Genetic Divergence of Ashwagandha (*Withaniasomnifera* (L.) Dunal) for growth and yield traits

ABSTRACT

Genetic divergence among 32 Ashwagandha (*Withaniasomnifera* (L.) Dunal) accessions of different geographic origins was assessed using Mahalanobis D^2 statistics. Observations revealed significant genotypic differences, and accordingly, genotypes were classified into six clusters. Cluster I was the largest, with twenty genotypes, followed by the cluster II, which has eight, and clusters III, IV, V, and VI contained only one most divergent genotype. The maximum divergence was observed between clusters II and III (D^2 = 47.90), followed by clusters V and VI (D^2 = 43.95), and clusters III and VI (D^2 = 42.29). Cluster IV had the least inter-cluster distance (D^2 = 22.82) with cluster VI. Clusters III, IV, V, and VI had only one genotype each, and hence, the intra-cluster distance was zero. The genotype of cluster V was unique as it had the highest values for fresh and dry root weight per plant, diameter and length of the main root, number of secondary roots per plant, number of berries per plant, and girth of stem with a high seed yield per plant. Cluster VI was desirable in respect of days to 50 per cent flowering and days to root harvest. Cluster II exhibited the highest value for height of plant and branches per plant. Cluster IV had the highest mean values for the number of seeds per berry. Thus, hybridization among these genotypes can generate desirable transgressive segregants.

Aim of studies: To assess the genetic divergen of*Withaniasomnifera*
Place and Duration of Study: Department of Horticulture, College of Agriculture, UAS, GKVK, Bengaluru from 2020-21 to 2021-22.

Methodology: The genetic divergence between the genotypes in the population was estimated using formula $D^2= p (Y^{it} - Y^{jt})$ and genetic divergence among 32 Ashwagandha (*Withaniasomnifera* (L.) Dunal) accessions of different geographic origins was assessed using Mahalanobis D^2 statistics. Observations revealed significant genotypic differences, and accordingly.

Results
Observations revealed significant genotypic differences, and accordingly, genotypes were classified into six clusters. Cluster I was the largest, with twenty genotypes, followed by the cluster II, which has eight, and clusters III, IV, V, and VI contained only one most divergent genotype. The maximum divergence was observed between clusters II and III (D^2 = 47.90), followed by clusters V and VI (D^2 = 43.95), and clusters III and VI (D^2 = 42.29). Cluster IV had the least inter-cluster distance (D^2 = 22.82) with cluster VI. Clusters III, IV, V, and VI had only one genotype each, and hence, the intra-cluster distance was zero. The genotype of cluster V was unique as it had the highest values for fresh and dry root weight per plant, diameter and length of the main root, number of secondary roots per plant, number of berries per plant, and girth of stem with a high seed yield per plant. Cluster VI was desirable in respect of days to 50 per cent flowering and days to root harvest. Cluster II

exhibited the highest value for height of plant and branches per plant. Cluster IV had the highest mean values for the number of seeds per berry

Conclusion of finding.

Significant genetic divergence and complementarity for most features has been shown by the genotypes of clusters II and III and clusters V and VI. This could lead to significant genetic improvement for particular characters and their selection for new varieties.

Keywords: Mahalanobis, Withania, genetic divergence, inter-cluster and genotypes.

INTRODUCTION

"Ashwagandha [*Withaniasomnifera* (L.) Dunal] belongs to the family Solanaceae with chromosome number 2n=48. Ashwagandha is one of the most popular medicinal crops being commercially cultivated as a dryland crop in late kharif season in India". [18] The crop has been assigned several names with distinct meanings such as "Indian winter cherry" or "Indian ginseng" in English, "Punir" or "Asgandh" in Hindi and "Hiremaddinagida" in Kannada. "The species name has been ascribed as '*somnifera'*, which means "sleep-inducer" in Latin, owing to its prodigious anti-stress characteristics. As the roots possess a characteristic 'wet horse' smell, it has been named as Ashwagandha. The economic part of ashwagandha is root which is rich in alkaloids, steroidal lactones and saponins. According to the research findings, the total alkaloid content in the Indian roots range between 0.13 per cent and 0.31 per cent. Withaferin A and Withanolide D are the two main withanolides which contribute to most of the biological activity of ashwagandha Matsuda" [11]."The therapeutic benefits of ashwagandha roots are immense, and they are used in many traditional Indian medical systems, including Ayurveda, Unani, and Siddha Sharma" [13]. According to Bhattacharya and Muruganandam [1], it possesses anti-stress qualities. Cytotoxic, antimicrobial, antifungal, immunosuppressive, and immunomodulatory qualities Singh [15]. As a sedative, for rheumatoid arthritis, for joint inflammation, for feminine diseases, for hiccups, against colds and coughs, for ulcers, as leprosy, etc. This plant's bruised leaves are used as an anti-inflammatory and to treat tumors and tubercular glands Jayaprakasam and Kaur [6&8]. Additionally, the leaves are hypnotically used to treat alcoholism. The leaves are applied externally to treat boils, painful eyes, and hand and foot edema. They work well as a pesticide to eradicate body lice. In fact, an ointment prepared by boiling the leaves heals effectively for wounds and bed sores. Anthrax pustules are a different illness for which the fresh leaf juice is used Farooqi and Sreeramu [2].

"D^2 statistics are crucial for morphological characterization in order to analyze and evaluate genotypes of ashwagandha and other plant species for genetic diversity studies. The D2 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 subsequent generations enabling further selection and improvement Singh" [14]. "The hybrids developed from the selected genotypes within the limit of compatibility of these clusters may produce desirable trasgressive segregants of higher magnitude of heterosis Joshi and Singh" [7 &14). As there hasn't been much work done to improve this significant medicinal plant, the current study's findings will be useful in

analyzinggenetic diversity based on morphological indicators and supplementary genetic markers from the decade to come. This will be of great significance for future crop improvement programmes

MATERIALS AND METHODS

The present investigation, comprising 32 genotypes of ashwagandha, evaluation was carried out during 2020-21 and 2021-22 late *kharif* at the Horticulture Research Station, Department of Horticulture, College of Agriculture, UAS, GKVK, Bengaluru, which geographically, lies at a latitude of 13°.05' N, a longitude of 77°.33' E and an altitude of 925 metres above mean sea level. The treatments included thirty-two genotypes of ashwagandha, with their sources of collection enlisted in Table 1. The experiment was conducted using Randomized Complete Block Design (RCBD) with three replications in Bengaluru conditions following 30 cm x 20 cm spacing with the individual gross plot size of 2.6 m^2. The recommended doses of organic manures and fertilizers were applied at the time of field preparation. Application of Farm yard manure at 10–12 tonnes,15 kg of nitrogen and 25 kg of phosphorus per hectare were applied at the time of land preparation of experimental plots. seeds were sown to a depth of 1 to 2 cm by line sowing method. "All agronomic practices were carried out in accordance with University of Horticultural Science, Bagalkot, package of practises and required preventative plant protection measures were taken to protect the crop from pests and diseases. According to the accepted statistical practise, the mean data on yield and parameters that contribute to yield were treated to an analysis of variance using a randomised block design. D^2 statistic was employed to measure the genetic distance between genotype Mahalanobis" [9] All the germplasm lines were evaluated systematically for grouping them into different clusters using Mahalanobis D^2. Statistical analysis using Tocher's technique, as defined by Rao [12], the genotypes of ashwagandha were categorised based on minimum generalised distance. The average inter- and intra-cluster distances as well as the contribution of various traits to genetic divergence were calculated using the Manivel [10] method.

RESULTS AND DISCUSSION

The analysis of variance in 32 ashwagandha genotypes indicated a highly significant difference among the genotypes for all 13 quantitative characters studied, indicating the existence of adequate genetic diversity among the genotypes. In order to assess the genetic diversity among the 32 genotypes, a D^2 statistic was carried out. The procedure suggested by Tocher Rao [14] was used to group 32 ashwagandha genotypes into various clusters by treating estimated D^2 values as the square of the generalized distance. The pattern of distribution of 32 genotypes into various clusters is indicated in Table 2 and Fig 1. Out of 6 clusters formed, cluster I is the largest group, comprising 20 genotypes (DWS-05, DWS-22, DWS-132, DWS-141, DWS-144, DWS-197, DWS-250, DWS-252, DWS-253, DWS-258, DWS-260, DWS-262, DWS-272, DWS-281, DWS-284, DWS-315, DWS-316, DWS-317, Arka Ashwagandha, and Poshita), followed by cluster II with 8 genotypes (DWS-09, DWS-41, DWS-259, DWS-270, DWS-280, DWS-296, DWS-309, DWS-319), whereas clusters III (DWS-40), IV (DWS-257), V (DWS-279), VI (DWS-143) were monotypic or solitary. Similarly, 37 diverse

genotypes of Ashwagandha were grouped into 8 clusters by Venugopal [17]. Gupta [4] carried out a similar type of genetic divergence study on 75 genotypes of ashwagandha and grouped them into 14 clusters using Tocher's method.

The relative contribution of different characters to genetic divergence (D^2) is given in Table 3. The number of berries per plant contributed the greatest (29.64%) to the total genetic diversity among the genotypes, followed by fresh root weight per plant (23.59%), dry root weight per plant (22.22%), height of plant (8.233%), days to root harvest (4.64%), length of main root (3.21%), number of seeds per berry (3.02%), days to 50 per cent flowering (1.81%), number of branches per plant (1.61%), diameter of main root (0.81%), seed yield per plant (0.81%), and girth of stem (0.40%). The characters that predominantly contributed to divergence in this study also happen to be the main components of yield. The results of the present study point out the positive contribution of genetic divergence for yield components, and this can be of considerable help in selecting yield and other economic traits.

The mean intra and inter-cluster D^2 values among the various clusters are presented in Table 4. The intra-cluster distance varied from 0.0 to 19.94. Among the six clusters, cluster II with eight genotypes showed maximum intra-cluster diversity (D^2 = 19.94), followed by cluster I (D^2 = 17.88). Clusters III, IV, V, and VI had only one genotype each, hence the intra-cluster distance was zero. Based on distance between clusters, i.e., inter-cluster distances, maximum divergence was observed between clusters II and III (D^2 = 47.90), followed by clusters V and VI (D^2 = 43.95), and clusters III and VI (D^2 = 42.29). Cluster IV had the least inter-cluster distance (D^2 = 22.82) with cluster VI. The inter-cluster distance was minimum between clusters IV and VI (D^2 = 42.29), indicating narrow genetic diversity, whereas the inter-cluster distance was maximum between II and III (D^2 = 47.90), followed by clusters V and VI (D^2 = 43.95), and clusters III and VI (D^2 = 42.29), indicating wider genetic diversity between these groups. The selection of parents from these diverse clusters for hybridization would help in achieving novel recombinants. The correlation between hybridization and novel recombinant genotypes lies in the increased genetic variation introduced through hybridization and the subsequent generation of novel combinations of alleles during genetic recombination. These processes contribute to genetic divergence, evolution, and potentially the formation of new species over time. Similar types of observations were reported by Gupta and Venugopal [4 & 17]. The clusters with a single genotype indicated their independent identity and importance due to the various unique characters they possessed.

The cluster means for each of 13 traits are presented in Table 5. The genotype belonging to cluster V found the highest mean girth of stem (16.15 mm), followed by cluster III (15.67 mm) and cluster II (15.45 mm), while genotypes belonging to cluster IV recorded the lowest average girth of stem (13.18 mm). The highest cluster mean for the number of berries per plant was observed in cluster V (558.90), followed by cluster II (447.55), and cluster VI (417.00). The lowest cluster mean was observed in cluster III (316.17). Genotypes belonging to cluster V reported the highest cluster mean for length of main root (21.67 cm), followed by cluster II (16.04 cm) and cluster IV (14.95 cm).

The lowest cluster mean was observed in cluster III (11.00 cm). The highest cluster mean for the diameter of the main root was observed in cluster V (2.71 cm), followed by cluster II (2.22 cm) and cluster IV (2.18 cm). The least cluster mean was observed in cluster VI (1.44 cm). Genotypes belonging to cluster V showed the highest cluster mean for secondary roots per plant (2.71), followed by cluster II (2.22) and cluster IV (2.18), while the lowest cluster mean was observed in cluster VI (1.44). The genotype belonging to cluster V reported a maximum cluster mean for seed yield per plant in cluster (11.89 g), followed by cluster II (9.77 g) and cluster IV (9.15 g), while a minimum cluster mean was observed in cluster III (3.20 g). The genotypes belonging to cluster V found the highest mean fresh root weight per plant (31.19 g), followed by cluster II (21.09 g) and cluster IV (17.66 g), while genotypes belonging to cluster III recorded the lowest average fresh root weight per plant (16.40 g).The genotypes belonging to cluster V registered the highest mean dry root weight per plant (6.80 g), followed by cluster II (5.68 g) and cluster IV (4.92 g), while genotypes belonging to cluster I recorded the least average dry root weight per plant (4.33 g). The genotypes belonging to cluster II recorded the maximum mean height of plant (66.44 cm), followed by cluster VI (62.50 cm) and cluster IV (53.44 cm), while genotypes belonging to cluster III recorded the minimum average height of plant (42.42 cm). The number of branches per plant was highest in cluster II (14.41), followed by cluster V (14.20) and cluster IV (14.00). However, the lowest cluster mean was observed in cluster I (10.83). The genotypes belonging to cluster IV exhibited the highest cluster mean for the number of seeds per berry (41.18), followed by cluster V (37.92) and cluster II (37.89), while genotypes belonging to cluster III reported the lowest average number of seeds per berry (22.46). The traits of days to 50% flowering recorded the maximum value in the genotypes of cluster IV and VI (84.00), followed by cluster III (79.00) and cluster I (72.55), while genotypes of cluster V (66 days) exhibited the minimum mean value for days to 50% flowering. Day to root harvest found the maximum value in the genotypes of cluster VI (176.00), followed by cluster III (165.00) and cluster IV (164.00), while genotypes of cluster V (148.00) exhibited the minimum mean value for days to root harvest. Genotypes belonging to clusters with maximum intra-cluster distance are genetically more divergent, and hybridization between genotypes in divergent clusters is likely to produce wide variability with desirable segregates Sharma [13].

The significant variation observed in mean values across clusters and characters studied points to extensive diversity among the examined genotypes. Therefore, in addition to opting for genotypes from clusters with high inter-cluster distances for hybridization, breeders may consider selecting parents based on their genetic divergence concerning a specific trait. For instance, if the goal is to enhance root yield, breeders can option for parents with significant dissimilarity in this trait. The decision of parents about heterosis breeding and expression is influenced by the genetic diversity they possess. Tomkowiak[16] demonstrated that while “genetic diversity is crucial for significant heterosis, it alone is not sufficient to guarantee it. Inter-crossing of diverse groups would lead to broad genetic base and greater opportunities for recombination to occur which intern may unlock hidden variability by breaking undesirable linkages”. Several reports suggest that hybrids from genetically diverse parents exhibit greater heterosis compared to those from more closely related parents

Gahalainand Jain [3 and 5]. In future crop improvement programs, this research will aid plant breeders in identifying, characterizing, and choosing verified genotypes. Consequently, these genotypes are expected to gain commercial significance due to their genetic content and medicinal value.

CONCLUSION

D2 analysis used morphological features to group all genotypes into six clusters. The results show that there is a high level of genetic variety in ashwagandha genotypes, with the inter cluster distance being greater than the intra cluster distance. strong genetic divergence between the genotypes of clusters II and III and clusters V and VI, which were complementary for most traits, might lead to strong genetic gain for certain features and make them candidates for selection to produce new varieties.

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Table 1: List of Ashwagandha genotypes

Treatments	Genotypes	Source of collection
T_1	DWS-05	DMAPR, Boriavi, Anand, Gujarat
T_2	DWS-09	DMAPR, Boriavi, Anand, Gujarat
T_3	DWS-22	DMAPR, Boriavi Anand, Gujarat
T_4	DWS-40	DMAPR, Boriavi, Anand, Gujarat
T_5	DWS-41	DMAPR, Boriavi, Anand, Gujarat
T_6	DWS-132	DMAPR, Boriavi, Anand, Gujarat
T_7	DWS-141	DMAPR, Boriavi, Anand, Gujarat
T_8	DWS-143	DMAPR, Boriavi, Anand, Gujarat
T_9	DWS-144	DMAPR, Boriavi, Anand, Gujarat
T_{10}	DWS-197	DMAPR, Boriavi, Anand, Gujarat
T_{11}	DWS-250	DMAPR, Boriavi, Anand, Gujarat
T_{12}	DWS-252	DMAPR, Boriavi, Anand, Gujarat
T_{13}	DWS-253	DMAPR, Boriavi, Anand, Gujarat
T_{14}	DWS-257	DMAPR, Boriavi, Anand, Gujarat
T_{15}	DWS-258	DMAPR, Boriavi, Anand, Gujarat
T_{16}	DWS-259	DMAPR, Boriavi, Anand, Gujarat
T_{17}	DWS-260	DMAPR, Boriavi, Anand, Gujarat

T_{18}	DWS-262	DMAPR, Boriavi, Anand, Gujarat
T_{19}	DWS-270	DMAPR, Boriavi, Anand, Gujarat
T_{20}	DWS-272	DMAPR, Boriavi, Anand, Gujarat
T_{21}	DWS-279	DMAPR, Boriavi, Anand, Gujarat
T_{22}	DWS-280	DMAPR, Boriavi, Anand, Gujarat
T_{23}	DWS-281	DMAPR, Boriavi, Anand, Gujarat
T_{24}	DWS-284	DMAPR, Boriavi, Anand, Gujarat
T_{25}	DWS-296	DMAPR, Boriavi, Anand, Gujarat
T_{26}	DWS-309	DMAPR, Boriavi, Anand, Gujarat
T_{27}	DWS-315	DMAPR, Boriavi, Anand, Gujarat
T_{28}	DWS-316	DMAPR, Boriavi, Anand, Gujarat
T_{29}	DWS-317	DMAPR, Boriavi, Anand, Gujarat
T_{30}	DWS-319	DMAPR, Boriavi, Anand, Gujarat
T_{31}	Arka Ashwagandha	IIHR, Bengaluru
T_{32}	Poshita	CIMAP Research Station, Bengaluru

Table 2: Cluster-wise distribution of thirty-two genotypes of Ashwagandha for growth and yield attributing traits

Sl. No.	Cluster	No. of genotypes	Genotypes
1	I	20	DWS-05, DWS-22, DWS-132, DWS-141,DWS-144 DWS-197, DWS-250, DWS-252, DWS-253, DWS-258, DWS-260, DWS-262, DWS-272, DWS-281, DWS-284, DWS-315, DWS-316, DWS-317, Arka Ashwagandha and Poshita
2	II	08	DWS-09, DWS-41, DWS-259, DWS-270, DWS-280, DWS-296, DWS-309, DWS-319
3	III	01	DWS-40
4	IV	01	DWS-257
5	V	01	DWS-279
6	VI	01	DWS-143

Table 3: Per cent contribution of different characters to the divergence in different genotypes of Ashwagandha

Sl. No.	Source	Relative contribution	
		Times Ranked 1st	Contribution (%)
1	Height of plant (cm)	34	8.23
2	Number of branches/plant	8	1.61
3	Girth of stem (mm)	2	0.4
4	Days to 50 % flowering	9	1.81

5	Days to root harvest	23	4.64
6	Number of berries/plant	147	29.64
7	Number of seeds/berry	15	3.02
8	Seed yield/plant (g)	4	0.81
9	Length of main root (cm)	16	3.21
10	Diameter of main root (cm)	4	0.81
11	Number of secondary roots/plant	0	0
12	Fresh root weight/plant (g)	117	23.59
13	Dry root weight/plant (g)	106	22.22

Table 4: Intra (diagonal) and inter-cluster distances for six clusters of *Withania somnifera*(L.) Dunal for growth and yield attributing traits.

Cluster	I	II	III	IV	V	VI
I	**17.88**	32.95	24.62	25.72	27.85	34.20
II		**19.94**	47.90	34.12	30.87	31.54
III			**0.00**	27.57	37.49	42.29
IV				**0.00**	33.74	22.82
V					**0.00**	43.95
VI						**0.00**

Table 5: Cluster means for various characters in 32 genotypes of *Withania somnifera* (L.)

Sl. No.	Character	I	II	III	IV	V	VI
1	Height of plant (cm)	52.95	66.44	42.42	53.44	51.26	62.50
2	Number of branches/plant	10.83	14.41	11.67	14.00	14.20	11.20
3	Girth of stem (mm)	13.44	15.45	15.67	13.18	16.15	15.14
4	Days to 50% flowering	72.55	71.00	79.00	84.00	66.00	84.00
5	Days to root harvest	160.60	153.87	165.00	164.00	148.00	176.00
6	Number of berries/plant	324.52	447.55	316.17	383.53	558.90	417.00
7	Number of seeds/berry	34.92	37.89	22.46	41.18	37.92	34.56
8	Seed yield per plant (g)	5.64	9.77	3.20	9.15	11.89	3.21

9	Length of main root (cm)	14.45	16.04	11.00	14.95	21.67	14.00
10	Diameter of main root (cm)	1.80	2.22	1.76	2.18	2.71	1.44
11	Number of secondary roots/plant	1.80	2.22	1.76	2.18	2.71	1.44
12	Fresh root weight/plant (g)	16.46	21.09	16.40	17.66	31.19	16.45
13	Dry root weight/plant (g)	4.33	5.68	4.60	4.92	6.80	4.80

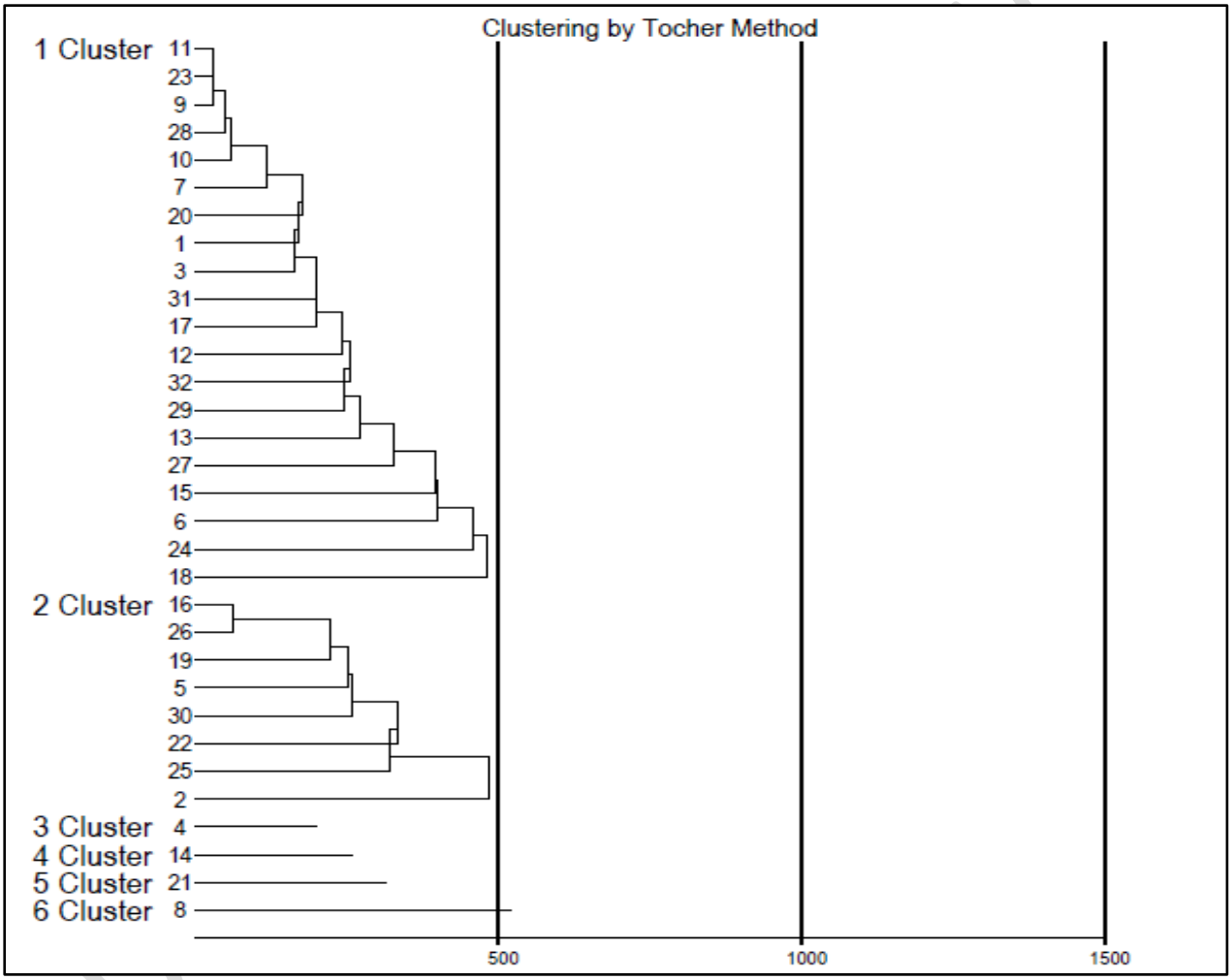

Fig.1: Dendrogram of genetic diversity of 32 ashwagandha genotypes by Tocher method

Sl.	Genotype	Sl.	Genotype	Sl.	Genotype	Sl.	Genotype

No.		No.		No.		No.	
1	DWS-05	9	DWS-144	17	DWS-260	25	DWS-296
2	DWS-09	10	DWS-197	18	DWS-262	26	DWS-309
3	DWS-22	11	DWS-250	19	DWS-270	27	DWS-315
4	DWS-40	12	DWS-252	20	DWS-272	28	DWS-316
5	DWS-41	13	DWS-253	21	DWS-279	29	DWS-317
6	DWS-132	14	DWS-257	22	DWS-280	30	DWS-319
7	DWS-141	15	DWS-258	23	DWS-281	31	Arka Ashwagandha
8	DWS-143	16	DWS-259	24	DWS-284	32	Poshita

Note: