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Application of RAPD markers for genetic distance analysis of hybrid rice parental lines

P. T. Naghia[1], J. P. S. Malik, M. P. Pandey and N. K. Singh[2]

Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture and Technology, Pantnagar 263 145

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Abstract

The genetic similarities of 19 hybrid rice parental lines were estimated using RAPD markers. Molecular genetic distances derived from dice's similarity coefficients were based on 133 RAPD markers. Cluster dendrograms were generated for molecular genetic distances. Genetic variability among thermo-sensitive genetic male sterile (TGMS) lines was much lower than that among cytoplasmic genetic male sterile (CMS) lines. PCR analysis based on 10 RAPD primers detected sufficient polymorphisms for the germplasm characterisation and genetic distance. The cluster analysis based on these markers revealed close genetic relationship among rice genotypes currently used in the hybrid rice breeding programmes.

Key words: *Oryza sativa*, RAPD, genetic divergence, molecular markers, male sterile line

Introduction

Rice hybrids utilising cytoplasmic male sterility (CMS) system give 15-20% higher yield from the best commercial cultivars. Recently, the thermosensitive genic male sterility (TGMS) system is considered a better option for raising the potential yield of hybrid rice in China [1-2]. An accurate classification of parental lines into heterotic groups is essential to facilitate selection of parents and predicting the performance of F_1 hybrids. Recent studies of genetic distance analysis of rice cultivars have focussed on molecular genetic markers [3-4]. The level of correlation between molecular markers based distances and hybrid performance is dependent on the germplasm used [5].

The intensive hybrid rice breeding programme at G. B. Pant University of Agriculture and Technology, Pantnagar developed several promising parental lines [6-7]. Exploitation of these lines in hybrid breeding required information on nature of association, heterosis of F_1 hybrid and genetic divergence of parents. Therefore, the present study aimed to investigate genetic divergence among hybrid rice parental lines by employed RAPD markers. This will aid the long term objective of predicting the level of heterosis in F_1 hybrids.

Materials and methods

Plant materials:

Nineteen rice genotypes including CMS, TGMS and the restorer lines bred either at the G. B. Pant University of Agriculture and Technology, Pantnagar or at the International Rice Research Institute (IRRI). Philippines were evaluated (Table 1) at Pantnagar during *Kharif* 1997. These genotypes possessed some of the very desirable features required for hybrid breeding programme like complete and stable male sterility (except restorer lines), higher out crossing rate, better grain quality, good plant type and adaptibility.

DNA extraction and RAPD analysis

For each genotype, 1 g of young leaves from seedlings were taken for DNA extraction using the method described by Dellaporta *et al.* [8]. The 25µ l polymerase chain reaction mixture contained 1 ng of template of DNA, 200 µM of each dNTP (Genei, Bangalore, India), 0.2 µM of decamer primer (Operon Technologies, see Table 2), 0.5 U Taq polymerase and 1 X reaction buffer containing 1.5 mM $MgCl_2$ (Genei, Bangalore, India). The thermocycler (Perkin - Elmer model) was operated for one cycle at 94°C for 5 min and then programmed for 45 cycles of 94°C (30 sec.), 35°C (1 min.) and 72°C (2 min.). It was followed by a final amplification step of 5 min. at 72°C. Amplified DNA samples were electrophoresed in polyacrylamide gel with 7.5% acrylamide. RAPDS were scored for presence

[1]Present address: CVV Long Rice Research Institute, Oman, Can Tho, Vietnam
[2]National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi 110 012

Table 1. List of different male sterile and CMS restorer lines of rice evaluated

S. No.	Accession	Parentage origin	Remarks	Origin
1.	IR58025A	IR48483A/8*Pusa167-120//Pusa167-120	CMS (WA)	IRRI
2.	IR62829A	IR46828A/7*IR29744/IR29744	CMS (WA)	IRRI
3.	PMS 2A		CMS (WA)	PAU
4.	IR54755A	IR1529-680/*O. officinalis*//IR1529-680	CMS (ARC)	IRRI
5.	Pant CMS2A	V97A/IET6223	CMS (WA)	PANT
6.	IR68897A	IR62829A/6*IR62856//IR62856	CMS (WC)	IRRI
7.	IR68281A	IR58025A/6*IR54718-C3//IR54718-C3	CMS (WC)	IRRI
8.	IR69617A	IR58025A/8*54718-C2//IR54718-C2	CMS (WC)	IRRI
9.	UPRI92-133	IR8/IR127-2	Restorer	IRRI
10.	IR31802	IR13168-143/IR12340-10//IR9129-209	Restorer	IRRI
11.	UPRI89-20	C681030/IR13429-57	Restorer	PANT
12.	UPRI89-43	IET4141/CR98-7216	Restorer	PANT
13.	UPRI95-140	Spontaneous Mutant	TGMS	PANT
14.	UPRI95-167	UPRI95-140/UPRI95-117	TGMS	PANT
15.	UPRI97-58	UPRI95-140/IR36	TGMS	PANT
16.	UPRI97-59	UPRI95-140/UPRI95-141	TGMS	PANT
17.	UPRI97-60	UPRI95-140/UPRI95-141	TGMS	PANT
18.	UPRI97-61	UPRI95-140/UPRI95-141	TGMS	PANT
19.	UPRI97-62	UPRI-140/UPRI95-141	TGMS	PANT

CMS (WA) = Cytoplasmic male sterile (Wild abortive)
IRRI = International Rice Research Institute
PANT = G. B. Pant University of Agriculture & Technology
PAU = Punjab Agricultural University, Ludhiana
TGMS = Thermo-sensitive genic male sterile

(1) or absence (0) of bands. The combined table of score bands obtained from 19 rice genotypes using 10 decamer primers was used for computing genetic distances [9].

$$GD = 1-[2Nij/N_i+N_j]$$

where, Nij is number of shared bands between two genotypes and N_i and N_j are the total number of bands for genotypes i and j respectively. Genetic distance were, then used to construct a cluster dendrogram by the UPGMA method.

Results and discussion

RAPD analysis

Ten randomly selected decamer primers were used to detect 133 RAPD markers among the 19 parental lines (Table 2). Out of these, 79.7% bands were polymorphic. The number of bands ranged between 4 to 23 with an average of 13.3 per primer. A primer OPDO8 was unique as it could distinguish the maximum of the genotypes tested (Fig. 1). However, in lane 4, no PCRed DNA samples were loaded and therefore, no amplified DNA fragments with primer UPD 08 was seen. This primer OPD08 has also been effectively used for genotype identification in earlier studies [4, 10]. Another primer OPJ13 was found particularly useful in discriminating among the seven TGMS genotypes that were phenotypically similar (Fig. 2). No single primer was found to produce distinct banding pattern for all the genotypes. However, it is interesting to observe that, by using primers OPD08 and OPJ13, all the 19 rice genotypes could be differentiated. Thus, these result shows that RAPD technique being technically simpler, quicker, relatively inexpensive and non-radioactive can detect sufficient polymorphisms for germplasm characterisation and genetic distance studies.

Table 2. Ten decamer primers used and number of RAPD marker detected by them

Primers	Sequence (5' to 3')	Total no. of RAPD markers	Polymorphic marker	
			No.	(%)
OPC 07	GTCCCGAGCA	14	13	92.86
OPC 15	GACGGATCAG	16	08	50.00
OPC 08	GTGTGCCCCA	23	23	100.00
OPJ 08	CATACCGTGG	08	05	062.50
OPJ 13	CCACACTACC	08	08	100.00
OPF 06	GGGAATTCGG	14	09	064.30
OPJ 13	CCACACTACC	17	08	047.06
OPF 14	TGCTGCAGGT	11	11	100.00
OPF 17	AACCCGGGAA	18	17	094.45
OPK 11	AATGCCCCAG	04	04	100.00
	Total	133	106	079.70

Cluster analysis based on RAPD

Analysis of the relationship based on 133 RAPD markers revealed that the genetic distances among 19 genotypes

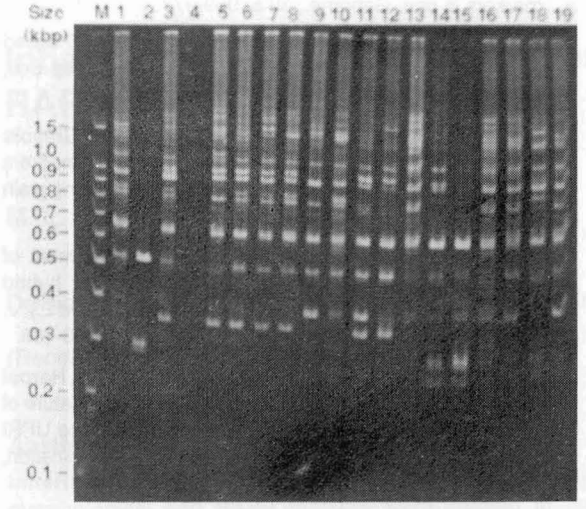

Fig. 1. RAPD profile of 19 hybrid rice parental genotypes obtained with primer OPD08. Lane M : DNA size marker "100 bp DNA ladder": Lane 1 : IR58025A, Lane 2 : IR62829A, Lane 3 : PMS2A, Lane 4 : IR54755A, Lane 5 : Pant CMS2A, Lane 6 : IR68897A, Lane 7 : IR68281A, Lane 8 : IR69617A, Lane 9 : UPRI92-133, Lane 10 : IR31802, Lane 11 : UPRI89-20, Lane 12 : UPRI89-43, Lane 13 : UPRI95-140, Lane 14 : UPRI95-167, Lane 15 : UPRI97-58, Lane 16 : UPRI97-59, Lane 17 : UPRI97-60, Lane 18 : UPRI97-61, Lane 19 : UPRI97-62

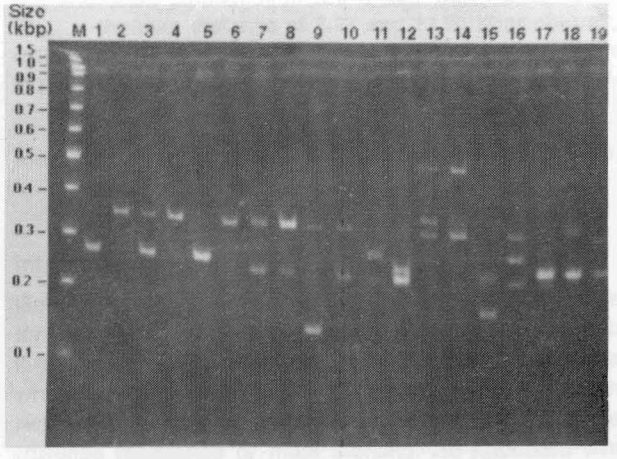

Fig. 2. RAPD profile of 19 hybrid rice parental genotypes obtained with primer OPJ13. Lane M : DNA size marker "100 bp DNA ladder". Lane 1 : IR58025A, Lane 2 : IR62829A, Lane 3 : PMS2A, Lane 4 : IR54755A, Lane 5 : Pant CMS2A, Lane 6 : IR68897A, Lane 7 : IR68281A, Lane 8 : IR69617A, Lane 9 : UPRI92-133, Lane 10 : IR31802, Lane 11 : UPRI89-20, Lane 12 : UPRI89-43, Lane 13 : UPRI95-140, Lane 14 : UPRI95-167, Lane 15 : UPRI97-58, Lane 16 : UPRI97-59, Lane 17 : UPRI97-60, Lane 18 : UPRI97-61, Lane 19 : UPRI97-62

ranged from 0.094 (90.6% similarity) to 0.344 (65% similarity) (data not shown). Genetic divergence among TGMS lines ranged from 0.094 to 0.269, while that

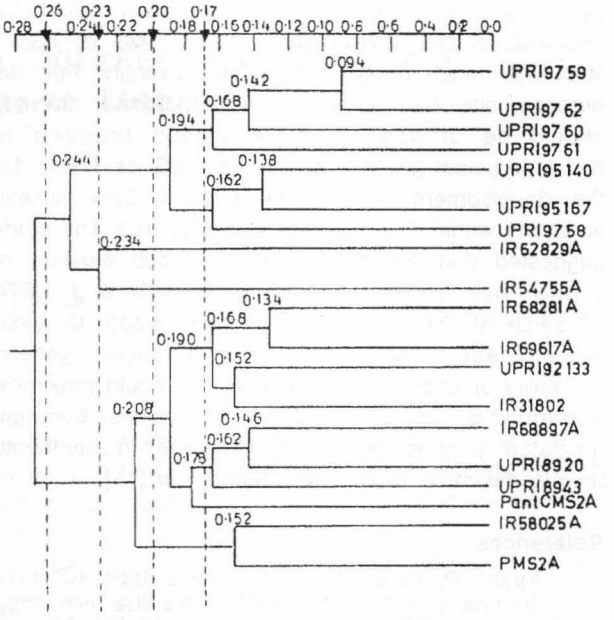

Fig. 3. Dendrogram of genetic distances of 19 hybrid rice parental genotypes constructed from 133 RAPD loci. Scale on the top is genetic distance derived from Dice's Coefficient of similarity

among restorers from 0.143 to 0.117. CMS group showed the most diversity that varied from 0.134 to 0.344. The RAPD cluster pattern is presented in Fig. 3. It showed five clusters at the cut of 0.20 genetic distance level and eight clusters at the cut of 0.17 genetic distance level. All the TGMS lines were grouped into one cluster at 0.20 level, but they were divided into two sub-clusters according to their parentage relationship at 0.17 level. Two CMS lines, IR 68281A and IR 69617A, which were developed through genome substitution of IR 58025A by repeated back crossing to male parents IR 54718-C_3 and IR 54718-C_2 respectively, were closely clustered together with the genetic distance of 0.134. These two CMS lines might be considered as sister lines derived from the same ancestral origin. Four leading CMS lines viz., IR 62829A, IR 54755A, IR 58025A and PMS 2A formed two clusters. The cluster of IR 54755A and IR 62829A was genetically much more closer to TGMS clusters compared to the cluster of IR 58025A and PMS 2A. However, despite being in the same cluster, the genetic similarity between IR 54755A and IR 62829A was only 76.6%. Pant CMS 2A was grouped into the same cluster with seven other genotype at 0.20 level but it was separated into distinct sub-cluster at 0.17 level. It is generally accepted that molecular markers such as RFLP and RAPD represent genetic variation at DNA level, providing more accurate measures of relationship between individuals without the influence of environmental variation.

In this study RAPD marker method has reliably revealed and grouped the genotypes according to their

pedigree relationships. This information could be exploited for the development of new CMS or TGMS lines with much broadened genetic diversity. For this purpose, only such elite lines with molecular diversity should be utilised by recombination breeding to developed new parental stocks of CMS or TGMS for the development of potential hybrids. Low genetic variability within TGMS group was revealed. The study suggested that genotypes from two sub clusters of TGMS viz., UPRI 97-58/UPRI 95-167 and UPRI 97-59/UPRI 97-62 should be hybridized to yield recombinant TGMS lines with enhanced genetic variability among them. This approach could maximize opportunities to obtain superior hybrids because unrelated parents would be expected to contribute unique desirable alleles at different loci [11].

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