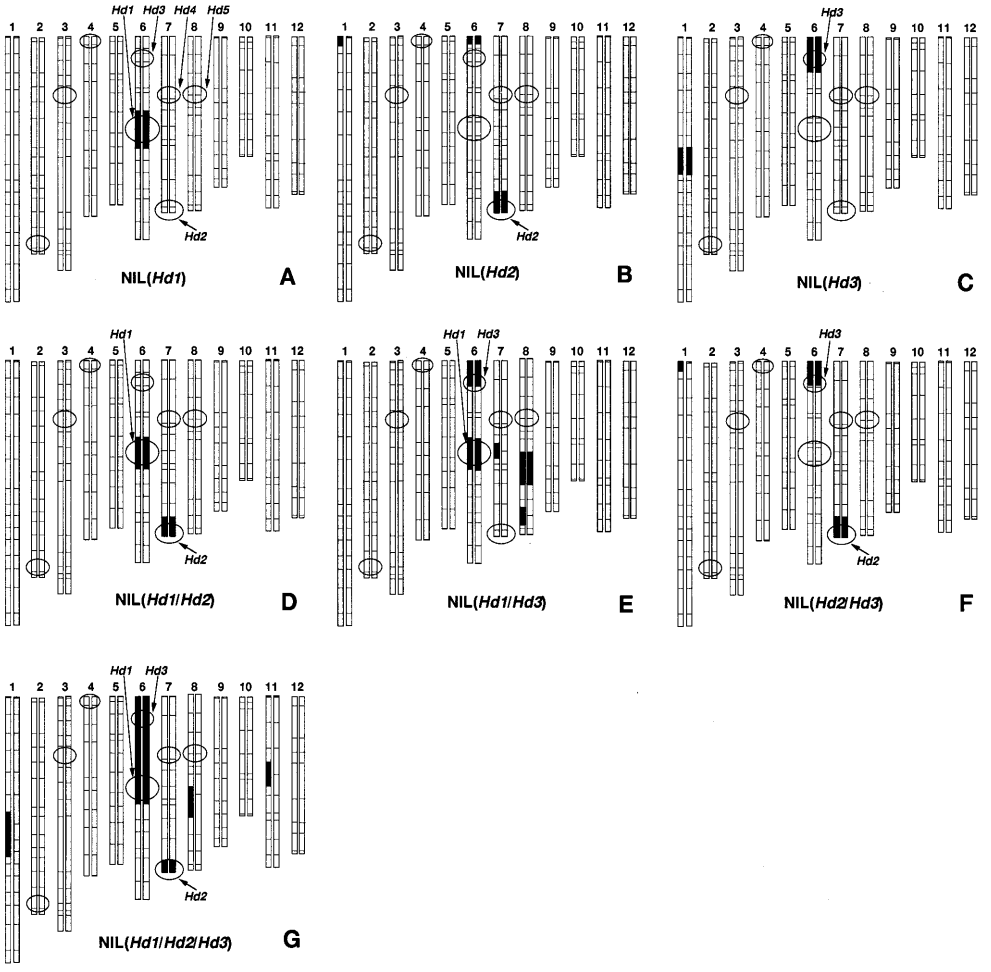

Fig. 1A–G Graphical genotypes of seven NILs: **A** NIL(*Hd1*), **B** NIL(*Hd2*) **C** NIL(*Hd3*), **D** NIL(*Hd1*/*Hd2*) **E** NIL(*Hd1*/*Hd3*), **F** NIL(*Hd2*/*Hd3*), **G** NIL(*Hd1*/*Hd2*/*Hd3*). The 12 pairs of bars represent the chromosomes, *numbered* at the *top*. The *horizontal lines* on the bars show positions of marker loci used in marker-assisted selection (MAS). The eight circles on the chromosomes represent regions for 8 QTLs detected in previous studies (Yano et al. 1997; Lin et al. 1998). *Open bars* and *solid bars* show segments of the chromosomes derived from Nipponbare and Kasalath, respectively

Nipponbare, by multiple comparisons with a common control by Dunnet's least significant difference test. To confirm the effect of individual QTLs, we carried out QTL analyses in the F_2 populations using MAPMAKER/QTL (Lander and Botstein 1989; Lincoln et al. 1993) and the one-way analysis of variance (ANOVA) of the PROC GLM module of SAS (SAS Institute 1988). Two-way ANOVA by PROC GLM was used to evaluate epistatic interactions between pairs of QTLs, as represented by the nearest marker loci in the F_2 populations. The program in GLM of SAS is as follows: proc glm data=exp; class m1 m2; model y=m1 m2 m1*m2; means m1 m2 m1*m2. The m1 and m2 are the nearest marker loci for QTL1 and QTL2, respectively. The *P* value in the source of variance for m1*m2 was used as the significance threshold-detected interaction between the two QTLs.

Results

QTL-NILs developed by marker-assisted selection

Figure 1 shows graphical genotypes of the seven NILs selected. These genotypes are homozygous for the Kasalath alleles at the target QTL regions and homozygous for the Nipponbare alleles in most other chromosomal regions, including other QTL regions. Small segments of Kasalath chromosomes including the target QTL were substituted in the Nipponbare genetic background in NIL(*Hd1*), NIL(*Hd2*), and NIL(*Hd3*). In the combined QTL-NILs, although introgression of some undesired Kasalath chromosomal segments did occur, the level of undesired introgression is minimal and never involves chromosomal regions for the heading date QTLs previously identified (Yano et al. 1997; Lin et al. 1998; Yamamoto et al. 2000) (Fig. 1). Thus, these lines can be used as NILs for further analysis.

Confirmation of QTLs

We observed a large variation of heading date in F_2 populations derived from crosses between three pairs of NILs with a single QTL under natural daylength conditions (Fig. 2). The existence of the 3 QTLs was confirmed by QTL analysis of these F_2 populations (Table 1). Days to heading were increased by the Nipponbare alleles at the *Hd1* and *Hd2* loci (Table 1, Fig. 2A–C) and the Kasalath allele at *Hd3* (Table 1, Fig. 2B, C). These results are consistent with those found in earlier reports (Yano et al. 1997; Yamamoto et al. 1998). Days to heading of the three NILs for *Hd1*, *Hd2*, and *Hd3* was 101.8, 106.6, and 122.4 days, respectively, and significantly different from that of the control (all $P < 0.01$) under the natural day-

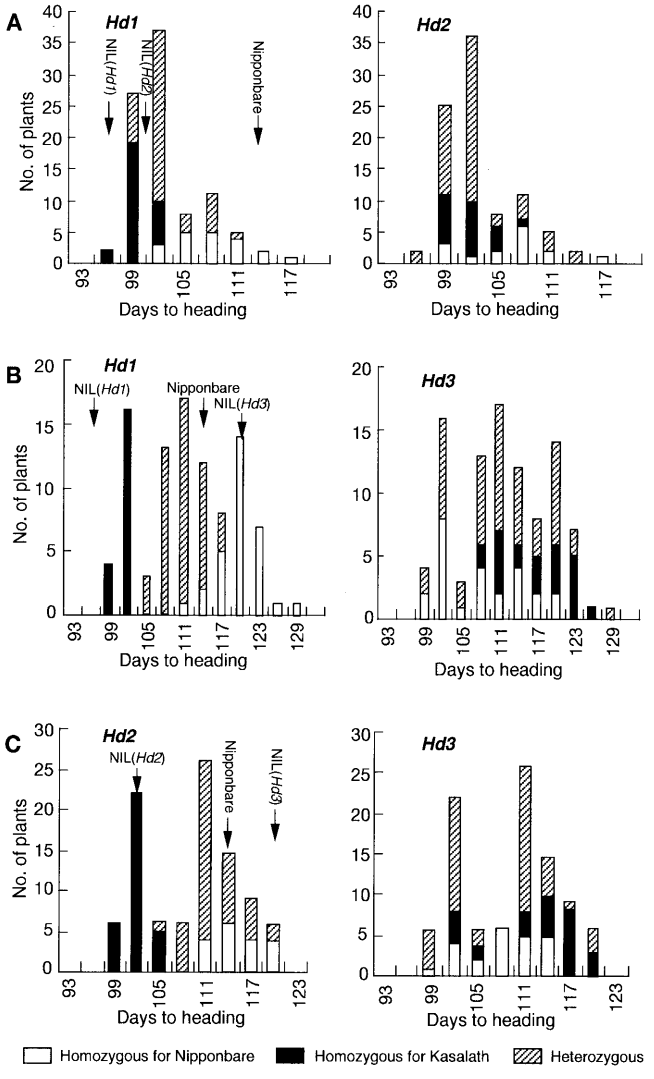

Fig. 2A–C Frequency distributions of days to heading in three genotype classes (homozygous for Nipponbare and Kasalath and heterozygous, respectively) of individual QTLs in three F_2 populations derived from crosses between QTL-NILs under natural conditions. **A–C** Frequency distributions of F_2 populations between: **A** NIL(*Hd1*) and NIL(*Hd2*), **B** NIL(*Hd1*) and NIL(*Hd3*), and **C** NIL(*Hd2*) and NIL(*Hd3*). *Arrows* indicate mean days to heading for Nipponbare and the QTL-NILs

length (ND) conditions of the field test (Table 2). These results support the existence and gene actions of *Hd1*, *Hd2*, and *Hd3*.

Characterization of *Hd1*, *Hd2*, and *Hd3*

Days to heading for NILs and Nipponbare was investigated under two different daylength conditions (10.0 and 13.5 h) (Table 2). Based on Dunnet's *t*-test, days to heading of these three QTL-NILs with a single QTL was significantly different from that of the isogenic control (P <0.01) under SD conditions. Days to heading of NIL(*Hd1*) and NIL(*Hd2*) increased under SD conditions but decreased under LD conditions. On the other hand, days to heading of NIL(*Hd3*) decreased under SD conditions but increased under LD conditions. This difference in days to heading between LD and SD illustrates a degree of photoperiod sensitivity (PS). The PS of NIL(*Hd1*) and NIL(*Hd2*) were smaller than that of Nipponbare and that of NIL(*Hd3*) was larger (Table 2). These results clearly indicate that *Hd1*, *Hd2*, and *Hd3* are involved in photoperiod sensitivity and also suggest that the Nipponbare alleles at the *Hd1* and *Hd2* loci and the Kasalath allele at the *Hd3* locus are functional in the enhancement of PS. Thus, Nipponbare alleles at *Hd1* and *Hd2* and the Kasalath allele at *Hd3* promote heading under SD conditions and inhibit heading under LD and ND condtitions.

Epistasis between QTLs revealed by F_2 analysis under natural daylength conditions

To clarify the epistatic interaction between the QTLs, we compared the average days to heading of different genotype classes in F_2 populations derived from crosses between QTL-NILs. For *Hd1* and *Hd2*, digenic interaction was detected based on two-way ANOVA (P <0.0001) (Fig. 3A). The effect of the Nipponbare allele at *Hd2* with increasing days to heading was observed in those sub-populations homozygous for the Nipponbare allele and heterozygous at *Hd1* but not in the sub-population homozygous for the Kasalath allele at *Hd1*. For *Hd2* and *Hd3*, epistatic interaction was also detected (P <0.0001) (Fig. 3C). The effect of the Kasalath allele at the *Hd3* locus, with increasing days to heading was observed in genotype classes homozygous for the Nipponbare allele and heterozygous at the *Hd2* locus but not in the class homozygous for the Kasalath allele at the *Hd2* locus. These results suggest that there are epistatic interactions between *Hd1* and *Hd2* and between *Hd2* and *Hd3*.

On the other hand, for *Hd1* and *Hd3*, the two-way ANOVA did not suggest the existence of digenic interaction (P=0.2912). *Hd1* and *Hd3* are located on the short arm of chromosome *6*. Owing to the loose linkage between these loci, no segregant homozygous for the Kasalath allele at both *Hd1* and *Hd3* was observed, even in the F_2 population (Fig. 3B). It was difficult to determine an epistatic interaction between *Hd1* and *Hd3* in the small segregating population.

Epistasis between QTLs revealed by NIL analysis under both natural and controlled daylength conditions

To confirm the existence of epistatic interaction between *Hd1*, *Hd2*, and *Hd3*, we investigated days to heading of QTL-NILs with 1, 2, or 3 QTLs under two different controlled daylength conditions. For *Hd1* and *Hd2*, the effect of the Nipponbare allele at *Hd2* on days to heading was not observed under LD and ND conditions when we compared NIL(*Hd1*) and NIL(*Hd1*/*Hd2*).

Table 1 QTLs detected for days to heading based on interval mapping (MAPMAKER/QTL) and one-way ANOVA

Cross	QTL	Chromosome	NML[a]	*P* value[c]	LOD[c]	PVE[d]	a[e]	d[f]
NIL(*Hd1*)/NIL(*Hd2*)	*Hd1*	6	S2539	<0.0001	14.4	50.8	–4.1	–1.1
	Hd2	7	C728	0.0008	3.1	14.8	–2.3	–1.6
NIL(*Hd1*)/NIL(*Hd3*)	*Hd1*	6	S2539	<0.0001	37.7	83.6	–9.1	0.2
	Hd3	6	C764	0.0001	4.0	17.5	4.2	–1.3
NIL(*Hd2*)/NIL(*Hd3*)	*Hd2*	7	C728	<0.0001	36.8	82.9	–6.8	3.4
	Hd3	6	C764	0.0070	2.3	10.1	2.0	–2.5

[a] The nearest marker locus to the QTL
[b] *P* value refers to the probability of the association between a QTL and the nearest marker locus estimated by one-way ANOVA
[c] $\log_{10}$ likelihood
[d] Percentage phenotypic variance explained
[e] Additive effect of the Kasalath allele on days to heading
[f] Dominant effect of the Kasalath allele
[c–f] Estimated by MAPMAKER/QTL

This result suggests that epistatic interaction is involved in phenotypic expression of *Hd1* and *Hd2* (Table 2) and agrees with the result revealed by F_2 analysis in under ND conditions (Fig. 3A). On the other hand, the phenotypic effect of the Nipponbare allele at *Hd2* was clearly observed under SD conditions when we compared NIL(*Hd1*) and NIL(*Hd1*/*Hd2*) (Table 2). This indicates that *Hd1* is epistatic to *Hd2* under both LD and ND conditions but not under SD conditions. Days to heading of NIL(*Hd1*/*Hd3*) and NIL(*Hd2*/*Hd3*) was smaller than that of NIL(*Hd1*) and NIL(*Hd2*) under SD conditions. On the other hand, no difference between days to heading of NIL(*Hd1*/*Hd2*) and NIL(*Hd1*/*Hd2*/*Hd3*) was observed under SD, LD or ND conditions. Hence, the phenotypic effect of the Kasalath allele at *Hd3* was observed when there was a Nipponbare allele at *Hd1* or *Hd2*. These results suggest that the Nipponbare alleles at the *Hd1* and *Hd2* loci are epistatic to the Kasalath allele at the *Hd3* locus. It also indicates that the Kasalath *Hd3* allele itself does not act on photoperiod sensitivity but does act on the response to photoperiod sensitivity of the Nipponbare alleles at *Hd1* and *Hd2*.

Fig. 3A–C Differences in mean days to heading for different genotype classes between *Hd1* and *Hd2* (**A**), *Hd1* and *Hd3* (**B**), and *Hd2* and *Hd3* (**C**) in three F_2 populations derived from crosses between QTL-NILs under natural conditions. Genotypes are represented by the nearest marker loci, S2539 (*Hd1*), C728 (*Hd2*), and C764 (*Hd3*). *P* was calculated by two-way ANOVA. *N*, *H*, and *K* indicate homozygous for Nipponbare allele, heterozygous, and homozygous for Kasalath, respectively

Discussion

In present study, the characterization of QTLs *Hd1*, *Hd2*, and *Hd3*, which control the heading date of rice, and the analysis of epistatic interaction among these three QTLs were performed using QTL-NIL developed by MAS. The results revealed that the 3 QTLs are involved in photoperiod sensitivity and provide concrete evidence for the existence of epistatic interactions among them. Yamamoto et al. (1998) reported the successful fine mapping for these 3 QTLs using selected advanced backcross progeny. An additinal QTL, *Hd6*, was detected and characterized by the same QTL-NIL strategy (Yamamoto et al. 2000). These results clearly indicate that the use of QTL-NILs is a powerful and effective method for characterizing QTLs in detail.

Twenty-four QTLs for heading date in rice have been identified in recent studies (Li et al. 1995; Xiao et al. 1995, 1996; Lin et al. 1996; Yano et al. 1997; Lin et al. 1998). Nevertheless, the functions of these QTLs – response to photoperiod and duration of basic vegetative growth – remain obscure. So far, 13 major gene loci for heading date have been identified (Kinoshita 1998; Ichitani et al. 1998). It is necessary to clarify the relationships between these major genes and the 24 QTLs. However, it is very difficult to identify allelic relationships among these mutant genes and QTLs owing to allelic differences in multiple genes controlling heading date. In this study, we tried to characterize response to photoperiod of three QTLs for heading date, *Hd1*, *Hd2*, and *Hd3*, us-

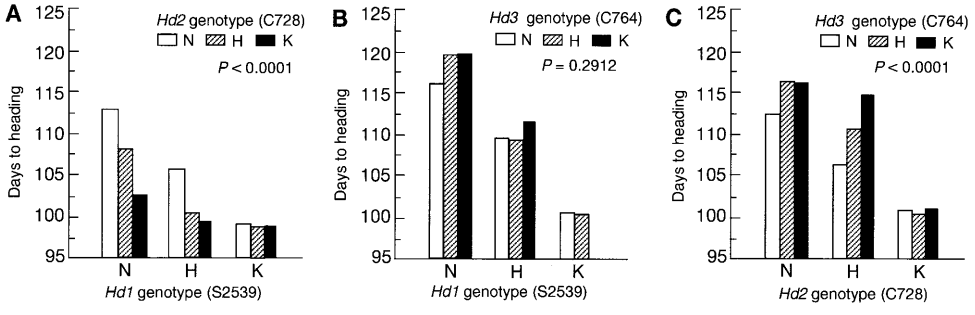

Table 2 Days to heading and photoperiod sensitivity (PS) of the QTL-NILs and control (Nipponbare) under two day-lengths and natural conditions

Lines	Short daylength (10.0 h)		Long daylength (13.5 h)		PS[c]	Natural daylength	
	Days to heading (mean±SD)[a]	Difference from control[b]	Days to heading (mean±SD)[a]	Difference from control[b]		Days to heading (mean±SD)[a]	Difference from control[b]
NIL(*Hd1*)	63.9±1.3 b	9.1**	79.6±2.4 ef	–9.4**	15.7**	101.8±1.5 e	–17.0**
NIL(*Hd2*)	58.2±1.2 c	3.4**	85.0±5.3 c	–4.0	26.8**	106.6±1.5 c	–12.2**
NIL(*Hd3*)	49.1±1.1 e	–5.7**	93.6±1.4a	4.6	44.5**	122.4±0.5 a	3.6**
NIL(*Hd1*/*Hd2*)	79.4±2.9 a	24.6**	80.6±3.0 de	–8.4**	1.2	101.8±0.8 e	–17.0**
NIL(*Hd1*/*Hd3*)	54.9±1.1 d	0.1	81.2±3.2 de	–7.8**	26.3**	103.6±1.1 d	–15.2**
NIL(*Hd2*/*Hd3*)	53.6±2.1 d	–1.2	81.8±1.8 de	–7.2**	28.2**	107.0±1.2 c	–11.8**
NIL(*Hd1*/*Hd2*/*Hd3*)	80.7±1.7 a	25.9**	82.0±3.8 cd	–7.0**	1.3	101.8±0.8 e	–17.0**
Control	54.8±0.6 d		89.0±2.9 b		34.2**	118.8±1.3 b	

*, ** Significance levels of $P \leqq 0.05$ and 0.01, respectively

[a] Means within a column followed by different letters are significantly different at $P \leqq 0.05$ by the least significant difference test

[b] All mean values of NILs were compared with the control (Nipponbare) by Dunnet's LSD test

[c] Photoperiod sensitivity (days to heading in 10-h treatment subtracted from that in 13.5-h treatment). A *t*-test was done

ing different daylength treatment experiments for NILs with the target region. Our results revealed that *Hd1*, *Hd2*, and *Hd3* are involved in photoperiod sensitivity. Several major genes have been determined for photoperiod sensitivity, including *Se1* and *En-Se1* (enhancer for photoperiod sensitivity 1) (Sano 1992). Based on their chromosomal positions, it has been suggested that *Hd1* and *Hd3* are the same loci as the genes, *Se1* and *En-Se1*, respectively (Yano et al. 1997). As we have clarified both *Hd1* and *Hd3* to be photoperiod sensitivity genes in this study, this suggestion is also supported by the function of the QTLs. Epistatic interactions between individual QTLs have been extensively evaluated in primary populations (such as F_2, BC_1, or RIL populations) of several crop plants, including tomato (DeVincente and Tanksley 1993), maize (Edwards et al. 1987; Stuber et al. 1992), and rice (Xiao et al. 1995; Li et al. 1997; Yano et al. 1997). However, only a limited number of epistatic interactions between QTLs were identified because the analysis of primary populations cannot provide much information about the real nature of epistatic interactions among QTLs (Yano and Sasaki 1997): background loci or other QTLs with a large effect interfere with the detection of epistatic interactions. However, by means of QTL- NILs, the frequencies of detecting epistatic interactions were higher than previously estimated in tomato (Eshed and Zamir 1996) and maize (Doebley et al. 1995). This is because QTL-NILs or populations derived from crosses between QTL-NILs with a uniform genetic background can minimize the background genetic effects on the analysis.

In a previous study, analysis for epistatic interactions among 5 QTLs (*Hd1–Hd5*) for rice heading date used an F_2 population from a cross between Nipponbare and Kasalath (Yano et al. 1997). The only epistatic interaction detected was between *Hd1* and *Hd2*. Our study confirmed this result. It also clearly showed epistatic interactions between *Hd1* and *Hd3*, and between *Hd2* and *Hd3*, based on the analysis of QTL-NILs. Previous studies did not detect an epistatic interaction between *Hd2* and *Hd3*, probably because of interference by *Hd1*, whose effect was too large (the percentage of total phenotypic variance explained is 66.7% in the primary population). In this study, the novel population made it possible to detect epistatic interaction between *Hd2* and *Hd3*. Moreover, epistasis between *Hd1* and *Hd2* and between *Hd1* and *Hd3* was different under SD, LD or ND conditions (Fig. 3, Table 2). These results also suggested that expression of genes involved in photoperiod response was may be differentially regulated under SD and LD conditions. More comprehensive analyses on epistasis between detected QTLs will be necessary to understand the genetic control system of heading date in rice. Koornneef et al. (1991, 1998) used late-flowering mutants and double mutants to analyze epistatic interactions among genes controlling flowering time in *Arabidopsis*. Based on these analyses, genetic control pathways of flowering time were proposed (reviewed by Weigel 1995; Levy and Dean 1998). In our study, to analyze epistatic interactions among alleles at the 3 QTLs for heading date in rice, we dealt with the combined QTL-NILs as double mutants. In contrast to mutant alleles, allelic differences at QTLs occur naturally, so it is difficult to assume which parental allele is responsible for the change in function. However, whatever the functional allele, the epistatic interactions between *Hd1*, *Hd2*, and *Hd3* revealed in this study gave us a preliminary indication of the genetic control pathway for heading date in rice. When we assume that the Nipponbare alleles at *Hd1* and *Hd2* enhance photoperiod sensitivity, we consider that the Kasalath allele at *Hd3* has an effect on enhancing photoperiod sensitivity, based on our analysis of NILs under different daylength conditions. However, without the Nipponbare alleles at *Hd1* and *Hd2*, the Kasalath allele at *Hd3* did not increase photoperiod sensitivity. We suggest that *Hd1* and *Hd2* might be genes acting in the downstream region of the genetic control pathway, while *Hd3* might be a gene

acting in the upstream region as a regulator gene controlling the expression of *Hd1* and *Hd2*.

Many genes involved in flowering time have been isolated in *Arabidopsis*, and molecular evidence for genetic interaction among them has been rapidly accumulated (reviewed by Levy and Dean 1998). We have shown clear evidence for epistatic interaction among QTLs for heading date in rice in this study. Our group is progressing with map-based cloning of genes at *Hd1*, *Hd2*, *Hd3*, and other QTLs for heading date. Molecular identification of the genes will be necessary to provide molecular evidence for epistatic interactions between the QTL alleles reported in this study.

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