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Linkage Analysis of Thermosensitive Genic Male Sterility Gene, *tms -2* in Rice (*Oryza sativa* L.)

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Summary

Environment-sensitive genic male sterility (EGMS) line is useful in hybrid rice seed production. A parental line of rice, Norin-PL12, has a thermosensitive genic male sterility (TGMS) gene, *tms -2*. We estimated the locus of *tms -2* using RFLP markers in F_2 population derived from a cross between Norin-PL12 and Aus variety, Dular. As a result of quantitative trait locus (QTL) analysis using RFLP markers, it was revealed that the *tms -2* is located between *R643A* and *R1440* on chromosome 7.

Key Words: *Oryza sativa* L., TGMS gene, linkage analysis, RFLP marker, QTL.

Introduction

One approach to increasing rice yields is the use of hybrid rice. The two-line method using environment-sensitive genic male sterility (EGMS), such as photosensitive genic male sterility (PGMS) or thermosensitive genic male sterility (TGMS), has been used for hybrid seed production mainly in China. EGMS has advantages over the three-line method which uses cytoplasmic male sterility (CMS) and a restorer gene, since, for example, seed multiplication of the EGMS line is much easier (Maruyama *et al.* 1991).

Recently, several TGMS lines have been reported (IRRI 1992, Maruyama *et al.* 1991, Sun *et al.* 1989). A parental rice line, Norin-PL12, induced by irradiation of a *japonica* variety, Reimei, shows TGMS which is sterile at high temperatures (above 31 °C) but is fertile at low temperatures in three weeks before heading (Maruyama *et al.* 1991). TGMS trait of Norin-PL12 is controlled by a single recessive gene, *tms-2* (Kinoshita 1992). But the map location of the gene have been not determined.

It is not possible to identify the locus of sterility gene using morphological markers even if it is single gene, because the evaluation of fertility which is easily influenced by the environmental factor and is expressed as quantitative trait is difficult. But quantitative trait locus (QTL) analysis using RFLP markers will be useful to map a sterility gene such as *tms-2*.

By bulked segregant analysis, RAPD markers linked to the unidentified TGMS gene derived from TGMS mutant line were found (Subudhi *et al.* 1995, Wang *et al.* 1995). In this paper, we report the result of linkage analysis of the TGMS gene, *tms-2* with RFLP markers using QTL analysis.

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Materials and Methods

Plant materials

The 249 F_2 plants derived from a cross between Norin-PL12 and an Aus variety, Dular, (Ikehashi and Araki 1987) was used as a mapping population to avoid the influence of hybrid sterility. The materials were transplanted on June 6th, in 1995 and cultivated in the field of National Agriculture Research Center in Japan.

Seed fertility of F_2 plants

The heading date of each plant of the F_2 population was recorded in order to confirm whether the material responded to high temperature or not. The seed fertility of F_2 plants were investigated by the mean seed set of three panicles.

RFLP analysis

Total DNA was extracted from frozen green leaves by the CTAB method (Murray and Thompson 1980) and digested with the eight restriction enzymes (*Bam* HI, *Bgl* II, *Eco* RV, *Hin* dIII, *Apa* I, *Dra* I, *Eco* RI and *Kpn* I). The digested DNA were separated by 0.7 % agarose gel electrophoresis and transferred to nylon membranes (Boehringer Mannheim). ECL direct nucleic acid labelling and detection systems (Amersham) were used for Southern hybridization. RFLP markers (Kurata *et al.* 1994) were used as DNA probes. The number of RFLP markers for which polymorphism was detected among the parents were 107 among 134 analyzed markers. These 107 markers are located on the rice RFLP map (Kurata *et al.* 1994) with about 15 centimorgans (cM) intervals as the average and almost cover all over the rice genome.

QTL analysis

We applied QTL analysis to the mapping of *tms-2*. As the first screening, RFLP markers linked to *tms-2* were determined using the 14 F_2 plants which showed less than 25 % seed fertility. We presumed that these F_2 plants were homozygous for the *tms-2* as in the parent Norin-PL12. Finally, we determined the genotypes of the RFLP markers which were linked to the *tms-2,* us-

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ing all the F_2 plants. The map location of *tms-2* was estimated using a computer program, MAPMAKER/EXP ver. 3.0 (Lander *et al.* 1987) and MAPMAKER/QTL ver. 1.1 (Lander and Botstein 1989). The value of the interval mapping was 0.1. The LOD score higher than 2.0 was used as threshold for putative locus. The mean seed fertility of the F_2 plants for the homozygous of Norin-PL12 at the putative locus, the additive effect and the dominance effect of the Dular allele, and the variance explained (R^2) by putative locus were calculated.

Results and Discussion

Seed fertility

Heading date, maximum temperature before heading date, and seed fertility of both the parents and the F_1 plants were shown (Table 1). Norin-PL12 showed high sterility because maximum temperature at three weeks before heading was sufficiently high for TGMS to be expressed. Seed fertility of the F_1 plants indicated the high value, 90.5 %. Therefore, it was confirmed that the cross combination used in this study did not recognized high hybrid sterility.

Frequency distribution of seed fertility in the F_2 population did not follow expected Mendelian segregation ratios (3 fertile: 1 sterile) due to the appearance of semi-sterile individuals (Fig. 1A).

QTL analysis of TGMS

As a result of first screening, the genetic distortion which almost highly sterile plants indicated Norin-PL12 homozygous genotype at the *R1440* locus on chromosome 7, was detected. Therefore, it is assumed that *R1440* link to the *tms-2* locus. *R1440* and the three RFLP markers linked to *R1440* on chromosome 7 were used for QTL analysis. As a result, it was confirmed the existence of QTL (LOD score=52.27) between *R643A* and *R1440* with a distance of 0.2 cM from *R643A*. Therefore, we estimated that *tms-2* is located between *R643A* and *R1440* on chromosome 7 (Fig. 2). At the *tms-2,* mean seed fertility of the F_2 plants for the homozygous of Norin-PL12 was 26.9 %. The additive effect and the dominance effect of the Dular allele were 29.19 and 25.04, respectively. These results suggest that the Dular allele of this QTL acts upon fertility. The variance explained at this QTL was 62.4 % (Table 2). This relative small value for the effect of one gene was explained that the seed fertility in the F_2 population was easily

Table 1. Characteristics of the parents and the F_1 plants

Materials	Heading date	Max. temperature at three weeks before heading (℃)	Seed fertility (%)
Norin-PL 12	14 Aug	35.0	1.2
Dular	11 Aug	26.3	95.7
F_1 (Norin-PL 12 /Dular)	31 Jul	32.5	90.5

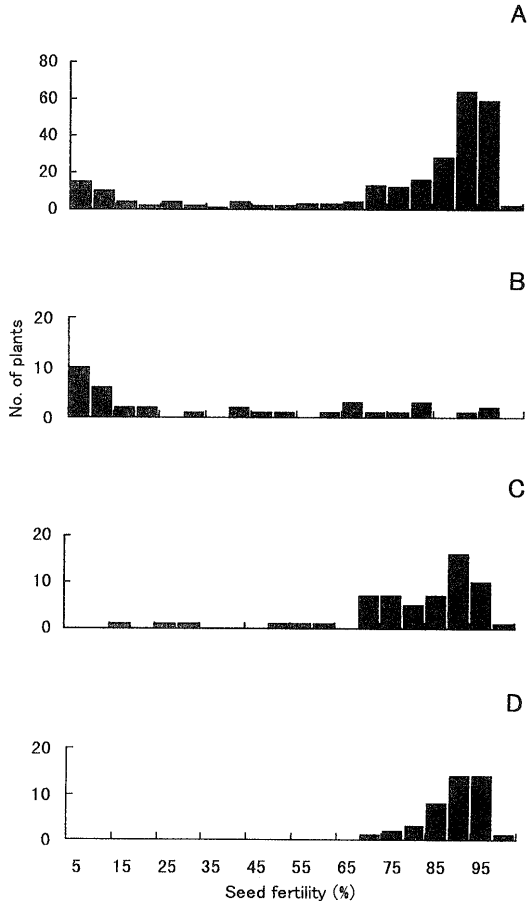

Fig. 1. The relationship between seed fertility and the genotype of the RFLP marker *R643A* in the F_2 plants (Norin-PL12/Dular) responded to high temperature (n =139).
A: All the F_2 plants (n=249)
B: Homozygous type of Norin-PL12 (n=37)
C: Heterozygous type (n=59)
D: Homozygous type of Dular (n=43)

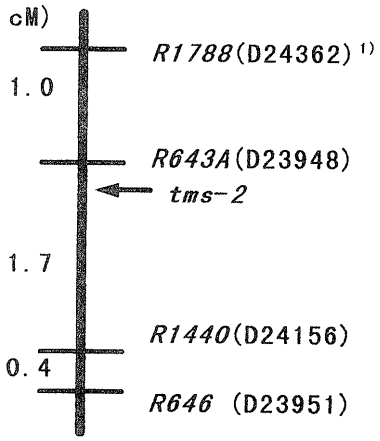

Fig. 2. Putative locus of *tms-2* on chromosome 7.
1)DDBJ No. is shown in parenthesis.

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Table 2. Effects of the estimated QTL

Marker	Mean[1)]	Additive[2)] effect	Dominance[3)] effect	LOD score	Variance explained (R^2)
R 643 A-R 1440	26.9 %	29.19	25.04	52.27	62.4 %

[1)] Mean seed set of the F_2 plants for the homozygous of Norin-PL 12 at the estimated QTL.
[2)] Effect of homozygote of the Dular allele.
[3)] Effect of heterozygote.

affected by the environmental factor. In order to confirm the result of QTL analysis, the relationship between seed fertility and the genotype of the RFLP marker, *R643A* in the F_2 plants responded to high temperature (n=139) was examined (Fig. 1B-D). Among the F_2 plants, some plants exhibited high seed fertility even though the genotypes of these plants were presumed to be homozygous for *tms-2* based on RFLP marker data. It is thought that some of such individuals were recombinants, and others were appeared by the environmental and unknown factor. Zhang *et al.* (1994) reported that the expression of PGMS was more stable in *japonica* than in *indica* genetic background. It is assumed that expression of TGMS is also variable according to the genetic background.

Subudhi *et al.* (1995) reported that molecular markers linked to the TGMS gene of IR32364TGMS were different from the TGMS gene which Wang *et al.* (1995) reported. Moreover Borkakati and Virmani (1996) showed the TGMS gene of IR32364TGMS was different from *tms-2*. From the results of the study reported here, *tms-2* is also different from the TGMS gene located on chromosome 8 reported by Wang *et al.* (1995).

The approximate recombination value between the *tms-2* locus and the RFLP markers calculated in this study. The F_3 test to detect the correct genotype is necessary to proceed with fine mapping of *tms-2*.

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