Analysis of Genetic Variability in Rice Varieties (*Oryza sativa* L) of Kerala using RAPD Markers

R Skaria*, S Sen, PMA Muneer
Molecular Biology and Genetic Engineering Research Unit (MBGERU), Mar Athanasios College for Advanced Studies, Tiruvalla (MACFAST), Kerala, India.

*Correspondence to: Reby Skaria, rebu2006@gmail.com
Accepted: January 14, 2011; Published: January 21, 2011

Abstract

The genetic diversity and phylogenetic relationships of eight different varieties of Rice (*Oryza sativa)* viz., MO 16, Ptb 43, Palakkaddan Matta, Ptb 40, MO 17, MO 6, Ptb 39 and KTR 2 from Kerala state, South India were analyzed by Random amplified polymorphic DNA (RAPD). Out of twenty 10-mer RAPD primers screened initially, eleven were chosen and used in a comparative analysis of different varieties of rice. Of the 101 total RAPD fragments amplified, 28 (27.72%) were found to be shared by individuals of all eight varieties. The remaining 73 fragments were found to be polymorphic (72.27%). The average gene diversity or heterozygosity for over all varieties was 0.224. This Study offered a rapid and reliable method for the estimation of variability between different accessions which could be utilized by the breeders for further improvement of the local rice varieties.

Keywords: *Oryza sativa*; RAPD markers; PCR; polymorphism; genetic variation.

1. Introduction

Rice (*Oryza sativa*) is a cereal foodstuff which forms an important part of the diet of more than three billion people around the world [1]. Wild relatives of cultivated rice play a very important role in rice breeding practically and theoretically [2]. It has most diversified crop species due to its adaptation to a wide range of geographical ecological and climatic regions [3]. South India has been the heartland of rice cultivation since long. Therefore, the indigenous rice germplasm of South India, including Kerala is enriched with wide genetic diversity and valuable gene system for yield attributes and adaptability [4]. A detailed understanding about the extent structure of genetic variation in different varieties of the same species is important for the development of appropriate and efficient strategies for collection, conservation, and preservation of cultivar relatives. In addition, the efficient use of genetic resources in all plant-breeding programs requires knowledge about genetic diversity. At present, several varieties have many synonyms in different regions which make identification difficult. Nevertheless, differentiation of varieties through morphological features is inefficient and inaccurate [5].

The random amplified polymorphic DNA (RAPD) fingerprinting method was first described by Welsh and McClelland [6] and Williams *et al.* [7] in which the decamer (10 nucleotide length) DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence and which are able to differentiate between genetically distinct individuals, although not necessarily in a reproducible way. They showed that variation of amplified fragments is often inherited in a Mendelian fashion. Because the RAPD technique surveys numerous loci in the genome, it is particularly attractive for analysis of genetic diversity and phylogeny [8]. RAPD has been used in the analysis of rice genotypes by several groups for (i) estimation of the genetic diversity and relatedness among different accessions [9-13]; (ii) detection of duplicates [14,15]; (iii) identification and classification of various cultivars [16-18] and (iv) determination of the genetic relationship between several species/varieties [19,21]. The present work was undertaken to estimate genetic variation in the germplasm of eight rice varieties using RAPD technique for future use in selection, hybridization, biodiversity assessment, evaluation and conservation of diverse gene pools, etc. However, there are no reports on the comparison of the genetic variations using RAPD markers in these eight varieties which we report in the present study. We found highly reproducible, polymorphic RAPD fingerprints for discrimination of genetic variation in these varieties.

2. Methods

2.1 Sampling

The seeds of eight varieties of rice (*O. sativa* L) include MO 16 (Uma), Ptb 43 (Swarnaprabha), Palakkadan Matta, Ptb 40 (Sabari), MO 17 (Revathi), MO 6 (Pavizham), Ptb 39 (Jyothi) and KTR 2 (Cheradi) from the different research institutions like Rice Research Institute, Mankomb; Kerala Agricultural University, Vellayani; and other paddy cultivating locations such as Kuttanad, Alapuzha and Palakad were selected for the genetic variability study. The seeds were germinated separately in plastic tray by labeling the names of variety. Leaves were collected after two weeks of growth for DNA extraction. Total DNA was extracted from the leaf samples following the standard CTAB method [22] after minor modification. After ethanol precipitation, DNA was resuspended in 100 µl of TE. For the removal of RNA, 10 mg of RNAse A was added to the DNA solution and incubated for 10 min at 37°C.

2.2 RAPD Reaction

RAPD amplification was performed in a reaction volume of 25 µl containing 1 X reaction buffer (100 mMTris, 500 mM KCl, 0.1% gelatin, pH 9.0) with 1.5 mM $MgCl_2$ (Genei, Bangalore, India), 5 pM of primer, 0.2 mM dNTPs (dATP, dCTP, dGTP and dTTP), 2 U Taq DNA polymerase (Genei, Bangalore, India) and 25 ng of template DNA. PCR amplification was performed in a PTC 200 thermal cycler (MJ Research, Inc., Watertown, Massachusetts, USA). The reaction mixture was pre-heated at 95^0C for 3 min followed by 40 cycles (94^0C for 3 min, 40^0C for 1.30 min, and 72^0C for 2 min). The reaction was then subjected to a final extension at 72^0C for 10 min.

Twenty decamer primers from the operon kits OPA, OPC and OPJ (Operon Technologies, Almeda, USA) were used for this study. Eleven primers (Table-1) out of 20 produced amplicons and they were selected by primary screening and were selected for genetic diversity analysis by taking into consideration the repeatability, sharpness and intensity of bands. The resulting products were electrophoretically analysed through 1.5 % agarose gels stained with ethidium bromide (5 µg /ml) in TBE buffer (90 mM Tris–borate and 2 mM EDTA, pH 8.0) and the gels were documented using Image Master 1D gel documentation system (Amersham Biosciences, New Jersey, USA). The pictures were used for the analysis of the amplified products. The molecular weights of the bands were calculated by using Image Master 1D Elite software (Pharmacia Biotech, USA) in relation to the molecular marker λDNA with EcoRI/HindIII double digest applied along with the samples. The reproducibility of the RAPD method was checked by running the different PCR samples of the same plant in agarose gel electrophoresis.

Table 1. Selected primers and genetic variations among eight varieties of rice in RAPD analysis.

S. No.	Primer Code	Sequence	Total no. of bands	App. range of size (bp)	Polymorphic bands		Monomorphic bands	
					Number	%	Number	%
1	OPA 14	TCTGTGCTGG	11	250-1800	9	81.82	2	18.18
2	OPC 01	CTCACCGTCC	8	250-1600	7	87.5	1	14.2
3	OPC 09	CTCACCGTCC	12	250-1600	11	91.67	1	9.09
4	OPJ 01	CCCGGCATAA	7	150-1800	2	28.57	5	71.43
5	OPJ 02	CCCGTTGGGA	9	200-1750	6	66.67	3	33.33
6	OPJ 03	TCTCCGCTTG	7	300-1600	5	71.43	2	28.57
7	OPJ 04	CCGAACACGG	8	350-1750	4	50.00	4	50.00
8	OPJ 05	CTCCATGGGG	9	350-950	8	88.89	1	11.11
9	OPJ 07	CCTCTCGACA	9	200-1500	7	77.78	2	22.22
10	OPJ 08	CATACCGTGG	10	200-1800	7	70.00	3	30.00
11	OPJ 09	TGAGCCTCAC	11	350-1150	7	63.64	4	36.36
Total	----	----	101	150-1800	72.2	74.26	28	27.72

2.3 Data Analysis

The stained gels were photographed and analyzed the banding patterns by counting the bands as seen in the photographs. Reproducible RAPD products were scored as the presence (1) or absence (0) of a fragment. Bands of equal molecular mass and mobility generated by the same primer were considered as of identical locus. Faint or poorly amplified fragments were excluded from the analysis. All RAPD fragments scored were two-allele system, i.e., presence (dominant) and absence (recessive) of bands [23]. The DNA marker applied along with RAPD samples helped to determine the molecular sizes of the DNA fractions of the rice samples. The genetic distances were assessed with Nei's unbiased genetic distance measure and converted to a dendrogram by unweighted pair group method using arithmetic averages (UPGMA). The robustness of the dendrogram was tested using 1,000 bootstrapping. All phylogenetic program described were routinely implemented in PHYLIP [24] using POPGENE ver. 1.31 [25].

3. Results and Discussion

Out of 101 bands generated, 28 (27.7%) were found to be shared by individuals of all eight varieties. The remaining 73 fragments were found to be polymorphic (72.27%) (Table-1). For overall varieties, the observed (na) and effective (ne) number of alleles were 1.66 and 1.37 respectively. The average gene diversity or heterozygosity (H) [26] for over all species (H) was 0.224. The genetic variation pattern of different rice varieties with primer OPJ-05 is shown in fig. 1. Nei's unbiased genetic identity and distance estimated between pairs of eight varieties of rice are presented in Table-2. According to the results, the varieties KTR 2 (Cheradi) and Palakkadan Matta are closely related having the genetic identity value 0.47 and less genetic distance (0.62). The less genetic identity (0.05) or more genetic distance (0.95) was shown between the varieties Ptb 43 (Swarnaprabha) and MO 6 (Pavizham).

Table 2. Similarity matrix of rice varieties analysed using Nei's original measures of genetic identity.

Rice Varieties	MO 16	Ptb 43	Palakkadan Matta	Ptb 40	MO 17	MO 6	Ptb 39	KTR 2
MO 16	****	0.346	0.394	0.279	0.267	0.315	0.253	0.393
Ptb 43		****	0.284	0.346	0.254	0.051	0.178	0.276
Palakkadan Matta			****	0.452	0.441	0.245	0.423	0.471
Ptb 40				****	0.284	0.417	0.212	0.393
MO 17					****	0.321	0.131	0.122
MO 6						****	0.247	0.354
Ptb 39							****	0.231
KTR 2								****

3.1 Cluster Analysis

UPGMA dendrogram (Fig. 2) based on genetic distance grouped eight varieties of rice into two clusters 1, 2 following geographical proximity. Cluster 1 includes 5 varieties namely MO16, Ptb 39, MO17, Ptb 40, Ptb43. This cluster is further differentiated into 2 subclusters with subcluster 1 including 2 rice varieties namely MO16 and Ptb 39 and subcluster 2 with varieties MO17, Ptb 40 and Ptb 43. The cluster-II includes two local varieties 'KTR 2' and 'palakkadan matta' which were closely related and formed an independent cluster with MO 6. The different varieties came from different hybrid (Table 3) which could be a reason for the high genetic distance among the varieties of rice. In addition, the samples were collected from different locations (geographically separated), which also be a factor for high genetic distance.

Table 3. Pedigree and salient features of rice varieties selected for the study.

S. No.	Name of the rice varieties	Pedigree/Salient features
1.	MO 16- Uma	MO6 X Pokkali / Dwarf grains: MB, tolerant to GM.
2.	Ptb 43- Swarnaprabha	Bhavani x Triveni/ Grains – long bold & tolerant to blast & BPH.
3.	Palakadan matta	Indigenous local variety/ Robust: semi dwarf, red, high content of nutrients.
4.	Ptb 40-Sabari	IR8/2 x Annapoorna / Grains: long bold, red, resistant to blast.
5.	MO17-Revathy	Cul. 1281 X MO6 / long bold Grains: MB, tolerant to BPH.
6.	MO 6-Pavizham	IR 8 x Karivenal/ Semi dwarf, grains: short bold, red.
7.	Ptb 39-Jyothi	Ptb-10 x IR-8 / Dwarf, grains: long bold, red, resistant to blast.
8.	KTR 2	Local cheradi / Semi-dwarf (111 cm), grains: MB, red, no major diseases & pests.

3.2 Variety-specific Markers

Several RAPD fragments showed fixed frequencies in a particular variety. These could be used as variety specific markers to distinguish the varieties. 21 RAPD fragments were detected as variety-specific markers in 10 primers in all the varieties. These 21 fragments were exclusively observed in particular varieties (Table 4). The primer OPJ 03 did not show any variety specific bands. Such specific RAPD markers can be generated as genetic tags for rice varieties in the future that would be helpful in cultivation and selection programs for the species/variety. The utility of individual marker systems and their combination was also discussed by Sarika et al [27].

Genetic variation in plants has proven valuable in plant conservation and management, for identification of populations, species and sub-species, and for estimating contributions to stock mixtures. In this study, the variety KTR 2 shows more similarity with Palakkadan Matta because both the varieties were local varieties and all the rest were hybrids. However, the problem that we face today is that these varieties are becoming endangered. Kerala has a rich tradition in rice farming. Nevertheless, of late many of the traditional varieties are not available. Genetic erosion might be the major reason to replace these traditional varieties by the high yielding rice varieties that are provided by government agencies [28]. A proper knowledge of the genetic make-up and variability of flora will help us in the management, improvement and conservation of endangered varieties. A lack of knowledge about the genetic structure of these varieties/populations may result in the differential harvest of the varieties/populations that will ultimately have a drastic and long-term effect [29]. To overcome this, there is always a need for investigations encompassing the genetic variations at the intra and inter-population levels as well as at the intra and inter-specific levels of the plant resources of any nation [30,31].

Random amplified polymorphic DNA is one of the common genetic marker, used for population genetic analysis, pedigree analysis and taxonomic discrimination [32-34]. Several authors have demonstrated that the RAPD-PCR method is a powerful tool in the assessment of discriminating differences at intra and inter-population level in a wide range of organisms including plants [19, 35-37]. In the present study, the genetic characteristics of different varieties of rice were analyzed for discriminating the different varieties by applying modern techniques of Polymerase Chain Reaction-random amplified polymorphic DNA (PCR-RAPD). The size of DNA fragments amplified in rice varieties ranged from 150-1800 bp and this conforms with the range of fragment sizes observed in traditional lowland rice varieties of Assam [38], wild rice in northern region of Thailand [39], rice genotypes of Pakistan [40].

Table 4. Variety specific RAPD markers with size in bp for each rice variety.

Primer	Fragment No.	Size (bp)	Variety
OPA 14	4	400	KTR 2
OPC 09	4	750	Ptb 39
	3	830	MO 6
OPJ 01	2	1420	Palakkadan matta
	9	200	Palakkadan matta
OPJ 02	2	1050	Ptb 40
	5	700	Ptb 39
	7	530	Ptb 40
OPJ 04	5	640	MO16
	6	1030	Palakkadan matta
OPJ 05	4	900	KTR 2
	6	450	Ptb 40
OPJ 07	5	500	Ptb 40
	7	1040	MO 16
	9	860	Ptb 43
OPC 01	4	450	KTR 2
	5	670	KTR 2
OPJ 08	7	550	Ptb 43
	9	950	MO 16
OPJ 09	3	400	MO6
	5	660	Palakkadan matta

Fig 1: RAPD pattern of different rice varieties with primer OPJ-05. Lanes: 1-2 MO 16, 3-4 Ptb 43, 5-6 Palakkadan Matta, 7-8 Ptb 40, 9-10 MO 17, 11-12 MO 6, 13-14 Ptb 39, 15 KTR 2. M-Molecular Weight marker (λDNA with EcoRI & HindIII double digest).

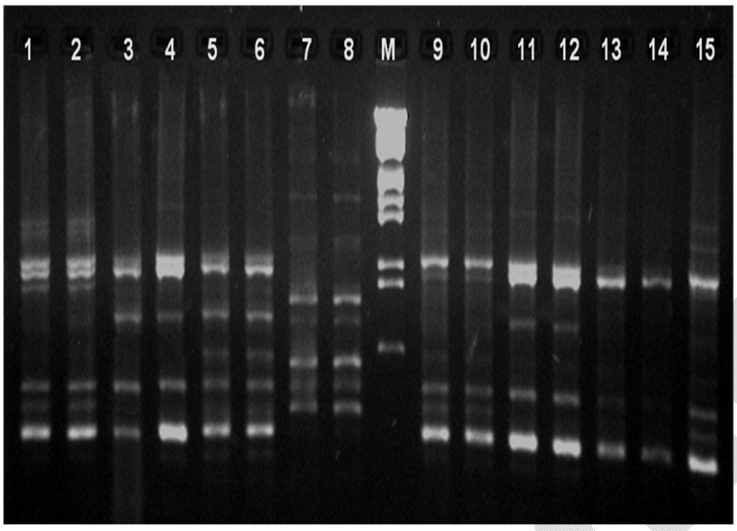
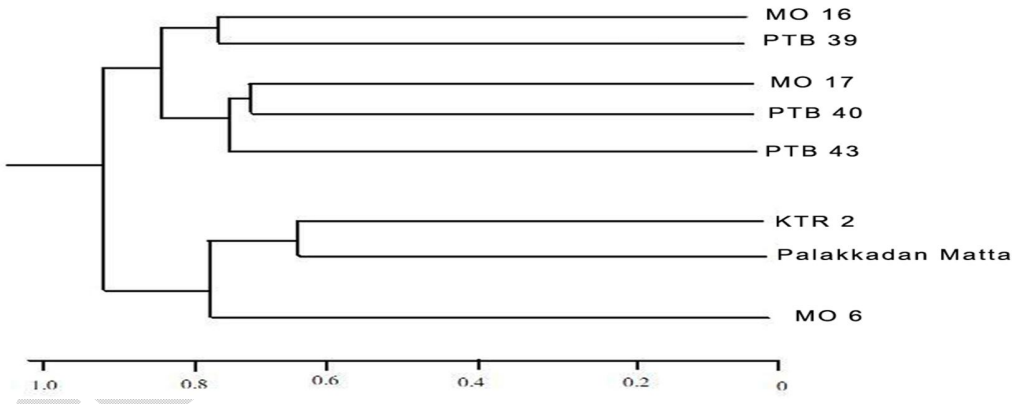


Fig 2: Dendrogram (UPGMA) pattern of RAPD analysis in different varieties of rice plant (*Oryza sativa*).



When gene flow is lower, then gene diversity is higher [41, 42]. In this experiment, all the rice varieties showed higher genetic distance (0.7417). Therefore, it can be commented that lower gene flow might have occurred in these genotypes. A recent study by Sarika et al revealed a high level of genetic diversity among the non-basmati groups that holds a promise in quest of conserving crop diversity and broadening gene pool for breeding [27]. The RAPD profiles in the present study displayed a high degree of polymorphism. This confirms suitability of RAPD markers for discrimination of different varieties of rice plants. In brief, the study yielded highly reproducible RAPD fingerprints, which were used as reliable and useful tool for discrimination of genetic variation in eight varieties of rice (*O. sativa*). Finally, the present findings of genetic divergence levels in rice suggest that all varieties are not drawn from the same randomly mating gene pool. This observation and the identification of unique specific markers (private alleles) are significant steps towards realizing the goal of variety-based management and cultivation and conservation of rice resource in Peninsular India.

4. Conclusion

RAPD marker was found to be powerful tool to analyze the genetic structure of different varieties of rice. These markers demonstrated striking genetic differentiation between pairs of rice varieties examined. Geographic isolation by land distance is likely to be the factor that contributed to the restricted gene flow between the varieties. This study reveals the average genetic variation among the rice varieties and emphasizes the need for stock/variety wise cultivation, conservation and propagation assisted-rehabilitation and selection of the natural populations of rice (*O. sativa*).

Abbreviations

CTAB: Cetyl trimethylammonium Bromide
RAPD: Randomly Amplified Polymorphic DNA
PCR: Polymerase Chain Reaction
UPGMA: Unweighted Paired Group Method Using Arithmetic Averages
DNTP: Deoxyribonucleotidase
MB: Medium Bold
BPH: Brown Plant Hopper

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

The work is original and it has been carried out by RS for MSc dissertation under supervision of PMA at Mar Athanasios College (Kerala), India. SS helped during the experimental work and sample collection.

Acknowledgement

The authors are grateful to Rev. Dr. Abraham Mulamoottil, Principal; Dr. C. Balagopalan, Dean, School of Biosciences and Biju Dharmapalan; MACFAST for encouragement, support and guidance. The authors also gratefully acknowledge Dr. A. Goapalakrishnan, Principal Scientist, NBFGR Cochin Unit, and CMFRI for providing the excellent facilities for gel documentation.

References

1) Cramer HH, 1967. Plant protection and world crop production. Pflan-zenschutz Nachrichten Bayer Crop Protection Advisory, Dep. of Farbenfabriken Bayer AG, Leverkusen: Germany.

2) Vaughan DA, 1994. The Wild Relatives of Rice. A Genetic Resources Handbook. IRRI, Manila, Philippines.

3) Chang TT, 1976. The origin, evolution, cultivation, dissemination and diversification of Asian and African rices. Euphytica, 25: 425-441.

4) Vanaja T, Randhawa, Mammootty K, 2006. Pedigree evaluation and molecular diversity of some true breeding rice (*Oryza sativa* L.) genotypes of Kerala**.** Journal of Tropical Agriculture, 44: 42-47.

5) Das BK, Jena RC, Samal KC, 2009. Optimization of DNA isolation and PCR protocol for RAPD analysis of banana / plantain (*Musa* spp.). International Journal of Agriculture Sciences, 1: 21-25.

6) Welsh J, McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research, 18: 7213-7218.

7) Williams JGK, Kubelik AR, Livak KJ, Reafalski JA, Tingey SV, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, 18: 6531-6535.

8) Clark AG, Lanigan CMS, 1993. Prospects for estimating nucleotide divergence with RAPDs. Molecular Biology and Evolution, 10: 1096-1111.

9) Davierwala AP, Chowdari KV, Kumar S, Reddy APK, Ranjekar PK, Gupta VS, 2000. Use of three different marker systems to estimate genetic diversity of Indian elite rice varieties. Genetica**,** 108: 269-284.

10) Raghunathachari P, Khanna VK, Singh US, 2000. RAPD analysis of genetic variability in Indian scented rice germplasm (*Oryza sativa* L.). Current Science, 79: 994-998.

11) Porreca P, Sabina MR, Martelli G, Sunseri F, Greco I, Pruneddu G, Spanu A, 2001. Genetic variability among Italian rice (*Oryza sativa* L.) cultivars investigated by RAPD analysis. Journal of Genetics & Breeding, 55: 349-355.

12) Neeraja CN, Sarla N, Siddique EA**,** 2002. RAPD analysis of genetic diversity in Indian landraces of rice (*Oryza sativa* L). Journal of Plant Biochemistry and Biotechnology, 11: 93-97.

13) Saker MM, Youseff SS, Abdallah NA, Bashandi HS, Sharkawi AM, 2005. Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. African Journal of Biotechnology**,** 4: 882-890.

14) Virk PS, Ford-Lloyd BV, Jackson MT, Newbury HJ, 1995. Use of RAPD for the study of diversity within plant germplasm collections. Heredity, 74: 170-179.

15) Verma SK, Khanna VK, Singh NK, 1999. Random amplified polymorphic DNA analysis of Indian scented basmati rice (*Oryza sativa* L.) germplasm for identification of variability and duplicate accessions, if any. Electrophoresis, 20: 1786-1789.

16) Ko HL, Cowan DC, Henry RJ, Graham GC, Blakeney AB, Lewin LG, 1994. Random amplified polymorphic DNA analysis of Australian rice (*Oryza sativa* L.) varieties. Euphytica*,* 80: 179-189.

17) Mackill DJ, 1995. Classifying japonica rice cultivars with RAPD markers. Crop Science**,** 35: 889-894.

18) Choudhury PR, Kohli S, Srinivasan K, Mohapatra T, Sharma RP, 2001. Identification and classification of aromatic rice based on DNA fingerprinting. Euphytica, 118**:** 243-251.

19) Bautista NS, Solis R, Kamjim O, Ishii T, 2001. RAPD, RFLP and SSLP analysis of phylogenetic relationships between cultivated and wild species of rice. Genes and Genetic Systems, 76: 71-79.

20) Ren F, Lu BR, Li S, Huang J, Zhu Y**,** 2003. Comparative study of genetic relationships among the AA genome *Oryza* species using RAPD and SSR markers. Theoretical and Applied Genetics, 108**:** 113-120.

21) Datta J, Lal N, Kaashyap M, Gupta PP, 2010. Efficiency of Three PCR based Marker Systems for Detecting DNA Polymorphism in *Cicer arietinum* L and *Cajanus cajan* L Millspaugh. Genetic Engineering and Biotechnology Journal, 2010: GEBJ-5.

22) Dellaporta SL, Wood J, Hicks JB, 1983. A plant DNA minipreparation: Version II. Plant Molecular Biology Reporter, 1: 19-21.

23) Sen S, Skaria R, Muneer PMA, 2010. Genetic diversity analysis in Piper species (Piperaceae) using RAPD markers. Molecular Biotechnology, 46: 72–79.

24) Felsenstein J, 1993. PHYLIP (Phylogeny Inference Package), version 3.5, distributed by the author. University of Washington, Seattle.

25) Yeh FC, Yang RC, Boyle T, 1999. POPGENE 32 – Version 1.31. Population genetics software. [http://www.ualberta.ca/fyeh/fyeh/].

E-ISSN: 21503516

26) Nei M, 1972. Genetic distance between populations. American Naturalist, 106: 283-292.

27) Mathure S, Altafhusain N, Narendra J, 2010. Diversity analysis in selected non-basmati scented rice collection (*Oryza sativa* L.). Rice Science, 17: 35–42.

28) Mathure S, Asif S, Renuka N, Kantilal W, Narendra J, Ratnakar T, Altafhusain N, 2010. Characterization of aromatic rice (*Oryza sativa* L.) germplasm and correlation between their agronomic and quality traits. Euphytica, DOI 10.1007/s10681-010-0294-9.

29) Rossetto M, Weaver PK, Dixon KW, 2008. Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera* (Proteaceae). Molecular Ecology, 4: 321-329.

30) Mani P, Bastin J, Arun Kumar R, Ahmed AAB, 2010. RAPD analysis of genetic variation of four important rice varieties using two OPR primers. ARPN Journal of Agricultural and Biological Science, 5: 12-15.

31) Morell MK, Peakall R, Appels R, Preston LR, Lloyd HL, 1995. DNA profiling technique for plant variety identification. Australian Journal of Experimental Agriculture, 35: 807-819.

32) Rodriguez JM, Berke T, Engle L, Nienhuis J, 1999. Variation among and within Capsicum species revealed by RAPD markers. Theoretical and Applied Genetics, 99: 147-156.

33) Muneer PMA, Gopalakrishnan A, Musammilu KK, Lal KK, Mohindra V, Basheer VS, Lakra WS, 2008. Genetic variation and population structure of endemic yellow catfish, *Horabagrus brachysoma* (Bagridae) among three populations of Western Ghat region using RAPD and microsatellite markers. Molecular Biology Reports, 36: 1779-1791.

34) Nair S, Keshavachandran R, 2006. Molecular diversity in chakkarakolli (*Gymnema sylvestre* R. Br.) assessed through isozyme and RAPD analysis. Journal of Tropical Agriculture, 44: 31-36.

35) Wu YJ, Chen Y, Wang J, Zhu CX, Xu BL, 2006. RAPD analysis of Jasmine rice specific genomic structure. Genome, 49: 716-719.

36) Archak S, Karihaloo JL, 2002. RAPD markers reveal narrowing genetic base of Indian tomato cultivars. Current Science, 82: 1139–1141.

37) Malviya N, Yadav D, 2010. RAPD analysis among Pigeon Pea [*Cajanus cajan* (L.) Mill sp.] cultivars for their genetic diversity. Genetic Engineering and Biotechnology Journal, 2010: GEBJ-1.

38) Bhuyan N, Borah BK, Sarma RN, 2007. Genetic diversity analysis in traditional lowland rice (*Oryza sativa* L.) of Assam using RAPD and ISSR markers. Current Science, 93: 967-972.

39) Ukoskit K, 2004. Genetic diversity of wild rice (*Oryza* spp.) in the northern region of Thailand. Thammasat International Journal of Science and Technology, 9: 11-20.

40) Arif M, Kousar S, Bajwa A, Arif A, Zafar Y, 2005. Genetic diversity among rice genotypes of Pakistan through random amplified polymorphic DNA (RAPD) analysis. Pakistan Journal of Botany, 37: 585-592.

41) Rahman SN, Islam MS, Alam MS, Nasiruddin KM, 2007. Genetic polymorphism in rice (*Oryza sativa* L.) through RAPD analysis. Indian Journal of Biotechnology, 6: 224-229.

42) Dinesh R, Kiran AG, Thomas G, 2010. Population genetic structure and conservation priorities of *Oryza rufipogon* Griff. populations in Kerala, India. Current Science, 98: 65-68.