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Analysis of Genetic Diversity of *Plumbago zeylanica* L.

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Abstract

Plumbago zeylanica is extensively used in herbal medicines and it has an anticancer compounds. The current investigation examines the genetic diversity of *Plumbago zeylanica* from five different populations of the Western Ghats using ISSR markers. Twenty primers were screened of which twelve were found to be the most informative. Based on Jaccard's coefficient, the *P. zeylanica* populations confirm a high level of genetic variability or heterozygosity. The PZ_1 (Kallar -Ooty) accession has the highest percentage (70.59 %) of polymorphism. The overall mean value of genetic diversity (H_T) in the group is 0.43 whereas the diversity within populations (*Hs*) is 0.30. The overall locus of Genetic differentiation (GST) between populations is 0.30. Inter population gene flow (*Nm* = 1.28) shows that the genetic exchange among populations is limited. Thus the ISSR marker is useful to study the genetic diversity of *Plumbago* populations.

Keywords: Genetic Diversity, Inter Simple Sequence Repeats (ISSR), *Plumbago zeylanica* L

1. Introduction

Biodiversity provides enormous direct benefits and indirect essential services for mankind through natural ecosystem function and stability. The Western Ghats is one of the diversity hot spots in India. It is considered as one of the treasure houses of several indigenous medicinal plants practiced in traditional and folk medicine. Most of these plants are proven to possess unique medicinal properties which can even cure dreadful diseases like cancer. However, traditional knowledge of medicinal plants has mostly been inherited traditionally and conserves these plants in its natural environment or cultivating it in favorable environments for future generations.

Genetic diversity measurements are important for selection of superior genotypes and for considering conservation of a particular species. It is also a best method to study the resources for conservation and utilization identification of the variants of economic value. Molecular markers help to assess the genetic diversity via DNA amplification. ISSR markers had also applied to study genetic variability of medicinal plant species [3, 7, 11].

Plumbago zeylanica L. is locally called Vellai Sidhirai Moolam which comes under the family Plumbaginaceae and is used to cure varies ailments and its root has rich amount of alkaloid (Plumbagin) which is used to prepare anticancer drug [9].

The current study is aimed to use ISSR markers for analyzing the molecular variations of *Plumbago zeylanica* in five selected geographic areas of the southern Western Ghats.

2. Materials and Methods

2.1 Plant Collection

The fresh and uninfected leaves of *Plumbago zeylanica* were collected from the Western Ghats of India (Figure 2) like Kallar (PZ_1), Senkottai gap (PZ_2), Oothu (PZ_3), Palagat gap (PZ_4), and Aralvoimozhi (PZ_5). The voucher

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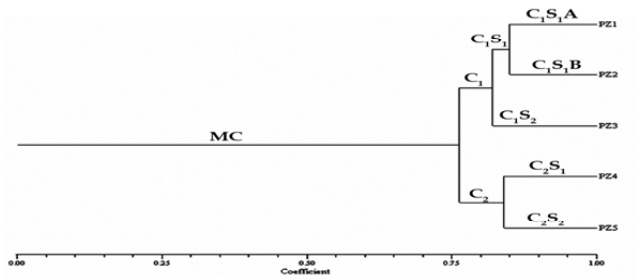

Figure 1. UPGMA dendrogram of *P. zeylanica*

Figure 2. *Plumbago zeylanica* L.

specimen (No: XCH: 28076-28080) was deposited at the St. Xavier's College Herbarium (XCH), Palayamkottai, Tamilnadu.

2.2 Isolation of Genomic DNA and PCR Amplification

Modified CTAB method was used to isolate genomic DNA [1] and it was preserved at –70° C for PCR amplification. A sum of 20 ISSR primers was screened, of which 12 primers produced reproducible band. For ISSR analysis, the annealing temperature varied at 34–56°C for 30 sec in amplification process. The amplified DNA was run on 1.5 % agarose gel containing ethidium bromide in electrophoresis and viewed under UV transilluminator.

2.3 Data Analysis

Five populations selected were compared with each other based on amplification profiles. Genetic similarity matrix of each plant was calculated using Standard coefficient method. A dendrogram was drawn in SHAN clustering module of NTSYS-pc software version 1.5 using UPGMA algorithm [6, 8]. The genetic diversity within and between populations was calculated using POPGENE package version 1.31software, based on Nei's formula.

3. Results and Discussion

Molecular markers (ISSR) have frequently been used for detecting the genetic variability in medicinal plants. The genetic variability and the relationships between five accessions of *Plumbago zeylanica* L. were analyzed with 12 primers which produced resolved amplified fragments that varied from 4 to 7 polymorphic bands. The differences in fragments size with different primers used for ISSR assay suggested that each of the primer amplified a different set of loci in the genome. A total of 68 ISSR fragments (Figure 3) its base pair varied from 0.2 to 1.0 kbp were observed. The mean genetic heterozygosity or diversity (*H*) ranged from 0.2103 to 0.3313. The PZ_2 population showed least diverse (0.21). The PZ_4 population showed highest variability (0.33) and the PZ_3 population revealed intermediate diversity (0.27) (Table 1). Nei's overall genetic diversity or heterozygosity was 0.2353. The genetic identity between the population ranges from 0.6028 to 0.7941 and genetic distance between the population ranged from 0.2305 to 0.5059. The observed and effective number of alleles was 1.5735 and 1.4163 respectively. The total polymorphic loci percentage was 57.35.

The study recorded an average genetic diversity of 0.3069 within populations (*Hs)*. The maximum gene diversity (*Hs)* is 0.3916. The minimum (*Hs*) is 0.2253. The

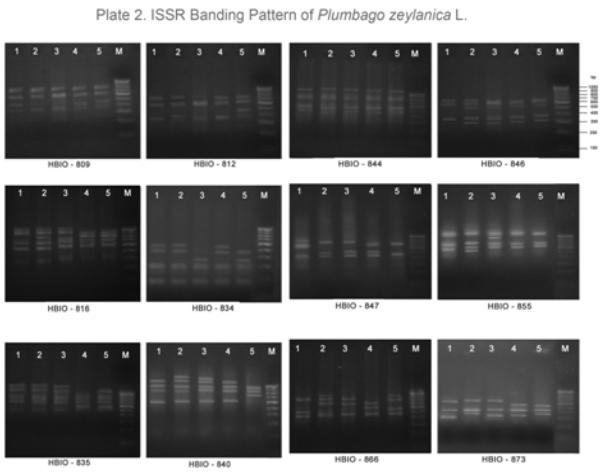

Figure 3. ISSR banding pattern of *Plumbago zeylanica* L.

total diversity (H_T) ranged from 0.3976 to 0.4775 with an average of 0.4369. The genetic differentiation (G_{ST}) ranged from 0.1602 to 0.4584 between populations with a mean value of 0.3073. The band frequency ranged from 0.6064 to 0.7318 and the average was 0.7092. The probable gene flow from one generation to other generation (*Nm*) was 1.2826 while the lowest was 0.5907 and highest was 2.6210 (Table 2). The highest percentage of polymorphism was 70.59%.

The present investigation revealed that *P. zeylanica* collected from Kallar (Ooty) showed high level of genetic variability based on ISSR markers. Using UPGMA algorithm a dendrogram was constructed to inter phylogenetic relationships between the five populations (Figure 1). Based on similarity index, a dendrogram was produced using Jaccard's coefficient matrix, which showed two major clusters with 77% similarity. Between the two major clusters, the upper cluster (C_1) comprised of PZ_1, PZ_2 and PZ_3 accessions respectively while the lower cluster (C_2) comprised of PZ_4 and PZ_5. Further, accessions of the upper cluster (C_1) was divided into two major sub clusters ($C_1 S_1$) and ($C_1 S_2$) having 81 % similarity. The upper major sub cluster 1 ($C_1 S_1$) again separated into two sub cluster $C_1 S_1$A and $C_1 S_1$ B, comprised of PZ_1 and PZ_2 having 85 % similarity. The lower major sub cluster (C_2) was further sub divided into two sub cluster $C_2 S_1$ from PZ_4 and $C_2 S_2$ from PZ_5 having 84 % similarity.

The ISSR markers were used for estimating molecular variation of *Perinereis aibuhitensis* [4], *Magnolia wufengensis* [5], *Vicia amoena* [10] and *Chrysanthemum* [2] by respective researchers.

Table 1. Genetic diversity within the populations of *P. zeylanica*

Accession	NPL	% of polymorphism	Na	Ne	H	I
PZ_1	48	70.59	1.7059	1.6245	0.3282	0.4632
PZ_2	32	47.06	1.4706	1.3945	0.2103	0.2995
PZ_3	42	61.76	1.6176	1.5239	0.2795	0.3972
PZ_4	48	69.32	1.7059	1.6353	0.3313	0.4667
PZ_5	39	57.35	1.5735	1.4849	0.2577	0.3666

PZ_1- Kallar; PZ_2- Senkottai gap; PZ_3- Oothu; PZ_4- Palagat gap; PZ_5- Aralvoimozhi;NPL- Number of Polymorphic Loci; *Na*- Observed number of alleles; *Ne*- Effective number of alleles; *H*-Gene heterozygosity; *I*- Shannon information index

Table 2. Genetic and gene diversity within and between the populations of *P. zeylanica*

S.No	Primers	No. of amplified fragments	Band Frequency	H_T	Hs	G_{ST}	Nm
1.	HBIO-809	5	0.7312	0.4291	0.2957	0.3150	1.0873
2	HBIO-812	5	0.7289	0.4737	0.3677	0.2403	1.5807
3	HBIO-816	7	0.7311	0.4220	0.2398	0.4302	0.6622
4	HBIO-834	5	0.7261	0.3976	0.2253	0.4343	0.6512
5	HBIO-835	7	0.7318	0.4096	0.2991	0.2926	1.2088
6	HBIO-840	6	0.7286	0.4565	0.3239	0.2951	1.1943
7	HBIO-844	4	0.7307	0.4728	0.2551	0.4584	0.5907
8	HBIO-846	6	0.6064	0.4172	0.2981	0.3016	1.1578
9	HBIO-847	6	0.7273	0.4172	0.2871	0.3242	1.0422
10	HBIO-855	6	0.6075	0.4634	0.3916	0.1602	2.6210
11	HBIO-866	6	0.7314	0.4775	0.3803	0.2067	1.9189
12	HBIO-873	5	0.7296	0.4062	0.3202	0.2297	1.6767

H_T -Total diversity; *Hs*- Genetic diversity within populations; G_{ST}- Genetic differentiation; *Nm*- Gene flow

4. Conclusion

Species adaptation is enhanced by high level of genetic diversity in this changing environment. Thus, the present study revealed that the population which exhibited high percentage of polymorphism was considered to be the superior genotypes. The PZ_1 accession collected from Kallar (Ooty), had the highest percentage of polymorphism. Hence, such a population could be collected and conserved for sustainable utilization.

5. Acknowledgement

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