

Original Research Article

Multivariate analysis of Genetic Diversity in the Ashwagandha (*Withania somnifera* (L.) Dunel) genotype

ABSTRACT

The present investigation was undertaken to decipher principal component analysis (PCA) among Sixty ashwagandha germplasm and 3 check were evaluated to study the diversity pattern among the collected accession. A randomized complete block design with three replications was used to conduct the trial. The Experiment was laid out late *kharif*-2017 at Rajasthan college of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur. The data of fifteen morphological traits were recorded and analyzed to find out principal components to reveal the diversity among the genotype. The principal component analysis (PCA) showed the first six principal component to reveal exhibited more than eigen values and accounted for 64.57 percent of the total variation that comprised of 16.32(PC 1), 11.63(PC 2), 11.04(PC 3), 9.23(PC 4), 8.65(PC5) and 7.66(PC 6). The genotype MPAS-57 along PCA 1 axis and MPAS along PCA 2 respectively, indicating that both are negatively associated. PC 1 contributes the maximum toward the total variability (16.32). The characters *viz.*, Days to 75% maturity, root length, dry plant weight, fresh plant weight, harvest index and 100 seed weight explained the maximum variance in PC 1.

Keywords: Multivariate analysis, Genetic diversity, *Withania somnifera*, Principal component analysis, Ashwagandha, Cluster analysis.

1. INTRODUCTION :

Ashwagandha [*Withania somnifera* (L.) Dunal] belongs to the Solanaceae family and is a cross pollinated crop having the chromosome number $2n = 48$ (Nigam and Kandalkar, 2006). It is popularly known as Indian Ginseng, gooseberry or winter cherry. It originates in North-Western and Central India as well as Mediterranean region of North Africa. Dry and subtropical climatic regions are best suited for growth of this crop. The genus *withania* comprises of 23 species among which only two (*Withania somnifera* and *withania coagulans*) are reported from India. Ashwagandha (*Withania somnifera*) is an important medicinal plant and its dried roots are used in traditional systems of medicine. The market price of roots is determined by physical (textural) quality. Brittle roots with high starch and low fiber are considered to be superior because of ease in grinding. (Kumar et al.,). Ashwagandha (*Withania somnifera*) is an important medicinal plant and its dried roots are used in traditional systems of medicine. The market price of roots is determined by physical (textural) quality. Brittle roots with high starch and low fiber are considered to be superior because of ease in grinding. It is also an ingredient of medicaments prescribed for curing disability and sexual weakness in males. Seeds are diuretic, warm leaves are used for providing comfort during eye disease (Nigam and Kandalkar, 2006). One of the well known withanolides, withaferin A

emerged as an important therapeutic molecules of ashwagandha due its anticancer properties (Koduru *et al.*, 2010; Lee *et al.*, 2010; Mayola *et al.*, 2011;). The total alkaloids content in the roots varied from 0.16-0.66%(Biennial Progress Report 2066-2008). The main alkaloids are withanolodes, somniferine, somniferinine, somine, withanine, pseudowithanolides, withanonine and witasomine (Covello and Ciampa (1960), Patel and Desai (2017)).

PCA has been applied continuously for analysis of genetic diversity in many crops such as ashwagandha .The current study was planned to select out the traits that sort the genotype into different group and suggest potential genotype that could be used as parents in the ashwagandha improvement program. The plant (Indian chemotype) is reported to contain 12 withanolides, five unidentified alkaloids, many free amino acids, chlorogenic acid, glycosides, glucose, condensed tannins, and flavonoids in the leaves. Ayurvedic and Unani systems use the leaves for treatment of tumours and tubercular glands as they possess antibacterial, fungal, and tumour properties. Ashwagandha has also found calm the mind, relieve weakness and nervous exhaustion, build sexual energy, and promote healthy sleep. The importance of multivariate analysis for the study of qualitative traits present in biological experimental populations has been shown in various research areas. Multivariate is commonly used to study genetic diversity. Among the multivariate techniques, principal component analysis (PCA) had been showed very significant in choosing the genotypes for selecting a breeding program.

2. MATERIALS AND METHOD

2.1 Plant Material: - The study was comprised of 60 diverse genotypes along with three standard checks *viz.*, JA-20 (Jawahar Ashwagandha-20), JA-134 (Jawahar Ashwagandha-134) and RVA-100 (Raj Vijay Ashwagandha). The diverse genotypes were collected from different agro climatic zone of Rajasthan India.

2.2 Experimentation: - The Experiment was laid out late *kharif*-2017 in the Botany fields at Rajasthan college of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur. Udaipur is situated at 24⁰-35' N latitude and 73⁰-42' E longitude and at an elevation of 582.17 meters above mean sea level. The climatic conditions of the area represent subtropical condition with humid climate.

2.3 Experimental Design:- Sixty three genotypes with three standard checks (JA-20, JA-134 and RVA-100) by growing them in a Randomized Block Design (RBD) with three replications a single row plot of 4.0 meter length maintaining a crop geometry of 30 X 5 cm at Instructional Farm Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, MPUAT, Udaipur during late *kharif*-2017. The recommended practices were applied for soil preparation, ploughing, levelling, irrigation and weeding.

2.4 Morphological Evaluation :- Observation were recorded on following fifteen traits day to flowering (DF), days to 75 percent maturity, (DTM) plant height (PH) (cm), number of primary branches per plant (PB), number of secondary branches per plant (SB), leaf area index (LAI), root length (RL) (cm), root diameter in collar region (RD) (mm), fresh root yield per plant (FRY) (g), dry root yield per plant (DRY) (g), fresh plant weight (FPW) (g/plant), dry plant weight (DPW) (g/plant), 100 seed weight (SW), harvest index (HI) and total alkaloid content (TAC) for present study on ten randomly selected plant competitive plants

from each genotype in each replication except for days to flowering and day to 75 per cent maturity, where observation were recorded on plot bases. Days to flowering were measured from the sowing date till the first flower initiate. Days to maturity were noted when the colour of the plant became yellow. The plant height was the length recorded from base to top of the plant with meter rod after maturation. The weight of resultant biomass was recorded where the whole plant was cutting into pieces and digital balance was used to record its mass. Seed yield per plant was determined after harvesting and threshing of all the spikelet. The average seed weight was calculated by taking 1000 seeds of each genotype and weighing it's through digital balance. Harvest index was calculated as the ratio between the grain yield and biological yield. To avoid border effect observation were not recorded on the first and last plant in the each row. Each observation on each variable from the value for the observation on the variable and dividing this by the standard deviation for the corresponding variable.

2.4 Statistical Analysis of Data:- PCA was done to transform the original variables into a limited number of uncorrelated new variables and to allow the visualization of differences among cultivars, the identification of groups, and the identification of relationships among cultivars and variables. The Eigen Values and Eigen Vectors were computed, which represent the variance and the loadings of the corresponding principal components (PCs). A biplot analysis was carried out based on the two most important PCs to visualize the pattern of total diversity within the germplasm studied. The degree of correlation between the traits and the percentage contribution of each trait to the total diversity were determined. The standard data were subjected to principal on minimum variable method of ward (1963) utilizing statistical software. The data was subjected to D^2 analysis (Mahalanobis, 1936) as elaborated by Murthy and Arunachalam (1966).

- 3. RESULT AND DISCUSSION:** Principal components analysis for 63 ashwagandha germplasm accessions for 15 attributing characters present in the table. PCA with high eigen value and variables considered as best representative of system attributes. In all germplasm accessions are categorised into six principal components (PCs) 64.57% cumulative variables of those PCAs. PCA1 has high percentage of variables that is (16.32%) after that PCA2 (11.63%), PCA3 (11.04%), PCA4 (9.23%), PCA5 (8.65%) and PCA6 (7.66%). The PCs having the eigen values more than one were considered as significant and hence not ignorance as they are likely to have any practical significance. 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).

Principal component analysis (PCA) was carried out based on fifteen quantitative morphological characters. The seven principal components account for 71.59% of the overall variability among the studied *withania somnifera* L. accessions for the total phenotypic variation (Table 1). Among these seven Principal components (PCs), the PCA-1 was found to have 16.32% out of the total variability. Dry root yield per plant (0.517) (g), day to flowering (0.130), plant height (0.063) (cm), number of secondary branches per plant (0.240), leaf area index (0.233), root diameter in collar region (0.070) (mm), total alkaloid content (0.137) contributed positively to first principal component. In contrast, number of primary branches

per plant (-0.010), root length (-0.066) (cm), days to 75 percent maturity, (-0.107), fresh root yield per plant (-0.224) (g), fresh plant weight (-0.486) (g/plant), dry plant weight (-0.505) (g/plant), 100 seed weight (-0.003), harvest index (-0.129) contributed negatively.

The contribution of PC-2 in total differences was 11.63% and positively associated with FRY (0.479), HI (0.365), RL (0.307) DTM (0.148), DRY (0.104) and DF (0.048), whereas PH (-0.057), PB (-0.163), SB (-0.178), RD (-0.281), DPW (-0.347), FPW (-0.181), LAI (-0.113), TAC (-0.313) and SW (-0.318) were negative associated with PC 2.

The PC-III showed 11.04% of the total agro-morphological variation and positively associated with day to flowering (0.482), plant height (0.458), Total alkaloid content (0.421), days to 75 percent maturity (0.284), root length (0.217), days to flowering (0.175), fresh root yield (0.125) and dry root yield (0.008), while negatively associated with leaf area index (-0.249), number of secondary branches per plant (-0.321), number of primary branch per plant (-0.023), root diameter in collar region (-0.101), dry plant weight (-0.119), fresh plant weight (-0.060) and harvest index (-0.100).

The PC-IV showed 9.23% of the total agro-morphological variation and positively associated with day to flowering (0.449), number of primary branches per plant (0.358), days to 75 percent maturity (0.319), root diameter in collar region (0.302) and plant height (0.073). Similarly traits *i.e.* number of secondary branches per plant (-0.059), root length (-0.028), fresh root yield (-0.070), dry plant weight (-0.046), fresh plant weight (-0.021), harvest index (-0.395), leaf area index (-0.301), total alkaloid content (-0.351), 100 seed weight (-0.293), dry root yield (-0.033) contributed negatively.

In the total variability the contribution of PC-V was observed 8.65% and the parameter *i.e.* days to 75 percent maturity (0.487), number of secondary branches per plant (0.255), 100 seed weight (0.232), day to flowering (0.224), fresh root yield (0.203), leaf area index (0.132), root diameter in collar region (0.117), dry plant weight (0.038), harvest index (0.005) were positively contributed. Whereas the characters *i.e.* plant height (-0.117), number of primary branches percent (-0.550), root length (-0.429), fresh plant weight (-0.021) total alkaloid content (-0.118) and dry root yield (-0.054) contributed negatively.

The PC-VI depicted proportion of variability as 7.66 and were positively associated with plant height (0.353), number of primary branches per plant (0.310), leaf area index (0.210), total alkaloid content (0.154), 100 seed weight (0.093), fresh root yield (0.060), days to 50% maturity (0.013) and dry plant weight (0.020). Similarly the traits *i.e.* plant height (-0.487), number of secondary branches per plant (-0.278), root length (-0.267), root diameter in collar region (-0.537), fresh plant weight (-0.077), harvest index (-0.119) and dry root yield (-0.043) were negatively associated with PC-VI.

PC-VII accounted 7.01% of total variation among agro-morphological traits. Days to flowering (0.408), plant height (0.358), harvest index (0.290), total alkaloid content (0.166) and fresh plant weight (0.140) were contributed positively to PC-VII Whereas days to 75 percent maturity (-0.395), number of primary branches per plant (-0.185), number of secondary branches per plant (-0.205), root length (-0.445), fresh root yield (-0.036), root

diameter in collar region (-0.045), dry plant weight (-0.126), leaf area index (-0.055), 100 seed weight (-0.306) and dry root yield (-0.157) were negatively associated with PC-VII.

The PCA scores for 60 ashwagandha genotype with three checks in the first three PCs were analyzed and considered three axes as X, Y, Z and squared distance of each genotype from these axes were computed (Table 2). The genotype identified on extreme positive side on both the axis were considered to be best genotypes *i.e.* genotype (8) MPAS-11 (11.70), MPAS-9(9.54), MPAS-5(8.65), MPAS-56(8.73) along PCA1 axis and genotype MPAS-55 (14.56), MPAS-3(11.35), MPAS-46(10.97), MPAS-25(8.49) along PCA 2 axis. This genotype might be exploited in future hybridization programme.

Cluster analysis or D² statistics: It includes 63 genotypes and 15 characters were used for study genetic diversity. Based the analysis values are arranged into 8 clusters (Table 3). This point towards substantial diversity is present in all the genotype estimated in the present study. The highest inter cluster distance was observed in between average inter cluster values were maximum between cluster I and VII (490.877) followed by cluster II and VIII (387.495) and cluster V and cluster VI (306.753) and cluster VI and cluster VII (133.77). on the rest of pairs, average divergence ranged from 215.099 (III and VI) and 120.571 (V and VI). The similar results were also reported by Joshi *et al.*, (2015). The average intra and inter cluster distance are given in table 4. Maximum intra cluster distance was in cluster-II (157.593) followed by III (99.913), I (95.138), VII (87.170), IV (85.313), VI (84.316), V (85.313) and VIII (82.010). The high intra-cluster distance in cluster II indicated the presence of wide genetic diversity among the genotype in this cluster. Highest inter cluster distance implied that the hybridization strategy including present from the clusters decided to have superior occurrence of the enhanced segregates. Minimum inter-cluster distance determines less heterogeneity in the genotypes. 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.

4.6.4 Cluster means: The cluster means (Table 4) indicated that cluster VIII was having maximum number of primary branch per plant (5.30), fresh root yield per plant (15.67), dry plant weight (53.03), fresh plant weight (212.41), cluster VI having maximum plant height (40.15), number of secondary branch per plant (10.08), leaf area index (0.97), total alkaloid content (0.45) cluster V having maximum days to flowering (102.37), harvest index (14.12) cluster III having maximum root length (21.07) cluster II having maximum day to 75 percent maturity (171.66) and cluster I shows maximum root diameter in collar region (9.52), 100-seed weight (0.218) and dry root yield per plant (3.23) and cluster VIII having maximum and therefore selection of genotypes for these characters may be made from these clusters.

Conclusion: - The diversity reported in a considerable number of ashwagandha germplasm originating from diverse agro-ecologies is exploited by the plant breeders for genetic improvement of this plant. To get an improved variety of given germplasm the difference in morphological and biochemical parameters are investigated which is the basic criterion for successful breeders. In this study, 63 genotypes were studied for differences in morphological

and biochemical traits. Cluster analysis or D^2 statistics include 63 genotype and fifteen characters were used for study genetic diversity. Based the analysis values are arranged into 6 cluster. This point towards substantial diversity is present in all the genotype estimated in the present study. This is in conformity in ashwagandha. Based on PCA analysis component explained over 71.59 % cumulative variables. PCA 1 has high percentage of variable (16.32).The first PCA account for the most of the variability of the data. The genotype identified on extreme positive side on both the axis were considered to be best genotypes *i.e.* genotype (8) MPAS-11 (11.70), MPAS-(9.54), MPAS-5(8.65), MPAS-56(8.73) along PCA1 axis and genotype MPAS-55(14.56), MPAS-3(11.35), MPAS-46(10.97), MPAS-25(8.49) along PCA 2 axis. This genotype might be exploited in future hybridization programme.

References

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Statistics	PC1	PC2	PC3	PC4	PC5	PC6	PC 7
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Table 1:- Eigen value, proportion of variance and traits that contributed to seven Principal component analysis (PCA) for morphological data.

DF	0.130	0.048	0.175	0.449	0.224	0.353	0.408
DTM	-0.107	0.148	0.284	0.319	0.487	0.013	-0.395
PH	0.063	-0.057	0.458	0.073	-0.117	-0.487	0.358
PB	-0.010	-0.163	-0.023	0.358	-0.550	0.310	-0.185
SB	0.240	-0.178	-0.321	-0.059	0.255	-0.278	-0.205
RL	-0.066	0.307	0.217	-0.028	-0.429	-0.267	-0.445
FRY	-0.224	0.479	0.125	-0.070	0.203	0.060	-0.036
RD	0.070	-0.281	-0.101	0.302	0.117	-0.537	-0.045
DPW	-0.505	-0.347	-0.119	-0.046	0.038	0.020	-0.126
FPW	-0.486	-0.181	-0.060	-0.021	-0.012	-0.077	0.140
HI	-0.129	0.365	-0.100	-0.395	0.005	-0.119	0.290
LAI	0.233	-0.113	-0.249	-0.301	0.132	0.210	-0.055
TAC	0.137	-0.313	0.421	-0.351	-0.118	0.154	0.166
SW	-0.003	-0.318	0.482	-0.293	0.232	0.093	-0.306
DRY	0.517	0.104	0.008	-0.033	-0.054	-0.043	-0.157
Eigene value (Root)	2.449	1.745	1.657	1.385	1.298	1.150	1.052
% Var. Exp.	16.328	11.639	11.047	9.237	8.656	7.668	7.015
Cum.Var.Exp.	16.328	27.967	39.015	48.253	56.909	64.578	71.593

Table 2:- PCA sources of 60 genotype with three checks of ashwagandha [*Withania somnifera* (L.)]

S.No.	Genotype	PC I (X vector)	PC II(Y Vector)	PCIII (Z Vector)
1.	MPAS 1	-5.651	-11.324	26.273
2.	MPAS 2	-6.831	-13.9170	24.180
3.	MPAS 3	-3.845	11.350	24.180
4.	MPAS 4	-3.226	-11.485	24.957
5.	MPAS 5	8.659	-11.251	23.749
6.	MPAS 6	-3.328	-9.911	26.350
7.	MPAS 7	-7.966	-8.895	22.255
8.	MPAS 8	-10.072	-11.403	19.015
9.	MPAS 9	-12.072	-11.403	19.015
10.	MPAS 10	7.980	-12.317	22.953
11.	MPAS 11	-4.162	-14.160	26.970
12.	MPAS 12	-2.135	-10.905	24.482
13	MPAS 13	-2.397	-11.638	24.948
14	MPAS 14	-2.624	-14.254	26.333
15	MPAS 15	11.708	-14.238	25.504
16	MPAS 16	-3.414	-11.843	26.803
17	MPAS 17	-6.254	-8.662	20.310
18	MPAS 18	-2.782	-15.129	27.879
19	MPAS 19	-2.800	-8.392	21.202
20	MPAS 20	-1.498	-13.131	27.732
21	MPAS 21	-7.718	-8.227	21.338
22	MPAS 22	-6.085	-11.145	23.547
23	MPAS 23	-5.469	-8.820	22.226
24	MPAS 24	9.547	-10.126	22.646
25	MPAS 25	-2.662	-8.489	21.002
26	MPAS 26	-5.966	-16.029	28.129
27	MPAS 27	-2.605	-12.319	26.204
28	MPAS 28	-5.411	-12.408	26.097
29	MPAS29	-4.264	-12.048	25.699
30	MPAS 30	-5.770	-10.860	25.201
31	MPAS 31	-2.486	-10.445	26.318
32	MPAS 32	-1.726	-10.786	26.800
33	MPAS33	-3.895	-10.974	24.562
34	MPAS 34	-4.094	-10.247	23.084
35	MPAS 35	-2.251	-11.884	26.915
36	MPAS 36	-3.958	-8.525	23.534
37	MPAS 37	-5.641	-6.723	25.887
38	MPAS 38	-4.129	-.957	28.020
39	MPAS 39	-3.309	-11.924	25.403
40	MPAS 40	-5.909	10.094	22.660
41	MPAS 41	-4.998	-12.361	25.232
42	MPAS 42	-1.459	-15.749	29.554

	I	II	III	IV	V	VI	VII	VIII
Cluster I	95.138	244.516	215.396	229.311	163.793	565.752	596.015	407.050
Cluster II		157.593	344.209	243.419	304.028	395.709	545.088	487.727
Cluster III			99.913	140.928	151.898	315.012	272.911	165.212
Cluster IV				85.313	132.907	205.884	200.780	163.112
Cluster V					83.891	390.644	322.138	205.953
Cluster VI						84.316	152.270	201.953
Cluster VII							87.170	135.949
Cluster VIII								82.010

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Table 4:- Cluster mean for fifteen characters in ashwagandha

Character	Day to Flowering	Day to 75% Maturity	Plant Height (cm)	Number of Primary Branches / plant	Number of secondary Branches / plant	Root Length (cm)	Fresh Root yield g/ plant	Root Diameter in collar Region	Dry plant weight/ Plant	Fresh plant weight/ plant	Harvest index	Leaf area Index	Total Alkaloid Content	100 seed weight	Dry Root Yield
Cluster I	99.80	170.46	38.35	4.99	9.51	21.20	21.20	9.52	51.27	143.34	6.32	0.94	0.32	.0218	3.23
Cluster II	99.33	171.66	37.04	5.10	9.50	18.55	18.55	9.23	47.80	116.80	4.50	0.92	0.44	0.211	2.08
Cluster III	100.46	171.06	37.62	4.75	9.06	21.07	15.66	8.05	31.10	109.41	10.51	0.89	0.30	0.199	2.93
Cluster IV	100.33	170.85	39.94	4.97	9.44	20.03	14.22	8.14	36.73	106.23	6.55	0.90	0.36	0.205	2.46
Cluster V	102.33	170.08	39.74	4.79	9.60	20.81	13.81	9.29	22.30	88.36	14.12	0.96	0.35	0.211	3.05
Cluster VI	100.33	168.28	40.15	5.25	10.08	19.61	11.93	9.20	30.83	104.43	6.56	0.97	0.45	0.208	2.02
Cluster VII	101.94	169.96	38.88	4.94	9.71	20.45	13.02	8.63	27.86	96.17	9.90	0.94	0.40	0.210	2.68
Cluster VIII	101.33	168.16	38.66	5.30	8.55	20.53	15.67	8.61	53.03	212.41	4.74	0.90	0.41	0.201	2.80

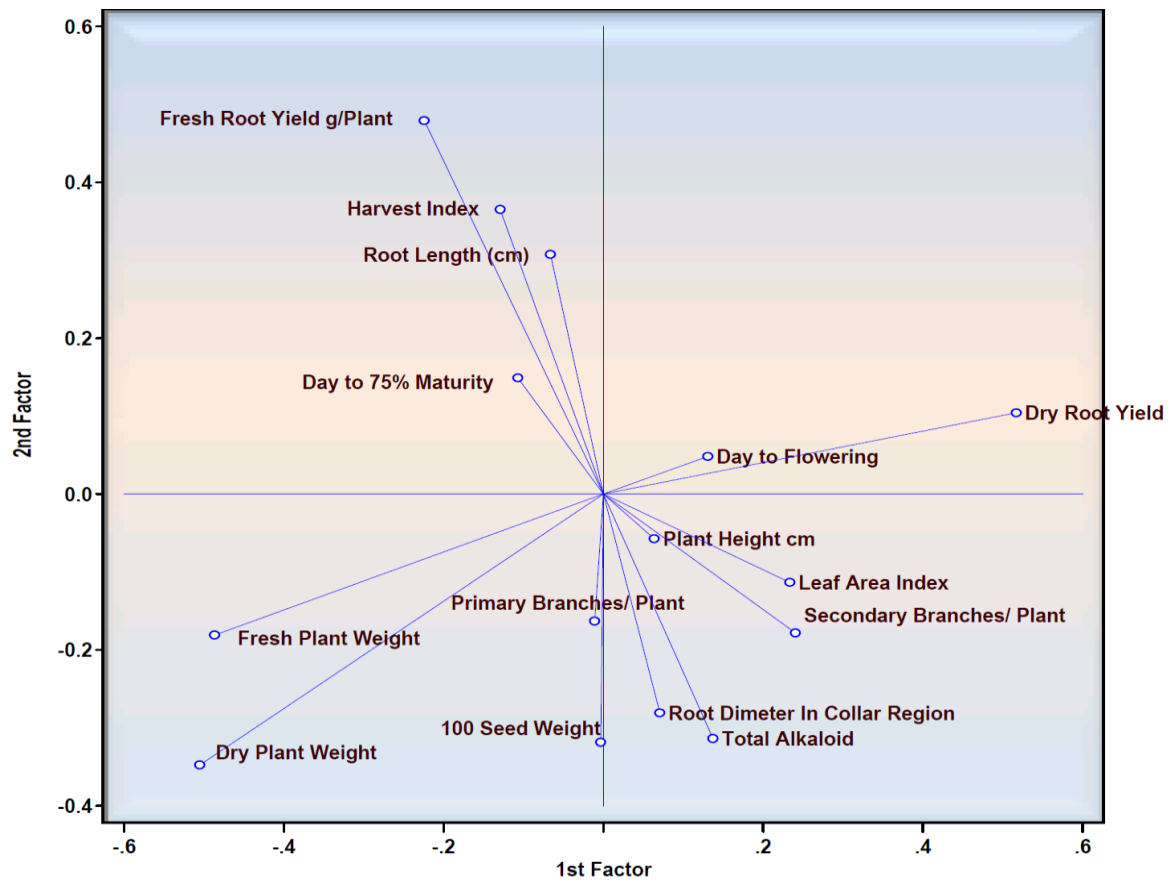


Figure 1. Plot of components weight of 15-morpho-agronomic traits of the ashwagandha cultivars studied.

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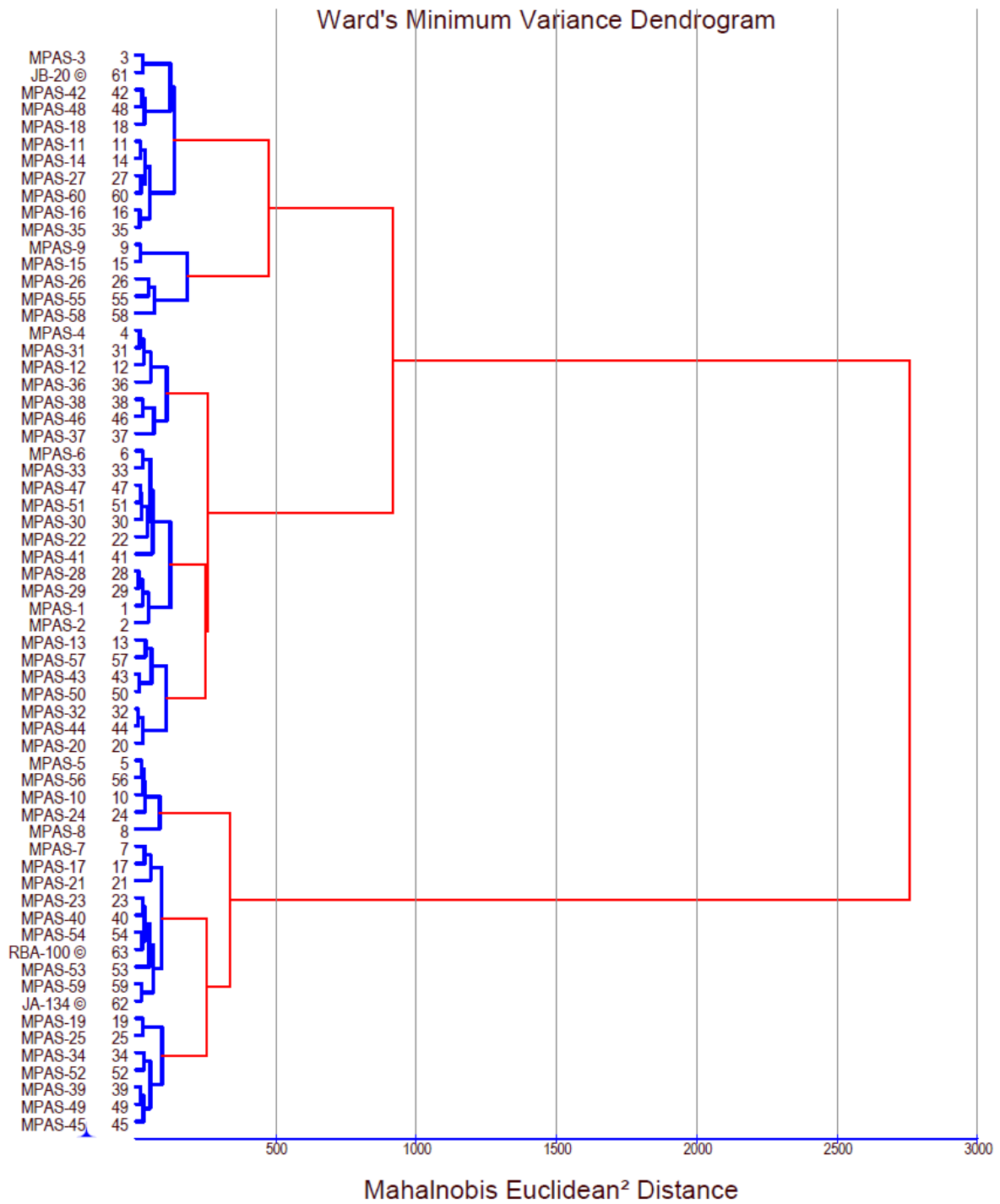


Fig 2 Dendrogram for different morphological in ashwagandha 63 genotypes based on root quality traits.