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Cluster and principal component analysis (PCA) in ashwagandha [*Withania somnifera* (L.) Dunal] for root traits

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Abstract
Present study of the research is based on genetic diversity in ashwagandha [*Withania somnifera* L. Dunal]. This study contains 43 ashwagandha germplasm accessions including 3 checks JA 20, JA 134 and RVA 100. The experiment was conducted at Research Cum Instructional Farm, Department of Genetics and Plant Breeding, College of Agriculture, IGKV Raipur (C.G.) during *rabi* 2018-19 and 2019-2020. Experiment was conducted in RCBD design with 2 replications. Most of the traits were found highly significant and shows the variability among the germplasm accessions. In genetic diversity study germplasm categorized into 6 clusters, highest cluster genetic distance was observed in between cluster V and cluster IV (105.4). It's implies that the hybridizations strategy including these genotypes from the clusters gives superior occurrence of the segregates. Highest number of genotypes occurs in cluster I with 18 genotypes. Based on PCA analysis component explained over 83.27% cumulative variables. PCA 1 has high percentage of variables (28.69%).The first PCA account for the most of the variability of the data.

Keywords: Genetic diversity, ashwagandha, cluster analysis and principal component analysis

Introduction

Most practitioners formulate and dispense their own recipes in the Indian medicine system, so this requires proper evaluation of documentations. There are about 45000 plant species in India and officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000. India is the largest producer of medicinal herbs and appropriately called the botanical garden of the world. Ashwagandha [*Withania somnifera* L. Dunal] belongs to family solanaceae and is a cross pollinated crop having the chromosome number 2n =48 (Nigam and Kandalkar 1995) [11]. It is popularly known as Indian Ginseng or Winter Cherry. Ashwagandha studies indicates that it's possesses antioxidant, anxiolytic, adaptogen, memory enhancing, antiparkinsdomia, antivenom, antinflamatory, author properties (Gupta and Rana 2000) [2]. Most of these pharmacological activities are attributed to a novel group of maturally occurring triterpenoid C-28 steroidal lactones called withanolides. One of the well known withanolides, withaferin A emerged as an important therapeutic molecules of *W.* ashwagandha due its anticancer properties (Koduru *et al.,* 2010 Lee *et al.,* 2010; Mayola *et al.,* 2011; Yang *et al.,* 2011) [4, 7, 9, 13]. Some herbalists refer to Ashwagandha as Indian ginseng, since it is used in Ayurvedic medicine in a way similar to that ginseng is used in traditional Chinese medicine. Ethno-medicinally, decoction of the roots is used for colds and chills; and to increase the tone of uterus after miscarriage or birth. An infusion of the root bark has been used for asthma, a use also common to traditional herbal practices in India. In Ayurvedic medicine, its root is used as an anti-inflammatory drug for swellings, tumours, scrofula and rheumatism; and as a sedative and hypnotic in anxiety neurosis (Krutika *et al.,* 2016) [5]. The medicinal utility of roots is due to presence of number of alkaloids. The total alkaloids content in the roots varied from 0.16 – 0.66% [Biennial Progress Report 2006-2008]. The main alkaloids are withanolides, somniferine, somniferinine, somine, withanine, pseudowithanolides, withanonine and withasomine [Covello and Ciampa (1960) Patel and Desai (2017)] [1, 12].
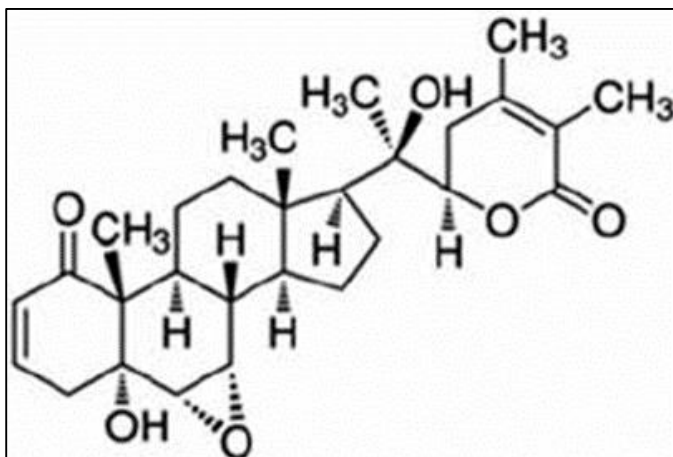
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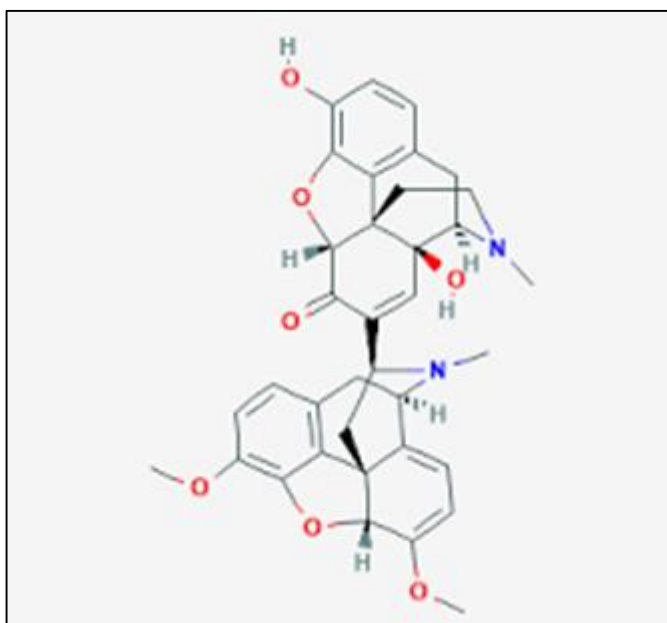
Fig 1: Chemical structure of Withanolides

Fig 2: Chemical structure of somniferine

Materials and Methods

The experimental material comprised of 43 germplasm accession including three checks JA 20, JA 134, RVA 100 and was conducted at research cum instructional farm, Department of Genetics and Plant Breeding, College of Agriculture, IGKV Raipur (C.G.) during *rabi* 2019-2020. Agro- climacterically Raipur is located in Chhattisgarh Plains with latitude of 21˚13'16' NL, longitude of 81˚143' EL and altitude is 298.5m over mean sea level. These accessions of germplasm will be tested in two replications in an RCBD Model and content will be planted with a spacing of 30 cm X 50 cm row to row and plant to plant in the second fortnight of October. In order to grow a safe harvest, all recommended packages of activities will be followed. Randomly five plants were selected from each treatment for recording observations in replications for following traits; days to 50% flowering, plant height (cm), numbers of berries per plant, number of primary branches, number of secondary branches, number of secondary roots, root length (cm), root girth (cm), dry root yield (gm), fresh root yield (gm), berry length/ berry width (cm).

In statistical analysis, analysis of variance, cluster analysis based on ward's method using squared Euclidian distance (Kumar *et al.*, 2009) [6] and identification the cutting point

using discriminate analysis and Multivariate analysis of variance (Mohammadi and Prasanna, 2003) [9] is used.

To calculate the genetic difference between genotypes, D^2 statistics were used (Mahalanobis, 1928) [8]. The statistics for D^2 were initially developed by P.C. Mahalanabis (1928). The use of this methodology for the evaluation of genetic divergence between populations was proposed by Rao (1952). The D2 between any two populations were estimated from the sample on the of P characters by the following formula:

$$D^2 = \sum_{i=1}^{p} \cdot \sum_{j}^{p} \cdot (\Delta ij)\Delta i. \Delta j$$

Where

1. (Δij) = reciprocal matrix of (ij) the pooled common dispersion matrix (*i.e.* error matrix)
2. Δi = difference in the mean value of ith character.
3. Δj = difference in the mean value of jth character.

Results and Discussions

Analysis of variance: The analysis of variance (ANOVA) was performed in Randomized Complete Block Fashion for the genotype accessions are screening highly significant difference among all characters of ashwagandha understudy. This indicates that there is high amount of variability present in the genotypes for the taken observation of all traits.

Genotypic and Phenotypic coefficient of variance: The RCBD analysis offers details on variance, i.e. genotypic variance (GCV) and phenotypic variance (PCV). In all features, PCV is greater than GCV, suggesting that the setting is more influential over all characters. The genotypic variation was lower than the phenotypic variance, suggesting that the climate has a masking effect on the expression of genetic variability. High PCV and GCV were reported by the character number of berries per plant followed by number of primary branches and number of secondary roots.

Heritability and Genetic Advance: In heritability (h^2) analysis and genetic advancement, high heritability brace with high genetic advancement is proof of heritability due to additive gene actions and choices for that unique desirable phenotype would be advantageous and successful. Maximal heritability paired with maximal genetic advance as percent of the population mean reported in root girth 75.10% and 77.40% respectively Minimum genetic advance suggests that the character of that selection was heavily affected by the environment in not being beneficial for that phenotype. Lowest genetic advance as percent of mean estimated in days to 50% flowering 2.50%. This specifies that traits were rule by non–additive genes and heterosis breeding programme is useful for this.

Cluster analysis or D^2 statistics: It includes 43 genotypes and 11 characters were used for study genetic diversity. Based the analysis values are arranged into 6 clusters. This point towards substantial diversity is present in all the genotypes estimated in the present study. The highest inter cluster distance was observed in between cluster V and cluster IV (105.4) afterwards cluster VI and cluster V (87.97) and cluster V and cluster I (72.63). Highest inter cluster distance implied that the hybridization strategy including parents from the clusters decided to have superior occurrence of the enhanced segregates. Minimum inter-cluster distance observed in Cluster I and Cluster III 19.38 followed by Cluster IV and

Cluster I 20.86 and Cluster VI and Cluster III 27.34 respectively.

Minimum inter-cluster distance determines less heterogeneity in the genotypes. The average maximum intra-cluster distance observed between clusters IV 22.72 followed by cluster III 14.33 and cluster II 13.5; the intra-cluster distance represented the low heterogeneity of the genotypes and the maximum intra-cluster distance indicated that the variability of the genotypes was low and that the selection character was less favourable.

Clustering for germplasm accessions: In total 6 genotypes cluster; cluster I have maximum number of genotypes which includes 18 genotypes, followed by cluster II consist 12 genotypes and cluster IV includes 7 genotypes. Besides this

cluster III has 4 genotypes, cluster V has 1 genotype and cluster VI has 1 genotype.

Principal component analysis (PCA): Principal component analysis for 43 ashwagandha germplasm accessions for 11 attributing characters presented in the table. PCA with high eigen values and variables considered as best representative of system attributes. In all germplasm accessions are categorised into 5 PCA and showed 83.270% cumulative variables of those PCAs. PCA 1 has high percentage of variables that is (28.69%) after that PCA 2 (25.28%), PCA 3 (13.82%), PCA 4 (9.57%) and PCA 5 (6.43%). First PCA account for much of the variability in the data as possible and each succeeding component accounts for as much of the remaining variability as possible.

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

S.V.	df	Days to 50 percent flowering	Plant height (cm)	No. of berries per plant	No. of primary branches	No. of secondary branches	No. of secondary roots	Dry root yield (gm)	Fresh root yield (gm)	Root length (cm)	Root girth (cm)	Berry Length/Berry Width (cm)
Replication	1	13.44	2.21	51866.79	0.42	1.41	3.77	5.53	78.19	3.24	3.01	0.48
Treatment	42	25.89*	246.29**	71728.83**	3.88**	15.11**	83.39**	38.44**	93.68*	46.65**	7.73**	1.12**
Error	42	15.27	133.36	31811.91	1.39	6.50	21.45	12.17	53.85	21.99	1.10	0.33

df - Degree of freedom
* - 5% level of significance
** - 1% level of significance

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

S no	Character	Mean	Range		GCV (%)	PCV (%)	h^2	h^2(%)	GA as a percent of the mean
			MIN	MAX					
1	Days to 50% flowering	96.605	85	106	2.40	4.70	0.258	25.80	2.50
2	Plant height(cm)	54.791	26.9	80.4	13.70	25.10	0.298	29.80	15.40
3	No of berries per plant	298.023	16	1014	47.40	76.30	0.386	38.60	60.60
4	No of primary branches	3.977	1	9	28.00	40.80	0.471	47.10	39.60
5	No of secondary branches	10.43	4	18	19.90	31.50	0.398	39.80	25.90
6	No of secondary root	12.488	1	29	44.60	58.00	0.591	59.10	70.50
7	Dry root yield(gm)	11.207	3	30	32.30	44.90	0.519	51.90	48.00
8	Fresh root yield(gm)	21.256	5	60	21.00	40.40	0.27	27.00	22.50
9	Root length(cm)	25.18	14.9	39.1	13.90	23.30	0.359	35.90	17.20
10	Root girth(cm)	4.186	0.6	7.9	43.50	50.20	0.751	75.10	77.70
11	Berry length/ berry width(cm)	6.581	4.7	8.2	9.50	13.00	0.541	54.10	14.40

Table 3: Grouping of 43 ashwagandha genotypes in different clusters.

| Cluster	Number of genotypes	Genotypes
I	18	ACC 57, ACC 86(RVA 100, ACC 66, ACC 84(JA 20), ACC 62, ACC 25, ACC 24, ACC 37, ACC 21, ACC 52, ACC 67, ACC 54, ACC 35, ACC 28, ACC 1, ACC 34, ACC 47, ACC 81(JA 134)
II	12	ACC 93, ACC 94, ACC 92, ACC 90, ACC 89, ACC 91, ACC 95
III	4	ACC 27, ACC 64, ACC 30, ACC 63, (ACC 94 X ACC 92), (ACC 91 X ACC 93), (ACC 90 X ACC 91), (ACC 92 X ACC 95), (ACC 92 X ACC 94)
IV	7	ACC 49, ACC 53, ACC 44, ACC 48, ACC 51, ACC 61, ACC 56
V	1	(ACC 90 X ACC 89)
VI	1	ACC 32
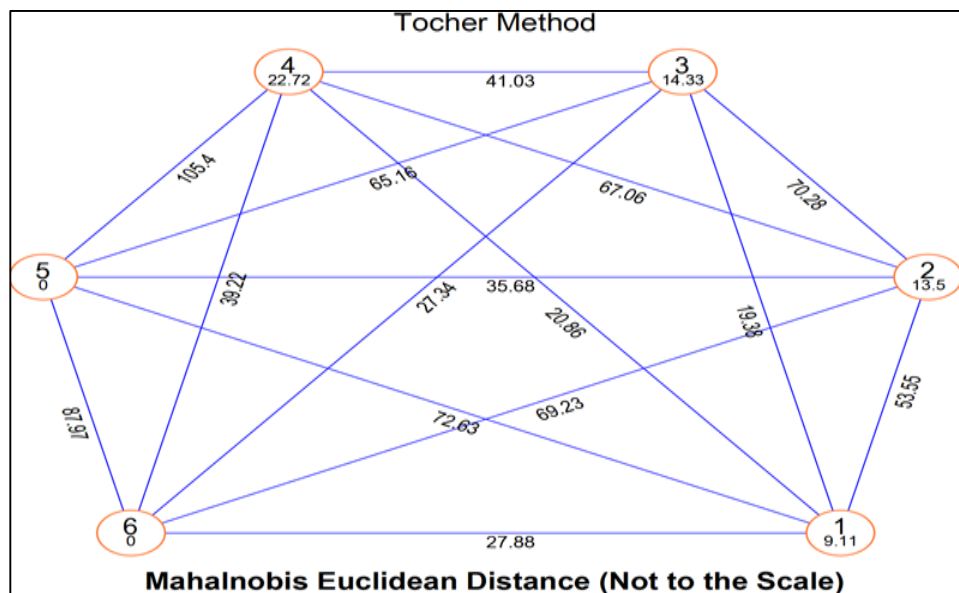
Fig 3: Cluster diagram by to Cher method

Table 4: Average intra-cluster (diagonal bold) and inter- cluster distances (D^2 values) among the 6 clusters in ashwagandha.

Cluster Distances						
	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Cluster 5**	**Cluster 6**
Cluster 1	9.11	53.55	19.38	20.86	72.63	27.88
Cluster 2		13.5	70.28	67.06	35.68	69.23
Cluster 3			14.33	41.03	65.16	27.34
Cluster 4				22.72	105.4	39.22
Cluster 5					0	87.97
Cluster 6						0

Table 5: Mean performance of genotypes in individual cluster for root trait in Ashwagandha.

Cluster Means: Tocher Method											
	Days to 50 percent flowering	Plant height (cm)	No. of berries/plant	No. of primary branches	No. of secondary branches	No. of secondary roots	Dry root yield (gm)	Fresh root yield (gm)	Root length (cm)	Root girth (cm)	Berry Length/Berry Width (cm)
Cluster 1	96.78	52.55	240.86	3.03	9.06	8.03	8.47	17.33	23.78	4.83	6.26
Cluster 2	96.63	49.61	240.33	4.96	11.88	20.58	13.83	23.88	26.09	1.59	6.81
Cluster 3	95.88	72.75	269.13	3.88	7.88	9.5	12.88	27.13	22.21	5.95	6.75
Cluster 4	96.64	58.96	562.29	4.21	12.79	11.43	9.93	20.14	30.11	6.12	6.84
Cluster 5	98.5	49.2	17.5	8	10.5	11	15	31	15.7	0.7	6.85
Cluster 6	94	61.85	565.5	4	11.5	16.5	27.5	35	26.25	6.65	6.95

Table 6: PCA analysis for root trait in 43 ashwagandha germplasm accessions

Canonical Roots Analysis (P. C. A.)					
	PCA 1	**PCA 2**	**PCA 3**	**PCA 4**	**PCA 5**
Eigene Value (Root)	3.157	2.782	1.461	1.053	0.707
% Var. Exp.	28.698	25.287	13.282	9.572	6.431
Cum. Var. Exp.	28.698	53.985	67.267	76.839	83.270
1. Days to 50 percent flowering	0.029	0.166	0.634	0.391	0.360
2. Plant height(cm)	0.188	0.218	-0.177	0.681	-0.488
3. No. Of berries/plant	0.053	-0.441	-0.226	0.170	0.534
4. No. Of primary branches	-0.442	0.065	0.150	-0.007	0.096
5. No. Of secondary branches	-0.403	-0.214	0.181	0.339	-0.214
6. No. Of secondary roots	-0.516	0.000	-0.132	-0.136	-0.042
7. Dry root yield (gm)	-0.268	0.324	-0.264	-0.035	-0.054
8. Fresh root yield (gm)	-0.036	0.517	0.047	-0.304	-0.045
9. Root length(cm)	-0.068	-0.524	-0.067	-0.097	-0.393
10. Root girth (cm)	0.493	0.027	-0.108	-0.145	-0.017
11. Berry length/berry width	-0.130	0.181	-0.595	0.310	0.362

Conclusion

Including all interpretations on genetic parameter, variability, co-efficient of variance, path and cluster analysis for dry root yield traits and its attributes traits in accessions of ashwagandha germplasm existence of maximum variability found in numbers of berries per plant, number of secondary roots, plant height, dry root yield and fresh root yield and low variability present in berry length/berry width, number of

primary branches and days to 50% flowering. Cluster analysis or D^2 statistics include 43 genotypes and eleven characters were used for study genetic diversity. Based the analysis values are arranged into 6 clusters. This point towards substantial diversity is present in all the genotypes estimated in the present study. This is in conformity with previous reports representing significant diversity in ashwagandha. Based on PCA analysis component explained over 83.27% cumulative variables. PCA 1 has high percentage of variables (28.69%). The first PCA account for the most of the variability of the data.

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