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Identification of superior rice (*Oryza sativa* L.) Germplasm from Bastar, using SSR molecular markers characterization

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Abstract
The experiment was conducted during *Kharif* 2016 in an RBD Design to assess the agro morphological characterization, genetic variability and genetic divergence among the ninety-four local landraces of rice and three popular standard checks namely MTU-1010, Danteshwari and CR-40. In the present investigation, out of 16 qualitative characters observed, basal leaf sheath colour, leaf blade colour, flag leaf angle, apiculus colour, awn length, awn colour, auricle colour, days to 50% flowering, plant height, panicle length, maturity(days) recorded the highest variation among genotypes. The analysis of variance for 20 quantitative characters showed that there were considerable inherent genetic differences for different characters. A wide range of genetic variability was observed for most of the quantitative traits. High magnitude of the coefficient of variation (more than 20%) in the entire genotypes was observed for grain yield per plant (27.4%), the number of effective tillers per plant (22.37%), test weight (21.14%), kernel length breadth ratio (20.59%). Total of 12 SSR markers (primers) was used for molecular characterization and discrimination of 28 genotypes of rice. After analysing the data generated from 12 microsatellite markers (SSR), a total of 22 alleles were detected in 28 rice genotypes. The number of alleles per locus generated by each marker ranged from 1 to 3 alleles with an average of 1.8 alleles per locus. Out of 12 SSR markers, 6 markers showed polymorphic reaction with polymorphism information content (PIC) values of 0.53 in RM125, 0.6 in RM161 and 0.75 in RM152, 0.84 in OCR13, 0.88 in RM413 and 0.89 in RM 408. Genetic similarity of genotypes of rice understudy the genetic similarity coefficient (Jaccard coefficient) ranged from 0.51-1.00 as revealed by UPGMA cluster analysis using the 12 SSR markers. A total of five distinct groups resulted in a cut-off similarity coefficient of 0.683 among the 28 rice genotypes.

Keywords: Bastar rice, SSR markers, genetic variability, heritability, genetic advance, quantitative traits

Introduction
Rice is one of the most important staple foods for more than half of the world's population (Anonymous, 2006) [2] and influences the livelihoods and economies of several billion people. Rice has been gathered, consumed, and cultivated by human world-wide from many years, longer than any other crop. Except of course for Antarctica, every continent of the planet produces rice, with over 122 countries currently growing the crop. Rice is grown from the equator to latitudes of 53^0 N (in China) and 35^0 N to elevations (in tropical regions) as high as 2400 meters above sea level (Kenmore, 2003) [9]. India is one of the centres for rice diversity and large diversity has been reported 100^{th} at inter and intraspecific levels.

The total area under rice cultivation is globally estimated to be 162 million hectares with annual global production for 2016 at 745.5 million tonnes (495.2 million tonnes, milled basis) (Anonymous, 2016) [3]. Rice is life, for most people living in Asia. Rice has shaped the cultures, diets, and economies of thousands of millions of people. For more than half of humanity rice is life. Considering its important position, the United Nations designated the year 2004 as the International Year of Rice. Devoting a year to a commodity was unprecedented in United Nations history. However, the 57^{th} session of the United Nations General Assembly noted that rice is the staple food of more than half the world's population, affirmed the need to heighten the awareness of the role of rice in alleviating poverty and malnutrition and reaffirmed the need to focus world attention on the role rice can play in providing food security and eradicating poverty and declared the year 2004 as the International Year of Rice. Approximately 50% of consumed calories by the whole population of humans depend on Wheat, Rice, and Maize (Gnanamanickam, 2009) [7].

The Rice Germplasm Section at Indira Gandhi Krishi Vishwavidyalaya, Raipur maintains a collection of more than 23,250 accessions, which is biggest in India and second-largest in the world after International Rice Research Institute, Philippines. And also the Germplasm Section at Shaheed Gundadhoor College of Agriculture & Research Station Kumhrawand, Jagdalpur maintains a collection of more than 390 accessions, which is biggest in Bastar. The collection was collected and conserved as biological treasure from overall Bastar division. The collection includes traditional cultivars like Safari, Gurmatia, Bhata Mokdo, Chudi Dhan, Kalimoonch, Laicha, etc. are according to the traditional healers of Bastar region many of these medicinal rice varieties are used in traditional medicine system for treatment of rheumatism, skin infections, paralysis, diabetes etc. (Das, and Oudhia, 2001) [6].

For the development of economically high-yielding varieties with all of the desirable agronomic traits, it is also important to consider other characteristics when selecting the parental material such as aspects related to the difference in grain type and shape, plant height, resistance to biotic and abiotic stresses. Yield is a complex trait being governed by a large number of cumulative, duplicate and dominant genes and directly or indirectly influenced by environment as well as response poorly to the direct selection. For the improvement of grain yield, the knowledge on the association between grain yield and its component characters will be helpful. Keeping in view the above facts, the investigation is very important for the exploration of hidden beneficial genetic characters of indigenous rice of Bastar. The old and existing landraces are important genetic resources of the region having the quality for climate-resilient agriculture in consideration of moisture stress, insects, diseases, etc. The diversity among landraces of any crop is required for improvement of the crop. This investigation may be helpful for researchers to identify the gene in combating biotic and abiotic stresses which are needed in the near future to achieve food security.

Materials and Methods

Phenotypic characterization

The Phenotypic characterization experimental materials comprised of ninety-four local landraces of rice and three popular standard checks. The experimental materials were received from rice breeding section of S.G. College of Agriculture and Research Station, Jagdalpur, Bastar, Chhattisgarh.

Molecular characterization

After Phenotypic characterization, on the basis of yield performance, top twenty-five Genotypes and three checks were selected for molecular analysis.

Total genomic DNA was extracted using modified CTAB (Mini-Prep) method Zheng *et al*. (1995) [20]. The quality of genomic DNA sample was assessed by 1% agarose gel electrophoresis at 5V/cm. For the Visualization of amplified products in gel electrophoresis two methods were used Agarose gel electrophoresis and Polyacrylamide gel electrophoresis. 2% agarose gels were used for separation and visualization of PCR amplified products of SSR markers. Gels were cast in electrophoresis unit. Loading dye (10x) was added to PCR products. Finally, the sample is loaded into the wells for facilitating the sizing of the various alleles. Known size markers, DNA ladder was loaded in the first well. The gel was run at 80 volts till the dye reached bottom of the gel for about 90-120 minutes. After completion of gel electrophoresis, gels were subjected to documentation with MultiImage Light Cabinet (Gel documentation system) and subsequently gel picture having DNA as a band was saved to the desktop connected with MultiImage Light Cabinet.

Detection of varietal polymorphism using simple sequence repeats (SSR) primers.

The varietal polymorphism was detected by using 12 SSR primers. The primers used for this purpose are presented in (Table 1).

Scoring and analysis of data:

The banding pattern of the population developed by each set of primer was scored separately. The size of amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers, DNA ladder. Particular base pair position was scored as "1" and absence of band for that particular base pair position was scored as "0" (zero). For analysis, XLSTAT 2017 software was used to construct a UPGMA (unweight pair group method with arithmetic averages) Jaccard's similarity coefficient dendrogram showing the distance-based interrelationship among the genotypes.

Polymorphism Information Content (PIC)

Genetic diversity was calculated at each locus for allelic Polymorphism Information Content (PIC), The PIC values for each SSR were estimated by determining the frequency of alleles per locus (Botstein *et al.*, 1980; Anderson *et al.*, 1993) [4, 1] using the following formula:

$$PIC = 1 - \Sigma X_i^2$$

Where x**i** is the relative frequency of the i^{th} allele of the SSR loci.

Table 1: 12 Microsatellite markers used for molecular characterization across 28 local genotypes of rice

S. No.	SSR Primers	Chromosome number	Primer sequences	
			Forward 5' → 3'	Reverse 5' → 3'
1	RM413	5	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC
2	RM161	5	TGCAGATGAGAAGCGGCGCCC	TGTGTCATCAGACGGCGCTCCG
3	RM162	6	GCCAGCAAAACCAGGGATCCG	CAAGGTCTTGTGCGGCTTGCGG
4	RM125	7	ATCAGCAGCCATGGCAGCGAC	AGGGGATCATGTGCCGAAGGC
5	RM408	8	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC
6	RM44	8	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
7	RM484	10	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
8	RM277	12	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG
9	RM338	3	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC
10	RM152	2	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG

11	RM154	2	ACCCTCTCCGCCTCGCCTCCC	CTCCTCCTCCTGCGACCGCCC
12	OSR 13	3	CATTTGTGCGTCACGGAGTA	AGCCACAGCGCCCATCTCTC

Results and Discussion

Molecular Characterization

Genetic characterization of crop plants has gained momentum with the advent of PCR based molecular markers. SSR markers are widely used for molecular characterization as is co-dominant distributed throughout the genome, highly reproducible, variable, reliable, easily scoreable, abundant and multi-allelic in nature. SSR markers have been used by many researchers for the characterization of rice varieties. These markers are highly polymorphic nature and give a better genetic diversity spectrum.

In the present study, the aim was to assess the trend in genetic diversity of rice germplasm SSR markers were employed to understand the genetic relationship amongst the varieties. 28 genotypes of rice were selected (25 germplasm line and three checks) based on grain yield per plant from the 97 rice genotype for molecular characterization and genetic diversity study presented in (Table 2).

A total of 12 SSR markers (primers) were used for molecular characterization and discrimination of 28 genotypes of rice. After analyzing the data generated from 12 microsatellite markers (SSR), a total of 22 alleles were detected in 28 rice genotypes. The number of alleles per locus generated by each marker ranged from 1 to 3 alleles with an average of 1.8 alleles per locus. The highest number of alleles (3) was detected in the locus RM413, RM408, RM152 and OSR13 the lowest number of alleles (1) was detected in each of locus RM162, RM44, RM484, RM227, RM338 and RM154. List of 12 microsatellite markers with their chromosomal locations, number of alleles, annealing temperature and allele size found among 28 rice genotypes are presented in (Table 3). Out of 12 SSR markers, 6 markers, RM162, RM44, RM484, RM227, RM338 and RM154 exhibited monomorphic reaction for all the genotypes whereas rest 6 showed polymorphic reaction with polymorphism information content (PIC) values of 0.53 in RM125, 0.6 in RM161 and 0.75 in RM152, 0.84 in OCR13, 0.88 in RM413 and 0.89 in RM408 (Presented in table 4). This suggests that these markers could be potentially used for molecular characterization of rice genotype from various sources. The gel images of amplified fragments produced by primers are presented in Fig. 1 to 7.

Microsatellite markers (SSR) are also used to detect the genetic similarity of genotypes of rice under study. The genetic similarity coefficient (Jaccard coefficient) ranged from 0.0.51-1.00 as revealed by UPGMA cluster analysis using the 12 SSR markers. A total of five distinct groups resulted in a cut-off similarity coefficient of 0.683 among the 28 rice genotypes. The dendrogram shows a clear separation of the rice genotypes into five groups (Fig. 8). But the genotype was divided into two major clusters, cluster I and cluster II. Cluster I had only one genotype Rago vati with the 51% genetic similarity with cluster II. Cluster II had divided into two sub-clusters IIA and IIB with the 57% genetic similarities. In the sub-cluster, IIA had only two genotype Band kari and Pat dhan with the 84% genetic similarity. Subcluster IIB had further divided in two sub-cluster IIB1 and IIB2 with the 62% genetic similarity and sub-cluster IIB1 had further divided in two sub-cluster IIB1 (i) and IIB1 (ii) with the 68% genetic similarity, sub-cluster IIB1 (i) had three genotypes Kala umari, Baso mati and Rang gada khuta, Baso mati and Rang gada khuta are 100% genetic similarity and Kala umari had 84% genetic similarity with Both genotype. Sub cluster IIB1 (ii) had divided in sub cluster of IIB1(ii) a and IIB1 (ii) b with the 72% genetic similarity and sub cluster IIB (ii) a had two genotype Barangi and Hathi panjra with the 84% genetic similarity. Sub cluster IIB1 (ii) b had further divided IIB1(ii) b* and IIB1(ii)b** with the 78% genetic similarity. IIB1(ii)b had only one genotype Bode bargi and IIB1(ii) b** had two genotypes Noni dhan and Bhata mokdo with the 84% genetic similarity.

Table 2: List of genotype selected for molecular analysis based on grain yield per plant

S. No.	Genotype Name	Grain Yield per plant (in Grams)
1	Rago vati	41.71
2	Hathi panjra	34.60
3	Ghdva phool	30.80
4	Baso mati	30.02
5	Sendur senga	26.40
6	Bhata mokdo	25.80
7	Narial	25.50
8	Pat dhan	24.90
9	Gogal sathka	24.87
10	Band kari	24.80
11	Rang gada khuta	24.73
12	Rami gali	24.56
13	Kala umari	23.83
14	Lankeshri	23.82
15	Kormel	23.68
16	Bode bargi	23.54
17	Pharsa phool	23.37
18	Baku dhan	23.23
19	Dumar phool	23.10
20	Noni dhan	22.40
21	Kari chudi	22.40
22	Mundra chudi	22.34
23	Rai kera	22.00
24	Barangi	21.80
25	Mudria	21.60
26	MTU1010	15.13
27	Danteshwari	15.00
28	CR40	28.00

Table 3: List of 12 microsatellite markers with their chromosomal locations, number of alleles and allele size found among 28 rice genotypes

S. No.	SSR Primers	Chromosome No.	Annealing temp.	No of alleles	Allele size (bp)
1	RM413	5	53	3	75, 80, 85
2	RM161	5	61	2	130, 150
3	RM162	6	61	1	130
4	RM125	7	63	2	120, 130
5	RM408	8	55	3	125, 130, 140
6	RM44	8	53	1	105
7	RM484	10	55	1	310
8	RM277	12	55	1	115
9	RM338	3	55	1	165
10	RM152	2	53	3	100, 110, 120
11	RM154	2	61	1	230
12	OSR 13	3	53	3	120, 130, 140

Table 4: List of 12 microsatellite markers with their polymorphic information content value

S. No.	SSR Primers	PIC value
1	OCR 13	0.84
2	RM 413	0.88
3	RM 125	0.53
4	RM 484	0
5	RM 338	0
6	RM 227	0
7	RM 44	0
8	RM 152	0.75
9	RM 161	0.6
10	RM 162	0
11	RM 408	0.89
12	RM 154	0

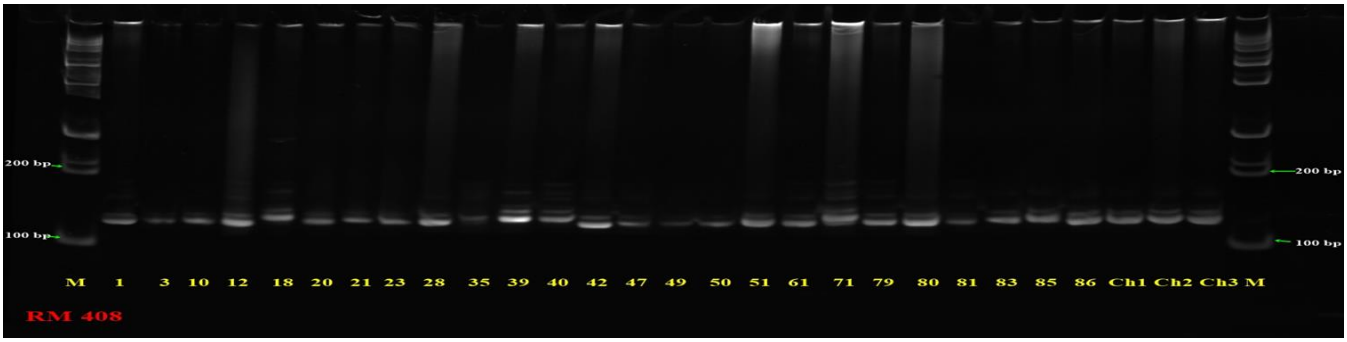

Fig. 1: Banding pattern generated using RM408 micro satellite primer

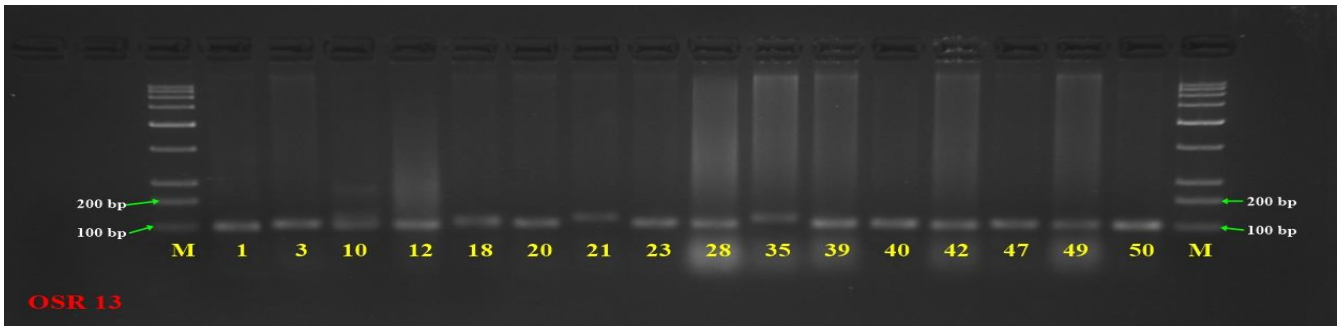

Fig 2: Banding pattern generated using OSR13 micro satellite primer

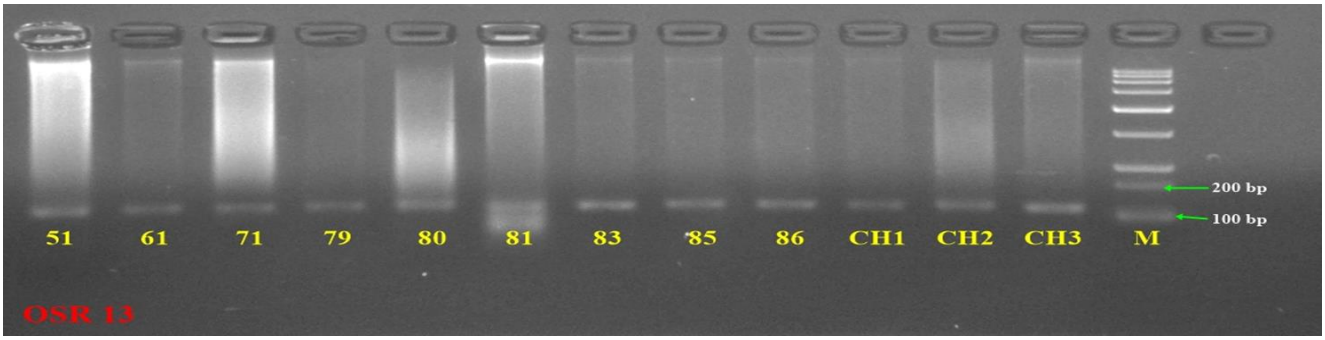

Fig 3: Banding pattern generated using OSR13 micro satellite primer

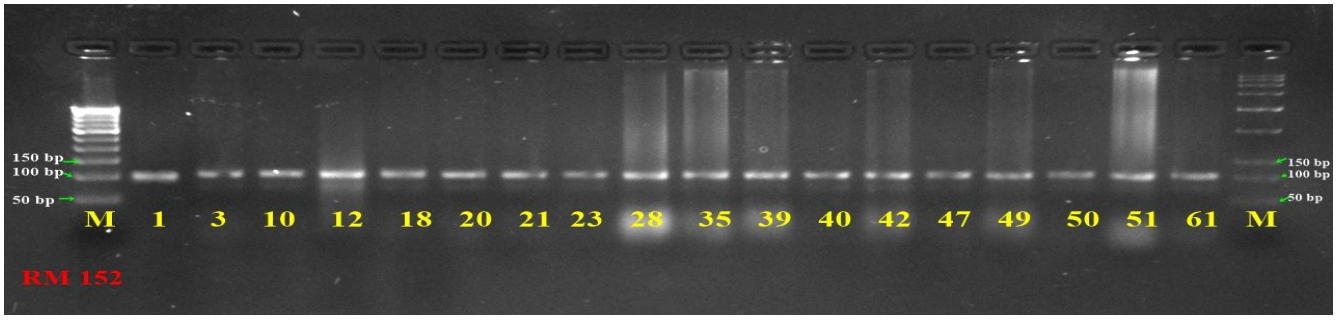

Fig 4: Banding pattern generated using RM152 micro satellite primer

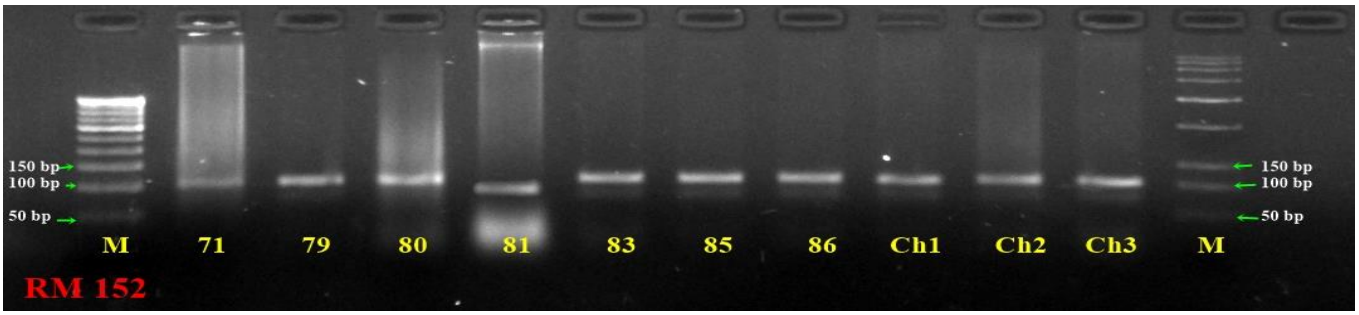

Fig 5: Banding pattern generated using RM152 micro satellite primer

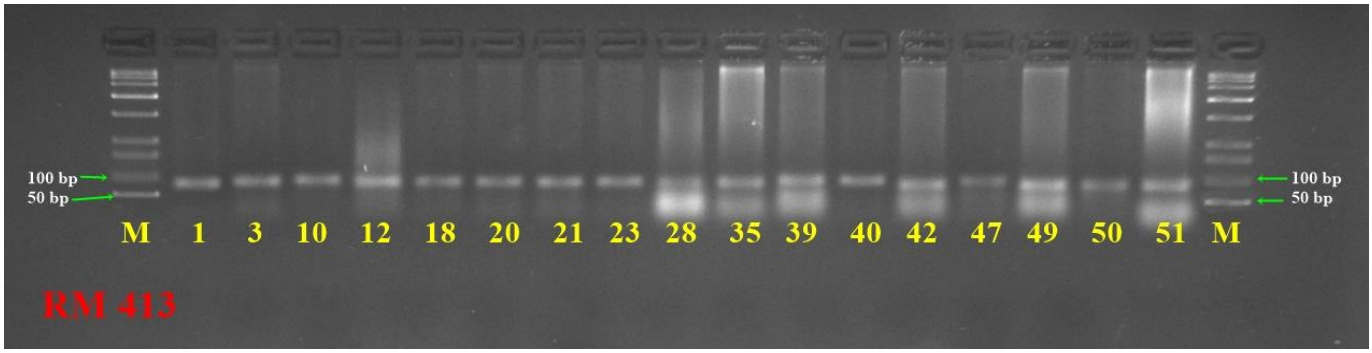

Fig 6: Banding pattern generated using RM413 micro satellite primer

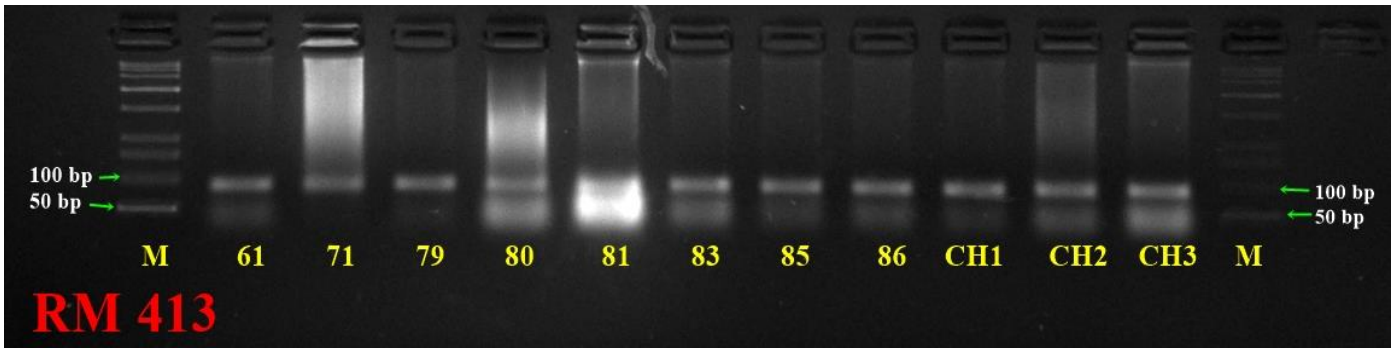

Fig 7: Banding pattern generated using RM413 micro satellite primer

Subcluster of IIB2 is divided into two sub-cluster IIB2(i) and IIB2(ii) with the 68% genetic similarity and IIB2(i) is further divided two sub-clusters with the 84% genetic similarity one sub-cluster contained two genotypes Mundra chudi and Ghdva phool with the 100% genetic similarity, another sub-cluster was contained five genotype Googl sathka, Kormel, MTU1010, Danteshwari and CR40 with duplicate (100% genetic similarity). Subcluster IIB2(ii) divided two sub-cluster IIB2(ii)a and IIB2(ii)b with the 79% genetic similarity, the IIB2(ii)a further divided two sub-clusters with the 84% genetic similarity one sub-cluster was contained four genotype Narial, Sendur senga, Kari chudi and Baku dhan with the 100% genetic similarity, another sub-cluster contained two genotypes Ramigali and Lankeshri with the 100% genetic similarity. Subcluster IIB2(ii)b had further divided two sub-clusters with 84% genetic similarity and one sub-cluster was contained only one genotype Dumar phool and another was contained three genotype Mudria, Rai kera and Pharsa Phool with the 100% genetic similarity (Fig. 8).

Thus, SSR markers provide acceptable power of resolution to discriminate between rice genotypes and it could serve as a probable tool in the identification and characterization of genetically distant cultivars from various sources.

The present investigation addresses the utilization of 12 microsatellite markers to reveal genetic polymorphism and ensures unambiguous identification of 28 genotypes of rice. The mean allele (1.8 alleles) across 12 loci obtained in our study was comparable with the result reported by Meti *et al.* (2013) [13].

In contrast, the mean value from our study is somewhat lower than the results observed in previous diversity studies, having similar result was observed in earlier report by Rahman *et al.* (2012) [15] who found an average of 4.18 alleles per locus and alleles per locus ranged from 2 alleles with an average of 3.33 alleles reported by Sajib *et al.* (2012), Kumar *et al.* (2014) [16, 10] reported SSR analysis with an average of 2.93, ranging from 1 to 4 alleles per locus. Patel *et al.* (2015) [14] conducted an experiment using SSR marker analysis and cluster analysis was performed using the Unweighted Paired Group of Arithmetic Means (UPGMA) using the Jaccard's similarity coefficient. The UPGMA dendrogram resolved the aromatic landraces of rice into two major clusters.

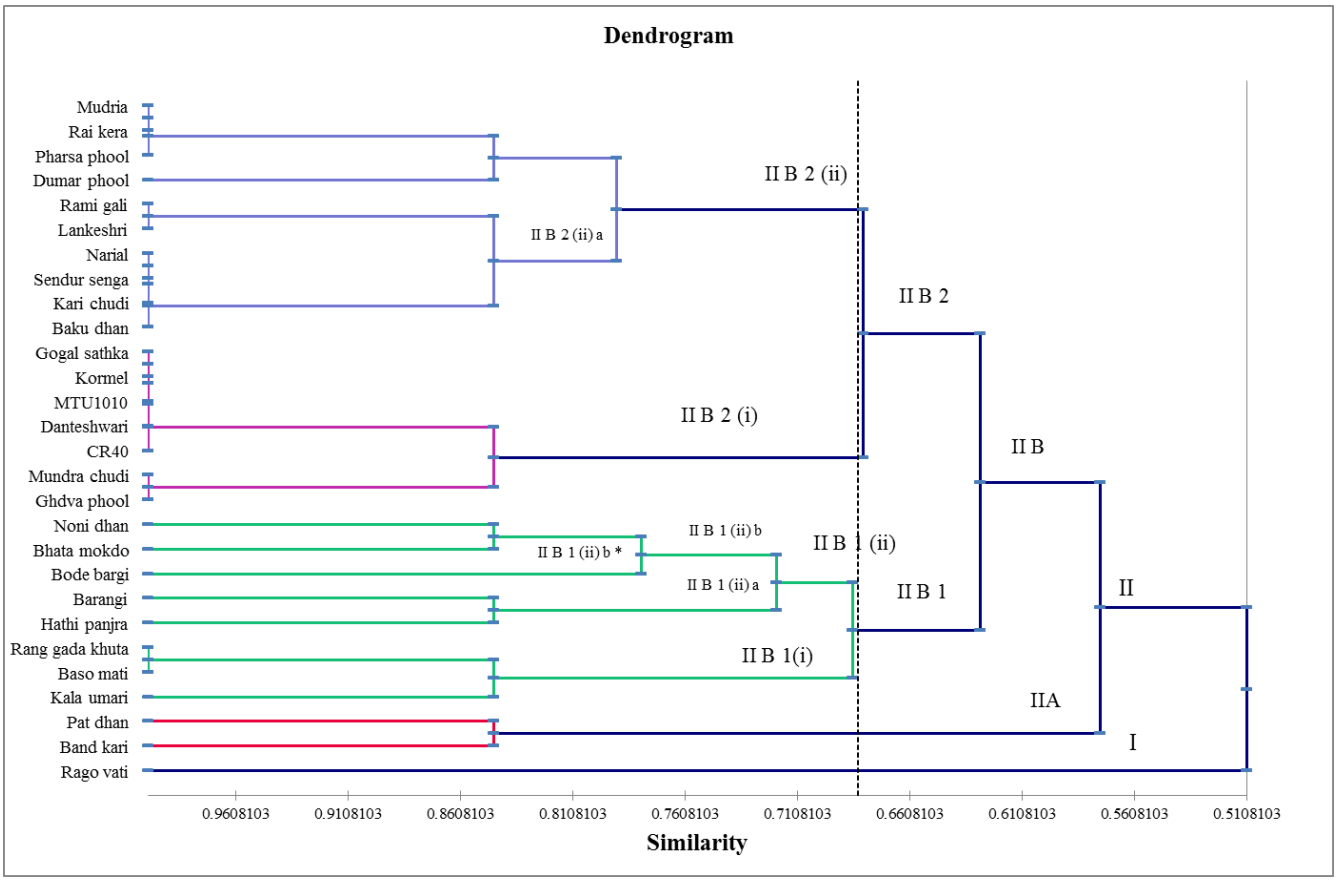

Fig 8: Molecular dendrogram depicting the distribution of genotypes

Out of 12 SSR markers used, 6 markers were polymorphic and should consistent bending patterns and amplification of each genotype and were chosen for assessing genetic diversity among the rice genotypes studied markers linked to RM 413 (plant height), RM161 (grain width, grain protein content, plant height), RM125 (flag leaf length, number of tiller/hill, number of productive tiller/hill), RM408 (drought tolerance), RM152 (aroma) and OSR13 (drought tolerance) characters were found to be respectively.

OSR13

Out of 28 genotypes used in study, 18 genotypes were (Rago vati, Sendur senga, Narial, Kari chudi, Baku dhan, Lankeshri, Rami gali, Rai kera, Dumar phool, Pharsa phool, Ghdva phool, Mundra chudi, Kormel, Mudria, Gogal sathka, Danteshwari, CR40 and MTU1010) gave us amplification of size 120bp while these were 3 genotypes (Band kari, Pat dhan and Bhata mokdo) with amplified fragmented of 130bp and 5 genotypes (Noni dhan, Bode bargi, Kala umari, Baso mati and Rang gada khuta) with 140bp size fragmented. This SSR reported to be linked to characters drought tolerance (Jha, 2015) [8].

RM125

Out of 28 genotypes used in study, 1 genotypes were (Rago vati) gave us amplification of size 120bp while these were 27 genotypes (Band kari, Pat dhan, Sendur senga, Bhata mokdo, Narial, Noni dhan, Kari chudi, Baku dhan, Bode bargi, Lankeshri, Rami gali, Rai kera, Mudria, Dumar phool, Pharsa phool, Hathi panjra, Barangi, Kala umari, Baso mati, Rang gada khuta, Ghdva phool, Mundra chudi, Kormel, Gogal sathka, MTU1010, Danteshwari and CR40) with amplified fragmented of 130bp. This SSR reported to be linked to characters flag leaf length, number of tiller/hill, number of productive tiller/hill (Chandel *et al.*, 2014) [5].

RM413

Out of 28 genotypes used in study, 13 genotypes were (Band kari, Pat dhan, Barangi, Kala umari, Baso mati, Rang gada, khuta, Ghdva phool, Mundra chudi, Kormel, Gogal sathka, MTU1010, Danteshwari and CR40) gave us amplification of size 75bp while these were 6 genotypes (Rago vati, Rai kera, Mudria, Dumar phool, Pharsa phool and Hathi panjra) with amplified fragmented of 80bp and 7 genotypes (Narial, Noni dhan, Kari chudi, Baku dhan, Bode bargi, Lankeshri and Rami gali) with 85bp size fragmented. This SSR reported to be linked to characters plant height (Mattar, *et al.*, 2016) [12].

RM152

Out of 28 genotypes used in study, 2 genotypes were (Ghdva phool and Mundra chudi) gave us amplification of size 100bp while these were 24 genotypes (Band kari, Pat dhan, Sendur senga, Bhata mokdo, Narial, Noni dhan, Kari chudi, Baku dhan, Bode bargi, Lankeshri, Rami gali, Rai kera, Mudria, Dumar phool, Pharsa phool, Hathi panjra, Barangi, Kala umari, Kormel, Gogal sathka, MTU1010, Danteshwari and CR40) with amplified fragmented of 110bp and 2 genotypes (Baso mati, Rang gada khuta) with 1120bp size fragmented. This SSR reported to be linked to characters aroma (Talukdar, *et al.*, 2017) [19].

RM408

Out of 28 genotypes used in study, 9 genotypes were (Rago vati, Pat dhan, Sendur senga, Narial, Kari chudi, Baku dhan, Rai kera, Mudria and Pharsa phool) gave us amplification of size 125bp while these were 11 genotypes (Band kari, Bhata mokdo, Noni dhan, Lankeshri, Rami gali, Dumar phool, Hathi

panjra, Barangi, Kala umari, Baso mati and Rang gada khuta) with amplified fragmented of 130bp and 8 genotypes (Bode bargi, Ghdva phool, Mundra chudi, Kormel, Gogal sathka, MTU1010, Danteshwari and CR40) with 140bp size fragmented. This SSR reported to be linked to characters drought tolerance (Jha, 2015) [8].

RM161

Out of 28 genotypes used in study, 25 genotypes were (Sendur senga, Bhata mokdo, Narial, Noni dhan, Kari chudi, Baku dhan, Bode bargi, Lankeshri, Rami gali, Rai kera, Mudria, Dumar phool, Pharsa phool, Hathi panjra, Barangi, Kala umari, Baso mati, Rang gada khuta, Ghdva phool, Mundra chudi, Kormel, Gogal sathka, MTU1010, Danteshwari and CR40) gave us amplification of size 130bp while these were 3 genotypes (Rago vati, Band kari and Pat dhan) with amplified fragmented of 150bp. This SSR reported to be linked to characters grain width, grain protein contain, plant height. (Chandel *et al.*, 2014 and Mattar *et al*., 2016) [5, 12].

In this study, the larger range of similarity values for cultivars revealed by microsatellite markers provides greater confidence for the assessments of genetic diversity and relationships, which can be used in future breeding programs. With the aid of microsatellite markers and clustering data, different distantly related rice genotypes may be combined by inter-crossing genotypes from different clusters to get hybrid varieties with the highest heterosis.

Conclusion

Molecular characterization using 12 microsatellite markers (SSR) revealed a total of 22 alleles in 28 rice genotypes with the number of alleles per locus generated by each marker ranging from 1 to 3 alleles with an average of 1.8 alleles per locus. Out of 12 SSR markers, 6 showed polymorphic reaction with polymorphism information content (PIC) values of 0.53 in RM125, 0.6 in RM161 and 0.75 in RM152, 0.84 in OCR13, 0.88 in RM413 and 0.89 in RM408. This suggests that these markers could be potentially used for molecular characterization of rice accession from various sources. The genetic similarity of genotypes of rice under study. The genetic similarity coefficient (Jaccard coefficient) ranged from 0.0.51-1.00 as revealed by UPGMA cluster analysis using the 12 SSR markers. A total of five distinct groups resulted in a cut-off similarity coefficient of 0.683 among the 28 rice genotypes.

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