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RFLP tagging of a gene for aroma in rice

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Summary. We report here the identification of a DNA marker closely linked to a gene for aroma in rice. The DNA marker was identified by testing 126 mapped rice genomic, cDNA, and oat cDNA, clones as hybridization probes against Southern blots, consisting of DNA from a pair of nearly isogenic lines (NILs) with or without the aroma gene. Chromosomal segments introgressed from the donor genome were distinguished by RFLPs between the NILs. Linkage association of the clone with the gene was verified using an F_3 segregating for aroma. Cosegregation of the scented phenotype and donor-derived allele indicated the presence of linkage between the DNA marker and the gene. RFLP analysis showed that the gene is linked to a single-copy DNA clone, *RG28*, on chromosome 8, at a distance of 4.5 cM. The availability of a linked DNA marker may facilitate early selection for the aroma gene in rice breeding programs.

Key words: Aroma – Rice (*Oryza sativa*) – RFLP markers – Grain quality

Introduction

Aromatic or scented rice (*Oryza sativa* L.) is highly valued in many areas of the world, and recently greater emphasis is being placed on the development of high-yielding scented cultivars (Tripathi and Rao 1979). Various techniques have been developed to evaluate and detect scent in order to study the inheritance of scent in rice. These techniques included chewing a half of a single seed (Berner and Hoff 1986) or a few seeds (Dhulappanavar 1976) from individual plants, heating leaf tissue in water and noting the aroma (Nagaraju et al. 1975), and eluting aroma from leaf tissue with dilute KOH (Sood et al. 1978). While these methods are useful, they are not error free and cannot unambiguously determine the genotype of a plant with respect to aroma. Inheritance of scent has been worked on by several investigators, and Jodon (1944) proposed a single gene (*Fgr*) control. However, data supporting these claims is equivocal since segregation ratios of 3:1 (scented:nonscented) (Kadam et al. 1938; Jodon 1944), 1:3 (Ghose et al. 1952; Sood et al. 1978; Berner and Hoff 1986), 9:7 (Tripathi et al. 1979), and 15:1 (Dhulappanavar 1976) have all been reported in F_2 populations from scented × nonscented cultivar crosses. Much of the conflicting information on the inheritance of scent may have arisen because of a failure to consider the endosperm nature of scent in rice seeds (Berner and Hoff 1986) and the unreliable and cumbersome methods for scent determination (Sood et al. 1978). Until now information on the linkage of genes for aroma with other genes has been very limited (Misro et al. 1966).

The recent development of restriction fragment length polymorphism (RFLP) techniques offers a new tool to monitor gene transfer in breeding programs and potentially to clone the genes whose products are not currently known (Tanksley et al. 1989). Nearly isogenic lines (NILs) have been used to rapidly identify regions of chromosomes where genes of interest are likely to reside (Young et al. 1988; Yu et al. 1991). Pairs of NILs are developed by introgression of genes of interest from a donor parent into a recurrent parent via backcrossing. Pairs of NILs, therefore, consist of the recurrent parent without the target gene and its counterpart with the target gene. These lines are purported to be genetically identical except for the presence or absence of the target gene, plus a small region of flanking DNA inherited

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along with the gene from the donor. If the genomes of the donor parent and the recurrent parent are divergent, it is possible to detect polymorphism between the pair of NILs. Markers that detect such polymorphisms will most likely be linked to the target gene.

With a recently constructed rice RFLP map (McCouch et al. 1988; Causse et al. in preparation) and a set of rice NILs developed at Beaumont, Texas, we conducted experiments to identify RFLP markers that are linked to gene(s) controlling the production of aroma in rice.

Materials and methods

Plant varieties and segregation analysis

A pair of rice NILs were used in this study: Lemont and Aromatic Lemont. Aromatic Lemont derives its scent allele from Della and has undergone five generations of backcrossing to Lemont, followed by a selfing generation. Since scent was believed to be due to a single recessive gene in Della (Berner and Hoff 1986), the scent gene was designated as *fgr* in the present study. One F_3 population, segregating for aroma and derived from the cross B8462T3-710 × Aromatic Lemont, was used for linkage mapping. Linkage analysis was performed with the program MAPMAKER (Lander et al. 1987).

Scent evaluation

Scent was evaluated according to the method of Berner and Hoff (1986). Leaf tissue and seed samples from plants of each F_3 family were placed in 0.3 mol L^{-1} KOH solution for aroma evaluation. In this way, homozygous non-scented F_3 families were identified. To determine whether the remaining F_3 families are fixed or segregating for aroma, individual F_2 seeds of those families were chewed and also, leaf tissue samples from each F_3 plant of those families were placed in 0.3 mol L^{-1} KOH solution for scent evaluation.

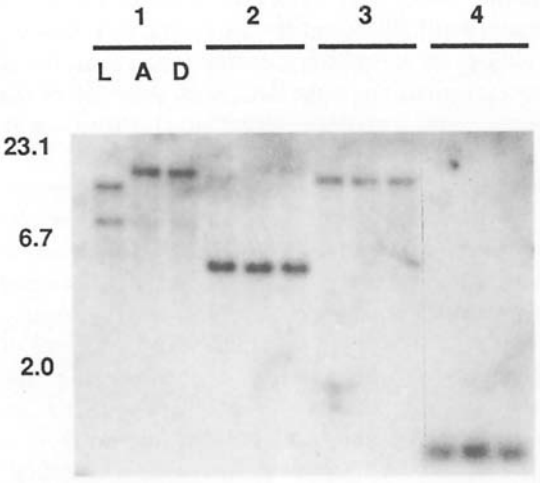

Fig. 1. Autoradiograph showing hybridization pattern of *RG28* in Della (*D*), Aromatic Lemont (*A*), and Lemont (*L*) DNA digested with the following restriction enzymes: *Bgl*II (*1*), *Bam*HI (*2*), *Eco*RI (*3*), and *Pst*I (*4*). Left-hand margin indicates molecular weight in kb. A total of 126 clones were tested for the presence of RFLPs between a pair of NIL

Screening of rice clones

An RFLP map of the rice genome constructed by McCouch et al. (1988) has been augmented with additional DNA markers to a total of more than 500 loci (Causse et al. in preparation). According to estimates of Hanson (1959), the average length of introgressed segments at B_5F_2 is at least 18 cM long. Therefore, 126 mapped clones, distributed on the map at approximately 20 cM interval, were chosen for the NIL survey.

DNA extraction, restriction digests, electrophoresis, and Southern analysis

Plant DNA was prepared from fresh-frozen leaf tissues according to McCouch et al. (1988). Total genomic DNA was digested with nine restriction enzymes (*Eco*RI, *Eco*RV, *Dra*I, *Xba*I, *Hin*dIII, *Sca*I, *Bgl*II, *Bam*HI, *Pst*I). For the F_3 verification filters, only those enzymes giving positive results were used. Electrophoresis and Southern analysis were according to McCouch et al. (1988).

Results and discussion

Identification of clones putatively associated with the scent gene

The percentage of clones detecting polymorphism between Lemont and Della was approximately 11.1% (data not shown). This rate is low compared to the 58% observed between IR34583 and Bulu Dalam (indica and javanica respectively, McCouch et al. 1988) or the 32% observed between Tetep and CO39 (both indica, Yu et al. 1991). The lower level of observed polymorphism is probably attributable to the fact that Della and Lemont are both japonica rice varieties and the polymorphism rate within rice subspecies (i.e., japonica or indica types) is generally lower than that between subspecies (Wang and Tanksley 1989). The majority of the polymorphic clones produced identical restriction fragment patterns with DNA from the pair of NILs. At such loci, the scented isogenic line inherits its allele from the recurrent parent rather than from the donor. Thus, the scent gene is not likely to be near these markers. However, two clones, *RG28* and *RG211*, exhibited different patterns between the NILs. The size of hybridizing restriction fragments was identical in both the donor and the scented isogenic line, but different from the non-scented isogenic line, Lemont. Figure 1 shows the scented NIL containing the same allele as the donor parent, Della, when probed with *RG28* (only with *Bgl*II). These two clones were considered potential positive markers associated with the scent gene.

Verification of the putative positive clones

Cosegregation of the two clones with the trait of interest was verified on populations segregating for scent. Confirmation of linkage between the RFLP marker and the scented genotype was obtained through the use of F_3

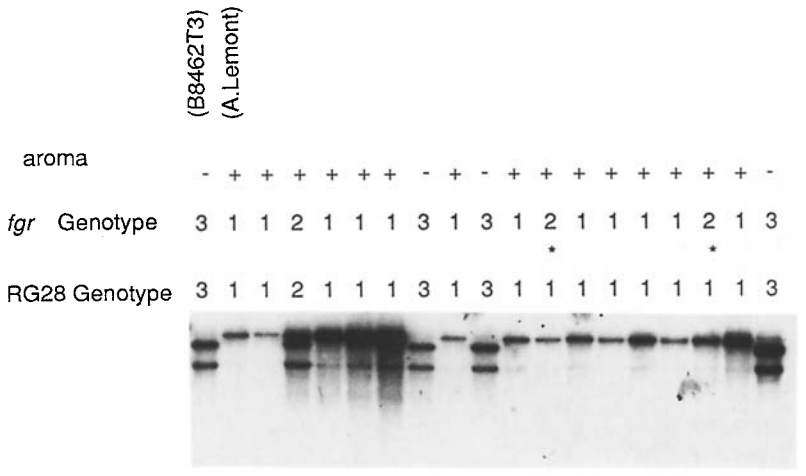

Fig. 2. Cosegregation of the *fgr* gene and the DNA clone *RG28* in the F_3 family derived from the B8462T3-710 × Aromatic Lemont (only 18 lines are shown). *Bgl*II-digested DNA was blotted onto the filter. Recombinant individuals are denoted by an *asterisk*. Genotypes: *1*, Aromatic Lemont/Aromatic Lemont; *2*, Aromatic Lemont/B8462T3-710; *3*, B8462T3-710/B8462T3-710. +, scented; −, non-scented

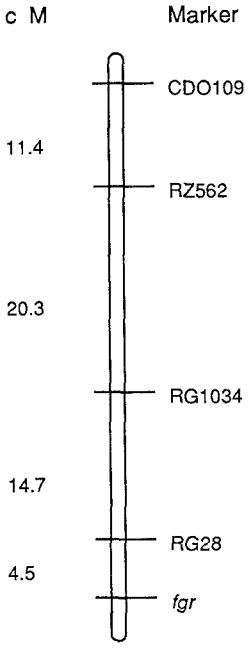

Fig. 3. RFLP map of *chromosome 8* of rice showing the location of the *fgr* and linked markers. A map of *chromosome 8* was developed by segregation analysis of RFLPs of rice genomic (*RG*), cDNA (*RZ*), and oat cDNA (*CDO*) clones based on 91 F_3 lines derived from the cross B8462T3-710 × Aromatic Lemont. All distances are given as cM. *RG1034*, *CDO109*, and *RZ562* were not polymorphic in the NIL survey, but were polymorphic between the mapping parents

families, derived from a single F_2 population. Ninety-one F_3 families were scored at Beaumont, Texas, for scent and remnant seeds from the same F_3 were grown at Cornell for fresh-tissue harvest. A segregation of the scored genotypes for scent and the restriction fragment was monitored after hybridization with the putative positive clones. If the donor-derived allele cosegregated with the scented genotype, and the Lemont-allele with the non-scented genotype, linkage would be confirmed. The map distance between the marker and the gene was estimated from the number of crossovers in the F_2 generation. *RG211* on *chromosome 11* proved to be a false positive upon testing of the F_3 families, as the donor-derived allele and the scented phenotype segregated independently (data not shown). This reflects a residual donor chromosomal segment, still present in the isoline, which is unrelated to the scent gene. Identification of such a marker can be used to purify the NIL by removing those introgressed DNA segments during further backcrossing. On the other hand, RFLP analysis has confirmed that *fgr* is closely linked to *RG28* on *chromosome 8*. It was possible to determine all three genotypes in the lines of an F_2 family, since heterozygotes could be identified by segregation for scent within a line (Fig. 2), and these data demonstrate recessivee behavior for the scented allele from Della. This marker can, therefore, be used to identify the heterozygous or homozygous condition at the scent locus in the F_2 generation of a cross without F_3 progeny testing. Seven crossovers (all single crossovers) were found in the F_2 generation among 91 F_3 lines (26 non-scented: 43 segregating: 22 scented). Figure 3 shows an RFLP map of rice *chromosome 8*, with the *fgr* gene and *RG28* linked at a distance of 4.5 centiMorgans. Although three clones (*CDO109, RZ562,* and *RG1034*) were not polymorphic in the NIL survey, they were polymorphic between the mapping parent, Aromatic Lemont, and B8462T3.

Conclusions

We report here that a single-copy clone, *RG28*, on chromosome 8 is closely linked to the scent gene (*fgr*). This marker can be used to facilitate early selection for the presence or absence of scent, and to identify the heterozygous or homozygous condition at the locus in a reliable manner; it may also be useful for the rapid incor-

poration of the scent character into breeding lines. Work is underway to find markers more closely linked to the scent gene. More linked markers may be achieved with additional polymorphic DNA clones and additional restriction enzymes. Tightly linked RFLP markers can be used to begin chromosome walking or to isolate very large DNA fragments containing the gene. Having mapped *fgr* to *chromosome 8* will allow a point of comparison for other studies of aroma inheritance and may lead to a better understanding of the genetic basis of polymorphism for aroma in rice varieties throughout the world.

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