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Molecular characterization of farmers' varieties of rice (*Oryza sativa*)

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ABSTRACT

A study was carried out for characterization of 60 farmers' varieties using grain characters and 40 SSR (Simple Sequence Repeat) markers. Only a set of 8 (20%) primers were polymorphic yielding 16 bands (alleles) among these 60 varieties. The size of amplicons ranged from 120 bp (RM238) to 240 bp (RM551). The number of alleles per microsatellite ranged from 1 (RM238, RM119 and RM120) to 3 (RM259, RM234 and RM551) with an average of 2 alleles per locus. Major allele frequency ranged from 0.51 to 0.97 averaging 0.74. Genetic diversity ranged from 0.045 to 0.588 with a mean of 0.34. Considering the entire genotypic array, the mean value for polymorphism information content (PIC) for all microsatellites was 0.27. Microsatellite RM122 with 2 alleles had the maximum PIC value (0.37) and the microsatellite RM205 with 2 alleles had the minimum (0.27) value. The UPGMA cluster analysis grouped varieties into three main clusters with 58% genetic similarity and homing 24, 23 and 13 varieties, respectively. Grain characteristic-based clustering was better than molecular markers as accessions were in expected cluster. However, few accessions showed scattering to other sub-clusters. The diversity analysis revealed the distinct nature of farmers' varieties in a large number of clusters indicating greater diversity, which could be exploited in breeding programmes to combine grain traits and early vigor.

Key words: Farmers' varieties, Rice, SSR markers, Seed characteristics

The Farmer's variety (FV), evolved by farmers/farming communities over several years, exhibits proven special features. Because of repeated propagation, progeny assessment and advancement, the FV is more homogenous and stable with distinct character(s) compared to their wild relatives. Such varieties are identifiable by unique characteristics and with a vernacular name often describing their unique features. The distribution or horizontal spread of such FVs with uniform otherwise distinct trait in their neighbourhood, as an unregistered variety, surmises that there has been a consumer acceptance for the produce. This proves that consumption driven selection was done by the farmers for such varieties (FVs). FV has been defined under the Plant Varieties and Farmers' Rights Act, 2001 as a variety that (i) has been traditionally cultivated and evolved by the farmers in their fields and (ii) is a wild relative or land race or a variety about which farmers possess common knowledge.

Traditional time consuming and expensive Distinctiveness, Uniformity and Stability (DUS) testing procedure involves recording the number of phenotypic characters by growing varieties side by side. Many of the characters used are polygenic or quantitative and their expressions are altered by environmental factors, which essentially require replications of observations. Further, the number of characteristics may not be adequate for sufficient discrimination of farmers' or extant varieties. Thus, it is imperative to find out more rapid and cost effective methods to corroborate this approach. Molecular markers assay the variation in the nucleotide sequences of DNA. Such differences remain unaffected across different growth stages, seasons, locations and agronomic practices. Varietal identification and differentiation, based on the molecular differences, therefore, are more reproducible and objective. The characteristics of a new variety based on DNA patterns can, therefore, be compared with the varieties of common knowledge in any part of the world for establishing its uniqueness. Among the various types of DNA markers, single Sequence Repeats (SSR) are preferred, being highly polymorphic, reproducible, co-dominant and distributed throughout the genome. Many researchers have checked the suitability of STMS markers for DUS testing in rice

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varieties (Singh *et al*. 2004). The present study was designed to characterize sixty farmers' varieties of rice using 40 SSR markers which are distributed across different chromosomes of rice genome.

MATERIALS AND METHODS

Seeds of sixty FVs of rice were collected from the farmers of major rice growing states of the country as well as from the NBPGR, New Delhi (Table 1). Most of the varieties are farmer's varieties, except few like Govind, HMT (PKV), Gontra 1, Gontra 2, Acharmati and MTU but these improved varieties may be considered as FV as these varieties are being grown by farmers for a long period of time. Each FV was raised in three replications at IARI, New Delhi. Phenotypic data for five seed morphological characterizations (seed length, seed width, seed length-width ratio, seed weight and grain color) were recorded on 10 seeds of all 60 lines for two seasons. For the assessment of colour chacteristics the latest Royal Horticultural Society (UK) colour chart (2005) was used. Correlation among the seed characteristics was estimated following standard statistical procedures (Panse and Sukatme 1985).

DNA was isolated according to the protocol of Doyle and Doyle (1990). PCR amplification of 40 markers distributed on all the 12 chromosomes was carried out in a 10 ml reaction mixture as described by Singh *et al.* (2004) with 25 ng template DNA. PCR reactions were in G-STORM thermo-cyclers using the following cycler parameters: initial denaturation at 94°C for 5 min., followed by 35 cycles of denaturation at 94°C for 1 min., annealing at 55°C for 1 min. and synthesis at 72°C for 2 min. and finally extension step of 7 min. at 72°C. The amplified products were electrophoresed on 3% percent metaphor agarose gels and amplicons were visualized under UV light and photographed using Gel documentation system.

A data matrix of 60 FVs from the means of five seed traits was constructed. Phenotype data were standardized first by using the standardization (subtracting the average and dividing by the standard deviation) program of NTSYSpc. Genetic distance (GD) was calculated using Euclidean dissimilarity and hierarchical clustering was done using the Unweighted Pair-Group Method of Arithmetic Averages (UPGMA) using NTSYS-pc 2.02 (Rohlf 1998).

All major DNA fragments were recorded as either 1 or 0 representing the presence or absence of the band, respectively, and data was entered in a binary data matrix as discrete variables. The pairwise genetic similarity coefficient (GS) was calculated using Jaccard coefficient by the SIMQUAL program of NTSYS-pc software version 2.02.In both dendrograms, average coefficient was used as cut-off value to define clusters. The Polymorphic Information Content (PIC) was calculated by applying the formula given in Singh *et al.* (2004).

RESULTS AND DISCUSSION

The assessment of genetic diversity is not only important for crop improvement efforts but also for efficient management and protection of germplasm resources. But the estimate of genetic diversity can be biased both by the choice of marker system and statistical methodology. In present study, we have utilized SSR markers on farmers' varieties to detect molecular variability to support rice breeding program.

Grain morphological characteristics and trait correlation

Among the yield attributes, the most important contributing characters in rice that are taken into consideration for drawing up an indication of grain yielding potential are seed length, seed breadth, seed length-breadth ratio and seed weight, whereas seed is important from consumer point of view. Five most important grain characteristics were studied for all sixty FVs of rice and it was observed that length of decorticated grain was maximum in Bharkhsh (7.648 cm) and was minimum in Kalo Jeero (3.991 mm). However, width of decorticated grain was recorded maximum in Suraniat (3.085 mm) and minimum in Kudarat 2 (1.433 mm), Karadhana showed highest (22.716 g) 1 000 grain weight while it was minimum (8.285 g) for Badshabhog. Grain L/B ratio was lowest in Acharamati (1.62) and highest in Lal Basmati (4.32). Based on grain colour varieties were grouped in four category, viz. White (38), Grey (18), Green (1) and Red (3) (Table 1).

Correlation among grain characters in germplasm revealed significant positive association of 1 000-grain weight with grain length and breadth. But it was negatively correlated with L/B ratio. The association was little stronger with grain breadth than grain length. On the contrary, grain width correlated negatively with L/B ratio. Similar results were earlier reported by many researchers (Vanaja and Babu 2006). Associations among grain characters in segregating populations were earlier reported by Govindaraj *et al*. (2005) and Hagiwara *et al*. (2006). Based on such results, it was concluded that L/B ratio could be effectively modified through selection in grain length and grain breadth (Rabiei *et al*. 2004). To examine the relatedness of morphological characters of seeds, seed length, seed width, 1 000 grain weight (g), L/B ratio and seed colour have often been used by the plant breeders for grouping of rice varieties. The availability of large number of farmers' varieties increased the task as well as the responsibilities of persons involved in seed testing and quality control. The varietal descriptions often relate to plant morphological characteristics but not to seed characters and are not enough to differentiate the variety at seed level. A significant positive correlation was detected between grain width and grain length. Grain weight was significantly positively associated with grain width as well as length, whereas it was negatively associated with L/B ratio. L/B ratio showed significant positive association with grain length and negative association with grain breadth (Table 2).

Marker polymorphism

In India PPV&FR Act, 2001 requires the registration of new varieties, including farmers-bred varieties, based on

Table 1 Grain morphological characteristics of sixty varieties of rice

Varieties	State	Decorticated grain length (mm)	Decorticated grain width (mm)	L/B	1 000 grain ratio	Grain weight(g)	Code color	Shades
Chandanchur	Bihar	6.15	1.94	2.95	14.424	White	159	A
Dhudhiya	Bihar	5.689	3.02	1.94	14.1885	Red	166	B
Ghuma	Bihar	6.005	1.929	3.27	13.774	Red	178	A
Patanjali	Bihar	6.179	2.28	2.74	17.165	Grey	164	B
Punjabia	Bihar	6.157	1.759	3.37	15.355	White	158	A
Shayam Jeera	Bihar	6.03	2	3.03	12.054	White	158	B
Aamachur	Madhya Pradesh	5.307	1.901	2.81	12.529	White	158	A
Balghati Luchai	Madhya Pradesh	6.242	2.05	2.89	16.765	White	159	A
Baspatri	Madhya Pradesh	5.575	3.001	1.87	18.515	White	157	A
Bedsar	Madhya Pradesh	4.835	2.689	1.86	8.933	White	157	A
Bharkhsh	Madhya Pradesh	7.648	1.5	3.54	16.995	White	158	B
Bogganda	Madhya Pradesh	6.36	2.166	2.94	18.378	Green	155	A
Budi Luchai	Madhya Pradesh	6.672	2.708	2.44	13.612	White	158	B
Chatry	Madhya Pradesh	6.346	2.868	2.13	10.474	Grey	161	C
Chipdo	Madhya Pradesh	5.65	2.05	2.75	15.857	Grey	162	C
Dugasuchia	Madhya Pradesh	6.64	2.565	2.53	16.411	White	157	A
Ekbiji	Madhya Pradesh	6.27	2.29	2.78	18.54	White	157	A
Gundro	Madhya Pradesh	5.545	2.295	2.4	14.912	Grey	162	C
Jeera Shankar	Madhya Pradesh	6.687	2.877	2.45	14.599	Grey	161	D
Karadhana	Madhya Pradesh	5.474	2.993	1.85	22.716	Grey	156	A
Kakeria	Madhya Pradesh	6.54	2.766	2.42	19.557	Grey	161	C
Lakna	Madhya Pradesh	5.663	2.767	2.06	16.64	White	158	B
Luchai	Madhya Pradesh	6.467	2.719	2.39	17.765	White	157	A
Nungi	Madhya Pradesh	5.477	2.606	2.17	13.737	White	159	B
Patel 3 (JNKVV)	Madhya Pradesh	5.092	2.04	2.39	10.99	White	159	B
Pili Luchai 1	Madhya Pradesh	5.654	2.811	2.01	12.998	White	157	A
Pili Luchai 2	Madhya Pradesh	5.713	2.777	2.05	15.291	Grey	160	C
Ramker 1	Madhya Pradesh	5.715	2.961	1.95	20.243	White	157	B
Ramker 2	Madhya Pradesh	5.844	2.866	2	19.515	Grey	160	D
Ranikajar	Madhya Pradesh	6.461	2.26	2.84	19.742	Grey	164	B
Safed Luchai 1	Madhya Pradesh	6.24	2.117	2.97	17.575	White	159	A
Safed Luchai 2	Madhya Pradesh	5.925	2.784	2.15	15.18	Grey	156	A
Salti	Madhya Pradesh	5.791	2.905	1.94	20.74	Grey	166	A
Sorna	Madhya Pradesh	6.387	2.974	2.09	19.267	White	157	A
Sulenads	Madhya Pradesh	6.275	3.073	1.96	19.359	White	157	A
Surajone	Madhya Pradesh	6.075	2.65	2.43	19.842	Grey	164	C
Suraniat	Madhya Pradesh	6.166	3.085	1.98	21.101	White	158	B
HMT (PKV)	Maharashtra	4.819	2.336	1.97	9.565	White	159	A
Patel-3	NBPGR, N. Delhi	5.464	2.144	2.63	9.779	Red	178	B
Acharmati	Orisa	4.216	2.48	1.62	11.894	Grey	161	D
Badshabhog	Orisa	4.882	1.959	2.44	8.285	White	158	B
Kala Jeera	Orisa	4.348	2.16	1.9	15.953	Grey	160	D
DPT52	Uttar Pradesh	6.056	2.588	2.3	17.529	Grey	160	C
IET17430 (GONTRA-1)	Uttar Pradesh	6.354	1.99	3.19	16.268	White	158	A
IET19571 (GONTRA-2)	Uttar Pradesh	6.281	2.223	2.72	9.94	White	158	B
Komal	Uttar Pradesh	6.301	2.434	2.55	16.909	White	157	A
Kudarat 2	Uttar Pradesh	6.275	1.433	3.21	9.28	White	158	B
Kudarat 5	Uttar Pradesh	5.759	1.959	3.08	9.774	White	158	A
MTU	Uttar Pradesh	5.578	2.634	2.13	12.26	White	158	A
Sonam	Uttar Pradesh	5.854	2.692	2.17	9.676	White	159	A

Contd.

Table 1. *(Continued)*

Varieties	State	Decorticated grain length (mm)	Decorticated grain width (mm)	L/B	1000 grain ratio	Grain weight(g)	Code color	Shades
Govind	Uttarakhand	6.513	1.824	3.51	19.668	White	159	A
Kalnamak	Uttarakhand	6.226	2.19	2.81	14.66	Grey	160	C
Lal Basmati	Uttarakhand	7.151	1.65	4.32	15.444	White	159	B
Tilak Chandan	Uttarakhand	4.922	2.06	2.41	16.594	White	157	A
HMT	West Bangal	5.284	1.712	3.08	9.845	Grey	161	C
Kalo Jeera	West Bangal	3.991	2.095	1.91	10.636	White	159	A
Kalonunia	West Bangal	5.344	1.61	3.59	12.343	White	159	A
Khasha	West Bangal	4.157	1.676	2.33	9.87	White	158	A
Tulaipanji	West Bangal	5.902	1.765	3.37	13.442	White	158	A
Crossa	-	5.66	2.144	2.68	18.678	White	158	A
Mean		5.831	2.558	2.51	15.067			
CV (%)		6.158	8.295	7.829	7.506			
CD (P= 0.01)		1.31	0.713	0.717	4.12			

Table 2 Correlation among grain characteristics

Characteristics	Grain	Grain width	Length/ breadth ratio	1000 grain weight (g)
Grain length	1			
Grain width	0.11**	1		
Length/breadth Ratio	0.49**	–0.27*	1	
1 000 grain weight (g)	0.42**	0.47*	–0.07(NS)	1

*,** Significant at P = 0.05 and P = 0.01, respectively.

the criteria of DUS of morphological characters. However, these morphological traits often fail to resolve the closely related varieties. The molecular markers can very well supplement the DUS data in such cases. Assessment of genetic diversity is an important criterion in germplasm characterization and conservation. In this study 40 SSR markers were chosen for the analysis of genetic diversity of 60 farmers' varieties of rice because several previous works have shown the power of such markers for differentiating individual germplasm accessions, particularly when they are closely related (Singh *et al*. 2004, Ghneim *et al*. 2009). In the present study, only 20% of the marker polymorphism was detected eventhough these were landraces and farmer varieties, which are expected to have diverse genetic background, which is rather small, as compared to previous reports (Sarao *et al*. 2009. Sivaranjani *et al*. 2010). This could be due to use of a limited number of markers (40). All 40 SSR primers gave successful amplification of the genomic DNA. A set of 8 (20%) primers were polymorphic and yielded 16 bands (alleles) from genomic DNA of all 60 accessions. The size of amplicons ranged from 120 bp (RM238) to 240 bp (RM551). The number of alleles per microsatellite ranged from 1 (RM238, RM119 and RM120) to 3 (RM259, RM234 and RM551) with an average of 2 alleles per locus. Major allele frequency ranged from 0.51 to 0.97 averaging 0.74 (Table 3). Genetic diversity ranged from 0.045 to 0.588 with mean of 0.34. Considering the entire genotype array, the mean value for polymorphism information content (PIC) for all microsatellites was 0.27. The marker RM122 with 2 alleles had the maximum (0.37) PIC while it was lowest (0.27) for RM205 (Table 3). Similarly, the mean value of alleles was also low. However, the PIC was comparable with earlier results (Sivaranjani *et al*. 2010) but was quite low compared with those reported for the worldwide collection and large scale studies (Yu *et al*. 2003). But this difference might be linked with selection of different markers and more diverse set of varieties. Similarly, the higher number of alleles detected by Nagaraju *et al*. (2002) was due to comparison between Basmati and non-Basmati varieties in their study. The amplification of all primers in all varieties suggests the conservation of primer binding sites in the traditional varieties used in the present study. The gene diversity was about half of the world estimate (H= 0.68). Similar low gene diversity have been reported by Hashimoto *et al. (*2004). The amplicon size of RM series markers was comparable with previous study (Tamilkumar *et al*. 2009), however, the absolute allele size estimated by different researchers in different gels varied by a few bases, as has been reported in different crops (Bredemeijer *et al*. 2002, Singh *et al*. 2004).

Table 3 Characterization of total bands obtained by SSR profiling of 60 rice varieties

SSR primer	Allele size (Range) bp	Major allele frequency	Genetic diversity	PIC
RM 259	170-180	0.5012	0.3883	0.3027
RM234	160-170	0.7455	0.3544	0.2847
RM205	120-150	0.7764	0.3338	0.2741
RM238	125	0.9167	0.1528	0.1411
RM119	150	0.9500	0.0950	0.0905
RM120	160	0.5610	0.9462	0.3713
RM122	230	0.5685	0.4889	0.3692
RM551	180-240	0.8222	0.2785	0.2334
	Mean	0.7388	0.3406	0.2720

Genetic similarity and cluster analysis

The morphological traits based Euclidean dissimilarity coefficient ranged from 0.0 to 0.19 with a mean of 0.06. Compared to Jaccard's coefficient, Euclidean distance coefficient yielded two clusters. Cluster I consisted of two sub-clusters with 25 varieties while cluster II had 35 varieties with two sub-clusters. Cluster II had maximum varieties from Madhya Pradesh with one variety each from Uttar Pradesh, Bihar and Uttarakhand. Whereas cluster I contained varieties from all locations with very high level of variation (Fig 1).

The ability to differentiate morphologically similar varieties is an important criterion in determining the efficiency of molecular markers in establishing distinctness. The UPGMA cluster analysis grouped 60 FVs into three main clusters with 58% genetic similarity. The genetic similarity among FVs ranged from 0.111 (Acharmati) to 1 (Kalajeera, Khasha and Nungi) with overall diversity was only 43%. Cluster I, II and III were homing 24, 23 and 13 FVs, respectively. According to SSR marker data, seven varieties in cluster I were showing 100% similarity. Similarly the remaining two clusters also showed 100% similarity among the varieties. Cluster II showed two sub-clusters at 62% similarity level (Fig 2).

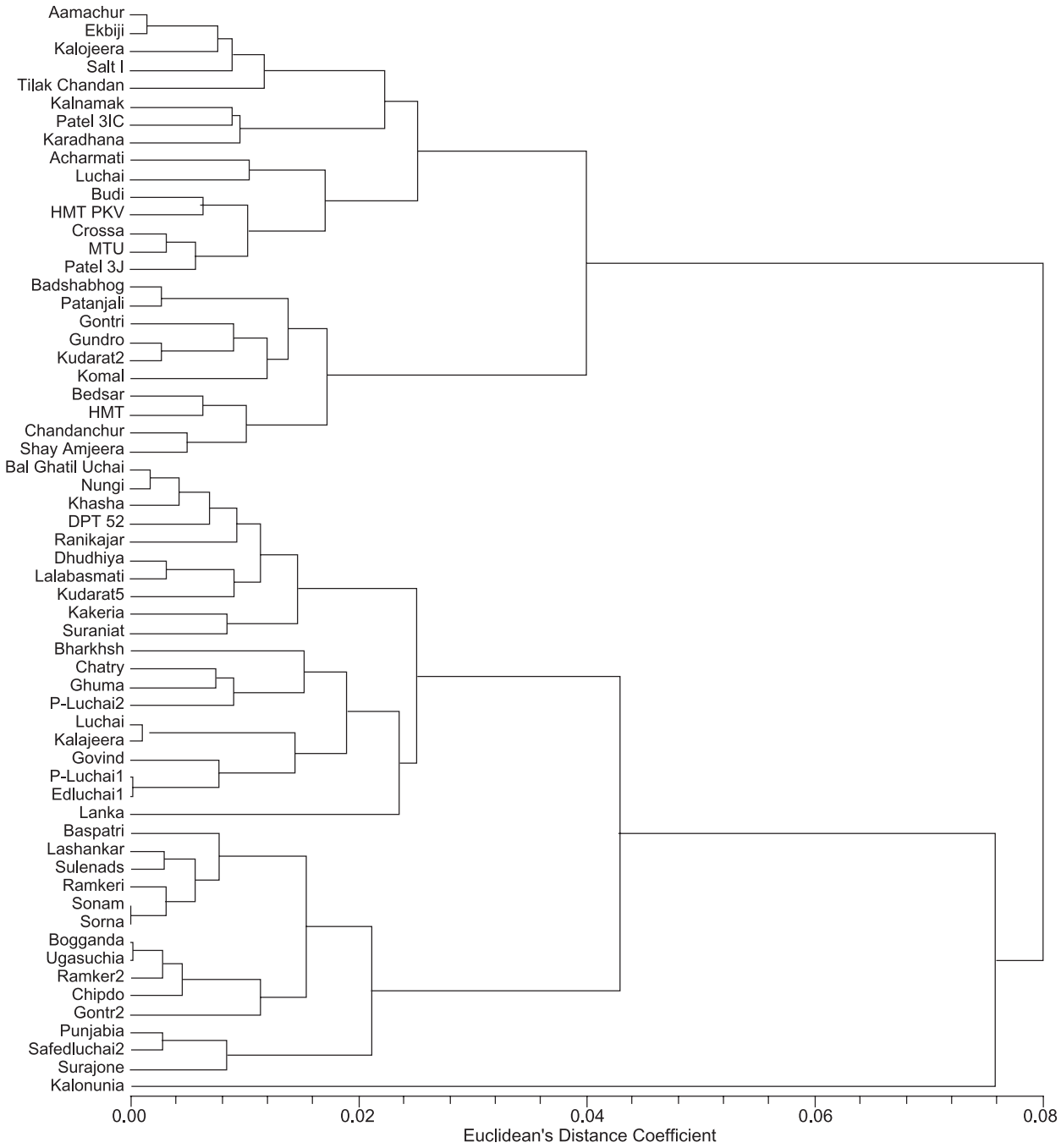

Fig 1 Dendogram derived from UPGMA cluster analysis using Euclidean dissimilarity coefficient based on morphological characteristics of sixty rice varieties

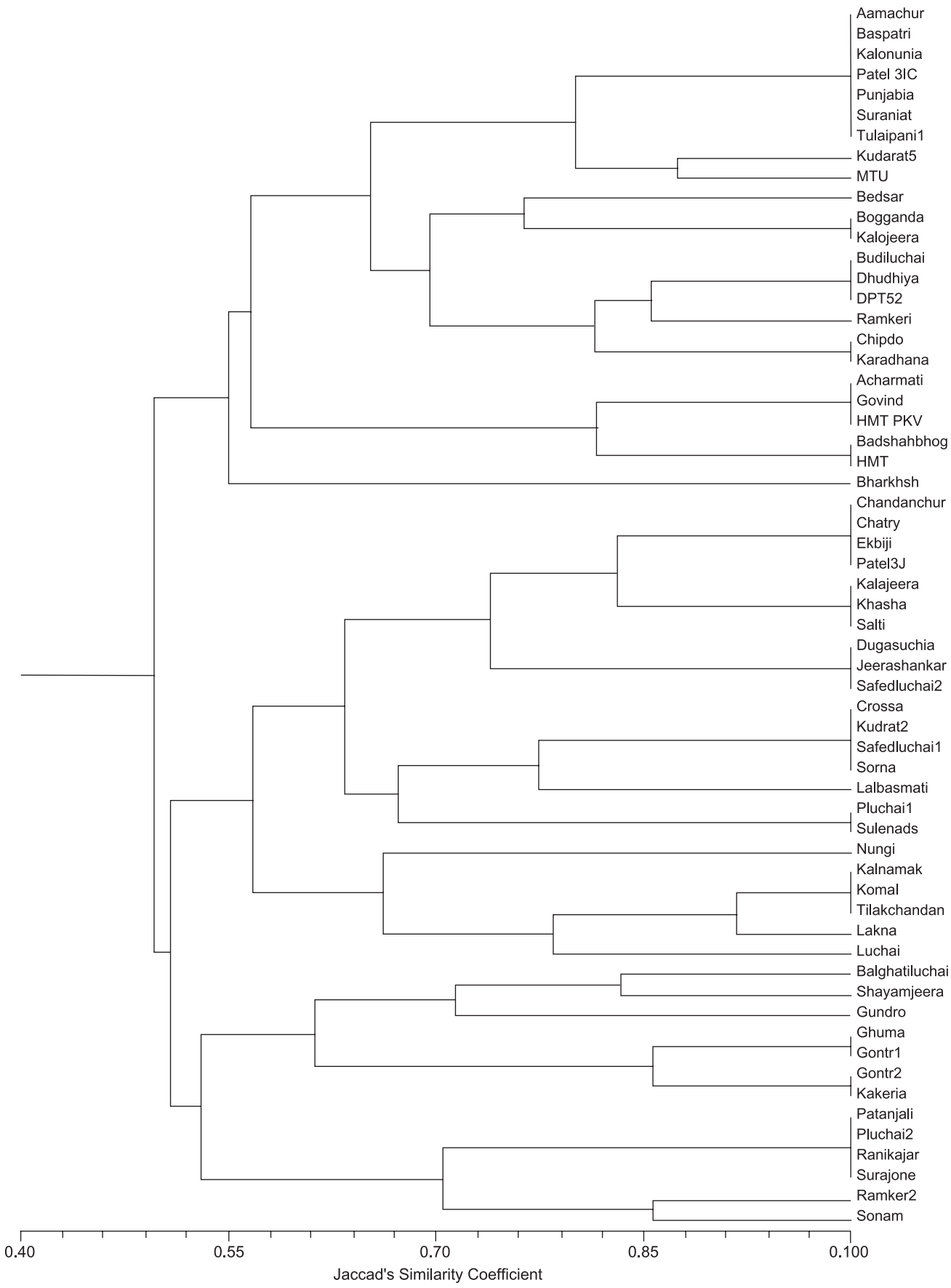

Fig 2 Jaccard's similarity co-efficient of 60 rice varieties using SSR markers

The UPGMA cluster analysis showed that the information generated by 8 SSR markers was not adequate to differentiate the present set of 60 FVs as clustering was also not as per the expected region. Many of these varieties used in this study have not been examined previously in terms of genetic relatedness using molecular markers. The low genetic diversity found among the varieties suggests a probable narrow genetic base or insufficient number of markers. Narrow genetic base in rice has also been previously reported by Gnheim *et al*. (2008). However, in similar studies, the varieties having similar pedigree could not be differentiated from each other in tomato (Bredemeijer *et al*. 2002). This may be due to the use of a small set of SSRs. Our results suggest that it is necessary to screen more number of SSRs to obtain polymorphic markers for more precise grouping of rice accessions. Molecular markers based correct grouping of germplasm can be further exploited in various breeding programmes. Wild germplasm and landraces are potential reservoir of many important alleles for crop improvement (McCouch 2007).

In the present analysis, distribution of varieties into a number of clusters indicated that there is exploitable variation among in FVs that can be used by breeders. Joshi and Chawla (2010) also reported that if the morpho-physiological DUS descriptors are not able to establish distinctiveness, then the biochemical and molecular markers may be used as additional/complementary descriptors for enhancing the distinctiveness resolution.

Though the SSR markers used in the present study alone cannot be much useful for DUS testing purpose of rice varieties, these could be of value in variety identification for consumer protection and resolving disputes concerning circulating seeds. The diversity analysis revealed the distinct nature of the farmers' varieties in large number of different clusters showing greater diversity, which could be exploited in developing high yielding varieties that combine desirable grain traits. However, the present study indicates the need for further screening of a larger set of primers to characterize the farmers' varieties.

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REFERENCES

Bredemeijer G M M, Cook R J, Ganal M W, Rpeeters P, Isacc Y, Noorijik and Randel S. 2002. Construction and testing of microsatellite data base containing more than 500 tomato varieties. *Theoretical and Applied Genetics* **105**: 1 091–126.

Doyle J J and Doyle J L. 1990. Isolation of plant DNA from fresh tissue. *Focus* **12**: 13–4.

Ghneim T H, Duina P D, Iris P A, Torrealba N, Alejandro J P, César P M and Joe M T. 2009. Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers. *Electronic Journal of Biotechnology* **11**(5): 1–14.

Govindaraj P, Arumugachamy S and Maheswaran M. 2005. Bulked segregant analysis to detect main effect QTL associated with grain quality parameters in basamti 370/ASD 16 cross in rice (*Oryza sativa* L.) using SSR markers. *Euphytica* **144**: 61–8.

Hagiwara W E, Onishi K, Takamure I and Sano Y. 2006. Transgressive segregation due to linked QTLs for grain characteristics of rice. *Euphytica* **150**: 27–35.

Hashimoto Z, Mori N, Kawamura M, Ishii T, Yoshida S, Ikegami M, Takumi S and Nakamura C. 2004. Genetic diversity and phylogeny of Japanese sake-brewing rice as revealed by AFLP and nuclear and chloroplast SSR markers. *Theoretical and Applied Genetics* **109:** 1 586–96.

Joshi A and Chawla H S. 2010. Biochemical and molecular markers for establishing distinctiveness of aromatic rice (*Oryza sativa* L.) varieties. *Indian Journal of Genetics and Plant Breeding* **70**(1): 58–64.

McCouch S, Sweeney M, Li J, Jiang H, Thomson M, Septiningsih E, Edwards J, Moncada P, Xiao J and Garris A. 2007. Through the genetic bottleneck: *O. rufipogon* as a source of trait-enhancing alleles for *O. sativa. Euphytica* **154**(3): 317–39.

Panse V G and Sukatame P V. 1999. *Statistical Methods for Agricultural Workers*. ICAR, New Delhi.

Rabiei B, Valizadeh M, Ghareyazie B, Moghaddam M and Ali A J. 2004. Identification of QTLs for grain size and shape in Iranian cultivars using SSR markers. *Euphytica* **137**: 325–32.

Rohlf F J. 1998. Numerical taxonomy and multivariate analysis system. Version 2.02. Exeter Software, Setauket, New York.

Singh R K, Sharma R K, Singh A K, Singh V P, Singh N K, Tiwari S P and Mohapatra T. 2004. Suitability of mapped sequence tagged microsatellite site markers for establishing distinctness, uniformity and stability in aromatic rice. *Euphytica* **135**: 135–43.

Sivaranjani A K P, Pandey M K, Sudharshan I, Kumar G R, Madhav M S and Sundaram R M. 2010. Assessment of genetic diversity among basmati and non-basmati aromatic rices of India using SSR markers. *Current Science* **99:** 221–6.

Tamilkumar P, Jerlin R, Senthil N and Ganesan K N. 2009. Fingerprinting of Rice Hybrids and their parental lines using microsatellite marker and their utilization in purity assessment of hybrid rice. *Research Journal of Seed Science* **2**(3): 40–7.

Vanaja T and Babu L C. 2006. Variation for grain and quality characteristics in rice (*Oryza sativa* L.). *Indian Journal of Genetics and Plant Breeding* **66**(1): 13–5.

Yu S B, Xu W J, Vijayakumar C H, Ali J, Fu BY, Xu J L, Jiang Y Z, Marghirang R, Domingo J, Aquino C, Virmani S S and Li Z K. 2003. Molecular diversity and multilocus organization of the parental lines used in the International Rice Molecular Breeding Program. *Theoretical and Applied Genetics* **108**: 131–40.