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Genetic Diversity Analysis in Ashwagandha [Withania somnifera (L.) Dunal] Genotypes

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ABSTRACT

Keywords

Withania somnifera, D² statistics, Cluster analysis, Genetic diversity, Inter and intra cluster

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Genetic diversity among 25 Ashwagandha (Withania somnifera) accessions of different geographic origin was assessed using Mahalanobis D² statistics. Observations revealed significant genotypic differences and accordingly genotypes were classified into five clusters. Cluster II was the largest comprising about 12 genotypes or 48% of the genotypes belonged to this cluster followed by cluster V and IV which contained 6 and 4 genotypes. The clusters I and III each had only 2 and 1 genotype, respectively. The highest intercluster distance was noted between clusters I and V (33.37) followed by cluster I and IV (27.41), III and I (26.66), I and II (25.84), V and III (22.14). The genotypes grouped in these clusters are found to be diverse. The least (10.83) inter-cluster distance was observed for cluster II and V followed by (12.21) inter cluster distance between II and IV, thereby indicating their genetic relatedness. The average intra-cluster distance between the genotypes was maximum (103.81) for the cluster II followed in descending order by cluster V (50.89), IV (28.83), I (13.85) and III (1.0), respectively. Cluster V had 6 genotypes over average values for number of primary branches per plant, days to 75% maturity, dry root yield per plant, total alkaloid content and total starch content and showed above average values for number of primary branches per plant, days to 75% maturity, dry root yield per plant, total alkaloid content and total starch content indicating their good potential for crop productivity. Thus, hybridization among these genotypes can generate desirable transgressive segregants.

Introduction

Withania somnifera also known as Ashwagandha, Indian ginseng, poison gooseberry or winter cherry belongs to family Solanaceae and is a cross pollinated crop with chromosome number of 2n=48 (Nigam and Kandalkar, 1995) and is one of the important medicinal plants (Mir *et al.*, 2013). It is a hardy and drought tolerant perennial shrub and grows naturally under subtropical dry climate (with an average rain fall of 600-750 mm) in well drained, sandy loam or light red soils that have a pH of 7.5 to 8.0. The genus *Withania* comprises of 23 species among which only two (*Withania somnifera* and *Withania coagulans*) are reported from India. It is a native herb of North-Western and Central India as well as Mediterranean region of North Africa. It grows wild in north western regions such as Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Punjab, Himachal Pradesh and Jammu. Withania coagulans (L) is a wildgrowing species of Withania. It is a late kharif crop and grows under dry climate or requires less irrigation for plant growth under rain fed cultivation. It is grown mostly on marginal lands of Neemuch and Mandsaur districts of Madhya Pradesh and Kota. Jhalawar. Pratapgarh, Chittorgarh and Baran districts of Rajasthan. The main active constituents are alkaloids and steroidal lactones. The important chemical constituents (Withanolides) are present in roots, leaf and berries. The dried roots of ashwagandha have been employed as source valuable of active medicinal ingredients in Indian traditional systems of medicine: Ayurveda, Siddha and Unani. It forms essential constituent or whole of 100 formulations of traditional medicinal pharmacies like Ayurveda, Unani and Sidha (Tuli and Sangwan, 2009). The roots of the plant are categorized as rasayanas, functions as a tonic for vitality and longevity (Singh et al., 2010) and have been used as antioxidant, adaptogen, aphrodisiac, liver tonic, antiinflammatory agent, astringent and more recently to treat ulcers, bacterial infections, venom toxins, senile dementia, hiccups, bronchitis, rheumatism, dropsy, several female disorders, stomach and lung inflammation, skin diseases, asthma, emaciation, insomnia, neurological disorders, Parkinson's disease. The medicinal utility of roots is due to presence of number of alkaloids. The total alkaloid content in the roots varied from 0.16 to 0.66 per cent (Biennial Progress Report, 2006-2008). alkaloids The main are withanolides, sominiferine, sominiferinine, somnine, withananine, pseudo withanolides, withananinine and withasomnine (Covello and Ciampa, 1960). The roots of ashwagandha have properties similar to ginseng roots, used for the maintenance and restoration of health:

hence it is also known as Indian ginseng (Singh and Kumar, 2010).

 D^2 For morphological characterization, statistics is important for analysis and assessment among genotypes of ashwagandha and also in other plant species for genetic diversity studies. The D^2 statistics and clustering suggested helps to search genetically diverse types for hybridization programme. The inter-cluster distance is higher than the intra-cluster, indicating the wide genetic diversity among the genotypes. The greater the distance between clusters, wider the genetic diversity between the genotype. Highly divergent genotypes would produce broad spectrum variability in generations enabling further subsequent selection and improvement (Singh, 2014). The developed hybrids from the selected genotypes within the limit of compatibility of these clusters may produce desirable trasgressive segregants of higher magnitude of heterosis (Singh et al., 2014 and Joshi et al., 2015).

Limited work has been done for improvement of this important medicinal plant and so results of the present study will help in analysis of genetic diversity based on morphological markers, supplemented by other genetic markers of the coming decade. This will be of great significance for future crop improvement programmes.

Materials and Methods

The experimental material consisted of twenty five genotypes of ashwagandha; the seeds of which were sown in randomized block design, in three replications, at the research farm of Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology Udaipur during *Kharif* 2013-14. Source and details of plant genetic material are given in Table 1. Seeds of twenty five genotypes were planted with a row-to-row spacing of 30 cm and a 10 cm plant-to-plant distance. Fertilizers were applied (@15 kg N/ha and 15 kg P_2O_5/ha) at the time of sowing. All the recommended agronomical practices and plant protection measures were adopted to raise a healthy crop canopy.

At maturity, five competitive plants were randomly selected from every replication of each genotype to record observations, except for days to 75 % flowering and 75 % days to maturity observations that were recorded on plot basis. The experimental material was evaluated for seven characters *viz.*, plant height, days to 75% flowering, days to 75 % maturity, dry root yield, number of primary branches per plant, root diameter and root length. The statistical analysis for quantitative characters was done on the basis of average values.

The data on morphological traits in the three replications was subjected to analysis of variance based on the method described by Panse and Sukhatme (1985) for individual characters. The data with replications were subjected to genetic divergence analysis using Mahalanobis's D^2 – statistics (Mahalanobis, 1936). In the present investigation, based on data of morphological and quality characters, grouping of all 25 genotypes of ashwagandha was done using Ward's minimum variance method. Intra- and inter-cluster distances generated were used to describe the relationship among the genotypes.

Results and Discussion

The mean values for performance and the origin of the 25 genotypes of ashwagandha are given in Table 1. Analysis of variance revealed highly significant difference for all characters studied indicating thereby considerable genetic variability among the

genotypes. Analysis of variance revealed highly significant differences for all characters studied, thus indicating considerable genetic variability among the genotypes. The extent of variability on the morphological characters were: plant height (28-50 cm), number of primary branches/plant (2-4), days to 75% flowering (79-113), days to 75% maturity (147-164), root length (12-22.4 cm), root diameter (0.86-1.45 cm), dry root yield (1.21-4.59 g/plant), total alkaloid content (0.08-0.96%), total starch content (4.4-15.7%). The variation among the individual genotypes is based on environmental/genetic effects (Rao, 1952). The twenty five genotypes from different regions of the country grown at a single location were subjected to same environmental conditions. Therefore, observed variations could be largely genetically based. The dry root yield/plant was maximum in UWS-134 followed by UWS-67 and UWS-13 (4.59, 4.58 and 4.57 g) respectively. The evaluation of these ashwagandha germplasm showed a large variation in the quantitative traits between the genotypes (Table 1).

The average mean squares from the ANOVA for different characters (Table 2) revealed that the mean squares due to genotypes were highly significant for all the characters observed, indicating the occurrence of significant genetic variability in the material.

The study provides the scope for selection and further subsequent utility of the genotypes in future crop improvement programmes. Hence the material appeared to be appropriate for further genetic analyses. Similarly, Singh *et al.*, (2014) revealed significant differences among different ashwagandha genotypes for all the traits studied.

Grouping the 25 genotypes of ashwagandha into 5 clusters by using D^2 statistics revealed that the genotypes within a cluster had a small or low D^2 values among themselves than those

placed in other clusters. Table 3 shows the cluster profile of these 25 genotypes followed by Euclidean² Distance values as shown in Figure 1.

Cluster II was the largest comprising 12 genotypes or 48% of the genotypes belonged to this cluster. Clusters V and IV followed next, containing 6 and 4 genotypes, while the clusters I and III each had only 2 and 1 genotype, respectively. Similarly Singh *et al.*, (2014) reported grouping of 21 genotypes of ashwagandha into 5 clusters. Misra *et al.*,

(1998) grouped genotypes of ashwagandha into 8 clusters. Fifty-five diverse genotypes of ashwagandha were grouped into ten different clusters by Jain *et al.*, (2007). Gupta *et al.*, (2011) carried out a genetic divergence study in 75 genotypes of ashwagandha and grouped them into fourteen clusters using Tocher's method. Kumar *et al.*, (2007) and Singh *et al.*, (2014) carried out genetic divergence studies in 25 accessions of ashwagandha and grouped them into 5 clusters by D^2 statistics (Table 4). Joshi *et al.*, (2015) report the grouping of ashwagandha genotypes into 6 clusters.

Table.1 Details of twenty five genotypes of ashwagandha used in present investigation

Genotypes	Source
UWS-10	Germplasm Collection AICRP in MAP&B, Udaipur
UWS-11	-do-
UWS-13	-do-
UWS-15	-do-
UWS-22	-do-
UWS-23	-do-
UWS-28	-do-
UWS-32	-do-
UWS-35	-do-
UWS-37	-do-
UWS-56	-do-
UWS-59	-do-
UWS-60	-do-
UWS-67	-do-
UWS-77	-do-
UWS-92	-do-
UWS-93	-do-
UWS-98	-do-
UWS-111	-do-
UWS-134	-do-
AWS2B	AAU, Anand
HWS-08-14	CCSHAU, Hisar
RVA-100	RVSKVV, Mandsaur
JA-20	-do-
JA-134	-do-

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Genotypes	Code	Plant Height(cm)	No. of primary branches/ plant	Days to 75% flowering	Days to 75% maturity	Root length (cm)	Root Diameter (cm)	Dry Root Yield (g)/plant	Total Alkaloid Content (%)	Total starch content (%)	Totalcrudefibrecontent(%)
UWS-10	1	41	2.3	111	150	16.8	1.25	1.53	0.17	8.9	31.2
UWS-11	2	32	2	91	156	18	.10	4.17	0.51	11.3	32
UWS-13	3	41	4	92	158	17	.12	4.57	0.27	14.9	33
UWS-15	4	44	3	94	151	19	.13	2.5	0.89	12.4	22.2
UWS-22	5	41	2	84	164	16.2	.95	4.17	0.9	5.8	28.7
UWS-23	6	39	2.7	93	148	15.7	.91	3.05	0.67	14.9	27.6
UWS-28	7	43	3	85	152	17	1.06	2.08	0.17	15	22.8
UWS-32	8	47	2	89	150	16.5	1.45	4.17	0.42	4.4	35.8
UWS-35	9	42	2	84	152	16	.10	3.33	0.28	10.5	31.8
UWS-37	10	50	3	79	148	21	.96	2.5	0.08	13.6	26
UWS-56	11	40	2	89	155	16	1.15	3.33	0.71	12.9	28.1
UWS-59	12	38	2	89	150	21	.95	2.92	0.96	15.7	29.9
UWS-60	13	43	3	86	157	13	1.21	4.17	0.5	10.7	29.8
UWS-67	14	28	2	87	148	12	.92	4.58	0.16	13.7	34.3
UWS-77	15	32	3	88	149	17	.95	2.5	0.42	10.3	23.3
UWS-92	16	36	3	79	155	14.5	1.22	2.08	0.18	13	18.7
UWS-93	17	32	3	80	154	12	.96	2.5	0.18	14.6	22.3
UWS-98	18	33	4	79	164	20	.86	2.5	0.7	15.1	21
UWS-111	19	49	2	79	157	16	1.3	3.33	0.5	12.1	33.8
UWS-134	20	38	2	78	158	12	.92	4.59	0.69	14.9	30.6
AWS2B	21	43.8	3.3	113	155	13.6	1.22	1.46	0.81	10.7	19.3
HWS-8-14	22	49	3	85	154	22.4	1.04	1.21	0.3	12.5	19.3
RVA- 100	23	39.3	3	89	153	15	1.11	2.92	0.28	5.5	26.2
JA-20	24	38	2	80	147	16.2	1.27	3.75	0.54	13.4	31.5
JA-134	25	33.3	3	80	155	15.1	1.11	3.33	0.61	15.7	30.3
Mean		39.54	2.65	87.39	153.50	16.36	1.087	3.09	0.48	12.22	27.4
Range		28-50	2-4	79-113	147-164	12-22.4	.86-1.45	1.21-4.59	0.08-0.96	4.4-15.7	18.7-35.8
Error		0.40	0.11	0.57	0.52	0.43	0.52	0.03	0.00	0.53	0.28
CD at 5 %		1.13	0.31	1.63	1.49	1.22	1.49	0.08	0.01	1.50	0.79
CV		1.73	7.03	1.14	0.59	4.55	8.33	1.49	1.63	7.49	5.07

Table.2 Mean performance of different genotypes for various characters in W. somnifera L. Dunal

S.No.	Characters	Source of variation				
	Degree of freedom	Replication	Treatment	Error		
		2	24	48		
1	Plant height	13.72	95.55**	0.47		
2	No. of Primary	0.02	1.21**			
	branches/plant					
3	Days to 75% flowering	0.69	242.38**	0.99		
4	Days to 75 % maturity	5.32	62.77**	0.82		
5	Root length	11.68	23.45**	0.56		
6	Root Diameter	5.32	6.67**	0.82		
7	Dry Root yield /plant	0.0012	3.05**	0.0021		
8	Total alkaloid content	0.01	0.21**	0.0001		
9	Total starch content	3.88	30.79**	0.84		
10	Total crude fibre	4.12	77.87**	0.87		
	content					

Table.3 ANOVA for the 10 chosen morphological characters in 25 genotypes of W. somnifera

df = degree of freedom

*, ** = Significant at 5% and 1% probability level, respectively

Table.4 Grouping of 25 genotypes of W. somnifera (L.) Dunal into various clusters on the basisof D^2 statistics

Cluster	Number of genotypes	Genotypes
Ι	2	UWS 10, AWS2B
Π	12	UWS 11, UWS 13, UWS 23, UWS 35, UWS 56, UWS 59, UWS 60, UWS 67, UWS 77, UWS 111, RVA 100, JA-20
III	1	UWS 32
IV	4	UWS 15, UWS 28, UWS 37, HWS-8-14
V	6	UWS 22, UWS 92, UWS 93, UWS 98, UWS 134, JA -134

Table.5 Average inter and intra- cluster	distance (D= D^2)	values in W	. somnifera
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Cluster	Ι	II	III	IV	V
Ι	13.85	25.84	26.66	27.41	33.37
II		103.81	13.97	12.21	10.83
III			1.00	17.24	22.14
IV				28.83	15.31
V					50.89

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Cluster	No. of Genotyp e	Plant heigh t (cm)	No. of primary branches /plant	Days to 75% flowerin g	Days to 75% maturit y	Root lengt h (cm)	Root Diamete r (mm)	Dry Root Yield (g)/plant	Total Alkaloid content (%)	Total root starch content (%)	Total crude fiber content (%)
Ι	2	42.4	2.8	112	152.5	15.2	12.35	1.49	0.49	9.8	25.25
Π	12	38.44	2.48	87.25	152.5	16.08	10.81	3.55	0.48	12.16	30.10
III	1	47	2	89	150	16.5	14.5	4.17	0.42	4.4	35.8
IV	4	46.5	3	85.75	151.25	19.85	10.9	2.07	0.36	13.38	22.5
V	6	35.55	2.83	80	158.33	14.97	10.03	3.20	0.54	13.18	25.26
Mean	5.00	41.98	2.62	90.8	152.92	16.52	11.72	2.90	0.46	10.58	27.78
TreatM	MSS	95.55	1.20	242.38	62.77	23.45	6.66	3.05	0.21	30.78	79.03
ErrM	ISS	0.47	0.03	0.98	0.82	0.55	0.82	0.00	0.00	0.83	1.94
F Ra	ıtio	5.0	0.94	22.10	2.95	2.59	2.87	5.69	0.26	2.59	4.53
Percent con towards va	ntribution ariability	0.005	0.461	0.00	0.045	0.068	0.049	0.003	0.896	0.068	0.015
Rar	ık	6	2	9	5	3	4	8	1	3	7

Table.6 Cluster means and average (overall) for various characters in 25 genotypes of W. somnifera (L.) Dunal

Fig.1 Intra and inter cluster distance for 5 groups of the 25 genotypes of W. somnifera







In this study, the highest inter-cluster distance was noted between clusters I and V (33.37) followed by cluster I and IV (27.41), III and I (26.66), I and II (25.84), V and III (22.14) and so on. The genotypes grouped in these clusters are found to be diverse. The least (10.83) inter-cluster distance was observed for cluster II and V followed by (12.21) intercluster distance between II and IV, thereby indicating their genetic relatedness. The average intra-cluster distance between the genotypes was maximum (103.81) for the cluster II followed in descending order by cluster V (50.89), IV (28.83), I (13.85) and III (1.0), respectively. Inter-cluster distances were greater than intra-cluster distances, showing a considerable degree of genetic diversity among the genotypes studied. Genotypes belonging to clusters with maximum inter-cluster distance are genetically more divergent and hybridization between genotypes in divergent clusters is likely to produce wide variability with desirable segregant (Maloo and Bhattacharjee, 1999).

Cluster mean for 10 characters for 25 *W. somnifera* (L.) Dunal genotypes are presented in Table 5 which revealed that differences in cluster means existed for all the characters studied. The cluster I comprising 2 genotypes was found to have above average values for plant height, number of primary branches per plant, days to 75% flowering, root diameter and total alkaloid content. Cluster II had 12 genotypes that indicated above average values for dry root yield/plant, total alkaloid content, total starch content and total crude fibre content. Cluster III comprised of 1 genotype that was found to have above average value for plant height, root diameter, dry root vield/plant and total crude fibre content. Cluster IV consisted of 4 genotypes that showed higher than average value for plant height, root length and total starch content. Cluster V had 6 genotypes that exhibited over average values for number of primary branches per plant, days to 75% maturity, dry root yield per plant, total alkaloid content and total starch content. Results indicate that genotypes belonging to Cluster V viz., UWS -22, UWS-92,UWS-93, UWS-98,UWS-134 and JA-134 showed above average values for number of primary branches per plant, days to 75% maturity, dry root yield per plant, total alkaloid content and total starch content indicating their good potential for enhancing crop productivity. The percent contribution total genetic divergence towards was maximum for the character total alkaloid content followed by no. of primary branches per plant, root length and total starch content.

Ward's hierarchical cluster analysis was carried out on the basis of 10 morphological characters, employed to measure genetic distances between the 25 *W. somnifera* (L.) Dunal genotypes (Figure 2). Cluster analysis revealed that the genotypes could be grouped into two clusters, cluster I and II that were apart at 25 rescaled values.

Cluster I included 23 genotypes *viz.*, UWS 56,UWS 60,UWS 35,RVA100, UWS 22,UWS 11,UWS 13,UWS 32,UWS 134,JA-134,JA-20,UWS 111,UWS 23,UWS 59,UWS 77,UWS 67,UWS 92,UWS93,UWS 98,UWS 28,HWS-8-14, UWS 15 and UWS 37. It

could further be divided into 3 subclusters. Subcluster I at 6 rescaled distances that included 12 genotypes viz., UWS 56, UWS 60, UWS 35, RVA 100, UWS 22, UWS 11, UWS 13, UWS 32, UWS 134, JA-134, JA-20 and UWS 111. It could be further subgrouped into 2 subgroups: Subgroup A and Subgroup B, both at 3 rescaled distances, respectively. Subgroup A can be further divided into subgroup A1 and Subgroup A2. Subgroup A1 at 3 rescaled distances accounted for a small group of 7 genotypes viz., UWS-56, UWS-60, UWS-35, RVA-100, UWS-22, UWS-11 and UWS-13 and a single genotype viz., UWS-32. Subgroup B consists of 4 genotypes viz., UWS-134, JA-134, JA-20 and UWS-111. Subcluster II at 3 rescaled distance consisted of 3 genotypes viz., UWS-23, UWS 59 and UWS 77. Subcluster III at 3 rescaled distances consists of 4 genotypes viz., UWS-28, HWS-8-14, UWS-15 and UWS-37. Cluster II included 2 genotypes viz., UWS-10 and AWS-2B at 3 rescaled distance.

This study will help plant breeders to identify, characterize and select authenticated genotypes in future crop improvement programmes, as a result, these genotypes will find commercial importance in their genetic content and medicinal value.

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