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Research Article

Genetic analysis for yield and quality contributing parameters in ashwagandha [*Withania somnifera* (L.) Dunal]

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Abstract
Fourty-eight ashwagandha [*Withania somnifera* (L.) Dunal] genotypes were studied for the genetic variability, heritability and trait association during 2018-19 based on 13 yield and quality traits. For all the 13 characters studied, highly significant ($P < 0.01$) differences were observed. Parameters such as withanolides, dry root yield per plant and crude fibre recorded higher estimates for GCV and PCV. High broad sense heritability and genetic advance were recorded for number of primary and secondary branches, number of berries, root length, number of secondary roots, fresh and dry root yield, starch, crude fibre and withanolides. These proved to be promising traits that would respond to selection of better genotypes owing to their high transmissibility and genetic variability. Character association study indicated that dry root yield had positive and significant association with the number of primary and secondary roots, fresh root yield and withanolides at both genotypic and phenotypic levels. Path analysis revealed that number of secondary roots and withanolides were major traits having high positive, direct effect and significant association with dry root yield. Hence, these traits would be quite effective when selected to improve dry root yield in ashwagandha.

Keywords: Genetic variability, heritability, genetic advance, correlation, path analysis

INTRODUCTION

Ashwagandha [*Withania somnifera* (L.) Dunal] belongs to the family Solanaceae, having chromosome number 2n = 48 which is a native of Central and North-Western India and also the Mediterranean part of North Africa (Kumar *et al.,* 2020) and is well known by its other names Indian ginseng (similar to `Panax ginseng'), Winter Cherry and Asgandh. It is largely self-pollinated because of high pollen load and stiff pollen competition (Mir *et al.,* 2013) but some extent of cross-pollination has also been reported (Nigam and Kandalkar 1995). Ashwagandha grows well in dry sub-tropical regions. In India, only two species *viz., somnifera* and *coagulans*, have been reported among 23 species so far identified in Withania genus. Major ashwagandha producing states in India are Rajasthan, Punjab, Haryana, Uttar Pradesh, Gujarat, Maharashtra and Madhya Pradesh (Bara *et al.,* 2016). Under domestication, the plant is mainly grown for its roots for its therapeutic use, although leaves and seeds also have medicinal properties. The roots are stout, cylindrical, whitish brown and they contain amino acids, alkaloids, steroids, starch, volatile oil, glycosides and reducing sugars (Uddin *et al.* 2012). Among different medicinal crops, it is used in various systems of medicines like Ayurveda, Unani and Siddha etc., (Chouhan *et al.*,

2018) as the root and leaf extracts possesses sedative, antistress, diuretic, anti-inflammatory, antitumor, immunomodulatory and antioxidant properties and so the cultivars are used in commercial production of alkaloids and novel sterols (Singh and Kumar, 1998). The Pharmacological activity of the root is attributed to alkaloids (like anaferine and isopelletierine), steroidal lactones (like withaferins and withanolides) and saponins with additional acyl group (Sitoindoside - VII and VIII) and withanolides with a glucose compound at C 27 (sitoindoside - IX and X) (Mishra *et al.,* 2000). Most of the biological activity of ashwagandha is contributed by two main withanolides *viz.,* Withaferin A and Withanolide D (Matsuda *et al.,* 2001). Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) are genetic parameters that are useful to detect the amount of variability existing in the germplasm. Environmental influences on the expression the genotype and reliability of characters are determined by heritability coupled with high genetic advance. The selection is affected by association of traits under selection and correlation and path coefficient analysis are active tools that pave the way to improve the efficacy in breeding programs. Therefore, the present investigation focuses to assess the genetic variability, heritability, correlation and path coefficient which in turn enhance the traits which attributes to improved yield and quality of ashwagandha.

MATERIALS AND METHODS

The experimental material containing 48 ashwagandha genotypes, obtained from MAPRS, AAU, Anand, Gujarat, were sown in RBD with three replications. The experiment was conducted during *Rabi*, 2018-19 at Medicinal and Aromatic Plant Research Station (M&AP), Anand Agricultural University (AAU), Anand, Gujarat, India. Each genotype was planted with a spacing of 30cm x10cm. Observations on various characters were observed in five competing plants from each replication selected randomly except days to 50% flowering, which was observed on population basis. Characters studied were days to 50% flowering, number of primary and secondary branches per plant, number of berries per plant, root length per plant (cm), number of primary and secondary roots per plant, fresh and dry root yield per plant (g), starch (%), crude fibre (%) and withanolides (%). The percentages of starch, crude fibre and withanolides were estimated in the properly dried root samples of all the 48 genotypes. Starch (%) and withanolides (%) were determined as per methodologies suggested by Hogde & Hofrieter (1962) and Mishra (1994), respectively. Crude fibre (%) was estimated by a method described in Association of Analytical Chemists (Anon 1980). The data on different morphological and biochemical characters were analysed statistically with ANOVA (Panse and Sukhatme, 1967), GCV and PCV (Burton, 1952), while classification of GCV and PCV were followed as per Sivasubramanian and Menon (1973), heritability in the broad sense and genetic advance (GA) (Allard, 1960), genetic gain expressed as mean % (Johnson *et al.,* 1955), phenotypic and genotypic correlation (Singh and Chaudhary, 1985) and analysis of path coefficient (Dewey and Lu, 1959).

RESULTS AND DISCUSSION

Variability Parameters: The ANOVA for thirteen characters presented in **Table 1**, revealed that the mean sum of squares for all characters studied for different genotypes was found to be significant ($P < 0.01$). It proved that considerable amount of diversity was present among the genotypes under study. The results on mean, range, PCV and GCV, heritability in broad sense and GA as mean % is

Table 1. Analysis of variance for morphological and quality traits of 48 ashwagandha genotypes

S. No.	Characters	Mean Sum of Square		
		Replications	Genotypes	Error
1.	Days to 50% flowering	7.500	25.247**	3.670
2.	Plant height (cm)	46.906	191.771**	24.731
3.	No. of primary branches plant^{-1}	0.199	1.437**	0.076
4.	No. of secondary branches plant^{-1}	0.660	9.026**	0.779
5.	Number of berries plant^{-1}	205.000	8754.702**	364.074
6.	Root length plant^{-1} (cm)	3.533	13.746**	1.162
7.	No. of primary roots plant^{-1}	0.066	0.077**	0.0233
8.	No. of secondary roots plant^{-1}	0.092	1.269**	0.075
9.	Fresh root yield plant^{-1} (g)	0.292	8.164**	0.249
10.	Dry root yield plant^{-1} (g)	0.062	2.287**	0.044
11.	Starch content (%)	0.344	12.038**	0.431
12.	Crude fibre (%)	1.682	57.507**	0.609
13.	Withanolides (%)	0.0001	0.054**	0.0001

Note: * and ** indicate significance at 5% level and 1% levels, respectively.

presented in **Table 2.** For all the traits, the values of PCV were higher in comparison with GCV which indicated that the environmental factors had influence in the expression of traits.

GCV and PCV: Estimates of PCV ranged from 3.74% (days to 50 % flowering) to 40.26% (withanolides) and values of GCV ranged from 3.04% (days to 50% flowering) to 39.76% (withanolides). Higher coefficients of variation estimates were registered for withanolides (GCV-39.76%; PCV-40.26%) followed by dry root yield (GCV-28.50%; PCV-29.33%) and crude fibre (GCV-21.24%; PCV-21.58%). The above traits had higher magnitude of GCV and PCV, which suggested the presence of high degree of variability. Hence, these traits have better potential for the improvement through simple selection. Similar findings were also reported by Kumar *et al.* (2021), Srivastava *et al.* (2018), Dev *et al.* (2015) and Sundesha and Tank (2013) observed high PCV and GCV for the dry root yield and withanolides, whereas Venugopal *et al.* (2021) and Kujur *et al.* (2021) observed high GCV and PCV for crude fibre. Further, moderate estimates of coefficients of variation were recorded for no. of berries (GCV-18.50%; PCV-19.67%), followed by no. of primary (GCV-15.61%; PCV-16.86%) and secondary (GCV-14.93%; PCV-16.91%) branches, starch (GCV-14.51%; PCV-15.30%), root length (GCV-11.05%; PCV-12.49%). and plant height (GCV-10.61%; PCV-12.75%). This indicated the presence of sufficient and significant inherent genetic variance by which selection could be very much effective. The results are in conformity with previous studies by Venugopal *et al.* (2021) for starch, Kumar *et al.* (2021) and Joshi *et al.* (2014) in plant height and root length. In contrast, low estimates of coefficients of variation were recorded only for days to 50% flowering (GCV-3.04%; PCV-3.74%), which indicated that those characters had low variability in the present experimental material and these traits have no much scope for the improvement. Kujur *et al.* (2021), Sundesha and Tank (2013) and Sangwan *et al.* (2013) reported similar results having low PCV and GCV values for the characters of days to 50% flowering. The remaining features like no. of secondary roots (PCV-21.69%; GCV-19.90%) and fresh root yield (PCV-20.07%; GCV-19.18%) had high PCV and moderate GCV whereas, no. of primary roots had moderate PCV and low GCV (PCV-11.37%; GCV-7.58%) which proved moderate to narrow genetic variability. The higher and lower values for fresh root yield respectively for phenotypic and genotypic coefficients of variation were recorded by Sangwan *et al.* (2013). All the traits showed significant difference between the estimates GCV and PCV indicating a substantial variation for these characters which was contributed by environmental factors.

Heritability: Heritability provides information regarding the transmission of traits from parents to progeny. The estimates of heritability for different characters were high and ranged from 44.48% to 97.53%. High heritability values were recorded for all the traits studied except for number of primary roots per plant which had moderate heritability indicating that the variation observed was mainly under genetic control and had less environmental influence and can be progressed from selection. The results are in resonance with the report of Kumar *et al.* (2021), Joshi *et al.* (2014), Yadav *et al.* (2008), Sangwan *et al.* (2013), Dev *et al.* (2015), Venugopal *et al.* (2021), Kujur *et al.* (2021), Sundesha and Tank (2013) and Srivastava *et al.* (2018).

Estimates of heritability (> 60%) along with genetic advance (> 20%) gives better prediction estimates than heritability alone when a gain under selection is predicted. From analysis, high heritability along with high GA as mean % was recorded in no. of primary (85.70% and 29.77%) and secondary branches (77.92% and 27.15%), no. of berries (88.48% and 35.84%), root length (78.30%

Table 2. Genetic analysis for morphological and qualitative parameters.

S. No.	Characters	Mean	Range	GCV (%)	PCV (%)	H^2_b (%)	GA% Mean
1.	Days to 50 % flowering	88.09	83.33-94.00	3.04	3.74	66.20	5.10
2.	Plant height (cm)	70.35	55.93-88.13	10.61	12.75	69.24	18.17
3.	No. of primary branches plant^{-1}	4.31	2.60-5.40	15.61	16.86	85.70	29.77
4.	No. of secondary branches plant^{-1}	11.10	8.37-14.53	14.93	16.91	77.92	27.15
5.	No. of berries plant^{-1}	280.89	190.33-402.00	18.50	19.67	88.48	35.84
6.	Root length plant^{-1} (cm)	18.52	14.07-22.40	11.05	12.49	78.30	20.14
7.	No. of primary roots plant^{-1}	1.77	1.47-2.07	7.58	11.37	44.45	10.40
8.	No. of secondary roots plant^{-1}	3.17	2.20-4.40	19.90	21.69	84.22	37.62
9.	Fresh root yield plant^{-1} (g)	8.46	5.44-11.79	19.18	20.07	91.37	37.77
10.	Dry root yield plant^{-1} (g)	3.03	1.62-4.35	28.50	29.33	94.41	57.04
11.	Starch (%)	13.55	9.59-17.82	14.51	15.30	90.00	28.35
12.	Crude fibre (%)	20.50	14.48-28.76	21.24	21.58	96.88	43.06
13.	Withanolides (%)	0.33	0.12-0.61	39.76	40.26	97.53	80.89

and 20.14%), no. of secondary roots (84.22% and 37.62%), fresh root yield (91.37% and 37.7%), dry root yield (94.41% and 57.04%), starch (90.00% and 28.30%), crude fibre (96.88% and 43.06%) and withanolides (9.53% and 80.89%). It proved better scope of improvement of the traits through direct selection, as additive genetic variance predominantly governs the traits. High heritability coupled with high GA was obtained by Venugopal *et al.* (2021) for starch, crude fibre, no. of secondary roots and dry root yield, Kujur *et al*. (2021) for root length and fresh root yield, Srivastava *et al*. (2018) and Joshi *et al.* (2014) for withanolides, Kumar *et al.* (2021) for no. of primary and secondary branches, Yadav *et al.* (2008) for no. of berries. On the other hand, high heritability along with moderate/low GA was noticed for days to 50% flowering, plant height and no. of primary roots. Similar results were obtained by Kumar *et al*. (2021), Dev *et al.* (2015) and Sangwan *et al*. (2013) which indicated the dominance of non-additive gene action for these traits and therefore, direct selection was not much effective.

Correlation studies: Genotypic correlation coefficients were higher than phenotypic correlation coefficients in magnitude which indicates a strong inherent association between the characters. Dry root yield depicted significant and positive association with the no. of primary roots (r_g=0.356; r_p=0.254), no. of secondary roots (r_g=0.834; r_p=0.742), fresh root yield (r_g=0.878; r_p=0.822) and withanolides (r_g=0.934; r_p=0.893) **(Table 3)** in genotypic and also phenotypic levels demonstrating that the dry root yield of ashwagandha was mostly influenced by these characteristics. Therefore, the selection practiced for improvement in characters would possibly result in improving the dry root yield. The results are in conformity with the report of Kujur *et al.* (2021) for fresh root weight and Kumar *et al*. (2011) for alkaloid content.

Days to 50% flowering manifested positive and a significant correlation with the no. of primary branches (r_g=0.255; r_p=0.210), no. of berries (r_g=0.257; r_p=0.217) and no. of secondary branches (r_g=0.189;r_p=0.173) at both the phenotypic and genotypic levels. Correlation among other traits revealed that plant height was significantly and positively correlated with root length (r_g=0.327;r_p=0.252), no. of primary (r_g=0.249;r_p=0.202) and secondary branches (r_g=0.215;r_p=0.129) and no. of berries (r_g=0.254;r_p=0.208) at both phenotypic and genotypic levels except for no. of secondary branches at phenotypic level. No. of primary branches was strongly and positively correlated with no. of secondary branches (r_g=0.443; r_p=0.365), no. of berries (r_g=0.439;r_p=0.369) and plant height (r_g=0.249; r_p=0.202) both at phenotypic and genotypic levels. No. of secondary branches noticed positive and highly significant association with the no. of berries (r_g=0.439;r_p= 0.369) at both genotypic and phenotypic levels. No. of primary roots was significantly and positively correlated with the number of secondary roots (r_g=465;r_p=0.262), fresh root yield (r_g=0.356;r_p=0.217) and withanolides (r_g=0.224;r_p=0.151) at both phenotypic and genotypic levels. No. of secondary roots had positive and significant correlation with fresh root yield (r_g=0.969;r_p=0.854), dry root yield (r_g=0.834;r_p=0.742) and withanolides (r_g=0.726;r_p=0.670) at both phenotypic and genotypic levels. Positive and significant association of fresh root yield was observed with dry root yield (r_g=0.878;r_p=0.822) and withanolides (r_g=0.834;r_p=0.786) at both genotypic and phenotypic levels. Such results indicated that the increase in one character will increase in the correlated character. Similar reports were found by Kujur *et al*. (2021), Srivastava *et al*. (2018), Sundesha *et al*. (2016), Gami *et al*. (2016), Dev *et al*. (2015), Joshi *et al*. (2014), Sangwan *et al.* (2013), Kumar *et al*. (2011) and Yadav *et al*. (2008).

Simple correlation does not give an exact contribution of the characters towards the yield and so path coefficient analysis was done to partition genotypic correlations into direct and indirect effects. Analysis of genotypic path coefficient showing direct and indirect effects of different parameters are presented in **Table 4**. The overall path coefficient analysis revealed that the no. of secondary roots (1.085) exhibited more direct effect towards the yield followed by withanolides (0.842) and no. of berries (0.691). No. of primary roots has a direct effect that was low, but it had an association with dry root yield which was positive and significant because of high indirect and positive effect through no. of secondary roots (0.504) and moderate indirect effect through withanolides (0.186). A high direct negative effect (-0.891) was expressed by fresh root yield which was counteracted by the higher positive indirect effects of no. of secondary roots (1.051) and withanolides (0.702) which resulted in higher correlation with dry root yield. The residual effect was found positive and very low (0.002), which explained the variability in dry root yield for which the characters included in the study are enough. The traits such as no. of secondary roots, withanolides and the no. of berries had a positive and direct effect towards the yield, and also exhibited a high positive correlation with yield except the no. of berries. Therefore, the selection of such characters would bring improvement in ashwagandha dry root yield. Similar results were found by Kandalkar *et al.* (1993), Kubsad *et al*. (2009), Kujur *et al.* (2021), Srivastava *et al.* (2018), Sundesha *et al.* (2016), Gami *et al*. (2016), Dev *et al.* (2015), Joshi *et al.* (2014) and Sangwan *et al*. (2013).

The current investigation on genetic variability, heritability and genetic advance proved that a greater scope existed for the improvement of dry root yield through selection in ashwagandha. High heritability coupled with high GA as mean % was noticed for number of primary and secondary branches, number of berries, root length, number of secondary roots, fresh and dry root yield, starch, crude fibre and withanolides, indicating selection may be effective for improvement. Correlation and path coefficients studies revealed the importance of number of berries, number of secondary roots and withanolides

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Table 3. Genotypic (r_g) and phenotypic (r_p) correlation coefficients for morphological and quality traits in ashwagandha

	Characters		Plant height (cm)	Number of primary branches plant^{-1}	Number of secondary branches plant^{-1}	Number of berries plant^{-1}	Root length plant^{-1} (cm)	Number of primary roots plant^{-1}	Number of secondary roots plant^{-1}	Fresh root yield plant^{-1} (g)	Dry root yield plant^{-1} (g)	Starch (%)	Crude fibre (%)	Withanolides (%)
1	Days to 50 % flowering	r_g	-0.104	0.255**	0.189*	0.257**	-0.087	0.180*	-0.176*	-0.217**	-0.295**	-0.016	-0.003	-0.368**
		r_p	-0.075	0.210**	0.173*	0.217**	-0.102	0.033	-0.092	-0.198*	-0.263**	-0.014	0.004	-0.297**
2	Plant height (cm)	r_g	**1.000**	0.249**	0.215**	0.254**	0.327**	0.145	0.074	0.071	0.081	-0.138	0.009	0.142
		r_p		0.202*	0.129	0.208*	0.252**	0.098	0.031	0.047	0.060	-0.124	0.005	0.101
3	Number of primary branches plant^{-1}	r_g		**1.000**	0.443**	0.439**	-0.108	-0.018	-0.094	-0.068	-0.008	0.112	-0.006	0.032
		r_p			0.365**	0.369**	-0.074	0.045	-0.084	-0.070	0.006	0.094	-0.006	0.039
4	Number of secondary branches plant^{-1}	r_g			**1.000**	0.955**	-0.071	0.127	0.112	0.153	0.122	0.157	-0.154	0.045
		r_p				0.809**	-0.059	0.036	0.081	0.112	0.090	0.134	-0.130	0.032
5	Number of berries plant^{-1}	r_g				**1.000**	-0.088	0.008	0.045	0.077	0.132	0.106	-0.085	0.027
		r_p					-0.063	0.005	0.033	0.078	0.110	0.104	-0.090	0.028
6	Root length plant^{-1} (cm)	r_g					**1.000**	-0.016	-0.040	-0.080	-0.107	-0.037	-0.157	-0.019
		r_p						-0.012	-0.038	-0.098	-0.097	-0.001	-0.155	-0.008
7	Number of primary roots plant^{-1}	r_g						**1.000**	0.465 **	0.356**	0.356**	0.103	-0.011	0.224**
		r_p							0.262**	0.217**	0.254**	0.071	-0.018	0.151**
8	Number of secondary roots plant^{-1}	r_g							**1.000**	0.969**	0.834**	0.093	0.125	0.726**
		r_p								0.854**	0.742**	0.081	0.117	0.670**
9	Fresh root yield plant^{-1} (g)	r_g								**1.000**	0.878**	0.075	-0.002	0.834**
		r_p									0.822**	0.074	-0.002	0.786**
10	Dry root yield plant^{-1} (g)	r_g									**1.000**	0.133	-0.072	0.934**
		r_p										0.117	-0.065	0.893**
11	Starch (%)	r_g										**1.000**	-0.200*	0.049
		r_p											-0.189*	0.042
12	Crude fibre (%)	r_g											**1.000**	-0.138
		r_p												-0.139
13	Withanolides (%)	r_g												**1.000**
		r_p												

Note: * and ** indicate significance at 5% level and 1% levels, respectively.

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Table 4. Genotypic path coefficient analysis showing direct (diagonal and bold) and indirect effects of different parameters on dry root yield per plant

Characters	Days to 50 % flowering	Plant height (cm)	Number of primary branches plant^{-1}	Number of secondary branches plant^{-1}	Number of berries plant^{-1}	Root length plant^{-1} (cm)	Number of primary roots plant^{-1}	Number of secondary roots plant^{-1}	Fresh root yield plant^{-1} (g)	Starch (%)	Crude fibre (%)	Withanolides (%)	Genotypic correlation coefficient with dry root yield plant^{-1} (r_g)
Days to 50 % flowering	**-0.082**	0.009	-0.005	-0.105	0.177	0.008	0.012	-0.191	0.192	-0.001	0.001	-0.311	-0.295**
Plant height (cm)	0.008	**-0.086**	-0.005	-0.120	0.175	-0.032	0.009	0.079	-0.063	-0.004	-0.001	0.120	0.081
Number of primary branches plant^{-1}	-0.021	-0.021	**-0.020**	-0.247	0.303	0.010	-0.001	-0.102	0.060	0.003	0.001	0.025	-0.008
Number of secondary branches plant^{-1}	-0.015	-0.018	-0.009	**-0.558**	0.660	0.007	0.008	0.121	-0.136	0.004	0.020	0.038	0.122
Number of berries plant^{-1}	-0.021	-0.021	-0.008	-0.533	**0.691**	0.008	0.001	0.049	-0.069	0.003	0.011	0.022	0.132
Root length plant^{-1}(cm)	0.007	-0.028	0.002	0.039	-0.060	**-0.100**	-0.001	-0.043	0.071	0.001	0.020	-0.014	-0.107
Number of primary roots plant^{-1}	-0.014	-0.012	0.001	-0.071	0.005	0.001	**0.068**	0.504	-0.317	0.003	0.001	0.186	0.356**
Number of secondary roots plant^{-1}	0.014	-0.006	0.001	-0.062	0.031	0.004	0.031	**1.085**	-0.864	0.002	-0.016	0.611	0.834**
Fresh root yield plant^{-1} (g)	0.017	-0.006	0.001	-0.085	0.053	0.008	0.024	1.051	**-0.891**	0.002	0.001	0.702	0.878**
Starch (%)	0.001	0.011	-0.002	-0.087	0.073	-0.003	0.007	0.100	-0.066	**0.031**	0.026	0.040	0.133
Crude fibre (%)	0.001	-0.001	0.001	0.08	-0.058	0.015	-0.001	0.135	0.002	-0.006	**-0.130**	-0.115	-0.072
Withanolides (%)	0.030	-0.012	-0.001	-0.025	0.018	0.001	0.015	0.787	-0.743	0.001	0.017	**0.842**	0.934**

Note: * and ** indicate significance at 5% level and 1% levels, respectively
(Residual effect = 0.02)

as selection criteria for effective yield improvement. Among these characters, number of secondary roots and withanolides had both high direct effects and positive association. Therefore, importance should be focussed on these traits during the selection in breeding program in order to increase dry root yield. Hence, based on these traits if selection is done, it can bring improvement in yield and quality attributing traits of ashwagandha.

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