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Fine linkage mapping enables dissection of closely linked quantitative trait loci for seed dormancy and heading in rice

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Abstract Two quantitative trait loci (QTLs) for seed dormancy (tentatively designated *Sdr1*) and heading date (*Hd8*) have been mapped to approximately the same region on chromosome 3 by interval mapping of backcross inbred lines derived from crosses between the rice cultivars Nipponbare (*japonica*) and Kasalath (*indica*). To clarify whether *Sdr1* and *Hd8* could be dissected genetically, we carried out fine-scale mapping with an advanced backcross progeny. We selected a BC_4F_1 plant, in which a small chromosomal region including *Sdr1* and *Hd8*, on the short arm of chromosome 3, remained heterozygous, whereas all the other chromosomal regions were homozygous for Nipponbare. Days-to-heading and seed germination rate in the BC_4F_2 plants showed continuous variation. Ten BC_4F_2 plants with recombination in the vicinity of *Sdr1* and *Hd8* were selected on the basis of the genotypes of the restriction fragment length polymorphism (RFLP) markers flanking both QTLs. Genotypes of those plants for *Sdr1* and *Hd8* were determined by advanced progeny testing of BC_4F_4 families. *Sdr1* was mapped between the RFLP markers R10942 and C2045, and co-segregated with C1488. *Hd8* was also mapped between C12534S and R10942. Six recombination events were detected between *Sdr1* and *Hd8*. These results clearly demonstrate that *Sdr1* and *Hd8* were tightly linked. Nearly isogenic lines for *Sdr1* and *Hd8* were selected by marker-assisted selection.

Keywords Seed dormancy · Heading date · QTL Linkage mapping · *Oryza sativa* L.

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Introduction

Seed dormancy is an important trait in rice breeding because it affects resistance to pre-harvest sprouting. Preharvest sprouting often occurs in hot, humid conditions at maturity, resulting in a reduction of grain quality. The degree of seed dormancy is determined by endogenous genetic factors and several environmental factors, such as the temperature during the ripening period and the degree of maturity (Roberts 1962; Anderson et al. 1993; Li and Foley 1997; Takahashi 1997; Wan et al. 1997; Lin et al. 1998; Cai and Morishima 2000). Wide variation in the degree of seed dormancy has been observed among rice cultivars (Seshu and Sorrells 1986; Wan et al. 1997).Wan et al. (1997) reported that the genes for seed dormancy were linked with isozyme locus *Pgi-1* on chromosome 3, morphological locus *C* (apiculus color), *Amp-3* and *Est-2* on chromosome 6, *Est-9* on chromosome 7, and *Acp-2* on chromosome 12. Cai and Morishima (2000) reported that they detected 17 putative quantitative trait loci (QTLs) for seed dormancy using DNA, isozyme, and morphological markers. However, further studies, such as fine genetic linkage mapping and characterization of putative genes, have not yet been conducted.

As a result of our previous study on seed dormancy, five QTLs for seed dormancy were identified on chromosomes 3, 5, 7 (two regions), and 8 using backcross inbred lines (BILs) derived from a backcross of Nipponbare (*japonica*)/Kasalath (*indica*)//Nipponbare (Lin et al. 1998). In addition, five QTLs for heading date were detected on chromosomes 2, 3, 4, 6, and 7 (Lin et al. 1998). Among these, a QTL designated *Sdr1*, which has major effects on seed dormancy, was mapped on the short arm of chromosome 3. Its chromosomal location was

coincident with that of the QTL designated *Hd8* for heading date (Lin et al. 1998; Yano et al. 2001). Furthermore, a significant phenotypic correlation between seed dormancy and heading date was observed in the BILs. We considered that this association between seed dormancy and heading date might be due either to a pleiotropic effect of one gene or to two tightly linked genes for seed dormancy and heading date (Lin et al. 1998). From the QTL analysis alone, it was difficult to conclude the genetic basis of this relationship between seed dormancy and heading date.

Therefore, to answer this question, we performed a fine-scale mapping of *Sdr1* and *Hd8* as single Mendelian factors by using advanced backcross progeny. As a result, we concluded that *Sdr1* and *Hd8* are tightly linked, but different, genes. This conclusion was also verified by the production and phenotype assay of nearly isogenic lines (NILs) of both *Sdr1* and *Hd8*.

Materials and methods

Plant materials

The scheme for constructing the palnt materials used in this study is summarized in Fig. 1. We obtained the F_1 plant from a cross between Nipponbare and Kasalath and subsequently backcrossed it with Nipponbare as the recurrent parent. Successive backcrossing and marker-assisted selection (MAS) allowed us to select one BC_4F_1 plant, in which the region of the short arm of chromosome 3, including *Hd8* and *Sdr1*, was heterozygous, and the other chromosomal regions were homozygous for Nipponbare. Self-pollinated progeny (BC_4F_2: 96 plants) of the plant were used as a mapping population. BC_4F_3 and BC_4F_4 seeds were obtained for progeny testing.

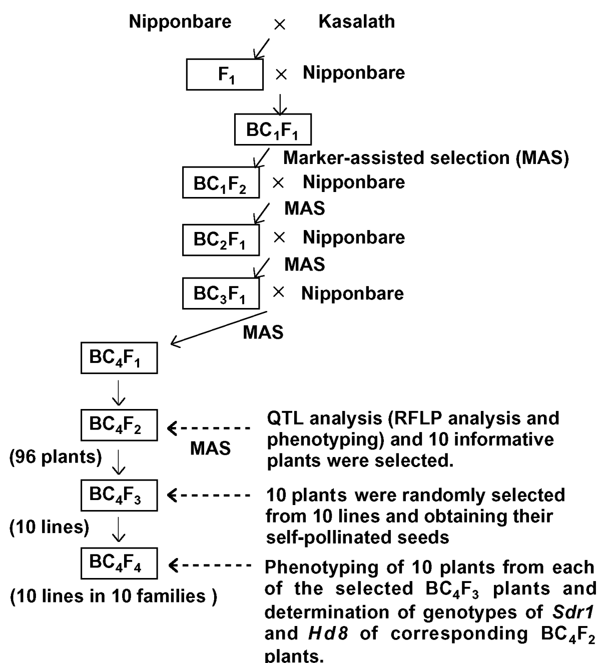

Fig. 1 Scheme for the development of plant materials used in this study

We produced three types of NILs. In NIL (*Sdr1*/*Hd8*), a Kasalath chromosomal region containing both *Sdr1* and *Hd8* was substituted in the Nipponbare genetic background; this NIL was selected from BC_4F_2 plants on the basis of the genotypes of restriction fragment ength polymorphism (RFLP) markers. In addition, we selected NIL (*Hd8*) and NIL (*Sdr1*) from BC_4F_3 plants by MAS; in these lines, a small chromosomal region containing *Hd8* or *Sdr1* was homozygous for the Kasalath allele. Two cleaved amplified polymorphic sequence (CAPS) markers, C12534S and C606, were used for the selection of NIL (*Hd8*) and NIL (*dr1*). Background genotypes of these NILs were checked with 207 RFLP markers (interval size from 5 cM to 15 cM) covering 12 rice chromosomes in backcross (BC) generations BC_3F_1 and BC_4F_1.

Phenotype assays

BC_4F_2 plants were cultivated in a paddy field at the National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan, in 1996 (April to September). Days-to-heading of each BC_4F_2 plant was measured as the number of days required from sowing to the emergence of the first panicle. The degree of seed dormancy was measured by the germination rates of seeds of two panicles from each plant. The panicles were harvested at 40 days after heading and were immediately placed under a temperature of 30 °C and 100% relative humidity. After 7 days, the number of germinated seeds was counted. Phenotype assays of BC_4F_3 and BC_4F_4 plants were same as that for the BC_4F_2 plants.

NIL (*Hd8*), NIL (*Sdr1*), NIL (*Sdr1*/*Hd8*) and their recurrent parent, Nipponbare, were cultivated in a paddy field in 1999. Days-to-heading and seed germination rate were measured in seven plants of each lines, and their means was compared by using the *t*-test.

DNA marker analysis

Two types of DNA markers, RFLP and CAPS (Konieczny and Ausubel 1993), were used to determine the genotype of each segregating chromosomal region. Total DNA of BC_4F_2 plants was extracted from the leaves by the CTAB method (Murray and Thompson 1980). Southern blotting and hybridization were performed according to the method described by Kurata et al. (1994). Fifteen RFLP markers in a target region containing *Hd8* and *Sdr1* were selected from a high-density genetic map (Harushima et al. 1998). One cDNA clone, R10942, was selected as a probe for RFLP analysis from the expressed sequence tag (EST) map (Wu et al. 2002).

CAPS analysis was performed according to the method of Lin et al. (2002). In brief, a small piece of rice leaf was ground in a 1.5-ml tube containing 300 μl of 100 m*M* Tris-HCl, 1 *M* KCl, and 10 m*M* EDTA. Crude DNA in the centrifuged supernatant was precipitated by isopropanol, and the pellet was re-dissolved in 50 μl 1/10 TE. One microliter of this DNA extract was used as a template for PCR amplification. A 10 μl volume of the PCR reaction mixture contained 1 μl of template DNA, 1 μl of 10× PCR buffer (Perkin Elmer, Norwalk, Conn, USA), 1 μl of 25 m*M* MgC_2, 1 μl of 2 m*M* each of dNTP (Boehringer Mannheim, Indian, IN, USA), 2 μl of 50% glycerol, 0.1 μl of 5 U *Taq* DNA polymerase (Perkin Elmer), 0.2 μl of 20-p*M* solutions of both primers, and 3.7 μl H_2O. Conditions for amplification by the GeneAmp PCR System 9600 (Perkin Elmer) were as follows: 30 cycles of 30 s of denaturation at 94 °C, 1 min of annealing at 60 °C, and 1 min of extension at 72 °C. To detect polymorphism, we first digested the amplified product, was digested with the appropriate restriction enzymes and then carried out electrophoresis on a 2.5% agarose gel. We converted two RFLP markers, C12534S and C606, to CAPS markers using their sequence information (Harushima et al. 1998). Based on 5′ and 3′ sequence data for the two clones, we designed unique primer pairs using Oligo 4.0 (National Biosciences, Plymouth, Minn.) to obtain specific amplicons of the C12534S and C606 regions. Restriction digestion of these fragments was subsequently carried

out using 28 enzymes to find enzymes for polymorphism detection. For C12534S, two primers, C12534SU (5′-AGA TGG TGG CGA TAA AGA A-3′) and C12534SL (5′-AAG GGC TCC AGG CAG AT-3′), were used to amplify the specific DNA fragment, and restriction digestion with *Hin*fI was performed to detect polymorphism. For C606, C606U (5′-CTC ATT GCC TCT CCA TCT C-3′), C606L (5′-CAA AAC CAT TGC CTC GTA TT-3′), and *Dra*I were used to detect polymorphism.

Linkage mapping and QTL analysis

Genotypes of each BC_4F_2 plant were determined for the 16 RFLP markers located on the target chromosomal region, and a linkage map was constructed with them. Linkage analysis was performed with MAPMAKER/EXP3.0 (Lander et al. 1987). QTL analysis was performed by using MAPMAKER/QTL (Lander and Botstein 1989).

For the fine mapping, we selected ten BC_4F_2 plants in which recombination had occurred in the vicinity of *Sdr1* and *Hd8*, on the basis of the genotype of the RFLP markers. We also selected three plants with no recombination in the region flanking both *Sdr1* and *Hd8* (homozygous for Nipponbare, homozygous for Kasalath, and heterozygous) as genotype references in the progeny testing. The self-pollinated progeny (BC_4F_3, 16 plants) of each of the ten BC_4F_2 plants were cultivated in a paddy field (Fig. 1). In the progeny testing of BC_4F_4, we randomly selected ten BC_4F_3 plants from each of the ten BC_4F_3 lines. Then 16 self-pollinated plants (BC_4F_4) from each of the 100 BC_4F_3 plants were cultivated in 1998 (70 BC_4F_4 lines) and 1999 (the other 30 BC_4F_4 lines), and days-to-heading and degree of seed dormancy were scored individually. The genotype of *Hd8* and *Sdr1* in the BC_4F_2 plants was determined from the segregation pattern of days-to-heading and seed germination rate in the BC_4F_4 lines.

Results

Verification of existence of *Sdr1* and *Hd8*

Self-pollinated progeny (BC_4F_2) showed a continuous variation in days-to-heading and germination rate. Days-to-heading ranged from 114 to 122 days, and the germination rate ranged from 0.7% to 95.9% (Fig. 2). QTL analysis for days-to-heading and germination rate was conducted on this population. For days-to-heading, one major QTL was detected in the marker interval between R10942 and C1488 (Fig. 3A). The additive effect of the Kasalath allele at the QTL was 1.8 days, and the percentage variance explained by this QTL was 53.1%. For seed dormancy, one QTL was detected in the marker interval between C12534S and R10942 (Fig. 3A), and the additive effect of the Kasalath allele and the percentage variance explained by the detected QTL were 23.5% and 37.9%, respectively. These results verified the existence of *Hd8* and *Sdr1* in this chromosomal region. However, it was difficult to exclude the possibility of a pleiotropic effect of one locus on days-to-heading and germination rate.

Fine-mapping of *Sdr1* and *Hd8*

Ten BC_4F_2 plants in which recombination occurred in the interval between the RFLP markers C68 and C606 were selected, and the self-pollinated progeny (BC_4F_3) of these plants were obtained for progeny testing (Fig. 3B). In progeny testing using BC_4F_3 lines, it was difficult to distinguish between plants that were either heterozygous or homozygous for the Kasalath allele (data not shown). Thus, we randomly selected ten plants in each line (BC_4F_3) to produce advanced progeny (BC_4F_4 families). Ten BC_4F_4 families (consisting of ten BC_4F_4 lines and 16 plants in each line) derived from ten informative BC_4F_2 plants were cultivated and scored individually for days-to-heading and germination rate. Days-to-heading in these BC_4F_4 families showed a range of 8 days in 1998 and 1999. Three types of segregation pattern were observed: either fixed early heading or late heading in all ten BC_4F_4 lines, or segregating from early to late heading among the ten BC_4F_4 lines (Table 1, Fig. 4A). It was clear that these three types of segregation pattern represented three genotype classes at *Hd8*, namely, Nipponbare homozy-

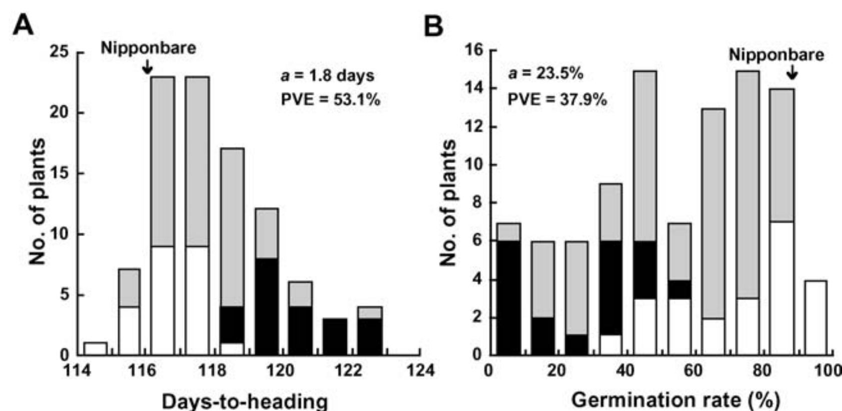

Fig. 2 Frequency distributions of days-to-heading (**A**) and germination rate (**B**) in the BC_4F_2 population. The mean values of the parental variety, Nipponbare, are indicated by the *arrow* in each figure. The additive effect of the Kasalath allele (*a*) and percentage of variance explained (*PVE*) are indicated in each figure. The genotypes of *Hd8* and *Sdr1* estimated by BC_4F_4 progeny tests are shown by the *white* (homozygous for the Nipponbare allele), *shaded* (heterozygous), and *black* (homozygous for the Kasalath allele) *blocks*

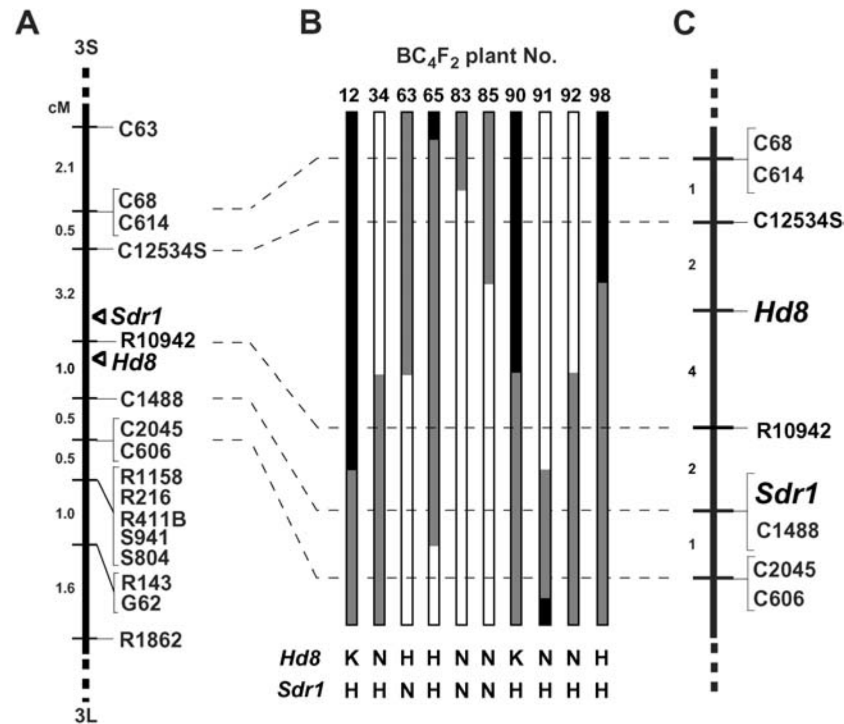

Fig. 3A–C Fine-scale mapping of two QTLs, *Sdr1* and *Hd8*, controlling seed dormancy and heading date. **A** Linkage map of the region on chromosome 3, showing the locations of the two QTLs. The *vertical bar* represents the linkage map constructed in this study. Putative QTLs for seed dormancy and heading date detected by using MAPMAKER/QTL are indicated by *triangles*. **B** Graphical genotypes of the region of chromosome 3 in ten BC_4F_2 plants. The BC_4F_2 plant number is shown at the *top*. *White blocks* Regions derived from Nipponbare, *shaded blocks* heterozygous regions, *black blocks* regions derived from Kasalath. The recombination point was arbitrarily determined at the mid-point between markers, which showed different genotypes. Genotypes of *Sdr1* and *Hd8* are shown at the *bottom*. The genotype of *Sdr1* or *Hd8* of each BC_4F_2 plant was classified as homozygous (designated as *N*) for the Nipponbare allele, homozygous (*K*) for the Kasalath allele, or heterozygous (*H*) on the basis of the status of segregation of germination rate and days-to-heading in corresponding BC_4F_4 lines (Table 1, Fig. 4). **C** The precise locations of two genes, *Sdr1* and *Hd8*, controlling seed dormancy and heading date, respectively, in ten recombinant plants. Numbers of recombinant plants are shown on the *left* of the *vertical bar*. Names of RFLP markers and genes are shown on the *right*

Table 1 Frequency distributions for days-to-heading and germination rate in BC_4F_4 families

BC_4F_2 plant	Days-to-heading			*Hd8* genotype[a]	Germination rate (%)			*Sdr1* genotype[a]
	Early	Segregating	Late		High	Segregating	Low	
12			10	K	1	4	5	H
34	10			N	5	3	2	H
63	5	4	1	H	10			N
65	6		4	H	2	5	3	H
83	10			N	10			N
85	10			N	10			N
90			10	K	2	6	2	H
91	10			N	3		7	H
92	10			N	4	3	3	H
98	1	5	4	H	5		5	H

[a] Genotypes of *Hd8* and *Sdr1* of BC_4F_2 plants were estimated from the segregation patterns for days-to-heading and germination rate, respectively, in the BC_4F_4 lines and are represented by N (Nipponbare homozygous), K (Kasalath homozygous), and H (heterozygous), respectively

gous, Kasalath homozygous, and heterozygous, respectively (Table 1). From the determination of these genotypes, *Hd8* was mapped in the interval between the RFLP markers C12534S and R10942 as a single Mendelian factor (Fig. 3C).

A large variation in germination rate was observed in BC_4F_4 families, and we classified BC_4F_4 families into three segregation patterns: fixed high germination rate or fixed low germination rate in all ten BC_4F_4 lines of the family, and segregating from low to high germination rate among the ten BC_4F_4 lines of the family (Table 1,

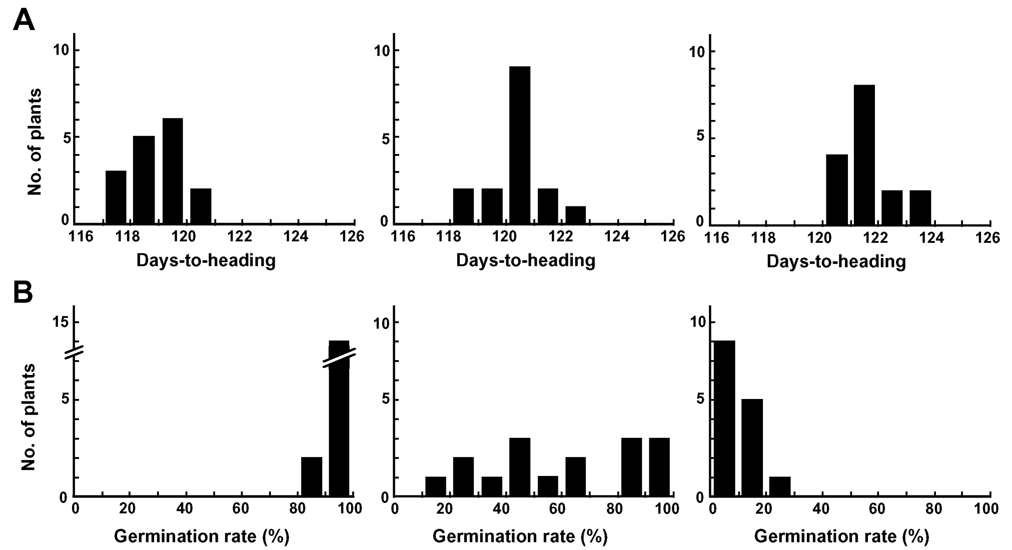

Fig. 4A, B Segregation patterns of days-to-heading and germination rate in BC_4F_4 lines. **A** Distributions for days-to-heading: *left* fixed early heading, *middle* segregating from early to late heading, *right* fixed late heading. **B** Distributions for germination rate: *left* fixed high germination, *middle* segregating from low to high germination, *right* fixed low germination

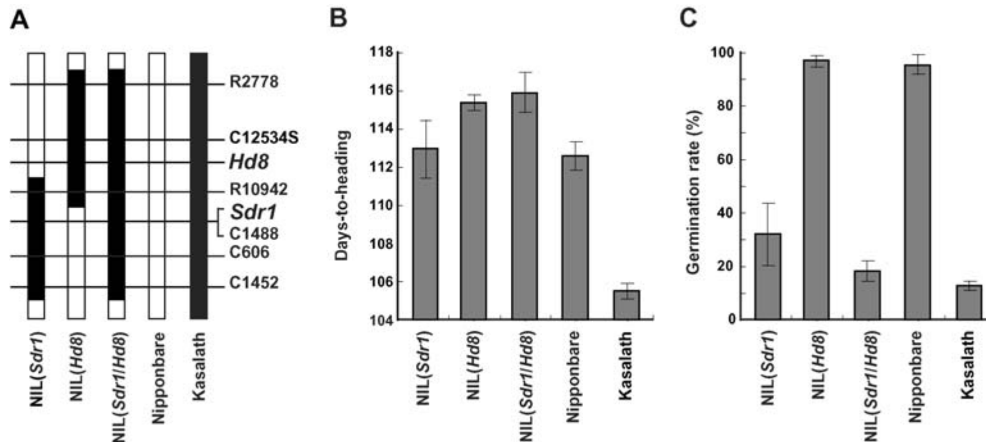

Fig. 5 Graphical representation of the genotypes of NIL(*Sdr1*), NIL(*Hd8*), NIL(*Sdr1*/*Hd8*), Nipponbare and Kasalath (**A**) and their days-to-heading (**B**) and germination rate (**C**). Days-to-heading and germination rate of each line represent the mean value of seven plants. The *error bar* indicated is the standard deviation of the mean. In graphical representation of the genotypes of three lines, the recombination point was arbitrarily determined at the mid-point between markers, that showed different genotypes

Fig. 4B). These three segregation patterns corresponded to three genotype classes at *Sdr1*: homozygous for the Nipponbare allele, homozygous for the Kasalath allele, and heterozygous, respectively (Table 1). Consequently, we mapped *Sdr1* as a single Mendelian factor that co-segregated with C1488 in the interval between the RFLP markers R10942 and C2045 (Fig. 3C).

On the basis of these results, both *Sdr1* and *Hd8* were mapped in different marker intervals and six recombination events between *Sdr1* and *Hd8* were detected (Table 1, Fig. 3). These results clearly demonstrated that *Sdr1* and *Hd8* were tightly linked but different loci.

Gene action of *Sdr1* and *Hd8*

To confirm our conclusion that two genes differentially determine heading date and seed dormancy, we produced three kinds of NIL. In NIL(*Sdr1*), a Kasalath chromosomal segment defined by the RFLP markers R10942 and C1452 was substituted in the genetic background of Nipponbare (Fig. 5A). In NIL(*Hd8*), a Kasalath chromosomal segment defined by R2778 and R10942 was substituted. In addition, we selected NIL(*Sdr1*/*Hd8*), in which the chromosomal segment including both *Sdr1* and *Hd8* was substituted.

Days-to-heading of NIL (*Hd8*) was 115.4 days and was later than that of Nipponbare (112.6 days) ($P < 0.01$) and NIL (*Sdr1*) (113.0 days) ($P < 0.05$) (Fig. 5B). There was no difference between that of NIL (*Hd8*) and NIL (*Sdr1*/*Hd8*) (Fig. 5B). The germination rate of NIL (*Sdr1*) was 31.7% and was markedly lower than that of both Nipponbare (95.6%) ($P < 0.001$) and NIL (*Hd8*) (95.8%) ($P < 0.001$) (Fig. 5C). There was no difference between that of NIL (*Sdr1*) and NIL (*Sdr1*/*Hd8*) (Fig. 5C). These results confirmed again the presence of

two different genes, *Sdr1* and *Hd8*, on the target region of chromosome 3.

Discussion

Fine-scale mapping of *Sdr1* and *Hd8*

In general, it has been very difficult to conclude from linkage studies whether different traits are controlled by two tightly linked genes or wether they are the pleiotropic effect of one gene. This has been the case for QTLs detected by statistical methods because QTL analysis can define only the chromosomal region controlling the target trait and cannot be used to map single Mendelian factors. In previous studies, we used advanced backcross progeny for fine mapping of the QTLs controlling heading date. Several QTLs for heading date – *Hd1, Hd2, Hd3a, Hd3b, Hd4, Hd5, Hd6*, and *Hd9* – have been mapped as single Mendelian factors by using advanced backcross progeny (Yamamoto et al. 1998, 2000; Lin et al. 2002, 2003; Monna et al. 2002). Furthermore, these analyses resulted in the molecular identification of three genes, *Hd1, Hd6*, and *Hd3a*, by a map-based strategy (Yano et al. 2000; Takahashi et al. 2001; Kojima et al. 2002). In this study, we performed fine linkage mapping of the QTLs for seed dormancy and heading date by using advanced backcross progeny. A discrete segregation pattern was expected in the BC_4F_2 population. However, the population showed continuous variation for germination rate in the two genotype classes homozygous for the Nipponbare or Kasalath alleles. Even though we determined background genotypes by using 207 RFLP markers, we could not rule out the involvement of another genetic factor in addition to *Sdr1*. However, it was assumed that such a continuous variation in the BC_4F_2 might be due to the effects of an environmental factor other than a gene because we observed clear discrete segregation patterns in the BC_4F_4 progeny testing (Fig. 4). Thus, two QTLs, *Sdr1* and *Hd8*, were mapped as single Mendelian factors, and precision mapping of these loci enabled us to distinguish two tightly linked QTLs detected in the same region (Fig. 3). This conclusion was supported by the production and phenotype analysis of NILs for each QTL (Fig. 4). We thus proved that an analysis of advanced backcross progeny could be effective in the fine mapping of QTLs for seed dormancy. This study also demonstrated that map-based cloning would be feasible for the identification of *Sdr1* as well as *Hd8*.

Comparison between QTLs detected in this study and those in previous reports

In this study, the QTL *Hd8* was verified and mapped on chromosome 3 as a single Mendelian factor. It is interesting to compare the chromosomal location of *Hd8* and QTLs for heading date in other studies. Five QTLs for heading date – *QHd3a* (Li et al. 1995), *dth3-2* (Xiao et al. 1995), *dth3* (Xiao et al. 1996), *Hd9* (Lin et al. 2002), and QTL3 (Zhou et al. 2001) – have been detected on the short arm of chromosome 3. Lin et al. (2002) concluded that the heading date QTL *Hd9* might be the same locus as *QHd3a, dth3-2*, and *dth3*, indicating that *Hd8* is not the locus of those QTLs. In addition, on the basis of a comparison of tightly linked RFLP markers (Zhou et al. 2001), QTL3 is not located in the same chromosomal region as *Hd8*. These results clearly indicate that *Hd8* is a new QTL for heading date in rice.

Several QTLs for seed dormancy have also been reported on chromosome 3 (Wan et al. 1997; Cai and Morishima 2000). Wan et al. (1997) found that a dormancy QTL was linked with an isozyme marker, *Pgi-1*, on chromosome 3. Cai and Morishima (2000) detected three QTLs for seed dormancy – qDOR-3-1 (on the short arm of chromosome 3), qDOR-3-2 (on the long arm of chromosome 3), and qDOR-3-3 (on the long arm of chromosome 3) – by using RFLP and isozyme markers. The *Sdr1* QTL detected in our study appears to coincide with qDOR-3-1. On the other hand, *Sdr1* might be a different locus from the QTL detected by Wan et al. (1997) because the isozyme locus *Pgi-1* has been located in the interval between RFLP markers C217 and G232 in the middle region of the long arm of chromosome 3 (Nagato and Yoshimura 1998). Further analyses, including allelic testing or cloning of genes at these QTLs, will be necessary to clarify the relationship between *Sdr1* and other dormancy genes.

Seed dormancy and heading date are independently regulated

A correlation between seed dormancy and heading date has been reported in rice (Yasue and Asai 1968; Lin et al. 1998). Yasue and Asai (1968) found a significant positive correlation. On the other hand, Lin et al. (1998) found a negative correlation in BILs derived from a backcross of Nipponbare/Kasalath//Nipponbare. Trait correlation often occurs from either pleiotropic effects of single QTLs or from tight linkage of several QTLs located in the same region. In this study, fine mapping of QTLs enabled us to genetically dissect *Sdr1* and *Hd8*. Thus, we concluded that the significant correlation between seed dormancy and heading date observed by Lin et al. (1998) was due to linkage of both *Sdr1* and *Hd8*.

The genetic dissection of two tightly linked QTLs for seed dormancy and heading date has implications for MAS in rice breeding programs. In general, some agronomically important genes, such as those for disease and insect resistance, have been introgressed from wild relatives or varieties of genetically distant relations (for example, *indica* and *japonica*) in elite modern rice varieties. In such a case, introgression of the target genes is often accompanied by introgression of undesirable traits in the elite variety. Fine mapping and the development of DNA markers have provided new opportunities to solve this problem. In fact, two tightly linked genes for

seed dormancy and heading date were separately introduced from the *indica* variety, Kasalath, into the *japonica* variety, Nipponbare, in the process of the establishing NILs in this study. Introgression of small chromosome segments might also be performed on the basis of phenotype-based selection. However, large-scale phenotyping will be required, and the reliability of phenotyping often varies depending on the environmental conditions, thereby preventing effective selection in breeding programs. MAS providces a more efficient and reliable selection system for developing new varieties with resistance to pre-harvest sprouting or late heading. In this study, we converted two RFLP markers, C606 and C12534S, which are tightly linked to *Sdr1* and *Hd8*, to PCR-based markers. These DNA markers will be very effective tools in MAS of *Sdr1* and *Hd8*.

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