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Assessment of Genetic Diversity among *Withania somnifera* Collected from Central India using RAPD and ISSR Analysis

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ABSTRACT

Withania somnifera is a multipurpose plant of immense therapeutic value and wide geographic distribution exhibiting extensive phenotypic and chemical variability. Characterization of plants using molecular markers is an ideal approach for improvement and conservation of plant genetic resources. Random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) molecular fingerprinting markers were employed as genetic markers to assay the genetic relationship of 16 accessions of *W. somnifera* collected from different locations of India. Randomly selected 25 decamer primers amplified 204 RAPD marker loci out of which 152 bands (74.5%) were polymorphic. Twenty eight ISSR primers were used to generate fingerprints and a total of 186 alleles were amplified, out of which 151 were polymorphic (81.1%). ISSR markers were more informative than the RAPD markers. Several ISSR markers amplified genotype-specific alleles that can be used for authentication and detection of adulteration in plant material. Similarity matrices were generated from RAPD and ISSR marker data separately using Jaccard's coefficient and dendrograms were constructed based on UPGMA clustering. Genotypes were clustered into two groups and the grouping was similar for both marker systems.

Keywords: DNA fingerprinting, ISSR, molecular markers, phylogenetic relationship, RAPD

INTRODUCTION

Withania somnifera (Hindi - *Ashwagandha*, English - Indian ginseng/winter cherry) is a multipurpose plant of immense therapeutic value having wide geographic distribution. It has been described in the sacred text of Ayurveda including *Charak Samhita* and is regarded as one of the most useful herbs having 'Vata' pacifying properties (Singh and Kumar 1998; Sangwan *et al.* 2004). Besides this, large numbers of medicinally important phytochemicals called withanolides are present in *W. somnifera* and there are reports of their use in diversity analysis (Kumar *et al.* 2007; Scartezzini *et al.* 2007; Chaurasiya *et al.* 2009). Plant-to-plant variation in the quantity and quality of active constituents exists; geographical conditions affect the active constituents and activity profiles in medicinal plants (Oleszek *et al.* 2002). Authentication of medicinal plants is a critical issue and it should occur from the harvesting of the plant material to the final product. Unfortunately, there is no single or superior method to assure proper authentication during the entire process but the goal can be achieved through the application of a variety of different methodologies.

Characterization of plants with the use of molecular markers is an ideal approach for conservation of plant genetic resources and genetic improvement (Rout and Mohapatra 2006). Molecular markers not only provide a useful method for characterization of cultivars but they also depict genetic relatedness, authentication of quality plant material, detection of adulteration and protection of intellectual property right issues (Joshi *et al.* 2004). Inter simple sequence repeats (ISSRs) and random amplified polymorphic DNA (RAPD) are ideal markers for population studies because of their abundance and high degree of polymorphism between individuals within a population of closely related genotypes (Agrawal *et al.* 2008). Since genomic information of *W. somnifera* is not well studied to date, these methods can be applied efficiently as they are rapid, inexpensive, simple to perform, do not require prior knowledge of DNA sequence and also require very little initial DNA template (Esselman *et al.* 1999). The objective of this study was to generate unique DNA fingerprints as well as to identify the genetic relationship among 16 *W. somnifera* genotypes using RAPD and ISSR markers.

MATERIALS AND METHODS

Plant materials and DNA isolation

Details of the 16 accessions used for the present study are given in **Table 1**. Genomic DNA was isolated from young leaves collected from plants maintained in a nethouse using the cetyltrimethyl

Table 1 *Withania somnifera* germplasms used in present study.

Germplasm	Accession Number	Place
G1	WS-90-100	Indore
G2	WS-90-104	Indore
G3	WS-90-117	Indore
G4	WS-90-134	Mandsore
G5	WS-90-105	Indore
G6	WS-90-125	Indore
G7	WS-90-126	Indore
G8	WS-90-135	Indore
G9	WS-JA-20	Mandsore
G10	WS-90-103	Indore
G11	WS-90-127	Indore
G12	WS-90-136	Indore
G13	WS-Poshita	CIMAP
G14	WS-local-1	Jabalpur
G15	WS-local-2	Jabalpur
G16	WS-local-3	Jabalpur

G - Germplasm, WS - *Withania somnifera*, JA - Jawahar Ashwagandha, CIMAP - Central Institute of Medicinal and Aromatic Plants

Received: 12 November, 2011. Accepted: 11 December, 2011.

Original Research Paper

ammonium bromide (CTAB) method as described by Doyle and Doyle (1990). The quantity and quality of the isolated DNA was determined by a UV visible spectrophotometer (JASCO, Tokyo, Japan).

PCR amplification

PCR amplifications were performed in a programmable thermal cycler (Thermo Hybaid Px2, Franklin, MA, USA). Each sample was amplified in a reaction mixture containing 50 ng genomic DNA, 1U *Taq* polymerase (Sigma Co., Missouri, USA), 10X PCR buffer with 2.5 mM $MgCl_2$ and 200 µM of dNTP mixture (Sigma Co.), 15 ρmol of RAPD or ISSR primers. Cycling parameters for ISSR were adjusted to 5 min at 94°C for pre-denaturation, 39 cycles each of 1 min at 94°C for denaturation, 1 min for annealing at 45/50/55°C, 2 min at 72°C for extension and a final extension at 72°C for 5 min. For RAPD marker, the PCR reaction mix and program profile was similar to ISSR markers except annealing temperature was adjusted to 37°C. PCR mixture was cooled to 4°C and stored at -20°C until electrophoretic analysis. Amplified products were separated on agarose gel (Sigma Co.) in 1X TAE buffer and the size of amplified DNA fragments was estimated on the basis of a 1 Kb plus DNA ladder (Fermentas, Glen Burnie, USA). Stained with 5µl ethidium bromide (0.01 % w/v solution) 100 ml agarose gels (1.5%) were run for 4 h at 65 V and photographed under a gel documentation system (Syngene, Cambridge, UK).

Data analysis

PCR products from ISSR and RAPD analyses were scored qualitatively for the presence or absence of bands. Only clear and apparently unambiguous bands were taken into account for analysis. Polymorphic information content (PIC) of each primer for both ISSR and RAPD was estimated using the formula: PIC = 1-Σ(Pij)2, where Pij is the frequency of the i^{th} pattern revealed by the j^{th} primer summed across all patterns revealed by the primers (Botstein *et al.* 1980). Genetic similarities between the accessions were measured by Jaccard's similarity coefficient based on the proportion of shared alleles using 'Simqual' sub-program of software package NTSYS-PC version 1.8 (Exeter Software, Setauket, NY, USA). The resultant distance matrix data was subjected to the unweighted pair-group method with an arithmetic average (UPGMA) subprogram of NTSYS-PC to construct dendrograms (Rohlf 1993). The correlation between the two matrices obtained with ISSR and RAPD was estimated by means of the Mantel test (Mantel 1967). In order to estimate the congruence among dendrograms, cophenetic matrices for each marker were computed and compared using Mantel's test. Principal coordinate analysis (PCA) was also performed with genetic similarity matrices using NTSYS-PC software in order to highlight the resolving power of the ordination.

RESULTS

ISSR analysis

Initially a sub-set of 100 ISSR primers (UBC primer set# 9-801 to 900) and three accessions were used to screen amplifiable ISSR primers. Amongst 100 ISSR markers, 30 amplified successfully and 27 were finally selected (**Table 2**) to generate fingerprints based on their reproducibility and amplification quality. These 27 ISSRprimers amplified 186 alleles, out of which 152 were polymorphic (81%) across all the genotypes. Primer 899 amplified maximum alleles (13) whereas primer 822 amplified only 2. The average number of bands per primer was 6.8 ± 0.57 whereas, the average number of polymorphic bands per primer was 5.5 ± 0.89 and average PIC was 0.429 ± 0.03 and ranged from 0.081 to 0.671 (UBC 854). A highly significant correlation was observed between total number of amplified bands and polymorphic bands (r = 0.905 at $P > 0.001$). A significant correlation was also observed between total number of bands amplified and PIC (r = 0.456 at $P > 0.05$) per primer. Among di-nucleotide repeat primers, primers having CT and AC repeats produced more bands with an average of 6.48 and 7.16 per primer, respectively as compared to the primers having AT, GT and AG repeats. The electrophoretic banding pattern of primer 844 is illustrated in **Fig. 1**. Primer 843 amplified a specific band of 450 bp in WS-90-134 which was absent in other accessions. Primer 899 amplified a unique band of 400 bp in WS-90-125. These primers can be used to differentiate specific accessions from others (**Table 3**).

Table 2 ISSR primers, their sequence, anchored end, repeat motif and data on DNA profile and polymorphism generated in *Withania somnifera*.

Primer	Sequence	TNB*	PB*	% Polymorphism	PIC*
801	ATA TAT ATA TAT ATA TT	5	2	80	0.134
807	AGA GAG AGA GAG AGA GT	6	3	50	0.348
808	AGA GAG AGA GAG AGA GC	5	3	60	0.185
811	GAG AGA GAG AGA GAG AC	4	3	75	0.405
814	CTC TCT CTC TCT CTC TA	7	5	71.42	0.521
820	GTG TGT GTG TGT GTG TC	6	6	100	0.575
822	TCT CTC TCT CTC TCT CA	2	2	100	0.178
823	TCT CTC TCT CTC TCT CC	3	3	100	0.257
825	ACA CAC ACA CAC ACA CT	11	11	100	0.610
843	CTC TCT CTC TCT CTC TRA	9	6	66.66	0.529
844	CTC TCT CTC TCT CTC TRC	10	7	70	0.534
845	CTC TCT CTC TCT CTC TRG	5	5	100	0.636
846	CAC ACA CAC ACA CAC ART	3	2	66.66	0.081
848	CAC ACA CAC ACA CAC ARG	10	8	80	0.573
851	GTG TGT GTG TGT GTG TYG	4	1	25	0.246
854	TCT CTC TCT CTC TCT CRG	9	9	100	0.671
857	ACA CAC ACA CAC ACA CYG	5	5	100	0.450
860	TGT GTG TGT GTG TGT GRA	4	3	75	0.575
873	GAC AGA CAG ACA GAC A	5	5	100	0.501
876	GAT AGA TAG ACA GAC A	7	6	85.71	0.384
881	GGG TGG GGT GGG GTG	5	5	100	0.443
885	BHB GAG AGA GAG AGA GA	10	10	100	0.415
888	BDB CAC ACA CAC ACA CA	6	6	100	0.497
889	DBD ACA CAC ACA CAC AC	10	6	60	0.270
891	HVH TGT GTG TGT GTG TG	4	4	100	0.603
895	AGA GTT GGT AGC TCT TGA TC	11	10	90.90	0.512
899	CAT GGT GTT GGT CAT TGT TCC A	13	12	92.30	0.481
900	ACT TCC CCA CAG GTT AAC ACA	7	4	57.14	0.180
Average		6.5 ± 0.57	5.2 ± 0.89	81.8 ± 7.71	0.429 ± 0.031

*TNB-Total number of bands, PB-Polymorphic bands, PIC-Polymorphism information content

Table 3 ISSR Markers amplified the specific allele in *Withania somnifera*.

ISSR primer	Allele 1	Allele 2
814	WS-90-127, WS-90-136	
820	Local-1, local-2	
825	Local-1	WS-90-104, WS-90-105
843	WS-90-134	
844	WS-90-117	WS-90-105
845	WS-90-134	WS-90-134, WS-90-125
848	Local-2, Local-3	Local-2, Local-3
851	Local-2, Local-3	
854	Local-3	Local-2
857	Local-1, local-2	
873	WS-90-127, WS-90-136	
881	WS-90-117, WS-90-135	
885	WS-Poshita	
889	Local-2, Local-3	
895	Local-2, Local-3	WS-90-103
899	WS-90-125	

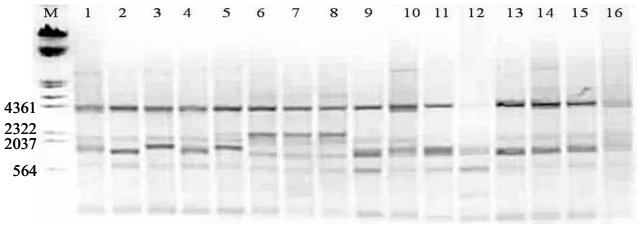

Fig. 1 Electrophoretic banding pattern of ISSR product amplified by primer UBC 844 resolved on 1.5% agarose gel. M = λ *Hind*III DNA ladder; lanes 1-16 *Withania somnifera* germplasm as described in **Table 1**.

Based on ISSR markers, pair-wise genetic similarity was estimated among 16 genotypes and a dendrogram was generated using UPGMA cluster analysis based on Jaccard's similarity coefficient with a range of 0.42 to 0.94 (**Table 4**). The cluster analysis grouped *W. somnifera* genotypes into two major groups (**Fig. 3A**). The first major group consisted of 13 accessions except for 3 local accessions which formed a second major group with a similarity coefficient of 0.723. The first major group was further divided into two subgroups and the first subgroup consisted of three accessions (WS-90-100, WS-90-104 and WS-90-117) exhibiting an intra-sub-group genetic similarity of 87%. The second subgroup was further subdivided into two sub-subgroups. The first sub-subgroup had seven accessions (WS-90-134, WS-90-105, WS-90-125, WS-90-126, WS-90-135, WS-JA-20 and WS-90-103) and the second sub-subgroup had only three (WS-90-127, WS-90-136 and Poshita) that revealed 85% genetic similarity with each other. Accessions WS-90-127 and WS-90-137 had the highest genetic similarity (0.94) compared to other pairs of accessions. PCA results were comparable to the cluster analysis and exhibited similar topology. The first three principal components PC1 (21.13%), PC2 (14.88%) and PC3 (11.75%) explained 47.76% of total variation.

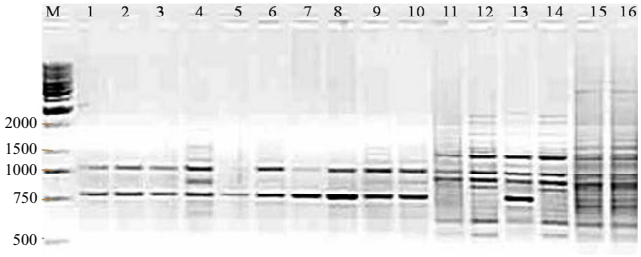

Fig. 2 Electrophoretic banding pattern of RAPD product amplified by primer OPAB-08 resolved on 1.5% agarose gel. M = 1 Kb DNA ladder; lanes 1-16 *Withania somnifera* germplasm as described in **Table 1**.

RAPD analysis

For RAPD analysis, initially 45 primers were screened and amongst them 25 decamer primers were selected on the basis of their clear banding pattern for final RAPD PCR analysis (**Table 5**). These 25 primers amplified a total of 204 RAPD marker loci and most bands (14) were scored by primer OPAB-07 whereas least bands (3) were produced by primer OPAA-01. Out of these 204 bands, 152 bands (74%) revealed to be polymorphic. The average number of bands per primer was 8.16 ± 0.60 whereas the average number of polymorphic bands per primer was 6.08 ± 0.51. Out of 25 RAPD primers, 23 amplified polymorphic bands while two (OPA-13 and OPAE-06) showed a monomorphic banding pattern. The average PIC was 0.448 ± 0.035 and ranged from 0 to 0.792 (OPC-06) (**Table 4**). Significant correlation ($r = 0.742$ at $P > 0.01$) was exhibited between total number of bands amplified and polymorphic bands, however it was non-significant between total number of bands and the PIC value. Specific bands were amplified by two primers, OPAB-08 amplified two specific alleles of 850 bp in WS-90-136 and WS-local-1 as well as 880 bp in WS-90-134 (**Fig. 2**) while OPAA-02 amplified a specific allele of 160 bp in WS-local-1.

On the basis of RAPD clustering, genotypes fell into two major groups with a Jaccard's similarity coefficient of 0.57 within a range of 0.44 to 0.941 (**Table 6**). The first cluster comprised 10 accessions whereas the second cluster incorporated the remaining six (**Fig. 3B**). Accessions WS-Local-2 and WS-Local-3 appeared to be closer to each other with a similarity coefficient of 0.91 and other two accessions WS-Local-1 and WS-90-117 proved to be the most

Table 4 Jaccard's similarity coefficient values among *Withania somnifera* accessions using ISSR primers.

Accessions	WS-90-100	WS-90-104	WS-90-117	WS-90-134	WS-90-135	WS-90-125	WS-90-136-1	WS-90-135-1	WS-JA-20	WS-90-103	WS-90-127	WS-90-136	WS-Poshita	WS-local-1	WS-local-2	WS-local-3
WS-90-100	1.00															
WS-90-104	0.88	1.00														
WS-90-117	0.85	0.84	1.00													
WS-90-134	0.71	0.70	0.70	1.00												
WS-90-135	0.74	0.74	0.71	0.78	1.00											
WS-90-125	0.69	0.71	0.65	0.80	0.80	1.00										
WS-90-136-1	0.75	0.76	0.70	0.79	0.86	0.90	1.00									
WS-90-135-1	0.71	0.73	0.66	0.76	0.80	0.81	0.91	1.00								
WS-JA-20	0.74	0.77	0.71	0.79	0.86	0.84	0.90	0.88	1.00							
WS-90-103	0.78	0.77	0.70	0.79	0.83	0.80	0.87	0.83	0.90	1.00						
WS-90-127	0.72	0.74	0.66	0.73	0.82	0.77	0.82	0.77	0.81	0.85	1.00					
WS-90-136	0.71	0.72	0.64	0.70	0.79	0.74	0.80	0.75	0.77	0.81	0.94	1.00				
WS-Poshita	0.69	0.68	0.62	0.70	0.77	0.74	0.79	0.72	0.77	0.76	0.79	0.82	1.00			
WS-local-1	0.48	0.47	0.45	0.56	0.57	0.54	0.57	0.59	0.56	0.59	0.63	0.63	0.66	1.00		
WS-local-2	0.43	0.44	0.42	0.52	0.52	0.50	0.52	0.52	0.52	0.53	0.58	0.59	0.57	0.71	1.00	
WS-local-3	0.46	0.47	0.46	0.54	0.55	0.52	0.56	0.53	0.54	0.55	0.58	0.59	0.61	0.65	0.81	1.00

WS - *Withania somnifera*, JA - Jawahar Ashwagandha

Table 4 Jaccard's similarity coefficient values among *Withania somnifera* accessions using ISSR primers.

Accessions	WS-90-100	WS-90-104	WS-90-117	WS-90-134	WS-90-135	WS-90-125	WS-90-136-1	WS-90-135-1	WS-JA-20	WS-90-103	WS-90-127	WS-90-136	WS-Poshita	WS-local-1	WS-local-2	WS-local-3
WS-90-100	1.00															
WS-90-104	0.88	1.00														
WS-90-117	0.85	0.84	1.00													
WS-90-134	0.71	0.70	0.70	1.00												
WS-90-135	0.74	0.74	0.71	0.78	1.00											
WS-90-125	0.69	0.71	0.65	0.80	0.80	1.00										
WS-90-136-1	0.75	0.76	0.70	0.79	0.86	0.90	1.00									
WS-90-135-1	0.71	0.73	0.66	0.76	0.80	0.81	0.91	1.00								
WS-JA-20	0.74	0.77	0.71	0.79	0.86	0.84	0.90	0.88	1.00							
WS-90-103	0.78	0.77	0.70	0.79	0.83	0.80	0.87	0.83	0.90	1.00						
WS-90-127	0.72	0.74	0.66	0.73	0.82	0.77	0.82	0.77	0.81	0.85	1.00					
WS-90-136	0.71	0.72	0.64	0.70	0.79	0.74	0.80	0.75	0.77	0.81	0.94	1.00				
WS-Poshita	0.69	0.68	0.62	0.70	0.77	0.74	0.79	0.72	0.77	0.76	0.79	0.82	1.00			
WS-local-1	0.48	0.47	0.45	0.56	0.57	0.54	0.57	0.59	0.56	0.59	0.63	0.63	0.66	1.00		
WS-local-2	0.43	0.44	0.42	0.52	0.52	0.50	0.52	0.52	0.52	0.53	0.58	0.59	0.57	0.71	1.00	
WS-local-3	0.46	0.47	0.46	0.54	0.55	0.52	0.56	0.53	0.54	0.55	0.58	0.59	0.61	0.65	0.81	1.00

WS - *Withania somnifera*, JA - Jawahar Ashwagandha

Table 5 RAPD makers used for generate fingerprints in *Withania somnifera*.

Primer	Sequence	TNB*	PB*	% Polymorphism	PIC*
OPAA-01	AGACGGCTCC	3	3	100	0.388
OPAA-02	GAGACCAGAC	8	6	75	0.362
OPAA-03	TTAGCGCCCC	13	9	69.23	0.333
OPAA-04	AGGACTGCTC	9	4	44.44	0.306
OPAA-06	GTGGGTGCCA	9	6	66.66	0.373
OP A-07	GAAACGGGTG	10	6	60	0.183
OPA-09	GGGTAACGCC	12	8	66.66	0.275
OPA-11	CAATCGCCGT	5	5	100	0.501
OPA-12	TCGGCGATAG	9	7	77.77	0.553
OPA-13	CAGCACCCAC	4	0	0	0.000
OPAB-07	GTAAACCGCC	14	8	57.14	0.402
OPAB-08	GTTACGGACC	12	12	100	0.735
OPAB-09	GGGCGACTAC	7	6	85.71	0.451
OPAE-06	GGGGAAGACA	5	5	100	0.000
OPAI-03	GGGTCCAAAG	6	5	83.33	0.587
OPB-01	GTTTCGCTCC	6	6	100	0.285
OPB-04	GGACTGGAGT	5	2	40	0.190
OPB-07	GGTGACGCAG	9	8	88.88	0.567
OPB-08	GTCCACACGG	10	8	80	0.534
OPB-17	AGGGAACGAG	9	7	77.77	0.483
OPB-18	CCACAGCAGT	12	10	83.33	0.495
OPBB-05	GGGCCGAACA	5	4	80	0.357
OPC-05	GATGACCGCC	10	7	70	0.284
OPC-06	GAACGGACTC	7	7	100	0.792
OPC-07	GTCCCGACGA	5	3	60	0.152
Average		8.16 ± 0.60	6.08 ± 0.51	74.6 ± 4.79	0.448 ± 0.035

*TNB-Total number of bands, PB-Polymorphic bands, PIC-Polymorphism information content

divergent accessions with a similarity coefficient of only 0.44. When cluster analysis were compared to the PCA analyses, genotypes that were grouped within the same cluster in the dendrogram also occupied the corresponding positions in 2-D and 3-D scaling by PCA using the pooled genetic distance matrix data (**Fig. 4**). The first three most informative principal components explained 47.76% of total variation.

Molecular markers for genotype differentiation

Both ISSR and RAPD marker systems were able to uniquely discriminate between sixteen genotypes of *W. somnifera* (**Table 3**). ISSR markers amplified maximum unique alleles from 3 local accessions (7 out of 14) as compared to other 13 genotypes. ISSR amplified more specific alleles than RAPDs as only two RAPD primers, OPAA-02 and OPAB-08 were able to amplify specific alleles. Primer OPAA-02 amplified a specific allele in WS-local-1 while OPAB-08 differentiated genotypes WS90-136 and WS90-134 from others. Judicious combination of several markers used in the present study will aid in differentiating all the genotypes of *W. somnifera*.

Combined ISSR and RAPD Data

Results of ISSR and RAPD were combined for PCA using the pooled genetic distance matrix data (**Fig. 4**). Matrices for ISSR and RAPD markers were also compared using Mantel's test. Correlation values between the matrices were significant (r = 0.623), which indicated a very good fit amongst RAPD and ISSR marker systems. First three most informative principal components explained 47.36% of the total variation. Results of PCA analysis were comparable to the cluster analysis as the genotypes under study also parted into two clusters.

The results suggested that genetic base is restricted and that introgression of genes from unexploited sources deserves attention. Conventional breeding efforts have been made for improvement of the cultivars belonging to several species of *Withania* but these efforts did not involve assessment and consideration of molecular diversity for the selection of parents. Knowledge of molecular marker aided genetic diversity profiles, parallel to morphological and biochemical relatedness and differences among the *W. somnifera*, could offer added advantages of strategic combination of traits and exploitation of the germplasm.

DISCUSSION

Assessment of genetic diversity is an essential step in germplasm characterization and in executing any organized plant improvement program. It is also important to know which type of marker(s) and how many of them truly represent variation in the entire genome and can be used in order to derive reliable estimates of diversity. During the present study, RAPD and ISSR marker systems were used for comparison and assessment of genetic relationship among the sixteen genotypes of *W. somnifera*. High genetic diversity was revealed in *W. somnifera* (~69% polymorphism) by these markers. The evolution of genotypes in distinct agroclimatic zones demonstrates significant levels of variation in response to the selection pressure in specific regions (Singh *et al*. 1998). It is, therefore, not surprising to find significant levels of polymorphism among the 16 genotypes of *Withania* investigated ISSR markers produced more polymorphic bands as compared to RAPD and also showed more discriminating power among *Withania* genotypes. ISSR marker has been reported to be more reproducible (Goulão *et al.* 2001) and to produce more complex marker patterns as compared to the RAPD (Parsons *et al.* 1997;

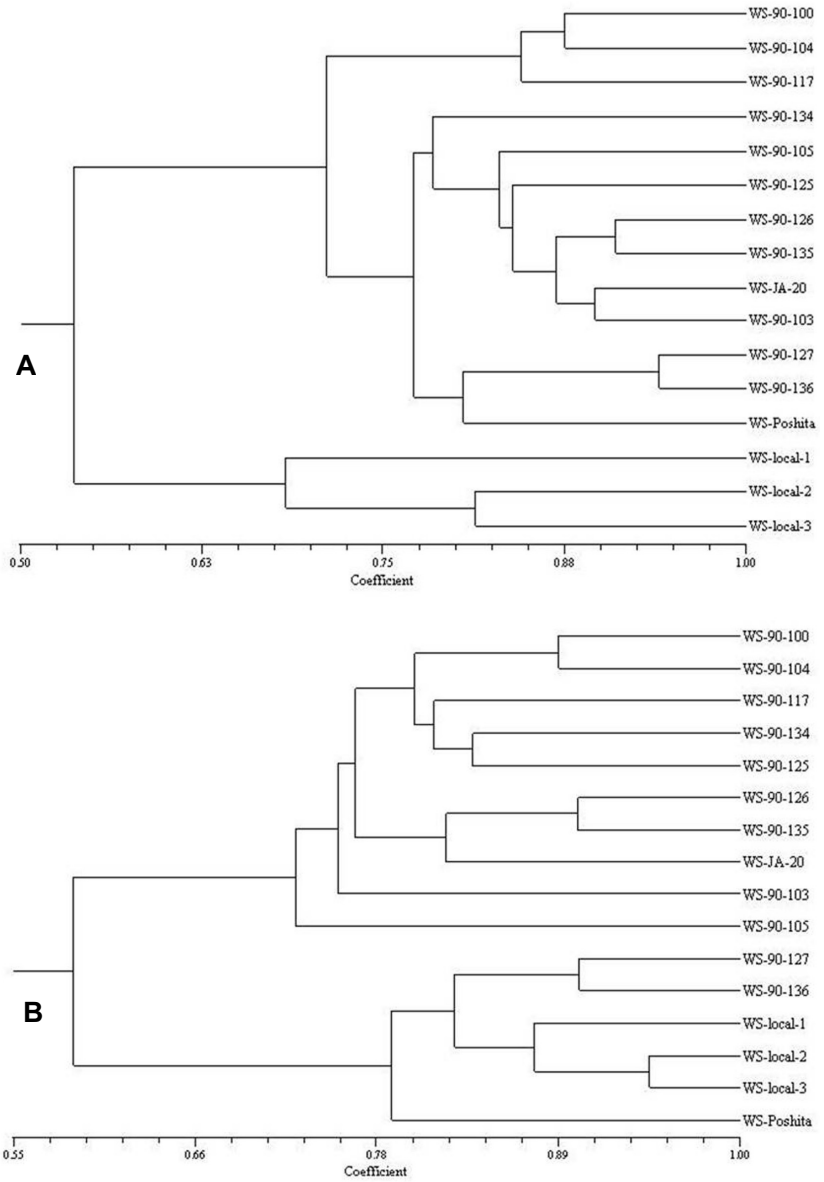

Fig. 3 Dendrograms (UPGMA, NTSYS-PC) showing genetic relationship among 16 *Withania somnifera* germplasms based on genetic distance matrix data obtained using (**A**) ISSR, (**B**) RAPD primers.

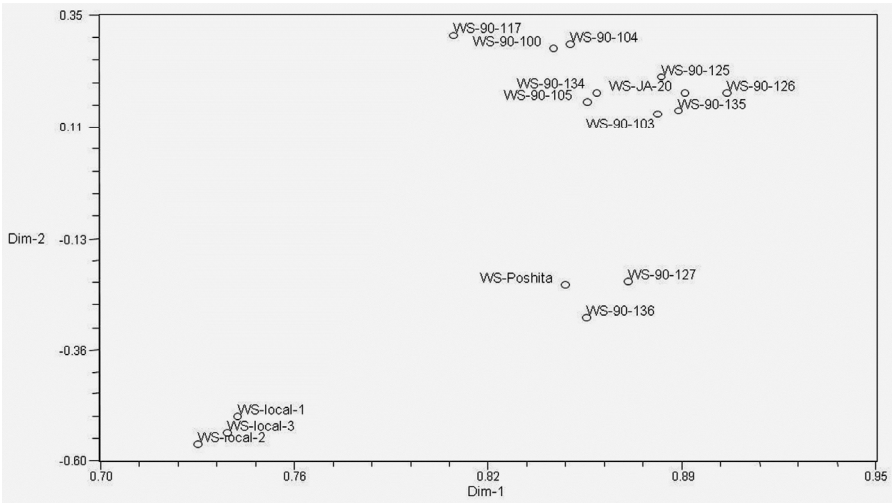

Fig. 4 Two dimensional scaling of 16 *Withania somnifera* germplasms by principal component analysis (PCA) using pooled genetic distance matrix data.

Chowdhury *et al.* 2002), which is advantageous at the time of differentiating closely related accessions.

Several studies have been carried out in *W. somnifera* to understand the diversity using RAPD, SAMPL (selective amplified microsatellite polymorphism loci), and AFLP (Dhar *et al.* 2006; Negi *et al.* 2006; Scartezzini *et al.* 2007;

Table 6 Jaccard's similarity coefficient values among *Withania somnifera* accessions using RAPD primers.

Accessions	WS-90-100	WS-90-104	WS-90-117	WS-90-134	WS-90-135	WS-90-125	WS-90-136-1	WS-90-135-1	WS-JA-20	WS-90-103	WS-90-127	WS-90-136	WS-Poshita	WS-local-1	WS-local-2	WS-local-3
WS-90-100	1.00															
WS-90-104	0.89	1.00														
WS-90-117	0.72	0.73	1.00													
WS-90-134	0.72	0.72	0.71	1.00												
WS-90-135	0.74	0.74	0.76	0.74	1.00											
WS-90-125	0.76	0.79	0.81	0.80	0.80	1.00										
WS-90-136-1	0.74	0.71	0.70	0.73	0.72	0.78	1.00									
WS-90-135-1	0.73	0.76	0.67	0.73	0.70	0.77	0.87	1.00								
WS-JA-20	0.66	0.68	0.69	0.69	0.69	0.70	0.71	0.74	1.00							
WS-90-103	0.65	0.66	0.62	0.64	0.66	0.66	0.65	0.69	0.70	1.00						
WS-90-127	0.58	0.56	0.53	0.58	0.56	0.59	0.63	0.63	0.62	0.55	1.00					
WS-90-136	0.54	0.51	0.48	0.56	0.51	0.55	0.58	0.60	0.57	0.59	0.87	1.00				
WS-Poshita	0.58	0.58	0.58	0.65	0.60	0.62	0.62	0.66	0.64	0.62	0.70	0.75	1.00			
WS-local-1	0.49	0.47	0.44	0.50	0.48	0.49	0.52	0.53	0.52	0.50	0.79	0.82	0.69	1.00		
WS-local-2	0.50	0.48	0.45	0.53	0.49	0.50	0.53	0.57	0.52	0.57	0.74	0.81	0.71	0.77	1.00	
WS-local-3	0.50	0.49	0.46	0.53	0.47	0.49	0.55	0.58	0.53	0.56	0.78	0.82	0.74	0.77	0.91	1.00

Chaurasiya *et al.* 2009; Mirjalili *et al.* 2009). These studies also suggest that there is an extreme degree of variability with respect to growth habit and morphological characteristics among *W. somnifera* accessions found in different parts of India (Atal *et al*. 1962; Anonymous 1976). This variability has been endorsed by the higher PIC values (ISSR 0.421 and RAPD 0.383), percent polymorphism (ISSR 81% and RAPD 69%) and clustering. In conformity, Dharmar and De Britto (2011) also found higher percentage (83.78%) of polymorphism among *W. somnifera* accessions collected from different geographical areas. On the contrary, Mir *et al.* (2011) reported low levels of genetic diversity revealed with RAPD (37.82%) and AFLP (43.94%) primers. ISSR markers proved to be marginally more informative than RAPD in the assessment of genetic diversity of *W. somnifera*. However, microsatellites based markers have been reported to be more informative as compared to other types of markers in many plant species including, neem (Singh *et al.* 2002), rice (Saini *et al.* 2004) and *Withania* (Negi *et al.* 2006). High level of variation observed in microsatellite region is due to replication slippage which is more frequent as compared to any other mutation generated.

Correlation between Jaccard's similarity values generated from both marker techniques was significant (r = 0.623) even though they target different portions of the genome. Clustering of *W. somnifera* accessions within groups was very similar when RAPD and ISSR derived dendrograms were compared. Irrespective of types of marker used, local genotypes of *W. somnifera* formed distinct cluster. Similar clustering pattern based on the geographical origin of the genotypes has been observed by Kumar *et al.* (2007). In fact, better understanding of the distribution of genetic variation at the intraspecific level is important for the identification of superior genotype(s) for cultivar improvement and to devise strategies for *in situ* and *ex situ* conservation (Bhutta *et al*. 2006; Basha and Sujatha 2007).

Results from present investigation demonstrated that the two marker assays i.e. RAPD and ISSR with different characteristics however both can be crucial for identification and estimation of the relatedness at the variety level. However, ISSR markers provide higher relative information as compared to RAPD (Nagaoka and Ogihara 1997). Likewise, during the present investigation ISSR markers proved to be the better choice for evaluation of diversity level and assessing the genetic relationships among *W. somnifera* germplasm with utmost accuracy. Nevertheless, the two marker systems provided almost comparable descriptions of diversity independent of geographical origin. In addition, low and significant correlation detected between genetic matrices allowed the efficacy and the utility of these two marker systems.

This study provides evidence that RAPD and ISSR polymorphisms could be used as efficient tools for the detection of similarities and phylogenetic relationships in *W. somnifera*. The RAPD and ISSR techniques can also be useful for authentication of specific accessions/genotypes of *W. somnifera*. The information obtained here could be valuable for devising strategies for conservation and management of this species.

ACKNOWLEDGEMENTS

Authors are thankful for grants from Madhya Pradesh Minor Forest Produce Cooperative Ltd., Bhopal, India.

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