The Andhra Agric. J 61(4): 809-814, 2014

Molecular Characterization of Drought Tolerant Lines in Rice using Microsatellite Markers

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ABSTRACT

Drought is one of the major abiotic stresses in rainfed rice which causes low yields (0.7 to 1.5 t/ha). Drought being the most devastating environmental stress, continuous efforts are needed to improve the crop productivity under water-limiting conditions. Molecular characterization of the genotypes and study on extent of variability among the genotypes for complex traits like drought is essential to incorporate such genotypes in the breeding programme. The experimental material consisted of 44 advanced breeding lines developed at APRRI & RARS, Maruteru in the APNL Biotechnology project. These 44 advanced breeding lines were characterized using a set of 30 microsatellites or SSRs (simple sequence repeat) spanning all the 12 rice chromosomes. The total number of alleles detected in the study was 46 and out of these 46 alleles, 29 alleles (63%) were polymorphic. The number of alleles detected at a single locus ranged from 1-3 with an average of 1.5 alleles per locus. UPGMA analysis has grouped the 44 genotypes into nine clusters. Clusters I to V had single genotype each, while cluster VII had two genotypes. Cluster VIII and Cluster VI had three and four lines respectively. Of all the clusters, Cluster IX is the largest having 30 genotypes. The coefficient of similarities based on random data among genotypes ranged from 0.46 to 0.59 with an average similarity index of 0.53.

Key words : Characterization, Drought, Markers, Microsatellite, Rice.

Rice is the world's second most widely grown cereal crop after wheat, and is the staple food for more than one half of the world's population. It is one of the most versatile crops grown under a wide range of agro-ecological conditions ranging from irrigated to rainfed lowland, upland, deep water and tidal conditions. India is the largest rice growing country (45.5 million hectares) and the second largest in production (99.18 million tons). Over 55% of the rice area is rainfed, where drought is a major constraint which depress yield by about 15-50%.

Drought being the most devastating environmental stress, efforts have been to improve the crop productivity under water-limiting conditions. More than 80 year long history of breeding for tolerances to drought has led us nowhere near development of ideal genotypes capable of resisting the stress, despite availability of reliable sources of tolerance to the stress in the germplasm.Like yield and its components, the traits that confer drought tolerance are controlled by many genes.

An understanding of the extent of genetic diversity is critical for the success of a breeding programme.Traditional methods using morphological characters for establishment of genetic diversity and relationships among accessions are largely unsuccessful due to strong influence of the environment. Recent advances in molecular biology have equipped scientists with a wide choice of marker assisted techniques to know the extent of molecular diversity. SSRs are highly effective in molecular characterization of the genotypes and also in assessing the genetic diversity existing in the genotypes as well as in genetic mapping studies. Potential markers and gene clusters have been identified for the various morpho-physiological indices of tolerance to moisture stress in rice (Lilley and Ludlow 1996; Lafitte *et al*., 2006, Bernier *et al.,* 2007 and Zhao *et al*., 2008).

Characterization of the genotypes and study on extent of variability among the genotypes for complex traits like drought is essential to incorporate such genotypes in the breeding programme. In the present investigation,

Table 1. List of Genotypes Studied.

S. No	Advanced Breeding Line	S. No	Advanced Breeding Line
1	124-22	23	124-55
2	124-23	24	124-57
3	124-24	25	124-60
4	124-26	26	124-65
5	124-27	27	124-66
6	124-28	28	124-71
7	124-29	29	124-74
8	124-31	30	124-84
9	124-32	31	124-110
10	124-33	32	124-111
11	124-34	33	124-112
12	124-38	34	124-113
13	124-39	35	124-114
14	124-41	36	124-115
15	124-42	37	124-119
16	124-43	38	124-126
17	124-44	39	124-127
18	124-45	40	124-128
19	124-47	41	124-129
20	124-49	42	124-133
21	124-50	43	124-134
22	124-52	44	124-168

characterization of 44 advanced breeding lines using 30 microsatellite markers was studied. Also the molecular genetic diversity was studied in these 44 advanced breeding lines.

MATERIAL AND METHODS

The experimental material consisted of 44 advanced breeding lines developed at APRRI & RARS, Maruteru in the APNL Biotechnology project (Table 1). These 44 advanced breeding lines were screened using a set of 30 microsatellites or SSRs (simple sequence repeats) spanning all the 12 rice chromosomes. DNA was extracted from 50 days old leaf tissue from all the genotypes using the modified Cetyl Tri Methyl Ammonium Bromide (CTAB) method of Dellaporta *et al.* (1983). The DNA quantification was done by using a Nanodrop spectrophotometer (Thermo Scientific) based on spectrophotometric measurement of UV absorbance at 260 nm since DNA has maximum absorbance at 260 nm as well as using known amount Lambda DNA (Bangalore Genei) as standards.

The amplification reaction with SSR primers was carried out in a final volume of 10 ìl in DNA Thermo cycler (Eppendorf Mastercycler Pro S). Each reaction mixture contained 1.0 ìl 10 X reaction buffer containing 1.5 mM $MgCl_2$, 1.0 U of Taq DNA polymerase (Bangalore Genei), 0.1 mM dNTP (Bangalore Genei), 10.0 picomoles each of forward and reverse primer (synthesized by Eurofins) and approximately 50 ng/ ìl of template DNA.

PCR amplification was carried out on thermal cycler as under:

Initial denaturation at 94^0C for 5 min

Denaturation at 94^0C for 30 sec,

Primer annealing at 55^0C for 45 sec, 45 cycles

Extension at 72^0C for 1 min,

Final extension of 72^0C for 7 min.

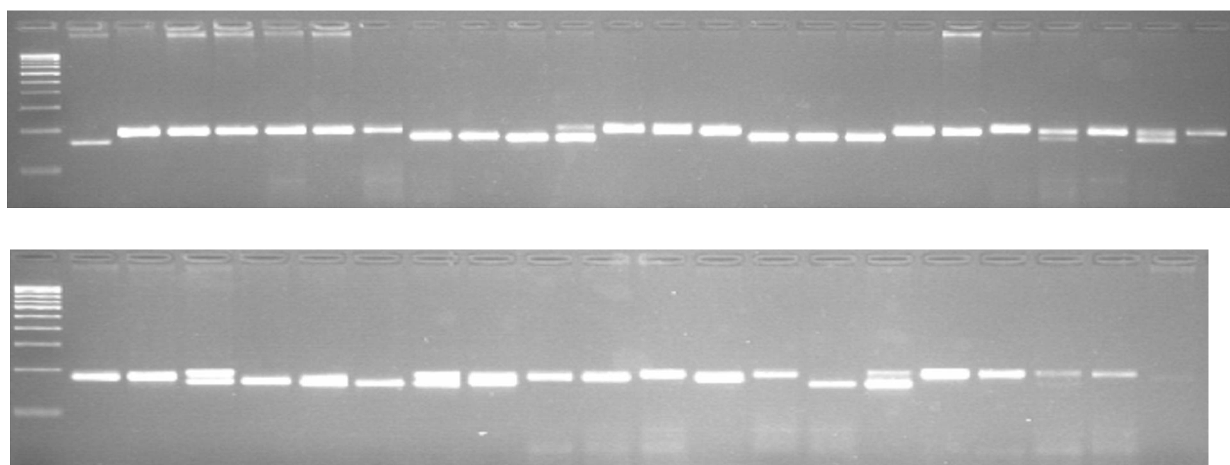
Table 2. Allele Variation for Microsatellite Loci (SSR) Studied in 44 Rice Genotypes.

S. No	SSR locus	Chromosome	Number of alleles	Amplicon size
1	RM 12135	1	I	365
			II	380
			III	398
2	RM 12184	1	I	376
3	RM 10886	1	I	126
4	RM 138	2	I	233
5	RM 208	2	I	286
6	RM 525	2	I	185
			II	197
7	RM 282	3	I	136
8	RM 14565	3	I	102
9	RM 15494	3	I	378
10	RM 335	4	I	100
			II	120
11	RM 5473	4	I	250
			II	266
12	RM 548	5	I	278
13	RM 440	5	I	200
14	RM 20648	6	I	165
			II	172
			III	186
15	RM 20168	6	I	420
			II	440
16	RM 8015	7	I	230
			II	264
17	RM 5100	7	I	200
			II	219
18	RM 248	7	I	271
19	RM 281	8	I	138
20	RM 210	8	I	140
21	RM 444	9	I	460
			II	482
22	RM 5122	9	I	450
			II	471
23	RM 171	10	I	328
24	RM 4771	10	I	140
			II	165
			III	180
25	RM 1812	11	I	265
26	RM 202	11	I	195
			II	205
27	RM 144	11	I	225
			II	245
28	RM 558	12	I	117
29	RM 512	12	I	197
30	RM 17	12	I	291

Table 3. Clustering Pattern among 44 Rice Genotypes.

S. No	Group	Number of lines	Genotype
1	I	1	168
2	II	1	57
3	III	1	126
4	IV	1	119
5	V	1	114
6	VI	4	42,71,74,111
7	VII	2	29,52
8	VIII	3	119,129,34
9	IX	30	26, 24, 34, 28, 23, 55, 60, 42, 47, 110, 65, 45, 41, 84, 50, 39, 133, 36, 128, 127, 112, 65, 44, 32, 31, 33, 27, 115, 45, 22

Fig 1. Molecular Profile of 44 Rice Genotypes with RM 202.



M- 100 bp Ladder

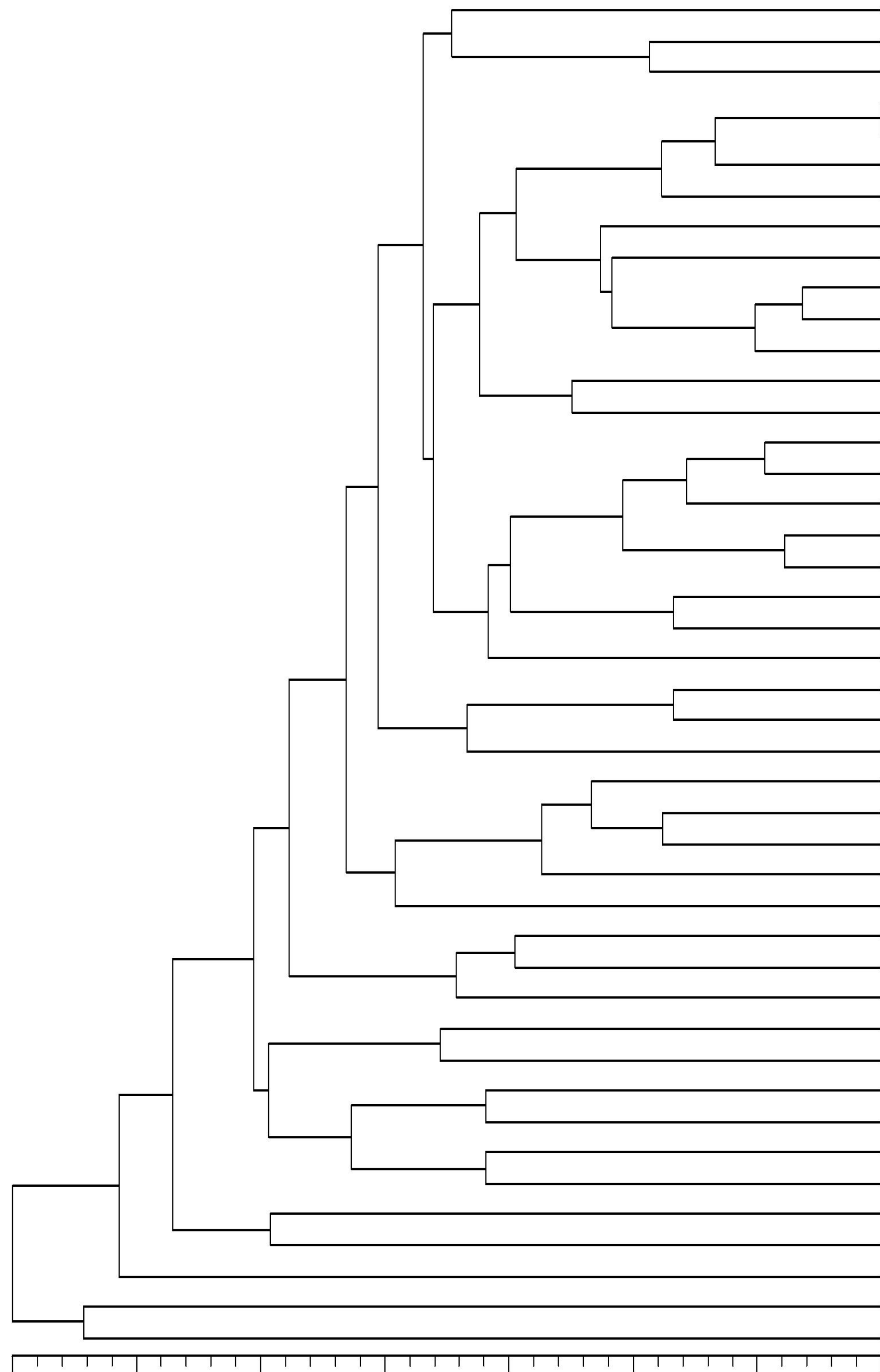
PCR samples were mixed with bromophenol blue (0.25% bromo phenol blue and 40% (w/v) sucrose mixed in water) and run on a 3% agarose gel (Sigma) containing ethidium bromide (10mg/ml) along with the marker 50bp ladder (MBI Fermentas, Canada) at 5.3V/cm (Bio-Rad Power Pac 300) for an hour in 1x Tris-Acetic acid-EDTA (TAE) buffer (242g Tris base, 57.1 ml Acetic acid 100ml 0.5M EDTA mixed and made up the volume to 1 litre with double distilled water and pH adjusted to 8.5). The resolved PCR bands were documented using Syngene Gel Doc System. The scoring of the population was done as presence or absence of the band. Cluster analysis and dendrogram construction was done by UPGMA analysis using ENTSYS software.

RESULTS AND DISCUSSION

Forty four advanced breeding lines (RILs) were screened using a set of 30 microsatellites or SSR (simple sequence repeat) spanning all the 12 rice chromosomes. The total number of alleles detected in the study was 45 and out of 45 alleles, 29 alleles (63%) were polymorphic. The number of alleles detected at a single locus ranged from 1-3 with an average of 1.5 alleles per locus. The allele size varied from 100 bp (RM 335) to 482 bp (RM 444). (Table 2) (Fig 1.). A positive correlation was found between the number of alleles per locus and the maximum number of repeats within a microsatellite marker.

UPGMA analysis has grouped the 44 rice genotypes into nine clusters. Clusters I to V had

Fig. 2. Cluster Analysis Dendrogram of 44 rice genotypes based on Jaccard's similarity index.



single genotype each *viz.,* I- 168, II- 57, III- 126, IV- 119 and V-114 while cluster VII had two genotypes (29 and 52). Cluster VIII and Cluster VI had three (119, 129, 34) and four (42,71,74 and 111) respectively. Of all the clusters, Cluster IX is the largest having 30 genotypes (26, 24, 34, 28, 23, 55, 60, 42, 47, 110, 65, 45, 41, 84, 50, 39, 133, 36, 128, 127, 112, 65, 44, 32, 31, 33, 27, 115, 45 and 22). The coefficient of similarities based on random data among genotypes ranged from 0.46 to 0.59 with an average similarity index of 0.53. (Fig 2)(Table 3). The results are in confirmation with Ni *et al*., 2002 who studied genetic diversity among 38 accessions utilizing 111 microsatellite markers.

The observed level of genetic diversity from the SSRs and its distribution pattern were generally consistent with those of most previous studies based on much larger samples (Glaszmann 1987; Li and Rutger 2000). SSR markers for this assay were chosen because of their obvious advantages such as abundance in the genome, high level of polymorphism, co-dominance and cost-effectiveness (Yang *et al.* 1994; McCouch *et al.* 1997; Ni *et al.* 2002), which were clearly demonstrated in this study. The level of polymorphism when SSR markers were utilized is high compared to other types of markers such as isozyme and RFLP loci.

Based on the UPGMA analysis, it can be concluded that variation was present among the genotypes as the genetic diversity showed a higher proportion of variation caused by predominant non-random associations.

LITERATURE CITED

Bernier J, Kumar A, Ramaiah V, Spaner D and Atlin G 2007 A Large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Science*, 47:505-516.

Dellaporta S C, Wood J and Hicks T B 1983 A plant DNA mini preparation: Version II Plant *Molecular Biology Report*, 1:19-21.

Glaszmann J C 1987 Isozymes and classification of Asian rice varieties. *Theoretical and Applied Genetics,* 74:21–30

Lafitte H R, Li Z K, Vijayakumar C H M, Gao Y M, Shi Y, Xu J L, Fu B Y, Yu S B, Ali A J, Domingo J, Maghirang R, Torres R and Mackill D J 2006 Improvements of rice drought tolerance through backcross breeding: Evaluation of donors and selection in drought nurseries. *Field Crops Research*, 97:77–86.

Li Z and Rutger J N 2000 Geographic distribution and multilocus organization of isozyme variation of rice (Oryza sativa L.). *Theoretical and Applied Genetics*, 101:379-387

Lilley J M and Ludlow M M 1996 Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. *Field Crops Research*, 48:185–197.

McCouch S R, Cho Y G, Yano M and Blinstrub M 1997 Report on QTL nomenclature. *Rice Genetics Newsletter,* 14:11-13.

Ni J, Colowit P M and Mackill D J 2002 Evaluation of genetic diversity in rice subspecies using microsatellite markers *Crop science,* 42 (2): 601-607.

Yang G P, Saghaimaroof M A, Xu C G, Zhang Q and Biyashev R M 1994 Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. *Molecular Genetics*, 245:187–194

Zhao X Q, Xu J L, Zhao M, Lafitte R, Zhu L H, Fu B Y, Gao Y M and Li Z K 2008 QTLs affecting morph-physiological traits related to drought tolerance detected in overlapping introgression lines of rice (*Oryza sativa* L.). *Plant Science,* 174: 618-625.

(Received on 18.02.2013 and revised on 19.04.2013)