Research Paper

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Molecular Characterization of Medicinal Plant Species of Acathaceae Family Using Rapd Molecular Markers

Biotechnology

KEYWORDS : Acanthaceae, Genetic diversity, RAPD, Dendrogram analysis.

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ABSTRACT

Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products. The history of plants being used for medicinal purpose is probably as old as the history of mankind. In this study the genetic diversity among 6 different species belonging to Acanthaceae family was evaluated using RAPD (Random Amplified polymorphic DNA) technique. Two arbitrary primers namely OP A8 and OP D18 were used. Both the primers exhibited polymorphism of 66.66 % and 58.62 % respectively. The dendrogram was constructed using unweighted pair group method with arithmetic averages (UPGMA), and the genotypes were differentiated into various clusters. These results indicate that RAPD is a useful tool for differentiating various species belonging to Acanthacea family.

1. INTRODUCTION

The large and primarily tropical plant family Acanthaceae contains at least 4000 species, placing it among the top 12 or so most diverse families of flowering plants. The group is part of the order Lamiales, which includes familiar plants such as snapdragons (*Antirrhinum*, Plantaginaceae), mints (Lamiaceae), African violets (Gesneriaceae), and monkey flowers (*Mimulus*, Phyrmaceae). Notably, as a result of the largely tropical distribution of Acanthaceae, species-level diversity (as well as some generic diversity) remains poorly understood, and there is little doubt that many new species remain to be discovered. A wide variety of essential and medicinally active ingredients have been identified and extracted from some members of the family. Many scientists have worked on some members of the family and have discovered some very essential active ingredients present in them [2].

Identification of plants at the species level is traditionally achieved by careful examination of the specimen's macroscopic and microscopic morphology. However, morphological identification is often not possible when the original plant material has been processed. Therefore, additional methods of identification at the species level have been sought and genome-based methods have been developed for the identification of medicinal plants starting in the early 1990 s. This work followed in the footsteps of the use of DNA for plant systematics in the preceding two decades and was greatly facilitated by the invention of the polymerase chain reaction (PCR) and the introduction of a heat-stable DNA polymerase from the thermophilic bacterium *Thermus aquaticus* [10]. Together, these two achievements have revolutionized the way scientists work with DNA and made molecular cloning and DNA-based analysis accessible to workers in virtually every field concerned with living matter. In fact, molecular taxonomists now envision cataloging all living species on earth using so-called DNA barcodes, the nucleotide sequence of a short DNA fragment

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. The chromatographic techniques and marker compounds which are used to standardize Ayurvedic formulations has limitations because of variable sources, chemical complexity and the amounts of secondary metabolites which is influenced by the environmental factors. DNA fingerprinting distinguishes different varieties according to their DNA variations at a set of genetic loci. Powerful and convenient molecular marker system, randomly amplified polymorphism of DNA (RAPD) analysis is widely used for the genetic mapping, taxonomic and polygenic studies of many plants. Their environmental stability and nearly unlimited availability have made RAPD markers an ideal tool for plant [3, 9]. RAPD maps have been or are being constructed for the genomes of various major crop plants. RAPD markers also provide useful tools for both evolutionary studies and the characterization of germ plasm stocks, [5,6].

2. MATERIALS AND METHOD

2.1 Sample Collection:

For the present study, six different species belonging to Acanthaceae family namely *Adathoda beddomei, Adathoda zeylanica, Barleria prionitis, Justicia gendarussa, Nigirianthus sp.,* and *Hemigraphis colorata* were collected. Keeping in view the criteria for the collection, fresh leaves were collected from similar environmental conditions for DNA isolation studies. Young leaves of the respective species were collected in sterile Ziploc bags and stored at -20 °C until further use.

2.2 Extraction of DNA

DNA was isolated from the leaf samples according to Ahmad et al., 2004 with modifications. 0.5 g of the leaf samples were ground well with 2 mL STE buffer (0.4 M sucrose; 2 mM Tris-HCl (pH8.0); 20 mM EDTA-Na2) in a sterile pestle and mortar. To this, 300 µl of 20 % SDS was added slowly by continuous grinding. The ground samples were transferred into 2 mL centrifuge tubes. To the tubes 200 µL of 8M LiCl, 20 µl of β-mercaptoethanol and a pinch of PVP were added and the contents were mixed thoroughly. Then the centrifuge tubes were incubated in a water bath at 60 °C for 45 minutes. After cooling the contents to room temperature, Chloroform:Isoamylalcohol (24:1) was added to all the centrifuge tubes and mixed well. The tubes were centrifuged at 13400 rpm for 10 minutes at 4 °C. After centrifugation, the supernatant was transferred into a new centrifuge without disturbing the middle protein layer. The Chloroform:Isoamylalcohol wash was repeated again and similarly the supernatant was removed & transferred into new tubes. To the supernatant ice cold ethanol was added in equivalent amounts to all vials in order to view the strands of DNA. Centrifugation at 10000 rpm was done again for 10 minutes. The obtained pellet was washed with 70% ethanol twice and centrifuged at 10000 rpm for 10 minutes. Then the supernatant was removed and pellet was air dried. The pellet was dissolved in 50 – 100 µl nuclease free water and stored at 4 °C until further use.

2.3 Estimation of DNA:

2.3.1 Qualitative estimation of DNA

The quality of the DNA was estimated using Agarose gel electrophoresis. The obtained DNA was run on 0.8 % Agarose gel stained with Ethidium bromide at 80 – 90 V for 1 hour followed by viewing the gel under UV transilluminator.

2.3.2 Quantitative Estimation of DNA

The quantity of the isolated DNA was further estimated by measuring the OD 260 and OD 280 or absorbance ratio. DNA yield and quality was calculated using a NanoDrop ND-1000 spectrophotometer (ThermoScientific) at 260 nm.

2.4 PCR Amplification using RAPD markers:

The seven DNA samples were subjected to PCR amplification with 2 RAPD primers namely OP A8 and OP D18. The total volume of the PCR reaction was 25 µL, which contained 1 µL of template DNA, 2.5 µL of 10x Taq buffer with $MgCl_2$ (10 mM), 1.5 µL of 2.5 mM dNTPs, 2 µL of Taq polymerase (Chromous), and 1 µL of 10 pmol RAPD primer. The PCR cycle was carried out with the initial denaturation at 94 °C for 2 min followed by 36 cycles of 94 °C for 45 s, 37 °C for 1 minute, 72 °C for 1 minute and a final extension of 72 °C for 10 minutes. The obtained PCR product was viewed in 1.5% agarose gel stained with ethidium bromide.

3. RESULTS

3.1 Estimation of DNA:

3.1.1 Qualitative estimation of DNA

The qualitative analysis of DNA was estimated using Agarose gel electrophoresis as shown in Figure-1. The obtained DNA showed sharp single bands on 0.8% agarose gel without any degradation or RNA contamination.

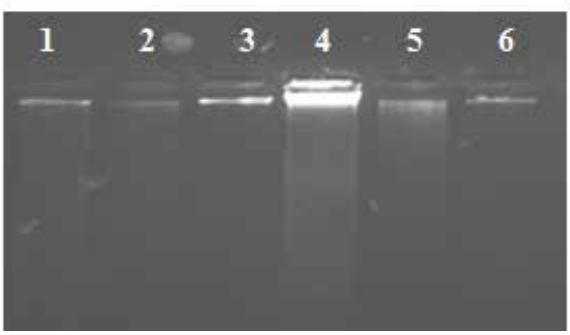

Figure 1: Isolated genomic DNA bands of various Acanthaceae species in the order (1) Adathoda beddomei, (2) Adathoda zeylanica, (3) Barleria prionitis, (4) Justicia gendarussa, (5) Nigirianthus sp., and (6) Hemigraphis colorata

3.1. 2 Quantitative estimation of DNA

Spectrophotometric analysis of the isolated DNA samples showed concentration in ng/µl ranging from 328.4 – 543.3 ng/µl. The 260/280 ratio of the samples obtained were found which indicates the presence of pure DNA (Figure-2)

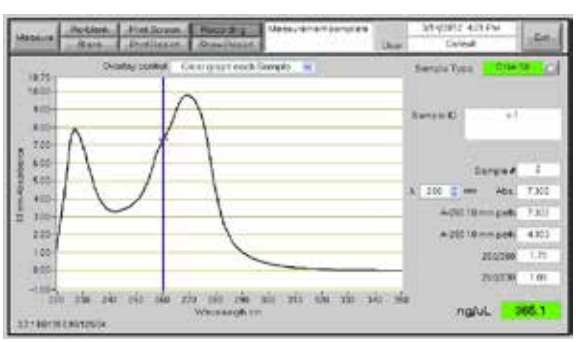

Figure 2: Spectra of genomic DNA obtained by Nanodrop

3.2 PCR Amplification using RAPD markers:

In the present study, for RAPD- PCR two primers namely OP A8 and OP D18 were used. The amplification observed using both the primers are shown in Figure-3 andFigure-5. The banding pattern score was based on the presence or absence of clear, visible and reproducible bands. 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. In this study, both the primers produced a total of 42 and 29 bands respectively.

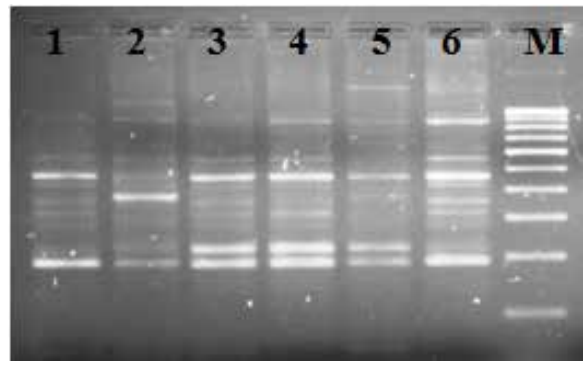

Figure 3: Amplification using OP A8

Among the bands obtained using primer OP A8, 66.66 % were polymorphic, 28.57 % were monomorphic and 4.76 % were unique Table-1. The obtained RAPD data was further utilized for construction of dendrogram and similarity matrix Figure-4.

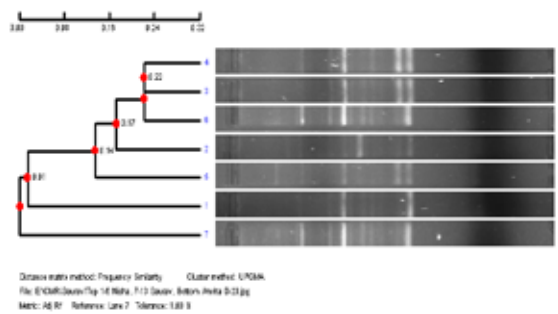

Figure 4: Dendrogram constructed using OP A8

	1	2	3	4	5	6
1	100.00	40.00	50.00	40.00	40.00	50.00
2	40.00	100.00	70.00	80.00	100.00	70.00
3	50.00	70.00	100.00	70.00	70.00	80.00
4	40.00	80.00	70.00	100.00	80.00	70.00
5	40.00	100.00	70.00	80.00	100.00	70.00
6	50.00	70.00	80.00	70.00	70.00	100.00

Table-1: Simlarity matrix using the RAPD data of OP A8

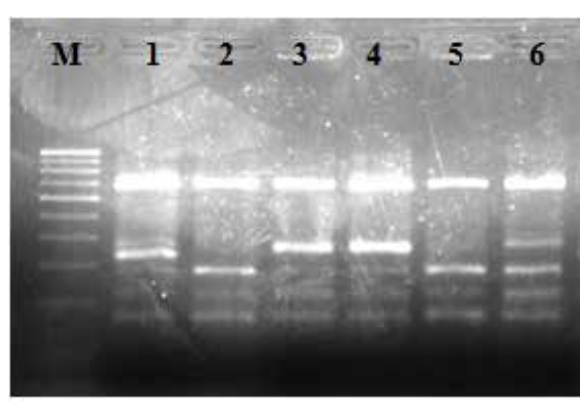

Figure 5: Amplification using OPD 18

Among the bands obtained, 58.62 % were polymorphic and 41.37 % were monomorphic Figure-6. This RAPD data was further used for dendrogram analysis.

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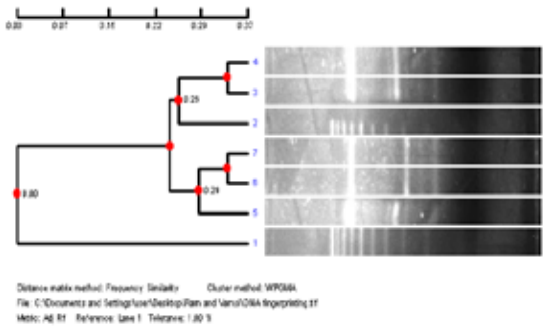

Figure 6: Dendrogram of OP D18

	1	2	3	4	5	6
1	100.0	75.00	81.25	81.25	62.50	68.75
2	75.00	100.0	75.00	68.75	75.00	81.25
3	81.25	75.00	100.00	93.75	81.25	87.50
4	81.25	68.75	93.75	100.00	81.25	87.50
5	62.50	75.00	81.25	81.25	100.00	75.00
6	68.75	81.25	87.50	87.50	75.00	100.0

Table-2: Simlarity matrix using the RAPD data of OP D18

The dendrogram obtained using primer OP A8 showed that, sample 4 (*Justicia gendarussa*) showed 80 % similarity with sample 5 (*Nigiranthus sp.*) and sample 2 (*Adathoda zeylanica*) respectively. Whereas sample 2 (*Adathoda zeylanica*) and sample 1 (*Adathoda beddomei*) exhibited the least similarity. Similarly, dendrogram using primer OP D18 showed that sample 3 (*Barleria prionitis*) and sample 4 (*Justicia gendarussa*) showed maximum similarity of 93.75 % and the least similarity of 62.50 % was observed among sample 1 (*Adathoda beddomei*) and sample 5 (*Nigiranthus sp.*) Table-2.

4. DISCUSSION

The present study deals with, establishing a phylogenetic relationship between various species of medicinal plant belonging to Acanthaceae family using RAPD markers. In this study six *Acanthaceae sp.* were selected. The novelty of this project lies in the use of different molecular markers with increasing order of specificity to study genetic diversity which will help in development of new herbal medicines and phytochemical drugs derived naturally in order to help the human in all ways.

The adversity surrounding the identification of herbs in traditional medicine has been reported in various studies [1, 11]. RAPD analysis has been widely used to differentiate between many medicinal species and their close relatives. The advantages of this technique include its speed, simplicity and the requirement for only small amounts of DNA [4]. Indeed, RAPD analysis has been successfully used for taxonomic and systematic classification, and phylogenetic or genetic diversity studies in plants [7]. Similar results using molecular markers were reported by [8] showing no genetic variation among *A. paniculata* accessions from nine areas throughout Thailand.

In the present study, primer OP A8 showed high degree of polymorphism compared to OP D18 suggesting that it could be used as a suitable molecular markers for diversity analysis The technique employed in this study has the advantage of being inexpensive to perform, and does not require a previous knowledge of the genome. The dendrograms obtained using the RAPD data separated the samples into different clusters. The primary clusters were then further sub divided into subclusters as seen in dendrograms. The percentage similarity observed among various samples gave a clear understanding about the diversity in Acanthaceae family.

5. CONCLUSION

The RAPD, analysis in the present study has proven to be useful in discrimination, characterization and differentiation among various medicinal plants belonging to Acanthaceae family. Despite the enormous and similar discriminating potential of the two markers used they showed some differences in their discrimination capacities. The genetic data collected during this work will guide the choice of genotypes to cross according to their lineage belonging or their level of diversity.

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