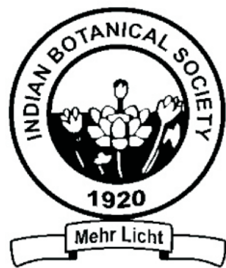

GENETIC DIVERGENCE ANALYSIS OF *WITHANIA SOMNIFERA* (L.) DUNAL GENOTYPES AS REVEALED BY RAPD MARKER

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Withania somnifera (L.) Dunal is an important medicinal plant and its pharmacological activities are attributed to its active principles known as withanolides. The objective of the present study was to investigate intraspecific variability observed between the intraspecific variants of Ashwagandha which include 35 selection lines and one released variety WS-20. This information was employed to estimate similarity matrix value based on Jaccard's similarity coefficients which was used to construct phenetic dendrogram. The dendrogram easily separated these genotypes into two major clusters 'A' and 'B'. The range of similarity extends from 0.220 between PWS-22 and PWS-8 to 0.930 between PW-2 and PWS-3. The PWS-4, PWS-17 and PWS-23 were three divergent lines obtained in major cluster 'B'. The cluster 'B' was subdivided into six minor subgroups B1 to B6. The released variety WS-20 was the part of 'B' which showed 62% similarity to PWS-35. This information reveals the efficiency of RAPD markers for rapid detection of genetic variations at intraspecific level and efficient management of plant genetic resource.

Key Words: Ashwagandha, medicinal plant, intraspecific variants, genetic divergence, RAPD.

Withania somnifera (L.) Dunal of Solanaccae, commonly known as Ashwagandha, a shrubby bush held in high repute in traditional Indian medicine recommended in Ayurveda (Dash and Junius 1983). It contains pharmacologically active compounds such as withanolides and alkaloids which attribute to its anti-cancerous, antioxidant, antibacterial, aphrodisiac etc. activities (Sharma and Dandiya 1992). The twenty-three known *Withania* species are widely distributed in the drier parts of tropical and subtropical zones, ranging from Canary islands, Mediterranean region and Northern Africa to South west Asia (Mirjalili *et al.*2009). In India, the distributional range of *W. somnifera* extends from 23^0N to 33^0N from 18-1700 m above sea level where the regions are extremely diversified geologically, ecogeographically, climatically and edaphically. *Withania somnifera* is cultivated in drier parts of India which include Mansa, Neemuch and Jawad tehsils of Mandsaur district of Madhya Pradesh, Punjab, Sind and Rajasthan (Anonymous 1976, Sharma 2004).

Various forms of Ashwagandha are different cultivars of the same species, since intra-specific variation and polymorphisms are usual phenomena in Solanaceae (Atal and Schwarting 1962). It was observed that considerable morphological and chemotypical variations observed in the species distributed in India and abroad (Glottor *et al.*1973). Hence there is a need for a systematic assessment of the various morphotypes of this important medicinal herb. Information on genetic diversity and relationship among and individuals, populations, plant verities and species are of importance to plant breeders for the improvement of crop plants (Dharmar and Britto 2011). Studies based on morphometric data have been analyzed in this crop (Misra *et*

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Table 1: Phenotypic characters of thirty six genotypes of *Withania somnifera* (L.) Dunal.

Name of genotypes	Plant height (cm)	Number of branches (no.)	Internodal distance (cm)	Plant canopy (cm^2)	Leaf area (cm^2)	Fresh root yield (gm)	Dry root yield (gm)	Root length (cm)	Root diameter (cm)
PWS-1	37	6	4	756	21.78	17	4.9	14.0	1.35
PWS-2	36	5	4	1085	19.8	11.2	3.92	14.8	1.25
PWS-3	34	4	4	930	24.81	15.3	2.38	12.3	1.19
PWS-4	38	5	4.5	1110	15.64	21.2	5.03	13.0	1.27
PWS-5	38	5	4	1140	14.31	18.3	6.47	14.0	1.50
PWS-6	30	4	2.5	780	13.96	7.8	1.42	8.0	0.90
PWS-7	31	5	4.5	750	12.98	28.4	6.5	13.5	1.45
PWS-8	37	4	4	850	14.80	24.3	6.2	14.5	1.23
PWS-9	45	3	3.5	1386	15.65	38.4	10.6	17.0	1.93
PWS-10	42	5	4.2	840	12.82	14.3	3.11	10.5	1.24
PWS-11	44	2	4.5	1020	23.63	29.5	7.86	10.0	1.85
PWS-12	31	6	6	960	12.04	9.4	2.05	11.6	0.87
PWS-13	30	8	5	1085	14.03	10	2.67	12.5	1.15
PWS-14	32	6	3.5	1050	10.80	12.5	1.92	12.5	1.14
PWS-15	40	6	5	1170	13.55	23.5	5.70	13.5	1.42
PWS-16	37	3	4.2	1050	13.00	19.6	4.02	12.8	1.23
PWS-17	35.5	3	4.2	1056	15.20	16.6	4.62	16.3	1.40
PWS-18	28	3	3	504	15.47	10.4	2.18	11.4	1.15
PWS-19	45	2	5.5	1230	19.15	36.0	8.8	14.5	1.94
PWS-20	38.5	3	3.5	930	14.66	30.1	6.61	12.5	1.89
PWS-21	28	7	4.5	825	14.70	6.3	1.30	12.6	0.87
PWS-22	24	2	3.5	460	14.05	4.3	2.42	10.5	0.89
PWS-23	34	4	5	682	20.24	14.1	3.38	9.0	1.04
PWS-24	33	2	3	667	20.63	12.8	3.14	11.0	1.27
PWS-25	36	4	4	930	18.06	10.1	2.18	10.0	0.75
PWS-26	21.5	2	2.6	462	14.05	3.6	0.600	7.0	.62
PWS-27	40	4	4.5	1302	18.31	15.8	3.47	11.2	0.92
PWS-28	43	7	5	1050	16.28	13.0	2.73	15.0	1.04
PWS-29	37	4	4	850	14.61	17.2	4.12	16.5	1.45
PWS-30	34	3	4	693	16.48	16.3	5.34	13.6	1.37
PWS-31	30.5	4	3.5	724	16.64	6.3	1.07	7.0	0.80
PWS-32	30	4	4	930	15.24	12.1	1.32	6.5	0.74
PWS-33	27	2	4.2	832	13.43	4.7	0.970	8.5	0.64
PWS-34	28	4	3.5	840	15.04	6.4	1.34	7.0	0.55
PWS-35	30	5	4	720	20.94	10.9	2.30	15.0	1.59
WS-20	25	4	3	693	17.80	8.6	1.98	14.6	1.98
Mean	34.049	3.879	3.8712	896.37	16.200	15.48	3.74	12.02	1.234
CD at 5%	2.0598	1.164	1.124	33.03	0.66912	2.136	0.26065	0.5269	0.6371

al. 1998). However such studies are unreliable as these are influenced by environmental effects. DNA based markers are much more efficient as they are developmentally stable, detectable in all tissues and remain uninfluenced by environmental factors (Negi *et al.* 2006).

In the present study we have used RAPD markers to assess the genetic variations in 36 morphotypes of ashwagandha, at intraspecific level, including one released variety WS-20 (Nigam *et al.*1991). The phenotypic characters of these morphotypes are summarized in Table 1.

MATERIALS AND METHODS

35 accessions were selected from a composite population grown at Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar. The source population was taken from Neemuch area of Madhya Pradesh (M.P.).These genotypes were cultivated for a period of complete vegetative growth and each one was selected on the basis of discrete phenotypic descriptors and Selection No. PWS (Pant *Withania* selection) was assigned for them. The one released variety WS-20 (from Manasa, M.P.) was used for a complete analysis of intraspecific genetic

Table 2: List of random primers used for RAPD analysis, revealing polymorphism.

Sl. No.	Primer used	Primer sequence (5′→3′)	Total no. of RAPD loci	Polymorphic loci	
				No.	%
1.	T-6	5′CGGCCACTGT3′	9	9	100
2.	BL-26	5′CTAGCTGACG3′	9	9	100
3.	Oligo-652	5′CCCAACACAC3′	8	8	100
4.	OPD-15	5′CATCCGTGCT3′	10	10	100
5.	OPG-15	5′ACTGGGACTC3′	4	4	100
6.	OPT-17	5′ACTGGGACTC3′	7	7	100
7.	Oligo-291	5′AGCTGAAGAG3′	7	6	85.6
8.	Oligo-402	5′CCCGCCGTTG3′	5	5	100
9.	Oligo-410	5′CGTCACAGAG3′	9	9	100
10.	Oligo-415	5′GTTCCAGCAG3′	7	7	100

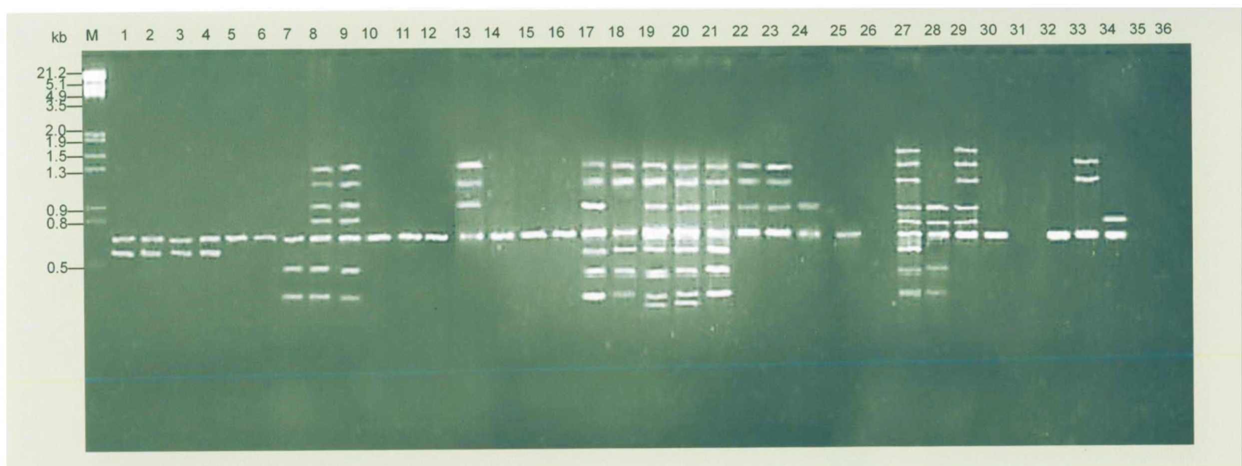

Figure 1: RAPD profile of *Withania somnifera* (L.) Dunal genotypes using Oligo-410 primer on agarose gel

variance. For RAPD analysis genomic DNA was isolated by SDS method (Dellaporta *et al.,* 1983). One gram of fine leaf powder sample was used, crushed with liquid nitrogen in a mortar with the aid of pestle, for DNA extraction using 500 ml of extraction buffer (1 M Tris-Cl, pH 8.0, 5 M NaCl, 0.5 M Na_2EDTA, 10% SDS). The homogenate was centrifuged to remove cell debris. The supernatant was treated with RNAse and DNA was precipitated by 95% alcohol. The quality was assayed by running DNA on a 0.8% Agarose gel while quality and quantity was assayed by spectrophotometrically. PCR amplification was carried out in 25 l reaction mixture containing 20 ng template DNA, 40 ng primer, 200 M of dNTPs, 1U Taq polymerse and 10 X assay buffer with 1X15 mM $MgCl_2$ (Banglore Genei Pvt. Ltd., Banglore, India). Initially 16 decamer random primers (Lifetech, New Delhi and Genei, Banglore) were used for RAPD amplification as described by Williams *et al.* (1990). The amplification cycle consisted of denaturation for 1 min at 94^0C, 2 min. annealing at 36^0C followed by extension upto 2.5 min at 72^0C and final extension after last cycle up to 10 min. The

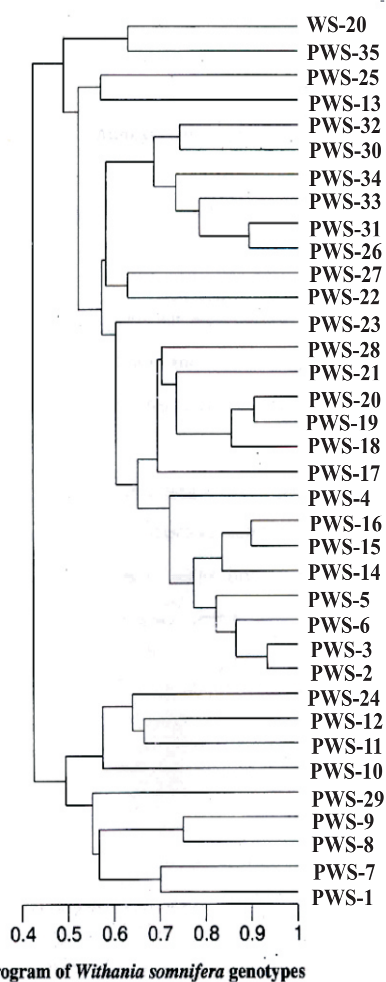

Figure. 2: Dendrogram of *Withania somnifera* genotypes as revealed by RAPD marker

PCR products were resolved by electrophoresis on 2% agarose gel, obtained from Sigma, in 1XTAE buffer and ethidium bromide stained gels were documented with gel dock (Alpha Imager™ 2200). Reproducibility of RAPD assay was confirmed by using duplicate reaction and only the reproducible bands were scored.

Data were scored on the basis of presence (1) or absence (0) of amplification products. Bands sharing data were used to calculate the genetic similarity based on Jaccard's similarity coefficient (Jaccard 1908) and UPGMA (unweighted Pair Group Method using Arithmetic Averages) algorithm was employed to determine the genetic relations of 36 genotypes of ashwagandha. All analysis were performed using NTSYS-PC 2.10 software (Rohlf 2002)

RESULTS AND DISCUSSION

Based on their amplification proficiency, ten decamer primers were used for the detection of RAPD loci among 36 genotypes ashwagandha. The percentage polymorphisms of ten decamer primers are summarized in Table-2. The amplification profile generated by a decamer primer(oligo-410) is depicted in Fig.1.

The total 76 RAPD loci were found to be amplified by 10 decamer primers. No monomorphic loci were detected in any primer. However, one unique band was observed in PWS-25 with respect to oligo-291 primer. The highest numbers of RAPD loci were recorded with primer OPD-15(10) and oligo-410 (9) and least number of bands were observed in OPG-15(4).This revealed 98.6% polymorphism with respect to 10 primers. Two primers oligo-410 and T-6 were observed to be the best from 10 since they showed amplification profiles for most of the genotypes with 100% polymorphism.

The similarity coefficient ranged from 0.220 between PWS-22 and PWS-8 to 0.930 between PWS-2 and PWS-3. The dendrogram readily separates the 36 genotypes into two major clusters 'A' and 'B' (Fig. 2). The major cluster, 'A' consisting of nine genotypes was further divided into two minor clusters 'A1' and 'A2'. 'A1' consists of five genotypes in which PWS-29 showed 54% similarity to other members of same sub-group. Similarly, 'A2' consists of four genotypes in which PWS-10 was 55% similar to the same sub-group members. 'A1' and 'A2' together showed 48% similarity. The major sub-group 'B' consisted of 27 genotypes including one released variety WS-20. This major group was sub-divided into six minor groups 'B1' to 'B6'. The 'B1' comprising seven genotypes in which PWS-2, PWS-3 and PWS-16, PWS-15 with 93% and

90% similarities were potential duplicates. The 'B2' includes five genotypes in which PWS-20 and PWS-19 showed 90% similarity. The PWS-4 and PWS-17 were neither part of 'B1' nor 'B2'. These were divergent lines in which PWS-4 showed 72% similarity to 'B1' and 66% similarity to PWS-17. Similarly PWS-17 showed 70% similarity to 'B2'. Together with 'B1' and 'B2' PWS-17 and 4 showed 60% similarity to another divergent line PWS-23. 'B3' consists of two genotypes PWS-27 and PWS-22 which were 64% similar. 'B4' consists of six genotypes in which PWS-31 and PWS-26 were 88% similar. 'B3' and 'B4' were 56% similar and together with PWS-23 were 52% similar to 'B5' and 50% to 'B6'. 'B5' contained PWS-25 and PWS-13 which were 56% similar. 'B6' consisted of one released variety WS-20 and PWS-35 which were 62% similar. Both 'A' and 'B' major groups were 42% similar.

These findings indicate that high level of polymorphism exhibited at intra-specific level in ashwagandha genotypes. Similar findings were reported by Negi (2000) while studying the genetic variation among and within *Withania* species. A similar observation was reported in *Withania somnifera* by Mir *et al*. (2010). Gilani *et al*. (2009) analyzed seven populations of *Withania coagulans* from the districts of Kohat and Karak in Pakistan. This information may be useful for the management of genetic resource collection, unequivocal identification, characterization, conservation and utilization of this important medicinal plant. However, the genetic diversity as revealed by RAPD based molecular markers, is yet to be supplemented with more sophisticated co-dominant markers such as AFLP, RFLP, SSR which will reveal broad spectrum variability existing in plant genotypes. Furthermore, these RAPD markers can be correlated with withanolides contents which have potential medicinal and pharmacological utility. From this account it is also clear that these selection lines have some divergent pool as comparison to variety, WS-20. Hence, these can be useful for breeding program to develop new varieties of *Withania somnifera* with some improved characters.

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