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Study of Genetic Divergence in Ashwagandha (*Withania somnifera* (L.) Dunal)

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Abstract: **Nineteen ashwagandha germplasm and 2 check were evaluated to study the diversity pattern among the collected accession. The genotype were grouped into five clusters. The distribution pattern indicate that the maximum no of genotypes (5) were grouped into cluster II, followed by 5 in cluster IV. The inter - cluster distance was higher than intra cluster distance indicating wide genetic diversity among the genotypes. The highest inter-cluster distance was observed between cluster III and I followed by III and IV showed wider diversity among the groups. The highest intra cluster distance was observed for cluster III followed by II. The accession with WS 90-141, MSW-302, MWS-314, RAS-23 can be used as potential donors for hybridization programme to develop variety with higher yield potential.**

Key word: Ashwagandha, diversity.

INTRODUCTION

Ashwagandha (*Withania somnifera*) is one of the important medicinal plants. It belongs to the family Solanaceae. It is placed at 3rd position among the 32 prioritized medicinal plants of India with a demand of 9127.5 tonnes and annual growth of 15 %. Directorate of Horticulture, government of C.G 2012-13, reported that 178 ha of land with production of 258 m.tones. In india the plant grows wild in north western regions such as

Gujrat, Rajsthan, Madhya Pradesh, Uttar Pradesh and Panjab plains extending to mountainous region of Panjab, Himachal Pradesh and Jammu. It forms essential constituent or whole of 100 medicinal formulations of traditional pharmacies like Ayurveda, Unani and Sidha (Tuli and Sangwan 2009). The withaniols, somnifirin and several other alkaloids present in the roots and to some extent leaves and seeds are used in Ayurvedic and Unani medicines particularly for hiccups, bronchitis, rheumatism, dropsy, several female disorders, stomach and lung inflammation and skin diseases. It has a characteristic odour and is bitter in taste. *Withania somnifera* (Ashwagandha) is a plant used in medicine from the time of Ayurveda, the ancient system of Indian medicine. The dried roots of ashwagandha (*Withania somnifera*) a dryland medicinal plant have been employed as valuable drug in Indian traditional systems of medicine: Ayurveda, Siddha and Unani. The roots of the plant are categorized as rasayanas, and have been used as antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently to treat ulcers, bacterial infections, venom toxins and senile dementia. Genetic parameters and character association provides information about expected response of various character and helps in developing suitable breeding procedure for their improvement nature and magnitude of variability in the existing plant material and the association among various character as per requisites for yield and selection of better plant type.

MATERIAL AND METHODS

The experimental material comprised of 19 germplasm and 2 check. These genotypes were evaluated in a RCBD in three replication during *rabi* 2011-12 at Research Farm, Indira Gandhi Krishi Vishwavidyala (IGKV), Raipur, Chhattishgarh (situated at $21^0$41' N latitude,$81^0$21, E longitude and at the height of 289.60 meters above the mean sea level). The material was planted in one row of 38 m length and 30 cm apart. The seeds were sown on 11/10/2012 and 5:12.5:0 kg/ha NPK was applied. All the recommended package of practices was adopted to raise the healthy crop. Five plants from middle of the row of each entry were randomly taken for observation on various 18 quantitative character viz. days to 50% flowering, plant height (cm), number of primary branches plant^{-1}, number of secondary branches plant^{-1}, number of capsules plant^{-1}, 1000 seed weight (g), seed yield plant^{-1} (g), biological yield plant^{-1} (g), harvest index plant^{-1} (%),root girth plant^{-1} (cm), root length plant^{-1} (cm), average diameter of root plant^{-1} (mm), surface area of root plant^{-1} (cm^2), number of secondary roots plant^{-1}, root volume plant^{-1} (cm^3), dry matter content of root plant^{-1} (%), fresh root yield plant^{-1} (g) , dry root yield plant^{-1} (g).

D^2 statistic was employed to measure the genetic distance between genotype (Mahalanobis, 1928). The D^2

statistics was originally developed by P.C. Mahalanabis (1928). Rao (1952) suggested the application of this technique for the assessment of genetic divergence between the populations. The D^2 between any two populations were estimated from the sample on the of P characters by the following formula

$$D^2 = \sum_{i=1}^{p}\sum_{j}^{p}(\overset{p}{\Delta}_{ij})\ \Delta_i\ \Delta_j$$

Where,

1. Δ_{ij} = reciprocal matrix of (ij) the pooled common dispersion matrix (i.e. error matrix)
2. Δ_i = difference in the mean value of i^{th} character
3. Δ_j = difference in the mean value of j^{th} character

RESULT AND DISCUSSION

A clear understanding of the extent of variability prevailing for each trait in germplasm is essential for the improvement of character through selection. In hybridization programme, selection of genetically diverse parent is important to get wide range of recombinants.

The analysis of variance (ANOVA) revealed highly significant differences aamong the genotype (Table 1) for all characters studied of considerable amount of genetic variation. The magnitude of variation between genotypes was reflected by high mean value and range of genotype traits studied (Table 2).

Table 1. Analysis of variance for fresh root yield and its components in ashwagandha

Source of variation	DF	Days to 50% flowering	Plant height (cm)	Number of primary branches plant^{-1}	Number of secondary branches plant^{-1}	Number of capsules plant^{-1}	1000 seed weight (g)	Seed yield plant^{-1} (g)	Biological yield plant^{-1} (g)	Harvest index plant^{-1} (%)	Root girth plant^{-1} (cm)	Root length plant^{-1} (cm)	Average diameter of root plant^{-1} (mm)	Surface area of root plant^{-1} (cm^2)	Number of secondary roots plant^{-1}	Root volume plant^{-1} (cm^3)	Dry matter content of root plant^{-1} (%)	Dry root yield plant^{-1} (g)	Fresh root yield plant^{-1} (g)
Replication	2	20.188	165.332	0.200	25.196	871.125	0.241	0.284	0.086	133.988	0.025	2.141	0.084	13.992	0.431	0.099	0.475	0.062	8.276
Treatment	20	41.877**	13.751**	0.379	6.659**	2779.327**	0.257	0.877	8.147**	92.047**	0.108	5.860**	6.767**	79.968**	0.907	5.770*	3.843	0.281	3.143
Error	40	27.224	10.387	0.111	1.081	223.977	0.182	0.312	1.449	21.820	0.044	5.444	0.336	29.769	0.295	0.376	8.745	0.072	1.404

DF = degree of freedom

* = Significant at 5% probability level

** = Significant at 1% probability level

Table 2. Estimation of genetic parameter for different traits in Ashwagandha

S.No.	Characters	GCV (%)	PCV %	H^2b (%)	GA	GA % of mean
1.	Days to 50% flowering	2.360	6.040	0.152	1.780	1.898
2.	Plant height (cm.)	2.730	8.750	0.097	0.680	1.754
3.	Number of primary branches plant^{-1}	10.780	16.180	0.444	0.410	14.813

4.	Number of secondary branches plant^{-1}	13.910	17.500	0.632	2.330	23.776
5.	Number of capsules plant^{-1}	17.050	19.160	0.792	53.500	31.259
6.	1000 seed weight (g)	5.730	16.520	0.120	0.110	3.990
7.	Seed yield plant^{-1} (g)	12.900	21.030	0.376	0.550	16.352
8.	Biological yield plant^{-1} (g)	6.030	7.750	0.607	2.400	9.691
9.	Harvest index plant^{-1} (%)	13.700	19.040	0.518	7.170	20.302
10.	Root girth plant^{-1} (cm)	3.680	6.470	0.325	0.170	4.299
11.	Root length plant^{-1} (cm)	1.600	10.160	0.025	0.120	0.516
12.	Average diameter of root plant^{-1} (mm)	29.220	31.430	0.865	2.800	55.883
13.	Surface area of root plant^{-1} (cm^2)	8.020	13.380	0.360	5.050	9.906
14.	Number of secondary roots plant^{-1}	13.450	21.030	0.408	0.590	17.583
15.	Root volume plant^{-1} (cm^3)	28.170	30.980	0.827	2.510	52.732
16.	Dry matter content of root plant^{-1} (%)	0.110	10.240	0.000	0.000	0.000
17.	Dry root yield plant^{-1} (g)	10.570	15.090	0.491	0.380	15.237
18.	Fresh root yield plant^{-1} (g)	8.760	16.210	0.292	0.850	9.782

The results (Table 2) revealed that estimates of PCV were slightly higher than those of GCV for all traits studied. The extent of the environment influence on traits is explained by the magnitude of the differences between PCV and GCV. Large differences between PCV and GCV values effect high environmental influence on the expression of traits. In this study, slight difference indicated minimum environmental influence and consequently greater role of genetic factor on the expression of traits. High GCV and PCV value were observed for average diameter of root plant^{-1} (29.220) (31.430), root volume plant^{-1} (28.170) (30.980) indicating the presence of ample variation for this character in the present material which indicate the possibility of yield improvements through selection on these traits.

Estimation of heritability and genetic advanced were high for average diameter of root plant^{-1} (86.5) (55.883) in genotypes indicating the predominance of additive gene action for these traits, hence direct selection may be highly effective. So these characters may be considered as important criteria for selection of the parent for hybridization program.

For quantitative character, 21 accession were grouped into 5 clusters by using D^2 statistics in such a way that the genotypes within a cluster had a small or low D^2 values than those of in-between characters. The composition of cluster has been presented in Table 3. Cluster- II (5) and cluster IV (5) had largest number of genotypes followed by cluster III (4), and cluster IV (4) whereas, cluster I had minimum number of genotype with 3. The inter-cluster distance is higher than intra-cluster, indicating the wide genetic diversity among the genotype Table 4. The highest inter-cluster distance varied from 6.779 to 3.478. The highest inter-cluster distance was observed between cluster III and I (6.779) (Table 4; fig. 1). On the other hand minimum distance was observed between cluster IV and V (3.478), indicating close relationship between these clusters would not provide good result. The greater the distance between clusters wider the genetic diversity between the genotype. Highly divergent genotype would produce a broad spectrum of variability in subsequent generations enabling further selection and improvement. The hybrids developed from the selected genotypes within the limit of compatibility of these clusters may produced desirable transgressive segregate or higher magnitude of heterosis. This would be useful in ashwagandha breeding programme to evolve miracle varieties with high root yield potential.

Table 3. Grouping of 21 ashwagandha genotypes in different clusters.

Cluster	No. of genotypes	Genotypes
I	3	RAS -15, WS 90-141, IGAU-1
II	5	RAS -25, RAS -16, JA-20, WS 90-12, JA-134
III	4	RAS -10, RAS -22, WS 90-111, MSW-302
IV	5	WS90-146, MWS 314, MWS 313, MSW 310, MSW-306
V	4	RAS -1, RAS -23, MWS 312, MWS 311

Table 4. Average intra-cluster (diagonal bold) and inter- cluster distances (D values) among the five clusters in ashwagandha.

Cluster	I	II	III	IV	V
I	**2.766**	4.322	6.779	4.919	4.991
II		**3.075**	3.977	3.965	4.672
III			**3.324**	5.032	4.168
IV				**2.754**	3.478
V					**2.876**

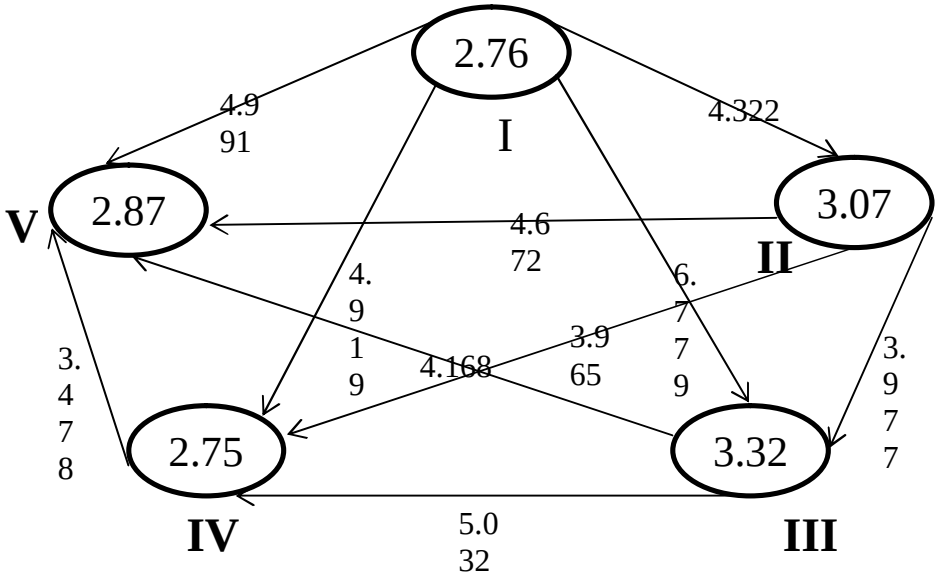

Figure 1. Intra and inter-cluster distance (D) in the ashwagandha genotype

The maximum intra cluster distance was observed for cluster III (3.324) followed by cluster II (3.075) and cluster V (2.876). (Table.4; Fig.1).It was reported that genotypes within the cluster with high degree of divergence would produce more desirable breeding material for achieving maximum genetic advance (Bose and Pradhan, 2005). The cluster minimum intra cluster distances was observed in I (2.766) followed by IV (2.754) indicating homogeneous nature of the genotypes, with less deviation between the genotype, therefore selection will be ineffective.

Table 5. Mean performance of genotypes in individual cluster for fresh root yield plant^{-1} and its components in ashwagandha

Cluster	Days to 50% flowering	Plant height (cm.)	Number of primary branches plant^{-1}	Number of secondary branches plant^{-1}	Number of capsules plant^{-1}	1000 seed weight (g)	Seed yield plant^{-1} (g)	Biological yield plant^{-1} (g)	Harvest index plant^{-1} (%)	Root girth plant^{-1} (cm)	Root length plant^{-1} (cm.)	Average diameter of root plant^{-1} (mm)	Surface area of root plant^{-1} (cm^2)	Number of secondary roots plant^{-1}	Root volume plant^{-1} (cm^3)	Dry matter content of root plant^{-1} (%)	Dry root yield plant^{-1} (g)	Fresh root yield plant^{-1} (g)
I	91.00	35.72	2.58	8.76	127.29	3.08	2.89	23.84	32.74	3.65	21.93	5.38	48.71	2.89	4.83	27.88	2.14	7.74
II	95.80	38.48	2.68	9.75	153.81	2.75	2.99	23.59	39.82	3.95	22.79	6.18	49.55	2.96	5.84	29.33	2.69	9.35
III	94.75	38.41	2.90	10.92	184.08	2.68	4.08	24.71	40.82	4.13	23.80	5.31	56.92	4.08	4.99	29.30	2.89	9.98
IV	95.13	41.38	2.74	8.92	190.68	2.81	3.09	26.13	31.23	3.99	23.60	4.39	48.03	3.09	3.44	29.13	2.40	8.19
V	90.67	38.51	2.92	10.63	188.38	2.53	3.80	25.27	31.22	3.97	23.91	3.75	52.20	3.80	4.78	28.28	2.23	7.91
CV	5.56	8.31	12.06	10.61	8.74	15.50	16.61	4.86	13.23	5.31	10.03	11.57	10.700	16.21	12.88	10.24	10.77	13.63

Cluster mean and coefficient of variation are presented in table 5. The coefficient of variation was highest in seed yield plant^{-1} followed by number of secondary root plant^{-1} and lowest was recorded in biological yield plant^{-1}. Cluster I showed highest cluster mean value for 1000 seed weight (3.08) along with root length plant^{-1} (21.93). Cluster II showed highest cluster mean value for days to 50% flowering along with average diameter of root plant^{-1} (6.18), root volume plant^{-1} (5.84), dry matter of content of root plant^{-1} (29.33). Cluster III showed highest cluster mean value for number of secondary branches plant^{-1} (10.92) along with seed yield plant^{-1} (4.08), harvest index plant^{-1} (40.82), root girth plant^{-1} (4.13), surface area of root plant^{-1} (56.92), number of secondary roots plant^{-1} (4.08), dry root yield plant^{-1} (2.89), fresh root yield plant^{-1} (9.98). Cluster IV showed highest cluster mean value for plant height (41.38) along with number of capsules plant^{-1} (190.68), biological yield plant^{-1} (26.13). Cluster V showed highest cluster mean value for number of primary branches plant^{-1} (2.92).

The results suggest that intercrossing of genotypes from different cluster showing good mean performance may help obtaining higher root yield. Inclusion of more diverse parents in hybridization is believed to increase the chances of obtaining better heterosis and give broad spectrum of variability in segregating generation.

The better accession can be selected for most of character on the basis of mean performance in the cluster. The best accession chosen for different character in presented in Table 6.

Table 6.Desirable genotypes based on cluster performance

Characters	Clusters				
	I	II	III	IV	V
Days to 50% flowering	RAS-15	RAS-16	RAS-22	MWS 314	RAS-23
Plant height (cm)	RAS-15	JA-20	RAS-22	MWS 313	RAS-23
Number of primary branches plant^{-1}	RAS-15	WS 90-12	MSW-302	MSW 310	RAS-23
Number of secondary branches plant^{-1}	RAS-15	JA-20	RAS-10	MWS 313	RAS-23
Number of capsules plant^{-1}	RAS-15	JA-134	RAS-10	WS 90-146	RAS-1
1000 seed weight (g)	WS 90-141	RAS-25	RAS-22	WS 90-146	RAS-23
Seed yield plant^{-1} (g)	RAS-15	RAS-25	RAS-10	WS 90-146	RAS-23
Biological yield plant^{-1} (g)	IGAU-1	JA-20	RAS-10	MWS 313	RAS-23
Harvest index plant^{-1} (%)	RAS-15	RAS-16	MSW-302	WS 90-146	RAS-1
Root girth plant^{-1} (cm)	RAS-15	RAS-25	RAS-22	MWS 313	RAS-23
Root length plant^{-1} (cm)	IGAU-1	JA-20	MSW-302	MWS 314	MWS 312
Average diameter of root plant^{-1} (mm)	IGAU-1	JA-134	MSW-302	MSW-306	RAS-1
Surface area of root plant^{-1} (cm^2)	IGAU-1	JA-20	MSW-302	MWS 313	RAS-1
Number of secondary roots plant^{-1}	RAS-15	RAS-25	RAS-10	WS 90-146	RAS-23
Root volume plant^{-1} (cm^3)	WS 90-141	RAS-25	WS 90-111	MWS 314	RAS-1
Dry matter content of root plant^{-1} (%)	WS 90-141	WS 90-12	WS 90-111	MSW-306	MWS 312
Dry root yield plant^{-1} (g)	WS 90-141	RAS-25	MSW-302	MWS 314	RAS-23
Fresh root yield plant^{-1} (g)	WS 90-141	RAS-16	MSW-302	MWS 314	RAS-23

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