RAPD TAGGING OF A SALT TOLERANCE GENE IN RICE

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Abstract

Salinity, which is critical in determining the growth and development of plants, is a major problem affecting ever-increasing areas throughout the world. A salt tolerant rice mutant (M-20) was obtained from accession 77-170 (*Oryza sativa*) through EMS mutagenesis and selection *in vitro* [1, 4]. The use of 220 10-mer RAPD primers allowed the identification of a new molecular marker, whose genetic distance from a salt tolerance gene is about 16.4 cM.

1. INTRODUCTION

Salinity is a major problem affecting ever-increasing areas throughout the world. s. Research on plant osmoregulation through molecular biology is accompanied by crop breeding programmes including marker-assisted selection and genetic engineering. It is currently an area of active investigation seeking salt tolerance-relevant genes and their linked molecular marker(s). A salt tolerant rice mutant (M-20) was obtained from rice accession 77-170 (*Oryza sativa*) through EMS mutagenesis and selection *in vitro* [1, 4]. Inheritance analysis demonstrated that a major salt tolerance gene was present in these materials, and that it was associated with a single copy RFLP probe, RG4. The genetic distance between the trait and the marker was shown to be 7.0 +/- 2.9cM [2, 3, 4]. The use of 220 10-mer RAPD primers allowed the identification of a new molecular marker, whose genetic distance from a salt tolerance gene is about 16.4 cM.

2. MATERIALS AND METHODS

2.1. Plant materials

Anthers of rice 77-170 (*Oryza sativa*) were used to obtain calli on N_6 medium, which were then mutagenized with EMS. The selection of NaCl-tolerant mutants was carried out on N_6 medium containing 1% NaCl. After continuous selection, five mutant lines were obtained, the salt tolerance of which has been stably inherited over nine generations [1].

2.2. Evaluation of salt tolerance

The F_1 hybrids of 77-170 X M-20 were planted under normal conditions. Since it is possible to split rice into two parts in the seedling stage, one part of each F_2 plant was planted under normal conditions and the other part was planted in the saline pool containing 0.5% NaCl.

2.3. Rice DNA extraction

Three to five grams of rice leaf tissue were ground into a fine powder in liquid nitrogen, to which was added 16 ml of a 65°C preheated extraction solution (100 ml Tris-HCl, pH8.0; 50 mM EDTA; 500 mM NaCl; 1.25% SDS(w/v), add 0.38g Na_2SO_4 per 100 ml just before use), mixed well by inverting the tube and incubated at 65°C for 20 minutes. To this was added 5 ml of 5M KAc, followed by gentle mixing and incubation on ice for 20 minutes.

This was centrifuged and the supernatant transferred to a new tube to which an equal volume of chloroform: isoamyl (24:1) was added to remove protein and pigment. To this, 2/3 volume of isopropanol was added to the upper layer to precipitate DNA. RNase was added to dissolve the RNA. The DNA was resuspended in TE buffer (50 mM Tris-HCl, pH8.0; 10 mM EDTA).

2.4. Construction of DNA pools

Pure resistant and pure sensitive individuals of the F_2 generation were selected to construct two DNA pools, a salt tolerant pool (+) and a salt sensitive pool (-). The "+" pool was composed of 10 highly salt tolerant individuals A31, A34, A44, A57, A60, A64, A84, A89, A106 and A107, while the "-" pool contained 7 salt sensitive individuals A26, A38, A43, A46, A82, A88 and A93.

2.5. RAPD primers

All of the 10-mer RAPD primers were purchased from OPERON TECHNOLOGY INC.

2.6. RAPD analysis

The reaction mixture (25microl/tube) included template DNA (30ng), 1 microl. 2.5 mM dNTPs each, 2 microl. 10-mer RAPD primer (50 pmol), 2.5 microl. 10X reaction buffer (500 mM Tris-HCl, pH 8.3: 500 mM KCl; 15 mM $MgCl_2$; 0.01% Gelatin), 0.9 unit of Taq DNA polymerase (Institute for Genetics laboratory). The mixture was overlaid with 25 microl. of paraffin oil.

Amplification was performed on a Perkin Elmer DNA Thermal Cycler 480. After initial denaturation (five cycles for 1 min at 94°C, 1 min at 36°C, 2 min at 72°C) the reaction was then continued for another 40 cycles with the following procedure: 94°C for 20 s, 36°C for 1 min, and 72°C for 2 min. Finally, the procedure ended at 72°C for 10 min. Amplification products were electrophoresed in 1.5% agarose gel (containing 1microg./ml EB). The results were observed under UV light and recorded by photography.

3. RESULTS

Backcrosses of the 11th generation of salt tolerant mutant line and the original F_1 generation line were planted under normal conditions in the field. Individuals selected from the F_2 generation (100) were split into two parts; one planted under normal conditions and the other in a saline pool containing 0.5% NaCl. There was no obvious segregation among different individuals of the F_2 under normal conditions. In the saline pool, 72 and 78 individuals set seed, when M-20 and 77-170 were the respective maternal plants. The segregation ratio is about 3:1 (Table I). The same results were obtained from reciprocal crosses indicating that the mutation is controlled by nuclear genes and there exists a major gene enabling seed setting under saline conditions.

The two DNA pools were used as templates to perform RAPD amplifications. Three primers, OPS-12, OPS-04, and OPO-09, showed polymorphism between the "+" and the "-" pools. The base sequences of these primers are listed in Table II. Figure 1 shows some results of the RAPD screening.

TABLE I. NACL-TOLERANT SEGREGATIONS IN F_2 POPULATIONS OF M-20 X 77-170 AND GOODNESS OF FIT TO MONOHYBRID RATIO

Generation	Crosses	No. of seed-setting plants		No. of plants not setting seed		P(1 df)	No. of plants
		Saline	Normal	Saline	Normal		
R_{11}	M-20 x170	72	100	28	0	~0.50	100
	170xM-20	78	100	22	0	~0.50	100

TABLE II. SEQUENCES OF RAPD PRIMERS THAT DISPLAY DIFFERENTIAL PATTERNS

RAPD primers	Sequences
OPS-12	CTGGGTGAGT
OPS-04	CACCCCCTTG
OPS-09	TCCCACGCAA

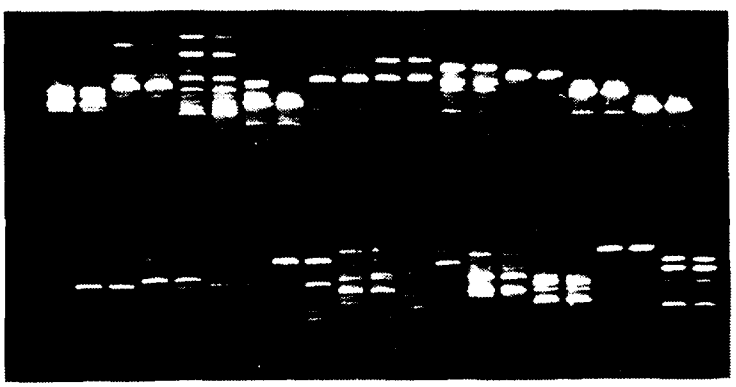

Fig. 1. Comparison of RAPD results from screening the "+" and "-" pools (the lanes alternate "+" and "-" pool; a different primer is used every two lanes).

Analysis of 80 individuals of the F_2 generation by use of the RAPD primers mentioned above. A 1 kb band was verified to be linked with the salt tolerance gene and the RFLP probe, RG4. Figure 2 shows this band and some of OPS-12 RAPD analysis in the F_2 generation.

The band shown in the figure is linked with RFLP probe RG4. Except for the two lanes noted, other lanes are all F2 individuals.

Fig. 2. The RAPD results of OPS-12 in the F_2 population.

The linkage between the RAPD marker, the RFLP probe RG4 and the locus relevant to salt tolerance was calculated with the program MAPMAKER. The result is shown in Figure 3. The genetic distance between the RAPD marker and RG4 is 5.0 cM, and between the RAPD marker and salt tolerance gene it is 16.4 cM.

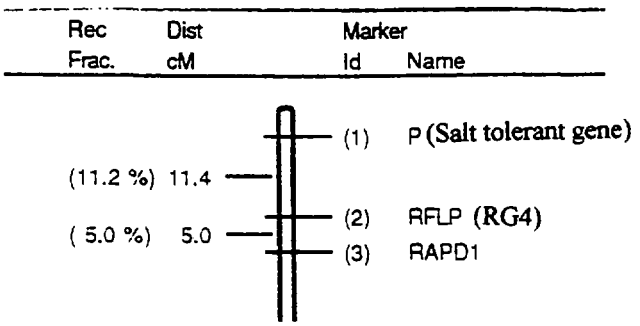

Fig. 3. The linkage between two probes and the locus relevant to salt tolerance.

3. DISCUSSION

Salt tolerance is a character controlled by multiple genes, and it has continuous phenotypic distribution. For this reason, it is regarded as a quantitative character. Despite this, there may exist a major gene responsible for a large part of the genetic variance for salt tolerance. In the F_2 generation of our materials, the ratio of salt tolerant to salt sensitive plants was about 3:1, where salt tolerance was evaluated on the ability to set seeds. This result indicated that salt tolerance is controlled by a major gene and modified by some other minor genes. Zhang et al. [3] tagged the major gene by a single copy probe, RG4, which is located on chromosome 7, at a genetic distance of 7.0 +/- 2.9 cM. We found a new marker near RG4, thus enhancing the possibility of mapping salt tolerance genes.

Although RAPD has some merits such as efficiency, simplicity and saving time, it still has a few shortcomings. Spurious bands often appear so that the amplification must be performed under specific conditions. The template DNA, polymerase concentration and the renaturation temperature should be kept in a suitable range. Moreover, we use individuals of pure character to construct the pools so as to diminish other factors irrelevant to salt tolerance.

We have to identify the phenotype of the F_2 by other criteria because of the nonexistence of an F_3 generation. It brings some uncertainty to our research and it is necessary for us to pay attention to DNA contamination and other sources of error. In the right part of Fig. 2, the pattern of the 6th lane is obviously different from others. It is suspected to be the result of DNA contamination.

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