DNA Fingerprinting and Genetic Diversity Analysis of Rice Cultivars using SSR markers

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Abstract:

Asia is the biggest rice producer and consumer, accounting 90% of the world's production and consumption of rice. India is the second most populous nation, stands first in area, second in production, followed and preceded by China on these two aspects. Increasing yield is still the most important objective of rice breeding programs in developing countries because of the growing demand for food resulting from population growth and a reduction in area devoted to rice production. In present study genomic fingerprinting and divergence analysis has been done in rice cultivars using SSR Markers. For this 10 rice cultivars were selected and 37 SSR primers were used for generating genomic finger prints and assessment of genetic diversity and identification of unique allele among them. The DNA amplification pattern revealed that a total number of 198 SSR loci were amplified with an average of 5.35 loci per primer and ranged from two (RM 242 and RM 331) to 9 (RM 488). All loci were polymorphic and were detected by Gene Tool software version 4.03.05.0. Out of 37 SSR primers, 22 primers amplified unique allele for the different rice cultivars. In the clustering pattern the dendogram generated based on SSR markers grouped the 10 rice cultivars into two clusters. Cluster I comprised of two sub-clusters. Sub-cluster I comprised of two cultivars i.e. JR 503 and Kranti. Sub-cluster II further divided into two groups, with the rice cultivars Mahamaya, Improved Jeerashakar (Group A) and JR 201, Improved Chinnor (Group B). Sub-cluster II comprised of four rice cultivars viz., NPT 65, JR 81 and MTU 1010.Cultivar NPT 29, which showed that this cultivar is totally divers for the rest of the 9 rice cultivars.

Key Words: Rice, Genomic Fingerprinting, Divergence analysis, SSR markers, Allele

Introduction

Rice, *Oryza sativa* (2n = 24) belonging to the family Graminae, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. It covered almost one-fifth of the total land area covered under cereals. Asia is the biggest rice producer and consumer, accounting 90% of the world's production and consumption of rice. India ranks first in the area of rice cultivation and second in the production, followed and preceded by china on these two aspects. The world's rice production has doubled during last 25 years, largely due to the use of improved technology such as high yielding varieties and better crop management practices **(**Byerlee, 1996). Knowledge of the genetic diversity and population structure of germplasm collections is an important foundation for crop improvement (Chakravarthi and Naravaneni 2006). Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level (Weir, 1990). Diversity based on phonological and morphological characters usually vary with environments and evaluation of these traits requires growing season and time consuming. Molecular Marker based Genetic Diversity Analysis (MMGDA) also has potential for assessing changes in genetic diversity over time and space (Duwick, 1984).

Several molecular markers viz. RFLP (Becker et al., 1995), RAPD (Tingey and Deltufo, 1993; Singh et al. 2013) , SSRs (Singh & Singh, 2012) , ISSRs (Joshi et al., 2000, Blair et al., 1999), AFLP (Vos et al., 1995; Zhu et al., 1998) and SNPs (Vieux,et al., 2002)

are presently available to assess the variability and diversity at molecular level . Like the RAPD markers, SSR markers were also employed in rice because of their polyallelic nature and greater allelic diversity over RFLPs (McCouch et al., 1997). It has been found that genetically mapped microsatellite markers cover the whole rice genome with at least one microsatellite for every 16 to 20 cM (Chen et al., 1997). Information regarding genetic variability at molecular level could be used to help, identify and develop genetically unique germplasm that compliments existing cultivars. Keeping that view, the present investigation was carried out to assess of the diversity among 10 rice cultivars and identification of unique allele for them.

Material And Methods:

Isolation of genomic DNA:

Genomic DNA from fresh leaves was isolated from 10 rice cultivars (table 1) at seedling stage following the CTAB (cetyl trimethyl ammonium bromide) procedure as described by Saghai Maroof et al. (1984) with some modifications. Random primers were selected for diversity analysis (table 2). Information regarding chromosomal location and repeat types can be found in web database (www.gramene.org). Quantification of DNA was accomplished by analyzing the DNA on 0.8% agarose gel stained with ethidium bromide using diluted uncut lambda DNA as standard.

PCR amplification:

Polymerase chain reaction (PCR) was performed in a 10µl volume reaction mixture containing 2X PCR assay buffer, 1.5mM $MgCl_2$, 100µM of each dNTPs, 12ng each of forword and reverse primers, 0.2 units of Taq DNA polymerase and 25 ng of genomic DNA template. Amplification reaction initiated with a 5-minute pre-denaturation steps at 94^0 C followed by 35 cycles of DNA denaturation at 94^0 C for 30 seconds, primer annealing at 50-55^0 C for 30 seconds and DNA extension at 72^0 C for 7 minutes was performed after 35 cycles. Amplified PCR products was separated on 2.5% of agarose gel at a voltage of 90V for the period of 45 minutes to 1 hour in 1X TBE buffer stained with ethidium bromide. The gel was visualized in UV trans illuminator and photograph

taken using Syngen gel documentation System (G-Box).

Data analysis:

Clearly resolved, unambiguous band were scored using Gene Tool software version 4.03.05.0 for their presence and absence with each primer by using size standard (100bp DNA ladder). Dendogram was prepared For Cluster analysis NTSYSpc, ver. 2.2 - Exeter Software was used. Dendogram was generated using Jaccard coefficient of similarity and UPGMA Method of cluster analysis.

Results And Discussion:

Efficiency of polymorphism detection:

A total of 37 SSR markers were evaluated for their efficiency of polymorphism across 10 rice cultivars (table 3). All the markers were found to be polymorphic. The average number of alleles established was 5.35 and ranged from two (RM 242 and RM 331) to 9 (RM 488).

Identification of variety specific primers:

Out of 37 SSR primers, 22 primers amplified unique allele for the different rice cultivars (table 3). For NPT 29, specific/ unique allele amplified by RM 152 (169 bp) and RM 171 (292 bp). Specific primers identified for NPT 65 were RM 137 (219 bp), RM 502 (157 bp) and RM 510 (118 bp). The only one primer RM 256 (141 bp) were identified for JR 81. For MTU 1010, identified specific primers was RM 228 (163 bp). A specific primer for identified for JR 201 was RM 16 (176 bp). Two primers i.e. RM 17 (131 bp) and RM234 (159 bp) were identified specific primers for JR 503. For Kranti, RM 147 (209), RM 235 (142 bp) and RM 438 (309 bp) were identified as specific primers. The four primers RM 201 (158 bp), RM 251 (141 bp), RM 488 (200 bp) and RM 539 (292 bp) were identified specific for rice variety Mahamaya. For the aromatic rice variety Improved Jeerashankar, the specific primers identified were RM 84 (116 bp), RM 233 (172 bp) and RM 154 (200 bp). The two primers RM 217 (108 bp) and RM 205 (282 bp) were found specific for the aromatic rice variety Improved Chinnor. These unique SSR profiles can be used for varietal profiling

and purity analysis, instead of using hundreds of random primers since SSR markers detect finer levels of variation among closely related lines.

Genetic relationship between rice cultivars:

Cluster analysis was used to group the varieties and to construct a dendogram. The dendogram generated based on SSR markers grouped the 10 rice cultivars into two clusters. Cluster I comprised of two sub-clusters. Sub-cluster I comprised of two cultivars i.e. JR 503 and Kranti. Sub-cluster II further divided into two groups, with the rice cultivars Mahamaya, Improved Jeerashakar (Group A) and JR 201, Improved Chinnor (Group B). Sub-cluster II comprised of four rice cultivars viz., NPT 65, JR 81 and MTU 1010.Cultivar NPT 29, which showed that this cultivar is totally divers for the rest of the 9 rice cultivars.   The similar results have been reported earlier by Nachimuthu et al., 2015, Kanawapee, et al., 2011.

Genetic diversity is the key determinant of germplasm utilization in crop improvement.

Population with high level of genetic variation is the valuable resource for broadening the genetic base in any breeding program. Molecular markers help us to understand the level of genetic diversity that exists among traditional races, varieties and exotic accessions which can be exploited in rice breeding programs. According to Manjarrez-Sandoral *et al.,* 1997 the accurate estimation of genetic diversity among germplasm sources may increase the efficiency of plant breeding / crop improvement. Similarly, evaluation of genetic diversity among cultivars can provide predictive estimate of genetic variation among segregating progeny for pure line cultivar development. (Barbosa-Nato et al. 1996 . It can also help in predicting the degree of heterosis or combining ability in the progeny of some parental combinations Moghaddam et al. 2005. Molecular approaches are more reliable for assessment of genetic divergence in rice   (Singh, 2011) and these are being used by various workers time to time (Singh et al., 2013, Koutu et al, 2017)

**Table 1: Cultivars subjected to DNA Fingerprinting and genetic diversity analysis**

| S. No.	Cultivars	S. No.	Cultivar
1	NPT 29	6	JR 503
2	NPT 65	7	Kranti
3	JR 81	8	Mahamaya
4	MTU 1010	9	Improved Jeerashanakr
5	JR 201	10	Improved Chinnor

Table 2: SSR primers used in this study

Primers	Reverse sequence	Forward sequence	Amplification temperature
RM 488	5'- CAGCTAGGGTTTTGAGGCTG-3'	5'- TAGCAACAACCAGCGTATGC-3'	55
RM 8	5'- GGCCAAACCCTAACCCTG-3'	5'- CACGTGGCGTAAATACACGT-3'	55
RM 469	5'- GACTTGGGCAGTGTGACATG-3'	5'- AGCTGAACAAGCCCTGAAAG- 3'	55
RM 16	5'-AAC ACA GCA GGT ACG CGC-3'	5'-CGC TAG GGC AGCATCTAA A-3'	55
RM 475	5'-ACGGTGGGATTAGACTGTGC-3'	5'-CCTCACGATTTTCCTCCAAC-3'	55
RM 17	5' GGTGATCCTTTCCCATTTCA-3'	5'- TGCCCTGTTATTTTCTTCTCTC-3'	55
RM 223	5'-GAAGGCAAGTCTTGGCACTG-3'	5'- GAGTGAGCTTGGGCTGAAAC-3'	55
RM 118	5'-CACATCCTCCAGCGACGCCGA G-3'	5'-CCAATCGGAGCCACCGGAGAG C-3'	55
RM 137	5'-CGGGTCGTCCCCGAGGATCTTG-3'	5'-GACATCGCCACCAGCCCACCA C-3'	55
RM 152	5'-GAAACCACCACACCTCACCG-3'	5'-CCGTAGACCTTCTTGAAGTAG-3'	55
RM 338	5'- GGCAAACCGATCACTCAGTC-3'	5- CACAGGAGCAGGAGAAGAGC-3'	55
RM 171	5'-AACGCGAGGACACGTACTTAC-3'	5'-ACGAGATACGTACGCCTTTG-3'	55
RM 84	5"- TTGCAAATGCAGCTAGAGTAC-3"	5"- TAAGGGTCCATCCACAAGATG-3"	52
RM 217	5'- GGGTGTGAACAAAGACAC- 3'	5'- ATCGCAGCAATGCCTCGT – 3'	55
RM 201	5'- CTACCTTTCTAGACCGATA-3'	5'- CTCGTTTATTACCTACAGTACC-3'	55
RM 242	5'TATATGCCAAGACGGATGGG-3'	5'- GGCCAACGTGTGTATGTCTC-3'	55
RM 495	5'- CAACGATGACGAACACAACC-3'	5'- AATCCAAGGTGCAGAGATGG-3'	55
RM 202	5'-CCAGACAGCATGTCAATGTA-3'	5'- CAGATTGGAGATGAAGTCCTCC-3'	55
RM 233	5'- GCATTGCAGACAGCTATTGA- 3'	5'- CCAAATGAACCTACATGTTG-3'	55
RM 228	5"- GCTTGCGGCTCTGCTTAC-3"	5"- CTGGCCATTAGTCCTTGG-3"	52
RM 154	5'-CTCCTCCTCCTGCGACCGCTCCC-3'	5'-ACCCTCTCCGCCTCGCCTCCTC-3'	55
RM 235	5'-TCACCTGGTCAGCCTCTTTC-3'	5'-AGAAGCTAGGGCTAACGAAC-3'	55
RM 251	5'- GAATGGCAATGGCGCTAG-3'	5'- ATGCGGTTCAAGATTCGATC-3'	55
RM 256	5'-GTTGATTTCGCCAAGGGC-3'	5'-GACAGGGAGTGATTGAAGGC-3'	55
RM 279	5'-GGCTAGGAGTTAACCTCGCG-3'	5'-GCGGGAGAGGGATCTCCT-3'	52
RM 502	5-CTGGCCCTTCACGTTTCAGTG-3'	5'-CTGGTTCTGTATGGGAGCAG-3'	55
RM 283	5'- CGGCATGAGAGTCTGTGATG-3'	5'-GTCTACATGTACCCTTGTTGGG-3'	55
RM 205	5'-CTGGCCCTTCACGTTTCAGTG-3'	5'-CTGGTTCTGTATGGGAGCAG-3'	55
RM 341	5'-CTCCTCCCGATCCCAATC-3'	5'- CAAGAAACCTCAATCCGAGC-3'	55
RM 438	5'-CTCTCTGCCACCGATCCTAC-3'	5'-CTTATCCCCCCGTCTCTCTC-3'	55
RM 331	5'- CATCATACATTTGCAGCCAG-3'	5'- GAACCAGAGGACAAAAATGC-3'	55
RM 488	5,- TAGCAACAACCAGCGTATGC-3'	5'- CAGCTAGGGTTTTGAGGCTG-3'	55
RM 212	5'-CACCCATTTGTCTCTCATTATG-3'	5'-CCACTTTCAGCTACTACCAG-3'	55
RM 510	5"-AACCGGATTAGTTTCTCGCC-3"	5"-AACCGGATTAGTTTCTCGCC-3"	55
RM 1	5'- GCGTTGGGCCCTGAC-3'	5'- GCGAAAACACAATGCAAAAA-3'	55
RM 234	5'-ACAGTATCCAAGGCCCTGG-3'	5'-CACGTGAGACAAAGACGGAG-3'	52
RM 539	5'- AGTAGGGTATCACGCATCCG- 3'	5'- GAGCGTCCTTGTTAAAACCG -3'	55

Table 3: Number of alleles obtained using SSR markers

S no.	Markers	Total allele	Polymorphic allele	Range	Unique allele	
					Cultivar	Size
1.	RM 488	9	9	180-259	-	-
2.	RM 8	3	3	245-260	-	-
3.	RM 469	4	4	100-113	-	-
4.	RM 16	4	4	166-188	JR 201	176
5.	RM 475	5	5	188-209	Kranti	209
6.	RM 17	6	6	131-165	JR 503	131
7.	RM 223	6	6	153-185	-	-
8.	RM 118	5	5	148-162	-	-
9.	RM 137	3	3	219-232	NPT 65	219
10.	RM 152	7	7	169-206	NPT 29	169
11.	RM 338	8	8	237-276	-	-
12.	RM 171	4	4	292-328	NPT 29	292
13.	RM 84	6	6	96-116	Improved Jeerashanakr	116
14.	RM 217	8	8	108-139	Improved Chinnor	108
15.	RM 201	7	7	100-171	Mahamaya	158
16.	RM 242	2	2	259-272	-	-
17.	RM 495	4	4	222-259	-	-
18.	RM 202	7	7	140-186	-	-
19.	RM 233	4	4	153-172	Improved Jeerashanakr	172
20.	RM 228	8	8	100-163	MTU 1010	163
21.	RM 154	7	7	158-200	Improved Jeerashanakr	200
22.	RM 235	5	5	100-153	Kranti	142
23.	RM 251	7	7	107-141	Mahamaya	141
24.	RM 256	5	5	107-141	JR 81	141
25.	RM 279	6	6	132-178	-	-
26.	RM 502	4	4	123-157	NPT 65	157
27.	RM 283	7	7	261-278	-	-
28.	RM 205	6	6	255-282	Improved Chinnor	282
29.	RM 341	7	7	335-370	-	-
30.	RM 438	4	4	309-331	Kranti	309
31.	RM 331	2	2	136-139	-	-
32.	RM 488	5	5	155-200	Mahamaya	200
33.	RM 212	6	6	108-134	-	-
34.	RM 510	4	4	107-118	NPT 65	118
35.	RM 1	3	3	100-114	-	-
36.	RM 234	4	4	149-164	JR 503	159
37.	RM 539	6	6	242-292	Mahamaya	292
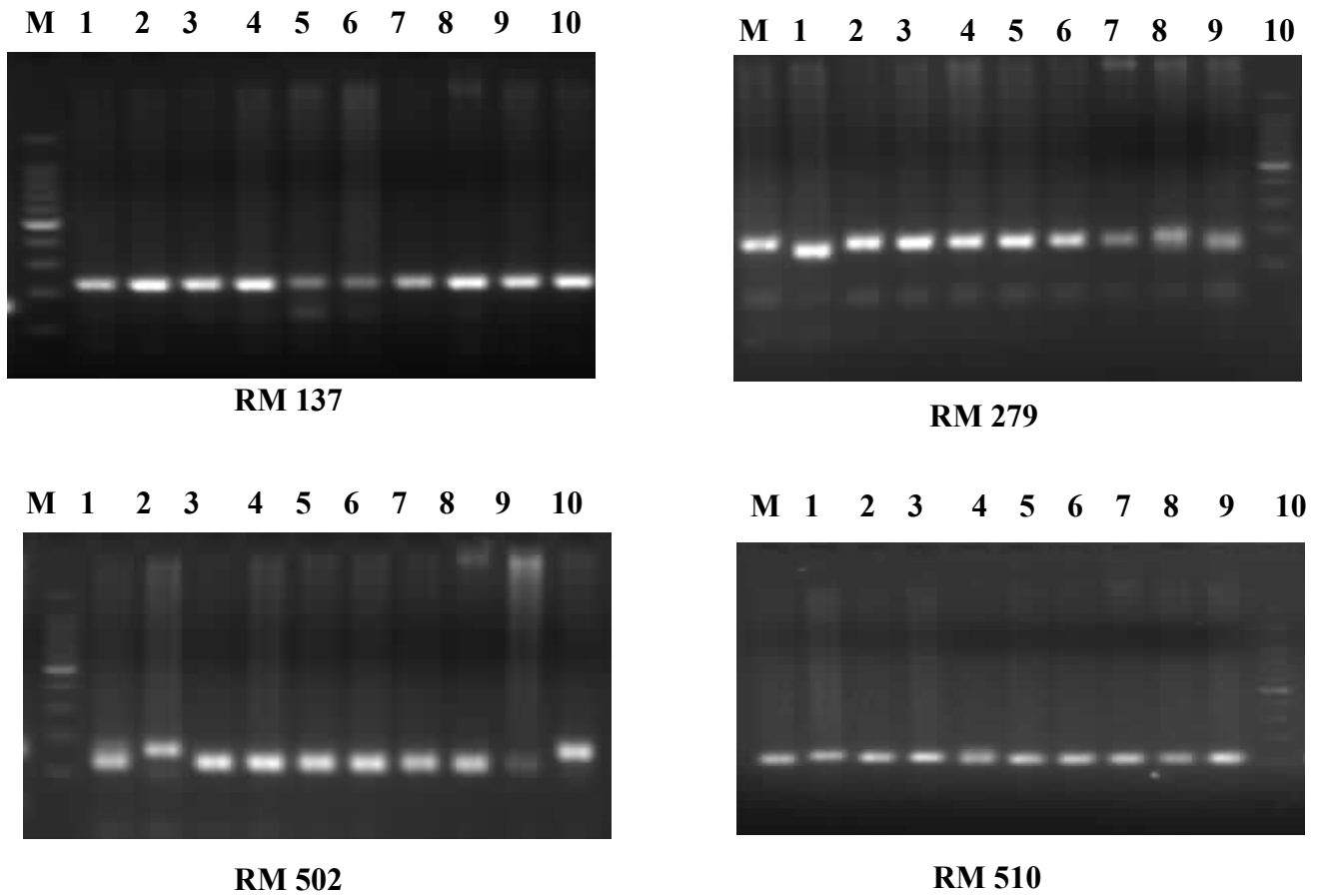
Fig 01: Fingerprinting of rice Variety NPT 65 using different SSR markers (M: 100 bp marker, 1: NPT 29, 2: NPT 65, 3: JR 81, 4: MTU 1010, 5: JR 201, 6: JR 503, 7: Kranti, 8. Mahamaya, 9: Improved Jeerashankar, 10: Improved Chinnor)
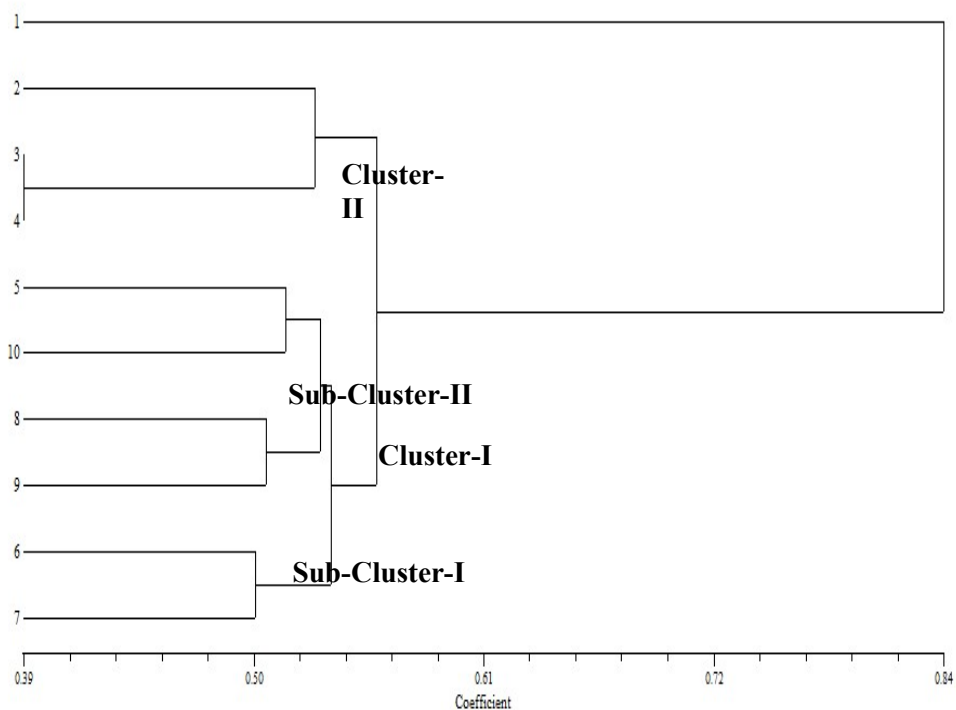
Fig 02: Dendogram of rice cultivars based on SSR markers

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