Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 3, 2013

Research Article

MOLECULAR ANALYSIS OF *MANIlKARA HEXANDRA* ROXB. AND *AVERRHOA CARAMBOLA L.* USING RAPD MARKERS HELPS TO UNDERSTAND GENETIC VARIATIONS

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Received: 17 Jun 2013, Revised and Accepted: 10 Jul 2013

ABSTRACT

Objective: It is to analyse the interrelationship and genetic polymorphism between *Manilkara hexandra* Roxb and *Averrhoa carambola L.* plants by using RAPD markers.

Methods: In this study, a set of 25 RAPD universal plant primers (RPI 1 - RPI 25) were used. Total Genomic DNA was extracted from the leaves for PCR reactions by using standardize protocol using 3B BlackBio Biotech kit.

Results: It was observed that the genetic pattern of the both species showed the distinct variation in polymorphism at amplicons level. Each of the plants reproduced six highly polymorphic bands.

Conclusion: It can be concluded that RAPD profile will help in identifying genetic variation among different species and developing ways to conserve the medicinal aspects of *Manilkara hexandra* Roxb and *Averrhoa carambola.*

Keywords: *Manilkara hexandra* Roxb, *Averrhoa carambola*, RAPD, UPGMA, Genetic diversity.

INTRODUCTION

Medicinal plants play a vital role to preserve our health and as the science makes improvement in its diverse field, the traditional methods are still followed to identify their potential. This grabs the attention towards the natural source available and efforts are being taken to explore these medicinal plants for their magnanimous potentials [1]. Indian medicinal plants are used since ancient times to treat different diseases as these natural products exert broad-spectrum actions [2]. In Ayurveda, plants are the main source for medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important aspect [3]. *Manilkara hexandra* belong to the Sapotaceae family. The fruit, leaves and bark of this plant is very well studied for its antioxidant potential. The plant is used to cure fever, colic, helminthiasis, traditionally. Furthermore, it is used as appetizer and as tonic, astringent [2]. Leaves are traditionally used as anti-inflammatory, diuretic, antiurolithiatic, analgesic, anti-pyretic, anti-microbial, tonic and febrifuge [4]. *Averrhoa carambola* Linn. (Oxalidaceae) also known as star fruit is cultivated extensively in India for its edible fruits. *Averrhoa carambola* is recommended as diuretic in kidney and bladder complaints. In Ayurveda, the ripe fruit is considered as digestive tonic and causes biliousness [5]. The plant has been studied extensively for its antioxidant capacity [6]. Considering the medicinal value of these plants, it is essential to conserve genetic diversity of plant species. Many techniques are available for assessment of diversity like morphological, biochemical and more recent DNA technology has become more reliable and acceptable.

The authentication of these plants with respect to the genetic polymorphism and relatedness is possible with random amplified polymorphic DNA (RAPD) technique. DNA fingerprinting distinguishes different varieties according to their DNA variations at a set of genetic loci. RAPD markers are accurate for determination of both interspecies and intra-species genetic variation in plants thus helping to understand about the phylogenetic and/or systematic of the plant [7]. RAPD markers are based on the amplification of unknown DNA sequences using single, short and random oligonucleotide primers. Hence, RAPD polymorphism is the reflection of variation of the whole genomic DNA and shall be effective in assessment of the genetic diversity of the important medicinal plants [1].

The present study is aimed to develop fingerprints and assess the inter-relation between the genes present in *Manilkara hexandra* Roxb and *Averrhoa carambola L.*

MATERIALS AND METHOD

Collection of Plant Materials

The leaves of *Manilkara hexandra* Roxb and *Averrhoa carambola* were collected from the Medicinal plants garden of National Research Ayurvedic Institute of Basic Ayurvedic Sciences, Pune. The plant materials were authenticated by Mrs. A. G. Mhase, the botanist and the specimens were preserved in the herbarium for reference.

DNA Extraction

The leaves samples were grounded in liquid nitrogen to a fine powder and were kept at -20°C until use**.** The total genomic DNA extraction from the leaves of both the plants was done by using 3B Black Biotech Biotools kit with approximately 100-120 mg of powdered sample of each. The DNA of both plants was then quantified by checking its absorbance at A_{260} nm with a UV Vis Spectrophotometer. The DNA was then subjected to 2 % agarose gel electrophoresis and presence of DNA was confirmed by visualization of the band. DNA was then kept at 4°C until further use.

RAPD- PCR

The reaction mixture for RAPD PCR was standardised to a total volume of 20µl containing nuclease free water (13µl), 10X PCR Buffer (2µl), $MgCl_2$ (1.5µl), dNTP (1µl), Primers (1µl), Template DNA (1µl), Taq polymerase (0.5µl). All the PCR reaction components and the Primers set were purchased from 3B Blackbio Biotech Biotools. The primers used for amplification of the genomic DNA are detailed in Table 1. Peltier P25$^+$ (Cyber lab) thermal cycler was used to carried out the PCR reaction step. The amplification conditions were 94^{0}C for 3 min, 94^{0}C for 45 sec, 44^{0}C for 30 sec, 72^{0}C for 1 min, 72^{0}C for 5 min, and 4^{0}C for the end hold. After the amplification step, the PCR product was analyzed by loading on a 2% Agarose gel along with DNA Marker ladder (100-1000bp), the run was carried out at 75 Volts for approximately 60 minutes. Along with the 25 different reaction mixtures for 25 different primers, a negative control (NC - No DNA template) and a positive control (PC - No primer) were placed in thermal cycler to rule out the possibility of contamination.

Statistical analysis

GelQuest® and Clustervis® software were used to construct dendrograms by the Unweighted Pair Group Method (UPGMA) with Arithmetical Averages by comparing the bands for the similarity between the genes.

Table 1: Plant RAPD primers used for the study

S. No.	Name of Primer	Accession Numbers	Sl. No.	Name of Primer	Accession Numbers
1	RPl 1	AM765819	14	RPl 14	AM773774
2	RPl 2	AM750044	15	RPl 15	AM773775
3	RPl 3	AM773310	16	RPl 16	AM773776
4	RPl 4	AM773769	17	RPl 17	AM911710
5	RPl 5	AM773770	18	RPl 18	AM765830
6	RPl 6	AM773771	19	RPl 19	AM773777
7	RPl 7	AM773312	20	RPl 20	AM773317
8	RPl 8	AM773773	21	RPl 21	AM765820
9	RPl 9	AM773315	22	RPl 22	AM911711
10	RPl 10	AM750045	23	RPl 23	AM911712
11	RPl 11	AM911709	24	RPl 24	AM765821
12	RPl 12	AM773316	25	RPl 25	AM750054
13	RPl 13	AM750046			

*(RPI 1 - RPI 25 indicates the Universal primers).

RESULTS AND DISCUSSION

The DNA obtained was 17.0 ng/µl (*Averrhoa carambola)* and 17.05 ng/µl (*Manilkara hexandra)*. The RAPD PCR resulted in amplicons from the specific sequences of the primers giving rise to bands in both plants.

Figure 1 shows the RAPD profile of *Averrhoa carambola*. The lane 2,7,8,10,11,14,15,16,17,18,19,21,23,24 show distinct band patterns for respective primers indicated in Table No.1 above. Figure 2 shows RAPD profile of *Manilkara hexandra.* The lane 1,2,3,4,7,8,11,12,13,15,16,17,,19,20,22,23,24 resulted in distinct band patterns for respective primers as indicated in Table 1. Only the primers which displayed reproducible, scorable and clear bands were considered for analysis. The results were analyzed based on the principle that a band is considered to be 'polymorphic' if it is present in some individuals and absent in others, and 'monomorphic' if present in all the individuals or accessions. In each lane, bands were scored present if their intensity was at least 10% of the monomorphic reference band within the same lane. Marker ladder was used as reference to outline the ancestral linkage between the two plants. RAPD profiling of the studied plants have not be yet reported. The Phylogenetic tree analysis by UPGMA method (Figure 3) and similarity index in present study showed genetic variations in the bands reproduced by both the plants. In this study, the large of similarity values revealed by RAPD markers provides greater confidence for assessment of genetic relationship among the species.

Fig. 1: RAPD Profile of *Averrhoa carambola* (M represent Marker ladder 100-1000bp, Lane 1,2,3,4,5,6,7,8,9,10,11,12,13,4,15,16,17,18,20,22,25 represents RPI Universal Primers).

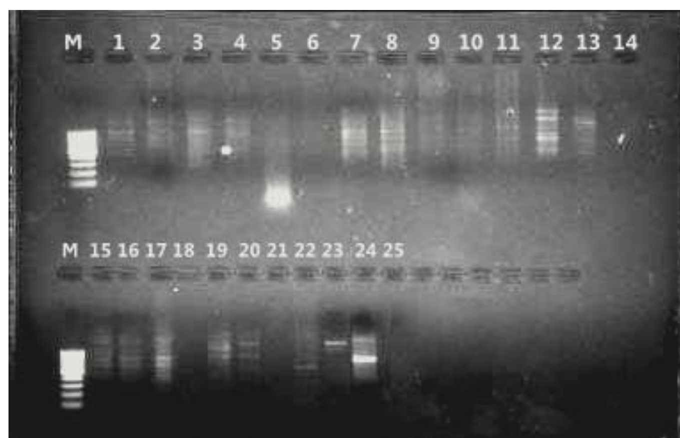

Fig. 2: RAPD Profile of *Manilkara hexandra* Roxb (M represent Marker Ladder, Lane 2,3,4,5,6,7,8,9,10,11,12,13,4,15,16,17,18,20,22,25 represents RPI Universal primers).

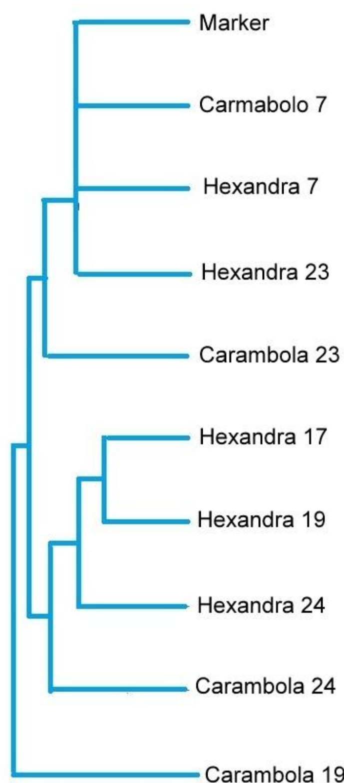

Fig. 3: The Phylogenetic tree representing the relationships between the five primers RPI 7, RPI 17, RPI 19, RPI 23, and RPI 24 constructed by UPGMA tree software

CONCLUSION

Based on the study the large range of similarity and dissimilarity values for the plants using RAPD provides the greater confidence for assessment of genetic diversity and relationships. Thus, this approach will be helpful in ranking the species according to their genetic inter-relatedness. This can provide a better platform for identification and authentication of *Manilkara hexandra* and *Averrhoa carambola.*

ACKNOWLEDGEMENT

The authors would like to express special thanks to the Director General, Central Council for Research in Ayurvedic Sciences, New Delhi for his support and encouragement to carry out the above mentioned study.

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