Oryza Vol. 48 No. 3, 2011 (206-210)

Studies on DNA polymorphism and genetic diversity among rainfed lowland rice genotypes

Elsa Marric* and J N Reddy

Ravenshaw University, Cuttack, Odisha

ABSTRACT

In the present study, 38 rainfed lowland rice genotypes differing in their submergence tolerance potential were used to study the DNA polymorphism and genetic diversity among these genotypes with the help of eleven RAPD markers employing PCR (polymerase chain reaction) based methods to identify divergent parents and generate mapping populations for tagging submergence tolerance loci in tolerant varieties using molecular markers in a long run.

Key words: *rice, submergence tolerance, DNA polymorphism, RAPD*

Rice is grown under a variety of conditions. Under rainfed lowlands, submergence is one of the most important abiotic stresses besides drought, that affects rice yield. Use of submergence tolerant cultivars is one of the ways for minimizing the losses caused by it. The development and application of restriction fragment length polymorphism (RFLP) technology in crop improvement were both reviewed by Tanksley *et al.* (1989). Williams *et al*. (1990) established a DNA polymorphism assay based on PCR amplification of random DNA fragments with single primers of arbitrary nucleotide sequence. Randomly Amplified Polymorphic DNA (RAPD) marker technology offers advantages in speed, technical simplicity, random coverage of genome and relatively higher level of polymorphism (Newbury *et al.*, 1993). RAPD markers are randomly scattered throughout the genome, which had made them particularly suitable to analyze population that would cover the genome to a greater extent (Tanksley *et al.,* 1989). A number of studies indicated PCR amplifications using arbitrary primer to be useful in genotype identification, population and pedigree analysis, phylogenetic studies and genetic mapping. (Welsh and Mcllelland, 1990; Vierling and Nguyen, 1992; Yu and Nguyen, 1994; Wang *et al*., 1996; Raghunathachari *et al*., 2000; Rajani *et al*. 2010).

In the present study, eleven RAPD markers were used to detect DNA polymorphism and genetic diversity employing PCR (polymerase chain reaction) based methods among the 38 rainfed lowland rice genotypes including land races, released varieties and improved cultures, those differ in submergence tolerance with the objective to identify diverse parental lines and also to generate segregating populations for tagging submergence tolerance loci in rice using molecular markers.

MATERIALS AND METHODS

Thirty eight rainfed lowland rice genotypes (Table 1) collected from the Crop Improvement Division of Central Rice Research Institute, Cuttack were grown in pots under green house conditions during the wet season, 2004 following standard agronomic practices. Total DNA was extracted from the leaves of 21 day old seedlings. A simple mini-prep method for DNA isolation (Zheng *et al*., 1995) was used, which yields about 1µg DNA from 2cm of rice leaf (Huang *et al*., 1997). DNA amplification reaction was performed in a volume of 25µl containing 10mM Tris HCL (pH 8.3), 50mM KCL, 2mM $MgCl_2$, 50mM each of dATP, dCTP, dTTP, dGTP, 10 ng of a single random primer (list of primers is given in Table 2), 1 unit of Taq Polymerase and 25ng of genomic DNA, overlaid with a drop of mineral oil. Amplification was performed in a Programmable Thermal Cycler (PTC-100, MJ Research Inc.). The reaction mixture was first

Table 1. List of rice varieties used for RAPD analysis

Sl No.	Variety	Reaction to submergence
1	Savitri	Susceptible
2	Gayatri	Susceptible
3	Pooja	Susceptible
4	Moti	Susceptible
5	Raghukunwar	Tolerant
6	Gangasiuli	Tolerant
7	Kusuma	Tolerant
8	Khoda	Tolerant
9	Khadara	Tolerant
10	LPR 116	Moderately tolerant
11	TCA 95-109-4-2-7-B	Moderately tolerant
12	RAU 95-1-1-B	Moderately tolerant
13	Boithalpakhia	Tolerant
14	Ravana	Tolerant
15	Hanseswari	Moderately tolerant
16	Matiaburush	Tolerant
17	Varshadhan	Moderately tolerant
18	TTB 202-3	Moderately tolerant
19	Kalaputia	Tolerant
20	Mayurkhanta	Tolerant
21	Nalimachakhanta	Tolerant
22	Doodh Khajara	Tolerant
23	Samba Mahsuri	Susceptible
24	Vijeta	Susceptible
25	Durga	Susceptible
26	Utkalprabha	Susceptible
27	Swarna	Susceptible
28	CR 673-475	Susceptible
29	IR 38784-15-19	Moderately tolerant
30	Ramchandi	Susceptible
31	Madhukar	Moderately tolerant
32	Sudhir	Moderately tolerant
33	FR 13A	Tolerant
34	Purnendu	Moderately tolerant
35	Sarala	Susceptible
36	IR 42	Susceptible
37	Atiranga	Tolerant
38	Makhara	Tolerant

denatured for 2 minutes at 92^{0}C and then subjected to 41 cycles of 1 minute denaturation at 92^{0}C, 1 min annealing at 37^{0}C, and 2 minute extension at 72^{0}C; and then a final extension for 5 minutes at 72^{0}C. Aliquots of 10µl of DNA products from PCR amplification were loaded in 1.5% agarose gel for electrophoresis in 0.5X TBE (pH 8.0)). At least one lane was loaded with 1 Kb Plus DNA ladder (from Gibco BRL). The gel was run at 75 volts (3V/cm) for 5 hrs. The gel was then stained with Ethidium Bromide (1µg/ml working solution) and photographed using a Gel Documentation System (Vilber Leurmat, France) equipped with Bio Capt Software.

Table 2. The random sequence decamer oligonucleotide primers (Operon Technologies, Inc.) used in this study and no. of polymorphic bands detected in 38 rainfed lowland rice genotypes

Sl. No.	Designation	5'-3' Sequence	RAPD markers detected	No. of polymorphic bands
1	OPD- 08	GTG TGC CCC A	8	8
2	OPE- 09	CTT CAC CCG A	6	5
3	OPW-19	TGG CAA GGC A	4	3
4	OPC- 20	ACT TCG CCA C	16	14
5	OPB- 11	GTG GAC CCG T	12	11
6	OPX- 11	GGA GCC TCA G	11	7
7	OPY- 17	GAC GTG GTG A	14	12
8	OPG- 03	GAG CCC TCC A	8	8
9	OPT- 17	CCA ACG TCG T	14	14
10	OPW-07	CTG GAC GTC A	3	1
11	OPW-15	ACA CCG GAA C	9	6

Data were scored for computer analysis on the basis of the presence or absence of the amplified products for each genotype-primer combination. The data entry was done into a binary data matrix as discrete variables. Data were analyzed and similarity matrix was constructed from binary data with Jaccard's coefficients and dendrogram was generated with unweighted pair group method arithmatic average (UPGMA) algorithm, using NTSYSpc 2.1 software (Rohlf, 2000).

RESULTS AND DISCUSSION

All the primers used in the present study could be amplified in all the varieties and all the genotypes exhibited unique fingerprinting pattern. In all, there were 106 bands amplified, out of which 90 were polymorphic (Table 2) which accounted for 84.91% of total number of bands. Singh *et al*. (2003) and Bhuyan *et al* (2007) also reported high percentage of polymorphic bands in

different varieties of lowland rice. Among the different primers used in the present study, two primers OPD-08 and OPT- 17 could give highest polymorphism among the 38 genotypes. Primer OPC-20, OPG- 03, OPB-11, OPY- 17, OPE- 09, OPX- 11, and OPW-15 showed good amplification and polymorphism. Specific bands were found in some tolerant cultivars (Table 3).

Primer OPD-08 could yield a total of 9 bands with the size varying from 1.1kb to 0.3kb. The number of bands varied in between 1 to 9, but most of the genotypes showed 4-6 bands. Only two bands having a size of 0.48kb and 0.30kb were present in the genotype Kalaputia. One band having the size of 0.40kb was found only in submergence tolerant varieties viz. Raghukunwar, Boithalpakhia, Matiaburush and FR 13A. With primer OPE-09, two bands with a size of 1.0kb and 0.8kb were found only in two tolerant varieties such as Nalimachakhanta and Doodh Khajara. With primer OPC- 20, Swarna, a high yielding variety showed two specific bands of 3.1 and 0.15 kb, whereas local submergence tolerant variety Khadara showed a specific band of 1.95kb size.

With primer OPB- 11, one band with 1.68kb size was found only in tolerant variety Kusuma and one susceptible variety Vijeta. The variety Moti showed only two bands, whereas CR 673-475 produced three bands. With the primer OPY-17, the number of bands varied between 6 and 10 in most of the varieties (Fig 1), the size of bands ranged from 1.1kb to 0.28kb, but cultivar Kalaputia had given only 3 bands. One specific band having the size of 0.28kb was found in 9 tolerant varieties and three moderately tolerant varieties. This band was absent in all the susceptible varieties.

Table 3. Amplification of specific DNA fragments in different genotypes

Primers	Size of DNA Fragments(in Kb)	Varieties
OPD-08	0.4	Raghukunwar, Boithalpakhia, Matiaburush and FR 13A
OPE-09	0.8	Nalimachakhanta and Doodh Khajara
	1.0	Nalimachakhanta and Doodh Khajara
OPC-20	1.95	Khadara
OPY-17	0.28	Raghukunwar, FR 13A, Nalimachakhanta, Doodh Khajara, Kusuma, Khadara, Ravana, Mayurkhanta, Makhara
	0.47	Gangasiuli, Makhara
OPT-17	0.625	Kusuma, Khadara, Boithalpakhia, Kalaputia, Nalimachakhanta, Doodh Khajara and FR-13A
	1.65	FR13A
OPW-15	0.5	Nalimachakhanta, Doodh Khajara and Atiranga

The primer OPT-17 could yield in good polymorphism with 14 polymorphic bands (Fig 2). Four to ten bands were found in each variety ranging from 1.65kb to 0.15kb size. A band of 1.65kb size was found only in tolerant variety FR13A. The high yielding variety Purnendu showed a specific band of 0.925kb size, which was not found in other genotypes. A distinct band of 0.625kb was found only in seven tolerant varieties i.e. Kusuma, Khadara, Boithalpakhia, Kalaputia, Nalimachakhanta, Doodh Khajara and FR-13A. With

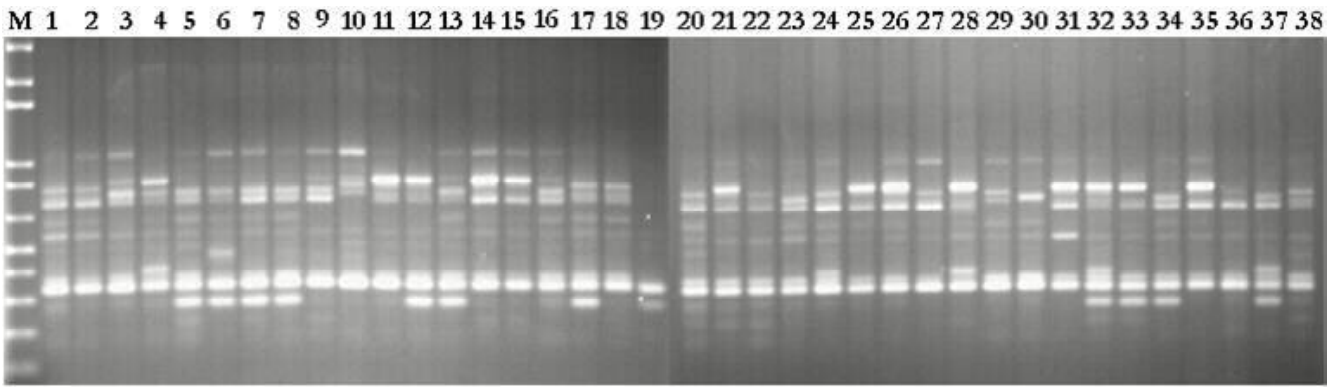

Fig. 1. Amplification products obtained with primer OPY 17, resolved by a 1.5% agarose gel. The numbers on top refer to the rice genotypes listed in Table 1.

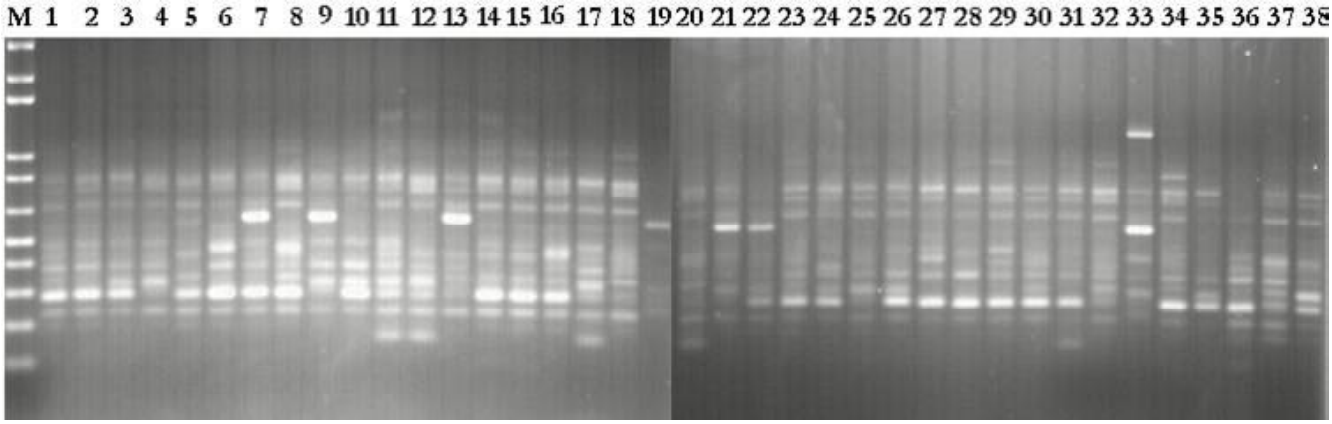

Fig. 2. Amplification products obtained with primer OPT 17, resolved by a 1.5% agarose gel. The numbers on top refer to the rice genotypes listed in Table 1.

primer OPW-15, one specific band with a size of 0.5kb was found only in three tolerant varieties viz., Nalimachakhanta, Doodh Khajara and Atiranga.

The dendrogram showed two major clusters, one containing a highly tolerant variety Kalaputia and a susceptible variety IR42 and other one containing all other varieties. At 80.2% similarity ten clusters were found, of which two were large clusters and eight were small clusters. Among the small clusters the three tolerant varieties Kalaputia, Mayurkhanta, and Atiranga, three susceptible varieties IR42, Swarna, and Utkalprava, and one moderately tolerant variety Sudhir singly formed independent clusters. Where as, two tolerant varieties Nalimachakhanta and Doodh Khajara grouped together into one cluster. Among the larger clusters one cluster contained four varieties viz., Ramachandi, Purnendu, FR13A and Makhara. The other cluster was the largest one containing the remaining 25 varieties. This cluster was divided into two subclusters, one containing varieties Sarala, IR 38784-15-19 and TTB-202-3, and another cluster was further subdivided. At 86.33% similarity the tolerant varieties viz., Gangasiuli, Khoda, Kusuma, Boithalpakhia, Matiaburush, Ravana and one moderately tolerant variety Hanseswari were grouped together into a single cluster.

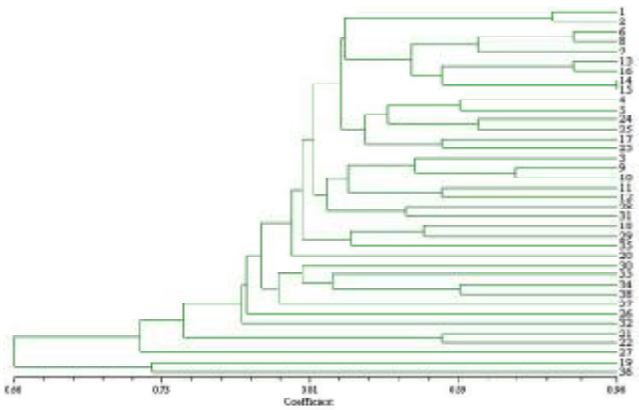

Fig. 1. Dendrogram of 38 rice genotypes screened with eleven primers.

Two varieties Ravana and Hanseswari were the closest varieties among all 38 varieties, where local tolerant variety Kalaputia and susceptible variety IR42 were most divergent from all other varieties. The cluster analysis also indicated that most of the tolerant varieties are closely related to each other, while the moderately tolerant varieties had shown relatedness with both the tolerant and susceptible varieties. This may give some indication about the changes at genetic level during the evolution of submergence tolerant varieties. But this needs further confirmation with large number of primers as well as varieties.

From the above results it can be concluded that the genetic materials used in the present study are highly variable. Though, the present study on 38 rainfed lowland rice genotypes including land races, released varieties and improved cultures, which differ in submergence tolerance with 11 RAPD markers gave some indications about the association of specific bands to some of the submergence tolerant genotypes like Kalaputia, Khadara, FR-13A, Doodh Khajara,

Nalimachakhanta, Boithalpakhia, Kusuma, etc., it needs further confirmation and screening with a large number of primers to map the submergence tolerant genes in these tolerant genotypes in the long run.

REFERENCES

Bhuyan N, Borah BK and Sarma RN 2007. Genetic diversity analysis in traditional lowland rice (Oryza sativa L.) of Assam using RAPD and ISSR markers. Curr. Sci. 93 (7): 967-972.

Huang N, Angeles ER, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadivel N, Bennett J and Khush GS 1997. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor. Appl. Genet. 95: 313-320.

Newbury JH, Ford Lloyd BV 1993. The use of RAPD for assessing variation in plants. Plant growth regulation, 12: 43-51.

Raghunathachari P, Khanna VK, Singh US and Singh NK 2000. RAPD analysis of genetic variability in Indian scented rice germplasm (Oryza sativa L.). Current Sci., 79: 994.

Rajani R, Das S, Choudhury PR and Mandal AB 2010. Genotyping of a mapping population derived from a salt susceptible and salt tolerant varieties (IR48 x Pokkali) of rice. Ind J. Biotech. 9: 147-152.

Rohlf FJ 2000. NTSYSpc- Numerical Taxonomy and Multivariate Analysis System, version 2.1. Applied Biostatistics Inc., New York, USA.

Singh S, Sarkarung S, Singh RK, Singh ON, Singh AK, Singh VP, Bhandari HS, Xu W and.Li Z 2003. Genetic diversity in rain fed lowland rice genotypes as detected by RAPD primers. Advances in Rice Genetics, P 200-202

Tanksley S D, Young ND, Paterson AH and Bonierbale MW 1989. RFLP Mapping in plant breeding: New tools for old science. Bio-technology 7: 257-264.

Vierling R and Nguyen HT 1992. Use of RAPD markers to determine the genetic relationships of diploid wheat genotypes. Theor. Appl. Genet., 84: 835-838.

Wang Z, Chien H, Yue P, Wandf X and Zhu L 1996. Polymorphism of Chinese common wild rice(O. rufipogon) and cultivated rice (Oryza sativa L.) as determined by RAPDs. J. Genet. & Breed. 50: 299-307.

Welsh T and Mcllelland M 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Res., 18: 7213-7218.

Williams JG, Kubelik AR, Livak KJ, Rafalski JA and Tungery SV 1990. DNA polymorphisms amplified arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Yu LX and Nguyen HT 1994. Genetic variation detected with RAPD markers among upland and lowland rice cultivars (Oryza sativa L.) Theor. Appl. Genet. 87: 668-672.

Zheng K, Huang N, Bennett J and Khush GS 1995. PCR-based marker-assisted selection in rice breeding. IRRI Discussion Paper, Intern. Rice Res. Inst., Philippines, 12: 24.