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Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.)

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Abstract. Genetic variation of nine upland and four lowland rice cultivars (*Oryza sativa* L.) was investigated at the DNA level using the randomly amplified polymorphic DNA (RAPD) method via the polymerase chain reaction (PCR). Forty-two random primers were used to amplify DNA segments and 260 PCR products were obtained. The results of agarose-gel electrophoretic analysis of these PCR products indicated that 208 (80%) were polymorphic. All 42 primers used in this experiment were amplified and typically generated one-to-four major bands. Only two primers showed no polymorphisms. In general, a higher level of polymorphism was found between *japonica* and *indica* subspecies while fewer polymorphisms were found between upland and lowland cultivars within the *indica* subspecies. A dendrogram that shows the genetic distances of 13 rice cultivars was constructed based on their DNA polymorphisms. Classification of rice cultivars based on the results from the RAPD analysis was identical to the previous classification based on isozyme analysis. This study demonstrated that RAPD analysis is a useful tool in determining the genetic relationships among rice cultivars.

Key words: RAPD – Genetic variation – Upland and lowland rice – Rice genetics

Introduction

Drought stress is the major limiting factor in rice production in about 70 million hectares of nonirrigated area (O'Toole and Chang 1979); hence drought resistance is an important objective in rice breeding programs (Chang et al. 1982; O'Toole 1982, 1989). O'Toole and Maguling (1981) screened 2,074 rice entries for drought resistance. They found that upland (dryland adapted) rice from West Africa, Brazil, and South and Southeast Asia have more desirable scores for drought-resistance traits than do lowland (wetland adapted) rice. Therefore, upland rice potentially can be used as genetic material for drought-resistance breeding and for tagging drought-resistant traits using molecular markers. Significant genetic variation for several root characteristics in rice associated with drought avoidance (dehydration avoidance) has been reported by investigators at the International Rice Research Institute (O'Toole and Chang 1979; O'Toole 1982; Chang et al. 1982; Ekanayake et al. 1985, 1986; O'Toole and Bland 1987). However, genetic improvement of root characteristics, such as root penetration ability, is difficult using conventional phenotypic selection criteria due to the underground nature of the root system. This difficulty may be overcome by molecular-marker-assisted selection (O'Toole 1989).

The development and application of restriction fragment length polymorphism (RFLP) technology in crop improvement were both reviewed by Tanksley et al. (1989). More recently, Williams et al. (1990) established a DNA polymorphism assay based on PCR amplification of random DNA fragments with single primers of arbitrary nucleotide sequence. This technique has been termed RAPD (randomly amplified polymorphic DNA). Recent studies have indicated that RAPD is a powerful method for genotype identification, population and pedigree analysis, phylogenetic studies, and genetic mapping (Welsh and McClelland 1990; Martin et al. 1991; Paran et al. 1991; Welsh et al.

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1991; Halward et al. 1992; Mazzarella et al. 1992; Reiter et al. 1992; Vierling and Nguyen 1992; Wilde et al. 1992).

In the present study, we have used RAPD markers to detect DNA polymorphisms and genetic diversity among upland and lowland rice genotypes which differ in drought-resistance characteristics. Our long-range objective is to identify diverse parental lines in order to generate segregating populations for tagging drought-resistance loci in rice using molecular markers.

Materials and methods

Plant materials

The 13 rice cultivars used in this experiment (Table 1) were kindly provided by the International Rice Research Institute (IRRI, Los Baños, Philippines). They were grown in a growth chamber under conditions of 28 °C day/ 25 °C night and with an irradiance of 800 μmoles m^{-2} s^{-1} for 12 h day^{-1}.

Primers

Forty-two 10-base (GC-rich) random primers were obtained from Operon Technologies (Alameda, Calif.). The primers were A-3, A-5, A-10, A-7, A-13, A-14, A-15, A-16, A-17, A-18, A-19, A-20, F-3, F-5, F-6, F-7, F-13, F-15, K-1, K-2, K-3, K-4, K-5, K-6, K-7, K-8, K-9, K-10, K-11, K-12, K-13, K-14, K-15, K-16, K-17, K-18, K-19, K-20, P-3, P-5, P-13, and P-20. The sequences of these primers are available from the above company.

Genomic DNA extractions

Rice leaves, at the sixth leaf stage, were collected and frozen in liquid nitrogen. Genomic DNA was extracted and purified according to McCouch et al. (1988).

Polymerase chain reaction

DNA amplification reactions were performed in a volume of 25 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 50 mM each of dATP, dCTP, dGTP and dTTP, 10 ng of a single random primer, 25 ng of genomic DNA and 2 units of Ampli *Taq* DNA polymerase (Perkin Elmer Cetus), topped with 50 μl of sterilized mineral oil. Amplifications were performed in a thermal cycler (Perkin Elmer Cetus) programmed for 45 cycles of 1 min at 94 °C, 1 min at 37 °C and 2 min at 72 °C.

Table 1. Name, International Rice Germplasm Center (IRGC) accession number, hydrological origin and country of origin of rice cultivars used for RAPD analysis

Cultivar	IRGC accession number	Hydrological origin	Country of origin
AZUCENA	328	Upland	Philippines
BPI-76 NS	9790	Lowland	Philippines
CO39	51231	Lowland	India
IAC25	19642	Upland	Brazil
IR20	11355	Lowland	Philippines
IR36	30416	Lowland	Philippines
IRAT13	28508	Upland	Ivory Coast
MGL-2	6218	Upland	India
MOROBEREKAN	12048	Upland	Guinea
OS4	11335	Upland	Nigeria
RIKUTO NORIN 21	7697	Upland	Japan
SALUMPIKIT	5423	Upland	Philippines
63-83	14725	Upland	Ivory Coast

Gel electrophoresis and photography

Aliquots of 10 μl of DNA products from the PCR amplification were loaded in either 1.4% agarose gels or in 5% polyacrylamide gels for electrophoresis in 1 × TBE (89 mM Tris, 89 mM boric acid and 2 mM EDTA). Gels were stained with ethidium bromide and photographed under UV light.

Data analysis

For each individual primer, the PCR products were sequentially designated "a", "b", "c" and so on. Data were scored for computer analysis on the basis of the presence or absence of the amplified products. If a product was present in a genotype it was designated "1"; if absent, it was designated "2". Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to generate similarity coefficients (Jaccard 1908). The similarity coefficients were then used to construct a dendrogram by UPGMA (unweighted pair-group method with arithmetical averages) using a computer program: Numerical Taxonomy and Multivariate Analysis System, Version 1.60 (Rohlf 1990).

Results

Forty-two primers of arbitrary nucleotide sequence were used to amplify DNA segments from the genomic DNA of 13 rice genotypes. For each primer evaluated, one-to-twelve DNA segments were amplified from a given sample of genomic DNA. Typically one-to-four major bands were amplified along with a number of bands of lesser intensity. A total of 260 DNA fragments were amplified and 208 (80%) of these showed polymorphisms. Fig. 1a shows the primer P-5 amplification products which were resolved by agarose-gel electrophoresis and visualized by staining with ethidium bromide. Overall more polymorphisms were generated with the K set of primers. Twelve bands were amplified with primer K-2 and all of them were polymorphic (Fig. 1b). The size of the amplified segments ranged from 0.5 to 4 kb. Polyacrylamide gels were also used to resolve amplification products as an alternate methodology. Fig. 1c shows the amplification products with primer A-19. A very clear banding pattern of amplified segments ranging from 0.3 to 1.5 kb were resolved using 5% polyacrylamide gels and ethidium bromide staining.

Amplified polymorphic DNA fragments were scored as described in Materials and Methods for computer analysis based on Jaccard's (1908) similarity coefficient. Table 2 shows the pair-wise similarity for 13 cultivars. The similarity coefficient ranged from 0.212

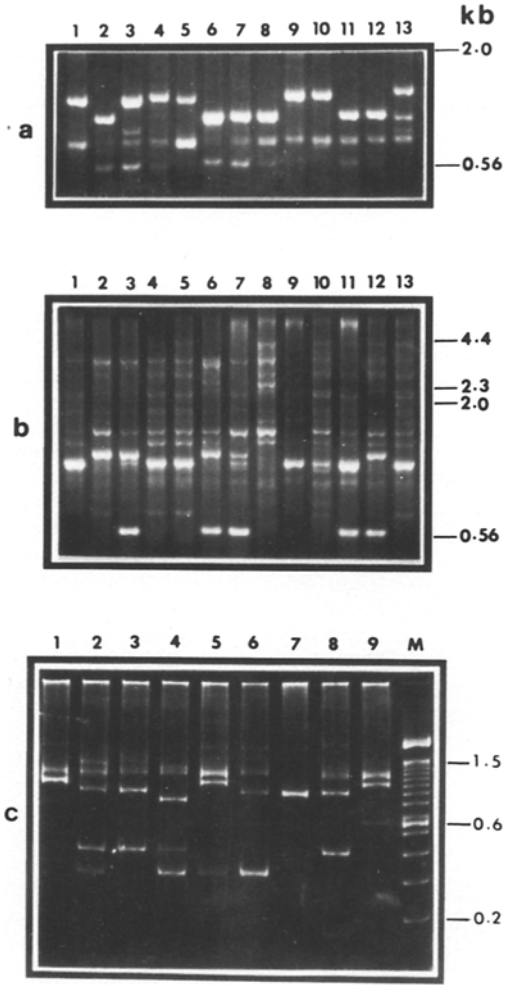

Fig. 1a–c. Polymorphic patterns detected in rice DNA from various cultivars using RAPD: Azucena (*lane 1*), BPI-76 NS (*lane 2*), CO39 (*lane 3*), IAC25 (*lane 4*), IRAT13 (*lane 5*), IR20 (*lane 6*), IR36 (*lane 7*), MGL-2 (*lane 8*), Moroberekan (*lane 9*), OS4 (*lane 10*), Rikuto Norin 21 (*lane 11*), Salumpikit (*lane 12*), 63-83 (*lane 13*), and DNA marker (*M*). Amplification products obtained with primer P-5, resolved by a 1.4% agarose gel (panel **a**); amplification products obtained with primer K-2, resolved by a 1.4% agarose gel (panel **b**); amplification products obtained with primer A-19, resolved by a 5% polyacrylamide gel (panel **c**)

to 0.798. The result of pair-wise comparisons indicated that CO39 was least similar to all other genotypes (less than 0.3 similarity coefficient when compared to all other genotypes except IR36). IAC25 and IRAT13 are highly associated as indicated by the large value of the similarity coefficient (0.798).

Associations among the 13 cultivars revealed by UPGMA cluster analysis are presented in Fig. 2. Upland and lowland cultivars were classified into two main clusters: *japonica* and *indica*. The *japonica* cluster was subdivided into two subclusters: subcluster 1, Azucena, Rikuto Norin 21 and Moroberekan; subcluster 2, IAC25, IRAT13, OS4 and 63-83. The *indica* cluster consisted of two subgroups: subgroup 1, BPI-76 NS and IR36; subgroup 2, IR20, MGL-2, and Salumpikit. CO39, a lowland genotype from India, diverged from other *indica* types. The results indicated that most upland rice cultivars clustered into the *japonica* group (Azucena, Rikuto Norin 21, Moroberekan, IAC25, IRAT13, OS4, and 63-83). The remaining six cultivars clustered into the *indica* group which was identical to that of isozyme analysis.

In general, large numbers of polymorphisms were found between *japonica* and *indica* rice. Moroberekan is a *japonica* upland rice collected in Guinea. Previous studies indicated that Moroberekan has a good capacity for drought avoidance (dehydration avoidance), due to its large root system, and good subsoil hard pan penetration ability. DNA amplification for this genotype showed higher levels of polymorphisms when compared to four *indica* lowland genotypes (BPI-76 NS, CO39, IR20 and IR36). The similarity coefficients between Moroberekan and CO39, IR36, IR20 and BPI-76 NS were 0.233, 0.292, 0.361 and 0.381, respectively. Large numbers of polymorphisms were also found between other upland and lowland genotypes such as OS4 (*japonica*, upland adapted) and CO39, IR20 or IR36; Rikuto Norin 21 (*japonica* and upland) and CO39, IR20 and IR36; IAC25 (*japonica* and upland) and CO39; IRAT13 (*japonica* and upland) and CO39, IR20 and IR36.

Discussion

Among rice cultivars, genetic variation was easily detected using RAPDs. Polymorphisms could be successfully scored and used for UPGMA analysis to construct dendrograms. Our RAPD results with 13 upland and lowland rice cultivars are highly consistent with the isozyme analyses of Glaszmann (1987) at the International Rice Research Institute (IRRI) in the Philippines. The isozyme information for these rice cultivars is available from IRRI's germplasm database. We do not think this is just a coincidence, since the use of the RAPD technique for detecting genetic variation among cultivars and identifying germplasms is well established (Welsh and McClelland 1990; Hu and Quirios 1991; Halward et al. 1992; Vierling and Nguyen 1992; Wilde et al. 1992).

In the present study, the application of agarose gels gave a satisfactory resolution of PCR amplification products. The use of a 1.4% agarose gel could resolve DNA products whose size ranged from 0.5 to 4 kb without any difficulty. Amplified products with molecular weights of less than 500 base pair could not be separated clearly in 1.4% agarose gels. To resolve low-molecular-weight DNA segments, we used 5% polyacrylamide gels and were able to successfully re-

Table 2. Similarity matrix for Jaccard's coefficient: range of values from 0 to 1.0, with values closer to 1.0 indicating increasing similarity[a]

Genotype	AZUCE	BPI-76	CO39	IAC25	IRAT13	IR20	IR36	MGL-2	MORO	OS4	RIKUTO	SALU	63-83
AZUCE	1.000												
BPI-76	0.384	1.000											
CO39	0.285	0.361	1.000										
IAC25	0.618	0.386	0.226	1.000									
IRAT13	0.606	0.350	0.212	0.798	1.000								
IR20	0.272	0.430	0.288	0.455	0.367	1.000							
IR36	0.391	0.415	0.410	0.383	0.345	0.438	1.000						
MGL-2	0.316	0.399	0.270	0.363	0.296	0.450	0.326	1.000					
MORO	0.624	0.381	0.233	0.602	0.602	0.361	0.292	0.323	1.000				
OS4	0.631	0.358	0.226	0.620	0.685	0.358	0.345	0.354	0.639	1.000			
RIKUTO	0.637	0.381	0.293	0.555	0.553	0.352	0.358	0.314	0.606	0.614	1.000		
SALU	0.370	0.400	0.279	0.391	0.338	0.423	0.336	0.423	0.358	0.409	0.426	1.000	
63–83	0.582	0.358	0.242	0.637	0.653	0.314	0.375	0.327	0.552	0.678	0.540	0.384	1.000

[a] The following abbreviations are used for the genotypes as indicated below:
AZUCE = Azucena, BPI-76 = BPI-76 NS, MORO = Moroberekan, RIKUTO = Rikuto Norin 21, SALU = Salumpikit

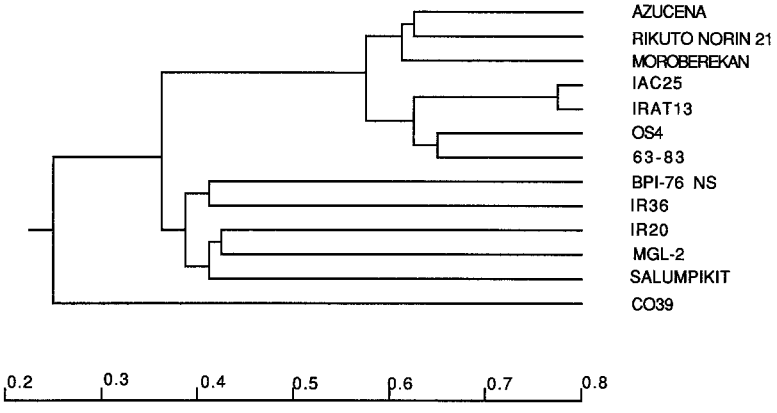

Fig. 2. Dendrogram of rice genotypes, constructed using UPGMA based on Jaccard's similarity coefficients. Scale on bottom is Jaccard's coefficient of similarity

solve DNA amplified products from 0.3 to 1.5 kb with very clear banding patterns (Fig. 1c). Our results suggest that polyacrylamide gels could be used to distinguish low-molecular-weight DNA products which are difficult to resolve in agarose gels.

This study indicated that the use of RAPD techniques to detect genetic variation at the level of DNA among rice cultivars was sensitive and powerful. This will be useful in the future for determining the best choice of parents in order to generate mapping populations for tagging drought-resistance traits. For example, Azucena and Salumpikit are both upland cultivars collected from the Philippines. Either of these genotypes can be used as an upland rice candidate for crossing with one of the lowland cultivars. However, the DNA amplification and UPGMA analyses indicated that Azucena was more distant to the other lowland rice than was Salumpikit. Thus, choosing Azucena as an upland parental line is likely to generate a higher level of polymorphism than would Salumpikit.

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