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Research Article

Genetic analysis in ashwagandha [*Withania somnifera* (L.) Dunal]

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Abstract
The present investigation was undertaken to decipher multivariate diversity, variability, genetic parameters and traits association in Ashwagandha (*Withania somnifera* (L.) Dunal), genotypes for the yield of a dry root, total alkaloids in root and agro-morphological traits. A total of 16 genotypes were evaluated at three experimental sites in Gujarat, India. A randomized complete block design with two replications was used to conduct the trial. Pooled ANOVA results showed highly significant differences ($p < 0.001$) among the genotypes (G), environments (E) and G x E interactions. Higher phenotypic and genotypic coefficient of variation values were recorded for yield and agro-morphological traits indicating variation and scope of improvement through selection based on phenotypes. Higher heritability associated with greater genetic advance for the majority of traits showed a prevalence of additive gene effect and thus, phenotypic selection will be more productive. Based on the similarity of traits within and between members of clusters, genotypes were divided into five clusters. The principal component analysis (PCA) showed that most of the differences (76.90%) were explained by the first four PCA. The correlation analysis implies that improving one or more components traits could result in enhancement in root yield and total alkaloid content in ashwagandha. Genetic variability was present for the traits under study in the tested genotypes. Hybridization of genotypes from different clusters could be able to yield new genotypes combining high yield and other desirable yield-contributing traits.

Key words: Multivariate diversity, variability, association analysis, cluster analysis, UPGMA, principal component, *Withania somnifera*

INTRODUCTION

Herbal plants are an important source of medicines and major health care providers across the globe since ancient times. In spite of incredible advances in modern science, approximately 80 per cent population still depends on traditional medicine mainly in underdeveloped and developing countries (Sharma *et al*., 2014). In India, the medicinal crop, ashwagandha is grown in an area of about 10,780 ha, and total dry root production of 8,429 tones and demand is increasing annually (Kumar *et al*., 2020b).

The roots of *W. somnifera* plants have medicinal values similar to roots of ginseng, which is used for health wellness and restoration in humans; hence, it is called as Indian ginseng as reported by Singh and Kumar (1998). The drugs derived from ashwagandha is an anti-cancerous, anti-epileptic, anti-ageing, anti-arthritic, anti-coagulant, anti-pyretic, diuretic and effective adaptogen used in immunomodulation, combating infectious agents, as a mood elevator, stress reliever, rejuvenator, hypoglycemia, and hypocholesterolemia (Dhama *et al*.,

2013; Mahima *et al*., 2012; Kumar *et al*., 2020 a). To boost the genetic yield potential of any crop it is important to identify sources with improved yield and other desirable yield contributing characters. Genetic variation is the basis of crop improvement programmes and provides a great array of genotypes that can be used in breeding high yielding cultivars. Further, to plan an efficient breeding programme reliable estimates of the degree of heritability, genetic advance and the extent and direction of genetic correlations among yield and agro-morphological traits is required. Heritability and genetic advance estimates could be valuable tools in predicting genetic improvement through selection. Principal component analysis (PCA) and cluster analysis (CA) are the two important multivariate statistical tools for studying the relatedness among the genotypes. Therefore, the present experiment was conducted to analyse multivariate genetic diversity, genetic variation, heritability and associations in 16 genotypes and to select important traits for further improvement.

MATERIALS AND METHODS

The experimental material consisted of 16 genotypes of ashwagandha along with one check (AWS-1). Trials were conducted over three consecutive years, 2016-17, 2017-18 and 2018-19 at three different locations (S.K.Nagar, Jagudan and Bhiloda) in the semi-arid conditions of Gujarat, India. The experiment was laid down in a randomized block design (RBD) with two replications. Seeds were sown directly during third week of October each year and thinned after 45 days of sowing. Each genotype was sown in 2 m long rows with a total number of 4 rows per plot per replication. Plant geometry of row-to -row and plant-to-plant distance of 30 and 10 cm, respectively were maintained in each plot. About, 150 days after sowing plants were uprooted carefully with intact roots; roots were cleaned in tap water and dried to a moisture level of 7-8 per cent of the initial content in the hot air oven for approximately 48 hours. Dried roots were packed in sealed polythene bags for biochemical study. Five random plants were chosen from each replication for each plot to report the yield of dry roots (g) and per cent of total root alkaloid material. The total root alkaloid content was calculated by the methodology suggested by Mishra (1998).

The genetic parameters were calculated using R statistical software, version 3.4.1 (R Development Core Team, 2017). GCV and PCVs were computed as per the formula suggested by Burton and DeVane (1953). The PCV and GCV values were categorized as high (20% and above), moderate (10-20%) and low (0-10%), as suggested by Sivasubramanian and Madhavamenon (1973).The heritability was classified as proposed by Robinson *et al*. (1949) as: high = 60% and above, moderate = 30-60% and low = 0-30%. Genetic advance and genetic advance as per cent of the mean were computed by the formula suggested by Johnson *et al.* (1955).Genetic advance as per cent of mean was categorized according to Falconer (1989) as: high = (>20%), moderate = (10-20%) and low = (<10%).

Hierarchical cluster analysis for root yield, agro-morphological characters and alkaloid content was performed to form a dendrogram based on the average distance between genotypes. The inter-genotypic divergence was computed using the unweighted pair group method of arithmetic averages (UPGMA). Cluster analysis and principal components analysis (PCA) was carried out using PAST software, version 3.25. Pearson's correlation coefficient (r) was used to measure the degree of inter-relationship among linearly related variables. The data for Pearson's correlations were analysed using R statistical software, version 3.4.1 (R Development Core Team, 2017).

RESULTS AND DISCUSSION

Pooled ANOVA showed considerable differences ($p<0.001$) among genotypes for all the characters considered (**Table 1**). This suggested that the genotypes were genetically diverse and considerable variation existed in the materials used. Such variation can be exploited by plant breeders in hybridization programs. The variance due to G x E interaction component was reported to be highly significant ($p<0.001$) for all the traits except days to maturity (significant at $p<0.05$) indicating significant differences among genotypes across the environments. The average performance, range, PCV, GCV and environmental variance, H^2 and genotypic advance calculated as per cent of mean for each character are given in **Table 2**. The number of days for 50% flowering ranged from 83.60 to 89.52 and the mean was found 85.92 days. The mean value for days to maturity was observed at 139.78 days with a range of 136.19-142.10 days. The agro-morphological traits *viz.,* primary branches ranged from 1.67 to 3.22, secondary branches 4.02 to 6.06, berries per plant from 98.63 to 196.60 and seeds per berry 23.83 to 31.59. The root morphometric traits *viz*., root branches, diameter and length ranged from 2.00 to 3.82, 9.94 to 12.04 mm and 14.38 to 18.37 cm, respectively. The average value of dry root yield per plant was 1.56 g with a range of 1.25 to 2.08 g. Total root alkaloid content ranged from 1.08 to 1.80 per cent with a mean value of 1.40 per cent. These genotypes can be further utilized in developing high yielding varieties of ashwagandha.

Higher values of PCV and GCV were recorded for the berries per plant (65.69:65.03%), root branches per plant (51.32:50.25%), primary branches per plant (50.57:49.74%), dry root yield per plant (45.39:44.14%), total root alkaloid content (40.90:38.19%), secondary branches per plant (37.16:31.13%) and the number of seeds per berry (27.23:26.22%) (**Table 2**). The magnitude of PCV was slightly higher than the GCV for all the characters under study that suggested little influence of

Table 1. Analysis of variance of twelve quantitative traits in ashwagandha

Sources of variation	DF	Days to 50% flower				Days to maturity				Plant height			
		Sum of square	Mean sum of square	F value	Pr(>F)	Sum of square	Mean sum of square	F value	Pr(>F)	Sum of square	Mean sum of square	F value	Pr(>F)
Genotype	15	746	49.70	23.48	< 2e-16***	977	65	26.79	<2e-16***	2906	193.70	22.89	< 2e-16***
Replication	1	6	5.60	2.62	0.108	0	0	0.15	0.695	5	4.70	0.55	0.46
Environment	8	3455	431.80	203.79	< 2e-16***	42695	5337	2194.90	<2e-16***	5310	663.70	78.41	< 2e-16***
Genotype x environment	120	952	7.90	3.74	6.21E-14***	430	4	1.48	0.013*	3358	28.00	3.31	7.72E-12***
Residuals	143	303	2.10			348	2			1210	8.50		
		Primary branches per plant				Secondary branches per plant				Berries per plant			
Genotype	15	40.02	2.67	60.86	<2e-16***	87.80	5.86	5.70	4.55E-09***	227477	15165	98.92	<2e-16***
Replication	1	0.01	0.01	0.18	0.669	0.20	0.18	0.18	0.676	77	77	0.50	0.479
Environment	8	70.55	8.82	201.21	<2e-16***	814.40	101.80	99.15	< 2e-16***	751942	93993	613.11	<2e-16***
Genotype x environment	120	36.46	0.30	6.93	<2e-16***	433.80	3.61	3.52	7.00E-13***	259711	2164	14.12	<2e-16***
Residuals	143	6.27	0.04			146.80	1.03			21923	153		

DF: Degree of freedom, Significance codes: '***'=0.001, '**'=0.01, '*'=0.05

Table 1. Continue...

Sources of variation	DF	Seeds per berry				Root branches				Root diameter			
		Sum of square	Mean sum of square	F value	Pr(>F)	Sum of square	Mean sum of square	F value	Pr(>F)	Sum of square	Mean sum of square	F value	Pr(>F)
Genotype	15	1602	106.80	26.46	< 2e-16***	61.59	4.11	47.43	<2e-16***	89.39	5.96	15.66	< 2e-16***
Replication	1	0	0.30	0.06	0.801	0.02	0.02	0.18	0.676	0.05	0.05	0.12	0.727
Environment	8	6376	797.00	197.37	< 2e-16***	268.32	33.54	387.42	<2e-16***	297.58	37.20	97.77	< 2e-16***
Genotype x environment	120	1818	15.10	3.75	5.65E-14***	97.56	0.81	9.39	<2e-16***	141.39	1.18	3.10	8.39E-11***
Residuals	143	577	4.00			12.38	0.09			54.41	0.38		
		Root length				Dry root yield				Total root alkaloid content			
Genotype	15	247.70	16.51	50.93	<2e-16***	14.62	0.97	35.66	<2e-16***	9.17	0.61	14.64	<2e-16***
Replication	1	0.00	0	0.01	0.926	0.10	0.10	3.56	0.0614	0.02	0.02	0.51	0.477
Environment	8	1428.60	178.58	550.82	<2e-16***	20.74	2.59	94.85	<2e-16***	14.29	1.79	42.78	<2e-16***
Genotype x environment	120	452.90	3.77	11.64	<2e-16***	19.36	0.16	5.90	<2e-16***	22.35	0.19	4.46	<2e-16***
Residuals	143	46.40	0.32			3.91	0.03			5.97	0.04		

DF: Degree of freedom, Significance codes: '***'=0.001, '**'=0.01, '*'=0.05

environment on the phenotypic expression of traits. The success of any crop improvement programme lies in exploiting genetic variability and the relative importance of genetic and non-genetic components in traits expression. High PCV and GCV for yield and related characters indicated considerable genetic variation for these characters and further scope of genetic gain through selection. Earlier, Arun Kumar *et al*. (2007), Chauhan *et al*. (2018), Das *et al*. (2011), Dubey *et al*. (2010), Gami *et al*. (2016), Gupta *et al*. (2011), Iqbal and Datta (2007), Jain *et al*. (2007), Joshi *et al*. (2014), Kandalkar *et al*. (1993), Manivel *et al*. (2017), Misra *et al*. (1998a), Misra *et al*. (1998b), Mohsina *et al*. (2007), Sangwan *et al*. (2013), Singh *et al*. (2014), SukhDev *et al*. (2015) Yadav *et al*. (2008) have also reported similar variations for these characters in ashwagandha.

The highest prediction of H^2 (98.00%) and genetic advance expressed as per cent of mean was reported for berries per plant (132.62%). Other traits also showed

Table 2. Mean, range, variance, coefficient of variations, heritability, genetic advance as per cent of mean of ashwagandha.

Characters	Mean	Range	PV	GV	EV	PCV (%)	GCV (%)	H^2 (%)	GAM
Days to 50% flower	85.92	83.60-89.52	25.93	23.81	2.12	5.93	5.68	91.83	11.21
Days to maturity	139.78	136.19-142.10	33.79	31.36	2.43	4.16	4.01	92.80	7.95
Plant height (cm)	56.13	47.16-59.83	101.09	92.62	8.46	17.91	17.15	91.63	33.81
Primary branches per plant	2.30	1.67-3.22	1.36	1.31	0.04	50.57	49.74	96.77	100.80
Secondary branches per plant	4.99	4.02-6.06	3.44	2.41	1.03	37.16	31.13	70.16	53.71
Berries per plant	133.22	98.63-196.60	7659.23	7505.92	153.30	65.69	65.03	98.00	132.62
Seeds per berry	27.34	23.83-31.59	55.44	51.40	4.04	27.23	26.22	92.72	52.02
Root branches	2.82	2.00-3.82	2.10	2.01	0.09	51.32	50.25	95.87	101.35
Root diameter (mm)	11.03	9.94-12.04	3.17	2.79	0.38	16.15	15.15	88.00	29.27
Root length (cm)	15.78	14.38-18.37	8.42	8.09	0.32	18.38	18.02	96.15	36.41
Dry root yield per plant (g)	1.56	1.25-2.08	0.50	0.47	0.03	45.39	44.14	94.54	88.40
Total root alkaloid content (%)	1.40	1.08-1.80	0.33	0.28	0.04	40.90	38.19	87.21	73.48
Mean									

PV= Phenotypic variance: GV= Genotypic variance: EV= Environmental variance: PCV= Phenotypic coefficient of variation: GCV= Genotypic coefficient of variation: H^2= Broad sense heritability: GAM= Genetic advance as per cent of mean

significantly high H^2 and genetic advance expressed as per cent of mean as presented in **Table 2** that indicated the prevalence of additive gene effects and thus, direct phenotypic selection for these characters will be effective for genetic improvement. High heritability coupled with high genetic advance for yield and related traits were previously reported in ashwagandha by Joshi *et al*. (2014), Manivel *et al*. (2017), Sangwan *et al*. (2013) and Singh *et al*. (2014).

The cluster analysis through the UPGMA method grouped the genotypes into five clusters with a different number of genotypes in each cluster (**Fig. 1**). Cluster I possessed 5 genotypes namely SKA-21, AWS-1, SKA-3, SKA-24 and SKA-25 with early flowering and dwarf stature. Cluster II also comprised of 5 genotypes *viz*., SKA-27, SKA-6, SKA-4, SKA-1 and SKA-10 with late maturity, more number of root branches, more number of primary branches, total alkaloid content (%) from roots and dry root yield and Cluster III was represented by four entries SKA-17, SKA-23, SKA-19 and SKA-26 which possessed tall plants with more primary and secondary branches per plant, the number of berries per plant, the number of seeds per berry and root diameter. Cluster IV constituted single genotype SKA-12 with late flowering, late maturity, taller plants, more number of secondary branches, the number of berries per plant, the number of seeds per berry, root diameter and total root alkaloid content (%). The single genotype SKA-11 was placed in cluster V on the basis of more yield and all agro-morphological traits except days to maturity. The distribution of 16 ashwagandha genotypes into five different clusters revealed substantial differences among genotypes for yield and various agro-morphological traits. The clustering of genotypes helps in the identification of potential parents from distant clusters for use in crossing programmes to generate worthy recombinants in segregating populations. Similar, clustering of ashwagandha genotypes based on quantitative data was reported by Joshi *et al*. (2015), Manivel *et al*. (2017) and Ramesh Kumar *et al*. (2012).

The PCA component based on correlation analysis determines the inter-relationships among the different traits and genotypes. Eigen values larger than 1 are considered important and component loadings > ±0.3 were deemed worthwhile. As a result, the analysis only used the first four PCs, and traits with loadings greater than 0.3 were chosen to represent the corresponding principal axis (Hair *et al*., 1998). The first four PCs having an eigen value greater than one were extracted from the mean of 12 traits and they explained 76.90 per cent variance in ashwagandha genotypes (**Table 3**). The PC1 was most significant as it accounted for 41.57 per cent of variation. The PC1 was highly correlated with days to 50% flower (0.306), plant height (0.697), primary branches number (0.804), secondary branches number (0.796), the number of berries per plant (0.750), the number of seeds per berry (0.375), the number of root branches (0.709), root diameter (0.710), dry root yield from a single plant (0.723) and total alkaloid from roots (0.682). A variance of 15.02, 10.73 and 9.58 per cent was derived from the second, third and fourth principal components, respectively (**Table 3**). The genotype-trait biplot based on two PCs were formed to represent the 2-D view of different genotypes and traits (**Fig.2**). The PCA study indicated the role of traits (specific to each PC) that contributed more towards total variation and in discriminating the genotypes. The current study was in accordance with the PCA traits analysis of Ramesh Kumar *et al*. (2012) in ashwagandha.

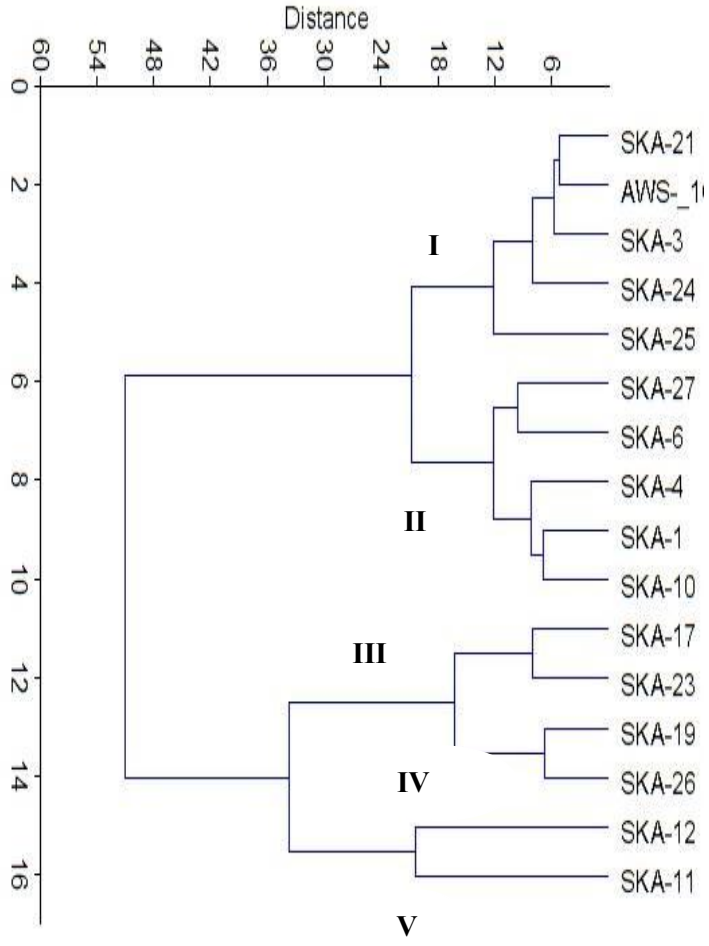

Fig. 1. Dendrogram showing genotypes of ashwagandha

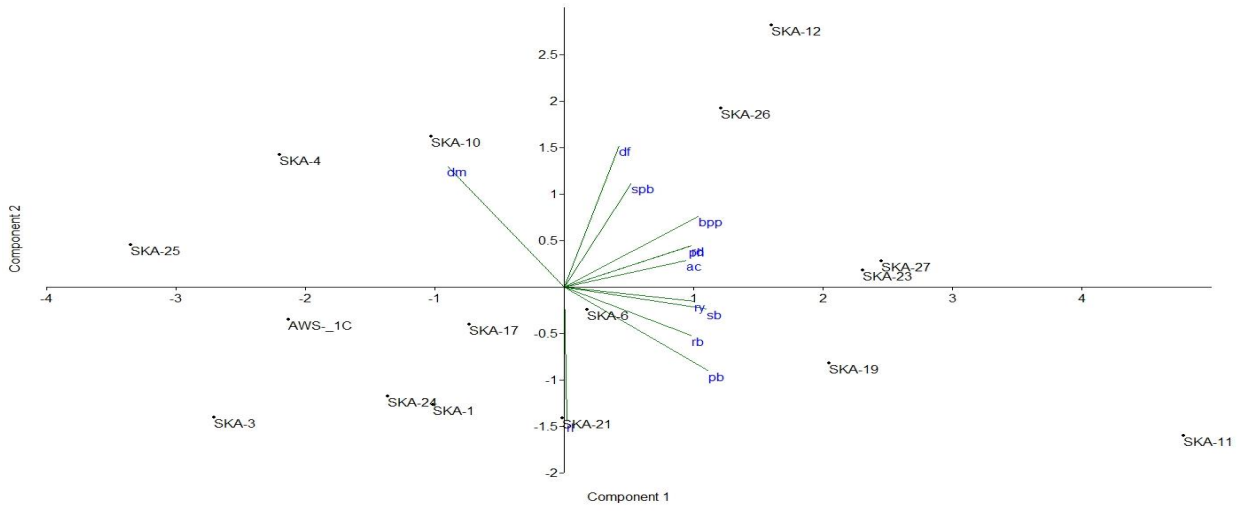

Fig. 2. PCA biplot showing twelve quantitative traits and 16 genotypes of ashwagandha

Table 3. Principal components of twelve quantitative traits in ashwagandha.

Principal Components	PC1	PC2	PC3	PC4
Eigen Value	4.99	1.80	1.29	1.15
% Variance	41.57	15.02	10.73	9.58
Loadings				
Days to 50% flowering	0.306	0.659	0.200	-0.406
Days to maturity	-0.657	0.564	0.075	-0.081
Plant height	0.697	0.191	0.079	-0.195
Primary branches per plant	0.804	-0.392	-0.010	-0.074
Secondary branches per plant	0.796	-0.102	0.379	-0.081
Berries per plant	0.750	0.332	0.399	0.117
Seeds per berry	0.375	0.484	-0.349	0.642
Root branches	0.709	-0.228	-0.258	-0.500
Root diameter	0.710	0.192	0.426	0.327
Root length	0.014	-0.631	0.350	0.358
Dry root yield per plant	0.723	-0.067	-0.409	0.047
Total root alkaloid content	0.682	0.124	-0.523	0.125

PC1, PC2, PC3 and PC4= Principal component 1, Principal component 2, Principal component 3 and Principal component 4 respectively

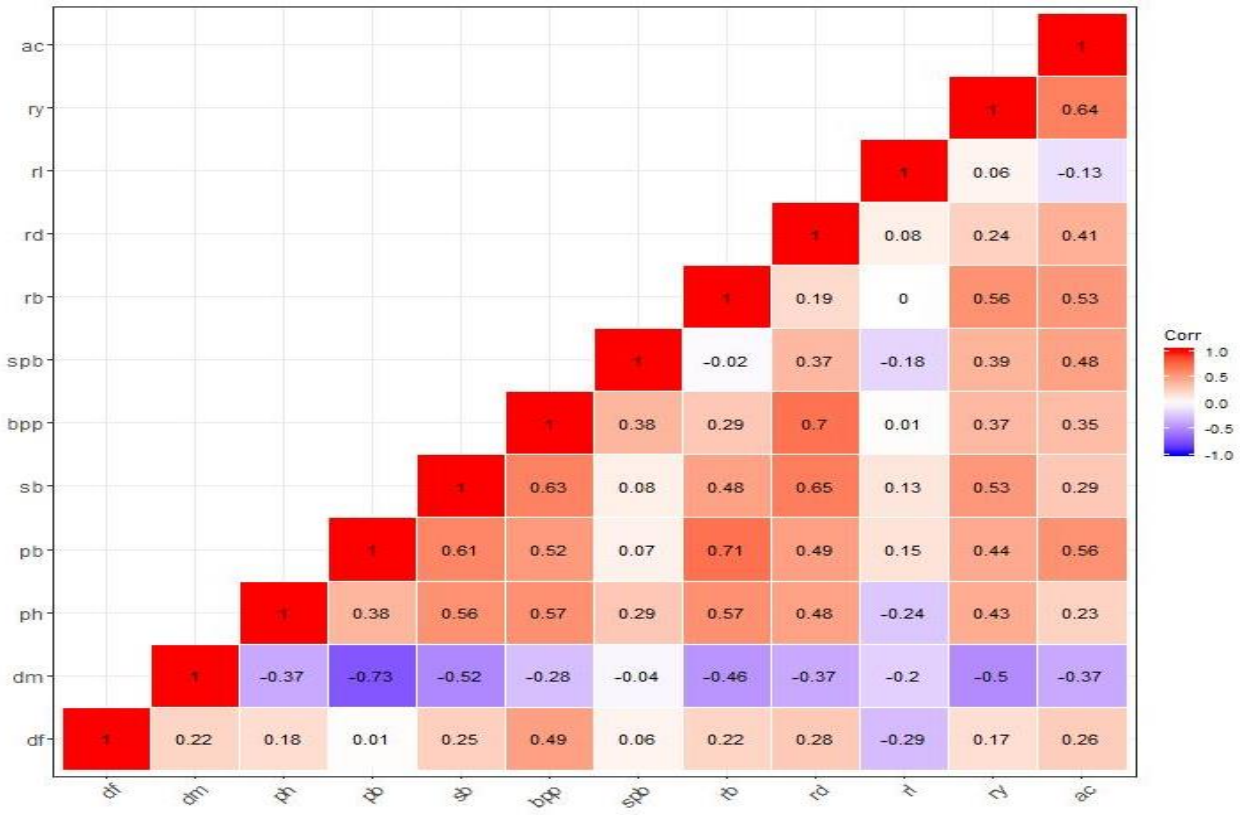

Fig. 3. Pearson's correlation among 12 quantitative traits in ashwagandha

df= Days to 50% flower: dm=days to maturity: ph= plant height: pb= primary branches per plant: sb= secondary branches per plant: bpp= berries per plant: spb= seeds per berry: rb= root branches: rd= root diameter: rl= root length: ry= dry root yield per plant: ac= total root alkaloid content .

Significant associations among various traits give an insight into the scope of simultaneous improvement of characters and their direct or indirect effects will lead to simultaneous improvement in yield. Conclusively, association studies among various traits are depicted in Figure 3. The significant positive association of dry root yield per plant and total root alkaloid content with other traits implied that improving one or more components traits could result in enhancement in root yield and alkaloid content in ashwagandha. Further plant height, the number of primary branches, the number of secondary branches, the number of berries per plant, the number of root branches, the diameter of roots, root length, dry root yield and total root alkaloid content showed a significant negative correlation with days to maturity. Early maturity is a preferred trait for cultivation as crops escape terminal heat and water stress and thus, allows ashwagandha cultivation in drier environments and also well suited for multiple cropping systems. The Pearson's correlations have been used for studying traits interrelationship by Alam *et al*. (2011), Ramesh Kumar *et al*. (2011) and Kumar *et al*. (2012) in ashwagandha.

From the study it is concluded that significant diversity existed among genotypes for all characters studied. Dry root yield per plant content had a strong positive association with total root alkaloid. Therefore, the characteristic of dry root yield can be exploited in selection programs to improve the root alkaloid content in ashwagandha. Moreover, a significant negative association of maturity duration with agro-morphological traits, dry root yield and total root alkaloid would facilitate in breeding short duration ashwagandha cultivars for hot and water stress climatic conditions of semi-arid regions.

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