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ORIGINAL PAPER

Detection of quantitative trait loci controlling extremely early heading in rice

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Abstract To clarify the genetic basis of extremely early heading in rice, we conducted quantitative trait locus (QTL) analyses using F_2 populations from two genetically wide cross combinations, Hayamasari/Kasalath (HaF_2) and Hoshinoyume/Kasalath (HoF_2). Hayamasari and Hoshinoyume are extremely early-heading *japonica* cultivars. Photoperiod sensitivity is completely lost in Hayamasari and weak in Hoshinoyume. Three QTLs, QTL(chr6), QTL(chr7), and QTL(chr8), for days-to-heading (DTH) in HaF_2 were detected on chromosomes 6, 7, and 8, respectively, and QTL(chr6) and QTL(chr7) were detected in HoF_2. On the basis of the chromosomal locations, QTL(chr6), QTL(chr7), and QTL(chr8) may be likely to be *Hd1*, *Hd4*, and *Hd5*, respectively, which had been detected previously as QTLs for DTH in an F_2 population of Nipponbare × Kasalath. Alleles of QTL(chr7) decreased DTH dramatically in both Hayamasari and Hoshinoyume, suggesting that QTL(chr7) has a major role in determining extremely early heading. In addition, allele-specific interactions were detected between QTL(chr6), QTL(chr7) and QTL(chr8). This result suggests that not only allelic differences but also epistatic interactions contribute to extremely early heading. QTL(chr8) was detected in HaF_2, but not in HoF_2, suggesting that it determines the difference in DTH between Hayamasari and Hoshinoyume. A major QTL was also detected in the region of QTL(chr8) in QTL analysis using an F_2 population of Hayamasari × Hoshinoyume. This result supports the idea that QTL(chr8) is a major factor that determines the difference in DTH between Hayamasari and Hoshinoyume, and is involved in photoperiod sensitivity.

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Introduction

Heading date (flowering time) is one of the key factors in the regional and seasonal adaptation of rice cultivars. Many studies have been conducted to reveal the genetic basis of heading date in rice. In particular, by enhancement of the resources derived from rice genome analysis (Sasaki 2003), the genetic and molecular bases of heading date have been greatly clarified during the last decade (reviewed by Yano et al. 2001; Izawa et al. 2003; Hayama and Coupland 2004). Many quantitative trait locus (QTL) analyses of heading date have been conducted so far (summarized in

Gramine; http://www.gramene.org/)). These studies have contributed on our understanding of genetic control of heading in rice and eventually four QTLs have been cloned at the molecular level (*Hd1*, Yano et al. 2000; *Hd6*, Takahashi et al. 2001; *Hd3a*, Kojima et al. 2002; *Ehd1*, Doi et al. 2004).

Rice cultivars show a wide range of natural variation in heading date and day-length response (photoperiod sensitivity; PS). Although knowledge of the genetic control of heading date in rice is being accumulated, the wide range of natural variation is not fully understood yet. Recently, several QTLs involved in extremely late heading have been identified by QTL analysis using an extremely late-heading cultivar, Nona Bokra, and a Japanese elite early-heading cultivar, Koshihikari (Uga et al. 2007). Some genetic studies have also been conducted on extremely early heading in rice (Okumoto et al. 1996; Ichitani et al. 1997, 1998a; Fujino 2003; Fujino and Sekiguchi 2005a, b; Gu and Foley 2007). Rice cultivars adapted to Hokkaido, in northern Japan, exhibit extremely early heading and weak or no PS. From analysis using genetic tester lines, Okumoto et al. (1996) considered that extremely early heading is due to a recessive allele at the *E1* locus on chromosome 7. Recently, it has been reported that two QTLs on chromosome 7–*qDTH-7-1* and *qDTH-7-2*—are involved in variation in heading date among cultivars adapted to the northern limit of cultivation, such as Hokkaido and Europe (Fujino and Sekiguchi 2005a, b). Both functional and non-functional alleles of *Se1* (*Hd1*), a major PS gene, are distributed among rice cultivars from Hokkaido (Ichitani et al. 1997). A newly identified gene for PS, *Se*(t), plays an important role in the variation in DTH among cultivars from Hokkaido (Fujino 2003). In addition, Gu and Foley (2007) detected two QTLs, $Se_{7.1}$ and $Se_{7.2}$, on chromosome 7 and one QTL, Se_8, on chromosome 8 using BC_1 population derived from a cross between day-neutral cultivar, EM93-1 and weedy rice. Although these studies have revealed several genetic factors that confer extremely early heading, the allelic relationships and epistatic interactions are not fully understood because of a lack of knowledge about the molecular structures and functions of the genes.

In this study, to more comprehensively understand the genetic basis of extremely early heading in rice, we conducted QTL analysis using two F_2 populations derived from crosses between extremely early-heading *japonica* cultivars, Hayamasari and Hoshinoyume, and an *indica* cultivar, Kasalath. We found that three QTLs located on chromosomes 6, 7 and 8. Two of them on chromosome 7 and 8 were found to be involved in extremely early heading. It was also suggested that allele-specific digenic interactions among these three QTLs are involved in extremely early heading.

Materials and methods

Plant materials

Two extremely early-heading *japonica* rice cultivars, Hayamasari and Hoshinoyume, and late heading *indica* cultivar Kasalath were genetically analyzed in this study. A *japonica* cultivar Nipponbare was also used as reference cultivar with strong PS. Each of Hayamasari and Hoshinoyume was crossed with an *indica* cultivar, Kasalath, to produce F_2 populations, HaF_2 (Hayamasari × Kasalath) and HoF_2 (Hoshinoyume × Kasalath). We grew 198 plants of HaF_2 and 197 of HoF_2 and their parental cultivars in an experimental field at the National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan (36.3°N). All seeds were sown on 21 April 1999, and seedlings were transplanted on 26 May 1999. Leaf samples of each plant were collected at heading for DNA extraction. An F_2 population was also produced from the cross between Hayamasari and Hoshinoyume. We grew 86 F_2 plants in an experimental field at Hokuren Agricultural Research Institute, Naganuma, Japan (43.3°N), and 180 at NIAS. Plants were sown on 24 April 2002 and transplanted on 24 May 2002 in Tsukuba, and on 26 April 2002 and 21 May 2002, respectively, in Naganuma.

Phenotypic evaluations

In order to evaluate a PS of cultivars used in this study, Hayamasari, Hoshinoyume, Kasalath, and Nipponbare (a control, strong PS cultivar) were grown in three different environmental conditions: natural summer field in Tsukuba, long-day (LD; 14.5-h light; 28°C for 12 h and 24°C for 12 h) and short-day conditions (SD; 10-h light; 28°C for 12 h and 24°C for 12 h) in a controlled-growth cabinet (Especmic TGEH-9, Tokyo, Japan). The number of days required from sowing to heading of the first panicle (days-to-heading: DTH) was scored for 10 plants per cultivar, and mean values were calculated for each cultivar. Difference between DTH under SD and LD conditions in each cultivar was tested by one-way ANOVA.

In the analysis of three F_2 populations, DTH was scored on each plant for the QTL analysis.

DNA marker analysis

RFLP (restriction fragment length polymorphism) analysis followed the procedures described by Kurata et al. (1994). Total DNA of plants was extracted by the CTAB (cetyltrimethylammonium bromid) method (Murray and Thompson 1980). Eight restriction enzymes—*Apa*I, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Kpn*I—

were used to digest the genomic DNA. The digested DNA was blotted onto a nylon membrane (Boehringer Mannheim, Mannheim, Germany). Southern hybridization and detection were done with an ECL (enhanced chemiluminescence) direct labeling and detection system (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK). A total of 102 RFLP markers covering the whole rice genome were used to construct the linkage map (Harushima et al. 1998; Rice Genome Research Program 2007). SSR (simple sequence repeat) marker GBR8001 (Fujino et al. 2004), which lies near marker R902 on chromosome 8, was used to estimate the genetic effect of any putative QTL in the F_2 population of Hayamasari × Hoshinoyume.

Linkage and QTL mapping

Linkage mapping was performed with MAPMAKER/EXP 3.0 (Lander et al. 1987). The Kosambi function was used to calculate genetic distances (in cm).

QTL analyses were performed by using composite interval mapping (Zeng 1993, 1994) as implemented by the program Zmapqtl (model 6), of the software package QTL Cartographer version 2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm; see also Basten et al. 2005). Genome-wide threshold values ($\alpha = 0.05$) were used to detect putative QTLs based on results of 1,000 permutations (Churchill and Doerge 1994).

Detection of epistatic interactions

For the analysis of digenic interactions among QTLs, we classified F_2 plants into nine genotype classes based on the genotypes of the two RFLP markers tightly linked to both target QTL. The mean DTH was compared with the nine genotype classes by two-way analysis of variance (ANOVA). The classes were determined by using the RFLP markers C235, C39 and R902, which were revealed to be tightly linked with putative QTLs detected in the primary QTL mapping.

Sequence analysis of genomic region of *Hd1*

Several genomic fragments of Hayamasari and Hoshinoyume were amplified by PCR with primers designed from the genomic sequence of *Hd1* (Yano et al. 2000). Amplified fragments (0.5–1.5 kb) were directly sequenced from both sides with appropriate primers in an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Sequences were compared with the *Hd1* of Nipponbare (Acc. No. AB041837) and Ginbozu (Acc. No. AB041840), which were found to be required to make rice a strong PS (Yano et al. 2000).

Results

Heading behavior of extremely early-heading cultivars

DTH was investigated under natural field conditions in Tsukuba and under two controlled day-length conditions, SD and LD. Under natural field conditions, Hayamasari and Hoshinoyume headed earlier than Nipponbare and Kasalath (Fig. 1).

Hayamasari showed a smaller DTH under LD than that of LD, and did not show delay of heading under LD conditions (no PS). DTH of Hoshinoyume under LD was a little bit larger than that under SD ($P < 9 \times 10^{-11}$ by ANOVA), suggesting that Hoshinoyume shows weak PS. On the other hand, although a difference in DTH was small between SD and LD conditions in Kasalath ($P < 0.0002$ by ANOVA), the DTH under LD was a little bit larger than that of SD, showing that Kasalath also has a weak PS.

Hayamasari headed 12.5 days earlier than Hoshinoyume under natural field conditions. The difference in PS between them may have resulted in the difference in DTH between them under natural field conditions.

QTLs detected in extremely early-heading cultivars

Frequency distributions of DTH for the HaF_2 and HoF_2 populations are shown in Fig. 2. Both populations showed a wide DTH range and a continuous distribution, ranging from 61 to 137 in HaF_2 and 64 to 128 in HoF_2. Both populations had many transgressive segregants with greater DTH than that of Kasalath, the late-heading parental cultivar.

We detected three QTLs for heading date in HaF_2 and two in HoF_2 (Table 1; Fig. 2). In HaF_2, QTLs were mapped

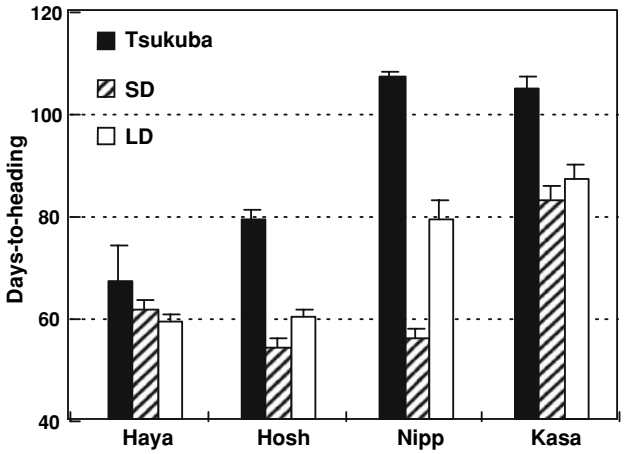

Fig. 1 DTH of four rice cultivars, Hayamasari (Haya), Hoshinoyume (Hosh), Nipponbare (Nipp), and Kasalath (Kasa), under three different environmental conditions: natural conditions in Tsukuba, and short daylength (10 h light) and long daylength (14.5 h light) in a growth chamber. Data are means and standard deviations (*error bars*)

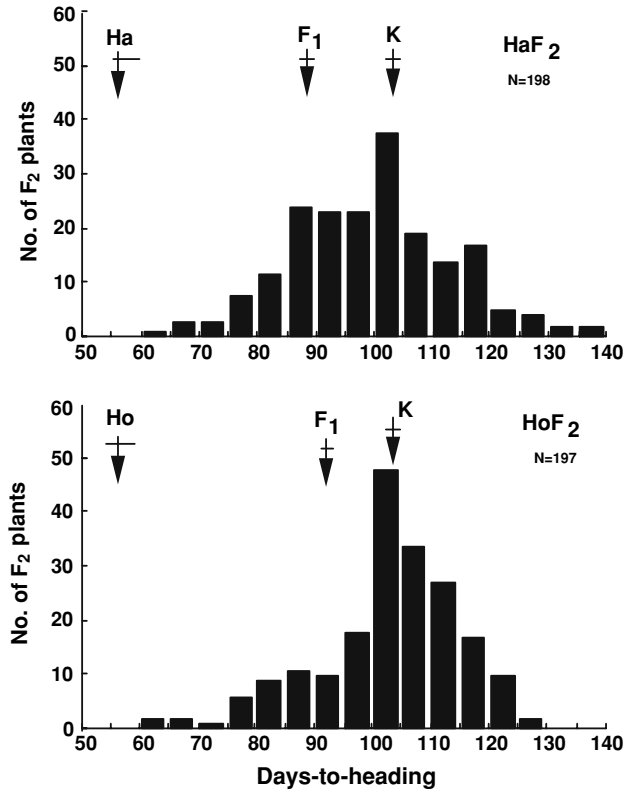

Fig. 2 Frequency distribution of DTH in two F_2 populations derived from Hayamasari × Kasalath (HaF_2) and Hoshinoyume × Kasalath (HoF_2). Plants were grown in a paddy field in Tsukuba, Japan. Means (*arrows*) and standard errors (*bars*) of parents are indicated

on chromosomes 6 (nearest RFLP marker C235), 7 (C39), and 8 (R902), and in HoF_2 on chromosomes 6 (C235) and 7 (C39). The QTLs located on chromosomes 6 and 7 in both populations were mapped in the same chromosomal regions and are most likely to be the same loci. The QTL on chromosome 7 (hereafter QTL(chr7)) explained about 26.6% of total phenotypic variation in HaF_2 and 69.3% in HoF_2, and the Hayamasari and Hoshinoyume alleles each decreased DTH. QTL(chr6) explained about 14.3 and 12.9% in HaF_2 and HoF_2, respectively, and the Hayamasari and Hoshinoyume alleles each increased DTH. QTL(chr8) explained about 25.3% of total phenotypic variation in HaF_2, and the Hayamasari allele decreased DTH.

Epistatic interaction between QTLs

Digenic interaction under natural field conditions was tested by ANOVA of DTH of nine genotype classes between pairs of QTLs. Epistatic interaction between QTL(chr6) and QTL(chr7) was detected in HaF_2 ($P = 0.0159$) (Fig. 3a). The effect of the Hayamasari allele at QTL(chr6) (i.e., increasing DTH) was observed in two genotype classes, homozygous for the Kasalath allele at QTL(chr7) and heterozygous, but not in the class homozygous for the Hayamasari allele (Fig. 3a). This interaction between QTL(chr6) and QTL(chr7) was also observed in HoF_2 ($P = 0.0003$) (Fig. 4). Epistatic interaction was observed between QTL(chr6) and QTL(chr8) in HaF_2 ($P = 0.0293$) (Fig. 3b). The effect of the Hayamasari allele at QTL(chr6) was observed in two genotype classes, homozygous for the Kasalath allele at QTL(chr8) and heterozygous, but not in the class homozygous for the Hayamasari allele (Fig. 3b). Allele specific digenic interaction was also observed in QTL(chr7) and QTL(chr8) ($P = 0.0001$) (Fig. 3c). Although genetic effect, delay of heading of Kasalath allele at QTL(chr8) was observed in all three genotype classes at QTL(chr7), relatively small effect was observed in genotype class homozygous of Hayamasari allele at QTL(chr7) compared with those in homozygous for the Kasalath allele at QTL(chr7) and heterozygous.

QTLs controlling difference in DTH among extremely early-heading cultivars

The results of the QTL analysis of HaF_2 and HoF_2 suggest that Hayamasari and Hoshinoyume have different alleles at QTL(chr8). To confirm that this allelic difference explained the difference in DTH between Hayamasari and Hoshinoyume, we performed genetic analyses using the F_2

Table 1 QTL for heading date detected in two F_2 populations, HaF_2 and HoF_2

Population	Marker interval	Nearest marker	Chr	LOD	PVE	AE	DE
HaF_2	R2171–R2123	C235	6	14.1	14.3	7.3	−5.2
	R2401–R1440	C39	7	22.4	26.6	−9.6	4.8
	R2285–C1121	R902	8	22.4	25.3	−10.2	7.2
HoF_2	R2171–R2123	C235	6	11.6	12.9	6.4	−2.4
	R2401–R1440	C39	7	52.3	69.3	−13.1	9.4

All genetic parameters were calculated by QTL Cartographer version 2.5 (Basten et al. 2005)

LOD thresholds for detection of QTL in composite interval mapping: 3.5 in HaF_2 and 4.1 in HoF_2

LOD Log-likelihood value, *PVE* Percentage of total phenotypic variance explained by the QTL, *AE* Additive effect of Hayamasari (HaF_2) or Hoshinoyume (HoF_2) allele on DTH. allele on DTH, *DE* Dominance effect of Hayamasari (HaF_2) or Hoshinoyume (HoF_2)

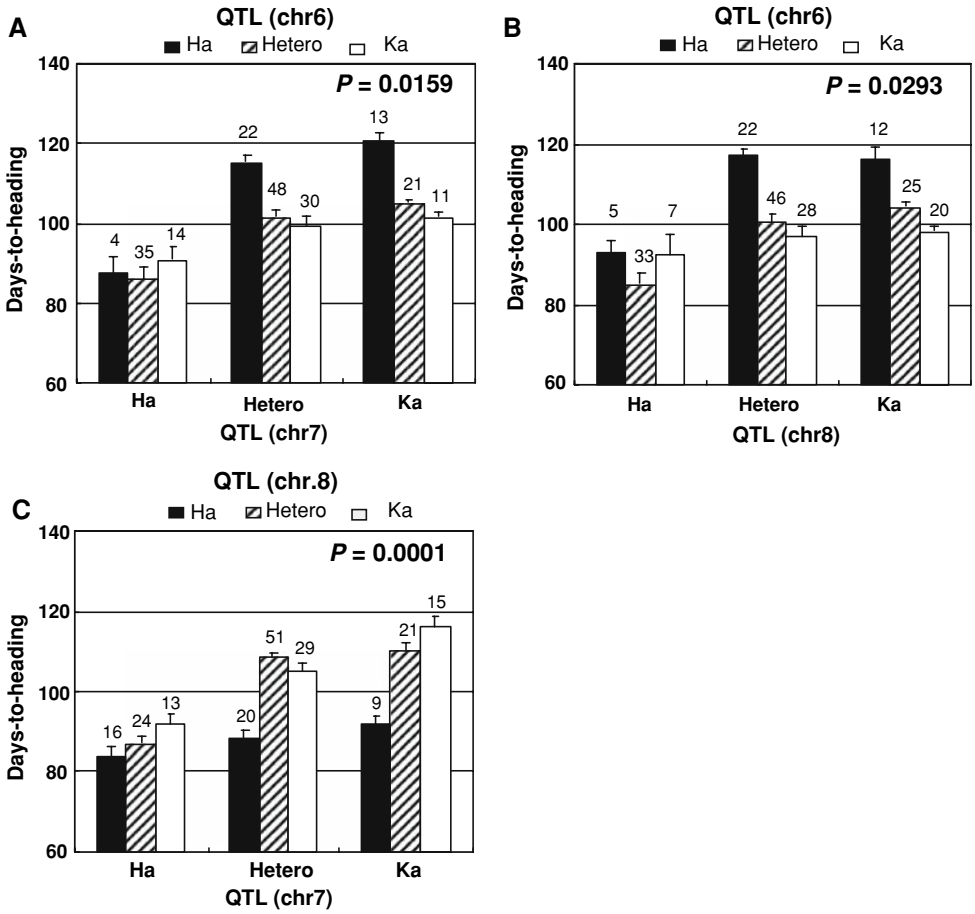

Fig. 3 Differences in mean DTH of nine genotype classes between three pairs of QTLs in F_2 plants of Hayamasari × Kasalath. **a** QTL(chr6) v. QTL(chr7), **b** QTL(chr6) v. QTL(chr8), **c** QTL(chr7) v. QTL(chr8). *Black, shaded, and white bars* indicate homozygous for the Hayamasari allele, heterozygous, and homozygous for the Kasalath allele, respectively. The *P*-value was calculated by two-way ANOVA. Data are means and standard deviations (*error bars*). Number of plants in each genotype class was indicated on the top of each vertical bar

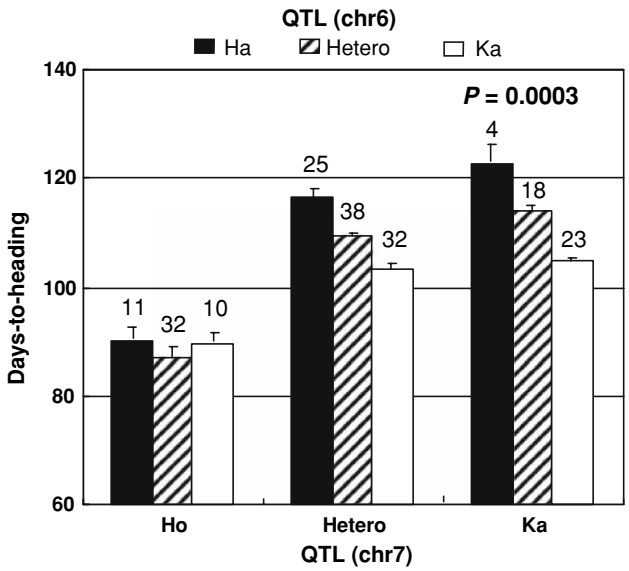

Fig. 4 Differences in mean DTH of nine genotype classes between QTL(chr6) and QTL(chr7) in the cross of Hoshinoyume × Kasalath. *Black, shaded, and white bars* indicate homozygous for the Hoshinoyume allele, heterozygous, and homozygous for the Kasalath allele, respectively. The *P*-value was calculated by two-way ANOVA. Data are means and standard deviations (*error bars*). Number of plants in each genotype class was indicated on the top of each vertical bar

population of Hayamasari × Hoshinoyume. In Naganuma, the F_2 population showed a continuous variation in DTH, ranging from 86 to 108 (Fig. 5a). In Tsukuba, DTH ranged from 58 to 90, also in a continuous distribution (Fig. 5b). A QTL for heading date was detected at SSR marker GBR8001 near QTL(chr8) at both Naganuma and Tsukuba. Most of the phenotypic variance in the F_2 population was explained by this QTL: 70.5% at Naganuma and 47.9% at Tsukuba. Although only one DNA marker was used in the QTL analysis in this population, most of the variation in DTH was attributable to the genetic factor at chromosome 8 (Fig. 5). These results demonstrate that QTL(chr8), linked to GBR8001, is the main factor responsible for the difference in DTH between Hoshinoyume and Hayamasari.

Sequence analysis of *Hd1* genomic region

The chromosomal location of QTL(chr6) suggests that this QTL is identical to *Hd1* (Yano et al. 2000). We determined about 4.8 kb genomic sequences corresponding to the *Hd1* of Hayamasari (DNA Data Bank of Japan, Accession No. AB353275) and Hoshinoyume (AB353276). In both

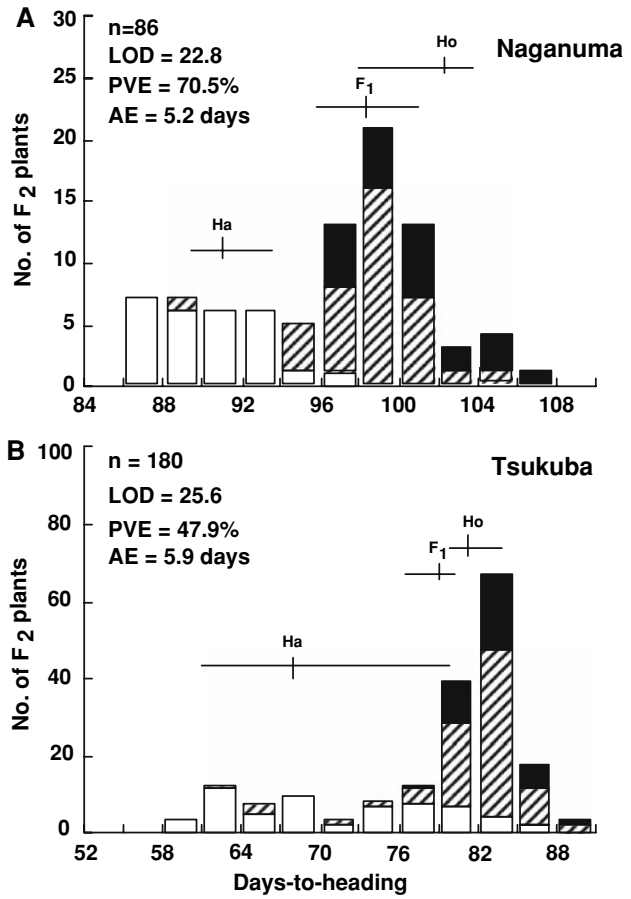

Fig. 5 Frequency distributions of DTH in F_2 population of Hayamasari × Hoshinoyume under two different environments, (**a**) Naganuma and (**b**) Tsukuba. The additive effect of the Hoshinoyume allele (AE), percentage of variance explained (PVE), and LOD score at marker GBR8001 are shown in both figures. *Black, shaded, and white bars* indicate homozygous for the Hoshinoyume allele, heterozygous, and homozygous for the Hayamasari allele, respectively

(early-heading) cultivars, the sequence was identical to that of Ginbozu, a late-heading cultivar with a functional allele of *Hd1* (Yano et al. 2000).

Discussion

Extremely early heading and very weak or no PS are essential for rice cultivars adapted to northern Japan and Europe. Several genetic analyses of heading date in cultivars adapted to such regions revealed the allelic composition of PS genes, *E1* (Okumoto et al. 1996); *Se9*(t) (Ichitani et al. 1997, 1998a); *Se*(t) (Fujino and Sekiguchi 2005); *qDTH-7-1* and *qDTH-7-2* (Fujino and Sekiguchi 2005b); $Se_{7.1}$, $Se_{7.2}$ and Se_8 (Gu and Foley 2007). On the basis of their chromosomal locations, it was suggested that *qDTH-7-2* and $Se_{7.2}$ might correspond with *Hd2* and *E1, qDTH-7-1, and* $Se_{7.1}$ might also correspond with *Hd4*, identified in the Nipponbare × Kasalath population (Yano et al. 1997; Yamamoto et al. 1998; Lin et al. 2003). Although these genetic studies revealed several genetic factors conferring extremely early heading, the allelic relationships and epistatic interactions of those factors have not been fully understood.

In this study, to understand the genetic control of extremely early heading in rice, we performed QTL mapping and epistatic interaction analyses using crosses between two extremely early-heading cultivars, adapted to Hokkaido, and the *indica* cultivar Kasalath. As a result, we detected three QTLs on chromosomes 6, 7, and 8. Comparison of their chromosomal locations suggests that the QTLs linked to markers C235, C39, and R902 are the same as *Hd1*, *Hd4*, and *Hd5*, respectively, which were detected in a QTL analysis of heading date using an F_2 population of Nipponbare × Kasalath (Yano et al. 1997; Yamamoto et al. 1998; Ichitani et al. 1998b; Lin et al. 2003). Furthermore, according to the chromosomal location, QTL(chr7) detected in this study may also be the same as the QTL, *qDTH-7-1* (nearest marker RM7110) detected by Fujino and Sekiguchi (2005b); $Se_{7.1}$ (RM214), (Gu and Foley 2007). In addition, QTL(chr8) was also considered to be same as Se_8 (RM25)(Gu and Foley 2007). Several other QTLs detected on chr.6, 7 and 8 were also seems to be located on the same chromosomal region as QTL(chr6), QTL(chr7), and QTL(chr8)(Gramine; http://www.gramene.org/). They might correspond these QTLs based on the comparison of chromosomal location.

Based on the comparison of chromosomal locations, it was difficult to clarify whether both genes are allele of same locus or tightly linked two genes. Thus, allelic relationships postulated in this study should be proved by the fine mapping and cloning of one of target QTLs. In fact, the *Hd1* sequences of both Hayamasari and Hoshinoyume were identical to that of the functional allele of *Hd1* in Ginbozu. Because Kasalath carries a non-functional allele of *Hd1* (Yano et al. 2000), QTL(chr6) is identical to *Hd1*.

Our results also suggest that QTL(chr8) plays an important role in determining PS. Interestingly, Hoshinoyume, but not Hayamasari, showed a weak PS (increase in DTH under LD condition), although both cultivars are adapted to northern Japan. The phenotypic variation in DTH of the Hayamasari × Hoshinoyume F_2 population could be fully explained by QTL(chr8) (Fig. 5). The difference in DTH between Hayamasari and Hoshinoyume is controlled by single gene, *Se*(t), which is involved in PS (Fujino 2003). Thus, it is very likely that *Se*(t) corresponds with QTL(chr8). An additional gene for PS, tentatively designated *Se9*(t) (Ichitani et al. 1998a), is involved in differences in DTH among cultivars adapted to Hokkaido. However, its chromosomal location is not determined yet, and the allelic relationship between QTL(chr8) and *Se9*(t) remains to be analyzed.

A large number of cultivars adapted to Hokkaido have a functional allele at *Hd1* but low or no PS (Ichitani et al. 1998a), so it has not been understood why *Hd1*, a strong PS gene, does not delay DTH in several cultivars in Hokkaido. In this study, we found digenic interactions among QTL(chr6), QTL(chr7) and QTL(chr8) (Figs. 3, 4). The QTL(chr6) alleles in both Hoshinoyume and Hayamasari increase DTH compared with that of Kasalath. Both HaF_2 and HoF_2 populations had many late transgressive segregants, which might be generated by a combination of the functional allele at *Hd1* from Hayamasari or Hoshinoyume and the Kasalath allele at QTL(chr7) and QTL(chr8) (Fig. 4). These results clearly suggest that epistatic interactions were involved in the expression of PS generated by QTL(chr6), QTL(chr7), and QTL(chr8). This might imply that QTL(chr7) and QTL(chr8) greatly affected the expression of the PS generated by QTL(chr6) in the PS control pathway.

Epistatic interaction has been detected between *Hd1* and *Hd5* in the Nipponbare × Kasalath population, but not between *Hd1* and *Hd4* (Lin et al. 2003). We detected epistatic interaction between QTL(chr6)/*Hd1* and QTL(chr7), and between QTL(chr6)/*Hd1* and QTL(chr8). If we assume that QTL(chr7) and QTL(chr8) correspond with *Hd4* and *Hd5*, respectively, result of this study supported the epistatic interaction between *Hd1* and *Hd5* in previous study (Lin et al. 2003). However, Lin et al (2003) and this study made a different conclusion in terms of epistatic interaction between QTL(chr6)/*Hd1* and QTL(chr7)/*Hd4* At present, we cannot make a clear explanation on this difference. One possibility might be due to the potential limitation of the detection of epistatic interaction using segregating population in both studies. It is also speculated that QTL(chr7) and *Hd4* are two different but tightly linked loci. Alternatively, the difference might be caused by a functional difference of the allele at QTL(chr7)/*Hd4*. Hayamasari and Hoshinoyume exhibited low or no PS, which suggests that both cultivars may carry a non-functional allele at QTL(chr7). In contrast, the small phenotypic difference (3–4 days) in *Hd4* between Nipponbare and Kasalath (Lin et al. 2003) suggests that the alleles in both cultivars may be functional. In this study, it was suggested that epistatic interactions were involved in the expression of three QTLs based on the analysis of F2 populations. In general, use of NILs for QTLs and their combined lines is more reliable to prove the interaction. With this regards, we are now developing NILs, in which relatively small chromosome segments containing QTL(chr6)/*Hd1*, QTL(chr7) and QTL(chr8) were substituted in the genetic background of Hayamasari and Hoshinoyume. Once we develop these lines, it will be necessary to measure DTH under different day-length conditions, to prove hypothesis for the epistatic interaction proposed in this study.

Epistatic interaction is one of important genetic basis of the PS in rice (Yamamoto et al 2000; Lin et al. 2000, 2003; Fujino and Sekiguchi 2005; Gu and Foley 2007). In particular, clear epistatic interactions have been observed among three QTLs, $Se_{7.1}$, $Se_{7.2}$ and Se_8, suggesting that these three genes function in the same pathway for the control of flowering (Gu and Foley 2007). In this study, we detected epistatic interactions between QTL(chr6)/*Se1* and QTL(chr7) and between QTL(chr6)/*Se1* and QTL(chr8), in addition to between QTL(chr7) and QTL(chr8). Based on the comparison of chromosomal locations, QTL(chr7) and QTL(chr8) were likely to be same loci with $Se_{7.1}$ and Se_8, respectively. Based on these assumptions, it might be suggested that four PS loci, QTL(chr6)/*Hd1*/*Se1*, QTL(chr7)/$Se_{7.1}$, $Se_{7.2}$, and QTL(chr8)/*Hd5*/Se_8, might be components of genetic control pathway of the PS on flowering. These hypotheses should be proved by cloning and sequence comparison of these genes.

Recently, several genes controlling PS have been cloned by a map-based strategy (Yano et al. 2000; Takahashi et al. 2001; Kojima et al. 2002; Doi et al. 2004). However, several other QTLs, such as *Hd4*, *Hd5*, and *Hd9*, remain to be identified, owing to the relatively small allelic differences between Nipponbare and Kasalath. In this study, we have genetically identified three QTLs. We observed a large allelic difference in QTL(chr7) between Hayamasari or Hoshinoyume and Kasalath, and the map location of the QTL(chr7) coincides with that of *Hd4*. We also observed a large allelic difference in QTL(chr8) between Hayamasari and Kasalath, and the map location of the QTL(chr8) coincides with that of *Hd5*. These relatively large allelic variations may allow us to perform effective map-based cloning. Molecular identification of these QTLs will enhance our understanding of the genetic control of heading date in rice. Molecular identification of these QTL will allow validating the epistatic interactions suggested by this study and other previous studies.

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