Genetic diversity study on *Pseudarthria. viscida* *(L.)* Wight &Arnott, athreatened medicinal plant in India using SSR markers

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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 genetic relatedness and diversity of 20 accessions of *P.viscida*collected from different parts of Kerala. 10 primers pairs used were found to be highly polymorphic showing 100 percentage polymorphism and an average PIC (Polymorphic Information Content) of 0.986, indicating high genetic variation among the accessions. A total of 126 alleles with an average of 12.6 alleles per locus were detected.The cluster analysis based on Jaccard's similarity coefficient using unweighted pair group method using arithmetic averages grouped the accessions into 4 clusters. Average genetic similarity coefficient of 0.09indicates that relatively high genetic diversity exists 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, immediate conservation measures are required. SSR markers could be used in future research on the genetic diversity of *P.viscida*.

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Keywords: Genetic diversity, SSR marker, Similarity

Introduction

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

*Pseudarthria*Wight & Arnott belonging to the family Fabaceae is a small genus thatcomprises of 4 -6 species spread across the Old World (Verdcourt 2000). *Pseudarthria. viscida*(L.) Wight & Arnott is the only species found in South and Southeast Asia (Baker& Bakhuizen 1963).The plant is perennial diffuse subshrubs, much branched with stems and branches with greyish-white hairs. It is known by the name '*Salaparni*' in Sanskrit. *Pseudarthriaviscida*is commonly used in many Ayurvedic medicines. It is one of the constituent of '*Dasamoola*'.They are useful in vitiated circumstances of cough, fever, hyperthermia, bronchitis, asthma, tuberculosis, hemorrhoids, helminthiasis, cardipathy, gout, and general debility(Warrier 1994). The plant is included in the group of high volume traded medicinal plants because of its high commercial value. The estimated annual trade of *Pseudarthriaviscida* is 200 - 500 MT (NMPB 2022). Due to indiscriminate and unsustainable harvesting from the wild, this plant species is under the threat of extinction. As per IUCN classification *Pseudarthriaviscida*species are assigned a ranked threat category ‘near threatened’ under the red list category (Gowthami*et al* 2021).

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 have been made on *Pseudarthriaviscida,* for its in-vitro propagation and biochemical characterization, genetic diversity is poorly studied. 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) (Irshad et al 2009), Amplified Fragment Length Polymorphism (AFLP) (17. Sarwat et al 2008),Simple Sequence Repeats (SSR) (Lal et al 2022), Inter Simple Sequence Repeats (ISSR) (Rajasekharan et al 2017), Single Nucleotide Polymorphisms (SNP) (Do et al 2019) etc. Simple sequence repeat markers have been used to determine genetic relatedness and diversity in order to conserve and utilise germplasm resources (Ahmad et al 2016). These markers are distinguished by their simplicity, effectiveness, abundance, hypervariability, reproducibility, codominant inheritance, and extensive genomic coverage (Powell et al 1996). It is anticipated that the result will provide a genetic information and a theoretical foundation for species protection and will help with germplasm monitoring in the future.

Materials and Methods

Survey and collection

A detailed survey was conducted to study the natural distribution of *Pseudarthriaviscida*population in different parts of Kerala (Table 1.) and accessions representing a wide range of morphological variation were collected. The collection sites' altitudes ranged from 7 metre (Athani, Kerala) to 157 metre (Kanalpirivu, Kerala). Geographical distribution map of *Pseudarthriaviscida* accessions collected for the study were created using DIVA GIS (Hijimans et al 2012), a program that is commonly used to map and analyse 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).

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DNA isolation and SSR amplification

DNA was isolated from all the 20 accessions using HiPurA Plant DNA Isolation Kit (CTAB Method). Genomic DNA concentration was checked in 1% Agarose gel. Information of SSR primer sequences were available from a previous work published (.Rajasekharan et al 2017)(Table). 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 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 were visualized in U.V transilluminator (Biometra).

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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 (Anderson et al. 1993), $PIC_i=1-\sum P^2 ij$ (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 (Rohlf, 1993) was used to analyse pairwise similarity co-efficients (Jaccard, 1908) 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 (Sneath and Sokal, 1973).The data was also analyzed using Principal Coordinates Analysis (PCoA)(Gower,1966), which clearly shows the multidimensional distributions of *Pseudarthriaviscida* accessions in a scatter plot.

Results

SSR polymorphism

Polymorphic Information Content (PIC), a measure of the informativeness of SSR markers, was calculated for each of the 10 SSR primerss using 20 *Pseudarthriaviscida* 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 was the most polymorphic with a PIC value of 0.99. Table 2 provides the data

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regarding the No. of polymorphic bands, allele number, percentage polymorphism and PIC value for the 10 primers studied on 20 *Pseudarthriaviscida* accessions.All the 10 primers pairs were found to be highly polymorphic showing 100 percentage polymorphism. A total of 126 alleles were generated by the 10 primers pairs.Allele number per locus ranged from 10 (SBT/2013/04) to 15(SBT/2013/07 and SBT/2013/08) with an average of 12.6 per locus.One representative SSR profile using primer SBT/2013/10 is shown in figure 2. SBT/2013/03 and SBT/2013/04 had the highest number of polymorphic bands (44) whilst SBT/2013/09 had the lowest (31) among the accessions.

Genetic relationships and diversity among accessions

The genetic similarity coefficient of the *Pseudarthriaviscida* 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 pair wise similarity coefficient among 20*Pseudarthriaviscida* 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 *Pseudarthriaviscida* accessions and the resulting dendrogram is shown in figure 3. In this study, all the *Pseudarthriaviscida*accessions could be grouped into three clusters, with a similarity coefficient of 0.07. Cluster I consisted of 13 accesssions 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 consists of two accessions each. While cluster IV consist of only one accession (PS-19).

Principal Coordinate Analysis

Principal Coordinate Analysis (PCoA) was applied to the SSR data in order 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 (Figure 4) obtained over the first two components showed the presence of genetic diversity even though the groups were not completely separated.

Discussion

Knowledge about the current genetic variations is necessary for an efficient conservation and recovery programme (Pierson et al 2016). There have been a very few attempts to uncover the genetic diversity of the *Pseudarthriaviscida* species. Prior to this work, the only report on an initial investigation into the morphological variation was available (Murugesan IBC). 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 (Frankham 1995). On the other hand, even within their highly restricted distributions, some threatened species exhibit high levels of genetic variation (Ellis et al 2006).

Co-dominance markers like SSR markers may give the estimates of genetic diversity that are more precise. SSRs have been used successfully in many medicinal plants to identify genetic diversity. (Feng et al 2016, Rajasekharanetal2017, Mohammad et al (2022). To qualify for diversity studies marker system should sample enough polymorphic loci (Luikart et al 2003). In this study primers pairs used were found to be 100% polymorphic which is similar to the polymorphic proportion of identified by SSR among *Chrysanthemum morifolium* cultivars (Feng et al 2016). (Nayak et al. (2006), reported that intra-specific variation may be the primary reason for a high level of polymorphism.According to (Bostein et al. (1980), PIC values above 0.5 are highly polymorphic and suitable for differentiating between alleles of a germplasm. The highest PIC values of the SSR markers utilised in the analysis of *Pseudarthriaviscida*accessions were 0.99, which is in line with previous reported values for Chrysanthemum cultivars (.Feng et al 2016), which showed that genetic diversity studies of *Pseudarthriaviscida* accessions can make use of these highly informative SSR markers.

Tosti and Negri (2008) 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 soyabean genotypes by.Rongwen et al (1995)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 *Pseudarthriaviscida* 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 byChauhan et al. (2015), the average genetic similarity coefficient was found to be lower, where they reported average genetic similarity coefficient of 0.26 among 48 soyabean genotypes using 21 SSR markers.

PCoA were 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 (Mohammadi and Prasanna (2003). The UPGMA cluster analysis and PCoA using SSR markers revealed that most of the accessions that were gathered from different locations were grouped together, mixing and grouping were not clearly based on geographic region. This finding suggests that there is some degree of gene-flow between accessions.In this study, there was no relationship between PCoA grouping and cluster analysis , which similar to the results of.Halilogu et al 2022 in forage pea.

Conclusion

Declining population and the loss of genetic diversity is threat to the existence of medicinal plants.*Pseudarthriaviscida* populations have become vulnerable in their natural habitat as a result of 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 *Pseudarthriaviscida* accessions.Considering the medicinal value of this species, the above discussed primer pairs are likely to be useful in assessing the genetic relationships of this naturally gifted species and the most diverse accessions can be used in upcoming breeding initiatives

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Table 1. Details of *Pseudarthriaviscida* 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	13.0

			76° 17' 12.715" E	
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 *Pseudarthriaviscida* 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

Table 3. Genetic similarity coefficient values of 20 *Pseudarthriaviscida*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

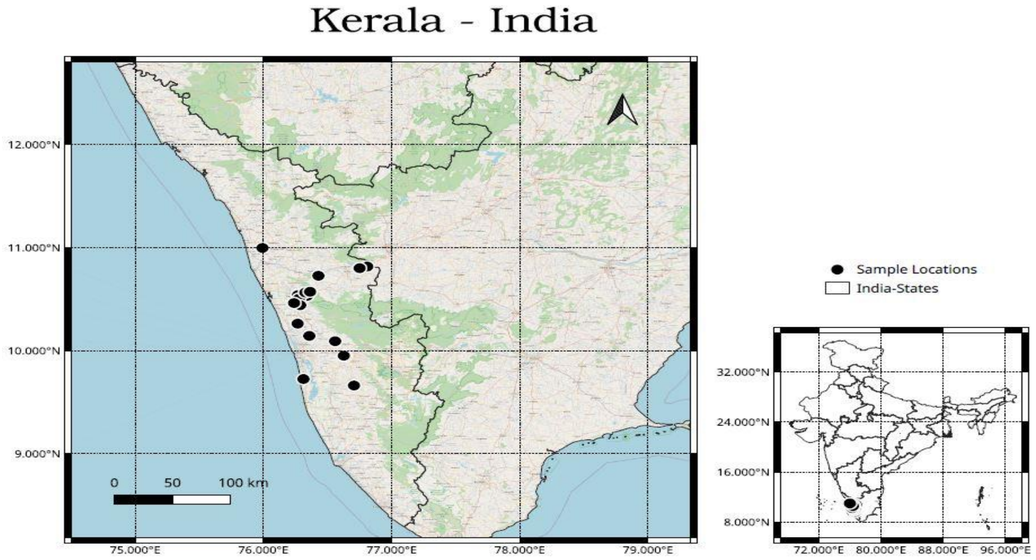

Figure 1. Geographical distribution map of *Pseudarthriaviscida*accessions collected for the study

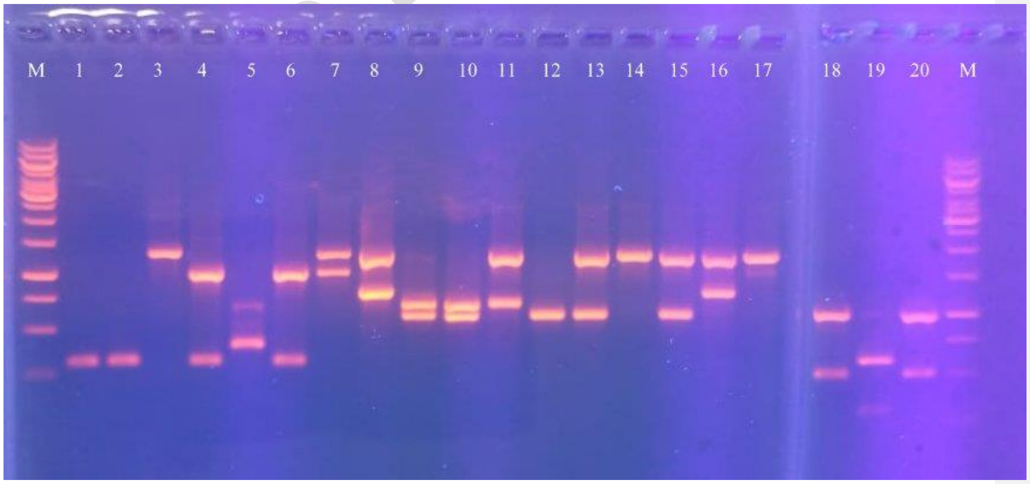

Figure 2. SSR profile of 20 accessions of *Pseudarthriaviscida*using primer SBT/2013/10

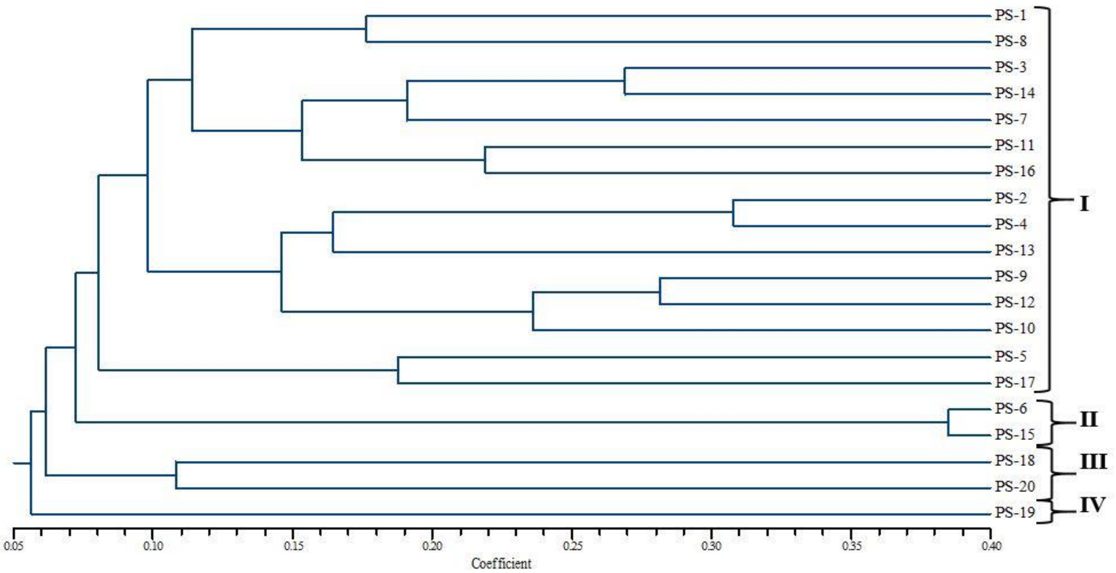

Figure 3. Dendrogram of *Pseudarthriaviscida*accessions constructed from UPGMA cluster analysis

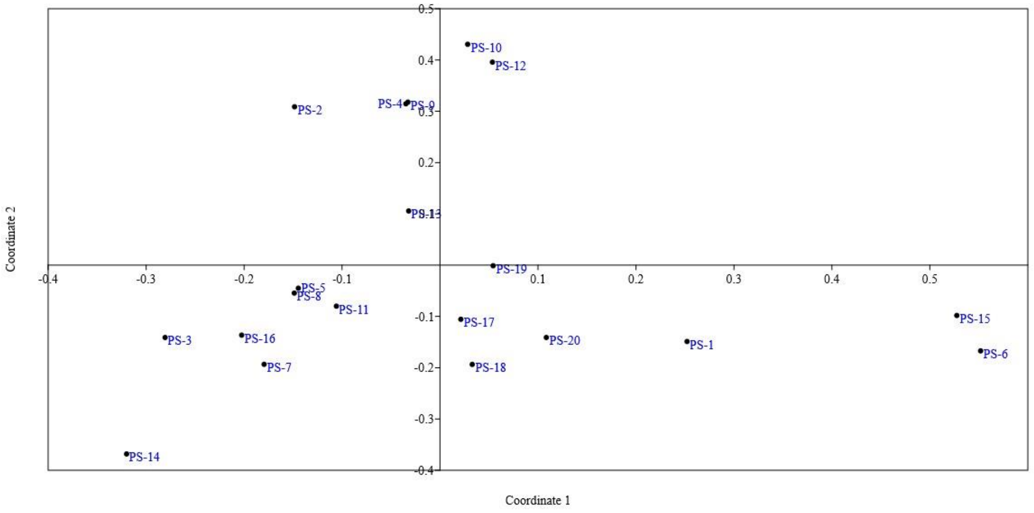

Figure 4. PCoA analysis of 20 *Pseudarthriaviscida*accessions