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Development of novel varieties of Ashwagandha (Withania somnifera L.) for high root yield and its medicinal quality parameters

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Development of novel varieties of Ashwagandha (*Withania somnifera* L.) for high root yield and its medicinal quality parameters

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ABSTRACT

Ashwagandha (*Withania somnifera*) one of the most important medicinal plants used as tonic for vitality and immunity. Present long term investigation (2015-23) was carried out by using the ten novel genotypes of ashwagandha + four checks to develop new high yielding varieties for root yield and its medicinal quality under arid and semi-arid regions of India. Analysis of variance revealed that the genotypes differed significantly. Over the years and locations, HWS 8-18 (593.53kg/ha) and HWS 12-12 (555.54kg/ha) were high dry root yielder as compared to best check, AWS 1 (551.19kg/ha). The average highest dry root yield of ashwagandha was recorded at Raipur (959.34kg/ha), followed by Coimbator (558.00kg/ha) and Mandsaur (473.54kg/ha). HWS 8-18 dry root yield was more than 10% higher as compared to best check ASW-1 at National level. HWS 8-18 was also superior in Withanolide-A (0.396 mg/g) content as compared to best check GAA-1 (0.379 mg/g) at National level. HWS 12-12 was more than 10% superior in quality parameters and also good in dry root yield. Therefore, HWS 8-18 and HWS 12-12 were also tested in large scale trials and found superior over the check thus recommended for commercial cultivation. As both the varieties are diverse in nature therefore, hybridization (HWS 8-18 x HWS 12-12) is suggested to combine high root yield and quality parameters for further development of new variety in future.

Keywords: Ashwagandha (*Withania somnirefa*), root yield, alkaloids, quality, characterization

Introduction

Since ancient times, Ashwagandha (*Withania somnirefa* (L.) Dunal (2n=48) is considered as one of the best medicinal herb utilzed in Ayurvedic and Unani medicines (Mishra, 2014; Behl et al., 2022). Its roots have similar bio-active compounds to the roots of ginseng , that's why people generally call it, Indian ginseng (Arya et al, 2025a). Its english name is winter cherry or gooseberry. It falls under the family Solanaceae. It was originated in Mediterranean area of North Africa and Central and North-Western part of India, more particularly the Nagour and adjoining area in Rajasthan. The sub-tropical dry climate region is found best for its cultivation. In India, *W. somnirefa* is grown in rained areas of (MP, Rajasthan, Punjab, Maharashtra, Gujarat, and UP) in about 10780 ha, with the production of 8,429 tones dry roots (Saran and Das, 2023). India is one of leading country to export the dry roots / roots powder. The major buyers of ashwagandha roots are USA, Australia and China (Anonymous, 2016).

Ashwagandha is the main ayurvedic tonic to enhance strength, vitality and longevity for the people of all age (Singh et al., 2010). It activates the human immune cells, phagocytes and lymphocytes, which ultimately reduces stress and also promotes wellness (Singh et al., 2001; Singh et al., 2003). It is also served as a good liver-tonic, astringent, as well as anti-inflammatory drug. It is to treat asthma, bronchitis, insomnia, emaciation, dementia, Parkinson's disease and neurological disorders (Arya et al., 2020). The significance of this ancient medicinal herb now increased several folds after COVID 19 (Arya et al., 2022a). It emerged out as an immunity-booster herb (Koli and Arya, 2021; Koli 2022). Moreover, its roots poweder / capsules were found effective to cure inflammation of joints, and rheumatic pains, (Al-Hindwani et al., 1992), ulcers, female disorders, hiccups, coughs and colds, leprosy, nervous disorders, as a sedative, etc. (Korduru et al., 2010, Arya et al., 2025b).

In ashwagandha roots, the total alkaloid content ranged from 0.13 to 0.31%, however, it may to high up to 4.3% (Anonymous, 1982 & 2007). The major bio-molecules found in this herb are withanolides (withaferin-A and withanolide-D) and steroidal-lactones responsible for medicinal properties (Sharma et al., 2011). According to Khare (2007), Indian chemo-type, 12 withanolides, and five unknown molecules, glycosides, glucose, several amino acids, chlorogenic acid, flavonoids and tannins, are found in the herb. Chopra (1994) reported that the ashwagandha leaves are utilized in Ayurvedic and Unani medicines to cure tubercular glands and tumors, because of its ability as anti-bacterial/fungal, and anti-tumor activities (Devi et al., 1993). It increases body power, and also relieves mental exhaustion, anxiety, depression, as well as promotes sleep with mental peace (Anonymous, 2013).

In ancient era, the medicinal herbs were found readily available in nearby forest, but with the passage of time forest area reduced very drastically which ultimately decreased their availability. Therefore, an urgent need is felt to develop the high yielding cultivars of medicinal herbs for the ayurvedic doctors and for the drug manufactures (Arya et al., 2025a). For the development of new high yielding cultivars of ashwagandha, germplasm with significant variability for root and its attributes is required. In India, huge genetic diversity have been reported in ashwagandha by several researchers (Yadav et al., 2008; Reddy et al., 2012, Koli et al., 2022a; Arya et al., 2025b), and very less research findings regarding to its genetic improvement are available. Keeping the importance of this medicinal herb in view, the present study was done with objective to identify the suitable high yielding genotypes of ashwagandha for commercial cultivation.

Materials and methods

Evaluation of genotypes

In this present investigation, at different locations, on basis of three years performance in station trials during 2010-11, 2011-12 and 2013-14, only 10 genotypes of ashwagandha (*Withania somnirefa* L.), viz. HWS 8-18 and HWS 12-12., I-WS-1, IWS 3, IWS32, MWS130, MWS90-14, UWS10, UWS23 and UWS37 were identified promising and recommended to be tested in multi-location trials. In Multilocation Trial, the best selected genotypes viz., HWS 8-18 and HWS 12-12, I-WS-1, IWS 3, IWS32, MWS130, MWS90-14, UWS10, UWS23 and UWS37 with check varieties, JA134, AWS-1, RVA100 and JA20 were evaluated at several locations (Fig. 1), i.e. Akola, Anand, DMAPR, IIHR, Hisar, Mandsaur, Raipur and Udaipur during 2015-16, 2016-17, 2017-18 & 2018-19, respectively, to develop the ashwagandha best high dry root yielding varieties for the semi-arid and arid climate. The experiments were planted in randomized block design with three replications. Each genotype was accommodated in six rows in plot of 4.0 m x 1.8 m having row-to-row spacing 30 cm and plant-to-plant 10 cm. All the recommended agronomic practices were followed to harvest a good crop (Patra et al., 2004). The observations were recorded on five competitive plants for plant height (cm), dry root yield (kg/ha) and its quality parameters. The roots of all the genotyeps were dug 200 DAS, and after washing roots were dried up to 8% moisture content.

During 2019-20, the seeds of two most promising genotypes viz., HWS 8-18 and HWS 12-12 were multiplied, and during 2020-21 and 2022-23, adoption trials with all the recommended package of practices were conducted with large plot size (50 m x 30 m) at five locations, i.e. Rewari, Mahendergarh, Bhiwani and Fatehabad and dry root (kg/ha) was recorded.

Statistical analysis

The data analysis was done by using the online Statistical Software, available on Website of CCS HAU, Hisar, as suggested by Sheoran et al. (1998). The pooled mean of all the traits across the locations were utilized for comparison.

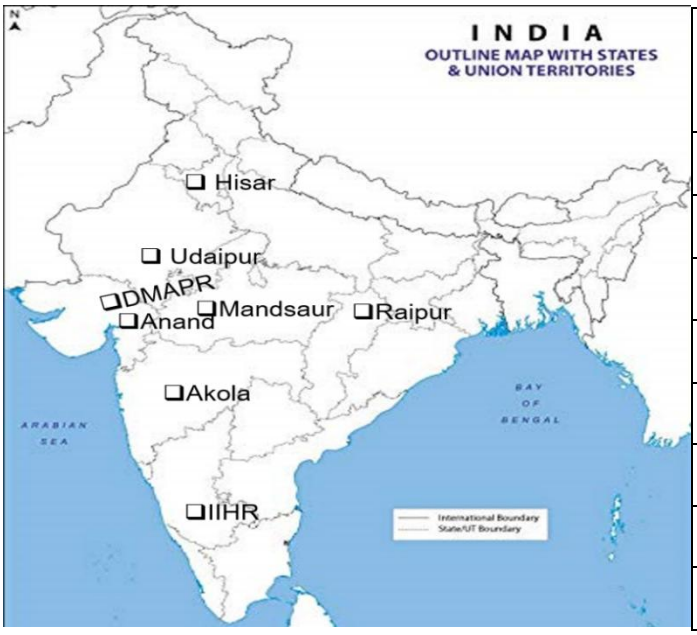
Extraction of total alkaloids and withanolides

The oven dried roots (at temperature 40-45°C) of ashwagandha were used for the estimation of total root alkaloid and different withanolides as suggested by Khajuria et al. (2004). First of all the dried roots were crushed into powder. For estimation, five gram powder of each genotype was taken separately with methanol (50 mL^3) in the test tube, and after filtration, evaporation process is carried out by using the Rotary evaporator (vacuum 337 mbar at 40°C). The remaining crude substance was then de-fatted with N-hexane (10 mL^3) and after that with 1% sulphuric acid (50 mL). The sulphuric acid solution was extracted with diethyl ether (30 mL^3) in a separating funnel. The two different layers observed and separated easily. The upper-layer containing ether soluble substance was dried over anhydrous Na_2SO_4, filtered, evaporated, and the crude withanolide content was calculated. The lower layer (acid soluble) was basified with ammonia and then extracted with $CHCl_3$ (30 mL^3) to separate two distinct layers (lower soluble layer and upper insoluble $CHCl_3$ layer). Using anhydrous Na_2SO_4, soluble layer was dried, filtered, and

evaporated on a rotary evaporator (vacuum 474 mbar for at 40C). This was estimated as the total alkaloid content and the upper layer was discarded.

Extraction of withanolides

For the extraction of withanolide A, and withastramonolide from the roots, reverse HPLC analysis was carried out at the analytical chemistry division of the institute. LC-10A gradient HPLC with two LC-10AD pumps controlled by a CBM-10 interface module, a model 7725 Imanual injector valve, a 20 mL sample loop, and a multi-dimensional UV-visible detector, SPD-10 was utilized for chemical analysis. For testing the peak purity of the chemical compound, a SPDM10AVP photodiode array detector was used. The solvents were filtered through a filtering system (Millipore, Bedford, MA, USA). According to HPLC method (Scartezzini et al., 2007), the genotypes of ashawagandha were analyzed with slight modifications (Srivastava et al., 2008) using a Waters Spherisorb C18 analytical column (4.6 mm 250 mm, 10 mm ODS); mobile-phase acetonitrile; 0.1% tri-flouro acetic acid in water (40:60; flow rate 1.0 mL/min; column temperature 26C, detector wavelength 220 nm.



Multi-locations trial sites	Latitude	Longitude	Altitude (above sea level)
Hisar	29.17° N	75.77° E	215.20 m
Udaipur	24.58° N	73.70° E	580.17m
DMAPR	22.61°N	72.92° E	42.00m
Mandsaur	24.06° N	75.09° E	442.00 m
Anand	22.56° N	72.95° E	45.11 m
Raipur	21.23° N	81.70° E	295.00m
Akola	20.43° N	77.00° E	307.42 m
IIHR	13.14° N	77.49° E	895.00m

Fig. 1. Multi-location trials testing sites all over India

Results

In the present investigation, several breeders working all over the country started the pre-breeding evaluation work on the newly collected genotypes of ashwagandha, to identify most desirable elite genotypes in their local station trials from 2010 to 2014 for further testing in multi-location trials as well as utilization in crossing program. Only identified 10 superior best performing elite genotypes viz. HWS12-12, HWS8-18, I-WS-1, IWS 3, IWS32, MWS130, MWS90-14, UWS10, UWS23 and UWS37 for high dry root yield and its contributing. The required care was taken to identify and select the most desirable plants from the germplasm of lines developed by them. These 10 novel genotypes severed as the material for the present investigation for multi-location testing.

Performance in Coordinated Trials

The newly developed and identified as the best performing 10 genotypes viz. HWS12-12, HWS8-18, I-WS-1, IWS 3, IWS32, MWS130, MWS90-14, UWS10, UWS23 and UWS37 (Fig. 1) alongwith checks (AWS-1, JA134, JA20 and RVA100) were evaluated in coordinated trails at several locations viz. Akola, Anand, DMAPR, IIHR, Hisar, Mandsaur, Raipur and Udaipur in IVT during 2015-16 AVT-I during 2016-17 and AVT-II during 2017-18 & 2018-19. During this period, observations were recorded on yield and its contributing traits, quality parameters and reaction against insect pest and diseases.

Plant height

The results of present investigation (Table 1) revealed that the performance of different genotypes varied from over the years and locations due to differences in soil and climatic conditions as well as agronomic management. During 2015-16, the average plant height over the locations revealed that the genotype HWS12-12was recorded tallest with plant height 66.46 cm followed by HWS8-18 (64.84 cm), UWS37 (59.98cm), HB-12-42 (22.81 q/ha), AWS-1 (59.20 cm) in plain zone at national level. The average plant height was recorded maximum 87.23cm at Anand, followed by DMAPR (68.37cm) Raipur (62.40cm), and Hisar (56.59cm). Likewise, during 2016-17, the average plant height (cm) over the locations revealed that the genotype HWS8-18 was recorded maximum plant height (63.85cm) followed by HWS12-12 (61.51cm), I-WS-1 (59.22cm), UWS23 (59.05cm), UWS37 (58.92cm) in plain zone at national level (Table 2). The average plant height was recorded maximum 95.64cm at Coimbatore, followed by Raipur (80.18cm), DMAPR (59.43cm) and Anand (57.09cm). Likewise, during 2017-18, the average plant height (cm) over the locations revealed that the genotype MWS90-14 was recorded maximum plant height (66.46cm) followed by HWS8-18 (63.29cm), HWS12-12 (62.33cm), UWS37 (62.16cm) and AWS-1© (61.63cm) in plain zone at national level (Table 3). The average plant height was recorded maximum (87.61cm) at Anand followed by Raipur (69.15cm), Coimbatore (64.05cm), Akola (62.00cm).

Table 1

Performance of Ashwagandha selections for plant height (cm) during 2015-16

Sr. No.	Ashwagandha plant height (cm)								
	Genotypes	Akola	Anand	DMAPR	Hisar	Mandsaur	Raipur	Udaipur	Mean
1	HWS12-12	52.80	121.63	73.83	61.67	34.40	77.57	43.33	66.46
2	HWS8-18	59.80	103.33	77.28	70.10	33.13	74.55	35.67	64.84
3	I-WS-1	48.33	77.45	64.59	68.53	33.67	56.13	30.67	54.20
4	IWS 3	54.27	79.17	62.23	44.00	35.47	58.07	41.00	53.46
5	IWS32	54.20	72.13	68.58	56.67	43.60	71.16	32.00	56.91
6	MWS130	58.80	81.57	68.25	52.43	36.33	52.27	41.00	55.81
7	MWS90-14	58.00	79.57	70.09	52.00	36.80	56.10	36.00	55.51
8	UWS10	59.20	87.73	66.32	49.13	34.07	56.19	45.67	56.90
9	UWS23	59.00	79.27	66.04	54.20	33.33	56.11	42.33	55.75
10	UWS37	53.47	90.40	66.44	57.13	38.20	65.87	48.33	59.98
11	AWS-1©	59.00	86.13	68.98	59.57	35.00	71.42	34.33	59.20
12	JA134©	50.53	82.23	71.11	59.00	37.27	58.78	44.33	57.61
13	JA20©	53.47	78.87	65.78	54.57	33.20	51.53	32.67	52.87
14	RVA100©	57.00	78.33	64.78	44.77	34.93	57.00	37.00	53.40
	Mean	55.79	87.23	68.37	56.59	35.90	62.40	39.60	57.98
	CV %	10.29	8.60	7.37	17.36	10.13	14.54	19.74	0.00
	CD 5%	9.59	12.35	8.44	16.32	6.07	15.04	12.88	0.00
	S. Em±	0.96	2.14	0.93	1.71	0.62	1.83	1.35	0.00

Table 2 Performance of Ashwagandha selections for plant height (cm) during 2016-17

Sr. No.	Ashwagandha plant height (cm)									
	Genotypes	Akola	Anand	Coimbatore	DMAPR	IIHR	Mandsaur	Raipur	Udaipur	Mean
1	HWS12-12	47.73	64.87	87.00	62.02	59.60	38.20	81.60	81.59	61.51
2	HWS8-18	45.73	64.90	92.20	59.23	56.53	40.00	91.87	91.87	63.85
3	I-WS-1	45.20	57.23	92.07	63.47	50.13	36.13	83.81	83.81	59.22
4	IWS 3	35.80	49.87	98.67	56.26	56.47	30.60	85.00	85.01	55.88
5	IWS32	36.87	55.10	98.20	58.13	40.07	32.53	82.00	82.00	56.12

6	MWS130	35.87	52.97	89.07	52.01	45.53	32.47	69.24	69.24	52.17
7	MWS90-14	41.13	53.70	96.33	57.07	47.93	36.00	77.92	77.92	56.38
8	UWS10	39.87	58.57	89.13	58.99	55.40	31.00	77.50	77.49	56.51
9	UWS23	36.20	61.80	105.40	56.71	52.27	33.47	82.25	82.25	59.05
10	UWS37	44.40	55.83	98.47	68.99	44.33	36.07	76.38	76.38	58.92
11	AWS-1©	41.20	59.90	105.87	70.36	56.50	40.80	81.13	81.13	63.10
12	JA134©	37.73	56.77	99.93	56.52	46.90	33.67	77.00	77.00	57.42
13	JA20©	35.47	53.50	93.73	53.11	45.63	33.27	77.53	77.53	53.92
14	RVA100©	38.13	54.30	92.93	59.11	47.27	38.67	79.34	79.34	55.17
	Mean	40.10	57.09	95.64	59.43	50.10	35.20	80.18	80.18	0.00
	CV %	14.34	11.79	10.71	9.64	18.88	9.42	10.52	10.52	0.00
	CD 5%	9.65	11.30	17.19	9.61	16.47	5.57	14.16	14.16	0.00
	S. Em±	3.32	3.89	5.91	3.31	5.46	1.92	4.87	4.87	0.00

Table 3 Performance of Ashwagandha selections for plant height (cm) during 2017-18

Sr. No.	Ashwagandha plant height (cm)									
	Genotypes	Akola	Anand	Coimbatore	DMAPR	IIHR	Mandsaur	Raipur	Udaipur	Mean
1	HWS12-12	54.13	91.87	65.60	60.27	40.40	44.90	74.48	41.33	62.33
2	HWS8-18	61.00	102.73	54.93	54.79	51.53	44.20	82.30	33.67	63.29
3	I-WS-1	63.07	86.53	70.40	61.79	41.27	46.53	67.64	35.67	61.02
4	IWS 3	55.00	86.47	60.00	49.86	33.80	40.07	57.19	39.00	54.95
5	IWS32	45.53	63.62	98.20	63.36	40.07	38.07	76.58	57.00	56.52
6	MWS130	59.07	82.20	66.87	56.35	38.80	40.73	76.83	43.67	61.24
7	MWS90-14	66.80	95.59	75.23	60.63	44.73	48.27	86.00	36.67	66.46
8	UWS10	63.20	95.41	49.90	56.69	38.27	41.27	60.63	32.33	56.83
9	UWS23	60.67	85.19	59.20	51.69	38.40	42.80	69.08	39.33	58.00
10	UWS37	68.40	84.19	79.10	63.66	32.60	44.67	72.40	37.00	62.16
11	AWS-1©	66.33	80.26	81.67	57.49	39.27	44.60	63.87	42.67	61.63
12	JA134©	64.00	91.13	49.80	56.67	35.07	45.00	64.05	40.67	57.81
13	JA20©	62.27	80.60	60.80	54.99	37.73	44.93	63.59	38.00	57.19
14	RVA100©	62.07	76.33	59.17	53.99	27.13	39.53	60.86	38.67	54.99
	Mean	62.00	87.61	64.05	56.84	38.38	43.65	69.15	38.36	59.85
	CV %	7.91	13.03	17.30	7.85	20.90	10.67	16.73	12.32	
	CD 5%	8.27	19.24	18.68	7.52	13.52	7.85	19.50	7.96	
	S. Em±	2.83	6.59	6.40	2.58	4.63	2.69	6.68	2.73	

Root length

The results of this study (Table 4, 5 & 6) revealed that the performance of different genotypes in relation to root length varied from over the years and locations due to differences in soil and climatic conditions as well as agronomic management. During 2015-16, the average root length over the locations revealed that the genotype HWS12-12 was longest in root length 21.05 cm followed by JA134 (20.41cm), HWS8-18 (64.84 cm), and AWS-1 (20.15cm) in plain zone at national level. The average root length was recorded maximum 23.86cm at Anand, followed by Mandsaur (23.49), Hisar (21.40cm), Raipur (20.30cm), Akola (19.68cm), DMAPR (16.61cm) and Udaipur (13.63cm). Likewise, during 2016-17, the average plant height (cm) over the locations revealed that the genotype HWS8-18 was recorded maximum root length (20.18cm) followed by HWS12-12 (19.97cm), IWS-3

(19.57cm), in plain zone at national level (Table 5). The average root length was recorded maximum 28.47cm at Coimbatore, followed by Raipur (23.82cm), DMAPR (19.27cm) and Akola (18.46cm). Likewise, during 2017-18, the average root length (cm) over the locations revealed that the genotype MWS90-14 was recorded maximum root length (21.56cm) followed by HWS12-12 (21.29cm), UWS10 (20.77cm), HWS8-18 (20.62cm), and JA 20© (20.45cm) in plain zone at national level (Table 6). The average root length was recorded maximum (26.03cm) at Mandsaur followed by Raipur (24.47cm), Akola (20.92cm) and Udaipur (19.46cm).

Table 4
Performance of Ashwagandha selections for root length (cm) during 2015-16

Sr. No.	Ashwagandha root length (cm)								
	Genotypes	Akola	Anand	DMAPR	Hisar	Mandsaur	Raipur	Udaipur	Mean
1	HWS12-12	18.53	24.30	16.34	22.23	28.80	23.79	13.33	21.05
2	HWS8-18	20.20	24.43	16.23	23.13	22.87	22.06	13.00	20.27
3	I-WS-1	19.07	23.75	17.07	21.67	23.13	17.87	11.00	19.08
4	IWS 3	21.60	24.20	15.95	19.67	23.87	21.90	13.33	20.07
5	IWS32	19.73	23.90	16.35	21.33	23.40	23.03	10.67	19.77
6	MWS130	20.93	24.17	15.55	21.13	24.27	18.13	14.33	19.79
7	MWS90-14	20.87	21.50	17.68	21.67	22.13	18.46	15.33	19.66
8	UWS10	19.60	23.30	17.45	20.77	21.67	20.41	16.00	19.88
9	UWS23	17.73	23.90	17.05	22.10	21.93	20.17	13.00	19.41
10	UWS37	18.53	25.10	16.45	20.33	22.87	17.15	16.33	19.54
11	AWS-1©	20.67	24.03	17.21	23.67	21.47	20.71	13.33	20.15
12	JA134©	20.53	22.90	18.08	18.90	24.67	21.14	16.67	20.41
13	JA20©	16.00	23.43	16.54	21.03	22.87	17.57	12.33	18.54
14	RVA100©	21.00	23.90	18.57	19.30	24.33	19.87	15.00	20.28
	Mean	19.68	23.86	16.61	21.40	23.49	20.30	13.63	19.85
	CV %	11.52	10.55	11.03	14.33	11.29	16.68	16.88	-
	CD 5%	**3.80**	**4.21**	3.13	5.10	4.44	5.64	3.92	-
	S. Em±	**0.38**	**0.34**	0.28	0.45	0.43	0.52	0.42	-

Table 5
Performance of Ashwagandha selections for root length during 2016-17

Sr. No.	Ashwagandha root length (cm)									
	Genotypes	Akola	Anand	Coimbatore	DMAPR	IIHR	Mandsaur	Raipur	Udaipur	Mean
1	HWS12-12	19.40	21.87	26.83	17.38	14.00	18.60	24.91	18.33	19.97
2	HWS8-18	17.87	17.47	27.27	15.25	20.27	18.47	29.83	18.67	20.86
3	I-WS-1	19.40	15.33	30.27	18.13	14.07	17.67	23.41	17.00	19.30
4	IWS 3	16.93	19.90	29.10	21.35	15.17	13.13	24.89	16.67	19.57
5	IWS32	20.07	17.13	28.50	19.98	12.47	15.47	23.05	20.67	19.53
6	MWS130	19.27	18.67	29.33	24.36	11.97	15.07	20.38	16.67	19.38
7	MWS90-14	16.87	16.57	26.47	19.99	12.00	16.40	26.99	16.67	18.97
8	UWS10	19.67	16.40	27.67	16.99	13.27	16.77	23.29	18.00	18.82
9	UWS23	17.73	17.57	28.30	21.60	14.13	15.60	20.83	19.33	19.44
10	UWS37	17.53	15.60	27.47	17.58	11.17	17.53	22.22	17.00	18.59
11	AWS-1©	17.40	17.17	29.03	18.51	17.87	16.97	25.00	17.33	20.18
12	JA134©	18.80	16.90	28.87	14.41	13.37	15.44	26.11	18.33	18.73
13	JA20©	18.13	17.57	29.57	22.57	11.17	17.01	20.96	20.33	19.54

14	RVA100©	19.40	15.87	29.93	21.68	13.20	16.60	21.61	16.33	19.13
	Mean	18.46	17.43	28.47	19.27	13.86	16.48	23.82	17.95	
	CV %	15.90	14.18	10.75	15.93	21.91	18.96	17.50	15.02	-
	CD 5%	4.93	4.15	5.14	5.15	5.40	5.25	7.00	4.53	-
	S. Em±	1.70	1.43	1.77	1.77	1.75	1.80	2.41	1.56	

Table 6
Performance of Ashwagandha selections for root length during 2017-18

Sr. No.	Ashwagandha root length (cm)									
	Genotypes	Akola	Anand	Coimbatore	DMAPR	IIHR	Mandsaur	Raipur	Udaipur	Mean
1	HWS12-12	19.93	17.87	18.87	18.01	18.40	22.40	24.44	20.33	21.29
2	HWS8-18	22.67	17.27	16.24	16.62	16.20	24.27	30.72	21.33	20.62
3	I-WS-1	19.47	16.43	18.31	16.17	13.73	25.87	25.94	21.67	19.95
4	IWS 3	21.60	19.03	18.23	16.45	12.83	22.20	22.27	22.00	19.52
5	IWS32	19.90	20.52	28.50	18.17	12.47	19.43	23.04	15.67	19.65
6	MWS130	21.33	17.00	16.61	20.92	13.43	23.60	22.77	17.33	19.68
7	MWS90-14	21.87	16.67	19.07	17.41	17.47	28.87	28.17	18.33	21.56
8	UWS10	20.53	16.10	13.89	29.57	17.07	27.07	21.38	21.00	20.77
9	UWS23	19.00	20.37	19.03	16.87	11.50	26.07	23.69	17.67	19.68
10	UWS37	21.20	14.93	22.46	16.31	11.33	27.00	28.57	19.67	20.44
11	AWS-1©	20.93	15.47	21.39	15.15	14.33	28.73	22.53	18.67	19.89
12	JA134©	19.13	20.97	11.87	17.63	13.63	26.53	23.87	15.67	18.93
13	JA20©	23.47	17.37	14.96	18.67	13.47	30.53	22.05	20.67	20.45
14	RVA100©	20.80	15.97	18.15	15.83	12.60	25.27	21.78	18.67	18.67
	Mean	20.92	17.34	17.62	18.12	14.31	26.03	24.47	19.46	20.12
	CV %	9.02	14.58	21.63	30.13	25.65	16.39	19.85	12.89	-
	CD 5%	3.18	4.26	6.42	9.20	6.19	7.19	8.19	4.23	-
	S. Em±	1.09	1.46	2.20	3.15	2.12	2.46	2.81	1.45	-

Dry root yield

The statistical analysis of data was done, which separates the total variation into different components. The mean sum of squares due to genotypes exhibited a significant differences in all the traits under study at 1 and 5% levels of significance. This encompasses the genetic differences among the genotypes of ashwagandha. The total variations in a plant population are considered as the outcomes mainly due to genotypic and environmental effects. That's why, the dry root yield of different genotypes of ashwagandha varies over the locations as well as over the years. The data on dry root yield of different genotypes of ashwagandha recorded over different locations during 2015-16, 2016-17, 2017-18 and 2018-19 is presented in Table 7, 8, 9, 10, respectively.

Table 7
Performance of Ashwagandha selections for dry root yield (kg/ha) during 2015-16

Sr. No.	Ashwagandha Dry root yield (kg/ha)								
	Genotypes	Akola	Anand	DMAPR	Hisar	Mandsaur	Raipur	Udaipur	Mean
1	HWS12-12	671.67	701.23	328.40	1051.20	352.67	951.10	284.33	620.09
2	HWS8-18	640.67	686.11	339.92	1096.80	353.00	988.88	425.67	647.29
3	I-WS-1	722.67	403.39	362.34	998.40	312.33	573.33	342.67	530.70

4	IWS 3	571.00	591.66	584.36	801.60	475.00	715.55	448.33	598.22
5	IWS32	454.33	600.31	633.33	1082.40	369.00	1148.88	559.00	692.47
6	MWS130	583.33	454.63	492.18	643.20	433.00	599.99	321.33	503.95
7	MWS90-14	595.00	458.02	451.44	576.00	542.00	686.66	429.67	534.11
8	UWS10	607.00	537.96	337.45	744.00	474.33	617.77	455.67	539.17
9	UWS23	524.33	421.60	313.99	739.20	444.00	583.33	369.67	485.16
10	UWS37	638.67	519.75	436.21	844.80	443.67	675.55	444.00	571.81
11	AWS-1©	601.00	570.68	544.45	906.00	584.33	844.44	414.67	637.94
12	JA134©	483.00	412.04	512.35	940.80	499.00	671.10	350.67	552.71
13	JA20©	602.00	524.69	443.62	722.40	283.33	564.44	359.67	500.02
14	RVA100©	512.33	502.47	520.16	582.00	388.67	617.77	385.33	501.25
	Mean	600.87	537.47	427.94	857.76	419.90	754.10	408.03	572.30
	CV %	25.98	12.20	26.77	28.45	29.33	31.38	28.37	-
	CD 5%	255.59	107.96	202.21	400.01	209.39	385.23	190.11	-
	S. Em±	23.28	16.60	21.56	40.90	22.34	40.01	18.40	-

Table 8

Performance of Ashwagandha selections for dry root yield (kg/ha) during 2016-17

Sr. No.	Ashwagandha Dry root yield (kg/ha)									
	Genotypes	Akola	Anand	Coimbatore	DMAPR	IIHR	Mandsaur	Raipur	Udaipur	Mean
1	HWS12-12	911.11	289.33	529.56	531.75	761.70	651.67	709.26	135.67	565.00
2	HWS8-18	800.00	478.73	505.33	733.05	715.17	622.00	779.63	220.33	606.78
3	I-WS-1	422.22	386.88	563.78	564.90	749.80	770.33	532.96	175.67	520.82
4	IWS 3	644.44	573.37	599.78	642.07	660.70	622.00	577.77	317.67	579.72
5	IWS32	511.11	509.35	444.67	578.81	661.83	592.33	618.52	369.33	535.74
6	MWS130	555.56	411.93	530.22	450.17	672.63	711.00	417.59	199.33	493.55
7	MWS90-14	511.11	384.10	466.22	432.86	729.20	592.33	492.22	176.67	473.09
8	UWS10	555.55	523.27	559.33	467.23	699.07	829.67	633.15	315.33	572.82
9	UWS23	466.67	478.73	573.33	559.28	673.47	681.33	523.89	203.33	520.00
10	UWS37	600.00	498.22	438.89	687.34	584.20	711.00	446.11	263.33	528.64
11	AWS-1©	533.33	509.35	543.11	535.00	620.77	651.67	487.04	323.33	525.45
12	JA134©	511.11	420.28	548.00	424.10	588.27	562.67	461.11	232.00	468.44
13	JA20©	444.44	414.72	543.11	464.76	604.13	622.00	579.45	178.00	481.33
14	RVA100©	466.67	439.77	473.33	567.32	711.60	681.33	486.67	231.00	507.21
	Mean	566.67	451.29	522.76	545.62	671.61	664.38	553.24	238.64	527.04
	CV %	36.16	15.68	17.27	22.12	17.09	18.00	29.13	31.95	0.00
	CD 5%	343.87	118.73	151.48	202.52	0.00	200.73	270.47	127.98	0.00
	S. Em±	118.29	40.84	52.11	69.67	66.26	69.05	93.04	44.03	0.00

Table 9

Performance of Ashwagandha selections for dry root yield (kg/ha) during 2017-18

Sr. No.	Ashwagandha Dry root yield (kg/ha)									
	Genotypes	Akola	Anand	Coimbatore	DMAPR	IIHR	Mandsaur	Raipur	Udaipur	Mean
1	HWS12-12	167.90	493.21	704.00	532.66	632.23	451.73	1392.17	350.67	590.57
2	HWS8-18	256.79	416.39	400.00	558.43	669.13	435.41	1463.30	429.00	578.56

3	I-WS-1	185.60	299.49	404.00	354.23	397.13	460.76	814.37	313.67	403.66
4	IWS 3	226.75	455.91	664.00	258.51	305.57	506.25	681.03	443.00	442.63
5	IWS32	482.71	554.82	444.67	558.43	661.83	480.66	883.70	429.00	561.99
6	MWS130	474.89	369.07	736.00	254.93	378.67	504.51	999.93	486.33	525.54
7	MWS90-14	542.38	384.10	694.00	305.78	611.37	498.26	1017.70	371.00	553.07
8	UWS10	432.92	384.66	380.00	272.12	465.33	429.98	755.50	342.00	432.81
9	UWS23	564.19	346.80	648.00	162.04	366.43	463.54	781.03	299.33	453.92
10	UWS37	379.01	264.42	676.00	277.27	284.00	468.40	1096.63	383.33	478.63
11	AWS-1©	509.05	311.18	672.00	216.36	442.23	534.37	928.83	313.33	490.92
12	JA134©	313.58	301.71	352.00	242.18	508.20	509.72	1085.50	350.33	457.90
13	JA20©	374.48	266.09	504.00	286.16	416.90	465.97	718.80	503.00	441.92
14	RVA100©	318.11	337.34	420.00	279.88	330.90	427.08	736.57	350.33	400.03
	Mean	365.05	356.18	558.00	307.74	446.78	473.54	959.34	379.64	480.78
	CV %	50.51	31.46	31.60	34.40	37.71	12.77	31.45	34.19	-
	CD 5%	310.74	188.85	297.16	178.40	283.94	101.92	508.37	218.76	-
	S. Em±	106.46	64.70	101.81	61.12	97.28	34.92	174.17	74.95	-

Source: Source: Annual Report 2017-18 AICRP on MAP&B, ICAR-DMAPR, Anand, Gujrat (Anonymous, 2018)

Table 10

Performance of Ashwagandha selections for dry root yield (kg/ha) during 2018-19

Sr. No.	Genotypes	IIHR	Anand	Mandsaur	Udaipur	Raipur	Mean
1	IWS 3	258.52	261.08	661.11	472.67	526.20	435.92
2	HWS8-18	566.52	392.45	634.26	608.00	506.20	541.49
3	HWS12-12	462.83	306.72	608.34	542.67	311.87	446.49
4	IWS 32	207.56	319.53	495.83	351.00	494.03	373.59
5	GAA-1©	166.22	408.59	541.67	361.00	484.73	392.44
6	JA20 ©	221.18	431.42	553.70	336.67	380.53	384.70
7	JA134©	113.19	347.36	513.89	322.00	608.00	380.89
8	RVA100 ©	102.22	385.77	478.70	315.67	571.37	370.75
	Mean	262.28	356.62	560.94	413.71	485.37	415.78
	CV%	47.42	-	-	26.08	-	
	CD (P=0.05)	217.81	NS	NS	188.98	NS	
	SE	87.94			76.30		

Source: Annual Report 2018-19 AICRP on MAP&B, ICAR-DMAPR, Anand, Gujrat (Anonymous, 2019)

Genotype performance

During 2015-16, the highest dry root yield (Fig. 2) was achieved by IWS 32 (692.47 kg/ha), followed by HWS 8-18 (647.29 kg/ha), AWS-1, Check (637.94 kg/ha) and HWS 12-12 (620.09 kg/ha). During 2016-17, the genotype HWS 8-18 (647.29 kg/ha), was the highest dry root yielder followed by IWS 3 (579.72kg/ha), UWS 10 (572.82 kg/ha) and HWS 12-12 (565.00 kg/ha) (Fig.3). During 2017-18, the genotype HWS 12-12 (590.57 kg/ha) was the highest dry root yielder followed by HWS 8-18 (578.56 kg/ha), IWS 3 (561.99 kg/ha), and MWS 90.14 (553.07 kg/ha) (Fig.4). During 2018-19, the highest dry root yield (Fig.5) was achieved by HWS 8-18 (541.49 kg/ha), followed by HWS 12-12 (446.49 kg/ha) and IWS-3 (435.92 kg/ha).

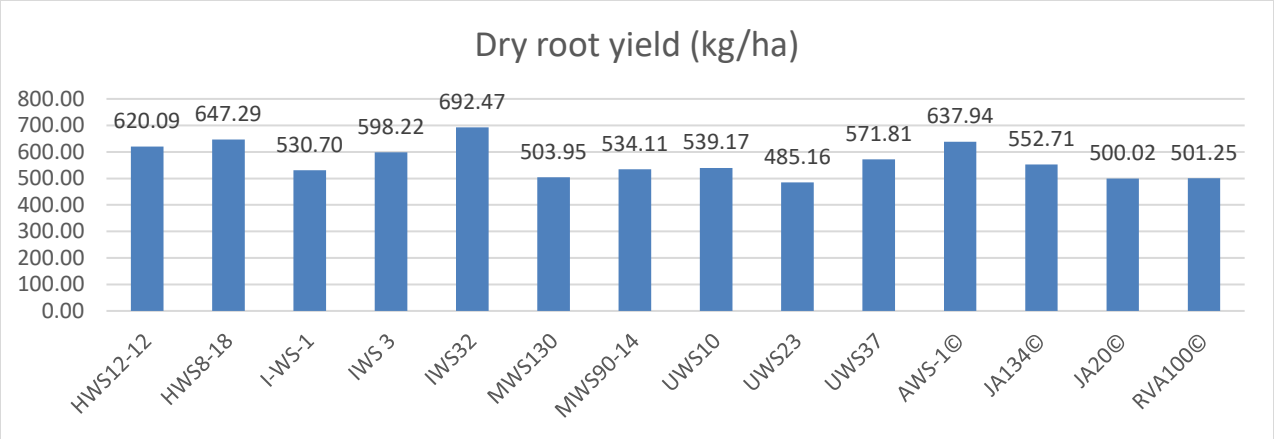


Fig. 2. Dry root yield performance of genotypes over the locations during 2015-16

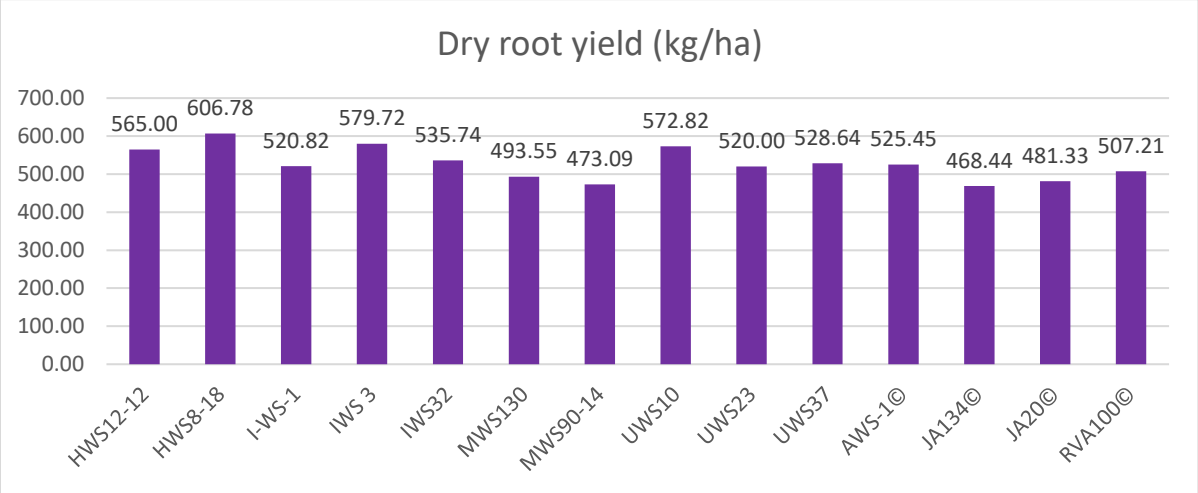


Fig. 3. Dry root yield performance of genotypes over the locations during 2016-17

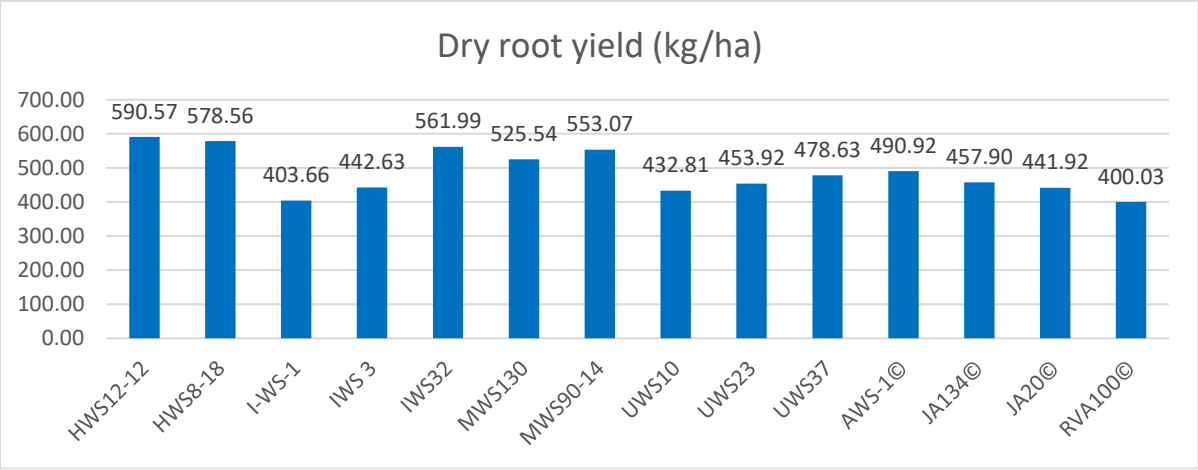


Fig. 4. Dry root yield performance of genotypes over the locations during 2017-18

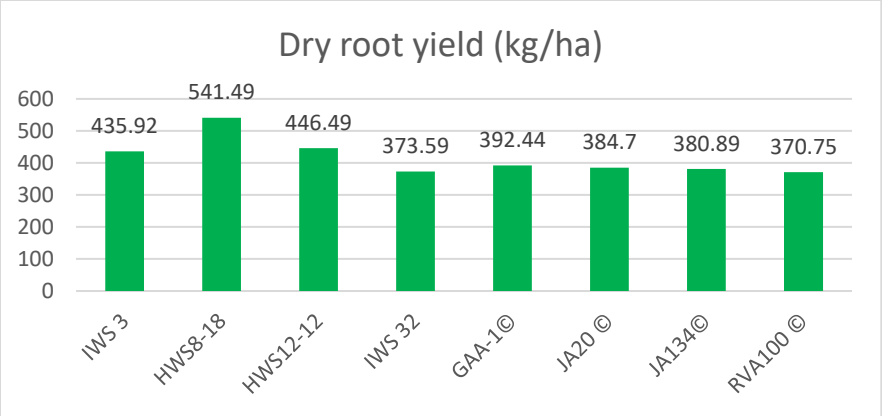


Fig. 5. Dry root yield performance of genotypes over the locations during 2018-19

The four best genotypes (HWS12-12, HWS8-18, IWS 3 and IWS32) were identified on the basis of the average dry root yield and further tested alongwith checks (GAA-1, JA20, JA134 and RVA100) during 2018-19 for dry root yield as well as the root alkaloid contents content in dried roots samples for comparison. The results of present study are presented in Fig. 5. It is clear from above graph that the genotypes HWS 8-18 was highest in dry root yield with average 541.49kg/ha and followed by HWS 12-12 (446.49kg/ha), and IWS 3 (435.92kg/ha) were having high dry root yield as compared to all the checks, GAA-1(392.44kg/ha), JA20(384.70kg/ha), JA134(380.89kg/ha) and RVA100 (370.75kg/ha), Whereas, genotypes IWS 32 (540.95) were found low yielder as compared to the checks.

Locations suitability

Under different environments, the performance on root yield and quality of different genotypes was found variable. It may be due to differential response of different genotypes under different soil and climatic conditions. The results on location suitability of present investigation are presented in Fig. 6 & 7. The maximum average dry root yield during 2015-16, was recorded at Hisar (857.76kg/ha), followed by Raipur (754.00kg/ha), Akola (600.87kg/ha), and Anand (537.42). During 2016-17, the highest average dry root yield was recorded at IIHR (671.61kg/ha), followed by Mandsaur (664.38kg/ha), Akola (566.67kg/ha), and Raipur (553.24kg/ha). But, again during 2017-18, the highest average dry root yield was recorded at Raipur (959.34kg/ha), followed by Coimbator (558.00kg/ha), IIHR (446.78kg/ha), and Mandsaur (473.54kg/ha). However, during 2018-19, Mandsaur dry root production was highest (560.94kg/ha) and followed by Raipur (485.37kg/ha), Udaipur (413.71kg/ha).

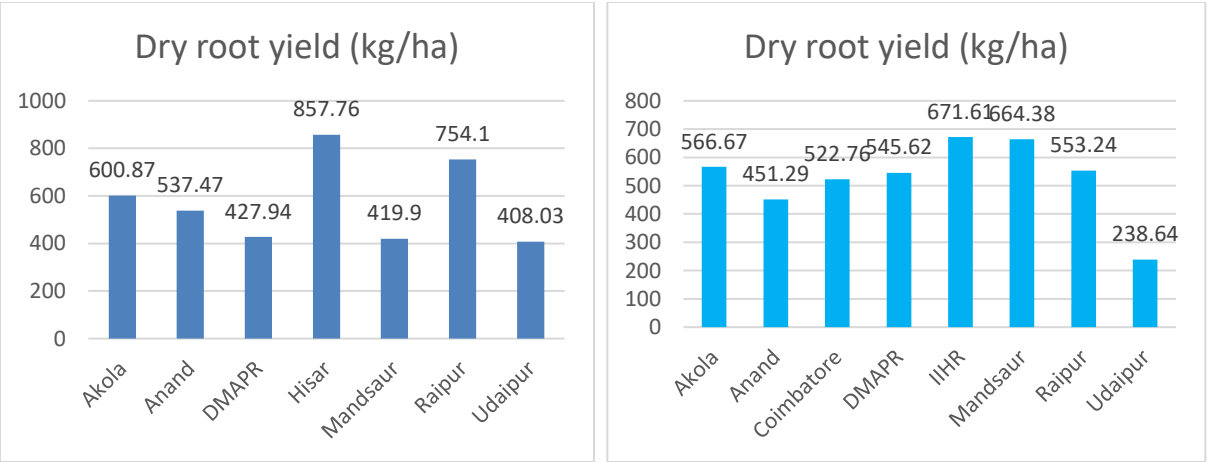


Fig. 6. Dry root yield performance of the locations over the genotypes during 2015-16 and 2016-17

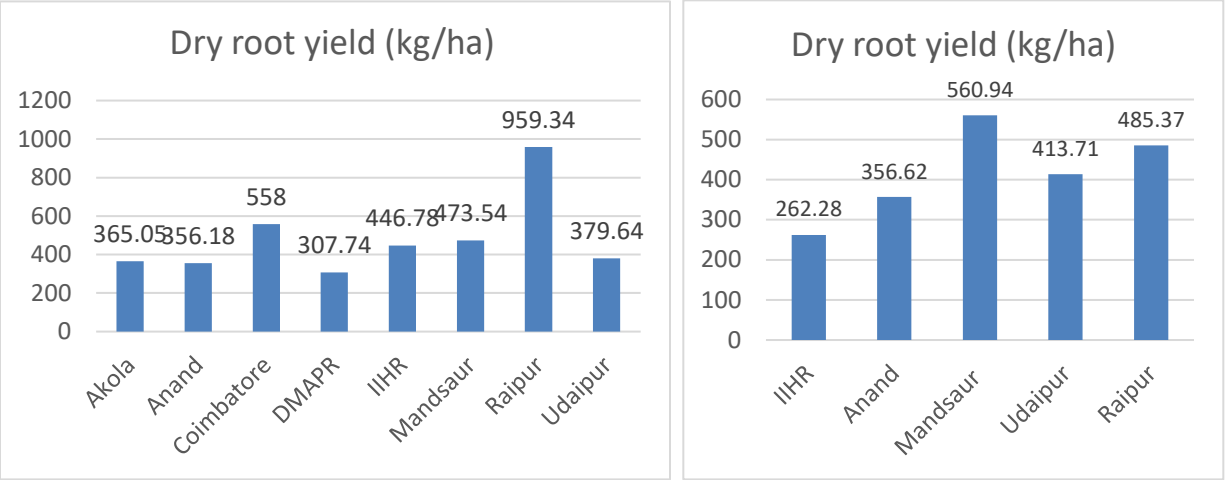


Fig. 7. Dry root yield performance of the locations over the genotypes during 2017-18 and 2018-19

Overall dry root yield performance

Based on overall dry root yield performance over all the location and years (Fig. 8), genotype HWS 8-18 and HWS 12-12 appears most promising for dry root yield and recommended for commercial cultivation for the semi-arid regions. Further, the some genotypes, found higher in specific bio-compound, may be utilized future breeding program as donor parents, e.g., HWS 12-12, IWS 32 IWS 3 and AWS 1 may be utilized for root yield and withanolide content in roots for further genetic improvement in breeding programs.

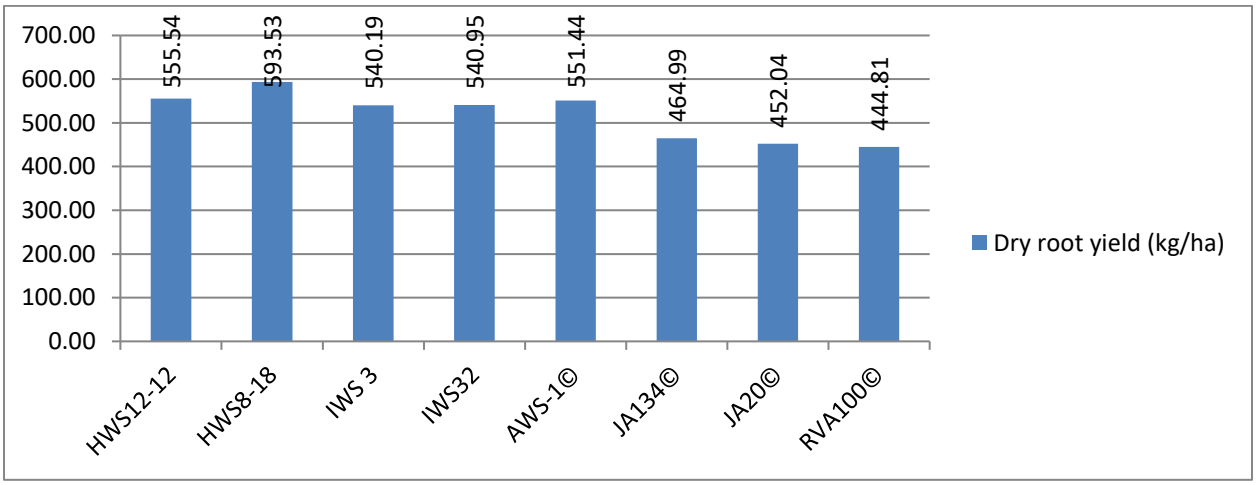


Fig. 8. Average dry root yield of Ashawagandha over four years of different locations

3.3.1 Quality

The data on quality parameters i. e. Withastramanolide (mg/g) and Withanolide-A (mg/g**)** is presented in Table 11. The ashwagandha genotypes showed differences in dry root quality parameters over the locations due to variations in location soils and climates. Udaipur was found best location for the maximum accumulation of Withastramanolide (0.208mg/g) and Withanolide-A (0.625mg/g) followed by IIHR. For Raipur location, HWS8-18 was most suitable genotype, as it produced maximum Withastramanolide (0.119mg/g) as well as Withanolide-A (0.406mg/g) contents. On basis of average over the locations, Withastramanolide (mg/g) content was found

maximum in genotype, HWS12-12 (0.147mg/g) followed by IWS 3 (0.143mg/g), GAA-1 (0.143mg/g), IWS 32(0.142mg/g). Likewise, on basis of average over the locations, Withanolide-A (mg/g) content was found maximum in genotype, HWS12-12 (0.457mg/g) followed by IWS 3 (0.397mg/g), HWS8-18 (0.396mg/g), GAA-1 (0.379mg/g), IWS 32(0.361mg/g). Therefore, keeping the alkaloids content of HWS12-12 in view, it may be utilized in breeding program for further improvement in ashwagandha quality parameters.

Table 11

The screening of ashawagandha roots of different centers for Quality parameters, during 2018-19

Sr. No.	Genotypes	IIHR	DMAPR	Anand	Mandsaur	Udaipur	Raipur	Mean
	Withastramanolide (mg/g)							
1	IWS 3	0.165	0.123	0.042	0.153	0.279	0.095	0.143
2	HWS8-18	0.137	0.094	0.047	0.153	0.149	0.119	0.117
3	HWS12-12	0.164	0.302	0.061	0.132	0.150	0.075	0.147
4	IWS 32	0.209	0.130	0.045	0.157	0.214	0.099	0.142
5	GAA-1©	0.211	0.133	0.051	0.156	0.218	0.086	0.143
6	JA20 ©	0.176	0.116	0.041	0.145	0.227	0.089	0.132
7	JA134©	0.168	0.118	0.067	0.120	0.228	0.099	0.133
8	RVA100 ©	0.191	0.136	0.047	0.144	0.195	0.092	0.134
	Mean	0.178	0.144	0.050	0.145	0.208	0.094	0.136
	CV%	2.290	0.920	3.790	1.850	1.130	1.720	
	CD (P=0.05)	0.007	0.002	0.003	0.005	0.004	0.003	
	Withanolide-A (mg/g)							
1	IWS 3	0.366	0.614	0.104	0.325	0.716	0.256	0.397
2	HWS8-18	0.436	0.405	0.127	0.470	0.532	0.406	0.396
3	HWS12-12	0.400	0.579	0.174	0.542	0.723	0.325	0.457
4	IWS 32	0.472	0.431	0.105	0.341	0.607	0.211	0.361
5	GAA-1©	0.587	0.393	0.123	0.352	0.607	0.212	0.379
6	JA20 ©	0.351	0.387	0.103	0.317	0.660	0.201	0.337
7	JA134©	0.331	0.386	0.202	0.287	0.583	0.234	0.337
8	RVA100 ©	0.528	0.403	0.125	0.321	0.570	0.213	0.360
	Mean	0.434	0.450	0.133	0.369	0.625	0.257	0.378
	CV%	1.930	1.020	5.130	1.560	1.150	1.150	
	CD (P=0.05)	0.015	0.008	0.012	0.010	0.013	0.005	

Source: Annual Report 2018-19 AICRP on MAP&B, ICAR-DMAPR, Anand, Gujrat (Anonymous, 2019)

Adaptation trials

The Analysis of variance results depicted highly significant differences in ashwahandha genotypes for root yield and quality traits. The locations and years impart important role in root yield production. Root yield of different genotypes varied in different environments, and only two genotypes, HWS 12-12 (511.5kg/ha) and HWS 8-18 (526.5kg/ha) yielded over all the check at national level. The photograph of plants, roots, berries and seeds of HWS 12-12 and HWS 8-18 presented in Fig. 9 & 10, respectively. The seed of HWS 12-12 and HWS 8-18 was multiplied at Hisar during 2019-20 for conducting large scale adaptation trials. Before the release of new varieties of ashwagandha for commercial cultivation, further evaluation of the best performing genotypes was conducted for dry root yield at large scale on farmers' fields on five different locations viz., Rewari, Mahendergarh, Bhiwani, Hisar and Fatehabad during 2020-21 and 2022-23. The results are presented in Table 12 revealed that HWS 12-12 and HWS 8-18 were 19.31 and 22.81% superior over the check variety, JA-20, respectively.

Table 12

Performance of Ashwagandha in Adaptation trials during 2020-21 and 2022-23

District / Locations	Dry root yield (Kg/ha)								
	HWS 12-12			HWS 8-18			JA-20 (Check)		
	2020-21	2022-23	Mean	2020-21	2022-23	Mean	2020-21	2022-23	Mean
Rewari	425.5	431.0	428.3	430.4	453.0	441.7	365.0	371.3	368.2
Mahendergarh	536.0	620.0	578.0	542.0	637.0	589.5	440.0	540.0	490.0
Bhiwani	460.0	502.4	481.2	500.0	524.0	512.0	402.4	440.0	421.2
Hisar	605.2	650.0	627.6	590.6	648.0	619.3	484.5	485.6	485.1
Fatehabad	450.0	435.0	442.5	472.0	468.0	470.0	397.0	361.0	379.0
Mean	495.3	527.7	511.5	507.0	546.0	526.5	417.8	439.6	428.7
Percent increase over check	18.55	20.04	19.31	21.35	24.20	22.81	-	-	-

Reaction to insect-pest

The evaluation data under natural field condition during 2022-23 of newly developed varieties (HWS 8-18 and HWS 12-12) for insect-pest were presented in Table 13, revealed that all insect-pest were under threshold limit. In addition to above insect –pests, some natural enemies e.g. Coccinellida predators and spiders were also reported in natural field conditions (Table 14). Likewise, data on disease reaction is also presented in Table 15. The novel variety, HWS 8-18 was found resistant to wilt disease, and other novel variety, HWS 12-12 was found moderately resistant.

Table 13

Reaction to insect- pests in novel varieties of Ashwagandha, HWS 8-18 and HAG-1 (HWS 12-12)

Name of proposed variety/hybrid: HWS 8-18 & HAG-1 (HWS 12-12) Adaptability zone: Plains Production condition: Irrigated			Insect-pest/5plants	
Year 2022-23	Condition	HWS 8-18	HAG-1 (HWS 12-12)	National Check-1 (JA-20)
Fruit borer *Helicovrpa armigera* Larvae/5 plants	Natural	0.67 (1.24)	1.0 (1.41)	1.67(1.58)
Hadda beetle: *Epilachana vigictopunctata*	Natural	1.33(1.52)	1.0(1.38)	1.33(1.47)
Aphid	Natural	5.33(2.51)	6.0(2.64)	7.33(2.87)
Stink Bug/5 Plants	Natural	4.67(2.38)	4.67(2.38)	5.33(2.51)
% Berry damage	Natural	5.33(13.34)*	5.0(12.92)*	6.33(14.53)*
% Defoliation	Natural	5.67(13.75)*	6.0(14.14)*	8.67(17.09)*

Note: Figures in parentheses are √n+1 values and Figures in parentheses with * are angular transformation values.

Table 14

Reaction to insect- pests' enemies in novel varieties of Ashwagandha, HWS 8-18 and HWS 12-12

Name of novel variety/hybrid: HWS 8-18 & HAG-1 (HWS 12-12) Adaptability zone: Plains, Production condition: Irrigated			Natural enemies /5plants	
Year 2022-23	Condition	HWS 8-18	HAG-1 (HWS 12-12)	National Check (JA-20)
Coccinellida predators/5 plants	Natural	1.69 (1.61)	1.67(1.55)	1.67(1.55)
Spiders	Natural	0.67(0.28)	0.33(1.14)	1.0(1.38)

Note: The values in parentheses are √n+1

Table 15

Reaction to major disease in novel varieties of Ashwagandha, HWS 8-18 and HAG-1 (HWS 12-12)

Name of proposed variety/hybrid: Proposed variety HWS 12-12(HAG 1) Year 2022-23			Adaptability zone: Plains Production condition: Irrigated	
Disease	Condition	HWS 8-18	HAG-1 (HWS 12-12)	National Check JA 20
Wilt disease (Severity %)	Natural	Resistant	Moderately Resistant	Susceptible
Any other disease	Natural	Nil	Nil	Nil

Note: 0-10% Resistance, 11-20% Moderately Resistant, 21-30% Moderately Susptible, >30% Susceptible
(Source: Release and notification proposal of ashwagandha variety HAG-I & HWS 8-18) (Anonymous 2023 & 2024)

Discussion

Generally, pre-breeding screening of the newly collected genotypes is carried out to identify most desirable genotypes for further testing in multi-location trials as well as utilization in crossing program (Arya et al., 2025a,b). In the present investigation, several breeder working all over the country collected the diverse genotypes of ashwagandha were evaluated and identified best performing 2-3 significanly superior ashwagandha genotypes for high dry root yield and its contributing. Like the present study, Luo et al., (2025) also collected the germplasm and studied the genetic diversity in *Gleditsia sinensis* and identified some promising lines. Koli et al. (2022) also evaluated ashwagandha genotypes and identified diverse high root yielding genotypes for further utilization in breeding and evaluation programme. All required practices were followed for identification and selection of the best performing plants from the germplasm of lines (Arya et al. 2022b,c).

Although, the different breeders at different locations doing ashwagandha breeding work and developing novel breeding lines, but evaluation of all these newly developed genotypes in co-ordinated trials is a challenging task. To obtain the best results, in present investigation, only the best performing and most capable novel genotypes of ashwagandha having more than 10% superiority in station trials for dry root yield were included in multi-location field trials (Anonymous 2018 & 2019). As, more than 10% superiority over the best performing released variety at that particular location is essential for included in co-ordinated trials (Raiger et al. 2023; Arya et al. 2025b).

In the present investigation, after thoroughly screening the 10 novel genotypes of ashwagndha (HWS12-12, HWS8-18, I-WS-1, IWS 3, IWS32, MWS130, MWS90-14, UWS10, UWS23 and UWS37) for dry root yield and other traits further screened over a wider climate range at several places in India. The design of response of novel genotypes of ashwagandha was not alike in all the environmental conditions due to difference in climatic conditions, soil properties and fertility. Moreover, ashwagandha is also sensitive to soil and climatic parameters (Saran and Das, 2023). As, yield is a polygenic trait, extremely affected by predominant environment, during plant growth development. Therefore, it is essential to identify the furthermost suitable elite genotype, in testing over the multiple sites and years. (Arya et al. 2025a). It is also revealed from the present investigation, that performance of varieties from location to location and year to year differ, therefore, it is suggested to grow the best performing varieties on a particular location (Arya et al., 2022a & 2023).

The genotype HWS 8-18 (593.53kg/ha) and HWS 12-12 (555.54kg/ha) were high dry root yield as compared to best check, AWS 1 (551.19kg/ha). Likewise, the best performing genotypes were also reported by Srivastava *et al*. (2018) on the basis of his study on ashwagandha dry roots yield and total alkaloid content (%).

Based on overall dry root yield performance over all the location and years, HWS 8-18 and HWS 12-12 appears most promising for dry root yield and recommended for commercial cultivation for the semi-arid regions (Anonymous 2019). Similar long term multi-location experiment were also conducted for identification of superior genotypes to release as a new variety in faba bean (Arya et al., 2022a & 2023). Similarly, Reddy et al. (2012) also compared the ashwagandha genotypes for dry root yield. Likewise, working on ashwagandha, Srivastava et al. (2018) also identified promising genotypes i.e. CWS-12 (0.58) and CWS-23(0.50) for good alkaloids and CWS-33 (0.55) and CWS-23 (0.52) for high withanolide content in roots. Further, the some genotypes, found higher in

specific bio-compound, may be utilized future breeding program as donor parents, e.g., HWS 12-12, IWS 32 IWS 3 and AWS 1 may be utilized for root yield and withanolide content in roots for further genetic improvement in breeding programs.

Root yield of different ashwagandha genotypes varied in different environments, and only two novel genotypes, HWS 12-12 and HWS 8-18 yielded best over all the check at national level. Before the release of new varieties of ashwagandha for commercial cultivation, further evaluation of the best performing genotypes was conducted for dry root yield at large scale on farmers' fields on five different locations during 2020-21 and 2022-23. The results revealed that HWS 12-12 and HWS 8-18 were 19.31 and 22.81% superior over the check variety, JA-20, respectively (Anonymous, 2024 & 2025). Adaptation trials are conducted for the confirmation of yield performance of a new variety on framers field (Arya *et al*., 2025b).

Recommendations

Therefore, HWS 12-12 named as Haryana Ashwagandha No.-1 (HAG-1) has been recommended and released for commercial cultivation in semi-arid conditions (Fig. 9). Its plants are erect, leaves are medium-dark green, lanceolate and berry colour is orange. The average dry root yield of this variety is 511.5 kg/ha in timely sown and under irrigated condition. It was found superior most variety in the quality parameters. It has moderate resistance to wilt disease and no major insect -pest's incidence was observed. Likewise, HWS 8-18 also recommended for released for commercial cultivation in semi-arid conditions (Fig. 10). Its plants are erect, leaves are medium-dark green, lanceolate leaves and red berry colour. The average dry root yield of this variety is 526.5 kg/ha in timely sown and under irrigated condition. It was found superior most variety in Withanolide-A content. It is resistant to wilt and other diseases, and no major insect -pest's incidence was observed. Both the new varieties cultivated commercially and sold in market. This research will also remain helpful designing crop improvement programs in future, as present research developed two diverse novel varieties of ashwagandha having high root yield and good quality (HWS 8-18 and HWS 12-12) which will the basis of the hybridisation to develop superior high yielding variety with excellent quality.



Fig. 9. HWS 12-12 variety plants, roots, seeds and berries



Fig. 10. HWS 8-18 variety plants, roots, seeds and berries

4. **Conclusions**

Under different environments, the performance on root yield and quality of different novel elite genotypes was found variable. It may be due to differential response of different genotypes under different soil and climatic conditions. The study lead to the development of high yielding variety of ashwagandha, HWS 8-18 and HWS 12-12 for commercial cultivation. As both the varieties (HWS 8-18 x HWS 12-12) are diverse in nature therefore, hybridization is suggested to combine high root yield and quality parameters or to get transgressive segregants for further development of new variety in future.

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Author contributions

RKA: conceived the study and designed the experiment & writing original draft. VK: Quality analysis, review & editing. PK & RP: Pathological studies. NK & HK: Entomological studies, RP, PK & MK: Farmer's field evaluation. HLR & K: data analysis and interpretation of results and co-wrote this paper.

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Declarations

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