RAPD-PCR ANALYSIS OF *BIXA ORELLANA* L. AND *COCHLOSPERMUM GOSSYPIUM* L. TO STUDY GENETIC DIVERSITY

V.E.VIJAYA SEKHAR, T. SUJATHA

Abstract: *Bixaorellana*L. and *Cochlospermum gossypium* L. are medicinal plants and native tropical trees of South 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 theseplants. Therefore, our aim was to analyse interrelationship and genetic polymorphism between these plants byRAPD Profiling. RAPD is a technique that is based on the amplification of DNA by the use of the polymerasechain reaction (PCR) with short nonspecific primers. RAPD results in amplification of genome regions flankedby 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 *Bixaorellana*L. and *Cochlospermum gossypium*L which are medicinally important and will develop ways to conserve the medicinal aspects of them.

Keywords: *Bixa orellana* L.,*Cochlospermum Gossypium* 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 potentialthe medicinal plants. One of the important medicinal plant *Bixaorellana* 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 beenreported that it could be used as an anti-tumorandbloodsugarloweringdrug,duetoitscarotenoidcontent.

Cochlospermum gossypium (*Bombax gossypium* L). hasbeen used as a tonic, blood purifier and to treat various diseases like amenorrhea aand dysmenorrhoea. Its root bark is used in gonorrhoea, rheumatism, skin diseases, asthma, ear diseases and hyperglycaemia.

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 *Cochlospermum gossypium* L.

Materials and Methods: The leaves of *Bixa orellana* L. Roxb and *Cochlospermum gossypium* L.were collected from the medicinal plants garden of National Research Ayurvedic Institute of Basic Ayurvedic Sciences, Pune. The plant materials were verified by Dr. Madhava Chetty 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 finger printing. The total genomic DNA extraction from the leaves of both the plants was done by using 3B Black Bio Biotech Biotools kit with approximately100-120mg 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 260nm with a UVV is Spectrophotometer. The quantity of extracted DNA was assessed using 2% agarose gel electrophoresis in TBE buffer at 75 Vfor 45minutes. The ethidium bromide (Amresco) was added into the molten agarose solution (2µl/25mlsolution) 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 usedto carry out the PCR reaction step. Best matched RAPD-PCR cycling conditions for *Bixaorellana* L and *Cochlospermum gossypium* 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 standardized to a total volume of 20µl containing

nuclease free water (13µl),10XPCR Buffer(2µl), $MgCl_2$ (1.5µl), dNTP (1µl), Primers (1µl),Template DNA (1µl), Taq polymerase (0.5µl).The amplification conditions were 94°C for 3min, 94°C for 45 sec, 44°C for 30 sec, 72°C for 1min, 72°C for 5 min, and 4°C 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 75V for 45minutes.

Screening of Primer: Along With the DNA, 25 different primers were procured form Black biobiotech 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 dendrogramsby 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 12.25ng/ml for *Bixaorellana* L.and 7.65 ng/ml for *Cochlospermum gossypium* 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 *Cochlospermum gossypium* L. The lanes which shows the distinct patterns of respective primersare3, 5, 7, 8, 10, 12, 13, 20, 22. Fig. 2 shows the RAPD Profiling of *Bixa orellana* L. in which the lane3, 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 with in 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 *Bixaorellana* L and *Cochlospermum gossypium* 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 finger printing 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. RAPD markers are based on the amplification of unknown DNA sequences using single, short and random oligo-nucleotides primers.

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 inbreeding programs to determine the relatedness of genotypes and in pedigree verification. RAPD also have attractive features such as relatively low cost, the far lesser quantity andquality of DNA needed and speed of this type of analysis. This technology also has a lot of potential in medical research, gene mapping, epidemiology, bacterial strain identification, examining inter-specific hybridization, and the study of genetic variation in natural populations.

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 finger printing and identification of *Bixa orellana* L. and *Cochlospermum gossypium* L from the adulterants and substitutes. This will also makes identification and characterization of genotype very easy.

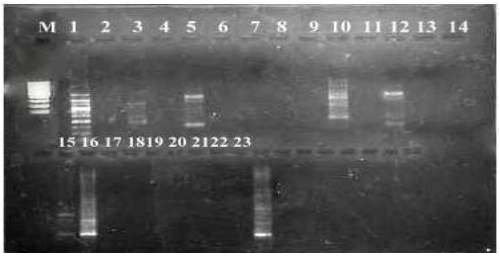

Figure 1: RAPD banding patterns of *Cochlospermum gossypium*L..

(M represents Marker (ladder) and1, 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.

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

Sl.No	Name of Primer	Accession Numbers	Sl.Name No.of Primer		Accession Numbers
1	RPl1	AM765819	14	RPl14	AM773774
2	RPl2	AM750044	15	RPl15	AM773775
3	RPl3	AM773310	16	RPl16	AM773776
4	RPl4	AM773769	17	RPl17	AM911710
5	RPl5	AM773770	18	RPl18	AM765830
6	RPl6	AM773771	19	RPl19	AM773777
7	RPl7	AM773312	20	RPl20	AM773317
8	RPl8	AM773773	21	RPl21	AM765820
9	RPl9	AM773315	22	RPl22	AM911711
10	RPl10	AM750045	23	RPl23	AM911712
11	RPl11	AM911709	24	RPl24	AM765821
12	RPl12	AM773316	25	RPl25	AM750054
13	RPl13	AM750046			

*(RPI1-RPI25indicates the Universal primers).

Abbreviations

RAPD: Random Amplification of PolymorphicDNA
PCR:PolymeraseChainReaction
dNTP: Deoxyribonucleotide triphosphateTaq: *Thermusaquaticus*
UPGMA: Unweighted Pair Group Method withArithmetical Averages

References:

1. Neetu,V., Sairkar,P.,*etal.*,(2009), Genetic variability in *Asparagus racemosus* (Willd.) from Madhya Pradesh, India by random amplified polymorphic DNA. African Journal of Biotechnology. 8(14): 3135-3140.
2. Raga,D.D., and Espiritu, R.A.(2011),

Abioactive sesquiterpene from *Bixaorellana*, Journal of Natural Medicine. 65:206-211.
3. Deokate,U.A., and Khadabadi, S.S.(2011),Phytopharmacological aspects of *Salaciachinensis* L., Journal of Pharmacognosy and Phytotherapy. 4(1): 1-5.
4. Gahlaut, A., Gothwal, A., *et al*., (2013), RAPDpatterns of some important medicinal plants andtheir substitutes used in Ayurveda to identify the genetic variations, International Journal of Pharmacy and Pharmaceutical Sciences.5(1):239-241.
5. Jondle,R.,(1992),Legalaspectsofvarietalprotectionusingmolecularmarkers,In:ApplicationsofRAPDtechnologytoplantbreeding,Jointplant breedingsymposiaseries,Minnesota, USA.: 50-52.
6. Hahn,W.J.,andGrifo,F.T.(1996),MolecularMarkersinPlantConservationGenetics,TheImpactofPlantMolecular Genetics, 113-136.
7. Bio Feedback.(1993), Artifactual Variation in Randomly Amplified Polymorphic DNA Banding Patterns, BioTechniques. 14: 14-17.

***

V. E.Vijaya Sekhar
Lecturer in Botany A. C. College, Guntur, A.P.
Email: ezravsekhar @gmail.com
T. Sujatha
Lecturer in Chemistry, Sri Sarathi Institute of Engineering & Technology,
Nuzividu, Krishna Dist, A.P.