Asian Journal of Biotechnology and Bioresource Technology

Volume 10, Issue 4, Page 27-36, 2024; Article no.AJB2T.122916
ISSN: 2457-0125

Assessment of Genetic Diversity in *Pseudarthria viscida* (L.) Wight & Arnott Using SSR Markers: Implications for Conservation and Breeding

Sindhu P. V. [a*], Nezam Ali E. [a] and Lekshmi Sekhar [a]

[a] AICRP on Medicinal Aromatic Plants and Betelvine, College of Agriculture, Vellanikkara, KAU, Thrissur - 680656, Kerala, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajb2t/2024/v10i4216

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/122916

Original Research Article

Received: 10/07/2024
Accepted: 14/09/2024
Published: 20/09/2024

ABSTRACT

Pseudarthria viscida (L.) Wight & Arnott is an important, high-volume, traded, threatened medicinal plant native to South and Southeast Asia. Simple Sequence Repeats (SSR) markers were used to determine the genetic relatedness and diversity of 20 accessions of *P. viscida* collected from different parts of Kerala. Ten primer pairs used were found to be highly polymorphic, showing 100 percentage polymorphism and an average Polymorphic Information Content (PIC) of 0.986, indicating high genetic variation among the accessions. A total of 126 alleles with an average of

**Corresponding author: E-mail: pv.sindhu@kau.in;*

Cite as: *P. V., Sindhu, Nezam Ali E., and Lekshmi Sekhar. 2024. "Assessment of Genetic Diversity in Pseudarthria Viscida (L.) Wight & Arnott Using SSR Markers: Implications for Conservation and Breeding". Asian Journal of Biotechnology and Bioresource Technology 10 (4):27-36. https://doi.org/10.9734/ajb2t/2024/v10i4216.*

12.6 alleles per locus were detected. The cluster analysis based on Jaccard's similarity coefficient using an unweighted pair group method using arithmetic averages grouped the accessions into 4 clusters. The average genetic similarity coefficient of 0.09 indicates relatively high genetic diversity among the accessions. Principal Coordinates Analysis (PCoA) showed the presence of genetic diversity. The three principal coordinates explained 29.55% of the total variation. *P. viscida* populations have become vulnerable in their natural habitat, and immediate conservation measures are required. SSR markers could be used in future research on the genetic diversity of *P. viscida*.

Keywords: Genetic diversity; SSR marker; similarity.

1. INTRODUCTION

India is endowed with abundant medicinal plants, which have long been used in several Indian traditional medical systems. The growing domestic and international demand for herbal products severely threatens the availability of indigenous medicinal plant resources. Many medicinal plant species' populations have shrunk to the point where their survival is now in danger.

The genus *Pseudarthria*, belonging to the family Fabaceae, is a small genus consisting of 4-6 species distributed across the Old World [1]. One of the species within this genus is *Pseudarthria viscida* (L.) Wight & Arnott, native to South and Southeast Asia [2]. The plant is a perennial diffuse subshrub, much branched with stems and branches with greyish-white hairs. It is known by the name '*Salaparni*' in Sanskrit. *P. viscida* is commonly used in many Ayurvedic medicines. It is one of the constituents of '*Dasamoola'.* They are useful in vitiated circumstances of cough, fever, hyperthermia, bronchitis, asthma, tuberculosis, hemorrhoids, helminthiasis, cardiopathy, gout, and general debility [3]. The plant is included in the high-volume traded medicinal plants group because of its high commercial value. The estimated annual trade of *P. viscida* is 200 - 500 MT [4]. Due to indiscriminate and unsustainable harvesting from the wild, this plant species is under threat of extinction. As per IUCN classification *P. viscida* species are assigned a ranked threat category 'near threatened' under the red list category [5].

Inadequacy of information related to the diversity of plant species being exploited will lead to genetic erosion, indicating its high necessity for diversity analysis, identification of superior genotypes and conservation. Despite numerous efforts on *P. viscida*, genetic diversity is poorly studied for its in-vitro propagation and biochemical characterization. DNA-based molecular markers are effective tools for assessing genetic diversity. Researchers have used various molecular markers in their studies on medicinal plants, including Random Amplified Polymorphic DNAs (RAPD) [6], Amplified Fragment Length Polymorphism (AFLP) [7], Simple Sequence Repeats (SSR) [8], Inter Simple Sequence Repeats (ISSR) [9], Single Nucleotide Polymorphisms (SNP) [10] etc. Simple sequence repeat markers have been used to determine genetic relatedness and diversity to conserve and utilize germplasm resources [11]. These markers are distinguished by their simplicity, effectiveness, abundance, hypervariability, reproducibility, codominant inheritance, and extensive genomic coverage [12]. It is anticipated that the result will provide genetic information and a theoretical foundation for species protection and will help with germplasm monitoring in the future.

2. MATERIALS AND METHODS

2.1 Survey and Collection

A detailed survey was conducted to study the natural distribution of *P. viscida* population in different parts of Kerala (Table 1), and accessions representing a wide range of morphological variations were collected. The collection sites' altitudes ranged from 7 meters (Athani, Kerala) to 157 meters (Kanalpirivu, Kerala). The geographical distribution map of *P. viscida* accessions collected for the study was created using DIVA GIS [13], a program that is commonly used to map and analyze biological distribution data. The collected accessions were maintained at experimental fields of the AICRP on Medicinal Aromatic Plants and Betelvine, College of Agriculture, Vellanikkara, Kerala Agricultural University, Kerala, India (10.5475¨N, 76.2822¨E).

2.2 DNA Isolation and SSR Amplification

DNA was isolated from all 20 accessions using the HiPurA Plant DNA Isolation Kit (CTAB Method). Genomic DNA concentration was checked in 1% Agarose gel. SSR primer

sequence information was available from a previous work published [11] (Table 2). PCR reaction containing 5 µl of template DNA (100 ng), 5 µl of 10 X complete PCR buffer, 5 µl each forward and reverse primers (2.5 pmole/µl), 5 µl of dNTPs (10 mM) and 1 µl of *Taq*DNA polymerase(Thermo Fisher Scientific) (1 units) and 24 µl of water. The initial denaturation of the template was done at 94°C for 2 min, followed by the denaturation step (94°C for 30 sec), annealing at 60 °C for 30 sec and extension at 72°C for 2 min. 40 cycles were performed. At the end of the last cycle, a final extension was carried out at 72°C for 5 min for the completion of truncated products. The amplified PCR products along with, along with a 1kb standard DNA ladder (Puregene) were separated by electrophoresis in 1.5 % (w/v) agarose gels with 1X TAE buffer stained by 0.5 µg/ml of ethidium bromide. The gel was visualized in a U.V transilluminator (Biometra).

Table 1. Details of *P. viscida* accessions collected from different geographical locations of Kerala

Sl. No.	Accession Code	Place of collection	GPS coordinates	Elevation (m)
1	PS-1	Ollur	10° 27' 47.86" N 76° 14' 22.30" E	25.0
2	PS-2	Vazhakkulam	9° 56' 44.215" N 76° 38' 15.01" E	38.1
3	PS-3	Athani	10° 8' 39.942" N 76° 21' 30.31" E	7.0
4	PS-4	Kottakkal	10° 59' 51.48" N 75° 59' 34.50" E	60.0
5	PS-5	Odakkali	10° 5' 35.052" N 76° 33' 35.29" E	60.0
6	PS-6	Vazhakkulam	9° 57' 9.863" N 76° 37' 39.4" E	26.0
7	PS-7	Pattikkad	10° 33' 55.94" N 76° 19' 39.05" E	35.0
8	PS-8	Poovarani	9° 39' 46.566" N 76° 42' 24.326" E	82.0
9	PS-9	Vellanikkara	10° 32' 50.814" N 76° 16' 50.233" E	32.0
10	PS-10	Kanalpirivu	10° 49' 3.206" N 76° 48' 36.398" E	157.0
11	PS-11	Kanjikode	10° 48' 5.99" N 76° 45' 2.47" E	124.0
12	PS-12	Mannuthy	10° 32' 26.037" N 76° 16' 11.576" E	17
13	PS-13	Pattanakkad	9° 43' 33.977" N 76° 16' 11.576" E	8.9
14	PS-14	Kuttanellur	10° 30' 19.925" N 76° 18' 45.508" E	21.3
15	PS-15	Peechi	10° 32' 0.33" N 76° 20' 27.354" E	47.0
16	PS-16	Vattanthra	10° 26' 33.446" N 76° 20' 27.354" E	21.0
17	PS-17	Vadama	10° 15' 47.477" N 76° 17' 12.715" E	13.0
18	PS-18	Vadama	10° 15' 47.477" N 76° 17' 12.715" E	13.0
19	PS-19	Thamaravellachal	10° 34' 21.348" N 76° 21' 52.146" E	73.0
20	PS-20	Thiruvilwamala	10° 43' 40.466" N 76° 25' 44.858" E	56.0

Table 2. Characteristics of the of SSR markers used to access genetic diversity in *P. viscida* accessions

S.No.	SSR Marker	Sequence	No. of polymorphic bands	No. of alleles	Polymorphism Percentage	PIC
1	SBT/2013/01	FP – AGCAGGAGTACCCATGAAAGTCC RP – TATCACAGCACGAAGCGATAGATG	36	10	100	0.99
2	SBT/2013/02	FP – CACAACTCCATCAGAGGACAGAGA RP – CTGCTACGACATACGCCAGGC	41	14	100	0.99
3	SBT/2013/03	FP – CCGAAGATAACCAAACAATAATAGTAGG RP – ACTGTACGCCTCCCCTTCTC	44	13	100	0.99
4	SBT/2013/04	FP – GCTCTATGTTATTCTTCAATCGGGC RP – GGTCGGTCGGTACTCTGCTCTA	44	10	100	0.99
5	SBT/2013/05	FP – TGCCACCACAGCTTTCTCCTC RP – TATGAGAGAAGCGGTTGGCACG	38	13	100	0.99
6	SBT/2013/06	FP – GGGAGGGTAGGGAAGCAGTG RP – GCGAACCACGTTCATGAATGA	34	13	100	0.96
7	SBT/2013/07	FP – TTTACGCACCGCAGCACCAC RP – TGGACTCATAGAGGCGCAGAAAG	32	15	100	0.99
8	SBT/2013/08	FP – ACCTAGAGCCTAATCCTTCTGCGT RP – GAATGTGAATATCAGAAAGCAAATGG	41	15	100	0.99
9	SBT/2013/09	FP – GGGTAGTAAAGGAAAGAGAAGAAAGAG RP – CCACCTTCTCGTACTGTTCCATG	31	12	100	0.99
10	SBT/2013/10	FP – GATGGACACCCTTCAATTTATGGT RP – TCCAAGTATCAGGCACACCAGC	33	11	100	0.98

2.3 Data Analysis

The band position in the SSR profile was determined using gel images for each accession and primer combination. The amplified fragments were scored as '1' for the presence of a band and '0' for the absence of a band, resulting in the 0 and 1 matrix. The Polymorphic Information Content (PIC) was calculated using the formula [14], $PIC_i=1-\sum P^2ij$ (j=1, 2,...n), where P_{ij} is the frequency of the j^{th} pattern for the i^{th} marker and the summation extends over (n) patterns. NTSYS-pc version 2.1 (Numerical Taxonomy and Multivariate Analysis System) software package [15] was used to analyse pairwise similarity coefficients [16] using the similarity for qualitative data (SIMQUAL) format. The SAHN module used the unweighted pair-group method and arithmetic average (UPGMA) to perform sequential, agglomerative, hierarchical, and non-overlapping clustering. SAHN data was converted into a dendrogram using the Tree Plot module. The Jaccard similarity coefficient was used to compute the pairwise distance matrix [17]. The data was also analyzed using Principal Coordinates Analysis (PCoA) [18], which clearly shows the multidimensional distributions of *P. viscida* accessions in a scatter plot.

3. RESULTS

3.1 SSR Polymorphism

Polymorphic Information Content (PIC), a measure of the informativeness of SSR markers, was calculated for each of the 10 SSR primers using 20 *P. viscida* accessions. Polymorphism Information Content (PIC) ranged from 0.99 to 0.96 with a mean of 0.986. The lowest PIC of 0.96 was observed in SBT/2013/06. Primers SBT/2013/01, SBT/2013/02, SBT/2013/03, SBT/2013/04, SBT/2013/05, SBT/2013/07, SBT/2013/08 and SBT/2013/09 were the most polymorphic with a PIC value of 0.99. Table 2 provides the data regarding the No. of polymorphic bands, allele number, percentage polymorphism and PIC value for the ten primers studied on 20 *P. viscida* accessions. All ten primer pairs were found to be highly polymorphic, showing 100 percentage polymorphism. The ten primer pairs generated a total of 126 alleles. Allele number per locus ranged from 10 (SBT/2013/04) to 15(SBT/2013/07 and SBT/2013/08), averaging 12.6 per locus. One representative SSR profile using primer SBT/2013/10 is shown in Fig. 2. SBT/2013/03 and SBT/2013/04 had the highest number of polymorphic bands (44), while SBT/2013/09 had the lowest (31) among the accessions.

3.2 Genetic Relationships and Diversity among Accessions

The genetic similarity coefficient of the *P. viscida* accessions (Table 3) was calculated using binary data matrices generated by SSRs. The genetic similarity coefficients found in the similarity matrix were relatively low, indicating that the accessions were quite diverse, with an average of 0.09. The pairwise similarity coefficient among 20 *P. viscida* accessions ranged from a maximum of 0.38 (between the accessions PS-6 and PS-15) to a minimum of 0.02 (between the accessions PS-13 and PS-20).

The UPGMA cluster analysis was performed using the corresponding genetic similarity coefficient to determine the relationship between the *P. viscida* accessions and the resulting dendrogram is shown in Fig. 3. In this study, all the *P. viscida* accessions could be grouped into three clusters with a similarity coefficient of 0.07. Cluster I consisted of 13 accessions viz; PS-1, PS-8, PS-3, PS-14, PS-7, PS-11, PS-16, PS-2, PS-4, PS-13, PS-9, PS-12, PS-10, PS-5 and PS-17. Cluster II, III consist of two accessions each. In contrast, cluster IV had only one accession (PS-19).

3.3 Principal Coordinate Analysis

Principal Coordinate Analysis (PCoA) was applied to the SSR data to obtain a different perspective on the genetic relationships among the accessions. The three primary coordinates of the basic coordinate analysis were found to account for 10.35, 9.97 and 9.23 percent of the genetic diversity, respectively. These first three components accounted for 29.55% of the diversity. The distribution of accessions on the 2-D diagram (Fig. 4) obtained over the first two components showed the presence of genetic diversity even though the groups were not completely separated.

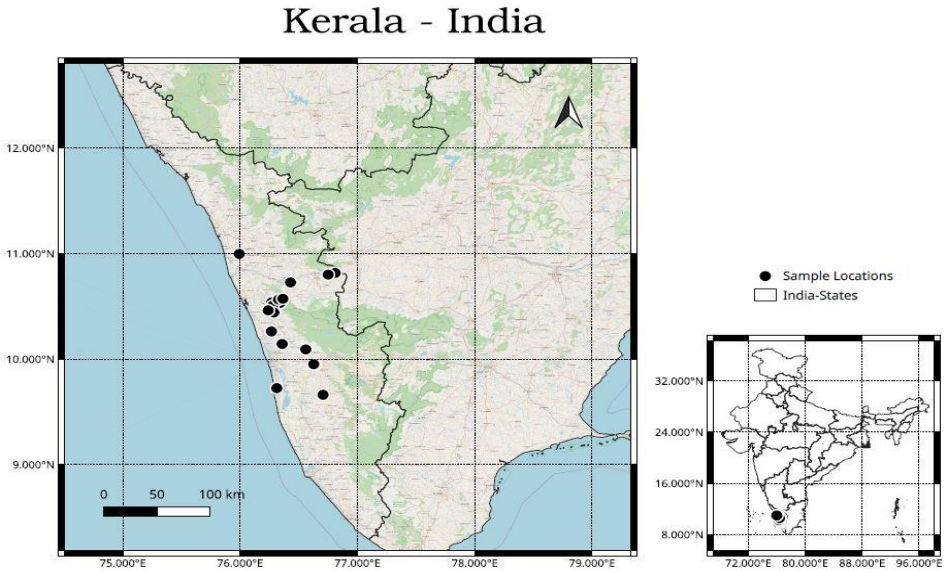

Fig. 1. Geographical distribution map of *P. viscida* accessions collected for the study

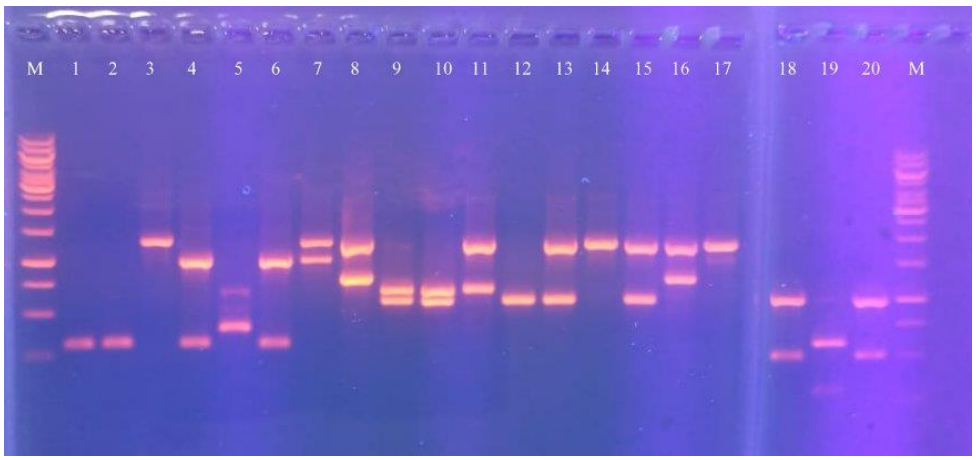

Fig. 2. SSR profile of 20 accessions of *P. viscida* using primer SBT/2013/10

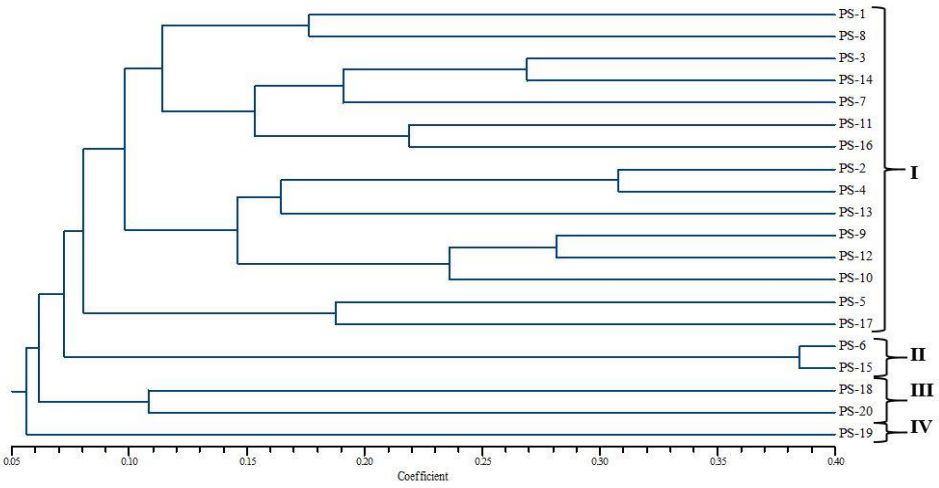

Fig. 3. Dendrogram of *P. viscida* accessions constructed from UPGMA cluster analysis

Table 3. Genetic similarity coefficient values of 20 *P. viscida* accessions

	PS-1	PS-2	PS-3	PS-4	PS-5	PS-6	PS-7	PS-8	PS-9	PS-10	PS-11	PS-12	PS-13	PS-14	PS-15	PS-16	PS-17	PS-18	PS-19	PS-20
PS-1	1																			
PS-2	0.06	1																		
PS-3	0.07	0.14	1																	
PS-4	0.1	0.31	0.1	1																
PS-5	0.03	0.19	0.06	0.12	1															
PS-6	0.17	0.03	0.06	0.13	0.03	1														
PS-7	0.09	0.06	0.2	0.06	0.11	0.08	1													
PS-8	0.18	0.14	0.15	0.11	0.1	0.08	0.16	1												
PS-9	0.08	0.11	0.12	0.14	0.1	0.05	0.22	0.15	1											
PS-10	0.03	0.1	0.07	0.18	0.09	0.06	0.03	0.15	0.26	1										
PS-11	0.06	0.06	0.22	0.09	0.03	0.06	0.11	0.14	0.14	0.1	1									
PS-12	0.09	0.17	0.18	0.17	0.03	0.03	0.08	0.05	0.28	0.21	0.13	1								
PS-13	0.11	0.15	0.12	0.18	0.08	0.08	0.1	0.15	0.15	0.15	0.08	0.14	1							
PS-14	0.09	0.06	0.27	0.09	0.15	0.03	0.18	0.17	0.05	0.03	0.13	0.03	0.14	1						
PS-15	0.13	0.03	0.06	0.03	0.06	0.38	0.03	0.05	0.11	0.06	0.13	0.16	0.11	0.03	1					
PS-16	0.06	0.09	0.2	0.12	0.11	0.03	0.08	0.16	0.1	0.06	0.22	0.08	0.16	0.18	0.11	1				
PS-17	0.06	0.06	0.06	0.06	0.19	0.09	0.05	0.03	0.08	0.13	0.03	0.06	0.03	0.16	0.06	0.08	1			
PS-18	0.09	0.03	0.09	0.06	0.08	0.05	0.11	0.05	0.07	0.03	0.05	0.08	0.16	0.11	0.05	0.05	0.05	1		
PS-19	0.1	0.1	0.03	0.06	0.06	0.03	0.06	0.05	0.03	0.07	0.03	0.06	0.09	0.03	0.03	0.09	0.03	0.09	1	
PS-20	0.06	0.06	0.03	0.09	0.11	0.06	0.05	0.05	0.05	0.03	0.06	0.03	0.02	0.06	0.06	0.03	0.06	0.11	0.03	1

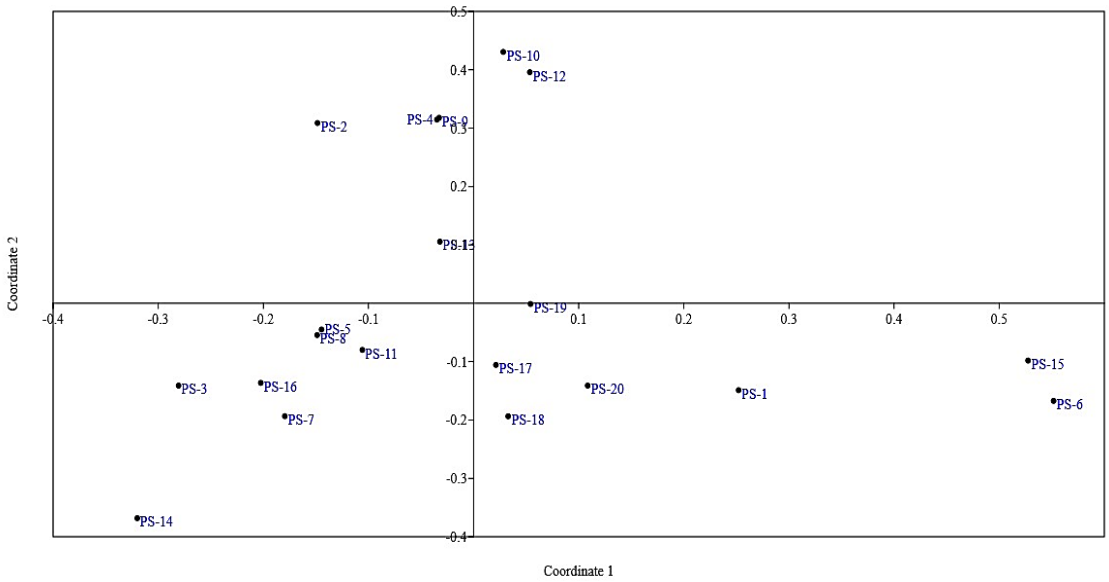

Fig. 4. PCoA analysis of 20 *P. viscida* accessions

4. DISCUSSION

Knowledge about genetic variations is necessary for an efficient conservation and recovery program [19]. There have been very few attempts to uncover the genetic diversity of the *P. viscida* species. Before this work, the only report on an initial investigation into the morphological variation was available [20]. The present study shows a high level of diversity among the accessions. Threatened plant species are generally thought to maintain a lower level of genetic diversity than common species [21]. On the other hand, even within their highly restricted distributions, some threatened species exhibit high levels of genetic variation [22].

Co-dominance markers like SSR markers may give more precise estimates of genetic diversity. SSRs have been used successfully in many medicinal plants to identify genetic diversity [23,24]. To qualify for diversity studies, the marker system should sample enough polymorphic loci [25]. In this study, primer pairs were found to be 100% polymorphic, similar to the polymorphic proportion identified by SSR among *Chrysanthemum morifolium* cultivars [23]. Intra-specific variation may be the primary reason for a high level of polymorphism [26]. According to [27], PIC values above 0.5 are highly polymorphic and suitable for differentiating between alleles of germplasm. The highest PIC values of the SSR markers utilised in the analysis of *P. viscida* accessions were 0.99, which is in line with previously reported values for Chrysanthemum cultivars [23], which showed that genetic diversity studies of *P. viscida* accessions could make use of these highly informative SSR markers. [28] identified 7.5 alleles per locus using 12 SSR markers in wild cowpea accessions. A higher value of 18.5 alleles per locus was observed in soybean genotypes [29], which was higher compared to the current study, which identified 12.6 alleles per locus using 10 SSR primers, proving that the SSR markers used in the current analysis were useful and seemed to be enough to assess genetic diversity.

In the present study, 20 accessions of *P. viscida* had an average genetic similarity coefficient of 0.09, reflecting the accessions' relatively high genetic diversity. Comparing the results of the current study to those reported by [30], the average genetic similarity coefficient was lower, where they reported an average genetic similarity coefficient of 0.26 among 48 soybean genotypes using 21 SSR markers.

PCoA was used to create a 2-dimensional scatter plot, where the geometrical distances between the accessions accurately reflect the genetic distances between them with less distortion [31]. The UPGMA cluster analysis and PCoA using SSR markers revealed that most accessions gathered from different locations were grouped together. Mixing and grouping were not based on geographic region. This finding suggests some degree of gene flow between the accessions. This study had no relationship between PCoA grouping and cluster

analysis, similar to the results of [32] in forage peas.

5. CONCLUSION

The declining population and the loss of genetic diversity threaten medicinal plants' existence. *P. viscida* populations have become vulnerable in their natural habitat due to significant population fragmentation, unsustainable harvesting practices and inadequate natural regeneration. As a result, immediate conservation measures are required. The PIC value of the primer pairs used in the analysis shows that they were found to be informative. The genetic similarity distribution, PCoA, and cluster analysis further demonstrate the genetic diversity of the studied *P. viscida* accessions. Considering the medicinal value of this species, the above-discussed primer pairs are likely to help assess the genetic relationships of this naturally gifted species and the most diverse accessions can be used in upcoming breeding initiatives.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENT

The authors greatly acknowledge the financial support from the National Medicinal Plant Board under the Ministry of Ayush, India and the physical support of Kerala Agricultural University, Vellanikkara.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Verdcourt B. Leguminosae-Papilionoideae. In: Flora Zambesiaca. Royal Botanic Gardens. Kew. 2000;3(6):25-27.
2. Baker CA, Bakhuizen Van Den Brink RC. Pseudarthria. In: Flora of Java. NVP Noordhoff. Groningen. 1963;609.
3. Warrier PS. Indian medicinal plants. Orient Longman Private Limited, New Delhi; 1994.
4. NMPB. Demand and supply of medicinal plants. National Medicinal Plants Board (NMPB); 2022. Available:https://nmpb.nic.in/medicinal_list.
5. Gowthami R, Sharma N, Pandey R, Agrawal A. Status and consolidated list of threatened medicinal plants of India. Genetic Resources and Crop Evolution. 2021;68(6):2235-2263.
6. Irshad S, Singh J, Kakkar P, Mehrotra S. Molecular characterization of *Desmodium* species an important ingredient of 'Dashmoola'by RAPD analysis. Fitoterapia. 2009;80(2):115-118.
7. Sarwat M, Das S, Srivasthava PS. Analysis of genetic diversity through AFLP, SAMPL, ISSR, and RAPD markers in Tribulus terrestris, a medicinal herb. Plant Cell Rep. 2008;27:519-528.
8. Lal M, Munda S, Bhandari S, Saikia S, Begum T, Pandey SK. Molecular genetic diversity analysis using SSR marker amongst high solasodine content lines of *Solanum khasianum* CB Clarke, an industrially important plant. Industrial Crops and Products. 2022;184:115073.
9. Rajasekharan PE, Kareem VKA, Ravish BS, Mini S. Genetic diversity in *Oroxylum indicum* (L.) Vent., a threatened medicinal plants from India by ISSR analysis. Indian Journal of Biotechnology. 2017;16(3):357-365.
10. Do HDK, Jung J, Hyun J, Yoon SJ, Lim C, Park K, Kim JH. The newly developed single nucleotide polymorphism (SNP) markers for a potentially medicinal plant, *Crepidiastrum denticulatum* (Asteraceae), inferred from complete chloroplast genome data. Molecular Biology Reports. 2019; 46(3):3287-3297.
11. Ahmad Haji RF, Tiwari S, Gandhi SG, Kumar A, Brindavanam NB, Verma V. Genetic diversity analysis among accessions of *Desmodium gangeticum* (L) DL with Simple Sequence Repeat (SSR) and internal transcribed spacer (ITS) regions for species conservation. J. Biodivers., Bioprospect. Dev. 2016;3:2-5. DOI: 10.4172/2376-0214.1000159
12. Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding. 1996;2(3):225-238.
13. Hijimans RJ, Guarino L, Mathur P. DIVA-Gis version 7.5. A geographic information system for the analysis of species distribution data; 2012.
14. Anderson A, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME. Optimizing

parental selection for genetic linkage maps. Genome. 1993;36(1):181-186.
15. Rohlf FJ. NT-SYS-pc: Numerical taxonomy and multivariate analysis system, Version 2.11 W, Exteer Software, Setauket; 1993.
16. Jaccard P. Nouvelles recherches sur la distribution florale Bulletin de la Société Vaudoise des Sciences Naturelles. 1908; 44:223-270.
17. Sneath PHA, Sokal RR. Numerical taxonomy. freeman press, San Francisco, CA, USA; 1973.
18. Gower JC. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika. 1966;53(3-4):325-338.
19. Pierson JC, Coates DJ, Oostermeijer JGB, Beissinger SR, Bragg JG, Sunnucks P, Schumaker NH, Young AG. Genetic factors in threatened species recovery plans on three continents. Frontiers in Ecology and the Environment. 2016;14(8): 433-440.
20. Murugesan. In: Book of Abstracts - Fourth Indian Biodiversiy Congress, 10-12 March 2017, Pondicherry niversity, Puducherry, India; 2017.
21. Frankham R. Conservation genetics, Ann Rev Genet. 1995;29:305-327.
22. Ellis JR, Pashley CH, Burke JM, McCauley DE. High genetic diversity in a rare and endangered sunflower as compared to a common congener. Molecular Ecology. 2006;15(9):2345-2355.
23. Feng S, He R, Lu J, Jiang M, Shen X, Jiang Y, Wang Z, Wang H. Development of SSR markers and assessment of genetic diversity in medicinal *Chrysanthemum morifolium* cultivars. Frontiers in Genetics. 2016;7:113.
24. Mohammad N, Dahayat A, Pardhi Y, Rajkumar M. Morpho-molecular diversity assessment of Indian kino (*Pterocarpus marsupium* Roxb.). Journal of Applied Research on Medicinal and Aromatic Plants. 2022;29:100373.
25. Luikart G, England PR, Tallmon D, Jordan S, Taberlet P. The power and promise of population genomics: From genotyping to genome typing. Nature Reviews Genetics. 2003;1-4(12):981-94.
26. Nayak S, Naik PK, Acharya LK, Pattnaik AK. Detection and evaluation of genetic variation in 17 promising cultivars of turmeric (*Curcuma longa* L.) using 4C nuclear DNA content and RAPD markers. Cytologia. 2006;71(1):49-55.
27. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics. 1980;32(3):314 -331.
28. Tosti N, Negri V. Efficiency of three PCR-based markers in assessing genetic variation among cowpea (*Vigna unguiculata* subsp. unguiculata) landraces. Genome. 2002;45(2):268-275.
29. Rongwen J, Akkaya MS, Bhagwat AA, Lavi U, Cregan PB. The use of microsatellite DNA markers for soybean genotype identification. Theoretical and Applied Genetics. 1995;90(1):43-48.
30. Chauhan DK, Bhat JA, Thakur AK, Kumari S, Hussain Z, Satyawathi CT. Molecular characterization and genetic diversity assessment in soybean [*Glycine max* (L.) Merr.] varieties using SSR markers. Indian Journal of Biotechnology. 2015;14(4):504-510.
31. Mohammadi SA, Prasanna BM. Analysis of genetic diversity in crop plants salient statistical tools and considerations. Crop Science. 2003;43(4):1235-1248.
32. Haliloglu K, Turkoglu A, Tan M, Poczai P. SSR-based molecular identification and population structure analysis for forage pea (*Pisum sativum* var. arvense L.) landraces. Genes. 2022;13(6): 1-16.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/122916