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# Detection of DNA Polymorphism in Five Varieties of Rice in Tirunelveli District in India

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**ABSTRACT** In order to ascertain the genetic variability among five varieties of rice, Random Amplified Polymorphic DNA (RAPD) fingerprints were analyzed by Polymerase Chain Reaction (PCR) of genomic DNA using random primers. These primers produced multiple band profiles with a number of amplified DNA fragments varying from 5 to 13. A total of forty-three polymorphic bands were observed. The genetic distance between the population ranged from 0.2341 to 0.7885 and the genetic identity ranged from 0.4091 to 0.7865. The overall observed and effective number of alleles is about 2 and 1.15 respectively. Nei (1978) overall genetic diversity is 0.5108. It is clear that there is distinct genetic variability among the five varieties of rice. This information could be very valuable in the management of genetic resources in such economically important crop species.

(Rice, Inter varietal, DNA polymorphism, genetic variability)

## INTRODUCTION

Bioprospecting and genetic prospecting are methods now widely adapted for valuing the genetic potential of the crop plant diversity. The basic characteristic of life is its unlimited diversity. No two individuals in sexually reproducing populations are the same. The underlying factors in this diversity is genetic, though other factors like age, sex, immune system, etc. do play their part in bringing about the observed differences. In order to prevent depletion of biodiversity due to man-made efforts or otherwise, it is necessary to understand how the diversity of life particularly in the genetic level is maintained under natural conditions. Based on this knowledge one can suggest appropriate strategies and policies for the conservation of biodiversity. Genetic diversity is the "raw material" permitting species to adjust to a changing world, whether these changes are due to natural or human factors. The level of similarity (homogeneity) or difference (heterogeneity) in the genetic makeup (genome) of populations of the same species indicates to what extent genetic material can be exchanged between populations and still maintain a species-specific gene pool. Understanding gene-exchange phenomena within a species can provide further insight into the role of diverse populations in maintaining a species genetic diversity, or in leading to the isolation and creation of distinctive new genomes and thus potentially new species.

There are many earlier reports on this type of study. By using eight RAPD primers, the genetic analysis of some Egyptian rice genotype was detected and the level of polymorphism as revealed by RAPD was 72.2% respectively [1]. 18 accessions from an Indian scented rice germplasm were detected with RAPD and it was found that 114 bands (95.1%) were polymorphic [2]. A transgenic molecular analysis of the genome of transgenic rice plant form Italian cultivars using RAPD was done [3]. Genetic diversity of *Oryza rufipogon* was detected for eight populations from China and Brazil, and found that the PPB values were 55.8% and 41.1% for the Chinease and Brazilian populations, respectively [4]. Rice is classified in the division Magnoliophyta, class Liliopsida, order Cyperales, family Gramineae. Native to the tropics and subtropics of Southeast Asia, rice is now cultivated in many localities throughout the world with favourable climatic conditions. More than 90% of the world rice production is in Asia, China and India being the largest producers. A cereal grass *(Oryza sativa)* is cultivated

extensively in warm climates for its edible grain. Although population studies of several cereal plants species have been examined genetically since early 1990 using the RAPD technique, the amount of genetic data concerning *Oryza sativa* varieties is relatively limited. Hence genetic diversity measurements are important for selection of superior genotypes with respect to longevity and for considering conservation and propagation of that particular variety. This study facilitates to identify the genetic variability in five varieties of *Oryza sativa*. The selected five varieties of rice have been produced through plant breeding techniques and introduced for use by the Department of Agriculture in Tamilnadu, India. People in this particular part of Tamilnadu widely use this variety as their staple food. They differ in their morphological features, duration of yield, taste of the grains etc. In order to find out the superiority among the five varieties, genetic variability study is necessitated. Hence, this study aims at studying the genetic variability among the five varieties of rice using RAPD-PCR technique.

## MATERIALS AND METHODS

The samples of *Oryza sativa* varieties (Table 1) were collected from Venkateswarapuram of the Tirunelveli District, Tamilnadu. Ten plant specimens from each variety were collected. The collected plant materials were transferred to plastic bags for transport from field to laboratory. Permanent storage was at -70$^0$ C until DNA isolation and RAPD analysis was carried out.

**Table 1.** Area of the study and Rice Varieties

| COLLECTED AREA | RICE VARIETIES |
|---|---|
| Ambasamuthram | KP |
| Tirunelveli | ADT 39 |
| Papanasam | ADT 43 |
| Tenkasi | ASD 18 |
| Palayamkottai | IR 20 |

A modified CTAB procedure [5, 6] was used for the extraction of genomic DNA. The DNA was isolated from individual young fresh leaves for each variety and pooled together. The leaf (5g) was ground to fine powder in a chilled mortar and pestle and transferred to 500μl of extraction buffer in a 2ml capacity eppendorf tube. The extraction buffer contained 1.21g Tris buffer, 14.0g NaCl, 0.5g EDTA (Ethylene Diamine Tetracetic Acid), 2g CTAB (N-acetyl –N, N, N trimethyl ammonium bromide), 1g PVP (Poly Venyl Pyrolidine) and 0.2% β-mercapto ethanol. The mixture was thoroughly stirred and incubated at 55$^0$C for 1 hour and then kept at room temperature for 10 - 15 minutes. The suspension was extracted with 500μl of chloroform:isoamylalcohol (24:1) to denature proteins and facilitate the phase separation. The mixture was then centrifuged at 15,000 rpm for 10 - 15 minutes at 5$^0$C. The top aqueous phase was carefully taken out in a fresh eppendorf tube (2ml) and 250μl of cold 7.5M ammonium acetate and 300μl of cold isopropanol was added to it. Then the mixture was thoroughly stirred and transferred in to deep freezer (-70$^0$C) for 5 - 10 hours and then kept at room temperature for 10 - 15 minutes. The precipitated DNA was pelleted by centrifugation at 15,000 rpm for 10 minutes. The pellet was washed in 500μl of cold ethanol (70%) and kept undisturbed for 5 minutes. Then the samples were centrifuged for 2 minutes at 15,000 rpm. The pellet was washed in 500μl of ethanol (95%) and kept undisturbed for 5 minutes. This mixture was then centrifuged at 10,000 rpm for 2 minutes. The pellet was collected and 100μl of distilled water was added. The mixture was stirred using cyclomixer. The amount of DNA and its quality were assessed by UV spectrophotometer.

The following reagents are used to amplify the plant template DNA.

| NO | CONSTITUENT | QUANTITY |
|---|---|---|
| 1. | Sterile water | 15 μl |
| 2. | dNTPs | 0.5μl |
| 3. | $MgCl_2$ | 2.5μl |
| 4. | PCR Buffer | 2.5μl |
| 5. | Primer | 1.2μl |
| 6. | Taq polymerase | 0.2μl |
| 7. | Plant DNA | 3.1 μl |
| 8. | Total | 25 μl |

Earlier, ten primers had been tested and five primers which produced reproducible bands were selected. The experiments were repeated three times and confirmed the reproducibility of bands.

**Table 2.** The RAPD – PCR reactions were conducted based on the following cycles.

| ACTIVITY | TEMPERATURE | TIME | NUMBER OF CYCLES |
|---|---|---|---|
| Initial denaturation | 94°C | 1 Min | One |
| Denaturation | 94°C | 1 Min | 35 |
| Annealing | 35 °C | 1 Min | |
| Extension | 72°C | 2 Min | |
| Final Extension | 72°C | 5 Min | One |
| Storage | 4°C | For ever | |

The amplification products were separated by electrophoresis in agarose gel (1.4%). The gel was visualized by UV transilluminator and photographed with gel documentation system (Alpha Imager 1200).

Based on the primary data (presence or absence of bands), pair wise genetic distance between samples was calculated using Pop gene package version 1.31.

## RESULTS AND DISCUSSION

Genetic relationship among the five rice varieties has been carried out using RAPD. Five primers generated reproducible, informative and easily scrabble RAPD profiles. These primers produced multiple band profiles with a number of amplified DNA fragments varying from 5 to 13. A total of forty-three polymorphic bands were observed (Table 3). The same type of bands occurred at different frequencies in all populations. There were many additional bands neglected which were not reproducible. The genetic distance between the population ranged from 0.2341 to 0.7885 and the genetic identity ranged from 0.4091 to 0.7865 (Table 3). The overall observed and effective number of alleles is about 2 and 1.15 respectively. Nei (1978) overall genetic diversity is 0.5108 [7].

Genetic variation in a population is measured by the heterozygosity or the degree of polymorphism. For the conservation of a species, genetic variability is of the utmost importance to preserve. Genetic variability among all species is important to maintain since it represents the 'blue print' for all of the living things on earth. The result obtained was analyzed in the Pop gene software 3.1 and the dendrogram obtained clearly indicates two clusters.

**Table 3.** Oligonucleotides used as random primers in rice varieties and their sequences

| NO. | PRIMER | SEQUENCE (5' TO 3') | NUMBER OF POLYMORPHIC BANDS |
|---|---|---|---|
| 1. | OPX - 4 | CCGCTACCGA | 9 |
| 2. | OPX - 7 | GAGCGAGGCT | 5 |
| 3. | OPX - 9 | GGTCTGGTTG | 8 |
| 4. | OPX - 16 | CTCTGTTCGG | 13 |
| 5. | OPX - 17 | GACACGGACC | 8 |

IR 20, ASD18, KP in one cluster and ADT 39, ADT 43 forms another cluster. In the first cluster KP forms a separate clade. Thus it is evident that there is clear variability with in the varieties. In the dendrogram based on Nei's genetic distance (UPGMA) obtained (Figure 1), the populations were highly differentiated by their own genetic distance. The clustering results of different accessions suggest that *Oryza sativa* undergoes major part of genetic variation by environmental factors. Genetic diversity refers to the variation at the level of individual genes (polymorphisms), and provides a mechanism for populations to adapt to their ever-changing environment. The more variation, the better the chance that at least some of the individuals will have an allelic

variant that is suited for the new environment, and will produce offspring with the variant that will in turn reproduce and continue the population into subsequent generations. In order to prevent depletion of biodiversity due to man-made efforts or otherwise, it is necessary to understand how the diversity of life particularly in the genetic level is maintained under natural conditions. Based on this knowledge one can suggest appropriate strategies and policies for the conservation of biodiversity. An understanding of the diversity of genes responsible for individual species adaptations and responses to their environment (intra specific diversity) is a foundation for understanding almost all ecological and evolutionary processes. Further analysis is necessary to find out the individual polymorphism in each variety and the variety which shows more heterozygosity may be considered to be the best and this data may be correlated with other features of the variety and the superior variety can be identified. Thus this type of genetic variability studies among varieties of crops would be ideal for the selection of the superior variety.

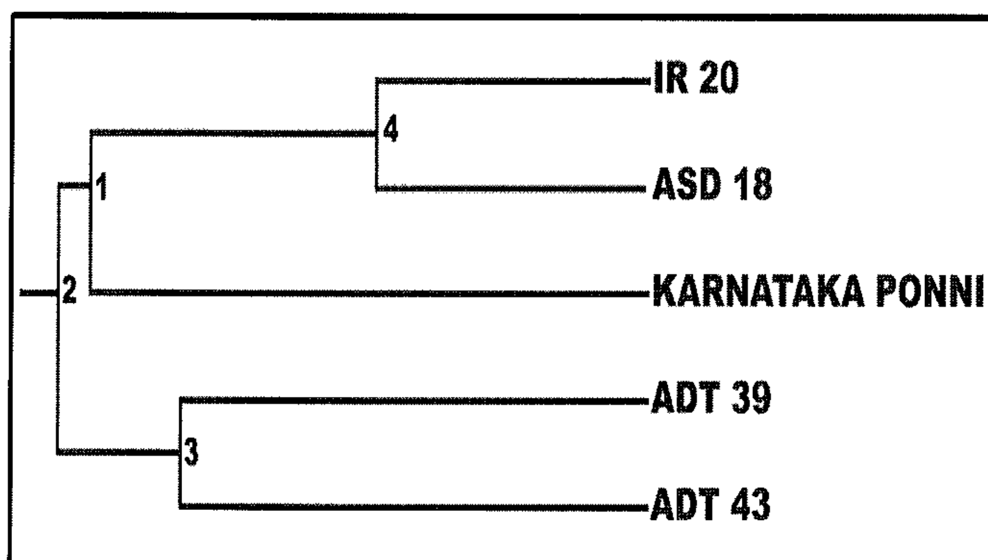


**Figure 1.** Dendogram based on UPGMA model calculated from genetic distance among five rice (*Oryza sativa* L.) varieties employing five RAPD primers

**Table 4.** Nei's Unbiased measures of genetic identity and distance

| RICE VARIETIES | KP | ADT 39 | ADT 43 | ASD 18 | IR 20 |
|---|---|---|---|---|---|
| KP | **** | 0.8765 | 0.4091 | 0.4321 | 0.5000 |
| ADT 39 | 0.2341 | **** | 0.7273 | 0.6364 | 0.4545 |
| ADT 43 | 0.3421 | 0.3185 | **** | 0.5455 | 0.6364 |
| ASD 18 | 0.3830 | 0.5432 | 0.6061 | **** | 0.7865 |
| IR 20 | 0.6543 | 0.7885 | 0.5432 | 0.4520 | **** |

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

**REFERENCES**

1. Saker, M.M., Youssef, S.S., Abdallah, N.A., Bashandy, H.S. and Sharkawy, A.M.E. (2005). Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. *African J. of Biotech* **4** (9): 882 - 890.
2. Raghunathachari, P., Khanna, V.K., Singh, U.S. and Singh, N.K. (2000). RAPD analysis of genetic variability in Indian scented rice germplasm (*Oryza sativa* L.). *Cur. Sci,* **79** (7): 994 - 998.
3. Arencibia, A., Gentinetta, E., Cuzzoni, E., Castiglione, S., Kohli, A., Vain, P., Leech, M., Christou, P. and Sala, F. (1998). Molecular analysis of the genome of transgenic rice (*Oryza sativa* L.) plants produced via particle bombardment or intact cell electroporation. *Mol. Breeding,* **4** (2): 99 - 109.
4. Ge, S., Oliveira, G.C.X., Schaal, B.A., Gao, L.Z. and Hong, D.Y. (1999). RAPD variation within and between natural populations of the wild rice *Oryza rufipogon* from China and Brazil. *Heredity*, **82**: 638 - 644.
5. Doyle, J.J. and Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem*, **19**: 11 – 15.
6. Williams, J.G.K., Kubelik, A.R., Livak, K.J. and Rafalski, J.A. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res,* **18**: 6531 - 6535.
7. Nei. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**: 583.