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RAPD-PCR ANALYSIS OF *BIXA ORELLANA* L. AND *SALACIA CHINENS* L. TO STUDY GENETIC DIVERSITY

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ABSTRACT

Bixa orellana L. and *Salacia chinensis* L. are medicinal plants and native tropical trees of Sri Lanka and Southern region of India which are used in Indian system of medicine to treat various diseases. There is a need to preserve and explore their quantum of genetic variation by analysing the polymorphism between the these plants. Therefore, our aim was to analyse interrelationship and genetic polymorphism between these plants by RAPD Profiling. RAPD is a technique that is based on the amplification of DNA by the use of the polymerase chain reaction (PCR) with short nonspecific primers. RAPD results in amplification of genome regions flanked by the specific priming sites. Our research work suggests that there is much lesser genetic variation between the two species. Both of these plants reproduced four highly monomorphic bands. Thus, study will help in determining genetic variation among *Bixa orellana* L. and *Salacia chinensis* L. which are medicinally important and will develop ways to conserve the medicinal aspects of them.

Key words: *Bixa orellana* L., *Salacia chinensis* L., RAPD, PCR, UPGMA, Genetic diversity

INTRODUCTION

Medicinal plants play a vital role to preserve our health, and though the science made improvements in diverse field, the traditional methods are still followed to identify potential the medicinal plants [1]. One of the important medicinal plant *Bixa orellana* L. (Family: Bixaceae) is being prescribed to cure gonorrhoea, dysentery and hepatitis. Its flowers are useful for treatment of cough, snakebites, irritable bowel and skin problems. It has been reported that it could be used as an anti-tumor and blood sugar lowering drug, due to its carotenoid content [2].

Salacia chinensis L. (Family: Celastraceae) has been used as a tonic, blood purifier and to treat various diseases like amenorrhea and dysmenorrhoea. Its root bark is used in gonorrhoea, rheumatism, skin diseases, asthma, ear diseases and hyperglycaemia [3].

Considering the medicinal importance of above mentioned plants, it is essential to explore, discover and conserve genetic diversity of these plant species. 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. The present research work is aimed to develop DNA fingerprints and to assess the possible inter-relation (if any) between the genes present in *Bixa orellana* L. and *Salacia chinens*is L.

MATERIALS AND METHODS

Plant material

The leaves of *Bixa orellana* L. Roxb and *Salacia chinensis* L. were collected from the medicinal plants garden of National Research Ayurvedic Institute of Basic Ayurvedic Sciences, Pune. The plant materials were verified by Mrs. A. G. Mhase, the botanist and the specimens were sterilized and preserved in the herbarium for reference.

DNA extraction

The leaves samples were crushed in liquid nitrogen to form a fine powder and were kept at -20°C until it can be assessed for generating DNA fingerprinting. The total genomic DNA extraction from the leaves of both the plants was done by using 3B BlackBio Biotech Biotools kit with approximately 100-120 mg of powdered sample of each.

DNA estimation

DNA quantification as well as quality assessment of both the plants was carried out spectrophotometrically using Biophotometer (Eppendorf). The absorbance of DNA was checked at 260 nm with a UV Vis Spectrophotometer. The quantity of extracted DNA was assessed using 2 % agarose gel electrophoresis in TBE buffer at 75 V for 45 minutes. The ethidium bromide (Amresco) was added into the molten agarose solution (2µl/25ml solution) before pouring. The visualization of the band of DNA was confirmed using UV trans-illuminator (GeNei) and photography was carried out using Gel Doc assembly (BioRad).

PCR Optimization

All the PCR reaction components were purchased from 3B BlackBio Biotech Biotools. Peltier P25^{+} (Cyber lab) thermal cycler was used to carry out the PCR reaction step. Best matched RAPD-PCR cycling conditions for *Bixa orellana* L. and *Salacia chinensis* L. was selected from four randomly selected Tm of DNA. The range of Tm was in between 37-45°C.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). The amplification conditions were 94^0C for 3 min, 94^0C for 45 sec, 44^0C for 30 sec, 72^0C for 1 min, 72^0C for 5 min, and 4^0C for the end hold. After the DNA was amplified, the PCR product was analyzed by loading on a 2% Agarose gel along with DNA Marker ladder (100-1000bp) and the run was carried out at 75 V for 45 minutes.

Screening of primer

Along With the DNA, 25 different primers were procured form Blackbio biotech Biotools and added in reaction mixture. The primers were screened and best primers producing multiple crispy bands were selected for constructing dendrograms for Phylogenetic 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.

RESULTS AND DISCUSSION

The quantities of the DNA extracted were 12.25 ng/ml for *Bixa orellana* L. and 7.65 ng/ml for *Salacia chinensis* L. The RAPD PCR was carried out as shown in fig 1 and 2 after the confirmation of undegraded DNA would be used as a template for amplification.

Fig 1 shows the RAPD Profiling of *Salacia chinensis* L. The lanes which shows the distinct patterns of respective primers are 3,5,7,8,10,12,13,20,22. Fig 2 shows the RAPD Profiling of *Bixa orellana* L. in which the lane 3,5,10,12 resulted in distinct band patterns for respective primers. The details of the primers which are used in RAPD Profiling are shown in table 1. The primers which showed the distinct, resourceful information and clear vision 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; if present, their intensity was at least 10% of the monomorphic reference band within the same lane. Marker ladder was used as a standard to outline the phylogenetic linkage between the two plants. In this study, the small similarity values revealed by RAPD markers provide greater confidence for assessment of genetic relationship among the *Bixa orellana* L. and *Salacia chinensis* L.

The DNA profiling is primarily used in plants for protection of biodiversity, identifying markers for traits, identification of gene diversity and variation etc. RAPD has become part of virtually every variation of the plethora of approaches used for DNA fingerprinting today. RAPD markers are also used for characterisation, estimation of genetic relatedness and determination of genetic diversity of plant germplasm. Tightly linked RAPD markers serve in turn as starting points for the characterization of genes without prior knowledge of their products or may render possible the physical characterization of large DNA fragments by pulsed field gel electrophoresis [4]. RAPD markers are based on the amplification of unknown DNA sequences using single, short and random oligonucleotides primers [1].

Even after several advancements in the area of genetic research and after the emergence of modern techniques like RFLP, AFLP; RAPD holds its special place and significance..One major application of molecular markers includes the use of fingerprint analysis in breeding programs to determine the relatedness of genotypes and in pedigree verification [5]. RAPD also have attractive features such as relatively low cost, the far lesser quantity and quality of DNA needed and speed of this type of analysis [6]. This technology also has a lot of potential in medical research, gene mapping, epidemiology, bacterial strain identification, examining interspecific hybridization, and the study of genetic variation in natural populations [7].

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. The practical approach developed in the study will be useful in DNA fingerprinting and identification of *Bixa orellana* L. and *Salacia chinensis* L. from the adulterants and substitutes. This will also makes identification and characterization of genotype very easy.

LIST OF ABBREVIATIONS

RAPD: Random Amplification of Polymorphic DNA
PCR: Polymerase Chain Reaction
dNTP: Deoxyribonucleotide triphosphate
Taq: *Thermus aquaticus*
UPGMA: Unweighted Pair Group Method with Arithmetical Averages

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FIGURES:

Figure 1: RAPD banding patterns of *Salacia chinensis* L.

(M represents Marker (ladder) and 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21 ,22,23 represent 25 universal primers containing lanes)

Figure 2: RAPD banding patterns of *Bixa orellana* L.

(M represents Marker (ladder) and 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21 ,22,23 represent 25 universal primers containing lanes)

TABLE:

Table 1: 25 Universal RAPD primers used for RAPD-PCR.

Sl. 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).

Figure 3: The Phylogenetic tree representing the relationships between the four primers RPI 3, RPI 5, RPI10, RPI 12, and RPI 15 by UPGMA Method.