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RFLP tagging of a new semidwarfing gene in rice

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Abstract A new rice semidwarfing gene which is not allelic to *sd1*, temporarily designated as *sdg*, might be of use as a new source of semidwarfism in rice breeding programs. We report here the identification of a DNA marker closely linked to this gene. The DNA marker was identified by testing 120 mapped rice RFLP makers as hybridization probes for Southern analysis of a pair of nearly isogenic lines with or without *sdg*. Linkage association of the marker with the gene was verified using a F_2 population segregating for semidwarfism. RFLP analysis showed that *sdg* is closely linked to a single-copy DNA clone RZ182 on chromosome 5, with a distance of 4.3 centiMorgans between them. This marker may facilitate early selection for the semidwarfing gene in rice breeding programs

Key words Semidwarfism · Restriction fragment length polymorphism · Rice (*Oryza sativa* L.) · DNA markers

Introduction

One of the most significant achievements in rice breeding has been the development of semidwarfism. Since isolation of the semidwarfing gene *Dgwg*, or *sd1*, from the Chinese semidwarf variety 'Dee-Geo-Woo-Gen', a great number of semidwarf stocks have been identified. However, about one-half of the stocks from the collection of the International Rice Research Institute have been found to be allelic to the *Dgwg* gene, while others have been found to possess many negative agricultural characters that limit their use in cross-breeding programs (Maluszynski et al. 1986). In southern China, the semidwarfing genes carried by the major short statured indica varieties of economic importance are found at the same locus as *sd1* (Gu and Zhu 1979; Xiong et al. 1988). Frequent use of the same semidwarfing gene may reduce genetic diversity and bring about genetic vulnerability. In addition, these principal semidwarf varieties also possess some negative agricultural characters (Xiong and Min 1988). Thus, new sources of semidwarfism are necessary to broaden the genetic basis of the high yielding varieties. Gu and Pan (1988) obtained a new semidwarf stock 'Xingui'ai' (XGA) from the progenies of 'Guiyang'ai'/'Nangjing 11'. The semidwarfism of XGA is controlled by a single recessive gene that is not allelic to *sd1*, temporarily designated as *sdg*. This gene might be used as a new source of semidwarfism in rice breeding. Through the use of marker gene lines and several trisomics of IR36, *sdg* has been mapped on chromosome 5 (Liang et al. 1993). However, the genetic distances between the gene and phenotype markers are very long.

The development of restriction fragment length polymorphism (RFLP) techniques offers a new tool by which to monitor gene transfer in breeding programs and potentially to clone genes whose products are not currently known (Tanksley et al. 1989). Nearly isogenic lines (NILs) provide a useful source of plant material for rapidly identifying regions of chromosomes where a gene of interest is likely to reside (Young et al. 1988; Yu et al. 1991; Ahn et al. 1992). Pairs of NILs are developed by introgressing a gene of interest from a donor parent into a recurrent parent via backcrossings. Consequently, a pair of NILs consists of the recurrent parent without the target gene and its counterpart with the target gene. The latter is genetically identical to the former except for the chromosome segment introgressed from the donor where the target gene resides. Thus, markers that detect polymorphisms between the pair of NILs are probably linked to the target gene. Using a rice RFLP map constructed at Cornell University (S. D. Tanksley, personal communication) and a pair of NILs, we conducted experiments to identify RFLP markers that are

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linked to the new semidwarfing gene, *sdg*. The results are reported here.

Materials and methods

Plant varieties

A pair of rice NILs were used in this study: 'Nangjing 6' (NJ6) and semidwarf NJ6. The donor of the semidwarfing gene is XGA. Semidwarf NJ6 obtained the *sdg* gene through five backcrossings followed by one selfing (B_5F_2). The B_5F_2 population segregating for semidwarfism, from which the semidwarf NIL was selected, was used for linkage confirmation. Linkage analysis was performed with the program MAPMAKER (Lander et al. 1987).

Screening of rice clones

An RFLP map of the rice genome constructed at Cornell University (S. D. Tanksley, personal communication) was used. One hundred and twenty mapped clones, including 14 on chromosome 5, distributed on the map in approximately a 20-cM interval were chosen for the NIL survey. All clones used in this experiment were generously supplied by S. D. Tanksley, Cornell University, New York, USA.

DNA extraction, restriction digests, electrophoresis, and Southern analysis

Rice DNA was prepaired from fresh-frozen leaf tissues according to McCouch et al. (1988). The DNA of the pair of NILs was digested with six restriction enzymes (*Eco*RI, *Eco*RV, *Dra*I, *Xba*I, *Hin*dIII, *Msp*I). For B_5F_2 segregation analysis, only *Msp*I was used. Electrophoresis and Southern analysis were conducted according to McCouch et al. (1988).

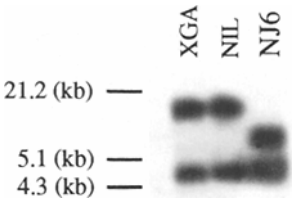

Fig. 1 Autoradiograph showing the hybridized restriction fragments of the DNA clone RZ182 with *Msp*I digests of the semidwarfing gene donor XGA (*left*), recurrent parent NJ6 (*right*), and one resulting NIL (*middle*). A total of 120 rice clones was used for detecting RFLPs in the NIL survey.

Results and discussion

Identification of RFLP markers putatively associated with *sdg*

According to Liang et al. (1993), *sdg* is located on chromosome 5. Of the 14 clones mapped on chromosome 5 chosen to detect polymorphism between NILs, only one, RZ182, exhibited different restriction fragment patterns. The 106 clones mapped on other chromosomes were also used for the NIL survey; approximately 14% showed polymorphisms between the donor and recurrent parents. However, no polymorphism was found between the NILs. Figure 1 shows the semidwarf NIL containing the same allele as the donor parent XGA, when probed with RZ182 (DNA was digested with *Msp*I). The size of the hybridized restriction fragments was identical in both the donor and the semidwarf NIL, but different from that in the tall NIL, NJ6. Thus, the chromosome segment represented by RZ182 in the semidwarf NIL is inherited from the donor rather than from the recurrent parent. RZ182 was considered a potential positive marker associated with *sdg*. Other clones which exhibited identical patterns between the NILs are more likely to represent the allele inherited from the recurrent parent. Therefore, they are not likely to be near the *sdg* gene.

Verification of linkage and estimation of map distance between the positive clone and the *sdg* gene

Confirmation of linkage between the RFLP marker and the semidwarf genotype was obtained through the use of the segregating B_5F_2 population. From the F_2 population 70 individuals were scored. The cosegregation of the scored semidwarf genotype with the restriction fragments was monitored after hybridizing with the putative clone, RZ182. If the donor-derived fragments cosegregated with the semidwarf genotype, and the NJ6-derived fragments with the tallness genotypes (including homozygous and heterozygous individuals), linkage would be confirmed. Figure 2 shows the cosegregation of the restriction frag-

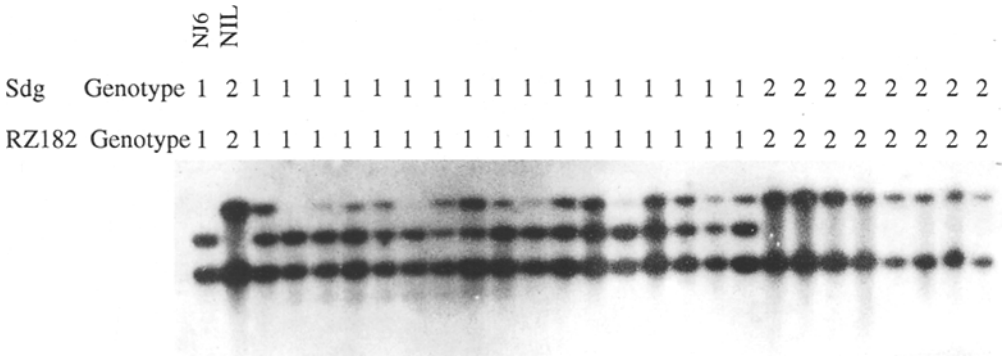

Fig. 2 Cosegregation of the DNA clone RZ182 and the *sdg* gene in the B_5F_2 population segregating for semidwarfism. *Msp*I was used for DNA digesting. No recombinant individual between the RZ182 locus and the *sdg* gene is shown. Genotypes: *1* NJ6/NJ6 or NJ6/XGA, *2* XGA/XGA

ments and the gene. Of the 70 individuals tested in the F_2 generation, 3 were found to be crossovers. The results from this genetic analysis shows that the *sdg* gene is linked to RZ182 on chromosome 5, with a distance of 4.3 centiMorgans between them.

Conclusions

We report here that a single-copy clone, RZ182, on chromosome 5 is closely linked to a semidwarfing gene (*sdg*). We further show that *sdg* is not allelic to the major semidwarfing gene *sd1*, which has been mapped on chromosome 1 by both traditional linkage analysis (Suh and Heu. 1978) and RFLP tagging (Yu 1991). The *sdg* gene will provide a new semidwarfism source for rice breeding. The RFLP maker linked to it can be used to facilitate early selection for the presence or absence of semidwarfism; this may be useful for the rapid incorporation of the semidwarf character into a breeding line. Work is underway to find markers more closely linked to *sdg*. Tightly linked markers can be used to isolate very large DNA fragments containing the gene. Since the semidwarfism caused by *sdg*, unlike that caused by *sd1*, is not sensitive to gibberellic acid (M.H. Gu, unpublished data), the isolation of this gene may lead to a better understanding of the genetic basis of semidwarfism.

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