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Assessment of Genetic Diversity and Fingerprinting of Rice Cultivars of Andhra Pradesh and Telangana with SSR Markers

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A B S T R A C T

Twenty three simple sequence repeat markers were used to study genetic relationship among fifty three rice varieties developed from Andhra Pradesh and Telangana states. All markers were polymorphic among 53 varieties. A total of 55 alleles were produced for 23 markers with fragment size ranging from 60bp (RM204) to 470bp (RGNMS1289). The number of alleles per marker were ranged from two (RM262) to four (RGNMS1776) with an average of 2.39 alleles/loci. The PIC value for this set of markers varied from 0.213 (RM5474) to 0.670 (RM152), while the gene diversity ranged from 0.228 (RM5474) to 0.720 (RM152). Cluster analysis revealed a total of four distinct clusters resulted out of analysis of pooled SSR marker data. First cluster contains eleven varieties, most of them released from ARI, Rajendranagar, Hyderabad. Second cluster was further divided into two sub-clusters, the varieties grouped into cluster IIa commonly had Mahsuri as one of the parents and cluster IIb had mostly varieties released from Maruteru and Warangal. The cluster III contained seven varieties, most of them developed from Maruteru and cluster IV had only two varieties i.e., Sumati and Nellore Mahsuri having a common character of very long slender grain type. Four SSR loci produced variety specific unique alleles for five varieties, which will be useful in in identification of these varieties. The results in the present study will be useful in understanding molecular variability of the varieties developed and in diversifying the genetic base of rice breeding program, identification of rice varieties in protection of IPR.

Introduction

Green revolution, mainly centered on high yielding varieties, has revolutionized rice production since the late 1960's. These varieties are characterized by higher yield potential, better grain quality, shorter growth duration, multiple resistance to diseases and insects and tolerance to problem soils. High yielding varieties are now planted in 70% of the world's rice lands (Khush, 1995). In India, to date 1,175 high yielding varieties have been released suitable for different ecologies (Shobha Rani *et al.*, 2014; DARE Annual Report, 2017-18). Rice was cultivated in an area of 21.6 lakh hectares and 10.46 lakh hectares in Andhra Pradesh and Telangana respectively during 2015-16 (DES, AP, 2017; DES, TS, 2017). Until the year 2014, approximately 55 and 34 high yielding rice

varieties were released from Andhra Pradesh and Telangana respectively (Shobha Rani *et al.,* 2014). A detailed understanding of the extent and structure of genetic diversity is necessary for effective management and use of crop germplasm resources. DNA-based markers such as SSR markers (or microsatellites), which are codominant and highly polymorphic, offer an easy, accurate, and quantifiable measure of the genetic variation within crop species (Litt and Luty, 1989; Tautz, 1989). For rice, nearly 52, 485 SSRs were developed by Zhang *et al.,* 2007 and these are being used to develop high-density genetic maps, genotype rice accessions, determine the genetic structure and diversity patterns, optimize the assembly of core collections, and for marker-assisted breeding (McCouch *et al.,* 2002; Yu *et al.,* 2003; Garris *et al.,* 2005). Apart from SSRs, around 19,555 rice Genic non-coding microsatellite (RGNMS) were developed and reported by Parida *et al*., (2009). Even though studies to assess the genetic diversity within *O. sativa* collections utilizing isozyme, restriction fragment length polymorphism (RFLP) and SSR markers have been conducted (Glaszmann, 1987; Yang *et al.,* 1994; Xu *et al.,* 2004; Lu *et al.,* 2005; Garris *et al.,* 2005, Chakravarthi *et al.,* 2006) there has been no comprehensive report on genetic diversity among varieties of Andhra Pradesh and Telangana at the DNA level. The objectives of this study were to use SSR markers to (i) quantify the allelic diversity, (ii) estimate the genetic diversity, and (iii) DNA fingerprinting of 53 rice cultivars including released and advance breeding material from different research institutes of Andhra Pradesh and Telangana.

Materials and Methods

Plant material

Fifty three rice cultivars (22 from Andhra Pradesh and 31 from Telangana) that include released varieties and advanced breeding material were selected for SSR screening (Table 1). This material includes rice varieties released and under minikit trials from different research stations of Andhra Pradesh and Telangana which are having special attributes like photoinsensitivity, quality, resistance to different pests and diseases. Seeds were kindly provided by seven main rice breeding stations of Andhra Pradesh and Telangana, which include Andhra Pradesh Rice Research Institute, Maruteru, Agricultural Reserch Institute, Hyderabad, Regional Agricultural Research Station, Warangal, Regional Agricultural Research Station, Jagtial, Regional Sugarcane and Rice Research Station, Rudrur, Agricultural Research Station, Bapatla and Agricultural Research Station, Nellore.

DNA extraction and SSR analysis

Plants were grown in a growth chamber under controlled conditions of light and temperature. Leaves of 15 days old seedlings were collected, frozen in liquid N_2, and stored at −80°C until used. DNA was isolated by CTAB (Cetyl- Tetra Methyl Ammonium Bromide) method (Murray and Thompson, 1980). Twenty-three SSR markers includng Rice Microsatellite (RM) markers and rice Genic noncoding microsatellite (RGNMS) markers linked to nine yield attributing traits representing the six of the twelve rice chromosomes, were used for genotyping (Table 2). PCR products were separated on a 3.5% Metapphor gels and DNA fragments were visualized using Gel star stain (BMA, USA). The size of the amplified fragments was determined by measuring migration distances of SSR alleles on gel photographs in relation to known fragment-length standard 50bp ladder (MBI Fermentas, Lithuania) which was run alongside the samples. When a null allele was detected, the result was confirmed by two independent PCR amplification reactions. Unique alleles were

defined as those detected in only one accession.

Data analysis

All the genotypes were scored for the presence and absence of the SSR bands. The data was entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of the character and this data matrix was subjected to further analysis. The Excel file containing the binary data was imported into NT Edit of NTSYS v. 2.02 (Rohlf, 1998). The 0/1 matrix was used to calculate Similarity as DICE coefficient using SIMQUAL subroutine in SIMILARITY routine. The resultant similarity matrix was employed to construct dendrograms using Sequential Agglomerative Hierarchical Nesting (SAHN) based Unweighted Pair Group Method with Arithmetic Means (UPGMA) to infer genetic relationships and phylogeny. In addition, GGT 2.0 (Berloo, 2008) was used to visualize chromosomal maps of 53 varieties with genotypic information of 23 SSR markers.

Results and Discussion

Assessment of genetic diversity is important for better utlization of breeding material. Results obtained in genetic diversity studies of *O. sativa* cultivars with RFLP and RAPD markers indicate that more genetic diversity exists in *indica* and *japonica* gene pools (Fuentes *et al.,* 1999; Qian *et al.,* 1995). Classical breeding affects genetic diversity within breeding programs. Selection increases the frequency of alleles or allelic combinations with favorable effects at the expense of others, eventually eliminating many of them (Cao *et al.,* 1998). In the present investigation, microsatellites (Rice microsatellites) or SSR markers (Simple Sequence Repeats) from chromosome numbers 2, 3, 4, 5, 6 and 8 were used to characterize and to assess genetic diversity among 53 rice cultures. A total of 23 microsatellite primers including 16 RM series primers and 7 RGNMS primers were utilized. All 23 primers showed polymorphism between 53 rice cultivars. A total of 55 alleles were scored with 23 markers for a set of 53 varieties where the allele size ranged from 60bp (RM204) to 470bp (RGNMS1289). The study revealed that the primer RGNMS1776 had maximum number (four) of alleles followed by three alleles each for RM3698, RM16, RM251, RM5709, RM431, RM190 and RM152, while rest of the fifteen markers had two alleles each, with the average being 2.39 alleles and the allele frequency ranged from 0.01 to 0.85. The PIC value for this set of markers varied from 0.213 (RM5474) to 0.670 (RM152), while the gene diversity ranged from 0.228 (RM5474) to 0.720 (RM152) (Table 2). A representative gel picture showing the banding pattern of these varieties with the markers has been depicted in Figure 1. Similar observations were made by Akagi *et al.,* (1997) that RM1 and RM3 and four other RM markers were less polymorphic. Many studies have also reported significantly greater allelic diversity of microsatellite markers than other molecular markers (McCouch *et al*., 2001).

Cluster analysis was used to group the varieties and to construct a dendrogram. The similarity matrix representing the DICE Co-efficient was used to cluster the data using the UPGMA algorithm. The UPGMA based dendrogram obtained from the binary data deduced from the DNA profiles of the samples analyzed adds a new dimension to the genetic similarity perspectives generated. A total of four distinct clusters resulted out of analysis of pooled SSR marker data (Fig. 2). This dendrogram revealed that the genotypes that are derivatives of genetically similar type clustered more together, i.e., the varieties derived from crosses having common parents were grouped together in a cluster thus reducing their diversity (Deepti *et al*., 2011; Gangaprasad *et al*., 2013).

Table.1 Information on pedigree, source station and status of genetic material

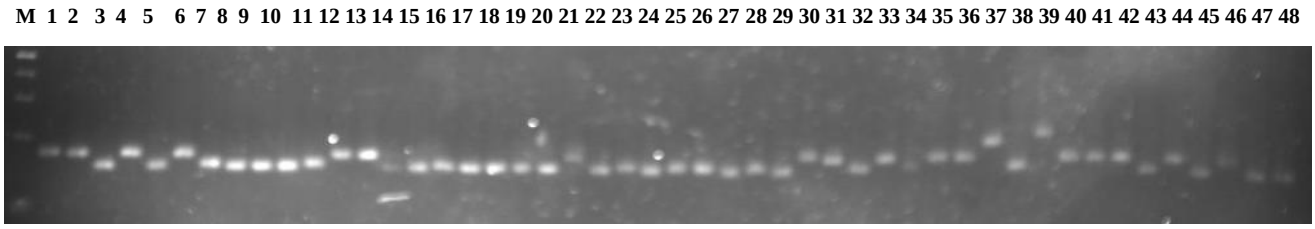
S.No	Name	Pedigree	Source Research Station	Year
1	Swarna	Vasistha/Mahsuri	A.P.R.R.I, Maruteru, Andhra Pradesh	1982
2	Prabhat	IR 8/MTU 3	A.P.R.R.I, Maruteru, Andhra Pradesh	1976
3	Vajram	MTU 4569/ARC 6650	A.P.R.R.I, Maruteru, Andhra Pradesh	1986
4	Chaitanya	Sowbhagya/ARC 5984	A.P.R.R.I, Maruteru, Andhra Pradesh	1988
5	Krishnaveni	Sowbhagya/ARC 5984	A.P.R.R.I, Maruteru, Andhra Pradesh	1989
6	Nandi	Sowbhagya/ARC 6650	A.P.R.R.I, Maruteru, Andhra Pradesh	1991
7	Vijetha	MTU 5249/MTU 7014	A.P.R.R.I, Maruteru, Andhra Pradesh	1995
8	Cottondora Sannalu	Krishnaveni/IR 64	A.P.R.R.I, Maruteru, Andhra Pradesh	2000
9	Indra (MTU1061)	PLA1100/MTU1010	A.P.R.R.I, Maruteru, Andhra Pradesh	2006
11	Amara (MTU1064)	PLA1100/MTU1010	A.P.R.R.I, Maruteru, Andhra Pradesh	2009
10	Pushyami (MTU1075)	MTU2716/MTU1010	A.P.R.R.I, Maruteru, Andhra Pradesh	2008
12	MTU1081	BPT5204/Ajaya	A.P.R.R.I, Maruteru, Andhra Pradesh	Adv material
13	Swarnamukhi	Cica 4 / IR 625-23-3-1//Tetep	A.R.S, Nellore, Andhra Pradesh	1991
14	Sriranga	RP 5-32/Mahsuri	A.R.S, Nellore, Andhra Pradesh	1991
15	Parthiva (NLR33892)	NLR27999/MTU4870	A.R.S, Nellore, Andhra Pradesh	2006
16	Bharani (NLR30491)	IR36/IET2508	A.R.S, Nellore, Andhra Pradesh	1997
17	Nellore Mahsuri (NLR34449)	IR72/BPT5204	A.R.S, Nellore, Andhra Pradesh	2009
18	Sona Mahsuri	Sona/Mahsuri	A.R.S. Bapatla, Andhra Pradesh	1982
19	Samba Mahsuri	GEB 24/TN1//Mahsuri	A.R.S. Bapatla, Andhra Pradesh	1986
20	Bapatla sannalu (BPT1768)	BPT3301/Mahsuri	A.R.S. Bapatla, Andhra Pradesh	2001
21	Akshaya (BPT2231)	Surya/BPT6844	A.R.S. Bapatla, Andhra Pradesh	2010
22	Bhavapuri sannalu(BPT2270	BPT5204/CR 15 MR 1523	A.R.S. Bapatla, Andhra Pradesh	2010
23	Tella Hamsa	HR 12/TN1	ARI, R. Nagar, Hyderabad, Telangana	1971
24	Rajendra	IJ 52/TN 1	ARI, R. Nagar, Hyderabad, Telangana	1976
25	Satya	Tella Hamsa/Rasi	ARI, R. Nagar, Hyderabad, Telangana	1987
26	Saleem	GEB24/Sgadis//IR8/RNR8102	ARI, R. Nagar, Hyderabad, Telangana	1987

27	Chandana	Sona/Manoharsali	ARI, R. Nagar, Hyderabad, Telangana	1989
28	Rajavadlu	Rajendra/IR30	ARI, R. Nagar, Hyderabad, Telangana	1993
29	Sagarsamba	IR8/Siam29//IR8/PTB21	ARI, R. Nagar, Hyderabad, Telangana	1993
30	Early samba (RNRM7)	Mutant of BPT5204	ARI, R. Nagar, Hyderabad, Telangana	2000
31	Sumati	Chandan / Pak. Basmati	ARI, R. Nagar, Hyderabad, Telangana	2001
32	Pothana	IR 579/WGL 12708	R.A.R.S, Warangal, Telangana	1988
33	Surekha	IR 8/Siam 29	R.A.R.S, Warangal, Telangana	1976
34	Divya	WGL 23022/Surekha	R.A.R.S, Warangal, Telangana	1989
35	Kavya	WGL 27120 // Mahsuri // WGL 17672 // Surekha	R.A.R.S, Warangal, Telangana	1991
36	Erramallelu	BC 5-55/W 12708	R.A.R.S, Warangal, Telangana	1991
37	Orugallu	OBS 677/IR 2070-423-2-5	R.A.R.S, Warangal, Telangana	1993
38	Bhadrakali	Phalguna/IR 36	R.A.R.S, Warangal, Telangana	1994
39	Kesava	WGL 28712/IR 36-1996	R.A.R.S, Warangal, Telangana	1997
40	Siva	Phalguna/IR 50	R.A.R.S, Warangal, Telangana	1997
41	Varalu	WGL 20471 / CR 544-1-2	R.A.R.S, Warangal, Telangana	2001
42	Warangal Sannalu	Divya/BPT5204	R.A.R.S, Warangal, Telangana	2006
43	Warangal Samba	BPT5204/ARC5984//Sonamahsuri	R.A.R.S, Warangal, Telangana	2000
44	Jagtial Samba (JGL3844)	BPT5204/ARC5984//kavya	RARS, Jagtial, Telangana	2009
45	JGL13595	MTU4870/JGL418	RARS, Jagtial, Telangana	Adv material
46	Pranahita (JGL11727)	JGL420/Vijetha	RARS, Jagtial, Telangana	2012
47	Manair Sona (JGL3828)	BPT5204/Aganni	RARS, Jagtial, Telangana	2009
48	Karimnagar Samba (JGL3855)	BPT5204/ARC5984//Kavya	RARS, Jagtial, Telangana	2010
49	Jagtial Mahsuri (JGL11470)	JGL418/G.Beton	RARS, Jagtial, Telangana	2010
50	Rudrama	HR19/TN1	R.S and R.R.S, Rudrur, Telangana	1991
51	Varsha	IR 50/Mahsuri	R.S and R.R.S, Rudrur, Telangana	1993
52	Indursamba	BPT 5204/Surekha	R.S and R.R.S, Rudrur, Telangana	1997
53	Pelalavadlu	OBS677/IR2070-423-2-5	R.S and R.R.S, Rudrur, Telangana	1998

Table.2 Genetic variability parameters of 23 SSR markers

Sl. No	Chr.	Marker	No. of Alleles	Allele frequency				PIC	Gene Diversity	Unique Alleles
				1	2	3	4			
1	2	RM262	2.0	0.406	0.594	-	-	0.366	0.482	
2	2	RGNMS3876	3.0	0.070	0.380	0.549	-	0.457	0.549	Chandana$_{(330bp)}$
3	3	RM7	2.0	0.571	0.429	-	-	0.370	0.490	
4	3	RGNMS1140	2.0	0.341	0.659	-	-	0.348	0.449	
5	3	RM3698	3.0	0.230	0.690	0.080	-	0.407	0.464	Indursamba$_{(200bp)}$
6	3	RM16	3.0	0.121	0.712	0.167	-	0.407	0.450	
7	3	RM251	3.0	0.223	0.277	0.500	-	0.553	0.624	
8	3	RGNMS1289	2.0	0.526	0.474	-	-	0.374	0.499	
9	3	RM5474	3.0	0.873	0.093	0.034	-	0.213	0.228	
10	4	RM273	2.0	0.411	0.589	-	-	0.367	0.484	
11	4	RM241	3.0	0.128	0.855	0.017	-	0.228	0.253	
12	4	RGNMS1539	4.0	0.494	0.458	0.036	0.012	0.441	0.545	
13	4	RGNMS3276	2.0	0.626	0.374	-	-	0.359	0.468	
14	4	RM5709	3.0	0.275	0.681	0.044	-	0.386	0.458	
15	4	RM1112	2.0	0.419	0.581	-	-	0.368	0.487	
16	5	RGNMS1776	3.0	0.176	0.365	0.460	-	0.547	0.625	
17	5	RM413	4.0	0.270	0.617	0.061	0.052	0.478	0.540	Krishnaveni$_{(130bp)}$, Vajram$_{(120bp)}$
18	6	RM6273	2.0	0.675	0.325	-	-	0.343	0.439	
19	6	RM190	3.0	0.067	0.723	0.210	-	0.378	0.429	
20	6	RM204	4.0	0.337	0.495	0.059	0.109	0.560	0.626	
21	6	RGNMS3878	2.0	0.839	0.161	-	-	0.233	0.270	Sona Mahsuri$_{(150bp)}$
22	8	RM152	4.0	0.162	0.180	0.378	0.279	0.670	0.720	
23	8	RM25	3.0	0.046	0.282	0.673	-	0.392	0.466	

Fig.1 Gel picture showing amplification pattern of RM413 in varieties



M- 100bp ladder; 1-48 – Variety numbers (Refer Table-1 for details)

Fig.2 Clustering of 53 Varieties of Rice based on 23 SSR markers

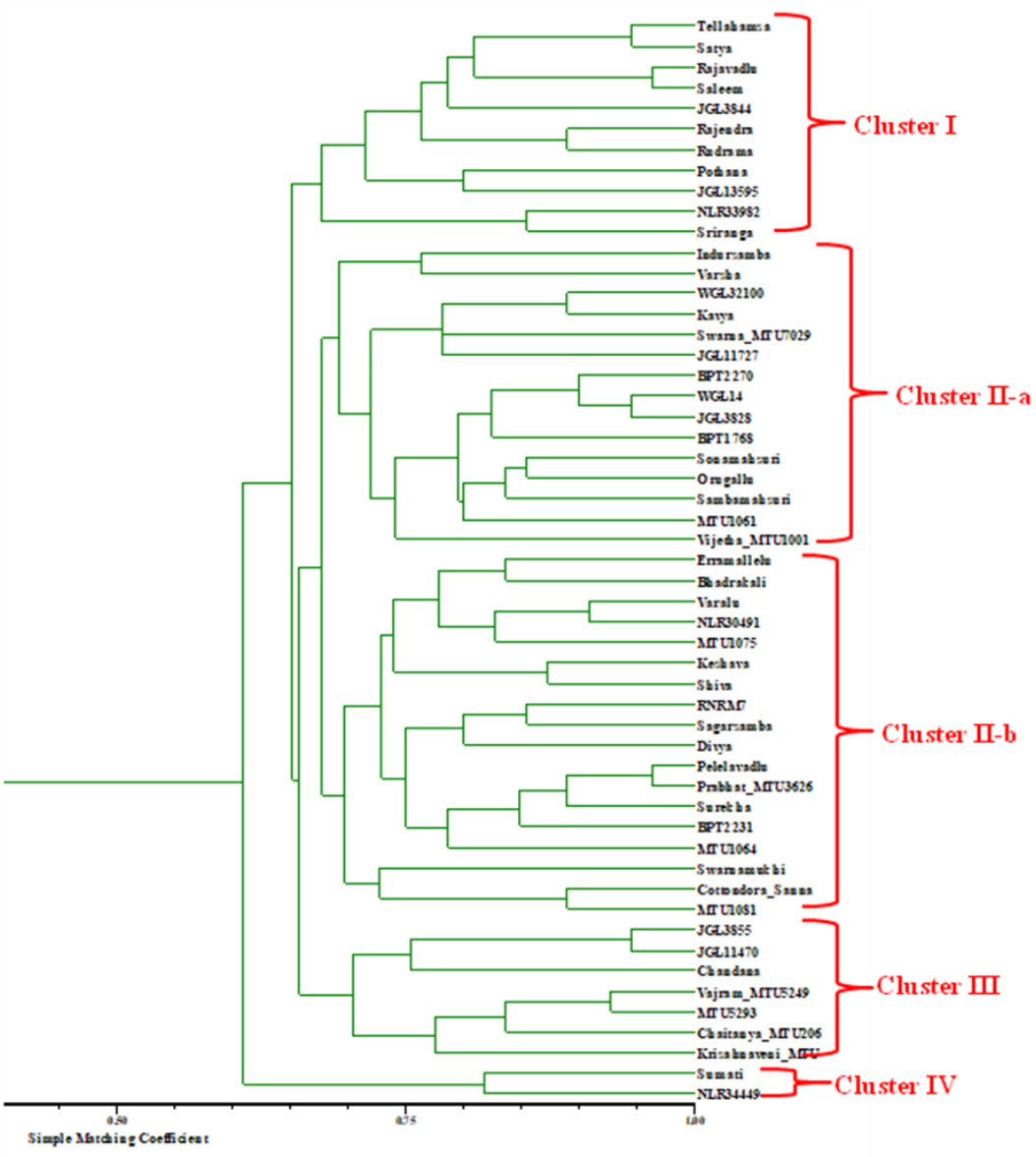
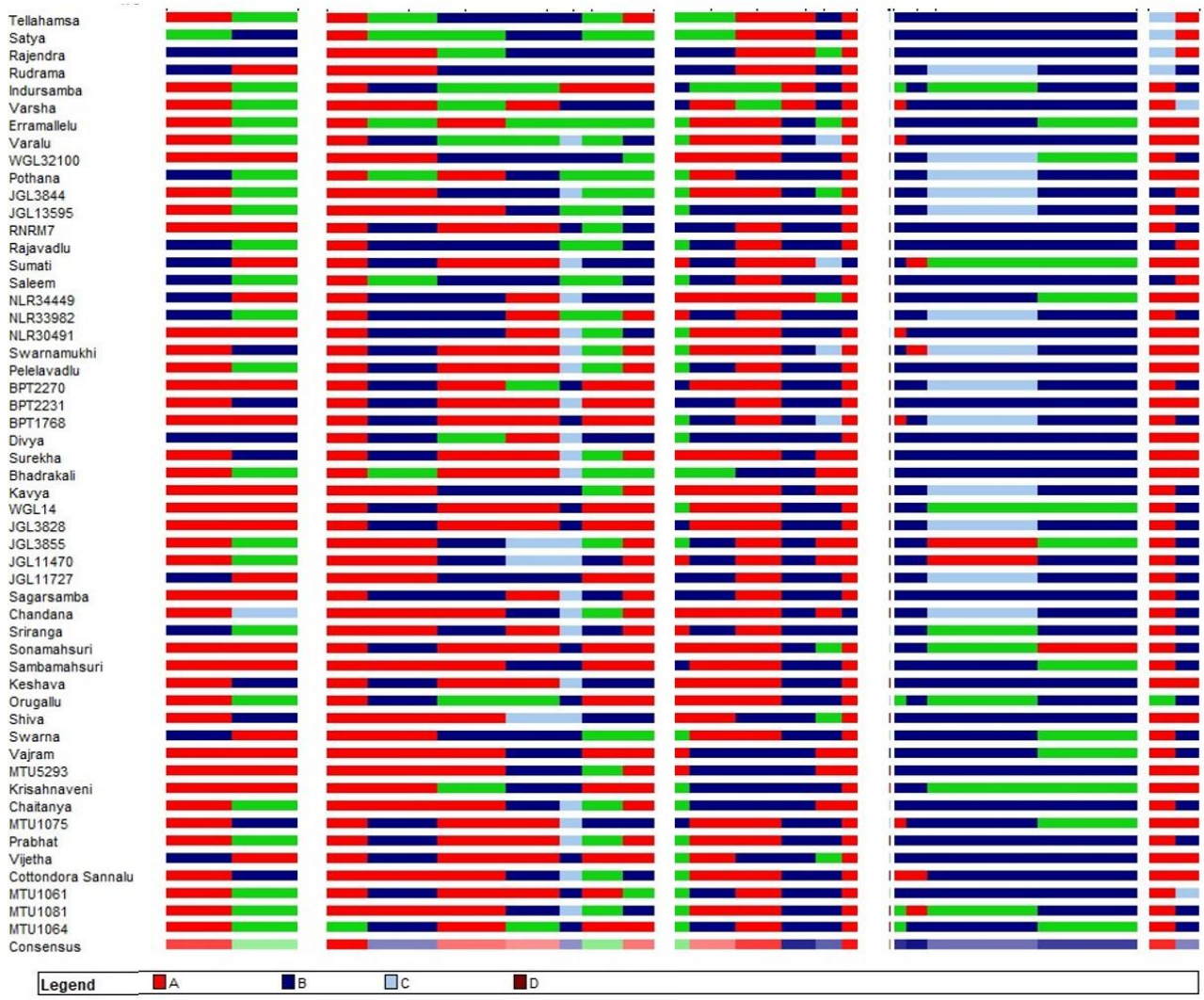


Fig.3 Graphical genotyping of 53 rice cultivars from Andhra Pradesh and Telangana based on 23 SSR markers



First cluster contains eleven varieties, most of them released from ARI, Rajendranagar, Hyderabad and others from Warangal, Jagtial, Rudrur and Nellore. The reason for these varieties clustering together might be their parentage. Second cluster was further seen to bifurcate into two sub-clusters containing fifteen and eighteen varieties respectively from all the research stations without a bias. It was observed that, the varieties grouped into cluster IIa commonly had Mahsuri as one of the parents, which might be the reason for these varieties to group together. On the other hand, cluster IIb had mostly varieties released from Maruteru and Warangal implying the repeated usage of same set of lines for crossing and release of new varieties. The cluster III contained seven varieties, most of them from Maruteru. Even in this cluster the parents used in crossing were the reason for grouping of varieties together. Finally, cluster IV had only two varieties (Sumati and Nellore Mahsuri) having a common character of very long slender grain type, which is a characteristic feature of Basmati type varieties. All the varieties are at least 65 %

similar to other meaning they are diverse for remaining 35% of the regions represented by the marker set used in the study.

Yu *et al*., 2003 carried out a similar study using 193 parental lines obtained from 26 countries and testing them with 110 well distributed SSR markers. An overall genetic diversity of 0.68 and an average of 6.3 alleles per locus were revealed, indicating a high level of genetic variation in these lines. Cluster analysis of the 193 accessions showed three major groups and nine subgroups. Group I corresponded to the classical indica subspecies, whereas groups II and III belong to the japonica subspecies.

Similar work was done on Indian scented and quality germplasm was done using fluorescently labeled SSR markers. A set of 69 rice varieties including 52 basmati and other quality varieties from different parts of India and 17 *indica* and *japonica* varieties that served as controls were used. These varieties were tested using a set of 30 SSRs as a result of which a total of 235 alleles were detected, of which 62 (26.4%) were present only in Basmati and other scented rice germplasm accessions. The number of alleles per locus ranged from 3 to 22, with an average of 7.8 and polymorphism information content (PIC) values ranged from 0.2 to 0.9, with an average of 0.6. Of the 30 SSR markers, 20 could distinguish traditional Basmati rice varieties, and a single panel of eight markers could be used to differentiate the premium traditional Basmati, cross-bred Basmati, and non-Basmati rice varieties having different commercial value in the marketplace. The results of this study indicated that Indian aromatic and quality germplasm is genetically distinct from other groups within *O. sativa* and is the product of a long, independent pattern of evolution (Jain *et al.*, 2004).

In another study carried out by Spada *et al*., 2004, a set of 96 rice cultivars was tested using AFLP and SSR markers. AFLP produced 461 fragments, 248 (53%) of which were polymorphic, SSR produced four to 11 alleles in the 12 genomic loci investigated. Both AFLP and SSR dendrograms coincided in splitting the cultivars into two main clusters: a small one, comprising four exotic accessions, and a larger one which could was split into four sub-groups. These were also analyzed on the basis of historical and pedigree information.

The practical approach developed in the study is useful in DNA fingerprinting also. It was found that five varieties could produce unique alleles with four markers, which could be used in identification of these varieties. Of the five varieties, two varieties (Krishnaveni$_{130bp}$ and Vajram$_{120bp}$) could be differentiated by a single marker, RM413. Rest of the three varieties could be differentiated by one marker each, RGNMS3876 (Chandana$_{330bp}$), RGNMS3878 (Sona Mahsuri$_{150bp}$) and RM3698 (Indursamba$_{200bp}$) (Table 2). The graphical representation of 53 rice varieties based on 23 SSR markers has been depicted in Figure 3. This fingerprinting makes identification and characterization of genotype very easy and further it will be of greater help in background selections during back cross breeding programs. The results in the present study will be useful in understanding molecular variability of the varieties developed from Andhra Pradesh and Telangana and in diversifying the genetic base of rice breeding program, identification of rice varieties in protection of IPR.

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