

Assessment of Genetic Variability in Ashwagandha [*Withania somnifera* Dunal]

MENKA, S.K. JAIN, KALYANI SRINIVASAN¹, S.K. LAL², R.L. SAPRA² AND C.P. SINGH

Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi 110 012
menka.smile@gmail.com

ABSTRACT The present paper makes an assessment of genetic variability in ashwagandha [*Withania somnifera* (L.) Dunal] based on morphological traits related to plant growth habit, root, leaf, flower, berry and seed. Mahalanobis D² statistics was used to classify the 20 accessions collected from different sources. The accessions investigated were grouped into 5 distinct clusters using Tocher's method. Cluster I being the largest comprised 6 genotypes, whereas cluster II, III, IV and V had 1, 3, 5 and 5 accessions, respectively. Clusters II and III were distantly located (13.387), whereas cluster I and III were closet (3.176).

Key words: Morphometric, genetic variability, accessions, cluster, ashwagandha

Ashwagandha is a multipurpose plant of immense therapeutic value and has a wide geographic distribution in India. It is among one of the ten most important medicinal crop plants designated by National Medicinal Plants Board. It is easy to cultivate and is ready for harvest within one year of growth. The plant contains the alkaloids withanine and somniferine, which are used to treat nervous disorders [1], intestinal infections and leprosy. All plant parts are used including the roots, bark, leaves [2], fruit and seed. Now a days leaves are being widely used to control obesity. Drug obtained from root is regarded as tonic and aphrodisiac. It has narcotic, anti-stress [3], anti-oxidant [4], immunomodulatory [4] and diuretic properties. The fruit is diuretic; it is useful in curing hiccup, reproductive disorders, rheumatism and dropsy.

The first and foremost step for improvement in any crop is to study the genetic diversity in its available germplasm. Ashwagandha displays an appreciable spectrum of variability in its morphological traits. Atal and Schwarting [5] documented five different morphological forms of

Withania somnifera in varied ecological regions of India. Khanna *et al.* [6] based on seed protein for morphometric characterization, identified five ashwagandha accessions. Kumar *et al.* [7] studied morphological and chemical variation in 25 elite accessions of wild and cultivated ashwagandha populations in India. Seven morphometric characters namely, plant height, number of branches/plant, number of seeds/berry, root yield, root length, root diameter and berry colour were investigated. They suggested that height of the plant, colour of the berry are closely associated with root yield and withanolide content. The cultivated plant type (cv. JA-20) was shorter in height [8]. The present study was an attempt to assess the detailed morphological variability among 20 accessions collected from different sources.

MATERIALS AND METHODS

Twenty genotypes of ashwagandha were collected from varied ecological regions of the country from different sources (Table 1). These were evaluated for assessing the morphometric

¹Division of Germplasm Conservation, NBPGR, New Delhi 110 012

²Division of Genetics, Indian Agricultural Research Institute, New Delhi 110 012

variability based on Minimal Descriptors of Agri-horticultural Crops [9]. Randomized block design (RBD) with two replications was used to evaluate their performance.

The data was statistically analysed for each trait. The "F" test was used to test the significant differences among genotypes, whereas critical

difference (CD) was used to identify the difference between means of any two accessions. The analysis of variance (ANOVA) was done, following procedure of Panse and Sukhatme [10]. The cluster analysis was performed to identify the diverse genotypes. Mahalanobis [11] generalized distance estimated by D^2 statistics was used for the estimation of genetic distance.

Table 1. Details on the genotype number and its source

Genotype No.	Variety/ Wild species/Genotype	Institution	Source	Year of collection
1	Poshita	CIMAP, Lucknow	Government Institute	2008
2	JA-20	JNKVV, Jabalpur	Government Institute	2008
3	JA-134	JNKVV, Jabalpur	Government Institute	2008
4	L-1	JNKVV, Jabalpur	Government Institute	2004
5	L-3	JNKVV, Jabalpur	Government Institute	2004
6	Genotype (Yellow berries)	IARI, New Delhi	Government Institute	2008
7	Genotype (Red berries)	IARI, New Delhi	Government Institute	2008
8	Genotype (Mixture of yellow+red berries)	IARI, New Delhi	Government Institute	2008
9	Wild spp	IARI, New Delhi	Government Institute	2007
10	Genotype	NRCMAP Anand	Government Institute	2007
11	Genotype	IARI, New Delhi	Government Institute	2007
12	Genotype	JNKVV, Jabalpur	Government Institute	2006
13	Genotype	JNKVV, Jabalpur	Government Institute	2005
14	Genotype	JNKVV, Jabalpur	Government Institute	2004
15	Genotype	NRCMAP, Anand	Government Institute	2004
16	Genotype	Patanjali, Haridwar	NGO's	2008
17	Genotype	Kharibaoli, Delhi (Maharashtra)	Seed Trader	2007
18	Genotype	Kharibaoli, Delhi (Rajasthan)	Seed Trader	2007
19	Genotype	Kharibaoli, Delhi, Madhya Pradesh	Seed Trader	2007
20	Genotype	Kharibaoli, Delhi (Madhya Pradesh bordering Rajasthan)	Seed Trader	2007

Note: Seed traders/NGO's, engaged in the line for the last two decades or more

Tocher's [12] method was used to classify the genotypes into distinct clusters.

RESULTS AND DISCUSSION

ANOVA for different morphometric traits showed that there were significant differences among the accessions for all quantitative traits studied except number of secondary roots/plants (Table 2). Below we describe the salient features in the variability for different traits.

Plant growth habit

All the genotypes including three aswagandha varieties planted in the field showed semi-erect nature of plant growth. In the early stages of growth it showed erect nature but at maturity it turns into semi-erect plant. There was wide variation in plant height of different accessions and varieties. The maximum value of plant height (116.0 cm) was registered for variety Poshita, whereas a minimum value (22.55 cm) was registered for variety JA-134 (Table 2). Two genotypes namely, JA-20 (74.10 cm), variety from JNKVV, Jabalpur and wild species (76.65 cm) were statistically at par. The maximum number of branches (6.8) was observed in Poshita, whereas minimum number of branches (2.5) was observed in genotype No. 10 and 12, *i.e.* genotype and a variety JA-20 from Directorate of Medicinal and Aromatic Plants, Anand.

Root characters

As mentioned earlier, ANOVA showed significant variations among all 20 accessions studied for root characteristics namely, root length, root diameter, fresh weight and dry weight (Table 2), except for number of secondary branches. However, all the 20 accessions were alike for its three qualitative characters, namely root colour, internal root colour and texture. Root length measured at the time of crop harvesting registered a maximum value (22.65 cm) for Poshita, a variety from CIMAP, Lucknow, whereas a minimum value (10.35 cm) for a strain from JNKVV, Jabalpur (Table 2). Poshita also registered more root diameter (2.83 cm), whereas variety JA-134 registered a minimum value (1.35 cm). Fresh and dry weight of root in aswagandha

registered maximum values (74.0 g and 25.13 g) for Poshita and minimum values (7.50 g and 3.00 g), respectively for variety JA-134. Results suggest that Poshita variety can be easily identified on the basis of root characters only.

Leaf characters

ANOVA also showed significant variations among all 20 accessions studied for its three quantitative characters, namely leaf length, leaf width and leaf area. They also differed in leaf shape. The maximum registered values for leaf length, leaf width and leaf area were 9.425 cm, 4.400 cm and 22.575 cm², respectively, for variety Poshita, whereas minimum values (4.450 cm, 1.300 cm and 4.675 cm²) were recorded for genotype No. 3 (from Kharibaoli, Delhi) (Table 2). Observation on leaf shape from 90 days old plant registered, in general, ovate shape, whereas accession No. 9, 16 and 18 registered ovate-round shape (Fig. 1). Visual scoring of leaf colour at flowering stage in general, registered light green except for wild species, whereas Poshita variety depicted dark green.

Flower characters

ANOVA also showed significant variation among 20 genotypes for days to flower initiation from seed sowing. The seeds registered a mean value of 93 days for flowering. Poshita variety took maximum number of days for flower initiation (102 days), followed by genotypes (100 days) either procured from JNKVV, Jabalpur, or seed traders from Delhi (the seed stocks either from Madhya Pradesh or Maharashtra). Rest of the strains including varieties of JNKVV, *i.e.* JA-20 and JA-134 registered 90 days for flower initiation from seed planting (Table 2). Inflorescence type, *i.e.* axillary inflorescence was similar among all the accessions studied. Flower colour was dull yellow among most of the genotypes and yellow in genotypes Nos. 18, 19 and 20 (procured from seed traders Kharibaoli, Delhi).

Berry characters

ANOVA also registered significant variation among all the 20 genotypes for berry characters

Table 2. Plant characters in ashwagandha genotypes

Genotype No.	Morphometric character in ashwagandha															
	1*	2**	3*	4*	5*	6*	7*	8*	9*	10***	11 ⁺	12 ⁺⁺	13 ⁺⁺⁺	14	15	16*
1	6.8	116.00	2.50	22.65	2.83	74.00	25.13	9.425	4.400	22.575	102	46	5.900	0.750	0.640	356.25
2	4.5	74.10	2.00	14.95	2.10	21.51	8.60	5.850	2.250	7.150	90	40	6.050	1.310	0.730	262.50
3	3.1	22.55	1.75	10.90	1.35	7.50	3.00	5.650	2.250	6.825	90	40	6.275	1.000	0.710	268.75
4	3.5	41.15	1.50	11.75	1.85	13.16	5.26	5.225	2.600	11.003	100	45	7.713	1.165	0.823	45.00
5	3.3	26.60	1.75	10.55	1.70	11.11	4.45	5.250	2.400	8.400	90	40	5.850	1.560	0.780	88.75
6	3.6	26.70	1.75	11.25	1.65	10.32	4.13	6.525	1.375	7.175	90	40	6.175	1.743	0.740	117.50
7	3.5	33.80	1.25	11.50	1.95	25.33	10.63	5.400	1.350	5.500	90	40	6.300	1.870	0.952	131.25
8	6.5	43.00	1.00	11.65	2.07	27.59	12.23	5.100	2.175	7.050	90	40	6.225	1.475	0.835	126.25
9	3.3	76.65	2.75	11.75	2.15	15.31	6.13	6.050	2.300	7.825	90	40	6.275	1.000	0.710	268.75
10	2.5	24.70	1.75	10.95	1.39	7.89	3.16	6.125	2.300	7.175	90	40	5.975	1.673	0.830	63.75
11	3.0	42.20	2.50	11.48	2.05	13.98	5.60	6.100	2.025	7.700	90	40	5.850	1.653	0.720	180.00
12	2.5	41.40	2.25	11.68	2.08	14.55	5.09	7.250	3.500	6.600	90	40	6.925	1.415	0.825	80.00
13	3.9	26.10	1.25	10.42	1.55	9.640	3.85	5.250	2.300	8.825	90	40	5.750	0.955	0.700	93.75
14	3.7	26.70	1.75	10.35	1.70	10.85	4.34	6.725	3.525	9.900	100	42	5.750	1.528	0.690	100.00
15	3.9	30.25	2.00	11.23	1.60	9.85	3.94	5.625	1.925	6.250	90	40	6.125	1.678	0.800	127.50
16	2.9	43.90	1.75	12.70	2.17	18.96	7.58	5.675	2.800	9.550	96	41	6.750	1.611	0.770	90.00
17	3.1	35.15	2.00	11.43	1.70	10.96	3.84	5.275	2.050	7.250	100	45	6.625	1.528	0.690	126.25
18	2.9	27.45	2.00	11.25	1.57	9.65	3.38	5.700	2.225	9.803	92	45	5.950	1.225	0.740	103.75
19	5.8	26.90	1.75	11.25	1.49	9.01	3.16	4.450	1.300	4.675	100	45	5.825	1.770	0.930	195.00
20	3.6	34.65	1.50	11.43	1.73	11.16	4.46	5.450	2.575	7.200	90	40	6.650	1.690	0.880	118.75
Mean	3.8	41.00	1.8625	12.021	1.833	16.6049	6.3979	5.905	2.406	8.572	93	41.45	6.2469	1.4300	0.7237	147.10
CD	1.1	5.83	NS	0.269	0.201	2.835	0.759	1.445	0.551	3.205	1.52	1.52	0.436	0.06	0.33	74.66

Where, 1: No. of branches/plant, 2: plant height, 3: No. of secondary branches in root, 4: root length (cm), 5: root diameter (mm), 6: root fresh weight (g), 7: root dry weight (g), 8: leaf length (cm), 9: leaf width (cm), 10: leaf area (cm²), 11: days to flower initiation, 12: days to berries formation, 13: berry diameter (mm), 14: berry fresh weight (g), 15: berry dry weight (g), 16: No. of berries/plant,

*Mean value from four replicates of five plants in each replicate; **Mean value from five plants each, ***Measured using leaf area meter (five leaves/replicate) were selected at random from different upper, middle and lower region

+Mean value from four replicates of five plants in each replicates showing over 50% flowering, ++Mean value from four replicates of 10 berries in each replicate, +++measured in mm., using screw gauge.

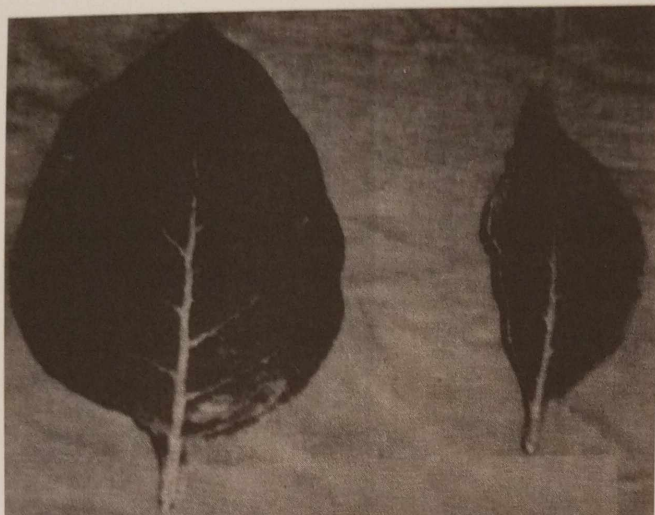


Fig. 1. Variation in leaf shape

(Table 2). Variety Poshita recorded maximum number of days (46 days). Genotypes Nos. 17, 18 and 19 procured from seed traders (Kharibaoli, Delhi) and one genotype No. 4 procured from JNKVV, Jabalpur, recorded 45 days to berry formation. Rest of the genotypes, in general required 40 days for berry formation. Berry diameter ranged between 5.750 mm (genotype Nos. 13 and 14) to 7.713 mm (genotype No. 4). Fresh weight ranged between 0.750 g (genotype No. 1: Poshita) to 1.870 g (genotype No. 7: Red berries purified and multiplied at IARI, New Delhi, during 2007-08). Dry weight of similar genotypes ranged between 0.640 g (Poshita) to 0.952 g (genotype No. 7). Poshita variety registered the maximum number of berries (356.25/plant), whereas genotype L-1 (No. 4) registered the minimum number of berries (45.00/plant). Visual scoring of berry colour at maturity, registered either yellow or red berries.

Seed characters

All the 20 accessions showed significant variation for seed quantitative characters, *i.e.* days to seed harvest, 1000-seed weight (TSW) and yield/plant. There was no variation in seed colour and seed shape. Mature seeds registered reniform shape and yellow colour. Data showed that days to seed harvest ranged between 180 and 186 days (Table 3). Seed yield/plant correspond to the number of branches/plant and plant height. It is the mean value from four replicates of five plants in each

replicate. Poshita registered maximum seed yield (29.03 g/plant), whereas JA-20 registered minimum seed yield (15.28 g/plant).

Cluster analysis

The 20 accessions could be grouped into 5 distinct

Table 3. Seed characters in ashwagandha

Genotype No.	DSH	Yield/plant* (g)	1000-seed weight (g)
1	185	29.03	1.95
2	180	15.28	2.05
3	180	24.83	2.00
4	185	19.70	1.86
5	180	18.50	1.96
6	180	20.78	2.13
7	180	18.68	1.87
8	180	20.28	2.02
9	180	16.13	2.37
10	180	20.78	2.25
11	180	18.95	2.20
12	180	20.25	2.32
13	180	17.17	2.31
14	185	18.50	1.80
15	180	16.18	2.50
16	180	21.00	2.08
17	186	19.40	2.28
18	186	17.25	2.37
19	186	17.55	2.40
20	180	24.70	2.16
Mean	181.65	19.61	2.14
CD	1.879	1.544	

Where, DSH: Days to seed harvesting from seed sowing,
*Mean value from four replicates of five plants in each replicate

Table 4. Clustering pattern of 20 ashwagandha genotypes

Cluster	No. of accession	Genotype No.	Geographical location
I	6	6, 7, 8, 10, 15, 20	New Delhi, Anand, Gujarat, Madhya Pradesh
II	1	Poshita	Lucknow, Uttar Pradesh
III	3	5, 13, JA-134	Jabalpur, Madhya Pradesh
IV	5	9, 11, 12, 16, JA-20	New Delhi, Jabalpur, Madhya Pradesh, Haridwar, Uttarakhand
V	5	4, 5, 17, 18, 19	Madhya Pradesh, Maharashtra, Rajasthan

Table 5. Cluster mean for different morphological characters of ashwagandha genotypes

Mean performance	Cluster				
	I	II	III	IV	V
Plant height (cm)	32.18	116.00	25.08	55.65	31.47
No. of branches/plant	3.93	6.75	3.41	3.22	3.79
Leaf area (cm ²)	6.72	22.58	8.02	7.77	9.13
Leaf width (cm)	2.01	4.40	2.32	2.58	2.44
Leaf length (cm)	5.70	9.42	5.38	6.19	5.47
Berry diameter (mm)	6.24	5.90	5.96	6.37	6.37
Berry fresh weight (g)	1.69	0.75	1.17	1.40	1.44
Berry dry weight (g)	0.80	0.64	0.50	0.73	0.77
No. of berries/plant	114.17	356.25	150.42	176.25	114.00
No. of seeds/berry	31.00	28.25	29.50	28.75	30.05
Days to flower initiation	90.00	102.50	90.00	91.25	98.50
Days to berry formation	40.00	46.00	40.00	40.25	44.40
Days to seed harvesting	180.00	185.00	180.00	180.00	185.60
Seed yield/plant (g)	17.96	29.02	16.98	21.86	19.02
Root length (cm)	11.33	22.65	10.62	12.51	11.07
Root diameter (cm)	1.33	2.43	1.13	1.73	1.26
Fresh root weight/plant (g)	15.36	7_4.00	9.42	16.86	10.73
Dry root weight/plant (g)	6.42	25.12	3.77	6.60	3.99
No. of secondary branches in roots	1.54	2.50	1.58	2.25	1.80

Table 6. Inter cluster and intra cluster distances among ashwagandha genotypes

Cluster	I	II	III	IV	V
I	2.429				
II	13.225	0.000			
III	3.176	13.387	2.142		
IV	3.325	11.268	3.898	2.558	
V	3.790	12.267	4.282	4.214	2.678

clusters. The largest cluster, *i.e.* cluster I comprised 6 genotypes, whereas cluster II, III, IV and V had 1, 3, 5 and 5 accessions, respectively (Table 4). The genotypes within a cluster have smaller D^2 values. The genotypes falling in highly divergent groups may help in identifying contrasting parents needed for carrying out the breeding programme and generating the variability.

The mean value of cluster showed the average performance for various characters studied. The genotypes tended to be grouped together in separate clusters on the basis of low, moderate or high mean values for different morphological characters (Table 5). Cluster means registered considerable differences among the clusters. Cluster I exhibited highest expression for the berry fresh and dry weight (0.80 g) and no. of seeds/berry, whereas traits, *viz.* leaf area, leaf width, berry diameter, days to flower initiation, days to early berry formation, days to seed harvesting and no. of secondary branches in roots had minimum expression (Table 5). The genotypes of Cluster II exhibited maximum plant height, number of branches/plant, leaf area, leaf length, leaf width, number of berries/plant, days to flower initiation, days to berry formation, seed yield/plant, root length, root diameter, fresh weight of root/plant and dry weight of root/plant, whereas it showed minimum expression for berry diameter, berry fresh weight and berry dry weight (Table 5). However, the remaining traits had moderate expression. Cluster III recorded minimum plant height, leaf width, leaf length, days to flower initiation, days to berry formation,

berry dry weight, days to seed harvesting, seed yield/plant, root length, root diameter, fresh and dry weight of root/plant, whereas the remaining traits had moderate expression. Cluster IV showed minimum number of branches/plant, leaf area and days to berry formation, whereas remaining traits had moderate expression. The genotypes of Cluster V exhibited minimum number of berries/plant and maximum days to seed harvest (Table 5), whereas rest of the characters showed moderate expression.

It was observed that average intra cluster distance ranged from 0.00 to 2.678, whereas inter cluster distance ranged between 3.176 and 13.387 (Table 6). Cluster II, which comprised of variety Poshita only, had no intra cluster distance, whereas cluster V had the maximum intra cluster distance ($D=2.678$). The highest inter cluster distance was between II and III (13.387), thus, indicating wide diversity among genotypes of these two clusters. The least inter cluster distance was between I and III (3.176). Therefore, the genotypes from cluster II and III may prove better in crop improvement for different traits by producing desirable hybrid combination. These genotypes may further be utilized in selection of ashwagandha varieties or hybrids for desirable economic characters. The clustering pattern indicated that the genotypes did not cluster as per geographical distribution (Table 4).

The present study showed extensive variation among the genotypes and varieties. Depending upon the aim of crop improvement, potential lines can be selected from different clusters as parents in hybridization programme. The contrasting parents from different clusters can be used in breeding programme and desirable traits can be incorporated in the improved cultivars.

ACKNOWLEDGEMENTS

Authors are thankful to the Head, Division of Seed Science and Technology, IARI, New Delhi, for facilities and first author, to ICAR, New Delhi, for awarding J.R.F.

REFERENCES

1. BHATTACHARYA, S.K., K.S. SATYAN & S. GHOSAL (1997). Antioxidant activity of

- glycowithanolides from *Withania somnifera*. *Indian J. Expt. Biol.* **35**(3): 236-239.
2. DAS, J.M. & P.A. KURUP (1964). Mechanism of antibacterial action of antibiotic isolated from leaves of *Withania somnifera*. I. Reaction between the antibiotic & glutathione. *Indian J. Biochem.* **1**(3): 157-158.
 3. ARCHANA, R. & A. NAMASIVAYAM (1999). Anti stress effect of *Withania somnifera*. *J. Ethnopharmacol.* **64**(1): 91-93.
 4. BUDHIRAJA, R.D. & S. SUDHIR (1998). Review of biological activity of withanolides (Antibacterial, antitumor, immunomodulating, antiinflammatory and insect antifeedcent). *J. Sci. Ind. Res.* **46**: 488-491.
 5. ATAL, C.K. & A.E. SCHWARTING (1962). Intraspecific variability in *Withania somnifera*: A preliminary survey. *Lloydia* **25**(2): 78-88.
 6. KHANNA, P.K., A. KUMAR, A. AHUJA & M.K. KAUL (2006). Seed protein characterization for morphotype identification in *Withania somnifera* (L.) Dunal. *Indian J. Plant Physiol.* **11**: 3.
 7. KUMAR, A., M.K. KAUL, M.K. BHAN, P.K. KHANNA & K.A. SURI (2007). Morphological and chemical variation in 25 collections of the Indian medicinal plant, *Withania somnifera* (L.) Dunal (Solanaceae). *Genet. Resour. Crop Evol.* **54**: 655-660.
 8. ANONYMOUS (2006). Morphological comparison of cultivated and wild plant types of ashwagandha. *Newsletter for NRCMAP*, July-December **2**: 2.
 9. SINGH, B.M., R.K. MAHAJAN, UMESH SRIVASTAVA & S.K. PAREEK (2003). *Minimal Descriptors of Agri-horticultural Crops. Part IV: Medicinal and Aromatic Plants*. NBPGR pp. 31-35.
 10. PANSE, V.G. & P.V. SUKHATME (1967). *Advanced Statistical Methods for Agricultural Workers*. ICAR, New Delhi pp. 58-67.
 11. MAHALANOBIS, P.C. (1936). On the generalized distance in statistics. *Proc. Natl. Inst. Sci. India* **2**: 49-55.
 12. MISRA, H.O., J.R. SHARMA & R.K. LAL (1998). Genetic divergence in ashwagandha (*Withania somnifera*). *J. Med. Aromatic Plant Sci.* **20**(4): 1018-1021.
 13. RAO, C.R. (1952). *Advanced Statistical Methods in Biological Research*. John Wiley and Sons Inc., New York pp. 390.