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GENETIC BASIS OF YIELD VARIATION IN SHALLOW LOWLAND RICE THROUGH RAPD ANALYSIS

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Abstract

The study was undertaken to assess the genetic diversity among shallow lowland rice genotypes using RAPD markers. Twenty five shallow lowland rice germplasm was selected for genetic diversity analysis using five RAPD markers. With an objective to evaluate the genetic variability, character association, genetic advance in shallow lowland rice collected from EIRLSBN project of IRRI, the experiment was laid out at Rice Research Station, O.U.A.T., Bhubaneswar during 2013 *kharif* season. A total of 32 bands were detected ranging in size from 200 bp to 2000 bp among the 25 genotypes. The number of bands produced with primer, OPC-20 is 8, OPD-11 is 4, OPM-3 is 6, OPK-1 is 6 and OPN-11 is 8 with a mean of 6.4 bands per primer. Of a total 32 RAPD bands, 17 (53.12%) were found to be polymorphic. The maximum number of polymorphic bands (8) was obtained using OPC-20 and OPN- 11 primer where as the minimum number of polymorphic bands (4) was obtained in Primer OPD-11. The cultivar pairs 'OR 2330-1' and 'OR 2397-1' showed the maximum similarity (1.00) among the 25 genotypes, whereas, 'NDR 8012' and 'Swarna 'as well as 'OR 2329-34' and 'Upahar', showed the least pair-wise similarity (0.69) in case of pooled data. The result of genetic diversity will be useful for the selection of parents for developing submergence tolerant and flash flood tolerant rice variety through molecular breeding program.

Key words : Shallow lowland rice, RAPD marker, genetic diversity.

Introduction

Rice (*Oryza sativa* L.) is the staple food for more than 70 per cent of Indians. Shallow lowland rice is grown in flash flooded conditions with water 0-30 cm deep. Many regions of India are flooded during rice cultivation season every year results in decrease in yield potential. The rice environments in India are extremely diverse. Of the 44.2 million ha of harvested area, about 30.8% are rainfed lowland, 44.9% irrigated, 17.4% rainfed uplands & 6.9% flood prone which profoundly influence the overall rice productivity of the country. Therefore, it is the time to select potential rice cultivars which shows good result in flash flood condition. Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationship within and among species. Random amplified polymorphic DNA is one of the most commonly used molecular marker technique. RAPD molecular marker technique is PCR based and cost effective technique. Single short oligonucleotide primer is arbitrarily selected to amplify a set of DNA segments distributed randomly throughout the genome. Compared to other markers, it is dominant and highly reproducible.

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Materials and Methods

The experiment was laid out at Rice Research Station, O.U.A.T., Bhubaneswar during 2013 *kharif* season. For planting material, seeds of twenty five rice genotypes was collected from EIRLSBN project of IRRI. Seeds were germinated at aseptic condition by incubating them at 30 degree Celsius grown in green house. Genomic DNA was isolated from young leaves of 21 days old plants. Extraction of total genomic DNA was carried out and the DNA samples were evaluated using spectrophotometer. PCR amplification of RAPD primer was carried out using five primers. The PCR mixture consisted of *Taq* DNA polymerase, PCR buffer, dNTPs, $MgCl_2$, oligonucleotide primers and genomic DNA. Optimization of concentration of PCR components was carried out for $MgCl_2$, *Taq* DNA polymerase, and genomic DNA concentration. To determine optimal amplification reaction conditions, a factorial experiment

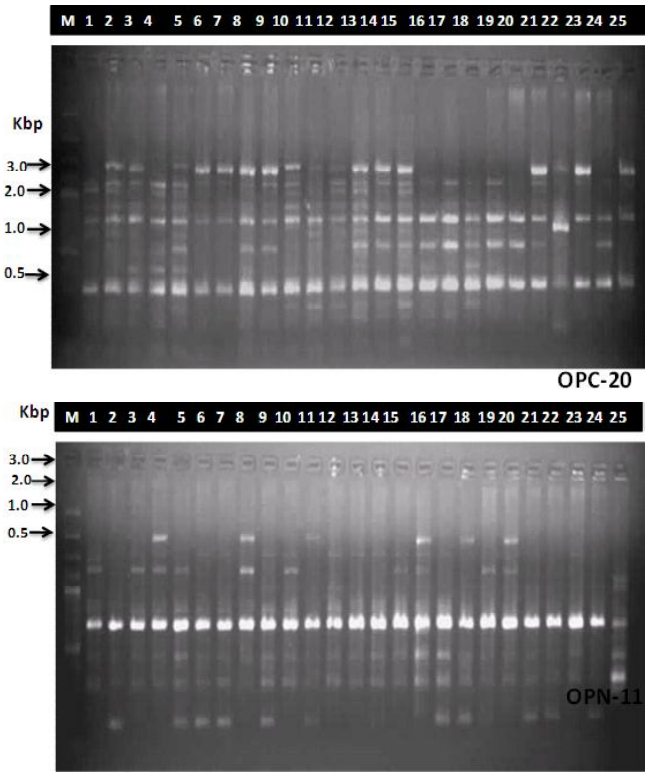

Fig. 1 : RAPD profile of 25 rice genotypes generated by the primer OPC-20 (5'-ACTTCGCCAC-3') and Primer OPN-11 (5'-TCGCCGCAAA-3').

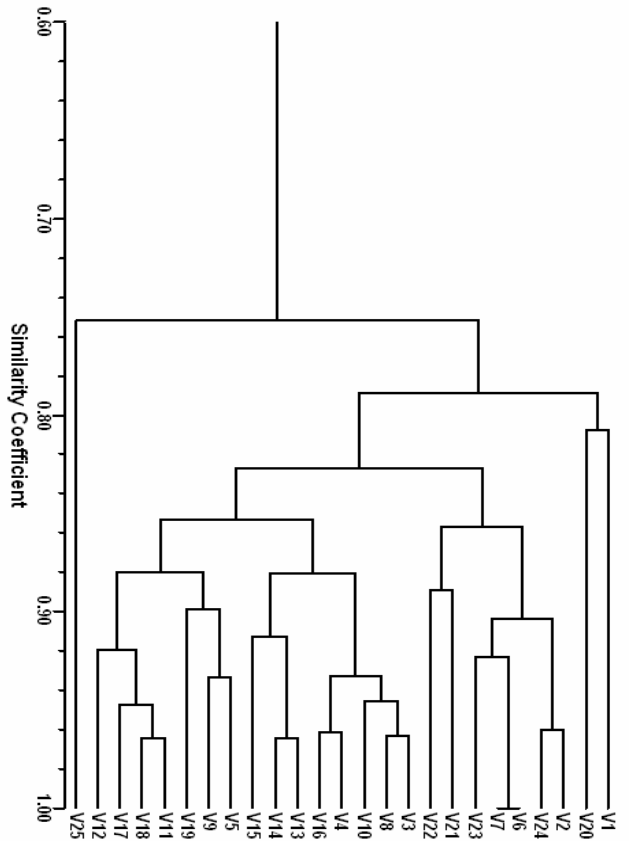

Fig. 3 : Dendrogram depicting genetic relationship among 25 shallow lowland rice genotypes based on RAPD profile.

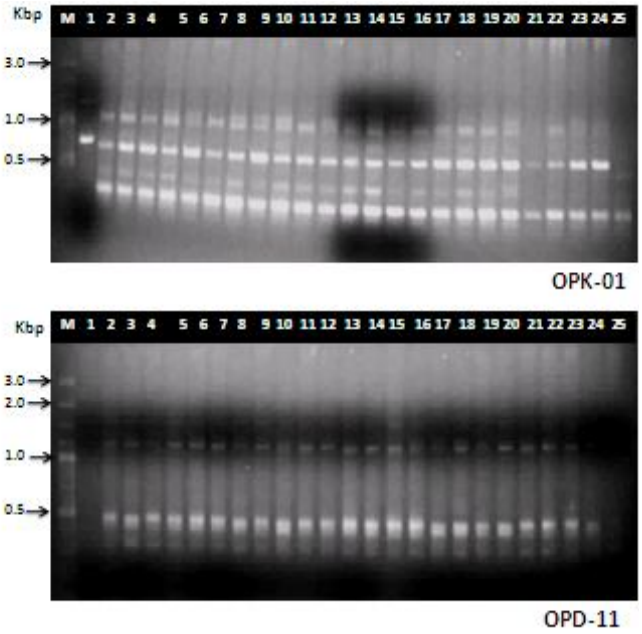

Fig. 2 : RAPD profile of 25 rice genotypes generated by the primer OPK-01 (5'-ACCTGGACAC-3') and Primer OPD-11 (5'-AGCGCCATTG-3').

was carried out at three concentrations of $MgCl_2$ (2.0 mM, 2.5 mM and 3.0 mM), three concentrations of *Taq* DNA polymerase (0.5 U, 1.0 U, 1.5 U), three concentrations of template DNA (10 ng, 25 ng, 50 ng) and 10 pmole primer in a volume of 25 µl. PCR was carried out using Thermal Cycler (Bio-Rad, USA), PCR conditions that gave better amplified DNA profile .were determined for RAPD analysis, all PCR were carried out in a final volume of 25 ml reaction mixture containing 50 ng template DNA, 200 µM each dNTPs, 2 mM $MgCl_2$, 10 pmoles primer, 1X *Taq* polymerase buffer and 1 unit of *Taq* DNA polymerase. Jaccard's similarity coefficient was calculated according to Jaccard (1908) as follows:

$$\text{Jaccard's similarity Coefficient} = \frac{n_{xy}}{n_1 - n_2}$$

n_{xy} = Number of bands common in sample a and b.

n_1 = Total number of bands present in all samples.

n_2 = Number of bands not present in sample a or b but found in other samples.

The similarity matrix was subjected to generate a dendrogram using software programme NTSYS pc Ver 2.1. Exeter Software, N.Y. (Rohlf, 2005).

Results and Discussion

A total of 32 bands were detected ranging in size from 200 bp to 2000 bp among the 25 genotypes. The number of bands produced with primer, OPC-20 is 8, OPD-11 is 4, OPM-3 is 6, OPK-1 is 6 and OPN-11 is 8

Table 1 : Details of rice germplasm for 25 lowland rice genotypes used in the study.

S. no.	Designation	Parentage
1.	NDR 8012	BKP 246/SABITA//CN 846-6-6
2.	NDR 8022	IR 49830-7-1-2-3/SABITA//IR 69502-6-SRN-3-UBN-1-3-1-3-2
3.	NDR 9452	BG90-2/IR 67962-84-2-2-2
4.	OR 2316-1	OR 1301-32/IR 65515-65
5.	OR 2329-34	OR 1530 -8/IR 68181-8-49
6.	OR 2330-1	OR 1530-1/NDR 8003
7.	OR 2397-1	OR 1530-1/IR 61040
8.	OR 2407-10	Indrawati/IR 62181-8-49
9.	CR 2565-278	Savitri/Naveen
10.	CN 1881	Sabita/Swarna
11.	RAU 1407-7-1-3-3	Sathi X Turanta
12.	TTB 283-3-38-2	Akisali/ Kushal
13.	IR 73727-20-R-TTB-1-21	RAJSHREE/IR 66879-19-1-B-5//BKP 246
14.	LPR 1123	SB 179/Ranjit
15.	LPR 1126	Panjasali/Ranjit
16.	IR 85041-SUB-18-3-2-1	IR 69502-6-SRN 3-UBN 1-3-1-3-2
17.	IR 85078-14-1-2-1	IRRI 141/IRRI 119
18.	IR 85078-42-2-2-2	IRRI 141/IRRI 119
19.	IR 85083-SUB-1-1-1-2	IR02N 255/IRRI 119
20.	IR 85085-SUB-17-3-3-2	IR02N 447/IRRI119
21.	Swarna	Vasistha/Mahsuri
22.	Swarna-Sub 1	Swarna *3/IR 49803-7-1-2-3
23.	Savitri	Pankaj/Jagannath
24.	IRRI 119	IR 43581-57-3-3-6/IR 26940-20-3-3-3-1// KHAO DWAK MALI
25.	Upahar	Mahalaxmi/IR 62

Table 2 : PCR constituents optimized for RAPD analysis.

Component	Stock	Quantity in µl	Final concentration in the reaction mixture
PCR Buffer with $MgCl_2$ (15mM)	10 x	2.5	1 x
dNTPs mix	10 mM	2.0	200 µM each
Taq DNA polymerase	I U/µl	1.0	1 unit
RAPD Primers	250 pM	4.0	10 pmoles
Sterile DNase, RNase free water		13.5	
Total		**23.0**	
Template DNA		2.0	30 ng

Table 3 : Primers used for RAPD analysis.

RAPD Primer

S. no.	Primer	Sequence 5'-3' →	T_M (°C)
1.	OPC-20	ACTTCGCCAC	37°C
2.	OPN-11	TCGCCGCAAA	37°C
3.	OPK-01	ACCTGGACAC	37°C
4.	OPD-11	AGCGCCATTG	37°C
5.	OPM-03	GGGGGATGTC	37°C

with a mean of 6.4 bands per primer. Of a total 32 RAPD bands, 17 (53.12%) were found to be polymorphic. The maximum number of polymorphic bands (8) was obtained using OPC-20 and OPN- 11 primer where as the minimum number of polymorphic bands (4) was obtained in Primer OPD-11. Genetic relationship between rice genotypes were determined on the basis of Jaccard's pair-wise similarity coefficients. The genetic similarity estimates among the genotypes are presented in table 4.

Table 4 : DNA profile and polymorphism generated in 25 rice genotypes using 5 RAPD primers.

Primer code	Total No. of bands	Total monomorphic band	Total polymorphic band	%age Polymorphism	Size Range (bp)
OPC-20	8	2	6	75.0%	300-1500
OPD-11	4	4	0	0	400-1000
OPM-3	6	5	1	83.3%	200-2000
OPK-1	6	1	5	16.6%	400-1000
OPN-11	8	3	5	62.5%	300-2000

The cultivar pairs 'OR 2330-1' and 'OR 2397-1' showed the maximum similarity (1.00) among the 26 genotypes, whereas, 'NDR 8012' and 'Swarna' as well as 'OR 2329-34' and 'Upahar', showed the least pair-wise similarity (0.69) in case of pooled data. The genotypes namely 'OR 2330-1' and 'OR 2397-1' exhibited the maximum similarity (100%). Two clusters (I-II) were identified from the dendrogram. The maximum number of genotypes (24) was included in the Cluster I whereas cluster II was represented by one genotypes. Cluster I was further subdivided into two sub-clusters (IA and IB). All the genotypes represented in Cluster I were similar with respect to most of the morphological characters. The sub-cluster IA was represented by 22 genotypes. Cluster IA further classified into two minor sub clusters AI and AII. AI was represented by 15 genotypes and AII was represented by 7 genotypes. Similarly, cluster IB is 2 genotypes. The genotypes 'Upahar' placed in cluster II was found to be the most divergent from rest of the genotypes.

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