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Evaluation of Genetic Diversity in Rice (*Oryza Sativa* L.) for Direct Seeding Traits Using SSR Markers

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ABSTRACT

Molecular markers are useful tools for evaluating genetic diversity and determining cultivar identity. The purpose of the present study was to evaluate the genetic diversity within a diverse collection of rice accessions, and to determine differences in the patterns of diversity. Forty eight rice genotypes of particular interest *i.e* to direct seeding were evaluated for genetic diversity using SSR markers linked to ten traits of direct seeding. The results indicated that among SSR markers used, 10 SSR loci were polymorphic and produced 36 alleles. The number of alleles per locus generated by each marker varied from 2 to 5 alleles with an average of 3.6 alleles per locus. The polymorphic information content (PIC) values ranged from 0.305 to 0.797 with an average of 0.628. Out of the 10 polymorphic SSR markers used, 8 markers were highly informative (PIC > 0.50), 2 markers were informative (0.25 < PIC < 0.50). Highly significant correlation coefficient was found between PIC values and the number of alleles detected per locus (r=0.925**). The SSR markers RM201, RM263 and RM5509 were the highest polymorphic markers in current study. The Jaccard's similarity coefficients among the studied genotypes ranged from 0.1 to 1. The 48 rice genotypes were grouped into six major clusters at 35 % similarity level. Cluster II was the largest with 11 genotypes followed by clusters III and I with seven and six genotypes respectively.

Key words: *Direct seeded rice, Molecular diversity, SSR markers.*

Rice is the main staple food crop of the world. About half of the world population depends on rice for their survival. It is cultivated in 114 countries across the globe, but 90 per cent of world's rice is grown in Asia. India has the largest area under rice among the rice growing countries in the world and ranks second in production after China.

Assessment of genetic diversity is important in plant breeding for its improvement. 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. Genetic diversity can be evaluated with morphological traits, biochemical and DNA markers. In contrast to morphological traits, molecular markers can reveal abundant differences among the genotypes at 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). SSR markers are efficient in detecting genetic polymorphisms and discriminating among genotypes from germplasm of various sources, even they can detect finer level of variation among closely related breeding lines within a same variety (Lapitan *et al*., 2007).

Many SSR markers have been reported to be linked to different traits or QTLs in rice such as yield under drought (Vikram *et al*., 2011), maximum root length (Steele *et al*., 2006), basal root thickness (Qu *et al*., 2008) and root dry weight (Kanbar and Shashidhar 2010).

The aim of this study is to investigate the genetic diversity among forty eight rice genotypes for direct seeding traits like anaerobic germination, seedling root length, seedling shoot length, rate of germination, culm diameter using ten SSR markers.

MATERIAL AND METHODS

The present study was carried out during *kharif*-2014 at Andhra Pradesh Rice Research Institute (APRRI) and Regional Agricultural Research Station (RARS), Maruteru, Andhra Pradesh, India. The molecular analysis was carried out at Biotechnology Laboratory, APPRI, Maruteru. The experimental material for this investigation comprised of 48 genotypes. A total of ten simple sequence repeat (SSR) markers, *i.e.,* RM206, RM7, RM 17, RM 231, RM 234, RM 263, RM 201, RM 341, RM5509 and RM20557 were used for studying molecular diversity. The details of SSR primers used are presented in Table 1.

Collection and Preservation of Leaf Material

Leaf samples of 48 genotypes were collected from 12 day old seedlings for DNA isolation and kept in labelled plastic cover and stored at -20ºC till further processing.

DNA Isolation and Quantification

DNA was isolated using CTAB method as described by Doyle and Doyle (1990). The quality and quantity of isolated DNA was checked through ethidium bromide stained 0.8% agarose gel electrophoresis.

DNA quality estimation

The DNA quality estimation was done using Biophotometer plus. The ratio of OD260/OD280 ratio was used to estimate the nucleic acid purity in the different DNA samples. A ratio of 1.8-2.0 denotes that the absorption in the UV range is due to nucleic acids. A ratio lower than 1.8 indicates the presence of proteins and/or other UV absorbers. A ratio higher than 2.0 indicates that the samples may be contaminated with chloroform or phenol. In either case (<1.8 or >2.0) samples were re-precipitated to purify the DNA.

Polymerase Chain Reaction (PCR)

The genomic DNA of the selected lines was subjected to PCR amplification as per the procedure described by Chen *et al.* (1997). PCR was carried out using a programmable thermocycler (Eppendorf, USA). The PCR reaction mixture containing 3µl DNA, 3.7 µl water, 1.0 µl Taq buffer A, 0.5 µl of 2.5 mM DNTP', 0.5 µl forward primer, 0.5 µl reverse primer and 0.8 µl Taq polymerase (10 µl reaction mixture) was subjected to the polymerase chain reaction.

Annealing temperature was determined based on the GC content of the primer as:

$$Tm = [2 \times (A+T) + 4 \times (G+C)] - 4$$

After the completion of the PCR, the products were stored at -20ºC until the gel electrophoresis was done.

Agarose gel electrophoresis

A 3% gel was prepared and the PCR product was loaded to check the amplification of SSR markers.

Gel documentation

After the gel run, the gel was visualized under UV light transmitted gel documentation system. The banding pattern was observed and recorded using gel documentation unit (Genaxy).

Scoring the PCR amplified fragments

Band position in comparative SSR profile for each genotype and primer combination was scored from the respective gel images. The amplified fragments were scored as '1' for the presence and '0' for the absence of a band generating the 0 and 1 matrix. These binary data matrix was then utilized to generate genetic similarity data among the 48 lines of rice genotypes.

SSR data analysis

The amplified bands were scored for each SSR marker based on the presence or absence of bands, generating a binary data matrix of 1 and 0 for each marker system. Effective alleles per locus (Aep) were calculated according to Weir (1989) ($A_{ep} = 1/ (1- H_e)$, where H_e, the genetic diversity per locus). Genetic diversity was calculated according to Nei, 1973. ($H_e=1-\acute{O}P_i^2$, where P_i is the frequency of the i^{th} allele). The data on polymorphism information content (PIC) of the prime pairs was obtained by calculating the value according to the formula as described by Anderson *et al* (1993) as follows:

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where, P_{ij} is the frequency of the j^{th} allele for i^{th} marker and summation extends over n alleles. The polymorphism was recorded on the basis of variation on their allelic size.

The data matrix were used to calculate genetic similarity based on Jaccard's similarity coefficients, and dendrogram displaying relationships among 48 rice genotypes was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

Cluster Analysis

The genetic associations and interrelationships among entries were analyzed by calculating the similarity coefficient for pair-wise comparisons based on the proportions of shared bands produced by SSRs. The process which was used for tree building analysis involves sequential agglomerative hierarchical non-overlapping (SAHN) clustering and is based on Jaccard's similarity coefficients. The dendrograms based on similarity indices were obtained by un-weighted pair-group method using arithmetic mean (UPGMA). The SIMQUAL programe was used to calculate the Jackard's coefficient. The analysis was performed with the help of NTSYS-pc software 2.02.

RESULTS AND DISCUSSIONS

Number of alleles and allelic diversity

The forty eight rice genotypes used in the present study were subjected to DNA polymorphism screening and assessment using ten SSR markers. All the ten markers showed polymorphism. The results indicated that among SSR markers used, 10 SSR loci were polymorphic and produced 36 alleles. The number

Table 1: The details of SSR primers used in the present study

S. No.	Trait	SSR marker	Forward primer	Reverse primer
1	Anaerobic germination	RM341	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC
2		RM206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
3	Seedling Root length	RM 201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA
4		RM 234	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG
5	Seedling Shoot length	RM 17	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA
6		RM 263	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG
7	Rate of germination	RM7	TTCGCCATGAAGTCTCTCG	CCTCCCATCATTTCGTTGTT
8		RM231	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG
9	Culm diameter	RM205	TCATGAGGGTTATGGGAGTCAAGG	AGTGGTCGTTGAAATCCTTGTGC
10		RM550	GATGATCCATGCTTTGGCC	TTCCAGCAGAAAGAAGACGC

Table 2: Polymorphic information content (PIC) values of the markers used in the study

Marker	Number of alleles	Expected Heterozygosity (He)	Polymorphic information content (PIC)
RM341	4	0.614	0.553
RM206	3	0.524	0.582
RM 201	5	0.789	0.755
RM 234	2	0.187	0.169
RM 17	4	0.689	0.636
RM 263	4	0.707	0.654
RM7	2	0.473	0.361
RM231	4	0.655	0.604
RM20557	4	0.685	0.636
RM5509	4	0.711	0.658
Mean	3.6	0.603	0.547

of alleles per locus generated by each marker varied from 2 to 5 alleles with an average of 3.6 alleles per locus. The polymorphic information content (PIC) values ranged from 0.305 to 0.797 with an average of 0.628 (Table.2). Out of the 10 polymorphic SSR markers used, 8 markers were highly informative (PIC > 0.50), 2 markers were informative (0.25 < PIC < 0.50). Highly significant correlation coefficient was found between PIC values and the number of alleles detected per locus (r=0.925**). The SSR markers RM201, RM263 and RM5509 were the highest polymorphic markers in current study. On the other side, the Jaccard's similarity coefficients among the studied genotypes ranged from 0.1 to 1. The 48 rice genotypes were grouped into six major clusters at 35 % similarity level. Cluster II was the largest with 11 genotypes followed by cluster III and I with seven and six genotypes respectively.

PIC value

PIC value refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and distribution of their frequency; thus, it provides an estimate of the discriminating power of the marker. As it shown in Table 2, the PIC values for the SSR used in this study varied from 0.305 to 0.797 with an average of 0.628. The largest PIC value was observed for locus RM 201 (0.797) followed by RM 263 (0.719) and lowest by RM 234 (0.305) followed by RM 7 (0.494). So PIC value ranged from 0.79 to 0.30 with a mean value of 0.63 indicating the potentiality of markers in differentiating genotypes.

Diversity analysis

A dendrogram (Figure 1) based on Jaccard's similarity coefficient were constructed using UPGMA based cluster analysis (Table.3). The dendrogram

Table 3: UPGMA based clustering of 48 genotypes

S.No.	Cluster No.	Sub Cluster	Number of genotypes	Genotypes included in cluster
1	I	IA	2	MTU 1010, MTU 1075
		IB	1	MTU 3626
		IC	3	MTU 2077, BPT 2270, MTU II 118-53-2-1
2	II	IIA	6	MTU 1156, SABITHA, RNR 15048, RATNA, JGL 17004, WAYRAREM
		IIB	2	IR 36, ANJALI
		IIC	3	MTU II 110-9-1-1-1, E412, KALINGA-III
3	III	IIIA	1	MTU 1121
		IIIB	3	MTU 1166, MTU 1112, MTU 1081
		IIIC	3	MTU 1061, BPT 3291, PLA 1100
4	IV		1	MTU 1140
5	V	VA	2	MTU 1064, MTU 1078
		VB	7	BPT 5204, NAVEEN, JGL 384, VANDANA, IR 64, RGL 2332, RGL 2537
6	VI		2	IRS-3, SHABAGIDHAN
7	VII	VIIA	3	IR 50, IR 72, ANNADA
		VIIB	5	AC 39416A, AC 34245, AC 39397, AC 34280, AC 34345
		VIIC	2	N22, AZUCENA
8	VIII	VIII	1	PS-140-1

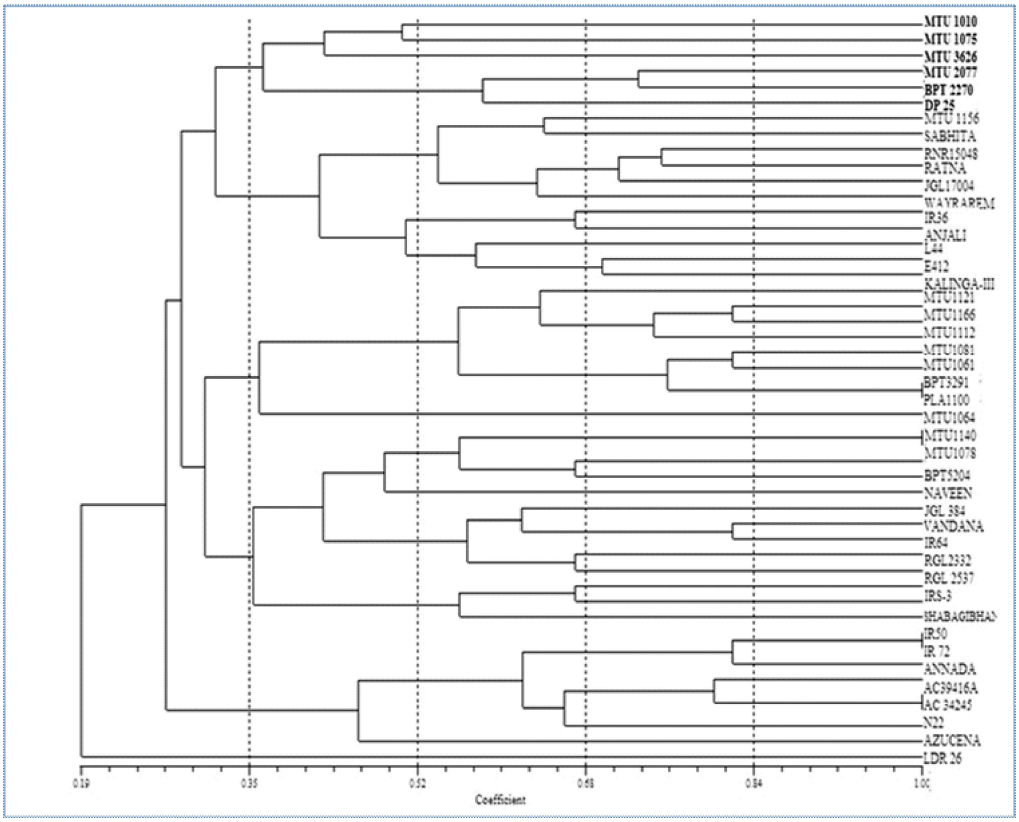

Figure 1. Dendogram (UPGMA) showing genetic diversity among 48 rice genotypes using direct seeding traits linked molecular markers

showed eight clusters at 35% similarity level. Cluster I comprised of six genotypes which were further sub divided into three sub clusters. Sub cluster I A had two genotypes, while, I B had single genotype *i.e* MTU 3626 and sub cluster I C had three genotypes.

Cluster II comprised with 11 genotypes. It was further sub grouped into three sub clusters. Six genotypes were in sub cluster II A, two in II B and three in II C. Cluster III comprised of seven genotypes. It was further sub grouped into three sub clusters. Sub cluster IIIA is unitary with MTU 1121, three genotypes were there in III B and three in II C. Cluster IV comprised of single genotype *i.e.,* MTU 1140. Cluster V was sub grouped into two sub clusters. Two genotypes were present in sub cluster V A, seven genotypes were there in V B. Cluster VI comprised of two genotypes. Cluster VII subdivided in to four sub clusters two, three, five and two genotypes were there in VIIA, VIIB, VIIC and VIID respectively. Cluster VIII is unitary with single genotype *i.e.,* PS-140-1. Cluster distribution given in Table 3. Hybridization between distantly related genotypes of different clusters would realize in good combinations for direct seeding traits. Earlier workers Sudharani *et al.* (2013), Girijarani (2015) and Ramadan (2015) assessed genetic diversity using molecular markers.

CONCLUSION

Based on the above results, we can conclude that all the 10 SSR markers used in the experiment were informative and useful in diversity analysis for traits related to direct seeding in rice. Selecting genotypes from different clusters will result in heterotic crosses as they are divergent at genotypic level for the concerned traits.

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LITERATURE CITED

Anderson J A, Churchill G A, Autrique, J E, Tanksley, S D and Sorrells, M E 1993 Optimizing parental selection for genetic linkage maps. *Genome.* 36:181–186.

Doyle J J and Doyle J L 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11-15.

Girijarani M 2015 Gene action and genotyping of elite rice lines for lodging resistance loci using molecular markers. *Ph.D. Thesis*, Acharya N.G. Ranga Agricultural University, Guntur.

Gracia A A F, Benchimol L L, Antonica M M, Geraldi I O and Deuza A P 2004 Comparison of RAPD, RFLP, AFLP and SSR marker for diversity studies in tropical maize inbred lines. *Euphytica*, 108: 53-63.

Kanbar A and Shashidhar H E 2011 Participatory selection assisted by DNA markers for enhanced drought resistance and productivity in rice (*Oryza sativa,* L.). *Euphytica*, 178(1): 137- 150.

Lapitan V C, Brar D S, Abe T and Redofia E D 2007 Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. *Breeding Science*. 57: 263-270.

Qu Y, Mu P, Zhang H, Chen C Y, Gao Y, Tian Y, Wen F and Li Z 2008 Mapping QTLs of root morphological traits at different growth stages in rice. *Genetica*, 133:187–200.

Ramadan E A, Elmoghazy A M, El-Mowafi, H F 2015 Molecular Markers based Genetic Diversity Analysis for Drought Tolerance in Rice (*Oryza Sativa,* L.) Using SSR Markers. *International Journal of Scientific Research in Agricultural Sciences*. 2: 137-146.

Steele K A, Price A H, Shashidhar H E and Witcombe J R 2006 Marker-assisted selection to introgress of rice QTLs controlling root traits and aroma into an Indian upland rice variety. *Theoretical and Applied Genetics*. 112: 208–221.

Sudharani M, Raghavareddy P and Hariprasadreddy G 2013 Identification of genetic diversity in rice (*Oryza sativa* l.) genotypes using microsatellite markers for salinity tolerance. *International Journal of Science Innovations and Discoveries*. 3 (1): 22-30.

Vikram P, Swamy B P M, Dixit S, Ahmed H U, Cruz M T S, Singh A K and Kumar A 2011 qDTY1.1, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genetics*, 12(89).

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