

Research Article

Evaluation of Genetic Diversity in *Cardiospermum halicacabum* based on Geographical Variation Using RAPD Markers

Annamalai A.^{1*}, Christina V.L. and Lakshmi P.T.V.²

¹Department of Biotechnology, School of Biotechnology and Health Sciences, Karunya University, Coimbatore, Tamil Nadu, India.

²Center for Bioinformatics, School of Life Sciences, Pondicherry University, Puducherry, India.

ABSTRACT: **Background:** Genetic diversity at the intra species level arises from differences in climatic conditions, geographical distribution and environmental stress. Thus the study of genetic diversity assists in determining the phylogeny of a particular plant species and the impact of geographical locations on the plant genome. **Objective:** To assess the genetic diversity and phylogeny of *Cardiospermum halicacabum* species' from different regions within the state of Tamil Nadu in India. **Materials and Methods:** Plant DNA was isolated and amplified using eight random primers. The presence of amplified DNA bands was recorded as a 1, while the absence of any bands was recorded as a 0. The percentages of polymorphism were determined using the ratio of similarity to dissimilarity of band pattern. The data was analysed using the VisionWork LS Analysis Software to construct a dendrogram to create a phylogenetic tree, for cluster analysis using UPGMA (Unweighed Pair Group Method with Arithmetical Mean). **Results:** RAPD analysis showed varied amplifications for each sample. The polymorphism was 29.41% and the number of polymorphic fragments ranged from 603 bp to 6557 bp. The dendrogram revealed two clusters, Cluster I included the sample from Chennai, while Cluster II included samples from Dharmapuri, Thanjavur, Thirunalveli, Coimbatore and Madurai, thus showing the extent of intra-specific variation. **Conclusion:** Variation in geographical plant location was consistent with genetic differences. Therefore DNA banding patterns may be used as a fingerprint marker for the identification of *Cardiospermum halicacabum* species.

KEYWORDS: Genetic diversity, RAPD, phylogenetic tree, *Cardiospermum halicacabum*.

INTRODUCTION

Genetic diversity is the reason for inter-species differences. This diversity is also observed within species, and in plants this variation can be a result of difference in climatic conditions, geographical distribution and environmental stress. The genetic diversity may not result in morphological changes to a great extent but creates variations in the phytochemical compositions of plants^[1-3] and their medicinal properties. Genetic diversity help detect the phylogeny of a particular plant species, and experiments such as RAPD (Random Amplified Polymorphic DNA) show the extent of genetic polymorphism observed within a particular species, despite morphological similarities.^[4-5]

In the present study, RAPD analysis was conducted on a single plant species *Cardiospermum halicacabum*, from various geographically distributed regions of Tamil Nadu, India. This analysis of the plant DNA is a simple and effective method of revealing the extent of polymorphism that can occur due to genetic variation. Since RAPD requires no prior information of the plant genome as is the case for VNTRs (Variable Number of Tandem Repeats) and ISSRs (Inter Single Sequence Repeats),^[6] it is an ideal method to analyze genetic polymorphisms in the plant genome. While RFLP and AFLP methods can also be implemented, these have proven to be a costly and time consuming method of analysis.

Cardiospermum halicacabum is a foliaceous creeper found in tropical regions, which belongs to the *Sapindaceae* family. It is a deciduous climber growing to 3m in height with tri-lobed, highly dentate leaves and flowers between July and August, with seeds ripening between August and October. The flowers are hermaphrodites and its 3 cm long, green fruit is an almost entirely spherical capsule containing a characteristic seed with a heart-shaped white

*Correspondence:

E-mail: aannamalai2001@yahoo.com;

drannamalai@karunya.edu

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region on the testa. The plant prefers moist soil and a sunny sheltered position,^[7] but succeeds in most soils.^[8]

The entire plant is diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific.^[9] External application of the oil from the leaves are effective for arthritis, while the juice of the leaves can be used for ear ache.^[10] The leaves are used as a poultice for skin eruptions while the while the seeds are used as a tonic for fever and rheumatism.^[11] It is occasionally used in the treatment of lumbago and nervous diseases.^[12] It is also known to be used to treat throat infection and headache.^[13]

MATERIALS AND METHODS

Plant material

Cardiospermum halicacabum was collected from six different geographical locations in Tamil Nadu, India (Chennai, Coimbatore, Dharmapuri, Madurai, Triunelveli and Thanjavur), shown in Figure 1. Fresh leaves from the tender parts of the plant were used for DNA extraction.

Reagents

Reagents required for DNA isolation includes, CTAB Extraction buffer consisting of 2% (w/v) CTAB; 20mM EDTA, pH 8.0; 100mM Tris-HCl, pH 8.0 and 1.4 M NaCl, CTAB / NaCl solution comprising of 10% (w/v) CTAB and 0.7M NaCl, CTAB precipitation solution of 1% (w/v) CTAB; 50mM Tris- HCl, pH 8 and 10mM EDTA, pH 8.0. Random primers mentioned in Table 1, were obtained from Sigma – Aldrich, (Chennai, India).

DNA Isolation

DNA was isolated from fresh samples using modified Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method.^[14,15] Briefly, 0.5g of fresh leaf tissue was ground into a fine power using liquid nitrogen, and freshly prepared

extraction buffer containing 3% (w/v) PVP and 2% (v/v) mercaptoethanol was added and the mixture incubated for 30 min at 65°C. After cooling to room temperature, an equal amount of chloroform - isoamyl alcohol (CI) (24:1) was added and samples were to the mixture and centrifuged at 10,000 rpm using an Eppendorf refrigerated centrifuge, for 10min at 25°C. Supernatant was treated with CTAB/NaCl soln at 65°C and again treated with CI (24:1) after centrifugation. DNA was precipitated using CTAB precipitation solution at 65°C. The pellet thus acquired after centrifugation at 2,700 rpm for 5 min was washed using 80% and 100% ethanol and re-suspended in TE Buffer. DNA concentration was determined spectrometrically by measuring the absorbance at 260nm and 280nm. 1.4% Agarose gel was also used to determine DNA concentration by comparing the band intensities using Lambda DNA marker.

PCR for RAPD

Eight random decamer primers, OPA01, OPA02, OPA04, OPA08, OPA11, OPC13, OPC14 and OPC15 were used.^[16] Isolated DNA was used as a template (50ng – 2.0µl) for PCR reactions carried out in 25µl tubes containing: 8.0µL Primer (2µM/µl), 5.0µl 10X Buffer, 5.0µl 2mM dNTP mix, 0.5µl Taq DNA Polymerase and 6.5µl sterilized distilled water. Samples were subjected to an initial temperature of 94°C for 5 min, followed by 45 cycles (denaturation at 94°C for 40 sec, annealing temperature of 36°C for 30 sec and elongation of 72°C for 90 sec) and a final extension of 72°C for 5 min.^[17] Amplified products were resolved by electrophoresis using 1.8% agarose gels in 1X TAE buffer with 0.5µg/ml ethidium bromide for 2-3 hrs at 60-80 volts. Fractionated DNA bands were viewed under UV light using a UV trans-illuminator and photographed.

RAPD Data analysis

The presence of DNA bands was recorded as 1 whereas absence of band as 0. Smear bands were not included. Therefore the percentage of polymorphism was determined via the ratio of similarity to dissimilarity of band patterns. The data obtained was used to construct a dendrogram using Phylip 3.69 software, to generate a phylogenetic tree,^[18] for cluster analysis using UPGMA (Unweighted pair group method with arithmetic average).



Figure 1: Geographical distribution of places from where the plant samples were collected

Table 1: Sequences of the decamer primers used for RAPD profiling

Primer	Nucleotide sequence
OPA01	5'CAGGCCCTTC3'
OPA02	5'TGCCGACCTG3'
OPA04	5'AATCGGGCTG3'
OPA08	5'GTGACGTAGG3'
OPA11	5'CAATCGCCGT3'
OPC13	5'AAGCCTCGTC3'
OPC14	5'TGCGTGCTTG 3'
OPC15	5'GACGGATCAG3'

RESULTS

Only two of the primers used in this study (OPC13 and OPC15) led to DNA amplification. The OPC15 primer yielded a variety of bands while primer OPC13 primer produced a single band from all samples. Thus for further studies banding patterns of OPC15 primer were analysed. Here the number of polymorphic fragments ranged from 603 bp to 6557 bp (Figure 2) and polymorphism was found to be 29.41%. A total of 51 bands were observed. Samples from Madurai and Chennai showed 0% polymorphism while other samples showed 20% - 47% polymorphism (Table 2). RAPD PCR results were analyzed using UVP Bioimaging, VisionWork LS Analysis Software. The amplified bands

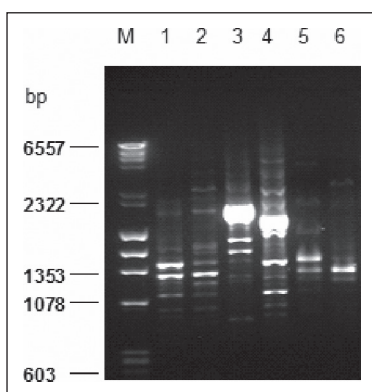


Figure 2: RAPD pattern obtained with primer OPC15, M- λ DNA marker, Lanes: 1 - C1, 2 - C2, 3 - C3, 4 - C4, 5 - C5, 6 - C6.

were used to generate a dendrogram (Figure 3), based on UPGMA (Unweighed Pair Group Method with Arithmetical Mean) analysis with Jaccard's similarity co-efficient to calculate the genetic distance among the 6 samples. Thus two clusters were observed. Cluster I which consisted of sample C6 alone with a genetic distance of 0.576 and Cluster II which consisted of samples C2, C4, C5, C1 and C3. However Cluster II further shows two sub-clusters showing a high rate of similarity among samples C2 and C4 with a genetic distance of 0.100 and among samples C5, C1 and C3 with a genetic distance of only 0.143 between C1 and C3.

DISCUSSION

Numerous plant species have been identified using RAPD analysis. In the present study numerous RAPD DNA markers were generated per for all 6 isolates. This simple technique permits genetic diversity to be identified in terms of the presence or absence of the number of bands.¹⁹ Thus RAPD analysis is a useful diagnostic tool for population strains at the species level,^{20,21} or in the construction of SCAR markers.²²

Our study revealed that the GC rich primer OPC15 generated the most products. This is in keeping with other reports that show that for good amplification of plant DNA, GC rich primers are more suitable.²³ By comparing the genetic distance we can conclude that samples C2 and C4 are closely related since the genetic distance between them is very less while sample C6 shows high degree of

Table 2: Summary of RAPD banding pattern obtained with primer OPC15

Sample	Polymorphic bands	Monomorphic bands	Total no. of bands	% of polymorphism
C1	0	6	6	0
C2	4	9	13	30.7
C3	2	6	8	25
C4	8	9	17	47
C5	1	4	5	20
C6	0	2	2	0

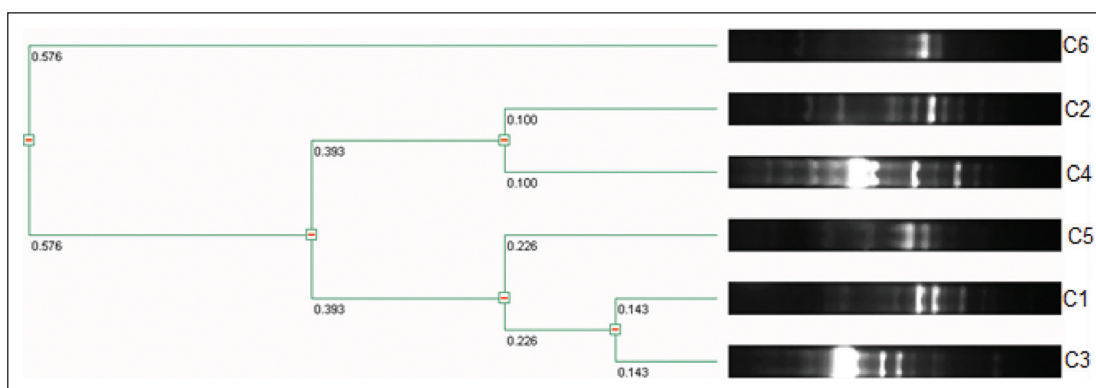


Figure 3 : Cluster analysis of *C. halicacabum* samples based on RAPD data using UPGMA. Values on the dendrogram branches represent the genetic distance among the samples.

Table 3: Details about the plant samples used in the study

Code number	Place of collection	Climate	Soil
C1	Madurai	hot, semi-arid	red, loamy
C2	Dharmapuri	hot, dry	red, loamy
C3	Thanjavur	warm	grey, clay loamy
C4	Tirunelvali	tropical	grey, sandy clay loamy
C5	Coimbatore	tropical wet and dry	fine loamy
C6	Chennai	hot, humid	clay

variance from the rest of the samples. The high variation in the Chennai sample can be explained to be due to the climatic and geographical variation of this region from the other five regions as shown in Table 3.

This study indicates that phylogenetic reconstruction is one approach in providing an understanding of the genetic diversity in medicinal plants and how climatic and geographical factors play a major role in species diversity. The genetic make-up of plants and their variation may also suggest a variation in the various phenotypic characteristics for which these genes may be responsible. Common DNA bands arising from RAPD analysis for these isolates can also be used in the identification of this particular plant species, thereby providing a molecular method for the detection of adulteration in the plant which takes place through substitution with morphologically similar species.

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