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Molecular Analysis and Study of Genetic Relationships among Species of *Desmodium* Desv. Using RAPD Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Author MM designed the study, performed the RAPD and cluster analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SNM and SDP managed the analyses of the study. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The present study was aimed to determine the genetic relatedness and diversity among accessions of *Desmodium* spp using RAPD markers.
Study Design: In order to achieve the aim authentic samples of *Desmodium dichotomum* (Willd.) DC., *Desmodium laxiflorum* DC., *Desmodium scorpiurus* (Sw.) Desv., and *Desmodium triflorum* (L.) DC., were analyzed and compared to illustrate the phylogenetic relationships among them.
Place and Duration of Study: National Research Institute of Basic Ayurvedic Sciences, Pune, India June – November, 2015.
Methodology: In this study, twenty five universal RAPD primer sets for plant were used. The amplified products were compared and the phylogenetic tree was drawn using UPGMA (Unweighted Pair Group Method with Arithmetic mean) by applying Jaccard's similarity coefficient.
Results: Twenty five random primers produced 218 loci. The primers RPI 14 and RPI 17 showed highest variability; the primers RPI 2 and RPI 9 were genetically closely linked. Identification of medicinal plants by their molecular signature is a well known and highly reliable tool to assess the genetic relatedness among species.

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Conclusion: There are less reports on genetic diversity studies of Desmodium, so the present study can be a baseline data for the development of robust and economic approach which may result in more inclusive *ex situ* conservation.

Keywords: PCR; phylogenetic; UPGMA; beggarweed.

1. INTRODUCTION

Desmodium Desv. (Fabaceae) is commonly known as beggarweed, with approximately 280 known species till date. Out of these around sixty are found in India. Hooker [1 reported 49 spp, and Cooke reported 14 spp, [2]. This plant mainly found growing in dry hilly areas of tropical and subtropical region. Most of the species of this genus known to play crucial role in sustainable agriculture [3]. *Desmodium* spp are being used as folk medicine in treating liver disorders, dysentery, ulcers and ocular problems [4].

DNA fingerprinting tool and genetic diversity studies are becoming mandatory as well as are gaining insight in the conservation biology [5,6]. Due to various reasons, such studies are still inadequate in terms of number, specifically regarding the Desmodium genus particularly at the gene level. Traditional methods in diversity study were based on morphological and / or agronomic traits. Such morphology traits and phenotypes are generally influenced by environmental factors [7].

Random amplified polymorphic DNA (RAPD) technique is reliable, easy, cost efficient and fastidious for the detection of genetic variations at primary level [8]. Though this method of genetic diversity screening has many advantages, it is usually criticized for reproducibility [9] and dominance [10]. Still RAPD is one of the commonly used techniques for the determination of genetic diversity in many tropical plants [11-13]. Therefore, in this study RAPD marker were used to analyze the genetic relatedness and to develop fingerprints among four *Desmodium* spp.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Healthy plant material (leaves) of *Desmodium dichotomum* (Willd.) DC., *Desmodium laxiflorum* DC., *Desmodium scorpiurus* Desv., and *Desmodium triflorum* (L.) DC. were collected from NRIBAS, Pune, India in the month of June-July, 2015. Plants were identified and authenticated using standard flora.

2.2 DNA Extraction

Fresh leaf tissue (100 mg) was used for DNA extraction using liquid nitrogen followed by homogenization. 3B Black Biotech Biotools kit was used for the extraction purpose. Biophotometer (Eppendorf, India) was used for measuring the amount DNA. The qualitative detection of DNA was made on 1% agarose gels against the known DNA marker ladder (Promega, USA). Presence of DNA was confirmed by visualization of band.

2.3 RAPD- PCR

For the polymerase chain reaction (PCR) analysis was performed in thermal cycler (Peltier P25+ (Cyber Lab)) for which mixture (total volume 20 µl) was prepared by using:

1. DNA template (10 ng);
2. Primer (0.2 µM)
3. dNTPs (100 µM)
4. PCR buffer (10 mM)
5. $MgCl_2$ (1.5 mM)
6. *Taq* Polymerase (1 Unit)

Program for PCR was as follows: Initial denaturation (3 min) at 94ºC, followed by 30 cycles of denaturation (45 sec) at 94ºC, then annealing (30 sec) at 36ºC; which was further followed by extension (1 min) at 72ºC; with final extension step (5 min) at 72ºC and end hold at 4ºC [14,15]. During PCR analysis a negative control (without DNA template) and positive control (with standard DNA template) was maintained for confirming the contamination free reactions. Chemicals used in PCR amplification were of Promega, USA and Primers from 3B Black Biotech. Amplified products were visualised and detected by gel electrophoresis (1% agarose) followed by documentation (BioRad, Germany). To avoid the controversy of reproducibility of banding patern, the PCR amplification was repeated twice.

2.4 Statistical Analysis

Amplicons were scored as discrete characters on the basis of presence or absence of the band using GelQuest® software. To calculate the Jaccard's similarity coefficients (JSCs) binary matrices resulted from the analysis were used with the aid of Clustervis® software. Dendrogram was generated based on JSCs using UPGMA [15,16].

3. RESULTS AND DISCUSSION

The DNA obtained was 27.0 ng/µl (*D. dichotomum*), 34.05 ng/µl (*D. laxiflorum*), 31.89 ng/µl (*D. scorpiurus*) and 28.66 ng/µl (*D. triflorum*).

Fig. 1 shows the RAPD profile of all *Desmodium* species used in the study. Those primers were considered in the analysis which was clear,

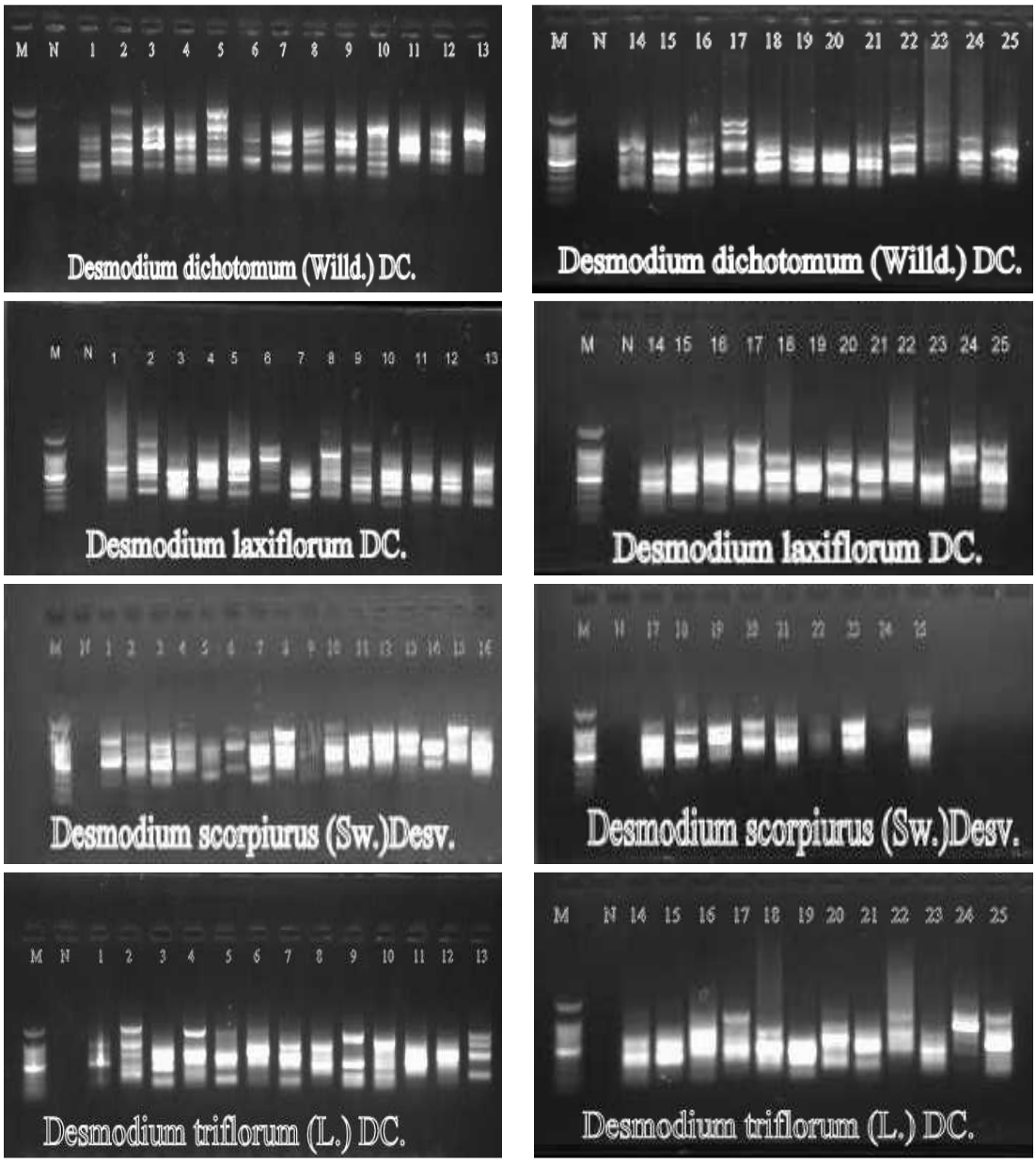

Fig. 1. RAPD profiles of plants studied

(M: DNA marker ladder; N: negative control; 1-25 numbers: Primers 1-25 as per table 1)

scorable and reproducible. The term 'polymorphic bands' was used if the band is present in some of the individuals and 'monomorphic' if found in all the individuals.

The RAPD PCR resulted in amplicons from the specific sequences of the primers giving rise to bands. In the analysis, total 25 primers were used that generated approximately 218 RAPD fragments for all the *Desmodium* species. In the RAPD analysis of *D. dichotomum*, 67 RAPD fragments were generated of which 80% were polymorphic. 57 RAPD fragments were observed in the analysis of *D. laxiflorum* of which 84% were polymorphic. *D. scorpiurus* analysis generated 42 fragments of which 68% were found to be polymorphic. 80% polymorphism was observed in the analysis of *D. triflorum* and total RAPD fragments generated were 52.

Table 1. Details of RAPD primers and banding pattern

Sr. no.	Primer code	Accession number	No. of polymorphic bands	Remarks
1	RPl 1	AM765819	2	Bands were observed only in *D. dichotomum*
2	RPl 2	AM750044	-	
3	RPl 3	AM773310	1	Band was observed only in *D. dichotomum*
4	RPl 4	AM773769	-	
5	RPl 5	AM773770	3	Bands were observed only in *D. dichotomum*
6	RPl 6	AM773771	2	Bands were observed in *D. dichotomum* and *D. scorpiurus*
			1	Band was observed in *D. laxiflorum* and *D. triflorum*
7	RPl 7	AM773312	-	
8	RPl 8	AM773773	-	
9	RPl 9	AM773315	1	Band was observed in *D. laxiflorum*
10	RPl 10	AM750045	-	
11	RPl 11	AM911709	-	
12	RPl 12	AM773316	-	
13	RPl 13	AM750046	1	Band was observed in *D. laxiflorum*
			1	Band was observed in *D. triflorum*
14	RPl 14	AM773774	1	Except D. triflorum band was present in others
15	RPl 15	AM773775	-	
16	RPl 16	AM773776	-	
17	RPl 17	AM911710	2	Bands were observed in *D. dichotomum*
18	RPl 18	AM765830	1	Band was observed in *D. laxiflorum*
19	RPl 19	AM773777	1	Band was observed in only *D. scorpiurus*
			1	Band was observed in *D. triflorum*
20	RPl 20	AM773317	1	Band was observed in *D. triflorum*
21	RPl 21	AM765820	1	Band was observed in *D. laxiflorum*
22	RPl 22	AM911711	1	Band was observed in *D. laxiflorum* and *D. triflorum*
23	RPl 23	AM911712	2	Bands were observed in *D. dichotomum* and *D. scorpiurus*
			2	Bands were observed in *D. laxiflorum* and *D. triflorum*
24	RPl 24	AM765821	1	Band was observed only in *D. dichotomum*
			2	Bands were observed in *D. laxiflorum* and *D. triflorum*
25	RPl 25	AM750054	1	Band was observed in *D. laxiflorum* and *D. triflorum*
			1	Band was observed in *D. dichotomum* and *D. scorpiurus*

In totality approximately 50% primers produced the polymorphic bands which were specific to the particular species. The primers which produced polymorphic bands can be used as identification tool during molecular analysis (Primer- RPI 1, RPI 3, RPI 17).

UPGMA cluster analysis: The dendrogram (Fig. 2) depicts a diverse and complex clustering. The cluster analysis using UPGMA method, showed considerable genetic variations in the *Desmodium* spp. The present study revealed that RAPD marker can be used for the assessment of genetic relatedness among the species. Accessions of *Desmodium* were classified into two main clusters. The primers RPI 14 and RPI 17 showed highest variability whereas; the primers RPI 2 and RPI 9 were genetically closely linked.

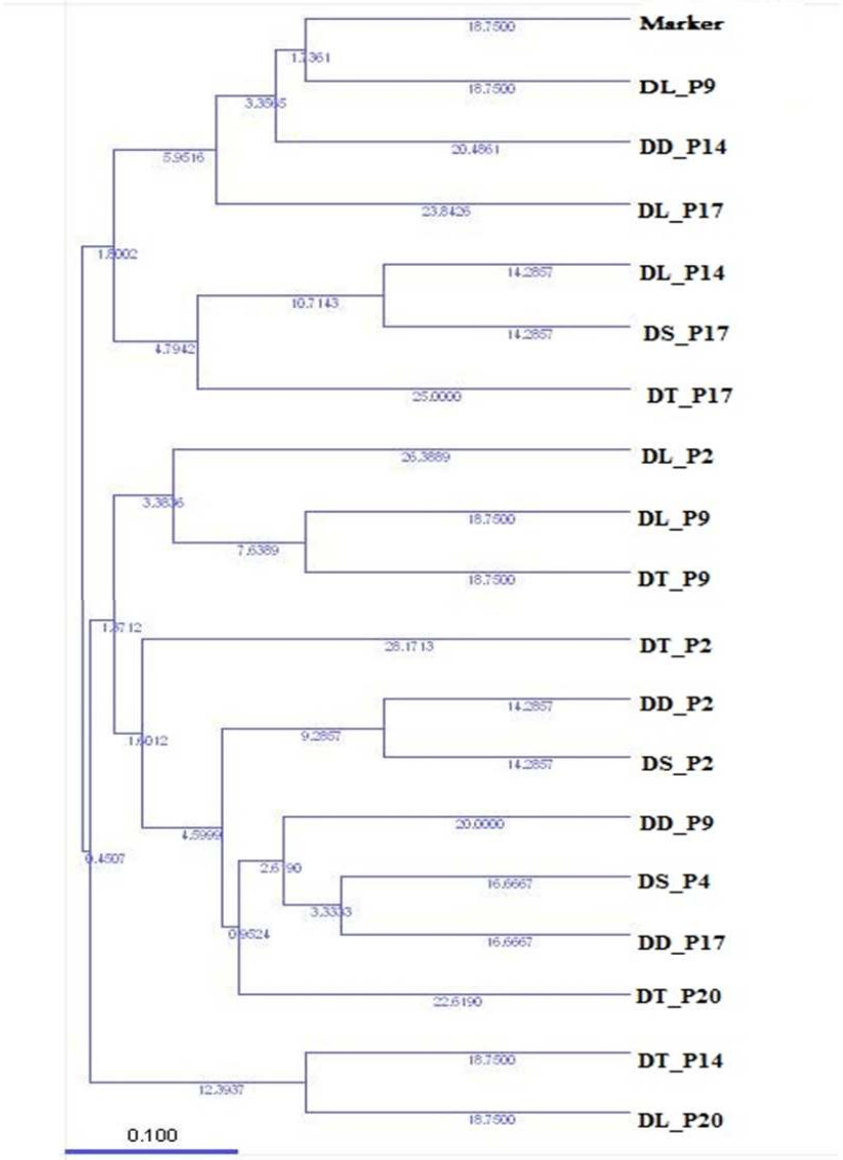

Fig. 2. Dendrogram generated using UPGMA method

(DL: Desmodium laxiflorum DC.; DD: Desmodium dichotomum (Willd.) DC; DS: Desmodium scorpiurus (Sw.) Desv.; DT: Desmodium triflorum (L.) DC. and P14, P20, P4, P9, P17 are primers used in the analysis; Values on the fork are similarity indices calculated by UPGMA software)

4. CONCLUSION

From the findings of this analysis it can be concluded that a higher similarity values could be obtained using RAPD technique, which specifically provides greater confidence for the assessment of genetic diversity and in all helps in identification of the individual species. Therefore this tool of molecular analysis is helpful in characterization of the species on the basis of genetic relatedness. As a result it provides a better understanding and authentication of *Desmodium* species studied.

CONSENT

Not applicable as this is the plant genomic analysis study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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