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Study on genetic diversity in the different populations of *Acalypha fruticosa* forssk by using RAPD analysis inhabiting foot hills of Southern Western Ghats

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Abstract
The random amplified polymorphic DNA (RAPD) analysis was made to know the level of genetic variation between the five populations of the medicinal shrub, *Acalypha fruticosa* (Euphorbiaceae) distributed in an environmental gradient. In the present study, of the nine random primers tested, one primer, GAATCTCAGG which gave polymorphism was used in RAPD analysis. The primer produced 47 scorable DNA fragments in the populations studied which showed the existence of genetic variation between the populations. A dendrogram was constructed based on Jaccard's coefficient to determine the degree of genetic relationship among the five populations and analyzed. It showed that the genetic similarity among the populations is varying from 70 to 91 %.

Keywords: *Acalypha fruticosa*, RAPD analysis, polymorphism, jaccard's coefficient

Introduction
Acalypha fruticosa (L.) Willd. Belongs to the family, Euphorbiaceae is a locally used medicinal plant in Coimbatore district of Tamil Nadu, India. The leaves are used for the treatment of Jaundice, dyspepsia, stomachic and skin problems. It is a woody bushy shrub, mainly distributed in tropical regions of India, Arabia, peninsular Burma and Africa. In India it is abundantly by present in the foot hills of the Western Ghats (Matthew, 1995) [13]. at different environmental conditions. The effectiveness of medicinal properties of certain populations of this species is more pronounced than that of the others (Sathishkumar, 2009; Anandakumar, 2008) [17]. In addition environmental factors, genetic constitution is also determined to be the factor for this fact in many species (Linhart and Grant, 1996; Via and Lande, 1985, 1987) [11, 20]. Hence for the present study RAPD analysis carried out to show the variation between the diverse populations at DNA level from various areas of Western Ghats.

Materials and Methods
Collection of Plant Material
The plant materials were collected from five different populations of Western Ghats. Climatically these populations are differentiated from arid region to humid environments. In Chennimalai it is highly arid, in Maruthamalai it is arid, in Palamalai semi-arid habitat, in Thadagai hills it is humid, and in Burliar, the habitat is highly humid (Fig 1).

Random Amplified Polymorphic DNA (RAPD) analysis
DNA isolation and primer screening
Tender leaves of the study plants were collected separately from each population and were washed well with double distilled water for few times and stored at 700 C in sealed polythene bags. Genomic DNA was isolated form 100mg of the tender leaf tissues using Gen elute plant genomic DNA purification kit following manufacturer's instructions.

Quality check and quantification of genomic DNA
About 2 µl of the genomic DNA isolated from 100mg of leaf tissue was subjected to electrophoresis on a 0.8% agarose gel containing 0.5 µg/ml of ethidium bromide. After the electrophoresis, the gel was viewed over a UV transilluminator (UVT- 40 M, Herolab) and the quality and quantity of the DNA were assessed by using undigested λ DNA as control. The genomic DNA was diluted 4ng/ µl and stored at 40 C as working solution while the stock DNA (undiluted) was stored at -200 C in aliquots.

RAPD Polymerase Chain Reaction (PCR)
The RAPD polymerase Chain Reaction (PCR) was carried out in a 20 µl reaction volume containing 28ng of genomic DNA, 1 unit of *Taq* DNA polymerase, 4 µl primer, 0.2mM d NTPs, 10mM Tris - HCL (pH 9.0), 1.5mM MgCl2, 50mM KCl and 0.01% gelatin. Ampilification was carried out in a thermocycler (Eppendorff) with an initial strand separation at 940 C for 4 minutes, followed by 40 cycles of 1 minute at 370 C and 1.5 minute at 720 C. After 40 cycles, there was a final extension step of 5 minutes at 720 C. A negative control without genomic DNA template was used for ampilification along with genomic DNA from 5 habitats with primers. This is to confirm the quality of primer dimmers or possible contaminants. Ampilification products were resolved on 1.2% agarose gel in 1 X TBE buffer stained with ethidium bromide (0.5 µg/ml). The gel were photographed using a gel documentation system (syngene).

Molecular Characterization
Each RAPD band was treated as a unit character and was scored manually as independent binary codes ('1' for presence and '0' for absence). Only distances and well resolved bands were scored. Two people did the scoring independently, and bands that had conflicting data between the two readings were eliminated form further analysis. The percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of

bands. The 1/0 matrix was prepared and the data were used to generate genetic similarity (GS) based on Jaccard's Coeffecient of similarity, GS(ij) = a/a+b+c, Where GS (ij) is the measure of genetic similarity between individuals i and j, 'a' is the number of polymorphic bands that are shared by i and j. 'b' is the number of bands present in i and absent j and c is the number of bands present in j and absent in i. To examine the genetic relationship among populations, a dendrogram was generated from distance values using the unweighted pair-group method of arithmetic averages (UPGMA), using the software, multivariate statistic package version (MVSP version) 3.13n (http: // www.kovcomp.com/mvsp).

Results and Discussion

DNA extracted from five population samples were examined for their PCR - RAPD patterns. Out of 9 primers screened one was selected based on robustness of amplification, reproducibility and scorability of banding pattern and used for diversity analysis in all the five populations. The GAATCTCAGG primer generated 11amplification products out of which 6 bands were polymorphic with 54.5% polymorphism (Fig 2). The number of polymorphic bands was ranging from 4 to 6 between the populations and reported the genetic diversity between the four populations of *Matricaria chamomilla* in South West Iran revealed 93.18% of polymorphism from RAPD analysis. A total number of 47 polymorphic bands observed in the five studied populations. The percentage polymorphism was varied between the five populations studied. In palamalai 75% polymorphism obtained followed by 66% for Thadagai hills, 55% for Maruthamalai and 45 and 40% for Chennimalai and Burliar respectively (Tables 1 and 2). The Jaccard's coefficient of genetic similarity matrix was prepared based on RAPD data. The genetic similarity coefficient among the five populations of *A. fruticosa* varied from 0.70 to 0.91 between the Thadagai hills and Palamalai cluster and Burliar and Chennimalai cluster (Fig. 3). The dendrogram (Fig. 7) obtained from the RAPD analysis showed two clusters between Thadagai hills and Palamalai and Maruthamalai to Burliar - Chennimalai sub cluster. The abiotic environmental factors induced genetic differentiation within and between populations have been reviewed by Linhart and Grant (1996) [11].

The genetic diversity evident from the RAPD analysis clearly indicates the interaction between the major abiotic factor, climate and other minor abiotic factors in shaping the genome to adapt to new environment. Thus the geographic distribution as explained by Aghdaei *et al.* (2006) [1] in *Rosa damascena* and Verma *et al.* (2009) [19] in *Trichodesma indicum*, might have influenced *A. fruticosa* genome in the process of adjusting to the climate. Hoglund (2009) [7] observed that the cold adapted species living in shrinking environments expanding their range by resorting to genetic variation because of warmer climate. The above observations agree with the present study were less genetic variation between highly arid Chennimalai population and highly humid population Burliar of *A. fruticosa.* Interestingly McKay (2003) [14]; Meyer (2001) [15] and Zapater *et al.* (2002) reported the synergistic effect of climate and other abiotic factor contributing to genetic variation. Via and Lande (1985, 1987) [20] observed morphological as well as genetic variation among the populations of *Ranunculus reptans*. Similarly, the present study also revealed the morphological and genetic variation in *A. fruticosa* populations separated by abiotic factors. Koorneef *et al.* (2004) [18] in their review on natural variation in *Arabidopsis* populations attribute the mechanism for variation induced by abiotic factors operating at metabolite as well as transcript level.

The observations of the present study along with the report of similar studies conducted on other parts of the world endorse the molecular changes in DNA level to adjust to the change in abiotic environment. This mechanism in turn reduces the risk of survival of plants due to the climatic change now being experienced, at least to a certain extent.

Large variation has been reported for tolerance to abiotic factors such as freezing temperatures, drought, UV light, high and low carbon dioxide salt, metals or oxidative factors. Developmental traits showing large variation include flowering time (Koorneef *et al.*, 1998), floral morphology (Juenger *et al.*, 2000) [8], leaf morphology trichome density venation pattern branching, plant size, and growth rate and cell division. Considerable genetic variation has also been analyzed for physiological traits such as seed dormancy circadian period components of plant growth such as nitrogen use efficiency or plant responses to light and hormone treatments (Borevitz *et al.*, 2002; Botto *et al.*, 2003) [3, 4]. Biochemical traits also differ among accessions, such as the content of seed lipids glucosinolates, epicuticular waxes seed oligosaccharides and enzyme activities for primary and secondary metabolism (Kliebenstein *et al.*, 2001; Camposde Quiros *et al.*, 2000) [9, 5]. Finally, genetic variation has been detected for complex genetic mechanisms such as chiasma frequency, DNA methylation and gene expression levels (Mckay *et al.*, 2003; Meyer *et al.*, 2001; Cooley *et al.*, 2001; Queseda *et al.*, 2002; Bentsink *et al.*, 2003; Loudet *et al.*, 2003; Sergeeva *et al.*, 2004) [14, 15, 6, 16, 2, 12, 18].

Conclusion

The study on genetic variations of *A. fruticosa* between the populations had significant variation with ecological factors, which meant that the genetic differentiation of *A. fruticosa* could not be affected by genetic drift, but by the natural selection pressure from the microenvironment at different elevations.

Meanwhile the present study along with the report of similar studies conducted on other parts of the world endorse the molecular changes in DNA level to adjust to the change in abiotic environment. This mechanism in turn reduces the risk of survival of plants due to the climatic change now being experienced, at least to a certain extent. Consequently, major attention should be paid to the scientific conservation for wild populations of *A. fruticosa* at different elevations when strategies for breeding and germplasm conservation will be implemented in the future.

Table 1: Matrix coded for bands obtained in RAPD analysis in *Acalypha fruticosa*

Population	Bands										
	1	2	3	4	5	6	7	8	9	10	11
Chennimalai	1	1	1	1	1	1	1	1	1	1	1
Maruthamalai	1	1	0	1	1	1	0	1	1	1	1
Palamalai	1	1	0	1	1	1	1	0	0	1	1
Thadagai hills	1	1	1	1	1	1	1	0	0	1	1
Burliar	0	1	1	1	1	1	1	1	1	1	1

'1'- Presence of a band and '0' - Absence of band

Table 2: Polymorphic and non-polymorphic bands for GAATCTCAGG primer in *Acalypha fruticosa* populations.

Population	Total no. of bands	Polymorphic bands	Percentage of polymorphism
Chennimalai	11	5	45
Maruthamalai	9	5	55
Palamalai	8	6	75
Thadagai hills	9	6	66
Burliar	10	4	40

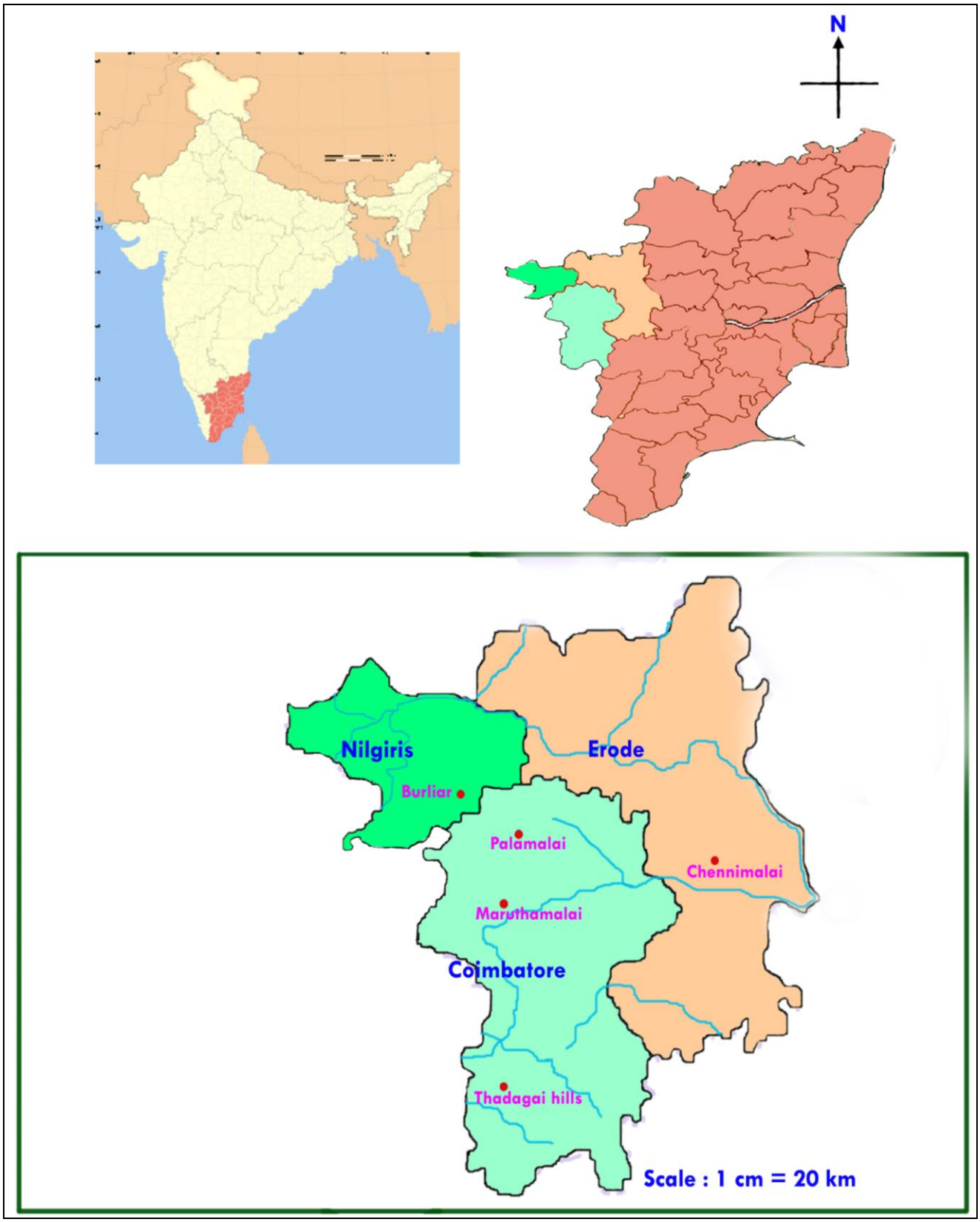

Fig 1: Location of Study Areas

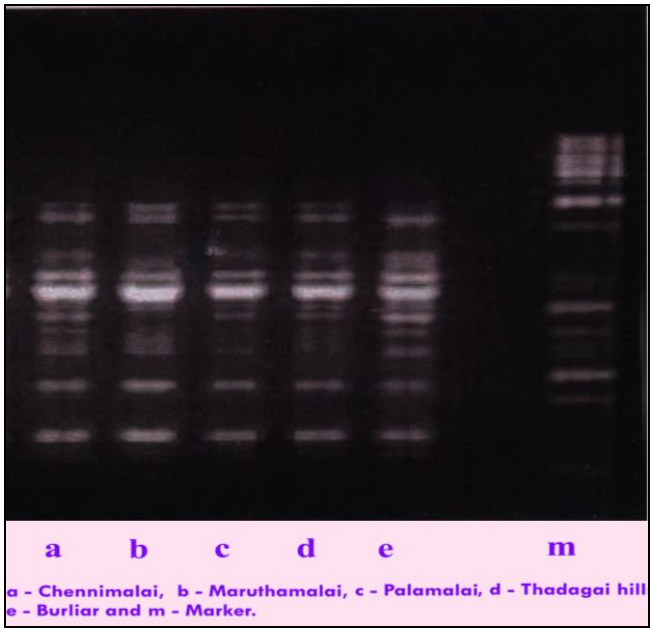

Fig 2: Amplification products obtained from DNA of the five different populations of *A. fruticosa* with UBC 807 - AGAGAGAGAGAGAGAGT primer.

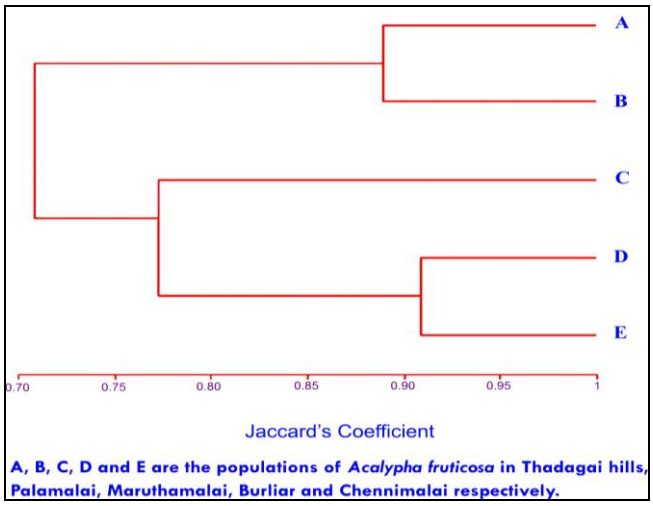

Fig 3: Dendrogram for the five populations of *A. fruticosa* developed from RAPD data using Unweighted Pair – Group Method of Arthmetic Averages (UPGMA).

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