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Study on Genetic Divergence for Yield and Yield attributing Traits in Ashwagandha (*Withania somnifera*)

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ABSTRACT: Ashwagandha (*Withania somnifera*) is a medicinal plant rich with medicinal properties but due to less research work on ashwagandha there is less information available about the diversity present among the ashwagandha genotypes and without this information a breeder cannot start a breeding program. To study the diversity among the ashwagandha genotypes an experiment was conducted at Agricultural Research Farm of Medicinal, Aromatic and Potential Crops Section of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India during *kharif* season 2019 and evaluated for fourteen quantitative characters using D^2 statistics of Mahalanobis. The genotypes were grouped into 3 clusters. Maximum number of genotypes (11) were grouped under cluster III followed by cluster I and II with 2 genotypes in each cluster. The maximum inter-cluster distance was recorded between I and II (6.73) followed by clusters I and III (6.49). Hence, the genotypes in cluster II had wider diversity with genotypes of cluster I and genotypes of cluster I had wider diversity with genotypes of cluster III and these lines may be utilized in further ashwagandha breeding program for the production of hybrids. The intra-cluster distance was maximum in cluster III (4.31) followed by cluster II (3.75) indicating hybridization involving genotypes of same clusters may provide a good cross combinations. The genotypes of cluster I were unique as cluster I having highest mean values for days of 50% flowering, test weight, number of seeds per berry, root length, average diameter of root, harvesting index, biological yield, number of roots and dry root yield. The cluster II was desirable in respect of plant height, number of primary branches, number of secondary branches, number of berries per plant and seed yield per plant. Thus, hybridization among these genotypes can generate desirable transgressive sergeants.

Keywords: Genetic diversity, ashwagandha, quantitative traits, D^2 analysis, divergence.

INTRODUCTION

India is rich in natural biodiversity and it is home of various types of medicinal plans. Western Ghats are famous for naturally grown medicinal plants. Medicinal plants are also grown commercially in India in different states. Among different-different medicinal plants ashwagandha is one the important medicinal plant cultivated at commercial level. It is a well-known herb from ancient time and it is an important part of Ayurveda and Unani health care system. Ashwagandha means "odor of the horse", probably originating from the odor of its root which resembles that of a sweaty horse (Ven Murthy *et al*., 2010). The scientific name of ashwagandha is *Withania somnifera* (L) and it is belongs to the *Solanceae* family. Ashwagandha belongs to the genus withania and withania is a group of 23 species. From genus withania only two species are grown in India (*Withania somnifera* and *Withania coagulans*). Ashwagandha is cross-pollinated crop having chromosome number 48 (2n=48) (Nigam and Kandalkar, 1995). In India, major ashwagandha producing states are Madhya Pradesh, Rajasthan, Punjab, Himachal Pradesh and Uttar Pradesh (Parita *et al.,* 2018). Winter cherry and Indian ginseng are the other name of ashwagandha (Deshpande, 2005). Roots are medicinally important part of ashwagandha contain total withanolides from 0.16 to.66 per cent [Biennial Progress Report (2006-2008)]. Roots of ashwagandha are important source of different withanolides like withasomnine, withaferine, sominiferine, sominiferinine, somnine and withananine (Covello and Ciampa, 1960; Bharti *et al.,* 2016). Ashwagandha can survive in adverse climatic conditions that's why it is a hardy and drought tolerant. Ashwagandha roots have different medicinal properties like memory enhancer, combating infectious agents, anti-cancer, anti-epileptic,

hypoglycemic, immunomodulation, antioxidant and hypocholesterolemic activities (Jadhav, 2003; Verma and Kumar, 2011; Giri, 2016; Koli *et al*., 2021). It was also found useful in neurodegenerative diseases such as Parkinson's, Huntington's and Alzeimer's diseases (Singh *et al*., 2011; Patnaik, 2016). The root powder of ashwagandha also helpful in boosting immunity to fight with COVID-19 disease (Shree *et al.*, 2020). The medicinal plants have lesser side effects as compared to allopathic medicine (Kala *et al*., 2006). The demand of medicinal plants are increasing day by day but the production of medicinal plants is still at constant stage. To increase the production of ashwagandha plants, improved verities are needed. To develop an improved variety breeding program is completely depends upon the diversity present among genotypes and traits of interest. To develop an improved verity, breeder have to select diverse genotypes for crossing. Therefore knowledge of genetic diversity present in genotypes is very necessary for a breeder. Plant breeders are interested in evaluating genetic diversity using morphological features since they are inexpensive, rapid and simple to score. D^2 analysis is one of the important tool to study the degree of genetic divergence present among the genotypes and traits. This method was developed by Mahalanobis (1936). D^2 analysis provides the information about inter and intra cluster distance that provide information of diversity at cluster level. Crossing between germplasm belongs to diverse cluster may give heterotic progeny (Meena and Bahadur, 2015). So this study can be useful for future breeding program in ashwagandha breeding.

MATERIALS AND METHODS

Fifteen genotypes of ashwagandha (*Withania somnifera*) were used in this study (Table 1). The genotypes were cultivated in randomized block design (RBD) design with three replications at Agricultural Research Farm of Medicinal, Aromatic and Potential Crops Section of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India during *kharif* season, 2019. Each ashwagandha genotype was sown with recommended 60x30 cm spacing between rows and plants, respectively. The fourteen morphological traits used in present study *viz*. dry root yield per plant (DRY) (gm), numbers of roots (NR), plant height (PH) (cm), days to 50% flowering (DF), numbers of primary branches (NPB), numbers of secondary branches (NSB), numbers of berries (TW), Test weight (TW) (gm), seed yield per plant (SYPP) (gm), biological yield (BY) (gm), harvesting index (HI) (%), number of seeds per berry (NSPB), root length (RL) (cm) and average diameter of root (ADR) (cm). Five randomly selected plants from each genotype were used for statistical analyses. Genetic divergence amongst different genotypes is assessed based on the estimated *inter-se* genetic distances amongst the genotypes. D^2 statistics of Mahalanobis (1928) is one of the most effective tools to measure the genetic distance between genotypes as measured by allelic frequencies at a sample of loci. Genetic similarity is defined as the converse of the genetic divergence i.e. the extent of gene similarity among the genotypes. The genotypes of ashwagandha were grouped into a number of clusters by Tocher's method described by Rao (1952).

RESULTS AND DISCUSSION

The results of analysis of variance showed that there is significance differences for all the fourteen quantitative traits under study. Under this study on the bases of D^2 value fifteen genotypes of ashwagandha were grouped into three clusters (Table 2). Clustering pattern indicated that eleven out of fifteen germplasms belong to the same cluster *i.e*. cluster III. On the other hand, two-two genotypes belong to cluster I and II. This study also supported by numerous researchers earlier (Bahadur and Choubey 2007; Mut, 2015; Jaipal and Shekhawat 2016; Kaur and Kapoor 2017). Intra and inter-cluster distances among the genotype i.e. D^2 values have been presented in the Table 3. Inter cluster distance was higher than intra cluster distance indicating wider genetic diversity among the genotypes (Singh *et al.*, 2017). The highest inter cluster distance was observed between cluster I and Cluster II which is about 6.73. The second largest inter cluster distance observed between cluster I and cluster III which is around 6.49 and lowest inter cluster distance observed between cluster II and cluster III (5.97). Its mean the genotypes in cluster II *viz*., HWS-106 and HWS-116 had wider diversity with RAS-16 and HWS-04-3 genotypes of cluster I and RAS-16 and HWS-04-3 genotypes of cluster I had wider diversity with HWS-04-2, HWS-08-4, HWS-08-6, HWS-110, HWS-100, HWS-101, HWS-105, HWS-08-18, HWS-104, HWS-108 and HWS-109 genotypes of cluster III. The highest intra-cluster distance was observed in the cluster III (4.31) which comprised of eleven germplasms followed by cluster II (3.75). Hence the selection of genotypes based on inter and intra cluster distance can be helpful for selection of diverse genotypes for ashwagandha breeding program. Similar results were observed by Singh *et al*. 2021); Chaudhary *et al.* (2021)**.** Mean values for different quantitative traits for different clusters have been presented in Table 4. The extreme mean values of the traits under study were observed to fall in different clusters. The cluster I showed highest values for days of 50% flowering (80.50), test weight (1.82), number of seeds per berry (20.73), root length (27.98), average diameter of root (1.25), harvesting index (30.94), biological yield (113.83), number of roots (2.83) and dry root yield (31.66). The cluster II showed highest mean value for the plant height (73.66), number of primary branches (5.28), number of secondary branches (21.91), number of berries per plant

(133.83) and seed yield per plant (7.65). The divergence in quantitative characters were also reported by Alam *et al.* (2007); Haydar *et al.* (2009); Kumar *et al.* (2017); Kasana *et al.* (2018); Haralayya *et al.* (2017). Thus, the germplasms in cluster I and II seems to be quiet promising for many of the characters under study (Kar et al., 2013; Kasana *et al*., 2018; Gadi *et al*., 2020; Priyanka *et al*., 2021). The highest average D^2 distance was observed in genotype RAS-16 (47.93) followed by HWS-116 (41.53), HWS-102 (37.63), HWS-100 (33.08) and HWS-04-3 (32.85) while lowest average D^2 distance was observed in genotypes HWS-08-18 (18.57) followed by HWS-110 (19.93) (Table 5). The genotypes present in cluster I and II havening high inter cluster distance, are divers genotypes and selection of these genotypes can be helpful for future breeding program in ashwagandha.

Table 1: List of germplasm of ashwagandha.

Sr. No.	Name of germplasm
1.	RAS-16
2.	HWS-04-2
3.	HWS-04-3
4.	HWS-08-4
5.	HWS-08-6
6.	HWS-08-18
7.	HWS-100
8.	HWS-101
9.	HWS-102
10.	HWS-104
11.	HWS-105
12.	HWS-106
13.	HWS-108
14.	HWS-110
15.	HWS-116

Table 2: Cluster information.

Cluster	Germplasm	Number
I	RAS-16, HWS-04-3	2
II	HWS-106,HWS-116	2
III	HWS-04-2, HWS-08-4, HWS-08-6, HWS-110, HWS-100, HWS-101, HWS-105, HWS-08-18, HWS-104, HWS-108, HWS-109	11

Table 3: Inter and Intra cluster distance.

	Cluster I	Cluster II	Cluster III
Cluster I	3.438	6.726	6.489
Cluster II		3.751	5.975
Cluster III			4.310

Table 4: Cluster mean.

	PH	DF	NPB	NSB	NB	TW	SPP	NSB	RL	ADR	HI	BY	NR	DRY
Cluster I	62.77	80.50	3.55	7.93	79.50	1.82	2.21	20.73	27.98	1.25	30.94	113.83	2.83	31.66
Cluster II	73.66	76.50	5.28	21.91	133.83	1.80	7.65	19.03	26.55	1.05	23.22	87.83	1.08	22.33
Cluster III	59.33	79.27	4.28	13.10	54.78	1.81	2.14	16.88	23.21	0.92	18.24	62.43	1.07	18.01

Where dry root yield per plant (DRY) (gm), numbers of roots (NR), plant height (PH) (cm), days to 50% flowering (DF), numbers of primary branches (NPB), numbers of secondary branches (NSB), numbers of berries (TW), Test weight (TW) (gm), seed yield per plant (SYPP) (gm), biological yield (BY) (gm), harvesting index (HI) (%), number of seeds per berry (NSPB), root length (RL) (cm) and average diameter of root (ADR) (cm).

Table 5: Distance Matrix Euclidean^2 Distance.

Sr. No.	Name of germplasm	Average D^2
1.	RAS-16	47.935
2.	HWS-04-2	23.829
3.	HWS-04-3	32.850
4.	HWS-08-4	23.807
5.	HWS-08-6	25.908
6.	HWS-08-18	18.570
7.	HWS-100	33.086
8.	HWS-101	19.865
9.	HWS-102	37.637
10.	HWS-104	22.207
11.	HWS-105	20.124
12.	HWS-106	29.498
13.	HWS-108	23.227
14.	HWS-110	19.937
15.	HWS-116	41.532

CONCLUSION

Genetic diversity analysis was carried out through D^2 analysis in order to assess the genetic divergence among genotype under study. Based on D^2 analysis fifteen genotypes of ashwagandha were grouped in three clusters. Maximum genotypes were found in cluster III while highest mean values for days of 50% flowering, test weight, number of seeds per berry, root length, average diameter of root, harvesting index, biological yield, number of roots and dry root yield were found in cluster 1. The maximum inter-cluster distance was observed between I and II followed by clusters I and III. The maximum intra-cluster distance was recorded in cluster III followed by cluster II. The genotypes belongs to these clusters can be utilized in further ashwagandha breeding programme for the production of hybrid. This diversity study will provide the knowledge about variation present among the genotypes under study and this knowledge about variation is basis requirement to start a breeding program. This study also provides information about different traits under study and their contribution in divergence. It will helpful for a breeder to select suitable breeding program for genetic improvement of ashwagandha.

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Conflict of Interest. None.

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