Indian J. Plant Physiol., Vol. 16, No. 3&4, (N.S.) pp. 340-345 (July-Dec., 2011)

SHORT COMMUNICATION

STUDIES ON GENETIC DIVERSITY AMONG RICE GENOTYPES USING RAPD AND SSR MARKERS

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Received on 30th May, 2011; Revised and accepted on 15th September, 2011

The genetic variation and relationships among 20 rice genotypes were evaluated using random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers. A high level of polymorphism were found with both RAPD and SSR markers and the mean polymorphism information content (PIC) values were 0.467 and 0.495 for RAPD and SSR markers, respectively. In RAPD analyses, 81 out of 94 bands (86.17%) were polymorphic. The number of alleles ranged from 3 to 7 per primer, with an average of 4.6 per primer. Cluster analysis based on RAPD banding pattern grouped the rice accessions into 2 major clusters. It also showed that N22 was distantly related to NDR18 with the Jaccard's coefficient of 0.63. Cluster analysis based on microsatellite data confirmed this clustering pattern of the accessions. SSR analysis also revealed that N22 was distantly related to Taraori with the Jaccard's similarity coefficient of 0.69. Combined dendrogram analysis showed that N22 was distantly related to Sahay Pasand with Jaccard's coefficient of 0.63. The two marker systems contrasted most notably in pair-by-pair comparisons of relationships. SSR analysis resulted in a more definitive separation of clusters of genotypes indicating a higher level of efficiency of SSR markers for the accurate determination of relationships between accessions that are too close to be accurately differentiated by RAPD markers.

Key words: **Genetic diversity, molecular markers, rice**

Rice is the staple food for more than half of the world's population and is model plant for genomic research (Sasaki and Burr 2000). Rice belongs to the grass family Poaceae, the genus *Oryza* 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 or derivation of plant lines. Moreover, the analysis of genetic diversity and relationship between or within different species, populations and individuals is a prerequisite towards effective utilization and protection of plant genetic resources (Weising *et al.* 1995). With DNA being the only basis of genetic differences between distinct organisms, DNA fingerprinting is presently the ultimate method of determination of biological diversification. Since all genetic differences between individuals are laid down in the primary sequence of their genomic DNA, the most straightforward method is identifying an individual sequence for genomes under comparison (Krawczak and Schmidtke 1994). Unlike the morphological and biochemical markers which may be affected by environmental factors and growth practices, DNA markers portray genome sequence composition,

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thus, enabling to detect differences in the genetic information carried by the different individuals. Restriction fragment length polymorphism (RFLP) was the first molecular marker, generated for genome analysis and mapping. However, the development of the polymerase chain reaction (PCR) technology has introduced a considerable number of useful molecular markers, e.g., RAPDs, AFLPs and SSRs. Because each marker system has specific advantages and disadvantages, the choice of the marker system to be used is very crucial.

The efficacies of different classes of PCR based markers (RAPDs, AFLPs and SSRs) were used to characterize rice and barley varieties (Virk *et al*. 2000, Saker *et al*. 2005). The objective of present investigation was to determine the genetic relationships among twenty rice genotypes and therefore, developing unique fingerprint for each variety. The leaf samples were collected from the rice genotypes screening experiment of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.), India from kharif season of 2010. The details of rice genotypes analyzed are presented in Table 1.

Total DNA was extracted from fresh leaves by the cetyl tri-methyl ammonium bromide (CTAB) method. Twenty 10-mer oligonucleotide random primers (Banglore Genei, India) were selected for analysis (Table 2). In case of SSR, a set of twenty primers (Banglore Genei, India) were used for analysis (Table 3). The RAPD-PCR products were analyzed directly on 1.5% agarose gels in TAE buffer while, SSR-PCR products were analyzed on 4% agarose gel, visualised by staining with ethidium bromide and transillumination under short-wave UV light. DNA ladder used in the electrophoresis was of 100bp.

Pair wise comparison of genotypes, based on the presence (1) or absence (0) of unique and shared polymorphic products was used to generate similarity coefficients of Jaccord's coefficient by NT-SYS-pc version 2.1 software (Rohlf 2000). The similarity coefficient was used to construct a dendrogram by the unweighted pair group method with arithmetic averages (UPGMA) according to (Rohlf 1993). The polymorphism information content (PIC) value described Anderson *et al.* (1993) for self-pollinated species was calculated as follows:

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where p_i equals the frequency of the *i*th allele and p_j the frequency of the allele. Only data from polymorphic loci were used for this analysis.

Among 20 RAPD primers, 9 primers showed 100% polymorphism among the twenty rice genotypes. The PIC values, derived from allelic diversity and frequency among the genotypes, were not uniform for all of the RAPD loci tested. The PIC value for 20 RAPD primers varied from 0.16 (OPA-10) to 0.84 (OPA-18). The 20 RAPD primers revealed 94 alleles. Out of 94 alleles 13 were monomorphic and 81 were polymorphic, thus revealing 86.17% polymorphism. The number of alleles per locus ranged from 3 to 7 with an average of 4.7 alleles per locus. The size of the detected alleles produced from the RAPD primer sets ranged from 1100-300 bp which reflect a large difference in the number of repeats between the different alleles. The cluster dendrogram with RAPD markers revealed 2 major clusters that were demarcated at a cut-off similarity coefficient level of 0.63, below which the similarity values narrowed conspicuously. Cluster II was the largest and included 18 genotypes while clusters I comprising 2 genotypes. Jaccord's coefficient of similarity revealed 80.1 % exists between P2511 and Taraori genotypes, where as N22 keeps very low level of similarity i.e. 53.1% with the HB1. The cluster analysis generated showed a significant genetic variation among the rice genotypes studied, with similarity coefficients ranging between 0.65 and 0.80. The dendrogram revealed 2 (I and II) major distinct clusters. The II major clusters further consist of 2 subclusture (IIA and IIB).The subcluster IIA consists of 2 genotypes, namely N22, and PS2. The similarity coefficient between N22, PS2 was 0.69. The subgroup IIB consists of a 16 genotypes, namely CSR-27, SP4, P1401, HB1, P1121, PD12, IRBB16, CSR10, VB, P2511, Taraori, Govind, PB1, CSR30, RB, SP with the similarity coefficients ranged between 0.67 and 0.80. Dendrogram revealed that the genotype N22 was distantly located to NDR18

Table 1. Details of selected rice genotypes used in the investigation.

S.No.	Varieties Name/ Code	Sources	Characteristic Features	Pedigree of genotypes
1	Nagina-22 (N22)	Nagina, U.P, India	Short and bold grains, susceptible to blast, BLB and resistant to drought	Selection from Rajbhog
2	CSR-27	CSSRI, Karnal Haryana, India	Grains: LS, resistant to blast and salinity	Nona Bokra X IR-5657
3	Pusa Sugandh -2 (PS2)	IARI, New Delhi, India	Basmati, Semi dwarf, medium duration, aromatic	Pusa-1238-1 X Pusa 1238-81-6
4	CSR -30	CSSRI, Karnal, Haryana, India	Super fine, semi dwarf and tolerant to salinity	BR-4-10 X Pakistani Basmati
5	Pusa -1121 (P1121)	IARI, New Delhi	Basmati, Scented variety	Pusa-614-1-2 X Pusa 614-2-4-3
6	Ranbir Basmati (RB)	Jammu & Kashmir, India	Basmati, tall, short duration, export quality, long slender, scented	Pureline selsction of Basmati-370-90-95
7	IRBB -16 (I16)	IRRI, Philippines	Semi dwarf, moderately resistant to bacterial blight	IRRI, Local Selection
8	Sarbati P – 4 (SP4)	Haryana, India	Basmati, Landrace, semi dwarf, short duration, lodging and disease resistance	Local Selection
9	Pusa-1401 (P1401)	IARI, New Delhi, India	Basmati, scented, dwarf variety	PB-1 X Pusa 1121-92-8-2-7-1
10	Sahay Pasand (SP)	Jharkhand, India	Tall and high tillering ability	Local Selection
11	CSR-10	CSSRI, Karnal, Haryana, India	Short and bold grains, tolerant to salinity and major diseases	M-40-431-24-114 X Jaya
12	CSR – 13	CSSRI, Karnal, Haryana, India	Long slender, resistant to blast and salinity	CSR-1 X Basmati-370 x CSR-5
13	Pusa-2511(P2511)	IARI, New Delhi, India	Fine scented variety	Pusa 3A X Haryana Basmati
14	Haryana Basmati-1 (HB1)	HAU, Haryana, India	Basmati, Super fine, semi dwarf, short duration	Sona X Basmati-370
15	Pusa Basmati-1 (PB1)	IARI, New Delhi, India	Basmati, Super fine, semi dwarf, long duration	Pusa -167 X Karnal local Basmati
16	Vallabh Bangani (VB)	SVPUA&T, Meerut, India	Dwarf, medium duration, violet color foliage	Local Selection
17	Pant Dhan – 12 (PD12)	GPBUA&T, Pantnagar, India	Dwarf, non scented, short duration	Govind X UPR-201-1-1
18	NDR - 18	NDUA&T, Faizabad, India	Dwarf, mid duration, Suitable for rainfed condition, direct sowing	Hansraj X IR-36
19	Govind (G)	GBPUAT&T, Uttarakhand, India	Dwarf, short-duration, aerobic, non-scented	IR-20 X IR-24
20	Taraori (T)	Haryana, India	Traditional Basmati, Tall, Long duration, Export Quality	Pure line selection of local Basmati

Table 2. Polymorphism Information Content (PIC) of RAPD Loci across various genotypes analyzed in the investigation

S.No.	Primer	Mol. wt. range (bp)	Total No. of alleles	No. of Polymorphic alleles	No. of monomorphic alleles	% of Polymorphism	PIC value
1.	OPA-04	750-300	5	5	0	100.00	0.37
2.	OPA-07	700-400	4	3	1	75.00	0.42
3.	OPA-08	700-300	5	5	0	100.00	0.76
4.	OPA-09	1100-900	4	3	1	75.00	0.46
5.	OPA-10	900-600	4	3	1	75.00	0.16
6.	OPA-11	80-600	5	5	0	100.00	0.47
7.	OPA-12	700-300	3	1	2	33.33	0.28
8.	OPA-13	750-350	4	3	1	75.00	0.49
9.	OPA-16	800-350	7	7	0	100.00	0.82
10.	OPA-17	750-400	6	6	0	100.00	0.62
11.	OPA-18	700-300	6	6	0	100.00	0.84
12.	OPA-19	700-400	4	3	1	75.00	0.32
13.	OPA-20	780-400	5	4	1	80.00	0.24
14.	OPB-06	780-400	6	5	1	75.00	0.46
15.	OPB-07	750-300	5	5	0	100.00	0.24
16.	OPB-08	700-350	4	4	0	100.00	0.49
17.	OPB-11	700-300	5	4	1	80.00	0.52
18.	OPB-13	700-380	4	4	0	100.00	0.57
19.	OPB-17	750-400	4	2	2	50.00	0.35
20.	OPB-18	700-350	4	3	1	75.00	0.44

with similarity coefficient 0.68. Based on dendrogram the genotype namely N22, P1401, HB1, CSR10, IRBB16, Govind, PB1, SP and NDR18 belongs to different clusters and they are genetically diverse. Based on study the large range of similarity values for related varieties using RAPDs provides greater confidence for the assessment of genetic diversity and relationships.

Twenty SSR primers pairs were used to investigate the level of polymorphism among different genotypes. SSR primers location on chromosome varies from 7 to 12. The PIC values, derived from allelic diversity and frequency among the genotypes, were not uniform for all of the SSR loci tested. The PIC value for 20 primers varied from 0.04 (RM286) to 0.99 (RM247).The 20 SSR primer sets which are distributed through 7 to 12 different rice chromosomes revealed 69 alleles (Table 3). All primers showed different levels of polymorphism except RM-47 which showed no polymorphism among the twenty rice genotypes. All of the alleles were polymorphic, thus revealing 100% polymorphism. The number of alleles per locus ranged from 2 to 6 with an average of 3.45 alleles per locus. The size of the detected alleles produced from the SSR primer sets ranged from 100- 570 bp which reflect a large difference in the number of repeats between the different alleles.

The cluster dendrogram using SSR markers revealed 2 major clusters that were demarcated at a cut-off similarity coefficient level of 0.65, below which the similarity values narrowed conspicuously. Cluster II was the largest and included 19 genotypes while clusters I comprising only one genotype (Taraori) with the similarity coefficient of 0.65. Cluster II consists of rest of the genotypes with the similarity coefficient ranges from 0.68 to 0.87. Jaccord's coefficient of similarity revealed 85.4 % exists between genotypes Vallabh Bangani and Govind. Whereas Taraori keeps very low level of

Table 3. Polymorphism Information Content (PIC) of SSR Loci across various genotypes analyzed in the investigation.

S.No.	Primer	Chromosomal location	Motifs	Mol. wt. range (bp)	Total No. of alleles	No. ofPoly-morphic alleles	No. of mono-morphic alleles	% Polymor-phism	PIC value
1.	RM21	11	(GA)18	350-570	6	6	0	100.00	0.97
2.	RM536	11	(CT)16	350-210	2	2	0	100.00	0.45
3.	RM206	11	(CT)21	200-120	4	4	0	100.00	0.88
4.	RM566	9	(AG)15	280-220	3	3	0	100.00	0.56
5.	RM320	7	(AT)11GTAT(GT13)	290-200	4	4	0	100.00	0.94
6.	RM247	12	(CT)16	210-110	5	5	0	100.00	0.99
7.	RM544	8	(TC)9	300-240	2	2	0	100.00	0.55
8.	RM346	7	(CTT)18	200-120	3	3	0	100.00	0.75
9.	RM547	8	(ATT)19	320-200	5	5	0	100.00	0.06
10.	RM519	12	(AAG)8	190-110	5	5	0	100.00	0.95
11.	RM286	11	(GA)16	160-100	6	6	0	100.00	0.04
12.	RM581	-	(GA)12	210-170	3	3	0	100.00	0.87
13.	RM222	10	(CT)18	270-210	4	4	0	100.00	0.63
14.	RM47	7	(AG)7(AG)11	-	0	0	0	0.00	Nil
15.	RM10	7	(GA)15	200-110	2	2	0	100.00	0.11
16.	RM19	12	(ATC)10	310-210	4	4	0	100.00	0.06
17.	RM20	12	(ATT)14	280-110	3	3	0	100.00	0.15
18.	RMR	-	-	350-230	4	4	0	100.00	0.08
19.	RM167	11	(GA)16	210-110	2	2	0	100.00	0.48
20.	RM264	8	(GA)27	200-130	2	2	0	100.00	0.37

similarity i.e. 65% to members of other clusters. Dendrogram revealed that the genotype N22 was distantly located Tarori with similarity coefficient 0.69. Based on dendrogram genotypes namely CSR27, PB1, SP, CSR10, PD12 and Taraori belong to different clusters and they are genetically diverse. Among the 2 distinct groups of clusters the genotypes of Basmati rice are also genetically distant. Based on study the large range of similarity values for related genotypes using microsatellites provides greater confidence for the assessment of genetic diversity and relationships. Among the 20 SSR primers studied, 19 primers spread over chromosome numbers 7-12 were found to be useful in fingerprinting of 20 genotypes.

The cluster dendrogram to study the combined genetic relationships revealed 2 major clusters (Fig.1) that were demarcated at a cut-off similarity coefficient level of 0.65, below which the similarity values narrowed conspicuously. Cluster II was the largest and included 19 genotypes while clusters I comprising one genotype. Jaccord's coefficient of similarity revealed 80 % exists between variety Vallabh Bangani and PD12. Whereas Sahay Pasand keeps very low level of similarity i.e. 63% to members of other clusters. The cluster analysis generated showed a significant genetic variation among the rice genotypes studied, with similarity coefficients ranging between 0.65 and 0.80. Cluster I consist of only one rice genotype Sahay Pasand with the similarity coefficient of 0.65. Cluster II consists of 19 rice genotypes with the similarity coefficient ranges from 0.67 to 0.80. Dendrogram revealed that the genotype N22 was distantly located to SP with similarity coefficient 0.63. Based on combined dendrogram analysis, the genotypes

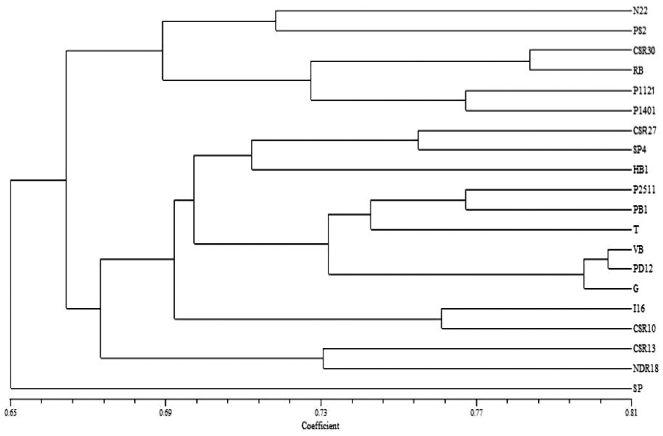

Fig. 1. Dendrogram showing clustering of 20 genotypes of rice based on combined SSR and RAPD data

N22, HB1, Taraori, IRBB16 and Sahay Pasand belong to different clusters and they are genetically diverse.

The RAPD marker systems did not produce the reproducible allelic profiles. However, SSR analysis resulted in a more definitive separation of clusters of genotypes indicating a higher level of efficiency of SSR markers for the accurate determination of relationships between accessions that are too close to be accurately differentiated by RAPD markers. Similar results were also reported by Ravi *et al.* (2003). As far as number of genotypes differentiated per assay is concerned, SSR had the maximum discriminatory power, followed by RAPD. SSRs showed higher percent polymorphism (100%) than RAPDs (86.17%). This was not unexpected because the SSR technique amplifies at least two microsatellite regions (regarded as highly polymorphic) as well as unique regions in between, and these results are consistent with Yu *et al.* (2005) in rice.

The results showed that SSR finger prints are more reliable because they are stable in genome localization. The inconsistency between RAPD and SSR analysis may be because reliability and reproducibility of RAPD is questionable, and it needs high level of standardization. Results obtained by RAPD and SSR marker analysis were not significantly correlated suggesting that the cluster analysis done in both approaches was not conserved. This may also be because genomic regions sampled by the two markers maintained different evolutionary processes. In current studies SSR primers RM21, RM247 and RM519 generated higher levels of polymorphism and any of them can be used to differentiate between the 20 rice genotypes. The SSR data sets generated from these primer combinations are sufficient for robust estimates and that additional data sets do not change the relationships among the 20 rice genotypes.

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