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Pedigree evaluation and molecular diversity of some true breeding rice (*Oryza sativa* L.) genotypes of Kerala

T. Vanaja[1]*, G.J. Randhawa[2], and K.P. Mammootty[1]

[1]Pepper Research Station, Kerala Agricultural University, Kanhirankad, P.O., Kannur, Kerala 670 142, India; [2]National Research Centre for DNA Fingerprinting, National Bureau of Plant Genetic Resources, New Delhi 110 01, India.

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Abstract

Genetic diversity analysis and pedigree evaluation were carried out using Random Amplified Polymorphic DNA (RAPD) molecular markers on 20 true breeding and high yielding rice genotypes of Kerala. Results show that the genetic base of rice varieties bred in Kerala is narrowing down, indicating the need to use genetically diverse genotypes in breeding programmes. We also identified markers that can prove the paternity of certain true breeding rice varieties/cultures of Kerala.

Keywords: Genetic diversity, Molecular markers, Random Amplified Polymorphic DNA, Paternity test.

Introduction

Variety identification has attained critical importance worldwide especially in the context of plant variety protection. Due to the proliferation of varieties in all major crop species, however, the number of combinations of morphological and physiological descriptors available to establish the uniqueness of a variety has narrowed down (Song et al., 1999). Hence in recent years, attempts were made to combine genetic markers to demonstrate the distinctness of crop cultivars. The development of polymerase chain reaction (PCR) has allowed the RAPD (Random Amplified Polymorphic DNA) approach for molecular analysis of genomes (Williams et al., 1990). The RAPD technique has several advantages such as sampling of a relatively unbiased portion of the genome, ease of use, lower cost, and use of a small amount of plant materials (Fritsch and Rieseberg, 1996). Pedigree evaluation and paternity testing through RAPD markers has paramount importance to resolve the disputes regarding germplasm identity, parentage, and ownership of varieties. Although several studies were made recently, no such attempt has been made on the rice (*Oryza sativa* L.) cultivars of Kerala. Hence, a study was undertaken to analyze the pedigree and molecular diversity within some of the true breeding rice genotypes of the state.

Materials and Methods

The materials include nine randomly selected true breeding rice genotypes of Kerala and their parents (Table 1). Seeds of the 20 genotypes were germinated and grown under aseptic conditions (*ca*. 30°C in a greenhouse) at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi during 2003. Genomic DNA was extracted from three-week-old seedlings following the protocol of Doyle and Doyle (1990) and quantified using the fluorimetric method (Brunk et al., 1978). Twenty four random decamer primers (primer kits A to Z; Operon Technology Inc., Alameda, California, USA) were used for screening the rice genotypes to identify highly polymorphic well-resolved and specific bands for pedigree evaluation. Polymerase Chain Reaction was performed in a 0.2 ml reaction tube. Each 20 ml reaction mixture consisted of 10 X assay buffer (2.5 ml), 12.5 ng template DNA, 0.2 mM each deoxynucleotide

*Author for correspondence: Phone 04602-227287; E-mail <prskan@sancharnet.in>.

Table 1. Pedigree of the rice varieties used for the study.

Name of varieties	Pedigree
Ptb 10	Pure line selection from 'Thekkan cheera'
'Annapoorna'	T(N)/Ptb 10
Ptb 15	Pure line selection from 'Kavungin poothala'
'Triveni'	'Annapoorna'/Ptb 15
IR 8	Peta x Dee Geo Woogen
Ptb 20	Pure line selection from 'Vadakkan chitteni'
'Karthika'	'Triveni'/IR 1539
'Bhadra'	IR 8/Ptb 20
'Jyothi'	Ptb 10/IR 8
'Kairali'	IR 36/'Jyothi'
'Aiswarya'	'Jyothi'/BR 51- 46-1
'Pavizham'	IR8/'Karivennel'
'Kanchana'	IR36/'Pavizham'
'Aruna'	'Jaya'/Ptb 33
'Remya'	'Jaya'/ Ptb 33
Culture 34	'Karthika'/'Bhadra'
Culture 34 S	'Karthika'/'Bhadra'
IR 36	IR1561-228//48*IR24/*O. nivara*///CR94-13
Ptb 33	Pure line selection from 'Arikkirai'
'Jaya'	TN-1 x T-141

triphosphate (dNTP), 5mM of 10-mer primer, and 1.0 unit *Taq* polymerase. PCR reactions were performed in a Perkin Elmer 9600 Thermal Cycler programmed for 40 cycles of standardized cycling conditions. Amplified DNA products were separated on a 1.6% agarose gel, using 1X TAE buffer stained with ethidium bromide. The amplification products were visualized and photographed under UV light using Polaroid 667 film. The PCR products were scored as present (1) or absent (0) for each primer-genotype combination and used to compute the measures of genetic distance. The data were statistically analysed using NTSYS software (ver. 2.1; Rohlf, 1993). Pair-wise comparisons of samples were used to estimate Jaccard's similarity coefficient (*GS*): *a/(n–d)*, where *a* = number of positive coincidences, *n*= total sample size, and *d*= number of negative coincidences. Genetic distances (*GD*), between pairs of lines were estimated as *GD = 1–GS* and dendrograms generated using Jaccard's similarity coefficients (Unweighted Pair Group Method with arithmetic mean; Sneath and Sokal, 1973).

Results and Discussion

Genetic diversity within some true breeding rice genotypes of Kerala

Twenty-four random primers used in the RAPD analysis of 20 true breeding varieties/cultures amplified 204 different reproducible amplicons (Table 2). The number of bands per primer ranged from 2 (OPU 02) to 15 (OPK 01), with an average of 8.5. The size of the amplified product varied from 0.25 to 6 Kb implying polymorphism among the cultivars. Out of the 204 bands scored, 104 bands (50.2%) were polymorphic and the rest monomorphic. Two largest number of polymorphic bands (11 and 10) were obtained with the primer OPB18 and OPB01 with an average of 5.2.

Similarity indices estimated based on all the 24 primers ranged from 0.74 to 0.95 with an average of 0.84 (Fig. 1). Similarity was generally high suggesting that the level of genetic diversity among the true breeding varieties/cultures is low, signifying a narrow genetic base of the breeding stock used. Indeed parents of most of the varieties/cultures evaluated in this study were previously released varieties and it suggests that future breeding programmes should focus on parents, which are genetically distant.

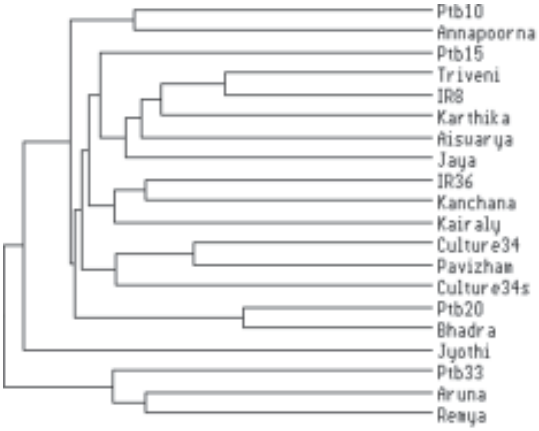

Figure 1. Dendrogram generated on 20 true breeding varieties/ cultures of rice based on 24 RAPD primers.

Table 2. Details of bands produced by 24 random primers with 20 true breeding varieties/cultures.

Name of primer	Nature of bands			Size range of band (bp)
	Monomorphic	Polymorphic	Total bands	
OPA 01	12	-	12	300–2000
OPA 05	5	-	5	350–2500
OPB 01	1	10	11	400–1900
OPB 05	1	5	6	1100–3250
OPB 11	5	-	5	350–2000
OPB 18	2	11	13	750–4000
OPC 07	3	-	3	300–2000
OPC 12	5	1	6	750–1750
OPC 13	11	-	11	300–2000
OPK 01	7	8	15	310–6000
OPK 05	-	4	4	900–1500
OPK 14	3	6	9	575–4500
OPM 06	4	5	9	300–1900
OPM 13	7	2	9	400–1750
OPM 15	5	9	14	250–3500
OPN 02	5	2	7	900–2000
OPN 05	2	9	11	600–2500
OPQ 02	2	7	9	850–2500
OPU 02	2	-	2	450–1500
OPU 11	3	7	10	375–2500
OPU 15	3	9	12	500–3000
OPW 01	4	2	6	375–2500
OPX 03	4	-	4	450–2000
OPY 01	5	7	12	300–2250

bp: base pair.

A comparison of the dendrogram (Fig. 1) further illustrates that varieties 'Remya' and 'Aruna' form a single group along with their male parent Ptb 33. This particular cluster is also distinct from other clusters with a similarity index of 78%. In this major cluster, however, Ptb 33 is sub-clustered separately from its progenies (similarity index: 86%). Previously Bansal et al. (1990) also reported that clustering pattern is influenced by the pedigree of the breeding lines. Nonetheless, Vanaja (1999) based on morphological characters, grouped 'Remya' and 'Aruna' in separate clusters. This lack of agreement between molecular clustering and morphological clustering patterns may be due to the differential genotype-environment interactions. Thus, both morphological and molecular clustering should be considered while grouping genotypes.

Among the 20 true breeding rice genotypes presently evaluated, maximum genetic distance was observed between varieties such as 'Remya' and 'Annapoorna' (0.26), followed by 'Remya' and 'Kairali', and 'Remya' and 'Aiswarya'. These varieties can, therefore, be selected as parents in recombination breeding.

Pedigree evaluation

Out of the 24 primers used for screening, four primers *viz.*, OPB 01, OPB 18, OPY 01 and OPM 15 showed high polymorphism (Figs 2–5), and these were used for pedigree evaluation. Details of the markers revealing the paternity of certain true breeding rice genotypes of Kerala are given in Table 3.

In the DNA fingerprint profile using the random primer OPB 01 (Fig. 2), it is seen that the prominent amplicon of size 900 bp of Culture 34 and Culture 34S is present in the male parent 'Bhadra' and not in the female parent 'Karthika'. Similarly, the prominent amplicon of size 700

Table 3. Markers revealing the paternity of certain true breeding rice genotypes of Kerala.

Name of genotypes	Pedigree	Parent marker	
		Male	Female
'Jyothi'	Ptb 10/IR 8	OPB 01_{500} OPB 18_{1300} OPM 15_{1250}	OPB 01_{900} OPB 18_{900} OPM 15_{1000}
'Triveni'	'Annapoorna'/Ptb 15	OPB 18_{1400} OPY 01_{800} OPM 15_{1250}	OPB 18_{1000} OPY 01_{1300} OPM 15_{1000}
'Aruna' and 'Remya'	'Jaya'/Ptb 33	OPB 01_{1000} OPY 01_{1000}	
'Bhadra'	IR 8/Ptb 20	OPB 18_{1750} OPB 18_{1000}	OPB 18_{1400}
'Kairali'	IR 36/'Jyothi'	OPB 01_{1200} OPB 01_{900} OPB 01_{750}	
'Kanchana'	IR36/'Pavizham'	OPB 01_{1900} OPB 01_{1500} OPB 01_{900} OPB 18_{2000}	
Culture 34 and Culture 34 S	'Karthika' x 'Bhadra'	OPB 01_{900}	OPB 01_{700} OPB 18_{1250} OPB 18_{750} OPY 01_{1300}

Culture 34 S: Segregant from Culture 34.

bp of Culture 34 was present in the female parent 'Karthika', but not in the male parent ('Bhadra'). Prominent bands of 1250 bp length and 750 bp (OPB 18; Fig. 3) and amplicon of size 1300 bp (OPY 01; Fig. 4) of the female parent 'Karthika' were also absent in Cultures 34 and 34S. The genetic difference between these cultures is obvious from the difference in the amplicon intensity of size 2000 bp and the absence of the amplicons of size1000 bp and 2250 bp in Culture 34S, which however, were present in Culture 34. In the DNA profile of the random primer OPM 15 (Fig. 5), the advance generation cultures (Culture 34 and Culture 34S) share a common amplicon of size 1000 bp with their female parent 'Karthika', which is absent in the

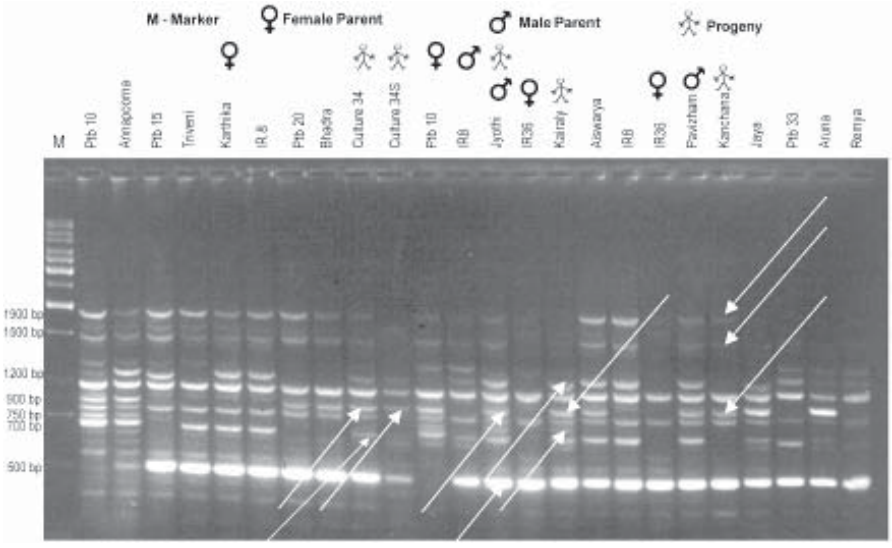

Fig. 2. Pedigree evaluation by random primer OPB 01.

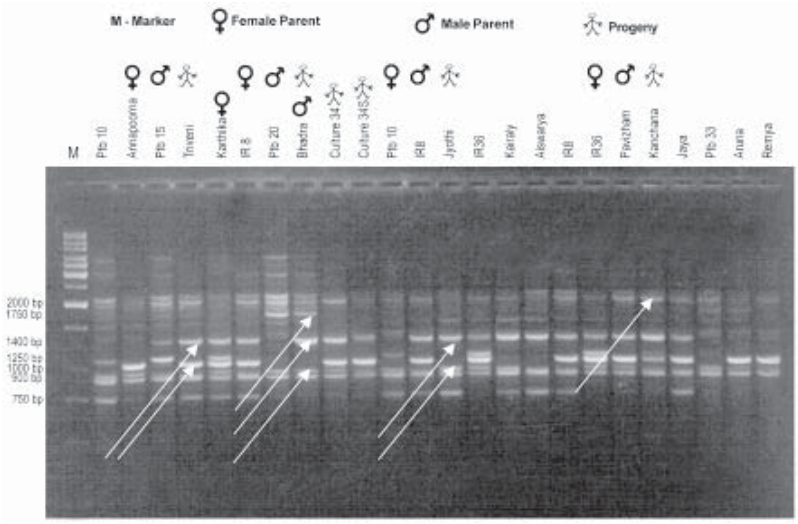

Fig. 3. Pedigree evaluation by random primer OPB 18.

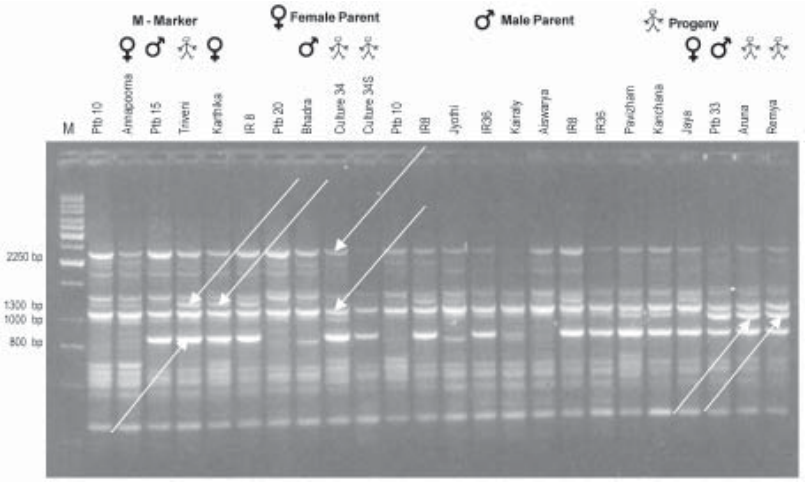

Fig. 4. Pedigree evaluation by random primer OPY 01.

male parent 'Bhadra'. All these molecular evidences confirm the parentage and recombinant nature of Culture 34 and Culture 34S.

We also used the DNA profiles to confirm the parentage of popular rice varieties of Kerala such as 'Jyothi', 'Kanchana', 'Triveni', 'Bhadra', 'Remya', and 'Aurna'. For 'Jyothi', the last prominent amplicon of 500 bp (Fig. 2) and the prominent amplicon of size 1400 bp (Fig. 3) are from its male parent (IR 8), while the amplicon of size 900 bp is derived from its female parent (Ptb 10). Similarly, the amplicons of size 1250 bp and 950 bp present in 'Jyothi' were derived from its male parent IR 8 and the female parent Ptb 10 respectively. For 'Kanchana', the 900, 1500, 1900, and 2000 bp amplicons in the DNA profile were from its male parent 'Pavizham' and these were absent in the female parent IR 36. As regards to 'Triveni', the DNA profiles of the primer OPB 18 (Fig. 3), OPY 01 (Fig. 4), and OPM 15 (Fig. 5) suggest that the amplicons of size 1250 and 1400 bp were from the male parent (Ptb 15) and the amplicons of size 1000 and 1300 bp were from the female parent ('Annapoorna'). The last bright amplicon (800 bp) of 'Triveni' is exclusively from the male parent. As seen from Fig. 3, the two DNA amplicons 1750 bp and 1000 bp present in 'Bhadra' are from its male parent (Ptb 20), which are absent in the female parent (IR8). However, the amplicon of 1400 bp length of 'Bhadra' was from the female parent. Likewise, the amplicons of size 1250 bp and 1750 bp of 'Bhadra' are from its female (IR 8) and male (Ptb 20) parents respectively (Fig. 5). The amplicon of size 1000bp of 'Remya' and 'Aruna' was from their male parent (Ptb 33), which was absent in the female parent 'Jaya' (Fig. 4). Similarly, the amplicons of size 1200 bp, 900bp and 750bp in the lane of 'Kairali' were from the male parent 'Jyothi' (Fig. 2).

In summary, the genetic base of rice varieties of Kerala is narrowing down. Similar deductions were made earlier too based on morphological markers. However, the present results provide a molecular basis for such a conclusion. These results also tell the breeders to use

genetically diverse rice genotypes as parents in future breeding programmes. This study also identified markers that can prove the paternity of certain true breeding rice varieties/ cultures of Kerala, which provide valuable descriptors for patenting these varieties.

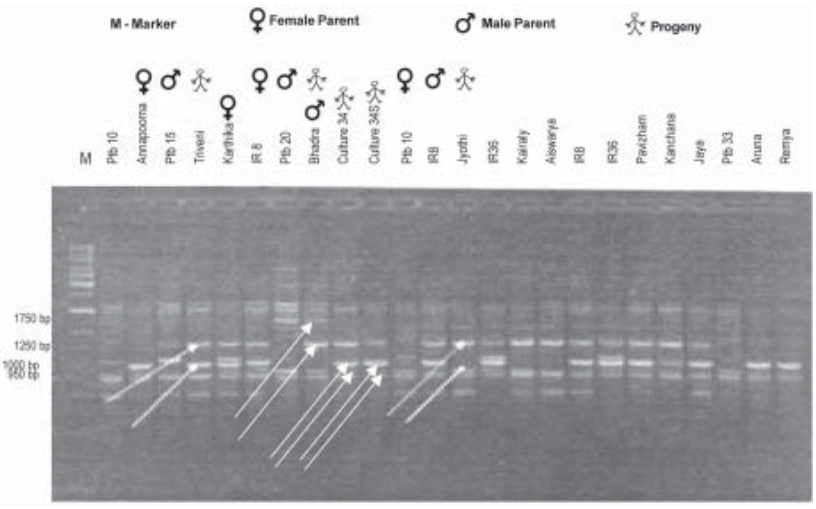

Fig. 5. Pedigree evaluation by random primer OPM 15.

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