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Detection of DNA Polymorphism by RAPD-PCR Fingerprint in *Plumbago zeylanica* L. from Western Ghats

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Studies were undertaken for detection of DNA polymorphism through Random Amplified Polymorphic (RAPD) DNA fingerprint in *Plumbago zeylanica* L. collected from ten locations in Western Ghats. A total of 27 DNA fragment (bands), ranging from 4 to 6 was observed. The genetic distance between the population ranged from 0.1178 to 0.8109 and the genetic identity ranged from 0.4444 to 0.8889. The overall observed and effective number of alleles is 0.3958 and 0.3599 respectively. Over all genetic diversity is 0 .1817 and percentage of polymorphic loci was 81.48. The genetic similarity analysis was conducted on the basis of presence or absence of bands which revealed a wide range of variability within the populations. The cluster analysis clearly showed that there was high degree of diversity within the population. The closest genetic distance existed within population. Thus these RAPD markers have the potential for conservation of identified clones and characterization of genetic relatedness among the population.

Key words: Gene diversity, RAPD-PCR analysis, *Plumbago zeylanica*

Plumbago zeylanica L. is an important medicinal herb. It has high medicinal properties. It belongs to the family Plumbaginaceae. Local name: Vellai sidhirai mulam and Kotivelli. *Plumbago zeylanica,* a rambling subscandent perennial herb or under shrub with green branches, stems somewhat cylindrical, spreading, terate, striate, glabous. Leaves opposite/alternate, ovate or oblong, petiole narrow, amplexicaul at the base and often dialted into stipule like auricles. Flowers white, in axillary and terminal elongated spikes, bisexual. Calyx densely covered with stalked, sticky glands, corolla white, very slender, tubular, stamens 5, free, ovary superior, 5-gonous, one celled, and ovule single and basal (John De Britto and Mahesh 2007).

The fruit, leaves, root of *Plumbago zeylanica* L. have high medicinal and economic value (Rajendran and Agarwal, 2007). It is used against constipation (Pande *et al*, 2007). The root of *Plumbago zeylanica* has numerous therapeutic uses. The root is known to be abortifacient and to have vesicant properties. It is used as appetizer and expectorant and used for dysentery, diarrhoea, diuretic and peptic ulcers. The root paste is applied externally for filarial diseases. It is used externally for early maturation, rupture and healing of abscess. The root powder taken orally along with honey gradually reduces hypercholostraemia and improves blood formation (anaemia). It is used to reduce obesity, vitiligo, splenomegaly, hepatomegaly and ascitis. It is also used to relieve coryza (running nose), hoarseness of voice and sore throat. It is used in the form of local applications for leucoderma, scabies, psoriasis, symptoms of leprosy and allied skin diseases. The decoction of the root is useful in checking and preventing spermatorrhoea (Madhava Chetty *et al,* 2006).

The conservation of plant population and species is mostly concerned with the number of individuals present in the populations in order to assess factors such as genetic drift, inbreeding depression and lack of mates in self compatible species (Barrett and Kohn, 1991).

Molecular techniques help researches not only to identify the genotypes but also in assessing and exploiting genetic variability through molecular markers. Random Amplified Polymorphic DNA (RAPD) analysis has proved for estimating genetic diversity particularly to assist in the conservation of rare species and plant genetic resources. RAPD analysis in particular has proven to be a rapid and efficient means of genome mapping and is well suited for the genetic resource characterization (Williams *et al*, 1990). The DNA fingerprinting generated by the polymerase chain reaction using arbitrary primers has provided a new tool for DNA polymorphism in number of herb species (Padmalatha and Prasad, 2006).

In the present study the detection of DNA polymorphism in *P. zeylanica* L. collected from ten locations of Western Ghats has been carried out using RAPD markers.

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Materials and Methods

The extensive field survey was carried throughout Western Ghats and ten accession of *P. zeylanica* L. were collected from Kannanur, Kalakad, Kothyar, Chunkankadai, Maruthamalai, Thiruparapu, Kollimalai, Aralvoimozhi, Karayar and Courtallum. Five plant samples from each location were collected. The plant materials were transferred to plastic bags for transport from field to laboratory. The samples were maintained in deep freezer at 70° C till the analysis was done. Young leaf tissues were used for extracting total genomic DNA, following protocol suggested by (Uta Pich and Ingo Schubert (1993).

The amount of DNA and its quality were assessed by UV spectrophotometer. The DNA was pure enough (OD260/280 =1.68), (Sambrook and Russel, 2000) for RAPD- PCR analysis (Williams *et al.*, 1990).

Earlier, ten primers were tested and five primers OPX 03 (GGAGGGTGTT), OPX 04 (CCGCTACCGA), OPX06 (AGGGGTCTTG), OPX12 (TCGCCAGCCA), OPX19 (TCTGTGCTGG) which produced reproducible bands and were selected. The experiments were repeated three times and confirmed the reproducibility of bands. PCR reactions were carried out in a total volume of 20 µl at a final concentration of 1 mM $MgCl_2$, 2 mM dNTP, *Taq* DNA polymerase enzyme (1ul/20 µl), with approximately 200 ng DNA as a template and a single random primer (0.2 mM). The PCR cycles were as follows: 94°C for 2 min, 94°C for 15 sec, 35°C for 15 sec, 72°C for 30 sec which were repeated in 35 cycles followed by 5 min-extension at 72°C. 8 µl of PCR product was subjected to electrophoresis at 80 V using 1.5 % agarose gel stained with ethidium bromide.The bands were viewed under UV transilluminator and photographed with gel documentation system Alpha Imager 1200.

Based on the primary data (presence or absence of bands), pair wise genetic distance between samples was calculated using POP GENE package version 1.31. (Yeh et al., 1999).

Results and Discussion

Analysis of ten accession of P. zeylanica L. revealed 22 polymorphic loci. Five primers generated reproducible, informative and easily scorable RAPD profiles. A total of 27 bands were scored for the 5 RAPD primers out of which 115 bands polymorphic with number of bands ranging from 4 to 6 (Plate 1). The same type of bands occurred at different frequencies in all populations. There were many additional bands neglected which were not reproducible. The percentage of polymorphic loci was 81.48. The genetic distance between the population ranged from 0.1178 to 0.8109 and the genetic identity ranged from 0.4444 to 0.8889 which are shown in Table 1. As it was

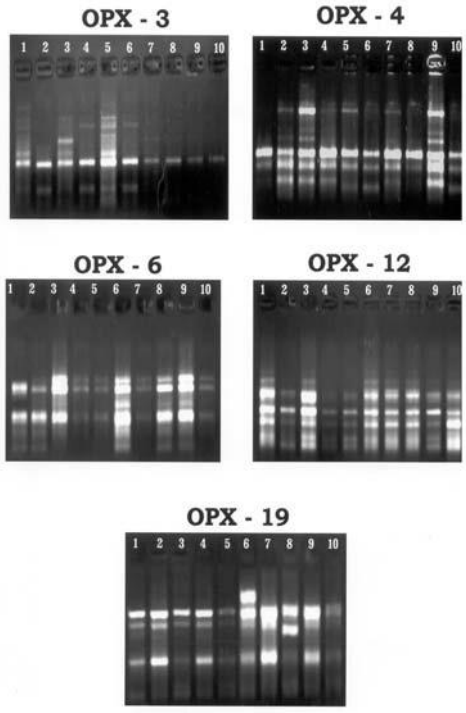

Plate 1. *Plumbago zeylanica* L.

observed in the Popgene software results, the overall observed and effective number of alleles was 1.8 and 1.3 respectively and overall genetic diversity was 0 .2719. The obtained result was analyzed in the POP GENE package 1.31 and the dendrogram obtained clearly indicates three clusters (Nei, 1978) (Fig 1).

Population 5 form a separate clade. Populations 1,6,2,4 and 7 form one clade and populations 3,9,8 and 10 form another clade. Three groups are distinctly formed in the first clade (populations 1 and 6,2 and 4 and 7). In the second clade again three groups are formed (populations 3 and 9, 8 and 10) (Fig 1).

The utility of RAPD markers in estimating genetic divergence has been demonsrated in several studies. The similar study was done in RAPD in in vitro regenerated leaf explants of Plumbago zeylanica L. (Rout, 2002). Genetic diversity analysis in Rauvolfia serpentina and Rauvolfia tetraphylla L, using RAPD Markers. (Padmalatha and Prasad 2007; Padmalatha and Prasad, 2006). Recently studies on molecular analysis in Urginea indica Kunth collected from different location of Karnataka was reported. (Harini et al., 2008).

The clustering results of different accessions suggest that Plumbago zeylanica L. undergoes major part of genetic variation by environmental factors. Genetic diversity refers to the variation at the level of individual genes (polymorphisms), and provides a mechanism for populations to adapt to their ever-changing environment. The genetic variability in P. zeylanica L. may be partly explained

Table 1. Nei's Original Measures of Genetic Identity and Genetic distance

pop ID	1	2	3	4	5	6	7	8	9	10
1	****	0.8519	0.7407	0.8148	0.5556	0.8519	0.8519	0.7778	0.7037	0.6667
2	0.1603	****	0.6667	0.8889	0.6296	0.7778	0.77778	0.7037	0.7778	0.5926
3	0.3001	0.4055	****	0.5556	0.4444	0.6667	0.6667	0.7407	0.8148	0.7778
4	0.2048	0.1178	0.5878	****	0.7407	0.7407	0.8889	0.6667	0.6667	0.6296
5	0.5878	0.4626	0.8109	0.3001	****	0.4815	0.6296	0.5556	0.4815	0.5185
6	0.1603	0.2513	0.4055	0.3001	0.7309	****	0.7778	0.7778	0.7037	0.5185
7	0.1603	0.2513	0.4055	0.1178	0.4626	0.2513	****	0.7778	0.7037	0.7407
8	0.2513	0.3514	0.3001	0.4055	0.5878	0.2513	0.2513	****	0.7778	0.6667
9	0.3514	0.2513	0.2048	0.4055	0.7309	0.3514	0.3514	0.2513	****	0.6667

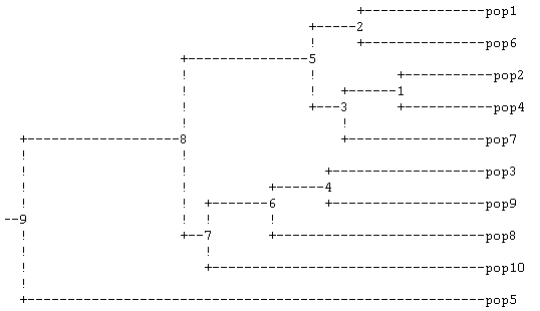

Figure 1. Dendrogram Based Nei's Genetic distance

as a result of abiotic- geographical, hydrographical connections, climatic differentiations - annual rainfall, temperature, pH of the soil, humidity etc., and also the various biotic factors.

Genetic variation in a population is measured by the heterozygosity or the degree of polymorphism. For the conservation of a species, genetic variability is of the utmost importance to preserve. Genetic variability among all species is important to maintain since it represents the 'blue print' for all of the living things on earth.

It is desired to maximize the preservation of alleles. Geographical, climatic or reproductive variables explain the partitioning of the diversity observed which may aid in improving the strategies for maximizing the efficiency of germplasm collection and preservation.

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