Int.J.Curr.Microbiol.App.Sci (2017) *6*(8): 2353-2361

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 8 (2017) pp. 2353-2361**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.608.278

RAPD Based Genetic Diversity Analysis in 25 Genotypes of *Withania somnifera* (L.) Dunal

Surya Chauhan*, Arunabh Joshi and Devendra Jain

Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur 31300, (Rajasthan), India
**Corresponding author*

Keywords

RAPD, Genetic diversity, 25 genotypes.

Article Info

Accepted:
21 June 2017
Available Online:
10 August 2017

A B S T R A C T

Genetic diversity was evaluated among 25 genotypes of *Withania somnifera* L. Dunal using RAPD marker. The 15 RAPD primers screened amplified 150 bands among which 141 bands (94%) were polymorphic. The Polymorphic Information Content (PIC) values ranged from 0.162 (OPA-05) to 0.422 (OPA-02) with an average of 0.310. A total of five primers detected in the study produced seven unique bands in seven genotypes Jaccards similarity coefficient values ranged from 0.29 to 0.85 with an average of 0.57. Cluster analysis based on Jaccards similarity coefficient using Un-weighted Pair Group Method with Arithmetic Averages (UPGMA) grouped all the 25 genotypes into two major groups. The cluster I is a major cluster consisting of 20 genotypes whereas cluster II which is minor consists of 5 genotypes. The results show that the level of genetic variation was high among the Ashwagandha genotypes.

Introduction

Ashwagandha (*Fam* = *Solanaceae*) [(ashwa = horse) and (gandha = smell), "horse like smell"], one of the most important medicinal plant also known as *Withania somnifera* L. Dunal, poison gooseberry, winter cherry and Indian ginseng (parallel to Panax Ginseng, of Chinese origin) (Tripathi *et al.*, 1996; Singh *et al.*, 1998). Due to its medicinal properties, it had been used since ancient times as "rasayana" and "medha rasayana" in Ayurveda (CSIR, 1976) and now-a-day paving pathway for preparing allopathy drugs. C-28 steroidal lactones known as with anolides are mainly responsible for vast medicinal properties such as anti-inflammatory, anticonvulsive, antitumor, immunosuppressive, antioxidant and adaptogenic action. The main part of plant which contributes for these with anolides and alkaloids are roots and leaves.

It is widely distributed from the Southern Mediterranean region to the Canary Islands and to South and East Africa; from Palestine up to North India, covering Israel, Jordan, Egypt, Sudan, Iran, Afghanistan, Baluchistan and Pakistan. It is widely distributed throughout the drier and subtropical parts of India. In India, it grows wildly/extensively in Madhya Pradesh, Uttar Pradesh, Andhra

Pradesh, Gujarat, Maharashtra, Rajasthan and Punjab extending to the mountainous regions of Himachal Pradesh and Jammu.

Molecular marker technique is a powerful tool for evaluating genetic variation and exploring evolutionary relationships among and within plant species (Hoisington *et al.*, 1998; Stuber, 1995). PCR (Polymerase Chain Reaction)-based markers i.e. RAPD are being used in the analysis of genetic diversity in crop plants because of their relative ease. There is no need of prior knowledge about the genome, which makes RAPD a common method for such studies in different plants. The RAPD technique (Williams *et al.,* 1990) is faster and cheaper for revealing genetic polymorphism. RAPD markers have been widely used for evaluating genetic variation and genetic relationships, genetic mapping, identifying genotypes, etc., with various plants.

RAPD markers have been used for genetic diversity analysis in ashwagandha by many workers (Arif *et al.,* 2010; Dharmar *et al.,* 2011; Mir *et al.,* 2011; Bhat *et al.,* 2012; Khatak *et al.,* 2013; Sairkar *et al.,* 2013; Udaykumar *et al.,* 2013; Khanna *et al.,* 2014; Khan and Shah, 2016; Tiwari and Shrivastava, 2016).

The objective of this work was to evaluate the extent and distribution of genetic diversity among 25 accessions of *Withania somnifera* using RAPD markers and to determine the phylogenetic relationship amongst them with the future goal of assisting the marker assisted crop improvement programme.

Materials and Methods

In the present investigation, seed of 25 diverse genotypes of *Withania somnifera* Dunal were procured from AICRP on MAP&B (Medicinal and Aromatic Plant and Betelvine). Source details of the genotypes used are given in table 1. Young fresh and healthy leaves were collected and DNA extraction was done following the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990).The extracted genomic DNA was analysed on 0.8% agarose gel and was diluted to an optimum concentration using TE for polymerase chain reaction (PCR). A total of 20 arbitrary decamer primers were initially used, out of which 15 primers showed clear, scorable and highly polymorphic bands (Table 2).

For RAPD analysis, the genomic DNA was amplified in a reaction volume of 20 ml containing 50 ng of template DNA,1x reaction buffer, 1 unit of Taq DNA polymerase, 200 mM each of dNTPs mix and 0.5 µM/reaction of primer using a programmable thermo-cycler DNA Engine (Biorad, Germany) with the following cycling parameters: initial denaturation at 94 °C for 4 min, followed by 44 cycles of denaturation at 94 °C for 45 sec, annealing at 32 °C for 2 min, and extension at 72 °C for 2 min. A final extension was done for 10 min at 72 °C with a hold temperature of 4 °C. The amplified PCR products were electrophoretically separated on 1.2% agarose gel prepared in 1x TAE buffer containing ethidium bromide (EtBr) staining dye. The gel was run for 3h at 50V.The size of the amplified DNA fragments were determined using low base pair and 500bp DNA ladders= (Bangalore Genie, India) as standard molecular size markers. DNA fragments were visualized under UV-trans-illuminator and photographed using gel documentation system. Scoring of amplicons obtained from different RAPD markers was done on the basis of presence (taken as 1) or absence (taken as 0) of bands for each primer. For banding pattern only clear and unambiguous bands were scored for each primer. Comparison of band position

was done relative to molecular weight of standard DNA ladders. Accordingly, a rectangular binary data matrix was obtained and statistical cluster analysis was performed using the NTSYS-pc version 2.02e (Rohlf, 1998). A pair wise similarity matrix was generated and the cluster analysis was performed via unweighted pair group method with arithmetic averages (UPGMA) to develop a dendrogram. A two-dimensional and three-dimensional principal component analysis (PCA) was constructed to provide another means of testing the relationship among the genotypes.

Results and Discussion

Among the 20 RAPD primers used for initial screening, 15 markers produced polymorphic, reproducible and scorable bands. A total of 150 bands were generated from 15 RAPD primers, of which 141 bands were polymorphic (94%) with an average of 12 polymorphic bands per primer (Table 2). The total number of amplified bands varied between 6 (primer OPA-02 and OPA-06) and 18 (primer OPB-05). The overall size of PCR amplified products ranged between 100 bp to 3000 bp. The percent polymorphism ranged from as low as 50 (OPA-02) to as high as 100 (C-19, OPA03, OPA06, OPB4, OPB05, OPB06, OPB07, OPC 05, P-20 and S-34).The average PIC was 0.310 ranging from 0.164 to 0.422. The lowest and the highest PIC value were recorded for primer OPA-05 and OPA-02 respectively. Figure 1 shows the amplification pattern obtained from primer OPA-05 produced 9 polymorphic bands.

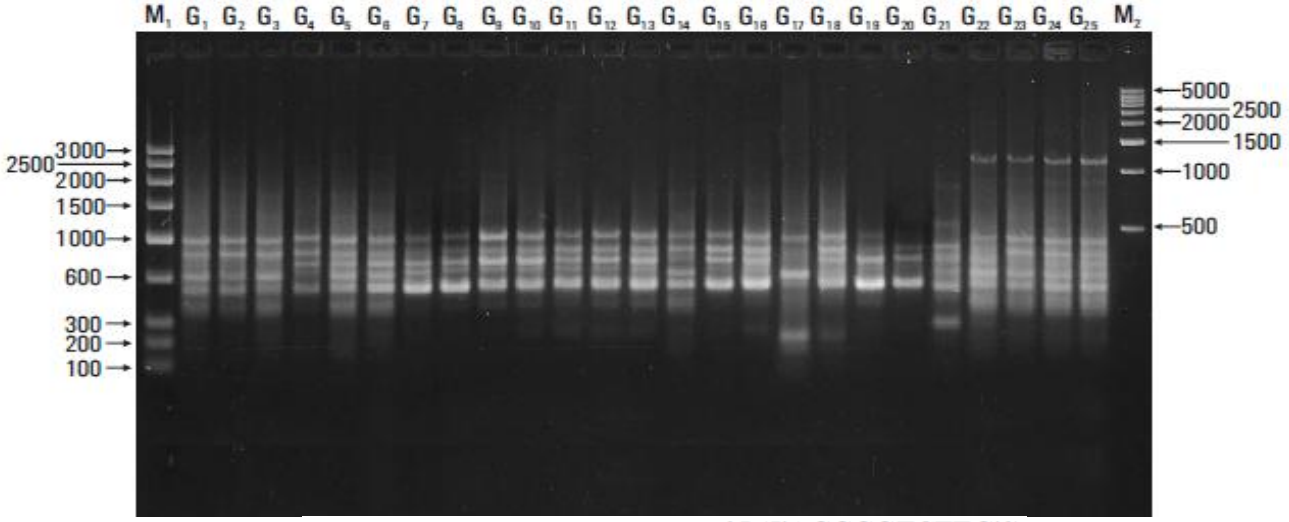
7 unique bands (band which is present in a particular genotype but absent in rest of the genotypes) were detected in 6 genotypes *viz.*, AWS2B, UWS 56, UWS 59, UWS 32, UWS 37 and UWS 22 with 5 RAPD primers (OPA-05 OPB-04, OPB-05, OPC-05 and S-34). The genotype UWS 32 gave maximum number of distinct bands *i.e.*, 2. The sizes of these unique bands ranged from 350 to 3000 bp (Table 3).

Based on RAPD similarity matrix data, the values of similarity coefficient ranged from 0.29 to 0.85 *i.e.*, 29-85% or genetic diversity ranged from 15 to 71%. The average similarity across all the genotypes was found out to be 0.57 showing that the genotypes were moderately diverse. Maximum similarity value of 0.85 was observed between genotypes UWS 23 and UWS 28 followed with a similarity coefficient value of 0.78 between UWS 11 and UWS 13. Similarly, minimum similarity value of 0.29 was observed between genotypes UWS 10 and UWS 67 and between UWS 10 and UWS 77 followed by a similarity coefficient of 0.3 between UWS 67 and UWS 15 (Table 4).

The RAPD cluster tree analysis of 25 *W. somnifera* L. Dunal genotypes showed that they could be mainly divided into 2 clusters at a similarity coefficient of 0.43 and 0.61 respectively (Fig. 2). The Cluster I is a major cluster including 20 genotypes and it can be divided into 2 sub-clusters i.e. sub cluster I and II at 0.54 and 0.49 similarity coefficients respectively. Sub cluster I can be further divided into 2 groups, subgroup A and B at 0.74 and 0.76 similarity coefficients.

Subgroups A and B includes 4 genotypes *viz.*, JA-134, JA-20, RVA 100 and HWS-8-14 from which JA-20 and JA-134 are related to each other whereas HWS-8-14 and RVA 100 are related to each other. Subgroup C and D at 0.68 and 0.73 similarity coefficient includes 5 genotypes *viz.*, AWS2B, UWS 134, UWS 111, UWS 98 and UWS 93.From these genotypes, UWS 134 and UWS 111 are related to each other. Similarly, UWS 98 and UWS 93 are related to each other at 0.73 similarity coefficient value. AWS2B is out grouped at 0.69 similarity coefficient.

Fig.1 RAPD profile of *W. somnifera* L. Dunal generated through OPA-05 primer



RAPD profile generated through OPA-05 (5'AGGGGTCTTG'3)

M_1= Low Base Pair Range DNA ladder; M_2= 500 bp DNA Ladder

G1-G25 represents following *W.somnifera* L. Dunal genotypes:

G1: UWS 10,	G2: UWS 11,	G3: UWS 13,	G4: UWS 15,	G5: UWS 22,
G6: UWS 23,	G7:UWS 22,	G8:UWS 23,	G9:UWS 28,	G10:UWS 32,
G11:UWS 35,	G12:UWS 37,	G13:UWS 56,	G14:UWS 59,	G15:UWS 60,
G16:UWS 67,	G17:UWS 92,	G18:UWS 93,	G19:UWS 98,	G20:UWS 111,
G21:UWS 134,	G22:AWS2B,	G23:HWS-8-14,	G24:RVA 100	G25: JA-20

Fig.2 Dendrogram constructed with UPGMA clustering method of 25 genotype of *W. somnifera* L. Dunal Using RAPD Primers

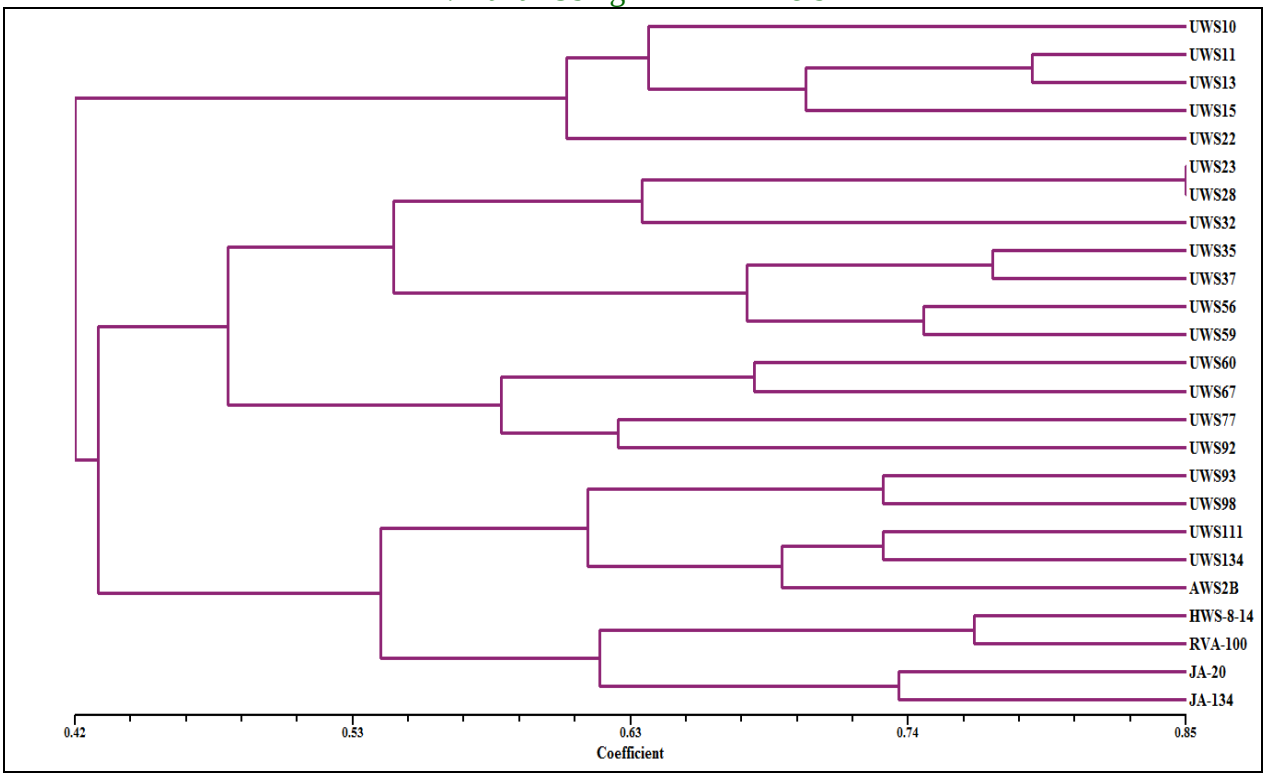
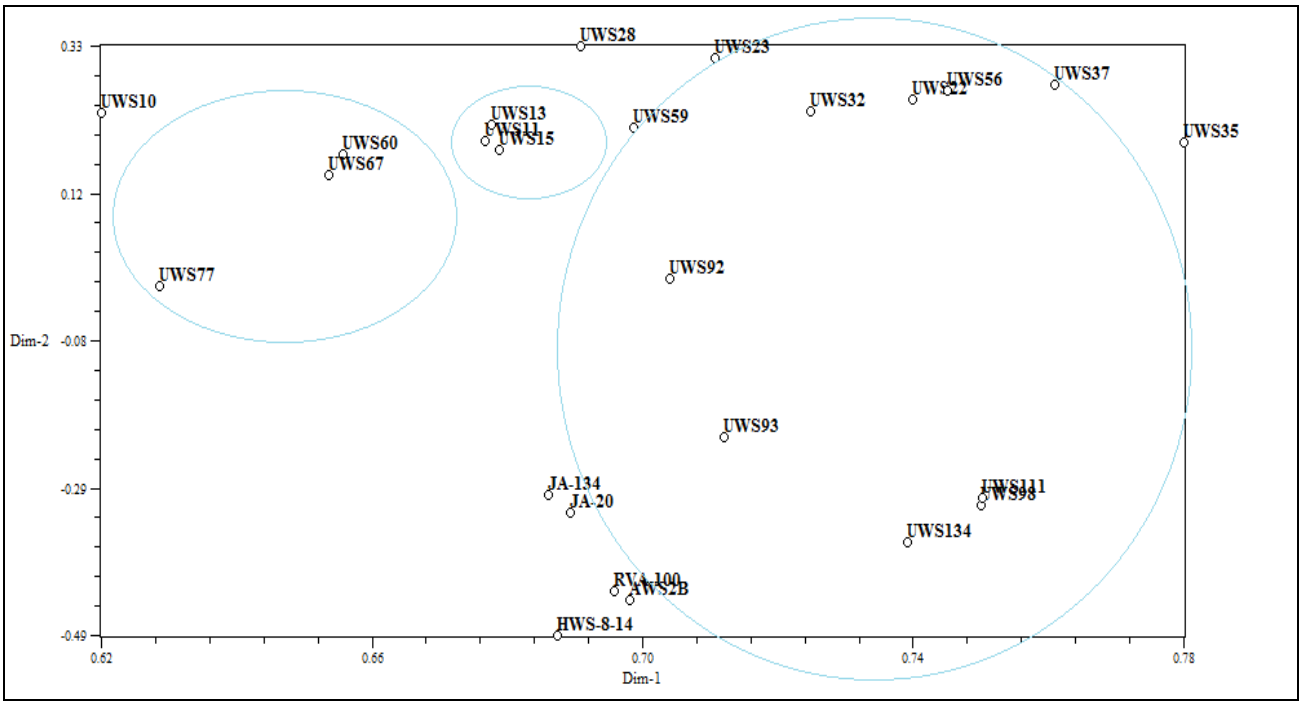


Fig.3 Two dimensional and three dimensional PCA (Principal Component Analysis) scaling of 25 genotypes of *W. somnifera* L. Dunal using RAPD markers



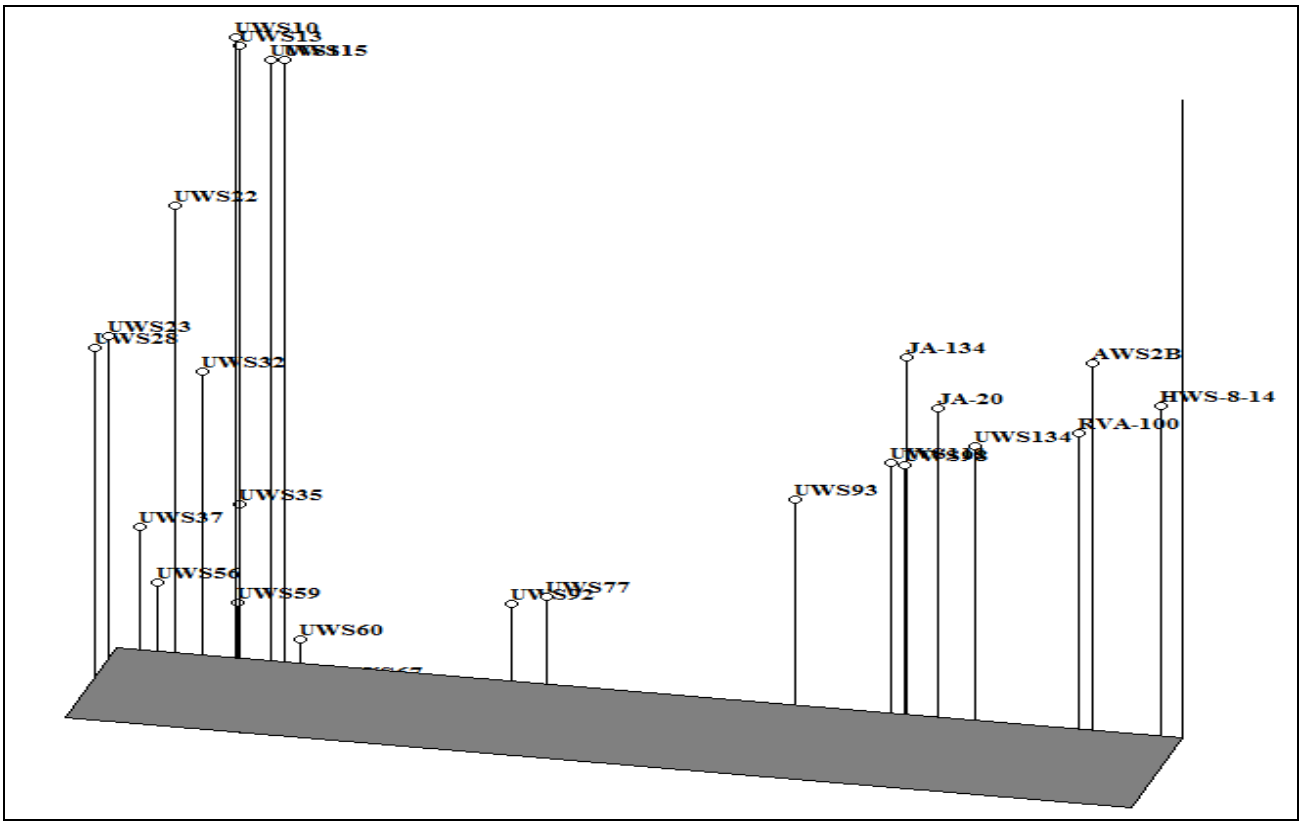


Table.1 Details of Twenty five genotypes of Ashwagandha used in present investigation

Genotypes	Source
UWS-10	Germplasm Collection AICRP in MAP&B, Udaipur
UWS-11	-do-
UWS-13	-do-
UWS-15	-do-
UWS-22	-do-
UWS-23	-do-
UWS-28	-do-
UWS-32	-do-
UWS-35	-do-
UWS-37	-do-
UWS-56	-do-
UWS-59	-do-
UWS-60	-do-
UWS-67	-do-
UWS-77	-do-
UWS-92	-do-
UWS-93	-do-
UWS-98	-do-
UWS-111	-do-
UWS-134	-do-
AWS2B	AAU, Anand
HWS-08-14	CCSHAU, Hisar
RVA-100	RVSKVV, Mandsaur
JA-20	-do-
JA-134	-do-

Table.2 DNA amplification profile and polymorphism generated in *W. somnifera* L. Dunal using 15 RAPD primers

S. No.	Primer Code *	Molecular weight range (bp)	Total no. of bands amplified (x)	Polymorphic bands		PIC*
				Number	Frequency (%)	
1.	C-19	2500-200	10	10	100	0.393
2.	OPA-02	1400-300	6	3	50	0.181
3.	OPA-03	2500-100	12	12	100	0.372
4.	OPA-05	3000-200	11	9	81.8	0.164
5.	OPA-06	2000-250	6	6	100	0.279
6.	OPA-07	2100-250	10	9	90	0.265
7.	OPA-08	2100-400	9	8	90	0.298
8.	OPB-01	2100-400	7	5	71.4	0.258
9.	OPB-04	3000-350	11	11	100	0.32
10.	OPB-05	2500-100	20	20	100	0.310
11.	OPB-06	2500-200	10	10	100	0.392
12.	OPB-07	2600-300	10	10	100	0.343
13.	OPC-05	3000-200	10	10	100	0.305
14.	P-20	2100-200	10	10	100	0.345
15.	S-34	2400-100	8	8	100	0.422
	Total		**150**	**141**	**92.21**	**0.310**

*Polymorphic Information Content, * Operon series code

Table.3 Genotype specific band as detected by 5 RAPD primers as shown by 4 different genotypes of *W. somnifera* L. Dunal

S. No.	Primer code	Total no. of unique bands	Genotype	Size of bands (bp)
1.	OPA-05	1	AWS2B	2000
2.	OPB-04	2	UWS 56 UWS 59	1200 350
3.	OPB-05	1	UWS 32	2400
4.	OPC-05	2	UWS 32 UWS 37	3000 2500
5.	S-34	1	UWS 22	1500
Total	**7**			

Table.4 Jaccards similarity coefficient for RAPD profile generated by Agarose gel electrophoresis

Genotypes	UWS 10	UWS 11	UWS 13	UWS 15	UWS 22	UWS 23	UWS 28	UWS 32	UWS 35	UWS 37	UWS 56	UWS 59	UWS 60	UWS 67	UWS 77	UWS 92	UWS 93	UWS 98	UWS 111	UWS 134	AWS 2B	HWS-8-14	RVA-100	JA-20	JA-134
UWS 10	1.00																								
UWS 11	0.71	1.00																							
UWS 13	0.64	0.78	1.00																						
UWS 15	0.56	0.64	0.76	1.00																					
UWS 22	0.58	0.56	0.6	0.68	1.00																				
UWS 23	0.43	0.47	0.48	0.51	0.62	1.00																			
UWS28	0.42	0.45	0.44	0.46	0.58	0.85	1.00																		
UWS 32	0.46	0.45	0.45	0.48	0.59	0.63	0.65	1.00																	
UWS 35	0.46	0.45	0.43	0.47	0.6	0.53	0.57	0.7	1.00																
UWS 37	0.46	0.44	0.43	0.44	0.6	0.53	0.53	0.63	0.77	1.00															
UWS 56	0.38	0.42	0.42	0.43	0.52	0.53	0.53	0.55	0.7	0.77	1.00														
UWS 59	0.34	0.4	0.4	0.38	0.44	0.44	0.44	0.51	0.61	0.64	0.75	1.00													
UWS 60	0.33	0.39	0.38	0.32	0.38	0.44	0.41	0.39	0.47	0.55	0.58	0.66	1.00												
UWS 67	0.29	0.32	0.33	0.3	0.39	0.43	0.42	0.43	0.51	0.53	0.57	0.6	0.68	1.00											
UWS 77	0.29	0.33	0.33	0.33	0.37	0.42	0.4	0.37	0.44	0.46	0.43	0.43	0.57	0.57	1.00										
UWS 92	0.33	0.39	0.4	0.38	0.42	0.48	0.47	0.44	0.51	0.51	0.52	0.47	0.56	0.63	0.63	1.00									
UWS 93	0.36	0.4	0.42	0.41	0.46	0.41	0.41	0.44	0.5	0.43	0.48	0.47	0.44	0.48	0.48	0.53	1.00								
UWS 98	0.39	0.45	0.46	0.47	0.46	0.4	0.41	0.44	0.53	0.46	0.47	0.45	0.41	0.46	0.46	0.54	0.73	1.00							
UWS 111	0.36	0.43	0.45	0.44	0.48	0.44	0.43	0.49	0.55	0.48	0.49	0.44	0.39	0.45	0.43	0.52	0.62	0.7	1.00						
UWS 134	0.41	0.43	0.4	0.41	0.47	0.42	0.4	0.48	0.53	0.48	0.44	0.42	0.39	0.41	0.43	0.53	0.57	0.64	0.73	1.00					
AWS2B	0.39	0.46	0.41	0.44	0.42	0.37	0.36	0.42	0.47	0.41	0.4	0.37	0.33	0.32	0.38	0.41	0.53	0.64	0.65	0.73	1.00				
HWS-8-14	0.34	0.38	0.35	0.39	0.41	0.38	0.36	0.45	0.44	0.41	0.38	0.37	0.34	0.34	0.38	0.43	0.49	0.55	0.58	0.65	0.69	1.00			
RVA-100	0.33	0.37	0.37	0.37	0.41	0.44	0.43	0.44	0.45	0.4	0.41	0.41	0.39	0.35	0.4	0.42	0.52	0.55	0.58	0.55	0.58	0.77	1.00		
JA-20	0.35	0.4	0.4	0.43	0.44	0.41	0.37	0.4	0.47	0.47	0.44	0.42	0.43	0.37	0.43	0.41	0.44	0.52	0.46	0.48	0.54	0.62	0.7	1.00	
JA-134	0.37	0.43	0.44	0.47	0.49	0.39	0.35	0.40	0.43	0.46	0.42	0.4	0.37	0.36	0.38	0.46	0.45	0.53	0.5	0.55	0.50	0.57	0.59	0.73	1.00

The sub cluster II at 0.49 similarity coefficient is divided into Subgroup E, F, G, H and I. Subgroup E and F at 0.67 and 0.62 similarity coefficients includes 4 genotypes viz., UWS 92, UWS 77, UWS 67 and UWS 60.From these genotypes, UWS 92 and UWS 77 are related to each other whereas UWS 67 and UWS 60 are related to each other. In Subgroup G and H at 0.75 and 0.77 similarity coefficient includes 4 genotypes viz., UWS 59, UWS 56, UWS 37 and UWS 35. From these genotypes, UWS 59 and UWS 56 are related to each other and UWS 37 and UWS 35 are related to each other. The Subgroup I at 0.64 similarity coefficient includes 3 genotypes *viz.,* UWS 32, UWS 28 and UWS 23. From these, UWS 28 and UWS 23 are related to each other.

Cluster II, a minor cluster included 5 genotypes *viz.,* UWS 10, UWS 11, UWS 13, UWS 15 and UWS 22. It could be divided into 2 subclusters. In subcluster IV, can be divided into 2, subgroups E and F.

Subgroup E has 3 genotypes *viz.,* UWS 11, UWS 13 and UWS 15 from which UWS 11 and UWS 13 were related to each other at 0.79 similarity coefficient. Genotype UWS 22 was out grouped from first cluster as Sub cluster III having similarity coefficient value of 0.61.

Two and three dimension principal component analysis based on RAPD data (Figs. 3 and 4, respectively) showed similar clustering of 25 genotypes as evident from cluster tree analysis. Dice similarity coefficients ranged from 0.62 to 0.78. Most of the genotypes tended to cluster mainly into two clusters.

Cluster I major cluster including 20 genotypes and minor cluster II included 5 genotypes. Genotypes UWS 10 and UWS 22 showed maximum genetic distance and therefore the most divergent genotypes.

Acknowledgements

The authors gratefully acknowledge the financial assistance from the RKVY project "Validation of important crop varieties through DNA fingerprinting".

References

Arif, I.A., Bakir M.A., Khan, H.A., AlFarhan, A.H., AlHomaidan, A.A., Bahkali, A.H., Al Sadoon, M. and Shobrak, M. 2010. Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment. Genetics and Molecular Research, 9(4):2191-2198.

Bhat, T.M., Kudesia, R. and Dar, S.A. 2012. Evaluation of Genetic Diversity among accessions of *Withania somnifera* (L.) Dunal using biochemical analysis and molecular markers. American-Eurasian Journal of Agriculture & Environmental Sciences, 12(7):983-990.

CSIR. *Withania somnifera* L. (Dunal) (Ashwagandha).1976. In: The Wealth of India -Raw Materials. Vol. X. Council of Scientific and Industrial Research, New Delhi, India, pp 81-85.

Dharmar, K. and Britto, A.J.D. 2011. RAPD analysis of genetic variability in wild populations of *Withania somnifera* (L.) Dunal. International Journal of Biological Technology, 2(1):21-25.

Doyle, J.J. and Doyle, J.L. 1990. Isolation of plant DNA from fresh tissue. Focus, 12:13-15

Hoisington D, Listman GM, Morris ML. 1998. Varietal development: applied biotechnology, In ML Morris, ed, Maize Seed Industries in Developing Countries. Lynne Rienner Publishers, Inc., Boulder, pp 77-102

Khan, S and Shah, R.A. 2016. *Withania somnifera* (L) Dunal using RAPD and ISSR markers. Research in

Biotechnology, 7: 01-10.

Khanna, P.K., Chandra, R., Kumar, A., Dogra, N.,Gupta, H., Gupta, G., Verma, V. 2014. Correlation between Morphological, Chemical and RAPD Markers for Assessing Genetic Diversity in *Withania somnifera* (L.) Dunal. Journal of Crop Science and Biotechnology, 17(1):27-34

Khatak, S., Dhillon, S., Yadav, O.P., Grewala & Sheokand R.N. 2013. Agro-Morphological and RAPD marker based characterization of genetic diversity in different genotypes of *Withania somnifera* L. Dunal. International Journal of Bio-Technology and Research (IJBTR), 3(4):1-16.

Mir, B.A., Koul, S., Kumar, A., Kaul, M.K, Soodan, A.S. and Raina, S.N. 2011. Assessment and characterization of genetic diversity in *Withania somnifera* (L.) Dunal using RAPD and AFLP markers. African Journal of Biotechnology, 10(66): 4746-4756.

Rohlf, F.J. 1993. NTSYS-pc. Numerical taxonomy and multivariate analysis system. Exeter Software, New York

Sairkar, P., Vijay, N., Batav, N., Silawat, N., Garg, R.K., Chouhan, S., Sharma, R. And Mehrotra, N.N. 2013. Genetic variability in two economically important medicinal plants of Madhya Pradesh, India and using RAPD markers *Withania somnifera* and *Rauwolfia serpentina*. Asian journal of Experimental Biological Sciences, 4(1):36-43.

Singh, S. and Kumar, S. 1998. In *Withania somnifera*: The Indian Ginseng, Ashwagandha. Central Institute of Medicinal and Aromatic Plants, Lucknow, India.

Smith JSC, Smith OS. 1989. Comparison of heterosis among hybrids as a measure of relatedness with that to be expected on the basis of pedigree. Maize Genetic Cooperation Newsletter 63: 86-87

Smith JSC, Smith OS. 1992. Fingerprinting crop varieties. Adv. Agron. 47: 85-140

Stuber CW. 1995. Mapping and manipulating quantitative traits in maize. Trends Genetics. 11: 477-481.

Tiwari, P and Shrivastava A. 2016. Efficacy of RAPD markers for molecular diversity analysis of *Withania somnifera* (L) Dunal in central India. International journal of advanced research in biological sciences 3(7): 126-130.

Tripathi, A.K., Shukla, Y.N. and Kumar, S. 1996. Ashwagandha (*Withania somnifera* Dunal) (Solanaceae): a status report. Journal of Medicinal and Aromatic Plant Sciences, 18(1):46-62.

Udayakumar, R., Kasthurirengan, S., Mariashibu, T. S., Sudhakar, B., Ganapathi, A., Kim, E. J., Jang, K. M., Choi C. W. and Kim, S. C. 2013. Analysis of genetic variation among populations of *Withania somnifera* (L.) in South India based on RAPD markers. European Journal of Medicinal Plants, 3(2):266-280.

Williams, G. K., Kubelik, A. R., Livak, K. L., Rafalshi, J. A. and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic acid research, 18:6531-6535.

How to cite this article:
Surya Chauhan, Arunabh Joshi and Devendra Jain. 2017. RAPD Based Genetic Diversity Analysis in 25 Genotypes of *Withania somnifera* (L.) Dunal. *Int.J.Curr.Microbiol.App.Sci.* 6(8): 2353-2361. doi: https://doi.org/10.20546/ijcmas.2017.608.278