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Research Article

Deciphering the combining ability and gene action for root yield and related traits in ashwagandha [*Withania somnifera* (L.) Dunal]

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Abstract
Genetics and crop improvement aspects of medicinal plants in Ashwagandha should be taken consideration to meet the accelerating pharmaceutical demands. For a better understanding of the genetic basis of root yield and related traits, a 9 × 9 diallel analysis excluding the reciprocals was carried out using SKA 27, SKA 11, SKA 6, SKA 24, SKA 10, SKA 26, JA 20, JA 134 and AWS 1 as parents. The σ^2gca/σ^2sca denotes that the majority of the traits considered except plant height and the number of secondary branches were governed by non-additive gene action. The fresh root yield was governed by almost equal proportions of additive and non-additive gene actions with a slight increment in additive proportion. Even though none of the parents was a good general combiner for all the traits considered, SKA 27 and SKA 11 appeared to be the best general combiners for root traits. The crosses JA 20 × AWS 1, SKA 24 × SKA 26 and JA 134 ×AWS 1 were found to be appropriate for the heterosis breeding on the basis of the estimates of SCA effects.

Key words
Ashwagandha, combining ability, GCA, SCA, gene action, half-diallel

INTRODUCTION

India is one of the twelve mega biodiversity centres in the world is gifted with the richness of medicinal plants. Ashwagandha [*Withania somnifera* (L.) Dunal] is one among them and is cultivated across Madhya Pradesh, Rajasthan, Gujarat, Punjab and Uttar Pradesh (Joshi *et al.,* 2014). Ashwagandha cultivation is a better choice for lands that is not suitable for food crops and this crop thrives well in a dry climate and low winter temperatures.

This solanaceous crop is globally represented by twenty-six species. Among that India hosts two species, *W. somnifera* and the wild *W. coagulans* (Mir *et al.*, 2012). A third species namely *W. ashwagandha* is also reported newly. Botanically ashwagandha is a short, woody, erect perennial shrub with tomentose branches which looks more like an eggplant. Leaves are simple, dull green, elliptical and usually up to ten centimetres long. Flowers are small, inconspicuous, greenish or lurid-yellow, axillary and the inflorescence is umbellate cyme. Fruit type is a berry and globous, yellow/orange-red/red in colour, enclosed in persistent calyx when mature. One berry contains an average of thirty to forty seeds. Seeds are yellow and reniform. The plant flowers and set fruits from November to February.

Ashwagandha can be considered as a wonder medicinal plant as its almost all parts are useful in diverse medicinal systems like Ayurveda, Siddha and Unani. This herb, especially the root powder possesses therapeutic value against a number of alignments such as arthritis, asthma, mental diseases, inflammation, rheumatism, infections, fever, anxiety, tuberculosis, male sexual disorders and even cancer.

The growing demand for the ashwagandha root powder in the drug industry necessitating the genetic analysis of this crop. Combining ability analysis and estimation of the nature of gene action governed by various traits is one such genetic tool which helps the breeder to design a productive breeding plan. Hence, a study was conducted to reveal the gene action and combing ability of root yield and related traits in ashwagandha using a 9 × 9 half-diallel analysis in the North-Gujarat region.

MATERIALS AND METHODS

The experimental material for the genetic analysis was generated by crossing the nine selected lines in diallel mating design excluding reciprocals at Botanical Garden, Department of Genetics and Plant Breeding, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat during *Rabi* 2018-19. The parental lines include SKA 27, SKA 11, SKA 6, SKA 24, SKA 10, JA 20, JA 134 and AWS 1 (check) (**Table 1**). The crosses were made by hand emasculation and pollination in order to generate thirty-six hybrids. The matured berries of the crosses were collected separately and the seeds were stored in proper conditions after drying and threshing. The hybrids generated along with their nine parental lines were evaluated in Randomized Block Design with three replications during *Rabi* 2019-20 at Agronomy Instructional Farm, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. A row length of 4 m and a spacing of 30 ×10 cm were adopted in the field.

During the evaluation in *Rabi* 2019-20, observations were recorded for different quantitative traits on randomly selected five competitive plants in each replication**.** The traits under study comprises the days to flowering, plant height (cm), the number of primary branches, the number of secondary branches, the number of berries per plant, the number of seeds per berry, root length (cm), root girth (cm), fresh root yield (g) and dry root yield (g). The data recorded were subjected to analysis of variance for combining ability according to the procedure developed by Griffing (1956) method II and model I. The test for significance of general and specific combing ability was carried out by using F-test. The linear mathematical model the combing ability analysis fixed effect (model I) is as follows:

$$Yijk= \mu + g_i + g_j + S_{ij} + 1/bc\ \Sigma k \Sigma_i e_{ijkl}$$

Where, $\mu$is the population mean, g_i and g_j are the general combining ability effects, S_{ij} is the specific combining ability effects of a cross between the i^{th} and j^{th} parents and e_{ijkl} is the error fraction. General combining ability effect for parents and specific combining effect for crosses were estimated as per the formulae given below:

$$gi = [1/p+2]\ [(Yi. + yij) – (2/p)\ Y..]$$
$$Sij = yij – [(1/p+2)]\ [Yi + yii + Y.j + yjj] + [2/(p+1)\ (p+2)]\ Y..$$

Where, gi is the gca effect of i^{th} parent and Sij is the sca effect of ij^{th} cross. The data obtained from this investigation were analysed through TNAUSTAT statistical package.

RESULTS AND DISCUSSION

The combining ability refers to the capacity of genotype to transmit a superior performance to its crosses and can serve as a useful guide for the identification of the best parents and cross combinations in crop breeding.

Analysis of variance for combining ability partitioned the total variance into the variance due to general combining ability (GCA) of parents and specific combining ability

Table 1. Origin and pedigree of selected parental lines used for analysis

S. No.	Parents	Pedigree/Source
1	SKA 27	IPS from MPAS 7,S. K. Nagar
2	SKA 11	IPS from MWS 101,S. K. Nagar
3	SKA 6	IPS from MWS 322,S. K. Nagar
4	SKA 24	IPS from MPAS 3,S. K. Nagar
5	SKA 10	IPS from MWS 309,S. K. Nagar
6	SKA 26	IPS from MPAS 5,S. K. Nagar
7	JA 20	Mandsaur, Madhya Pradesh
8	JA 134	Pedigree selection from JA 20 and wild types Mandsaur, Madhya Pradesh
9	AWS 1	AAU, Gujarat

Table 2. Analysis of variance and the mean square estimates and mode of gene action of root yield and related traits in Ashwagandha

Sources of variation	d. f	DF	PH	PB	SB	BPP	SPB	RL	RG	FRY	DRY
GCA	8	40.30**	3948.14**	1000.47**	1623.94**	5.55**	23091.67**	23736.39**	48.60**	306.92**	1.26**
SCA	36	8.98**	231.32**	135.20**	72.18**	2.26**	8168.68**	4418.73**	48.12**	26.45**	0.54**
Error	88	3.13	19.38	8.36	2.46	0.11	34.74	9.07	1.99	0.42	0.04
σ^2 gca		3.37	357.16	90.19	147.40	0.49	2096.08	2157.02	4.23	27.86	0.11
σ^2 sca		5.84	211.93	126.83	69.72	2.14	8133.94	4409.65	46.13	26.03	0.50
σ^2gca/σ^2sca		0.57	1.68	0.71	2.11	0.23	0.25	0.48	0.09	1.07	0.22
Gene action*		N-A	A	N-A	A	N-A	N-A	N-A	N-A	A	N-A

** $P \leq 0.01$

N-A: Non-additive gene action, A: Additive gene action

DF: Days to flowering PH: Plant height PB: Number of primary branches SB: Number of secondary branches BPP: Number of berries per plant SPB: Number of seeds per berry RL: Root length RG: Root girth FRY: Fresh root weight DRY: Dry root **yield**

(SCA) of the crosses (**Table 2**). The mean sum of squares due to GCA as well as SCA were highly significant ($P \leq 0.01$) for all the traits under study. This reveals that both additive and non-additive components of heritable variance are involved in the inheritance of all these traits. Moreover, leaves a hint towards the significant differences among the GCA and SCA effects. The predominance of non-additive genetic variance in all the traits except plant height, the number of primary branches and fresh root yield were evident from the higher magnitude of SCA variance. The SCA variance is proved to be more important than that of GCA for various yield attributing traits in ashwagandha from the experiments conducted by Dhuri (2016).

Good general combiner is the parent with significant GCA effects in a desirable direction and can serve as a better parent of choice. The estimates of GCA effects from this study pointed out that none of the parents was a good general combiner for all the yield attributing traits (**Table 3**). Although, SKA 27 found to be a good combiner for the majority of the traits *viz.,* days to flowering, plant

Table 3. General combining ability effects nine parents for root yield and related traits in Ashwagandha

Parents	DF	PH	PB	SB	BPP	SPB	RL	RG	FRY	DRY
SKA 27	-4.60** (G)	-48.98** (G)	23.17** (G)	31.57** (G)	-1.70** (P)	-117.14** (P)	117.83** (G)	4.36** (G)	13.78** (G)	0.17 ** (G)
SKA 11	-0.87 (A)	-5.56** (G)	5.36** (G)	3.22** (G)	0.49** (G)	16.36** (G)	16.42** (G)	1.52** (G)	0.98** (G)	-0.30 ** (P)
SKA 6	1.61 (A)	6.89** (P)	-8.52** (P)	-4.88** (P)	0.03 (A)	-7.44** (P)	-29.96** (P)	0.78 (A)	-1.57** (P)	-0.14 * (P)
SKA 24	-0.01 (A)	8.53** (P)	-5.70** (P)	-5.16** (P)	-0.17 (A)	1.80 (A)	-18.49** (P)	0.15 (A)	-1.86** (P)	0.38 ** (G)
SKA10	1.65** (P)	9.20** (P)	-4.44** (P)	-5.63** (P)	0.39** (G)	25.08** (G)	-32.39** (P)	-1.69** (P)	-2.58** (P)	0.58 ** (G)
SKA 26	0.34 (A)	4.47** (P)	-4.89 (A)	-5.02** (P)	0.00 (A)	25.50** (G)	-6.18** (P)	-0.49 (A)	-2.28** (P)	0.08 (A)
JA 20	1.32** (P)	8.17** (P)	-3.21** (P)	-4.55** (P)	0.03 (A)	6.64** (G)	-10.67** (P)	-2.98** (P)	-2.69** (P)	-0.08 (A)
JA 134	0.06 (A)	8.08** (P)	-1.76** (P)	-4.99** (P)	0.10 (A)	14.30** (G)	-17.87** (P)	-1.02* (P)	-2.27** (P)	-0.49 ** (P)
AWS 1	0.48 (A)	9.20** (P)	-0.01 (A)	-4.55** (P)	0.84 (A)	34.89** (G)	-18.69** (P)	-0.63 (A)	-1.50** (P)	-0.20 ** (P)
S.E. (g_i)	0.50	1.25	0.82	0.44	0.09	1.67	0.85	0.40	0.18	0.06

*$P \leq 0.05$, ** $P \leq 0.01$

DF: Days to flowering PH: Plant height PB: Number of primary branches SB: Number of secondary branches BPP: Number of berries per plant SPB: Number of seeds per berry RL: Root length RG: Root girth FRY: Fresh root weight DRY: Dry root yield

[Letters in the parenthesis indicates class of parent according to respective gca effects: G: Parent having significant GCA effects in desired direction (Good general combiner), A: Parent having either positive or negative but non-significant GCA effects (Average general combiner), P: Parent having significant GCA effect in undesired direction (Poor general combiner)]

Table 4. Specific combining ability effects of thirty-six hybrids for root yield and related traits in Ashwagandha

Crosses	DF	PH	PB	SB	BPP	SPB	RL	RG	FRY	DRY
P1 × P2	1.57 *	1.50	8.22 **	11.12 **	0.74 **	-27.17 **	205.30 **	0.75	10.95 **	-0.16
P1 × P3	-1.70 *	-12.91 **	-0.89	0.96	-0.87 **	-6.92 **	-79.85 **	2.92 **	3.89 **	-0.44 **
P1 × P4	0.53	-13.97 **	1.28	11.18 **	0.57 **	-11.33 **	28.12 **	13.72 **	11.57 **	0.22 **
P1 × P5	-0.92	-11.98 **	5.02 **	-0.55	-0.46 **	-37.47 **	-90.35 **	3.45 **	-1.18 **	0.98 **
P1 × P6	-1.48 *	-8.89 **	11.47 **	8.22 **	-0.31 *	-36.73 **	161.56 **	3.96 **	0.67 **	0.20 *
P1 × P7	-2.34 **	-14.27 **	15.79 **	14.24 **	0.06	-17.16 **	115.09 **	0.75	-2.40 **	-0.50 **
P1 × P8	-1.44 *	-13.88 **	10.35 **	9.53 **	-1.27 **	-26.46 **	25.55 **	14.96 **	2.66 **	-0.39 **
P1 × P9	0.03	-11.95 **	3.26 **	11.66 **	-0.65 **	-46.52 **	55.74 **	-3.89 **	12.50 **	-0.82 **
P2 × P3	-2.30 **	-6.72 **	2.55 *	1.76 **	0.40 **	4.17	-26.47 **	6.06 **	-4.25 **	-0.30 **
P2 × P4	0.57	16.80 **	-10.13 **	-8.13 **	0.78 **	30.14 **	-27.75 **	-4.11 **	-4.09 **	0.96 **
P2 × P5	-1.02	6.14 **	-10.27 **	-7.16 **	0.82 **	100.06 **	-17.21 **	-5.80 **	-2.98 **	-1.04 **
P2 × P6	3.22 **	26.53 **	1.34	-6.27 **	2.16 **	175.83 **	-47.82 **	4.76 **	-2.68 **	0.33 **
P2 × P7	4.60 **	3.16	-5.02 **	-9.27 **	2.61 **	115.97 **	-49.18 **	5.02 **	-0.64 *	1.13 **
P2 × P8	1.34 *	20.92 **	-5.88 **	-8.63 **	-0.79 **	-13.39 **	-33.46 **	-6.41 **	-2.69 **	-0.03
P2 × P9	-1.51 *	21.13 **	-23.53 **	-8.40 **	-0.74 **	-63.62 **	-33.31 **	-3.03 **	-3.28 **	0.59 **
P3 × P4	2.80 **	12.35 **	-4.51 **	-0.57	0.46 **	-42.27 **	19.85 **	-6.90 **	-0.31	0.31 **
P3 × P5	-1.58 *	10.02 **	11.69 **	1.94 **	0.67 **	-35.61 **	30.91 **	4.70 **	0.58 *	-0.29 **
P3 × P6	5.90 **	-8.93 **	-2.93 **	-1.44 *	-0.05	10.76 **	6.49 **	-2.37 **	1.29 **	0.24 **
P3 × P7	1.30	12.05 **	-0.68	-0.74	1.36 **	61.93 **	4.78 **	-1.41 *	0.23	0.17 *
P3 × P8	-3.96 **	4.81 **	-0.70	0.12	-0.67 **	-48.89 **	1.18	-6.43 **	1.19 **	-0.26 **
P3 × P9	-4.78 **	-5.99 **	5.64 **	-0.99	0.93 **	83.34 **	8.16 **	7.08 **	-1.78 **	-0.35 **
P4 × P5	-2.59 **	-1.29	12.21 **	0.11	0.13	-12.28 **	5.10 **	-1.98 **	-1.79 **	-0.59 **
P4 × P6	3.32 **	-0.90	13.71 **	1.36 *	1.29 **	47.02 **	-12.16 **	-3.81 **	0.83 **	0.26 **
P4 × P7	-0.55	-0.60	4.36 **	-0.92	-0.33 *	71.76 **	-10.43 **	1.39 *	0.06	-0.21 *
P4 × P8	-1.27	0.50	-18.82 **	-1.49 *	-1.97 **	36.90 **	4.77 **	-0.63	-1.72 **	-0.90 **
P4 × P9	3.08 **	-12.96 **	6.14 **	0.88	1.72 **	24.67 **	-2.95 *	14.41 **	0.35	-0.23 **
P5 × P6	0.03	-11.23 **	-11.54 **	-0.55	-1.00 **	-64.42 **	-0.40	-4.32 **	0.75 **	0.51 **
P5 × P7	6.25 **	-0.93	3.26 **	3.68 **	0.18	50.05 **	9.75 **	-1.50 **	2.44 **	-0.79 **
P5 × P8	1.44 *	-5.84 **	11.29 **	0.74	1.36 **	207.43 **	21.18 **	1.78 **	1.87 **	-0.53 **
P5 × P9	-3.58 **	7.04 **	0.94	-0.59	-0.80 **	-81.77 **	6.66 **	-2.09 **	-2.32 **	0.32 **
P6 × P7	-2.14 **	12.79 **	-8.49 **	-1.33 *	-1.68 **	-86.58 **	-27.57 **	-6.04 **	-0.84 **	-0.29 **
P6 × P8	-1.72 *	-9.78 **	10.27 **	1.11	0.46 **	-31.44 **	-8.97 **	-6.26 **	-0.78 **	-0.32 **
P6 × P9	-2.00 **	6.76 **	10.84 **	2.41 **	3.30 **	241.25 **	-15.86 **	-7.01 **	0.11	-0.92 **
P7 × P8	-0.63	-3.47 *	13.13 **	0.26	2.41 **	19.73 **	-6.44 **	-0.70	0.93 **	-0.29 **
P7 × P9	0.19	-9.60 **	2.86 *	-2.46 **	-2.01 **	-68.96 **	-10.63 **	3.45 **	0.14	0.22 **
P8 × P9	5.60 **	0.83	-5.92 **	-0.29	1.32 **	-61.69 **	3.47 **	-0.25	-1.03 **	2.65 **
S. E. (S_{ij}) ±	0.67	1.67	1.10	0.59	0.12	2.24	1.14	0.53	0.24	0.08

*P ≤ 0.05, ** P ≤ 0.01

DF: Days to flowering PH: Plant height PB: Number of primary branches SB: Number of secondary branches BPP: Number of berries per plant SPB: Number of seeds per berry RL: Root length RG: Root girth FRY: Fresh root weight DRY: Dry root yield

height , the number of primary branches, the number of secondary branches, root length , root girth, fresh root yield and dry root yield . While considering the root traits, the parent SKA 11 appeared to be a good combiner other than SKA 27. The check variety AWS 1 was a good combiner for the number of seeds per berry and an average combiner for days to flowering and the number of berries per plant.

Significant specific combiners could be the best cross combinations for exploiting hybrid vigour. Majority of the crosses showed significant SCA effects. However, none of the crosses exhibited positive significance for SCA effects in all the traits considered (**Table 4**). The values of SCA effects revealed the crosses SKA 27 × SKA 24, SKA 27 × SKA 26, SKA 27 × JA 134 and SKA 10 × JA 134 were the best possible combinations for the improvement of root length , root girth and fresh root yield . The hybrids with significant SCA effects were not only resulted from the good general combiners but also possible by other combinations. The best specific combiners were combinations of good × good, good × average, good × poor, average × average, poor × average and poor × poor general combiners (**Table 3**). Epistatic effects or more specifically the dominance × dominance type of epistatic effect could be the possible reason behind the greater SCA effects exhibited by poor × poor combinations. Sahu (2015) Dhuri (2016) and Tirkey (2016) also reported the best parents and cross combinations for the improvement of one or more yield attributing traits in ashwagandha.

As explained by Wright (1935) gene action is of two types *viz.,* additive and non-additive gene action. The relative proportion of GCA and SCA variance indicates the type of gene action governed by the trait. The predominance of GCA variance specifies the additive gene action. Whereas, SCA variance specifies the non-additive gene action. The nature of gene action governed by various traits provides a clear-cut idea about the choice of parents and the procedures that to be followed in a breeding programme.

The variance due to GCA was less than that of the SCA for the majority of the traits except for plant height, the number of secondary branches and fresh root yield (**Table 2**). The traits like days to flowering, the number of primary branches, the number of berries per plant, the number of seeds per berry, root length , root girth and dry root yield appeared to be governed by non-additive gene action which is in agreement with Dhuri (2016). As the ratio σ^2gca/σ^2sca was more than unity for plant height and a number of secondary branches, these were proven to be governed by additive gene action. For the fresh root yield trait, the ratio σ^2gca/σ^2sca is slightly higher than unity (1.07). Even though there was a scant increment in the GCA variance, an adequate amount of non-additive fraction of gene action was also visible. Gami *et al.* (2015) reported the preponderance of additive gene action for plant height which is comparable with the present results.

In conclusion, the results derived from this investigation provide an insight into the breeding approaches that can be followed for genetic improvement in Ashwagandha. The fresh root yield of the plant holds paramount importance and the analysis revealed the additive gene action of the trait with a nearly equal proportion of non-additive gene action. Both the selection and heterosis breeding can markedly improve the fresh root yield in ashwagandha. The parent SKA 27 and SKA 11 are found to be the best general combiners. Hence, could be the right choice of parents for a hybridization programme. The presence of additive gene action broadens the success in early generation testing and transgressive breeding. The crosses JA 20 × AWS 1, SKA 24 × SKA 26 and JA 134 × AWS 1 are proper cross combinations to exploit the hybrid vigour referring to the estimates of SCA effects. Other yield attributing traits, plant height and a number of seeds per berry are governed by additive gene action and are possible to improve by selection. In the case of dry root yield, the cross JA 134 × AWS 1 can be utilized for hybrid development because of good specific combining ability. The high SCA effects displayed by a poor general combiner in combination with a good general combiner may because of its epistatic effects.

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