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Assessment of Rice Genotypes for Drought Tolerance Using SSR Markers

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ABSTRACT

Phenotypic response of the 24 rice genotypes to drought tolerance and recovery ability was evaluated at flowering stage under field conditions. The results indicated that for drought tolerance, the genotypes were classified into five groups from score 1 to score 9 and for recovery ability, none of the genotypes scored 1 and 9. The genotypes were classified into 3 groups score 3, 5 and 7. A total of 70 alleles were detected by 24 polymorphic markers with an average of 2.92. Polymorphic information content (PIC) value varied from 0.980 to 0.990 with an average of 0.985. An efficient separation of 25 rice genotypes based on SSR data into two groups was achieved by using unweighted pair group method with arithmetic means (UPGMA) clustering procedure based on genetic similarity expressed by the Jaccard similarity coefficient (JSC). Genotypes that are derivatives of genetically similar type clustered more together. The present study provided an overview of the genetic diversity of the 24 rice cultivars for drought tolerance. Since the SSR markers are neutral and co dominant, they are powerful tools to assess the genetic variability of the cultivars under study .The information about genetic diversity of these cultivars will be very useful for proper selection of parents in rice breeding programs especially for gene mapping and eventually for the application of marker assisted selection (MAS).

Key words : Drought, PIC,Polymorphison, Rice, SSR Markers

Genetic improvement for adaptation to drought is addressed through the conventional approach by selecting for yield and its stability over locations and years. Such selection programs are slow in attaining progress because drought is sporadic and broad-sense heritability (H) of yield under stress is assumed to be lower than in non-stress environments (Rosielle and Hamblin, 1981). Alternatively, yield improvements in water-limited environments could be achieved by identifying secondary traits contributing to drought resistance and selecting for those traits in a breeding program. Root characteristics such as length, thickness, volume, dry weight and root shoot ratio, Chlorophyll Content Index (CCI) have been associated with drought avoidance in rice (Nguyen *et al.,* 1997). But, these traits are rarely selected for in crop improvement programs because phenotypic selection most of the root traits is difficult and labour intensive. Considering these limitations to efficient selection, molecular marker technology is a powerful tool for selecting such traits.

DNA based molecular markers have been used extensively to assess the genetic diversity of most crop species. Due to high efficiency, reproducibility, easy-to-use, co-dominance nature and high degree of polymorphism, microsatellite markers or simple sequence repeats (SSRs) are widely-used as molecular markers for fingerprinting germplasm to assess genetic diversity, pedigree analysis, evolutionary studies and genome mapping (Nagaraju *et al*., 2002; Kumar *et al*., 2005 and Sundaram *et al*., 2007). So screening of the genotypes having desirable shoot and root characters by using SSR markers is less time consuming and accurate procedure to identify desirable genotypes. The present study was taken up with a goal to identify the parental polymorphism present in the 25 rice genotypes coupled with the tolerance to moisture stress.

MATERIAL AND METHODS

Plant material and Screening for drought tolerance

The experimental material was consisted of 25 rice genotypes *viz.,* Moroberekan, Azucena, N-22, Lalnakanda-41, Bhadraj, MTU 1006, Dular, Chennangi, Varalu, MTU 1010, Tulasi, Govind, Vandana, Prasanna, Anjali, Rasi, NLR 145, IR 64, MTU 1042, MTU 1061, MTU 1038, BPT 5204, MTU 1081, MTU 7029 and MTU 1001. Genotypes included upland and lowland, both indica and japonica varieties having varied response to moisture stress (table 1). The field screening for evaluation of

Table 1.Scoring of rice genotypes for drought tolerance at flowering stage

Genotype	Parents	Source	Drough tolerance	Recovery %
Moroberekan	Landrace	West Africa	1	3
Azucena	Landrace	Philippines	1	3
N-22	A selection from Rajbhog	Uttar Pradesh	1	5
Lalnakanda-41	Landrace	Punjab	3	5
Bhadraij	Landrace	Orissa	3	5
MTU 1006	Pureline selection from Oodasannalu	Maruteru	3	5
Dular	Landrace	West Bengal	3	5
Chennengi	Landrace	Karnataka	5	5
Varalu	WGL 20471 / CR 544-1-2	Warangal	3	5
MTU 1010	Krishnaveni / IR64	Maruteru	5	5
Tulasi	Rasi / Fine gora	CRRI	3	5
Govind	IR20 / IR 24	Orissa	3	5
Vandhana	C22 / Kalakeri	DRR	3	5
Prasanna	IRAT8 / N-22	DRR	3	5
Anjali	PR-19-2 / RR-149-1129	CRRI	1	5
Rasi	TN 1 / Co 29	DRR	1	5
NLR 145	CICA-4 / IR- 625-23-3-1 /Tetop LS	Nellore	7	7
IR 64	IR 5857-33-2-1 / IR 2061-465-1-5-5	Philippines	5	7
MTU 1042	MTU 7029 / IR 64	Maruteru	5	7
MTU 1061	MTU 1010 / PLA 1100	Maruteru	7	7
MTU 1038	Pure line selection from Darukaselam	Maruteru	5	7
BPT 5204	GEB- 24 / TN1 / Mahsuri	Bapatla	7	7
MTU 1081	BPT 5204 / Ajay	Maruteru	7	7
MTU 7029	Vasishtha / Mahsuri	Maruteru	7	7
MTU 1001	MTU 5249 / MTU 7014	Maruteru	7	7

responses to moisture stress at flowering stage was followed. The genotypes were grouped under 1, 3, 5, 7 and 9 score categories on the basis of drought tolerance and recovery ability from stress. Visual scoring was done according to standard evaluation systems of rice, IRRI.

DNA isolation and PCR amplification

Genomic DNA of 25 genotypes was isolated by modified IRRI protocol (Zheng *et al.,* 1995). The quality of the DNA was checked on an agarose gel (0.8%, w/v). Fourty five SSR markers which are distributed on entire rice genome were used for this study. SSR primers were obtained from Sigma Aldrich, Bangalore. The PCR reactions were performed in 10-µl volumes using eppendorf Master cycler Gradient. The reaction mixture contained 25 ng template DNA, each 0.5 µM of forward and reverse primers, 125 µ M dNTPs, 1x PCR buffer (20 Mm Tris HCl, 15mM MgCl2), and 0.05U/µl Taq DNA polymerase. The amplification profile was 94°C for 5 min followed by 35 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 1 min with a final extension of 7 min. at 72°C. Amplified PCR products were electrophoretically resolved on a 3% agarose gel using 1x TBE buffer. DNA banding patterns were visualized using Syngene Bio-Imaging gel documentation system.

Data Analysis

Only clear and unambiguous SSR markers were scored. All the genotypes were scored for the presence (1) and absence (0) of the SSR bands. and the data was entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of the character and this data matrix was subjected to further analysis. The Excel file containing the binary data was imported into NT Edit of NTSYS-pc 2.02 (Rohlf, 1999). Genetic similarities were estimated from the matrix of binary data using Jaccards coefficient. The resultant similarity matrix was employed to construct dendrogram using

Table 2. Number of alleles and polymorphism information content (PIC) value of SSR markers for 25 rice genotypes

S.No	SSR marker	No.of polymorphic alleles	SSR motif	Chromo-some location	Amplification size (bp)	PIC value
1	RM 242	2	(CT)26	9	225	0.987
2	RM 324	3	(CAT)21	2	175	0.987
3	RM 16	2	(TCG)5(GA)16	3	181	0.981
4	RM 404	3	(GA)33	8	236	0.981
5	RM 351	4	(CCG)9(CGAAG)4	7	134	0.980
6	RM 70	2	(ATT)33	7	170	0.988
7	RM 493	2	(CTT)9	1	211	0.980
8	RM 400	2	(ATA)63	6	321	0.990
9	RM 138	4	(GT)14	2	233	0.982
10	RM 259	4	(CT)17	1	162	0.982
11	RM 490	4	(CT)13	1	101	0.987
12	RM 143	3	(CGG)7	3	207	0.990
13	RM 293	2	(GT)20	3	207	0.982
14	RM 552	3	(TAT)13	11	195	0.987
15	RM 289	2	G11(GA)16	5	108	0.981
16	RM 336	3	(CTT)18	7	154	0.987
17	RM 340	4	(CTT)8T3(CTT)14	6	163	0.990
18	RM 282	2	(GA)15	3	136	0.981
19	RM 171	2	(GATG)5	10	328	0.981
20	RM 221	2	(TC)4T3C3(TC)(CT)2	2	192	0.982
21	RM 520	4	(AG)10	3	247	0.990
22	RM 564	4	(GT)14	6	228	0.990
23	RM 231	4	(CT)16	3	182	0.990
24	RM 470	3	(CTT)14	4	83	0.990

Sequential Agglomerative Hierarchical Nesting (SAHN) based on Unweighted Pair Group Method with Arithmetic Means (UPGMA) to their genetic relationships and phylogeny.

Polymorphic information content

The term polymorphism information content (PIC) refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency. In the present study, PIC value of a marker was calculated according to a simplified version after Anderson *et al*. (1993).

$$PIC = 1 - €^{n}_{j=1} \; P^{2}_{ij}$$

Where Pij is the frequency of the j^{th} allele for the i^{th} marker and summed over n alleles.

RESULTS AND DISCUSSION

The results of field screening of rice genotypes to drought tolerance indicated the varied genotypic responses. For drought tolerance none of the genotypes scored 0 (no symptoms) and 9 (all the plants apparently dead). The genotypes were classified into four groups from slight tip drying (score 1), tip drying extended up to ¼ length in most leaves (score 3), one-fourth to ½ of all leaves fully dried (score 5) and more than 2/3 of all leaves dried (score 7). For recovery ability, none of the genotypes scored 1 (90-100% recovery) and 9 (0-19% recovery). The genotypes were classified into 3 groups from 70-89% recovery (score 3), 40-69% recovery (score 5) and 20-39% recovery (score 7). Moroberakan and Azucena showed good drought tolerance and recovery ability when compared to other varieties.

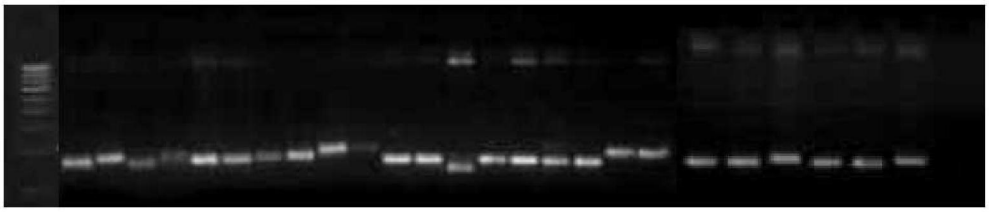
Number of alleles and polymorphism information content values of SSR markers for 25 rice genotypes were showed in table 2. The lowest amplicon size was produced by RM 470 (83bp) while highest amplicon size was belonged to RM 171 (328bp). Out of forty five markers used, 24 SSR markers showed polymorphism by revealing 70 alleles. The number of alleles per locus varied from 2 (RM 242, RM 16, RM 70, RM 493, RM 400, RM 293, RM 289, RM 282, RM 171, RM 221) to 4 (RM 351, RM 138, RM 259, RM 490, RM 340, RM 520, RM 564, RM 231) with an average of 2.92. Many studies have also reported significant differences in allelic diversity among various microsatillite loci (Ni *et al.,* 2002).

The polymorphic information content (PIC) value, a reflection of allele diversity and frequency among the cultivars also varied from 0.980 to 0.990 with an average of 0.985, which confirms that SSR markers used in this study were highly informative, because PIC value higher than 0.5 indicate high polymorphism. The polymorphic banding pattern of RM 336 and RM 493 markers in 25 rice genotypes is presented in fig 1. The higher mean PIC value indicate that the genotypes used in the present study were more diverse due to differences in origin, ecotype and speciation. The genetic diversity of each SSR locus appeared to be associated with the number of alleles detected per locus. The higher the mean PIC value of the locus, higher the number of alleles detected. This observed pattern was consistent with report of Yu *et al.* (2003).

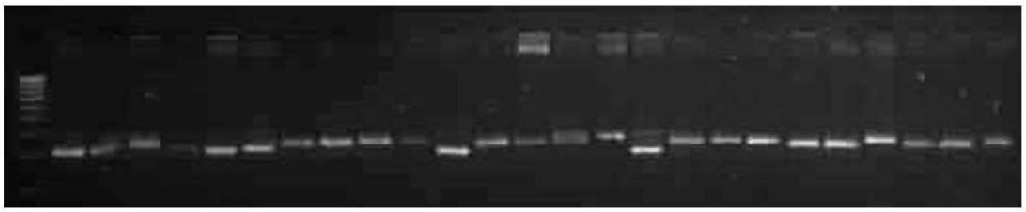
A total of two major clusters (japonica and indica) resulted out of analysis of pooled SSR marker data (fig 2). This dendrogram revealed that the genotypes derived from a genetically similar type clustered together. Group one comprised of Moroberekan and Azucena which are of japonica type. Group two comprised of indica genotypes. These findings were consistent with those reported by Saker *et al.* (2005). The parental phenotypic evaluation is in sinmilarity with genotypic evaluation using SSR markers.

The present study provided an overview of the genetic diversity of the 24 rice cultivars for drought tolerance. There was large range of similarity values for related cultivars using micro satellites provide greater confidence for the assessment of polymorphism.

Fig 1. Polymorphism observed using RM 336 and RM 493 in the 25 rice genotypes.



RM 336



RM 493

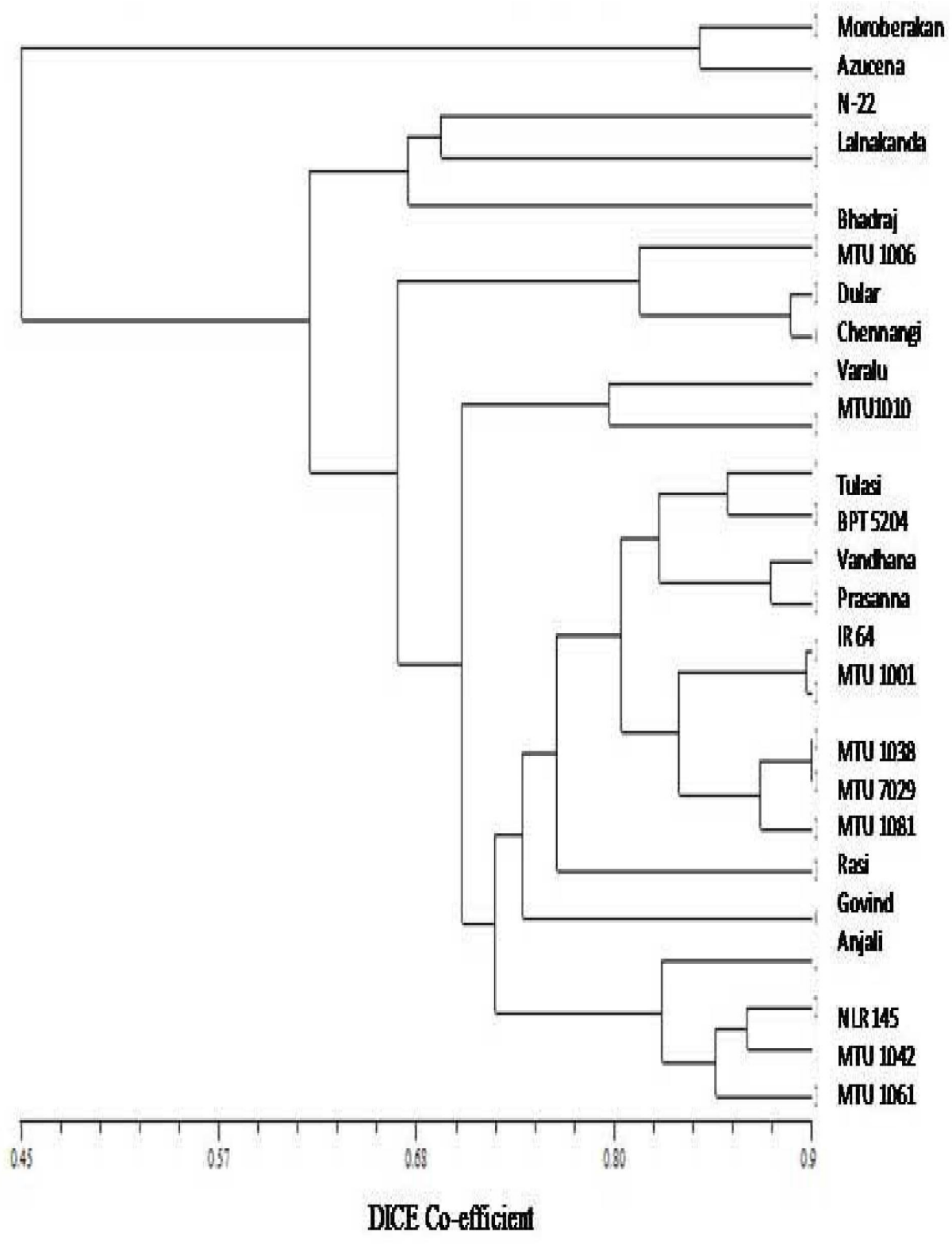


Fig 2. Denodrogram of the 25 ruce gebittoes deruved from UPGMA culster analysis using Jaccard's Co-efficient based on 24 polymorphic SSR markers.

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