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Genetic diversity in ashwagandha (W*ithania somnifera* (L.) Dunal)

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Abstract
Genetic divergence among 40 ashwagandha (*Withania somnifera* (L.) Dunal) accessions of different geographic origin was assessed using Mahalanobis D^2 statistics. Observations revealed significant genotypic differences and accordingly genotypes were classified into six clusters. Cluster I was the largest with thirty genotypes followed by II and III clusters which have four and three genotypes, respectively. Cluster IV, V and VI contained only one most divergent genotype. The maximum inter-cluster distance (1538.09) was found between cluster II and VI, followed by that between II and III (983.03). The minimum inter-luster distance was observed between cluster Iand II (285.09). Theintra-cluster distance (D) ranged from 142.22 (cluster-III) to 192.10 (cluster-I). The three clusters (IV, V and VI) contained single genotype each and therefore, their intra-cluster distances were zero. The genotype of cluster VI was unique as it was having highest values for leaf width, diameter of root at collar region with high dry root yield. The cluster II was desirable in respect of days to flower initiation and days to maturity and also had highest value for number of primary branches per plant, withanoloide content (%) and starch content. The cluster III exhibited highest value for plant height and leaf length. The cluster IV had highest mean values for number of secondary branches per plant and root length. Thus, hybridization among these genotypes can generate desirable transgressive segregants.

Keywords: D^2 statistics, cluster, genetic divergence, cluster composition.

Ashwagandha (*Withania somnifera* (L.) Dunal.), also known as Indian ginseng, (poison) gooseberry or winter cherry is a plant of the *solanaceae* family (Mir *et al*., 2013). The chromosome number is recorded as 2n=48. It is native of North-Western and Central India as well as Mediterranean region of North Africa, In India, two species of genus Withania *viz., Withania somnifera* (L.) Dunal (Ashwagandha) and *Withania coagulants* (L.) Dunal (Panir) are found. Ashwagandha is cultivated mainly in Madhya Pradesh, Rajasthan, Gujarat, Maharashtra, Punjab and Uttar Pradesh, whereas, *Withania coagulants* (L.) found in wild. It is late *kharif*crop and grows under dry climate or requires less irrigation for plant growth and rainfed cultivation. Mostly it is grown on marginal lands of Neemuch and Mandsaur districts of Madhya Pradesh and Kota, Jhalawar, Pratapgarh, Chittorgarh and Barandistricts of Rajasthan. Among the various medicinal plants, ashwagandha is cultivated over an area of 15,000 ha with a production of 60,000 q/ha and productivity of 4.00 q/ha (Patidar,2012)

in India. In Madhya Pradesh it is cultivated over an area of 8,000 ha with a production of 32,000 q and productivity of 4.00 q/ha. In Ayurveda, the roots of ashwagandha known to possess health maintenance and restoration properties, which are similar to ginseng roots, hence it is called as Indian ginseng. It is an adoptogenic herb and its roots, seeds and leaves are used in Ayurvedic and Unani medicines. The root drug finds an important place in treatment of rheumatic pain, inflammation of joints, nervous disorders, female disorders, hiccup, cold, cough, as a sedative, ulcers and leprosy etc.The main active constituents are alkaloids and steroidal lactones. The leaves contain the steroidal lactones, withanoloide. Ashwagandha root contains 0.4 - 1.2% alkaloids, 40-65% starch, 40-65% fibers and minor quantity of oil. The important chemical constituents are alkaloids (Withanoloide) that are present in roots, leaf and berries. Limited breeding work has been done in this important medicinal crop for developing high yielding varieties. There are strong possibilities to develop genotypes having high dry root yield and withanoloide content. ThereforeCharacteristic quantification of genetic variability has been a long major goal in breeding.Assessment of variability present in ashwagandha accessions is an important aspect for improvement of this crop.

The experimental material consisting of forty genotypes of ashwagandha were sown in randomized block design comprising three replications with two rows plot each of 3.0 m length, maintaining crop geometry of 45 x 10 cm at the research farm of Medicinal and Aromatic Plants Project, Anand Agricultural University, Anand during *Kharif* 2012-13. The recommended agronomical practices were adopted to raise a healthy crop. The experimental material was evaluated for twelve characters *viz.,* plant height

(cm), leaf length (cm), leaf width (cm), days to flower initiation, days to maturity, number of primary branches per plant, number of secondary branches per plant, root length (cm), diameter of root at collar region (cm), dry weight of root (g/plant), total withanoloide content (%) and starch content (%). The statistical analysis for quantitative characters was done on the basis of averages. Mahalanobis D^2(1936) statistic was employed to determine the degree of differentiation among n(n-1)/2 = 780 pairs of n=40 population. Grouping of genotypes into various clusters was done according to Tocher's method.

The analysis of variance revealed that the differences among the mean square due to genotypes were significant for all the characters indicating the presence of high amount of genetic variability for all the characters studied. The D^2values between all 780 pairs ranged from 32.32 (between RAS-23 and RAS-37) to 1664.21 (between MWS-202 and AWS-57), which indicated the presence of high genetic diversity among the genotypes for all the traits (these D^2 values were taken from matrix table, which is not given here).

Grouping of the genotypes was carried-out with the assumption that the genotypes within cluster have smaller D^2-values among themselves than those from groups belonging to different clusters. In all, 6 clusters were formed from 40 genotypes. The composition of clusters is given in Table 2. The cluster I was the largest cluster having 30 genotypes. Cluster II was the second largest which contained 4 genotypes. The cluster III was the third largest which contained 3 genotypes. The cluster IV, V and VI contained one genotype each. Similarly diverse genotypes of ashwagandha grouped in 8 clusterMisra *et al.,* (1998). Fifty-five diverse genotypes of ashwagandha were grouped into ten different clusters by Jain *et al.,* (2007). Gupta *et al.,* (2011) carried out similar type of genetic divergence study in 75 genotypes of ashwagandha and grouped them into fourteen clusters using Tocher's method. Inter and intra-cluster distances are shown in Table 3. The maximum inter-cluster distance (1538.09) was found between cluster II and VI, followed by that between II and III (983.03). The minimum inter-cluster distance was observed between cluster I and II (285.09). The intra-cluster distance (D) ranged from 142.22 (cluster-III) to 192.10 (cluster-I). The three clusters (IV, V and VI) contained single genotype each and therefore, their intra-cluster distances were zero. In the present investigation the inter cluster distance were higher than that of intra cluster distance which indicated substantial diversity among the parents and there may be a greater opportunity for obtaining the rare but superior segregants from crosses between plants with more diverse genotypes.

The cluster means for 12 characters are presented in Table 4. Estimates of inter and intra cluster variances, along with ratio (R^2) of inter cluster variance to the total variance and inter cluster coefficient of variation (CV_b) for 12 characters were worked out. Maximum value of R^2 (0.89) was observed for leaf length and dry weight of root, followed by leaf width and root length and minimum value for R^2 (0.33) was observed for number of primary branches per plant. From inter cluster coefficient of variation (CV_b) it was revealed that the dry weight of root per plant contributed maximum (56.24%) towards the total divergence in yield. The next major contribution came from the leaf width (27.24%) towards divergence in yield followed by root length (25.68 %), starch content (24.48 %)and leaf length (24.24 %) respectively. Apart from above mentioned traits, other characters *viz.,* plant height (17.95%), days to flower initiation (11.14%), number of primary branches per plant (13.61%), number of secondary branches per plant (20.06) had moderate to low contribution towards the total divergence, while days to maturity (5.44%) contributed negligible towards the total divergence in yield.

Wide ranges of mean values among the clusters were recorded for different traits. The cluster VI had the highest mean values for leaf width (5.76 cm), diameter of root at collar region (1.32 cm) and dry weight of root (3.93 g). The cluster II was desirable in respect of phonological characters like days to flower initiation (58.83) and days to maturity (124.41) and also had highest value for number of primary branches per plant (5.93), withanoloide content (0.376%) and starch content (32.17%). The cluster III exhibited highest value for plant height (61.28 cm) and leaf length (10.93 cm) The cluster IV had highest mean values for number of secondary branches per plant (3.20) and root length (23.10 cm). The results obtained in the present study are in accordance to the findings of Misra *et al.,* (1998), Jain *et al.,* (2007). It has been well established fact that morethe genetically diverse parents used in hybridization programme, the greater will be the chances of obtaining high heterotic hybrids and broad spectrum variability in segregating generations. It has also been observed that the most productive varieties may come from high yielding parents/selections with a high genetic diversity. Therefore, in the present investigation, based upon high yielding genotypes and large inter-cluster distances, it is advisable to attempt crossing/ selection of the genotypes from clusters II (RAS-65, MWS-202, MWS-203, MWS-208) and III

(HWS-08-14, AWS-32, AWS-63) with the genotype of cluster VI (AWS-57).

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Table 1: Grouping of 40 genotypes of Ashwagandha in various clusters on the basis of D^2 statistics

Cluster	No. of genotypes	Name of genotypes
I	30	IC 283966, IC 310620 (A), RAS-10, RAS-23, RAS-28, RAS-29, RAS-37, RAS-39, RAS-46, RAS-51, RAS-120, RAS-143, MWS-100, MWS-132, MWS-206, MWS-214, MWS-318, MWS-327, HWS-08-18, AWS-1, AWS-27, AWS-42, AWS-45, AWS-53, AWS-56, AWS-65, AWS-66, JA-20, JA-134, RVA-100
II	4	RAS-65, MWS-202, MWS-203, MWS-208
III	3	HWS-08-14, AWS-32, AWS-63
IV	1	AWS-60
V	1	RAS-63
VI	1	AWS-57

Table 2: Average inter and intra- cluster distance ($D=\sqrt{D^2}$) values in Ashwagandha

Clusters	I	II	III	IV	V	VI
I	192.10	285.09	647.27	344.08	364.87	917.77
II		147.68	983.03	527.74	568.68	1538.09
III			142.22	433.02	303.94	336.72
IV				0.00	287.40	809.74
V					0.00	519.56
VI						0.00

Note: Diagonal values are Intra cluster distance

Table 3: Cluster means for different characters in Ashwagandha

Clusters	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Days to flower initiation	Days to maturity	No. of Primary branches per plant	No. of Secondary branches per plant	Root length (cm)	Diameter of root at collar region (cm)	Dry weight of root (g/plant)	Withanoloide content (%)	Starch content (%)
I	46.52	8.11	3.95	67.68	131.64	4.79	2.84	16.13	1.11	1.86	0.278	25.46
II	46.40	7.53	3.75	58.83	124.41	5.93	3.16	15.30	1.00	1.43	0.376	32.17
III	61.28	10.93	5.29	75.88	139.77	4.62	2.77	22.43	1.30	3.26	0.291	31.79
IV	46.90	9.31	4.69	63.00	133.66	5.00	3.20	23.10	0.91	1.80	0.194	29.01
V	43.60	10.33	5.64	69.66	141.00	4.33	1.26	16.63	1.13	1.80	0.330	26.71
VI	54.00	10.88	5.76	73.33	138.33	4.73	2.33	18.70	1.32	3.93	0.187	13.85
Mean	47.74	8.42	4.13	67.49	131.98	4.89	2.82	16.77	1.11	1.97	0.285	26.44
S.Em.	2.47	0.32	0.19	3.52	3.68	0.42	0.27	0.77	0.04	0.17	0.027	2.78
C.V.%	11.55	8.58	10.68	11.65	6.22	19.47	21.88	10.32	9.61	19.61	21.60	23.46
$C.D._{0.05}$	6.93	0.90	0.55	9.88	10.31	1.19	0.77	2.17	0.13	0.48	0.077	7.79
R^{2*}	0.71	0.89	0.87	0.48	0.43	0.33	0.46	0.86	-	0.89	-	0.52
CV_b %	17.95	24.24	27.24	11.14	5.44	13.61	20.06	25.86	-	56.24	-	24.48

* $R^{2:}$ Ratio of the inter cluster variance to the total variance, - : Not estimated due to –ve variance, CV_b : Inter cluster coefficient of variation