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Genetic diversity analysis in ashwagandha (*Withania somnifera* L.)

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Abstract
The experiment was laid out in a completely Randomized Block Design with 29 ashwagandha accessions as treatments during *Kharif*, 2018 at Medicinal and Aromatic Plant Research Station, Sri Konda Laxman Telangana State Horticultural University, Rajendranagar, Hyderabad. Each treatment was randomly replicated thrice. All the germplasm lines were evaluated systematically for grouping them into different clusters using Mahalanobis D^2 statistical analysis. The results indicated highly significant difference among the genotypes and these genotypes were classified into 12 clusters. Cluster I is the largest group comprising of 9 genotypes followed by cluster II with 8 genotypes, cluster VII with 3 genotypes, whereas clusters III, IV, V, VI, VIII, IX, X, XI and XII were monotypic or solitary. The intra cluster distance varied from 0.0 to 2326.3. Cluster VII recorded maximum D^2 value (2326.3) followed by cluster II (1546.1) and cluster I (1248.2). Intra cluster distances were not observed in cluster III, IV, V, VI, VIII, IX, X, XI and XII. The inter cluster D^2 values revealed that the highest inter cluster distance (23780.9) was between cluster VII and XII, while the lowest (1189.3) was between cluster IV and V. The inter cluster distance was minimum between cluster IV and V (1189.3) indicating narrow genetic diversity, whereas the inter cluster distance was maximum between VII and XII (23780.9) followed by VI and XII (22140.8) indicating wider genetic diversity between these groups. Selection of parents from these diverse clusters for hybridization would help in achieving novel recombinants. Maximum mean value for dry leaf weight per plant was recorded in cluster IX (67.32 g) followed by cluster V (41.56 g). The highest dry root weight per plant recorded in the genotypes of cluster IX (13.78 g) followed by cluster VIII (9.81 q), while genotypes of clusters III (0.90g) recorded the lowest dry root weight per plant. The highest alkaloid was noticed in cluster VII (0.39) followed by cluster IX (0.34) and the lowest alkaloid was recorded in the genotypes of cluster X (0.20). The wide range of mean values among the clusters and the characters studied indicates the presence of wide variation among the genotypes studied.
Therefore, in the present investigation, based upon high yielding and high alkaloid genotypes with large intra and inter-cluster distances, it is advisable to attempt crossing between the genotypes from clusters IX (NMTLI-101), cluster XI (CIM-Chetak) and the genotype of cluster VII (RAS-65, MWS-218, Poshita).

Keywords: Ashwagandha, genetic diversity, intra and inter cluster distance

Introduction
Ashwagandha (*Withania somnifera* L.) belongs to the family Solanaceae with chromosome number 2n = 48. Ashwagandha is one of the most popular medicinal crops being commercially cultivated as a dry land crop in late *kharif* season in India. It is commonly known as Indian Winter Cherry, Asgandh and Indian Ginseng. The origin of ashwagandha is North-Western and Central India as well as Mediterranean region of North Africa (Srivastava *et al*., 2017) [17]. The plant is an evergreen erect under shrub which is 30-150 cm tall and it produces flowers indeterminately round the year with a peak of flowering between March and July (Mir *et al*., 2012) [12]. High pollen load on the stigma and stiff pollen competition within a flower strongly favours self-pollination (Mir *et al*., 2012) [12].
The economic part of ashwagandha is root which is rich in alkaloids, steroidal lactones and saponins. The medicinal properties of the root are attributed to the chemical quality, i.e., alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins) and saponins containing an additional acyle group (Sitoindoside VII and VIII) content (Gupta and Rana, 2007) [8]. The total alkaloid content in the Indian roots range between 0.13% and 0.31%. Withaferin A and Withanolide D are the two main withanolides which contribute to most of the biological activity of ashwagandha (Matsuda *et al*., 2001) [10]. The commercial value of roots depends upon the physical (textural) quality and root morphology.

Brittle, robust and lengthy roots have high market value (Misra *et al*., 1998) [13].

Ashwagandha roots have a tremendous medicinal value and constituent of various formulations in the traditional Indian medical systems such as Ayurveda, Unani and Siddha. (Sharma *et al*., 2014) [15]. It has anti-stress (Bhattacharya and Muruganandam, 2003) [3], immunomodulatory, cytotoxic, anti-bacterial, antifungal, and immunosuppressive properties (Atta-ur-Rahman *et al*., 1998) [2], treatment of rheumatic pain, inflammation of joints, female disorders, hiccups, coughs and colds, ulcers, leprosy, as a sedative *etc*, (Al-Hindwani *et al*., 1992) [1]. The bruised leaves of this plant are used in the treatment of tumors, tubercular glands and as an anti-inflammatory agent (Jayaprakasam *et al*., 2003; Chopra, 1994) [9, 4] due to its antibacterial, antifungal, and antitumor properties (Devi *et al*., 1993) [6].

One of the important factors restricting the large-scale production and development of better varieties is the availability of meagre information about the genetic diversity, inter and intra-specific variability and genetic relationship among ashwagandha genotypes. Evaluation of germplasm has an immense importance in genetic improvement of the crop for achieving higher yields and productivity. Genetic diversity analysis assists in interpreting the genetic background and breeding value of the germplasm.

Materials and Methods

The experiment was laid out in a completely Randomized Block Design with 29 ashwagandha accessions as treatments during *Kharif*, 2018 at Medicinal and Aromatic Plant Research Station, Sri Konda Laxman Telangana State Horticultural University, Rajendranagar, Hyderabad. Each treatment was randomly replicated thrice.

The experimental material comprised of 29 germplasm lines of ashwagandha were obtained from DMAPR, Anand, Gujarat; CIMAP, Lucknow, Uttar Pradesh and PDKV, Akola, Maharashtra (Table 1.). The recommended agronomical practices were adopted to raise a healthy crop. The experimental material was evaluated for 25 characters *viz*., Plant height (cm), number of branches per plant, leaf length (cm), leaf width (cm), days to flower initiation, days to fruit formation, days to root harvest, fresh leaf weight per plant (g), dry leaf weight per plant (g), number of berries per plant, berry diameter (cm), number of seeds per berry, seed yield per plant (g), seed yield (q ha-1), main root length (cm), diameter of root (cm), number of secondary roots per plant, fresh root weight per plant (g), dry root weight per plant (g), fresh root yield (q ha-1), dry root yield(q ha-1), crude fiber estimation (%), starch estimation (%), starch and fiber ratio, total alkaloid content (%). All the germplasm lines were evaluated systematically for grouping them into different clusters using Mahalanobis D^2 statistical analysis.

Results and Discussion

The analysis of variance in 29 ashwagandha genotypes indicated highly significant difference among the genotypes for all the 25 quantitative characters studied, indicating the existence of adequate genetic diversity among the genotypes. In order to assess the genetic diversity among the 29 genotypes, D^2 statistic was carried out. Procedure suggested by Tocher (Rao, 1952) was used to group 29 ashwagandha genotypes into various clusters by treating estimated D^2 values as the square of the generalized distance. The pattern of distribution of 29 genotypes into various clusters is indicated in Table 2.

Out of 12 clusters formed, cluster I is the largest group comprising of 9 genotypes (AKAS-13, MWS-100, Red berry, MWS-132, RAS-67, AKAS-02, AKAS-11, RAS-57, IC-310620(B)) followed by cluster II with 8 genotypes (IC-283662, IC-310595, IC-286632, IC-283966, AKAS-10, CIM Pratap, RAS-28, RAS-7), cluster VII with 3 genotypes (RAS-65, MWS-218, Poshita), whereas clusters III (IC-310620(A)), IV (BHM-42), V (JA-134), VI (MWS-323), VIII (NMITLI-118), IX (NMITLI-101), X (MWS-324), XI (CIM-Chetak) and XII (IC-283942) were monotypic or solitary. Similarly, 37 diverse genotypes of ashwagandha were grouped into 8 clusters by Misra *et al*., (1998) [13]. Gupta *et al*., (2011) [7] carried out similar type of genetic divergence study in 75 genotypes of ashwagandha and grouped them into 14 clusters using Tocher's method.

The mean intra and inter cluster D^2 values among the various clusters are presented in the Table 3. The intra cluster distance varied from 0.0 to 2326.3. Cluster VII recorded maximum D^2 value (2326.3) followed by cluster II (1546.1) and cluster I (1248.2). Intra cluster distances were not observed in cluster III, IV, V, VI, VIII, IX, X, XI and XII. The inter cluster D^2 values revealed that the highest inter cluster distance (23780.9) was between cluster VII and XII, while the lowest (1189.3) was between cluster IV and V. The inter cluster distance was minimum between cluster IV and V (1189.3) indicating narrow genetic diversity, whereas the inter cluster distance was maximum between VII and XII (23780.9) followed by VI and XII (22140.8) indicating wider genetic diversity between these groups. Selection of parents from these diverse clusters for hybridization would help in achieving novel recombinants. Similar type of observations was reported by Misra *et al*. (1998) [13] and Gupta *et al*. (2011) [7]. The clusters with single genotype indicated their independent identity and importance due to various unique characters possessed by them.

The cluster means for each of 25 traits are presented in table 4. The genotypes belonging to cluster IX recorded the highest mean plant height (119.09 cm) followed by cluster XII (107.28 cm), while genotypes belonging to cluster IV recorded the lowest average plant height (47.20 cm). Number of branches per plant was maximum in cluster XII (7.20) followed by cluster VII (5.94), while minimum number of branches per plant was recorded in cluster XI (3.96). The maximum leaf length was recorded in genotypes of cluster XII (10.19 cm) followed by cluster IX (9.72 cm), whereas minimum leaf length was registered in genotypes of cluster X (4.41 cm). The highest leaf width was noticed in cluster XII (1.70 cm) followed by cluster IX (6.16 cm) and the lowest leaf width was recorded in the genotypes of cluster X (2.59 cm).

The traits, days to flower initiation recorded maximum value in the genotypes of cluster XI (119.9 days) followed by cluster VII (112.64 days)while genotypes of cluster V (61.22 days) exhibited minimum mean value for days to flower initiation, day to fruit formation recorded maximum value in the genotypes of cluster III (39.12 days) followed by cluster IV (39.06 days), while genotypes of clusters XII (30.89 days) exhibited minimum mean value for days to flower initiation and days to root harvest was minimum in cluster V (189.56 days) followed by cluster III (189.68 days) with maximum number of days to root harvest in cluster X (208.63 days).

The genotypes of cluster IX recorded the highest average fresh leaf weight per plant (295.92 g) followed by cluster XII

(177.84 g), while the genotypes of cluster X (32.72 g) recorded the lowest fresh leaf weight. Maximum mean value for dry leaf weight per plant was recorded in cluster IX (67.32 g) followed by cluster V (41.56 g). The minimum mean value recorded for dry leaf weight in genotypes of cluster X (9.24 g). The genotypes belonging to cluster V (378.86) recorded the highest mean number of berries per plant followed by cluster II (258.14), whereas the lowest number of berries per plant was recorded in cluster VIII (58.46). Maximum berry diameter was recorded in cluster VI (0.73 cm) followed by cluster VII (0.70 cm) with minimum berry diameter in cluster III (0.52 cm).

The number of seeds per berry was the highest in cluster XII (41.25) followed by cluster VIII (39.17), whereas the lowest number of seeds per berry was exhibited by genotypes belonging to the cluster IV (24.24). The genotypes of cluster V recorded the highest average seed yield per plant (6.72 g) followed by cluster II (6.02 g), while the genotypes of cluster IV (1.56 g) recorded the lowest seed yield per plant. The highest average seed yield per hectare was recorded in cluster V (14.93q) followed by cluster II (13.5 q). The lowest seed yield per hectare recorded in genotypes of cluster VI (3.46 q).

The maximum main root length was recorded in genotypes of cluster IX (18.4 cm) followed by cluster VIII (15.64 cm), whereas minimum main root length was registered in genotypes of cluster XII (8.59 cm). The highest root diameter was noticed in cluster IX (2.64 cm) followed by cluster VIII (2.15 cm) and the lowest root diameter was recorded in the genotypes of cluster IV (0.69 cm).Maximum number of secondary roots per plant was noticed in cluster XI (7.1) followed by cluster IX (6.35) and minimum number of secondary roots per plant was recorded in the genotypes of cluster III (1.94).

The highest fresh root weight per plant was exhibited in the genotypes of cluster IX (29.70 g) followed by cluster VIII (20.79 g), while genotypes of cluster III (2.68g) exhibited the lowest fresh root weight per plant. The highest dry root weight per plant recorded in the genotypes of cluster IX (13.78 g) followed by cluster VIII (9.81 q), while genotypes of clusters III (0.90g) recorded the lowest dry root weight per plant. The highest fresh root weight per hectare exhibited in the genotypes of cluster IX (66.00 q) followed by cluster VIII (46.20 q), while genotypes of cluster III (5.96 q) exhibited the lowest fresh root weight per hectare. The highest dry root weight per hectare recorded in the genotypes of cluster IX (30.63 q) followed by cluster VIII (21.80 q), while genotypes of clusters III (2.00 q) recorded the lowest dry root weight per hectare

The genotypes belonging to cluster III (24.29) recorded the lowest fiber content followed by cluster I (29.64), whereas the highest fiber content recorded in cluster IX (42.30). Maximum starch content was recorded in cluster VIII (15.87) followed by cluster IX (14.80) with minimum starch content in cluster III (9.51). The maximum starch fiber ratio was recorded in genotypes of cluster VII (0.45) followed by cluster VI (0.44), whereas minimum starch fiber ratio was recorded in genotypes of cluster XII (0.28). The highest alkaloid was noticed in cluster VII (0.39) followed by cluster IX (0.34) and the lowest alkaloid was recorded in the genotypes of cluster X (0.20). The wide range of mean values among the clusters and the characters studied indicates the presence of wide variation among the genotypes studied.

Hence, apart from selecting genotypes from the clusters which have high inter cluster distance for hybridization, one can also think of selecting parents based on extent of genetic divergence in respect to a particular character of interest. This means that, if breeders' intention is to improve root yield, he can select parents which are highly divergent with respect to these characters.

The choice of parents for heterosis breeding and expression of heterosis is influenced by genetic diversity of parents. Cress (1966) [5] demonstrated that 'genetic diversity' is necessary for significant heterosis but not sufficient to guarantee the same. Several reports indicate that hybrids between genetically diverse parents manifest greater heterosis than those between more closely related parents (Ram and Panwar, 1970 and Singh and Sharma, 1989) [14, 16]. In fact, such a conclusion is based upon a rather restricted range of genetic diversity and may not hold over the entire range of divergence encountered in a species. In general, the level of heterosis increases with the increase in parental diversity up to some limit and decreases with further increase in parental diversity owing to cross ability barriers. Thus, maximum heterosis occurs at an optimal or intermediate level of parental diversity. Further, the occurrence of heterosis cannot be predicted on the basis of genetic divergence alone (Matzinger and Werusman, 1958) [11]. Apart from the high degree of divergence, the mean performance of genotypes and the characters with maximum contribution towards divergence should also be given due consideration.

Therefore, in the present investigation, based upon high yielding and high alkaloid genotypes with large intra and inter-cluster distances, it is advisable to attempt crossing between the genotypes from clusters IX (NMTLI-101), cluster XI (CIM-Chetak) and the genotype of cluster VII (RAS-65, MWS-218, Poshita).

Table 1: List of genotypes used for evaluation along with their sources

S. No	Accession. No	Genotype	Source
1	A_1	AKAS-13	PDKV, Akola
2	A_2	AKAS-11	PDKV, Akola
3	A_3	AKAS-10	PDKV, Akola
4	A_4	AKAS-02	PDKV, Akola
5	A_5	MWS-324	DMAPR, Gujarat
6	A_6	MWS-100	DMAPR, Gujarat
7	A_7	MWS-132	DMAPR, Gujarat
8	A_8	MWS-323	DMAPR, Gujarat
9	A_9	MWS-218	DMAPR, Gujarat
10	A_{10}	RAS-7	DMAPR, Gujarat
11	A_{11}	RAS-28	DMAPR, Gujarat
12	A_{12}	RAS-57	DMAPR, Gujarat
13	A_{13}	RAS-65	DMAPR, Gujarat
14	A_{14}	RAS-67	DMAPR, Gujarat

15	A_{15}	IC-310620(A)	DMAPR, Gujarat
16	A_{16}	IC-310620(B)	DMAPR, Gujarat
17	A_{17}	IC-283662	DMAPR, Gujarat
18	A_{18}	IC-286632	DMAPR, Gujarat
19	A_{19}	IC-283966	DMAPR, Gujarat
20	A_{20}	IC-283942	DMAPR, Gujarat
21	A_{21}	IC-310595	DMAPR, Gujarat
22	A_{22}	Red berry	DMAPR, Gujarat
23	A_{23}	BHM-42	DMAPR, Gujarat
24	A_{24}	JA-134	DMAPR, Gujarat
25	A_{25}	NMITLI-118	CIMAP, Lucknow
26	A_{26}	NMITLI-101	CIMAP, Lucknow
27	A_{27}	CIM-Chetak	CIMAP, Lucknow
28	A_{28}	CIM-Pratap	CIMAP, Lucknow
29	A_{29}	Poshita	CIMAP, Lucknow

Table 2: Distribution of 29 genotypes of ashwagandhain different clusters (Tocher's method)

Cluster	***Kharif*, 2018**
I	AKAS-13, MWS-100, Red berry, MWS-132, RAS-67, AKAS-02, AKAS-11, RAS-57, IC-310620(B),
II	IC-283662, IC-310595, IC-286632, IC-283966, AKAS-10, CIM-Pratap, RAS-28, RAS-7,
III	IC-310620(A)
IV	BHM-42
V	JA-134
VI	MWS-323
VII	RAS-65, MWS-218, Poshita
VIII	NMITLI-118
IX	NMITLI-101
X	MWS-324
XI	CIM-Chetak
XII	IC-283942

Table 3: Average intra (bold) and inter-cluster D^2 values of twelve clusters for 29 genotypes of ashwagandha (Tocher's method)

	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**
I	1248.2	3512.9	2432.9	2305.5	2378.1	2981.8	3487.8	6073.7	8870.1	4921.2	11676.9	14403.1
II		1546.1	6556.1	2703.7	3591.6	8790.3	6245.8	4682.4	6419.5	5206.0	6921.3	9252.7
III			0.00	2394	1898.5	4268.7	5179.9	10897.4	13587.9	8749.0	19230.2	17155.4
IV				0.00	1189.3	7257.5	4671.2	5756.9	9750.0	3670.0	10026.8	12378.2
V					0.00	6112.3	3002.5	8019.1	10953.8	6109.2	14711.1	16298.0
VI						0.00	3981.4	8702.5	10201.7	10202.7	17310.6	22140.8
VII							2326.3	7103.4	10695.2	6618.0	14386.3	23780.9
VIII								0.00	2590.7	5732.00	2890.6	12297.9
IX									0.00	13549.3	6232.2	9572.3
X										0.00	7094.4	18371.6
XI											0.00	12334.0
XII												0.00

* Bold diagonal values indicate intra cluster distance, rest of the values show the inter cluster distances

Table 4: Mean values of twelve clusters for 25 characters in 29 genotypes of ashwagandha. (Tocher's method)

Cluster	**PH**	**NBRP**	**LL**	**LW**	**DFLI**	**DFF**	**DRH**	**FLWP**	**DLWP**	**NBEP**	**BD**	**NSPB**	**SYP**	**SYH**	**MRL**	**RD**	**NSRPP**	**FRWP**	**DRWP**	**FRWH**	**DRWH**	**CFE**	**SE**	**SFR**	**TA**
I	65.62	4.10	6.10	3.71	62.94	35.53	192.65	69.22	21.12	177.07	0.64	31.25	3.21	7.13	11.09	1.16	2.45	3.54	1.90	7.87	4.21	29.64	11.16	0.38	0.30
II	73.85	5.26	6.35	3.66	70.87	34.13	198.98	98.37	28.32	258.14	0.67	35.33	6.08	13.50	10.78	1.57	3.88	8.04	3.36	17.86	7.47	32.97	11.73	0.36	0.33
III	50.23	4.02	5.82	3.54	63.04	39.12	189.68	69.52	19.60	186.86	0.52	27.12	3.12	6.92	9.74	0.69	1.94	2.68	0.90	5.96	2.00	24.29	9.51	0.39	0.31
IV	47.20	4.54	5.89	3.62	83.38	39.06	208.32	92.24	23.28	215.37	0.56	24.24	3.75	8.32	9.58	0.69	3.06	4.60	2.15	10.22	4.77	29.77	10.02	0.34	0.26
V	78.31	4.90	6.50	3.87	61.22	35.65	189.56	139.40	41.56	378.86	0.66	31.92	6.72	14.93	14.49	1.01	4.55	11.34	3.68	25.22	8.18	34.22	11.71	0.34	0.23
VI	87.83	4.82	6.61	3.28	66.31	36.60	199.64	111.88	30.92	78.93	0.73	37.01	1.56	3.46	13.65	1.61	3.29	7.84	2.94	17.42	6.53	31.68	14.03	0.44	0.26
VII	63.98	5.34	6.38	3.67	72.67	34.21	198.47	128.28	30.60	230.98	0.70	34.44	5.32	11.82	13.46	1.66	3.96	12.80	6.71	28.44	14.90	32.51	14.62	0.45	0.39
VIII	102.17	5.94	9.22	5.62	112.64	33.49	205.93	144.18	38.34	58.46	0.65	39.17	1.99	4.42	15.64	2.15	3.73	20.79	9.81	46.20	21.80	41.80	15.87	0.38	0.29
IX	119.09	5.76	9.72	6.16	107.91	32.91	205.41	295.92	67.32	79.72	0.63	30.51	2.20	4.88	18.40	2.64	6.35	29.70	13.78	66.00	30.63	40.30	14.80	0.37	0.34
X	57.84	4.46	4.41	2.59	90.29	35.22	208.63	32.72	9.24	198.14	0.58	36.32	2.18	4.85	12.51	1.47	3.29	4.24	1.84	9.42	4.09	29.77	10.83	0.36	0.20
XI	107.28	3.96	10.19	7.77	119.90	30.89	208.52	129.96	37.80	66.43	0.67	41.25	2.13	4.73	14.41	2.07	7.10	16.56	8.17	36.79	18.16	42.30	13.93	0.33	0.33
XII	89.46	7.20	5.94	3.20	62.13	35.47	192.23	177.84	39.60	204.17	0.70	31.55	2.42	5.37	8.59	1.86	4.11	11.88	3.78	26.40	8.40	34.38	9.78	0.28	0.24

PH - Plant height (cm); NBRP - Number of branches per plant; LL - Leaf length (cm); LW - Leaf width (cm); DFLI - Days to flower initiation; DFF - Days to fruit formation; DRH - Days to root harvest; FLWP - Fresh leaf weight per plant (g); DLWP - Dry leaf weight per plant (g); NBEP - Number of berries per plant; BD - Berry diameter (cm); NSPB - Number of seeds per berry; SYP - Seed yield per plant (g); SYH - Seed yield (q ha^{-1}); MRL - Main root length (cm); RD - Diameter of root (cm); NSRPP - Number of secondary roots per plant; FRWP - Fresh root weight per plant (g); DRWP - Dry root weight per plant (g); FRWH - Fresh root yield (q ha^{-1}); DRWH - Dry root yield (q ha^{-1}); CFE - Crude fiber estimation; SE - Starch estimation; SFR - Starch and fiber ratio; TA - Total alkaloid.

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