Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 3065-3069
Received: 04-07-2018
Accepted: 06-08-2018

B Jalandhar Ram
Seed Officer, APSSDCL, Vijayawada, Andhra Pradesh, India

G Suresh Babu
HOD, Department of Genetics and Plant Breeding, Naini, Agricultural Institute, SHUATS, Allahabad, Uttar Pradesh, India

ON Singh
Technical Member, Plant Variety Protection Appellate Tribunal, Intellectual Property Appellate Board, Guna Complex Annex-I, 2nd floor, 443 Anna Salai, Teynampet, Chennai, Tamil Nadu, India

G Roopa Lavanya
Associate Professor, Department of Genetics and Plant Breeding, Naini Agricultural Institute, SHUATS, Allahabad, Uttar Pradesh, India

K Madhu Kumar
Scientist, RARS, Anakapalle, Andhra Pradesh, India

Correspondence
B Jalandhar Ram
Seed Officer, APSSDCL, Vijayawada, Andhra Pradesh, India

Molecular characterization of rice genotypes using microsatellite markers

B Jalandhar Ram, G Suresh Babu, ON Singh, G Roopa Lavanya and K Madhu Kumar

Abstract
Rice is the most important staple food crop for more than half of the population in the world. Crop improvement in rice depends on the magnitude of genetic variability and the extent to which the desirable genes are heritable. A total of thirty SSR markers were used to assess the extent of polymorphism and genetic diversity across the 34 rice genotypes. Out of the 30 SSRs, 25 markers were found to be polymorphic, whereas remaining was monomorphic. A total 86 alleles were detected from 25 SSR markers with an average of 4.12, which ranged from 2 (RM 10123, RM20354 and RM223) to 7 (RM232 and RM18571). The average PIC value was 0.50 at a range from 0.11 (RM 10123) to a maximum of 0.75 (RM232). Jaccard's coefficient results showed that high amount of diversity existed between BPT2411*NDR359 and MTU1001 (81%), followed by the IR64 with BPT5204 and BPT2411*NDR359. The dendrogram based on the UPGMA analysis of NTSYS software grouped the studied rice genotypes into five major clusters. The grouping pattern showed that the clustering was done mostly according the parentage involved. Also there is a vide diversity existed between the parents and F1 progeny. Sufficient polymorphism revealed by the 25 SSR markers among the 34 rice genotypes in the present study justifies their use in genetic improvement programme, which depends on the extent of genetic variation. The genotypes which are found to be diverse based on both morphological and molecular diversity could be used for further breeding programme.

Keywords: Rice, genetic diversity, SSR markers, dendrogram

Introduction
Rice is the most important staple food crop for more than half of the population in the world. The slogan 'Rice is life' is the most appropriate for India, as this crop plays a livelihood for millions of rural households. India has largest area under rice (44.6 million ha) with a production of about 104.70 million tons (Directorate of Economics and Statistics, 2016-17). It is estimated that the demand for rice will be 121.2 million tons by the year 2030 for internal consumption (Agriculture Statistics at glance (2016) [1]. The target is no doubt a challenging task, but it is not unachievable given the potential opportunities and avenues yet to be exploited and rapid advances being made in crop improvement research.

True to the strategy of converting constraints into opportunities, existence of wide yield gaps found across ecologies and zones should be regarded as potential opportunities for raising the yield level and achieving thereby the future targets. Molecular marker assisted selection has been successfully applied in rice breeding programs to improve the effectiveness and precision of the selection process, as well as shorten the turnaround time and lower the costs of implementation. Molecular marker assisted selection can help in enhancing breeding efficiency through: Transfer of economically important traits across genus/species barrier into the rice gene pool (i.e. broadening the genetic base) Manipulation of target trait without disruption to the non-target regions of the rice genome (i.e. increasing efficiency in selection), and Shortening the breeding cycle Enhancing precision in rice breeding.

A critical survey of the genetic variability, correct understanding of the gene effects and knowledge on the extent of heritability of these traits would help in planning an effective breeding programme. Heterosis and Combining ability are thus, excellent tools which help discern the goal and direction in a breeding programme. Inclusion of more diverse parents in hybridization is supposed to increase the chance of obtaining maximum heterosis and gives broad spectrum of variation in segregating generations an investigation was performed to identify an ideal SSR marker. Molecular marker technology is the powerful tool for determining genetic variation in rice varieties (Xu and Wang, 1974). In contrast to morphological traits, molecular markers can reveal abundant difference among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation, Management and untouched by environmental influence.

Among various PCR based markers, SSR markers are more popular in rice because they are highly informative, mostly monolocus, codominant, easily analyzed and cost effective (Gracia *et al*., 2004) [3]. With this aim, the present study aimed at Studying the genetic diversity among parents and their hybrids and identification of desirable crosses through molecular markers (SSR Markers) for future hybridization Programme.

Materials and Methods

Plant materials and DNA extraction Seeds of 34 rice genotypes including parents and hybrids were taken for the present study. The details of the rice genotypes given in table 1. The genomic DNA was isolated from the fresh young leaves following Murray and Thomson (1980). Thirty SSR markers were used to detect polymorphism among the 34 rice genotypes spread evenly over all the chromosomes. The polymerase chain reactions and gel documentation were carried out using standard procedures, and the amplified products were resolved on 3.5% agarose gel [Super Fine Resolution (SFR) Agarose; Amresco, USA] and scoring was carried out manually.

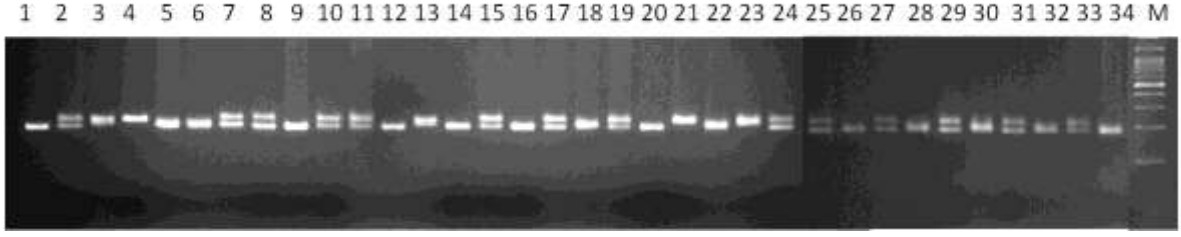
Table 1: The details of the rice genotypes

S. No	List of genotypes	Used as F_1
1	MTU 1010X NDR 359	F_1
2	MTU 1010 X BM 71	F_1
3	MTU 1010 X SHIATS Dhan 1	F_1
4	MTU 1001 X NDR 359	F_1
5	MTU-1001 X BM-71	F_1
6	MTU-1001 X SHIATS Dhan 1	F_1
7	Sahabhaagi dhan X NDR 359	F_1
8	Sahabhagi dhan X BM 71	F_1
9	Sahabhagi dhan X SHIATS Dhan 1	F_1
10	BPT 5204X NDR 359	F_1
11	BPT 5204 X BM71	F_1
12	BPT 5204 X SHIATS Dhan1	F_1
13	BPT 2411 XNDR 359	F_1
14	BPT 2411X BM 71	F_1
15	BPT 2411 XSHIATS Dhan1	F_1
16	BPT-2615 X NDR-359	F_1
17	BPT 2615X BM 71	F_1
18	BPT 2615X SHIATS Dhan1	F_1
19	NLR-34449 X NDR359	F_1
20	NLR 34449 XBM-71	F_1
21	NLR 34449 X SHIATS Dhan 1	F_1
22	MTU 1010	Line
23	MTU 1001	Line
24	Sahabhagi dhan	Line
25	BPT 5204	Line
26	BPT 2411	Line
27	BPT 2615	Line
28	NLR 34449	Line
29	NDR 359	Tester
30	BM 71	Tester
31	SHIATS Dhan 1	Tester
32	Jaya	Check
33	IR 64	Check
34	Anjali	Check

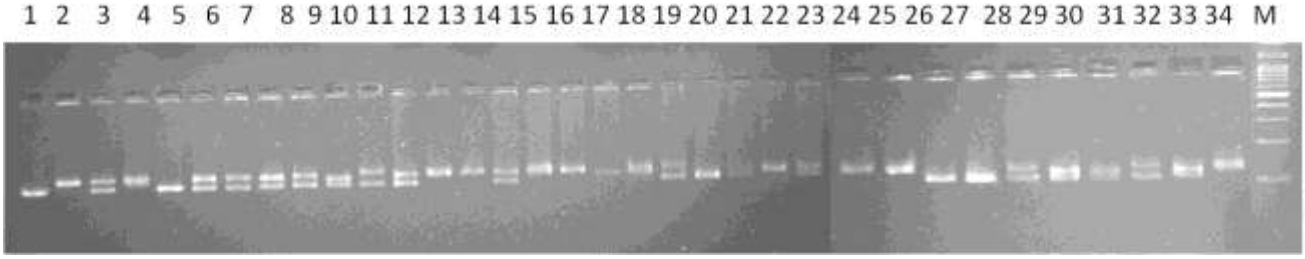
Results

A total of thirty SSR markers were used to assess the extent of polymorphism and genetic diversity across the 34 rice genotypes. Out of the 30 SSRs, 25 markers were found to be polymorphic, whereas remaining were monomorphic. The level of polymorphism among the 34 rice genotypes was evaluated by allele number and PIC values for all the SSR loci. A total 86 alleles were detected from 25 SSR markers with an average of 4.12, which ranged from 2 (RM 10123, RM20354 and RM223) to 7 (RM232 and RM18571). The banding pattern of SSR markers among the 34 rice genotypes given in figure 1.

The genotyping profiling of the 34 rice genotypes using the primer RM572.



The genotyping profiling of the 34 rice genotypes using the primer RM22321



The details of the SSR markers with their number of alleles, and PIC values are given in table 2. Polymorphism information content (PIC) denotes the polymorphic in formativeness of a marker. Among the set of 34 rice genotypes, six rare alleles were found. The PIC values, which are a reflection of allele diversity and frequency among the genotypes, were not uniformly high for all the SSR loci tested. The average PIC value was 0.50 at a range from 0.11 (RM 10123) to a maximum of 0.75 (RM232). This high amount of PIC values was an indication that SSR markers are more polymorphic and of more diversity existed among the genotypes. The primers which showed more number of alleles were also found to contain high amount of PIC also. These results showed that the significant correlation existed between the PIC values and the number of alleles. Sufficient polymorphism revealed by the 25 SSR markers among the 34 rice genotypes in the present study justifies their use in genetic improvement programme, which depends on the extent of genetic variation.

Table 2: Parameters for genetic analysis of 25 SSR loci across the 34 rice accessions.

S. No.	SSR loci	Chromosome	Alleles	PIC
1	RM 10123	1	2	0.11
2	RM550	2	4	0.28
3	RM21918	7	6	0.60
4	RM24152	9	3	0.31
5	RM19456	6	5	0.61
6	RM232	12	7	0.75
7	RM20773	6	4	0.54
8	RM22321	8	4	0.62
9	RM572	1	3	0.37
10	RM28521	8	3	0.39
11	RM20354	6	2	0.32
12	RM223	12	2	0.45
13	RM27644	11	5	0.65
14	RM18571	5	7	0.72
15	RM21478	7	6	0.64
16	RM23181	8	3	0.45
17	RM 18434	5	3	0.38
18	RM24240	9	4	0.57
19	RM11361	1	3	0.34
20	RM12119	2	4	0.60
21	RM22283	9	5	0.65
22	RM18337	5	6	0.61
23	RM11184	1	4	0.52
24	RM26118	10	4	0.49
25	RM22881	8	4	0.50
	Mean		4.12	0.50

The hybrid MTU1010*NDR359 showed 48% dissimilarity from the parent mtu1010, whereas 59% dissimilarity from the NDR359 parent. Likewise the F1 hybrid MTU1010*SHIATS Dhan 1 showed 72% dissimilarity with MTU1010 and 61% dissimilarity with the parent SHIATS Dhan 1. Jaccard's coefficient results showed that high amount of diversity existed between BPT2411*NDR359 and MTU1001 (81%), followed by the IR64 with BPT5204 and BPT2411*NDR359. The hybrid BPT2411*NDR359 showed 79% dissimilarity with IR64 at molecular level using SSR markers. Presented in table 3

Table 3: Highly diverse genotypes based on SSR profiling

Hybrid/ genotype	Dissimilarity percentage	With hybrid/ genotypes
BPT2411*NDR359	81%	MTU1001
BPT2411*NDR359	74%	MTU1001*NDR359
IR64	79%	BPT5204
IR64	77%	BPT2411
IR64	79%	BPT2411*NDR359
NLR34449*NDR359	78%	BPT2411*NDR359
BPT5204*NDR359	85%	ANJALI
BPT5204*BM71	82%	ANJALI

In otherwise the Jaccard's coefficient of similarity ranged from 0.15 (BPT5204*BM71 and Shabagidhan*Shiats Dhan1) to 0.81 (MTU1010*NDR359, and MTU1001) with an average of 0.42, suggesting nature of the genotypes under study. These results highlighted the presence of diversity at genomic level among the 34 rice genotypes. It is however suggested to increase the number of markers for efficiency in further characterization of the genotypes into distinct groups.

The dendrogram based on the UPGMA analysis of NTSYS software grouped the studied rice genotypes into five major clusters (Figure 3). The cluster I comprised of 11 genotypes, cluster II comprised of nine genotypes, whereas cluster III comprised of four genotypes. The cluster IV and V comprised of seven and three genotypes respectively. The cluster I comprised of MTU1010*NDR359, MTU1001, MTU1010*BM71, MTU1001*NDR359, MTU1001*SHIATS Dhan 1, Shahabagidhan*BM71, MTU1010* SHIATS Dhan 1, BPT2615, ANJALI, Shahabagidhan* SHIATS Dhan 1, Shahabagidhan. The cluster III consisted of three varieties and one hybrid viz., NDR359, SHIATS Dhan 1, BM71, BPT2411*BM71. The cluster II consisted of hybrids except the variety Jaya. The cluster IV comprised of seven including varieties and hybrids viz., MTU1001*BM71, MTU1010, IR64, Shahabagidhan* NDR359, NLR34449, NLR34449* NDR359, BPT2615* NDR359.

Table 4: List of genotypes categorised into different clusters based on SSR profiling

Cluster	Total number	Genotypes/ hybrids
Cluster I	11	MTU1010*NDR359 MTU1001, MTU1010*BM71 MTU1001*NDR359, MTU1001*SHIATS Dhan 1, Shahabagidhan*BM71, MTU1010* SHIATS Dhan 1, BPT2615, ANJALI, Shahabagidhan* SHIATS Dhan 1, Shahabagidhan
Cluster II	9	BPT2615* SHIATS Dhan 1, BPT2411* SHIATS Dhan 1, BPT5204, BPT2411, BPT2411*NDR359, JAYA, NLR34449* SHIATS Dhan 1, NLR34449*BM71, BPT2615*BM71
Cluster III	4	NDR359, SHIATS Dhan 1, BM71, BPT2411*BM71
Cluster IV	7	MTU1001*BM71, MTU1010, IR64, Shahabagidhan*NDR359 NLR34449, NLR34449*NDR359, BPT2615*NDR359
Cluster V	3	BPT5204*NDR359 BPT5204*BM71 BPT5204* SHIATS Dhan 1

Discussion

The level of polymorphism among the 34 rice genotypes was evaluated by allele number and PIC values for all the SSR loci. In the present study, 25 markers were found to be polymorphic, whereas remaining 5 were monomorphic. A total 86 alleles were detected from 25 SSR markers with an average of 4, which ranged from 2 to 7. Rathi and Sarma (2012) obtained 181 alleles from 37 SSR markers ranging from 2 to 13 with an average of 4.90 alleles per marker while studying genetic diversity of 106 glutinous rice land races of Assam. Ram *et al.* (2007) [5] obtained 3 to 8 alleles with an average of 4.86 alleles per marker. In other studies, Pachauri *et al.* (2013) assessed 41 rice collected from different part of India using 24 SSR markers and detected 2 to 4 alleles per locus with an average of 2.79 alleles per SSR locus. Becerra *et al* (2017) assessed the genetic diversity in temperate japonica rice germplasm using SSR markers among 1200 accessions, mainly temperate japonica rice accessions, well adapted to the local conditions. Total number of alleles scored across 249 genotypes was 183 with an overall mean of 6.1 alleles per locus, ranging 2-14. Jayamani *et al.* (2007) [4] studied 176 rice accessions originating from 19 countries in Portuguese working germplasm using 24 SSR markers and found 3 to 16 alleles per locus with an average of 7.7 alleles. These reports are similar to the present study on allelic diversity in the 34 rice genotypes using 25 SSR markers. However, the high number of alleles per marker could probably be a result of large number of accessions used (Jayamani *et al.,* 2007 and Choudhary *et al.*, 2013) [4].

In the present study, average PIC value was 0.51 at a range from 0.23 to a maximum of 0.75. Low PIC values for some other primers were earlier reported by Juneja *et al.,* (2006). The average PIC value of 0.51 indicated that SSR markers used in this study were highly informative because 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 a specific locus (DeWoody *et al*., 1995). The PIC of an SSR marker, which is also defined as its capacity to discriminate genotypes depends on the allelic diversity (Ribeiro-Carvalho *et al*., 2004). A strong positive correlation between gene diversity of an SSR locus and the number of alleles detected is also reported by Yu *et al*. (2003) and Onaga *et al*. (2013). Thus SSR analysis has considerable potential for studying the genetic diversity of rice (Bligh *et al*., 1999; Jeung *et al*., 2005; Xu *et al*., 2004).

Diversity analysis using molecular markers determines the degree of relatedness and helps in accurate grouping of genotypes, identification of parents for exploitation of heterosis and detection of duplicate genotypes. It has potential for assessing changes in genetic diversity over time and space (Duwick, 1984). The grouping pattern showed that the clustering was done mostly according the parentage involved. Also there is a vide diversity existed between the parents and F1 progeny. The F1 line MTU1010*NDR359 was clustered in A1, whereas their parents are in different clusters. The parent MTU1010 was in cluster B, whereas NDR359 under cluster A2. The results showed that high amount of heterosis and diversity existed. Likewise BPT5204 existed in cluster A2, whereas its progeny was in B cluster. The results showed that the F1 progeny obtained from different crosses are highly divergent with the parents.

In order to determine the degree of correlation of the morphological and molecular data Mantel test was performed. The Mantel matrix correspondence test was used to compare the similarity matrices. The test indicated that clusters produced based on morphological and SSR markers were correlated as the value indicated as 0.45. similar results were also reported by Autrique *et al.* (1996) in their genetic diversity study in durum wheat using RFLP and agronomic traits calculated a moderate correlation (0.47) which was a result of using wide range of genotypes representing more than one ecotype, where it was reverse in other reports in rice (Taran *et al.,* 2005; Rahman *et al.,* 2011) [6] and other crops (Moghaddam *et al.,* 2005; Rana *et al.,* 2005; Garcia *et al.,* 2007). Correspondence between molecular and agronomic diversity might be improved by analyzing more morphological and DNA markers (Martinez *et al*., 2005). The clustering pattern was observed to be similar broadly. The genotypes MTU1010*NDR359, MTU1001*NDR359, MTU1001*SHIATS Dhan1 were grouped together by both morphological and molecular dendrogram methods. Swain *et al* (2017) [7] studied the genetic diversity of Wild Rice of

Eastern India Using SSR Markers on 26 accessions of *O. rufipogon* and *O. nivara* collected from different districts of Orissa, West Bengal and Tripura. They concluded that concluded that the SSR markers used were found to be equally informative for the genetic diversity study between and among the accessions of two wild species such as *O. rufipogon* and *O. nivara* collected from different locations of Orissa, West Bengal & Tripura.

Combination of morphological and molecular markers is useful in studying genetic diversity of rice for conservation, breeding and other crop improvement activities (Rahman *et al*., 2011) [6]. The best measure to analyze genetic diversity among genotypes would be with the use of all information, both from morphological characters and DNA based markers. Molecular marker data and morphological data subjected to various numerical and taxonomical techniques measures the relationship among the genotypes (Kumar *et al.*, 2003). The genotypes which are found to be diverse based on both morphological and molecular diversity could be used for further breeding programme.

References

1. Agriculture Statistics at glance Directorate of Economic and Statistic Agricultural Government of India, 2016
2. Directorate of Economics and Statistics, 2016-17.
3. Gracia AAF, Benchimol LL, Anotonica MM, Geraldi IO, Deuza AP. Comparision of RAPD, RFLP, AFLP and SSR marker for diversity studies in tropical maize inbred line. Euphytica, 2004; 108(2):53-63.
4. Jayamani P, Negrao S, Martins M, Macas B, Oliveira, MM. Genetic Relatedness of Portuguese Rice Accessions from Diverse Origins as Assessed by Microsatellite Markers. Crop Science. 2007; 47(4):879-884.
5. Ram SG, Thiruvengadam V, Vinod KK. Genetic diversity among cultivars, land races and wild relatives of rice as revealed by microsatellite markers. Journal of Applied Genetics. 2007; 48(4):337-345.
6. Rahman MM, Rahman MA, Hossain A, Rasul G. Comparative study on morphological, physiological and molecular genetic diversity analysis in rice (*Oryza sativa* L.). Libyan Agriculture Research Center of International Journal. 2011; 2(2):85-93.
7. Swain Rosalin S, Mohapatra S, Roy Singh ON, Meher J, Dash SK, Rao GJN. Et al. Assessment of Genetic Diversity in Wild Rice of Eastern India Using SSR Markers. Journal of Agricultural Science. 2017; 9(6):6-12