Original Research Article

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

ABSTRACT

The current study was conducted to decipher principal component analysis (PCA) among 60 ashwagandha germplasmas and 3-check was evaluated to study the diversity pattern among the collected accessions. A randomized complete block design with three replications was used to perform the experiment. The experiment was posted late kharif-2017 at Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur. Data from fifteen morphological traits were recorded and analyzed to find out main components to reveal the diversity among the genotype. The main component analysis (PCA) showed that the first six main components revealed exhibited more than intrinsic values and accounted for 64.57 percent of the total variation, which consisted 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 the PCA 1 axis and MPAS along the PCA 2, respectively, indicates that both are negatively linked. PC 1 contributes maximum to the overall variability (16.32). The grades, namely days to 75% maturity, root length, dry plant weight, fresh plant weight, harvest index and 100 seed weights 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, 1995). 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., 2012). Ashwagandha (*Withania somnifera*) is an important medicinal plant and its dried roots are used in traditional systems of medicine. It is also an ingradient 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, 1995). 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 2006-2008). 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 (Srivastava *et al.*, 2018). 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 24^0-35' N latitude and 73^0-42' 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:** 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), number of secondary branches per plant (0.240), leaf area index (0.233), total alkaloid content (0.137) day to flowering (0.130), root diameter in collar region (0.070), plant height (0.063) (cm), (mm), contributed positively to first principal component. In contrast dry plant weight (-0.505) (g/plant), fresh plant weight (-0.486) (g/plant), fresh root yield per plant (-0.224) (g), harvest index (-0.129), days to 75 percent maturity, (-0.107), root length (-0.066) (cm), number of primary branches per plant (-0.010), 100 seed weight (-0.003) 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 DPW (-0.347), SW (-0.318), TAC (-0.313), RD (-0.281), FPW (-0.181), SB (-0.178), PB (-0.163), LAI (-0.113) and PH (-0.057) 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 number of secondary branches per plant (-0.321), leaf area index (-0.249), dry plant weight (-0.119), root diameter in collar region (-0.101), harvest index (-0.100), fresh plant weight (-0.060) and number of primary branch per plant (-0.023).

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.* harvest index (-0.395), total alkaloid content (-0.351), leaf area index (-0.301), 100 seed weight (-0.293), fresh root yield (-0.070), number of secondary branches per plant (-0.059), dry plant weight (-0.046), dry root yield (-0.033) root length (-0.028), fresh plant weight (-0.021), 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.* number of primary branches percent (-0.550), root length (-0.429), total alkaloid content (-0.118), plant height (-0.117), dry root yield (-0.054) and fresh plant weight (-0.021) 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.* root diameter in collar region (-0.537), plant height (-0.487), number of secondary branches per plant (-0.278), root length (-0.267), harvest index (-0.119), fresh plant weight (-0.077) 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 root length (-0.445), days to 75 percent maturity (-0.395), 100 seed weight (-0.306), number of secondary branches per plant (-0.205), number of primary branches per plant (-0.185), dry rot yield (-0.157), dry plant weight (-0.126), leaf area index (-0.055), root diameter in collar region (-0.045) and fresh root yield (-0.036) 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 computes (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-(9.54), MPAS-56(8.73), MPAS-5(8.65) 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 D^2 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 (83.891) 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 studies 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-56(8.73), MPAS-5(8.65) 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.

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Table 1:- Eigen value, proportion of variance and traits that contributed to seven Principal component analysis (PCA) for morphological data.

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

Statistics	PC1	PC2	PC3	PC4	PC5	PC6	PC 7
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
Eigen 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

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
43	**MPAS 43**	-5.180	-11.191	26.627
44	**MPAS 44**	-2.238	-11.753	27.457

45	**MPAS 45**	-3.268	-8.845	23.543
46	**MPAS 46**	-3.618	10.197	28.356
47	**MPAS 47**	-6.038	-11.051	26.254
48	**MPAS 48**	-3.268	-15.924	30.781
49	**MPAS 49**	-4.070	-11.889	24.279
50	**MPAS 50**	-3.224	-12.021	26.626
51	**MPAS 51**	-5.518	-10.828	25.370
52	**MPAS 52**	-3.421	-10.756	22.279
53	**MPAS 53**	-5.589	-6.822	22.020
54	**MPAS 54**	-6.680	-8.659	23.125
55	**MPAS 55**	-6.213	14.563	29.410
56	**MPAS 56**	8.739	-12.197	23.345
57	**MPAS 57**	-0.736	-12.891	25.433
58	**MPAS 58**	-8.269	-16.387	26.652
59	**MPAS 59**	-5.979	-8.151	23.889
60	**MPAS60**	-4.300	-12.210	27.918
61	**JB-20©**	-3.846	-11.938	30.544
62	**JA-134©**	-5.516	-7.081	23.689
63	**RBA-100©**	-6.648	-9.587	24.333

Table 3:- Average intra and inter cluster D^2 values in63 genotypes of ashwagandha ($Euclidean^2$: Cluster distance: Ward)

	Cluster I	**Cluster II**	**Cluster III**	**Cluster IV**	**Cluster V**	**Cluster VI**	**Cluster VII**	**Cluster VIII**
Cluster	**95.138**	244.516	215.396	229.311	163.793	565.752	596.015	407.050

I								
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**

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

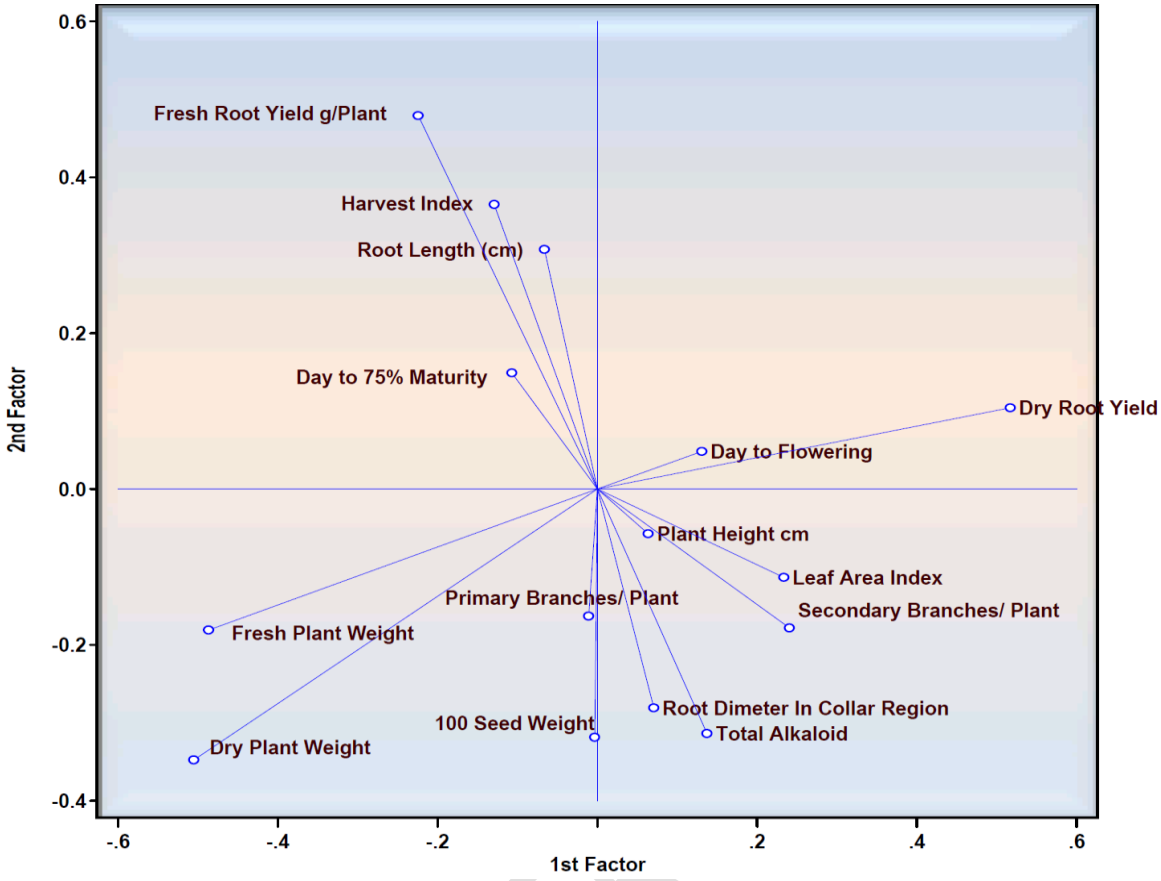

Figure 1. Plot of components weight of 15-morpho-agronomic traits of the ashwagandha cultivars studied.

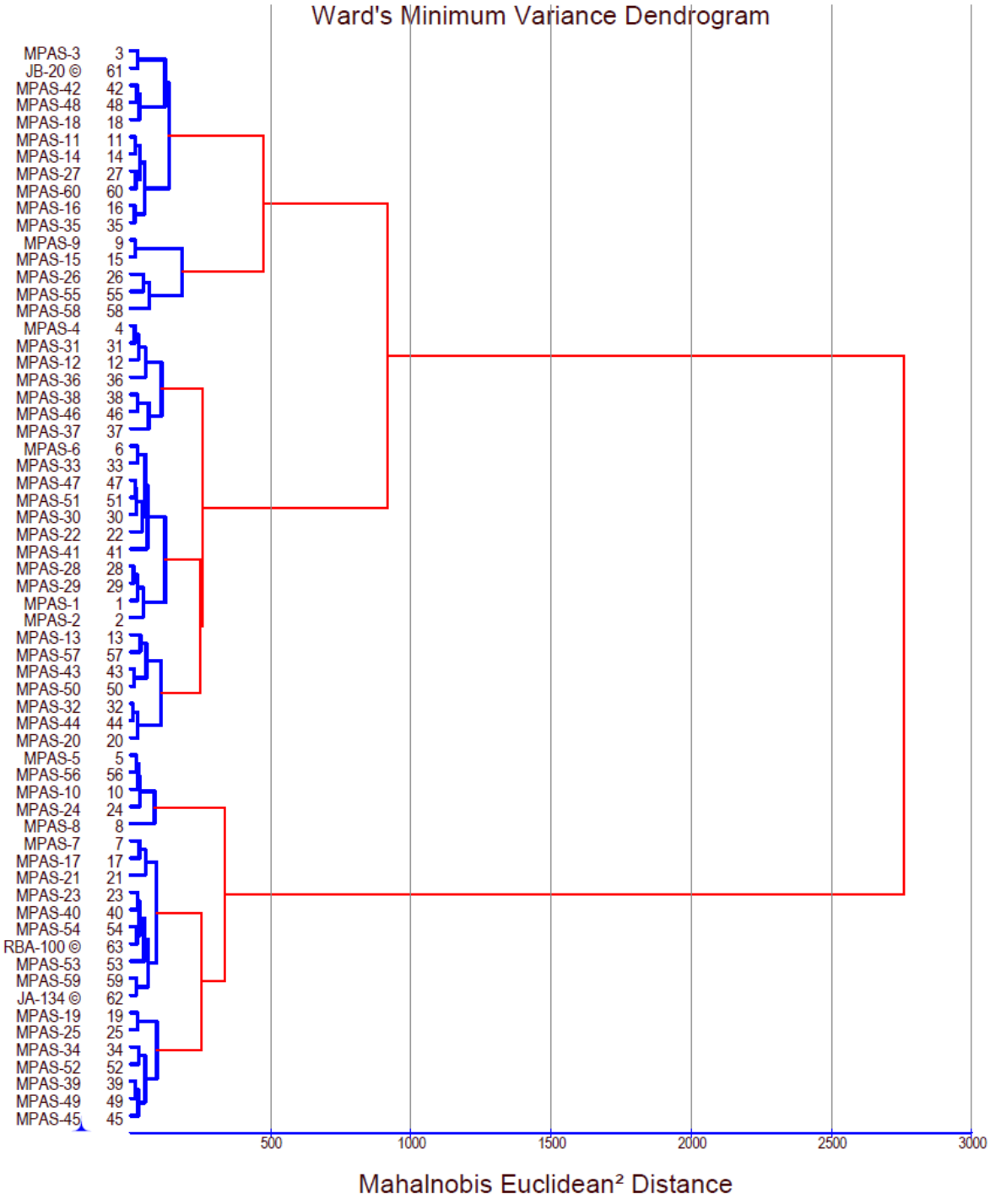

Fig 2 Dendogram for different morphological in ashwagandha 63 genotypes based on root quality traits.