Analysis of Molecular Diversity and Differentiation of Red and White Kernel Rice Varieties

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Colour of the rice kernel is an important characteristic in commerce. Rice kernels having white colour are preferred in the national and international markets. The red colour of the kernels is preferred by consumers in some regions of India particularly in the southern state, Kerala. Therefore, development of varieties with specific kernel colour is an important breeding objective. A number of red and white kernel varieties have been bred. No systematic attempt has been made to understand the extent of diversity within and between these two distinct phenotypic classes. In the present study, RAPD markers were employed to analyze the genetic relationship among 20 varieties of each kernel colour classes and to identify marker (s) that can be used to differentiate the red and white kernel types from each other. The genetic diversity analysis among red and white kernel rice varieties using different DNA amplicons grouped 40 varieties into two major clusters. The maximum diversity was observed between two red kernel varieties, *i.e.*, 'Ptb 5' and 'Jyothi' followed by distance between two white kernel varieties 'Rohini' and 'Ptb 14', and two red kernel varieties 'Remanika' and 'Jyothi'. These varieties may be selected as parents for hybridization progrmme. DNA pooling strategy was adopted to identify molecular marker(s) for kernel colour. A total number of 145 random decamer primers were screened to identify highly polymorphic well-resolved specific bands for kernel colour. OPC-12_{1200} was indicated as a putative molecular marker for red kernel colour and OPK-14_{1000} as a putative molecular marker for white kernel colour in rice.

Key Words: Rice, Kernel colour, RAPD marker, Jaccard's similarity index, Cluster analysis

Introduction

As kernel aroma and kernel elongation, colour of the rice kernel is also an important characteristic in commerce. Rice kernels having white colour are preferred in the national and international markets. The red colour of the kernels is preferred by consumers in some regions of India particularly in the southern state, Kerala. Therefore, development of varieties with specific kernel colour is an important plant breeding objective. Even though a number of red and white kernel varieties have been bred, no systematic attempt has been made to understand the extent of diversity within and between these two distinct phenotypic classes. Study of germplasm diversity has significant impact on the improvement of crop plants. It is necessary to investigate the genetic diversity in rice germplasm in order to broaden the genetic variation in future rice breeding. Morphological traits can be used for assessing genetic diversity, but they are very often influenced by the environment. Similarly, isozyme analysis represents only a part of the genome.

The use of molecular markers for the evaluation of the genetic diversity is receiving lot of attention. The development of PCR has allowed the introduction of the RAPD (Random Amplified Polymorphic DNA) approach (Williams *et al*., 1990) to the molecular analysis of the genome. The RAPD technique has several advantages such as a relatively unbiased portion of the genome sampled, simplicity of use, lower cost and the use of a small amount of plant material (Fritsch *et al*., 1996). Chaudhury *et al.* (2001) employed RAPD analysis for identification and classification of aromatic rice genotypes. Phong *et al.* (2001) demonstrated existence of somaclonal variation in rice plants regenerated from callus which survived the desiccation treatment using RAPD method. Xie *et al.* (2000) investigated the genetic diversity of three salinity tolerant rice varieties using RAPD. Identification of a linked marker to a specific trait of interest is imperative in molecular marker aided selection programme. Identification of a molecular marker linked with rice kernel colour will be useful for rice breeders for the selection of desirable progenies from the segregating population at the seedling stage. A study was under taken with an objective to differentiate red and

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white kernel rice varieties and to identify putative RAPD marker(s) associated with kernel colour in rice. In this study, RAPD markers were employed to analyze the genetic relationship among 20 varieties of each kernel colour classes and to identify marker(s) that can be used to differentiate the red and white kernel types from each other.

Materials and Methods

Plant Material

Forty rice varieties were procured from Kerala and Gene bank of NBPGR, New Delhi. The pedigree and characters of the varieties are given in (Table 1).

Twenty varieties were red kernel type and 20 were white kernel type. Seeds of these varieties were germinated and grown under aseptic conditions at about 30°C in green house of NBPGR, New Delhi.

DNA Extraction

Three weeks old seedlings were taken for the isolation of genomic DNA following the protocol of Doyle and Doyle (1990). The DNA was quantified using TKO 100 Fluorometer (Hoefer, San Fransisco, CA).

Preparation of Bulk DNA and RAPD Analysis

DNA pooling can be an effective strategy for detecting genetic marker differences among groups of individuals with similar genotypes or phenotypes (Michelmore *et al*., 1991). DNA bulks were constituted by mixing equal amount of (5 ng ¼l $^{-1}$) DNA from 10 different varieties each having the same kernel colour. Thus, there were two DNA bulks of white kernel coloured rice and two DNA bulks of red kernel coloured rice. Initially DNA bulks of red kernel colour and white kernel colour were used as target DNAs for RAPD analysis. A total number of 145 random decamer primers (from Operon Technology Inc., Alameda, California, USA) (primer kits A to Z) were screened to identify highly polymorphic well-resolved specific bands for kernel colour. The repeatability of those primers, which showed polymorphism among bulks, was tested. Those primers, which showed repeatability with respect to polymorphic bands in red and white bulks, were used to screen individual rice varieties, which formed the red and white bulks.

PCR Conditions

PCR was performed in a 0.2 ml reaction tube. Each 25 ¼l reaction mixture consisted of 10x assay buffer 2.5 ¼l, 12.5 ng templates DNA, and 0.2 mM each deoxy nucleotide triphosphate (dNTP), 5 ¼M of 10- mer primer and 1.0 unit *Taq* DNA polymerase. PCR reactions were performed in a Perkin Elmer 9600 Thermal Cycler programmed for 40 cycles of standardized cycling conditions. The each cycle consisting of, denaturation 94°C for one minute, primer annealing at 35°C for one minute, primer extension at 72°C for two minutes and final extension at 72°C for 5 minutes. Amplified DNA products were separated on a 1.6% agarose gel, using loading dye, in 1X TAE buffer and stained with ethidium bromide. The amplification products were visualized and photographed under UV light using Polaroid 667 film.

Data Analysis

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 for all pairs or individuals. The data entry was done into a binary data matrix as discrete variables. The statistical analysis was carried out using NTSYS software (version 2.1) (Rohlf, 1993). Pair-wise comparisons of samples were used to estimate Jaccard's similarity coefficients (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. Jaccard's similarity coefficients were used to generate dendogram using Unweighted Pair Group Method with Arithematic Average (UPGMA) (Sneath and Sokal, 1973) and relationships between accessions were depicted in the dendrogram.

Results and Discussion

Level of Genetic Diversity

Thirteen RAPD primers used for DNA profiling of 40 rice varieties, which included 20 red kernel and 20 white kernel varieties, amplified 126 different reproducible bands (Table 2).

Number of bands per primer ranged from 1 (OPU-11 and OPU-9) to 17 (OPK-01), the average bands per primer being 9.07. The size of the amplicon varied 0.25 kb to 5 kb. Out of 126 bands that were scored, 72.2% bands were found to be polymorphic. Maximum numbers of polymorphic bands were detected with the primers OPK-01, OPK-14 and OPU-10. The average number of polymorphic bands per primer was 7.

Based on information generated from thirteen RAPD primers on forty varieties of rice, cluster analysis was performed. The genetic diversity of 40 rice varieties revealed by 13 primers is depicted in dendogram (Fig. 1).

Table 1. Pedigree and specific characters of rice varieties

S.No.	Name of varieties	Kernel colour	Pedigree	Other specific characters
1	Ptb 14	white	Pure line selection from Maskathi	Photo insensitive
2	Ptb 15	white	Pure line selection from Kavunginpoothala	Photo sensitive
3	Ptb 16	white	Pure line selection from Kavunginpoothala	Photo sensitive, moderately resistant to sheath blight
4	Rohini	white	Ptb 10/IR 8	Photo insensitive
5	Aswathy	white	Ptb 10/Dee–Geo-woogen	Photo insensitive
6	Triveni	white	Annapoorna (TN-1 x Ptb10)/Ptb 15	Photo insensitive
7	Suvarnamodan	white	Pure line selection from ARC 11775	Photo insensitive, drought tolerant, moderately resistant to blast
8	Swarnaprabha	white	Bhavani/Triveni	Photo insensitive, moderately resistant to blast, stem borer and drought but, susceptible to sheath blight and bacterial blight
9	Jayathi	white	Triveni/IR 2061	Photo insensitive, moderately resistant to blast and blight
10	Neeraja	white	IR 20/IR 5	Photo insensitive, resistant to blast, sheath rot, neck blast and leaf folder but susceptible to sheath blight
11	Suraksha	white	Sasyasree/CR57-MR1523	Resistant/tolerant to blast and bacterial leaf blight
12	IR 36	white	IR1561-228-1-2/IR 1737//CR94-13	Resistant/tolerant to blast and bacterial leaf blight
13	Pranava	white	Vikram/Benong 3	Resistant/tolerant to blast
14	Akasi	white	IR8/N22	—
15	Ajaya	white	IET 4141/CR98 – 7216	Resistant/tolerant to blast and bacterial leaf blight
16	IR 8	white	Peta/Dee-geo-woo-gen	Resistant/tolerant to blast
17	Vivekdhan 62	white	China 4/BG 367 – 4	Resistant/tolerant to blast and stem borer
18	Triguna	white	Swarnadhan/RP1579 -38	Resistant/tolerant to brown plant hopper and gall midge
19	Kannagi	white	—	—
20	TRY–1	white	BR 153-2B-10-1-3/	—
21	Jyothi	Red	Ptb 10/IR 8	Photo insensitive, moderately resistant to BPH but susceptible to blast and blight.
22	Sabari	Red	Annapoorna (TN-1xPtb10)/IR 8/2	Photo insensitive
23	Bharathy	Red	Ptb 10/IR 8	Photo insensitive, moderately resistant to blast
24	Mattatriveni	Red	Reselection from Triveni	Photo insensitive, moderately resistant to BPH but susceptible to blast and blight.
25	Kairaly	Red	IR 36/Jyothi	Photo insensitive, moderately resistant to blast, sheath blight, gall midge and leaf folder
26	Kanchana	Red	IR 36/Pavizham	Photo insensitive, moderately resistant to blast, brown spot and ungro virus
27	Aathira	Red	BR 51–46–1/Cul.23332–2	Photo insensitive, moderately resistant to blast, sheath blight, BPH and gall midge
28	Aiswarya	Red	Jyothi/BR 51 – 46 -1	Photo insensitive, moderately resistant to blast, sheath blight, BPH and gall midge
29	Bhadra	Red	IR 8/Ptb 20	Photo sensitive, resistant to BPH but susceptible to sheath blight
30	Asha	Red	IR 11–66/Kochuvithu	Photo insensitive, moderately resistant to sheath blight, sheath rot, bacterial blight and BPH
31	Pavizham	Red	IR 8/Karivenel	Photo insensitive, moderately resistant to sheath blight, sheath rot, stack burn and BPH
32	Karthika	Red	Triveni/IR 1539	Photo insensitive, moderately resistant to sheath blight, sheath rot, bacterial blight and BPH
33	Aruna	Red	Jaya/ptb 33	Photo insensitive, moderately resistant to sheath blight, brown spot, bacterial leaf blight, gall midge, stem borer and BPH
34	Makom	Red	ARC 6650/Jaya	Photo insensitive, moderately resistant to sheath blight, sheath rot, brown spot, gall midge, stem borer, BPH and leaf folder
35	Remya	Red	Jaya/ptb 33	Photo insensitive, resistant to BPH, moderately resistant to sheath blight, sheath rot, blast and gall midge
36	Kanakom	Red	IR 1561/Ptb 33	Photo insensitive, highly resistant to BPH, moderately resistant to sheath blight, sheath rot, bacterial leaf blight, blast, stem borer and gall midge
37	Remanika	Red	Mutant of MO1	Photo insensitive, resistant to BPH, moderately resistant to gall midge biotype 5
38	Ptb 5	Red	Pure line selection from Velutharikayama	Photo insensitive, moderately resistant to major pests and diseases
39	Ahalya	Red	Ptb10/TN1/TN 1	Photo insensitive
40	Hraswa	Red	IR 8/T140	Photo insensitive, suited to summer crop with lift irrigation and for areas requiring extra short duration varieties due to water scarcity or flash floods

Table 2. Details of nature of bands produced by 13 random primers with 40 (20 white and 20 red kernel coloured) rice genotypes

S.No	Primer	Nature of bands			Size range of bands(bp)	% Polymorphism
		Monomorphic (number)	Polymorphic (number)	Total bands (number)		
1	OPC-12	4	4	8	320-1750	50
2	OPK-01	4	13	17	350-5000	76
3	OPK-14	2	12	14	350-5000	86
4	OPM-10	4	9	13	375- 2500	69
5	OPM-13	2	11	13	400-1900	85
6	OPM-15	3	8	11	250-2200	73
7	OPN-02	6	0	6	450-1400	0
8	OPN-05	5	5	10	500-2400	50
9	OPQ-02	0	11	11	625-2500	100
10	OPU-09	1	0	1	400	0
11	OPU-10	0	12	12	400-3500	100
12	OPU-11	1	0	1	500	0
13	OPU-15	3	6	9	800-2500	67

Jaccard's similarity coefficient estimated on the basis of all the 13 primers ranged from 0.67 to 0.87 with an average of 0.72. The maximum similarity was observed between the varieties 'Jayathi' and 'Kairaly' and between 'Kannakam' and 'Remya' with 88% similarity index. But they are different in their kernel colour. This indicated that the clustering pattern was generated irrespective of kernel colour. The most genetically distant varieties revealed from genetic distance measurement are 'Ptb 5' and 'Jyothi' (0.43) followed by 'Rohini' and 'Ptb 14' (0.41), and 'Remanika' and 'Jyothi' (0.41). The distant group varieties are of same kernel colour. These divergent varieties can be used as parents for recombination breeding without disturbing kernel colour. At 72% similarity index, the 40 rice varieties were grouped into two major clusters, and the variety 'Suvarnmodan' was out grouped. 'Suvarnamodan' is a drought tolerant high yielding variety with moderate resistance to blast disease.

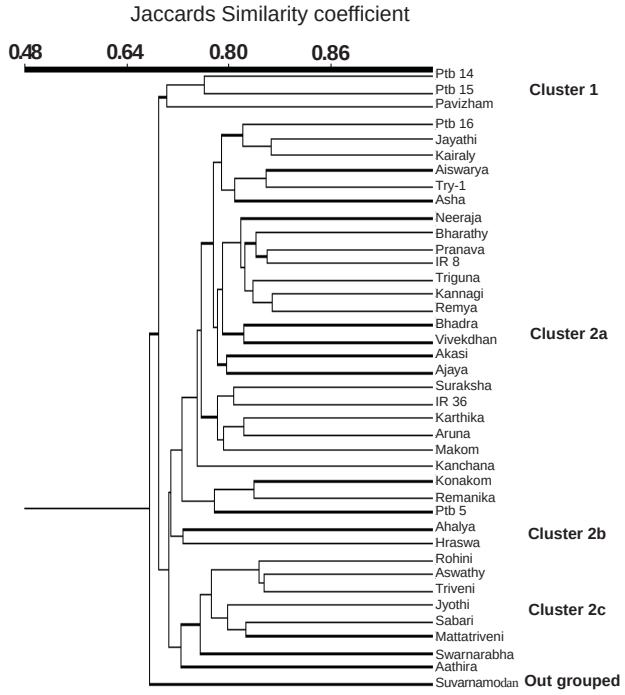

Fig. 1: Dendogram generated for 40 rice genotypes (20 red kernel coloured and 20 white kernel coloured) using RAPD markers

In cluster 1 'Pavizham'–a photo insensitive, short bold grained, high yielding variety, moderately resistant to sheath blight, brown plant hopper, sheath rot and stack burn was grouped with 'Ptb 14' and 'Ptb 15'. Remaining thirty-six varities were grouped together in cluster 2. This cluster can be further subdivided into three sub clusters. In sub cluster 2a twenty six varieties were grouped but grouping was neither based on kernel colour nor pedigree.

In sub cluster 2b, two varieties of red kernel colour namely, 'Hraswa' and 'Ahalya' were grouped together. 'Hraswa' is an extra short duration, high yielding variety suited to summer crop with lift irrigation and for areas requiring extra short duration varieties due to water scarcity or flash floods. 'Ahalya' is a photo insensitive high yielding variety with long bold grain. The variety 'Ptb 10' was one of the parents/ancestral parents for all the eight varieties that were grouped in sub cluster 2c. 'Ptb 10' is a variety from Kerala which is resistant to the pests, brown plant hopper, gall midge and stem borer and have high photosynthetic efficiency. This indicated that some of the varieties were clustered based on pedigree. The diversity analysis indicated that there is much

diversity among the varieties which could not be differentiated based on kernel colour. Several other factors might have played major role in the pattern of clustering.

Differentiation of Red and White Kernel Rice Varieties

Out of 145 RAPD primers used for screening DNA bulks, to identify highly polymorphic well-resolved specific bands for kernel colour, 13 RAPD markers showed repeated consistent difference between DNA bulks of white kernel rice and red kernel rice. When these polymorphic primers were used to analyze individual varieties of red and white bulk, the DNA profile with the primer OPC-12 showed that the amplicon of 1200 bp was totally absent in the 1st set (Fig. 2A) as well as in the 2nd set (10 individual varieties) with white kernel colour varieties (Fig. 2B), but same amplicon ($OPC\text{-}12_{1200}$) was amplified in 60% of individual varieties of red kernel colour in the 1st set (Fig. 2A) and 40% of individual varieties of red kernel colour in the 2nd set (Fig. 2B). Considering both sets together, there was complete absence of this RAPD marker in white kernel coloured rice varieties and presence of this marker in 50% of rice varieties with red kernel colour. The varieties, which showed presence of band for red kernel colour, were 'Jyothi', 'Sabari', 'Bharathi', 'Mattatriveni', 'Kairaly', 'Aiswarya', 'Karthika', 'Remya', 'Remanika' and 'Hraswa'.

The DNA profile with primer OPK-14 showed a bright thick amplicon of 1000 bp in 50% of 1st set 10 individual of varieties with white kernel, which was totally absent in the 1st set of 10 individual varieties of red kernel colour (Fig. 3A). Similar result was observed with the 2nd set in which 30% of white kernel varieties amplified bright thick amplicon of same size which was absent in all red kernel coloured individuals with similar thickness and brightness (Fig. 3B). Considering both sets together, there was complete absence of bright thick amplicon of 1000 bp in 20 red kernel colored rice varieties and present in 40% of rice varieties with white kernel color. The varieties, which showed presence of band for white kernel colour were 'Ptb 14', 'Ptb 15', 'Suvarnamodan', 'Swarnaprabha', 'Neeraja', 'Suraksha', 'Triguna' and 'Kannagi'.

Complete absence of molecular marker $OPC\text{-}12_{1200}$ in all white kernel coloured varieties, and absence of thick bright amplicon of size 1000 bp with the primer OPK-14 in all red kernel coloured varieties indicated that the character kernel colour in rice may not be controlled by a single gene. The variation in red kernel viz., deep red, brownish red and light red (Fig. 4) also emphasized that the character kernel colour in rice might be controlled by more than one gene or alleles. Amplification of separate molecular markers, through RAPD, which is a dominant molecular marker leads to the inference that each red and

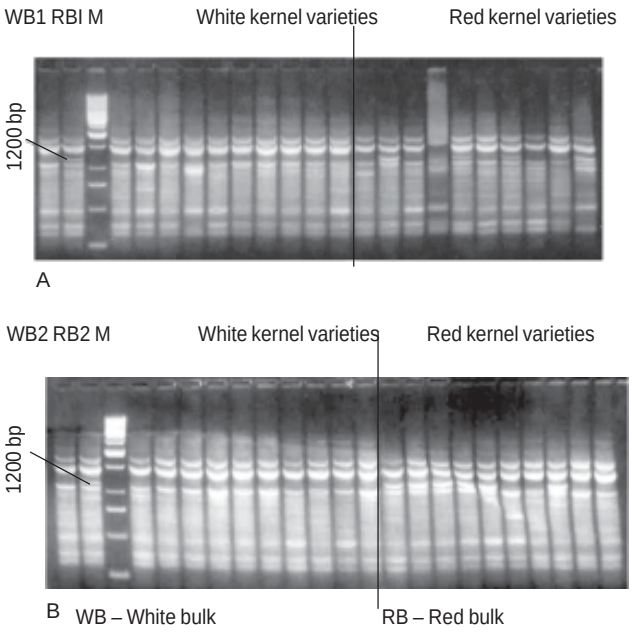

Fig. 2(A&B): Specific marker for red kernel colour revealed by the decamer primer OPC-12

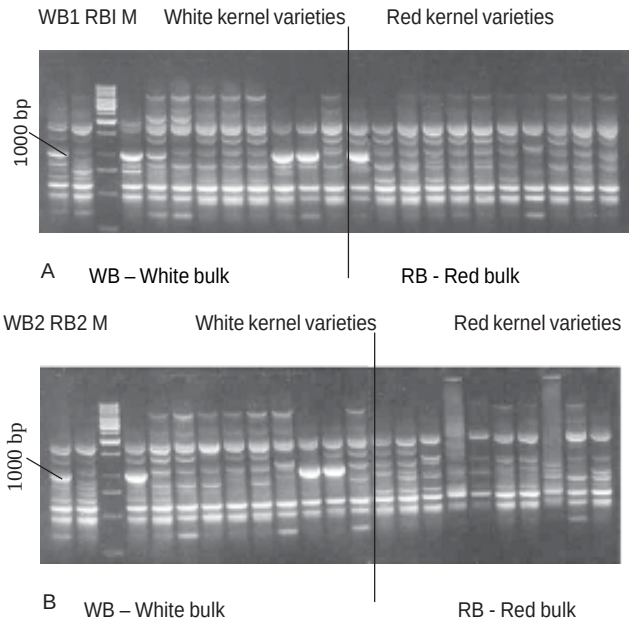

Fig. 3(A&B): Specific marker for white kernel colour revealed by the decamer primer OPK-14

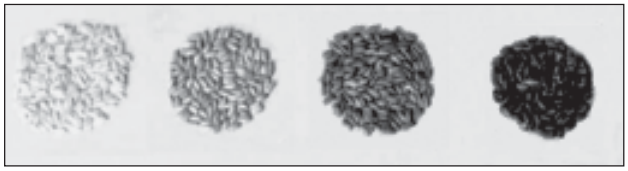

Fig. 4: Colour variation of rice kernel

white kernel colour, which is believed to be dominant and recessive character respectively, was separately controlled by more than one gene. Recessive forms of genes controlling red colour is not the only factor for the production of white kernel colour. The evolutionary process might have changed the recessive form of gene controlling red kernel colour into dominant in behaviour. The present result from molecular studies support the results of Vanaja (1999) based on F_2 segregation analysis for kernel colour in rice.

Segregation pattern of kernel colour in rice in F_2 generations of 19 crosses studied by Vanaja (1999) revealed that one dominant inhibitory gene and another colour gene appeared to be responsible for the inheritance. Gene governing red kernel colour found to exhibit inhibitory epistatic gene action over white kernel colour. Within the red kernel colour there was variation as light red, yellowish red and deep red both in F_2 generations and in back cross generations. The segregation ratio for red colour and white colour suggested that, each red and white colour may be separately controlled by two or more sets of major genes and there may be action of minor genes also. Among the dihybrid modified mendelian ratios, the phenotypic ratio 13:3 was found to fit in majority of the segregating populations, which suggested predominance of inhibitory type gene interaction.

Present investigation revealed lack of correspondence between molecular based RAPD markers and red kernel colour. More divergent types were found within red kernel colour class than white kernel colour class. The study also identified two molecular markers that can be used to differentiate the red kernel types from white kernel types and so can be used for transfer of this trait when these varieties are used as donors for kernel colour in breeding programmes. However, the kernel colour is not controlled by single gene and thus additional efforts are needed to validate these markers using a large germplasm set.

Acknowledgements

The research was supported by NATP, ToE project of NBPGR, New Delhi. We thank Dr. JL Karihaloo, former Project Director, National Research Centre on DNA Finger Printing, Dr. SK Pareek, Project Co- ordinator, NATP and Dr. BS Dhillon, former Director, NBPGR for the financial support.

References

Choudhary RP, S Kohli, K Srinivasan, T Mohapatra and RP Sharma (2001) Identification and classification of aromatic rices based on DNA finger printing. *Euphytica* **118:** 243-251.

Doyle JJ and JL Doyle (1990) Isolation of plant DNA from fresh tissues. *Focus* **12:** 13-14.

Fritsch P and LH Rieseberg (1996) The use of random amplified polymorphic DNA (RAPD) in conservation genetics. *In:* TB Smith and RK Wayne (eds). *Molecular Genetic Approaches in Conservation*. Oxford University Press, London. pp 54-73.

Michelmore RW, I Paran and RV Kesseli (1991) Identification of markers linked to disease-resistance gene by bulked sergeant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA* **88:** 9828-9832.

Phong DT, LT Muoi and LT Binh (2001) RAPD variability in rice (*Oryza sativa* L.) *Euphytica* **121:** 297-303.

Rohlf FJ (1993) NTSYS-pc *Numerical Taxonomy and Multivariate Analysis System*. V. 2.1.

Setauket NY, PH Sneath and RR Sokal (1973) Exeter Software, *Numerical taxonomy* WH Freeman & Company. San Francisco. **573:** 11.

Williams JG, AR Kubelik, KJ Livak, JA Rafalski and SV Tingey (1990) DNA polymorphisms amplified by arbitrary primers as useful genetic markers. *Nucleci Acid Res*. **18:** 6531-6535.

Xie JH, FJ Zapata-Arias, M Shen and R Afza (2000) Salinity tolerant performance and genetic diversity of four rice varieties. *Euphytica* **116:** 105-110.

Vanaja T (1999) Genetic analysis of high yielding rice varieties of diverse origin. Ph.D. thesis submitted to Kerala Agricultural University, Kerala.