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Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes for heat tolerance using SSR markers

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Abstract

Genetic diversity among a set of 48 rice lines which included 24 heat tolerant genotypes and 24 heat susceptible genotypes was investigated with the help of a set of 16 rice SSR markers. All 16 primers were polymorphic amplifying a total of 127 alleles and mean number of 7.9 alleles per locus. The polymorphism information content (PIC) values ranged from 0.921(RM7075) to 0.995 (RM27289) with an average of 0.965 per locus. The rice lines were grouped into two major clusters and a clear separation of tolerant genotypes and susceptible genotypes into different clusters was observed. The results obtained from the study could be useful for selection of donors for heat tolerance for development of new rice varieties with heat stress tolerance

Keywords: Genetic diversity, heat tolerance, SSR markers, heat susceptible, genotypes

Introduction

Rice (*Oryza sativa L.*) occupies 23% of the total area under cereal production in the world (FAO. 2003) and is the staple food for nearly half of the world's population (Maclean *et al.,* 2002; Wassmann *et al.,* 2009). Most of the world's rice is grown and consumed in Asia where the production levels are estimated to decline by 4% due to climate change (Jagadish *et al.,* 2007). High temperature at anthesis stage in rice crop (which is the most sensitive stage to temperature) is expected to occur more frequently in the future (Jagadish *et al*., 2010). Climate change would affect the growth and developmental aspects of rice significantly. Additionally, yield reduction, head rice recovery (HRR), chalkiness, amylose content and gelatinization temperatures will also get affected (Subba rao *et al*., 2010). Therefore, it is necessary to identify the genotypes with heat tolerance. DNA markers that differentiate genotypes are reliable and convenient also complementing the phenotype process (Zeng *et al.,* 2004). Among various PCR based markers, SSR markers have many advantages due to their multi-allelic and co-dominant nature of amplification, high degree of polymorphic information content, high reproducibility, abundance etc. Hence this work was taken up with an objective to analyze a set of rice lines possessing varied level of tolerance to heat stress using a set of rice SSR markers and identify possible associations between marker amplification pattern and tolerance.

Materials and methods

Plant material: The experimental plant material for genetic diversity assessment of forty eight rice genotypes (detailed in Table 1) were collected from ICAR-IIRR, Hyderabad, India.

DNA extraction and SSR marker analysis: The DNA of 48 selected lines for genotyping was isolated from young leaves harvested after 15 days of sowing using CTAB method as described by Doyle and Doyle (1990). The genomic DNA of these genotypes was subjected to PCR amplification using a set of 16 rice SSR markers (Table 1) as per the procedure described by Chen *et al*., (1997). PCR reactions were carried out in thermal cycler with the total reaction volume of 20µl containing, 2µl of genomic DNA, 10X assay buffer, 10mM of dNTPs, 25mM $MgCl_2$, 5pmol of forward and reverse primer and 3U Taq polymerase enzyme and nano pure water. The PCR cycles were programmed as 95°C for 5 min, 94°C for 20 sec, 55°C for 30 sec, 72°C for 45 sec for 35 cycles and an additional temperature of 72°C for 7 min for final extension. The amplified products were separated on 3.5 per cent agarose gel prepared in 1X TAE buffer. The gel was run in 1X

Table 1: List of forty eight rice genotypes used for diversity analysis

S.No.	Genotype	S.No.	Genotype	S.No.	Genotype	S.No.	Genotype
1	GP:7858	13	GP:8109	25	E-191	37	E-8
2	GP:8001	14	GP:7868	26	E-750	38	GP:8551
3	GP:8067	15	E-849	27	E-224	39	GP:7862
4	GP:7860	16	GP:8058	28	E-186	40	GP:8219
5	GP:8706	17	E-888	29	E-179	41	GP:8205
6	E 147-145	18	GP:8709	30	E-743	42	GP:8178
7	GP:8182	19	GP:8600	31	E-156	43	GP:8170
8	GP:8595	20	GP:8130	32	GP:8447	44	GP:8338
9	E-846	21	GP:8716	33	GP:8331	45	E-321
10	E-437	22	E-851	34	GP:8309	46	GP:8441
11	E-929	23	GP:8142	35	E-283	47	GP:8028
12	E-601	24	GP:7880	36	E-729	48	GP:8762

TAE buffer at constant voltage of 80 V for a period of 100 minutes and stained with ethidium bromide.

Data analysis: Bands were scored as present (+/1) or absent (-/0). The size of each allele was determined by running simultaneously a DNA ladder by using a software (Uvitec, Fire-reader software version 15.12). The data was used for similarity based analysis using the programme NTSySPC (Rohlf, 2000). Similarity coefficients were used to construct UPGMA (unweighted pair group method with average) to generate dendrogram. Distance matrix and dendrogram was constructed based on diversity coefficient generated from pooled data by using Unweighted Pair Group Method of Arithmetic Means (UPGMA), a computer programme for distance estimation. Polymorphism Information Content (PIC) values were determined as per the procedure described by Senior *et al.* (1998)

Results and discussion

Polymorphism of SSR markers

Sixteen microsatellite markers were utilized for the determination of genetic diversity among 48 lines. All were observe to be polymorphic as presented in Table 2. A total of 127 alleles were detected by the 16 SSR markers. The number of alleles per locus varied from 4 to 17 with a mean of 7.9 alleles per locus. The PIC values of the 16 polymorphic SSR primers varied from 0.921 (RM 7075; Figure 1A) to 0.995 (RM 27289; Figure 1B) with an average of 0.969 per locus. Similar results were reported by Madhavi *et al*., (2011).

The SSR markers have significantly superior allelic diversity as compared to other co-dominant markers like RFLPs (McCouch *et al*., 1997). The average number of alleles per locus was 7.9 and this indicates a greater magnitude of variability among the plant materials analyzed in this work. These results agree with those of Olufowote *et al.,* (1997) who reported an average of 7.4 alleles per locus among 71 cultivars of rice. The results obtained in this study indicate that the rice lines selected for the present study have sufficient genetic divergence for possible use in future breeding programmes.

Polymorphism information content (PIC) value reflects the allele diversity and frequency among the genotypes. The informativeness of each SSR marker was established by using PIC value. The usefulness of a marker for different purposes like mapping, molecular bredding and germplasm evaluation is indicated by PIC value. DNA markers with greater PIC values have greater potential to indicate allelic variation. The markers analyzed in this study showed average PIC value of 0.969, indicating that the SSR markers utilized in this work were very informative and are helpful in identifying the polymorphism among rice lines. Higher PIC values reported in this study could be due to diverse origin of the used lines. The higher the mean PIC value of the locus, larger the number of alleles detected. This observation was consistent with report of Yu *et al.,* (2003), wherein SSR markers were noticed to exhibit high PIC valve because of their co-dominant nature and multi-allelic amplification pattern..

Table 2: List of primers and their chromosome location, product size and PIC values of 48 genotypes of rice

S. No	Name of the primer	Chromosome location	Product size (bp)	No. of alleles	PIC content
1	RM 7075	1	376	4	0.921
2	RM 3763	2	194	5	0.948
3	RM 12349	2	297	7	0.967
4	RM 1024	5	190	7	0.966
5	RM 19983	6	176	5	0.953
6	RM 542	7	201	11	0.99
7	RM 22524	8	176	6	0.969
8	RM 22710	8	182	15	0.994
9	RM 24035	9	231	8	0.984
10	RM 1026	9	195	7	0.98
11	RM 27258	11	220	9	0.984
12	RM 27289	11	194	17	0.995
13	RM 206	11	288	7	0.978
14	RM 27973	12	498	6	0.95
15	RM 28157	12	175	5	0.96
16	RM 16216	12	493	8	0.978
	Mean			**7.9**	**0.969**

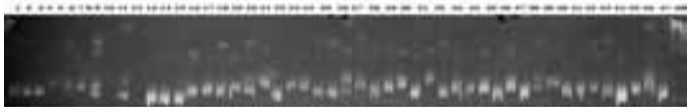

Figure 1A: Amplification pattern of 48 rice genotypes obtained by SSR marker RM7075

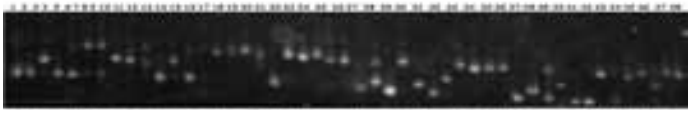

Figure 1B: Amplification pattern of 48 rice genotypes obtained by SSR marker RM27289

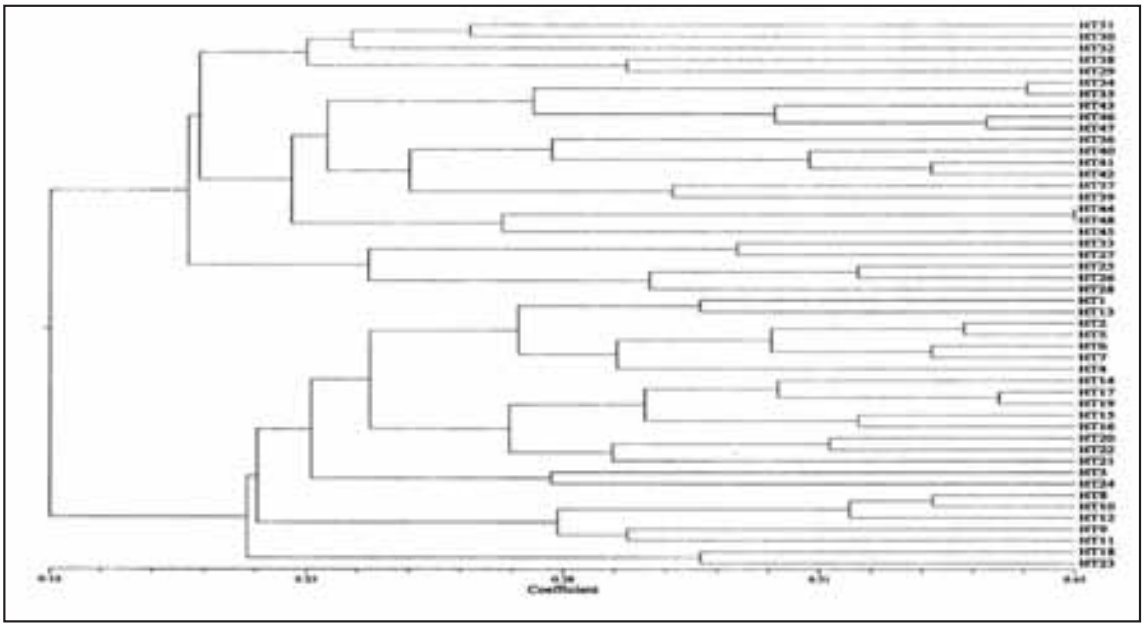

Figure 2. . Dendrogram resulting from UPGMA cluster analysis of 48 genotypes of rice

Genetic diversity Pattern

A dendrogram constructed based on UPGMA cluster analysis grouped the 48 rice lines into two clusters (Figure 2). The Jaccard's similarity coefficient ranged between 0.12 and 0.65. At the genetic similarity of 12%, the lines were grouped into two different clusters. Cluster I included all the 24 genotypes which were heat susceptible and cluster II had 24 heat tolerant genotypes. These findings were consistent with those reported by Saker *et al*., (2005). The results obtained clearly indicate that the genotypes belonging to cluster II can be used for development of heat tolerant varieties. It can also be concluded that the 16 carefully selected hyper-variable SSR markers are able to distinguish among tolerant and susceptible genotypes. Thus, marker-based identification and selection of the diverse genotypes could be helpful for the plant breeders and farmers for the improvement of new high yielding rice varieties.

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