Molecular Distinguishness among rice (Oryza sativa L.) landrace of Central India using microsatellite markers

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DOI: https://doi.org/10.22271/chemi.2020.v8.i4h.9741

Abstract
Molecular characterization of the genotypes gives precise information about the extent of genetic diversity which helps in the development of an appropriate breeding program. A total of 16 microsatellite (SSR) markers distributed across the rice genome were used for molecular characterization and discrimination of 100 local landraces of rice. The molecular data revealed a total of 24 alleles in 100 rice accessions. The number of alleles per locus generated by each marker ranged from 1 to 5 alleles with the mean of 1.5 alleles per locus and an average polymorphism information content (PIC) of 0.387. This suggests that these markers could be potentially used for molecular characterization of rice accession from various sources. Microsatellite markers (SSR) are also used to detect the genetic similarity of accessions of rice under study. The genetic similarity coefficient ranged from 0.76-1.00 as revealed by UPGMA cluster analysis using the 16 SSR markers. A total of four distinct groups resulted at a cut-off similarity coefficient of 0.83 among the 100 rice accessions. Allelic variability among the SSR markers was high enough to categorize landraces and to catalogue the genetic variability observed for future use. The information about the genetic diversity will be very useful for proper identification and selection of appropriate parents for breeding programs, including gene mapping, and ultimately for emphasizing the importance of marker-assisted selection (MAS) in rice improvement.

Keywords: Genetic diversity, microsatellite markers, PIC values, rice landraces

Introduction
Rice is the world’s largest food crop, providing the daily caloric needs of more than half of the global population. South Asia, one of the major centers for rice domestication, has been described as the “food basket” and “food bowl” of Asia. Cultivated rice is one of the most polymorphic crop species, and is composed of several ecological groups, frequently referred to as subspecies (Oka, 1988) [7]. One of the important approaches to rice breeding is hybridization and subsequent selection. Parents’ choice is the first step in plant breeding program through hybridization. In order to benefit transgressive segregation, genetic distance between parents is necessary. Asian rice (Oryza sativa L.) has been cultivated for an estimated 10,000 years (Liu et al., 2007) [3] and currently feeds more than one third of the world’s population. Growth and development of agricultural resources is mostly depending on genetic diversity among different crop plants and it is estimated that not even 15% of the potential diversity has utilized. This implies that thousands of valuable allelic variations of traits of economic significance remain unutilized (Hossain et al., 2007) [2]. Therefore, landraces of distinct genetic structure are a good promise for the future rice crop improvement. Thus, identification of genotypes and their inter-relationships is vital.

Landraces harbor a great genetic potential for rice improvement. Unlike high-yielding varieties (whose variability is limited due to homozygosity), the landraces maintained by farmers are endowed with tremendous genetic variability, as they are not subjected to subtle selection over a long period of time. This aids in the adaptation of landraces to wide agro ecological niches and they also have unmatched qualitative traits and medicinal properties. This rich variability of complex quantitative traits still remains unexploited. Landraces are also important genetic resources for resistance to pests and fungal diseases. Collection and evaluation of landraces are an integral part of the pre-breeding process carried out by rice breeders (Vaughan 1991; Siddiq et al., 2005) [15, 13].
Simple sequence repeat is an important tool for genetic variation identification of germplasm (Powell *et al*., 1996; Ma *et al*., 2011) [18, 42]. The morphological marker characters may be qualitative or quantitative in nature as they may be governed by one or more genes. The quantitative characters are influenced by environment, which indicates that such characters are not stable hence cannot be used as marker trait whereas; qualitative traits may be used as morphological markers with low reliability in characterization of germplasm because they are less influenced by environment. The molecular markers are DNA based marker and it represents the genetic constitution of any individual. DNA of any individual does not influenced by environment; hence the DNA based markers are supposed to be stable marker to diagnose any trait. Molecular markers are powerful tools in the assessment of genetic variation, in the elucidation of genetic relationships within and among species, and have demonstrated the potential to detect genetic diversity and to aid in the management of plant genetic resources. In the present study, 100 accessions of rice are used for molecular characterization and genetic diversity study. The present study addresses the utility of SSR markers in revealing genetic relationships at the molecular level among local landraces of rice collected from Bastar plateau zone of Chhattisgarh, India which is a hot spot of biodiversity. In Bastar region of Chhattisgarh rice is grown predominantly during kharif season as rainfed crop having 2.39 million hectare area but the productivity of this crop is very low, 08.53 q/ha. Rice based cropping systems are in existence and farmers raise traditional rice varieties and still adopt organic farming. Safari, Gurmatia, Sathka, Bhata Mokdo, Chudi Dhan etc. are among these traditional varieties. 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. (Oudhia, 2006) [8]. The Bastar region is also known for its rich floristic diversity and tribal culture. The rice cultivated in the region is globally known for variability in grain size, aroma, and medicinal value. The interaction between tribal farmers and the terrestrial heterogeneity of the landscape, presenting diverse ecologies, has resulted in the evolution of a very large number of landraces and farmer’s varieties with variability for most traits and suitability for diverse agroecologies in staple food crops such as rice, converting the region into a very important center of genetic diversity for rice (Singh, 2013) [14].

**Materials and Methods**

The present investigation was carried out during the *Kharif*, 2015 at Research cum Instructional Farm, S.G. College of Agriculture and Research Station, Kumhraward, Jagdalpur, Bastar, Chhattisgarh, India. The latitude and longitude of Jagdalpur can be projected as 19° 40’ N and 82° 20’ E, respectively. The city is nestled on the Bastar Plateau and is positioned at a height of around 552 meters from the sea level. The experimental materials comprised of hundred local landraces of rice collected from Bastar Plateau zone (Table 1). A detailed study of morphological differences among this landraces is done and also subjected to molecular characterization to judge molecular diversity.

### Table 1: List of hundred local landraces of rice used in the study

| S. No. | Accession Name | S. No. | Accession Name | S. No. | Accession Name | S. No. | Accession Name |
|-------|----------------|-------|----------------|-------|----------------|-------|----------------|
| 1     | Olesar         | 29    | Ram-Laxman     | 57    | Dhadhar Dhan   | 85    | Phara dhaan    |
| 2     | Lochai         | 30    | Aasanchudi     | 58    | Baadichudi     | 86    | Baans Kontiya |
| 3     | Pakhiya Dhaan  | 31    | Sonpuri        | 59    | Pakhiyadhaan   | 87    | Kurso Bhog     |
| 4     | Khui Dhaan     | 32    | Idiraghotiya   | 60    | Begnidhaan     | 88    | Muthiya        |
| 5     | Baadshah Bhog  | 33    | Safurolochayi  | 61    | Motilur        | 89    | Umarchudi      |
| 6     | Kukdimundi     | 34    | Mayurddhaan    | 62    | Rakhidhaan     | 90    | Khudbudi       |
| 7     | Gongel         | 35    | Pandri Lochai  | 63    | Machhripoti    | 91    | Kaatamechar    |
| 8     | Sofa Kaanan    | 36    | Haldigaathi    | 64    | Laalbargi      | 92    | Aanjan         |
| 9     | Madras Chudi   | 37    | Vishnuhob      | 65    | Dogarkaabhi    | 93    | Laal Banso     |
| 10    | Kumda Phool    | 38    | Pulkosnai      | 66    | Shivdharohar   | 94    | Rani Kaajar    |
| 11    | Gada Khuta     | 39    | Gangababaru    | 67    | Guthiya        | 95    | Kusum Jhopa    |
| 12    | Kaakad Kado    | 40    | Jhumra         | 68    | Bhokva dhaan   | 96    | Photki Dhaan   |
| 13    | Naani Chudi    | 41    | Masurideres     | 69    | Jordonakati    | 97    | Jatiya         |
| 14    | Baasta Bhog    | 42    | Mokdo          | 70    | Badekuhi       | 98    | Kaalamaali     |
| 15    | Milkooor Mail  | 43    | Dubraj         | 71    | Kabrodhon      | 99    | Gechi Dhaan    |
| 16    | Baudi          | 44    | Hansa Dubraj   | 72    | Bhatamokdo     | 100   | Goydi          |
| 17    | Bagdi Chudi    | 45    | Kurludhaan     | 73    | Sendursenga    |       |                |
| 18    | Turejagadkhatu | 46    | Madiadhana     | 74    | Sela           |       |                |
| 19    | Bluyar         | 47    | Teenkormiai    | 75    | Kolyara        |       |                |
| 20    | Sonasaari      | 48    | Baadigoydi     | 76    | Rangchadi      |       |                |
| 21    | Baadlochayi    | 49    | Haldijeeera    | 77    | Mehraldhan     |       |                |
| 22    | Jeerdhaan      | 50    | Pandrisatika   | 78    | Denzichudi     |       |                |
| 23    | Gurmatioya     | 51    | Kukdi          | 79    | Mundrichu      |       |                |
| 24    | Haldigodi      | 52    | Bahiyakhuta    | 80    | Basomati       |       |                |
| 25    | Adgdhaan       | 53    | Kaalumaari     | 81    | Manki dhaan    |       |                |
| 26    | Ghotiya Dhaan  | 54    | Chirdhaan      | 82    | Sargipholo     |       |                |
| 27    | Keraphool      | 55    | Dhaagun        | 83    | Kantabargi     |       |                |
| 28    | Sorchuabaadi   | 56    | Karigraass     | 84    | Rang gadaakhuta|       |                |

**Table 1**

**Genomic DNA isolation**

Total genomic DNA was extracted and purified from 15 day old seedling leaves collected from at least 2-3 seedlings from each lines, using modified CTAB method described by Zheng *et al*., 1995. The quality of genomic DNA sample was assessed by 1% agarose (Sigma A9539) gel electrophoresis at 5V/cm. Sixteen SSR markers (Table 2) were selected from the
list of panel of 30 SSR markers displayed at the Rice Genes web site; http://www.gramene.org/microsat/ssr.html. The Polymerase Chain Reaction (PCR) was conducted in a reaction solution of total 10 μl prepared by mixing 1 μl of 50 ng per μl concentration of template DNA, with 9 μl of cocktail (Table 3).

**Table 2**: Sixteen microsatellite markers used for molecular characterization across 100 local landraces of rice.

| S. No | SSR Primers | Chromosome number | FORWARD 5′ → 3′ | REVERSE 5′ → 3′ |
|-------|-------------|-------------------|-----------------|-----------------|
| 1     | RM495       | 1                 | AATCCAAGGTGCAGAGATGG | CAACGATGACGAACACAACC |
| 2     | RM283       | 1                 | GTCTACATGTACCTTGTTGG | CGGCGATGAGATCTGTAGTG |
| 3     | RM514       | 3                 | AGATTTGATGTCCTCCATTCCC3 | CACGACGATATTACCTAGTGG |
| 4     | RM124       | 4                 | ATCGTCTGGTTGGCCTGCTG | CATGGATACCGAGCTCCCCCGC |
| 5     | RM145       | 5                 | GGGCGATTCGATGAAAGAAGGAG | TCCCCACAAATCTTTCTCTTC |
| 6     | RM161       | 5                 | TGGAGATGAGAAGCCGGCCCTC | TGTGTCATACGAGCGCGGCTC |
| 7     | RM133       | 6                 | TTGGAATGTGTTTGCTTGCTG | GGGAGCAGGGCTGCGAAGG |
| 8     | RM162       | 6                 | GCCAGCAAACCACGGATCCGG | CAAGGCTTTGTGGCATCGG |
| 9     | RM125       | 7                 | ATCGACGACATGCGACGCACC | AGGGGATGATCTGGCCAGAGCC |
| 10    | RM455       | 7                 | AACAGAGCCACACCTGCTCT | AGAAGGAAAGGATGGATCT |
| 11    | RM408       | 8                 | GACAGAGCAACTCCGGTCC | ACCTGCTACTGGTACGTGAC |
| 12    | RM152       | 8                 | GAAACCCACACACCTCCCGG | CGTGAGACCTTCTTGAGTAG |
| 13    | RM44        | 8                 | AGCGGCAATTGGGAAACACC | TGGGGAAAACCTACCTAC |
| 14    | RM447       | 8                 | CCGGCTGACGGGACGGCC | ACGGGATCTCTTGCTC |
| 15    | RM484       | 10                | TCTCCCTCCTCTACATGTC | TCTGGTCCTCTCTCTCCTCCTC |
| 16    | RM277       | 12                | CGGTCAAAATCATCACCTGAC | CAGGGCTTGCAAGGGAAG |

**Table 3**: PCR mix for one reaction

| Reagent | Stock concentration | Volume (μl) |
|---------|---------------------|-------------|
| PCR buffer with 15 mM MgCl₂ | 10X | 1.0 |
| dNTPs (Mix) | 1mM | 0.8 |
| Nuclease Free Water | - | 5.95 |
| Tag polymerase | 3 U/μl | 0.25 |
| DNA template | 50 ng/μl | 1.0 |
| Total 10 |

Electrophoretic separation and visualization of amplified products

5% polyacrylamide gels (vertical) were used for better separation and visualization of PCR amplified microsatellite products After electrophoresis gel stained with Ethidium Bromide (10 μl in 200 ml distilled water) were visualized under UV.

SSR data statistical analysis

Only clear and unambiguous SSR markers were scored. The banding pattern of 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, 100 base pairs (bp) DNA ladder. Particular base pair position was scored as “1” and absence of band for that particular base pair position was scored as “0”. Polymorphism information content (PIC) was calculated, according to the method of Anderson et al. (1993):

\[
PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2
\]

Where, \( P_{ij} \) is the frequency of the \( j^{th} \) allele for the \( i^{th} \) marker, and is summed over \( n \) alleles. Genetic similarities were estimated from the matrix of binary data using Jaccard coefficient. The similarity coefficients were used for cluster analysis of the rice cultivars utilizing the Unweighted Pair Group Method with Arithmetic Averages (UPGMA). The analysis and dendrogram showing the distance-based interrelationship among the genotypes construction were performed using the NTSYS-pc version 2.02 (Rohlf, 1999).

Results and Discussions

SSR Polymorphisms

A total of 16 SSR markers (primers) were used for molecular characterization and discrimination of 100 accessions of rice. After analysing the data generated from 16 microsatellite markers (SSR), a total of 24 alleles were detected in 100 rice accessions. The number of alleles per locus generated by each marker ranged from 1 to 5 alleles with an average of 1.5 alleles per locus. The highest number of alleles (5) was detected in the locus RM152 and the lowest number of alleles (1) was detected on each of locus RM16, RM455, RM495, RM447, RM484, RM413, RM283, RM125, RM277, RM133, RM124 and RM162. only three SSR marker (RM44, RM408 & RM152) showed polymorphic reaction with polymorphism information content (PIC) values of 0.04, 0.53 and 0.59, respectively. There is a co-relation also found in our study between higher number of alleles and PIC values. Similar results of PIC values was also found by Nadia et al. 2014. This suggests that these markers could be potentially used for molecular characterization of rice accession from various sources. The gel images of amplified fragments produced by primer RM152, RM44 and RM408 are presented in Fig. 1.
Clustering of landraces
Microsatellite markers (SSR) are also used to detect the genetic similarity of accessions of rice under study. The genetic similarity coefficient ranged from 0.76-1.00 as revealed by UPGMA cluster analysis using the 16 SSR markers. A total of four distinct groups resulted at a cut-off similarity coefficient of 0.83 among the 100 rice accessions. The dendogram shows a clear separation of the rice accessions into four groups (Fig. 2). The accessions that are derivatives of genetically similar dropped in one group. Group I had maximum accessions (61). Sonpuri, Bhatamokdo and Jondranakti formed Group II. On the other hand, Group III has 32 accessions. In Group I Haldigodi, Pandrilochai, Ghotiyadhaan and Keraphool were found in duplicate (i.e. 100% similarity) while they exhibited 84% similarity with rest of the

**Table 4: List of 16 microsatellite markers with their chromosomal locations, number of alleles and allele size found among 100 rice accessions**

| S. No | SSR Primers | Chromosome number | Annealing temperature | Number of alleles | Allele size (bp) |
|-------|-------------|-------------------|-----------------------|------------------|-----------------|
| 1     | RM495       | 1                 | 55                    | 1                | 170             |
| 2     | RM283       | 1                 | 61                    | 1                | 120             |
| 3     | RM514       | 3                 | 55                    | 1                | 110             |
| 4     | RM124       | 4                 | 67                    | 1                | 110             |
| 5     | RM413       | 5                 | 53                    | 1                | 120             |
| 6     | RM161       | 5                 | 61                    | 1                | 160             |
| 7     | RM133       | 6                 | 63                    | 1                | 160             |
| 8     | RM162       | 6                 | 61                    | 1                | 140             |
| 9     | RM125       | 7                 | 63                    | 1                | 130             |
| 10    | RM455       | 7                 | 57                    | 1                | 160             |
| 11    | RM408       | 8                 | 55                    | 4                | 100, 120, 130, 140. |
| 12    | RM152       | 8                 | 55                    | 5                | 100, 110, 115, 120, 130. |
| 13    | RM44        | 8                 | 53                    | 2                | 100, 120        |
| 14    | RM447       | 8                 | 55                    | 1                | 135             |
| 15    | RM484       | 10                | 55                    | 1                | 140             |
| 16    | RM277       | 12                | 55                    | 1                | 110             |

**Fig 1:** Images of gel obtained from UV transilluminator

**Fig 2:** Molecular dendrogram depicting the distribution of genotypes
accessions of group I. Machripoti is nearly 84.3% similar to rest of the accessions of group I (i.e. from Olesar to Kurludhan). This group is further divided into two subgroups. With 87.5% genetic similarity group I A included accessions from Olesar to Bagdirchidi whereas group I B included accessions from Naanichudi to Kurludhan. In Group II, Jondranakti is nearly 87.7% similar with Sonpuri and Bhatamokdo whereas Sonpuri and Bhatamokdo are duplicate (i.e. 100% similarity). Group III is further divided into two sub groups. With 86.8% genetic similarity Group III A included accessions from Turejugadakhuta to Bhokvadhan, Whereas Group III B included Kursobhog to Lalbanso. In Group IV, Hansadubraj, Teenkormail, Madiadhaan and Baadigodyi were found in duplicate (i.e. 100% similarity). Thus, SSR markers provide adequate power of resolution to discriminate between rice accessions and it could serve as a potential tool in the identification and characterization of genetically distant cultivars from various sources.

The present investigation addresses the utilization of 16 microsatellite markers to reveal genetic polymorphism and ensures unambiguous identification of 100 accessions of rice. The mean allele (1.5 alleles) across 16 loci obtained in our study was comparable with the result reported by Meti et al. (2013) [3]. In contrast, the mean value from our study is somewhat lower than the results observed in previous diversity studies, having 3 to 9 alleles, with an average of 4.53 alleles per locus for 30 microsatellite markers (Hossain et al., 2007) [2]. Similar result was observed in earlier report by Rahman et al. (2012) [10] who found an average of 4.18 alleles per locus. 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 makers and clustering data, different distantly related rice genotypes may be combined by intercrossing genotypes from different clusters to get hybrid varieties with the highest heterosis (Sajib et al., 2012) [12].

Conflict of Interest: The authors declare that they have no conflict of interest.

References
1. Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME. Optimizing parental selection for genetic linkage maps. Genome. 1993; 36:181–186.
2. Hossain MZ, Rasul MG, Ali MS, Iftekharuddaula KM, Mian MAK. Molecular characterization and genetic diversity in fine grain and aromatic landraces of rice using microsatellite markers. Bangladesh J Genet. Pl. Breed. 2007; 20(2):1-10.
3. Liu L, Lee GA, Jiang L, Zhang J. The earliest rice domestication in China. Antiquity 2007; 81:313.
4. Ma H, Yin Y, Guo ZF, Cheng LJ, Zhang L, Zhong M, Shao GJ. Establishment of DNA fingerprinting of Liaojing series of japonica rice. Middle-East Journal of Scientific research. 2011; 8(2):384-392.
5. Meti N, Samal KC, Bastia DN, Rout GR. Genetic diversity analysis in aromatic rice genotypes using microsatellite based simple sequence repeats (SSR) marker. African Journal of Biotechnology. 2013; 12(27):4238-4250.
6. Nadia I, Mohiuddin AKM, Sultana S, Ferdous J. Diversity analysis of indica rice accessions (Oryza sativa L.) using morphological and SSR markers. Annals of Biological Research.2014; 5(11):20-31.
7. Oka HI. Origin of cultivated rice. Japan Scientific Societies Press. Elsevier, Tokyo, 1988.
8. Oudhia P. Ignored Medicinal Rice in Chhattisgarh needing immediate attention. http://www.Ecoport.org. 2006.
9. Powel W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A. Comparison of RFLP, RAPD, AFLP and SSR markers for germplasm analysis. Molecular Breeding. 1996; 2(3): 225-238.
10. Rahman MM, Rasaul MG, Hossain MA, Iftekharuddaula KM, Hasegawa H. Molecular Characterization and Genetic Diversity Analysis of Rice (Oryza sativa L.) Using SSR Markers. Journal of Crop Improvement. 2012; 26(2):244-257.
11. Rohlf FJ. NTYS Spec: Numerical Taxonomy System, ver. 2.1. Exeter Publishing, Ltd. Setauket, NY. 1999.
12. Sajib MA, Hossain MM, Mosnaz ATMJ, Hossain H, Islam MM, Ali MS et al. SSR marker-based molecular characterization and genetic diversity analysis of aromatic landraces of rice (Oryza sativa L.). J Bio Sci. Biotech. 2012; 1(2):107-116.
13. Siddiq EA, Saxena S, Malik SS. Plant genetic resources: food grain crops. In: Dhillon BS, Saxena S, Agrawal A, Tyagi RK, eds. Indian Society of Plant Genetic Resources, New Delhi, Narosa Publishing House, New Delhi, India. 2005, 27–57.
14. Singh A K. Probable Agricultural Biodiversity Heritage Sites in India: XV. The Bastar Region. Asian Agri-History. 2013; 17(1):3–24.
15. Vaughan DA. Choosing rice germplasm for evaluation. Euphytica 1991; 54: 147–154.
16. Zheng K, Huang N, Bennet J, Khush GS. PCR-based marker assisted selection in rice breeding. IRRI Discussion Paper Series No. 12, International Rice Research Institute, P.O. Box 933, Manila, Philippines, 1995, 16-18.