Oryza Vol. 47. No.3, 2010 (173-178)

Genetic diversity of saline rice cultivars based on RAPD markers

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ABSTRACT

RAPD markers were used to evaluate genetic relationships in 20 cultivars of saline tolerant rice including few tolerant varieties. Twenty primers generated 207 highly reproducible and discernible loci, among which 160 were polymorphic. Percentage of polymorphism varied from 50 to 100 with an average of 74.8. Two primers, OPBA-03 and OPBC-10 showed 100 percent polymorphism among the accessions studied. A relatively high genetic diversity was detected among all the samples with the similarity coefficient value ranging from 0.45 to 1.00. The uweighed pair group method with arithmetic averages (UPGMA) dendrogram clustered 20 accessions in four clusters at 0.75 coefficient level. Principal coordinate analysis (PCA) further indicated that the genetic diversity of saline rice cultivars was presented by a clustered distribution pattern. Thus, the results from the UPGMA which grouped the accessions into 4 clusters lies more or less on par to the results of PCA. The cluster II and IV consisted of 6 and 10 accessions, respectively and cluster I and III consisted of 1 and 3 accessions, respectively. The similarity co-efficient was maximum between IR 61920-3B-22-2-1 and IR 42 (1.00) indicating less divergence among them. Lower similarity coefficient indices were observed between IR 61920-3B-22-2-1 and HP 3319-2WH-6-3-1-1-3 (0.26), indicating more divergence. Neither the UPGMA dendrogram analysis nor the PCA exhibited strict relationship with geographic distribution for the characters studied.

Key words: *saline rice genetic diversity, RAPD markers.*

More than 7 m. ha of the cultivable land is affected by salinity problem (IRRI, 1998). This ultimately reduces the yield as the salt concentration increases. High yielding rice varieties of irrigated ecosystem has not been productive under saline conditions. Hence, it is imperative to identify high yielding and stable genotypes with increased salt tolerance. This requires new genetic sources of salt tolerance and more efficient techniques for identifying salt tolerant germplasm. As a valuable genetic resource, landraces and wild relatives comprise an extraordinarily important gene pool for breeding. Knowledge on the extent and distribution of genetic variation within a species is fundamental for conservation of genetic resources and breeding (Ayana *et al*. 2000). DNA markers made possible a fast survey of a species, genetic diversity and genetic structure. Randomly amplified polymorphic DNA (RAPD) technique is a simple and powerful DNA marker tool.

The genetic diversity studies using D^2 statistics of Mahalanobis (1936) is being used for selection of prospective parent for hybrid production. Molecular genetic polymorphism is also used to analyse the variability which has been the most efficient approach in plant genetics. RAPD analysis is widely used to study the genetic diversity of various cultivars and lines. RAPD assay is rapid and easy to perform and also requires only limited amount of DNA. In the present investigation the assessment of genetic diversity in saline tolerant lines of rice has been studied with a new for their utilization in breeding programme.

MATERIALS AND METHODS

Twenty accessions collected from different sources (Table 1) were used for RAPD marker analysis in the present study. These accessions were grown in nursery beds of 3x1m in the Plant Breeding Farm, Annamalai University, Tamil Nadu. A total of five leaves were collected from 25 days old seedling in each accession.The seedlings were raised under natural saline condition. The salinity level of the experimental field was EC 4.0dsm^{-1} and pH 7.8.

Table 1. List of rice cultivars studied under natural saline condition.

Accession No.	Name of the accessions	Orgin /source
1	HP 3319-2WH-6-3-1-1-3	Himachal Pradesh, India
2	IR47547-3B-26-2B-1	IRRI, Philippines
3	IR 6199-313-24-3	IRRI, Philippines
4	IR 5931-110-1	IRRI, Philippines
5	IR 6192-3B-15-2-2	IRRI, Philippines
6	IR 28	IRRI, Philippines
7	IR 29	IRRI, Philippines
8	PY 5	Pondicherry, India
9	IR 63311-B-3R-B-24-3	IRRI, Philippines
10	IR 61920-3B-22-2-1	IRRI, Philippines
11	POKKALI	Kerala, India
12	IR 36	IRRI, Philippines
13	IR 42	IRRI, Philippines
14	IR 61920-3B-22-2-1	IRRI, Philippines
15	IR 71657-5R-B-129	IRRI, Philippines
16	IR 7500-59-1-1-B	IRRI, Philippines
17	ADT 38	Tamil Nadu, India
18	ADT 45	Tamil Nadu, India
19	CR 1009	Tamil Nadu, India
20	TRY 1	Tamil Nadu, India

DNA was extracted following the protocol described by Williams *et al.* (from frozen leaves stored at -180ºC, 1990) with modification. The leaves cut into pieces were completely homogenized in liquid nitrogen. Extraction buffer (100mM Tris HCl (pH 8.0), 20 mM EDTA, 1.4 mM NaCl, 2 % cTAB per litre) was added in 50 ml tubes filled with leaf powder to a volume of 15 ml and mixed well. The tubes were incubated at 65ºC for 30 minutes with repeated shaking. Equal volume of chloroform: isoamylalcohol mix (24:1) was added and mixed thoroughly for 15 minutes, followed by centrifugation at 4000 rpm for 30 minutes. Equal volume of isopropanol was added to the supernatant. DNA was hooked out after half an hour and washed in 70% ethanol and suspended in 500 μ l of TE buffer (pH 8.0). The DNA was incubated with 10-15 μ l (10 μ g/ μ l concentration) of RNAse for 30 minutes. To this, equal volume of chloroform: isoamylalcohol was added and centrifuged at 12,500 rpm for 10 minutes. Twice the volume of absolute ethanol and1/10th volume of 3M sodium acetate were added to the acqueous layer and kept over night. The contents were centrifuged at 12500 rpm for 15 minutes and supernatant was discarded. The pellet was washed with 70 percent ethanol and air-dried. Then, the pellet was dissolved in 500 μ l of TE and stored at -20ºC. The quality and quantity were checked through 0.8% agarose gel by electrophoresis. DNA concentretain for PCR reaction was estimated, by comparing the band intensity produced by the known dilution that gave good amplification.

Genomic DNA was used as template for PCR amplification as described by Williams *et al*., (1990). A set of 20 arbitrary primers (OPERON technologies. Inc. California, USA) was used to produce distinct marker profiles for twenty parental lines (Table 2). Amplification reactions were in the volumes of 20 μ l containing 10mM Tris HCl (pH8.3), 50mM KCl, 1.5mM $MgCl_2$, 0.001% gelatin, dATP, dCTP, dGTP and dTTPs each at 0.1mM, 0.2mM primes, 25-30 ng of genomic DNA mol 0.5 unit of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore). Amplification was performed with thermal controller (MJ Research Inc.) programmed for 40 cycles. After initial denaturation for two minutes at 94ºC, each cycle consisted of one minute at 94ºC, one minute at 36ºC and two minutes at 72ºC. The 40 cycles

Table 2. List of random primers used in the genetic diversity analysis of saline rice cultivars.

SI. No.	Primers name	Nucleotide sequence 5'to 3'
1	OPBA – 01	5' – TTCCCCACCC – 3'
2	OPBA – 02	5' – TGCTCGGCTC – 3'
3	OPBA – 03	5' – GTGCGAGAAC – 3'
4	OPBA – 04	5' – TCCTAGGCTC- 3'
5	OPBA – 05	5' – TGCGTTCCAC- 3'
6	OPBA – 06	5' – GGACGACCGT – 3'
7	OPBA – 07	5' – GGGTCGCATC – 3'
8	OPBA – 08	5' – CCACAGCCGA – 3'
9	OPBA – 09	5' – GGAACTCCAC – 3'
10	OPBC – 10	5' – AACGTCGAGG – 3'
11	OPBA – 11	5' – CCACCTTCAG – 3'
12	OPAX – 06	5' – ACACTCGGCA – 3'
13	OPAK – 10	5' – CAAGCGTCAC – 3'
14	OPAY – 18	5' – AACTTGGCCC – 3'
15	OPBA – 15	5' – GAAGACCTGG – 3'
16	OPBA –16	5' – CCACGCATCA – 3'
17	OPBA – 17	5' – TGTACCCCTG – 3'
18	OPBA – 18	5' – CTCGGATGTC – 3'
19	OPBA – 19	5' – CCATCCGTTG – 3'
20	OPBA – 20	5' – GAGCGCTACC – 3'

were followed by seven minutes of final extension at 72ºC. PCR amplified products were subjected to elctrophoresis on 1.5% agarose gel in 1XTBE buffer at 120V for 4 hrs using Hoefer Super Submarine electrophoresis unit (Pharmacia Biotech, USA). The electronic images of ethidium bromide stained gels were captured using Kodak digital science DC 120 digital camera (Rochester, USA) and gels were documented using electrophoresis Documentation and Analysis System (EDAS 120).

All the accessions were subjected to RAPD analysis. The DNA's were isolated by adopting cTAB method and then amplified using PCR. The amplified products were then loaded under gel electrophoresis. The amplified regions were visualized by means of adding suitable dye under trans-illuminator and documentated through gel documentation system. The data generated from the polymorphic fragments were scored as present (1) or absent (0) for each of the twenty accessions. In order to detect whether genetic diversity of the rice accessions is evenly distributed, principal component analysis was performed based on the data matrix from the DNA amplification products. The diversity among the lines was worked out by subjecting the RAPD scores to cluster analysis. Sequential Agglomerative Hierarchic Non-overlapping (SAHN) clustering was performed on simple matching similarity matrix by Unweighed Pair Group Method with Arithmetic Averages (UPGMA). The data analysis was done using the software NTSYS pc version 2.02 (Rohlf, 1998).

RESULTS AND DISCUSSION

Two hundred seven bands were detected of which 22.71% bands were monomorphic across all the cultivars studied. The remaining 160 bands (77.29%) were polymorphic with the average number ranging from 3 to 14 per primer with a mean of 8.0, which indicated the high level of polymorphism expressed by arbitrary primers (Table.3). The primers OPBC-10 produced maximum number of fragments (14) followed by OPBA-03 and OPAX-06 each with 13 and 12 fragments, respectively. The primer OPBA-08 recorded minimum number of fragments (3). The analysis of genetic distance between pair wise individuals revealed a considerable genetic diversity of the rice accessions, presenting a clustered distribution pattern. These

Table 3. The polymorphism obtained with random primers among all the cultivars of rice

Sl. No.	Primer	Total bands	Polymorphic bands	Polymorphism (%)
1.	OPBA-01	10	7	70.0
2.	OPBA-02	9	6	66.6
3.	OPBA-03	13	13	100.0
4.	OPBA-04	9	6	66.6
5.	OPBA-05	10	7	70.0
6.	OPBA-06	11	10	90.9
7.	OPBA-07	9	6	66.6
8.	OPBA-08	6	3	50.0
9.	OPBA-09	10	6	60.0
10.	OPBC-10	14	14	100.0
11.	OPBA-11	10	6	60.0
12.	OPX6-12	13	12	91.6
13.	OPAK-10	12	11	91.6
14.	OPAY-18	12	10	83.3
15.	OPBA-15	8	5	62.5
16.	OPBA-16	10	7	70.0
17.	OPBA-17	9	6	66.6
18.	OPBA-18	12	11	91.6
19.	OPBA-19	10	7	70.0
20.	OPBA-20	10	7	70.0
	Total	207	160	-
	Mean	10.35	8.0	74.8

scattered plots, based on the first two principal co-ordinates accounting for the total variation, exhibited relatively significant differentiation between the accessions (Fig.1). Group I, group II and group III were mainly clustered rice accessions. The two accessions, IR 61920-3B-22-2-1 and HP 3319-2WH-6-3-1-1-3 (0.26), showed a distant genetic relationship with other accessions, indicating sexual hybridization of rice accession either within the same type or between different types may possibly cause desirable heterotic effects. Three accessions namely HP 3319-2WH-6-3-1-1-3 from Himachal Pradesh, India, IR 61920-3B-22-2-1 from IRRI, Philippines and TRY 1 from Tamil Nadu, India showed different genetic patterns. The Indian accession (accession 8) was grouped together with those accession-sharing genes with the IRRI accessions.

The sample matching similarity was calculated using RAPD score and dendrogram was constructed using unweighed pair group method with arithmetic averages (UPGMA) employing sequential, agglomerative hierarchic and non-overlapping clustering (SAHN). In UPGMA tree, high genetic diversity was

Table 4 . Similarity coefficient among 20saline rice accessions based on RAPD analysis.

Accessions	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20
G1	1.000																			
G2	0.631	1.000																		
G3	0.736	0.789	1.000																	
G4	0.578	0.736	0.842	1.000																
G5	0.631	0.789	0.894	0.947	1.000															
G6	0.631	0.789	0.684	0.736	0.789	1.000														
G7	0.473	0.736	0.736	0.894	0.842	0.736	1.000													
G8	0.578	0.736	0.736	0.684	0.736	0.736	0.684	1.000												
G9	0.421	0.789	0.684	0.631	0.684	0.684	0.631	0.736	1.000											
G10	0.263	0.631	0.526	0.578	0.526	0.526	0.578	0.578	0.842	1.000										
G11	0.421	0.789	0.684	0.631	0.684	0.684	0.631	0.736	0.894	0.842	1.000									
G12	0.368	0.736	0.631	0.684	0.736	0.736	0.684	0.684	0.842	0.684	0.842	1.000								
G13	0.368	0.736	0.631	0.578	0.631	0.631	0.578	0.684	0.947	0.789	0.842	0.894	1.000							
G14	0.368	0.736	0.631	0.578	0.631	0.631	0.578	0.684	0.947	0.789	0.842	0.894	1.000	1.000						
G15	0.421	0.684	0.578	0.526	0.578	0.578	0.526	0.631	0.894	0.736	0.789	0.842	0.947	0.947	1.000					
G16	0.421	0.789	0.684	0.631	0.684	0.684	0.631	0.736	0.894	0.736	0.894	0.947	0.947	0.947	0.894	1.000				
G17	0.473	0.736	0.631	0.578	0.631	0.526	0.578	0.578	0.842	0.684	0.736	0.789	0.894	0.894	0.947	0.842	1.000			
G18	0.578	0.736	0.736	0.789	0.736	0.736	0.789	0.789	0.736	0.684	0.736	0.684	0.684	0.684	0.736	0.736	0.684	1.000		
G19	0.578	0.736	0.736	0.684	0.736	0.736	0.684	0.894	0.736	0.578	0.736	0.684	0.684	0.684	0.736	0.736	0.684	0.894	1.000	
G20	0.526	0.789	0.789	0.631	0.684	0.684	0.631	0.736	0.789	0.736	0.894	0.736	0.736	0.736	0.684	0.789	0.631	0.736	0.736	1.000

G1=HP 3319-2WH-6-3-1-1-3, G2=IR47547-3B-26-2B-1, G3=IR 6199-313-24-3, G4=IR 5931-110-1, G5=IR 6192-3B-15-2-2, G6=IR 28, G7=IR 29, G8=PY 5, G9=IR 63311-B-3R-B-24-3, G10=IR 61920-3B-22-2-1, G11=POKKALI, G12=IR 36, G13=IR 42, G14=IR 61920-3B-22-2-1, G15=IR 71657-5R-B-129, G16=IR 7500-59-1-1-B, G17=ADT 38, G18=ADT 45, G19=CR 1009, G20-TRY 1

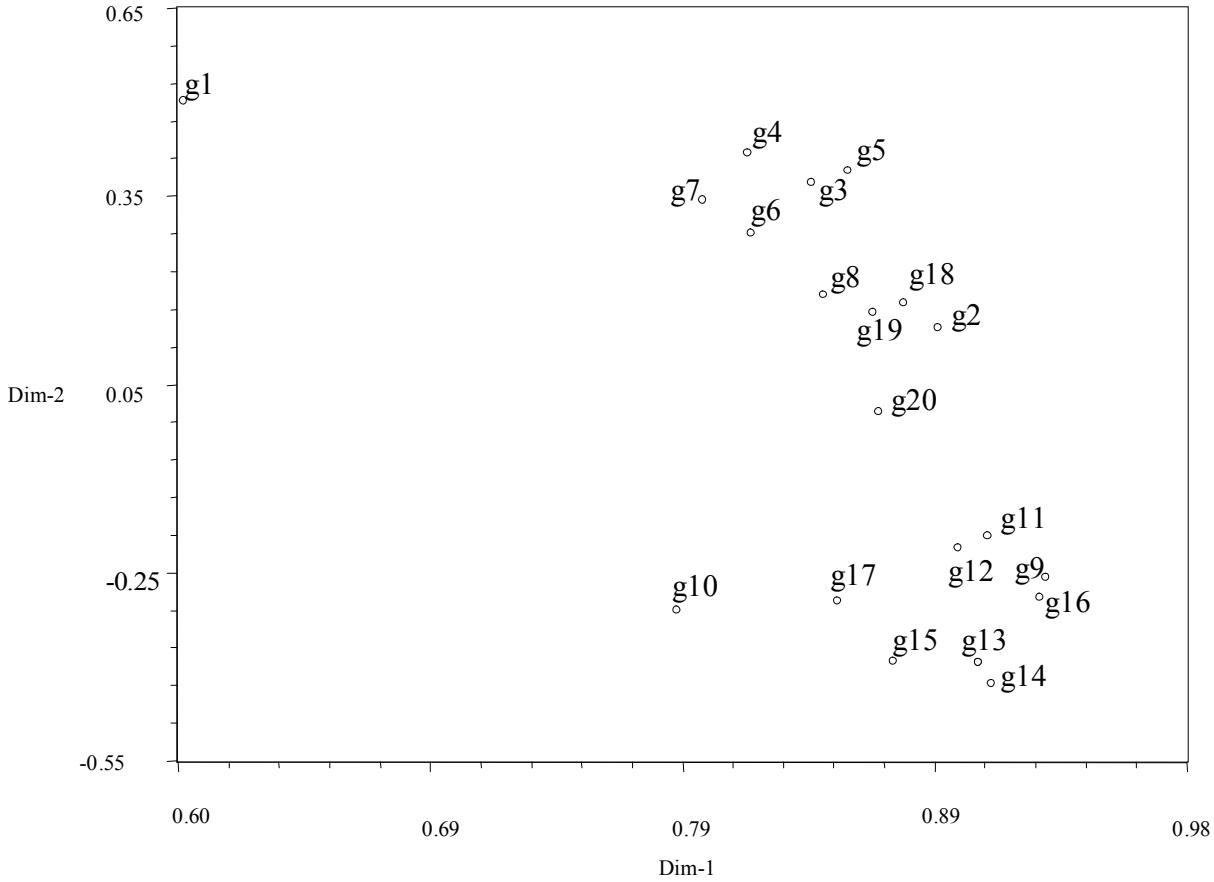

Fig. 1. PCA plot of the first two principal coordinates of PCA based on RAPD (Number at each dot refers to the corresponding genotype in Table 1.

revealed in all the rice accessions with the Jaccard similarity coefficient values varying from 0.45 to 1.00. The accessions were grouped into four clusters at 0.75 similarity co-efficient level (Fig.2).

Cluster I consisted of one accession i.e. accession 1, indicating different genetic pattern from other accessions. Cluster II had six accessions of IRRI. Only three accessions (accession 18, 8 and 19) were grouped in cluster III. Cluster IV included 10 accessions mainly from the IRRI accessions. In addition, all the four clusters in dendrogram did not show any strict relationship with geographical distribution, morphotypic classification, though some accessions exhibited their genetic differentiations among all the accessions. The similarity co-efficient was maximum between accession IR 61920-3B-22-2-1 and accession IR 42 (1.00). While, minimum similarity was observed between accession IR 61920-3B-22-2-1 and accession HP 3319-2WH-6-3-1-1-3 (0.263) (Table 4). Molecular marker analysis of the twenty accessions using 20 RAPD primers produced polymorphism for most of the loci studied. As per the similarity index, the accessions were grouped into 4 clusters. Crossing between the accessions with low similarity co-efficient will manifest high heterosis.

Our RAPD survey of 20 saline rice accessions indicated a high level of genetic diversity with 74.8% of bands being polymorphic. The high genetic diversity of saline rice accessions may be attributed to two reasons. firstly, the geographic isolation and genetic variation of saline rice accession, though this result did not show strict geographic relationship among all accessions. Secondly, the sexual hybridization between two accessions also can develop special cultivar or breeding lines. Although attempts were made to disclose the relationship between morphophyte and molecular markers, the result was not satisfactory, which may be

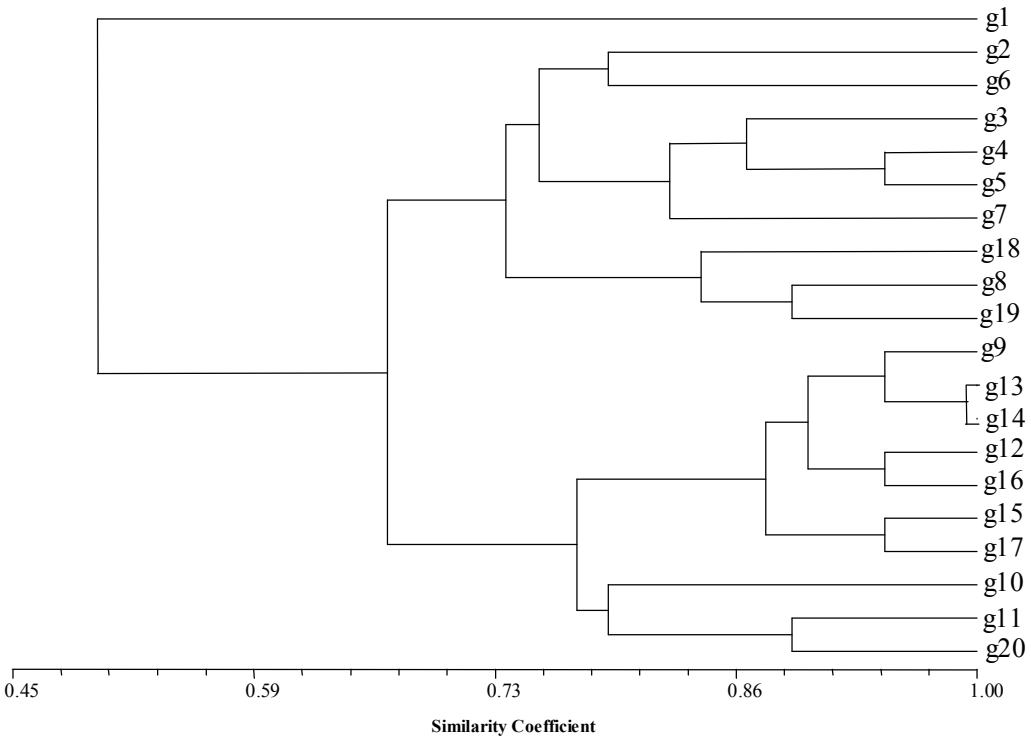

Fig. 2. Dendrogram based on genetic similarities among 20 saline accessions of rice.

attributed to the dominant RAPD markers and the few variations of morphological characteristics, that are controlled by quantitative genes and influenced by environment.

Genetic diversity of all the accessions was obviously lower than that of total accessions, indicating some loss of genetic diversity during domestication. The preliminary study provides a much-needed knowledge for future basic and applied research efforts related to saline tolerant rice.

REFERENCES

Ayana A, Bekele AE and Bryngelsson T. 2000. Genetic variation in wild sorghum (*Sorghum bicolor* ssp. Verticilliflorum Moench) germplasm from Ethiopia assessed by random amplified polymorphic DNA (RAPD). Hereditas 132:249-254.

Bolaric S, Barth S, Melchinger AE and Posselt UK. 2005. Genetic diversity in European perennial ryegrass cultivars investigated with RAPD markers. Plant breed. 124: 161-166.

IRRI. 1998. Program Report for 1997. International Rice Research, Los Banos. Philippines.

Lynch M and Milligan BG. 1994. Analysis of population genetic structure with RAPD markers. Mol Ecol 3:91-99.

Mackil DJ. 1995. Classifying japonica rice cultivars with RAPD markers. Crop Sci. 35: 889-894.

Mahalanobis PC. 1936. On the generalized distance in statistic. Proc. Nat. Inst. Sci., 24: 49-55.

Rohlf FJ. 1998. NTSYS-PC numerical taxonomy and multivariate analysis system. Exeter software, Applied Biostatistics Inc., New York.

Williams JGK, Kubelik AP, Livak KJ and Tingey SV. 1990. DNA polymorphism amplified by arbitrary primers as useful as genetic markers. Nucl. Acids Res. 18: 6531-6535.