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# Molecular and phytochemical characterization of *Acalypha indica* L. in Tirunelveli hills

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In the present study, *Acalypha indica* L. was collected from ten locations in Tirunelveli hills and the genetic variability was investigated using RAPD-PCR fingerprint and the population which showed high percentage of polymorphism was selected. The selected populations were further subjected to phytochemical analysis. The active principle in these plants was quantified by HPLC analysis. The population which exhibited both high percentage of polymorphism and high amount of active principle was considered as the superior genotype.

Key words : *Acalypha indica*, Genetic variability, RAPD, Active principle, HPLC, Superior genotype

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## INTRODUCTION

An herbal medicine may consists of hundreds of phytochemicals, and their contents vary depending on climate, regions of cultivation and seasons of harvest which make it difficult to ensure batch-to batch uniformity. The quality control of standardized herb extracts is essential for the therapeutic reproducibility, efficacy and safe application of extract. HPLC method is gaining importance for qualitative and quantitative analysis of plant extracts, being useful for quality control of phytochemical compounds (Daniele *et al.,* 2006).

Phytochemical differences are directly or indirectly linked to the genetic diversity. DNA markers based fingerprinting can distinguish species rapidly using small amounts of DNA and therefore, can assist to deduce reliable information on their phylogenetic relationships. Various approaches are available for DNA fingerprinting such as AFLP (Amplified fragment length polymorphism), SSR (Simple sequence repeats) and RAPD (Random amplified polymorphic DNA). RAPD is convenient to conduct with good polymorphism and can be used in analyzing genetic diversity and the relation between species. It is been used in analyzing the relationships and genetic diversity in many plants, especially medicinal plants (Lanying *et al.,* 2009).

Although RAPD is of dominant nature, several strategies have been put forth to minimize the dominance effects on genetic variation analysis (Stewart and Excoffier, 1996). In occasional cases, RAPD is poor in reproducibility but this can usually be solved by optimization of reaction conditions. RAPD analysis requires only a small amount of genomic DNA and can produce high level of polymorphism and may facilitate more effective diversity analysis in plants (Szmidt *et al.,* 1996).

Assessment of genetic diversity prevalent in the germplasm needs immediate attention for the improvement of a species (Lakhanpaul *et al.,* 2003). To understand the effective management of plant genetic diversity from a conservation point of view, it is essential to consider the variation richness and distribution at intra and interspecific levels. Information about genetic diversity is important not only for study of the flora, but also to elaborate strategies of conservation and rational use of genetic resources (Viccini *et al.,* 2004).

*Acalypha indica* Linn. belongs to the family Euphorbiaceae, it is an erect, annual herb. The whole plant is used as medicine to treat skin diseases, constipation, ulcers and bronchitis. The plant contains a cyanogenetic glucoside and two alkaloids *viz.*, acalyphine and triacetonamine. The other constituents are n-octasosanol,

beta-sitosterol, kaempferol, quebrachitol, tannin, resin and essential oil.

In the present investigation, molecular and phytochemical characterization of the populations of *Acalypha indica* collected from ten different geographical locations of Tirunelveli hills based on RAPD markers and HPLC.

## RESEARCH METHODOLOGY

The tender leaves of *Acalypha indica* were randomly collected from ten different locations: Thirugurungudi, Kalakad, Manimuthar, Ambasamuthram, Papanasam, Courtallum, Kadayanalloor, Krishnapuram, Vasudevanallor and Sivagiri in Tirunelveli hills. The distance between two populations was 15 km. Each population consisted of ten plants within a radius of 5 m.

Tender unfolded leaf samples were collected from five different locations and stored immediately at -70° C for DNA extraction. The total genomic DNA was extracted from the stored leaf samples using the modified CTAB method (Doyle and Doyle, 1987) and purified according to standard method followed by Sambrook and Russel (2000). Concentration of the purified genomic DNA in each case was adjusted to 10 ng/ µl in different aliquots and stored at -70° C for use in PCR amplification.

Five RAPD primers and their sequences used for PCR amplification of the genomic DNA of *A. indica* (Williams *et al.,* 1990) are OPX03-TGGCGCAGTG; OPX12- TCGCCAGCCA; OPX18-GACTAGGTGG; OPX19-TGGCAAGGCA; OPX20- CCCAGCTAGA. PCR reactions were carried out in a final volume of 25 µl, which contained 2.5 µl 10 x taq polymerase buffer, 2.0 µl of deoxyribonucletides (dNTPs), 3.5 µl $MgCl_2$, 0.1 µl of taq DNA polymerase, 2.0 µl of deca oligonucleotide primer, 2.0 µl of tamplate DNA and 12.9 µl of sterile dis.$H_20$.

The reaction mixture was subjected to programmed PCR-amplification in a Perkin-Elmer Gene Amp PCR system (model 2400). Amplification process included, initial denaturation of DNA at 95ºC for 5 minutes, denaturation 94ºC for 30 seconds, annealing at 35ºC for 1 minute and extension at 72ºC for 2 minutes followed by thirty five cycles and final extension at 72ºC for 5 minutes followed by storage at 4ºC till electrophoresis.

The amplification products were resolved by electrophoresis on 1.5 per cent agarose gel containing ethidium bromide along with 1 Kb ladder DNA as a standard molecular weight size marker. The gels were visualized under UV transilluminator and image was captured using gel documentation system Alpha Imager 1200.

Based on the primary data (presence or absence of bands), pair wise genetic identity and genetic distance between samples were calculated using Popgene package version 1.31. A dendrogram was constructed and analyzed the phylogenetic relationship among each population.

The wild, fully grown *A. indica* leaves were shade dried for a week and powdered and extracted with 100 ml of methanol. Standard stock solutions (500 µg/ml) were prepared by dissolving 50 µg acalyphin 5 ml of warm methanol and was made up to 100 ml with distilled water and sonicated for 20 minutes. Standard solutions were prepared by diluting the stock solution with 50 per cent methanol to obtain the desired concentration. The methanol extract of each sample was used for HPLC analysis.

## RESULTS AND ANALYSIS

The five primers used to analyze genetic variation in *Acalypha indica* produced 59 polymorphic bands (Plate 1). The same type of bands occurred at different frequencies in all populations. The genetic distance between the population ranged from 0.1214 to 0.8286 and the genetic identity ranged from 0.6000 and 0.8857 (Table 1). The overall observed

**Table 1 : Nei's unbiased measures of genetic identity and genetic distance in *Acalypha indica***

| Pop ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | **** | 0.6571 | 0.6571 | 0.8286 | 0.8286 | 0.6571 | 0.7143 | 0.7143 | 0.6571 | 0.7143 |
| 2. | 0.4199 | **** | 0.8857 | 0.6000 | 0.7143 | 0.7714 | 0.6571 | 0.6571 | 0.7714 | 0.6571 |
| 3. | 0.4199 | 0.1214 | **** | 0.6000 | 0.7143 | 0.7714 | 0.6571 | 0.6571 | 0.7714 | 0.6571 |
| 4. | 0.1881 | 0.5108 | 0.5108 | **** | 0.8857 | 0.7143 | 0.7714 | 0.8286 | 0.6571 | 0.7143 |
| 5. | 0.1881 | 0.3365 | 0.3365 | 0.1214 | **** | 0.7714 | 0.8286 | -.8286 | 0.7714 | 0.7143 |
| 6. | 0.4199 | 0.2595 | 0.2595 | 0.3365 | 0.2595 | **** | 0.7714 | 0.7714 | 0.7714 | 0.7143 |
| 7. | 0.3365 | 0.4199 | 0.2595 | 0.2595 | 0.3365 | 0.2595 | **** | 0.8286 | 0.7714 | 0.8286 |
| 8. | 0.3365 | 0.4199 | 0.4199 | 0.1881 | 0.1881 | 0.2595 | 0.1881 | **** | 0.8286 | 0.7143 |
| 9. | 0.4199 | 0.2595 | 0.2595 | 0.4199 | 0.2595 | 0.2595 | 0.2595 | 0.1881 | **** | 0.7143 |
| 10. | 0.3365 | 0.4199 | 0.4199 | 0.3365 | 0.3365 | 0.3365 | 0.1881 | 0.3365 | 0.3365 | **** |

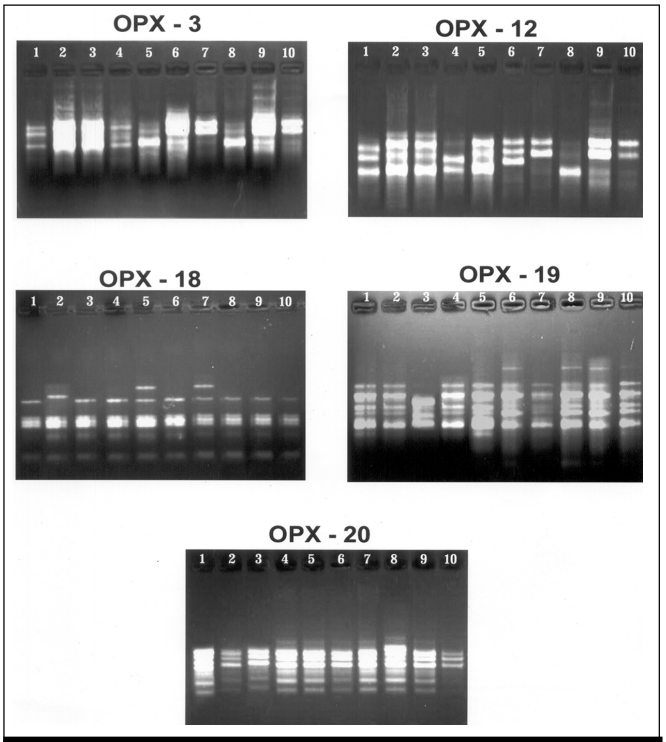


**Plate 1 : RAPD-PCR banding pattern of *Acalypha indica***

and effective number of alleles is about 1.68 and 1.39, respectively. Nei (1978) overall gene diversity is 0.2366.

The dendrogram of *Acalypha indica* (Fig.1) produced three clusters. Cluster 2 was the largest cluster among the three, containing population 7, 8, 9 and 10. Here population 7, 8 and 9 are closely together than population 10. In the first cluster population 1 form a separate clade and population 4 and population 5 are closely together. In the third cluster population 2 and population 3 are close together and population 6 forms a separate clade. It is understood that there is considerable amount of genetic variability between the 10 populations of *Acalypha indica*.

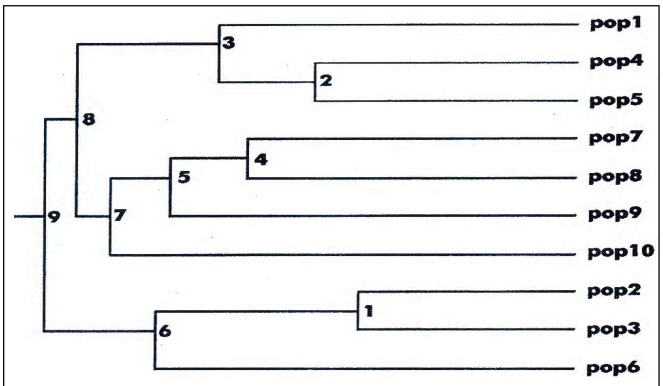


**Fig. 1 : UPGMA dendrogram of Acalypha indica based on Nei's genetic distance derived from RAPD data**

The number of polymorphic loci and percentage of polymorphism (Table 2) was calculated by using the software Popgene package version1.31. Among these ten populations, populations 1, 4, 7, 8, 10 (Thirugurungudi, Ambasamuthram, Manimuthar, Papanasam and Courtallum) showed highest polymorphism. Among these five, percentage of polymorphism was higher in population 10.

**Table 2 : Number of polymorphic loci and percentage of polymorphism in *Acalypha indica***

| Pop | Number of polymorphic loci | Percentage of polymorphic loci |
|---|---|---|
| 1. | 14 | 40.00 |
| 2. | 8 | 22.86 |
| 3. | 10 | 28.57 |
| 4. | 12 | 34.29 |
| 5. | 8 | 22.86 |
| 6. | 8 | 22.86 |
| 7. | 10 | 28.57 |
| 8. | 10 | 28.57 |
| 9. | 6 | 17.14 |
| 10. | 16 | 45.71 |

Since a population needs variation, the measure of the amount of heterozygosity across all genes can be used as a general indicator of the amount of genetic variability and genetic health of a population. Variations that enable individuals to produce more offspring are considered to be 'most fit'. These variations become more frequent with each generation. Speciation occurs when individuals become genetically isolated from other groups by conditions that prevent inter-breeding. Genetic variability among all species is important to maintain since it represents the 'blueprint' for all of the living things on earth. It is important to point out that the genetic variation that a population of organisms possesses is the fuel that allows them to be able to change or evolve in response to changing environmental conditions. Genetic variability within a population can sometimes allow a species to adapt to a changing environment, it leads to long term survival of a species, and it comes to the rescue of a species at crucial situations by lending genes that impart resistance, surveillance and higher productivity. Species with little or no genetic variability will have greater tendency to go extinct when a new disease, a new predator, or some other change occurs in the environment.

In order to scrutinize further, phytochemical analysis was done. The five populations, which showed the highest polymorphism, were selected for further

**Table 3 : Quantification of acalyphin in *Acalypha indica* dry plant samples carried out with MS/MS detection**

| Sample | Weight for extraction (g) | Residuum after evaporation (g) | Determined w (%) in residuum | Amount of acalyphin in 1 g of dry plant (µg/g) |
|---|---|---|---|---|
| Thirugurungudi | 5 | 2.99 | 0.188 | 1.80 ± 0.23 |
| Ambasamuthram | 5 | 2.96 | 0.083 | 0.95 ± 0.14 |
| Manimuthar | 5 | 2.13 | 0.070 | 1.03 ± 0.33 |
| Papanasam | 5 | 1.23 | 0.126 | 2.28 ± 0.49 |
| Courtallum | 5 | 2.00 | 0.190 | 3.25 ± 0.20 |

Results are expressed as mean ± SD, n = 2.

HPLC. Acalyphin quantification (Table 3) was based on the analysis of kvazimolecular ion m/z = 361.12 with an internal standard caffeine. Among these five, (1, 4, 7, 8, and 10) population 10 which was collected from Courtallum, showed the highest amount of acalyphin (3.25µg ± 0.20µg), followed by pop. 8, which was collected from Papanasam (2.28µg ± 0.49µg).

In the wake of the current pace of habitat loss and depletion of plant genetic resources in the tropics, it is essential for the developing countries which are rich in biological diversity, to evolve strategies and conscious efforts to scrutinize their resources and identify the variants of economic value for conservation and utilization. Molecular and phytochemical characterization has been carried out in medicinal plants in various parts of India and in the world. In an investigation on *Withania somnifera*, the dendrogram generated by UPGMA distinguished *Withania somnifera* from *W. coagulans* and formed two major clusters. The dendrogram further separated *W. somnifera* into three subclasses corresponding to Kashmiri and Nagori groups and an intermediate type (Nagi *et al.,* 2000). Genetic diversity of 54 populations from 22 species of *Medicago* collected from Iranian natural habitat was studied (Ghanavati and Mozafari, 2005). The morphological, chemical and genetic differences of 12 tree basil (*Ocimum gratissimum* L.) accessions was studied to determine whether volatile oils and flavonoids can be used as taxonomical markers and to examine the relationship between RAPDs to these chemical markers (Roberto *et al.,* 2001). The collection of *Phyllanthus amarus* was made from various parts of India to determine the extent of genetic variability using analysis at DNA level. RAPD profiling of 33 collections from different locations was generated (Jain *et al.,* 2003). Three albanian ecotypes of *Oreganum vulgare* were undergone RAPD analysis and GC analysis. Genomic DNA was isolated from leaves of individual plants and PCR amplifications were carried out. The composition of the essential oil was completed via GC. Based on the results of the RAPD analysis, essential oils composition, and their attitude toward the *in vitro* propagation conditions, it was concluded that these ecotypes differ from each other and that they cannot be grouped into groups of similarity (Bacu *et al.,* 2005).

## Conclusion:

Hence, based on the molecular and phytochemical characterization, the plants collected from Courtallum and Papanasam were considered to be the superior to other populations of other areas. It provided information that can help to define the distinctiveness of species and phylogenetic relationships at molecular level and phytochemical level.Use of such techniques for germplasm characterization may facilitate the conservation and utilization of plant genetic resources, permitting the identification of unique genotypes or sources of genetically diverse genotype.

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