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ORIGINAL RESEARCH

Fine Mapping of *qHD8-1*, a QTL Controlling the Heading Date, to a 26-kb DNA Fragment in Rice (*Oryza sativa* L.)

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Abstract Heading date is one of the importance agronomic traits. A library consisting of 1,123 single segment substitution lines (SSSLs) in the same genetic background of an elite rice variety Huajingxian 74 (HJX74) was evaluated for heading date (HD). From this library, the SSSL W06-26-35-1-5-2 with the substituted interval of PSM152–PSM154–PSM155–RM25–RM547–RM72–RM404 was found having a gene, which performed stable and late heading in the different environments of Shandong and Hainan provinces. To map the gene governing heading date, the SSSL W06-26-35-1-5-2 was crossed with the recipient HJX74 to develop an F_2 segregating population. The distribution of late and early heading plants in this population fitted a segregation ratio of 3:1, indicating the late heading was controlled by a dominant gene. The gene locus for heading date was tentatively designated as *qHD8-1*. Using a random sample of 460 individuals from the F_2 population, the *qHD8-1* was narrowed down to a region flanking by two SSR markers PSM155 and RM547. For fine mapping of *qHD8-1*, a large $F_{2:3}$ segregating population of 3,000 individuals were developed from F_2 plants heterozygous in the PSM155–RM547 region. Recombinants analysis further mapped *qHD8-1* to an interval of region 26 kb with markers RM22492 and P23 bounded on the left and right sides, respectively. Sequence analysis of this 26-kb fragment revealed that it contains five putative open reading frames, which were regarded as candidates of *qHD8-1*. These results will be useful in cloning of the *qHD8-1* gene.

Keywords Rice (*Oryza sativa* L.) · Heading date (HD) · Single segment substitution line (SSSL) · Physical mapping · Sequence information

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Introduction

In rice (*Oryza sativa* L.), heading date (HD) is a critical trait for rice adaptation to diverse cultivation areas and cropping seasons and is a key factor to attain the desired yield level. Control of HD is one of the leading objectives in rice breeding. HD in rice is a typical quantitative trait locus (QTL) with complex inheritance and is controlled by polygene. HD is one of the classes of traits (yield, plant height, and heading date) that have been extensively investigated, and hundreds of QTLs have been reported for these traits in the literature (Xue et al. 2008). With the advance of molecular marker-based mapping, we performed QTL analyses for HD. The recent advances in molecular marker technology and developments of high-density molecular marker linkage maps in rice have provided powerful tools for elucidating the genetic bases of quantitatively inherited traits, including most of the agriculturally important traits (Yu et al. 2002). Fifteen QTLs controlling HD (*Hd1–Hd3a*, *Hd3b–Hd14*) in rice have been identified from the same cross between a *japonica*

variety, Nipponbare, and an *indica* variety, Kasalash (Li et al. 1995; Xiao et al. 1995; Lin et al. 1996, 1998, 2002; Yano and Sasaki 1997; Xiong et al. 1999; Yamamoto et al. 2000; Monna et al. 2002). At present, six QTLs for HD, *Hd1*, *Hd3a*, *Hd6*, *Ehd1*, *Ehd2*, and *Ghd7* (Yano et al. 2000; Kojima et al. 2002; Takahashi et al. 2001; Doi et al. 2004; Kazuki et al. 2008; Xue et al. 2008) have been cloned by map-based cloning strategy, and sequence comparison revealed that underlying genes from rice share a high degree of similarity with those from *Arabidopsis*, although 2*Arabidopsis* (*Arabidopsis thaliana* L.) and rice (*O. sativa* L.) are model long- and short-day plants, respectively.

Growth period from sowing to heading is composed of the vegetative growth phase and reproductive phase. The vegetative growth phase consisting of the basic vegetative phase and photoperiod-sensitive phase is controlled mostly by the genetic factors and environmental conditions (Chang et al. 1969; Hosoi 1981; Sato and Takahashi 1983; Leang et al. 2005), and meanwhile, the reproductive phase makes no differences across cultivars. It was reported that in the tropics, advanced HD caused the shortening of the vegetative growth of rice which actually increased grain yield reduction considerably and delayed heading let the crop have sufficient vegetative growth to give better grain yield and/or biomass (Kawano and Tanaka 1968; Akita 1989; Wada and Sta Cruz 1989; Peng et al. 1999). Therefore, it makes great sense to breed rice varieties possessing genes for late heading.

As previous studies on HD, primary populations like F_2, F_3, double haploid, and recombinant inbred lines are not suitable for fine mapping of single QTL because of the whole parental chromosomal segments segregation simultaneously (Yamamoto et al. 2000). For the purpose of avoiding genetic background noise, QTL analysis in advanced populations is necessary for a good understanding of their characteristics. Single segment substitution lines (SSSLs) have been proven to be an ideal population for QTL fine mapping and cloning. SSSLs are developed through successive backcrossing of the recipient parent with the donor genotype followed by marker-assisted selection. Each SSSL can be used to separate the complexity of polygenic traits into a set of monogenic loci. Excepting carrying a substituted segment from a donor, each SSSL contains homozygous genetic background with its recipient, and the epistatic effects from the donor parent are eliminated. So SSSLs developed recently are useful tools for dissecting genetic mechanisms of QTLs and elucidating gene functions in plants (Liu et al. 2003, 2004; He et al. 2005a, b). In addition, genetic allele mining depends on the combination of the parental lines obviously.

With completely sequenced rice genome, nearly unlimited numbers of molecular markers can be developed from the known sequence information available in public databases. Utilization of chromosome walking in the progress of map-based gene isolation has been greatly reduced or even avoided. The Rice Genome Automated Annotation system (http://RiceGAAS.dna.affrc.go.jp), combined with high resolution of gene fine mapping, is helpful in identifying the candidate gene. To date, hundreds of QTLs have been mapped in crops during the last two decades, and the emphasis of QTL research is being gradually changed from mapping to cloning.

In our previous study, a SSSL library with an elite genetic background of Huajingxian 74 (HJX74) introgressed from 24 different rice varieties has been developed by applying simple sequence repeat (SSR) marker-assisted selection. The total length of substituted segments in this SSSLs library is 21,674 cM, which is about 14 times the size of rice genome; the average length in each SSSL is 19.3 cM (Zhang et al. 2004). SSSL is a powerful tool for functional genomics and molecular breeding and genetic dissection of QTLs in rice (Liu et al. 2003, 2004; He et al. 2005a, b; Xi et al. 2006). From this library, one SSSL W06-26-35-1-5-2 with the substituted interval of PSM152–PSM154–PSM155–RM25–RM547–RM72–RM404 on chromosome 8 was found to have significant difference in HD from the recipient HJX74. The gene locus for HD was tentatively designated as *qHD8-1*. For fine mapping of the *qHD8-1* locus, the SSSL W06-26-35-1-5-2 was crossed with the recipient HJX74 to develop a large F_2 secondary segregating population, and the *qHD8-1* locus was finally mapped, by recombinants analysis, to a 26-kb DNA fragment flanked by left marker RM22492 and the right marker P23. This result provides a solid base for molecular cloning of the gene. The sequence analysis of the corresponding region using the Rice Genome Automated Annotation system (http://RiceGAAS.dna.affrc.go.jp) predicted that there are five candidate genes. The result will be very useful in molecular cloning of the gene for HD.

Materials and Methods

Mapping Population

From the library of SSSLs constructed by our group, the SSSL W06-26-35-1-5-2 with the substituted interval of PSM152–PSM154–PSM155–RM25–RM547–RM72–RM404 on chromosome 8 was found having a gene for extremely late HD which performed stable and late heading in the different environments of Shandong and Hainan provinces.

In order to fine map this QTL, the SSSL W06-26-35-1-5-2 was crossed with the recipient HJX74 to development an F2 segregating population. The resultant F_1 plants were selfed to produce F_2 seeds. The gene for HD was mapped

on chromosome 8 between two SSR markers PSM155 and RM547 from the F_2 segregating population. Ten F_2 plants, in which the region around the target gene locus was heterozygous, were used to develop an F_3 segregating population containing 3,000 individuals for high-resolution linkage mapping of the *qHD8-1* locus.

Field Materials and Investigation of Heading Date

The corresponding parents and the segregating population were planted in the normal rice growing season on the farm of the High-Tech Research Center in May–October 2008 and 2009, Shandong Academy of Agriculture Science, Jinan, People's Republic of China. Each entry was planted at a spacing of 16 cm from plant to plant and 30 cm from row to row. Days to heading of each plant was scored when the first panicle was 1 cm of emergence. Phenotype of HD in the segregating populations revealed a large variation.

Detection of QTLs

QTLs were detected using the *t* test on difference of days to heading between SSSL W06-26-35-1-5-2 and control HJX74. A probability level of $P \leq 10^{-6}$ was used as the threshold for the presence of putative QTL. QTL nomenclature was conducted using the method described by McCouch et al. (1997).

DNA Extraction

Rice genomic DNA was extracted from fresh leaves harvested from each plant using a microisolation method described by Zheng et al. (1995) with a minor modification. The leaf samples were cut into small pieces 1 cm long and placed into a 1.5-ml tubes, some nitrogen was added, and samples were ground with a small plastic pestle. Then, 1 ml of isolation buffer (100 mmol/L Tris–HCl (pH 8.0), 10 mmol/L EDTA (pH 8.0), and 1 mol/L KCl) was added to the powdered tissue, incubated at 75°C for 30 min, and centrifuged at 12,000 rpm for 10 min. The supernatant was collected and DNA precipitated with cold absolute ethanol.

PCR Amplification

PCR amplification was conducted as described by Panaud et al. (1996) with a slight modification. The PCR was performed in 20-μl reactions volume containing 30 ng of template DNA, 0.15 μl of 10 mmol/L dNTPs, 1.5 U of *Taq* DNA polymerase, 2 μl of 10× PCR buffer (50 mmol/L KCl, 10 mmol/L Tris–HCl (pH 8.3), 1.5 mmol/L $MgCl_2$, and 0.01% gelatin), and 1.5 μl of 2 μmol/L forward and reverse primers. Cycling conditions were 5 min at 94°C followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products were subjected to electrophoresis on 6% polyacrylamide gel. After completion of the electrophoresis, the gels were silver stained as per Li et al. (2002).

Marker Selection and Primer Design

SSR markers were used to determine the genotypes of marker loci for each plant. Known SSR markers were adopted from the public database released by the International Rice Microsatellite Initiative (www.gramene.org); other newly developed SSR and InDel markers were from the Plant Molecular Breeding Research Center, South China Agricultural University, Guangzhou, China. The sequence between two flanking markers, downloaded from a publicly rice genome sequence (www.ncbi.nlm.nih.gov), was exploited to design new SSR markers by using (www.gramene.org/db/searches/ssrtool) and the primer premier version 5.0. Sequence diversities between *indica* "93–11" and *Japonica* "Nipponbare" were used to develop InDel markers. All primer pairs flanking SSRs or InDels were designed in light of the following parameters: 18–25 nucleotides in length, devoid of secondary structure, a GC content around 50%, and a melting temperature around 55°C.

Map Construction

The genetic map of the gene locus was developed using MAPMAKER/EXP version 3.0 based on genotypic and phenotypic data for 460 segregating individuals in F2 population (Lander et al. 1987). The genetic distance (centimorgans) was calculated using the Kosambi function (Kosambi 1944).

Fine Physical Mapping of the Gene for Heading Date

An F_3 segregating population comprising 3,000 individuals was used to fine map the gene locus. SSR markers, which flanked the target gene, were first used to detect the recombinants in these plants. Additional PCR-based markers were subsequently developed according to the sequence information of the reference 93-11 and Nipponbare. Physical map of the target gene was constructed by using bioinformatics analysis. Molecular markers linked with the target gene were landed on the BAC or PAC clones of the reference Nipponbare and released by International Rice Genome Sequencing Project using the sequence homology search tool BLASTN (www.blast.ncbi.nlm.nih.gov/BLAST.cgi). Sequences of these clones were downloaded and aligned using the sequence alignment tool pairwise BLAST (boast.ncbi.nlm.nih.gov/blast.cgi/b12seq/b12.html) for constructing the BAC/PAC contigs spanning the target gene locus. At the same time, the genotype and phenotype of the

recombinants and their progenies were analyzed in the present study.

Results

Identification of QTLs for Heading Date

SSSL W06-26-35-1-5-2 showed a significant difference from HJX74 in days to heading (Table 1); it had a substituted segment on chromosome 8 with an estimated length of 29.9 cM from PSM152 to RM404. SSSL W06-26-35-1-5-2 had heading occurred later than recipient HJX74 in two different environments. *t* test showed that days to heading between control HJX74 and W06-26-35-1-5-2 had a significant difference, indicating that there was a QTL for HD at the interval of PSM152–PSM154–PSM155–RM25–RM547–RM72–RM404, and the QTL was named as *qHD8-1*. The allele from donor Zihui100 at the locus delayed flowering under natural conditions.

Frequency Distribution and Inheritance of Heading Date

The frequency distribution for days to heading in the F_2 segregating population exhibited a bimodal distribution and showed transgressive segregation. The days to heading of HJX74 varied from 106 to 110 days, while for the F_2 population it ranged from 128 to 133 days. Among the 460 segregating plants, individuals were classified into early and late heading, and the average difference among segregating early and late progenies is 21 days. This segregation agreed with 3 (late):1 (early) ratio $\left(\chi^2 = 1.11 \chi^2_{0.05,1} = 3.84\right)$, indicating that the late heading was controlled by a single dominant gene from donor Zihui100.

Construction of the Genetic Map for the *qHD8-1* Locus

Sixteen pairs of SSR markers located at the substituted interval were used to screen parents, HJX74 and W06-26-3-5-1-5-2, of which five pairs exhibited polymorphism. Selected 460 F_2 individuals were used as the initial mapping population; the individuals were genotyped using the five positive markers. Based on the genotypes of marker loci, a linkage map was constructed using MAPMAKER analysis, and the target gene locus for HD was mapped between RM22475 and RM22548, with genetic distances of 1.0 and 3.6 cM, respectively (Fig. 1a).

Fine Genetic and Physical Mapping of the *qHD8-1* Locus

For fine genetic and physical mapping of the *qHD8-1* locus, a large F_3 segregating population containing 3,000 individuals developed from F_2 plants with the heterozygous region around the *qHD8-1* locus was used to identify recombinant events between the *qHD8-1* locus and tightly linked markers. The two flanking SSR markers RM22475 and RM22548 were used to identify recombinants. In total, 87 recombinants were identified in the 460 F_2 and 3000 F_3 individuals between two markers, RM22475 and RM22548. Based on mapping results, it was clear that three types of segregation pattern represented three genotype classes: HJX74 homozygous (1/1), W06-26-3-5-1-5-2 homozygous (2/2), and heterozygous (1/2).

To delimitate the genomic region containing the *qHD8-1* locus, four polymorphic SSR markers RM22492, RM22499, RM22502, and RM22517 between RM22475 and RM22548 were used to examine these 87 recombinants. Among these recombinants, 14 early HD recombinants showed heterozygous alleles (1/2) at the four right-border markers (RM22499, RM22502, RM22517, and RM22548) and homozygous alleles (1/1) at the two left-border markers (RM22475 and RM22492); meanwhile, 15 late HD recombinants showed heterozygous alleles (1/2) at the two left-border markers (RM22475 and RM22492) and homozygous alleles (1/1) at the four right-border markers (RM22499, RM22502, RM22517, and RM22548), indicating that the *qHD8-1* locus was located upstream of RM22499. Eight late recombinants showed homozygous alleles (1/1) at the left-border markers (RM22475 and RM22492) and heterozygous alleles (1/2) at the right-border markers (RM22499, RM22502, and RM22517), indicating that the *qHD8-1* locus was located downstream of RM22492 (Table 2). It can be deduced that the *qHD8-1* locus is located between RM22492 and RM22499. Sequence analysis indicated that the physical distance between RM22492 and RM22499 was approximately 73 kb in length (Fig. 1b).

In an attempt to pinpoint the qHD8-1 locus, the sequence between RM22492 and RM22499 was downloaded from publicly available rice genome sequence (http://www.ncbi.nlm.nih.gov) and new SSR markers were designed by using the primer premier V. 5.0 and SSRIT procedures

Table 1 Heading date comparison between W06-26-35-1-5-2 and HJX74

Line	Mean of days to heading in Shandong (μ±SD)	*p* value
W06-26-3-5-1-5-2	129.43±0.79	1.81E−44
HJX74	108.76±0.56	
The difference of days to heading	20.67	

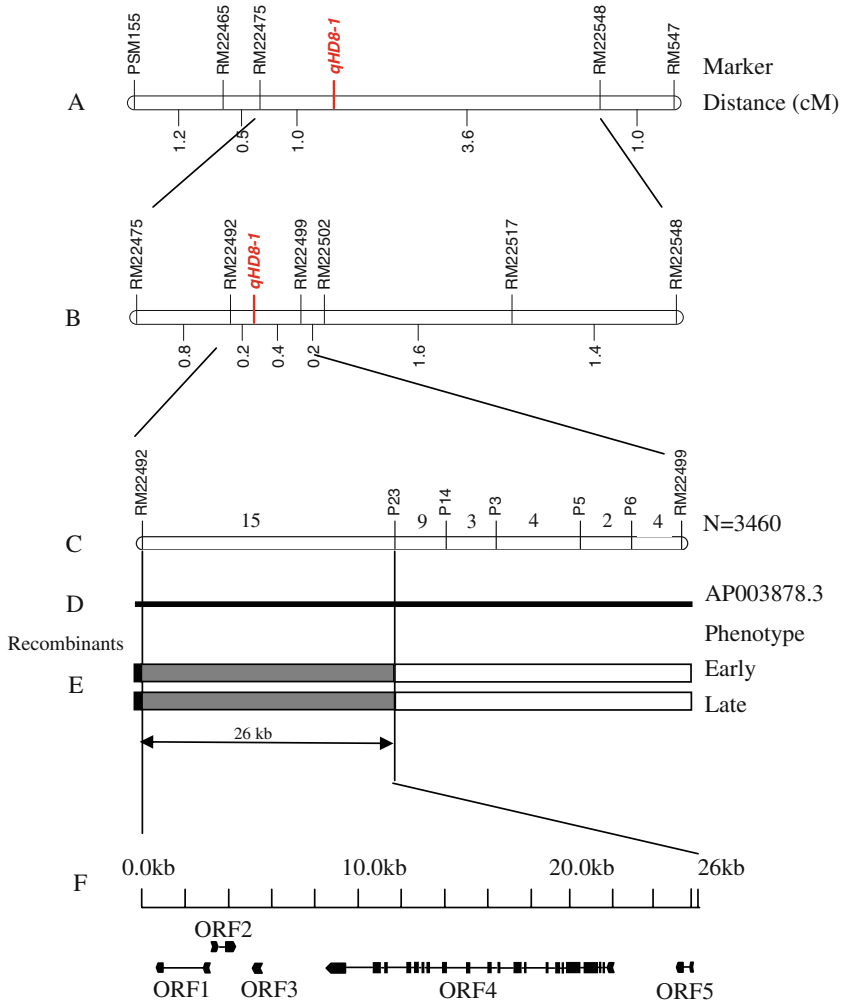

Fig. 1 Physical map of the *qHD8-1* locus. **a** Location of the *qHD8-1* locus on rice chromosome 8. **b** High-resolution linkage map of the *qHD8-1* locus generated using 3,000 F_3 plants. **c** The number of recombinants between the adjacent markers is shown above the linkage map. **d** BAC clones containing the region between markers RM22492 and P23. **e** Analysis of overlap mapping of the *qHD8-1* locus with key recombinants. The *qHD8-1* locus was finally narrowed down to a less than 26-kb DNA fragment. *Open*, *solid*, and *shaded bars* represent homozygous fragments from W06-26-35-1-5-2 segments, HJX74, and possible interval of crossover, respectively. **f** *Arrows* represent predicted ORFs using Rice GAAS

(http://www.gramene.org/db/searches/ssrtool). Meanwhile, sequence diversities between the *indica* cv. 93–11 and the *japonica* cv. Nipponbare were used to develop InDel markers. Among them, three SSR markers and two InDel markers showed polymorphism between the parents. Primer sequences, map position, and amplified length of these newly developed polymorphic markers are listed in Table 3. These polymorphic markers were used to screen the 37 recombinants between RM22492 and RM22499 to identify the crossover point and primer sequences are listed in Table 3. Genotypes of recombinants labeled with seven polymorphic SSR markers were showed in Table 4.

Among 37 recombinants, eight late heading date recombinants showed heterozygous alleles (1/2) at the right-border markers P23 and homozygous alleles (1/1) at the left-border markers RM22492, indicating that the *qHD8-1* locus was located downstream of RM22492. Seven of early heading date recombinants showed heterozygous alleles (1/2) at the right-border markers P23 and homozygous early heading date alleles (1/1) at the left-border markers RM22492, indicating that the *qHD8-1* locus was located upstream of P23. The closest crossover point to the *qHD8-1* locus occurred between the two markers RM22492 and P23. The genomic region containing the *qHD8-1* locus was further narrowed down to an interval bounded by RM22492 and P23. A physical map of the region between RM22492 and P23 covering the *qHD8-1* locus based on the Nipponbare sequence was constructed (Fig. 1c). The physical distance between RM22492 and P23 was approximated 26 kb in length (Fig. 1e; Table 4).

Candidate Genes in the 26-kb Region

Gene prediction analysis of the 26-kb DNA fragment using the Rice Genome Automated Annotation System (ricegaas.dna.affrc.go.jp/) identified five putative open reading frames (ORFs; Fig. 1). Among these ORFs, ORF3 encodes a transcript of 189 bp, classified as an unknown protein similar to Os08g0177400. ORF4 transcripts a message RNA of 6,033 bp containing 23 exons and translates a putative DNA binding protein. ORF1, ORF2, and ORF5 encode unknown proteins.

Table 2 The genotypes of the 59 recombinants at six polymorphic SSR markers in the RM22475–RM22548 region harboring the *qHD8-1* gene

No.	Recombinants	SSR markers						Phenotype
		RM22475	RM22492	RM22499	RM22502	RM22517	RM22548	
14	AQ284, AQ286, AQ328, AQ556, AQ657, AQ728, AQ927, AA40, AR141, AR263, AR300, AR347, NN4, NN130	1/1	1/1	1/2	1/2	1/2	1/2	Early
8	AQ110, AQ339, AQ465, AQ720, AQ862, NN122, NN321, NN554	1/1	1/1	1/2	1/2	1/2	1/2	Late
15	AQ195, AQ289, AQ322, AQ429, AQ506, AQ744, AQ890, AA35, AA55, NN275, NN392, AR1, AR338, AR364, AR464	1/2	1/2	1/1	1/1	1/1	1/1	Late
3	AQ67, AQ949, NN35	1/2	1/2	1/2	1/1	1/1	1/1	Late
2	AQ625, AR333	1/2	1/2	1/2	1/2	1/1	1/1	Late
3	AQ402, AA128, NN171	1/2	1/1	1/1	1/1	1/1	1/1	Early
4	AQ139, AQ971, AA103, AR41	1/1	1/1	1/1	1/1	1/2	1/2	Early
3	AQ701, AA284, AR222	1/1	1/1	1/1	1/2	1/2	1/2	Early
7	AQ391, AQ837, AA412, AA623, AR123, AR437, NN56	1/1	1/2	1/2	1/2	1/2	1/2	Late

1/1 the early homozygote, both alleles were derived from the HJX74; *1/2* the heterozygote, one allele was derived from the early parent, HJX74; the other was derived from the late parent W06-26-3-5-1-5-2

Discussion

Among the agronomic traits, HD is one of the most important traits and varies widely in rice; thus, cultivars are bred for specific geographical regions and seasons (Ding et al. 2010). Clear making of genetic basis affecting HD is important for different cultivation areas and crop seasons of the adaptation of rice. HD is controlled by many genes and various environmental conditions such as temperature and day length (Wang et al. 2009). Therefore, comprehensive research work has been done on the rice HD, which mapped many HD-associated genes/QTLs. Collecting data published on Gramene in 2008 (http://www.gramene.org) showed that there are 618 HD-associated QTLs were mapped and distributed in 12 chromosomes.

With the recent advances in molecular marker technology and developments of high-density molecular marker linkage maps in rice (Causse et al. 1994; Harushima et al. 1998), there are many HD genes that were located on chromosome 8 (Yano et al. 2001; Zhou et al. 2001; Lin et al. 2003; Liu et al. 2004; Zhang et al. 2006; Nonoue et al. 2008; Zhang et al. 2008). For instance, a QTL for HD in rice, *Hd5*, was finally mapped to a region between C166 and R902 on the short arm of chromosome 8 by using advanced backcross progeny derived from a cross between a *japonica* rice variety, Nipponbare, and an *indica* variety, Kasalath. Compared with that of Nipponbare, days to heading of *Hd5* increased under long-day and natural-field conditions.

Using a chromosome segment substitution line population, Yang et al. (2010) reported that a QTL for HD in rice

Table 3 Names and sequences of newly developed polymorphic primers from BAC sequence data of Nipponbare on chromosome 8 at the RM22492–RM22499 region

Primer	Physical position on the BAC clone (n)	Primer sequence	Motif	Predicted size (bp)
P3F	AP003878.3 (70634–70657)	AGAGCACCCTATTTTACCTTTACT	InDel	191
P3R	AP003878.3 (70801–70824)	CTGAATGTCTGAATATGTCCTCAT		
P5F	AP003878.3 (77784–77805)	GAGAAAAAGTAGCGGCAAAAAA	InDel	143
P5R	AP003878.3 (77905–77926)	ATGACCCTAGTGGGCTCAAAATC		
P6F	AP003878.3 (83460–83483)	GAACGTACTAACAAGTTGAGATGG	InDel	205
P6R	AP003878.3 (83641–83664)	ATTAAAAGGAGAAATTAACAGGGG		
P14F	AP003878.3 (64909–64929)	AAAGCAGTTGACGACCTGACA	InDel	182
P14R	AP003878.3 (65069–65089)	AAGGACGTTCTTGTGCAGTGA		
P23F	AP003878.3 (60259–60282)	TATGGATGACAAAGAGCGTAGGAG	InDel	130
P23R	AP003878.3 (60364–60388)	TCGGTGTATTTTTGGACAGAGAGGT		

n the positions of forward and reverse primers on the BAC clones AP003878.3

Table 4 The genotypes of the 37 recombinants at nine polymorphic SSR markers in the RM22492–RM22499 region harboring the *qHD8-1* gene

No.	Recombinants	SSR markers							Phenotype
		RM22492	P23	P14	P3	P5	P6	RM22499	
7	AQ284, AQ556, AQ927, AR141, AA40, NN4, NN130	1/1	1/2	1/2	1/2	1/2	1/2	1/2	Early
8	AQ110, AQ339, AQ465, AQ720, AQ862, NN122, NN321, NN554	1/1	1/2	1/2	1/2	1/2	1/2	1/2	Late
9	AQ195, AQ289, AQ429, AQ744, AR364, AA35, AA55, NN275, NN392	1/2	1/2	1/1	1/1	1/1	1/1	1/1	Late
2	AQ286, AR263	1/1	1/1	1/1	1/1	1/1	1/1	1/2	Early
2	AQ328, AR300	1/1	1/1	1/1	1/1	1/1	1/2	1/2	Early
3	AQ657, AQ728, AR347	1/1	1/1	1/1	1/2	1/2	1/2	1/2	Early
2	AQ322, AR1	1/2	1/2	1/2	1/2	1/2	1/2	1/1	Late
4	AQ506, AQ890, AR338, AR464	1/2	1/2	1/2	1/2	1/1	1/1	1/1	Late

1/1 the early homozygote, both alleles were derived from the HJX74; *1/2* the heterozygote, one allele was derived from the early parent, HJX74; the other was derived from the late parent W06-26-3-5-1-5-2

was mapped between RM4058 and RM8271 on the chromosome 8 and named *qHD-8*. The *qHD8-1* locus in this work was mapped to chromosome 8 between RM22492 and p23, and an overlapping interval was detected between C166–R902, RM4085–RM8271, and RM22492–P23. The *qHD8-1* is likely to be the same locus as the QTLs *Hd5* and *qHD-8.* However, it is difficult to establish these relationships conclusively because of the inherent low resolution of QTL mapping. Detection of QTLs for HD has allowed further genetic analyses, such as analyses of epistatic interaction among QTLs and map-based cloning. Therefore, cloning and sequence comparison of these QTLs will be required to clarify these relationships.

In this study, the *qHD8-1* locus for HD was fine mapped to a 26-kb genomic region, and this mapping result was performed by using rice SSSL and their resultant population. Gene prediction analysis of the 26-kb DNA fragment using the Rice Genome Automated Annotation System predicted five ORFs. Among these ORFs, ORF4 is a gene with a transcript of 6,033 bp, having 23 exons and encoding a putative DNA binding protein. ORF1, ORF2, and ORF5 encode unknown proteins. HD is determined mainly by two factors: duration of the basic vegetative growth and photoperiod sensitivity in rice. Map-based cloning of major QTLs that are known to affect HD in rice has revealed that the components of the photoperiod pathway are conserved in *Arabidopsis* and rice, although rice is a short-day plant (Yano et al. 2001; Hayama and Coupland 2004). Lin et al. (2003) detected interaction between *Hd5* and *Hd1*, a key photoperiod sensitivity QTL, on the basis of an analysis of the F_2 population. Results of Lin et al. (2003) suggested that *Hd5* is involved in photoperiod sensitivity and may act downstream or upstream of *Hd1* in the same photoperiodic pathway. Molecular and genetic studies of *Arabidopsis* have shown that the *CONSTANS* (*CO*) gene is a central regulator in the photoperiod pathway, which is one of the floral pathways (Putterill et al. 1995; Imaizumi and Kay 2006; Kobayashi and Weigel 2007). The *CO* gene encodes a B-box-type zinc-finger transcriptional activator with a CCT (*CO*, *CO-like*, and *TOC1*) domain near the carboxy1 terminus (Kim et al. 2008). *CONSTANS* is a member of 16 *CO-Like* (*COL*) genes that have been identified by *Arabidopsis* genome analysis (Robson et al. 2001). A family of *CO-Like* (*COL*) regulatory or putative regulatory genes has been identified in *Arabidopsis* and other species, members of which may function as transcriptional activators through DNA binding or protein–protein interactions. These studies have observably improved our understanding of the *qHD8-1*-controlling mechanisms in rice.

The *qHD8-1* gene reported here may be a new gene, and the ORF4 that encodes a putative DNA binding protein maybe the candidate gene. Although six QTLs for HD, *Hd1*, *Hd3a*, *Hd6*, *Ehd*1, *Ehd2*, and *Ghd7* (Yano et al. 2000; Kojima et al. 2002; Takahashi et al. 2001; Doi et al. 2004; Kazuki et al. 2008; Xue et al. 2008) have been map-based cloning in rice, little is known about the *qHD8-1* gene. In rice (*O. sativa* L.), two independent floral pathways—*Hd1* (a *CO* ortholog)-dependent and *Ehd1*-dependent pathways—control *Hd3a* (an *FT* ortholog) and flowering time. There is an antagonistic action between *Hd1* and *Ehd1* in the control of flowering time under long-day conditions because *Hd1* represses floral transition whereas *Ehd1* promotes it (Takeshi Izawa 2007). Previous studies and QTL analyses of flowering-time genes in rice have revealed several key genes involved in floral transition and have provided enough information to allow study adaptation during the domestication of rice. It will be interesting to analyze *qHD8-1* genes which may provide insights into the adaptation of rice in Shandong province. Meanwhile, it is useful to search orthologs of *qHD8-1* gene in other species, such as

Arabidopsis, and to examine their roles in flowering. There is no doubt that the existence of an *Arabidopsis* gene might give us some hints and provide a molecular probe for rice.

We have shown clear evidence for *qHD8-1* for rice HD in this study. Functional analysis of the candidate genes by transformation and other strategies is ongoing. This result will be very useful in molecular cloning of the *qHD8-1* gene.

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