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T. Amutha Priya
Lab of Developmental Biology and Plant Biotechnology SPK Centre for Environmental Sciences Manonmaniam Sundaranar University, Tamil Nadu, India

V. Manimekalai
Lab of Developmental Biology and Plant Biotechnology SPK Centre for Environmental Sciences Manonmaniam Sundaranar University, Tamil Nadu, India

P. Ravichandran
Lab of Developmental Biology and Plant Biotechnology SPK Centre for Environmental Sciences Manonmaniam Sundaranar University, Tamil Nadu, India

Correspondence:
T. Amutha Priya
Lab of Developmental Biology and Plant Biotechnology SPK Centre for Environmental Sciences Manonmaniam Sundaranar University, Tamil Nadu, India

Intra Specific Genetic Diversity Studies on C*alotropis gigantea* (L) R. Br. - Using RAPD Markers

T. Amutha Priya, V. Manimekalai, P. Ravichandran

Abstract
Calotropis gigantea (L.) (Asclepiadaceae) is an important medicinal plant which exhibits variations in flower color, morphology and anatomy. Taxonomists treat these variants as two different forms due to contrasting floral features. Hence, a molecular marker based study using random amplified polymorphic DNA (RAPD) was carried out to ascertain the origin of morphological variations as well as to check whether the floral variation is due to phenotypic or genotypic factor. Further the study was aimed to assess the genetic diversity of these two forms collected from ten different accessions. Genomic DNA was isolated and purified from flower petals using the procedure described by Jobes *et al*., with minor modifications. The DNA content was quantified and purity was checked using Spectrophotometer. Twenty decamer random operon primers were used to amplify the DNA and RAPD-PCR was done. High level of genetic similarity was observed between the white and pink form accessions. Ten random primers, each with 10 bases generated a total of 16 polymorphic bands out of 209 total bands. In addition to the morphological variations a significant level (16 %) of polymorphism of was observed. Overall genetic similarity based on 10 random primers was 84 %. Cluster analysis was carried out based on UPGMA Jaccard coefficient. The results reveal relatively a rich source of genetic diversity of the selected variants. The observed variation and polymorphism among the morphological forms deemed to be worth for conservation of *C. gigantea*. The molecular variations and data obtained from these two forms can be of taxonomic importance. Further analysis involving ISSR or mini/micro satellite markers is required to confirm the results obtained.

Keywords: Genetic diversity, *Calotropis gigantea*, RAPD-PCR, primers, ISSR

Introduction
Calotropis gigantea is a common xerophytic perennial shrub. This plant is a soft-wooded, evergreen, perennial shrub. *C. gigantea* ranges from 8 to 10 feet in height, and grows in various climates and soils, and sometimes in habitats where nothing grows. Consequently, it is a common wasteland weeding [1]. *Calotropis* is used many diseases such as fevers, rheumatism, indigestion, cough, cold, eczema etc [2]. *Calotropis gigantea* yields a durable fibre (commercially known as bowstring of India) useful for roots, carpets, fishing nets, and sewing thread. Molecular techniques have found to be more useful and accurate for determination of both intra and inter specific variations in plants. Randomly amplified polymorphic DNA (RAPD) markers, in particular, have been successfully employed for determination of intra-specific genetic diversity in several species. These include date palm [3]. Detection and analysis of genetic variation can help us to understand the molecular basis of various biological phenomena in plants. Since the entire plant kingdom cannot be covered under sequencing projects, molecular markers and their correlation to phenotypes provide us with requisite landmarks for elucidation of genetic variation. Molecular marker based study using random amplified polymorphic DNA (RAPD) was carried out to ascertain the origin of morphological variations as well as to check whether the floral variation is due to phenotypic or genotypic factor.

Materials and Methods
Calotropis gigantea plants were collected from Ambur, Alwarkurichi, Tenkasi, and Papanasam, along the road sides and wastelands of Tirunelveli district, South India. The plants were maintained in the centre's nursery for tissue culture and further genetic diversity studies.

Plant Material and DNA extraction
Calotropis gigantea two forms collected genomic DNA was isolated from flower petals by [4] with minor modifications.

RAPD analysis
Twenty decamer random operon primers were used to amplify the DNA and RAPD-PCR was done according to the method described by [5] with minor modifications. Amplifications were carried out in volumes of 25µL containing 40 ng of template DNA, 100µM of each dNTP, 1.5mM $MgCl_2$, 25 picomoles, Oligonucleotide primer, 0.5U Taq DNA polymerase (Gene) in 1X PCR Buffer (10 mM Tris-HCl, 50mM KCl) In Biorad thermal cycler programmed for: 5 min of initial denaturation (94 ^{0}C), 42 cycles of 1min at 94^{0}C, 1min at 37^{0}C and 2 min at 72^{0}C, with final extension at 72^{0}C for 7 min. A negative run was added in each run to test for contamination.

Agarose gel electrophoresis
Amplified products were electrophoresed on 1.5% agarose gel using 1X TBE buffer (89mM Tris-borate, 2.5mM EDTA) and visualized by ethidium bromide staining. The patterns were photographed and stored as digital pictures in gel documentation system (Bio rad). Low range PCR Ladder were used to establish molecular weight of the product the reproducibility of the amplification was confirmed by repeating each experiment three times.

Data analysis
DNA banding patterns generated by RAPD were scored for the presence (1) or for absence (0) of each amplified band. All RAPD assays were repeated twice and only the reproducible bands were scored. For considering a marker as polymorphic, the absence of an amplified product in at least one species was used as a criterion. For genetic distance analysis, using NTSYS software Cluster analysis was based on similarity matrices using the unweighted pair group method analysis (UPGMA) program in the software package. The Jaccard coefficient was used for dendrogram construction.

Results and discussion
In RAPD analysis using 20 random primers, only 10 random primers could be amplified. The results reveal relatively a rich source of genetic diversity of the selected variants. High level of genetic similarity was observed between the white and pink form accessions. Ten random primers, each with 10 bases generated a total of 16 polymorphic bands out of 209 total bands (table1). In addition to the morphological variations a significant level (16 %) of polymorphism of was observed. Overall genetic similarity based on 10 random primers was 84 %. Cluster analysis was carried out based on UPGMA Jaccard coefficient. The results reveal relatively a rich source of genetic diversity of the selected variants. The observed variation and polymorphism among the morphological forms seemed to be worth for conservation of *C. gigantea* (Figs. 1 – 4 & Dendrogram).

Table: 1 Details of primers used for amplification

S. No	Name of Primer	Sequence of the primer	Amplications size range (300-1500bp)	Total no. of amplified bands	Total No. of polymorphic bands	% polymorphism
1.	OPH-18	GAATCGGCCA	550-1500bp	16	6	38%
2.	OPM-12	GGGACGTTGG	400-1500bp	33	10	30%
3.	OPM-13	GGTGGTCAAG	550-900bp	14	4	29%
4.	OPM-14	AGGGTCGTTC	550-300bp	19	1	5%
5.	OPAL-11	GTCACGTCCT	200-300bp	21	3	4%
6.	OPD-14	CATGCCAGAC	300-1500bp	18	0	0%
7.	OPA-04	AATCGGGCTG	550-300bp	23	0	0%
8.	OPAK-14	CTGTCATGCC	200-1000bp	28	3	11%
9.	OPM-15	GACCTACCAC	200-8000bp	17	2	12%
10.	OPU-13	GGCTGGTTCC	200-900bp	20	4	20%
Total				209	33	16%

Calotropis gigantea (L.) (Asclepiadaceae) is an important medicinal plant which exhibit variations in flower color, morphology and anatomy. Taxonomists treat these variants as two different forms due to contrasting floral features. But till date no report on the molecular data, except for assessment for genetic diversity in *Calotropis procera* [6]. Hence, a molecular marker based study using random amplified polymorphic DNA was done as a initial step to check feasibility of variants. The molecular variations and data obtained from these two forms can be of taxonomic importance. Further analysis involving ISSR or mini/micro satellite markers is required to confirm the results obtained.

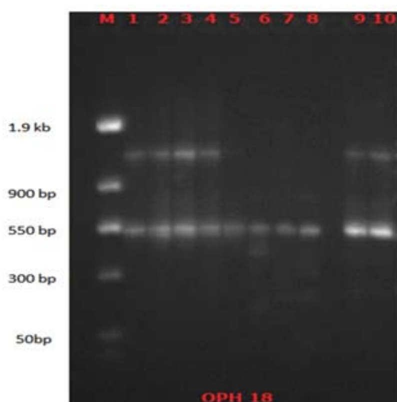


Figure 1

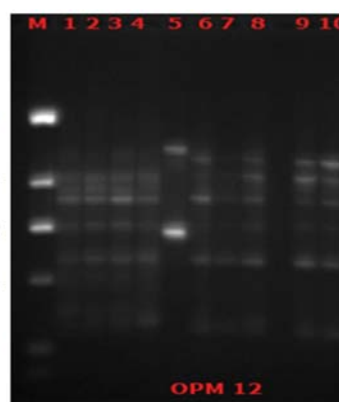


Figure 2

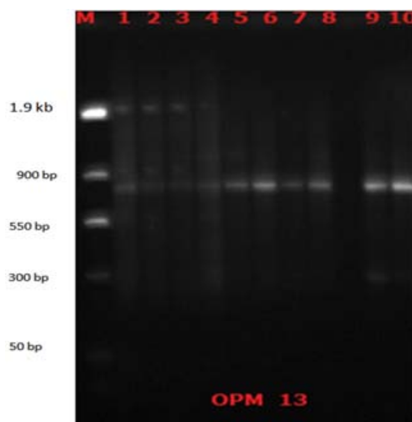


Figure 3

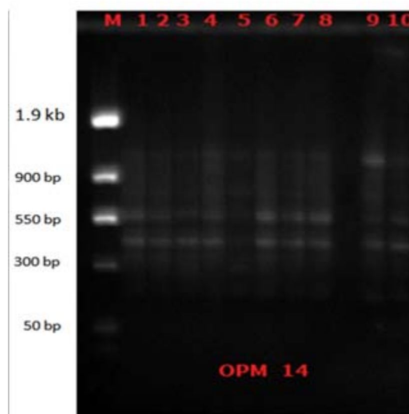
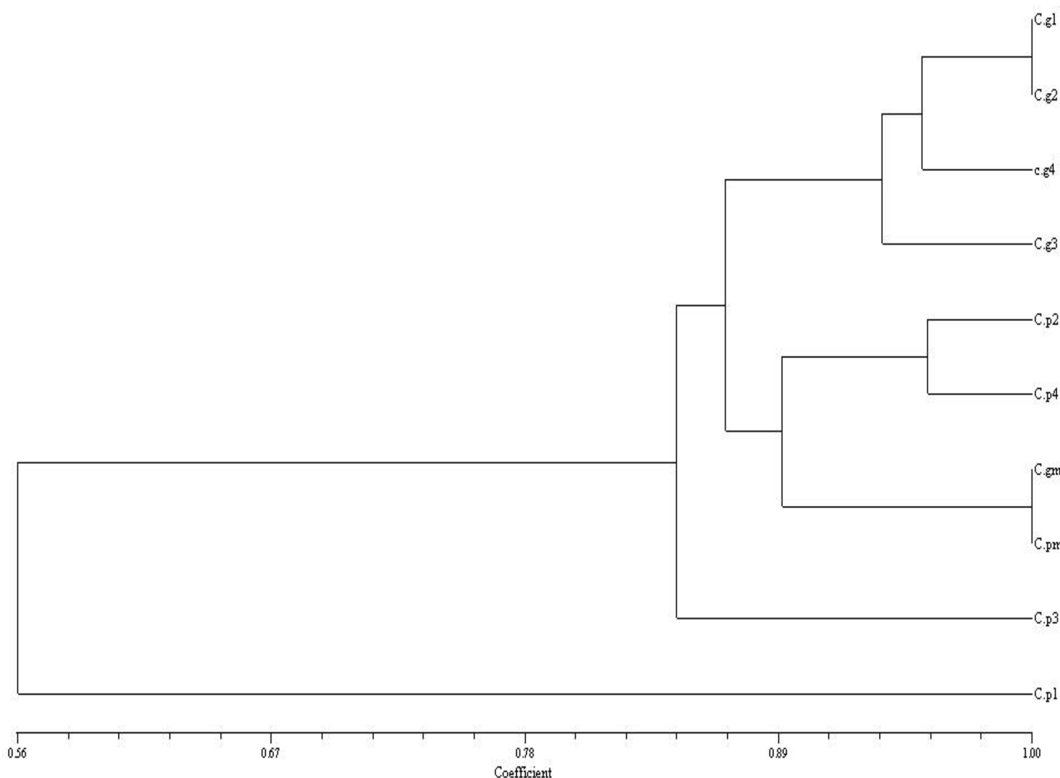


Figure 4



Dendrogram

Conclusion

This study was carried out to check the feasibility of understanding the variants using RAPD markers though, the two forms exhibit two distinct clusters there could be a significant sequence of DNA which may be responsible for different colour patterns. The study reveals that the similarity index is among these populations collected from different locations is very high indicating that the variants can be retained as a single species. The polymorphism and dissimilarity is lesser however the clustering pattern is unique which will help distinguish the elite populations as well as genetically rich groups.

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