Genotyping of rice accessions using SSR markers

Sahu Shail, NK Rastogi, PK Chandrakar and Ritu R Saxena

DOI: https://doi.org/10.22271/chemi.2020.v8.i4c.9882

Abstract

Molecular characterization of germplasm accessions of rice revealed genetic polymorphism and ensured unambiguous identification. A total of 24 SSR markers were used covering all the chromosomes of rice for their molecular characterization and discrimination. After analysis of the data generated, a total of 69 alleles were detected in 24 accessions of rice. The number of alleles per locus generated by each marker ranged from 1 to 6 alleles with an average number of 2.87 alleles per locus. The highest number of alleles (6) was detected in the locus RM 22565 while, lowest number of alleles (1) detected on each of locus OSR 13, RM 431, RM 454 and Xa5s. The PIC value ranged from 0.00 (OSR 13, RM 431, RM 454 and Xa5s) to 0.76 (RM 12146). Microsatellite markers (SSR) are also used to detect the genetic similarity of germplasm accessions of rice. The genetic similarity coefficient ranged from 0.24 to 1.00 as revealed by UPGMA cluster analysis using the 24 SSR markers. A total of five distinct groups resulted at a cut-off similarity coefficient of 0.46 among the 24 rice accessions, below which the similarity values narrowed conspicuously. Coefficient of similarity revealed that the rice accessions of cluster I were genetically distant from cluster IV. Thus, Peeleeluchai (135131) and Mahuwadeta Lal of cluster I; whereas, Kadamphool and Ram Karoni of cluster IV seems to be promising and should be utilized in hybridization programme. Molecular markers like RM 1, RM 12146, RM 215, RM 22710 RM 154 and RM 25 could potentially be used for molecular characterization of rice germplasm accessions from various sources on the basis of polymorphic reactions and high PIC values.

Keywords: Accessions, DNA fingerprinting, polymorphic, rice, SSR

1. Introduction

Genetic diversity probably serves as insurance against crop failure (Subba Rao et al. 2001) [7]. Collection and characterization of the germplasm is not only important for utilizing the appropriate attitude based on donors in breeding programme but it is essential in the present era for protecting the unique rices. The amount of genetic variation in germplasm accessions and genetic relationships between genotypes are important consideration for designing breeding programme. Advances in plant genetics and molecular biology have led to the development of many types of molecular markers which can be used to characterize germplasm. Different types of DNA markers are available now-a-days, each method differing in principle, application, types and amount of polymorphism detected, cost and requirement. Simple Sequence Repeats (SSR) is codominant, abundant and highly reproducible and exhibits a high degree of allelic variations. SSR are excellent molecular marker system for many types of genetic analysis including linkage mapping, germplasm surveys and polygenetic studies. Microsatellites (also known as SSR) are simple, tandem repeated, di- to tetra nucleotide sequence and valuable as genetic markers because they detect high level of allelic diversity, coordinate easily and economically assayed by PCR and are easily automated. Characters may be qualitative or quantitative in nature. They may be governed by one or more genes. The quantitative traits are influenced by environment, which indicates that such characters are not stable and hence, cannot be used as marker traits whereas, qualitative traits may be used as morphological markers with low reliability because they are less influenced by environment. On the other hand, molecular markers are DNA based markers 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 markers to diagnose any trait. The molecular markers are powerful tools in the assessment of genetic variation and having potential to detect genetic diversity in the management of plant genetic resources. In the present study, 24 rice germplasm accessions were used for molecular characterization and genetic diversity study.
2. Materials and Methods

2.1 Plant Material

A Field experiment was conducted during Kharif 2016 at Research cum instructional Farm, IGKV, Raipur. The experimental material consisted of 47 rice germplasm accessions including checks namely, Danteshwari, Indira Aerobic-1, IGKV R-1, IGKV R-2, and Safri 17 (Table 1).

2.2 Genomic DNA isolation

Total genomic DNA was extracted using CTAB method (Zheng et al. 1995) [16].

2.3 Procedure

1. Young plant leaves were collected at seeding stage, about one gram of leaves bits were cut by scissors and put in 2 ml of Eppendorf tube.

2. Add 700 µl of CTAB extraction buffer.

3. Grind the leaves with the help of tissue lyzer. After grinding add 300 µl of CTAB extraction buffer.

4. Keep it in water bath at 65 °C for 20 minutes.

5. Add 700 µl of Chloroform: Isoamyl alcohol (24:1).

6. Vertex the sample.

7. Centrifuge it for 10 min at 14000 rpm in centrifuge machine.

8. Transfer the supernatant in 1.5 ml of fresh Eppendorf tube and repeat the protocol twice form step 5-8.

Each entry was transplanted in a single row at spacing of 20 cm between rows and 15 cm between plants. The standard agronomical practices were adopted for normal crop growth. All the 47 accessions were used for molecular characterization using 24 SSR and 31 ISSR primers to determine the genetic diversity.

Table 1: List of germplasm accessions of rice

| S. No. | IC Number | Name of Accessions/ Genotypes | District |
|--------|------------|-------------------------------|----------|
| 1.     | IC 132668  | Lali Ajan                     | Bastar   |
| 2.     | IC 132671  | Ajaniya                       | Bastar   |
| 3.     | IC 132694  | Badshah Bhog (B)              | Durg     |
| 4.     | IC 132695  | Badshah Bhog                  | Raigarh  |
| 5.     | IC 132702  | Badshah Bhog (II)             | Shahdol  |
| 6.     | IC 132708  | Jogi Bhog                     | Mahasamund |
| 7.     | IC 132732  | Vishnu Bhog                   | Sarguja  |
| 8.     | IC 132740  | Anur Guchcha                  | Durg     |
| 9.     | IC 132746  | Anjan (II)                    | Shahdol  |
| 10.    | IC 132747  | Anjan (I)                     | Mandla   |
| 11.    | IC 132748  | Anjani                        | Mandla   |
| 12.    | IC 132750  | Anjan                         | Mungeli  |
| 13.    | IC 132920  | Dhabli Banko (I)              | Raipur   |
| 14.    | IC 132935  | Lal Banko                     | Raipur   |
| 15.    | IC 132398  | Mudi Banko                    | Sarguja  |
| 16.    | IC 132939  | Raj Banko                     | Bastar   |
| 17.    | IC 132942  | Sugujibi Banko                | Bilaspur |
| 18.    | IC 132957  | Banskupi                      | Shahdol  |
| 19.    | IC 135043  | Luchai                        | Mandla   |
| 20.    | IC 135128  | Peeleeluchai                  | Balaghat |
| 21.    | IC 135131  | Peeleeluchai                  | Balaghat |
| 22.    | NA         | Mauhawateta Lal               | Mandla   |
| 23.    | IC 135209  | Mauhakuchi                    | Raipur   |
| 24.    | IC 135221  | Makado                        | Bastar   |
| 25.    | IC 135253  | Malpa (I)                     | Raipur   |
| 26.    | IC 135256  | Mancha                        | Bastar   |
| 27.    | NA         | Patel 27 Type 4               | Durg     |
| 28.    | IC 135769  | Kadamphool                    | Bastar   |
| 29.    | IC 135855  | Rajniti (A)                   | Shahdol  |
| 30.    | IC 214037  | Ram Karoni                    | Balaghat |
| 31.    | IC 214410  | Surmatia                      | Bastar   |
| 32.    | NA         | X.5 Bhairamgarh               | Bastar   |
| 33.    | IC 124706  | Anjan (A)                     | Raipur   |
| 34.    | IC 113998  | Barangi                       | Bastar   |
| 35.    | IC 113999  | Barangi                       | Raipur   |
| 36.    | IC 114049  | Moti Basmati                  | Seoni    |
| 37.    | IC 114050  | Baspan                        | Raipur   |
| 38.    | IC 114073  | Bauwara                       | Raipur   |
| 39.    | IC 114093  | Bhaya                         | Raigarh  |
| 40.    | NA         | Bhujanhi                      | Durg     |
| 41.    | NA         | Bhurkund                      | Raigarh  |
| 42.    | IC 125488  | Chitarboti                    | Bastar   |
| 43.    | IC 124706  | Danteshwari (Check)           | IGKV Raipur |
| 44.    | IC 113998  | Indira Aerobic 1 (Check)      | IGKV Raipur |
| 45.    | IC 113999  | Rajeshwari (Check)            | IGKV Raipur |
| 46.    | IC 114049  | Durgeshwari (Check)           | IGKV Raipur |
| 47.    | IC 114050  | Safri 17 (Check)              | IGKV Raipur |
9. Add 70 µl of sodium acetate and about 400 µl of pre-chilled isopropanol (equal volume of the supernatant transferred) in this and kept it for incubation at 4 °C for 2 hrs or -20 °C for overnight.

10. Centrifuge it for 3 min @ 1400 rpm.

11. Decant the solution and add 50 µl of 70% ethanol for washing and centrifuged at 14000 rpm for 5 minutes.

12. Decant the solution and dry the pellet for 2 hours or overnight until the smell of ethanol was evaporated.

13. Finally dissolved the pellets in 50 µl of TE buffer.

14. Stored at -20 °C until use.

2.4 PCR amplification and electrophoresis Data analysis

PCR Reaction: 2 µl of diluted template DNA of each genotype was dispensed at the bottom of PCR plate. Separately cocktail was prepared in an Eppendorf tube as described in Table 2. About 8 µl of cocktail were added to each sample and the PCR was set up as the profile depicted in Table 3.

| Table 2: PCR mix for one reaction |
|----------------------------------|
| Reagent                          | Stock Concentration | Volume (µl) |
| Sterile and nanopure H2O         | -                  | 5.2         |
| PCR Buffer A                     | 10 X               | 1.0         |
| dNTPs (Mix)                      | 2.5 mM             | 0.5         |
| Primer (Forward+reverse)         | 5 pmol             | 1.0         |
| Taq polymerase                   | 3 U/µl             | 0.3         |
| DNA Template                     | 50 ng / µl         | 2.0         |
| Total                            |                    | 10.0        |

### Table 3: Temperature profile used for PCR amplification using micro-satellite markers

| Steps | Temperature (°C) | Duration (min.) | Cycles | Activity |
|-------|------------------|-----------------|--------|----------|
| 1     | 94               | 5               | 1      | Denaturation |
| 2     | 94               | 0.5             | 35     | Annealing |
| 3     | 55               | 0.5             |        | Extension |
| 4     | 72               | 1               | 1      | Final Extension |
| 5     | 72               | 7               |        | Storage |
| 6     | 4                | ∞               |        |           |

### Table 4: Detection of varietal polymorphism using simple sequence repeats (SSR) markers

| S. No. | Name of Primers | Forward Sequence | Reverse Sequence |
|--------|-----------------|------------------|------------------|
| 1      | RM 25           | GGGAAGAATGATCTTTCATGG | CTACCATCTAAACCAATGTTC |
| 2      | RM 19           | CAAAAACAGCAGACATGAC | CTCAAGATGGAAGCAGAACAG |
| 3      | RM 1            | GCGAAAACACATGCAAAA | GGTGGTGGAGCTGACATAG |
| 4      | RM 11           | TCTCTCTTCCTCCCCGATC | ATAGGCGGAGGGCTTACAG |
| 5      | RM 161          | TCCGGCGAATGCAGAGTGG | AGACGAAACACCGTGACACAG |
| 6      | RM 431          | TCTCTGTGGGCGAGACTAATCG | ATGACGGAACACGCTGACC |
| 7      | RM 152          | GAAGCACCACACCTACACCC | CGGTAGATCTTCTGAGTAG |
| 8      | OSRqw 13        | CATTGTTGGATGCAGTGA | AGGCACGGCAGGCGCATC |
| 9      | RM 408          | CACCGAGCTAATCTGGGCTCC | ACTGCAATCTTGGGACACAG |
| 10     | RM 171          | AGCAGGAGCGGCAGCCACCG | ATGCAGACGACACCTGACC |
| 11     | RM 154          | ACCCTCTCCGCGCTGCTCTCTCTC | CTCCGAGTTCCTGAGCAGCTC |
| 12     | RM 527          | GGGTCGATCTAGAAGAATCG | TTGAGCAAGTGAGCTGAGAG |
| 13     | RM 433          | TGCAGTGAACGTAAACACAGC | AGCAGAAGCCAGAGCACAG |
| 14     | RM 22710        | CGCGGAGGAGTAATTCTG | CTTGAGCTTGAGGAGGTCTTACC |
| 15     | RM 22565        | TCCACCGGATTTGGTTAGAAATTAAGC | AGCCAGGACCACTGAAACAC |
| 16     | RM 287          | TCCCTGTGGAGACGAGAACAG | GTGTATTGGTGAGAACAAAC |
| 17     | RM 316          | CATGTTGCGGCTAGCATGAGC | AGGGATATATGATGAGTACAG |
| 18     | RM 447          | CCCCTTGCGCTGCTTCTTC | ACGGGCTCCTCCTCCTCCTC |
| 19     | RM 215          | CAAATGAGGAGACGCAAGAC | TGAGCAGCTTCTTCTGAG |
| 20     | RM 454          | CTGACGAGTGCTGCTGCTG | GTGATGCTGAGCCCATACG |
| 21     | Xa 13           | GGCATGCAATGATGAG | GAGCTTTAGAGCTGAGCAG |
| 22     | Xa 5x           | GGCTCAATGATAAGTCGCC | ATACAGAGGGCTGAGCTAG |
| 23     | RM 12146        | AGTATGCCCCTGCCCAGCAG | CAGGGAGATGGAAGACACAG |
| 24     | RM 242          | GCCCAACGTACGGAGTA | AGGCCAGACGCGGCATC |

### Table 5: List of 24 microsatellite markers with their chromosome locations, number of alleles, allelic size and PIC value found among 24 rice accessions

| SSR Primers | Chromosomal locations | Number of Alleles | Allele Size (bp) | PIC value |
|-------------|-----------------------|-------------------|-----------------|-----------|
| RM242       | 9                     | 2                 | 200, 250        | 0.079     |
| RM11        | 7                     | 2                 | 130, 140        | 0.079     |
| RM527       | 6                     | 3                 | 133, 120, 125   | 0.288     |
| RM25        | 8                     | 3                 | 140, 150, 155   | 0.621     |
| OSR13       | 3                     | 1                 | 100             | 0.365     |
| RM1         | 1                     | 4                 | 100, 117, 125, 130 | 0.725     |
| RM22710     | 8                     | 4                 | 145, 147, 150, 175 | 0.635     |
| RM22565     | 8                     | 6                 | 220, 223, 275, 290, 293, 295 | 0.569 |
| RM19        | 12                    | 3                 | 290, 300, 315   | 0.482     |
3. Results and Discussion

A total of 24 SSR markers (Simple Sequence Repeats) were used for molecular characterization and discrimination of 24 rice germplasm accessions with the aim to access the genetic diversity (Table 4). After analysis of the data generated from 24 microsatellite markers (SSR), a total of 69 alleles were detected in 24 accessions of rice (Table 5). The number of alleles per locus generated by each marker ranged from 1 to 6 alleles with an average number of 2.87 alleles per locus. The value is lower than that of 3.02 alleles per locus (Anand et al., 2011) [1] and 5.66 alleles per locus (Haque et al., 2014) [3]. In the present study, 2.87 alleles per locus were higher than 2.6 alleles (Joshi et al., 2017) [2] and 2.17 alleles (Gour et al., 2017) [2]. The highest number of alleles (6) was detected in the locus RM 22565 while, lowest number of alleles (1) detected on each of locus ORS 13, RM 431, RM 454 and Xa5s. The PIC value ranged from 0.00 (ORS 13, RM 431, RM 454 and Xa5s) to 0.76 (RM 12146). Markers with PIC value of 0.50 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at specific locus (De Woody et al. 1995) [8]. Markers having polymorphic reaction along with high PIC value viz., RM I and RM 12146 should be potentially used for molecular characterization of rice accessions from various sources. On the other hand, four markers ORS 13, RM 431, RM 454 and Xa5s exhibited monomorphic reaction for all the accessions, as a potential tool in the identification and characterization of genetically distant cultivars from various sources.

The present study addresses the utilization of 24 microsatellite markers to reveal genetic polymorphism and ensures unambiguous identification of 24 accessions of rice. The mean allele (2.87) across 24 loci in the present study was comparable with the result reported by Anand et al. (2012) [1] for 3.02 alleles per locus in 25 rice hybrids; Singh et al. (2016) [15] for 3.11 alleles per locus in 729 rice varieties; Meti et al. (2013) [14] who found an average of 2.08 alleles per locus among 48 traditional indigenous aromatic rice germplasm using SSR Markers with the range of 1 to 5 alleles per locus. Gour et al. (2017) [2] found an average of 2.17 alleles in the range of 1 to 3 alleles per locus with a total of 26 alleles among 19 accessions. Sajib et al. (2012) [16] detected 3.3 alleles per locus who used a total of 24 SSR markers across 12 elite aromatic rice genotypes for their characterization and discrimination. The number of alleles per locus ranged from 2 to 6 alleles. In another study, Kumar et al. (2014) [17] found an average of 2.93 alleles per locus among 72 rice genotypes using 15 polymorphic SSR markers. The number of alleles ranged from 1 to 4 per locus. Whereas, Hossain et al. (2007) [10] reported 3 to 9 alleles per locus with an average of 4.53 alleles per locus for 30 microsatellite markers; Marathi and Maliha (2018) [13] reported 2 to 4 alleles per locus with an average of 2.39 alleles per locus for 23 SSR markers in 53 varieties. Similarly, Rahman et al. (2012) [21] found an average of 4.18 alleles per locus. In another study, Jain et al. (2004) [11] found 3-22 alleles per locus with the average of 7.8 for 30 SSR markers.

| RM154 | 2   | 4   | 175, 183, 186, 190 | 0.635 |
|-------|-----|-----|-------------------|------|
| RM431 | 1   | 1   |                   |      |
| RM316 | 9   | 4   | 200, 205, 212, 215 | 0.583 |
| RM152 | 8   | 3   | 150, 155, 175     | 0.468 |
| RM287 | 11  | 2   | 90, 100           | 0.375 |
| RM433 | 8   | 3   | 295, 300, 305     | 0.569 |
| RM408 | 8   | 3   | 110, 127, 132     | 0.402 |
| RM171 | 10  | 3   | 395, 400, 405     | 0.531 |
| RM447 | 8   | 2   | 105, 115, 125     | 0.496 |
| RM161 | 5   | 2   | 170, 185          | -0.001 |
| RM215 | 9   | 3   | 110,130,133       | 0.692 |
| RM12146|  1  | 5   | 60, 72, 83, 86, 90 | 0.760 |
| RM454 | 6   | 1   | 205               | 0     |
| Xa13  | 8   | 3   | 225, 230, 237     | 0.402 |
| Xa5s  | 5   | 1   | 275               | 0     |
Larger range of similarity values for cultivars revealed by microsatellite markers provides a greater confidence for the assessment of genetic diversity and relationships, which can be used in future breeding programme. 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 hybrids with the highest heterosis.

The present study revealed a wide variation among the accessions. The results indicated that SSR markers are neutral and co-dominant and could be a powerful tool to assess the genetic variability of the accessions. Coefficient of similarity revealed that the rice accessions of cluster I were genetically distant from cluster IV. Thus, Peeleeluchai (135131) and Mahuwadeta Lal of cluster I; whereas, Kadampoo and Ram Karoni of cluster IV seems to be promising and should be utilized in hybridization programme on the basis of kernel L:B ratio and grain yield. Molecular markers like RM 1, RM 12146, RM 215, RM 22710 RM 154 and RM 25 could potentially be used for molecular characterization of rice germplasm accessions from various sources on the basis of polymorphic reactions and high PIC values. The information about the genetic diversity will be very useful for proper identification and selection of appropriate parents for breeding programme, including gene mapping and ultimately for emphasizing the importance of marker assisted selection in germplasm of rice improvement worldwide.

Fig 1: An UPGMA cluster dendogram showing the genetic relationships among 24 germplasm accessions of rice based on the alleles detected by 24 SSR markers

4. References
1. Anand D, Prabhu KV, Singh AK. Analysis of molecular diversity and fingerprinting of commercially grown Indian rice hybrids. Journal of Plant Biochemistry and Biotechnology. 2012; 21(2):173-79.
2. Gour Lokesh, Koutu GK, Singh SK, Mishra DK. Genetic potency identification of indigenous rice (Oryza sativa L.) lines using quality assessment and SSR marker analysis. Indian Journal of Chemical Studies. 2017; 5(4):97-101.
3. Haque S, Pradhan SK, Anandan A, Singh ON. Monomorphic diversity studies in rice genotypes for yield and yield attributing characters under drought. The International Journal of Science & Technology. 2014; 2(8):139-42.
4. Kumar S, Tantwai K, Kottapalli PR, Katiyar SK. Genetic diversity analysis of rice genotypes collected from different villages of Chhattisgarh using simple sequence repeat (SSR) markers. Advances in Plant Sciences. 2014; 25(2):419-22.
5. Rahman MM, Rasaul MG, Hossain MA, Iftekharuddaula KM, Hasegawa H. Molecular characterization and genetic diversity analysis of rice using SSR markers. Journal of Crop Improvement. 2012; 26:244-57.
6. Sajib, AM, 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.). Journal of Biosciences. 2012; 1(2):107-16.
7. Subba Rao LV, Prasad GSV, Chiranjivi M, Chaitanya U, Surender R. DUS characterization for farmer varieties of rice. IOSR Journal of Agriculture and Veterinary Science 2013; 4(5):35-43.
8. De Woody JA, Honeycutt RL, Skow LC. Microsatellite markers in white-tailed deer. J. Hered. 1995; 86:317-319.
9. Hoque A, Begum SN, Hassan L. Genetic diversity assessment of rice (Oryza sativa L.) germplasm using SSR markers. Res. Agric. Livest. Fish. 2014; 1:37-46.

10. Hossain MZ, Rasul MG, Ali MS, Iftekharuddaula KM, Mian MAK. Molecular characterization and genetic purity in fine grain and aromatic landraces of rice using microsatellite markers. Bangladesh Journal of Genetics and Plant Breeding. 2007; 20(2):1-10.

11. Jain S, Jain RK, Mc Couch SR. Genetic analysis of Indian aromatic and quality rice (Oryza sativa L.) germplasm using panels of fluorescently-labeled microsatellite markers. Theor. Appl. Genet. 2004; 109:965-977.

12. Joshi M, Singh PK, Pallavi, Waza SA, Singh A. Estimation of genetic diversity among the rice (Oryza sativa L.) genotype using microsatellite markers. Indian J. Plant Genet. Resour. 2017; 30(2):130-135.

13. Marathi, Balram, Allugundu, Maliha. Assessment of genetic diversity and fingerprinting of rice cultures of Andhra Pradesh and Telangana with SSR markers. Int. J Curr. Microbiol. App. Sci. 2018; 7(12):2553.

14. Meti N, Samal KC, Bastia DN, Rout GR. Genetic diversity analysis in aromatic rice genotypes using microsatellite based simple sequence repeats (SSR) marker. African J of Biotech. 2013; 12(27):4238.

15. Singh N, Choudhary DR, Tiwari G, Singh AK, Kumar S, Srinivasan K. Genetic diversity trend Indian rice varieties: An analysis using SSR markers. BMG Genetics 2016; 17:1.

16. Zheng K, Subudhi PK, Domingo J, Maopanty G, Huang N. Rapid DNA isolation for marker assisted selection in rice breeding. Rice Genet. Newsl. 1995; 12:255.