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Genetic diversity among aerobic rice accessions using RAPD markers

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Investigation was carried out to evaluate genetic diversity between 103 aerobic rice (Oryza sativa L.) accessions using RAPD markers. Eighty random primers were used to generate the RAPD profiles of 103 aerobic rice accessions. Fourteen markers that showed reproducibility were used to detect polymorphism. Of the 94 bands obtained, 74 (78.72%) were polymorphic. The pair wise similarity ranged from 0.62 to 0.94 with a mean of 0.78. The PIC (polymorphic information content) ranged from 0.01 (OPE7) to 0.36 (OPC14 and OPD9) with an average PIC 0.26. The genotypes were grouped into clusters and sub-clusters. The genotype 109 (DGI 155) was found distinct from all other accessions. A group of RAPD primers showing 100% polymorphism can be used in genetic diversity studies of rice germplasm.

Key words: Genetic diversity, RAPD, Rice.

INTRODUCTION

Rice is a staple food for more than 2.5 billion people around the world (IRRI, 2004). Growing rice is the largest single use of land for producing food, covering 9% of the earth's arable land (IRRI, 2002). Asia itself accounts for over 90% of the world's production of rice. About half the total world rice area is rain fed, where drought is a major constraint (Fukai Cooper *et al*., 1998), so cultivation of drought tolerant rice varieties under such conditions is the only alternative. One of the major future challenges for agriculture is to produce more food with less water. Rice is mainly grown in the submerged conditions but there is a need to find out the strategy for growing rice under aerobic conditions to decrease water use in rice production.

In rice, molecular markers have been used to identify accessions (Olufowote *et al*., 1997; Virk *et al*., 1995) to determine the genetic structure and pattern of diversity for cultivars of interest (Akagi, *et al*., 1996). Compared to morphological analysis, molecular markers reveal differences among accessions at the DNA level and thus provide a more direct, reliable and efficient tool for germplasm conservation and management. Molecular markers are useful tools for evaluating genetic diversity and determining cultivar identity (Ni *et al.*, 2002).

Agriculture relies heavily on the genetic diversity of crop plants, during the process of domestication and cultivation of crop plants, a wealth of genetic diversity has been utilized and partly preserved. Thousands of valuable allelic variations of traits of economic significance remain unutilized in nearly all crop plants. Approximately 15 per cent of the potential diversity in crop plants has been utilized for developing new varieties or hybrids. These can be discovered and effectively used to meet the existing and emerging challenges that threaten world food security. The objective of the present study was to evaluate the genetic variation within a collection of aerobic rice accessions and to reveal genetic relationships among them using RAPD markers.

MATERIALS AND METHODS

Plant Materials :

103 Aerobic rice accessions used in the present study were obtained from International Rice Research Institute (IRRI) Philippines. Details of genotypes are shown in Table 1.

Plant DNA extraction :

The genotypes were grown in an experimental field of University of Agricultural Sciences, GKVK, Bangalore. The genotypes were grown in aerobic conditions without water stagnation and irrigation was given at an interval of five days. The leaves were collected at maturity and dried properly in an oven at 55^0C. Dried leaves were then powdered in a mixer, and the fine powder was used for the DNA extraction. DNA was extracted by following the Porebski *et al*. (1997) method with certain modifications. 100mg of leaf powder in 2.0ml of pre-warmed extraction buffer (100mM Tris pH 8.0 containing

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20mM EDTA, 1.4M NaCl, 1% 2-ME, 3% CTAB) was incubated in water bath at 65^{0}C for 30 min with periodic shaking. Equal volume of chloroform: Isoamylalcohol (24:1v/v) was added and vortexed gently. Centrifuged at 12,000 rpm for 20 min at 4^{0}C. The aqueous phase was repeatedly washed with equal volume of chloroform: Isoamylalcohol (24:1 v/v). To the aqueous phase equal volume of chilled isopropanol and half the volume of 5M NaCl was added. Mixed gently. Kept at –40^{0}C for overnight to accentuate the precipitation of DNA. Centrifuged at 12,000 rpm for 20 min at 4^{0}C to recover DNA pellet. The pellet was washed first with 70% aqueous ethyl alcohol and then with 100% ethyl alcohol, air dried. The pellet was dissolved in 100µl of TE buffer and incubated with 3 µl of RNase overnight at 37^{0}C. Washed with equal volume of phenol: chloroform: Isoamylalcohol (25:24:1v/v) and chloroform: Isoamylalcohol (24:1 v/v). The DNA was precipitated by addition of equal volume of chilled isopropanol and kept at –40^{0}C for 2 hr. Centrifuged at 12,000 rpm for 20 min to pellet the DNA. The DNA pellet was dissolved in 300 µl of TE buffer and stored at –40^{0}C. DNA quantification was done at OD_{260nm} and was diluted to a final concentration of 12.5 ηg μl^{-1} and 2µl of this DNA was used for the PCR amplification.

RAPD amplification :

Eighty RAPD primers (Operon technologies, USA) were employed to generate polymorphism among the diverse rice genotypes which 14 showed reproducibility and were used for the detailed study. The primers were diluted to a concentration of 5pmol/ìl by using sterilized Milli-Q water. The primers belonging to the OPA9, OPA11, OPB8, OPC11, OPD3, OPD5, OPD8, OPD9, OPD11, OPE1, OPE 2, OPE 4, OPE 7 and OPE13 series were used for the RAPD amplification.

Amplification was carried out in a 20µl reaction mixture containing 1X PCR buffer, 3mM $MgCl_2$, 200µM dNTPs, 1U *Taq* polymerase, 25pmol primers and 25 ng of DNA template. The amplification reaction was carried out in a thermal cycler (MJ Research Inc. USA). The first cycle consisted of denaturation of template DNA at 94^{0}C for 5 min, primer annealing at 36^{0}C for 1 min and primer extension at 72^{0}C for 2 min. In next 40 cycles the period of denaturation was reduced to 1 minute while the time for primer annealing and extension remained as in the first cycle. The last cycle consisted of only primer extension at 72^{0}C for 12 min.

PCR products were separated on a 1.5 % agarose gel containing ethidium bromide. TBE (1X) buffer was used for preparation of gel and for running the gel. It was run for 2.5 hr at 85V. The size of the fragments were determined by using 100bp and 500 bp DNA ladder marker and the gel was visualized under ultraviolet light and documented using gel documentation system (Herolab GmbH Laborgerate).

Data analysis :

The RAPD bands were scored qualitatively as present (1) and abscent (0). Data matrices were created and used to obtain Jaccard's similarity indices (SI) (Jaccard, 1908). Constructing dendogram and calculating genetic relationship was done based on SI matrix using software NTYSYS-PC (Rohlf, 1998). The PIC (Polymorphic information content) for each RAPD marker was calculated, as proposed by Salazar *et al.* (2006) as $PIC_i = 2f_i(1-f_i)$, where PIC_i is the polymorphic information content of marker i, f_i is the frequency of the marker band present and $(1-f_i)$ is the frequency of the marker band absent.

RESULTS AND DISCUSSION

Amplification products using fourteen RAPD primers showing reproducible results were scored to determine the genetic diversity among rice genotypes. Totally, 94

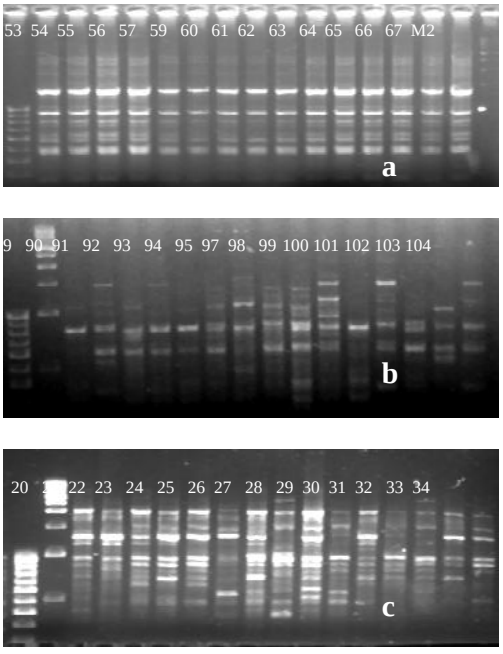

Fig. 1: RAPD profiles of selected rice genotypes using random primes OPC 11(a), OPD 5(b), OPD 9(c). Lane M1 (100bp DNA ladder plus) and Lane M2 (500bp ladder plus). Other numbers on various lanes represent the diverse rice accessions details on Table 1.

Table 1 : Rice accessions used in the present study.

Genotype No.	Designation	Source	Genotype No.	Designation	Source
1	AUS 196	A03WS-09	60	IR 77078-B-17-3-2	A03WS-01
2	AZUCENA	A03WS-09	61	IR 77080-B-6-2-2	A03WS-01
3	B 6144F-MR-6	A03WS-10	62	IR 77298-12-7	A03DS-03
4	C 22	A03WS-09	63	IR 77298-14-1-2	A03DS-03
5	CT 13370-12-2-M	A03WS-09	64	IR 77298-5-6	A03DS-03
7	CT 13382-8-3-M	A03WS-10	65	PR 27699-B-D808-4-4	A03DS-03
8	CT 6510-24-1-2	A03WS-09	66	PSBRC 9	A03WS-01
9	CT 6516-24-3-2	A03WS-09	67	UPL RI 7	A03DS-03
10	DINORADO	A03WS-10	68	Yunlu 29	A03DS-03
13	IR 47686-30-3-2	A03WS-09	70	IR 76558-188-1-1-1	A04DS-02
14	IR 55419-04	A03WS-09	71	IR 76558-156-4-1-3	A04DS-02
15	IR 55423-01	A03WS-10	73	IR 76569-259-1-1-3	A04DS-02
16	IR 60080-46A	A03WS-10	74	IR 76569-243-2-1-4	A04DS-02
18	IR 65907-116-1-B	A03WS-09	76	IR 76558-152-2-2-1	A04DS-02
19	IR 66417-18-1-1-1	A03WS-09	77	IR 76569-166-4-2-2	A04DS-02
20	IR 66421-062-1-1-2	A03WS-09	78	DGI-196	Binam
21	IR 66424-1-2-1-5	A03WS-09	79	DSL-89-3	BG300
22	IR 68702-072-1-4-B	A03WS-09	80	DSL-104-1	Jhna349
23	IR 70358-84-1-1	A03WS-09	81	DGI-195	Binam
24	IR 70360-38-1-B-1	A03WS-09	83	IR64-e7	
25	IR 71524-44-1-1	A03WS-09	85	DSU-16-3	Lemont
26	IR 71525-19-1-1	A03WS-09	87	DSU-10-3	Basmati
27	IR 72768-15-1-1	A03WS-09	88	RF-53-20	Binam
28	IRAT 170	A03WS-10	89	DSU-4-4	Feng-Ai-Zan
29	IRAT 177	A03WS-09	90	DSU-4-18	Feng-Ai-Zan
30	IRAT 212	A03WS-10	91	DSU-8-1	Babaomi
31	IRAT216	A03WS-09	92	DSL-109-3	MR 106
32	MARAVILHA	A03WS-09	93	DGI-296	FR 13A
33	PALAWAN	A03WS-09	94	DSL-101-3	Ptb33
34	PSBRC 80	A03WS-09	95	DSL-89-10	BG300
35	PSBRC 82	A03WS-09	97	DGI 21	STYH
36	UPL RI 5	A03WS-09	98	DSU-4-11	Feng-Ai-Zan
37	UPL RI 7	A03WS-09	99	DGI 71	BR24
38	VANDANA	A03WS-09	100	DGI-143	Type3
41	WAB 638-1	A03WS-09	101	DGI-138	Type3
42	WAB 96-1-1	A03WS-09	102	DGI 32	STYH
43	WAY RAREM	A03WS-09	103	DSU-4-7	Feng-Ai-Zan

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Genotype No.	Designation	Source	Genotype No.	Designation	Source
45	IR 55419-04	A03DS-03	105	DSL-81-1	Cisedane
46	IR 64	A03WS-10	106	DSU-18-6	OM 1706
47	IR 70210-39-CPA-7-1-1-4-2	A03WS-01	107	DSL-78-10	IR72
48	IR 71525-19-1-1	A03DS-03	108	DSU-10-5	Basmati
49	IR 72667-16-1-B-B-3	A03WS-01	109	DGI-155	Type3
50	IR 72875-94-3-3-2	A03DS-03	110	DSL-111-4	MR 167
51	IR 74371-3-1-1	A03DS-03	112	DSL-69-6	Hua-Gen_Xian74
52	IR 74371-46-1-1	A03DS-03	113	DGI 28	STYH
53	IR 74371-54-1-1	A03DS-03	114	IR 64	
54	IR 74371-70-1-1	A03DS-03	115	DGI 81	BR24
55	IR 74371-78-1-1	A03WS-01	118	IR 79907-B-3	A04DS-03
56	IR 74963-262-5-1-3-3	A03WS-01	260	IR 79907-B-145	A04DS-03
57	IR 75003-95-5-1-3	A03WS-01	292	IR 79907-B-177	A04DS-03
59	IR 77076-B-21-1-2	A03WS-01			

Table 2 : Illustration of percent polymorphism generated from each marker.

Marker	DNA Band		% Polymorphism	PIC
	Monomorphic	Polymorphic		
OPE 13	1	3	75.00	0.35
OPE 4	2	6	75.00	0.21
OPE 2	1	6	85.70	0.26
OPE 7	5	4	44.44	0.01
OPE 1	1	4	80.00	0.31
OPD 5	0	4	100.00	0.35
OPD 8	0	5	100.00	0.35
OPD 9	0	6	100.00	0.36
OPC14	4	3	42.85	0.36
OPD 3	0	8	100.00	0.26
OPC 11	1	6	85.71	0.09
OPB 8	1	6	85.71	0.24
OPA 9	4	7	63.63	0.16
OPA 11	0	6	100.00	0.30

bands amplified, out of which 74 (78.72%) were polymorphic. The band size ranged from 350bp-2.4Kb. Total number of fragments amplified per primer ranged from 4 to 11 with an average of 6.7. Among the primers employed for amplification, OPA9 amplified maximum fragments (11) and OPD5 amplified minimum fragments (4). Amount of polymorphism that was observed in OPD3, OPD5, OPD8, OPD9 and OPA11 was 100% and for OPD11 was only 42.85% with an average of 78.72%. The PIC (polymorphic information content) ranged from 0.01 (OPE7) to 0.36 (OPC14 and OPD9) with an average PIC 0.26. The other primers having higher PIC values include OPD5, OPD8, OPE13, OPE1 and OPA11 (Table 2).

More diversity among the amplified fragments of

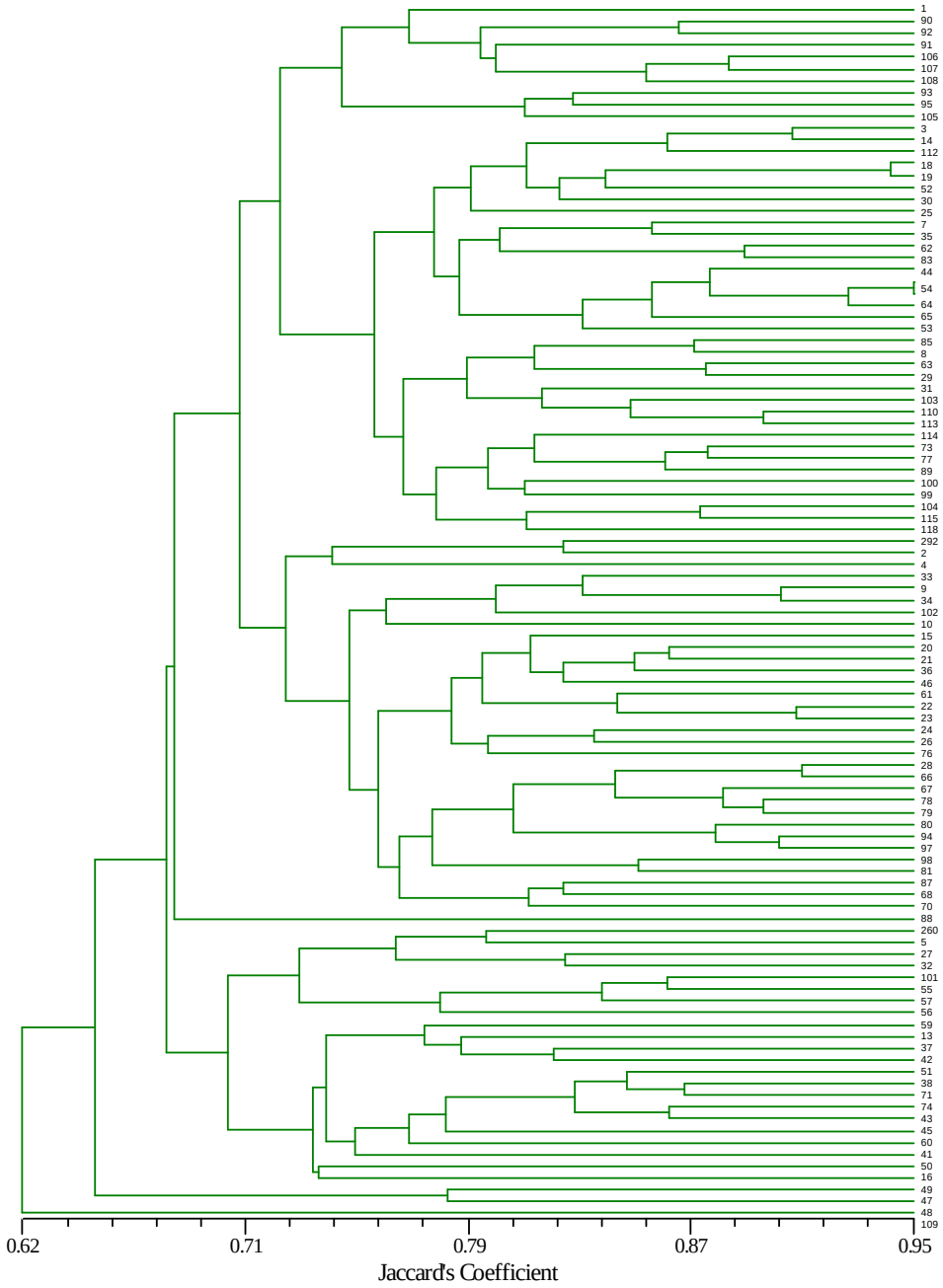

Fig. 2: Dendrogram showing the similarity among 103 rice accessions using RAPD markers.

various genotypes was observed with primers of OPD3, OPD5, OPD8, OPD9, OPA11, OPC11, OPB8 and OPE2 (Figure 1) as such these primers are useful in molecular diversity analysis of rice germplasm.

The Jaccard's coefficient of similarity for all pair wise comparisons ranged from 0.62 to 0.94 with a mean of 0.78. The genotype 109 (DGI 155) was found distinct from all other genotypes with a similarity co-efficient of 0.62 (Figure 2).

RAPD involves the use of random, largely dominant markers and can be used for evaluating genetic diversity among closely related rice cultivars. The use of molecular markers is considered best for the analysis of genetic diversity and identification of variety, as there is no effect of stage of development, environment or management practices. Among various classes of the molecular

markers the RAPD markers (Williams *et al.,* 1990) despite some limitations (Penner, 1996), have been widely used because of its speed, simplicity, and amenability of automation. In rice, RAPD analysis has been used extensively for exploitation of genetic diversity among the accessions (Virk *et al.,* 1995a) duplicate identification (Virk *et al.,* 1995b, Verma *et al.,* 1999) and identification of hybrids (Qian *et al.,* 1996).

In our study we observed 78.72 % polymorphism among aerobic rice accessions. Sarma and Bahar (2006) observed 82% polymorphism among rice accessions from Assam with RAPD markers. The Jaccard's coefficient of similarity for all pair wise comparisons in our study ranged from 0.62 to 0.94 with a mean of 0.78, a similar average Jaccard's coefficient of 0.78 was being found in a similar study on genetic diversity among Brinjal accessions using RAPD markers (Koundal *et al*., 2006). Salazar *et al*. (2006) in a study to findout the relaiability of the RAPD technique for germplasm analysis of Sesame (*Sesamum indicum*) found out an average PIC value of 0.37%, and in our study the PIC (polymorphic information content) ranged from 0.01 (OPE7) to 0.36 (OPC14 and OPD9) with an average PIC 0.26. The other primers having higher PIC values include OPD5, OPD8, OPE13, OPE1 and OPA11 (Table. 2).

The five RAPD primers which have generated very high polymorphism among the rice accessions can be used for analyzing genetic diversity among the rice germplasm.

The genetically divergent accessions identified in this study can be used for cross hybridization for their further improvement.

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