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RESEARCH ARTICLE

Morphological characterization and DNA barcoding of farmers' varieties of rice (*Oryza sativa* L.) of central India

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Abstract

Assessment of variety distinctness is important for the registration and protection of traditional rice landraces. The present study was conducted to develop a procedure for the assessment of genetic diversity and relatedness among farmers' varieties of rice collected from tribal farmers of Madhya Pradesh. To distinguish and characterize the 100 rice genotypes, 22 SSR markers were applied, which detected a total of 67 polymorphic alleles. The results suggested that the primer RM144, followed by RM263, RM16, RM341, RM122, RM11 and RM234, maybe the best markers for identification and estimating diversity. The polymorphic information content (PIC) values ranged from 0.02 (RM8 and RM510) to 0.617 (RM144), with an average of 0.255. The allelic richness per locus varied from 2 (about 10) to 6 (RM144), with an average of 3.05 alleles per locus. The major allele frequency per locus varied from 45% (RM341 and RM144) to 99% (RM8 and RM510), with an average of 80.7%. The pairwise genetic dissimilarity indices revealed the highest genetic dissimilarity of 72.7% between Khuddi and Pasai Dhan and the lowest genetic dissimilarity of 3.4% was recorded between Bhata makadas and Ranikajal. Colored DNA fingerprint was produced by 22 SSR markers, which can identify the 100 farmers' varieties of rice under study.

Keywords: Farmers' variety, polymorphism, rice, SSR markers, DNA barcoding

Introduction

Rice (*Oryza sativa* L.) cultivation in Asian countries is, perhaps, the most significant and major food hotspot for the larger part of the global population. Among all the Asian nations, India is a noticeable rice-producing nation, representing about 20% of all world rice production. Madhya Pradesh harbors an enormous asset of rice cultivars that are lesser known at the market front and hold extraordinary importance to the farmers as well as to the local consumers. Farmers' varieties has been defined under the Plant Varieties and Farmers' Rights Act, 2001, as a variety that (a) has been traditionally cultivated and evolved by the farmers in their fields and (b) is a wild relative or landrace or a variety about which farmers possess common knowledge (Kumar et al. 2015). They play a very important role as genetic resources. Assessment of such genetic wealth is vital, and different genes need to be conserved in cultivated crop species to fulfill the forever-changing objectives of plant breeding.

Characterization of untrapped germplasm based on morphology is a powerful taxonomical tool that can be utilized for the preliminary grouping of cultivars prior to their profiling using more robust marker technologies. Molecular markers are DNA segments associated with agronomically important traits which act as selection tools for breeders for varietal identification (Wang et al. 2015), crop improvement (Singh et al. 2011), genome analysis (Bhattarai et al. 2021), genetic purity (Vashisht et al. 2014, Singh et al. 2017, Rajesh et al. 2012), phylogenetic (Jain et al. 2008) and population diversity study. Simple sequence repeats (SSR) markers are regarded as the preferred molecular marker among the various DNA markers used for molecular characterization and genetic diversity studies in plants

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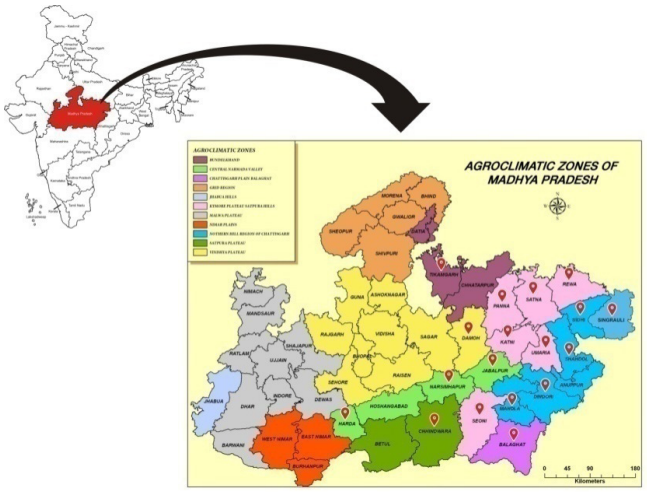

Fig. 1. Map showing collection site of Farmers' variety of rice

due to their prevalence, high rate of polymorphism, and excellent reproducibility.

The objective of the present investigation was aimed at the phenotype of 100 farmers' varieties of rice grown in various pockets of Madhya Pradesh and to produce a unique DNA profile using a set of SSR markers for varietal identification. The data thus obtained can be used for the protection of the farmers' variety of Madhya Pradesh and to generate information to facilitate plant breeding objectives.

Materials and methods

Collection and maintenance of farmers' variety

An extensive survey was done by the scientists of the Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV) Jabalpur, Madhya Pradesh, India, in the rice growing district of Madhya Pradesh in order to collect and conserve the landraces and traditional knowledge regarding paddy. Eight out of eleven agro-climatic zones of Madhya Pradesh were included in the study. Information was gathered from the local people on the salient features of their varieties, which they have been selecting over the years and passed on to generations. Germplasm conservation of 100 such farmers' varieties (Table 1) collected from 18 districts of Madhya Pradesh (Fig. 1) was maintained at Seed Breeding Farm, JNKVV, Jabalpur, to study the biological integrity and provide farmers' varieties with validated phenotypic and genetic descriptions. Each entry was planted in a plot consisting of four rows, each measuring three meters with a distance of 20 cm between rows and 15 cm between plants, with three replications using a Randomized Complete Block Design (RCBD). The present investigation was carried out during *Kharif*, 2017 and 2018. *In vivo* gene bank was also maintained to protect a core collection of all the entries by storing the seed of farmers' variety of rice at -20°C

Table 1. A list of Farmers' variety of rice

S. No.	Rice farmers' variety	S. No.	Rice farmers' variety	S. No.	Rice farmers' variety	S. No.	Rice farmers' variety
1	JamunSurki	26	Jeeraphool	51	Kishori Dhan	76	Khada
2	Luchai	27	Gurmatiya	52	Uresa	77	Monyakali
3	Lohandi	28	Vanskupee	53	Safed saraiya	78	Bahurupi
4	LalDhan	29	Oraikant	54	Karman	79	Hariya Pouni
5	Nawari	30	Kali Saraiya	55	Karmayalndra	80	Galgaliya
6	Bhaisan	31	ChhotiLuchai	56	Bahurupi	81	Lohandi
7	Gurmatiya	32	BadaiLuchai	57	Kalimooch Pouni	82	badaliDhan
8	Kshatriya	33	Haruhan	58	Manesh Dhan	83	Dubrajlallu
9	KarondaBhudh	34	PeeliLuchai	59	Bhata makadas	84	Benamdhan
10	Basmati Purani	35	Chhinoor(Lohara)	60	Karanphool	85	Mangar(raimun)
11	Tinpakhia	36	AssanChuni	61	Kushiyari	86	Kalikammo
12	Methichoor	37	Jeera Shankar	62	Dungu dhan	87	Tendhaniya dhan
13	Bagri Sarethi	38	Chhinoor	63	Ishwarya	88	Ranikajal
14	Bharaphool	39	ChhotaSathiya	64	Gurmatiya	89	Dhansingh dhan
15	Jeeraphool	40	UraiBoota	65	Bode nunga	90	Bedhaar Papra
16	Hanskana	41	ChhotiNunga	66	Badlapur	91	Gundali Ram
17	Biranjphool	42	MamokDhan	67	Nungi	92	Harudhan
18	Brajbhog	43	JalDhan	68	Sukdas	93	Haathi
19	PasaiDhan	44	Kardhana	69	Chintamandhan	94	Galari

20	SikiaKallu	45	Nagkeshar	70	Dhan khamera	95	Chhindikapoor
21	DhaurDhan	46	Assam Koti	71	Lakhoa	96	Khadda
22	Shera	47	Kakehari	72	Badlidhan	97	Lahi Khairwa
23	Khuddi	48	Bagboot	73	Sitri	98-	Dhaniya Dhan
24	Kadambhog	49	SarsariDhan	74	Sultho	99	kaliKamad
25	SafedJeerashankar	50	Biranjphool	75	Layachi	100	Padma

in a deep freezer for long-term use. For assessing the morphological characterization and grain quality, a group of plants or specific plant parts were evaluated using a visual observation with the standard protocol as prescribed by the Indian Institute of Rice Research, Hyderabad, India (Formerly Directorate of Rice Research) was followed.

Isolation of genomic DNA

Green, young and healthy leaves were collected one month after sowing (before flowering) in the morning hours from the field during *kharif* 2018 from the phenotypically identical plants for DNA extraction using the procedure of CTAB (cetyl trimethyl ammonium bromide) method (Murray and Thompson 1980) with minor alteration. The DNA was purified using 3 µL (10 µg/µL) of RNase and incubated for 45 mins at 37°C. Purified DNA was quantified on agarose gel of 0.8% concentration. The concentration of DNA in an individual sample was identified on the basis of the intensity of the bands. The quantified DNA was diluted according to the DNA quantity in each sample for PCR amplification in sterile double distilled water to prepare the working solution with the 25 ng/µL concentration, followed by storing at 4°C.

PCR amplification

Polymerase chain reaction (PCR) for the specific SSR marker analysis was done in 10µl reaction volume. The reaction mixture of 10 µL was made by adding 1-µL of 25 ng/µL template DNA, 1-µL of forward primer, 1-µL of reverse primer, 5 µL master mix and 2 µL nuclease-free water was added to make the final volume. All the primers were amplified using Esco PCR Thermocycler using the following cycler parameters: initial denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 50 to 60°C for 30 seconds and elongation at 72°C for 30 seconds and finally elongation step of 5 minutes at 72°C.

Gel electrophoresis and visualization of amplicons

The amplified PCR products were electrophoresed on 2% agarose gel along with 100 bp ladder for the generation of micro-satellite fingerprints. Fragments of amplicons were visualized under UV-light and photographed using a gel documentation system (Syngene, UK). Power Marker version 3.25 (Liu and Muse 2005) was used to calculate the average number of alleles, gene diversity, heterozygosity and polymorphic information content (PIC) values. A phylogenetic tree was generated on the basis of an unweighted pair group method with arithmetic averages (UPGMA) implemented in Power Marker.

Results and discussion

Phenotypic traits

Out of 21 qualitative characters under study except coleoptile color, presence of leaf collar, presence of leaf ligule, shape of ligule and presence of male sterility, all the other 16 characters were found to be polymorphic, *i.e.,* time of panicles heading, length of steam and panicle, panicle exertion, attitude of flag leaf, distribution of awns and pigmentation in coleoptiles, ligule, auricle, stigma, steam node, apex of lemma, awn, leaf sheath, leaf blade, sterile lemma, decorticated grain. The grain morphology varied significantly in 100 farmers' varieties with respect to awn color, size of the grain, length of panicle, presence and absence of furrow, spots and pubescence in lemma and palea, primary and secondary branching in panicles (Figs. 2(a-d). Three genotypes, namely, Nagkeshar, Monya Kali and Padma, had uniform purple coloration of a leaf (Fig. 2a) and looked like ornament plants. One novel trait was identified in two varieties, Galari and Galgaliya, having very long sterile lemma and palea (Fig. 2b) on both sides of each spikelet. It is important to note that Bahurupi, Haathi and Tendhaniya dhan showed a clustered arrangement of 3 to 4 spikelets on the panicle (Fig. 2c). Genotypes Hariya Pouni possesses dark purple kernel color (Fig. 2d) whereas Kardhana bears brown kernel color. Records of these preliminary evaluations could be used as a general approach for assessing genetic diversity among morphologically distinguishable farmer's variety of rice.

Overall microsatellite diversity

In the present study, a total of 28 SSR markers were analyzed. Twenty-two SSR loci were found to be highly polymorphic (Table 2) in the genetic material across 100 farmers' varieties of rice. Markers with monomorphic (RM105, RM536, RM484, RM307, RM455 and RM124) banding patterns were excluded from further statistical analysis. In total, 67 alleles were detected for the 22 polymorphic SSR loci (Fig. 3(a-e)). The allelic richness per locus varied from 2 to 6 alleles, with an average of 3.05 alleles. Among the polymorphic markers, 10

Fig. 2a. Distribution of anthocyanin coloration on leaf blade

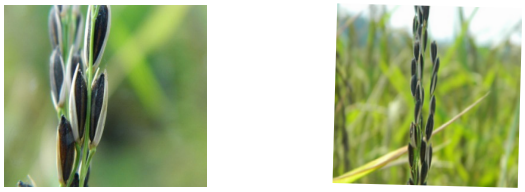

Fig. 2b. Very long sterile lemma and palea

Fig. 2c. Secondary branching on panicle

markers, namely, RM283, RM5, RM212, RM8, RM55, RM231, RM161, RM510, RM215 and RM201 produced two alleles each, while 5 SSR markers, RM154, RM475, RM25, RM223 and RM209 produced three alleles each, 4 markers, *i.e.*, RM341, RM122, RM11 and RM234 generated four allele each and 2 markers namely RM263 and RM16 produced 5 allele each, however, maximum allele, *i.e.*, 6 was produced by only one marker RM44. The variability in allele number per locus is consistent with the frequency of the most common allele at each locus, ranging from 45% (RM341 and RM144) to 99% (RM8 and RM510). On average, 80.7% of 100 farmers' varieties of rice shared a common major allele at any given locus. Based on the allele frequencies, the PIC (polymorphism information content) values were estimated for different SSR loci analyzed. PIC is the measure that reflects the discriminating power of the markers. It is a measure of allele diversity at a locus. Loci that are highly informative have PIC values> 0.5, reasonably informative loci have PIC values between 0.25 and 0.5, and slightly informative loci have PIC values <0.25. Out of the 22 SSR markers used in this study, only 3 markers showed PIC values > 0.5 (Table 2).

The highest PIC value 0.617 was obtained by RM144 followed by RM341 (0.589) and RM263 (0.530). These 3 SSR markers were found to be highly informative and the best markers for the identification of 100 rice landraces, as revealed by PIC values. The primers showed an average PIC value of 0.255, which indicates that SSR markers used in this study are reasonably informative and could detect a diversity value of more than 0.5. Singh et al. 2011 also attributed the high PIC value of RM144 to the polymorphism of the ATT motif in rice. A high PIC value of RM341 was reported by Nihad et al. 2021, which shows polymorphism of the CTT motif in rice. The genetic diversity of these 22 loci for 100 landraces ranged from 0.0198 to 0.6720, with an average being 0.2859, indicating a moderate level of genetic diversity. Similar studies were carried out by Kumar et al. 2018 showing the potential utility of molecular markers RM263 which generated a greater percentage of unique alleles.

Clustering of farmers' varieties of rice

Based on the electrophoretic banding pattern of SSR markers, pairwise genetic similarity amongst selected rice farmers' varieties was estimated and a dendrogram was generated by the neighbor-joining method implemented in Power Marker Version 3.25 software to increase the accuracy of group classification by minimizing the impact of human factors.

Fig. 2d. Decorticated grain color

Table 2. Allelic distribution and PIC values of 22 microsatellite markers across 100 genotypes

S. No.	Marker	Chr. No.	Repeat motif	No. of Allele	Allele Frequency	Gene Diversity	PIC
1	RM283	1	(GA)18	2	0.950	0.095	0.090
2	RM5	1	(GA)273	2	0.960	0.077	0.074
3	RM212	1	(AG)14	2	0.970	0.058	0.057
4	RM8	2	(GA)317	2	0.990	0.020	0.020
5	RM341	2	(CTT)20	4	0.450	0.656	0.589
6	RM154	2	(GA)12	3	0.790	0.348	0.313
7	RM263	2	(CT)34	5	0.590	0.581	0.530
8	RM475	2	(TATC)8	3	0.710	0.450	0.403
9	RM16	3	(GA)53	5	0.900	0.186	0.179
10	RM55	5	(GA)17	2	0.730	0.394	0.317
11	RM231	3	(CT)16	2	0.910	0.164	0.150
12	RM122	5	(GA)11	4	0.610	0.516	0.433
13	RM161	5	(AG)20	2	0.900	0.180	0.164
14	RM510	6	(GA)15	2	0.990	0.020	0.020
15	RM11	7	(GA)397	4	0.760	0.374	0.318
16	RM234	7	(GA)25	4	0.840	0.277	0.252
17	RM25	8	(GA)18	3	0.880	0.217	0.203
18	RM223	8	(CT)25	3	0.790	0.336	0.286
19	RM215	9	(CT)16	2	0.980	0.039	0.038
20	RM201	9	(CT)17	2	0.880	0.211	0.189
21	RM209	11	(CT)18	3	0.730	0.419	0.368
22	RM144	11	(ATT)11	6	0.450	0.672	0.617
	Total			67			
	Mean			3.05	0.807	0.286	0.255

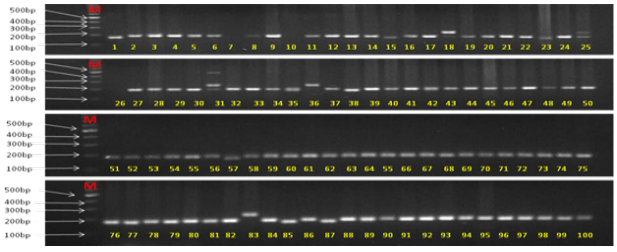

Fig. 3a. PCR amplification of 100 farmers' variety of rice with SSR primers RM16

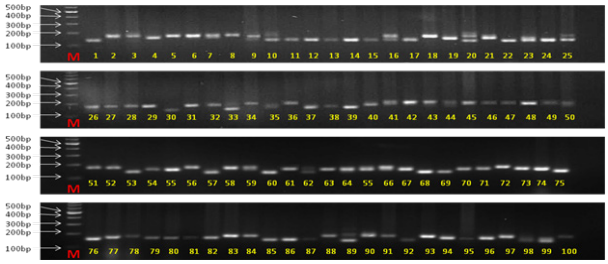

Fig. 3c. PCR amplification of 100 farmers' variety of rice with SSR primers RM 341

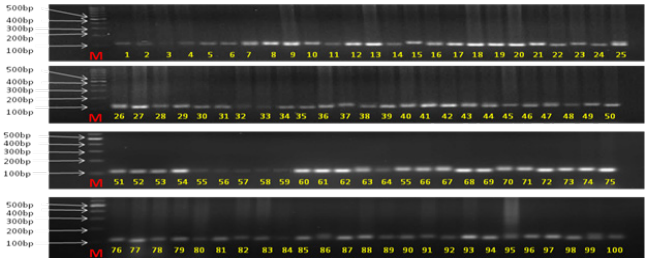

Fig. 3b. PCR amplification of 100 farmers' variety of rice with SSR primers RM5

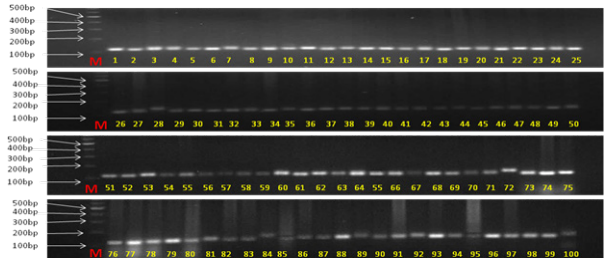

Fig. 3d. PCR amplification of 100 farmers' variety of rice with SSR primers RM212

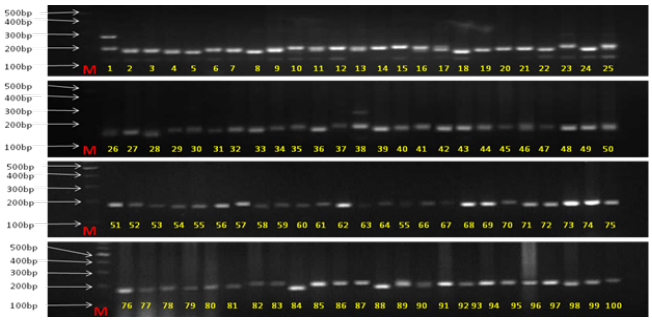

Fig. 3e. PCR amplification of 100 farmers' variety of rice with SSR primers RM 154

The dendrogram showed (Fig. 4) that the 100 landraces of rice fall into two clusters, *i.e.,* cluster I and cluster II. Cluster I comprises of two farmers' varieties, namely Jamun Surki and Khuddi, showing more genetic relatedness among them as compared to cluster II. These two varieties were found to be totally divergent from the other 98 varieties included in the study. It was observed that the clustering based on the allelic data was conserved to some extent for some genotypes. Cluster II was further subdivided into subgroups IIA and IIB. Cluster IIA was monogenotypic, consisting of a variety Sukdas. Variety Sukdas was found to be different from other 97 landraces in cluster IIB. Cluster IIB was again subdivided into cluster IIB-1 and IIB-2. Cluster IIB-1 consisted of two landraces namely Brajbhog and Assan Chuni. Cluster IIB-2 could be again subdivided into cluster IIB-2a and cluster IIB-2b. Cluster IIB-2a is multigenotypic and comprises of 82 landraces of rice. Cluster IIB-2b is also polygenotypic, comprising of 13 genotypes, namely, Jeeraphool, Shera, Bharaphool, Biranjphool, Kadambhog, Harudhan, Safed Jeerashankar, Kali Saraiya, Haruhan, Layachi, Pasai Dhan, Tinpakhia and Bagri Sarethi as depicted in Fig. 4. The results clearly demonstrated the genetic relationships between the cultivars, which provide the required theoretical support for breeding research despite the farmers' varieties not being strictly grouped according to their place of origin or phenotypic traits reflecting the broad genetic base in the farmers' varieties of rice of Madhya Pradesh. Results from the present study support the observations of Rathi et al. (2014) and Singh and Singh (2008).

Pairwise genetic dissimilarity

A dissimilarity matrix was used to determine the level of relatedness among the studied accessions. The genetic similarity among the farmer's variety varied from 0.034 to 0.727. The pairwise genetic dissimilarity indices revealed the highest genetic dissimilarity of 72.7% between Khuddi and Pasai Dhan followed by Jamun Surkhi and Assan Chuni (68.2%), Sukdas and Chhinoor (68.1%) and the lowest genetic dissimilarity of 3.4% was found between Bhata makadas and Ranikajal. The genetic dissimilarity between Khuddi (from

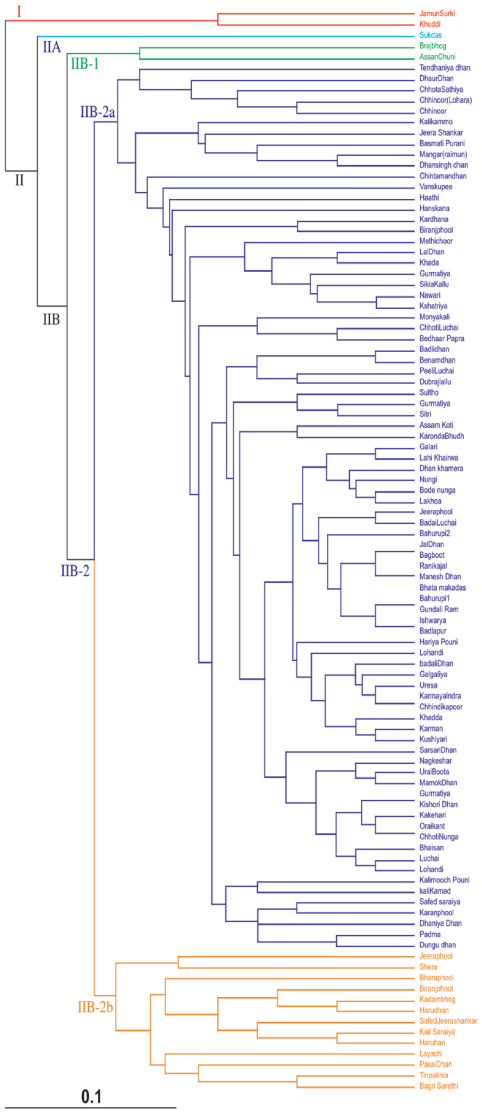

Fig. 4. Dendrogram of 100 selected farmers' varieties of rice based on twenty two polymorphic SSR marker

Dindori district) and rest of the accessions was comparatively high. Therefore, SSR markers provide an adequate power of resolution to discriminate between the farmers' varieties and it could serve as a potential tool in the identification and characterization of genetically distant genotypes from different sources. A similar study on genetic dissimilarity indices was also reported by Sarkar et al. (2014) on wheat cultivars and Bhardwaj et al. (2018) on pearl millet.

DNA barcoding/fingerprints

The similarity index has been producing in the form of colored barcodes. A computer-based program that provides a specific color to different amplicon size for each marker. The 22 polymorphic SSR markers was used to generate a unique barcodes/fingerprint profile for each of the 100 farmers' varieties of rice, which may act as a reference barcode for accurate identification with a visual

representation of the number and size of alleles, allowing easy identification between cultivars. Rare alleles were also observed. An allele is considered to be rare when it is revealed in less than 5% of the genotypes being studied. Each rice variety had of unique color pattern with the same set of 22 SSR markers (Fig. 5). Large-scale DNA barcode data can be generated to make a reference library of *Oriza sativa,* which can provide an open access gateway for researchers from the different arena of biodiversity studies. In the recent past, DNA barcoding has been evidenced as an effective supplementary tool for accurate species identification and biodiversity research. Hence, an integrated approach of both morphology and DNA barcoding can become a useful approach for differentiating rice varieties, similarities among the genotypes and identification of species complexes. The current study also enriches the global database with DNA barcode data of Indian rice and aims at research associated with biodiversity and systematic research.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Jamun Surki	Luchai	Lohandi	LalDhan	Nawari	Bhaisan	Gurmatiya	Kshatriya	Karonda Bhudh	Basmati Purani	Tinpakhia	Methichoor	Bagri Sarethi	Bharaphool Karwahi	Jeeraphool	HanskanaK arwahi	Biranjphool Shivram	Brajbhogj agdish	Pasai Dhan	SikiaKallu
RM8	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252
RM122	233	233	233	233	233	233	233	233	253	220	253	233	233	253	253	253	253	253	253	233
RM16	169	180	180	180	180	180	180	180	180	180	180	180	180	180	169	180	180	200	180	180
RM283	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	185	157
RM5	111	111	111	111	111	111	111	111	111	111	111	111	111	111	125	111	111	111	111	111
RM55	226	226	226	226	226	210	226	226	226	226	210	226	210	226	226	210	210	226	210	226
RM215	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157
RM341	133	172	172	154	172	172	172	172	172	133	133	133	133	133	133	172	133	172	154	172
RM510	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122
RM25	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130
RM201	133	152	152	152	152	152	152	152	152	132	132	132	152	152	152	152	152	152	152	152
RM209	159	132	132	132	159	132	159	132	132	159	159	132	159	132	132	159	159	132	159	132
RM212	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118
RM144	210	210	210	215	210	210	210	210	226	235	226	210	226	215	235	235	210	215	226	215
RM154	200	185	185	174	174	185	185	174	185	200	200	200	200	200	200	200	185	174	185	185
RM161	200	187	187	187	187	187	187	187	187	187	187	187	187	187	200	187	187	187	187	187
RM263	169	169	169	169	200	169	169	200	169	169	169	200	169	169	169	169	169	200	200	200
RM475	200	200	200	174	174	200	174	174	174	200	174	174	174	200	174	174	174	200	174	174
RM223	160	169	160	169	169	169	169	169	169	169	160	169	160	160	160	169	169	169	160	169
RM11	125	140	140	140	140	140	140	140	140	125	125	140	125	125	125	140	125	140	125	140
RM234	131	157	157	157	157	157	157	157	131	157	157	157	131	131	157	157	157	157	157	157
RM231	182	182	182	182	182	182	182	182	182	182	182	182	182	182	182	182	182	160	182	182

	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
	Shera2	Khuddi	Kadambhog	Safed Jeerashankar	Jeeraphool	Gurmatiya	Vanskupee	Oraikant	Kali Saraiya	Chhoti Luchai	Badai Luchai	Haruhan	Peeli Luchai	Chhinoor(Lohara)	Assan Chuni	Jeera Shankar	Chhinoor	Chhota Sathiya	UraiBoota
RM8	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252
RM122	233	233	253	253	253	233	233	233	253	253	253	253	253	253	253	253	233	233	233
RM16	180	169	180	180	180	180	180	180	180	200	180	180	180	180	200	180	180	180	180
RM283	157	157	157	157	157	157	157	157	157	157	157	157	157	157	185	157	157	157	157
RM5	111	125	111	111	111	111	111	111	111	111	111	111	111	111	111	125	111	111	111
RM55	210	226	226	210	210	226	226	226	210	210	210	210	210	210	210	226	210	210	226
RM215	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157
RM341	133	133	133	133	172	172	172	172	133	172	172	133	174	154	174	154	154	154	172
RM510	122	122	122	122	122	122	132	122	122	122	122	122	122	122	122	122	122	122	122
RM25	130	130	130	130	130	130	130	130	120	130	130	130	130	130	130	130	130	120	130
RM201	152	132	152	152	152	152	152	152	152	152	152	152	152	152	132	152	132	132	152	152
RM209	132	132	159	132	132	132	132	132	159	132	132	159	132	132	132	132	132	132	132
RM212	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118
RM144	210	210	215	210	210	235	210	235	210	210	210	210	235	235	235	235	235	210	235
RM154	185	200	185	200	185	185	174	185	185	185	185	185	185	185	200	200	185	185	185
RM161	200	200	187	200	187	187	187	187	187	187	187	187	187	187	187	200	200	200	187
RM263	169	169	169	169	200	169	200	169	169	169	200	200	200	169	200	169	169	169	215
RM475	174	200	174	200	200	200	200	200	200	200	200	200	200	200	220	200	200	220	200
RM223	160	169	169	169	169	160	169	169	169	169	169	169	169	169	169	169	169	169	169
RM11	125	133	125	125	140	140	140	140	125	140	140	125	140	140	140	125	140	140	140
RM234	157	131	140	131	157	157	157	157	131	157	157	131	157	131	157	131	131	157	157
RM231	182	182	182	182	182	182	182	182	182	160	182	182	160	182	160	182	182	182	182

	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
	Chhoti Nunga	Mamok Dhan	JalDhan	Kardhana	Nagkeshar	Assam Koti	Kakehari	Bagboot	Sarsari Dhan	Biranjphool	Kishori Dhan	Uresa	Safed saraiya	Karman	Karmayaln dra	Bahurupi	Kalimooch Pouni	Manesh Dhan	Bhata makadas	Karanphool
RM8	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252
RM122	233	233	233	233	233	253	233	233	253	233	233	253	253	253	253	253	260	253	253	253
RM16	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	169	180	180	180
RM283	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157
RM5	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111
RM55	226	226	226	226	210	226	226	226	226	210	226	226	226	226	226	226	226	226	226	226
RM215	157	157	157	169	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157
RM341	172	172	172	172	172	172	172	172	172	172	172	172	133	154	154	172	132	172	172	133
RM510	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122
RM25	130	130	130	130	130	130	130	130	130	130	130	130	140	130	130	130	130	130	130	120
RM201	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152
RM209	132	159	132	159	132	132	159	132	159	159	132	132	159	132	132	132	132	132	132	159
RM212	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118
RM144	235	235	210	210	235	210	235	210	235	210	235	235	210	210	235	210	210	210	210	210
RM154	185	185	185	185	185	185	185	185	185	185	185	185	185	185	185	185	185	185	185	185
RM161	187	187	187	187	187	187	187	187	187	187	187	187	187	187	187	187	187	187	187	187
RM263	169	215	169	169	215	169	169	169	169	200	169	169	169	169	169	215	169	169	169	169
RM475	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200
RM223	169	169	169	169	169	169	169	169	160	169	160	169	169	169	169	160	169	169	169	169
RM11	140	140	140	125	140	125	140	140	140	125	140	140	125	140	140	140	140	140	140	140
RM234	157	157	157	157	157	131	157	157	157	157	157	157	157	157	157	157	157	157	157	157
RM231	182	182	182	182	182	182	182	182	182	182	182	182	182	182	182	182	182	182	182	182

	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
	Kushiyari	Dungu dhan	Ishwarya	Gurmatiya	Bode nunga	Badlapur	Nungi	Sukdas	Chintama ndhan	Dhan khamera	Lakhoa	Badlidhan	Sitri	Sultho	Layachi	Khada	Monyakali	Bahurupi	Hariya Pouni	Galgaliya
RM8	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252	252
RM122	260	260	253	253	253	253	233	268	253	253	253	253	253	233	253	233	235	253	253	255
RM16	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180
RM283	157	157	157	157	157	157	157	185	157	157	157	157	157	157	157	157	157	157	157	157
RM5	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111
RM55	226	226	226	210	226	226	226	226	226	226	226	226	210	210	210	226	210	226	226	226
RM215	157	157	157	157	157	157	157	157	157	157	157	157	157	157	169	157	157	157	157	157
RM341	154	133	172	154	154	172	154	133	133	154	154	172	154	154	133	154	172	172	154	154
RM510	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122
RM25	130	130	130	130	130	130	130	120	130	130	130	130	130	130	130	130	130	130	120	130
RM201	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152
RM209	132	159	132	132	132	132	132	159	159	132	132	132	132	132	159	132	132	132	132	132
RM212	118	118	118	118	118	118	118	118	118	118	118	140	118	118	118	118	118	118	118	118
RM144	210	210	210	235	235	210	210	210	235	210	210	235	210	210	210	235	210	210	210	235
RM154	185	185	185	185	185	185	185	185	185	200	185	185	185	185	185	185	185	185	185	185
RM161	187	187	187	187	187	187	187	187	187	187	187	187	187	187	187	187	187	187	187	187
RM263	169	169	215	200	215	215	215	200	152	215	215	200	169	169	200	169	200	200	200	200
RM475	200	200	200	200	200	200	200	200	200	200	200	200	200	200	174	174	174	200	200	200
RM223	169	169	169	160	169	169	169	160	169	169	169	169	160	160	160	169	169	148	169	169
RM11	140	140	140	140	140	140	140	125	140	140	140	140	140	140	125	140	140	140	140	140
RM234	157	157	157	157	157	157	157	157	148	157	157	157	157	157	157	157	157	157	157	157
RM231	182	182	182	182	182	182	182	160	160	182	182	182	182	182	182	182	160	182	182	182

	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
	Lohandi	badali Dhan	Dubraj lallu	Benam dhan	Mangar (raimun)	Kalikammo	Tendhaniya dhan	Ranikajal	Dhansingh dhan	Bedhaar Papra	Gundali Ram	Harudhan	Haathi	Galari	Chhindikapoor	Khadda	Lahi Khairwa	Dhaniya Dhan	kali Kamad	Padma
RM8	252	252	252	252	252	252	240	252	252	252	252	252	252	252	252	252	252	252	252	252
RM122	253	253	253	253	253	253	253	253	253	253	253	253	253	253	253	253	253	253	253	260
RM16	180	180	220	180	169	180	169	180	180	180	180	180	180	180	180	180	180	180	180	180
RM283	157	157	157	157	157	185	157	157	157	157	157	157	185	157	157	157	157	157	157	157
RM5	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111
RM55	226	210	226	226	226	226	226	226	226	226	226	226	226	226	226	226	226	226	226	226
RM215	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157
RM341	154	154	172	172	154	154	154	172	133	172	172	133	172	154	154	154	172	133	133	172
RM510	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122
RM25	140	130	130	130	130	130	120	130	130	130	130	130	130	130	130	130	130	130	120	130
RM201	152	152	152	152	132	152	132	152	132	152	152	152	152	152	152	152	152	152	152	152
RM209	132	132	132	132	132	132	132	132	132	132	132	159	132	132	132	159	132	159	132	159
RM212	118	118	118	140	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118	118
RM144	215	235	235	235	235	235	210	210	235	210	210	235	215	210	235	210	210	210	210	210
RM154	185	185	185	185	200	200	185	185	200	185	185	185	185	185	185	185	185	185	185	185
RM161	187	187	187	187	187	187	187	187	187	187	187	187	187	200	187	187	200	187	187	187
RM263	169	169	200	200	169	169	169	169	169	169	215	169	215	215	169	169	215	215	169	215
RM475	200	200	200	220	200	200	220	200	200	220	200	200	220	200	200	200	200	220	200	200
RM223	169	169	169	169	169	169	169	169	169	169	160	169	160	169	169	169	169	169	160	169
RM11	140	140	140	140	140	125	140	140	140	140	140	125	140	140	140	140	140	140	140	140
RM234	157	157	157	157	157	157	157	157	157	157	157	140	157	157	157	157	157	157	131	157
RM231	182	182	160	182	182	182	182	182	182	160	182	182	182	182	182	182	182	182	182	182

Fig. 5. Coloured DNA Barcoding of farmers' variety of rice from 22 SSR marker

Authors' contribution

Conceptualization of research (GKK, MJK); Designing of the experiment (GKK, MJK, SKS); Contribution of experimental material (GKK); Execution of field/lab experiments and data collections (MJK, GKK, YS, SKS); Analysis and interpretation of data (YS, MJK, GKK), Preparation of the manuscript (MJK, YS).

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