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Molecular diversity analysis among *Withania somnifera* genotypes collected from Madhya Pradesh using RAPD markers

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ABSTRACT

Indian Ginseng or Ashwagandha [Withania somnifera (L) Dunal] is a highly valued medicinal plant in Ayurveda. In spite of being one of the major medicinal plants in the world, it has received very little attention from geneticists, cytogeneticists and molecular biologists. Systematic molecular characterization of W. somnifera germplasm is required for future programmes of quality improvement. In the present study, analysis of genetic diversity of Withania somnifera collected from different climatic regions of Central India has been carried out using RAPD technique. Randomly selected 53 decamer primers were used to genetic diversity analysis by RAPD marker and they amplified 379 RAPD marker loci. The size of amplified markers ranged from 100-4000 bp. Out of these 379 bands, 234 (61.74%) bands were polymorphic. Cluster analysis revealed that all the genotypes of Withania somnifera under study were divided into two groups. On the basis of RAPD analysis, all genotypes constituted different cluster other than their respective places of collection. The results of the present study indicate that the level of genetic variation was high among the Ashwagandha genotypes. The genetically diverse genotypes may be used for future improvements in Ashwagandha crop.

Keywords: Ashwagandha, genetic diversity, medicinal plant, electromorph, RAPD, allele

INTRODUCTION

Indian Ginseng or Ashwagandha [*Withania somnifera* (L) Dunal] is a highly valued medicinal plant in Ayurveda, used either singly or in combination with other herbs. Plants growing under different agro climatic conditions often show qualitative and quantitative variations in their phytoconstituents. There are five primary chemotypes in *W. somnifera* which originate from different areas and are categorized as Form I to Form V. Form I is cultivated in Madhya Pradesh and is the primary source of commercial material in India. In India, it grows wild in Madhya Pradesh, Uttar Pradesh Andhra Pradesh, Gujarat, Maharashtra, Rajasthan and Punjab extending to the mountainous regions of Himachal Pradesh and Jammu up to an elevation of 1500m (Singh and Kumar, 1998). Some of the major constituents include Withanolide A, Withanone and Withaferin A. *W. somnifera* displays a appreciable spectrum of morphological and phytochemical variability (Kumar *et al.*, 2007). Several withanolides are identified in W. somnifera including withanolides A-Y (Tripathi *et al.*, 1996). In spite of being one of the major medicinal plants in the world, it has received very little attention from geneticists, cytogeneticists and molecular biologists. Systematic morphochemical and molecular characterization of *W. somnifera* germplasm is required for future programmes of quality improvement. 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). Random amplified polymorphic DNA (RAPD) is an ideal marker system for population studies because of abundance and high degree of polymorphism between individuals within a population of closely related genotypes (Agrawal *et al.*, 2008). In the present study, analysis of genetic diversity of *Withania somnifera* collected from different climatic regions of Central India has been carried out using RAPD technique.

MATERIALS AND METHODS

The plants of Ashwagandha [*Withania somnifera* (L.) Dunal] were collected from different places of Madhya Pradesh. A total of 21 genotypes of Ashwagandha were collected and grown in pots to obtain the tissue samples for the isolation of genomic DNA. Genomic DNA

was isolated using a standardized method (Saghai-Maroof *et al.*, 1984) with required modifications. The quality of DNA was also checked by horizontal submarine gel electrophoresis on 0.8% agarose gel. The quantity, quality and integrity of isolated DNA were also checked by gel electrophoresis. After quantification the DNA was diluted by distilled water. The final concentration of DNA was maintained upto 25 ng/µl.

RAPD analysis

Random Amplified Polymorphic DNA analysis of Ashwagandha germplasm was done by using decamer nucleotide primer obtained from OPERON Technologies, Almeda, California, USA in low stringency conditions as described by Williams *et al.* (1990). The amplification of genomic DNA was done by using 53 random decamer nucleotide primers (Table 1). PCR products were electrophoresed on 1.5% agarose gel electrophoresis. After electrophoresis the amplicons were visualized under gel documentation system.

Scoring of RAPD bands

Total number of bands generated by each primer were counted and each band was numbered according to their molecular weight in decreasing order. Bands were scored as '1' for presence and '0' for absence of particular band and data were entered in the data input format for software NTSYS-pc (Rholf, 2002).

RESULTS AND DISCUSSION

Randomly selected 53 decamer primers were used to genetic diversity analysis by RAPD marker and they amplified 379 RAPD marker loci. The size of amplified markers ranged from 100-4000 bp. Out of these 379 bands, 234 (61.74%) bands were polymorphic. Specific bands were amplified by some of primers i.e. OPAH-03, OPB-08, OPB-10 and OPB-18 primers. Primer OPB-08 amplified a specific electromorph only in WS-12 accession of molecular weight ~450 bp, similarly, OPAH-03 amplified a specific electromorph only in WS-13 accession of molecular weight about 600 bp and OPB-10 amplified 2 specific bands in WS-4 accession of molecular weight about ~400 bp and ~2000 bp. These primers could be used to differentiate the specific isolates from others one. Coefficient values for *Withania somnifera* were calculated by Jaccard's coefficients (Table 2) method using NTSYS-pc programme. The range of genetic similarity based on RAPD primers was 0.161- 0.960 indicating that there is significant genetic variability among the *Withania somnifera* accessions collected from various geographical regions of Madhya Pradesh. A dendrograme was generated by unweighted pair group method with "UPGMA" sub programme of "NTSYS-pc" (Fig.1). Cluster analysis revealed that all the accessions of *Withania somnifera* under study were divided into two groups (Chauhan *et al.* 2017).

The first major group contained 17 accessions and second group contained only 4 accessions. The first major group was divided into two subgroups. First subgroup contained only two accessions namely WS-1 and WS-2 while, second subgroup consisted fifteen accessions. Among fifteen accessions WS-4 formed separate subgroup and remaining fourteen accessions namely WS-3, WS-5, WS-7, WS-12, WS-9, WS-6, WS-10, WS-13, WS-16, WS-19, WS-18, WS-15, WS-21 and WS-8 were grouped together. The second major group consisted of four accessions WS-11, WS-14, WS-17 and WS-20. These four accessions were highly diverse among all accessions. On the base of RAPD analysis, all accessions constituted different cluster other than their respective districts. Although, out of two accessions from Jabalpur district WS-13 and WS-14 were unable to group together, these both accessions showed maximum diversity among all studied accessions. WS-13 was collected from Tropical forest research institute showed 91.0% similarity with WS-10 accession collected from Shivpuri, and WS-14 was the collection from herbal garden department of plant physiology, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur showed maximum similarity (91.8%) with WS-11 collected from Satna. Among the studied accessions highest genetic similarity 96.0% was shown between WS-7 and WS-12. These both accessions clustered together and were collected from Rewa and Singrauli respectively. The accession WS-7 also showed 95.0% relatedness with WS-9 (Amarkantak). The accessions WS-11 and WS-13 showed

Table 1: Details about RAPD primers used in the study

S. N.	Primer	Sequences 5'-3'	GC %	TB	MB	PB	PP	PIC
1	OPA-03	AGTCAGCCAC	60%	7	5	2	28.57	0.273729
2	OPA-07	GAAACGGGTG	60%	10	4	6	60.00	0.273438
3	OPA-09	GGGTAACGCC	70%	8	1	7	87.50	0.635601
4	OPA-11	CAATCGCCGT	60%	5	0	5	100.00	0.675001
5	OPA-12	TCGGCGATAG	60%	6	3	3	50.00	0.435752
6	OPA-13	CAGCACCCAC	70%	4	4	0	0.00	0.000000
7	OPAA-01	AGACGGCTCC	70%	3	0	3	100.00	0.378021
8	OPAA-02	GAGACCAGAC	60%	8	2	6	75.00	0.486816
9	OPAA-03	TTAGCGCCCC	70%	13	4	9	69.23	0.406851
10	OPAA-04	AGGACTGCTC	60%	9	5	4	44.44	0.306424
11	OPAA-06	GTGGGTGCCA	70%	9	3	6	66.66	0.431858
12	OPAB-07	GTAAACCGCC	60%	14	6	8	57.14	0.397321
13	OPAB-08	GTTACGGACC	60%	8	1	7	87.50	0.632086
14	OPAB-09	GGGCGACTAC	70%	7	1	6	85.71	0.681362
15	OPAC-12	GGCGAGTGTG	70%	8	8	0	0.00	0.000000
16	OPAC-13	GACCCGATTG	60%	6	1	5	83.33	0.640590
17	OPAC-14	GTCGGTTGTC	60%	11	2	9	81.82	0.571429
18	OPAD-05	ACCGCATGGG	70%	10	9	1	10.00	0.048980
19	OPAE-06	GGGGAAGACA	60%	5	0	5	100.00	0.708594
20	OPAH-01	TCCGCAACCA	60%	8	7	1	12.50	0.084184
21	OPAH-02	CACTTCCGCT	60%	9	4	5	55.55	0.355505
22	OPAH-03	GGTTACTGCC	60%	7	5	2	28.57	0.154843
23	OPAI-03	GGGTCCAAAG	60%	10	6	4	40.00	0.241950
24	OPAI-05	GTCGTAGCGG	70%	10	7	3	30.00	0.167347
25	OPAI-06	TGCCGCACTT	60%	8	8	0	0.00	0.000000
26	OPB-01	GTTTCGCTCC	60%	6	0	6	100.00	0.550130
27	OPB-04	GGACTGGAGT	60%	5	3	2	40.00	0.189844
28	OPB-07	GGTGACGCAG	70%	9	1	8	88.88	0.566840
29	OPB-08	GTCCACACGG	70%	6	1	5	83.33	0.640590
30	OPB-10	CTGCTGGGAC	70%	11	2	9	81.82	0.571429
31	OPB-17	AGGGAACGAG	60%	6	0	6	100.00	0.622071
32	OPB-18	CCACAGCAGT	60%	6	2	4	66.67	0.455782
33	OPBB-05	GGGCCGAACA	70%	5	1	4	80.00	0.357031
34	OPC-05	GATGACCGCC	70%	10	3	7	70.00	0.364453
35	OPC-06	GAACGGACTC	60%	10	0	10	100.00	0.726531
36	OPC-07	GTCCCGACGA	70%	4	0	4	100.00	0.709184
37	OPC-09	CTCACCGTCC	70%	6	1	5	83.33	0.640590
38	OPC-12	TGTCATCCCC	60%	4	4	0	0.00	0.000000
39	OPD-01	ACCGCGAAGG	70%	3	0	3	100.00	0.399121
40	OPD-04	TCTGGTGAGG	60%	8	2	6	75.00	0.486816
41	OPD-06	ACCTGAACGG	60%	5	3	2	40.00	0.189844
42	OPD-10	GGTCTACACC	60%	9	1	8	88.88	0.566840
43	OPE-03	CCAGATGCAC	60%	4	4	0	0.00	0.000000
44	OPE-06	AAGACCCCTC	60%	3	0	3	100.00	0.388021
45	OPE-08	TCACCACGGT	60%	6	3	3	50.00	0.435752
46	OPK-12	TGGCCCTCAC	70%	7	5	2	28.57	0.273729
47	OPM-11	GTCCACTGTG	60%	8	8	0	0.00	0.000000
48	OPM-13	GGTGGTCAAG	60%	6	0	6	100.00	0.550130
49	OPR-03	ACACAGAGGG	60%	6	1	5	83.33	0.620590
50	OPR-07	ACTGGCCTGA	60%	6	0	6	100.00	0.622071
51	OPS-05	TTTGGGGCCT	60%	6	2	4	66.67	0.455782
52	OPW-06	AGGCCCGATG	70%	3	0	3	100.00	0.278021
53	OPW-07	CTGGACGTCA	60%	8	2	6	75.00	0.486816
Average				7.15	2.74	4.42	-	0.405

TB – Total Bands, MB – Monomorphic Bands, PB – Polymorphic Bands, PP – Percentage, Polymorphism, PIC – Polymorphism Information Content

Table 2: Genetic similarity among *Withania somnifera* accessions based on RAPD analysis

	WS-1	WS-2	WS-3	WS-4	WS-5	WS-6	WS-7	WS-8	WS-9	WS-10	WS-11	WS-12	WS-13	WS-14	WS-15	WS-16	WS-17	WS-18	WS-19	WS-20	WS-21
WS-1	1.000																				
WS-2	0.733	1.000																			
WS-3	0.403	0.534	1.000																		
WS-4	0.470	0.571	0.618	1.000																	
WS-5	0.418	0.515	0.672	0.627	1.000																
WS-6	0.486	0.597	0.698	0.650	0.878	1.000															
WS-7	0.449	0.545	0.718	0.639	0.908	0.907	1.000														
WS-8	0.466	0.504	0.598	0.582	0.689	0.759	0.750	1.000													
WS-9	0.448	0.533	0.706	0.627	0.880	0.862	0.950	0.755	1.000												
WS-10	0.403	0.516	0.640	0.622	0.823	0.821	0.867	0.687	0.856	1.000											
WS-11	0.282	0.243	0.196	0.235	0.202	0.212	0.200	0.202	0.204	0.173	1.000										
WS-12	0.422	0.541	0.731	0.636	0.890	0.889	0.960	0.717	0.930	0.865	0.195	1.000									
WS-13	0.371	0.480	0.688	0.570	0.813	0.811	0.874	0.661	0.845	0.910	0.161	0.910	1.000								
WS-14	0.257	0.210	0.181	0.212	0.181	0.190	0.178	0.188	0.181	0.166	0.918	0.180	0.161	1.000							
WS-15	0.407	0.504	0.675	0.578	0.748	0.775	0.798	0.650	0.772	0.735	0.244	0.829	0.802	0.244	1.000						
WS-16	0.401	0.493	0.614	0.611	0.750	0.734	0.754	0.631	0.744	0.843	0.216	0.752	0.786	0.216	0.674	1.000					
WS-17	0.278	0.242	0.213	0.266	0.217	0.227	0.201	0.211	0.205	0.169	0.894	0.196	0.164	0.841	0.272	0.266	1.000				
WS-18	0.423	0.518	0.659	0.615	0.798	0.782	0.820	0.648	0.794	0.744	0.240	0.851	0.778	0.232	0.774	0.847	0.290	1.000			
WS-19	0.371	0.460	0.645	0.565	0.744	0.728	0.763	0.609	0.737	0.791	0.209	0.791	0.862	0.216	0.733	0.882	0.253	0.843	1.000		
WS-20	0.256	0.213	0.199	0.244	0.198	0.207	0.181	0.198	0.185	0.163	0.832	0.183	0.166	0.903	0.267	0.260	0.935	0.276	0.254	1.000	
WS-21	0.412	0.493	0.651	0.562	0.707	0.705	0.722	0.604	0.698	0.667	0.294	0.748	0.724	0.294	0.867	0.778	0.337	0.856	0.802	0.331	1.000

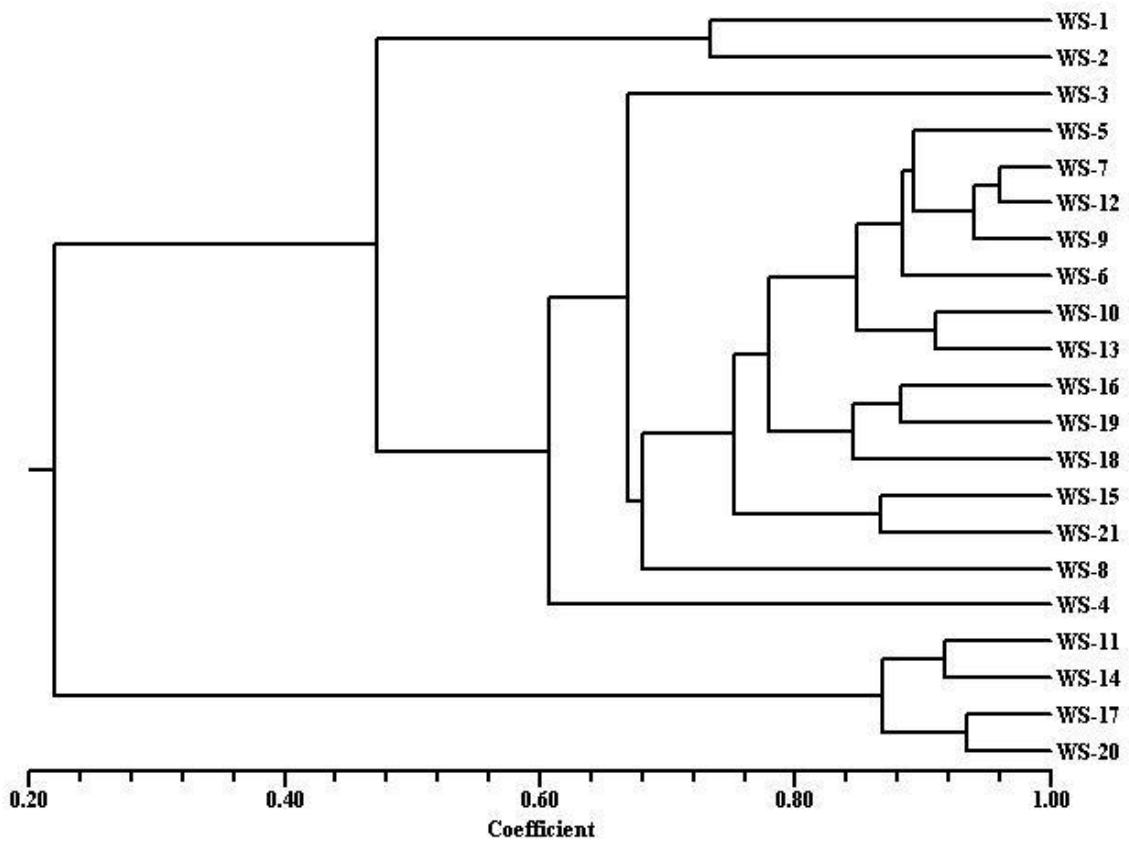

Fig. 1: Dendrogram generated using UPGMA analysis showing relationship among *Withania somnifera* genotypes using RAPD markers

maximum divergence with each other similarly, WS-13 and WS-14 grouped separately and showed maximum distant with each other. First subgroup consisted WS-1 and WS-2 with 73.3% genetic similarity. The second major group contained four accessions namely WS-11, WS-14, WS-17 and WS-20. Among these four accessions WS-11 and WS-14 were found 91.8% genetically similar with each other. WS-17 and WS-20 showed 93.5% similarity and grouped together. Scartezzini *et al.,* (2007) also got same types of result when analyzed seven samples by using RAPD markers. The accessions which were considered in similar group were placed in closely related position in two-dimensional and three dimensional scaling analyses, WS-11, WS-14, WS-17 and WS-20 formed separate group as in dandogram and WS-1 and WS-2 accessions were placed distanly from other accessions.

RAPD markers represent an efficient and inexpensive way to generate molecular data and thus have been used successfully in various taxonomic and phylogenetic studies (Tripathi *et al.*, 2012). RAPD markers are generated by PCR amplification of random genomic DNA segments with single primer in an arbitrary sequence. They are usually dominant markers with polymorphism between individuals defined by the presence or absence of a particular RAPD band. The results of the present study indicate that the level of genetic variation was high among the Ashwagandha genotypes.

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