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ORIGINAL PAPER

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Mapping of QTLs conferring extremely early heading in rice (*Oryza sativa* L.)

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Abstract Two genes related to extremely early heading were identified in populations derived from crosses between *Hoshinoyume*, a variety adapted to the northernmost limit of rice cultivation (Hokkaido), and *Nipponbare*, a variety adapted to the temperate region of Japan. The segregations for heading date clearly revealed that a two-gene model determined the extremely early heading in the F_2 and BC_1F_1 populations under natural field conditions in Hokkaido. Using molecular markers corresponding to ten known quantitative trait loci (QTLs) for heading date, we carried out QTL analysis in the BC_1F_1 population and detected two QTLs, qDTH-7-1 and qDTH-7-2, both on chromosome 7, and observed epistatic interaction between them. We conclude that the recessive alleles of these two genes contribute to extremely early heading for the adaptation to Hokkaido environment and to stable rice production in Hokkaido. The relationships between the two QTLs identified in this study and known QTLs are discussed.

Introduction

Heading date is controlled by many genes and by environmental conditions such as temperature and daylength. The interaction between genotype and environment is a fundamental component influencing quantitative traits. Quantitative trait loci (QTLs) with a relatively strong effect on heading date in rice might not be detected under different environmental conditions, including differences in latitude (Lu et al. 1996). Consequently, important aims of rice breeding programs are to evaluate the response of a genotype under the conditions in which the variety will be grown and to determine whether gene effects differ under different environmental conditions.

Natural variation has recently become an efficient resource for the genetic and molecular analysis of complex traits in rice (Yano and Sasaki 1997; Yano 2001). Hokkaido, Japan (42–45°N latitude) is the northernmost limit of rice cultivation in Japan and is distinctive with respect to the environmental conditions for rice cultivation, such as low temperature and long natural daylength during the rice-growing season. Only extremely early-heading varieties are adapted to this region. These varieties have been subjected to substantial characterization, including several genetic studies. It is known that a photoperiod-insensitive allele at the *E1* locus is essential for the success of these varieties (Okumoto et al. 1996). A photoperiod-sensitivity QTL on chromosome 7, qDTH-7, differentiates the varieties in the northernmost limit of rice cultivation into two genetic bases—Europe and Hokkaido (Fujino and Sekiguchi 2005). These authors suggested that the germplasms from different varieties adapted to the northernmost limit of rice cultivation might be a good source for controlling heading date within the optimum range. In support of this suggestion, Ichitani et al. (1998a) identified a novel gene involved in photoperiod sensitivity—the response to a 24-h daylength condition. There might be yet unidentified allelic variation affecting heading date.

Some studies of early heading have been carried out under environmental conditions different from those in Hokkaido. It is possible that under those conditions the gene(s) and genetic effect(s) of the gene(s) controlling early heading might not be identifiable. The objective of the study reported here was to identify the gene(s) responsible for extremely early heading. We performed segregation analysis under field conditions in Hokkaido, to which only extremely early heading varieties are

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adapted and identified two genes related to extremely early heading by segregation and QTL analyses.

Materials and methods

Plant materials

Hoshinoyume, a variety adapted to the northernmost limit of rice cultivation in Japan, Hokkaido, was crossed with *Nipponbare*, a variety adapted to the temperate region of Japan. Both varieties are temperate *japonica*-types in Japan. The F_2 population consisting of 279 plants was obtained from the self-pollination of the resultant F_1 plants of the two varieties. The F_1 plants were crossed with *Hoshinoyume* to produce the BC_1F_1 population consisting of 95 plants. Both the F_2 and BC_1F_1 populations together with the parental varieties and F_1 plants were cultivated in a paddy field at the Hokkaido Green-Bio Institute (Naganuma, Hokkaido, Japan, 43°03′N latitude) in 2003. Germinated seeds were sown in nursery beds in a greenhouse, and 4-week-old seedlings were transplanted into paddy fields at a spacing of 35 × 2.5 cm. Sowing and transplanting were performed on April 25 and on May 23, 2003. Days to heading of the earliest heading panicle among the individual panicles was recorded for each plant as the number of days required from sowing to heading.

DNA marker and QTL analysis

Total DNA was extracted from leaves of the BC_1F_1 plants following the CTAB method (Murray and Thompson 1980) with minor modifications. The genotypes of each simple sequence repeat (SSR) marker in the BC_1F_1 population were determined. It was difficult to develop polymorphic markers over the entire genome as *Hoshinoyume* and *Nipponbare* are quite closely related. We also attempted to develop polymorphic markers corresponding to the 11 known QTLs for heading date listed in Table 1. These targeted QTLs were identified by using several types of progeny derived from crosses between a *japonica* variety, *Nipponbare*, and an *indica* variety, *Kasalath* (Yano et al. 1997; Lin et al. 1998; Yamamoto et al. 2000; Yano et al. 2001; Lin et al. 2002). To amplify the genomic DNA to detect SSRs, we used 50 markers from the International Rice Microsatellite Initiative (IRMI) (McCouch et al. 2002) and the previously developed SSR markers (Fujino et al. 2004). In addition, using the sequence of *Hd1* (Yano et al. 2000), we developed a PCR primer pair for *Hd1* to amplify structural changes of the sequence (unpublished data) (forward: 5′-CGACGTGCAGGTGTACTCCG-3′; reverse: 5′-ACCTCCTCGTCCTTGTCGCC-3′). To detect polymorphism, we electrophoresed the PCR-amplified product on a 2% or 3% agarose gel, or on a 6% polyacrylamide denaturing gel. PCR amplification, gel electrophoresis and detection of the amplified products were performed according to the method described by Fujino et al. (2004).

For the QTL analysis of heading date, we compared the mean value of days to heading in the BC_1F_1 population between each genotype class by ANOVA. The detection threshold for QTLs in this study was $P = 0.001$.

Table 1 Number of SSR markers surveyed for polymorphism between rice varieties *Hoshinoyume* and *Nipponbare*

Target QTL[a]	Chromosome	Number of SSR primers tested	Number of polymorphisms
Hd7	2	3	0
Hd9	3	4	1
Hd8	3	5	4
Hd6	3	2	1
Hd3	6	14	6
Hd4	7	6	3
Hd2	7	3	1
Hd5	8	2	1
Hd12	8	2	0
Hd14	10	8	2
Hd13	12	1	1
Total		50	20

[a] Yano et al. (1997, 2001), Lin et al. (1998, 2002) and Yamamoto et al. (2000)

Detection of epistatic interaction

For the analysis of epistatic interaction, the appropriate 93 BC_1F_1 plants were classified into four classes based on the genotypes of the two SSR markers used to detect the QTLs RM7110 and RM1306. The genotype class of A/A, which represents the genotype RM1306/RM7110, contained 31 plants, while genotype classes of A/H, H/A and H/H contained 26, 16 and 20 plants, respectively. A and H represent the homozygous and heterozygous *Hoshinoyume* allele, respectively. The trait of mean days to heading under natural field conditions was compared among the four genotypes classes by 2-way analysis of variance (ANOVA).

Results

Phenotypic variation

A large variation was observed for days to heading between *Hoshinoyume*—107.4 days (range: 106–110 days)—and *Nipponbare*—estimated at 155 days (range: 148–165 days). The average days to heading of F_1 plants was 144 days. The F_2 and BC_1F_1 population showed distinct segregation patterns for heading date (Fig. 1). Based on the range of heading dates obtained for *Hoshinoyume*, we concluded that the F_2 population contained two distinct classes of plants, 18 of the extremely early type corresponding to *Hoshinoyume* and 261 of the late type,

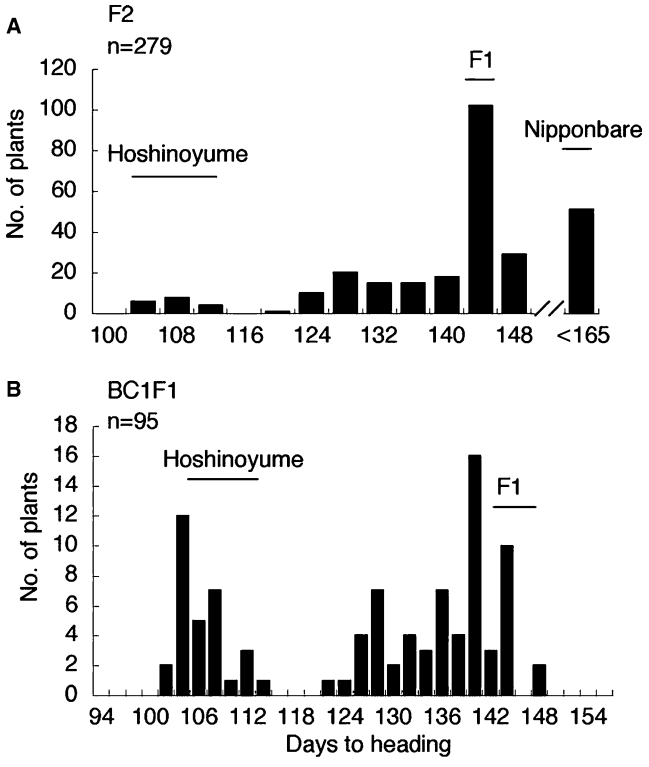

Fig. 1 Frequency distributions of days to heading in the F_2 (**a**) and BC_1F_1 (**b**) populations derived from the crosses between rice varieties *Hoshinoyume* and *Nipponbar*e. The *horizontal lines* represent the ranges for the parents and F_1 plants

with 116 days as the boundary (Fig. 1a). The ratio fitted a two-gene segregation (1:15, $\chi^2 = 0.017$, $0.90 > P > 0.70$). The BC_1F_1 population exhibited a 1:3 ratio—31 extremely early-heading plants and 64 late-heading plants, with 118 days as the boundary (1:3, $\chi^2 = 2.94$, $0.10 > P > 0.05$) (Fig. 1b). These results indicate that the two genes are related to the difference in heading date between *Hoshinoyume* and *Nipponbare*.

QTL analysis

Of the 50 SSR markers analyzed, 20 corresponding to nine known QTLs showed polymorphism between *Hoshinoyume* and *Nipponbare* (Table 1). The genotypes of the nine SSR markers for the nine known QTLs and one PCR marker for *Hd1* in the BC_1F_1 population were determined. The QTL analysis for heading date was performed by ANOVA using the genotypes of the markers and days to heading under natural field conditions in the BC_1F_1 population. Two QTLs controlling heading date were detected (Table 2). The *Nipponbare* alleles for both QTLs increased the value of days to heading. The QTL with the largest effect, qDTH-7-1, was detected on marker RM7110 on the long arm of chromosome 7 and accounted for a difference in days to heading between the two genotypic classes of 20.9 days. The other QTL, qDTH-7-2, was detected on marker RM1306 on the most distal end of the long arm of chromosome 7 and accounted for a difference in days to heading between the two genotypic classes of 12.2 days.

On the basis of the genotype of the RM7110, we classified the BC_1F_1 plants into two classes. Of the 47 heterozygous plants, 38 headed later (134–146 days) than 42 of the 47 plants homozygous for *Hoshinoyume* (102–132 days) (Fig. 2a). In addition, among the phenotypic group (102–132 days), 18 of the 19 plants heterozygous at the RM1306 locus headed later than 30 of the 31 plants homozygous for the *Hoshinoyume* allele (Fig. 2b). These results clearly reveal the existence of two QTLs.

Epistatic interaction

To clarify whether qDTH-7-1 and qDTH-7-2 show epistatic interaction, we compared days to heading under natural field conditions in the BC_1F_1 population

Table 2 The QTLs of heading date in the BC_1F_1 population derived from the cross between *Hoshinoyume* and *Nipponbare* as determined by ANOVA

Target QTL[a]	Marker[b]	Chromosome	Position (cM)[c]	Mean of days to heading			Probability
				A[d]	H[d]	Difference	
Hd9	GBR3002	3	3	128.5	124.4	4.1	0.23
Hd8	RM5639	3	40	127.4	126.8	0.6	0.86
Hd6	RM5924	3	139	126.4	127.0	0.6	0.85
Hd3	RM4608	6	16	129.2	125.5	3.7	0.25
Hd1	*Hd1*	6	54	128.1	125.4	2.7	0.39
Hd4	RM7110	7	56	116.2	137.1	20.9	0.0000**
Hd2	RM1306	7	116	121.7	133.9	12.2	0.0001**
Hd5	GBR8001	8	36	127.4	126.0	1.4	0.66
Hd14	RM1873	10	48	126.4	127.9	1.5	0.65
Hd13	RM5653	12	47	122.5	130.6	8.1	0.01

**The detection threshold for QTLs was $P = 0.001$

[a]Yano et al. (1997, 2001), Lin et al. (1998, 2002) and Yamamoto et al. (2000)

[b]The markers RM and GBR are from McCouch et al. (2002) and Fujino et al. (2004), respectively

[c]Position of the markers in the high-density linkage map (Harushima et al. 1998)

[d]A and H indicate homozygous and heterozygous *Hoshinoyume* allele, respectively

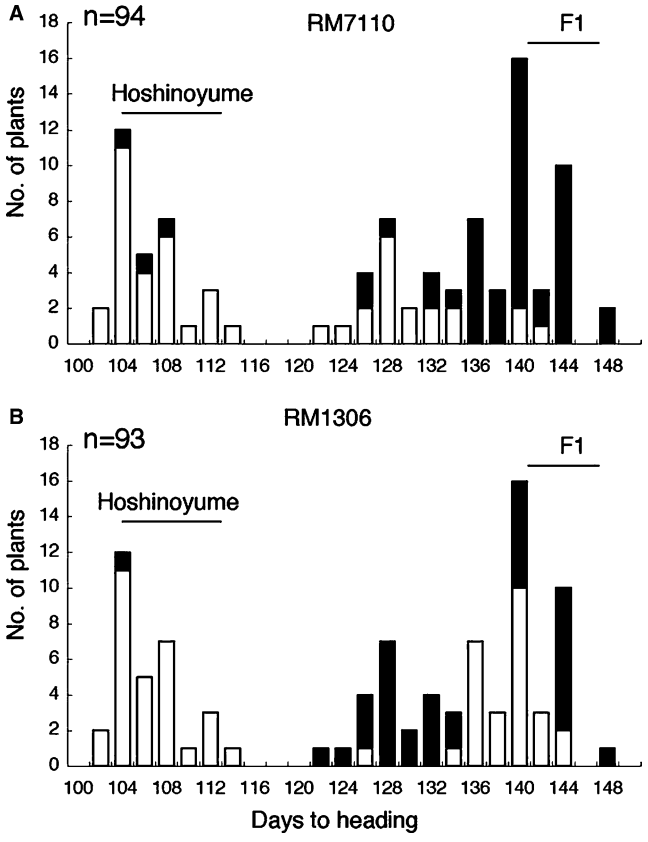

Fig. 2 Classifications with the marker genotype for frequency distributions of days to heading in the BC_1F_1 population derived from the cross between *Hoshinoyume* and *Nipponbare*. The *horizontal lines* represent the ranges for the parents and F_1 plants. The *black* and *white bars* indicate the number of plants that were heterozygous and homozygous for the *Hoshinoyume* allele, respectively, at each locus. The SSR markers RM7110 (**a**) and RM1306 (**b**) were used to detect the QTLs qDTH-7-1 and qDTH-7-2, respectively

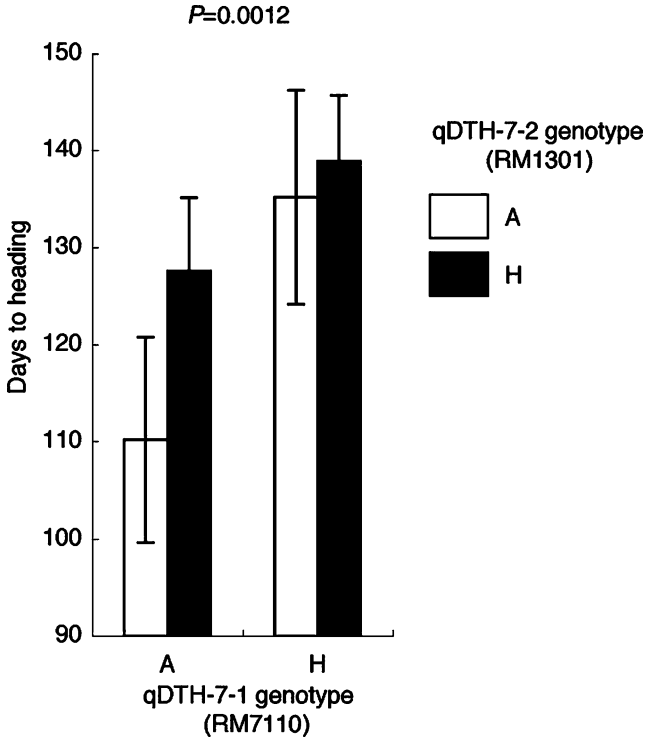

Fig. 3 Differences in mean days to heading for the four genotypic classes in the BC_1F_1 population derived from the cross between *Hoshinoyume* and *Nipponbare* under natural field conditions. Genotypes were determined using the markers RM7110 (qDTH-7-1) and RM1306 (qDTH-7-2). The genotype class of A/A, which represents the genotype RM1306/RM7110, contained 31 plants; the genotype classes of A/H, H/A and H/H contained 26, 16 and 20 plants, respectively. *A* and *H* indicate the homozygous and heterozygous *Hoshinoyume* allele, respectively. The *P*-value was calculated by using a 2-way analysis of variance (ANOVA). The data are presented as means ± standard deviation

among the four-genotype classes for each QTL. Epistatic interaction between qDTH-7-1 and qDTH-7-2 was detected ($P = 0.0012$) by 2-way ANOVA (Fig. 3). The effect of the *Nipponbare* allele at qDTH-7-2 (i.e. increased days to heading) was observed in plants homozygous for the *Hoshinoyume* allele at the qDTH-7-1 locus, but not in the plants heterozygous at the qDTH-7-1 locus.

Discussion

Heading date is controlled by many genes and various environmental conditions, such as temperature and daylength. The segregation analysis in this study was performed under natural field conditions in Hokkaido, and the genotype of extremely early heading was evaluated under the same conditions. The segregation for heading date in the crosses between *Hoshinoyume* and *Nipponbare* clearly revealed that a two-gene model determined extremely early heading (Fig. 1). Segregation analysis revealed the presence of two QTLs, qDTH-7-1 and qDTH-7-2, both on chromosome 7 (Table 2). Recessive alleles of these two genes contribute to the adaptation of rice varieties to growing conditions in Hokkaido and to stable rice production in Hokkaido.

Comparisons of the chromosomal locations suggested that qDTH-7-1 and qDTH-7-2 may be the same as *Hd4* and *Hd2*, respectively (Yano et al. 1997). *Hd4* may be the same locus as *E1* (Okumoto et al. 1992b; Ichitani et al. 1998b; Lin et al. 2003). *E1* is a photoperiod-sensitive gene, and it is known that the photoperiod-insensitive allele at the *E1* locus might be essential to extremely early heading (Okumoto et al. 1996). *Hd2* is a key photoperiod-sensitivity gene in *Nipponbare* (Yano et al. 1997; Yamamoto et al. 1998; 2000). Both *Hd4* (*E1*) and *Hd2* are photoperiod-sensitive genes. The QTL qDTH-7-2 may be the same locus as qDTH-7, which has been shown to differentiate the varieties adapted to the northernmost limit of rice cultivation into two genetic bases, Europe and Hokkaido (Fujino and Sekiguchi 2005). This QTL may be involved in photoperiod sensitivity (Fujino and Sekiguchi 2005). Because the photoperiod sensitivity of *Hoshinoyume* was quite low (Fujino 2003), both qDTH-7-1 and qDTH-7-2 in *Hoshinoyume* may have photoperiod-insensitive alleles. In addition to

the photoperiod-insensitive allele at the *E1* locus (Okumoto et al. 1996), the recessive allele of the *Hd2* gene may be essential to extremely early heading. Further clarification of the relationships between the QTLs identified in this study and known QTLs is required.

Epistatic interaction between qDTH-7-1 and qDTH-7-2 was detected (Fig. 3). The *Nipponbare* allele at the qDTH-7-2 locus increased days to heading only in plants homozygous for the *Hoshinoyume* allele at the qDTH-7-1 locus. However, *Hd4* showed no epistatic interaction with *Hd2* (Lin et al. 2003). As *Hoshinoyume* has a photoperiod-insensitive allele at qDTH-7-1, the allelic difference at this locus may have led to the different results. Also, we only compared the two genotypes, homozygous and heterozygous *Hoshinoyume* alleles. More comprehensive characterization of the QTLs identified in the present investigation should be performed to identify the manner of genic interaction for these QTLs using near-isogenic lines (Lin et al. 2000, 2002, 2003). This results of this study demonstrated that varieties adapted to different environmental conditions are useful for discovering previously unidentified allelic variation. Rice, *Oryza sativa* L., is distributed throughout the world, and the many varieties adapted to unique environmental conditions would provide a wide range of allelic variation.

Both qDTH-7-1 and qDTH-7-2 are located on the long arm of chromosome 7. *E1*, corresponding to qDTH-7-1, is distributed throughout Japan—with the exception of Hokkaido (Okumoto et al. 1991, 1992a). Although the distribution of *Hd2*, corresponding to qDTH-7-2, is not known, *Nipponbare* has a functional allele at this locus. Days to heading of the plants that did not show extremely early heading in the F_2 and BC_1F_1 populations were completely out of the range for adaptation to Hokkaido. The two genes identified here or the chromosomal region harboring these two genes may reduce the breeding efficiency in progeny being developed in rice breeding programs in Hokkaido using exotic germplasm . In the rice breeding programs for extremely early-heading plants, there may be strong selection pressure on this chromosomal region. *qCT-7*, which can be found in this chromosomal region, controls cool-temperature tolerance at the booting stage in *Koshihikari* (Takeuchi et al. 2001). The appropriate use of the gene(s) located in this region would exploit the genetic variation among varieties adapted to the northernmost limit of rice cultivation.

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