RFLP Mapping of a Shattering-Resistance Gene in the Mutant Line, SR-1 Induced from an *indica* Rice Variety, Nan-jing 11

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Summary

A shattering-resistance gene in the mutant line, SR-1 induced from an *indica*, semi-dwarf rice variety, Nan-jing11 was mapped on chromosome 1 using seven RFLP markers. It was also found that the shattering-resistance mutant gene was linked to a semi-dwarf gene, *sd-1*, derived from Nan-jing11. Eighty plants of a F₂ population derived from the crossing between SR-1 and a *javanica* rice variety, Dinalaga with shattering habit and long culm, were investigated for the degree of shattering, culm length and genotypes by RFLP markers. Although the segregation for the shattering degree and culm length was complex in the F₂ population, a significant correlation ($r = -0.57^{**}$) between the shattering degree and culm length was detected. Thus the shattering-resistance gene in SR-1 is likely to be linked to the semi-dwarf gene, *sd-1*, which has been mapped on chromosome 1 using RFLP markers (Ogi *et al.*, 1993). Moreover, the results of a quantitative trait loci (QTL) analysis for the shattering degree and the culm length by a computer program, MAPMAKER/QTL suggested that the genes for both the shattering-resistance and *sd-1* in SR-1 were located on chromosome 1, and linked most closely to *c 86* among the RFLP markers used. It was also assumed that the loci of the shattering-resistance gene in SR-1 corresponded to those of the recessive shattering gene *sh-2*. In this paper, QTL analysis using the RFLP markers was used for the single locus that expresses quantitative phenotypes in the segregation of the F₂ population derived from wide cross between an *indica* and a *javanica* variety, and the usefulness of the QTL analysis was demonstrated.

Key Words : RFLP mapping, shattering-resistance gene, mutant, semi-dwarf, QTL analysis.

Introduction

The shattering habit is one of the most important characters in harvesting, and many genetic analyses for the shattering habit have been carried out in rice (*Oryza sativa* L.).

Three shattering genes have already been mapped on rice chromosomes 1, 4 and 11. The recessive gene *sh-1* located on chromosome 11 (Nagao and Takahashi 1963), and *sh-2* was on chromosome 1 (Oba *et al.* 1989, Ogi *et al.* 1993). The dominant gene *Sh-3* that originated from a

common wild rice, was located on chromosome 4 (Eiguchi and Sano, 1990). It was also observed that the recessive gene *sh-2* was linked to the semi-dwarf gene *sd-1* which was one of the most important genes related to high yield in rice (Ogi *et al.* 1993, Oba *et al.* 1989).

Although the shattering habit itself may be controlled by major gene(s), it has been difficult to analyse the genetic mechanism of the shattering habit, since the shattering degree expresses a quantitative phenotype in the segregation of a hybrid population derived from the combination between varieties with different genetic backgrounds.

The development of RFLP markers and RFLP linkage maps enables to analyse the loci for a quantitative trait (QTL) in detail (Lander and Botstein, 1989). These methods can also be applied to a single Mendelian locus that expresses the phenotype like a quantitative trait (Doebley and Stec 1993, Dorweiler *et al.*, 1993). The RFLP linkage maps and RFLP markers have also been developed in rice (McCouch *et al.* 1988, Saito *et al.* 1991, Nagamura *et al.* 1993).

Fukuta *et al.* (1994 a) have produced five shattering-resistant mutant lines from an *indica* rice variety, Nan-jing 11 that was semi-dwarf, highly prone to shattering and high yielding, in order to improve the shattering habit and analyse the mechanism of the shattering habit. These five mutant lines were semi-dwarf like the original variety, Nan-jing11 and, a mutant line, SR-1 is the most resistant to shattering among them.

It was also found that these five shattering-resistant mutant lines were controlled by a single recessive gene, respectively, and that these mutant genes acted incompletely for the resistance to shattering. Moreover the five genes could be classified into three groups based on the results of allelism test, namely group 1, SR-1, group 2, SR-2, and group 3, SR-4, SR-5 (Hokuriku 143) and SR-6 (Fukuta *et al.*, 1994 b).

In this paper, the mapping of a shattering-resistance mutant gene and semi-dwarf gene in SR-1 was reported.

Materials and Methods

Eighty plants of the F₂ population and four F₁ plants derived from the cross between the shattering-resistant mutant lines, SR-1 induced from an *indica*, semi-dwarf rice variety, Nan-jing 11 and a *javanica* rice variety, Dinalaga with a shattering habit and a long culm, were used to localize the shattering-resistance mutant gene and the semi-dwarf gene on the RFLP linkage map.

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The F_1 and F_2 plants were cultivated under a 16 hr. daylight for the first 40 days after seeding, and then they were subjected to an 8 hr. daylight in a greenhouse, in order to synchronize the heading of panicle.

The shattering degree and the culm length of the F_1 and F_2 plants were determined, and the linkage among the characters and RFLP markers was examined. The shattering degree of each plant was determined using a shattering habit tester (TR-II, Ichikawa *et al.* 1990). The average value of the breaking tensile strength (gf) of top ten spikelets on a main panicle represented the shattering degree of each plant. Culm length (cm) was measured for the tallest tiller.

After heading, green leaves of the F_2 plants were collected, and the total DNA was extracted by the CTAB method (Murray and Thompson 1980). The extracted DNA was digested with five restriction enzymes (*Bgl* II, *Dra* I, *EcoR* V, *Hind* III, *Xho* I), and separated by agarose gel (0.8 %) electrophoresis. Southern analyses were carried out with the seven labeled RFLP markers on chromosome 1 (Saito *et al.* 1991, Nagamura *et al.* 1993) as the probes. A non-radioactive method (ECL system : Amersham) was used for labeling and detection.

The QTL analysis was carried out to localize the shattering-resistance mutant gene and the semi-dwarf gene on the RFLP linkage map using the computer

programs, MAPMAKER/EXP ver. 3.0 (Lincoln *et al.* 1992 a) and MAPMAKER/QTL ver.1.1 (Lincoln *et al.* 1992 b). The RFLP linkage map used in this analysis was presented in Kosambi cM units. A likelihood map based on the LOD scores for the shattering degree and the culm length was developed by interval mapping (Lander and Botstein, 1989). In this analysis, the value of the LOD score indicates the strength of the data supporting the hypotheses about the existence of the loci of the shattering and culm length gene. When the LOD score of the point was higher than 3.0, it was postulated that the locus of the RFLP marker was linked at the loci of shattering and culm length, and that the maximum likelihood position of the two loci corresponded to the highest point of the LOD score's curves. Thoughts, the average value of F_2 plant phenotypes for SR-1/SR-1 homozygotes, the additive effects and the dominance effects of the Dinalaga allele and the variances explained by QTL (R^2 value) was calculated at the putative loci.

Results

Segregations for shattering degrees and culm length in the F_2 population

Segregation for the shattering degree in the F_2 plants

Fig. 1. Segregation of the shattering degree and culm length in the three genotypes of RFLP marker (*c 86*) on chr. 1 in the F_2 population derived from the cross between the shattering-resistant mutant line SR-1 induced from an *indica* rice variety Nan-jing 11 and a *javanica* variety Dinalaga.

A-D : Segregation patterns of the shattering degree

E-H : Segregation patterns of the culm length

A, E : Distribution in the whole F_2 population,

B, F : Homogeneous type for Dinalaga

C, G : Heterogeneous type

D, H : Homogeneous type for SR-1

— : Means and ranges of the parents and F_1

showed a continuous and wide distribution (Fig. 1 A). The average value of the breaking tensile strength (gf) of SR-1 was 332 and that of Dinalaga was 151. The F_1 showed a value of 205 which was intermediate between those of SR-1 and Dinalaga. The breaking tensile strength (gf) in the F_2 population was distributed continuously from the values for the prone to shattering habit to the resistant to shattering habit, and the distribution consisted of three groups, a group highly resistant to shattering, an intermediate group and a group prone to shattering which may correspond to SR-1, F_1 and Dinalaga. The group prone to shattering segregated transgressive plants with a much more prone to shattering habit than that of Dinalaga.

The segregation for the culm length(cm) in the F_2 population showed a continuous distribution consisting of two groups with short and long culm(Fig. 1 E), respectively. The culm length(cm) of the semi-dwarf line, SR-1 and long culm variety, Dinalaga was 57.2 and 113.8, respectively. The F_2 population was distributed within the range of the two parents.

The correlation was significant between the shattering degree and culm length ($r = -0.57^{**}$, Fig. 2). The higher the breaking tensile strength of the plants, the shorter the culm length.

QTL analysis of the shattering-resistance mutant gene using RFLP markers.

Based on the results of the QTL analyses for the shattering degree and the culm length using seven RFLP markers on chromosome 1, it was observed that the shattering-resistance mutant gene and the semi-dwarf gene in SR-1 were located close to the RFLP marker *c 86* (Fig. 3). A likelihood map was drawn based on LOD scores for the shattering degree and the culm length, throughout the 98.8 cM distance spanned by the seven RFLP markers. In the likelihood map, the highest LOD scores for both characters were recorded close to *c86*. The peak of the LOD score for the shattering degree was recorded at the point between RFLP markers *c86*

Fig. 2. Relationship between shattering degree and culm length among the F_2 population derived from the crosses between the shattering-resistance mutant line, SR-1 induced from an *indica* variety, Nan-jing 11 and a *javanica* variety, Dinalaga.

Fig. 3. Mapping of the shattering-resistance mutant gene and the semi-dwarf gene in a mutant line, SR-1 induced from an *indica* rice variety, Nan-jing 11. The likelihood map is indicated by LOD scores for the shattering degree (solid line) and culm length (dotted line), throughout the 98.8 cM distance spanned by the seven RFLP markers. The RFLP linkage map used in the analysis is calibrated along the abscissa, in Kosambi cM units. The maximum likelihood position of two loci for shattering-resistance and semi-dwarfism corresponds the highest point in the LOD score curves. The dotted line at the LOD score, 3.0 indicates a significant level.

and *ald / XNpb113*, with a distance of 0.2 cM from *c 86*. The LOD score of 11.3 at that point was very high compared with the value of the LOD threshold(3.0). At the putative locus, the average value of the breaking tensile strength (gf) of the F_2 plants for the SR-1/SR-1 homozygous type corresponded to the value for a the very high resistance, 316.05 which the additive effect of the Dinalaga allele was -118.14, and the dominance effect for the Dinalaga allele was -23.62. The absolute value for the additive effect was much higher than that for the dominance effect, and the value of the variance explained by QTL was 50.4 %. These results indicate that the putative gene of SR-1 and the allele of Dinalaga act incompletely on resistance to shattering and shattering, respectively. In the distribution for the shattering degree in the three genotypes of the RFLP marker *c 86*, it was also confirmed that the segregants with the Dinalaga/Dinalaga homozygous type tended to distribute in the classes of shattering, (the value of low breaking tensile strength (gf)), which those with the SR-1/SR-1 homozygous type tended to distribute in the classes of resistance to shattering, (high strength value) (Fig 1 B, C, D). The highest LOD score for culm length (13.8) was also recorded at the point between *c86* and *ald / XNpb 113* with a distance of 2.2 cM from *c86*. At the putative loci, the average value of the culm length (cm) of the F_2 plants for the SR-1/SR-1 homozygous type was 66.34. The values of the additive effect and dominance effect for the Dinalaga allele were 18.63 and 7.05, respectively. The additive effect was much more pronounced than the dominance effect and the value of

the variance explained by QTL was 62.1 % (Table 1). The effects calculated for the putative loci of culm length indicate that the gene of SR-1 and the allele of Dinalaga act incompletely the short and long culm, respectively, at the putative locus. Regarding the culm length, it was also confirmed that the distribution of the plants with Dinalaga homozygous and SR-1 homozygous types tended to cluster in the long and short length classes, respectively (Fig 1 F, G, H).

Discussion

It was suggested that there was a linkage between the shattering-resistant mutant gene and semi-dwarf gene in SR-1 based on the correlation coefficient between the breaking tensile strength(gf) and the culm length(cm).

Kobayashi (1973) reported that a shattering gene was linked to a single recessive semi-dwarf gene with a recombination's value 7.6-15.3 (%). Kikuchi *et al.* (1984) also reported that a recessive shattering gene was linked to a semi-dwarf gene. Oba *et al.* (1989) reported that the recombination's value between the locus of the shattering gene and that of the semi-dwarf gene *sd-1* was 13.1 (%). Ogi *et al.* (1993) mapped the semi-dwarf gene *sd-1* and a shattering gene *sh-2* on chromosome 1 using the RFLP markers. Thus, it had already been demonstrated that the shattering gene *sh-2* was linked to the semi-dwarf gene *sd-1* on chromosome 1.

In this experiment, QTL analysis using RFLP markers chromosome 1 was performed to localize the shattering-resistance mutant gene and a semi-dwarf gene in SR-1 more precisely.

The value of the LOD scores and the variance explained by QTL at the putative loci of the shattering degree and the culm length was high. Moreover, it was confirmed that the most remarkable distortion from the expected distribution of F₂ segregation for the shattering degree and culm length occurred in each of the three genotypes for the RFLP marker *c86* that was linked most closely at the putative loci. These results indicate that both the shattering-resistance mutant gene and the

semi-dwarf gene located on chromosome 1 close to *c86*.

The shattering-resistance mutant gene and the semi-dwarf gene in SR-1 were located in the same chromosome region where Ogi *et al.* (1993) mapped *sh-2* and *sd-1*. Moreover, Taniguchi *et al.* (1989) reported that the locus of the semi-dwarf gene in Nan-jing 11 was the same as that of *sd-1*. These facts suggest that the loci of the shattering-resistance mutant gene and the semi-dwarf gene in SR-1 correspond to *sh-2* and *sd-1*, respectively.

The distance between the shattering-resistance mutant gene and the semi-dwarf gene in SR-1 is 2.0cM, a value which is, however, lower than that reported by Kobayashi (1973), Oba *et al.* (1989) and Ogi *et al.* (1993). The differences in the recombination's value may be due to the small F₂ population size used in this experiment.

Kikuchi *et al.* (1984) reported that *sh-2* was a recessive gene for shattering, based on the analysis using the isogenic lines derived from continuous backcrossings to introduce a shattering gene from an *indica* variety, Taichung Native 1 into the genetic background of a *japonica* variety, Norin 29. Fukuta *et al.* (1994 b) reported that the resistance to shattering of SR-1 was controlled by a single recessive gene. The fact that the resistance mutant gene of shattering in SR-1 and *sh-2* occupy the same locus, suggest that the difference in the phenotypes between two genes originating from the different alleles, or other genetic factor(s) in the *indica* and *japonica* background influenced the segregation for the shattering degree in the analyzed population. In the latter case, the estimation for the shattering habit may be different, since it was difficult to determine the shattering degree as a quantitative trait. It will thus be necessary to analyze the allelism between the shattering-resistance mutant gene in SR-1 and *sh-2*.

The segregation pattern in the F₂ population was complicated, since the distribution was continuous from the prone to shattering habit to the resistant to shattering habit and contained the transgressive plants that was much more prone to shattering than Dinalaga. The segregation suggests that the induced resistance mutant gene in SR-1 was not the only factor controlling the

Table 1. Effects of putative loci in the shattering degree and culm length

Trait	LOD score ¹⁾	Mean ²⁾	Additive ³⁾ effect	Dominance ⁴⁾ effect	Variance explained
Shattering degrees	11.30	316.05 (gf)	-118.14	-23.62	50.4%
Culm length	13.79	66.34 (cm)	18.63	7.05	62.1%

The 80 F₂ plants derived from the cross between the mutant line "SR-1" resistant to shattering and "Dinalaga" were analyzed using the seven RFLP markers on Chromosome 1.

The putative loci for shattering degree and culm length corresponded to the maximum likelihood positions indicated by LOD scores.

¹⁾ Strength of the data supporting the hypothesis about the existence of loci for shattering-resistance and semi-dwarf genes.

²⁾ Mean values of the trait for SR-1/SR-1 individuals at putative loci.

³⁾ Genotypic value of homozygote of "Dinalaga" allele.

⁴⁾ Genotypic value of heterozygote.

shattering degree in the F₂ population, but that other factors may coexist in the genetic background of Dinalaga and SR-1. The mutant line SR-1 may harbour also the two other shattering genes originating from Nan-jing11 in addition to the shattering-resistant mutant gene, since Nan-jing 11 had two different shattering loci, in addition to the mutant locus in SR-1 (Fukuta *et al.* 1994 b).

In this paper, the other genes originating from Dinalaga and Nan-jing 11 were not analyzed, since the RFLP analysis covered only a limited region on chromosome 1. In the future, analysis of the remaining chromosome region using the RFLP markers and the other mutant lines to detect other factors relating to the genetic mechanism of the shattering habit should be performed.

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