Random Amplified Polymorphic DNA Markers in Polyploid Rice

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Abstract

RAPD technique was accomplished by pcr-amplified DNAs followed by separation on 5% polyacrylimide gel. Then, gel was dried for 20 min. The objective of this study RAPD technique was used on identical polyploid rices. Polymorphism in RAPD markers were observed. Polymorphism presented in 4 of 26 primers tested. The polyploid lines were didtinct in RAPD patterns. This kind of markers is useful in case of interspecific hybridiazation to identify the true hybrids.

1. Introduction

The potential use of RAPD markers is not only for evaluating a source of genetic markers to study genetic varitation but also for applying to study genetic diversity and defining gene pool. Modern molecular techniques such as, Restriction Fragment Length Polymorphism (RFLP), Amplification Fragment Length Polymorphism (AFLP) and Random Amplified Polymorphic DNA (RAPD), have been used in many laboratories dependent on the objective of the program. If the experimental materials are closely related, it's better to use RAPD or AFLP.

RAPD technique was developed to identify random-amplified-polymorphic DNA (RAPDs) in several organisms. This approach to generate markers used to establish genetic linkage maps uses PCR to produce random-amplified-polymorphic DNA (RAPD) markers [8]. RAPD markers are generated using short DNA primers and polymerase chain reaction (PCR). DNA has been separated on poly-acrylamide gel with variation in denaturation conditions [7] . This technique has been utiliized to study genetic variation in various organisms, including crop plants such as tomato[4] wheat [2] potato [5] and sorghum [6].

The objective of this study was to etermine the genetic differences among polyploid ines with their parents.

2. Materials and Methods

-*Oryza minuta* (BBCC genome) as a male parent; IR70 (AA genome) as a female parent and 4 polyploid lines namely PTT-KU-91-9, PTT-KU-91-11, PTT-KU-91-12 and PTT-KU-91-14 (from the cross of IR70 x *O.minuta* and chromosome was double by colchicine treat-ment). (Table 1)

-Young leaves from 1 month old seedlings were collected for DNA extraction.

-RAPD technique.

Genomic DNA was isolated using the procedure Laboratory Crop Genome Analysis with modification. Dry leaf was ground in liquid nitrogen in a 1.5 ml microcentrifuge tube. 600 ul of DNA Ext. buffer (32.86 g Sucrose, 3.00 ml of 1 M Tris (pH 9.0), 15 ml of 0.5 M EDTA (pH 8.0), and 4.5 ml of 5 M NaCl per liter) and 20 ul of 10% SDS, were added. Tubes were incubated at 65°C for 10 minutes and extracted with chloro-form: phenol of 1:1 (v/v) then centrifuged at 16000 rpm for 5 minutes. Used P1000 and pipeted the aqueous layer. Care was taken not to disturb the interface. The aqueous layer was

transferred to a clean 1.5 ml microcentrifuge tube. 100 ul of 3 M NaOAc (pH 5.2) was added and 1000 ul of cold 100% ETOH was added. The tube was placed at -70°C for 30 minutes or -20°C overnight. Tubes were centrifuged at 16000 rpm for 5 minutes to percipitate DNA. DNA pellets were washed with 70% ETOH and resuspended in 30 ul of 1xTE 1M Tris HCL pH 8.0 and 0.5 M. EDTA pH 8.0)

DNA concentration was determined using Hoechst dye 33258 [1] (1 ul of dye: 10 ml of 1xTE) and a TKO 100 fluorometer (Hoefer Scientific Instruments.) DNA quality was checked on a 0.6% agarose gel.

Samples were diluted to a concentration of 5 ng/ul in sterile water. PCR reaction for RAPD were performed. PCR per reaction contained; 1.5 ul of 10xPCR buffer; 2.5 ul of M_gCl_2 (25 mM); 1.5 ul of dATP (1mM); 1.5 ul of dGTP (1mM); 1.5 ul of dTTP (1mM); 1.5 ul of dCTP (1mM); 1.5 ul of primer (annex 1); 0.075 ul of Taq DNA polymerase;2 ul of genomic DNA and 1.425 ul of water. The final volumn should be 15 ul. Performed PCR in PE 9600 or similar type machine. The conditions were 96°C, held for 2 minutes; 3 temperature cycle programme; 94°C for 30 seconds; 36°C for 1 minute; 72°C for 1 minute with ramp of 15 second; 72°C held for 10 minutes; and final 4°C held forever.

PCR - amplified DNA (2.5 ul) was mixed with 1.5 ul of Manual Sequencing Loading buffer solution and denatured at 95°C for 3 minutes. Samples (4 ul) were loaded on to a 5% polyacrylamide gel. Run at 60 W for 1.5 hours (or until the bromophenol blue reached the bottom.) Gel was stained by silver staining and dried for 20 minutes.

2. Result

A total of 312 scorable RAPD markers varying in size were generated with the four primers (Table 2). The three primers namely OPC-05, OPAL-01 and OPAL-13 generated almost the same number of markers. Primer OPAL-12 generated more markers (94).

Variation among the parents and polyploid lines.

The RAPD markers generated by the parents and polyploid lines for each primer are shown in figure 1. The total and unique number of markers varied between parents and polyploid lines. Among the four primers studied, primer OPAL-12 generated the most number of markers (94) followed by OPAL-01 (81) OPC-05 (70) and OPAL-01 (67). The polyploid lines shared the same markers of their male parent and some markers of the female parent. They also had some markers unique to them. This kind of unique marker is highly useful in cases of interspecific hybridization to identify the true hybrids by comparing the hybrid pattern with their parents. The primers OPC-05 and OPAL-01 produced two markers each (figure A and B) that were unique to polyploid lines while the primer OPAL-12 and OPAL-13 generated one marker (figure C and D) that was unique to polyploid lines.

Variation among the polyploid lines.

Among the four polyploid lines studied here the marker pattern for all the four primer were similar except some of them varied in intensity.

3. Acknowledgements

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Table 1. plant materials used in this study.

No.	Varieties/lines
1.	*Oryza minuta*
2.	IR70
3.	PTT-KU-91-9 (IR70x*O.minuta*)
4.	PTT-KU-91-11 (IR70x *O.minuta*)
5.	PTT-KU-91-12 (IR70x *O.minuta*)
6.	PTT-KU-91-14 (IR70x*O.minuta*)

Table 2. Nucleotide sequences of the 4 oligonucleotide primers and the total number of RAPD markers generated by them.

Primer	Nucleotide sequence (5'-3')	Number of markers
OPC-05	GATGACCGCC	70
OPAL-01	TGTGACGAGG	81
OPAL-12	CCCAGGCTAC	94
OPAL-13	GAATGGCACC	67

Annex 1 40 primers were used in RAPD.

1. AI17 OPAI-17 5'-ACCCCCTATG-3'
2. AJ18 OPAJ-18 5'-GGCTAGGTGG-3'
3. AJ19 OPAJ-19 5-'ACAGTGGCCT-3'
4. AL04 OPAL-04 5'-ACAACGGTCC-3'
5. AL07 OPAL-07 5'-CCGTCCATCC-3'
6. AL12 OPAL-12 5'-CCCAGGCTAC-3'
7. AL13 OPAL-13 5'-GAATGGCACC-3'
8. AL15 OPAL-15 5'-AGGGGACACC-3
9. AL17 OPAL-17 5'-CCGCAAGTGT-3'
10. AK01 OPAK-01 5'-TCTGCTACGG-3'
11. AK02 OPAK-02 5'-CCATCGGAGG-3'
12. AK03 OPAK-03 5'-GGTCCTACCA-3'
13. AK04 OPAK-04 5'-AGGGTCGGTC-3'
14. AK05 OPAK-05 5'-GATGGCAGTC-3'
15. AK06 OPAK-06 5'-TCACGTCCCT-3'
16. AK07 OPAK-07 5'-CTTGGGGGAC-3'
17. AK08 OPAK-08 5'-CGGAAGGGTG-3'
18. AK09 OPAK-09 5'-AGGTCGGCGT-3'
19. AK11 OPAK-11 5'-CAGTGTGCTC-3'
20. AK12 OPAK-12 5'-AGTGTAGCCC-3'
21. AK13 OPAK-13 5'-TCCCACGAGT-3'
22. AK14 OPAK-14 5'-CTGTTCATGCC-3'
23. AK15 OPAK-15 5'-ACCTGCCGTT-3'
24. AK16 OPAK-16 5'-CTGCGTGCTC-3'
25. AK17 OPAK-17 5'-CAGCGGTCAC-3'
26. C-05 OPC-05 5'-GATGACCGCC-3'

Table 3. Number of RAPD markers unique to parents and polyploid lines, markers similar to female parent, markers similar to male parent and number of markers unique to polyploid lines.

Primer	No of markers unique to varieties/line	Similar markers to female parent				Similar markers to male parent				No of markers unique to polyploid lines
		3	4	5	6	3	4	5	6	
OPC-05	70	10	6	-	9	4	3	-	5	2
OPAL-01	81	7	8	7	9	1	4	3	4	2
OPAL-12	94	13	11	10	9	6	6	8	3	1
OPAL-13	67	8	8	8	7	3	5	8	4	1

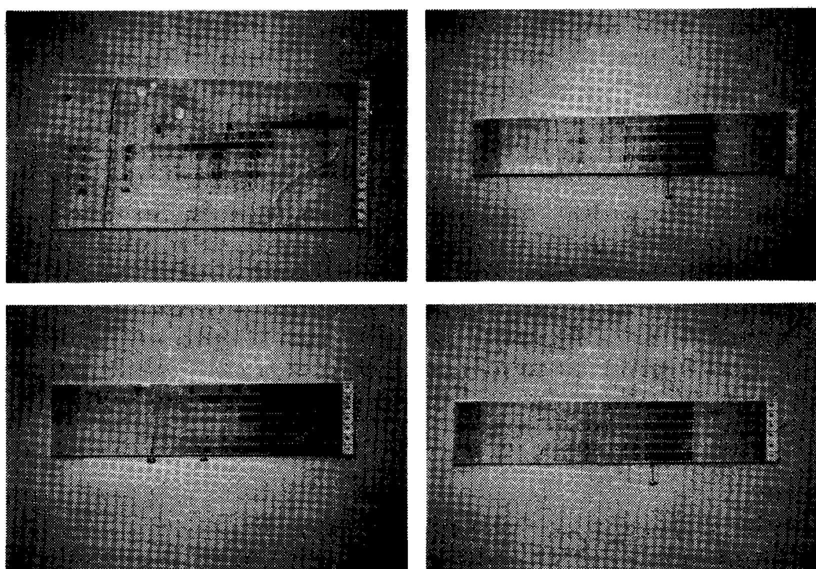

Figure 1. Polyacrylamide gel eletrophoresis patterns of RAPD markers generated by the primers OPC-05 (A), OPAL-01 (B), OPAL-12 (C) and OPAL-13 (D) for the parents and polyploid lines in rice. Lane 1. Kb ladder, lane 2. O minuta, lane 3. IR70, lane 4-7 polyploid lines.