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Assessment of genetic variation among *Asparagus racemosus* genotypes using molecular and biochemical markers

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Abstract
Asparagus racemosus is an important over-exploited endangered medicinal plant. The genetic variability of 60 genotypes collected from different geographical region of India based on ISSR molecular markers and its active compound, saponin was determined. Saponin content varied from 33.02 mg/g to 53.46 mg/g and the 60 genotypes could be grouped into high (42.87-53.46 mg/g), low (33.02-35.58 mg/g) and intermediate (35.70-41.48 mg/g) groups. Sixty three ISSR markers generated a total of 448 polymorphic and 110 monomorphic loci. The size of amplicons ranged from 130 bp to 2.0 kb. The genetic variation measured for the three saponin groups with respect to estimated percentage of the polymorphic loci was 28.57%, 65.93% and 70.33%; Nei's gene diversity was 0.1129, 0.1469 and 0.1699; Shannon's information index was 0.1658, 0.2364 and 0.2782 respectively. The estimated overall percentage of polymorphic loci (80.22%), Nei's gene diversity (0.1527) and Shannon's information index (0.2512) for the 60 genotypes of *Asparagus racemosus* indicates a high degree of genetic variability which can potentially be exploited for the production of improved genotypes and conservation practices.

Keywords: *Asparagus racemosus*, genetic polymorphism, ISSR, saponin

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 [1]. *Asparagus racemosus* is a well known Ayurvedic rasayana [2] and has been used in nervous disorders, bronchitis, inflammation, dyspepsia, diabetes, diarrhoea etc. [3, 4, 5]. It has also been reported to be used in treatment of kidney disorders, chronic fevers, stomach ulcers and liver cancer [6]. Furthermore, it is recommended for threatened abortion, lactation in women and to normalize the hormonal changes that occur during pregnancy [7, 8, 9].

The pharmacological and medicinal activities of medicinal plants are generally attributed to the secondary metabolites. The secondary metabolites reported to date in *Asparagus racemosus* include flavonoids [10], oligosaccharides [11], amino acid derivatives [12], asparagamine, racemosol, racemofuran, adscendin and steroidal saponins [7, 13, 14]. Of all these secondary metabolites, steroidal saponins known as shatavarins, are the major constituents and the bio-active compound in *Asparagus racemosus*. The demand for *Asparagus racemosus* has been on the rise due to its multiple medicinal uses, and its indiscriminate exploitation has markedly reduced supply. The plant is now considered 'endangered' in its natural habitat and therefore sustainable conservation is now a priority [15, 16]. However, the successful recovery of an endangered species requires a good knowledge of genetic diversity available in the existing germplasm.

Traditionally, the assessment of genetic diversity is conducted through morphological and biochemical markers. However, these markers are influenced by environmental factors as well as developmental stages of the plant. In contrast, molecular markers like ISSR (inter simple sequence repeats), SSR (simple sequence repeats), AFLP (amplified fragment length polymorphism), SCARs (sequence characterized amplified regions) etc. are ideal for germplasm characterization due to their plasticity, ubiquity, stability and independence of the environmental factors as well as the developmental stages of the plant [17]. Specifically ISSR

markers are approximately 20 to 23 nucleotides long and have comparatively ease, relatively low cost and less amount of template DNA required for analysis [18,19,20]. Moreover, their greater primer length and higher annealing temperature make them highly reproducible and stringent. Previously ISSR markers have been successfully used for the analysis of repeat motifs in mung bean [21], genetic relationships in chickpea [22, 23], and varietal identification in black gram [24]. Thus in this study we have used ISSR markers to investigate the genetic variation among the 60 *Asparagus racemosus* genotypes pertaining to the conservation and management strategies.

Materials and Methods

Plant materials and DNA extraction

A total of 60 *Asparagus racemosus* genotypes (Table 1) were collected from different geographical regions of India and maintained at Herbal Garden, CCSHAU, Hisar and Herbal Garden, Rohtak. Genomic DNA was isolated from young leaves using CTAB method with slight modification. The quantitative and qualitative analysis of DNA was done by Nano-Drop spectrophotometer (ND-100) and 0.8% agarose gel electrophoresis. The 60 genotypes were categorised into three groups according to their saponin content (Table 2).

Table 1: *Asparagus racemosus* genotypes with their respective accession numbers and saponin contents.

S. No.	Genotype	Accession No.	Saponin Conc. (mg/g)	S. No.	Genotype	Accession No.	Saponin Conc. (mg/g)
1	AR1	HAR 1	38.91	31	AR31	KR-100-VII	33.68
2	AR2	HAR 2	39.95	32	AR32	KR-110	37.85
3	AR3	HAR 3	40.26	33	AR33	KR-120	39.43
4	AR4	HAR 4	40.45	34	AR34	CONTROL	38.18
5	AR5	HAR 5	40.48	35	AR35	KR60MA1	35.40
6	AR6	HAR 6	38.18	36	AR36	KR60MB1	51.32
7	AR7	HAR 7	40.74	37	AR37	KR60MC1	37.63
8	AR8	HAR 8	40.42	38	AR38	KR60MD1	36.12
9	AR9	HAR 03-1	41.48	39	AR39	KR60-1M-1	42.87
10	AR10	HAR 03-2	41.35	40	AR40	KR120-1M-1A	35.57
11	AR11	HAR 03-3	40.98	41	AR41	KR120-1M-1B	33.64
12	AR12	HAR 03-4	40.52	42	AR42	KR120-1M-1C	39.90
13	AR13	HAR 03-5	40.59	43	AR43	KR100-5M-1	37.65
14	AR14	HAR 03-6	42.18	44	AR44	KR60A-4M-1	35.70
15	AR15	HAR 03-7	51.96	45	AR45	KR60B-4M-1	33.02
16	AR16	HAR 03-8	49.25	46	AR46	KR60C-4M-1	44.98
17	AR17	SP-1-03-9	38.57	47	AR47	KR60D-4M-1	44.97
18	AR18	SP-2-03-10	44.33	48	AR48	KR60E-4M-1	39.87
19	AR19	SP-3-03-11	37.36	49	AR49	ARHMV1	39.72
20	AR20	SP-5-03-12	37.73	50	AR50	ARHMV2	36.05
21	AR21	HAR 03-13	36.10	51	AR51	ARHMV3	53.46
22	AR22	HAR 03-14	37.35	52	AR52	ARHMV4	39.10
23	AR23	HAR 03-15	36.14	53	AR53	ARHMV5	40.51
24	AR24	HAR 03-16	36.13	54	AR54	ARHMV6	41.04
25	AR25	KR-100-I	37.39	55	AR55	ARHMV7	45.04
26	AR26	KR-100-II	37.6	56	AR56	ARHMV8	45.66
27	AR27	KR-100-III	36.85	57	AR57	ARHMV9	36.14
28	AR28	KR-100-IV	37.63	58	AR58	ARHMV10	37.61
29	AR29	KR-100-V	38.33	59	AR59	ARHMV11	43.50
30	AR30	KR-100-VI	35.58	60	AR60	ARHMV12	43.66

Table 2: Groupings of *Asparagus racemosus* genotypes on basis of their saponin contents

S. No.	Groups*	*Asparagus racemosus* genotypes
1.	Group 1 (High)	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)	KR-100-VI, KR-100-VII, KR60MA1, KR120-1M-1A, KR120-1M-1B, KR60B-4M-1
3.	Group 3 (Intermediate)	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-IV, KR-100-V, KR-110, KR-120, CONTROL, 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.

Saponin Estimation

The saponin content of all the genotypes was estimated by method of Sirohi *et al*. [25] with slight modification. Briefly, two grams of finely powdered asparagus root was taken in a 250 ml conical flask consisting of 20 ml of aqueous methanol. The flask was tightly sealed and kept in a shaker at 25°C and 120 rpm for 24 h. and filtered with whatman no. 1 filter paper. The filtrate (0.25 ml) was added to a tube containing 1.75 ml ethyl acetate. To it 1.0 ml of reagent A (0.5 ml anisaldehyde + 99.5 ml ethyl acetate) and reagent B (Concentrated H_2SO_4 and ethyl acetate, 1:1) were added, thoroughly mixed and incubated in water bath maintained at 60°C for 20 minutes. After cooling to room temperature, the absorbance of the solution was measured at 430 nm and the amount of saponin was estimated.

ISSR analysis

A total of 63 ISSR primers (Integrated DNA Technologies and Alpha DNA) were used, out of which 39 ISSR primers that gives good amplification were screened to study genetic diversity among the 60 asparagus genotypes. The PCR amplification was carried out in thermal cycler (MJ Research) with a reaction volume of 15 µl that consist approximately 30 ng template DNA, 0.2 mM dNTPs, 3.5 mM $MgCl_2$, 20 pico moles primer and 1.0 Units of Taq DNA polymerase (Sigma-Aldrich). The PCR condition was programmed as follows: initial denaturation (94 °C) for 5 minutes and 1 minute for the rest 35 cycles, annealing (36.4-52.0 °C) for 1 minute, extension (72 °C) for 1 minute and final extension (72 °C) for 10 minutes and then storage at 4 °C. The amplified products were electrophoresed on 1.8% (w/v) agarose gels in 1x TAE buffer at constant voltage (100 V) for 1 h and documented under ultraviolet light using Gel Documentation System (SynGene, Gemany).

Data Analysis

Only the reproducible bands were selected for scoring. The individual bands of different sizes were scored as 0 (when it is absent) and 1 (when it is present). The Jaccard similarity coefficient was used to calculate the pairwise similarity matrix among the genotypes [26] and was subjected to cluster analysis based on UPGMA (Unweighted Pair Group Method with Arithmetic average) method using NTSYS-pc program [27]. POPGENE software was used to calculate Nei's unbiased genetic distance among different genotypes. 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 [28]. Further, both within group diversity (Hs) and total genetic diversity (Ht) were calculated using POPGENE software [29]. The ISSR data were subjected to a hierarchical analysis of molecular variance (AMOVA) [30], using three hierarchical levels; individual, population and grouping based on their saponin content. The resolving power of the primer was calculated according to Prevost and Wilkinson [31], Rp = ∑ 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 the marker system [32]. DI for the genetic marker was calculated from the sum of squares of allele frequencies as DIn=1 - ∑ 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 as $DI_{av}=\sum Di_n/n$, (where n represents the number of the 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 according to Anderson *et al*., [33] based on the allele pattern of all the *Asparagus* genotypes by employing the following formula:

$$PIC = 1 - \sum_{i=1}^{n} Pi^2$$

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 performed for cluster analysis of genotypes based on the EIGEN program analysis using NTSYS-pc software [26].

Results

Saponin content

The saponin content in the root extract of 60 *Asparagus racemosus* genotypes (AR1 to AR 60) varied from 33.02 mg/g (AR 45) to 53.46 mg/g (AR 51). Based on the saponin content, all the asparagus genotypes were categorized into three major groups; high (42.87-53.46 mg/gm), low (33.02-35.58 mg/gm) and intermediate (35.70-41.48 mg/gm) consisting of 12, 6 and 42 cultivars respectively (Table 1).

ISSR analysis

Thirty nine primers (Table 3) generated a total of 558 loci (an average of 14.31 loci per primer) out of which 448 loci (an average of 11.48 loci per primer) were polymorphic and 110 loci were monomorphic. The size of the bands varied from 130 bp to 2.0 Kb. The PIC value of the primers varied from 0.727 (P-813) to 0.951 (P-16). Similarly the resolving power of the primers ranged from 4.07 (P-813) to 32.53 (P-16). The pairwise Jaccard's similarity coefficient between genotypes ranged from 0.33 to 0.96

Table 3: Sequence of ISSR primers, their GC content, annealing temperature (TA), Total number of loci (TL), Number of polymorphic loci (NPL), Percentage of polymorphic loci (PPL), Total fragments amplified (TF), Resolving power (R_p) and Polymorphic Information Content (PIC).

Primer	Primer Sequence (5'-3')	GC (%)	T_A	TL	NPL	PPL (%)	TF	R_P	PIC
P-1	5′-$(CA)_8$AT-3′	44.4	53.4	13	10	76.9%	497	17.33	0.911
P-2	5′-$(CA)_8$AC-3′	50.0	54.4	7	5	71.4%	281	9.37	0.821
P-3	5′-$(CA)_8$GT-3′	50.0	52.4	11	9	81.1%	346	11.53	0.885
P-4	5′-$(CA)_8$GAC-3′	52.6	54.3	15	13	86.7%	538	17.93	0.922
P-5	5′-GGT$(CA)_7$C-3′	55.5	54.0	17	13	76.5%	812	27.07	0.936
P-6	5′-CGT$(CA)_7$C-3′	55.5	52.8	15	12	80.0%	705	23.5	0.925
P-7	5′-CAG $(CA)_7$C-3′	55.5	56.6	15	11	73.3%	630	21.0	0.928
P-8	5′-CAG$(CT)_7$C-3′	55.5	52.6	18	15	83.3%	754	25.13	0.938
P-9	5′-GAG$(TC)_9$-3′	52.3	54.1	22	20	90.9%	751	24.97	0.949
P-10	5′-$(CT)_9$G-3′	52.6	47.5	9	7	77.8%	189	6.3	0.853
P-12	5′-GTC $(ACC)_6$AC-3′	65.2	62.8	18	15	83.3%	717	23.9	0.934
P-15	5′-$(GA)_7$RC-3′	53.1	47.6	19	15	78.9%	833	27.77	0.942
P-16	5′-$(CTC)_4$RC-3′	67.8	51.4	23	18	78.3%	977	32.53	0.951
P-17	5′-$(CAC)_4$RC-3′	67.8	49.8	20	18	90.0%	733	24.43	0.936

P-18	5′-(GAG)$_7$RG-3′	67.3	62.0	22	17	77.3%	864	28.77	0.946
P-19	5′-(GTG)$_4$RC-3′	67.8	54.5	21	15	71.4%	825	27.5	0.944
P-20	5′-(CT)$_8$RG-3′	52.7	46.8	11	9	81.8%	356	11.93	0.882
P-21	5′-(CT)$_8$TG-3′	50.0	49.1	8	7	87.5%	214	31.0	0.831
P-22	5′-(CT)$_8$RC-3′	52.7	46.6	13	11	84.6%	441	15.07	0.908
P-23	5′-(GT)$_6$AY-3′	46.4	41.0	17	15	88.2%	452	15.03	0.911
P-807	5′-(AG)$_8$T-3′	47.1	53.5	19	16	84.2%	529	18.4	0.933
P-810	5′-(GA)$_8$T-3′	47.1	54.0	20	17	85.0%	723	24.1	0.939
P-812	5′-(GA)$_8$A-3′	47.1	53.0	15	11	73.3%	599	19.17	0.923
P-813	5′-(CT)$_8$T-3′	47.1	48.4	8	6	75.0%	122	4.07	0.727
P-814	5′-(CT)$_8$A-3′	47.1	48.1	7	5	71.4%	203	6.73	0.802
P-815	5′-(CT)$_8$G-3′	52.9	50.0	14	12	85.7%	300	10.03	0.889
P-816	5′-(CA)$_8$T-3′	47.1	53.0	12	10	83.3%	337	11.23	0.886
P-817	5′-(CA)$_8$A-3′	47.1	52.3	8	6	75.0%	250	8.33	0.824
P-818	5′-(CA)$_8$G-3′	52.9	55.8	14	12	85.7%	355	12.3	0.902
P-822	5′-(TC)$_8$A-3′	47.1	51.6	8	4	50.0%	297	9.9	0.860
P-823	5′-(TC)$_8$C-3′	52.9	54.9	10	8	80.0%	342	11.4	0.879
P-824	5′-(TC)$_8$G-3′	52.9	50.0	8	6	75.0%	195	6.57	0.796
P-825	5′-(AC)$_8$T-3′	47.1	52.6	13	11	84.6%	309	10.3	0.895
P-826	5′-(AC)$_8$C-3′	52.9	55.0	14	12	85.7%	434	14.47	0.916
P-827	5′-(AC)$_8$G-3′	52.9	52.9	10	8	80.0%	237	7.9	0.845
P-834	5′-(AG)$_8$YT-3′	50.0	53.8	10	8	80.0%	293	9.77	0.866
P-835	5′-(AG)$_8$YC-3′	55.5	55.0	16	11	68.8%	701	23.37	0.932
P-836	5′-(AG)$_8$YA-3′	50.0	50.4	21	16	76.2%	733	24.4	0.940
P-840	5′-(GA)$_8$YT-3′	50.0	52.3	17	14	82.4%	533	17.77	0.920
TOTAL				558	448	80.28%	19409		

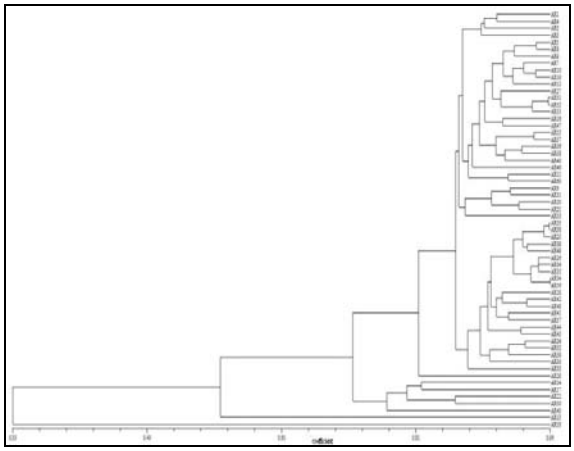

Fig 1: UPGMA generated dendrogram by ISSR analysis.

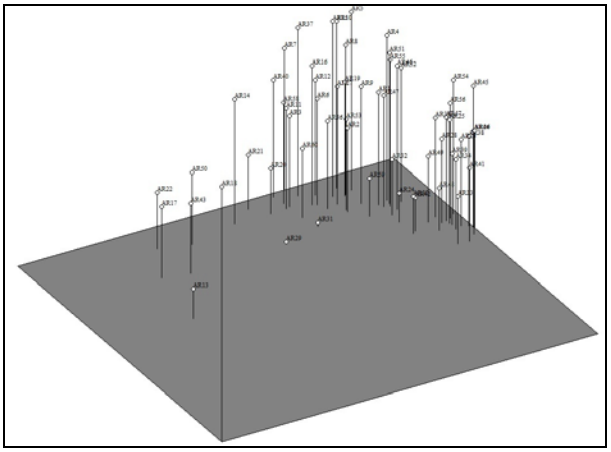

Fig 2: Three dimensional PCA by ISSR analysis.

The dendrogram (Fig. 1) generated based on UPGMA grouped all the 60 asparagus cultivars into three major clusters, two of which were further subdivided into mini clusters. The 3-D PCA analysis (Fig. 2) also corroborates the similar grouping pattern.

The POPGENE analysis (Table 4) with respect to low, intermediate and high saponin containing genotypes showed wide variation in the percentage of the polymorphic loci (28.57%, 65.93% and 70.33% respectively).

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

Saponin content	Sample size	Na	Ne	H	I	Ht	Hs	NPL	PPL (%)
Low	6	1.2857 (0.4543)	1.1983 (0.3442)	0.1129 (0.1867)	0.1658 (0.2695)	0.1129 (0.0349)	0.0	26	28.57
High	12	1.7033 (0.4593)	1.2523 (0.2715)	0.1699 (0.1504)	0.2782 (0.2192)	0.1699 (0.0226)	0.0	64	70.33
Intermediate	42	1.6593 (0.4766)	1.2332 (0.3147)	0.1469 (0.1696)	0.2364 (0.2416)	0.1469 (0.0287)	0.0	60	65.93
All 60 cultivars	60	1.8022 (0.4005)	1.2377 (0.3064)	0.1527 (0.1631)	0.2512 (0.2278)	0.1527 (0.0266)	0.1481 (0.0251)	73	80.22

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

Nei's gene diversity was found to be 0.1129, 0.1469 and 0.1699, whereas the Shannon's information index (I) was detected to be 0.1658, 0.2364 and 0.2782, respectively for the low, intermediate and high saponin containing genotypes. The overall percentage of polymorphic loci (80.22%), Nei's gene diversity (0.1527) and Shannon's information index (0.2512) indicated a high degree of genetic polymorphism among the genotypes. The results obtained are in accordance with the principle that the number of individuals used to estimate average heterozygosity can be very small if a large number of loci are studied. Also, the higher values of the Effective multiplex ratio (14.04) and Marker Index (0.983) well supported the high genetic polymorphism revealed by ISSR marker system. Further, the AMOVA (analysis of molecular variance) analysis measured the percentage similarity among genotypes as 99.16%by ISSR analysis.

Discussion

The saponin content estimated in *Asparagus racemosus* showed wide variation among the genotypes. While the existing variation in the saponin content among the genotypes was correlated with the genetic diversity, a very low level of correlation was obtained. This might be due to the environmental factors which play a critical role in secondary metabolite production. The concentration of different saponins was also estimated in *Asparagus racemosus* [34]. The root extracts of *A. racemosus* was found to contain major steroidal saponins viz. shatavarin I (19%), shatavarin IV (44%) and a newly identified shatavarin V (2.3%) [35]. The amount of shatavarin IV was also estimated to be 0.23 mg/100 mg [36]. However, the above data do not resemble to the present findings where the saponin content was found to be in quite higher amount. The possible reasons for this variation could be due to differences in soil type, genotypes in question, agro-climatic conditions, fractions of saponin evaluated, methods used for analysis etc. Further, all these studies collectively reported steroidal saponins to be a major bioactive compound responsible for the medicinal activities of the plant. More specifically, shatavarin I and shatavarin IV were found to be the major shatavarins present in the roots of *A. racemosus* plants. When compared with the shatavarin content of other medicinal plants, *A. racemosus* is supposed to be a rich source of shatavarin and therefore has numerous pharmacological activities.

Various approaches ranging from morphological to molecular techniques have been used to infer patterns of diversity and relationships among plant species. Out of these, ISSR markers represent efficient and inexpensive marker systems to explore the genetic data and have been successfully used in *A. officinalis* and *A. acutifolius*. The present study used ISSR markers for the first time in *Asparagus racemosus* which deduced higher level of genetic polymorphism within individual genotypes as well as among the 3 groups. All the members of the high saponin content group were found in a single mini cluster except ARHMV8, ARHMV11, KR60-1M-1 and SP-2-03-10. The group containing low saponin content was present in another mini cluster except KR-100-VII and KR120-1M-1A, whereas the group with intermediate saponin content was found scattered in the dendrogram. ISSR markers used in the present study revealed a greater level of polymorphism which corroborates the earlier result [37] where ISSR markers have been used efficiently to study genetic diversity among *Asparagus acutifolius* populations. This suggested the highly informative nature of ISSR primers regarding the genetic analysis studies. Other genetic diversity studies by ISSR markers on different plants such as *Solancium tuberosum* [38], *Toiticum dicoccoides* [39], *Cicer arietinum* [22], *Oryza officinalis* [40], mangrove populations [41] etc. also supported the polymorphic nature of ISSR marker's. Furthermore, greater polymorphism (80.22%), higher value of marker index and effective multiplex ratio showed ISSR markers to be more polymorphic as revealed by the grouping pattern in the UPGMA generated dendrogram and PCA analysis. The possible reason for higher and reproducible polymorphism detected by ISSR might be because of the greater coverage of genome by the primers, higher annealing temperature and higher GC content. Our study demonstrated ISSR marker as an efficient marker system in resolving the genetic diversity among *A. racemosus* cultivars because of its capacity to detect high level of polymorphism and reliability in reconstructing phylogenies.

Towards conservation aspects of *A. racemosus*, it is very important to assess the saponin content of the existing genotypes to select the best chemotypes. In this study, the cultivars with high saponin content include HAR 03-7, HAR 03-8, ARHMV12, KR60MB1, KR60C-4M-1, KR60D-4M-1, ARHMV3 and ARHMV7. As the chemical synthesis of pure saponin is very expensive commercially, most of the pharmaceutical industries depend upon the available germplasm for saponin. As more and more uses of this plant are identified, the pressure on existing natural populations will increase further. Since the value of medicinal plants depends mainly on the active principle present in it; consistency in quality and quantity of planting material assumes paramount importance. This can be ensured by identifying elite genotypes through the application of molecular marker techniques and chemo profiling followed by mass multiplication using both conventional and biotechnological approaches. The superior genotypes selected on the basis of saponin content can also be used for molecular characterization of the gene corresponding to saponin production, its isolation and integration in other plant or micro-organism for the production of even higher quantity of saponin.

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