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Inter Population Genetic Variability in *Tinospora cordifolia* (wild.) Miers ex Hook. F. & Thoms. (Menispermaceae), through RAPD marker

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Abstract. *Tinospora cordifolia*(Guduchi) is a widely used shrub in folk and ayurvedic systems of medicine. The notable medicinal properties reported are anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities. In this study, the RAPD (Random Amplified Polymorphic DNA) technique was employed for determination of the genetic variability of *Tinospora cordifolia* among the selected populations. The number of polymorphic loci is 19. The percentage of polymorphic loci is 46.34. The overall genetic distance and the genetic identity ranges from 0.1872 to 0.4555 and 0.6341 to 0.8293 respectively. The overall observed and effective number of allele is 0.5049 and 0.3902. Nei's overall gene diversity is 0.2114.
Keywords: *Tinospora cordifolia*, RAPD-PCR and genetic variation

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1 Introduction

Around the world many billions of people still use plants as their primary source of medicine. Much of these uses are based on knowledge passed down through generations. In addition, nearly 40% of the modern medicines are derived from natural products. India is bestowed with enormous biodiversity of medicinal plant species. Among them, *Tinospora* with a wide array of bioactive principles as well as proven medicinal use has received considerable scientific attention. *Tinospora cordifolia*(Willd.) Miers ex Hook. F. & Thoms is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae [1, 2]. It is distributed throughout tropical Indian subcontinent, Sri Lanka and China, ascending to an altitude of 1,200m. It thrives easily in the tropical region, often attains a great height, and seems to be particularly fond of climbing up the trunks of large neem trees. The part used for medicinal purposes are the stem, root, leaves and starch obtained from the roots and stem. It is widely used in veterinary folk medicine/ ayurvedic system of medicine for its general tonic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-allergic and anti-diabetic properties [2, 3, 4, 5, 6]. The plant is used in ayurvedic, ”Rasayanas” to improve the immune system and the body resistance against infections. The root of this plant is known for its anti-stress, anti-leprotic and anti-malarial activities [6, 7]. A large number of compounds have been isolated from the whole plant, aerial part, stem and roots of *T. cordifolia*. They belong to different classes such as alkaloids, diterpenoids, lactones, glycosides, steroids, sesquiterpenoid, phenols, aliphatic compounds and polysaccharides. The identification of this medicinal plant having enormous variation in its population is very much needed for this time. The presence of superior variety for the effective identification can then only be correctly identified. While having a wide variety of molecular marker techniques for the determination of genetic variability, RAPD is used in this study due to its implicational benefits. The method known as RAPD (Random Amplified Polymorphic DNA), which is simple and faster than other DNA fingerprinting techniques, uses a single oligonucleotide primer in a PCR (Polymerase Chain Reaction) with low stringency. The technique requires no sequence information prior to analysis and only a minute amount of DNA (Welsh and McClelland, 1990; [8]. DNA markers seem to be the best candidates for efficient eval-

uation and selection of plant material. Unlike protein markers, DNA markers segregate as single genes and they are not affected by the environment as morphological markers. Genetic diversity may be measured through genetic markers. These have been used to determine evolutionary relationship within and between species, genera or higher taxonomic categories [9]. The aim of this work is to evaluate genetic variation and relatedness of *Tinospora cordifolia* by Random Amplified Polymorphic DNA (RAPD) technique. The study was conducted to determine the genetic variation between the populations, to determine the genetic distance between five populations and to select the suitable accession for the isolation of medicinally important chemical constituents.

2 Materials and methods

The leaf samples of *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms were collected from Alangulam, Sankarankoil, Tenkasi, Ambasamudram and Tirunelveli from TIrunelveli District in Tamilnadu (Table 1). The plants were collected from these localities and identified by the Herbarium specimen deposited in St. Xavier's College Herbarium. The plant materials were stored in deep freezer at -70°C (Remi quick freezer) for RAPD work.

Table 1: . Areas of collection and accession ID of *Tinospora cordifolia*.

S. No	Area	Accession ID
1.	Alangulam	Pop 1
2.	Sankarankoil	Pop 2
3.	Tenkasi	Pop 3
4.	Ambasamudram	Pop 4
5.	Tirunelveli	Pop 5

Pop1, Pop2, Pop3, Pop4 and Pop5 are the ID numbers
given to the areas of collection of *Tinospora cordifolia*

DNA Isolation: Genomic DNA from the young leaves was isolated following CTAB method [10]. Quantity was also checked spectrophotometrically from the absorbance

data of the sample DNA at 260 and 280 nm. The purity of DNA sample was calculated from OD 260/OD 280 and its ratio ranged from 1.5 to 1.9 [11]. Random amplification was performed following a modified PCR method of Schweder [12]. The reaction was carried out in a thermal cycler. Five primers, procured from OPERON Technologies(Alameda,CA,USA), viz. OPX-20 (5'CCCAGCTAGA3'), OPX 18 (5' GACTAGGTGG 3'), OPX-6 (5'ACGCCAGAGG3'), OPX-12 (5'TCGCCAGCCA3') and OPX-14 (5'ACAGGTGCTG3') were used. The PCR amplification mix consisted of 10X reaction buffer (2.5 μl), 1.5 mM $MgCl_2$ (0.3 μl), 10 mM dNTPs (4.0 μl), 1 unit *Taq*polymerase (Genei, Bangalore), 2.4 μl of random primer and 2.0 μl of template DNA. The volume was made to 25 μl with sterile double-distilled water. The solution was mixed gently and amplification was carried out for 35 cycles. After initial heat denaturation of the DNA at 94 $^\circ$ C for 1 min, the thermal cycling was performed with the following temperature regimes 94 $^\circ$ C for 1 min, 37 $^\circ$ C for 1 min. and 72 $^\circ$ C for 1min. The final extension step was performed at 72 $^\circ$ C for 5 min followed by cooling to 4 $^\circ$ C for completion of the programme. The products were separated on 1.5% agarose gel in 1X TAE buffer (40 mM Tris acetate, pH 8.2; 1 mM EDTA). The gel was visualized using ethidium bromide stain and photographed in a gel documentation system (Velbert Lourmat, France).

Data Analysis: The PCR protocol was adopted in the study resulted in reproducible pattern of amplicons using specific combination of accession and primer. Only the primers which displayed reproducible, scoreable and clear bands were considered for analysis. The image profiles of banding patterns were recorded. The banding patterns were scored based on the presence or absence of clear, visible and reproducible bands [8]. The similarity index between individuals was calculated following the method by Nei and Li [13]. The pair wise genetic distance between the populations was calculated using Pop gene package version 1.31.

3 Results and Discussion

The five primers used to analyze genetic variation in **Tinospora cordifolia** produced 41 polymorphic bands (Fig. 2). The same type of bands occurred at different frequencies in all populations. The number of polymorphic loci is 19. The percentage of polymorphic

Table 2: Nei's Unbiased Measures of Genetic Identity and Genetic distance in *Tinospora cordifolia*.

pop ID	1	2	3	4	5
1	****	0.7317	0.8049	0.7561	0.8293
2	0.3124	****	0.7805	0.8293	0.7561
3	0.2171	0.2478	****	0.8049	0.6341
4	0.2796	0.1872	0.2171	****	0.7317
5	0.1872	0.2796	0.4555	0.3124	****

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

loci is 46.34. The overall genetic distance and the genetic identity are ranged from 0.1872 to 0.4555 and 0.6341 to 0.8293 respectively (Table 2). The overall observed and effective number of allele is 0.5049 and 0.3902. Nei's [14] overall gene diversity is 0.2114.

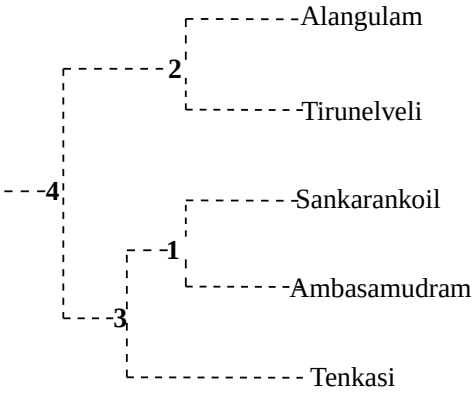

Figure 1: Dendrogram of Five different locations of *Tinospora cordifolia* generated by UPGMA based on RAPD markers.

Dendrogram based on Nei's [15], genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the five accessions. All data were analyzed by a computer programme, POPGENE (Version 1.31) [16]. The UPGMA dendrogram (Fig. 1) shows that the five accessions are divided in three

main clusters. Cluster 1 consisted of pop2 and pop4 and the 2nd cluster consisted of pop1 and pop5 with more similarity. Pop3 differed from other accessions with more variation in 3rd cluster. The dendrogram based on the pooled similarity indices is indicative of low to medium level of polymorphism among the 5 accessions. This result demonstrated existence of low level of genetic variability in the species in a small geographical area. The dendrogram showed three clusters between five populations. This low level of RAPD variation observed indicates the need for detailed studies on the interplay of sexual and/or vegetative modes of reproduction and natural selection forces operating on the populations of this region. Generally, the observations of this study indicate that there is little variation among the different accessions of *Tinospora cordifolia* collected from different population of Tirunelveli District of Tamilnadu. It may be noted here that the microenvironments of the habitats of the species are largely similar in geographically distant locations as the plant is observed to grow near the banks of water bodies. Therefore, wide heritable phenotypic and chemotypic variation observed in the collection of accessions might be due to qualitative genetic differences. The lack of RAPD variation is possible due to highly heterozygous plants but that would not be uniform across the different populations throughout the District and in this case the accessions used represent the natural populations from various agro-climatic zones of India. This technique has frequently been used for the detection of genetic variability in plants.

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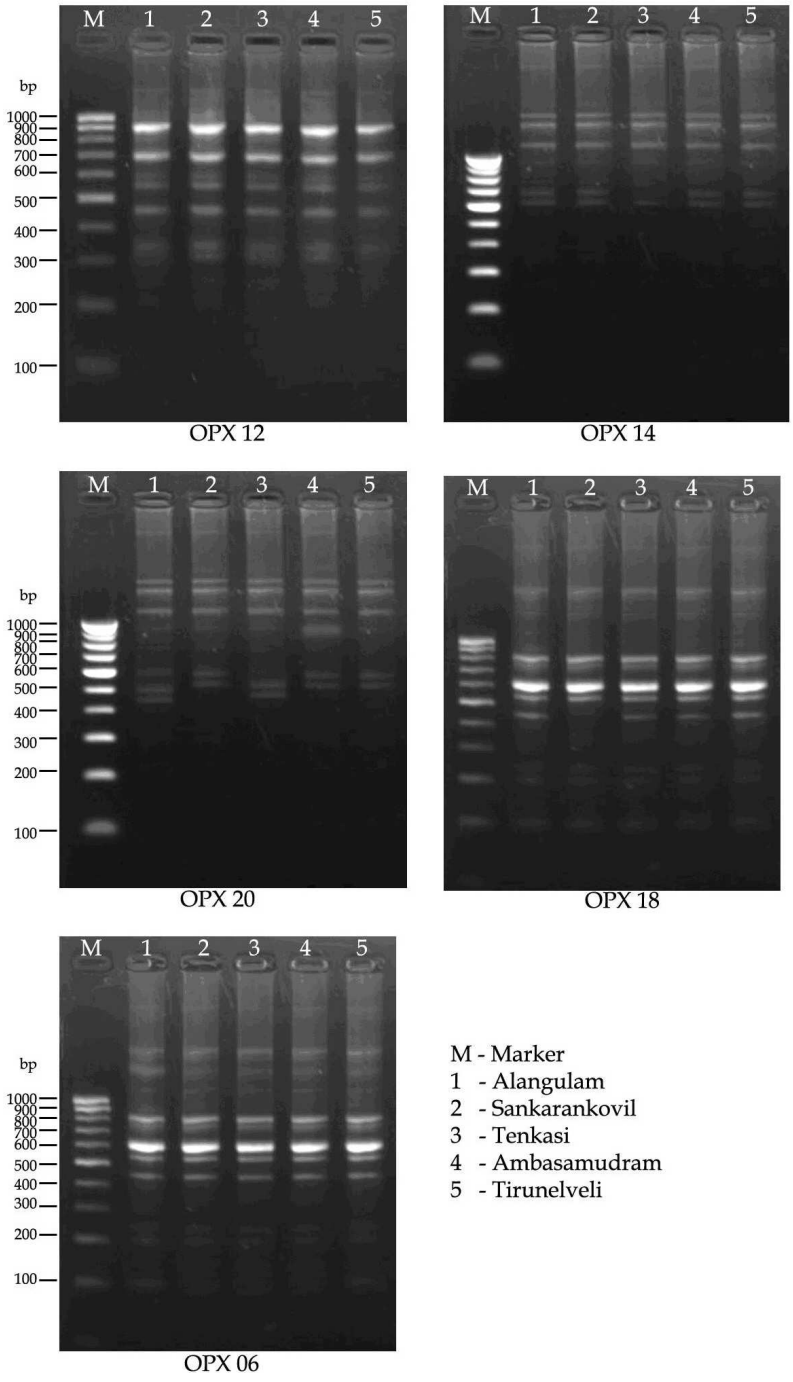

Figure 2: RAPD-PCR banding pattern in *Tinospora cordifolia*.