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Genetic variability, heritability and genetic advance in Ashwagandha (*Withania somnifera* L.) promising lines

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Abstract

Information regarding genetic relationships among different promising lines are very valuable in crop improvement initiatives. This study aimed to identify the best promising ashwagandha lines out of the 8 under investigation by assessing genetic variability, broad-sense heritability, and genetic advancement in terms of growth, yield, and quality traits. The research involved eight ashwagandha promising lines and was conducted in a Randomized Block Design with three replications at the Medicinal and Aromatic Plants Research Station in Rajendranagar, Hyderabad, SKLTSHU during the late *Kharif* season of 2022-23. Analysis of variance unveiled significant differences among the promising lines, signifying substantial level of variability in all 24 evaluated traits. The minimal disparity between Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) suggested that environmental factors had limited influence on trait expression. Traits such as plant height, number of branches per plant, leaf width, days to flower initiation, seed yield per plant, main root length, main root diameter, number of secondary roots per plant, fresh root weight per plant, dry root yield per plant, fresh root weight per plot, dry root yield per plot, fresh root weight per hectare, and dry root yield per hectare exhibited notably high PCV and GCV estimates.

High heritability, along with a substantial genetic advance as a percentage of the mean, indicated the prevalence of additive gene action. Promising lines can be effectively selected based on traits like plant height, number of branches per plant, leaf length, days to flower initiation, seed yield per plant, seed yield per plot, main root length, main root diameter, number of secondary roots per plant, fresh root weight per plant, fresh root weight per plot, fresh root weight per hectare, dry root yield per plant, dry root yield per plot, dry root yield per hectare, starch content, crude fiber content, starch-to-fiber ratio, and total alkaloid content, all of which displayed elevated heritability and genetic advancement as a percentage of the mean.

The study identified four superior promising lines, namely NMITLI-101, CIM-CHETAK, IC-286632, and NMITLI-118, exhibited superior performance in terms of both yield and quality traits. These lines hold significant potential for future utilization in crop improvement programs.

Keywords: Ashwagandha, variability, heritability and genetic advance

Introduction

Ashwagandha is a significant medicinal plant that has been grown for commercial purposes for a long time. *Withania somnifera* (L.), commonly known as ashwagandha, has chromosome $2n = 48$ and is belongs to Solanaceae family. Ashwagandha, commonly referred to as Indian ginseng, is also known by names such as "poison gooseberry" and "winter cherry." It derives its name from its root, which emits an odor reminiscent of a horse and is believed to impart the strength and vigor associated with a horse. Ashwagandha is native to northwestern and central India, as well as the Mediterranean region of Africa (Srivastava *et al.*, 2017) [15]. This versatile plant is recognized by various names, including "Indian Ginseng," "Asgandh," and "Indian Winter Cherry," as documented by Deshpande in 2005. This hardy and drought-tolerant perennial shrub thrives naturally in subtropical arid climates, where the average annual rainfall ranges from 600 to 750 mm. It prefers well-drained sandy loam or light red soils with a pH level of 7.5 to 8.0 (Kukreti *et al.*, 2013) [7]. The plant is characterized by its upright growth, evergreen nature, and a height ranging from 30 to 150 cm. It features stellate tomentose branches, simple ovate green leaves, small green flowers, ripe orange-red fruits, reniform yellow seeds, and fleshy whitish-brown roots (Chaurasia *et al.*, 2022) [3].

Ashwagandha is categorized as a medicinal crop suitable for dryland conditions, and its roots hold significant value in traditional medicine systems. Interestingly, the market worth of ashwagandha roots is influenced by their physical attributes, particularly their texture. Roots that are long, brittle, and sturdy are considered to have a higher market value, as noted by Mishra *et al.*, in 1998 [13].

Ashwagandha roots exhibit significant therapeutic potential and are integral components of various formulations in Ayurvedic, Unani, and Siddha traditional medicine systems. This herb possesses therapeutic efficacy against a wide range of health conditions, including but not limited to arthritis, asthma, mental disorders, infections, fever, anxiety, tuberculosis, male sexual disorders, and cancer, as reported by (Mirjalili *et al.*, in 2009 and Anjani devi *et al.*, in 2022) [11, 1]. Ashwagandha may be capable to inhibit COVID-19's M pro by inhibiting SARS-CoV-2, according to (Tripathia *et al.*, 2021) [16], and it may also have an antiviral effect on nCoV. The plant is known as Indian ginseng because of its extensive medical uses and potential for growth due to the spread of illnesses and viruses like COVID-19. There are many opportunities to grow this plant commercially because of widespread interest in it and the great demand for its roots. Therefore, there is a pressing need to identify suitable and high-yielding varieties among the existing promising lines in terms of their growth, yield, and quality attributes.

The extent of variability and the impact of environmental factors on traits can be examined through the Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV). In this regard, the present investigation was undertaken to evaluate the Genetic Variability, Heritability, and Genetic Advancement within eight promising lines of ashwagandha. This research aims to provide valuable insights for future breeding programs focused on enhancing growth, yield, and quality.

Materials and Methods

The study on the growth, yield, and quality attributes of ashwagandha was conducted at the Medicinal and Aromatic Plants Research Station in Rajendranagar, Hyderabad, during the late *kharif* season of 2022-23. The study followed a Randomized Block Design and involved eight promising ashwagandha lines, each replicated three times randomly. These eight ashwagandha lines were planted in the field, with each line spaced at 40 cm x 50 cm within a 3.0 m x 2.0 m plot. Standard agricultural practices were employed to ensure the healthy growth of the crops, and necessary preventive measures were taken to protect the promising lines from pests and diseases. The primary objective of this comprehensive study was to systematically evaluate all the promising ashwagandha lines and identify the best performers in terms of growth, yield, and quality.

A total of 24 characteristics were examined in the experimental material, including parameters like plant height (in cm), number of branches per plant, leaf length (in cm), leaf width (in cm), days to flower initiation, days to berry formation, days to root maturity, days to root harvest, number of seeds per berry, seed yield per plant (in grams), seed yield per plot (in kilograms), main root length (in cm), main root diameter (in cm), number of secondary roots per plant, fresh root weight per plant (in grams), fresh root weight per plot (in kilograms), fresh root weight per hectare (in quintals), dry root yield per plant (in grams), dry root yield per plot (in kilograms), dry root yield per hectare (in quintals), starch content (in percentage), crude fiber content (in percentage), starch-to-fiber ratio, and total alkaloid content (in percentage). Crude fiber content was determined using a modified method based on Maynard (1970) [9], while starch content was estimated following the method outlined by Mc Cready *et al.* (1950) [10]. The total alkaloid content was quantified using the

method developed by Mishra (1996) [12].

The analysis of variance was performed using the approach recommended by Panse and Sukhatme (1985) [14] to assess the variability of different traits. Variability for various characteristics was estimated using the method proposed by Burton and De Vane (1953) [2]. Furthermore, heritability and expected genetic advancement were calculated as per the methodology outlined by Johnson *et al.* (1955) [5].

Results and Discussion

Analysis of variance

The analysis of variance indicated notable distinctions among the promising lines, signifying the existence of a significant degree of variability across all 24 traits under investigation (Table 1). Wide range of variability was observed for plant height (1984.13) followed by days to flower initiation (1471.05), days to root harvest (791.03), days to root maturity (707.73) and fresh root weight per plant (192.28) indicating the scope for selection of initial experimental material for further improvement. These results of analysis of variance are in concurrence with the findings reported by Anjanidevi *et al.* (2022) [1] in growth, yield and quality of ashwagandha.

Estimation of genetic parameters

The values for the coefficient of variation, broad-sense heritability, and genetic advancement for all 24 traits examined are presented in Table 2 and Fig. 1, and these findings are elaborated upon below.

Phenotypic and genotypic coefficients of variation

The investigation demonstrated that Phenotypic Coefficient of Variation (PCV) slightly exceeded Genotypic Coefficient of Variation (GCV) for all traits, suggesting that environmental factors had some influence on each trait. Nevertheless, there was generally good concordance between the Genotypic Coefficient of Variation and Phenotypic Coefficient of Variation for all the characteristics. The outcomes of these coefficients are summarized as follows.

All of the characteristics showed a wide range of phenotypic coefficient of variation (PCV), which varied from 8.63 (days to root harvest) to 50.68 (fresh root weight per hectare). The Phenotypic Coefficient of Variation (PCV) exhibited its highest values for fresh root weight per hectare (50.68), followed by seed yield per plot (48.84), dry root yield per plant (47.69), seed yield per plant (47.47), number of secondary roots per plant (40.79), plant height (36.23), fresh root weight per plant (32.64), fresh root weight per plot (32.16), days to flower initiation (29.16), main root length (29.00), dry root yield per hectare (27.32), dry root yield per plot (26.21), main root diameter (23.89), number of branches per plant (22.09), and leaf width (21.60).

The Genotypic Coefficient of Variation (GCV) exhibited a range from 6.97 (for days to berry formation) to 50.44 (for fresh root weight per hectare). Among the traits examined, the highest GCV values were observed for fresh root weight per hectare (50.44), followed by dry root yield per plant (47.37), seed yield per plant (46.87), seed yield per plot (45.22), number of secondary roots per plant (38.31), plant height (35.99), fresh root weight per plot (31.88), fresh root weight per plant (31.51), main root length (28.21), days to flower initiation (28.55), dry root yield per hectare (26.96), dry root yield per plot (25.66), and number of branches per plant (21.57).

Across various characteristics examined, it was observed that, on average, high Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) values were most prominent for plant height, number of branches per plant, leaf width, days to flower initiation, seed yield per plant, seed yield per plot, main root length, main root diameter, number of secondary roots per plant, fresh root weight per plant, dry root yield per plant, fresh root weight per plot, dry root yield per plot, fresh root weight per hectare, and dry root yield per hectare. This suggests a substantial degree of variability within these traits among the promising ashwagandha lines studied. These findings align with previous research by Srivastava *et al.* (2017) [15], Venugopal *et al.* (2021) [17], and Anjani devi *et al.* (2022) [1] in the context of ashwagandha."

In contrast, the Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) estimates were moderate to low for traits such as leaf length, leaf width, days to berry formation, days to root maturity, days to root harvest, number of seeds per berry, starch content, crude fiber content, starch-to-fiber ratio, and total alkaloid content. This suggests a relatively modest to narrow range of variability within these traits. These findings are consistent with the observations made by Venugopal *et al.* (2021) [17] and Anjani devi *et al.* (2022) [1] in their studies on ashwagandha.

Heritability and genetic advance

High levels of heritability in broad sense were observed across all the studied traits, ranging from 62.50 (for starch-fiber ratio) to 99.10 (for dry root yield per plot).

Heritability estimates for various traits are valuable tools for researchers in devising effective strategies for crop improvement. They are often employed by breeders to anticipate the response to selection. While high heritability suggests that selection based on phenotypic performance can be effective, it does not necessarily guarantee a high genetic advancement as a percentage of the mean for a particular trait. Therefore, considering both heritability and genetic advancement as a percentage of the mean is more crucial for assessing the effectiveness of selection compared to heritability alone. As noted by Johnson *et al.* (1955) [5], estimating heritability values alongside genetic advancement is more useful in determining the best individual through

selection.

In this study, traits such as plant height (cm), number of branches per plant, leaf length (cm), days to flower initiation, seed yield per plant (g), seed yield per plot (kg), main root length (cm), main root diameter (cm), number of secondary roots per plant, fresh root weight per plant (g), fresh root weight per plot (kg), fresh root weight per hectare (q), dry root yield per plant (g), dry root yield per plot (kg), dry root yield per hectare (q), starch content (%), crude fiber content (%), starch-to-fiber ratio, and total alkaloid content (%) exhibited high genetic advancement as a percentage of the mean. In contrast, leaf width, days to berry formation, days to root maturity, days to root harvest, and number of seeds per berry showed moderate to low genetic advancement as a percentage of the mean.

The presence of high heritability coupled with high genetic advancement indicates the predominance of additive gene action. This was observed in traits such as plant height, number of branches per plant, leaf length, days to flower initiation, seed yield per plant, seed yield per plot, main root length, main root diameter, number of secondary roots per plant, fresh root weight per plant, fresh root weight per plot, fresh root weight per hectare, dry root yield per plant, dry root yield per plot, dry root yield per hectare, starch content, crude fiber content, starch-to-fiber ratio, and total alkaloid content.

The findings of Srivastava *et al.* (2017) [15], Manivel *et al.* (2017) [8], Venugopal *et al.* (2021) [17], and Anjani devi *et al.* (2022) [1] reported high heritability coupled with high genetic advancement as a percentage of the mean for traits such as plant height, number of branches per plant, seed yield per plant, main root length, main root diameter, fresh root weight per plant, fresh root weight per hectare, dry root yield per plant, dry root yield per hectare, and crude fiber content.

Additionally, Joshi *et al.* (2014) [6] reported similar findings of high heritability coupled with high genetic advancement as a percentage of the mean for starch content and total alkaloid content.

In contrast, traits with high heritability and moderate genetic advancement indicate the influence of both additive and non-additive genes, as well as the favorable impact of the environment on trait expression. This pattern was observed in leaf width, days to berry formation, days to root maturity, days to root harvest, and number of seeds per berry.

Table 1: RBD-Analysis of Variance-(ANOVA) of 8 promising lines of ashwagandha

S. No	Character	Mean Sum of Squares		
		Replication (DF=2)	Treatments (DF=7)	Error (DF=14)
1	Plant height (cm)	19.80	1984.13**	8.54
2	Number of branches per plant	0.03	4.95**	0.08
3	Leaf length (cm)	2.30	8.86**	0.62
4	Leaf width (cm)	1.73	3.70**	0.47
5	Days to flower initiation	28.80	1471.05**	19.66
6	Days to berry formation	16.26	23.32**	3.62
7	Days to root maturity	15.22	707.73**	7.54
8	Days to root harvest	15.34	791.03**	14.96
9	Number of seeds per berry	3.74	54.18**	7.32
10	Seed yield per plant (g)	0.75	19.91**	0.17
11	Seed yield per plot (kg)	0.00	0.02**	0.001
12	Main root length (cm)	2.02	58.22**	1.08
13	Main root diameter (cm)	0.03	0.41**	0.07
14	Number of secondary roots	0.03	6.04**	0.29
15	Fresh root weight per plant (g)	3.58	192.28**	4.55
16	Fresh root weight per plot (kg)	0.00	0.17**	0.001
17	Fresh root weight per hectare (q)	0.08	61.31**	0.20

18	Dry root yield per plant (g)	0.07	48.87**	0.24
19	Dry root yield per plot (kg)	0.00	0.02**	0.00
20	Dry root yield per hectare (q)	0.08	7.46**	0.06
21	Starch content (%)	0.04	9.84**	0.12
22	Crude fibre content (%)	0.89	67.85**	2.43
23	Starch Fibre ratio	0.00	0.006**	0.001
24	Total alkaloid content (%)	0.00	0.009**	0.00

** Significant at 5% level of significance respectively

Table 2: Estimates of variability, heritability and genetic advance as percent of mean for 24 characters in 8 promising lines of ashwagandha

S. No	Characters	PCV (%)	GCV (%)	h ² bs (%)	Genetic Advance	GA as percent of mean
1	Plant height (cm)	36.23	35.99	97.82	52.54	73.67
2	Number of branches per plant	22.09	21.57	95.40	2.56	43.39
3	Leaf length(cm)	17.66	15.93	81.40	3.07	29.60
4	Leaf width(cm)	21.60	17.98	69.30	1.78	30.85
5	Days to flower initiation	29.16	28.55	95.90	49.01	57.60
6	Days to berry formation	8.67	6.97	64.53	4.31	11.53
7	Days to root maturity	8.83	8.70	96.90	30.96	17.63
8	Days to root harvest	8.63	8.36	93.80	32.05	16.69
9	Number of seeds per berry	13.97	11.52	68.10	7.05	20.59
10	Seed yield per plant (g)	47.47	46.87	97.5	5.21	95.32
11	Seed yield per plot(kg)	48.84	45.22	85.70	0.14	86.24
12	Main root length(cm)	29.00	28.21	94.61	10.50	50.53
13	Main root diameter(cm)	23.89	20.92	72.70	0.62	34.63
14	Number of secondary roots per plant	40.79	38.31	88.20	2.69	74.12
15	Fresh root weight per plant(g)	32.64	31.51	93.20	15.99	62.67
16	Fresh root weight per plot(kg)	32.16	31.88	98.30	0.48	65.10
17	Fresh root weight per hectare(q)	50.68	50.44	99.05	8.11	64.24
18	Dry root yield per plant(g)	47.69	47.37	98.66	6.76	56.93
19	Dry root yield per plot(kg)	26.21	25.66	99.10	0.18	52.86
20	Dry root yield per hectare(q)	27.32	26.96	97.50	3.19	54.81
21	Starch content (%)	14.40	14.14	96.40	4.14	28.61
22	Crude fibre content (%)	14.10	13.37	89.96	9.30	26.12
23	Starch Fibre ratio	12.60	10.96	62.50	0.10	24.43
24	Total alkaloid content (%)	16.22	14.22	90.90	0.11	29.30

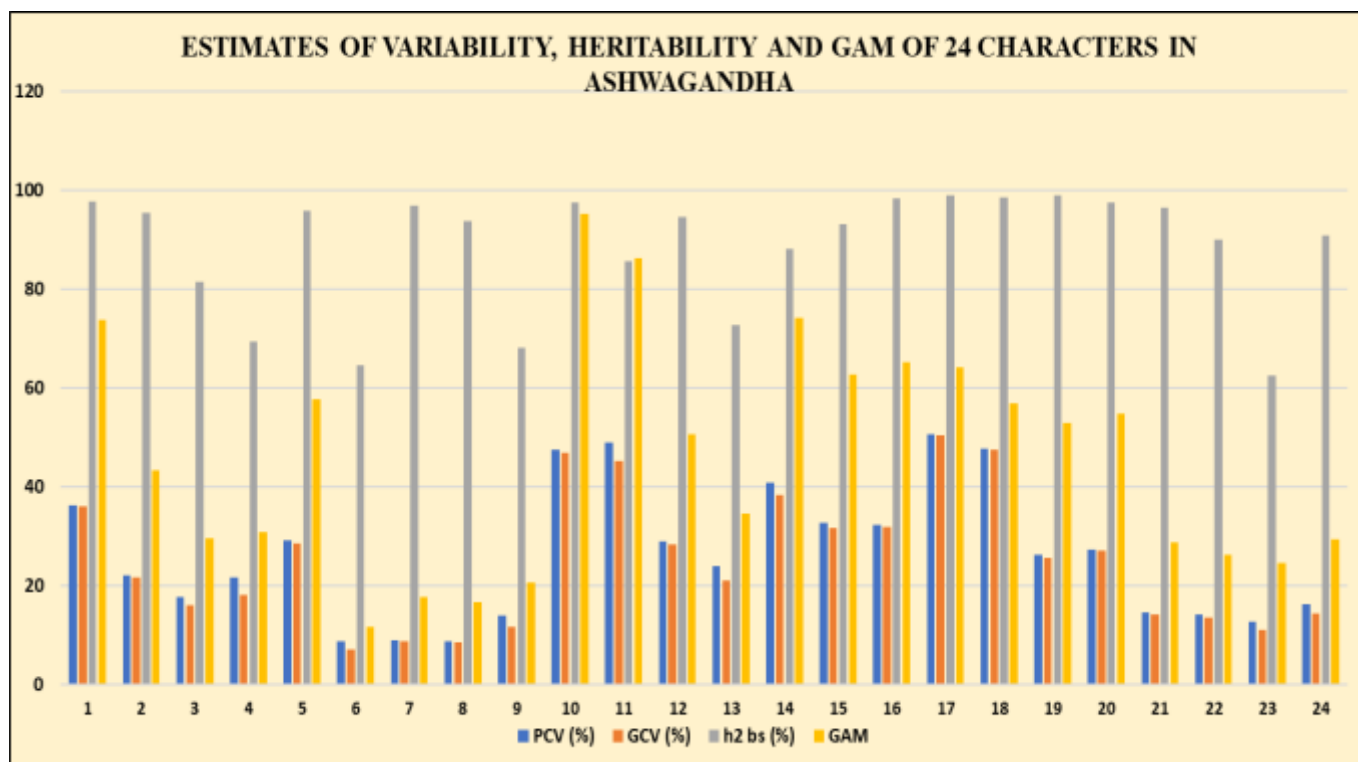


Fig 1: Estimates of Variability, Heritability and GAM of 24 characters in ashwagandha

Table 3: Mean performance of 8 promising lines of ashwagandha for different characters

S. No	Promising lines	Plant height (cm)	Number of branches per plant	Leaf length (cm)	Leaf width (cm)	Days to flower initiation	Days to berry formation	Days to root maturity	Days to root harvest	Number of seeds per berry	Seed yield per plant (g)	Seed yield per plot (kg)	Main root length (cm)	Main root diameter (cm)
1	IC-286632	54.08	7.20	8.70	4.41	59.93	36.93	172.80	196.23	38.53	7.08	0.24	18.79	1.83
2	NMITLI-101	114.84	6.73	12.95	6.85	104.13	37.80	189.80	204.33	30.53	2.60	0.09	25.02	2.27
3	NMITLI-118	92.64	6.27	12.79	7.54	118.73	43.40	198.20	215.60	36.07	2.35	0.08	24.82	1.98
4	RAS-65	48.15	7.80	9.41	5.15	84.47	40.27	172.13	186.13	41.20	8.97	0.27	18.43	1.54
5	CIM-CHETAK	96.26	4.20	10.38	5.99	121.27	40.93	186.13	204.13	35.07	3.07	0.08	25.51	2.15
6	MWS-218	56.07	5.07	9.83	5.26	61.87	31.80	149.13	165.20	28.80	8.26	0.22	17.06	1.13
7	POSHITA © 1	53.06	4.67	10.80	6.34	59.73	33.67	169.60	186.33	33.13	5.83	0.20	18.93	1.82
8	NAGORE © 2	55.20	5.33	8.37	4.57	70.67	34.87	167.13	178.33	30.93	5.64	0.19	17.78	1.57
	Grand Mean	71.29	5.91	10.40	5.77	85.10	37.46	175.62	192.04	34.28	5.47	0.17	20.79	1.79
	SEm ±	1.687	0.163	0.458	0.398	2.522	1.247	1.586	2.222	1.563	0.239	0.018	0.656	0.15
	CD (P=0.05)	5.118	0.493	1.389	1.21	7.649	3.783	4.809	6.682	4.741	0.724	0.054	1.989	0.455
	Range Minimum	48.15	4.20	8.37	4.41	59.73	31.80	149.13	165.20	28.80	2.35	0.08	17.06	1.13
	Range Maximum	114.84	7.80	12.95	7.54	121.27	43.40	198.20	215.60	41.20	8.97	0.27	25.51	2.27

Conclusion

Thus, considering the assessments of genetic parameters, including GCV, Heritability (in broad sense) and Genetic Advancement as a percentage of the mean, it becomes evident that traits such as plant height, number of branches per plant, leaf length, days to flower initiation, seed yield per plant, main root length, main root diameter, number of secondary roots per plant, fresh root weight per plant, dry root yield per plant, fresh root weight per plot, dry root yield per plot, fresh root weight per hectare, dry root yield per hectare, starch content, crude fiber content, starch-fiber ratio, and total alkaloid content hold significant importance. Focusing on the selection of these traits would prove to be more effective in enhancing dry root yield in ashwagandha. The study identified four best promising lines, namely NMITLI-101, CIM-CHETAK, IC-286632, and NMITLI-118, exhibited superior performance in terms of both yield and quality traits. These lines hold significant potential for future utilization in crop improvement programs.

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