British Biotechnology Journal
10(2): 1-11, 2016, Article no.BBJ.19173
ISSN: 2231–2927, NLM ID: 101616695

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Genetic Variations in *Asparagus racemosus*, an Endangered Medicinal Herb Endemic to India Using RAPD Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MK and Sarla performed the experiments and wrote the first draft of the manuscript. Author PKN performed the analysis and compilation of data. Author VC conceptualize and designed the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2016/19173
Editor(s):
(1) Giovanni DalCorso, Department of Biotechnology, University of Verona, Italy.
Reviewers:
(1) Akansha Singh, GN Khalsa College of Arts, Science and Commerce, Mumbai, India.
(2) Sandeep Sharma, Indian Agriculture Research Institute, Pusa Campus, New Delhi, India.
(3) Manoj K. Yadav, SVP University of Agriculture and Technology, India.
Complete Peer review History: http://sciencedomain.org/review-history/11560

Original Research Article

Received 28th May 2015
Accepted 25th August 2015
Published 27th September 2015

ABSTRACT

Aim: To study the genetic diversity in *Asparagus racemosus* germplasm using RAPD molecular markers for its better conservation and utilization.
Study Design: RAPD markers used to check genetic diversity in *Asparagus racemosus* using different softwares.
Place and Duration of Study: Department of Bio & Nano Technology, Guru Jambheshwar University of Science & Technology, Hisar-125001 between May 2013 to June 2014.
Methodology: A total of 60 RAPD markers used to check polymorphism at genetic level among 60 asparagus genotypes. PCR amplified bands were scored as 0 and 1 for absence and presence. The binary data so obtained used to reveal genetic polymorphism via NTSYS, POPGENE and

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AMOVA analysis.
Results: A significant level of genetic diversity (81.48%) among all genotypes was assessed by using RAPD molecular markers. Out of 60, 49 RAPD primers produced 425 polymorphic loci. The value of Jaccard's coefficient varied from 0.48 to 0.97 for RAPD. OPB-15 primer proved to be the most polymorphic marker among all used. The POPGENE analysis revealed 44.44, 79.01 and 64.20% polymorphism for RAPD analysis in groups with low, intermediate and high saponin content. The overall value of Shannon's index and Nei's genetic diversity was 0.3402 & 0.2169 for RAPD marker system.
Conclusion: These results showed RAPD marker system useful in detecting significant genetic polymorphism among genotypes which can be used for production and conservation of improved genotypes.

Keywords: Asparagus racemosus; genetic polymorphism; RAPD; endangered medicinal plant.

1. INTRODUCTION

The genus Asparagus has gained importance as a vegetable (*Asparagus officinalis*), a medicinal herb (*Asparagus racemosus*) and also as an ornamental plant (*Asparagus plumosus*). It includes about 300 species around the world. Out of the 22 species of *Asparagus* recorded in India, *Asparagus racemosus*, commonly known as "Shatavari" is a much branched, spinous under shrub found growing wild in tropical and sub-tropical parts of India up to an altitude of 1500 m. The World Health Organization has estimated that 80% population of developing countries, being unable to afford pharmaceutical drugs, relies on traditional medicines, mainly plant based, to sustain their primary health care needs [1]. The root extracts of *Asparagus racemosus* were also found to possess antiulcer [2], antioxidant [3], antidiarrhoeal [4,5], anticarcinogenic, antiulcerogenic, immunostimulant and hepatoprotective activities [6] and is also used in nervous disorders, bronchitis, inflammation, dyspepsia [7]. A study of ancient classical Ayurvedic literature also claimed several therapeutic attributes for the root of *A. racemosus* and has been specially recommended in cases of threatened abortion and as a galactogogue. With an increasing realization that hormone replacement therapy with synthetic oestrogens is neither as safe nor as effective as previously envisaged, the interest in plant-derived oestrogens has increased tremendously making *A. racemosus* more important [8]. The plant also has some economic use including the plant tubers that are used to prepare chogaru, a colorant to give colour to areca nut [9].

The increasing demand of *Asparagus racemosus* for medicinal uses has caused a serious reduction in native populations due to over harvest and deforestation. This plant is now considered endangered in its natural habitat and has also been recognized as 'vulnerable' [10]. Hence analysis of genetic diversity becomes essential for the development of suitable conservation, management and multiplication strategies for the available germplasm. Genetic diversity studies have traditionally been through morphological and biochemical markers which are rather less effective and are influenced by environmental factors as well as developmental stage. Recently molecular markers have become an ideal tool for germplasm characterization, because of their plasticity, ubiquity and stability. By using molecular markers, breeders can bypass the traditional phenotype based selection methods which involves growing plants to maturity and closely observing their physical characteristics in order to infer underlying genetic makeup. PCR technology has now led to the development of simple, quick and efficient techniques like RAPD (random amplified polymorphic DNA) [11] which in contrast to allozyme/protein markers is independent of the environmental factors and the developmental stages of the plant. RAPD markers are usually 10-23 nucleotides long and generate randomly amplified fragments of DNA. The RAPD technique has several advantages such as the ease and rapidity of analysis, relatively low cost, does not require any radioactive compounds, requirement of lesser amount of template DNA for analysis, no prior requirement of genetic information and availability of large number of primers [11-13]. The technical simplicity and the advantages associated with RAPDs have made them a favourite marker technique in mapping and determination of the phylogenetic relationships. RAPD analysis also makes it possible to measure the genetic diversity among accessions of different species and among accessions within species.

Although extensive studies have been carried out on genetic diversity in different *Asparagus* species and especially *Asparagus officinalis* using RAPD markers [14-26] but little attention have been paid so far on genetic diversity studies in *Asparagus racemosus*. Hence, the present study was proposed to analyze the genetic polymorphism among *Asparagus racemosus* genotypes using RAPD markers for better utilization of available germplasm.

2. MATERIALS AND METHODS

2.1 Plant Materials and DNA Extraction

A total of 60 *Asparagus racemosus* cultivars (Table 1) were collected from Herbal Garden, CCSHAU, Hisar and Herbal Garden, Rohtak. Genomic DNA was isolated from young leaves using slightly modified CTAB method. The quantitative and qualitative analysis of DNA was done by Nano-Drop spectrophotometer (ND-100) and 0.8% agarose gel electrophoresis.

2.2 RAPD Analysis

A total of 60 RAPD primers (Integrated DNA Technologies) were used belonging to three series namely OPB, OPC and OPD each having 20 primers. The PCR amplifications was carried out in thermal cycler (MJ Research) which consisted of reaction volume of 15 µl each consisting of approximately 30 ng of template DNA, 1X PCR buffer, 0.2 mM dNTPs, 3.5 mM $MgCl_2$, 20 pico moles primer and 1.0 Units of Taq DNA polymerase enzyme (Sigma-Aldrich). The thermo cycler for the RAPD amplification was programmed as: initial denaturation (94℃) for 3 minutes followed by 35 cycles of denaturation (94℃) for 1 minute, annealing (33-44.5℃) for 1 minute, extension (72℃) for 1 minute and final extension (72℃) for 10 minutes followed by storage step (4℃) till use.

2.3 Agarose Gel Electrophoresis

The amplified products were electrophoresed on 1.8% (w/v) agarose gels in 1x TAE buffer at

Table 1. *Asparagus racemosus* genotypes with their respective accession numbers

S. no.	Genotype	Accession no.	S. no.	Genotype	Accession no.
1	AR1	**HAR 1**	31	AR31	**KR-100-VII**
2	AR2	**HAR 2**	32	AR32	**KR-110**
3	AR3	**HAR 3**	33	AR33	**KR-120**
4	AR4	**HAR 4**	34	AR34	**CONTROL**
5	AR5	**HAR 5**	35	AR35	**KR60MA1**
6	AR6	**HAR 6**	36	AR36	**KR60MB1**
7	AR7	**HAR 7**	37	AR37	**KR60MC1**
8	AR8	**HAR 8**	38	AR38	**KR60MD1**
9	AR9	**HAR 03-1**	39	AR39	**KR60-1M-1**
10	AR10	**HAR 03-2**	40	AR40	**KR120-1M-1A**
11	AR11	**HAR 03-3**	41	AR41	**KR120-1M-1B**
12	AR12	**HAR 03-4**	42	AR42	**KR120-1M-1C**
13	AR13	**HAR 03-5**	43	AR43	**KR100-5M-1**
14	AR14	**HAR 03-6**	44	AR44	**KR60A-4M-1**
15	AR15	**HAR 03-7**	45	AR45	**KR60B-4M-1**
16	AR16	**HAR 03-8**	46	AR46	**KR60C-4M-1**
17	AR17	**SP-1-03-9**	47	AR47	**KR60D-4M-1**
18	AR18	**SP-2-03-10**	48	AR48	**KR60E-4M-1**
19	AR19	**SP-3-03-11**	49	AR49	**ARHMV1**
20	AR20	**SP-5-03-12**	50	AR50	**ARHMV2**
21	AR21	**HAR 03-13**	51	AR51	**ARHMV3**
22	AR22	**HAR 03-14**	52	AR52	**ARHMV4**
23	AR23	**HAR 03-15**	53	AR53	**ARHMV5**
24	AR24	**HAR 03-16**	54	AR54	**ARHMV6**
25	AR25	**KR-100-I**	55	AR55	**ARHMV7**
26	AR26	**KR-100-II**	56	AR56	**ARHMV8**
27	AR27	**KR-100-III**	57	AR57	**ARHMV9**
28	AR28	**KR-100-IV**	58	AR58	**ARHMV10**
29	AR29	**KR-100-V**	59	AR59	**ARHMV11**
30	AR30	**KR-100-VI**	60	AR60	**ARHMV12**

constant voltage (100 V) for 1 h, visualized by staining with ethidium bromide (@ 0.5 µg /ml) and 2 µl of 6X gel loading dye (40% sucrose, 0.25% Xylene cyanol and 0.25% Bromophenol Blue) was added to each reaction tube before loading into agarose gel. The electrophoresis was followed by documentation under ultraviolet light using Gel Documentation System (SynGene, Germany). 100 bp and 1.0 kb ladders were used as molecular size markers. All PCR reactions were run in duplicates and only clear and reproducible bands were scored for polymorphism study.

2.4 Data Analysis

Only the reproducible bands were selected for scoring and the scoring of the bands of different sizes is done as 0 (absent) and 1 (present) of a particular sized band, each of which was considered to be an independent character. The Jaccard similarity coefficient was used to calculate the pairwise similarity matrix of the accessions [27]. The similarity matrix was subjected to the cluster analysis by using UPGMA (Unweighted Pair Group Method with Arithmetic average) and dendrogram was generated by NTSYS-pc program [28]. POPGENE software was used to calculate Nei's unbiased genetic distance among different genotypes with all markers. Data for observed number of alleles (Na), effective number of alleles (Ne), Nei's gene diversity (H), Shannon's information index (I), number of polymorphic loci (NPL) and percentage of polymorphic loci (PPL) across all the 60 genotypes were analysed [29]. Within group diversity (Hs) and total genetic diversity (Ht) were calculated within the species and within three major groups (based on the saponin contents of genotypes i.e. group with low, intermediate and high saponin contents) by using POPGENE software [30]. The RAPD data were subjected to a hierarchical analysis of molecular variance (AMOVA) [31], using three hierarchical levels; individual, population and grouping based on their saponin content. The resolving power was calculated according to [32], where the resolving power of a primer is: $Rp = \sum IB$ where IB (band informativeness) takes the value of: 1-[2 X (0.5 - P)], P represents the proportion of 60 genotypes having that band.

The diversity index (DI), effective multiple ratio (EMR) and marker index (MI) were calculated to determine the utility of each of the marker systems [33]. DI for the genetic markers was calculated from the sum of squares of allele frequencies: $DIn=1 - \sum pi^2$ (where pi is the allele frequency of the i^{th} allele). The arithmetic mean heterozygosity, DI_{av}, was calculated for each marker class: $DI_{av}=\sum Di_n/_n$, (where n represents the number of the markers (loci) analyzed). The DI for the polymorphic marker is: $(DI_{av})p = \sum Di_n / n_p$ (where np is the number of polymorphic loci and n is the total number of loci). EMR (E) is the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay $EMR (E) = n_p(n_p / n)$. MI is defined as the product of the average diversity index for polymorphic bands in any assay and the EMR for that assay, $MI = DI_{avp} X E$. The PIC (Polymorphism Information Content) was calculated based on the allele pattern [34] of all the asparagus accessions by employing the following formula:

$$PIC_i = 1 - \sum_{j=1}^{n} Pij^2$$

Where, p_i is the frequency of an individual genotype generated by a given primer and summation extends over *n* alleles.

The two dimensional and three dimensional PCA (Principle Component Analysis) were also constructed for accurately testing the relationships among 60 *Asparagus racemosus* accessions using the EIGEN program analysis using NTSYS-pc software [27].

3. RESULTS AND DISCUSSION

Forty nine RAPD primers out of sixty gave reproducible amplification pattern (Fig. 1) and scored a total of 522 loci (an average of 10.65 loci per primer) out of which 425 loci (an average of 8.67 loci per primer) were found to be polymorphic (81.42%) and 97 loci were monomorphic (Table 2). The size of the amplified fragments varied from 130 bp to 3.0 Kb. The value of Jaccard's similarity coefficient varied from 0.48 to 0.97 and the value of coephenetic correlation coefficient was found to be 0.9854.

The resolving power of the RAPD primers varied from 0.8 (OPB-17) to 25.17 (OPB-15). The value of PIC varied from 0.497 (OPB-17) to 0.945 (OPB-15). The UPGMA generated dendrogram (Fig. 2) consisted of 5 mega clusters, out of which two were further extensively divided into mini clusters. These clustering were also confirmed by 2 dimensional and 3 dimensional PCA analysis (Figs. 3 and 4).

Table 2. Sequence of RAPD primers, their GC content, annealing temperature (T_A), total number of loci (TL), number of polymorphic loci (NPL), percentage of polymorphic loci (PPL), total fragments amplified (TF), resolving power (Rp) and polymorphic information content (PIC)

Primer	Primer sequence (5'-3')	GC (%)	T_A	TL	NPL	PPL (%)	TF	R_P	PIC
OPB-1	5´-GTTTCGCTCC-3´	60	34.3	12	9	75.0%	277	9.23	0.872
OPB-2	5´-TGATCCCTGG-3´	60	33.4	10	9	90.0%	144	4.8	0.819
OPB-3	5´-CATCCCCCTG-3´	70	38.3	5	3	60.0%	171	5.7	0.782
OPB-4	5´-GGACTGGAGT-3´	60	32.4	13	10	76.9%	326	10.87	0.899
OPB-7	5´-GGTGACGCAG-3´	70	37.6	17	12	70.6%	602	20.07	0.930
OPB-8	5´-GTCCACACGG-3´	70	38.8	15	10	66.7%	418	13.83	0.910
OPB-9	5´-TGGGGGACTC-3´	70	38.8	10	10	100.0%	149	4.97	0.887
OPB-10	5´-CTGCTGGGAC-3´	70	38.8	11	8	72.7%	422	14.07	0.895
OPB-11	5´-GTAGACCCGT-3´	60	35.5	19	11	57.9%	722	24.03	0.941
OPB-12	5´-CCTTGACGCA-3´	60	37.0	14	10	71.4%	425	14.13	0.919
OPB-15	5´-GGAGGGTGTT-3´	60	35.8	20	13	65.0%	764	25.17	0.945
OPB-17	5´-AGGGAACGAG-3´	60	35.8	3	3	100.0%	24	0.8	0.497
OPB-19	5´-ACCCCCGAAG-3´	70	40.3	8	8	100.0%	202	6.73	0.863
OPC-1	5´-TTCGAGCCAG-3´	60	35.0	13	10	76.9%	438	14.6	0.914
OPC-2	5´-GTGAGGCGTC-3´	70	35.0	13	8	61.5%	389	12.93	0.910
OPC-4	5´-CCGCATCTAC-3´	60	35.0	10	9	90.0%	202	6.73	0.883
OPC-5	5´-GATGACCGCC-3´	70	38.6	9	7	77.7	299	9.97	0.864
OPC-6	5´-GAACGGACTC-3´	60	32.8	6	6	100.0%	91	3.03	0.819
OPC-7	5´-GTCCCGACGA-3´	70	37.0	7	4	57.1%	259	8.67	0.831
OPC-8	5´-TGGACCGGTG-3´	70	38.0	10	7	70.0%	290	9.93	0.871
OPC-9	5´-CTCACCGTCC-3´	70	38.4	9	6	66.6%	338	13.07	0.874
OPC-10	5´-TGTCTGGGTG-3´	60	55.6	8	8	100.0%	187	6.23	0.859
OPC-11	5´-AAAGCTGCGG-3´	60	36.3	13	9	69.2%	426	14.2	0.907
OPC-13	5´-AAGCCTCGTC-3´	60	38.0	10	9	90.0%	190	6.33	0.864
OPC-14	5´-TGCGTGCTTG-3´	60	37.0	12	10	83.3%	344	11.47	0.897
OPC-15	5´-GACGGATCAG-3´	60	32.8	12	12	100.0%	186	6.23	0.905
OPC-16	5´-CACACTCCAG-3´	60	34.0	10	10	100.0%	141	4.7	0.878
OPC-17	5´-TTCCCCCCAG-3´	70	37.0	7	7	100.0%	101	3.37	0.810
OPC-18	5´-TGAGTGGGTG-3´	60	34.6	7	6	85.7%	129	4.3	0.792
OPC-19	5´-GTTGCCAGCC-3´	70	37.5	13	13	100.0%	280	9.4	0.908
OPC-20	5´-ACTTCGCCAC-3´	60	37.0	11	8	72.7%	317	10.57	0.878
OPD-1	5´-ACCGCGAAGG-3´	70	43.1	10	10	100.0%	128	4.27	0.871
OPD-2	5´-GGACCCAACC-3´	70	39.0	13	10	76.9%	331	10.4	0.910
OPD-3	5´-GTCGCCGTCA-3´	70	42.6	11	8	72.7%	487	16.2	0.898
OPD-4	5´-TCTGGTGAGG-3´	60	32.9	8	8	100.0%	105	3.5	0.850
OPD-5	5´-TGAGCGGACA-3´	60	39.1	7	6	85.7%	153	5.1	0.792
OPD-6	5´-ACCTGAACGG-3´	60	37.1	6	6	100.0%	70	2.33	0.781
OPD-7	5´-TTGGCACGGG-3´	70	43.6	11	9	81.8%	271	9.03	0.892
OPD-8	5´-GTGTGCCCCA-3´	70	39.6	11	11	100.0%	168	5.6	0.888
OPD-10	5´-GGTCTACACC-3´	60	33.3	10	10	100.0%	133	4.43	0.894
OPD-11	5´-AGCGCCATTG-3´	60	38.6	13	13	100.0%	180	6.0	0.896
OPD-12	5´-CACCGTATCC-3´	60	31.9	7	6	85.7%	153	5.1	0.836
OPD-13	5´-GGGGTGACGA-3´	70	41.5	10	8	80.0%	276	9.2	0.873
OPD-14	5´-CTTCCCCAAG-3´	60	34.3	5	5	100.0%	40	1.33	0.744
OPD-15	5´-CATCCGTGCT-3´	60	38.1	15	11	73.3%	505	16.83	0.921
OPD-16	5´-AGGGCGTAAG-3´	60	35.4	12	11	91.7%	277	9.23	0.902
OPD-18	5´-GAGAGCCAAC-3´	60	32.9	10	6	60.0%	400	13.33	0.894
OPD-19	5´-CTGGGGACTT-3´	60	30.0	10	10	100.0%	190	6.3	0.892
OPD-20	5´-ACCCGGTCAC-3´	70	36.5	16	12	75.0%	528	17.6	0.925
Total				522	425	81.42%	13648		

In a previous study, all the 60 cultivars were grouped into three groups according to their saponin content as: group with low, intermediate and high saponin content (Table 3) [35]. The POPGENE analysis (Table 4) revealed that percentage of polymorphic loci were 44.44% (low saponin group), 64.20% (high saponin group) and 79.01% (intermediate saponin group).

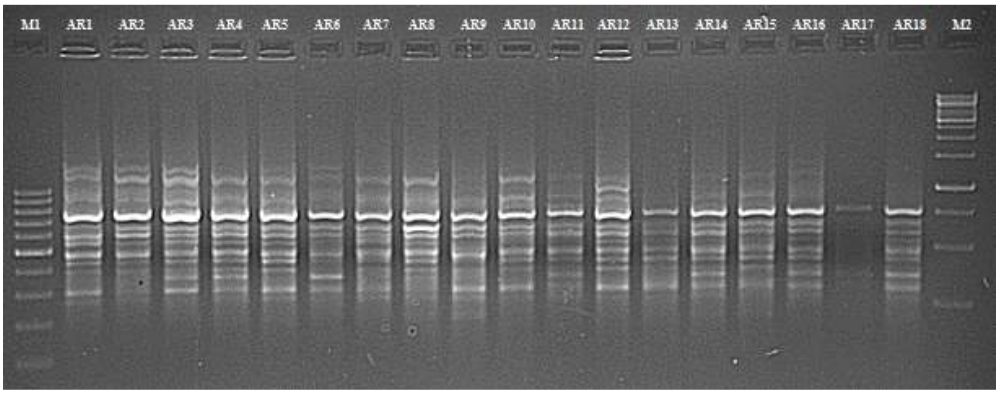

Fig. 1. A representative agarose gel pattern of PCR product amplified with OPB-7 random primer [M1 and M2 were the DNA ladders of 100 bp and 1.0 Kb size]

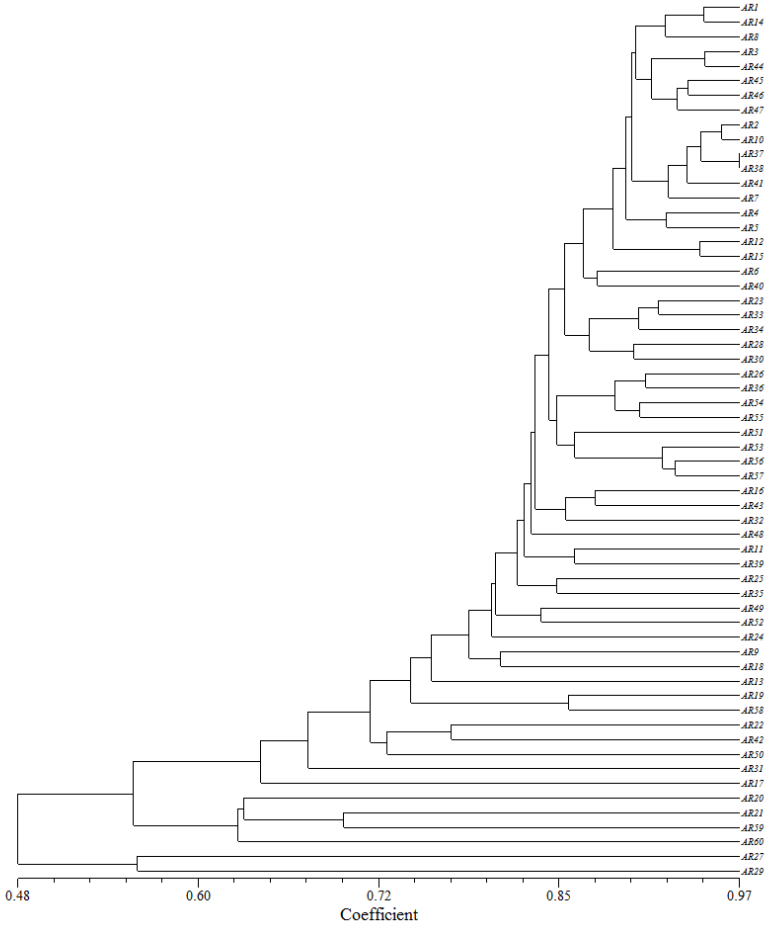

Fig. 2. UPGMA generated dendrogram by RAPD analysis

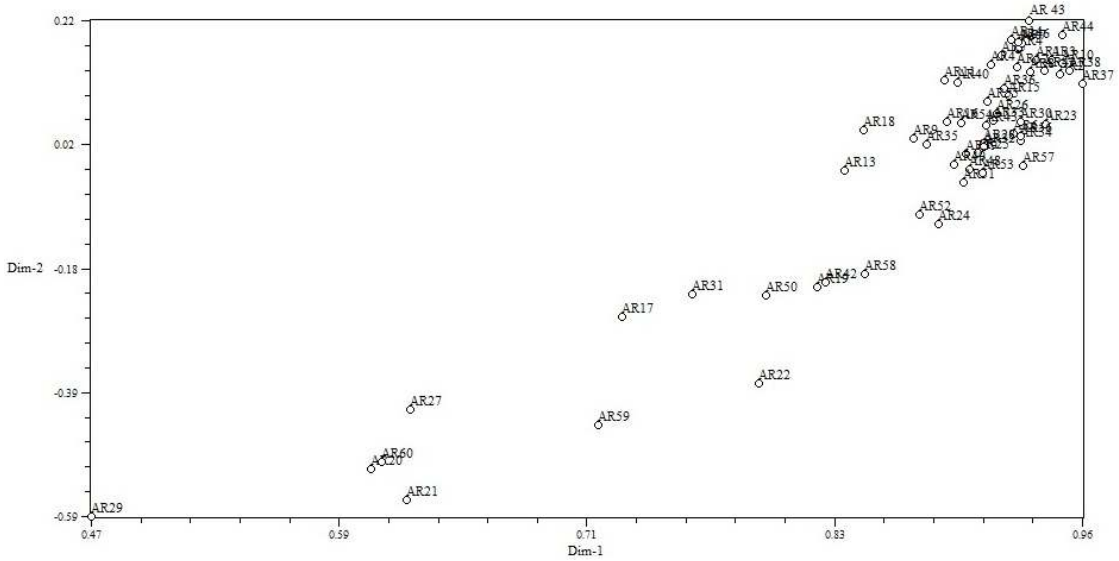

Fig. 3. 2-D PCA clustering by RAPD analysis

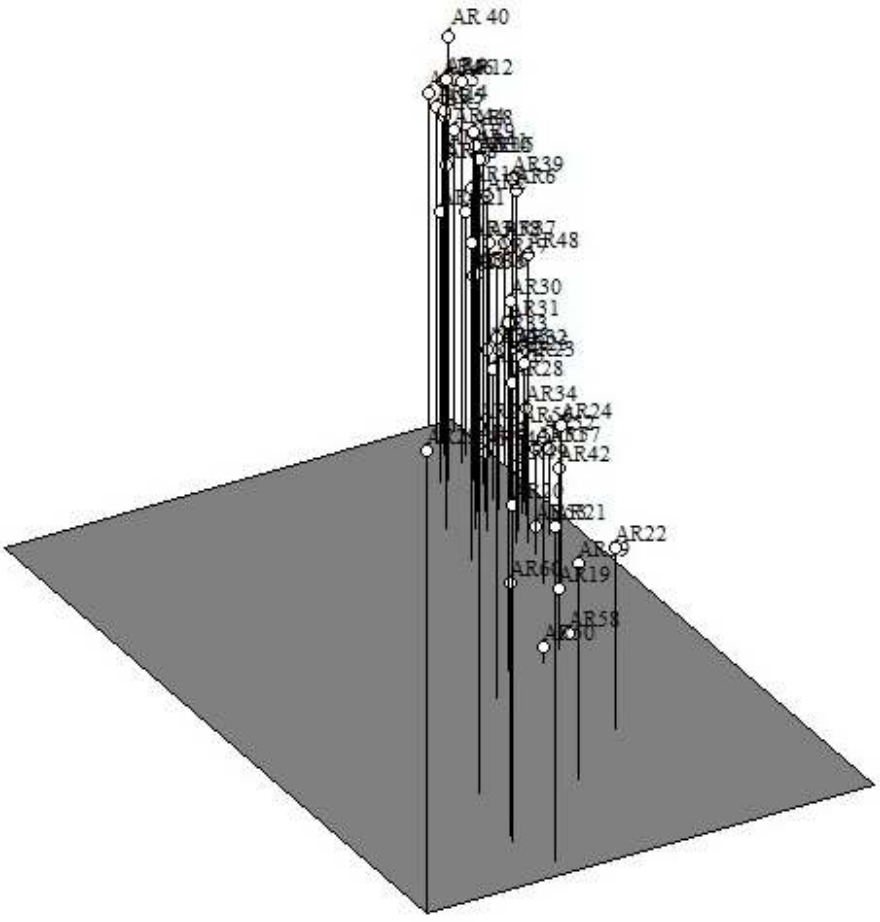

Fig. 4. 3-D PCA clustering by RAPD analysis

The three groups containing cultivars with low, intermediate and high saponin contents showed the values of Nei's genetic diversity (H) : 0.1639, 0.2151 and 0.2154 respectively and of Shannon's information index (I) 0.2449, 0.3381 and 0.3260 respectively. The overall value of Shannon's index (0.3402) and Nei's genetic diversity (0.2169) showed that although a complete genetic isolation of the cultivars did not occur but a very high level of polymorphism was observed among the asparagus cultivars. The significant level of genetic diversity was also supported by the AMOVA analysis (Table 5).

Table 3. Groupings of *Asparagus racemosus* genotypes on basis of their saponin contents

S. no.	Groups*	*Asparagus racemosus* genotypes
1.	Group 1 (high saponin content) (42.87-53.46 mg/gm)	HAR 03-7, HAR 03-8, ARHMV12, KR60MB1, KR60-1M-1, KR60C-4M-1, KR60D-4M-1, ARHMV3, ARHMV7, ARHMV8, ARHMV11, SP-2-03-10
2.	Group 2 (low saponin content) (33.02-35.58 mg/gm)	KR-100-VI, KR-100-VII, KR60MA1, KR120-1M-1A, KR120-1M-1B, KR60B-4M-1
3.	Group 3 (intermediate saponin content) (35.70-41.48 mg/gm)	HAR 1, HAR 2, HAR 3, HAR 4, HAR 5, HAR 6, HAR 7, HAR 8, HAR 03-1, HAR 03-2, HAR 03-3, HAR 03-4, HAR 03-5, HAR 03-6, SP-1-03-9, SP-3-03-11, SP-5-03-12, HAR 03-13, HAR 03-14, HAR 03-15, HAR 03-16, KR-100-I, KR-100-II, KR-100-III, KR-100-IVKR-100-V, KR-110, KR-120, CONTROL, KR60MB1, KR60MC1, KR60MD1, KR120-1M-1C, KR100-5M-1, KR60A-4M-1, KR60E-4M-1, ARHMV1, ARHMV2, ARHMV4, ARHMV5, ARHMV10, ARHMV9, ARHMV6

** Asparagus genotypes were categorized into three groups such as high (42.87-53.46 mg/gm), low (33.02-35.58 mg/gm) and intermediate (35.70-41.48 mg/gm) saponin contents [low (< 20 mg/g), intermediate (20-50 mg/g) and high (>50 mg/g)]*

Table 4. Summary of genetic variation statistics for all loci of RAPD among the *Asparagus racemosus* cultivars with respect to their shatavarin content

Shatavarin content	Sample size	Na	Ne	H	I	Ht	Hs	NPL	PPL (%)
Low	6	1.444 (0.500)	1.2779 (0.3591)	0.1639 (0.1958)	0.2449 (0.2848)	0.1639 (0.0383)	0.0	36	44.44
High	12	1.6420 (0.4824)	1.3629 (0.3676)	0.2154 (0.1939)	0.3260 (0.2753)	0.2154 (0.0376)	0.0	52	64.20
Interme diate	42	1.7901 (0.4098)	1.3406 (0.3169)	0.2151 (0.1699)	0.3381 (0.2385)	0.2151 (0.0289)	0.0	64	79.01
All 60 cultivars	60	1.8148 (0.3909)	1.3467 (0.3246)	0.2169 (0.1732)	0.3402 (0.2412)	0.2169 (0.0300)	0.2100 (0.0281)	66	81.48

Value are represented as mean (standard deviation), Na = observed number of alleles; Ne = effective number of alleles; H = Nei's gene diversity; I = shannon's information index; Ht = heterogeneity; Hs = homogeneity

Table 5. Summary of nested analysis of molecular variance (AMOVA) based on RAPD among the cultivars of *Asparagus racemosus* (levels of significance are based on 1000 iteration steps, d.f.: degree of freedom; S.S.D.: sum of square deviation; P-value: probability of null distribution)

Source of variation	d.f.	S.S.D.	M.S	Variance component	Percentage	P-value
Among groups	2	16.740	8.370	0.042	0.469	-
Among cultivars	57	510.310	8.953	8.953	99.53	< 0.001

Various approaches ranging from morphological to molecular techniques have been used to infer patterns of diversity and relationships among plant species. The RAPD-PCR has been efficiently employed for varietal characterization of *Asparagus officinalis* [36,21,22,24] and also among four species of the genus Asparagus: *Asparagus racemosus*, *Asparagus falcatus*, *Asparagus officinalis* and *Asparagus plumosus* and helped in authentic *A. racemosus* identification from its adulterants [37]. The low level of genetic diversity among different accessions of *Asparagus racemosus* was reported earlier [8] showing low level of polymorphism (54.92%) in comparison to present study showed low genetic diversity in *Asparagus racemosus*, which might be due to restricted distribution in a particular area, non effective gene flow, low fecundity, low pollen flow, local selection procedure (environment and struggle for existence) and inbreeding systems [38]. Furthermore, RAPD-PCR was also used for genetic diversity evaluation among different *Asparagus* species and populations as it was necessary for crop improvement and conservation of gene pool [39]. In the present study, RAPD markers were used for genetic diversity analysis in *Asparagus racemosus* and deduced higher level of genetic polymorphism within individual genotypes and among all three groups.

The present and previous studies suggested RAPD as an appropriate marker for studying genetic polymorphism in loosely and closely related genotypes; however the problems have been reported concerning the reproducibility of results. Most of these problems seem to be due to mis-priming of the short decamers at the relatively low temperatures (35℃) common in RAPDs and competition between different DNA fragments for amplification [11,40]. At such low temperatures maintenance of consistent reaction profile is critical for reproducible RAPD profiles. To minimize the possibility of mis-priming in the present study, primers with a higher G/C content were used, allowing a high annealing temperature and more reproducibility. Furthermore, greater polymorphism in case of RAPD markers (81.48%) may be due to the fact that out of 60 primers used 49 random primers showed amplification. Furthermore, the higher level of genetic polymorphism was also supported by the values of MI (Marker Index), DI (Diversity Index) and EMR (Effective Multiplex Ratio) i.e. 0.952, 0.09 and 10.58 respectively. Our study demonstrated that the RAPD markers are efficient in resolving the genetic diversity among *A. racemosus* cultivars.

4. CONCLUSION

As more and more uses of this plant are identified, the pressure on existing natural populations will get increased further. Since the value of medicinal plants depends mainly on the active principle present in it (saponins in *Asparagus racemosus*); consistency in quality and quantity of planting material assumes paramount importance. The development of better conservation strategies can be ensured by identification of elite genotypes via molecular marker techniques and chemo profiling followed by their mass multiplication using both conventional and biotechnological approaches.

ACKNOWLEDGEMENT

The first author is highly thankful to University Grants Commission, New Delhi for providing financial assistance as Senior Research Fellowship for the successful completion of this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Singh A, Sinha B. *Asparagus racemosus* and its phytoconstituents; an updated review. Asian Journal of Biochemical and Pharmaceutical research. 2014;4(4):230-240.
2. Sairam KS, Priyambada NC, Goel RK. Gastroduodenal ulcer protective activity of *Asparagus racemosus*: an experimental, biochemical and histological study. J. Ethnopharmacol. 2003;86(1):1-10.
3. Kamat JP, Boloor KK, Devasagayam TP, Venkatachalam SR. Antioxidant properties of *Asparagus racemosus* against damaged induced by gamma radiation on rat liver mitochondria. J. Ethanopharmacol. 2000; 71:425-435.
4. Venkatesan N, Thiyagarajan V, Narayanan S, Arul A, Raja S, Gurusamy S, Kumar V, Rajarajan T, Perianayagam JB. Antidiarrhoeal potential of *Asparagus racemosus* willd root extracts in laboratory

animals. J. Pharm. Pharma. Sci. 2005; 8(1):39-45.
5. Visavadiya N, Narasimhacharya RL. Hypolipidemic and antioxidant activities of *Asparagus racemosus* in hypercholesteremic rats. Ind. J. Pharmacol. 2005;37(6):376-380.
6. Singh A, Sinha B. Pharmacological significance of Shatavari; The Queen of herbs. International Journal of Phytomedicine. 2014;6:477-488.
7. Garabadu D, Krishnamurthy S. *Asparagus racemosus* attenuates anxiety-like behaviour in experimental animal models. Cellular and Molecular Neurobiology. 2014;34(4):511-521.
8. Vijay N, Sairkar P, Silawat N, Garg RK, Mehrotra NN. Genetic variability in *Asparagus racemosus* (Willd.) from Madhya Pradesh, India by random amplified polymorphic DNA. African Journal of Biotechnology. 2009;8(14): 3135-3140.
9. Prakasha HM, Krishnappa M. People's knowledge on medicinal plants in Sringeri taluk, Karnataka. Indian Journal of Traditional Knowledge. 2006;5(3):353-357.
10. Warner PK, Nambiar VPK, Ganapathy PM. Some important medicinal plants of the Western Ghats, India: A profile. International Development Research Centre, Artstock, New Delhi, India; 2001.
11. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA Polymorphisms amplified by arbitrary primers and useful as genetic markers. Nucl. Acids Res. 1990;18:6531-6535.
12. Whitkus R, Doebley J, Wendel JF. Nuclear DNA markers in systematics and evolution. In: Phillips, R.L. and Vasil, I.K. (eds) DNA based markers in plants. Kluwer Academic Publishers; 1994.
13. Weising K, Nybom H, Wolf K, Meyer D. DNA fingerprinting in plants and fungi. CRC Press, Inc, Boca Raton. 1995;159-200.
14. Singh B, Yadav R, Singh H, Singh G, Punia A. Studies on Effect of PCR-RAPD conditions for Molecular Analysis in *Asparagus* (Satawari) and Aloe Vera Medicinal Plants. Australian Journal of Basic and Applied Sciences. 2010;4(12): 6570-6574.
15. Ikinci N. Genetic diversity in two *Lilium* (Liliaceae) species from different regions of Greece based on a random amplified polymorphic DNA (RAPD) analysis. Journal of Medicinal Plants Research. 2010;4(18):1888-1894.
16. Eimert K, Reutter G, Strolka B. Fast and reliable detection of doubled haploids in *Asparagus officinalis* by stringent RAPD-PCR. Journal of Agricultural Science. 2003;141:73–78.
17. Jiang C, Sink KC. RAPD and SCAR markers linked to the sex expression locus M in asparagus. Euphytica. 1997;93(3): 329-333.
18. Pontaroli AC, Camadro EL. Somaclonal variation in *Asparagus officinalis* plants regenerated by organogenesis from long-term callus cultures. Genetics and Molecular Biology. 2005;28(3):423-430.
19. Raimondi JP, Masuelli RW, Camadro EL. Assessment of somaclonal variations in asparagus by RAPD fingerprinting and cytogenetic analysis. Scientia Horticulturae. 2001;90:19-29.
20. Hollingsworth WO, Christie CB, Nichols MA, Neilson HF. Detection of variation among and within asparagus hybrids using random amplified DNA (RAPD) markers. New Zealand Journal of Crop and Horticultural Science. 1998;26:1-9.
21. Hollingsworth WO, Christie CB, Nichols MA, Behboudian MH. Detection of variation within asparagus embryogenic calli using RAPD markers. Proc. 9th International Asparagus Symposium; 1999; 121-125.
22. Jaag T, Langsdorf A, Snowdon RJ, Koehler W, Hartmann HD. Random amplified polymorphic DNA variation among German and Dutch asparagus cultivars. Plant Breeding. 1998;117(3): 290–292.
23. Spada A, Caporali E, Marziani G, Portaluppi P, Restivo F M, Tassi F, Falavigna A. A genetic map of *Asparagus officinalis* based on integrated RFLP, RAPD and AFLP molecular markers. Theor Appl Genet. 1998;97:1083-1089.
24. Khandka DK, Nejidat A, Golan-Goldhirsh A. Polymorphism and DNA markers for asparagus cultivars identified by random amplified polymorphic DNA. Euphytica. 1996;87:39-44.
25. Irshad M, Idrees M, Saeed A, Muhammad, Naeem R. Genetic diversity among *Asparagus* species and cultivars of *Asparagus officinalis* L. using random amplified polymorphic DNA (RAPD) markers. Int. J. Biodivers. Conserv. 2014; 6(5):392-399.

26. Lal S, Mistry KN, Vaidya PB, Shah SD, Thaker RA. Genetic diversity among five economically important species of asparagus collected from central Gujarat (India) utilizing RAPD markers (random amplification of polymorphic DNA). Int. J. Adv. Biotechnol. Res. 2011;2(4):414-421.
27. Sneath PHA, Sokal K. Numerical Taxonomy. WHF. San Francisco. 1973; 100-308.
28. Rohlf FJ. NTSYS-PC: Numerical taxonomy and multivariate analysis system version 2.0. State University of New York (Stony Brook, New York); 1992.
29. Zhao WG, Zhang JQ, Wangi YH, Chen TT, Yin Y, Huang YP, Pan Y, Yang Y. Analysis of genetic diversity in wild populations of mulberry from western part of North East China determined by ISSR markers. J. Genet. Mol. Biol. 2006;7:196-203.
30. Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 1978;89:583-590.
31. Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 1992;131:479-491.
32. Prevost A, Wilkinson MJ. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor. Appl. Genet. 1999;98:107-112.
33. 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. Mol. Breed.1996;2: 225-238.
34. Anderson JA, Churchill GA, Antique JE, Tanksley SD, Sorrells ME. Optimising parental selection for genetic linkage maps. Genome. 1993;36:181-186.
35. Kumar M, Sarla, Yadav OP, Chhokar V. Estimation of saponin content in Asparagus (*Asparagus racemosus* Willd.) roots by colorimetric method. Annals of Biology. 2011;27(2):115-120.
36. Moreno R, Espejo JA, Cabrera A, Millan T, Gil1 J. Ploidic and molecular analysis of 'Morado de Huetor' asparagus (*Asparagus officinalis* L.) population; A Spanish tetraploid landrace. Genetic Resources and Crop Evolution. 2006;53:729–736.
37. Singh A, Rai VP, Singh M, Singh AK, Sinha B. Molecular diversity analysis of *Asparagus racemosus* and its adulterants using Random amplified polymorphic DNA (RAPD). J. Medicinal Pl. Res. 2013;7: 1050-1056.
38. Loveless MD, Hamrick JL. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. 1984; 27:237-277.
39. Ray S, Mukhopadhyay MJ, Mukhopadhyay S. Phylogenetic relationship among six economically important species of *Asparagus* utilizing RAPD, ISSR and isozyme polymorphism. Bioresearch Bulletin. 2010;3:157-164.
40. Hallden C, Hansen M, Nilsson NO, Hjerdin A, Sall T. Competition as a source of errors in RAPD analysis. Theor. Appl. Genet. 1996;93:1185–1192.

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