Indian J. Plant Genet. Resour. 31(3): 310–314 (2018)
DOI 10.5958/0976-1926.2018.00036.0

RESEARCH ARTICLE

Molecular Characterization of Rice Accessions Using Microsatellite Markers

Anjali Toppo*, NK Rastogi, AK Sarawgi and Ritu R Saxena
Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur–492012, Chhattisgarh, India.

(Received: 21 November 2016; Revised: 13 December 2017; Accepted: 26 March, 2018)

Genetic improvement mainly depends on the extent of genetic variability present in the population. The molecular marker is a useful tool for assessing genetic variations and resolving cultivar identities. The objective of this study was to evaluate the genetic divergence of 24 rice accessions using SSR markers. A total of 48 alleles were detected in 24 accessions and the number of alleles per locus ranged from 1 to 5 with an average of 2 alleles per locus. The results revealed that out of twenty four, fourteen primers showed distinct polymorphism. The PIC value ranged from 0.07 to 0.79 with an average of 0.23. Markers having polymorphic reaction along with high PIC value *i.e.* RM 287 (0.79) and RM 22565 (0.69) should be potentially used for molecular characterization of accessions from various sources. The dendrogram based on SSR marker analysis grouped the rice accession into three clusters, where cluster II was the largest with 15 accessions. The cluster analysis showed higher level of genetic variation among the genotypes. Similarity coefficients ranged from 0.53 to 0.92. Coefficient of similarity revealed that the rice accessions of cluster I *viz.,* Kala jira and CGR: 7144; Jhingo of cluster II and Cross 116 of cluster III were genetically distant from rest of the accessions as shown by low genetical similarity coefficient. 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 to get better heterosis. The information obtained from the DNA fingerprinting studies helps to distinctly identify and characterize the various genotypes.

Keywords: Dendrogram, Genetic divergence, SSR markers

Introduction

Rice is the staple food for more than half of the world's population and it is a model plant for genomic research. Rice belongs to the grass family Poaceae, the genus having 21 wild and 2 cultivated species. It has rich genetic diversity in the form of thousands of land races and progenitor species. From the commercial point of view, DNA fingerprinting is a useful tool for varietal protection to prove ownership of plant lines. Moreover, the analysis of genetic diversity and relationship between or within different species, populations and individuals is a prerequisite for effective utilization and protection of plant genetic resources (Weising *et al.,* 1995). As DNA being the basis of genetic differences between distinct organisms, DNA fingerprinting is an effective method of biological differentiation. In principle, genetic uniqueness is brought about by two factors inheritance and new mutations. Genetic differences between individuals are laid down in the primary sequence of their genomic DNA; the most straight forward method is identifying an individual sequence for genomes under comparison (Krawczak and Schmidtke, 1994). Molecular marker technology is the powerful tool for determining genetic variation in rice varieties (Xu *et al.,* 1974). Among various PCR based markers, SSR markers are more popular in rice because they are highly informative, mostly mono locus, codominant, easily analyzed and cost effective (Gracia *et al.,* 2004). Information regarding genetic variability at molecular level could be used to help, identify and develop genetically unique accessions that compliments existing cultivars. Therefore, in the present context an attempt was made to evaluate the genetic divergence of 24 rice accessions with 24 SSR markers.

**Author for Correspondence: Email- anjali.toppo205@gmail.com*

Materials and Methods

Plant Material

A field experiment was conducted during *Kharif* 2015 at Research cum Instructional Farm, IGKV, Raipur. A total of twenty four rice accessions were used for the molecular analysis (Table 1).

Genomic DNA Isolation

DNA was isolated from fresh leaves by using CTAB method of DNA extraction (Zheng *et al*., 1995). The DNA samples were quantified using Nano Drop Spectrophotoscopy (NANODROP 2000). After

Table 1. List of the 24 rice accessions, their popular name and location of collection

S.No.	Collector's No.	IC No.	Accessions name	Village	Block	District
1	CGR:6323	IC-125558	Kanak chudi	Kurandi	Jagdalpur	Bastar
2	CGR:6366	IC-125601	Cross 116	Ludenga	Patthalgaon	Raigarh
3	CGR:6416	IC-125648	Deshi safri	Peeparkhar	ChhuiKhadan	Rajnandgaon
4	CGR:6644	IC-125878	Gada khuta	Muli	Bakawand	Bastar
5	CGR:6711	IC-125945	Gedrei	Buranga	Bagicha	Raigarh
6	CGR:7133	IC-114277	Jhaler genda	Kopa	Bagicha	Raigarh
7	CGR:7139	IC-114281	Jhili	Sarnadi	Balrampur	Sarguja
8	CGR:7142	NA	Jhilli	Siwani	Balod	Durg
9	CGR:7144	NA	Jhilli	Bancharoda	Abhanpur	Raipur
10	CGR:7159	IC-114289	Son jhilli	Marga	Jashpur Nagar	Raigarh
11	CGR:7168	IC-114295	Jhilli paragi	Femse	Duldula	Raigarh
12	CGR:7176	IC-114303	Jhingo	Sarjhoka	Baikundpur	Sarguja
13	CGR:7187	IC-114309	Jhumaki	Durgukondal	Durgukondal	Bastar
14	CGR:7189	IC-114311	Jhuna	Kewra	Lakhanpur	Sarguja
15	CGR:7209	IC-114325	Kala jira	Kalwa	Bagicha	Raigarh
16	CGR:7210	NA	Kanak jira	Dharampura	Dhamdha	Durg
17	CGR:7211	IC-114326	Maiphal jira III	Chotpan	Geedam	Bastar
18	CGR:7306	IC-114362	Kakadiha	Boda	Saraipali	Raipur
19	CGR:7367	IC-114403	Kali muchh	Chakserai	Kailaras	Muraina
20	CGR:7472	IC-114470	Kanthdudgi	Dharam Jai Garh	Dharam Jai Garh	Raigarh
21	CGR:7565	IC-114505	Kera ghul	Hukuram	GharGhoda	Raigarh
22	CGR:7593	IC-114518	Ketaki 11	Mallaur	Manendragarh	Sarguja
23	CGR:7629	IC-114539	Khira sar	Kewri	Lakhanpur	Sarguja
24	CGR:7634	IC-114543	Khira sar	Bilsiya	Surajpur	Sarguja

quantification, the DNA was diluted with TE such that the final concentration of DNA was approximately 40-50 ηg/µl for PCR amplification.

PCR Amplification and Electrophoresis

A set of 24 microsatellite markers distributed over 12 chromosomes of rice were used. 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. Cocktail of 8 µl was added to each sample and the PCR was set up as the profile depicted in Table 3. Five per cent polyacrylamide gels (vertical) were used for better separation and visualization of PCR amplified products, since polyacrylamide gel (PAGE) have better resolution for amplified products.

Data Analysis

Clearly resolved unambiguous bands were scored visually for their presence or absence with each primer. The scores were obtained in the form of matrix with'1'and'0', which indicate the presence and absence of bands in each accessions, respectively. Polymorphic information content (PIC) values were calculated for each of the SSR loci using the formula developed by Nei (1973).

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

Where, Pi is the allele frequency for the i^{th} allele.

Results and Discussion

Assessment of genetic diversity is an essential component in germplasm characterization and conservation. Selection increases the frequency of alleles or allelic combinations with favorable effects at the expense of others, eventually eliminating many of them (Cao *et al*., 1998).

In the present investigation 24 SSR markers (Simple Sequence Repeats) were used to characterize and assess genetic diversity among 24 rice accessions, out of which 14 markers showed polymorphism (Table 4). After analysis the data generated from 24 markers, a total of 48 alleles were detected in 24 rice accessions. The number of alleles per locus generated by each marker ranged from one to five alleles with an average number

Table 2. PCR mix for one reaction

Reagent	Stock concentration	Volume (µl)
Sterile and nanopure H_2O	-	5.2
PCR buffer A	10 X	1.0
dNTPs (Mix)	2.5 mM	0.5
Primer (forward+ reverse)	5 ρmol	1.0
Taq polymerase	3 U/µl	0.3
DNA template	50 ηg/µl	2.0
Total		10

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		Denaturation
3	55	0.5	35	Annealing
4	72	1		Extension
5	72	7	1	Final Extension
6	4	∞		Storage

Table 4. List of 24 microsatellite markers with their number of alleles, allele size and PIC value found among 24 rice accessions

S. No.	Marker	Number of alleles	Allele size (bp)	PIC Value
1	RM 1	3	85, 105, 110	0.45
2	RM 11	1	135	0
3	RM 19	2	225, 250	0.46
4	RM 25	2	150, 155	0.07
5	RM 152	2	95, 105	0.15
6	RM 154	1	170	0
7	RM 161	1	170	0
8	RM 171	1	340	0
9	RM 215	2	150, 155	0.48
10	RM 242	1	250	0
11	RM 287	3	100, 118, 123	0.79
12	RM 316	2	150, 158	0.07
13	RM 408	2	125, 135	0.49
14	RM 431	1	310	0
15	RM 433	2	250, 275	0.48
16	RM 447	3	60, 70, 85	0.49
17	RM 454	1	225	0
18	RM 527	3	90, 95, 120	0.46
19	RM 22565	5	140, 175, 225, 235, 250	0.69
20	RM 22710	1	140	0
21	OSR 13	2	86, 90	0.27
22	Xa 13	1	240	0
23	Xa 21	2	766, 916	0.07
24	Xa 5s	1	250	0
	Total	48		
	Average	2		0.23

of two alleles per locus. The value is lower than that of 5.89, 5.66 and 2.6 alleles per locus as reported by Lapiton *et al.* (2007), Hoque *et al.* (2014) and Joshi *et al.* (2017), respectively. The highest number of alleles (five) was detected in the locus RM 22565. The PIC value ranged from 0.07 (RM 25, RM 316 and Xa 21) to 0.79 (RM 287) with an average of 0.23. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at specific locus (DeWoody *et al.,* 1995). The early studies on PIC values ranged from 0.24 to 0.94 with an average of 0.61 (Jain *et al.,* 2004), 0.19 to 0.90 with an average of 0.75 (Borba *et al.,* 2009), 0.356 to 0.798 with an average of 0.543 (Hoque *et al.,* 2014), which is relatively higher than that of present study. Markers having polymorphic reaction along with high PIC value *i.e.* RM 287 and RM 22565 should be potentially used for molecular characterization of accessions from various sources.

Microsatellite markers (SSR) are also used to detect the genetic similarity of rice accessions under study. The genetic similarity coefficient ranged from 0.53-0.92 as revealed by UPGMA cluster analysis using the 24 SSR markers. Jaccard coefficient showed a cut-off similarity coefficient level of 0.60, below which the similarity values narrowed conspicuously (Fig. 1). This dendrogram revealed that the accessions those are derivatives of genetically similar type clustered more together. The dendrogram revealed 3 clusters among the accessions. Cluster I had eight accessions and consisted of Kanak chudi, Deshi safri, Gada khuta, Jhaler genda, IC-114281, Jhumaki, Kala jira and CGR: 7144 whereas, cluster III had only one accession namely Cross 116. On the other hand, cluster II had maximum accessions (15) consisting of Gedrei, CGR: 7142, Kanak jira, Maiphal jira III, Kanthdudgi, Jhilli paragi, Jhuna, Kakadiha, IC-114539, Kali muchh, IC- 114543, Ketaki II, Son jhilli, Kera ghul and Jhingo.

Jaccard's coefficient of similarity revealed that high degree of similarity (92%) was observed between CGR: 7142 and Kanak jira, thus, are genetically similar. Whereas, Cross 116 showed low degree of similarity (59%), thus, is distant from members of other clusters. Similarity coefficient ranged between 0.62 and 0.88 in cluster I with other clusters of dendrogram. Cluster II with the similarity coefficient ranged between 0.61 and

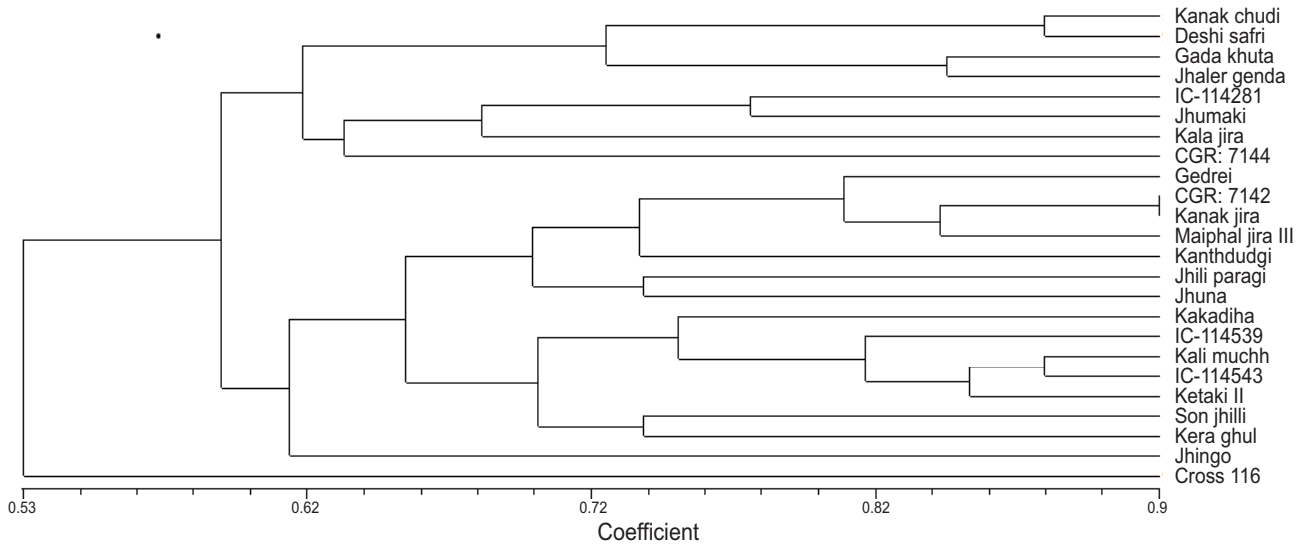

Fig. 1. UPGMA dendrogram showing the genetic relationships among 24 accessions of rice based on 24 SSR markers

0.92. While cluster III with the similarity coefficient ranged between 0.62 and 0.94.

In cluster I, coefficient of similarity revealed that high degree of similarity (>70%) was observed between Kanak chudi and Deshi safri; Gada khuta and Jhaler genda; IC-114281 and Jhumaki, thus, are genetically similar. On the other hand, low degree of similarity was observed between Kala jira and CGR: 7144, thus, are distant from each other.

In cluster II, high degree of similarity was observed between CGR: 7142 and CGR: 7210; CGR: 7211 with CGR: 7142 and CGR: 7210; CGR: 6711 with CGR: 7142, CGR: 7210 and CGR: 7211; CGR: 7472 with CGR: 6711, CGR: 7142, CGR: 7210 and CGR: 7211; CGR: 7168 and CGR: 7189; CGR: 7367 and CGR: 7634; CGR: 7593 with CGR: 7367 and CGR: 7634; CGR: 7634with CGR: 7367, CGR: 7629 and CGR: 7593; CGR: 7306 with CGR: 7367, CGR: 7634, CGR: 7593 and CGR: 7629; CGR: 7159 and CGR: 7565. On the other hand, CGR: 7176 showed low degree of similarity with rest of the accessions of cluster II, thus, is distant from rest of the accessions.

Cross 116 of cluster III showed low degree of similarity with rest of the accessions of cluster I and II, thus, is distant from rest of the accessions.

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 microsatellite markers to reveal genetic polymorphism and ensures unambiguous identification of 24 accessions of rice. The mean allele (2 alleles) across 48 loci obtained in our study was comparable with the result reported by Meti *et al.,* (2013) who found an average of 2.08 alleles per locus among 48 traditional indigenous aromatic rice germplasm using SSR markers with the range of one to five alleles per locus. They also found 25 bands as polymorphic and three as monomorphic bands.

Hossain *et al.* (2007) reported three to nine alleles per locus with an average of 4.53 alleles per locus for 30 microsatellite markers. Similarly, Rahman *et al.* (2012) found an average of 4.18 alleles per locus.

In the present 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 programme. With the aid of microsatellite markers 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.

The present study revealed a wide variation among the accessions. Coefficient of similarity revealed that the rice accessions of cluster I *viz.,* Kala jira and CGR:

7144; Jhingo of cluster II and Cross 116 of cluster III were genetically distant from rest of the accessions as shown by low genetical similarity coefficient. 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 worldwide.

References

Borba TCO, RPV Brondani, PHN Rangel and C Brondani (2009) Microsatellite marker-mediated analysis of the EMBRAPA rice core collection genetic diversity. *Genetica* **137**: 293-304.

Cao T, E Duprez, KLB Borden, PS Freemont and LD Etkin (1998) Ret finger protein is a normal component of PML nuclear bodies and interacts directly with PML. J. *Cell Sci.* **111**:1319-1329.

DeWoody JA, RL Honeycutt and LC Skow (1995) Microsatellite markers in white-tailed deer. *J. Hered*. **86**:317-319.

Gracia AAF, LL Benchimol, MM Antonica, IO Geraldi and AP Deuza (2004) Comparison of RAPD, RFLP, AFLP and SSR marker for diversity studies in tropical maize inbred lines. *Euphytica*, **108**: 53-63.

Hoque A, SN Begum and L Hassan (2014) Genetic diversity assessment of rice (*Oryza sativa* L.) germplasm using SSR markers. *Res. Agric. Livest. Fish.* **1**: 37-46.

Hossain MZ, MG Rasul, MS Ali, KM Iftekharuddaula and MAK Mian (2007) Molecular characterization and genetic diversity in fine grain and aromatic landraces of rice using microsatellite markers. *Bangladesh J. Genet. Pl. Breed.*, **20**: 1-10.

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

Joshi M, PK Singh, Pallavi, SA Waza and A Singh (2017) Estimation of Genetic Diversity among the Rice (*Oryza sativa* L.) Genotypes using Microsatellite markers. *Indian J. Plant Genet. Resour.* **30**: 130-135.

Krawczak M and J Schmidtke (1994) Introduction in DNA fingerprinting. Bio scientific publishers Ltd, Oxford, UK. pp. 1-16.

Lapiton VC, DS Brar, T Abe and ED Redofia (2007) Assessment of genetic diversity of Philippine Rice cultivars carrying good quality traits using SSR markers. *Breed. Sci.* **57**: 263-270.

Meti N, KC Samal, DN Bastia and GR Rout (2013) Genetic diversity analysis in aromatic rice genotypes using microsatellite based simple sequence repeats (SSR) marker. *African J. of Biotech.,* **12**: 4238-4250.

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA **70**: 3321-3323.

Rahman MM, MG Rasaul, MA Hossain, KM Iftekharuddaula and H Hasegawa (2012) Molecular characterization and genetic diversity analysis of rice using SSR markers. *Journal of Crop Improvement*, **26**: 244-257.

Weising K, H Nybom, K Wolff and W Meyer (1995) DNA fingerprinting in plants and fungi. An Arbor CRC Press Inc. Boca Raton pp.1-3.

Xu XH, GC Wang, XB Zheng and HX Wang (1974) A report on the vertical distribution of the rice varieties in Simao, Yunnan. *Acta Botanica Sinica,* **16**: 208-222.

Zheng K, PK Subudhi, J Domingo, G Maopanty and N Huang (1995) Rapid DNA isolation for marker assisted selection in rice breeding. *Rice Genet. Newsl.,* **12**: 255-258.