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Molecular Characterization of *Mangifera indica* by Using RAPD Marker

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ABSTRACT

Genetic variation and relationship of mango germplasm were analyzed using Random Amplified Polymorphic DNA (RAPD) for ***Arka Anmol*** and ***Ratna***. The amplified DNA fragments (amplicons) obtained and compared by agarose gel electrophoresis. Isolated specific RAPD fingerprints were obtained. Out of many primers screened, one was selected and 5 and 6 bright bands of ***Arka Anmol*** and ***Ratna*** were found out repectively. Out of which 4 bands were shared and others were unique. RAPD is highly polymorphic nature and genetic diversity was studied accordingly. *Arka Anmol* and *Ratna* are very close to each other and showed a minimum dissimilarity of 1%. The data suggest that RAPD may be of value by virtue of its rapidity, efficiency and reproducibility in generating genetic fingerprints.

INTRODUCTION

Mango (*Mangifera indica* L.) is the most important species of the 'Anacardiaceae' family and is said to have originated in Indo-Burma region (Mukherjee, 1951). Mango is a "King of fruit" and "National fruit of India" because of the flavor and nutritional qualities. RAPD (Random Amplified Polymorphic DNA) assay, detects nucleotide sequence polymorphisms in DNA amplification based assay using only a single primer of arbitrary nucleotide sequence. In this reaction, a single species of primer binds to the genomic DNA at two different sites on opposite strands of the DNA templates. If these primary sites are within an amplifiable distance thermo cyclic amplification occurs. The presence of each amplification product identifies complete or partial nucleotide sequence homology, between the genomic DNA and oligonucleotide primer at each end of the amplified product. On an average, each primer will direct the amplification of several discrete loci in the genome, making the assay an efficient way to screen for nucleotide sequence polymorphism between individuals. The DNA amplification product is generated from a region that is flanked by a part of 10-base pair priming site in the appropriate orientation. Genomic DNA from two different individual often produces different amplification pattern (RAPDs). A particular fragment generated from one individual, but not for other represents DNA polymorphism and can be used as Genetic marker.

Arka Anmol: Originated as a cross between 'Alphanso and 'Janardhan Pasand' at Indian Institute of Horticultural Research (IIHR), Bangalore, India. Tree is semi vigorous bearing regular and prolific fruiting free from spongy tissue (Iyer and Mukunda, 1998).

Ratna: This cultivar was developed at Regional Fruit Research Station (R.F.R.S.), Vengurla Sindhudurg district of Maharashtra, by crossing 'Neelum' and 'Alphonso'.

RAPD markers were used to identify cultivars of *Mangifera indica* L. and validate genetic relationships among them. Controlled pollination is difficult to make because of the small flower size. The long juvenility period of mango would make RAPD markers an extremely useful tool for identification of cultivars. This would also aid in the management of germplasm collections of mango, where identical cultivars often have different names (Schnell *et. al.*, 1995). There are several advantages associated with RAPD, like no prior requirement for DNA sequence information of the genome is required; the protocol is also relatively quick and easy to perform and uses florescence in live of radioactivity; it is simple and dominant in nature; only nanogram quantity of DNA is required and automation is feasible; and the procedure involves extraction of DNA by any one of the standard protocol and amplifying the DNA by PCR using random primers.

MATERIALS AND METHODS

Apparatus Used

The apparatus used for the research work were the gel electrophoresis unit, cool centrifuge, program thermo cycler, deep freezer (-20^{o}C and -80^{o}C), refrigerator, hot water bath, hot oven, pH meter, autoclave, gas stove, electronic weighing balance, micropippette, DNA, UV transilluminator, mortar and pestle, tips and eppendorf tubes

DNA Extraction Solution

It has C.TAB, Tris base, NaCl, EDTA (disodium), Chloroform: Isoamyl alcohol,β mercaptoethanol ,PVP – 1% (Poly vinyl pyrrolidine).

PCR Ingredients

Template DNA, dNTPs, 10x PCR buffer, $MgCl_2$, Taq DNA polymerase, Mineral oil, Primer OPC 11- AAAGCTGCGG

I. Reagents: 1.5% agarose, loading buffer – sucrose, Xylene cyanol, Bromophenol blue Ethidium bromide

II. Running buffer (5x) or TBE / TAE: Tris Base (pH 8.0), Boric acid, EDTA

Method for RAPD Analysis

DNA is extracted by meshing the leaves of the plant in mortar and pestle and treated with different reagents, followed by verifying it by electrophoresis in 1.2% agarose gel. DNA is subjected to selective amplification in PCR where primer is amplified and further treated with different reagents of PCR. Target sequence is amplified and is then subjected to agarose gel electrophoresis and stained with ethidium bromide. The gel was photographed and RAPD profile was obtained (Fig. 2).

OBSERVATION AND DISCUSSION

DNA was isolated and amplified using PCR from *Arka Anmol* and *Ratna* varieties of Mango. The yield and quality of DNA obtained was adequate and consistent (Fig. 1).

Fig.1: Isolation and amplification of DNA using PCR followed by Electrophoresis. (***Abbrv***. L1: *Arka Anmol*, L2: *Ratna*)

The concentrations of DNA thus obtained were calculated by using Hoefer's Dyna Quant Fluorometer measured as 60ng/µl and 27.5ng/µl for *Arka Anmol* and *Ratna* respectively. Fingerprint profile for commercial mango varieties using RAPD markers were established with the help of primers. The banding patterns of *Arka Anmol* and *Ratna* revealed 5 and 6 bands respectively Fig. 2. Out of the these bands, 4 bands were shared and others were unique.

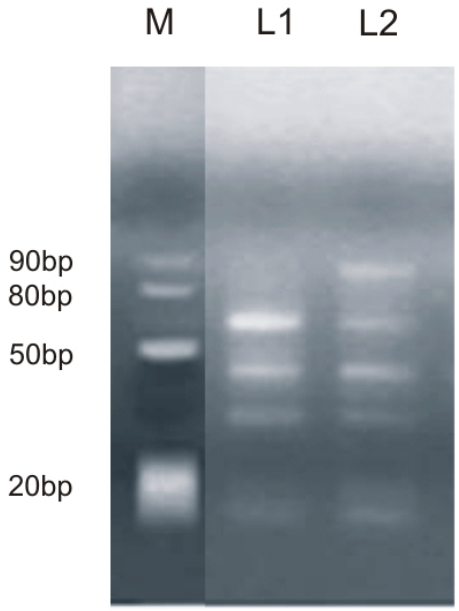

Fig. 2: RAPD profile for *Arka Anmol* and *Ratna* varieties of Mango (***Abbrv***. M: Markers 100bp, L1: Amplified samples of *Arka Anmol* variety; L2: Amplified samples of *Ratna* variety)

RAPD analysis is shown to be very efficient in identifying markers linked to the targeted region of the genome. The extent of polymorphisms detected by RAPD method is better than that of RFLP since it is a dominant marker. RAPD is less expensive, fast and reliable when compared to RFLP, which is more expensive, tedious and involves radioisotopes. The results of the present study indicated that the RAPD analysis could be utilized by breeders for further improvement of mango varieties.

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