Molecular Diversity Analysis of Traditional Rice Varieties of Kerala Using RAPD Markers

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Thirty traditional landraces collected from nine ecological zones of Kerala were subjected to molecular studies. Twenty oligonucleotide primers used for the study produced 222 amplicons with a polymorphism of 82%. The amplification products derived with random primers had size ranging from 2.0 kb to less than 0.5 kb. Of the twenty primers OPF-04 gave maximum number of polymorphic products and also produced two unique positive products in the accession Cheruvirippu (size between 1.0 kb and 1.5 kb) and in the accession Njavara yellow (size of less than 0.5 kb). This primer has the highest resolving power (24.52) and Effective Multiplex Ratio (15.0) and so is selected as the best primer for distinguishing rice varieties in this study. Clustering based on Jaccard's similarity coefficient revealed the highest similarity between the accessions Chettivirippu and Pokkali (0.825). Both these accessions are suited to the saline sodic soils of Kerala. The Njavara group of accessions (medicinal rice), Njavara yellow and Njavara black, clustered at a similarity value of 0.707. The primer OPB-05 and OPF-01 could distinguish the Njavara accessions from others. OPB-05 produced unique product with a size of less than 1.0 kb and OPF-01 produced product at size of less than 0.5 kb unique for the Njavara accessions. Even though these accessions were from different ecological zones location specific clustering was not observed pointing the uniqueness of the collection at the micro level.

Key Words: Diversity, RAPD, Rice

Introduction

Kerala is considered as one of the centres of diversity of rice and the antiquity of rice cultivation here dates back to 3000 BC (Manilal, 1990). Rice covers a vast array of ecological niches and a vast diversity of germplasm of both cultivated and wild rice exist in Kerala (Kumary and Francies, 2002). Diversity at ecosystem level, inter-specific levels in addition to ethnic diversity is the highest in this stretch of land. These traditional varieties may be containing favourable genes, especially genes for resistance to biotic and abiotic stresses. Conservation and characterization of these varieties is essential for future genetic improvement of rice.

Characterization based only on morphological and physiological parameters with clear cut features of distinctness are not always possible. The morphologies reflect not only the genetic constitution of the individual but also the interaction of the genotype with the environment within which it is expressed (Patterson and Weatherup, 1984). Characterization with molecular markers is more reliable and DNA based molecular markers are in abundance and clearly allow the comparison of genetic material avoiding any environmental influence on gene expression. RAPD (Randomly amplified polymorphic DNA) markers (Williams *et al*., 1990) are PCR based and is widely in use for molecular analysis of plant genome. RAPD analysis is a simple technique with lower cost using a small amount of DNA and has the advantage of hitting the genome randomly.

The present investigation was done to assess the molecular diversity of the thirty genotypes collected from different ecological zones of Kerala using RAPD markers.

Materials and Methods

The experimental material consisted of 30 traditional rice accessions of Kerala chosen from the material collected under NATP project on Sustainable Management of Biodiversity, at KAU (Table 1). The seed material was procured from Rice Research Station, Moncompu, Alappuzha.

Isolation of Genomic DNA

Isolation of genomic DNA was done from all the 30 samples following the procedure of Regowsky *et al.* (1991) with required modifications.

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Table 1. Morphological characters of the selected 30 rice accessions

S. No.	Name	Source	Leaf			Days to heading	Panicle		
			Length (cm)	Width (cm)	Blade colour		Length (cm)	Type	Secondary branching
1	Njavara (yellow)	Allepey	49	0.7	Green	58	18.5	Compact	Light
2	Njavara (black)	Palakkad	61.6	1.0	Pale green	60	26.0	Intermediate	Light
3	Cheeravithu	Palakkad	51.5	1.1	Purple blotch	91	24.5	Intermediate	Light
4	Jeerakasala	Wayanad	35	1.1	Pale green	92	36.5	Compact	Heavy
5	Chempan	Trivandrum	48.5	0.9	Pale green	104	29.5	Intermediate	Light
6	Kannikayama	Kannur	52.5	0.8	Green	96	27.2	Compact	Light
7	Thowan	Kannur	42.2	1.0	Green	102	23.3	Compact	Light
8	Kozhivalan	Kannur	41.7	1.3	Pale green	121	23.4	Compact	Heavy
9	Kururayima	Kannur	64.51	1.2	Green	107	27.2	Compact	Light
10	Karuthacheera	Perumbavur	53.5	0.9	Green	89	26.7	Compact	Light
11	Kalladi Aryan	Kannur	35.5	0.7	Green	101	22.5	Compact	Light
12	Veluthittaryan	Palakkad	59	1.5	Green	126	22.5	Intermediate	Light
13	Athikiramundakan	Allepey	45.5	1.2	Green	116	20.6	Intermediate	Light
14	Anakodan	Palakkad	39.1	1.2	Green	125	22.3	Compact	Light
15	Veluthakattamodan	Kannur	63.7	1.1	Green	90	28.8	Intermediate	Light
16	Kattamodan	Palakkad	79.4	1.1	Pale green	100	27.3	Compact	Light
17	Allikannan	Kannur	40.5	1.0	Green	104	25.2	Compact	Light
18	Parambuvattan	Palakkad	32.5	0.6	Dark green	94	23.3	Compact	Light
19	Vellakkoli	Thrissur	45.2	0.7	Green	89	23.5	Intermediate	Light
20	Vellamundakan	Allepey	35.9	0.8	Dark green	76	17.4	Compact	Light
21	Cheruvirippu	Ernakulam	58.5	1.3	Green	103	26.0	Open	Light
22	Choottupokkali	Ernakulam	65	1.4	Green	104	27.5	Intermediate	Light
23	Chenthadi	Wayanad	31.5	0.6	Green	87	23.1	Intermediate	Light
24	Kazhama	Kannur	54.3	1.1	Pale green	96	29.8	Intermediate	Light
25	Mundon	Malapuram	57.5	0.8	Dark green	88	27.2	Intermediate	Light
26	Chettivirippu	Ernakulam	43	1.0	Dark green	100	26.5	Intermediate	Clustering
27	Pokkali 3	Ernakulam	48	0.9	Dark green	102	23.5	Intermediate	Light
28	Ponkuruka	Ernakulam	49.5	1.2	Dark green	95	25.2	Compact	Light
29	Pandivella	Trivandrum	52	1.1	Green	99	28.0	Compact	Heavy
30	Thavalakannan	Palakkad	43.2	1.1	Green	105	24.0	Compact	Light

Random Amplified Polymorphic DNA (RAPD)

The procedure of Williams *et al*. (1990) was used for the amplification of DNA. The amplification was done using 20 reported arbitrarily designed primers, which included primers from different Operon primer series *viz.* OPA-5, OPB-05, OPB-08, OPB-10, OPC-07, OPC-15, OPD-03, OPD-18, OPF-01, OPF-03, OPF-04, OPF-05, OPF-06, OPF-13, OPG-18, OPG-19, OPH-19, OPK-14, OPK-19 and OPL-17 [Barooah and Sarma (2004); Raghunathachari *et al.* (2000); Saker *et al.* (2005)].

The reaction was carried out in 25 µl reaction mixture containing 20 ng template DNA, 2.5µl of 10x PCR buffer, 2 µl of 2.5 mM $MgCl_2$, 1 µl of 10 mM dNTP mix, 1 unit of Taq DNA polymerase and 1 µl of 10 pmoles primer. Amplification was done in a thermocycler (MJ Research Inc., USA) with PCR cycle as follows:

An initial denaturation at 94ºC for 5 min followed by 40 cycles of denaturation at 94ºC for 1 min, annealing at 36ºC for 1 min and extension at 72˚C for 90 sec. The synthesis step of final cycle was extended further by 7 min. Finally the products of amplification were stored at 4ºC. Amplified products were separated by agarose gel electrophoresis using 1.4% gel as described earlier and photographed using gel documentation system.

Data Analysis

The reproducible bands were scored for their presence (1) or absence (0) for all the genotypes studied. A genetic similarity matrix was constructed using the Jaccard's coefficient method (Jaccard, 1908).

Each RAPD band was treated as an independent character and was scored as present (1) or absent (0). The resulting binary data matrix was used to compute a parwise distance matrix using the DICE coefficient utilizing the software WINBOOT (Yap and Nelson 1996).The distance matrix was subjected to cluster analysis employing UPGMA using SAHN (Sequential Aglomerative Heirarchial and nested cluster) module of the software NTSYS pc (Rohlf 2002).The robustness of the clusters was estimated by performing a bootstrap analysis on 1000 data replicates using WINBOOT (Yap and Nelson 1996). Principal coordinate analysis was conducted using the EIGEN procedure of NTSYS pc in order to cluster the genotypes. The value of each of the 20 primers was assessed using two indices PIC which

Table 1. continued

S. No	Name	Grain							Maturity days
			Lemma and palea		100-grain wt	Seed coat colour	Endosperm		
		Awning	Colour	Pubescence			Type	Scent	
1	Njavara (yellow)	Absent	Brown furrows on straw	Hairs on upper portion	2.01	Brown	Non waxy	Non scented	80
2	Njavara (black)	Absent	Black	Hairs on upper portion	2.18	Brown	Non waxy	Non scented	98
3	Cheeravithu	Absent	Brown furrows on straw	Hairs on upper portion	2.65	Brown	Non waxy	Non scented	109
4	Jeerakasala	Absent	Straw	Hairs on upper portion	2.27	White	Non waxy	Lightly scented	123
5	Chempan	Absent	Straw	Hairs on upper portion	2.52	Brown	Non waxy	Non scented	121
6	Kannikayama	Absent	Brown furrows on straw	Hairs on upper portion	3.5	Brown	Non waxy	Non scented	122
7	Thowan	Absent	Straw	Short hairs	3.33	Red	Non waxy	Non scented	132
8	Kozhivalan	Absent	Straw	Hairs on upper portion	2.36	Brown	Non waxy	Non scented	148
9	Kururayima	Absent	Gold and/or gold furrows on straw background	Short hairs	2.7	Brown	Non waxy	Non scented	133
10	Karuthacheera	Absent	Straw	Short hairs	2.65	Brown	Non waxy	Non scented	109
11	Kalladi Aryan	Absent	Brown furrows on straw	Hairs on upper portion	2.51	Brown	Non waxy	Non scented	109
12	Veluthittaryan	Absent	Gold and/or gold furrows on straw background	Hairs on upper portion	2.86	Red	Non waxy	Non scented	150
13	Athikiramundakan	Absent	Brown	Hairs on lemma keel	2.24	Brown	Non waxy	Non scented	147
14	Anakodan	Absent	Straw	Hairs on lemma keel	2.41	Red	Non waxy	Non scented	147
15	Veluthakattamodan	Long and fully awned	Gold and/or gold furrows on straw background	Hairs on upper portion	3.25	Brown	Non waxy	Non scented	132
16	Kattamodan	Absent	Brown furrows on straw	Short hairs	2.14	Light brown	Non waxy	Non scented	130
17	Allikannan	Absent	Gold and/or gold furrows on straw background	Hairs on upper portion	3.03	White	Non waxy	Non scented	135
18	Parambuvattan	Long and partly awned	Black	Hairs on upper portion	2.84	Light brown	Non waxy	Non scented	120
19	Vellakkoli	Absent	Brown furrows on straw	Short hairs	2.45	Brown	Non waxy	Non scented	130
20	Vellamundakan	Absent	Brown furrows on straw	Hairs on upper portion	2.62	Red	Non waxy	Non scented	117
21	Cheruvirippu	Short and partly awned	Brown furrows on straw	Hairs on upper portion	3.36	Brown	Non waxy	Non scented	135
22	Choottupokkali	Absent	Straw	Hairs on upper portion	2.72	Brown	Non waxy	Non scented	135
23	Chenthadi	Absent	Straw	Hairs on upper portion	3.32	Brown	Non waxy	Non scented	127
24	Kazhama	Absent	Gold and/or gold furrows on straw background	Hairs on upper portion	1.52	Brown	Non waxy	Non scented	135
25	Mundon	Absent	Straw	Hairs on upper portion	2.33	Brown	Non waxy	Non scented	129
26	Chettivirippu	Absent	Straw	Hairs on upper portion	1.8	Red	Non waxy	Non scented	122
27	Pokkali 3	Absent	Straw	Short hairs	2.63	Brown	Non waxy	Non scented	136
28	Ponkuruka	Short and partly awned	Brown	Hairs on upper portion	2.38	Brown	Non waxy	Non scented	121
29	Pandivella	Absent	Gold and/or gold furrows on straw background	Hairs on upper portion	1.91	Red	Non waxy	Non scented	122
30	Thavalakannan	Absent	Gold and/or gold furrows on straw background	Hairs on lemma keel	2.91	Light brown	Non waxy	Non scented	134

* Data obtained from NATP on Sustainable Management of Biodiversity [Principal Investigator, Dr S Leenakumary]

is same as diversity index (DI) and Rp (Prevost and Wilkinson 1999).PIC was estimated as PIC= ∑ (1-pi ^{2})/n where n is the number of band positions analyzed in the set of accessions, pi is the frequency of the ith pattern. The ability of the primers to distinguish between accessions was assessed by calculating their resolving power as, Rp= ∑Ib where Ib is the band informativeness and Ib=1- (2x0.5-pi) where pi is the proportion of individuals containing the band (Prevost and Wilkinson, 1999). Marker Index was calculated as the product of DI and EMR (Effective multiplex ratio). EMR of a primer is defined as the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay (Milbourne *et al*., 1997).

Results and Discussion

The 20 oligonucleotide primers randomly selected produced multiple band profiles with a number of amplified fragments ranging from 2.0 kb to less than 0.5 kb with mean number of 11.10 amplicons per primer. The primer OPF-04 gave the maximum number of amplicons (15) (Fig. 1) while the lowest number (7) was amplified by the primer OPG-18 (Table 2).

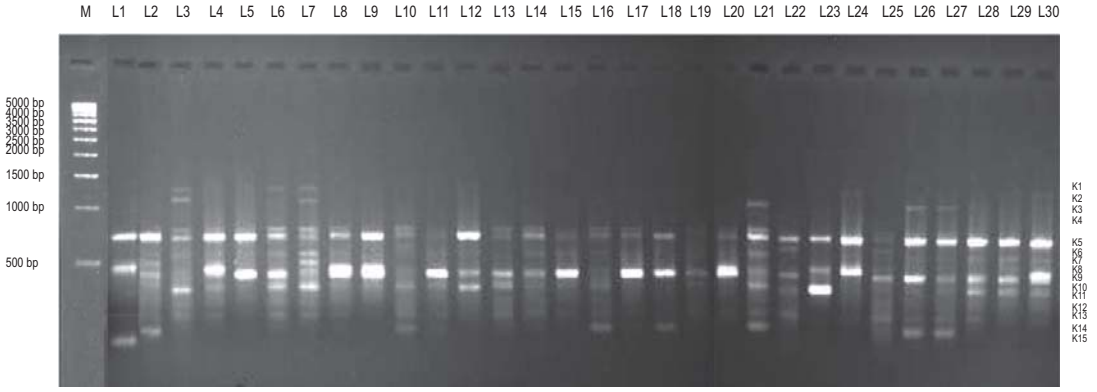

Fig. 1: Amplification profile of the DNA of 30 rice accessions using the primer OPF-04

L1 = Njavara yellow, L2 = Njavara Black, L3 = Cheeravithu, L4 = Jeerakasala, L5 = Chempan, L6 = Kannikayama, L7 = Thowan, L8 = Kozhivalan, L9 = Kururayima, L10 = Karuthacheera, L11 = Kalladi Aryan, L12 = Veluthittaryan, L13 = Athikiramundakan, L14= Anakodan, L15 = Veluthakattamodan, L16 = Kattamodan, L17 = Allikannan, L18 = Parambuvattan, L19 = Vellakkoli, L20 = Vellamundakan, L21= Cheruvirippu, L22 = Choottupokkali, L23 = Chenthadi, L24 = Kazhama, L25 = Mundon, L26 = Chettivirippu, L27 = Pokkali, L28 = Ponkuruka, L29 = Pandivella, L30 = Thavalakannan

Table 2. Base sequence of RAPD primers, number of amplicons and percentage of polymorphism in rice genomic DNA

Primer name	Sequence	Number of amplicons	Number of polymorphic amplicons	Number of monomorphic amplicons	Polymorphism (%)	PIC	Rp	EMR
OPA-05	AGGGGTCTTG	9	6	3	66.66	0.42	7.46	3.96
OPB-05	TGCGCCCTTC	12	10	2	83.33	0.59	15.50	8.30
OPB-O8	GTCCACACGG	9	8	1	88.88	0.52	6.71	7.04
OPB-10	CTGCTGGGAC	11	6	5	54.54	0.29	6.42	3.24
OPC-07	GTCCCGACGA	10	9	1	90.00	0.66	12.48	8.10
OPC-15	GACGGATCAG	12	10	2	83.33	0.52	12.42	8.30
OPD-03	GTCGCCGTCA	8	6	2	75.00	0.39	6.34	4.50
OPD-18	GAGAGCCAAC	11	10	1	90.90	0.57	12.70	9.00
OPF-01	ACGGATCCTG	14	12	2	85.71	0.58	18.52	10.20
OPF-03	CCTGATCACC	9	6	3	66.66	0.37	6.76	3.96
OPF-04	GGTGATCAGG	15	15	0	100.00	0.65	24.52	15.00
OPF-05	CCGAATTCCC	10	9	1	90.00	0.67	13.48	8.10
OPF-06	GGGAATTCGG	14	13	1	92.85	0.65	18.58	11.96
OPF-13	GGCTGCAGAA	10	6	4	60.00	0.32	6.42	3.60
OPG-18	GGCTCATGTG	7	5	2	71.42	0.43	6.14	3.55
OPG-19	GTCAGGGCAA	11	9	2	81.81	0.46	10.32	7.25
OPH-19	CTGACCAGCC	14	13	1	92.85	0.53	14.88	11.96
OPK-14	CCCGCTACAC	12	7	5	58.33	0.41	9.72	4.07
OPK-19	CACAGGCGGA	14	13	1	92.85	0.70	15.02	6.96
OPL-17	AGCCTGAGCC	10	9	1	90.00	0.81	6.90	8.10

The average number of polymorphic bands per primer was found to be 9.10. A total of 222 amplicons were produced which showed a polymorphism of 81.98%. The polymorphism produced by the different primers ranged from 100% to 54.54%. Forty cultivated varieties of rice were evaluated for polymorphism after amplification with thirty six decamer RAPD primers by Ravi *et al.* (2003) and a total of 499 RAPD markers were produced with a polymorphism percentage of ninety. He *et al*. (2004) had reported 69.4% polymorphism while using 12 RAPD primers in assessment of genetic diversity of allelopathic rice germplasm.

Of the 20 primers studied, 13 gave a polymorphism of above 80%. This shows the effectiveness of the selected primers in genetic analysis of the different accessions. Primer OPF-04 gave 100% polymorphism in the studied accessions. Barooah and Sarma (2004) had reported 100% polymorphism for the primer OPK-19. In the present study, this primer produced 92.85% polymorphism. Barooah and Sarma (2004) had reported that the primer OPK-14 gave the least percentage of polymorphism (33.33) in the genetic diversity analysis of traditional Sali rice. In the present study this primer produced 58.33% polymorphism.

The 20 primers used in this study produced a total of 222 amplicons with mean number of 11.10 bands per primer. Raghunathachari *et al*. (2000) reported an average of 11.4 bands per primer in the study of genetic variability in Indian scented rice germplasm using RAPD analysis. Saker *et al.* (2005) had reported on an average 10.90 bands per primer in the study of RAPD analysis in Egyptian rice genotypes.

In this RAPD analysis of rice accessions the amplicons produced had a molecular weight ranging from 2.0 kb to less than 0.5 kb. Raghunathachari *et al*. (2000) had reported amplicons of size ranging from 4.0 kb to 0.5 kb. Wu *et al*. (2002) had reported amplicons of size 2.5 kb to 0.5 kb. Neeraja *et al*. (2002) reported amplicons of size 3.9 kb to 0.25 kb whereas Monna *et al*. (1994) had reported amplicons of size 2.0 kb to less than 0.1 kb.

Nine unique positive amplicons were obtained by the primers OPC-15, OPD-03, OPF-03, OPF-04, OPF-05, OPG-18 and OPK-19 (Table 3). These bands could distinguish Njavara yellow, Njavara black, Cheeravithu, Chempan, Thowan, Cheruvirippu and Allikannan. These markers can be used as fingerprints for these accessions. Primers OPB-05 and OPF-01 produced amplicons for the Njavara group alone at less than 1000 bp. Njavara group includes Njavara yellow and Njavara black, two ecotypes of the medicinal rice Njavara.

Comparative ability of different primers to detect diversity in 30 accessions was depicted from the PIC, Rp and EMR values (Table 2). The mean PIC value for the twenty primers was 0.5 .The primer OPB-10 showed the least PIC value and primer OPF05 showed the highest PIC value (0.673).The mean Rp value for the twenty primers was 11.56 with OPF 04 having the highest (24.52) and OPG 18 (6.14) the lowest. EMR (Effective Multiplex ratio) of the primers ranged from 15 (OPF 04) to 3.55 (3.55) and that value was also highest for OPF 04. Among the 20 primers studied OPF 04 is the best primer for distinguishing the varieties with highest Rp value, high PIC value and highest EMR.

Table 3. Unique positive and negative markers identified in the RAPD analysis of the 30 accessions of rice

Accession	Unique positive markers			Unique negative markers			
	Size of marker (bp)	Primer	Total No. of markers/variety	Size of marker (bp)	Primer	Total No. of markers/ variety	Grand total
Njavara yellow	< 500	OPF-04	1	-	-	-	2
	500 - 1000	OPK-19	1	-	-	-	
Njavara black	< 500	OPD-03	1	-	-	-	1
Cheeravithu	~ 1000	OPF-05	1	-	-	-	1
Chempan	< 1000	OPF-05	1	-	-	-	1
Thowan	500 - 1000	OPG-18	1	-	-	-	1
Kozhivalan	-	-	-	< 500	OPH-19	1	1
Athikiramundakan	-	-	-	~ 500	OPL-17	1	1
Allikannan	~ 500	OPF-03	1	< 1000	OPL-17	1	2
Vellakkoli	-	-	-	< 500	OPG-19	1	2
	-	-	-	< 1000	OPL-17	1	
Cheruvirippu	1000-1500	OPF-04	1	-	-	-	2
	~ 1500	OPC-15	1	-	-	-	
Choottupokkali	-	-	-	~ 1000	OPD-03	1	1
Chenthadi	-	-	-	< 500	OPK-19	1	1

In the similarity indices, worked out based on Jaccard's coefficient analysis, accessions Chettivirippu and Pokkali 3 had the highest similarity index of 0.825. These accessions formed a cluster with maximum similarity in UPGMA clustering (Fig. 2). These two accessions are the landraces of Ernakulam district suited especially for saline sodic soils of Kerala (Pokkali). The least similarity coefficient (0.451) was observed between Njavara yellow and Choottupokkali. Njavara yellow is the medicinal rice and Choottupokkali is an accession suited for saline soil. Njavara yellow and Njavara black, the two ecotypes of the medicinal rice Njavara, had a similarity coefficient of 0.707. The clustering of these two accessions had a high bootstrap value of 85.

Jaccard's similarity coefficient values were used for the nested clustering of the genotypes to develop dendrogram (Fig. 2). At a similarity coefficient of 59% the accessions clustered into two main clusters. The first cluster (I) consisted of three accessions *viz.* Mundon, Choottupokkali and Vellakkoli while all the other twenty seven accessions formed the second cluster (II).

In the dendrogram, Jeerakasala and Chempan formed a cluster with 80% similarity. These two accessions were collected from two ecological zones of Kerala state, Wayanad and Trivandrum. The two accessions Kannikayama and Kozhivalan from Kannur district clustered at 78% similarity and these accessions also had similar morphological characters. Cheruvirippu and Chettivirippu, both from Ernakulam district suited to saline soils also clustered at 78%. Even though Karuthacheera and Kalladi Aryan clustered at 78% these are from two different ecological zones *viz.*

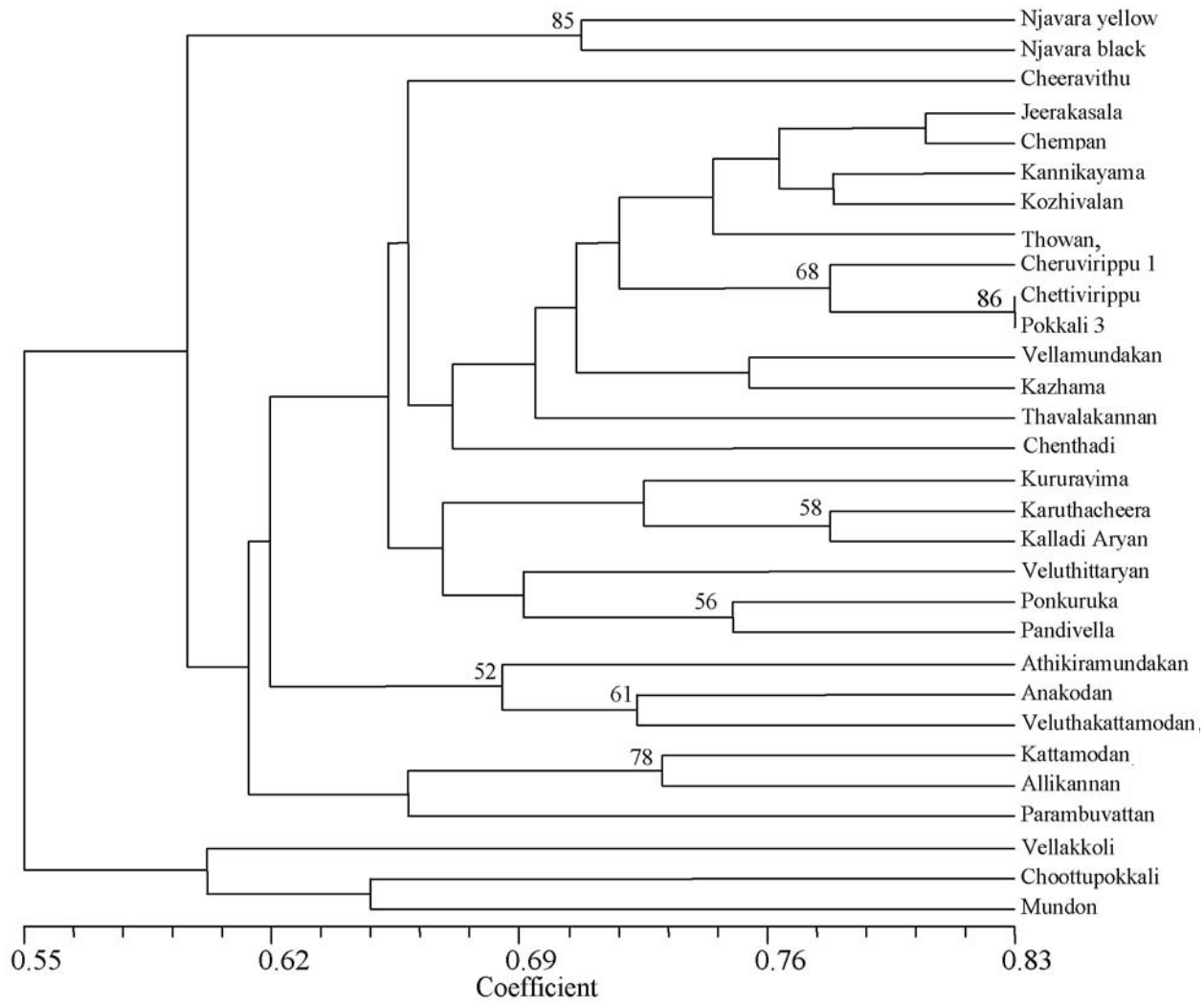

Fig. 2. Dendrogram of rice cultivars based on RAPD markers (Bootstrap values above 50 written at the nodes)

Perumbavur (Central Kerala) and Kannur (North Kerala). Ponkurukka and Pandivella which clustered at 75% are from nearby locations, Ernakulam and Trivandrum. At 73% similarity Kururayima from Kannur district clustered with Karuthacheera from Perumbavur and Kalladi Aryan from Kannur. But the other accession from Kannur district did not show much similarity with these. Accessions Kattamodan and Allikannan, which clustered at 73% with a bootstrap value of 78, were also from two different ecological zones, Palakkad and Kannur. Anakodan and Veluthakattamodan clustered at 72%. These accessions are also from Palakkad and Kannur, respectively. Vellamundakan from Allepey district and Kazhama from Kannur district clustered at 76%. But Athikiramundakan the other accession from Allepey apart from Njavara Yellow, the medicinal rice from Allepey, was distant from Vellamundakan. This shows that the accessions from a single location were distinct from each other and the collections were representative of the ecological zone at microlevel.

The principal co-ordinate analysis gave a better picture of the groupings. In this the medicinal rice accessions njavara yellow and njavara black were distinctly placed (Fig. 3)

Location Specific Similarity

In order to assess the location specific similarity of the accessions the similarity indices of the 30 accessions were grouped. The 30 accessions were collected from nine locations in Kerala State (Fig. 4). Of this Thrissur, Perumbavur and Malappuram had only one accession each. Wayanad and Trivandrum had two accessions each. Similarity indices within each location with more than two accessions were grouped for analysis. The two accessions from Wayanad, Jeerakasala and Chenthadi, showed a similarity of 67.6%. The accession from Trivandrum, Pandivella and Chempan, showed a similarity of 65.2%. Within the accessions from Ernakulam district only Pokkali 3 and Chettivirippu had above 80% similarity. Within the accessions of Kannur district Kannikayama and Thowan, Kannikayama and Kozhivalan, Thowan and Kozhivalan, Thowan and Kazhama, and Kozhivalan and Kazhama had above 70% similarity. Among the accessions within Palakkad and Allepey district, none had similarity above 70%. In this study no marker was obtained for grouping the accessions within the location.

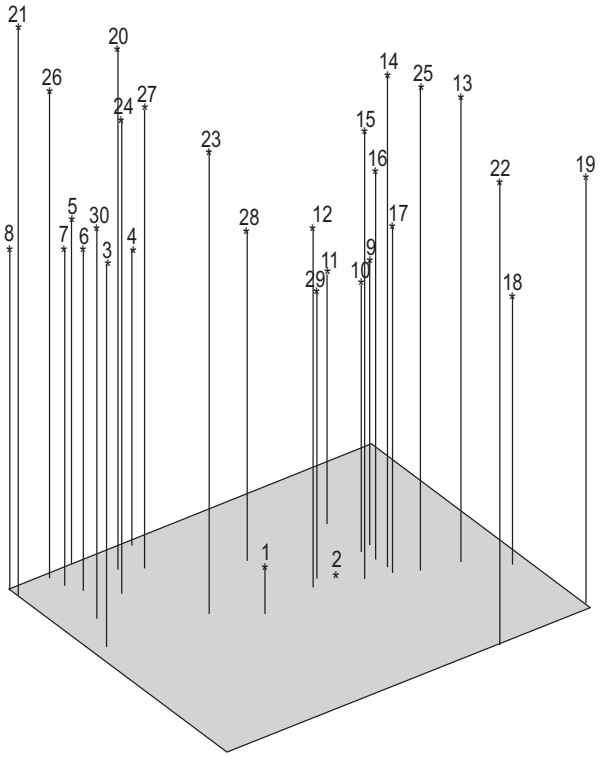

Fig. 3. Ordination 3D Projection of the rice genotype

A1 = Njavara yellow, A2 = Njavara black, A3 = Cheeravithu, A4 = Jeerakasala, A5 = Chempan, A6 = Kannikayama, A7 = Thowan, A8 = Kozhivalan

A9 = Kururayima, A10 = Karuthacheera, A11 = Kalladi Aryan, A12 = Veluthittaryan, A13 = Athikiramundakan, A14 = Anakodan, A15 = Veluthakattamodan,

A16 = Kattamodan, A17 = Allikannan, A18 = Parambuvattan, A19 = Vellakkoli, A20 = Vellamundakan, A21 = Cheruvirippu, A22 = Choottupokkali

A23 = Chenthadi, A24 = Kazhama, A25 = Mundon, A26 = Chettivirippu, A27 = Pokkali 3, A28 = Ponkuruka, A29 = Pandivella, A30 = Thavalakannan

RAPDs for Kernel Colour

Kernel colour or seed coat colour in rice is a complex character and the accessions in this study included 19 accessions with brown seed coat colour, two with white, three with light brown and six with red seed coat colour (Table 1) Vanaja *et al*. (2008) reported that with primer OPK-14 a bright thick amplicon of size 1000 bp was detected in 50% of white kernelled varieties, which were totally absent in red kernelled varieties. In the present study with the primer OPK-14 an amplicon at 1000 bp was detected but the total absence of the amplicon in red kernelled accessions was not seen (Fig. 5). The variation in seed coat colour as red, brown, light brown and white shows

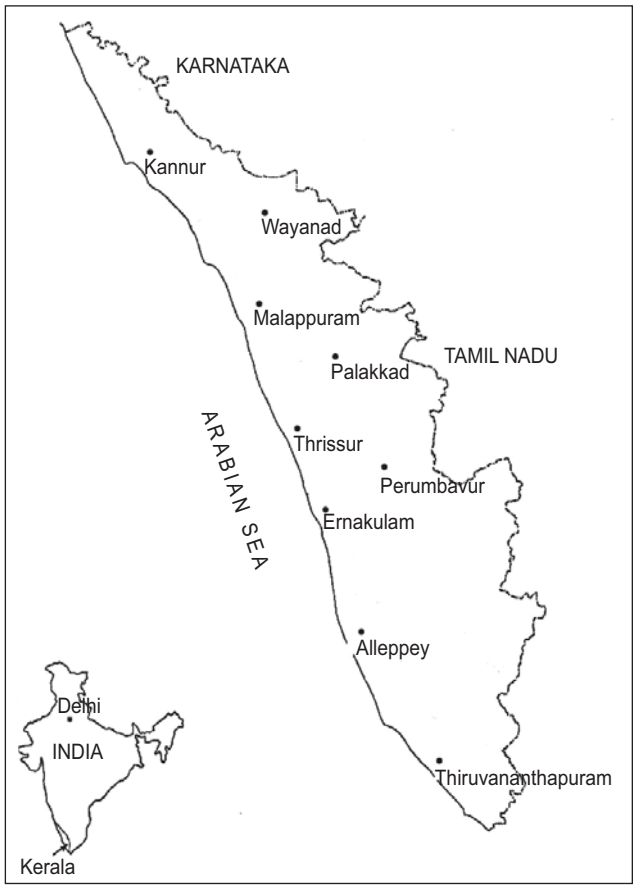

Fig. 4. Locations of the sites of germplasm collections used in Kerala

that this character kernel colour in rice is controlled by more than one gene or alleles so tagging this gene requires more studies by screening large germplasm with robust markers.

A good germplasm collection can offer diverse sources of gene donors in plant breeding programmes and thereby result in higher level of diversity in the cultivated varieties and agricultural systems. The 30 genotypes used in this study were collected from different locations of Kerala under project on "Sustainable Management of Biodiversity". The molecular diversity analysis of these accessions showed that the accessions were representative of the location specific micro environment and the molecular diversity is high enough for use in crop improvement programmes.

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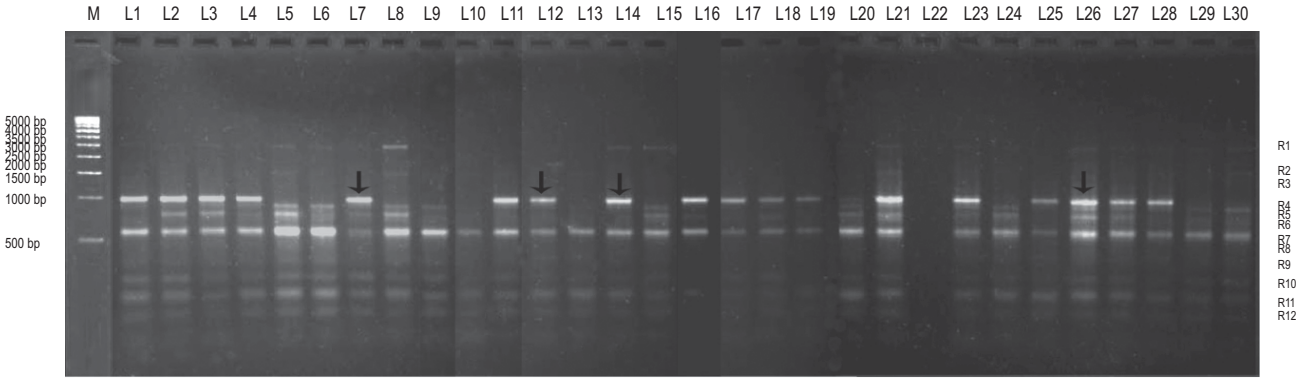

Fig. 5. Amplification profile of the DNA of 30 rice accessions using the primer OPK-14

↓– Red Kernelled accessions with amplicon at 1000bp

L1 = Njavara yellow, L2 = Njavara Black, L3 = Cheeravithu, L4 = Jeerakasala, L5 = Chempan, L6 = Kannikayama, L7 = Thowan, L8 = Kozhivalan, L9 = Kururayima, L10 = Karuthacheera, L11 = Kalladi Aryan, L12 = Veluthittaryan, L13 = Athikiramundakan, L14= Anakodan, L15 = Veluthakattamodan, L16 = Kattamodan, L17 = Allikannan, L18 = Parambuvattan, L19 = Vellakkoli, L20 = Vellamundakan, L21= Cheruvirippu, L22 = Choottupokkali, L23 = Chenthadi, L24 = Kazhama, L25 = Mundon, L26 = Chettivirippu, L27 = Pokkali, L28 = Ponkuruka, L29 = Pandivella, L30 = Thavalakannan

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