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# Genetic diversity in *Azima tetracantha* (Lam) assessed through RAPD analysis

B. Thendral Hepsibha[1], V. Premalakshmi [2] and T. Sekar [3]

[1] *Dept. of Biotechnology, Alpha Arts & Science College, Chennai- 600 116, India*
[2] *Dept. of Biotechnology, Mother Teresa Women's University, Kodaikanal - 624 102, India*
[3] *Dept. of Botany, Pachiayappa's College, Chennai - 600 030, India*
thendraljoel@yahoo.com

## Abstract

*Azima tetracantha* is a shrub known for its various therapeutic properties. Genetic diversity was analysed using RAPD primers among six accessions collected from different locations of Tamilnadu, India. Genetic distances were calculated using Nei's coefficient. Dendrogram was constructed on the basis of the similarity matrix data by unweighted pair group method with average (UPGMA) cluster analysis. The analysis with RAPD markers revealed wide variation within *A. tetracantha* that reflected a high level of diversity within this species.

**Keywords:** *Azima tetracantha*, genetic diversity, RAPD.

## Introduction

*Azima tetracantha* known as 'Mulchangan' in Siddha is a rambling spinous shrub belonging to the family Salvadoraceae. The presence of quadrangular spines at the nodes is considered to be a unique character (Kirtikar & Basu, 1984) of this species. The shrub grows widely in most parts of south India, Ceylon, Philippines and in Burma. The ethno botanical survey reveals the usage of this plant for various ailments (Jaswanth *et al.,* 2001; Hebbar *et al.,* 2004; Mohamed *et al.,* 2007; Ignacimuthu *et al.,* 2008). It has been reported for its antimicrobial (Mohamed *et al.,* 2007), analgesic (Nandgude *et al.,* 2007), anti inflammatory (Ismail *et al.,* 1997) and wound healing activity (Jaswanth *et al.,* 2001). The roots and leaves were used as stimulant and tonic.

Botanical additives and their natural compounds have therapeutic effects and have become popular among many consumers (Xie *et al.,* 2005; Xu *et al.,* 2005). However, herbal medicine safety is an important issue that both the government and the researcher must address (Chrubasik *et al.,* 2005; Yoo *et al.,* 2005). Plant wealth is greatly exploited for its therapeutic potential and medicinal efficacy to cure various ailments since time immemorial. For this reason, they were identified and defined by taxonomist at the very early ages of human history, although the taxonomic studies about the genus are limited and especially morphological characters were used in the genus key which can be influenced by environmental conditions (Sumer Aras *et al.,* 2003). In the evolutionary history of a species, the distribution of populations could be constricted or expanded due to the environmental changes (Jin-Ming *et al.,* 2008). Molecular markers could reflect the difference between species directly without affecting the environment (Wang *et al.,* 1996). Several molecular markers particularly the Random Amplified Polymorphic DNA (RAPD), Restriction fragment length polymorphism (RFLP) and Variable Number of Tandem Repeats (VNTR) have been proven useful in detecting genetic diversity. RAPD technique has several advantages such as speed, low cost and the usage of small amounts of plant materials (Jain *et al.,* 1994; Heun *et al.,* 1994; Becerra Velasques & Gepts, 1994). So far analysis of genetic diversity in *A. tetracantha* has not been carried out. The aim of the present study was to analyse the genetic diversity as well as their relationship in the diminishing wild populations of *A. tetracantha* using the RAPD markers.

## Materials and methods

### *Plant materials*

A total of six accessions (Table 1) of *Azima tetracantha* were collected from various locations in Tamilnadu, India. Young leaves were harvested and placed in sealable plastic bag with appropriate label. The collected leaves were used immediately for DNA extraction, while excess leaf materials were stored in -80°C for future use.

### *DNA extraction*

Total genomic DNA was extracted from leaves using a modified CTAB method based on the protocol of Doyle & Doyle (1990). Quality and concentration of total DNA was verified by UV Spectrophotometry at 260 nm and 280 nm. Further quality of DNA was tested by submerged horizontal agarose gel (0.8%) electrophoresis and visualized under UV light, gel documentation system.

### *RAPD analysis*

Out of 19, Random decamer primers (Operon technologies, USA) of OPA, OPB & OPN series were used individually as primers for RAPD analysis. The PCR amplification was carried out in MJ Research, Inc. PTC-150 minicycler. PCR reactions were performed in reaction mixture with a total volume of 25 µl containing different


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combinations [DNA con. (25ng & 50ng), primer conc. (0.2, 0.25 & 0.3 µM) and Taq DNA polymerase conc. (0.6, 0.8, 1.0 & 2.0 U) were tried to optimize the PCR condition. PCR amplification was carried out with pre-denaturation at 94°C for 3 min, denaturation at 94°C for 1 min, primer annealing at 36°C for 1 min and primer extension at 72°C for 2 min followed by 40 cycles of amplification and final extension at 72°C for 7 min. PCR products were kept at 4°C. Gel electrophoresis was carried out on the amplified products using 2% agarose, stained with ethidium bromide and visualized under UV illumination. The 1kb DNA ladder was used as a molecular weight marker and the amplifications were repeated twice to confirm the results.

*Table 1. Plants collected from the various districts of Tamilnadu*

| Population ID | Area of the study / District |
|---|---|
| Loc1 | Red Hills, Chennai, Thiruvallur Dt. |
| Loc2 | Periyapalayam, Chennai, Thiruvallur Dt. |
| Loc3 | Karisangal , Chennai, Chengalpattu Dt. |
| Loc4 | Kallagam , Trichy Dt. |
| Loc5 | Sirumalai Hills, Dindugal Dt. |
| Loc6 | Sukkambar , Tanjore Dt. |

*Table 2. RAPD primers data and the percentage of polymorphic bands*

| Primer Code | Nucleotide Sequence | Size of fragments(bp) | Polymorphic bands | Monomorphism % | Polymorphism % |
|---|---|---|---|---|---|
| OPA7 | GAAACGGGTG | 250-2000 | 5 | 16.66 | 83.33 |
| OPA10 | GTGATCGCAG | 500-1000 | 4 | - | 100 |
| OPA18 | AGGTGACCGT | 400-1500 | 7 | - | 100 |
| OPN9 | TGCCGGCTTG | 500-1500 | 4 | 20 | 80 |
| OPN6 | GAGACGCACA | 100-2500 | 7 | - | 100 |

*Data analysis*

Evaluation of fragment patterns was carried out by similarity index. Reproducible bands were scored manually as '1' or '0' for presence or absence of the bands. The data was used for similarity – based analysis using the software program NTSYS (2.20). RAPD analyses were analyzed using the Nei genetic similarity index (Nei and Li, 1979). On the basis of the equation,

$SI = 2N_{ij} / (N_i + N_j)$

Where $N_{ij}$ is the number of common bands shared between samples i and j, $N_i$ and $N_j$ are the total number of DNA bands for genotypes i and j, respectively. A dendrogram was constructed (Fig.1) on the basis of the similarity matrix data by un weighted pair group method with average (UPGMA) cluster analysis.

## Results and discussion

The RAPD technique had been successfully used in variety of taxonomic and genetic diversity studies (Rodriguez *et al.,* 1999: Alam *et al.,* 2009) and it was found suitable for use with *A. tetracantha* genotype because of its ability to generate reproducible polymorphic bands. A total of 19 RAPD OPERON primers were screened of six *A. tetracantha* accessions. Out of these, only 5 of the primers (Table 2) that showed reproducible results were chosen to amplify the whole six accessions (Fig 2) A total of 29 bands were amplified among 6 accessions using 5 primers and the polymorphic bands were 27. Monomorphic bands are those which are present in all individuals, polymorphic are present in one or more but not all individuals and unique ones are present in at least one individual not in any other (Mehetre *et al.,* 2004). The mean percentage of polymorphic bands was 92.66% with molecular sizes ranged from 0.100 to 2.5 Kb. Only 2 bands of the 29 bands were commonly detected in all the samples which reflected certain homology of the sample. The lesser similarity value of 0.085 indicates high diversity between Loc 1 and Loc 6 species (Table 3). The genetic similarity index between Loc 2 and Loc 4 was highest with a value of 0.900.

A dendrogram was constructed using neighbor joining method of cluster analysis separated all the six accessions into two clusters at 0.09 similarity coefficient. Cluster 1 has only Loc 1 accession. Cluster 2 contained the remaining five accessions separated into 3 sub clusters. Loc 2, 4, 5 are in a clad. Loc 3 and Loc 6 are in a separate clad (Fig 1).

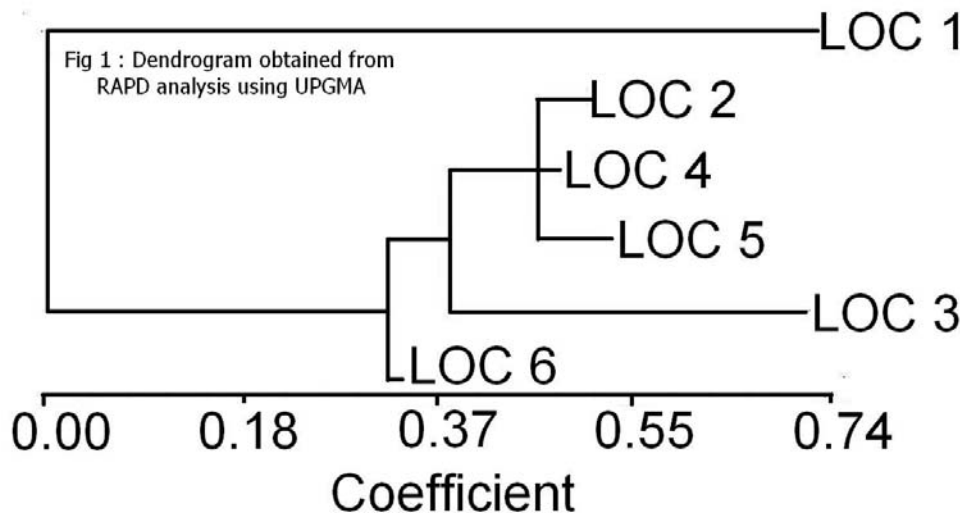


Fig 1 : Dendrogram obtained from RAPD analysis using UPGMA

The genetic structure of plant populations reflects the interactions of many different processes such as the long-term evolutionary history of the species (e.g., shifts in distribution, habitat fragmentation, and/or population isolation), mutation, genetic drift, mating system, gene flow, and selection (Slatkin, 1987; Schaal

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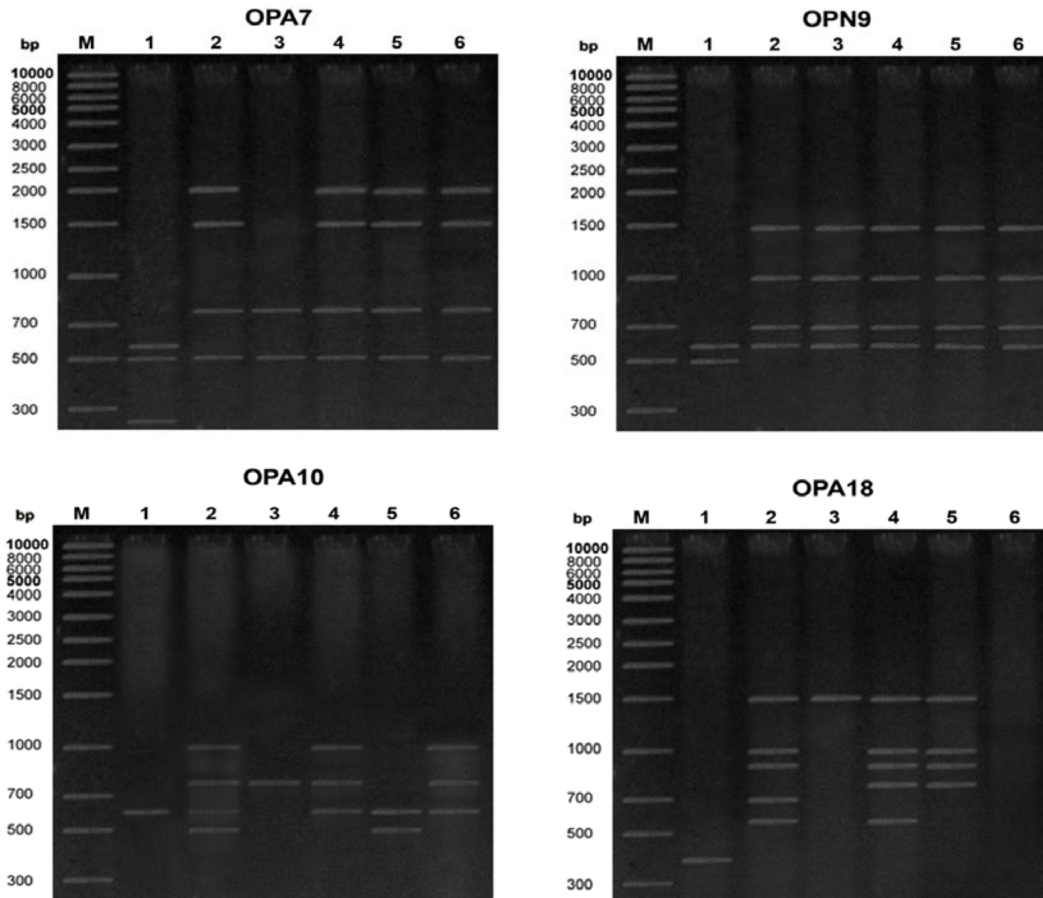
*et al.,* 1998). All of these factors can lead to complex genetic structuring within populations. Genetic diversity is of great importance to the sustainability of plant populations (Wang *et al.,* 2007). Based on the above results there are collections with high similarity index, even though they may be belonging to geographically different locations. High similarity indices suggest that the individuals in the population have close genetic relation among them. This situation can rise in natural populations when there is a possibility of free/random pollen flow and fertilization. The genetic similarity of the samples slightly correlated with their close geographic locations (Sayed *et al.,* 2009).

*Fig 2. Polymorphic band generated by different RAPD primers*




Sources of polymorphism in RAPD assay may be due to deletion, addition or substitution of base within the priming site sequence (Willams *et al.,* 1990). High diversity is the reflection of adaptation to environment, which is beneficial to its propagation, resources conservation, the domestication of wild species and the screen of specified locus. The accessions collected from Loc 2, Loc 4, Loc 5, Loc 3 and Loc 6 are from different geographical location, but free of pollution. Geographically isolated individuals tend to accumulate genetic variations during the course of environmental adaptations (Sarwat, 2008). Accession from Loc 1 is collected from an area surrounded by industries and posed to environmental pollution and the resulting genetic adaptation. In the conservation management of a species, knowledge of interspecies genetic variations may help to assess extinction risks such as inbreeding and evolutionary potential in a changing world (Hedrick, 2001). Effective conservation of a vulnerable species depends largely on the knowledge of patters of genetic variation. For example, the spatial structure of genetic variation can provide information for sampling strategies for ex situ or in situ conservation (Torre *et al.,* 2008). The herbs type of plant group may provide the best model systems for future studies aimed at illuminating the role of the Quaternary climatic changes in driving diversification and speciation. Short-lived herbs, as opposed to long-lived trees, experience a higher number of life cycles within a given time period, and may have responded more quickly to environmental change on Quaternary time-scales (Comes & Kadereit, 1998). This study was an attempt to establish the genetic diversity background in *A. tetracantha* with RAPD markers. High levels of polymorphism found in the present work showed that RAPD markers as a suitable tool for genetic diversity studies. This study could pave the way for detailed research to understand all the aspects of this divergence.

*Table 3. Genetic similarity index from RAPD data of different populations*

| Sample | Loc 1 | Loc 2 | Loc3 | Loc 4 | Loc 5 | Loc 6 |
|---|---|---|---|---|---|---|
| Loc 1 | 1.000 | | | | | |
| Loc 2 | 0.287 | 1.000 | | | | |
| Loc 3 | 0.498 | 0.517 | 1.000 | | | |
| Loc 4 | 0.287 | 0.900 | 0.517 | 1.000 | | |
| Loc 5 | 0.237 | 0.839 | 0.434 | 0.896 | 1.000 | |
| Loc 6 | 0.085 | 0.800 | 0.587 | 0.797 | 0.693 | 1.000 |

*Nei's Similarity Coefficient generated by UPGMA analysis*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