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Molecular and Morphological Characterization of Aromatic Farmer's Rice Varieties Collected from Different Districts of Madhya Pradesh, India

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A B S T R A C T

In the present study, sixty four aromatic landraces of rice collected from different districts of Madhya Pradesh were analysed for genetic variation using SSR markers. Specially, the objective of the study was DNA fingerprinting and genetic diversity analysis of aromatic landraces to requisite to study the unique feature and to identify the duplication among them and to assist in broadening the germplasm base of future aromatic rice breeding programs. Very high variation was observed for the trait sterile lemma colour in the classes of straw (76.56%), purple (10.94%), gold (7.81%) and 4.69%). Similar trend was also observed for grain weight of 1000 fully developed grains in the classes of low (28.13%), medium (25.00%), very high (20.31%), very low (18.75%) and high (7.81). The experiment for analysing genetic relationship using 31 SSR primers revealed that all primers showed polymorphism with reproducible and informative profiles. The DNA amplification pattern revealed that a total number of 594 SSR loci were amplified with an average of 19.31 loci per primer.

Introduction

Rice (*Oryza sativa* L.) is a staple food crop and a primary food source for more than a third of world's population. Among all the Asian countries, India occupies the largest area under cultivation and accounting for about 20% of all world rice production..Aromatic rice forms a separate group and, is nature's gift exclusive to Indian sub-continent (Glaszman 1987). Aromatic rice is known for its characteristic fragrance when cooked due to chemical 2-acetyl-1-pyroline. The aromatic landraces found in Madhya Pradesh constitute a special group of rice that is known for the best in quality. Landrace refers to domesticated plants adapted to the natural and cultural environment in which they

live (or originated) and, in some cases, work. Landraces have been shown to be excellent sources of genes for novel alleles (McCouch *et al.,* 1997; Hoisington *et al.,* 1999). No systematic studies have been done for characterization of these aromatic landraces and no clear cut identification features of these landraces are yet known. Since at different places, the same landrace is grown with different names. Many farmers bring some varieties from distant places and start cultivating them with local names. So, Genetic study of these aromatic landraces is the pre requisite to study the unique feature and to identify the duplication among them.

The molecular and phenotypic characterization could reveal their phylogeny and this information would be quite useful for identification of diagnostic traits. The uniqueness of a particular variety is to be established by the test called DUS (Subba Rao *et al.,* 2013**).** DUS test has been established to be the foundation of plant variety protection and also to identify a new variety from reference collection (Kwon *et al.,* 2005). Requirement of DUS is assessed on the basis of characteristics. The characteristics are a feature of whole plant or part of plant. Moreover, the existing methods are time consuming, which have altogether led to more necessity for developing a substitutionary, less costly system. Thus, the studies on the use of molecular markers in DUS testing proving the expected capability of molecular markers have encouraged International Union for the Protection of New Varieties of Plants (UPOV) to contemplate the introduction of molecular markers to the DUS testing system (Pourabed *et al.,* 2015).

Molecular Marker based Genetic Diversity Analysis has the potential for assessing changes in genetic diversity over time and space (Duwick, 1984). DNA markers are predominantly used in molecular characterization and diversity studies due to their abundance and repeatability (McCouch *et al.,* 1997). Several molecular marker techniques viz. Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphism (AFLP) and Single Nucleotide Polymorphisms (SNPs) have been used to assess genetic diversity of various rice cultivars throughout the world (Joshi *et al.,* 2000). They are more reliable and remain unaffected across growth stages, seasons, locations and agronomic practices. These markers have been recently utilized for many purposes including genome mapping, gene tagging, estimation of genetic diversity, varietal differentiation, resolution of uncertain parentage and purity testing **(**Olufowote *et al.,* 1997**;** Coburn *et al.,* 2002; Ni *et al.,* 2002, Singh,*et al.,* 2008, Singh and Singh,2012). Among different PCR based markers, the microsatellite markers based on simple sequence repeats (SSRs) are preferred over other molecular markers due to their ease of application, high reproducibility, rapid analysis, low cost, easy scoring patterns and greater allelic diversity (Chen *et al.,* 1997).

More than 2200 microsatellite markers have been mapped to specific locations in rice genome (Mc Couch *et al.,* 2002). A random set of these mapped markers providing genome wide coverage should facilitate an unbiased assay of genetic diversity and thus giving a robust, unambiguous molecular description of rice cultivars (Nagraju *et al.,* 2002).

In the present study, sixty four aromatic landraces of rice collected from different districts of Madhya Pradesh were analyzed for genetic variation using SSR markers. Specially, the objective of the study was DNA fingerprinting and genetic diversity analysis of aromatic landraces to requisite to study the

unique feature and to identify the duplication among them and to assist in broadening the germplasm base of future aromatic rice breeding programs.

Materials and Methods

Germplasm Collection and Morphological Characterization

A total of sixty four farmers varieties collected from different districts of Madhya Pradesh (Table 1) were grown in a randomized complete block design with three replications at Seed Breeding Farm, JNKVV Jabalpur in kharif 2015 and kharif 2016, each entry was sown in three rows of 2m length at spacing of 20 cm between rows and 15 cm between plants. Crop was raised following recommended package of practices. Observations were recorded on five randomly chosen plants of each genotype per replication for twenty five traits, according to the National Test Guidelines for DUS test in rice which was developed by Directorate of Rice Research, Rajendarnagar, Hyderabad (Shobha Rani *et al.,* 2004). The observation of various characteristics was recorded at different stages of growth with appropriate procedures as per the DUS test guidelines of PPV & FR Act, 2001.

DNA Extraction

Total genomic DNA was isolated from fresh young leaves of 64 farmers varieties collected from different districts of Madhya Pradesh following the CTAB (cetyl trimethyl ammonium bromide) procedure as described by Saghai Maroof *et al.,* (1984) with some modifications. Quantification of DNA was accomplished by analyzing the DNA on 0.8% agarose gel stained with ethidium bromide using diluted uncut lambda DNA as standard. Final concentration was adjusted to 50ngμl^{-1} for further uses in PCR analysis.

PCR amplification

A total of 31 SSRs primer pairs, distributed across the genome of rice were used (www.gramenae.org.in). The details of SSR markers, their sequences and motifs are given in table 2. DNA was amplified by PCR using our previously standardized method (Sahu *et al.,* 2012) in a total volume of 10 µl containing 2X PCR assay buffer, 1.5mM $MgCl_2$, 100µM of each dNTPs, 12ng each of forword and reverse primers, 0.2 units of Taq DNA polymerase and 25 ng of genomic DNA template. Amplification reaction initiated with a 5-minute pre-denaturation steps at 94^0 C followed by 35 cycles of DNA denaturation at 94^0 C for 30 seconds, primer annealing at 50-55^0 C for 30 seconds and DNA extension at 72^0 C for 7 minutes was performed after 35 cycles. Amplified PCR products was separated on 2.0% of agarose gel at a volage of 90V for the period of 45 minutes to 1 hour in 1X TBE buffer stained with ethidium bromide. The gel was visualized in UV transilluminator and photograph taken using Alpho Digidoc gel documentation instrument.

SSR allele scoring and data analysis

The presence or absence of SSR fragment in each accession was recorded for all the polymorphic SSR markers. The SSR bands appearing without ambiguity were scored as 1 (present) and 0 (absent) for each primer. The size of the amplified product was calculated on the basis of its mobility relative to molecular mass of marker (100 bp DNA ladder). The genetic similarity among genotypes was estimated based on Jaccard's similarity coefficient. The resulting similarity matrix was further analysed using the unweighted pair-group method arithmetic average (UPGMA) clustering algorithm for construction of dendrogram; the computations were carried out using NTSYSpc version 2.2 (Rohlf 2000).

Results and Discussion

Morphological characterization of farmer's varieties

Twenty two (88%) out of 25 phenotypic traits showed variation among the 64 aromatic farmers varieties of rice, whereas no variation was observed for the traits like leaf: auricle, leaf: shape of ligule and stem: anthocyanin colouration of nodes. The summarised data of all the traits for farmers varieties are presented in table 2.

Most of the farmer's varieties exhibited green (90.63%) and light purple (7.81%) basal leaf sheath colour. One farmers variety Nagkesar showed purple basal leaf sheath colour. Weak (35.94% & 85.94%) and medium (64.06% & v14.06%) pubescence on blade surface and lemma of the spikelet showed by all the farmers varieties, respectively. Colourless leaf anthocyanin colouration of auricles expressed by most of the farmers varieties (93.75%) followed by light purple (6.25%).

White (92.19%) and light purple (7.81%) ligule colour exhibited by farmers varieties. Most of the farmer's varieties exhibited medium (56.25%), late (26.56%) and early (17.19%) time of heading. Most of the lines showed erect (87.50% & 15.63%), semi erect (12.50% & 68.75%) and horizontal (0.00% & 15.63%) flag leaf attitude of blade in early and late observation, respectively. Lemma anthocyanin colouration is absent in most of the lines (85.94%), while rest of the lines (14.07%) expressed weak, medium, strong and very strong anthocyanin colouration of lemma. White stigma colour expressed by most of the lines (84.38%) followed by purple (14.06%) and light purple (1.56%).

High variation observed in the character stem length (excluding panicle) in the classes of very long (39.06%), long (25.00%), medium (23.44%) and short (12.50%) and same is also for panicle length in the classes of long (57.81%), medium (28.13%), very long (9.38%) and short (4.69%). Deflexed panicle curvature exhibited by most of the lines (85.94%) followed by drooping (10.94%) and semi straight (3.13%). Most of the lines had medium (54.69%) number of panicle per plant followed by few (40.63%) and many (4.69%). Yellowish white colour of tip of lemma showed by most of the lines (54.69%) followed by brown (25.00%), black (12.50%), white (6.25%) and purple (1.56%). Panicle awns present in 65.63% of the farmers varieties, out of which 69.04% showed yellowish white awn colour followed by yellowish brown (21.42%) and reddish brown (9.52%) and their distribution in upper half only (50.00%), whole length (45.24%) and tip only (4.76%). Most of the lines expressed erect to semi erect (78.13%) attitude of secondary branches in panicle followed by semi erect (20.31%) and semi erect to spreading (1.56%). Farmers varieties showed well exserted (84.38%) and mostly exserted (15.63) type of panicle exsertion. Very high variation was observed for the trait sterile lemma colour in the classes of straw (76.56%), purple (10.94%), gold (7.81%) and 4.69%). Similar trend was also observed for grain weight of 1000 fully developed grains in the classes of low (28.13%), medium (25.00%), very high (20.31%), very low (18.75%) and high (7.81).

Table.1 DUS Characterization of aromatic cultivars collected from different districts of Madhya Pradesh

S no.	Cultivar	Place of collection	2	8	9	10	14	15	20	21	22	25	27	29	30	33	34	35	36	37	39	40	42	45	46	49	50
1	Pusa Basmati	Umariya	1	5	9	1	3	1	5	1	3	1	1	3	1	9	3	5	5	2	9	1	5	5	7	1	5
2	Badalphool	Umariya	3	3	9	2	3	2	3	1	3	5	5	5	1	5	1	5	5	6	9	4	1	3	7	3	3
3	Luchai	Umariya	1	3	9	1	3	1	5	1	3	1	1	9	1	5	3	5	3	2	1	-	-	3	7	1	5
4	Chinnor	Umariya	1	5	9	1	3	1	7	1	3	1	1	9	1	5	3	5	3	2	1	-	-	3	7	1	1
5	Jeeraphool	Umariya	1	5	9	1	3	1	7	1	3	1	1	7	1	5	3	5	5	2	1	-	-	3	7	1	1
6	Basmatiya	Umariya	1	3	9	1	3	1	3	1	3	1	1	5	1	5	1	5	3	2	9	1	3	3	7	1	3
7	Badalphool	Umariya	3	3	9	2	3	2	3	1	3	5	5	5	1	5	1	5	5	6	9	4	1	3	7	3	3
8	Karhani	Umariya	1	3	9	1	3	1	3	1	5	1	1	5	1	3	1	3	5	2	9	1	5	3	5	1	9
9	Sukaraphool	Shahdol	1	3	9	1	3	1	5	1	3	9	1	9	1	7	3	7	3	5	9	4	5	3	7	4	5
10	Kapoorsar	Shahdol	1	5	9	1	3	1	5	3	5	9	1	9	1	9	5	7	3	6	1	-	-	3	7	4	3
11	Vishnubhog	Shahdol	1	5	9	1	3	1	5	1	3	1	1	9	1	7	3	5	5	3	9	1	3	3	7	2	3
12	Lohandi Choti	Shahdol	1	3	9	1	3	1	5	3	3	1	1	9	1	7	3	5	3	3	1	-	-	3	7	1	3
13	Vishnubhog	Shahdol	1	5	9	1	3	1	5	1	3	1	1	9	1	7	3	5	5	3	9	2	3	3	7	2	3
14	Kandha Jal	Shahdol	1	3	9	1	3	1	5	1	5	1	1	7	1	5	3	5	5	2	9	1	3	7	7	1	7
15	Jeeraphool	Shahdol	1	5	9	1	3	1	7	1	3	1	1	7	1	5	3	5	5	2	1	-	-	3	7	1	1
16	Laxmibhog	Dindori	1	3	9	1	3	1	5	1	5	1	1	9	1	7	5	5	3	3	1	-	-	3	7	1	1
17	Kshatriya	Dindori	1	5	9	1	3	1	7	1	3	1	1	9	1	7	3	5	3	2	9	2	5	3	7	1	3
18	Khuddy	Dindori	1	5	9	1	3	1	7	1	5	1	1	9	1	7	5	5	3	3	1	-	-	3	7	3	5
19	Luchai	Dindori	1	3	9	1	3	1	5	1	3	1	1	9	1	5	3	5	3	2	1	-	-	3	7	1	5
20	Kodo Kapoor	Dindori	1	5	9	1	3	1	5	3	3	1	1	9	1	7	5	7	3	3	1	-	-	3	7	1	1
21	Shrikamal	Dindori	1	5	9	1	3	1	7	1	3	5	1	9	1	9	5	7	3	6	1	-	-	5	7	4	1
22	Chota Luchai	Dindori	1	3	9	1	3	1	5	1	3	1	1	7	1	3	5	5	5	1	1	-	-	3	7	1	7
23	Vishnubhog	Dindori	1	5	9	1	3	1	5	3	3	1	1	9	1	7	3	5	5	3	9	2	3	3	7	2	3
24	Jeerashankar	Mandla	1	5	9	1	3	1	7	1	3	3	1	9	1	9	3	7	5	6	1	-	-	3	7	4	1
25	Ranikajar	Mandla	3	3	9	1	3	2	3	1	3	7	5	3	1	5	3	5	5	6	9	4	5	3	5	4	3
26	Akash	Mandla	1	3	9	1	3	1	3	1	5	1	1	3	1	7	3	5	5	2	1	-	-	3	5	1	5
27	Lakha Luchai	Mandla	1	3	9	1	3	1	5	1	3	1	1	7	1	5	3	5	3	3	1	-	-	3	7	1	5
28	Chota Luchai	Mandla	1	3	9	1	3	1	5	1	3	1	1	7	1	3	5	5	5	1	1	-	-	3	7	1	7
29	Durgawati	Mandla	3	3	9	1	3	2	3	1	3	1	5	7	1	7	3	5	3	1	9	1	5	3	7	1	5
30	Mahuadheta	Mandla	1	5	9	2	3	1	5	1	3	1	5	9	1	7	1	5	3	3	9	2	3	3	7	1	9
31	Mahuadheta	Mandla	1	5	9	1	3	1	5	1	3	1	5	9	1	7	1	5	3	3	9	2	3	3	7	1	9
32	Durgawati	Mandla	3	3	9	1	3	2	3	1	3	1	5	7	1	7	3	5	5	1	9	1	5	3	7	1	5
33	Nagkesar	Mandla	4	5	9	2	3	3	3	1	5	1	4	7	1	7	1	5	3	3	1	-	-	3	7	1	7
34	Nungi	Mandla	1	5	9	3	3	1	3	1	5	1	5	7	1	5	3	5	3	3	9	2	3	3	5	1	9
35	CSR 30	Hosangabad	1	5	9	1	3	1	5	1	3	1	1	7	1	7	5	5	5	2	9	1	5	3	5	1	5
36	Pusa 1121	Hosangabad	1	5	9	1	3	1	5	1	3	1	1	5	1	7	3	5	7	2	9	1	3	5	7	1	9

37	CSR 30	Hosangabad	1	5	9	1	3	1	5	1	3	1	1	7	1	7	5	5	5	2	9	1	5	3	5	1	5
38	Pusa 1121	Hosangabad	1	5	9	1	3	1	5	1	3	1	1	5	1	7	3	5	5	2	9	1	3	5	7	1	9
39	Pusa 1	Hosangabad	1	5	9	1	3	1	5	1	3	1	1	5	1	7	3	5	5	2	9	1	5	3	7	1	5
40	Menka	Hosangabad	1	5	9	1	3	1	5	3	3	1	1	3	1	7	3	5	3	2	9	1	3	5	5	1	9
41	Desi Basmati	Hosangabad	1	5	9	1	3	1	7	1	3	1	1	7	1	7	5	5	5	3	9	1	3	3	7	2	3
42	Pusa 1121	Hosangabad	1	5	9	1	3	1	5	1	3	1	1	5	1	7	3	5	7	2	9	1	3	5	7	1	9
43	Sugandha	Raisen	1	5	9	1	3	1	5	1	3	1	1	3	1	7	3	5	3	2	9	1	3	5	5	1	9
44	Pusa 1121	Raisen	1	5	9	1	3	1	5	1	3	1	1	5	1	7	3	5	5	2	9	1	3	5	7	1	9
45	Pusa 1	Raisen	1	5	9	1	3	1	5	1	3	1	1	5	1	7	3	5	5	2	9	1	5	3	7	1	5
46	Pusa 1121	Raisen	1	5	9	1	3	1	5	1	3	1	1	5	1	7	3	5	5	2	9	1	3	5	7	1	9
47	Kshatriya	Raisen	1	5	9	1	3	1	7	1	3	1	1	9	1	7	3	5	3	2	9	2	5	3	7	1	3
48	Pusa 1121	Raisen	1	5	9	1	3	1	5	1	3	1	1	5	1	7	3	5	5	2	9	1	3	5	5	1	9
49	Pusa 1121	Raisen	1	5	9	1	3	1	5	1	3	1	1	5	1	7	3	5	5	2	9	1	3	5	7	1	5
50	Pusa 1	Raisen	1	5	9	1	3	1	5	1	3	1	1	5	1	7	3	5	5	2	9	1	5	3	7	1	5
51	Menka	Raisen	1	5	9	1	3	1	5	3	3	1	1	3	1	7	3	5	3	2	9	1	3	5	5	1	9
52	Pusa 1	Raisen	1	5	9	1	3	1	5	1	3	1	1	5	1	7	3	5	5	2	9	1	5	3	7	1	5
53	Kshatirya	Katni	1	5	9	1	3	1	7	1	3	1	1	9	1	7	3	5	3	2	9	2	5	3	7	1	3
54	Madhuri	Katni	1	3	9	1	3	1	5	1	3	1	1	3	1	5	1	5	5	2	9	1	5	3	7	1	3
55	Dubraj	Katni	1	3	9	1	3	1	7	1	3	1	1	7	1	5	3	5	3	3	9	1	5	3	7	1	3
56	Vishnubhog	Balaghat	1	5	9	1	3	1	5	3	3	1	1	9	1	7	3	5	5	3	9	2	3	3	7	2	3
57	Jeerashankar	Balaghat	1	5	9	1	3	1	7	1	3	3	1	9	1	9	3	7	5	6	1	-	-	3	7	4	1
58	Chinnor	Balaghat	1	5	9	1	3	1	7	1	3	1	1	9	1	5	3	5	3	2	1	-	-	3	7	1	1
59	Makram	Balaghat	1	3	9	1	3	1	5	3	3	1	1	3	1	5	1	5	7	2	9	1	3	3	7	1	3
60	Chinnor Desi	Balaghat	1	3	9	1	3	1	7	1	3	1	1	9	1	7	3	5	5	2	9	1	5	3	7	1	1
61	Dubraj	Balaghat	1	3	9	1	3	1	7	1	3	1	1	7	1	5	3	5	5	3	9	1	5	3	7	1	1
62	Jeerashankar	Balaghat	1	5	9	1	3	1	7	1	3	3	1	9	1	9	3	7	5	6	1	-	-	3	7	4	3
63	Chinnor	Balaghat	1	5	9	1	3	1	7	1	3	1	1	9	1	5	3	5	3	2	1	-	-	3	7	1	1
64	Tulsi	Balaghat	1	3	9	1	3	1	3	1	5	1	5	7	1	7	1	3	5	2	1	-	-	5	7	1	7

2-Basal leaf: sheath colour, 8- Leaf: pubescence of blade surface, 9- Leaf: auricles, 10- Leaf: anthocyanin colouration of auricles, 14- Leaf: shape of ligule, 15- Leaf: colour of ligule, 20-Time of heading, 21- Flag leaf attitude of blade (early observation), 22- Spikelet: density of pubescence of lemma, 25- Lemma: anthocyanin colouration of area below apex, 27- Spikelet: colour of stigma, 29- Stem: length (excluding panicle), 27- Stem: anthocyanin colouration of nodes, 28- Stem: intensity of anthocyanin colouration of nodes, 30-Stem: anthocyanin colouration of nodes, 33- Panicle: length of main axis (cm), 34- Flag leaf attitude of blade (late observation), 35- Panicle: curvature of main axis, 36-Panicle: number per plant, 37- Spikelet: colour of tip of lemma, 39- Panicle: awns, 40- Panicle: colour of awns, 42- Panicle: distribution of awns, 45- Panicle: attitude of branches, 46- Panicle: exsertion, 49- Sterile lemma colour, 50- Grain weight of 1000 fully developed grains.

Table.2 SSR markers with their sequences selected for the study

Primers	Reverse sequence	Forward sequence	Amplification temperature (^{o}C)
RM 1	GCGTTGGTTGGACCTGAC	GCGAAAACACAATGCAAAAA	55
RM 5	GCATCCGATCTTGATGGG	TGCAACTTCTAGCTGCTCGA	55
RM 8	AACACAGCAGGTACGCGC	CGCTAGGGCAGCATCTAAA	55
RM 11	ATAGCGGGCGAGGCTTAG	TCTCCTCTTCCCCCGATC	55
RM 16	AACACAGCAGGTACGCGC	CGCTAGGGCAGCATCTAAA	55
RM 17	GGTGATCCTTTCCCATTTCA	TGCCCTGTTATTTTCTTCTCTC	55
RM 30	TCACCTCACCACACGACACG	GGTTAGGCATCGTCACGG	55
RM 137	CGGGTGGTCCCCGAGGATCTTG	GACATCGCCACCAGCCCACCAC	55
RM 152	CCGTAGACCTTCTTGAAGTAG	GAAACCACCACACCTCACCG	55
RM 154	CTCCTCCTCCTGCGACCGCTCC	ACCCTCTCCGCCTCGCCTCCTC	55
RM 201	CTACCTCCTTTCTAGACCGATA	CTCGTTTATTACCTACAGTACC	55
RM 202	CCAGCAAGCATGTCAATGTA	CAGATTGGAGATGAAGTCCTCC	55
RM 217	GGGTGTGAACAAAGACAC	ATCGCAGCAATGCCTCGT	55
RM 219	CATATCGGCATTCGCCTG	CGTCGGATGATGTAAAGCCT	55
RM 223	GAAGGCAAGTCTTGGCACTG	GAGTGAGCTTGGGCTGAAAC	55
RM 228	GCTTGCGGCTCTGCTTAC	CTGGCCATTAGTCCTTGG	55
RM 231	CACTTGCATAGTTCTGCATTG	CCAGATTATTTCCTGAGGTC	55
RM 234	CACGTGAGACAAAGACGGAG	ACAGTATCCAAGGCCCTGG	55

RM 237	TGGGAAGAGAGCACTACAGC	CAAATCCCGACTGCTGTCC	55
RM 242	TATATGCCAAGACGGATGGG	GGCCAACGTGTGTATGTCTC	55
RM 251	ATGCGGTTCAAGATTCGATC	GAATGGCAATGGCGCTAG	55
RM 256	GTTGATTTCGCCAAGGGC	GACAGGGAGTGATTGAAGGC	55
RM 260	GAACAATCCCTTCTACGATCG	ACTCCACTATGACCCAGAG	55
RM 263	GCTACGTTTGAGCTACCACG	CCCAGGCTAGCTCATGAACC	55
RM 271	TCGGTGAGACCTAGAGAGCC	TCAGATCTACAATTCCATCC	55
RM 25	CTACCATCAAAACCAATGTTC	GGAAAGAATGATCTTTTCATGG	55
RM 283	CGGCATGAGAGTCTGTGATG	GTCTACATGTACCCTTGTTGGG	55
RM 338	GGCAAACCGATCACTCAGTC	CACAGGAGCAGGAGAAGAGC	55
RM 488	TAGCAACAACCAGCGTATGC	CAGCTAGGGTTTTGAGGCTG	55
RM 255	CGAAACCGCTCAGTTCAAC	TGTTGCGTGTGGAGATGTG	55
RM 277	CAAGGCTTGCAAGGGAAG	CGGTCAAATCATCACCTGAC	55

Table.3 Number, polymorphic and unique alleles and allele size n involving SSR markers

S no.	Primers	Number of alleles	Polymorphic alleles	Unique alleles	Allele size range (bp)
1	RM 1	25	25	10	91-225
2	RM 5	24	24	10	91-230
3	RM 8	14	14	05	240-292
4	RM 11	23	23	07	100-200
5	RM 16	19	19	05	115-196
6	RM 17	13	13	04	141-200
7	RM 30	15	15	03	80-132
8	RM 137	17	17	05	163-244
9	RM 152	17	17	03	116-224
10	RM 154	36	36	07	111-385
11	RM 201	23	23	10	121-293
12	RM 202	15	15	05	122-200
13	RM 217	15	15	02	112-165
14	RM 219	27	27	10	115-248
15	RM 223	16	16	01	142-231
16	RM 228	16	16	06	100-175
17	RM 231	21	21	02	135-274
18	RM 234	26	26	10	103-279
19	RM 237	15	15	03	82-165
20	RM 242	17	17	07	205-292
21	RM 251	16	16	05	90-158
22	RM 256	15	15	01	85-140
23	RM 260	14	14	06	91-148
24	RM 263	20	20	03	140-289
25	RM 271	11	11	05	83-180
26	RM 25	34	34	19	86-248
27	RM 283	13	13	01	126-183
28	RM 338	11	11	02	125-183
29	RM 488	17	17	07	165-250
30	RM 255	22	22	09	117-204
31	RM 277	11	11	01	113-154

Fig.1 SSR Profiling of Rice varieties using RM 25 SSR markers

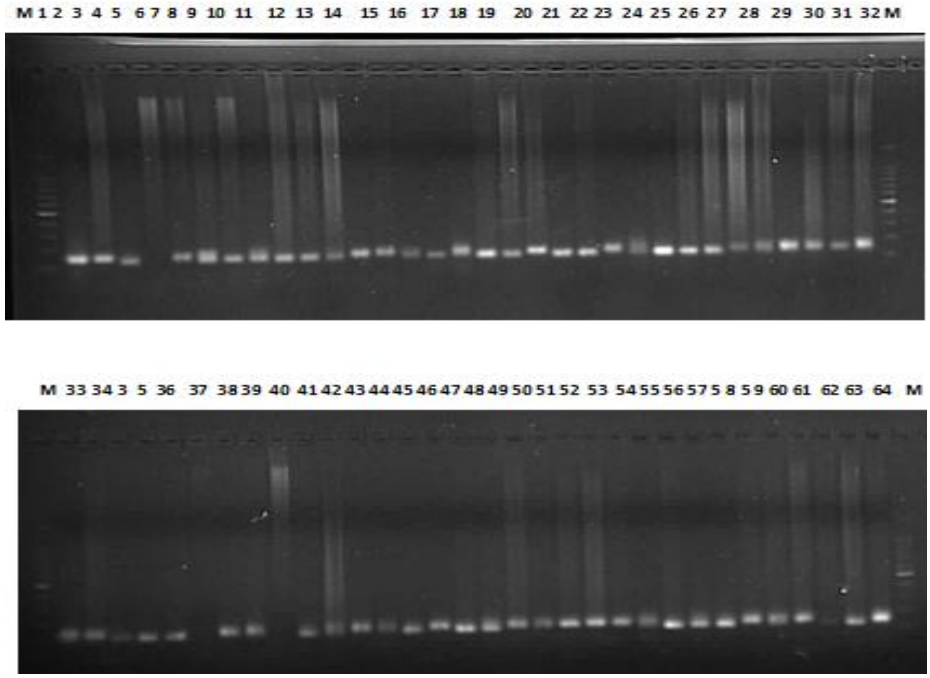
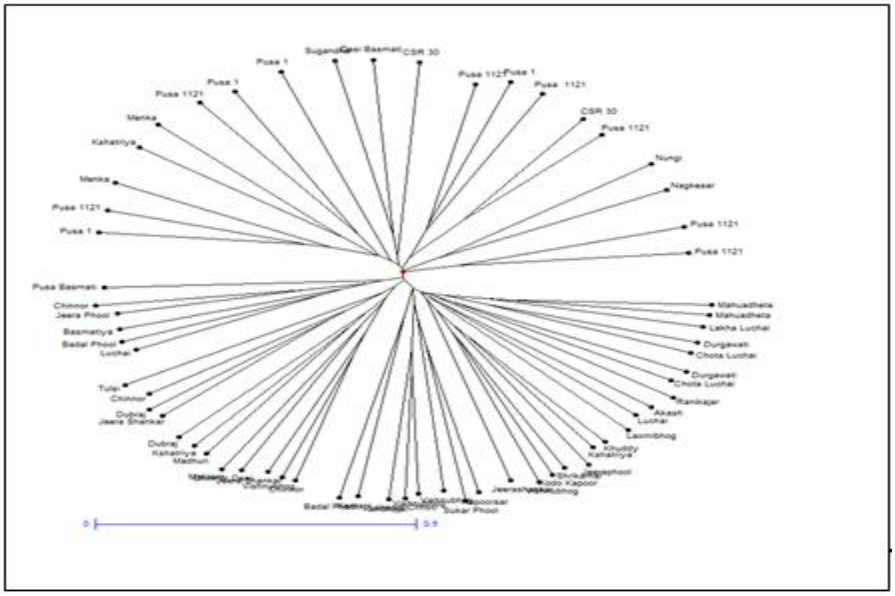


Fig.2 Unrooted Dendogram of Rice varieties based on SSR markers



Molecular characterization

In the present investigation, 31 SSR markers were employed to assess the genetic diversity among 64 varieties of rice. The experiment for analysing genetic relationship using 31 SSR primers revealed that all primers showed polymorphism with reproducible and informative profiles (Figure-1). The DNA amplification pattern revealed that a total number of 594 SSR loci were amplified with an average of 19.31 loci per primer Table-3). Unrooted cluster analysis also depicted quiet variation among all rice varieties. (Figure-2). Molecular approaches are more reliable for assessment of genetic divergence in rice (Yogendra Singh (2011, Singh and Singh,2008): and these are being used by various workers time to time (Singh *et al.*,2013, Koutu *et al.,* 2017. Koutu *et al.,* 2019)

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