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Genetic relationships among a collection of Indica rice (*Oryza sativa*) genotypes of Kerala revealed by SSR markers

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ABSTRACT

A study was conducted during 2005 to understand genetic relationship at molecular level among a collection of rice (*Oryza sativa* L.) varieties and land races of Kerala using SSR markers. Twenty eight SSR markers were used to screen the genotypes. The 28 SSR markers loci revealed 73 alleles in 76 cultivars with an average of 2.61 alleles/locus. The number of alleles/locus varied between 1 and 8. Out of 28 SSR markers, 4 were monomorphic hence percentage polymorphism was 85.7. The size of allele amplified among the 76 cultivars varied from 130 to 1 180 bp. Cluster analysis was done using pair-wise Jaccard's similarity co-efficient which grouped 76 rice genotypes into 4 major clusters. Whereas 1 scented land race of Kerala, namely 'Gandhakasala' and the brown plant hopper resistant variety 'Ptb 20' were not grouped with any cluster. The genotypes 'Vytilla 2', 'Ptb 20', 'Deepti' and 'Malakkaran' were grouped in different clusters and observed to be genetically distant.

Key words: Cluster analysis, Genetic diversity, Jaccard's similarity index, Rice, SSR markers

Narrow genetic diversity in the existing rice (*Oryza sativa* L.) varieties is problematic in breeding for adaptation to biotic and abiotic stresses. Therefore it is necessary to investigate the genetic diversity in rice germplasm to broaden the genetic variation in future rice breeding. Morphological traits can be used for assessing genetic diversity but are often influenced by the environment. Similarly Isozyme analysis represents only a part of the genome. The use of molecular markers for the evaluation of genetic diversity is receiving lot of attention.

Among the polymerase chain reaction (PCR)-based markers, SSR markers are becoming more popular, both for genetic diversity and breeding research as they are robust markers compared to RAPD and AFLP markers. Certain rice varieties of Kerala are well known as a source of valuable genes for certain biotic and abiotic stresses and for red kernel colour. But at molecular level rice varieties of Kerala has not been well studied. Hence a genetic relationship among 76 rice genotypes, which included 67 genotypes from Kerala including land races was studied using SSR markers for selection of parents for recombination as well as heterosis breeding. Certain land races of Kerala included in this study have therapeutic value, some are scented rice and some are tolerant to salinity.

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MATERIALS AND METHODS

The materials consisted of 51 released varieties (both pure lines and hybrid derivatives), 14 land races and 2 advanced breeding lines of Kerala, 7 released varieties from other parts of India and 2 well known varieties from IRRI, Philippines. The details of genotypes are given in Table 1. Seeds of the 76 rice genotypes were germinated and grown under aseptic conditions at about 30°C in the greenhouse of NBPGR. Genomic DNA was extracted young seedling following the protocol of Doyle and Doyle (1990).

Twenty-three STMS markers and 5 STS markers were used for screening 76 rice genotypes. The details of each marker loci are given in Table 2. Forward and reverse primers were synthesized according to information given in original papers (Temynkh *et al*. 2001, Katiyar *et al*. 2001, Murai *et al*. 2001, Liu *et al*. 2002).

Polymerase Chain Reaction (PCR) was performed in a Perkin Elmer 9600 Thermal Cycler programmed for 35 cycles of standardized cycling conditions as described by Temnykh *et al*. 2001. The basic profile was 5 min. at 94°C, 35 cycles of 1 min. at 94°C, 1 min. at 55°C, 61°C or 67°C (depending upon primer amplification temperature), were used to amplify specific primer sets, and primer extension at 72°C for 2 min. This was followed by a final extension step at 72°C for 7

Table 1 Rice genotypes used for SSR analysis

Rice genotypes	Variety/land race/ breeding line	Specific features	Geographical source
‘Ptb 7’	Variety	Photo insensitive, red kernel, resistant to gallmidge, stem borer and foot rot and withstands drought	Kerala, South India
‘Ptb 21’	Variety	Photo sensitive, red kernel, resistant to gallmidge, stem borer and 3 bio-types of BPH	Kerala, South India
‘Panchami’	Variety	Red kernel, resistant to gallmidge biotype 5	Kerala, South India
‘Uma’	Variety	Red kernel, resistant to gallmidge biotype 5 and BPH	Kerala, South India
‘Ptb 10’	Variety	Photo insensitive, red kernel, resistant to gallmidge, stem borer and BPH and high photosynthetic efficiency	Kerala, South India
‘Annapoorna’	Variety	Photo insensitive, red kernel susceptible to BPH, blast and sheath blight	Kerala, South India
‘Ptb 15’	Variety	Photo sensitive, white kernel, moderately resistant to low flooding	Kerala, South India
‘Triveni’	Variety	Photo insensitive, white kernel, moderately resistant to BPH, susceptible to blast and sheath blight	Kerala, South India
‘Karthika’	Variety	Photo insensitive, red kernel moderately resistant to BPH, sheath blight, sheath rot and bacterial blight	Kerala, South India
‘IR 8’	Variety	Resistant/tolerant to blast	IRRI, Phillippines
‘Ptb 20’	Variety	Photo sensitive, red kernel, resistant to brown plant hopper	Kerala, South India
‘Bhadra’	Variety	Weakly photo sensitive, red kernel, resistant to brown plant hopper but susceptible to sheath blight	Kerala, South India
‘Culture 34’	Breeding line		Kerala, South India
‘Culture34s’	Breeding line		Kerala, South India
‘Jyothi’		Photo insensitive, red kernel, moderately resistant to BPH, but susceptible to blast and blight	Kerala, South India
‘IR 36’	Variety	Resistant to gallmidge	IRRI, Phillippines
‘Kairaly’	Variety	Photo insensitive, red kernel, moderately resistant to blast, sheath blight, gall midge and leaf folder	Kerala, South India
‘Aiswarya’	Variety	Photo insensitive, red kernel, moderately resistant to BPH, gall midge, blast	Kerala, South India
‘Pavizham’	Variety	Photo insensitive, red kernel, moderately resistant to BPH, sheath blight, sheath rot and stack burn	Kerala, South India
‘Kanchana’	Variety	Photo insensitive, red kernel, moderately resistant to blast, brown spot and thungro virus	Kerala, South India
‘Jaya’	Variety		South India
‘Ptb 33’	Variety	Photo sensitive, red kernel, resistant to BPH, thrips, leaf roller and sheath blight	Kerala, South India
‘Aruna’	Variety	Photo insensitive, red kernel, moderately resistant to sheath blight, BPH, stem borer, gall midge, brown spot and bacterial leaf blight	Kerala, South India
‘Remya’	Variety	Photo insensitive, red kernel, resistant to BPH, moderately resistant to sheath blight, sheath rot, blast and gall midge	Kerala, South India
‘Ptb 8’	Variety	Photo insensitive, red kernel, resistant to green leaf hopper	Kerala, South India
‘Renjini’	Variety	Red kernel, resistant to BPH and blast, moderately resistant to sheath blight, sheath rot and stem borer	Kerala, South India
‘Revathy’	Variety	Red kernel,resistant to BPH	Kerala, South India
‘Kanakom’	Variety	Photo insensitive, red kernel, resistant to BPH, moderately resistant to sheath blight, sheath rot, stem borer, gall midge, blast and bacterial leaf blight	Kerala, South India
‘Remamika’	Variety	Photo insensitive, red kernel, resistant to BPH, moderately resistant to gall midge, biotype 5	Kerala, South India
‘Basmati’	Variety		North India
‘Basmati-IC298157’	Variety		North India
‘Gandhakasala’	Land race	Aromatic land race	Kerala, South India
‘Jeerakasala’	Land race	Aromatic land race	Kerala, South India
‘Nivara-red’	Land race	Medicinal rice	Kerala, South India
‘Nivara-black’	Land race	Medicinal rice	Kerala, South India
‘Chitteni’	Land race		Kerala, South India
‘Kayama’	Land race	Have good cooking quality	Kerala, South India

(Contd...)

(Table 1. concluded)

Rice genotypes	Variety/land race/ breeding line	Specific features	Geographical Source
'Malayudumban'	Land race		Kerala, South India
'Mavilan'	Land race	Land race	Kerala, South India
'Malakkaran'	Land race	Have brown ring at inter-nodal region	Kerala, South India
'Puncha'	Land race		Kerala, South India
'Allikkannan'	Land race	Have good cooking quality	Kerala, South India
'Kuthiru'	Land race	Tolerant to salinity	Kerala, South India
'Orkayama'	Land race	Tolerant to salinity	Kerala, South India
'Rajkayama'	Land race	Tolerant to salinity, good cooking quality and aroma	Kerala, South India
'Vytilla 1'	Variety	Saline resistant	Kerala, South India
'Vytilla 2'	Variety	Saline resistant	Kerala, South India
'Vytilla 3'	Variety	Saline resistant	Kerala, South India
'Vytilla 4'	Variety	Saline resistant	Kerala, South India
'Vytilla 5'	Variety	Saline resistant	Kerala, South India
'Onam'	Variety	Photo insensitive, red kernel, moderately resistant to blight, blast and tolerant to drought in the early stage	Kerala, South India
'Kumbam'	Variety	Photo sensitive, red kernel, tolerant to lodging	Kerala, South India
'Sagara'	Variety	Photo sensitive, red kernel, moderately resistant to stem borer,sheath blight and blast but susceptible to leaf roller, tolerant to salinity and flood	Kerala, South India
'Deepthi'	Variety	Suitable to high ranges	Kerala, South India
'WND 1'	Variety	Suitable to high ranges	Kerala, South India
'WND 2'	Variety	Suitable to high ranges	Kerala, South India
'Suraksha'	Variety		North India
'Pranava'	Variety		North India
'Ajaya'	Variety		North India
'Triguna'	Variety		North India
'Ptb 1'	Variety	Photo sensitive, red kernel, moderately resistant to blast	Kerala, South India
'Ptb 4'	Variety	Photo sensitive, red kernel, moderately resistant to water logging	Kerala, South India
'Ptb 5'	Variety	Photo insensitive, red kernel, lmoderately resistant to major pests and diseases	Kerala, South India
'Ptb 14'	Variety	Photo insensitive, white kernel	Kerala, South India
'Ptb 16'	Variety	Photo sensitive, white kernel, moderately resistant to blast	Kerala, South India
'Ptb 22'	Variety	Photo insensitive, red kernel, moderately resistant to blast	Kerala, South India
'Ptb 25'	Variety	Photoinsensitive, red kernel, moderately resistant to blast	Kerala, South India
'Ptb 28'	Variety	Photo insensitive, red kernel, moderately resistant to blast and drought	Kerala, South India
'Ptb 2'	Variety	Photo sensitive, red kernel	Kerala, South India
'Ptb 3'	Variety	Photo sensitive, red kernel, moderately resistant to drought	Kerala, South India
'Ptb 6'	Variety	Photo sensitive, red kernel, moderately resistant to major pests and diseases	Kerala, South India
'Ptb 9'	Variety	Photo insensitive, red kernel, moderately resistant to major pests and diseases	Kerala, South India
'Ptb 17'	Variety	Photo insensitive, red kernel, moderately resistant to blast	Kerala, South India
'Ptb 18'	Variety	Photo sensitive, red kernel, moderately resistant to blast	Kerala, South India
'Ptb 19'	Variety	Photo sensitive, red kernel, moderately resistant to sheath blight and gall fly	Kerala, South India
'Ptb 26'	Variety	Photo insensitive, red kernel, moderately resistant to blast	Kerala, South India

min. followed by storage at 4°C until electrophoresis. PCR products were separated on 3% (W/V) metaphore agarose gel prepared in 1×TBE buffer (*p*H 8.0) stained with gel star dye @ 0.5 mg/ml. The amplification products were visualized under UV transilluminator and photographed using Polaroid 665 film. Scoring was done for size of the amplified products based on the molecular weight markers.

For each marker system, the amplified fragments were scored for their presence (1) and absence (0). Genetic similarity values were computed based on Jaccard's similarity co-efficient using NTSYS-PC version 1.70. Cluster analyses were performed based on similarity matrices using UPGMA (unweighed pair group method with arithmetical averages) and relationships between cultivars were visualized as dendrograms. Polymorphism Information Content (PIC) value of each marker was also calculated.

RESULTS AND DISCUSSION

Twenty-eight primer pairs were used for the fingerprinting of 76 cultivars of rice amplified 1–8 loci/primer pair (Table 3). PIC values for each marker was calculated and it varied from 0.0 to 0.92 (Table 3). The 28 SSR marker loci

Table 2 Maker ID, rice chromosome number and references of SSR markers used in the study

Marker ID	Rice chromosome	Anealing temperature (°C)	Specific character (gene responsible)	Reference for primer
RM 226	1	55	Not specified	Temnykh *et al*. (2001)
RM 165	1	67	Not specified	Temnykh *et al*. (2001)
RM 102	1	61	Not specified	Temnykh *et al*. (2001)
RM 112	2	55	Not specified	Temnykh *et al*. (2001)
RM 145	2	67	Not specified	Temnykh *et al*. (2001)
RM 154	2	61	Not specified	Temnykh *et al*. (2001)
RM 135	3	61	Not specified	Temnykh *et al*. (2001)
RM 132	3	61	Not specified	Temnykh *et al*. (2001)
RM 335	4	55	Not specified	Temnykh *et al*. (2001)
RM 185	4	61	Not specified	Temnykh *et al*. (2001)
RG 476	4	55	Gall midge resistance [*Gm 6*(*t*)]	Katyar *et al*. (2001)
RG 329	4	61	Gall midge resistance [*Gm 6*(*t*)]	Katyar *et al*. (2001)
RM 146	5	55	Not specified	Temnykh *et al*. (2000)
RM 122	5	55	BLB resistance	Blair and Mc Couch (1997)
RM 103	6	55	Not specified	Temnykh *et al*. (2001)
RM 150 C	6	61	Not specified	Temnykh *et al*. (2001)
pB8	6	61	Blast resistance [*pi2* (*t*)]	Liu *et al.* (2002)
RM 180	7	55	Not specified	Temnykh *et al*. (2001)
RM 182	7	67	Not specified	Temnykh *et al*. (2001)
RM 149	8	55	Not specified	Temnykh *et al*. (2001)
RG 136	8	55	BLB resistance [*Xa 13*]	Sanchez *et al*. (1999)
RM 189	9	61	Not specified	Temnykh *et al*. (2001)
RM 105	9	55	Not specified	Temnykh *et al*. (2001)
RM 171	10	55	Not specified	Temnykh *et al*. (2001)
RM 181	11	67	Not specified	Temnykh *et al*. (2001)
OSR 1	11	55	Not specified	Akagi *et al*. (1996)
OSR 20	12	55	Not specified	Akagi *et al*. (1996)
KAM 4	12	61	BPH resistance [*bph 2*]	Murai *et al*. (2001)

revealed 73 alleles in 76 cultivars. The average number of alleles/ locus was 2.61. Panaud *et al*. (1996) detected 2 to 9 alleles for micro satellite markers in 22 Japonica and Indica cultivars and Yang *et al*. (1994) reported 3 to 25 alleles for 10 microsatellite markers among 238 accessions of Indica and Japonica cultivars and land races. The two OSR prefixed markers, viz OSR 1 and OSR 20 used in this study amplified 3 and 4 alleles, respectively (Fig 1). Allelic variation of 7 for OSR 1 and 5 for OSR 20 was reported by Akagi *et al*. (1997) in a collection of 59 Japonica cultivars. In the present study OSR 20 was more polymorphic than OSR 1 unlike in Akagi's study which indicates the difference in polymorphism level between Japonica and Indica rice cultivars with respect to OSR prefixed micro satellite markers. Out of 28 SSR markers, 24 markers showed polymorphism within the 76 genotypes and rest 4 were monomorphic. The allelic variation detected by OSR 20 and RM 180 are shown in Figs 1 and 2, respectively.

RM 135, based on a poly (CGG) trinucleotide repeat and RM 189, based on a poly (AT) dinucleotide repeat, had the largest allele size range (100–1000bp and 250–1100bp respectively) and the highest number of alleles (8 and 7 respectively). The allele (1000bp) with RM 135 marker loci was detected for the varieties 'Aiswarya', 'Kanchana',

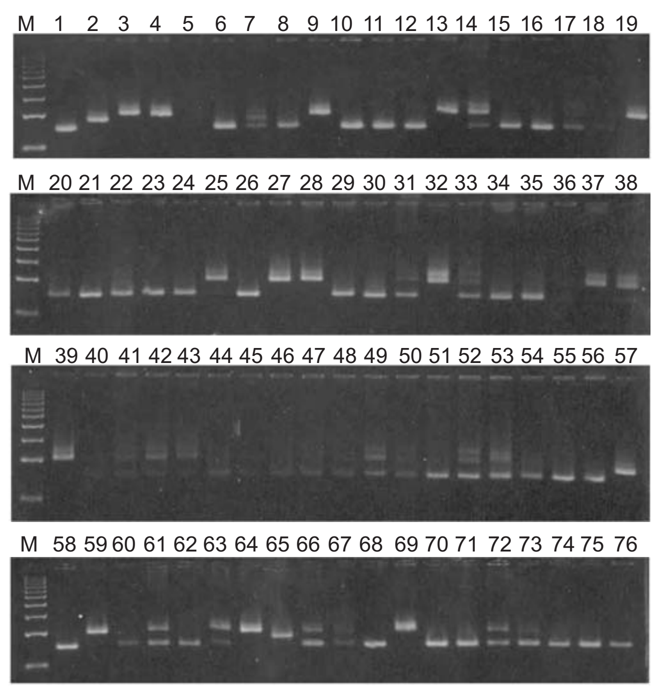

Fig 1. Finger print pattern generated in 76 rice genotypes using the STMS marker OSR 20 (*see* Table 1 for details of lanes 1 to 76)

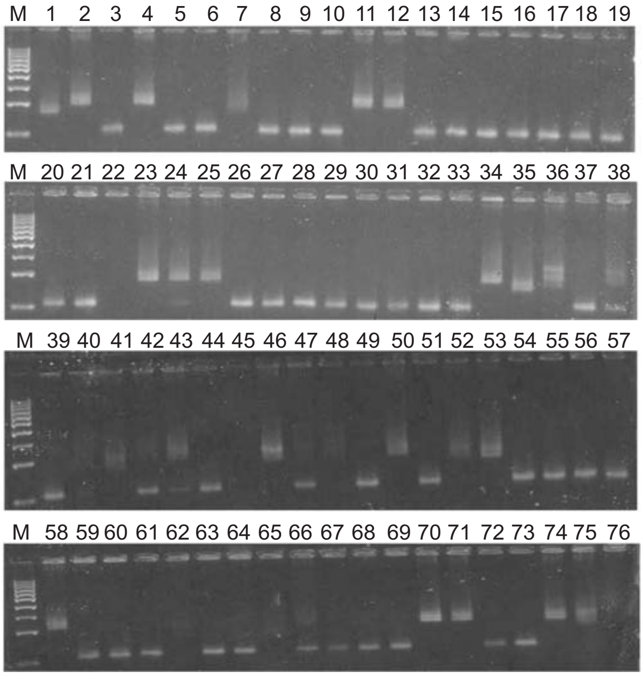

Fig 2 Discrimination of 4 alleles detected in the STMS RM180 among 76 rice genotypes (*see* Table 1 for details of lanes 1 to 76)

'Njavara'-red, 'WND 2' and 'Basmati'. These rice genotypes would be of interest to include in an expanded standard germplasm set when testing allele size ranges prior to the multiplexing of microsatellites. The land races, 'Chitteni', 'Malakkaran', 'Puncha', 'Orkayama' and 'Rajkayama' possess small size micro satellite alleles only of size 70bp detected by RM 132. 'Njavara'-red (the medicinal land race) and the land races 'Malayudumban' and' Mavilan' possess only largest alleles.

The Polymorphic Information Content (PIC) values are related to the number of alleles and allele combinations. As a measure of the informativeness of SSR loci, the average PIC value was with the range of '0' to 0.927. High gene diversity within 76 indica genotypes had been observed for the marker loci pB8, RM 150, RM 185, and RM 102 as 0.927, 0.834, 0.816, 0.816, respectively demonstrating the rich source of bio-diversity in rice genotypes of Kerala (Table 3). The highly polymorphic SSR marker loci identified in the present study might be useful for identifying closely related rice cultivars. The percentage of amplification of PCR products among 22 STMS markers ranged from 32.9 to 100 and among STS markers the range was 14.5–97.4.

A dendrogram was constructed based on Jaccard similarity co-efficient of 76 rice genotypes and is presented in Fig 3. Jaccards similarity co-efficient based on all the 28 primers ranged from 0.27 to 0.88 with an average similarity of 0.54. At 55% similarity index 76 rice genotypes were grouped into 4 major clusters. 'Ptb 20', which is a brown plant hopper resistant variety of Kerala was most distinct genotype and it

Table 3 Number of alleles, PIC values, percentage of amplification and allele size range found among 76 indica rice genotypes

Marker ID	No. of alleles detected	PIC value	Amplification (%)	Allele size range (bp)
RM 226	4	0.503	97	200 – 300
RM 165	2	0.467	97.4	200 – 220
RM 102	2	0.816	32.9	480 – 500
RM 112	1	0	100	130
RM 145	3	0.636	85.5	160 – 200
RM 154	3	0.662	98.7	180 – 200
RM 135	8	0.783	98.7	100 – 1 000
RM 132	2	0.546	93.4	70 – 85
RM 335	4	0.641	94.7	95 – 170
RM 185	4	0.816	63.2	190 – 400
RG 476	3	0.746	76.3	790 – 850
RG 329	2	0.723	55.3	1 000 – 1 100
RM 146	1	0	100	350
RM 122	2	0.467	97.4	220 – 230
RM 103	1	0	100	350
RM 150 C	3	0.834	36.8	200 – 350
PB8	2	0.927	14.5	500 – 550
RM 180	4	0.747	92.0	110 – 200
RM 182	1	0.092	90.8	375
RM 149	2	0.401	100	250 – 270
RG 136	3	0.768	69.7	1 000 – 1 100
RM 189	7	0.741	100	250 – 1 100
RM 105	4	0.783	81.6	140 – 170
RM 171	4	0.658	94.7	370 – 400
RM 181	3	.632	96	275 – 300
OSR 1	3	0.663	96.	250 – 1 180
OSR 20	4	0.608	96.1	160 – 230
KAM 4	2	0.783	43.4	300 – 350

shared only 37% similarity index with other rice genotypes and could not be grouped in to any cluster. Similarly at 43% similarity index, 'Gandhakasala', a land race from high range of Kerala was also distinctly grouped. In cluster 1, 5 genotypes are grouping together out of which 4 varieties are from outside Kerala and 'Triveni' variety from Kerala is grouping in this cluster probably due to 'TN 1', the ancestral parent of 'Triveni.' 'TN 1', might have played a role in the clustering of 'Triveni' along with varieties of different geographical origin. In cluster 2, fourteen genotypes are grouping together. This cluster can be further sub-divided in to cluster 2a and cluster 2b. Cluster 2b consisted of 5 genotypes and all are saline resistant varieties developed in Kerala ('Vytila 1' to 'Vytila 5'). This is in consistent with the result of Vanaja *et al.* (2003) based on morphological characters, indicating that the SSR markers used in the present study may be the flanking markers of some of the morphological characters. Cluster 2a consisted of 9 genotypes which included 5 varieties from Pattambi Rice Research Station, Kerala and 4 land races of Kerala. Hence these land races, viz 'Puncha', 'Chitteni',' Malakkaran' and 'Rajkayama' may have common genetic characters with the

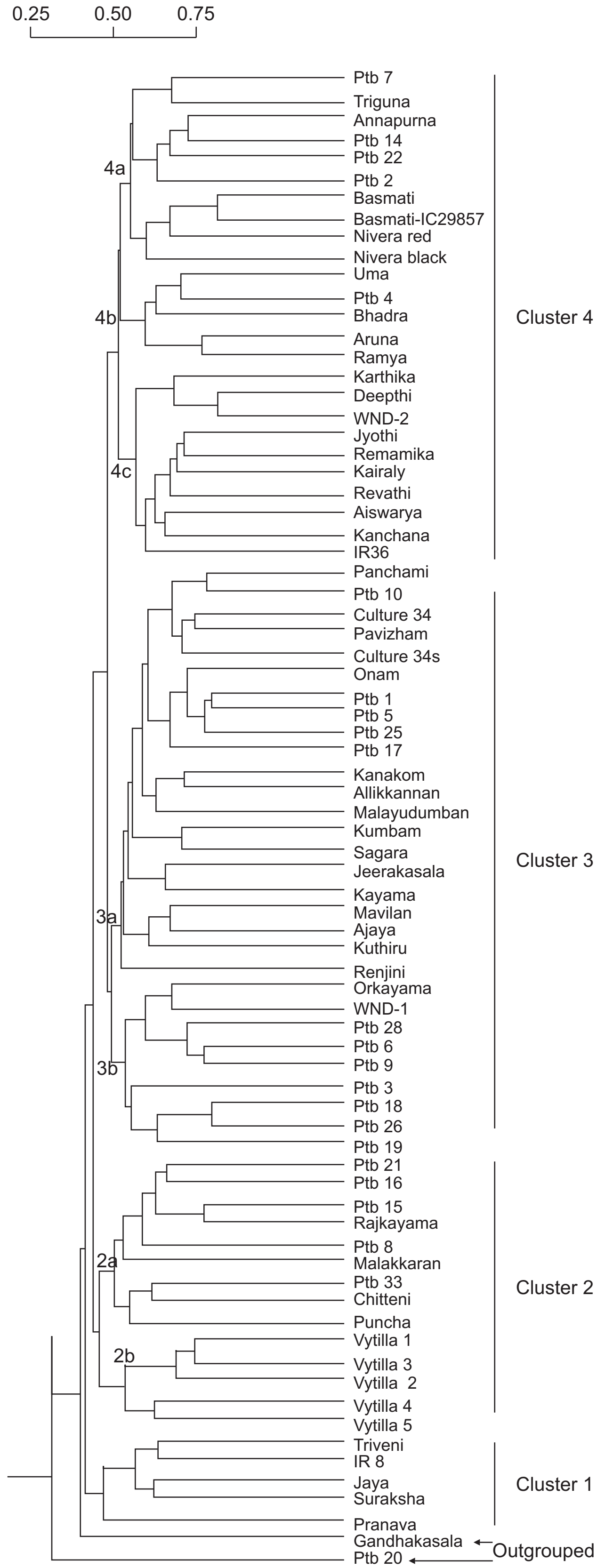

Fig 3 Dendogram generated for 76 genotypes of rice based on 28 SSR markers

Pattambi varieties namely, 'Ptb 33', 'Ptb 8', 'Ptb 15', 'Ptb 16 'and 'Ptb 21'. In cluster 3, thirty genotypes are grouping together and this is the largest cluster. This cluster can be further sub-grouped in to 3a and 3b. In cluster 3b 9 genotypes are grouping together out of which 7 genotypes are from Pattambi Rice Research Station of Kerala. In cluster 3a, 14 varieties/cultures from different rice research stations of Kerala were grouped together along with one North Indian Variety, 'Ajaya' and 6 land races from Kerala showing the genetic similarity of these rice genotypes. Cluster 4 consisted of 25 genotypes and can be further sub-grouped into 3 sub-clusters. Sub-cluster 4 c consisted of one IRRI variety 'IR 36', 4 Pattambi varieties, 3 Mancompu varieties, and 2 high range varieties ('WND 2', 'Deepthi'), whereas sub-cluster 4b consisted of 4 Mancompu varieties along with 1 Pattambi variety 'Ptb 4'. Sub cluster 4a consisted of 10 genotypes which includes 2 medicinal land races from Kerala ('Njavara' red and 'Njavara' black), 5 Pattambi varieties, 2 'Basmati' accessions and one North Indian variety 'Triguna'.

The aromatic land races of high range areas of Kerala, 'Gandhakasala' and 'Jeerakasala' were grouped in different clusters of similarity index 48 and 72% respectively. When 'Gandhakasala' clustered as distinct, 'Jeerakasala' grouped along with a land race of low range area. The overall clustering pattern leads to the inference that, factors other than geographical diversity might be responsible for grouping and there was no similarity between geographical distribution and genetic diversity (Singh *et al.* 1980). Similar observations of non-correspondence of genetic divergence and geographical diversity based on morphological characters had been made by Roy and Panwar (1993), and Vanaja *et al.* (2003).

The advanced generation cultures, Culture 34 and Culture 34s from Kerala have the same pedigreee as 'Karthika' female parent and 'Bhadra' male parent. But these 2 cultures clustered in 2 different sub-clusters with similarity index 83 and 78% respectively. This indicates their distinctness from each other even though having same pedigree. Similar results were reported by Selvakumar *et al.* (1989), Mall and Maurya (1992) and Vanaja *et al.* (2003) based on morphological characters. At the same time, the varieties 'Aruna' and 'Remya' having the same parentage of 'Jaya' and 'Ptb 33' were grouped in a single cluster. But on the basis of morphological characters, Vanaja *et al.* (2003) reported the clustering of these varieties in different groups. Similarly the varieties 'Kairaly', 'Aiswarya' and their parents 'IR 36' and 'Jyothi' were grouped in a single cluster 4c. This result is in consistent with the report of Bansal *et al.* (1990) who suggested that clustering pattern was influenced by the pedigree of the breeding lines. The parents and progenies of all other pedigrees analyzed in this study were grouped in different clusters.

The varieties 'Kanchana', 'Jyothi' and 'Karthika' which were grouped in the 4c cluster, based on molecular markers were also grouped together in a single cluster based on morphological characters (Vanaja *et al.* 2003). This also confirms the co-linearity between the morphological characters evaluated and marker alleles detected in the present study.

The magnitude of heterosis was largely depended on the degree of genetic diversity in the parental lines. The greater the distance between the clusters, the wider will be the genetic diversity between progenies. In the present study the maximum genetic distance was observed between the varieties 'Vytilla 2' and 'Ptb 20' with genetic distance 0.75, followed by 'Deepthi' and 'Malakkaran' with genetic distance 0.72. All these genotypes are from Kerala. For pedigree breeding, intercrossing between those parents from same geographic region which were divergent among themselves are more desirable than choosing the parents from other regions (Kumar and Subramanian 1992). Hence the varieties 'Vytilla2', 'Ptb 20', 'Deepthi' and 'Malakkaran' can be utilized for heterosis breeding as well as for recombination breeding.

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