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Study of combining ability over the environments in line x tester crosses in Ashwagandha (*Withania somnifera* L.)

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Abstract

Three different locations were chosen to conduct the experiment to get information on combining ability including 65 genotypes of Ashwagandha. Analysis of variance indicated that experimental material contained considerable variability and both specific combining ability along with general combining ability effects are involved in the genetic expression of characters; furthermore, SCA, as well as GCA, were highly influenced by the environment. The GCA effects over the environments disclosed that among parents, L_9, L_5, L_{14}, L_{15}, and T_3 were excellent hence these are identified as good general combiners in terms of several root attributing traits besides root yield at dry basis, while, six crosses L_8xT_1, $L_{10}xT_1$, $L_{12}xT_1$, $L_{15}xT_2$, L_1xT_3, and L_4xT_3 were reported superior specific combiner for economic important trait dry root yield per plant. The above selected genotypes may be useful for further crop improvement breeding programs.

Keywords ashwagandha, dry root yield, environments

Introduction

Ayurveda-‘wisdom of life’ the Indian traditional medicinal science was developed mainly because of the huge experience and wisdom of our ancestors, which is being practiced for over 5000 years [1]. Ashwagandha (*Withania somnifera* L. Dunal), the royal herbal plant, known as “Indian winter cherry” is widely used in herbal medicinal formulations, belongs to the family Solanaceae [2], contains 48 chromosomes, and its reproductive biology reported as a self-pollinated plant [3-4]. The root is the main part of the plant, fragile, sturdy, and longer root has good market value, furthermore, starchy, brittle roots have high market price due to their easy powder making feature and also have been quoted to be characteristic root texture [5]. It is a versatile herb, furthermore, the beneficial medicinal effects have already been reported on different systems of the human body viz., immune system, reproductive as well as neurological systems along with the system of endocrine and energy production [6]. It is an adaptogen-a substance that assists the body to adapt to stressors and is best known for its anti-anxiety (anxiolytic) properties. It is beneficial to mitigate stress-produced insomnia, depression, and immuno-suppression, reduces cortisol levels as well as helpful in reducing low density-lipoprotein cholesterol (LDL-C), enhances physical performance in athletes as well as in sedentary people, and may be helpful to treat Alzheimer's disease [7]. The favorable result of a designed crop improvement program depends mainly on the selection of parents, which are then brought into the single genetic background will result in the development of suitable hybrids. The parent selection in the crossing program only based on high mean performance and adaptation to a particular environment sometimes does not provide fruitful results. This necessitates the investigation of combining ability studies [8]. It is a powerful tool in breeding programs to distinguish between good and bad combinations and select suitable parent material and also provides information about the behavior of genes involved in the inheritance of various traits. Furthermore, the L x T biometrical analysis technique [9] has been found excellent technique in terms of large-scale screening of genotypes with fast speed as well as reasonable accuracy [10]. The usefulness and requirement of combining ability for the selection of parents in the crossing program had been reported by several workers [10-11, 12].

It is very important to evaluate the identified parents and newly developed cross for the yield of the root and its traits. Therefore, this study was conducted over the environments to identify good combiners and promising crosses for better future accomplishments in Ashwagandha.

Methodology

The field trials, including 65 treatments were conducted at three environments/ locations viz., Rajasthan College of Agriculture (RCA-Udaipur; E_1), Krishi Vigyan Kendra (KVK- Chittorgarh, E_2), and Agriculture Research Sub-Station- (ARSS-Vallabhnagar, E_3). At each location three replications were also included by using randomized block design of experiment during *Kharif* 2018-19. The crossing was done during *Khari*f 2017-18 at the breeding field, Rajasthan College of Agriculture (RCA-Udaipur), which included fifteen females (lines) and three males (testers) by using LxT design of matting [9] to develop 45 F_1 experimental hybrids. The experimental material included 3 testers viz., UWS--10(T1), WS--90--146(T2), RVA--100(T3) and 15 lines viz., UWS--301(L1), UWS--302(L2), UWS--303(L3), UWS--304(L4), UWS--305(L5), UWS--306(L6), UWS--307(L7), UWS--308(L8), UWS--309(L9), UWS--310(L10), UWS--311(L11), UWS--312(L12), UWS--313(L13), UWS--314(L14), UWS--315 (L15) along with their resultant 45 F1's crosses as well as 2 checks viz., JA-20 and JA-134. Observations for these trials were noted on the following characters viz., days to 50 % flowering (DTFF), number of primary branches /plant (NPBP), root length (RL), dry plant weight (DPWT), and dry root yield (DRYP). The pooled data of all 3 different locations of the above traits had been subjected to the statistical analysis for combining ability (CA-effect) estimation as per the model [9].

Results and Discussion

Analysis for combing ability divulged (Table 1) that the mean sum of square because of lines, testers, and LxT was of significance for all the traits in all three locations excluding because of testers for days to 50 % flowering in the E_2 environment, whereas results on the pooled basis (Table 2) indicated that mean squares ascribed to line, tester and LxT were significant for all characters. The interaction due to lines with environments, testers with environments, and the LxTxE was reported significant for all the traits excluding days to 50 % flowering due to testers with environments, this indicates that the influence of environment was high for the estimation of GCA as well as SCA effect for all characters under study. Similar results were also reported [13, 6] in Ashwagandha and [10] in safflower and several other workers in different crops such as [14] in potato [15] and [16] in brinjal. The trait i.e., days to 50 % flowering is important in Ashwagandha as it contributes to earliness. The estimate of the GCA effect for DTFF (Supplementary Table 1) indicated that on the pooled basis 6 lines viz., L_{12}, L_{13}, L_4, L_{10}, L_6, and L_{15} showed a significantly negative GCA effect, out of which, line L_{12} showed the highest negative significant GCA effects (-6.10) in E_1, (-7.30) in E_2, (-8.44) in E_3 and (-7.28) on a pooled basis, while among the testers, T_1 showed significantly negative GCA effect on the pooled basis as well as in two environments viz., E_1 and E_3, which signaled their superiority in the transmission of desirable genes for early flowering. Among the crosses, 2 crosses viz., L_8xT_3 (-6.74), as well as $L_{15}xT_2$ (-5.16), exhibited significantly negative SCA effects on a pooled basis. However, maximum negative and significant SCA effects were shown by crosses viz., $L_{14}xT_2$ (-10.29) in E_1, L_8xT_3 (-9.31) in E_2, (-8.83) in E_3 as well as (-6.74) on a pooled basis. Out of 15 lines, 5 lines viz., L_5, L_9, L_{14}, L_1, and L_{15} reported best for the number of primary branches based on significant GCA effect over the environments. Line L_5 showed the highest positive significant GCA effects (0.88) in

Table 1. Analysis of variance in each individual environment for different characters in Ashwagandha

SN.	Characters	Env.	Rep.	Genotypes	Parents	Crosses	Testers	Lines	L X T	Error
			[2]	[64]	[17]	[44]	[2]	[14]	[28]	[128]
1	Days to 50 per cent flowering	1	41.09	98.49**	118.43**	96.99**	173.17**	122.81**	78.64**	31.57
		2	47.82	97.30**	100.94**	94.85**	68.89	178.37**	54.95**	27.20
		3	32.19	121.28**	141.64**	119.79**	191.87**	211.45**	68.80**	25.31
2	Number of primary branches per plant	1	0.11	1.70**	2.76**	1.33**	3.14**	2.58**	0.57**	0.05
		2	0.05	1.62**	3.13**	1.07**	2.20**	2.66**	0.19**	0.05
		3	0.09	1.97**	3.50**	1.37**	2.92**	2.99**	0.44**	0.03
3	Root length	1	2.45	48.70**	44.08**	50.51**	82.81**	104.42**	21.25**	2.14
		2	7.51	42.54**	53.87**	38.67**	101.59**	77.29**	14.86**	2.60
		3	6.77*	38.09**	39.52**	39.84**	36.58**	89.17**	15.41**	2.00
4	Dry plant weight.	1	1.50	131.89**	189.00**	104.51**	246.61**	239.38**	26.93**	5.81
		2	6.55	161.90**	179.23**	150.68**	569.35**	321.40**	35.42**	8.62
		3	10.85	130.12**	135.39**	127.53**	549.87**	224.29**	48.99**	8.81
5	Dry root yield	1	0.07	2.12**	2.45**	1.97**	6.11**	4.79**	0.27**	0.04
		2	0.03	2.25**	3.15**	1.81**	5.05**	4.52**	0.22**	0.05
		3	0.13	2.07**	2.40**	1.92**	8.38**	4.29**	0.27**	0.06

*, ** Significant at 5% and 1%, respectively

Table 2. Pooled analysis of variance for different characters in Ashwagandha

SN.	Source	Df	Days to 50 % Flowering	No. of primary branches/ plant	Root Length	Dry Plant weight	Dry root yield
1.	Environment	2	10607.51**	3.12**	141.84**	46.58**	2.17**
2.	Rep./Env.	6	40.37	0.08	5.58*	6.30	0.08
3.	Genotype	64	204.17**	4.87**	111.80**	372.62**	6.06**
	Tester	2	392.84**	7.95**	210.11**	1304.16**	18.95**
	Line	14	368.27**	7.96**	241.73**	703.52**	13.01**
	L x T	28	101.31**	0.87**	36.74**	67.12**	0.51**
4.	T x E	4	75.21*	0.29**	7.99**	4.29	0.09
	L x E	28	58.00**	0.36**	6.24**	17.96**	0.21**
	L x T x E	56	50.54**	0.17**	7.39**	22.11**	0.13**
5.	Pooled Error	384	28.03	0.04	2.25	7.74	0.05

*, ** Significant at 5% and 1%, respectively

E_1 and (0.87) on a pooled basis, whereas line L_9 (0.79) in E_2 and (1.00) in E_3. The GCA for testers ranged from -0.21 (T_1) to 0.26 (T_3) on a pooled basis, out of which, T_3 was found significantly superior for primary branches on the pooled basis as well as all three environments. Among the hybrids, 14 hybrids were found significantly superior, out of which the following three hybrids viz., L_4xT_3 (0.58), L_8xT_1 (0.44) with L_7xT_1 (0.36) disclosed the highest positive as well as significant SCA effect across the locations. The highest positive as well as significant SCA effects were recorded in crosses viz., L_8xT_1 (0.79) in E_1 and (0.41) in E_2, whereas L_4xT_3 (0.74) in E_3 and (0.58) on a pooled basis, (Supplementary Table 2).

Root length is an important yield contributing factor to achieving higher root yield in Ashwagandha. The data in estimates of GCA effects for root length varied from -4.34 (L_3) to 4.42 (L_{14}) on a pooled basis. Five lines viz., L_{14}, L_{15}, L_9, L_5, and L_8 on pooled basis exhibited significant values of GCA effects in the desirable direction. The lines L_5 (5.63) in E_1, L_9 (4.23) in E_3, L_{14} (5.38) in E_2, and (4.42) on pooled basis were reported with the highest significant GCA effect in a positive direction. Among the testers, T_3 exhibited significant GCA effects in the positive direction on the pooled basis as well as in all the environments (Supplementary Table 3). Among the hybrids, 12 hybrids were found significantly superior, out of which following five hybrids viz., $L_{12}xT_1$ (3.17), L_7xT_2 (2.43), L_3xT_3 (2.10), L_8xT_1 (1.92) and $L_{14}xT_1$ (1.89) were disclosed significant and highest SCA effects in the positive direction across the environments. The significant and highest positive SCA effect was noted in hybrids viz., $L_{12}xT_1$ (5.22) in E_1 and (3.17) on a pooled basis, whereas L_7xT_2 (3.21) in E_2 as well as L_3xT_3 (3.40) in E_3 location/environment. GCA effects among lines across the environments ranged between -5.76 (L_{10}) to 9.38 (L_5). Out of the 15 lines, 6 lines viz., L_5, L_9, L_{14}, L_{15}, L_1, and L_{12} were found superior based on the GCA effect for dry plant weight. The line

L_5 showed the highest positive and significant GCA effects (9.51) in E_1, (10.15) in E_2, (8.47) in E_3 as well as (9.38) on a pooled basis, out of 3 testers, tester T_3 was found to superior based on GCA effect on the pooled basis as well as over the environments. The values of SCA effects for dry plant weight among crosses ranged from -4.18 (L_1 x T_1) to 5.42 (L_1 x T_3) on a pooled basis. Among the hybrids, 5 hybrids were found significantly superior, viz., L_1xT_3 (5.42), L_4xT_3 (4.90), L_8xT_2 (3.21), $L_{14}xT_1$ (2.76), and L_5xT_1 (2.52) with positive along with significant SCA effects across the locations. The highest significant SCA effects in desired direction were recorded in hybrids viz., L_{10} x T_2 (5.06) in E_1, L_4 x T_3 (6.05) in E_2, L_1 x T_3 (9.19) in E_3 as well as over the environments (5.42), (Supplementary Table 4). The range of GCA effects among lines varied from -0.72 (L_{11}) to 1.31 (L_9) on the pooled basis and five lines viz., L_9, L_5, L_{14}, L_{15}, and L_1 were reported significantly superior for dry root yield. Line L_9 exhibited the highest significantly positive GCA effects (1.28) in E_2 and (1.31) on a pooled basis, while it was shown by L_5 (1.49) in E_1 and L_{14} (1.22) in the E_3 environment. The GCA for testers ranged from -0.38 (T_1) to 0.37 (T_3) on a pooled basis, out of which tester T_3 was found significantly superior for dry root yield. The estimates of SCA effects for dry root yield among crosses ranged from -0.40 (L_1xT_1 and L_8xT_3) to 0.50 (L_1xT_3) on a pooled basis. Among the crosses, 6 hybrids were found significantly superior viz., L_1xT_3 (0.50), $L_{15}xT_2$ (0.33), L_8xT_1 (0.29), L_4xT_3 (0.27), $L_{10}xT_1$ (0.24) and $L_{12}xT_1$ (0.22) with significantly positive SCA effect across the locations. The maximum value of positively significant SCA effect was recorded in crosses viz., L_1xT_3 (0.75) in E_1, (0.60) in E_3, and (0.50) on a pooled basis, while hybrid $L_{13}xT_1$ (0.37) in E2 environment, (Supplementary Table 5). Parents classified based on significant GCA effects (Supplementary Table 6), as well as crosses showed significant SCA effects in desirable directions over the environments (Supplementary Table 7).

Conclusion

Based on the experimental trial, the following parental material were identified as superior general combiners over the environments for the trait of interest viz., 6 lines (L_{12}, L_{13}, L_4, L_{10}, L_6, and L_{15}) and tester (T_1) for early flowering; 5 lines (L_5, L_9, L_{14}, L_1, L_{15}) and tester (T_3) for the number of primary branches per plant; 5 lines (L_{14}, L_{15}, L_9, L_5, and L_8) and tester (T_3) for root length; 6 lines (L_5, L_9, L_{14}, L_{15}, L_1 and L_{12}) and tester (T_3) for dry plant weight; 5 lines (L_9, L_5, L_{14}, L_{15}, and L_1) and tester (T_3) for dry root yield. Whereas among the crosses the following crosses were found excellent specific combiner across the locations viz., two hybrids (L_8xT_3 and $L_{15}xT_2$) for earliness; while 14 crosses for primary branches per plant, out of which the following three hybrids viz., L_4xT_3, L_8xT_1 and L_7xT_1 with highest positive significant SCA effects; 12 crosses, out of which the following five crosses viz., $L_{12}xT_1$, L_7xT_2, L_3xT_3, L_8xT_1, and $L_{14}xT_1$ disclosed highest positively significant SCA effect for root length; 5 hybrids viz., L_1xT_3, L_4xT_3, L_8xT_2, $L_{14}xT_1$ and L_5xT_1 for dry plant weight; for dry root yield, the hybrid combinations viz., L_1xT_3, $L_{15}xT_2$, L_8xT_1, L_4xT_3, $L_{10}xT_1$, and $L_{12}xT_1$ were identified superior based on SCA effects in a positive direction.

The overall picture based on GCA effects revealed that among the parents, four lines viz., L_9, L_5, L_{14}, L_{15}, and one tester T_3 have been reported as an excellent general combiner for root yield at dry basis as well as for most of the yield attributing characters over the environments. On the basis of significant and positive SCA effect over the environments, the crosses viz., L_8xT_1, $L_{10}xT_1$, $L_{12}xT_1$, $L_{15}xT_2$, L_1xT_3, and L_4xT_3 were identified superior for dry root yield per plant.

Conflict of interest

The authors declare no conflict of interest.

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