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Exploring genetic variability and trait relationships in Ashwagandha (*Withania somnifera* Dunal L.) for improved yield

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Abstract

This study estimated genetic variability and interrelationship among traits in 50 genotypes of Indian Ashwagandha, with the objective of identifying superior genotypes for improvement. Significant differences ($p < 0.01$) among genotypes were observed for 12 morphological and yield-related traits. High heritability estimates (>90 %) were coupled with high genetic advance as % of the mean (>40 %) for most traits. The highest genotypic and phenotypic coefficients of variation (106.143 % and 106.548 % respectively) and a genetic advance as a % of the mean of 217.823 % were recorded for root dry weight per plant. Root dry weight was strongly positively correlated with root fresh weight, root diameter and root length. Seed yield was positively correlated with berry dry weight and traits related to branching, but negatively correlated to root dry weight, suggesting a trade-off between seed and root production. Root fresh weight is the primary contributor to root dry weight. Cluster analysis grouped the genotypes into four clusters, with Cluster 2 showing the maximum potential for improvement in Ashwagandha root yield. The first three principal components explained 85.58 % of the total variation; PC1 primarily explained root-related traits, while PC2 explained branching and reproductive traits. In the chemical quality analysis of eight genotypes in Cluster 2, the germplasm WS 2 (IC - 0604214-X) exhibited the highest withaferin A (0.318 %), alongside moderate biochemical characteristics such as fiber, carbohydrate and protein content. This study provides valuable insights in developing breeding strategies to enhance economically important traits in Ashwagandha but also calls highlights the challenges involved in simultaneously improving both root and seed yield.

Keywords

Ashwagandha; *Withania somnifera*; genetic variability; heritability; genetic advance

Introduction

Ashwagandha (*Withania somnifera* Dunal L.) (Solanaceae, 2n = 48) is an important commercial medicinal plant of India, also referred as "poison gooseberry" and "winter cherry", It originated from the Mediterranean region of Africa and northwest and central India. Its name derives from its root, which has a horsey smell and is said to confer the vitality and strength of a horse (1). The plant possesses numerous medicinal properties and is extensively used in all Indian system of medicine, primarily as a mainly to as

vital tonic, immune booster, antioxidant, adaptogen and aphrodisiac. It is also utilized to treat ulcers, inflammation, cancer, venom toxins, liver ailments, bacterial infection and is known for its astringent properties (2, 3). Additionally, Ashwagandha is used to address bronchitis, asthma, emaciation, insomnia, dementia, neurological disorders, inflammation, Parkinson's disease, contributing to overall health maintenance and restoration (4). The overall alkaloidal content of Ashwagandha is reported to range between 0.13 and 0.6 %. India is the largest exporter of Ashwagandha extract in the world market, primarily in the form of root extracts, with demand increasing significantly after the Covid 19 pandemic due to its recognition as a natural remedy for stress relief and wellness. This growth supports rural livelihoods and significantly contributes to the Indian economy while enhancing Ashwagandha as a global standing in the herbal supplement market.

Initially collected from wild, Ashwagandha has been brought into cultivation due to its increased demand, with approximately 10,768 ha of land dedicated to Ashwagandha farming (5). Mostly in Rajasthan, Gujarat, Uttar Pradesh, Punjab, Haryana, Andhra Pradesh, Maharashtra, Karnataka, Andra Pradesh, Telangana and Tamil Nadu. This resilient and drought-tolerant herb requires 600 -750 mm of average annual rainfall and grows well in light red soils with a pH of 7.5 to 8.0 or well-drained sandy loams (6).

The future demand for Ashwagandha must be met through cultivation, necessitating the availability of improved varieties and good agricultural practices as per WHO guidelines. Several varieties of *Withania somnifera*, such as JA-134, Poshita, CIMAP-Pratap, CIM-Pushti and NMITLI-101, offer distinct advantages for cultivation and medicinal applications. These varieties exhibit traits such as high starch content, disease resistance and enhanced levels of bioactive compounds like withanolides and alkaloids (7). Hence there is an urgent need to develop improved varieties of Ashwagandha with high yield coupled with physical and chemical qualities. The success of any plant breeding program largely hinges on the genetic variability for yield and its component characters. A wide range of variability increases the breeder's chances of choosing the right material.

In addition to understanding variability, an extensive knowledge of how traits are related to yield is also required. The linkage between these traits can be elucidated using correlation analysis, which provides a basis for a shared selection program aimed at the mutual enhancement of two desired traits. Analysing path coefficients makes it easier to assess how much each trait directly and indirectly contributes in relation to yield. There is a dearth of information regarding the genetic diversity of *W. somnifera* (8). Some ongoing research focused on the genetic improvement of *Withania somnifera* to enhance its bioactive compounds and yield (7-11). The focus is on utilizing modern plant breeding techniques, such as marker-assisted selection to identify key genetic traits associated with both high yield and bioactive compoud, particularly withaferin A for therapeutic efficacy. Therefore, a study was conducted to assess the variability in Ashwagandha and evaluate the extent of genetic associations among different traits in relation to the plant's root yield from the set of Ashwagandha germplasm obtained from the national gene bank at ICAR-NBPGR (National Bureau of plant Genetic Resources, New Delhi.

Materials and Methods

Fifty Ashwagandha germplasm accessions (Table 1), obtained from ICAR-NBPGR, New Delhi, were sown in Randomized Block Design (RBD) with 3 replications during Rabi season, 2023-24 at the farm of ICAR- Central Tobacco Research Institute-Regional Station, Vedasandur, Tamil Nadu (latitude 10° 32'N longitude 77° 57'). The soil was sandy gravel, having a pH of 8.1, medium in organic C (0.46 %), low in available N (168 kg/ha), medium in P (9.1 kg/ha) and high in K (170 kg/ha). Each genotype was planted with a spacing of 30 x 10 cm, both row to row and plant to plant. Data were recorded randomly from 5 plants for days of flowering, plant height (cm), number of primary branches per plant, number of secondary branches per plant, root length (cm), root diameter (cm), leaf dry weight per plant (g), stem dry weight per plant (g), berry weight per plant (g), seed yield per plant (g), root fresh weight per plant (g) and root dry weight per plant (g). The total carbohydrate content was estimated using the anthrone reagent method (12) and the protein content of Ashwagandha root was estimated by Kjeldahl (13). The fiber content in the root was determined by acid-alkali digestion (14). The analysis of withaferin A content of best accession by using HPTLC involved methanol extraction of shade-dried, coarsely ground roots using Soxhlet extraction, with the extract concentrated under reduced pressure, dried and redissolved in methanol to a concentration of 20 mL^{-1}. Stock solutions of withaferin A standards were prepared in HPLC-grade methanol (1 mL^{-1}) and working standards at 50 ng μL^{-1} were applied in graded volumes (2, 4, 6 and 8 µL) on HPTLC plates to generate 4-point calibration curves. Linear ascending development was performed in a pre-saturated CAMAG twin trough chamber using a mobile phase of toluene, ethyl acetate and formic acid (5:5:1) and the chromatogram was developed to 80 mm. Quantitative analysis at 524 nm was conducted in absorption reflection mode, with baseline correction and imaging via a digital camera.

Statistical analysis

Recorded data were statistically analysed using packages in R (version 4.2.3). ANOVA was applied to the mean values of each of the twelve characters in accordance with the standard protocol (15). Variability metric, including Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV), were calculated (16), with classification based on established categories (17). Heritability (broad sense) and genetic advance (GA) were estimated (18), with genetic gain expressed as mean Percentage (19). Additionally, phenotypic and genotypic correlations and analysis of path coefficient were performed to understand trait relationships (20). To calculate the genetic difference between genotypes, cluster statistics were used (21).

Table 1. Origin of accession of Ashwagandha used in the study.

Acc. No.	IC Number	Place of Collection	Acc. No.	IC Number	Place of Collection
WS 1	IC 0646383	Katni, Madhya Pradesh	WS 26	IC - 0615346	Pratapgarh, Rajasthan
WS 2	IC -0604214-X		WS 27	IC - 0615347	Pratapgarh, Rajasthan
WS 3	IC - 0649168	Erode, Tamil Nadu	WS 28	IC - 0615348	Pratapgarh, Rajasthan
WS 4	IC - 0282714	Churu, Rajasthan	WS 29	IC- 0615349	Chittorgarh, Rajasthan
WS 5	IC – 0281147	Sikar, Rajasthan	WS 30	IC - 0615350	Pratapgarh, Rajasthan
WS 6	IC – 0421053	Chittoor, Andhra Pradesh	WS 31	IC - 0615351	Chittorgarh, Rajasthan
WS 7	IC – 0310620	Amreli, Gujarat	WS 32	IC - 0615354	Chittorgarh, Rajasthan
WS 8	IC – 312580	Pune, Madhya Pradesh	WS 33	IC - 0615357	Chittorgarh, Rajasthan
WS 9	IC – 0418292	Jhunjhunu, Rajasthan	WS 34	IC - 0614772	DMAPR, Anand, Gujarat
WS 10	IC – 0361042	Tehri Garhwal, Uttarakhand	WS 35	IC - 0210633	Thrissur, Kerala
WS 11	IC – 0361043	Tehri Garhwal, Uttarakhand	WS 36	IC - 0262380	Bikaner, Rajasthan
WS 12	IC – 590838	DMAPR, Anand, Gujarat	WS 37	IC - 0262381	Bikaner, Rajasthan
WS 13	IC – 0588697	DMAPR, Anand, Gujarat	WS 38	IC - 0262383	Didwana, Nagaur, Rajasthan
WS 14	IC – 0614010	Gurgaon, Haryana	WS 39	IC - 0262384	Nagaur, Rajasthan
WS 15	IC – 0614019	Jhajjar, Haryana	WS 40	IC - 0262386	Bikaner, Rajasthan
WS 16	IC – 0615336	Chittorgarh, Rajasthan	WS 41	IC - 0262387	Bikaner, Rajasthan
WS 17	IC – 0615337	Chittorgarh, Rajasthan	WS 42	IC - 0262388	Bikaner, Rajasthan
WS 18	IC – 0615338	Jhalawar, Rajasthan	WS 43	IC - 0262389	Bikaner, Rajasthan
WS 19	IC – 0615339	Jhalawar, Rajasthan	WS 44	IC - 0262390	Bikaner, Rajasthan
WS 20	IC – 0615340	Jhalawar, Rajasthan	WS 45	IC - 0262393	Nagaur, Rajasthan
WS 21	IC – 0615341	Chittorgarh, Rajasthan	WS 46	IC - 0262394	Nagaur, Rajasthan
WS 22	IC – 0615342	Jhalawar, Rajasthan	WS 47	IC - 0262396	Nagaur, Rajasthan
WS 23	IC – 0615343	Jhalawar, Rajasthan	WS 48	IC - 0262397	Nagaur, Rajasthan
WS 24	IC – 0615344	Chittorgarh, Rajasthan	WS 49	IC - 0272705	Visakhapatnam, Andhra Pradesh
WS 25	IC – 0615345	Pratapgarh, Rajasthan	WS 50	IC - 0273842	Cuddalore, Tamil Nadu

(Acc. No., accession number; CIMAP: Central Institute of Medicinal and Aromatic Plants)

Results and Discussion

Significant differences were observed for all the trait studies among the accessions. The genetic parameters (Table 2) showed that the coefficient of variation ranged from 0.48 % for days of flowering to 9.28 % for root dry weight per plant, demonstrating a high degree of genetic diversity. In particular, root dry weight exhibited significant variability, with a high mean square value of 243.5** (data not shown) and a range of 3.1 g (WS 6) to 53.85 g (WS 4) (Table 2). The substantial genetic diversity and notable genotype-to-genotype variation in root dry weight indicates the possibility of selecting superior genotypes to enhance production. The wide variation in root fresh weight, ranging from 9.75 to 135.7 g, which correlated strongly with root dry weight, further supports the substantial variability for this trait. The findings indicate that considerable genetic variability exists across the genotypes of Ashwagandha, which could be utilized for crop improvement through breeding and selection activities.

Genotypic and Phenotypic Coefficient of Variation

For all traits, the GCV and PCV values nearly identical, supporting the idea that genetic factors play a significant role in determining phenotypes. Root dry weight showed the highest GCV (106.143 %) and PCV (106.548 %), indicating significant genetic diversity. Other characteristics with high GCV and PCV values were the seed yield, berry dry weight and root fresh weight. Root diameter and root length displayed moderate GCV values. The close relationship between each trait's GCV and PCV values indicates that the environment has little effect on these traits (22), hence we can use them as selection criteria for finding the superior germplasm. However, the minimal differences between PCV and GCV for most traits indicate very minimum environmental influence on these characters, thus probably allowing effective phenotypic selection.

Genetic Advancement over mean (GAM)

All traits, except days to flowering, plant height and root diameter showed high GAM values (>50 %), suggesting a strong potential for improvement through selection. The highest GAM was found in the root dry weight per plant (217.82 %), which was followed by the seed yield per plant (128.69 %) and the root fresh weight per plant (157.02 %). These qualities can be improved by phenotypic selection because of their high GAM values and high heritability estimates, which show that they are mainly controlled by additive gene action (1).

Heritability

High heritability is crucial in breeding programs as it indicates that the genetic component significantly influences trait expression, ensuring that selected traits are reliably passed to the next generation. This enhances the efficiency of selection and accelerates genetic gains in breeding populations (23). The broad-sense heritability estimates for all traits were high, ranging from 90.78 % for plant height to 99.39 % for days of flowering. These estimates were all over 90 %. This suggests that environmental influences the majority of the observed phenotypic variance. This high heritability implies an efficient transfer of these traits to progeny, which is important for breeding programs. Thus, the combined consideration of heritability and genetic advance is crucial in predicting the effect of selection on phenotypic expression of traits (24).

Table 2. Table of SED, Heritability, GCV and PCV for 50 Ashwagandha germplasm accessions.

Response Variable	Minimum	Maximum	SED	Heritability	GCV	PCV	Gen-Advance	Gen-Adv % Means
Days of flowering (DF)	69 (WS 5)	82(WS 40)	0.51	99.39	5.23	5.249	8.25	10.74
Plant height (cm) (PH)	50 (WS 31)	118(WS 1)	6.73	90.78	15.54	16.31	25.59	30.50
Number of primary branches	3 (WS 9)	5 (WS 1)	0.65	96.76	30.23	30.73	4.43	61.26
Number of secondary	3 (WS 9)	19 (WS 35)	1.10	97.44	36.17	36.64	8.52	73.55
Root length (cm) (RL)	14.5 (WS 35)	39 (WS 15)	2.04	95.59	24.88	25.45	11.78	50.11
Root diameter (cm) (RD)	14.2 (WS 31)	37.1 (WS 4)	2.01	93.07	21.60	22.39	9.03	42.93
Leaf dry weight per plant (g)	2.3 (WS 31)	58.08 (WS 4)	1.80	98.58	45.21	45.54	18.91	92.48
Stem dry weight per plant (g) (SDW)	7.15 (WS 31)	118.68 (WS 4)	3.81	98.49	43.02	43.35	38.82	87.96
Berry dry weight per plant (g) (BDW)	4.18 (WS 31)	65.24 (WS 46)	2.27	99.09	57.04	57.31	30.06	116.98
Seed yield per plant (g) (SD)	3.39 (WS 31)	59.42 (WS 46)	2.06	99.11	62.75	63.03	27.53	128.69
Root fresh weight per plant (g)	9.75 (WS 31)	135.7 (WS 4)	3.12	99.29	76.49	76.77	46.76	157.02
Root dry weight per plant (g) (RDW)	3.1 (WS 6)	53.85 (WS 4)	1.27	99.24	106.14	106.54	18.46	217.82

Correlations coefficients among the 12 quantitative characters in Ashwagandha

Genotypic correlation analysis indicated significant associations among various morphological traits in Ashwagandha (Fig. 1). Root dry weight had positive correlation with root fresh weight ($r = 0.9774$ at $p < 0.01$), root diameter ($r = 0.7857$ at $p < 0.01$) and root length ($r = 0.5879$ at $p < 0.01$) data. Thus, selection for larger, longer and heavier roots may enhance the production of dry roots. Strong positive correlations were observed between seed yield and berry dry weight, number of primary branches and number of secondary branches ($r = 0.9961$, $p < 0.01$; $r = 0.6976$, $p < 0.01$ and $r = 0.6476$, $p < 0.01$), indicating that increased branching is associated with increased seed yield. However, a negative correlation was found between root dry weight and seed yield, presenting a challenge in selecting genotypes that balane improvement in both traits. Thus, index selection systems may be necessary to address this challenge (25).

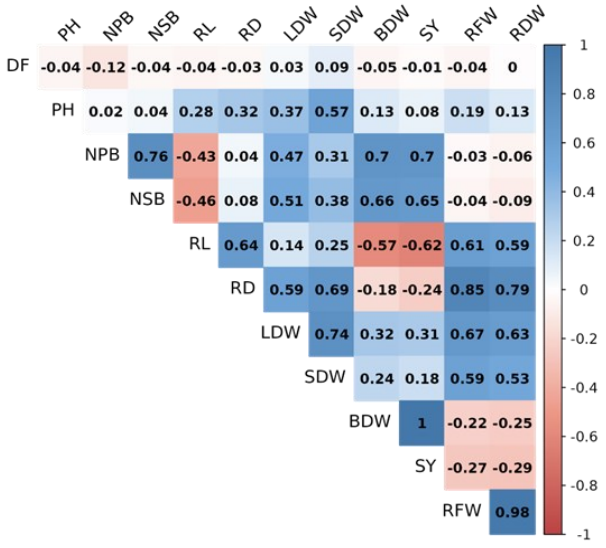

Fig. 1. Genotypic correlation among 12 characters in 50 Ashwagandha accessions.

Path analysis

The path coefficient analysis revealed that five traits showed positive direct effects (Fig. 2). Number of primary branches per plant, leaf dry weight per plant, stem dry weight per plant, berry dry weight per plant and root fresh weight per plant and the effect was highest forroot fresh weight per plant. Five traits showed negative direct effects, which were plant height, number of secondary branches per plant, root length, root diameter and seed yield per plant. For indirect effects, root fresh weight per plant had the greatest positive indirect influence on quite a few traits, mainly on root diameter, leaf dry weight per plant, root length and stem dry weight per plant. Most other indirect effects in the analysis were rather small, even a slightly negative. The closely linked traits, especially root fresh weight showing high positive direct influence of 1.052 which most likely translates into root dry weight, can be employed to deduce its importance. An interesting relationship is the one held between root diameter and root dry weight: it has a negative direct effect (-0.119), even though it is paired with a large positive indirect effect through root fresh weight (0.861) suggesting that increases in root diameter may indirectly increase dry weight because of an increase in fresh weight. A similar trend could be viewed with regard to root length, which also has a positive indirect effect (0.628), although this time paired with a negative direct effect (-0.012). Additionally, above-ground characteristics play an aspect. Leaf dry weight has a favourable impact of 0.023 and plant height has a significant indirect effect of 0.187, indicating the importance of the entire plant architecture in influencing root biomass. It's interesting to note that seed yield and berry dry weight, 2 reproductive qualities, have a negative indirect effect on root fresh weight (-0.279 and -0.235 respectively). This finding might be related to a trade-off between the development of root biomass and reproductive output. These findings highlight the need for a comprehensive strategy to be implemented when creating breeding programs to raise root dry weight. demonstrated that different features had both direct and indirect effects on the buildup of root biomass in such plants (26).

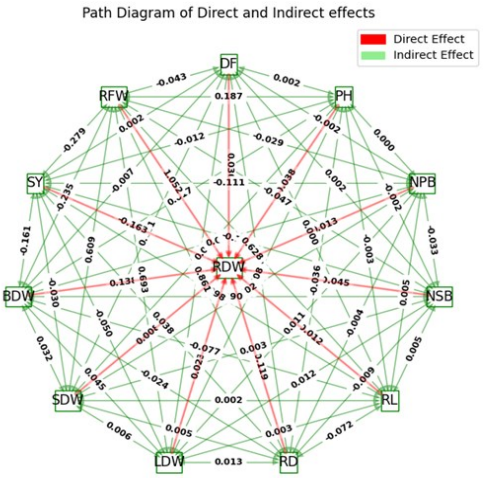

Fig. 2. Path coefficient (genotypic) values of various characters showing direct (red-bold) and indirect effects on root dry weight in 50 accessions of Ashwagandha germplasm.

Principal component analysis

The correlation analysis-based PCA component establishes connections between the various traits and genotypes (Table 3). Eigenvalues greater than one are considered significant, while component loadings greater than ±0.3 were regarded as valuable. As a result, the analysis focused on the first 3 PCs, with traits having loadings greater than 0.3 selected to represent the corresponding principal axis (27).

PCA was conducted to understand the underlying structure of variability among the 50 Ashwagandha genotypes (Table 3, Fig. 3 and 4) The first 3 principal components (PC1, PC2 and PC3) explained 39.87 %, 36.06 % and 9.66 % of the variance respectively and accounted for 85.58 % of the overall variation. This suggests that the majority of the genetic diversity present in the population under study is extracted by these factors with great success. High positive correlations between PC1 and variables connected with roots, like root fresh weight, 0.93, root dry weight, 0.90 and root diameter, 0.89, suggested that PC1 is important in explaining the variance in root yield. PC2 was significantly positively connected with the number of primary branches (0.84), the number of secondary branches (0.85) and the seed yield (0.82), as PC2 primarily related to the traits associated with branching and reproduction. This validates the results who found a relationship between branching features and seed yield (28). Plant height (0.86), had a significant impact on PC3, which explains plant design (Fig. 3). The biplot's relationships make it evident which

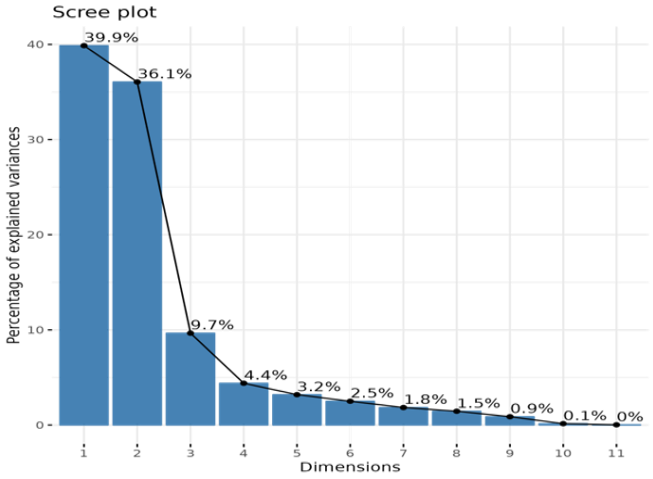

Fig. 3. Scree plot for 50 Ashwagandha germplasm accessions.

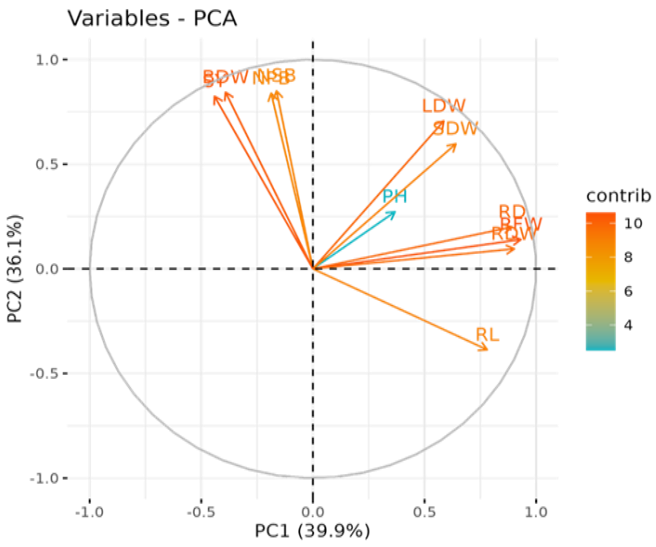

Fig. 4. Correlation between PCs for 50 Ashwagandha germplasm accessions.

Table 3. Total variances explained by different principal components and factor loading of different characters with respect to different principal factor in 50 Ashwagandha accessions.

	PC1	PC2	PC3
Eigen value	4.39	3.97	1.06
Proportion variance %	39.87	36.06	9.66
Cumulative variance %	39.87	75.92	85.58
Plant height	0.37	0.27	0.86
Number of primary branches per plant	-0.19	0.84	-0.20
Number of secondary branches per plant	-0.16	0.85	-0.16
Root length	0.78	-0.39	0.14
Root diameter	0.89	0.20	-0.06
Leaf dry weight per plant	0.59	0.71	-0.08
Stem dry weight per plant	0.64	0.60	0.26
Berry dry weight per plant	-0.39	0.84	0.08
Seed yield per plant	-0.44	0.82	0.05
Root fresh weight per plant	0.93	0.14	-0.25
Root dry weight per plant	0.90	0.10	-0.30

characteristics are tied to roots and which are related to branching and reproduction. This highlights the challenges of concurrently improving seed and root yield while emphasizing the need for focused breeding techniques to achieve balanced improvements in several desirable qualities. In the biplot description, it is crucial to clarify which characteristics are specifically tied to root traits, such as root length and diameter and which are related to branching and reproductive traits, such as branch number and flowering time, to make the distinctions clearer (29).

Diversity analysis

The 50 Ashwagandha accessions were grouped into 4 distinct clusters based on their genetic similarities (Table 4) like Cluster I: Contains 13 genotypes (WS 1, WS 7, WS 14, WS 16, WS 18, WS 19, WS 20, WS 24, WS 26, WS 32, WS 33, WS 36, WS 37). Cluster II: Contains 8 genotypes (WS 2, WS 3, WS 4, WS 5, WS 6, WS 8, WS 10, WS 11). Cluster III: Contains 12 genotypes (WS 9, WS 12, WS 13, WS 15, WS 17, WS 21, WS 22, WS 23, WS 25, WS 28, WS 31, WS 48). Cluster IV: Contains 17 genotypes (WS 27, WS 29, WS 30, WS 34, WS 35, WS 38, WS 39, WS 40, WS 41, WS 42, WS 43, WS 44, WS 45, WS 46, WS 47, WS 49, WS 50) (Fig. 5).

The genotypes have been classified into 4 separate clusters (Table 5). Cluster 2 indicated the strongest potential for large root yield, as shown by its mean root dry weight of 24.98 g and root fresh weight of 71.10 g. Additionally, the cluster performed better in terms of root diameter and length, with mean values of 30.25 and 27.80 cm respectively. It's important to note that although Cluster 4 had the largest berry dry weight of 39.05 g and the highest yield of seeds of 34.39 g, its root dry weight was only the second lowest at 4.24 g. It made it clear that challenge is to simultaneous increase seed yield and root dry weight (16). For the majority of the qualities that were measured, performance of cluster 1 was moderate. Cluster 3 had the lowest root dry weight (4.24 g). The genotype genetic could be seen in dendrogram (Fig. 5). Cluster 2 registered the most diversity, it offered the scope for selective breeding to increase root yield; however, it is not recommended to increase seed and root yield simultaneously as this needs to be carefully considered beforehand (22, 30).

Table 4. Grouping of 50 Ashwagandha accessions in different clusters.

Cluster	Number of accessions	accessions
I	13	WS 1, WS 7, WS 14, WS 16, WS 18, WS 19, WS 20, WS 24, WS 26, WS 32, WS 33, WS 36, WS 37
II	8	WS 2, WS 3, WS 4, WS 5, WS 6, WS 8, WS 10, WS 11.
III	12	WS 9, WS 12, WS 13, WS 15, WS 17, WS 21, WS 22, WS 23, WS 25, WS 28, WS 31, WS 48
IV	17	WS 27, WS 29, WS 30, WS 34, WS 35, WS 38, WS 39, WS 40, WS 41, WS 42, WS 43, WS 44, WS 45, WS 46, WS 47, WS 49, WS 50

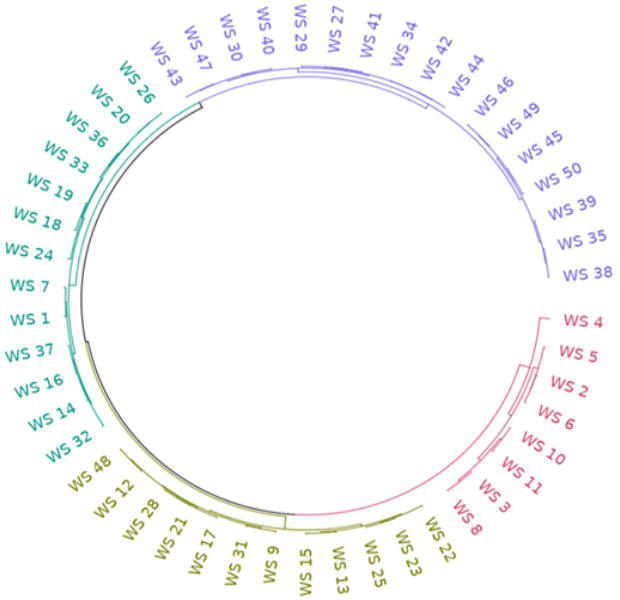

Fig. 5. Dendrogram based agglomerative clustering in 50 germplasm accessions of Ashwagandha.

Biochemical profiling of high yielding accessions

The biochemical traits of Ashwagandha, such as fiber, protein and total carbohydrate, are crucial for its applications in nutrition and pharmacology. Crude fiber supports digestive health, regulates blood sugar and aids in weight management. Protein contributes to tissue repair, stress adaptation and immune function. Total carbohydrates provide energy, enhancing stamina and vitality. Together, these traits make Ashwagandha valuable for promoting overall well-being and addressing specific health conditions. Table 6 presents the key biochemical components of 8 Ashwagandha germplasm lines, showing significant variation. The fibre content ranged from 10.65 to 26.18 % with WS 11 having the highest value (26.18 %) and WS 10 the lowest (10.65 %). There was an 18.54 % to 31.32 % variance in the carbohydrate content. Among all the accessions, WS 4 had the highest percentage at 31.32 % and WS 8 was the lowest at 18.54 %. The percentage of protein varied from 4.06 % to 7.38 %. With 7.38 %, WS 10 had the highest protein % and WS 5 had the lowest (4.06 %).

Conversely, one of the main bioactive substances in Ashwagandha is withaferin A, ranging from 0.048 to 0.318 %. Among these 8 accessions, WS 2 recorded the highest withaferin A (0.318 %), while WS 3 had the lowest concentration (0.048 %) (Fig. 6). These results are consistent with prior studies (31) that reported the different biochemical profiles in Ashwagandha germplasm.

This biochemical profiling suggests that there is potential for selection to enhance some of the desirable characteristics, such as increasing the amount of Withaferin-A for therapeutic uses or enhancing the nutritional value of fibre and carbohydrates. In order to generate acceptable cultivars for a range of uses, future research could look at linking these biochemical markers with agronomic features and medicinal capabilities (32).

Table 5. Cluster analysis for 50 Ashwagandha germplasm accessions.

Traits	Cluster	Minimum	Maximum	Mean	Std. Dev.
Days of flowering	1	72	79	76.08	2.33
	2	69	81	76.00	3.59
	3	75	78	76.42	1.24
	4	71	82	76.35	2.62
Plant height (cm)	1	80	118	96.92	11.28
	2	59	104	83.25	14.97
	3	50	98	75.83	13.82
	4	73	88	80.06	3.90
Number of primary branches per plant	1	4	11	6.85	2.03
	2	5	9	6.50	1.60
	3	3	9	5.92	1.88
	4	4	11	8.76	2.02
Number of secondary branches per plant	1	6	16	11.23	3.88
	2	4	16	9.25	4.13
	3	3	16	9.50	3.71
	4	8	19	14.53	3.28
Root length (cm)	1	15.2	35	25.48	5.30
	2	24	33	30.25	2.96
	3	18	39	23.58	5.71
	4	14.5	25	18.83	3.18
Root diameter (cm)	1	18.1	25.9	22.47	2.67
	2	22.9	37.1	27.80	4.68
	3	14.2	25.5	18.53	3.53
	4	16	23.5	18.58	2.40
Leaf dry weight per plant (g)	1	14.1	30.34	20.61	4.44
	2	14.7	58.08	27.88	14.89
	3	2.3	21.12	12.42	5.05
	4	12.82	36.89	22.49	7.29
Stem dry weight per plant (g)	1	43.18	91.5	58.49	12.81
	2	27.25	118.68	52.71	30.05
	3	7.15	46.55	27.91	10.40
	4	21.27	63.56	40.56	10.10
Berry dry weight per plant (g)	1	10.58	46.16	27.29	11.34
	2	8.16	23.12	15.01	5.92
	3	4.18	24.47	12.20	5.36
	4	20.65	65.24	39.05	11.97
Seed yield per plant (g)	1	6.65	34.07	21.74	9.48
	2	5.98	17.78	10.79	4.62
	3	3.39	18.4	9.70	4.24
	4	17.3	59.42	34.39	11.25
Root fresh weight per plant (g)	1	17.17	49.41	29.39	8.53
	2	37.65	135.7	71.10	30.33
	3	9.75	28.1	16.94	5.99
	4	13.24	30.43	19.71	5.14
Root dry weight per plant (g)	1	4.09	16.82	7.36	3.42
	2	13.07	53.85	24.98	12.48
	3	2.11	9.86	4.24	2.18
	4	3.1	6.21	4.54	1.09

Table 6. Biochemical profiling of high yielding 8 genotype in Ashwagandha.

Accession number	Fiber content (%)	Carbohydrate content (%)	Protein content (%)	Withaferin-A (%)
WS 2	13.65	24.08	6.23	0.318
WS 3	16.71	20.16	4.65	0.048
WS 4	20.44	31.32	6.69	0.112
WS 5	11.18	18.63	4.06	0.124
WS 6	18.62	25.12	7.11	0.130
WS 8	22.52	18.54	5.18	0.217
WS 10	10.65	27.18	7.38	0.084
WS 11	26.18	24.65	4.87	0.054

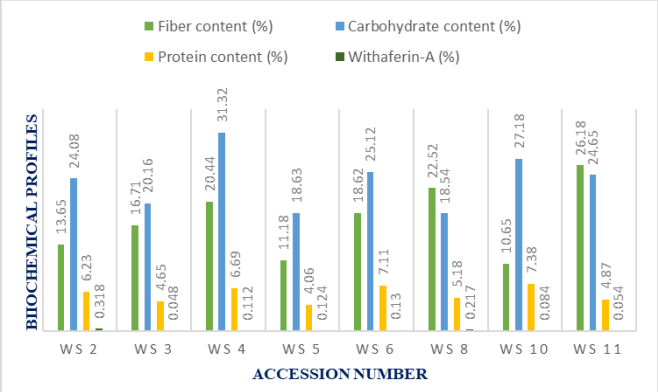

Fig. 6. Graph representing the content of fiber content (%), carbohydrate content (%), protein content (%), withaferin-A (%) of the 8 best Ashwagandha germplasm accessions selected based on dry root yield.

Conclusion

The analysis of genetic variability among 50 genotypes of Ashwagandha revealed a considerable level of diversity, indicating high improvement potential for all traits studied. High heritability estimates and substantial genetic advance further suggest that phenotypic selection can lead to effective improvements in these characteristics. Notably, all root-related traits correlated strongly and positively with one another, suggesting that selection for larger roots may result in higher dry root yield. The analysis of genetic variability among 50 genotypes of Ashwagandha revealed a considerable level of diversity, indicating high improvement potential for all the traits studied. High heritability estimates and genetic advance further suggest that phenotypic selection can lead to effective improvement in these characteristics. Notably, all the root-related traits correlated strongly and positively with one another; suggesting that selection for bigger roots may bring about increased dry root yield. These findings have important implications for future research and breeding programs, particularly in efforts to enhance root yield and quality. By focusing on traits with strong genetic control and positive correlations, breeders can efficiently target improvements. This work aligns with broader agricultural goals, such as sustainability, by optimizing plant resources and reducing the need for external inputs. Additionally, given the medicinal value of Ashwagandha, improving root traits can significantly impact the production of bioactive compounds, contributing to both economic and therapeutic applications. However, the negative correlation between root dry weight and seed yield presents a challenge for simultaneous improvement, requiring a careful balance in breeding strategies. Cluster and principal component analyses identified promising genotype groups while elucidating the variability structure.

These findings provide valuable insights for developing targeted breeding strategies with emphasis on a balanced approach for the improvement of economically important traits in Ashwagandha. There exists the significant variation in chemical content also among the selected high yielding accessions. These accession with higher yield and chemical content can be utilized for the future improvement of Ashwagandha.

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Authors' contributions

GP carried out the experiment, took observations and analysed the data and writing the original graft. S T guided the research by formulating the research concept, helped in securing research funds and approved the final manuscript. MP guided the research by formulating the research concept, helped in securing research funds and approved the final manuscript. VM contributed by developing the ideas, reviewed the manuscript and helped in procuring research grants. SVP contributed by imposing the experiment, helped in editing, summarizing and revising the manuscript. KM helped in summarizing and revising the manuscript. SM helped in editing, summarizing and revising the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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