Journal of Research in Biology

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# Genetic and phytochemical variability in *Acalypha indica* L.

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**ABSTRACT:**

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. The population which showed high percentage of polymorphism was selected. The samples from this population were chosen for further phytochemical analysis and the active principle has been quantified. The population which exhibited both high percentage of polymorphism and high amount of active principle was considered to be the superior genotype.

**Keywords:**
*Acalypha indica*, phyto chemical genetic variability, superior genotype.

**Web Address:**
http://jresearchbiology.com/Documents/RA0139.pdf.

**Article Citation:**
**John De Britto and Rekha GS.**
Genetic and phytochemical variability in *Acalypha indica* L.
Journal of research in Biology (2011) 7: 477-482

**Dates:**
**Received:** 09 Sep 2011 /**Accepted:** 21 Oct 2011 /**Published:** 02 Nov 2011





Journal of Research in Biology

## INTRODCUTION:

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.

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. Genetic variability among all species is important to maintain since it represents the 'blueprint' for all of the living things on earth. Due to various environmental, physico-chemical factors, soil conditions and climatic factors, the quality and quantity of active principles may vary from population to population. Hence, phytochemical characterization along with the molecular characterization gains significance in order to find out the superior genotypes.

*Acalypha indica* L. comes under the family Euphorbiaceae. 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. This is distributed through out the Western Ghats. With this background, in order to find out the superior genotypes among the various populations in Tirunelveli hills, the molecular and phytochemical characterization of Acalypha indica was carried out.

## MATERIALS AND METHODS:

Ten locations in Tirunelveli hill area such as Thirugurunkudi, Kalakad, Manimuthar, Abasamudram, Papanasam, Courtallum, Kadayanallur, Krishnapuram, Vasudevanallur and Sivagiri were selected for collection of species. Young leaves from ten individuals of Acalypha indica L. from each location collected in separate vials for RAPD analysis and stored in -70$^0$C until DNA was extracted from all ten individuals of each location and pooled. Leaf samples were also collected for HPLC anlaysis. DNA was extracted from these plants by CTAB (modified) technique Doyle JJ and Doyle JL, 1987. The A260/A280 reading of DNA ranged from 1.5 to 1.9.

For RAPD analysis Williams *et al.,* (1990) (modified) method was followed. The reaction was performed by 50ng of DNA, 15 picomoles of a single decamer random primer, PCR mixture and water to a total volume of 25µl in 2.5ml PCR tubes. The amplification was carried out in a thermal cycler (**Table. 1**).

**Table.1. PCR amplicfication programme**

| Activity | Temperature ($^0$C) | Time | Number of cycles |
|---|---|---|---|
| Initial Denaturation | 94 | 5 Min | One |
| Denaturing | 94 | 1 Min | 35 |
| Annealing | 37 | 1 Min | |
| Extension | 72 | 1 Min | |
| Final Extension | 72 | 5 Min | One |
| Storage | 4 | For ever | |

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. Dendrogram was constructed and analyzed.

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 % methanol to obtain the desired concentration. The methanol extract of each sample was used for HPLC analysis following the standard protocol given by Tikhomiroff and Jolicoeur, 2002.

## RESULTS AND DISCUSSION:

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. The overall observed and effective number of

Table. 2. Nei's Unbiased Measures of Genetic Identity in *A. indica*

| Pop ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | **** | | | | | | | | | |
| 2 | 0.4199 | **** | | | | | | | | |
| 3 | 0.4199 | 0.1214 | **** | | | | | | | |
| 4 | 0.1881 | 0.5108 | 0.5108 | **** | | | | | | |
| 5 | 0.1881 | 0.3365 | 0.3365 | 0.1214 | **** | | | | | |
| 6 | 0.4199 | 0.2595 | 0.2595 | 0.3365 | 0.2595 | **** | | | | |
| 7 | 0.3365 | 0.4199 | 0.2595 | 0.2595 | 0.3365 | 0.2595 | **** | | | |
| 8 | 0.3365 | 0.4199 | 0.4199 | 0.1881 | 0.1881 | 0.2595 | 0.1881 | **** | | |
| 9 | 0.4199 | 0.2595 | 0.2595 | 0.4199 | 0.2595 | 0.2595 | 0.2595 | 0.1881 | **** | |
| 10 | 0.3365 | 0.4199 | 0.4199 | 0.3365 | 0.3365 | 0.3365 | 0.1881 | 0.3365 | 0.3365 | **** |

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 two major clusters. The first cluster is with populations 2, 3 and 6. This is further divided into two sub clusters with populations 2 and 3 together leaving population 6 separaet. The rest of the populations groups into another cluster. In this cluster, there are two sub clusters. The first sub cluster is with populations 1, 4 and 5. Here again population 1 is dfferent from populations 4 and 5. The second sub cluster is with populations 7, 8, 9 and 10. Here again populations 7, 8 and 10 form a separate cluster leaving population 10 separate. Populations 7 and 8 form a sub cluster leaving population 9 separate. From the dendrogram, it is understood that there is considerable amount of genetic variability between the 10 populations of *Acalypha indica*. The poulation 10 collected from Courtallum forms a unique cluster differing from all other populations.

The number of polymorphic loci and percentage of polymorphism (**Table. 4**) was calculated by using the software Popgene package version1.31. Among these ten populations, populations 1, 4, 7, 8, 10 (Thirugurungudi,

Table. 3. Nei's Unbiased Measures of Genetic distance in *A. 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 | | **** | 0.8857 | 0.6000 | 0.7143 | 0.7714 | 0.6571 | 0.6571 | 0.7714 | 0.6571 |
| 3 | | | **** | 0.6000 | 0.7143 | 0.7714 | 0.6571 | 0.6571 | 0.7714 | 0.6571 |
| 4 | | | | **** | 0.8857 | 0.7143 | 0.7714 | 0.8286 | 0.6571 | 0.7143 |
| 5 | | | | | **** | 0.7714 | 0.8286 | -.8286 | 0.7714 | 0.7143 |
| 6 | | | | | | **** | 0.7714 | 0.7714 | 0.7714 | 0.7143 |
| 7 | | | | | | | **** | 0.8286 | 0.7714 | 0.8286 |
| 8 | | | | | | | | **** | 0.8286 | 0.7143 |
| 9 | | | | | | | | | **** | 0.7143 |
| 10 | | | | | | | | | | **** |

**Nei's genetic identity (above diagonal) and genetic distance (below diagonal)**

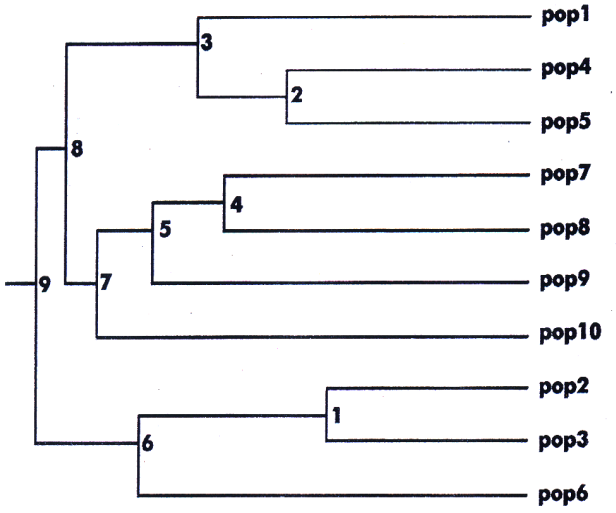


**Fig 1. UPGMA dendrogram of *Acalypha indica* based on Nei's Genetic distance derived from RAPD data.**

**Table. 4. Number of polymorphic loci and percentage of polymorphism in *Acalypha indica***

| Population no | 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 |

Ambasamuthram, Manimuthar, Papanasam and Courtallum) showed highest polymorphism. Among these five, percentage of polymorphism was higher in population 10**.**

In order to scrutinize further, phytochemical analysis has been done. The five populations, which showed the highest polymorphism, were selected for further HPLC. Leaves from the plants of these five populations were collected and shade dried and powdered. Acalyphin quantification (**Table.5**) 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).

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

**Table.5. 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) |
|---|---|---|---|---|
| AI -1 Thirugurungudi | 5 | 2.99 | 0.188 | 1.80 ± 0.23 |
| AI - II Ambasamuthram | 5 | 2.96 | 0.083 | 0.95 ± 0.14 |
| AI - III Manimuthar | 5 | 2.13 | 0.070 | 1.03 ± 0.33 |
| AI - IV Papanasam | 5 | 1.23 | 0.126 | 2.28 ± 0.49 |
| AI - V Courtallum | 5 | 2.00 | 0.190 | 3.25 ± 0.20 |

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

when a new disease, a new predator, or some other change occurs in the environment. Phytochemical characterization will provide additional information about the impact of environmental, climatic and edaphic factors on the quality and quantity of active principles in medicinal plants. One could predict the superior genotypes by combining the quality and quantity of active principle and the percentage of polymorphism in a species. Molecular and phytochemical characterization has been carried out in medicinal plants in various parts of India and in the world. In an investigation the genetic variability of *Withania somnifera* was done Nagi *et al.,* 2000.

Genetic diversity of 54 populations from 22 species of *Medicago* collected from Iranian natural habitat was studied Ghanavati F and Mozafari J, 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 Vieira *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 Jain *et al.,* 2003. The bisbolol and chamzulene content being of special importance for the oil quality of chamomile (*Chamomilla recutina* was analyzed Wagner *et al.,* 2003. Three albanian ecotypes of *Oreganum vulgare* were undergone RAPD analysis and GC analysis Bacu *et al.,* 2005.

In the present study, it is clear from the results that distinct variations exist between the populations of Acalypha indica distributed in Tirunelveli hills. Combining both the polymorphism which is the sign of variability, and the quantity of active prinicple we can predict the superior genotype. Hence, based on the molecular and phytochemical characterization, the plants collected from Courtallum and Papanasam are considered to be the superior to other populations in Tirunelveli hills. The samples from this population could be further collected and better exploited for optimum mediicnl uses.

**ACKNOWLEDGEMENT**

The authors are grateful to University Grants Commission, New Delhi and Indian National Science Academy, New Delhi for the financial assistance to carryout this research work. The authors are very grateful to Dr.Vilim Simanek and Dr.Pavel Kosina, Department of Chemistry, Palacky University, Olomouc, Czech Republic for helping in analyzing the samples using HPLC.

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