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Evaluation of Genetic Diversity Studies in *Gymnema sylvestre* Assessed through RAPD Markers

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ABSTRACT

Genetic diversity studies among Gymnema sylvestre accessions were analyzed using randomly amplified polymorphic DNA (RAPD) markers. A total of twenty random decamer primers were used to analyze the diversity studies among twenty four G.sylvestre accessions which generated a total of 140 bands. Jaccard's similarity coefficient showed a wide range (00.12 to 0.95) of variability among all twenty four accessions. UPGMA cluster analysis grouped the G.sylvestre variants into two major clusters, cluster-A and cluster-B. RAPD studies revealed that 74% genetic similarity has been recorded among the accessions collected from different regions. This marker studies will be helpful in conservation of Gymnema species.

Key words: *Gymnema sylvestre, anti diabetic, genetic diversity, molecular markers, RAPD markers.*

Abbreviations:

RAPD: Random Amplified Polymorphic DNA, PCR:Polymerase chain reaction, DNA:Deoxy ribonucleic acid, CTAB: Cetyl trimethylammonium bromide, PCA:Principal component analysis. UPGMA: Unweighted Pair Group Method with Arithmetic Mean.

INTRODUCTION

Gymnema sylvestre, commonly known as "Periploca of the woods" belongs to the family Asclepiadaceae is a large woody climber distributed throughout all parts of India & Srilanka. *Gymnema sylvestre* is being used in Ayurvedic traditional medicine for curing diabetes, it is popularly known as Madhunashni or Gudmar also called as sugar destroyer for inhibiting the taste of sweetness because of the presence of a group of oleanane-type triterpenoid saponins known as "gymnemic acids" known for its anti diabetic, lipid lowering, antimicrobial as well as anti-hypercholesterolemial properties[1,3,4,7-9,13,15,16,18]. The plant is also known for its free radical scavenging, cytotoxic and wound healing activities [6, 12, 17].

Because of its high commercial value and demand in pharmaceutical industry the plant is getting over exploited from natural habitat and becoming endangered.

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Molecular characterization helps in crop improvement, development of new variety, germplasm conservation within and between species and populations. Various types of PCR based markers are used for characterizing and evaluating genetic diversity, assessing genetic relationship during last few years among which random amplified polymorphic DNA (RAPD) markers are proved better for their high degree of polymorphism between individuals within a population of closely related genotypes [2,5,19]. Considering *G.sylvestre* medicinal value and increasing demand there is a need for conserving the genetic diversity of this plant species. The main objective of this work is to develop a RAPD-DNA marker to study the genetic diversity /similarity among twenty four accessions of *G.sylvestre.*

MATERIALS AND METHODS

DNA isolation

A total of twenty four *Gymnema sylvestre* accessions were collected from different places of Andhra Pradesh and Telangana (Table 1). Genomic DNA was extracted from one gram tender leaves from all the twenty four accessions with modified CTAB protocol. DNA concentration and purity were determined by measuring the absorbance of diluted DNA solution at 260 nm and 280 nm and samples were stored at -20°C. The quality of the DNA was determined using agarose gel electrophoresis stained with ethidium bromide.

Primer selection

A total of twenty decamer random primers GSRAPD-1, GSRAPD-2, GSRAPD-3, GSRAPD-4, OPJ-03, OPJ-07, OPJ-10, OPJ-15, OPJ-20, OPH-01, OPH-03, OPH-06, OPH-12, OPH-16, OPM-02, OPM-05, OPM-10, OPM-13, OPM-19, OPU-07 were used for RAPD amplification.

PCR amplification and conditions

PCR was performed in a DNA Thermal Cycler (Master Cycler, Eppendorf,USA) and PCR reaction was carried out in 25 µl reaction tubes. Each reaction mixture contained 1X PCR buffer, 1.5mM $MgCl_2$, 0.5 U of Taq DNA polymerase, 200 µM each of deoxynucleotide triphosphate (dNTP), 10 pM of primer, nuclease free water and 50 ng of template genomic DNA. PCR was performed using the following conditions: 94°C for 5 min (initial denaturation), 94°C for 1 min (denaturation), 35°C for 1 min (annealing), 72°C for 2 min (elongation), repeat 35 cycles, 72°C for 10 min (final extension). Amplified products were separated by 1.2% agarose gel, stained in ethidium bromide and visualized under UV transillumination.

Data Analysis

To examine the genetic relationship among 24 *Gymnema* accessions, a dendrogram was constructed using a UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) analysis as implemented by NTSYS-pc, Version 2.02c (Rohlf, 1997). A pair wise different matrix between genotypes was determined according to Jaccard's (Jaccard, 1908) similarity. The PCR data generated from the twenty four accessions were scored into 0 and 1. For each genotype, the presence of a band (1) or its absence (0) was scored. The binary data was also subjected to principal component analysis (PCA) using the "statistical" package. Principal components analysis is described as combining two correlated variables into one factor.

RESULTS

To estimate the genetic diversity/similarity among twenty four accessions of *G. sylvestre* collected from different regions, twenty random primers were designed to amplify the genomic DNA and study the banding pattern generated from the individuals. Twenty random primers generated a total number of 140 bands out of which 34 were polymorphic bands and 8 were monomorphic bands. Out of twenty random primers used four primers viz.GSRAPD-1(Fig 1B), GSRAPD-2 (Fig 1C), OPJ-20 (Fig 1D), OPM-05 (Fig 1E) showed good amplification. Except GSRAPD-4, OPH-06, OPM-13, OPM-19, OPU-07 all the other primers showed amplication generating an average of 2 to 15 bands that ranged from 100-4000bp (Table 2). Maximum numbers of 15 bands were obtained with primer GSRAPD-1 (Fig 1A) and OPH-16 produced a minimum number of 2 bands (Fig 1F).

Primer GSRAPD-1 has showed the highest size of the amplified product of 4000bp and the lowest size of 100 bp amplified products were obtained with GSRAPD-2, OPJ-03, OPJ-20, OPH-03 primers (Table 2). OPJ-20 recorded highest of

72.7% polymorphism, OPJ-15 recorded 62.5% polymorphism, GSRAPD-1 revealed 53.3% polymorphism and OPH-12 recorded lowest of 8.3% polymorphism (Table 2). The mean polymorphism information content (PIC) calculated from the frequency of polymorphic bands across all genotypes ranged between 0.08 to 0.21with an average of 0.14 per fragment. The primer OPM-2 revealed the highest PIC value of 0.21 followed by the primers OPM-5 (0.19) and OPJ-7 (0.17) and the lowest PIC value was recorded for GSRAPD-1(0.08). Majority of the fragments showed PIC value between 0.10- 0.17(Table 2). Among twenty four *G.sylvestre* accessions, the range of genetic similarity was from 0.161 to 0.874 (Table 3). Genetic similarity of 98% was observed within the species collected from same habitat. 74% of similarity index was observed among the *G.sylvestre* accessions collected from various geographically distinct locations. Jaccard's similarity coefficient showed a wide range (00.12 to 0.95) of variability among all twenty four accessions (Table 3). UPGMA cluster analysis of the Jaccard's similarity coefficient generated a dendrogram that showed the distinctiveness of the clusters exhibiting two major clusters Cluster-A and cluster-B. Cluster A contained 2 accessions and cluster B was the largest group with 22 accessions (Fig 2). The maximum genetic distance was between PR2 (0.74) and CB3(0.54), while MBN-1(0.60) and MBN-2 (0.59) has high genetic similarity values as calculated by Jaccard's similarity coefficient (Fig 2). In the present study, the fragments showed an average PIC value of 0.143 from the different fragments obtained, which were detected in 50-60% of accessions.

The Principal Component Analysis (PCA) was conducted in this study using the program NTSYS-pc vers. 2.2. In PCA, twenty four accessions were separated into five distinct groups in which Group 1 has 8 accessions, OSM-1,OSM-2,OSM-3,CB-1,CB-2,CB-3,WRL-2 and WRL-3, Group 2 has 7 accessions VJA-1, VJA-2,VJA-3, NGL-1, NGL-2, NGL-3 and WRL-1, Group 3 has 4 accessions HBG-1, HBG-2, HBG-3 and MBN-3, Group 4 has also 4 accessions PR-1, PR-3, MBN-1 and MBN-2, Group 5 has only 1 accession PR-2. The overall grouping pattern of PCA corresponded well with the clustering pattern of the dendrogram (Fig 3).

Table 1: List of *G.sylvestre* accessions collected from different places of Andhra Pradesh and Telangana

S. No.	Accession Number	Collection area
1	PR-1	Pragathi Resorts, Hyderabad
2	PR-2	Pragathi Resorts, Hyderabad
3	PR-3	Pragathi Resorts, Hyderabad
4	MBN-1	Mahabubnagar
5	MBN-2	Mahabubnagar
6	MBN-3	Mahabubnagar
7	HBG-1	Khammam
8	HBG-2	Khammam
9	HBG-3	Khammam
10	NGL-1	Nalgonda
11	NGL-2	Nalgonda
12	NGL-3	Nalgonda
13	VJA-1	Vijayawada
14	VJA-2	Vijayawada
15	VJA-3	Vijayawada
16	WRL-1	Warangal
17	WRL-2	Warangal
18	WRL-3	Warangal
19	OSM-1	Osmania University, Hyderabad
20	OSM-2	Osmania University, Hyderabad
21	OSM-3	Osmania University, Hyderabad
22	CB-1	Guntur
23	CB-2	Guntur
24	CB-3	Guntur

Table 2: List of primers screened for RAPD study, number of amplified products, polymorphism percentage and polymorphism information content

Primer	Sequence	Mol. Weight	GC[%] Content	Total Bands	Total No. of Bands	Polymorphic Bands	Monomorphic Bands	Percentage Polymorphism	PIC	Range of Molecular SIZE[bp]
GSRAPD-1	TGCAGGAACC	92.33	60	15	290	8	0	53.3	0.082	200-3000bp
GSRAPD-2	GCGGGGGTCT	119.2	80	13	256	5	0	38.5	0.107	100-4000
GSRAPD-3	GAGAGGGGCG	111.2	80	12	172	2	0	16.7	0.122	200-3000
GSRAPD-4	TTGAGAAATG	97.77	30							
OPJ-03	TCTCCGCTTG	103.8	60	7	110	1	0	14.3	0.164	100-2500
OPJ-07	CCTCTCGACA	115.4	60	9	73	0	0	0.0	0.179	200-1500
OPJ-10	AAGCCCGAGG	92.82	70	7	86	0	0	0.0	0.156	250-2000
OPJ-15	TGTAGCAGGG	98.43	60	8	100	5	0	62.5	0.116	250-2000
OPJ-20	AAGCGGCCTC	99.42	70	11	226	8	1	72.7	0.117	100-3000
OPH-01	GGTCGGAGAA	116.3	60	8	85	3	1	37.5	0.127	400-3000
OPH-03	AGACGTCCAC	118.5	60	7	72	0	2	0.0	0.128	100-3000
OPH-06	ACGCATCGCA	117.9	60	-	-	-	-	-	-	-
OPH-12	ACGCGCATGT	100.9	60	12	122	1	3	8.3	0.163	250-3500
OPH-16	TCTCAGCTGG	95.9	60	2	26	0	0	0.0	0.167	250-1500
OPM-02	ACAACGCCTC	101.6	60	10	101	0	1	0.0	0.213	250-3000
OPM-05	GGGAACGTGT	120.1	60	11	142	1	0	9.1	0.196	250-2500
OPM-10	TCTGGCGCAC	118.6	70	8	124	0	0	0.0	0.109	250-3000
OPM-13	GGTGGTCAAG	110.3	60	-	-	-	-	-	-	-
OPM-19	CCTTCAGGCA	111.2	60	-	-	-	-	-	-	-
OPU-07	CCTGCTCATC	116.6	60	-	-	-	-	-	-	-

Table 3: Similarity matrix calculated by Jaccard's coefficient of 24 accessions of *G. sylvestre*

	PR1	PR2	PR3	MBN1	MBN2	MBN3	HBG1	HBG2	HBG3	NGL1	NGL2	NGL3	VJA1	VJA2	VJA3	WRL1	WRL2	WRL3	OSM1	OSM2	OSM3	CB1	CB2	CB3
PR1	1	0.253	0.614	0.608	0.595	0.402	0.452	0.598	0.515	0.474	0.52	0.5	0.462	0.519	0.578	0.621	0.5	0.408	0.373	0.427	0.449	0.5	0.367	0.46
PR2		1	0.185	0.182	0.172	0.429	0.206	0.169	0.167	0.4	0.198	0.206	0.429	0.296	0.301	0.247	0.176	0.226	0.244	0.449	0.407	0.194	0.297	0.161
PR3			1	0.806	0.793	0.374	0.636	0.781	0.707	0.344	0.674	0.574	0.363	0.436	0.416	0.538	0.599	0.485	0.504	0.339	0.367	0.565	0.338	0.616
MBN1				1	0.874	0.352	0.631	0.791	0.728	0.376	0.644	0.535	0.331	0.438	0.45	0.531	0.629	0.489	0.507	0.328	0.368	0.606	0.348	0.611
MBN2					1	0.364	0.606	0.765	0.794	0.387	0.682	0.535	0.341	0.45	0.462	0.531	0.629	0.523	0.496	0.328	0.368	0.594	0.359	0.611
MBN3						1	0.404	0.325	0.317	0.547	0.355	0.306	0.394	0.462	0.427	0.414	0.327	0.437	0.344	0.661	0.531	0.35	0.382	0.347
HBG1							1	0.672	0.62	0.446	0.593	0.477	0.264	0.477	0.491	0.393	0.454	0.536	0.544	0.386	0.408	0.453	0.421	0.515
HBG2								1	0.795	0.403	0.697	0.56	0.358	0.421	0.455	0.511	0.619	0.46	0.447	0.292	0.331	0.585	0.343	0.614
HBG3									1	0.42	0.78	0.547	0.35	0.438	0.45	0.508	0.643	0.489	0.453	0.294	0.344	0.571	0.389	0.647
NGL1										1	0.431	0.333	0.456	0.532	0.57	0.461	0.419	0.404	0.308	0.639	0.567	0.377	0.418	0.317
NGL2											1	0.616	0.393	0.474	0.475	0.593	0.631	0.48	0.442	0.364	0.409	0.543	0.361	0.565
NGL3												1	0.408	0.452	0.441	0.544	0.612	0.483	0.48	0.304	0.36	0.548	0.386	0.57
VJA1													1	0.357	0.33	0.537	0.392	0.225	0.24	0.388	0.342	0.337	0.265	0.314
VJA2														1	0.805	0.611	0.491	0.515	0.466	0.513	0.6	0.45	0.512	0.437
VJA3															1	0.609	0.478	0.531	0.481	0.513	0.618	0.477	0.494	0.394
WRL1																1	0.602	0.444	0.379	0.459	0.518	0.532	0.388	0.484
WRL2																	1	0.509	0.432	0.336	0.41	0.673	0.348	0.608
WRL3																		1	0.604	0.432	0.489	0.509	0.453	0.524
OSM1																			1	0.354	0.392	0.504	0.392	0.556
OSM2																				1	0.737	0.333	0.472	0.311
OSM3																					1	0.368	0.521	0.341
CB1																						1	0.407	0.609
CB2																							1	0.458
CB3																								1

Fig. 1: RAPD profiles of Twenty four accessions of *Gymnema sylvestre* generated with different primers

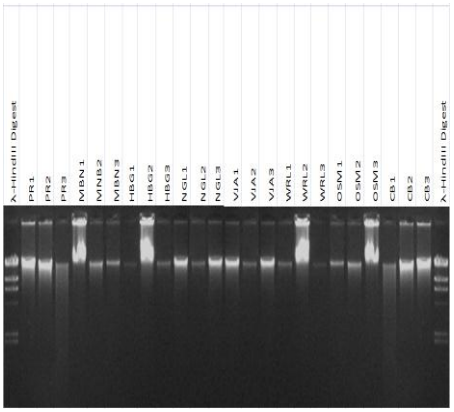


Fig. 1A : Extraction of DNA from 24 accessions of *G. sylvestre* accessions

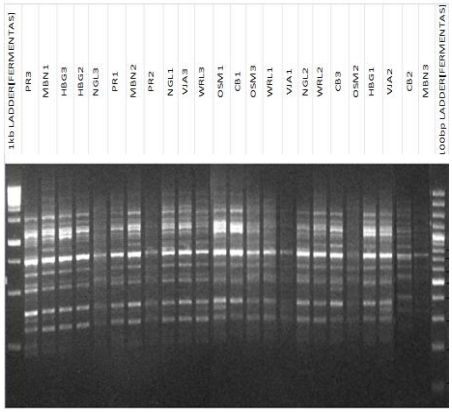


Fig. 1B: RAPD profile generated for 24 accessions of *G. sylvestre* using primer GSRAPD-1

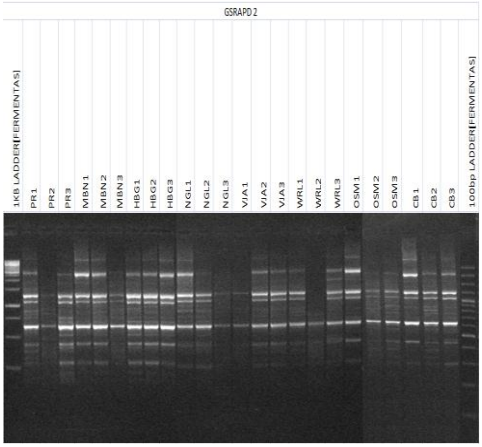


Fig 1C :RAPD profile generated for 24 accessions of *G. sylvestre* using primer GSRAPD-2

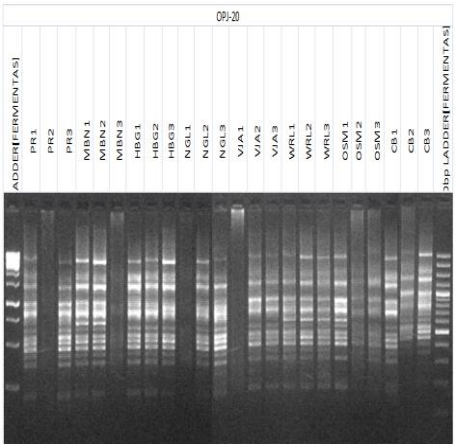


Fig 1D: RAPD profile generated for 24 accessions of *G. sylvestre* using primer OPJ-20

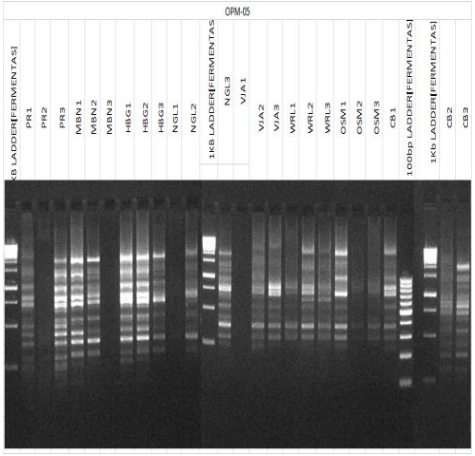


Fig 1E:RAPD profile generated for 24 accessions of *G. sylvestre* using primer OPM-05

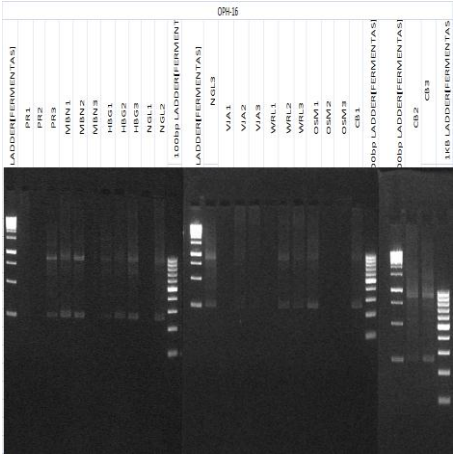


Fig 1F: RAPD profile generated for 24 accessions of *G. sylvestre* using primer OPH-16

Fig 2: Dendrogram (UPGMA) cluster analysis of 24 *G. sylvestre* accessions collected from different regions

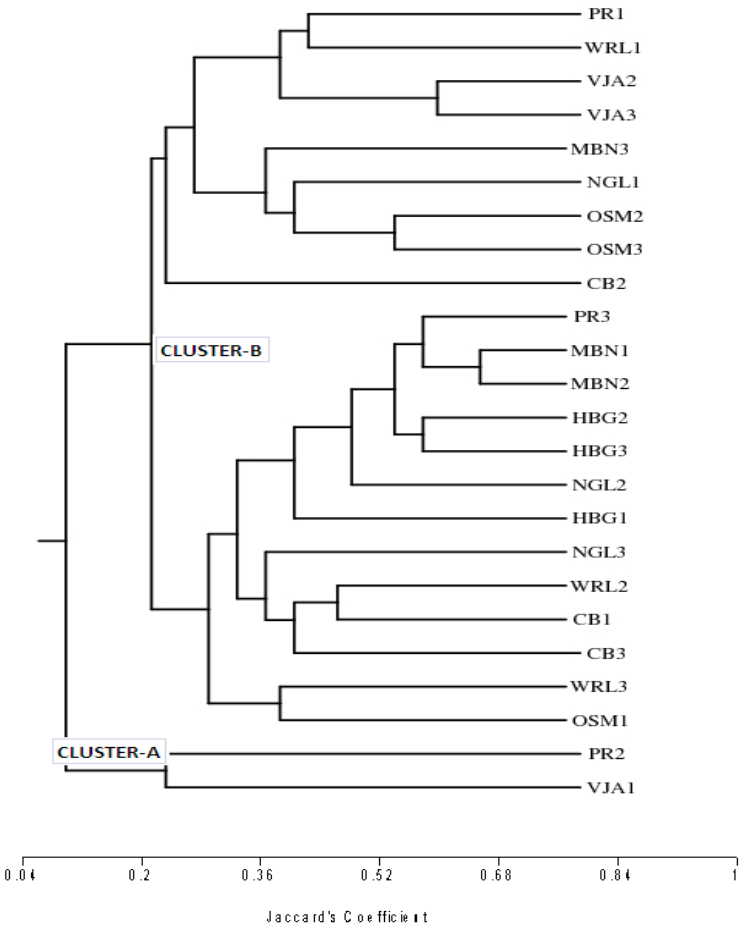
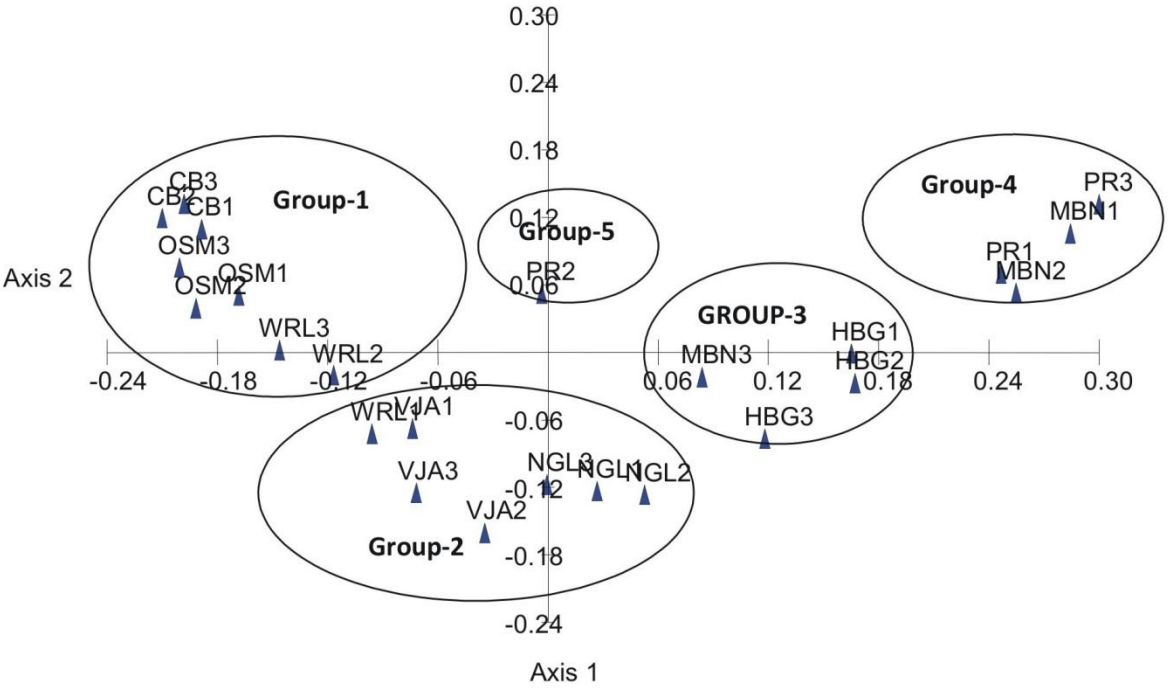


Fig 3: Principal Coordinate Analysis (PCA) of Twenty four *G.sylvestre* accessions based on Jaccard's similarity matrix



DISCUSSION

Medicinal plants are becoming endangered due to over exploitation by pharma industries and also due to the effect of diverse environmental factors and natural hazards. Genetic variability is influenced by the plant species adaptability to the prevailing environmental conditions. *Gymnema sylvestre* is an important medicinal plant whose demand is increasing day-by-day in herbal and Ayurvedic medicines because of its wide biological activity and higher safety margin than synthetic drugs. Studying the genetic diversity in different populations of this species may help in conservation strategies.

In the present study RAPD primers selected generated a good number of polymorphic markers and banding patterns that could enable to discriminate between the accessions by each primer pair. The banding pattern generated by the primers reveal that is an extreme degree of variability with respect to growth habit and morphological characteristics among *G. sylvestre* accessions found in different parts of AP and Telangana. This genetic variation was plotted in the UPGMA dendrogram and the PCA generating a number of clusters that were grouped according to their genetic variation.

Earlier genetic variation in *G.sylvestre* were also studied revealed a considerable genetic variation (73.2%) in the RAPD banding pattern in the *Gymnema* germplasm among 18 accessions of *Gymnema* collected from different parts of Kerala[11]. The level of polymorphism detected by RAPD in the present study is also comparable with the molecular studies carried out [10]. They studied nomenclature, taxonomy, geographical distribution, morphological and genetic diversity among 22 accessions of G. *sylvestre* populations spread across Western Ghats of Maharashtra by using RAPD and ISSR molecular markers.

CONCLUSION

The main emphasis of the present study was to analyze the genetic diversity among twenty four accessions of *Gymnema sylvestre* and also help in devising strategies to protect the genetic diversity of this species. The results could be used for identification of ideal genotypes for extraction of drugs by correlating the molecular fingerprints. The results of the present study clearly suggested that *Gymnema sylvestre* can be characterized through RAPD markers and superior accessions can be identified for product development in control of diabetes.

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Conflict of interest

The author(s) have no conflict of interest in this study.

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