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# GENETIC DIVERSITY USING RAPD MARKERS AMONG SELECTED SPECIES OF *DESMODIUM* FROM MELGHAT REGION OF WESTERN GHATS INDIA

**[1]Mushtaq Ahmad Dar, [2]Varsha D. Hutke,**

[1,2]P.G. Department of Botany, Govt. Vidarbha Institute of Science and Humanities (Autonomous), Amravati.

## ABSTRACT

The genus *Desmodium* is an important member of family fabaceae and is represented by more than 27 species and majority of these species are nutritionally, medicinally and economically important. The species of *Desmodium* are used traditionally for treatment of various ailments and are also used as sources of food, nutritional supplements like oxidants etc. Six species of genus *Desmodium* (viz. *D. triflorum, D. latifolium*, *D. dichotomum, D. rotundifolium, D. gangeticum* and *D. laxiflorum*) available were collected from Melghat region of Western Ghats to study their diversity and relevance using RAPD markers. A total of twenty markers were used in the study. The results of diversity and relativeness were developed by calculating the presence and absence (Binary digit system- 1 & 0, respectively) of bands on Agarose gel. For every primer, the polymorphic information content (PIC) value was closer to 0.43. Using Neighbor Joining and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) techniques, the calculated and observed data was transformed into phylogenetic trees.In Neighbor Joining method six species analysed were grouped into two major clads (Clad I- *D. triflorum, D. latiflolium*, *D. dichotomum, D. gangeticum*;and Clad II- *D. rotundifolium,* and *D. laxiflorum)* and showed a type of congruency in majority of markers. In UPGMA analysis the phylograms obtained were diverse and showed a prompt change in the taxa positioning in different markers. It was concluded from the data, the Neigbour joining method to calculate the distance matrix and similarity pattern is reliable and specific. This was also concluded that the species of *Desmodium* has changed and diverging from each other with course of time, which is also reflected in the morphology of species.

**Keywords:** *Desmodium***,** RAPD, Genetic diversity, PIC, Molecular markers.

## 1) Introduction

The *Papolionoideae* subfamily of *Fabaceae* family, which includes the clades of *Phyllodium*, *Lespedeza*, and *Desmodium*, includes the tribe Desmodieae, which is a sizable member of the Phaseoloid clade (Jabbour et al., 2018). Many species from this tribe are used as medicinal herbs in various Asian countries because of their high levels of antioxidant and anti-inflammatory compounds. (Lai et al., 2010; Li et al., 2014; Ma et al., 2011).

*Desmodium* Desv. (Beggarweed) with about 280 species now known and about sixty of these are in India. Hooker and Cooke reported 14 and 49 species respectively (Hooker, 1999; Cook, 1967). These plant species are mostly found growing in arid, hilly regions that are tropical or subtropical in nature. Most of this species are known to be important to sustainable agriculture (Gu J et al., 2007). Folk medicine uses *Desmodium* spp. to treat ulcers, diarrhea, liver ailments, and eye issues (Allen and Allen, 1981).

 

Genetic resources from medicinal plants are essential to the long-term advancement of human therapeutic uses. However, the unrelenting and uncontrolled exploitation of biodiversity resources results in significant limitations on genetic variety. Therefore, appropriate and practical methods for the preservation of these genetic resources must be created.

To comprehend the underlying causes of genetic variety, molecular markers are employed. Diverse investigators have employed distinct markers in their research, including single nucleotide polymorphisms (SNPs), amplified fragment length polymorphisms (AFLP) (Thomas et al., 1995; Vos et al., 1995; Zhu et al., 1998), random amplified polymorphic DNAs (RAPD) (Rafalski and Tingey, 1993), restriction fragment length polymorphisms (RFLP) (Becker et al., 1995), simple sequence repeats (Levinson and Gutman, 1987), simple sequence repeats (SSR) (Vieux et al., 2002; Varshney et al., 2006; Kashi and King, 2006; Morgante 2002), etc. Simple sequence repeat markers are used to determine genetic diversity and relatedness as well as to manage and maintain germplasm resources (Da Maia et al., 2008; Homolka et al., 2010; Hou et al., 2011; Wang et al., 2011).

The approach known as random amplified polymorphic DNA (RAPD) is a dependable, simple, economical, and meticulous method for identifying genetic variants at the basic level (Williams et al., 1990). Despite the many benefits of this genetic diversity screening approach, it is frequently criticized for its dominance (Lynch and Milligan, 1994) and reproducibility (Jones et al., 1997). Nonetheless, RAPD is one of the methods frequently employed to assess genetic diversity in a wide range of tropical plants (Gillies and Abbott, 1998; Juárez-Muñoz et al., 2002; Liu, 1996). Consequently, six *Desmodium* species were studied in this study using RAPD markers to determine their genetic diversity.

## 2) Materials and methods

During the month of July the leaves from healthy plants belonging to *Desmodium gangeticum (L.)DC., Desmodium latifolium Wight, Desmodium laxiflorum DC., Desmodium triflorum (L.)DC., Desmodium dichotomum (Willd.)DC.* and *Desmodium rotundifolium DC.* were collected from the Melghat region of Maharashtra, India. Standard flora was used to identify and certify the plants.

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**Image Illustration**

**A** *D. dichotomum* **B** *D. rotundifolium* **C** *D. latiflolium*
**D** *D. triflorum* **E** *D. laxiflorum* **F** *D. gangeticum*

**2a) DNA Extraction**

Fresh leaf tissue (100 mg) was utilized to extract DNA. For the extraction process GeneElute$^{TM}$ plant genomic DNA miniprep kit was utilized. DNA concentration was determined by DNA quantifications performed using UV spectrophotometric measurements as well as agarose gel analysis. Using 1% agarose gels and a recognized DNA marker ladder (Promega, USA), DNA was qualitatively detected. The band's appearance verified the presence of DNA.

**2b) RAPD- PCR**

Minor adjustments are made to Williams et al. (1990)'s suggested technique while creating the RAPD profile. In an Eppendorff thermo-cycler, amplification processes were conducted (master cycler personal). A total of 20 OPA and OPB decamer primers (Operon Tech., Alameda, USA) and Taq DNA polymerase (Native), both obtained from Thermo Fisher Scientific, were employed in the current investigation. Each primer has no self-complimentary ends and at least 60 to 70% GC content.

**2c) RAPD amplification reaction:**

Using 20 RAPD primer sets, RAPD amplification was carried out using genomic DNA that was isolated and purified from the six *Desmodium* species. Three microliters of template DNA (30–50 ng), two microliters of 10X Taq Buffer with 20 mM MgCl2 (Fermentas), two microliters of 2.5 mM MgCl2 (Fermentas), two microliters of 10 mM each dNTP mix (Fermentas), 0.3 microliters of 5U/µl of Taq DNA polymerase (native, Fermentas), one microliter of each primer (10 ρmoles), and 9.7 microliters of nuclease-free PCR grade water were all included in the reaction mixture.

 

**2d) PCR reaction conditions for the amplification**

The PCR master cycler was programmed with an initial denaturation temperature of $94^0$C for 5 min, 40 seconds of denaturation at $94^0$C, 45 sec of annealing at $34^0$C, 90 sec of extension at $72^0$C, followed by 40 cycles, and finally 10 minutes of extension at $72^0$C to ensure the completion of the primer extension with final termination at 4°C. The PCR product was then amplified and measured on an agarose gel at 1.2 to 1.5%. Amplified DNA fragments were quantified and then kept for future analysis at -20°C. Amplified products were visualised and detected by gel electrophoresis (1% agarose) followed by documentation. To avoid the controversy of reproducibility of banding pattern, the PCR amplification was repeated twice.

**3) 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 (Tusamda et al., 201;Shah et al., 2015).

**4) Observation and Result**

The aim of the present study was to know and discriminate between the selected species of *Desmodium* collected from Melghat Forests of Vidharbha region of Maharashtra and evaluate the genetic difference among selected six species available in the region. The primary genetic studies were conducted using the RAPD analysis using 20 random primers. Six species of *Desmodium* namely *D. gangeticum, D. latifolium, D. laxiflorum, D. triflorum, D. dichotomum,* and *D. rotundifolim* were collected, identified and used in the present study to observe the genetic affinity between the species. A total of twenty primers *viz*. OPA-07, OPH-02, OPA-06, OPD-08, OPK-19, OPA-08, OPB-07, OPA-01, OPR-02, OPA-13, OPM-01, OPF-08, OPE-02, OPA-03, OPA-04, OPA-10, OPE-08, OPC-15, OPE-01, OPB-02 were used for this analysis, out of twenty primers only thirteen primers were polymorphic *viz*. OPA-07, OPH-02, OPA-06, OPD-08, OPK-19, OPB-07, OPA-01, OPE-02, OPA-03, OPE-08, OPC-15, OPE-01, OPB-02 and considered for the study and gave a total of 406 RAPD fragments. For every primer employed in this investigation, the number of resolved amplified fragments ranged from 1 to 11. These variations in fragment sizes between the assay's several primers imply that each primer amplified a distinct collection of the genome's genetic loci. Out of 406 bands 277 bands were conserved and 130 bands varied between the species using all polymorphic primers.

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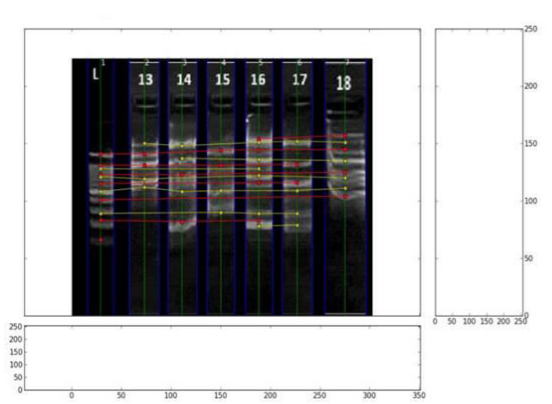
**OPA-06**



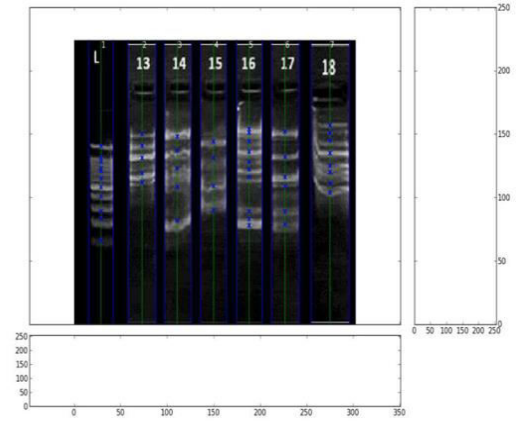
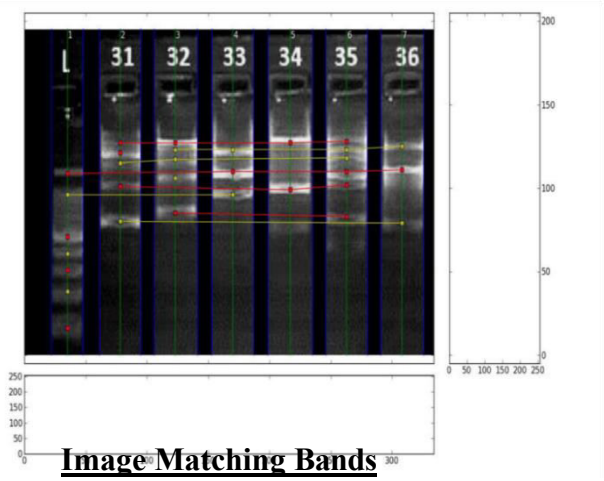




**Image Matching Bands**

**Image for Band Count**

**OPB-07**



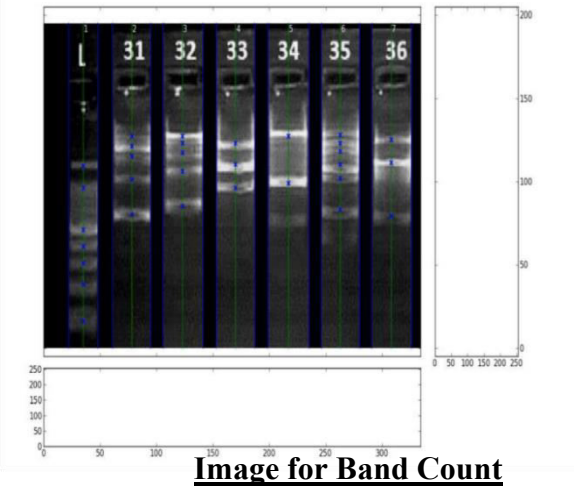




**Image Matching Bands** **Image for Band Count**

**Table 1: Polymorphism Information Content (PIC) of Primers used in this analysis**

| Marker | Present | Absent | Present Frequency | Absent Frequency | Allele Frequency | PIC |
|---|---|---|---|---|---|---|
| OPA-07 | 41 | 61 | 0.40 | 0.59 | 0.99 | 0.48 |
| OPH-02 | 33 | 69 | 0.32 | 0.67 | 0.99 | 0.43 |
| OPA-06 | 38 | 52 | 0.42 | 0.57 | 0.99 | 0.48 |
| OPD-08 | 38 | 76 | 0.33 | 0.66 | 0.99 | 0.44 |
| OPK-19 | 24 | 42 | 0.36 | 0.63 | 0.99 | 0.46 |
| OPA-08 | 22 | 68 | 0.24 | 0.75 | 0.99 | 0.36 |
| OPB-07 | 24 | 39 | 0.38 | 0.61 | 0.99 | 0.47 |
| OPA-01 | 32 | 52 | 0.38 | 0.61 | 0.99 | 0.47 |
| OPR-02 | 14 | 46 | 0.23 | 0.76 | 0.99 | 0.35 |

| | | | | | | |
|---|---|---|---|---|---|---|
| OPA-13 | 20 | 64 | 0.23 | 0.76 | 0.99 | 0.36 |
| OPM-01 | 24 | 54 | 0.30 | 0.69 | 0.99 | 0.42 |
| OPF-08 | 18 | 42 | 0.3 | 0.7 | 0.9 | 0.42 |
| OPE-02 | 26 | 52 | 0.33 | 0.66 | 0.99 | 0.44 |
| OPA-03 | 32 | 52 | 0.38 | 0.61 | 0.99 | 0.47 |
| OPA-04 | 23 | 55 | 0.29 | 0.70 | 0.99 | 0.41 |
| OPA-10 | 16 | 44 | 0.26 | 0.73 | 0.99 | 0.39 |
| OPE-08 | 20 | 34 | 0.37 | 0.62 | 0.99 | 0.46 |
| OPC-15 | 31 | 53 | 0.36 | 0.63 | 0.99 | 0.46 |
| OPE-01 | 33 | 69 | 0.32 | 0.67 | 0.99 | 0.43 |
| OPB-02 | 37 | 53 | 0.41 | 0.58 | 0.99 | 0.48 |

On comparison of *Desmodium* species with different phylogenetic tools *viz*. Neighbour Joining (NJ) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA), these species clearly depicted their relationship and divergence through period. The species like *D. gangeticum* are center of this investigation, which are important related to their chemicals, economy etc. and are generally adulterated because of the economic values and need to be identified properly to avoid the adulteration in the available products of this species.

Considering the values from the highly polymorphic primers, the *D. gangeticum* has often grouped with *D. latifolium* and *D. dichotomum* along with *D. triflorum* species in this study. The other species *D. laxiflorum* and *D. rotundifolium* are kept in the different clad. The grouping of *D. gangeticum* with *D.latifolium* is often seen with multiple primers and supported by the morphological data also. In UPGMA phylogenetic tree (Fig. 1) a diverse and complex clustering was observed. The clustering analysis by UPGMA method, gave a considerable genetic variations in the *Desmodium* species and shown that RAPD marker can be used for the assessment of genetic relatedness among the species of genus. Different accessions obtained in this study were classified into two main clusters *viz*. *D. gangeticum, D. latifolium*, *D. dichotomum*, *D. triflorum* and *D. laxiflorum, D. rotundifolium*. The primers showed highest variability and close relations between the specie of genus *Desmodium*. The values on branches are the branch length, helps in understanding the course of evolution and divergence during period of time, maximum the value the more divergence of species. The NJ analysis has shown congruency with UPGMA tree.

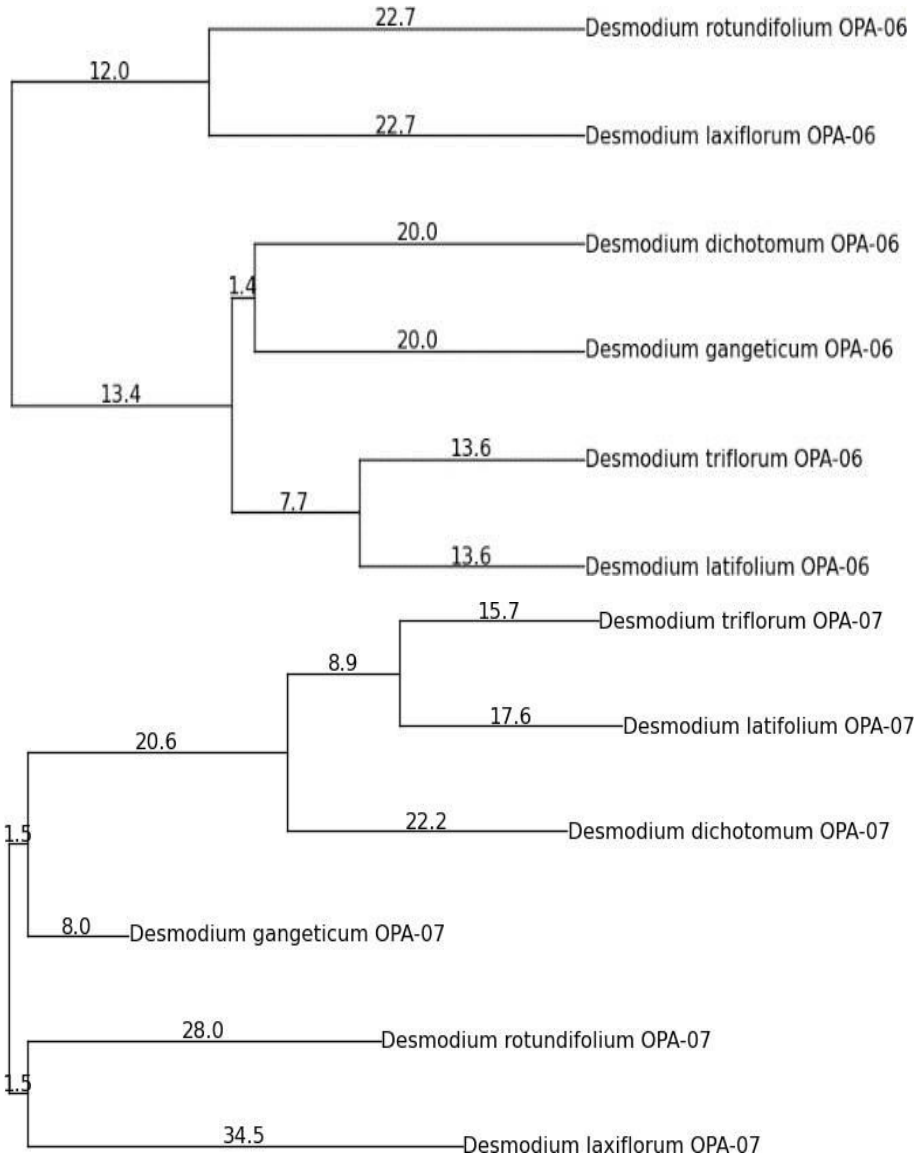



**Fig. 1:** Phylogenetic tree obtained through UPGMA and Neighbour Joining analysis of 06 *Desmodium* species collected from Melghat region of Maharashtra, India.

## 5) Discussion

Suman Singh et al. (2016) discovered a distinct separation between *Desmodium gangeticum* and *Desmodium laxiflorum* based on the results of the RAPD study. In the modern period, RAPD analysis is a widely used technique for identifying genetic variability in plants. This approach has the advantages of being quick, easy to use, and not requiring any prior genetic knowledge about the plant. No matter the age or source of the plant, RAPD patterns are constant (Welsh et al., 1990; Micheli et al., 1994). Additionally, it has been effectively applied to the identification of herbal medicinal components (Shinde et al., 2007) and medicinal plants (Tochika-Komatsu et al., 2001; Um et al., 2001). Because RAPD analysis reflects both coding and non-coding sections of the genome (Vanijajiva et al., 2005), RAPD primers can differentiate taxa below the species level (Choo et al., 2009). On the other hand, a number of RAPD-related issues concern repeatability, primer design, and amplification of RAPD-PCR products. Suman Singh et al., 2016 found that using ten primers together

improved resolution. These results support previous research showing that using more than one primer combination improves resolution (Temiesak et al., 1993; Vanijajiva et al., 2005). According to Arif and Khan (2009), RAPD analysis is less costly and time-consuming while still being effective in verifying the identity of medicinal plant species. The NJ analysis has shown congruency with UPGMA tree.

The results of this study were in line with earlier research on the genetic diversity of *Desmodium* species. In their 2018 study, Ohashia et al. examined the *Desmodium* group of the Leguminosae Tribe Desmodieae. Their findings, which included 33 taxa in the Leguminosae/Fabaceae family, is reclassified into 26 genera, including the establishment of new genera such as *Desmodiopsis, Tateishia, Bouffordia, Oxytes*, and *Huangtcia*, based on molecular phylogenetic analyses and morphological characteristics, resolving the polyphyly of *Desmodium* and proposing taxonomic treatments for several species. Malgaonkar et al., 2016, studied that the utilized RAPD markers to generate 218 loci, with primers RPI 14 and RPI 17 displaying the highest variability and primers RPI 2 and RPI 9 indicating close genetic linkage, highlighting the efficacy of molecular signatures for assessing genetic relationships among *Desmodium* species, particularly useful in medicinal plant identification. Also suggested that the RAPD technique offers higher similarity values, enhancing confidence in assessing genetic diversity and aiding in the identification and characterization of individual *Desmodium* species. Suman Singh et al., 2016 utilized RAPD method to genetically fingerprint two *Desmodium* species, *Desmodium gangeticum* and *Desmodium laxiflorum*, finding 60-65% similarity between them, suggesting potential substitution between the species and highlighting the need for further pharmacological and clinical studies. Using RAPD markers, Rahman et al. (2017) examined genetic variation and molecular relationships among eight taxa of *Desmodium* Desv. They found that there were significant genetic distances between species, with D. triflorum exhibiting the greatest genetic distance from other taxa, as shown in the constructed UPGMA dendrogram, followed by *D. gangeticum* and *D. motorium*.

In their 2009 study, Heider et al. used RAPD markers to examine the genetic relationships between four species of *Desmodium* and related genera from Northeast Vietnam. They found moderate to high levels of inter-accession diversity and stressed the significance of baseline data for marker-assisted research and conservation efforts.

The current study revealed the relationships and evolutionary divergence of the *Desmodium* species were shown by comparing them using various phylogenetic methods, including Neighbour Joining (NJ) and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Notably, both genetic and morphological evidence supports the grouping of *D. gangeticum*, a species of economic importance, with *D. latifolium* and *D. dichotomum*. Significant genetic variability across *Desmodium* species were revealed in a complicated clustering pattern in UPGMA phylogenetic trees. According to the study, RAPD markers may be helpful in determining the genetic relatedness of species within the *Desmodium* genus. The close relationships and significant variability between species were reinforced by the classification of accessions into two primary clusters. Longer branches in the phylogenetic tree indicate greater divergence. The branch length values in the phylogenetic tree show the level of species divergence over time. In order to avoid adulteration in related

products, accurate species identification is crucial, especially when it comes to commercially significant species like *D. gangeticum*.

## 6) Conclusion

The analysis's findings provide a strong conclusion: applying the RAPD technique leads in noticeably greater similarity values. This improved accuracy and consistency supports the evaluation of genetic diversity and species differentiation. As a result, the RAPD method becomes a potent instrument for molecular analysis, greatly improving the capacity to define species according to their genetic relatedness. This has far-reaching implications since it provides a better understanding of the genetic complexities of the examined *Desmodium* species and raises the bar for their authentication and distinction. The usefulness of RAPD markers for determining genetic relatedness within the *Desmodium* genus is shown by this study. The majority of the accessions were grouped into two clusters, which highlights the strong ties and great variety among species. The phylogenetic tree's branch length values, where larger branches denoted greater divergence, quantified the degree of species divergence across time.

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