High Density Molecular Map of Semidwarfing Gene, *sd-1*, in Rice (*Oryza sativa* L.)

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Summary

High density molecular map of semidwarfing gene, *sd-1*, was constructed using 178 F₂ plants derived from the cross between the semidwarf near-isogenic line and its recurrent parent, *japonica* cultivar "Shiokari". The total size of the map including nine molecular markers (five RAPD and four RFLP markers) and *sd-1* was 6.4 cM. The most closely linked RAPD markers in both sides of *sd-1* were OPV10₅₀₀ and OPAE14₁₀₀₀, and the distance between them was 1.7 cM. Since *sd-1* is a recessive gene, these closely linked RAPD markers will be very powerful to monitor the gene for large scale selection in breeding program.

Key Words: *Oryza sativa*, semidwarfing gene, linkage analysis, molecular map, RAPD marker, RFLP marker.

Introduction

In rice, there are about 60 dwarfing and semidwarfing genes which are designated *d-1* to *d-60* (review; Kinoshita 1990). Among them, *d-12*, *d-60* and *sd-1* are practically used to improve the cultivars with lodging resistance in rice breeding. Especially, *sd-1* is known as one of the important genes to produce high yielding cultivars. This gene is originated from a Chinese cultivar 'Dee-Geo-Woo-Gen' and transferred to many high-yielding cultivars, such as Taichung Native 1, IR8 and IR36.

Since the *sd-1* gene has greatly contributed to the success of producing high yielding cultivars, a lot of linkage analyses have been carried out to identify the location of the gene in the genome and to monitor the gene. Suh and Heu (1978) were the first to locate *sd-1* on chromosome 1 based on the linkage with some morphological markers, such as purple auricle and purple node. Later, shattering habit was also reported to be linked to *sd-1* (Yokoo and Saito 1986, Oba *et al.* 1989). On the basis of the biochemical study, Cho *et al.* (1994a) showed close linkage relationship between *sd-1* and esterase locus, *Estl-2*, and Nakamura *et al.* (1991, 1995) found the gene locus for seed protein linked to *sd-1* using two-dimensional electrophoresis. The effect of *sd-1* on culm and internode lengths were investigated by

Murai *et al.* (1995), indicating incomplete recessiveness of *sd-1*.

Recently, many morphological markers have been tagged with molecular markers after the advent of the molecular linkage maps (McCouch *et al.* 1988, Saito *et al.* 1991, Causse *et al.* 1994). Regarding the semidwarfing gene, Ogi *et al.* (1993) and Cho *et al.* (1994b) identified some RFLP markers which closely linked to *sd-1*. They confirmed the location of *sd-1* on chromosome 1, however, the linked markers were clustered at the one side of the *sd-1* gene in both studies. For further marker-based selection, it is practical to construct high density map covering both sides of the gene (Hittalmani *et al.* 1995). Therefore, in this study, we carried out RAPD and RFLP analyses to find new molecular markers closely linked at the both sides of the *sd-1* gene, and constructed high density map which enable to support large scale selection of the *sd-1* gene.

Materials and Methods

Plant materials

A *japonica* cultivar "Shiokari" (SH) and a near-isogenic semidwarf line (ID47) were used in this study. The latter, which has homozygote *sd-1* gene, was derived from the BC₇ plant between the recurrent parent, Shiokari, and the donor accession, IR8. One hundred seventy eight F₂ plants were made from the cross between SH and ID47 for linkage analysis. Eight F₃ plants from each F₂ line were grown to determine the F₂ genotypes of *sd-1* gene.

RAPD analysis

Total DNA was extracted from the small amount of leaves of SH, ID47 and F₂ plants by a CTAB method (Doyle and Doyle 1987) and used as a template for PCR. Amplification reaction was performed in the volume of 10 µl, containing 10 ng total DNA, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2 mM MgCl₂, 0.1 % Triton X-100, 0.2 mM primer, 100 mM each dNTP and 0.2 unit *Taq* DNA polymerase (TOYOBO, Japan). In total, 900 decamer primers (KIT A-J, P-Z, AA-AX; Operon Co., USA) were separately used for each reaction. Thermal cycling was carried out in BioOven III (BioTherm Corp., USA) programmed for 45 cycles of 30 sec at 95 °C, 1 min at 36 °C and 2 min at 73 °C, and end-

Received April 19, 1996. Accepted August 28, 1997.

ing with 2 min at 73 °C. Amplified products were electrophoresed in 1.6 % or 2.0 % agarose gel, and the banding patterns were observed.

RFLP analysis

Total DNA was extracted from the leaves of both parents and F₃ bulks of eight plants according to the method of Dellaporta *et al.* (1983). Extracted DNA was digested with seven restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, *Pst*I, *Xba*I), subjected to electrophoresis (3–5 µg per lane) in 0.8 % agarose gel, and blotted to Nylon membrane (Hybond-N+, Amersham, UK). Since *sd-1* is known to locate on the chromosome 1, 31 probes on the same chromosome were selected. Out of them, 22 probes were provided from Rice Genome Research Program, STAFF Institute, Japan, and the rest nine were from Cornell University, USA. Non-radioactive probe DNA labeling with digoxigenin, Southern hybridization and immunological detection were carried out according to the supplier's instruction (Boehringer Mannheim, Germany).

Evaluation of semidwarfism

The culm length of 178 F₂ plants was measured after heading to evaluate the morphological types of semidwarfism. Eight F₃ plants from each F₂ line were also examined to determine *sd-1* genotype of the F₂ plant.

Linkage analysis

Segregation for DNA markers and semidwarfing gene was analyzed to determine linkage relationships. Linkage values were calculated using the MAPMAKER program (Lander *et al.* 1987).

Results

Parental survey and F₂ segregation of RAPD markers

In total, 900 primers were surveyed to detect polymorphisms between SH and semidwarf NIL, ID47. Among them, polymorphic patterns were revealed by the five primers, OPB04, OPC19, OPV10, OPAE14 and OPAK02 (Table 1 and Fig. 1). The polymorphic bands with OPB04 and OPC19 primers were detected in the amplified products of SH at the position of 450 bp and 550 bp, respectively, and that with OPAE14 was detected in ID47 at the position of 1000 bp. On the other hand, OPV10 and OPAK02 gave a pair of polymorphic bands from each parent at the position of 500 bp and 1050 bp, respectively. Based on the primer code and the size of molecular weight, they were designated as OPB04₄₅₀, OPC19₅₅₀, OPV10₅₀₀, OPAE14₁₀₀₀ and OPAK02₁₀₅₀. The segregation of these markers was examined with F₂ population (Fig. 2). All of them showed a good fit to 3 : 1 or 1 : 2 : 1 ratio (Table 2). This indicates OPB04₄₅₀, OPC19₅₅₀ and OPAE14₁₀₀₀ are dominant markers, and OPV10₅₀₀ and OPAK02₁₀₅₀ are co-dominant.

Table 1. Molecular markers showing polymorphisms between Shiokari and semidwarf near-isogenic line, ID47

(A) Nucleotide sequences of the primers showing RAPD markers		(B) Polymorphic combinations of RFLP probes and restriction enzymes	
Primer	Sequence (5' to 3')	Probe	Restriction enzyme
OPB04	GGACTGGAGT	C385	<i>Dra</i> I
OPC19	GTTGCCAGCC	C949	<i>Dra</i> I, <i>Eco</i> RI
OPV10	GGACCTGCTG	RG109	<i>Eco</i> RV
OPAE14	GAGAGGCTCC	RG220	<i>Xba</i> I
OPAK02	CCATCGGAGG

Fig. 1. RAPD banding patterns of Shiokari and ID47 using five decamer primers. M: lambda DNA digested with *Pst*I. 1: Shiokari (recurrent parent). 2: ID47 (near-isogenic semidwarf line). Asterisks indicate the polymorphic bands between Shiokari and ID47.

Fig. 2. RAPD banding patterns in F₂ population of a cross between Shiokari and ID47 using primer OPAK02. M: lambda DNA digested with *Pst*I. 1: Shiokari (recurrent parent). 2: ID47 (near-isogenic semidwarf line) 3: IR8 (*sd-1* donor). Arrow heads indicate the codominant RAPD marker (OPAK02₁₀₅₀) segregating F₂ population.

Parental survey and F₂ segregation of RFLP markers

Using 31 probes on chromosome 1, RFLP survey was carried out with SH and ID47. Polymorphic patterns were detected with four markers, C385, C949, RG109 and RG220 (Table 1). Further, their segregation was examined with F₂ population. All these RFLP markers

Table 2. Segregation of five RAPD and four RFLP markers and *sd-1* gene in F₂ population derived from the cross between SH and ID47

Locus	Genotype ¹⁾			Total	χ^2
	A/A	A/B	B/B		
OPB04 ₄₅₀	125		53	178	2.16
OPC19 ₅₅₀	126		52	178	1.69
OPV10 ₅₀₀	38	87	53	178	2.62
OPAE14 ₁₀₀₀	39		139	178	0.91
OPAK02 ₁₀₅₀	39	86	53	178	2.40
C385	38	88	52	178	2.22
C949	39	86	53	178	2.40
RG109	39	87	52	178	1.99
RG220	39	87	52	178	1.99
<i>sd-1</i>	39	86	53	178	2.40

¹⁾ A/A and B/B show the genotypes for SH and ID47, respectively.

showed normal segregation in a ratio of 1:2:1 (Table 2).

Segregation analysis of culm length in F₂ population

The F₂ population of 178 plants derived from the cross between SH and ID47 was evaluated for culm length. The mean culm lengths for SH and ID47 were 77 and 47 cm, respectively, and those of F₂ plants were ranged from 39 to 84 cm. In order to determine the genotype of *sd-1* in F₂ population, culm lengths of eight F₃ plants derived from each F₂ individual were examined. Based on these data, 39, 86 and 53 F₂ plants were found to be normal, heterozygous semidwarf and homozygous semidwarf, respectively. This segregation was fit to the ratio of 1:2:1 (Table 2).

Linkage analysis between *sd-1* gene and molecular markers

Based on the parental survey between SH and ID47, polymorphisms were detected with nine molecular markers (five RAPD and four RFLP markers). In order to identify their linkage relationships, segregation for *sd-1* gene and these markers in F₂ population was examined. From these results, all nine molecular markers were turned out to be associated with *sd-1*. Fig. 3 shows the high density molecular map of *sd-1* constructed with RAPD markers and two kinds of RFLP markers from Rice Genome Research Program (Rice Genome Research Program 1993, Kurata *et al.* 1994) and Cornell University (Tanksley *et al.* 1992).

Discussion

Chromosomal segment containing *sd-1* gene in near-isogenic line

ID47 is a near-isogenic semidwarf line which has homozygote *sd-1* derived from the BC₇ plant between SH and IR8. After most of the backcrossings, BC plants were further selfed to confirm the existence of this recessive gene. Therefore, the remaining segment from IR8 should be very small. In fact, polymorphisms between SH and ID47 were detected by only five RAPD markers out of ca. 5000 amplified bands using 900

Fig. 3. High density map of *sd-1* gene with RAPD and RFLP markers on rice chromosome 1. Chromosomal locations of mapped RFLP markers are shown by arrows. A and B: Molecular maps of rice chromosome 1 constructed by Rice Genome Research Program (Rice Genome Research Program 1993, Kurata *et al.* 1994) and Cornell University (Tanksley *et al.* 1992), respectively. Numbers to the right of the chromosome represent clone designation, and the last column indicates whether polymorphism between Shikari and ID47 is found (+) or not (-).

primers. For RFLP markers, polymorphisms were detected by four among 31 markers on the chromosome 1. Since all these molecular markers were associated with *sd-1* gene, other portions in 11 chromosomes might have been replaced by those of recurrent parent during the backcrossing. The distance between two markers (OPAK02₁₀₅₀ and OPB04₄₅₀) at both ends of the linkage group was 6.4 cM (Fig. 3). Since the total map distance of saturated molecular map was reported to be 1491 cM by Causse *et al.* (1994), the remaining segment containing *sd-1* gene in ID47 might be equivalent to about 0.5 % of the total genome.

High density molecular map of *sd-1*

Previously, Ogi *et al.* (1993) and Cho *et al.* (1994b) reported RFLP markers linked to *sd-1*. However, in both studies, the linked markers were clustered at the one side of the *sd-1* gene. In this study, besides two RFLP markers (RG109 and RG220) reported by Cho *et al.* (1994b), seven new molecular markers (two RFLP and five RAPD markers) were identified to be associated with *sd-1*. In the map of Rice Genome Research Program (Kurata *et al.* 1994), two newly identified RFLP

markers (C385 and C949) are located near the markers reported by Ogi *et al.* (1993). In the high density molecular map, *sd-1* gene was mapped inside the linkage group. However, the linkage order of *sd-1* and two RFLP markers (RG220 and RG109) did not agree with previous one by Cho *et al.* (1994b). This may be caused by the few recombination events detected between these markers in this study. Therefore, in order to determine the precise linkage order, a larger number of plants are needed to be analyzed in mapping population.

Since the most popular dwarf character in breeding varieties is controlled by a single recessive gene, *sd-1*, DNA markers linked to this gene are very useful for the selection in breeding program. In this study, molecular markers RG220 and OPV10₅₀₀ were found to be closely located in both sides of *sd-1* gene. However, RG220 is a RFLP marker and RFLP analysis requires more steps and time than RAPD analysis. Therefore, it may be practical to use a RAPD marker, OPAE14₁₀₀₀, instead of a RFLP marker, RG220. The distance between these two RAPD markers located in both sides of *sd-1* gene is 1.7 cM. If these RAPD markers are detected with other breeding varieties, they will be powerful markers for large scale selection of *sd-1* from IR8.

Acknowledgements

We would like to thank Rice Genome Research Program, Tsukuba, Japan, and Dr. S. R. McCouch, Cornell University, USA, for providing rice DNA probes. We are also grateful to Drs. S. V. Tingey, Du Pont Co., USA, and E. S. Lander, Whitehead Institute, USA, for supplying us MAPMAKER program.

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