RESEARCH PAPER

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Characterization of genetic variability in different rice lines using DNA markers

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Rice is one of the important staple food crop in the world. Which has been extensively studied both the genetic and molecular level. New technique based on DNA profiling provides novel approaches to varietal characterization which offer advantage over traditional morphological comparisons. Identification of plant varieties/ cultivars and determination of their genetic variability through direct DNA analysis not only help to identify molecular marker across the chromosome for mapping purpose, it also help in gene tagging as well as molecular marker aided selection (MAS), because of several advantages of the molecular method over the biochemical or morphological methods to identify, characterize and determine genetic variability among rice lines, an attempt has been made to characterize different rice lines containing known genes of blast resistance and some of local cultivar with an objective to study the level of DNA polymorphism among different rice lines using PCR based DNA marker and to estimate the genetic variability among 23 rice lines during the year 2003 at NRCPB, New Delhi. PCR based Random Amplified Polymorphic DNA (RAPD), UPR, ISSR and STMS markers were used for characterization of genetic variability among 23 rice lines. Total 83 primers were used, out of which 30 primers gave polymorphism. A total 1276 number products amplified. Dendrogram was constructed on the basis of the result obtained through PCR. It showed that 23 lines were clustered in to 7 groups. The first group contains 2 isogenic blast resistant lines, second group contains four isolines having different genes of P14. The third cluster contains three rice lines which all contains different genes of PiK, HPU-741 which is local variety, distinguished from the other three variety they having 55 similarity in cluster 4. Three japonica type lines were grouped in cluster five. Cluster 6 having two rice lines which are japonica type having dark nature. Cluster 7 having two varieties which are susceptible for blast.

Key words : Rice, DNA, RAPD, ISSR, UPR, STMS, Polymorphism, Dendrogram

INTRODUCTION

Identification of plant varieties/cultivars and determination of their genetic variability through direct DNA analysis not only helps to identify marker variation across the chromosomes, which is useful for mapping and tagging agronomical useful genes as well as marker aided selection (MAS) programmes.

There are diversified molecular approaches adapted to generate DNA fingerprinting and thus identifying crop varieties. These techniques include polymerase chain reaction (PCR) based random amplified polymorphic DNAs (RAPDs), AFLPs and ISSR etc. RAPDs are primarily used for their simplicity. Although reproducibility of RAPD is less with changing parameters, these can be stabilized by maintaining constant experimental procedures or by maintaining greater stringency of experiment. Because of several advantages of these molecular methods over the biochemical or morphological methods to identify, characterize and determine genetic variability among rice lines, an attempt has been made to characterize different rice lines which harbor known genes for blast resistance and some of local cultivars with an objective to study the level of DNA polymorphism among different rice lines using PCR based DNA markers and to estimate the genetic variability among the different rice lines.

MATERIALS AND METHODS

Plant material and DNA extraction

A total of 23 rice (*Oryza sativa* L) lines were selected for this study. The list of materials is indicated below:

Young leaves of these plants were collected from glass house of National Research, Centre on Plant

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Reaction to blast	Name of cultivars
Resistant	Tadukan (Pi b gene), Usen, Zenith, K-60, Sh. Teao-Trao, Kanto, Tetep, Kusabue, Shin 2, C102 PKT, C 101 A51, C 101LAC, C 105 TTP
Susceptible	Nipponbare, HP-2216, HPU-741, Bhrigudhan, Jattoo, 25-Matali, Deval Red, Deval White, Norin-18, Nagardhan

Biotechnology, Indian Agricultural Research Institute, New Delhi. DNA was isolated from young leaves following the CTAB method. with slight modifications. The crude DNA was purified by successive RNase (100 ug/ml) treatments followed by Phenol-Chloroform extraction. The DNA was ethanol precipitated and dissolved in TE buffer.

Assessment of DNA quality and quantity:

The quality and quantity of the extracted DNA was checked by running 2 µl of each DNA sample on 0.8% agarose gel in 0.5 x TAE buffer (at 100v for 30 min.) with lambda uncut DNA at 30 ng/µl. Ethidium bromide @ 0.5 µl/100ml was added to Agarose at the time of gel casting. After the completion of electrophoresis, the gel was visualized on a UV transilluminater and the gel image was taken by using gel documentation system (Multi Images Light Cabinet, Alpha Innotech Corporation, USA). The concentration of DNA was assessed by comparing with the intensity of known quantity of lambda uncut DNA band co-electrophoresed with the samples based on the assessment, the DNA samples were diluted to a final concentration of 30 µg/µl.

Primers:

Total 83 primers were utilized for the diversity analysis. It included 25 RAPD, 12 URP, 9 ISSR and 37SSR primers. Primers were synthesized from Operon Technologies (Alameda, Calif, USA).

Polymerase chain reaction:

For RAPD, URP, ISSR primers, the PCR reaction was set up in 25 µl reaction mixture contained 1 µl of 15-20 µg of template DNA, 2.5 µl of 10 x PCR buffer (500 mM KCl, 15 mM $MgCl_2$, 100 mM Tris – HCl pH 8.0) 5 µl of 10 mM dNTP Taq polymerase (Genei, Bangalore, India) 1 µl of 0.2 URP primer and 19.8 µl Milli – Q water.

In case of PCR, 25 µl reaction mixture contains 1 µl of 25-30 ng of template DNA, 2.5 µl of 10 X PCR buffer (500mM DCL, 15 mM $MgCl_2$, 100mM Tris – HCl PH 8.0), 0.5 µl of 10mM dNTP mix (Genei, Bangalore, India), 0.2 µl of 1 unit Taq polymerase (Genei, Bangalore, India). 0.5 µl of 0.2 µl of forward and reverse primer each (Operon Technologies) and 19.8 µl Milli-Q water. For RAPD, URP and ISSR, PCR reactions were performed on the thermocycler (T-Gradient Biometra) programmed for initial DNA denaturation of 94^0C for 5 min followed by 35 cycles at 94^0C (1 min) for DNA denaturation, 42-61^0C (1 min) for primer annealing, 72^0C (2 min) for primer extension and a final extension at 72^0C for 7 min was also performed.

All the amplified DNA product were resolved on 1.6% metaphore gel (with 1 x TBE) in case of STMS. The gel stained with ethidium bromide and was performed for 2 hours at 80 volts. Then photographs of the gels were taken in gel documentation system.

Data analysis:

Gel photographs were scored for the presence (1) or absence of bands of various molecular sizes. Binary matrices consisting of 1 and 0 were analyzed to obtain Jaccards coefficients among the genotypes using NTSYS – PC (Version exterior Biological software Setanket, NY). Jaccard Coefficient were clustered to generate tree using the SAHN clustering program selecting the unweighted pair group method with arithmetic average (UPGMA).

RESULTS AND DISCUSSION

Genomic DNA from rice lines were isolated, purified and quantified on Agarose gel using molecular weight marker. Total 83 primers used in this study comprised of 25 RAPD, 12 URP, 9 ISSR, and 37 STMs. Out of these primers, 12 URP, 4 ISSR, 1 RAPD and 13 STMs primers were found polymorphic with rice lines. URP 2R, ISSR I, RAPD C-11 were found to be superior over all primer in discriminating the different rice lines. (Fig. 1) indicated pattern of polymorphism among various rice lines using different PCR based markers.

When all primers each gel was scored based on presence absence of bands and binary matrix was

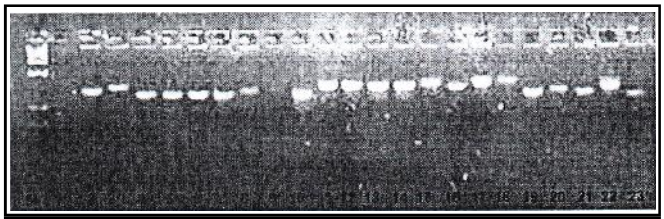

Fig. 1. A: PCR analysis of 23 rice lines performed with STMS primer RM 206 (M: 100 bp ladder)

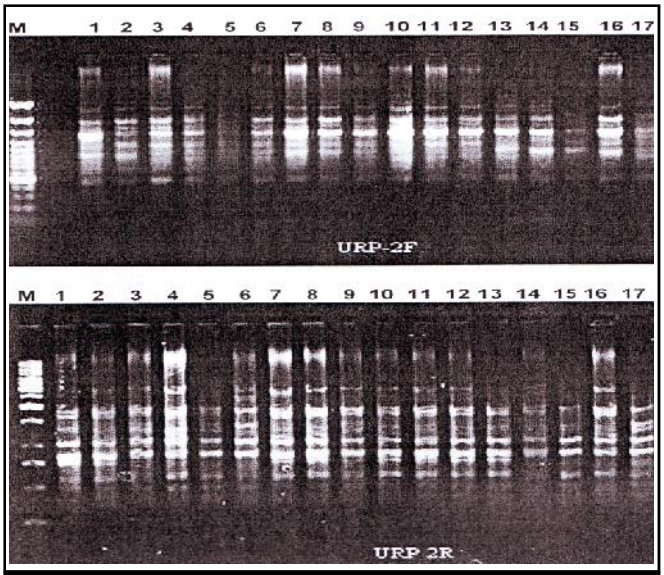

Fig. 1. B: DNA profiles of 23 rice lines with URP primer 2F and 2R electophoreses on 1.5% agarose gel, M: 100 bp ladder

constructed and data were analyzed by using NTSYS software. Clustering was done on the basis of UPGMA method. Using 23 rice lines diversity analysis was made using 83 different markers and total numbers of amplified products are 1276. Using this dendrogram was made (Fig.2). Dendrogram showed that 23 lines were clustered into 7 groups. The first group contains two isogenic blast

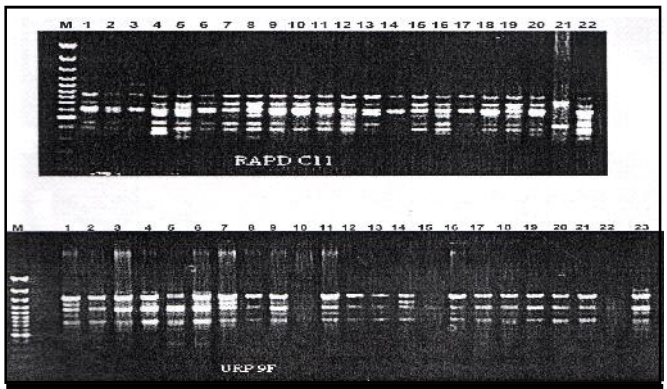

Fig. 1 C (i) RAPD profiles of 23 rice lines with primer C11 PCR product were of 1.5% agarose gel M: 100 bp ladder
(ii) DNA fingerprint profiles of 23 rice lines with URP 9F primer electrophoresis of PCR product was performed on 1.5% agarose gel. M: 100 bp ladder

resistance lines. Second group contains four isolines having different genes of PI4.The third cluster contains three rice lines which all contains different genes of PIK. HPU-741 which was a local variety distinguished from the other three varieties they having 55 similarities in

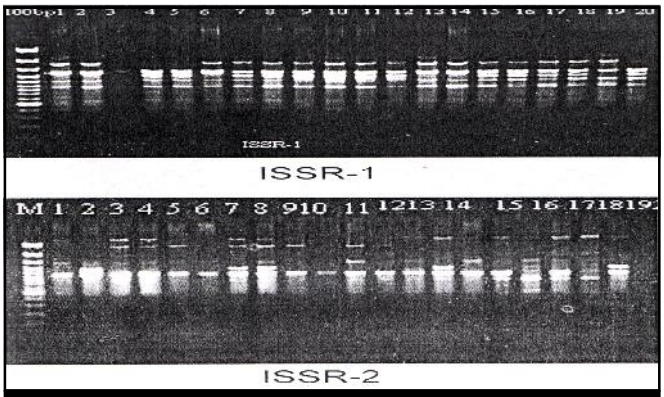

Fig. 1 D. DNA profiles of 23 rice lines obtained with primers ISSR-1 & ISSR-2 electrophoresis was performed on 1.5% agarose gel, M: 100 bp ladder

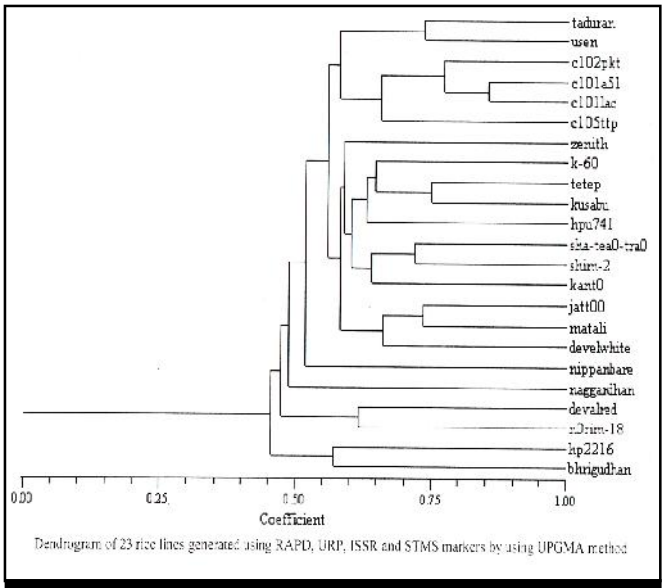

Fig. 2: Dendragram of 23 rice lines constructed 7 groups

cluster 4. Three japonica type rice lines were grouped in cluster 5. Cluster 7 having two varieties which are released from H.P.A.U., which were susceptible for blast.

Using 83 different PCR based markers, total number of 1276 products were amplified. Twenty three rice lines with varying specificity to rice blast have been classified into 7 groups. All the isogenic lines containing *Pi k*-blast resistance gene formed separate group.

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