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DUS Characterization of the Most Promising High Root Yielding Genotype HWS 8-18 of Ashwagandha (*Withania somnirefa*)

Ganesh Kumar KOLI[1] **Rajesh Kumar ARYA**[1*]

[1] MAP Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar- 125004 (India)

[*] Corresponding author e-mail: rakarayogi@gmail.com

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ABSTRACT

Present study on DUS characterization is about the most promising high root yielding genotype HWS 8-18 of Ashwagandha (*Withania somnifera*) was done during the late *Kharif*, 2018 and 2019 and established its distinctness among different genotypes. The genotype HWS 8-18 was tested for several morphological descriptors. The ashwagandha genotype HWS 8-18 has been found superior in dry root yield as compared to ASW-1 (best check) 10.78% at National level and 40.99% higher in the Haryana State. The mean seed yield of HWS 8-18 was 610.87 kg/ha compared to 551.43 kg/ha of ASW-1 (best check) recorded at National level while at Hisar the mean seed yield was found to be 767 kg/ha as against ASW-1 (544 kg/ha). The genotype HWS 8-18 has given numerically high seed yield (579.41 kg/ha) and at par with best check, AWS-1 (541.05 kg/ha). The morphological characters, lenceolate, smooth, medium dark green leaves, orange yellow berry colour and semi-erect are the marker characters of HWS 8-18. In addition to this, the characterization information will be helpful to maintain the genetic as well as proper identification. Moreover, high root yield and seed yield have greater importance and would be utilized in hybridization for further genetic improvement.

Keywords**:** Ashwagandha (*Withania somnirefa*), characterization, diversity, DUS testing, descriptors

Introduction

Globally, Ashwagandha (*Withania somnifera* L.) Dunal (2n=48) is most demanding medicinal herbs and known for the medicinal utility of its root alkaloids as immunity booster against COVID. Now-a-days, due to spread of COVID pandemic all over the world, importance of this herb has been increased several folds. Ashwagandha is most valuable herbal plant for Indian culture because it is an important part of Ayurveda and Unani systems of medicine since ancient times (Koli et al. 2021). It is most interesting to note down that the cultivated plants have sizable differences in their therapeutic action. Ashwagandha belongs to the family Solanaceae and genus Withania. In Ashwagandha, only two species viz., *Withania somnifera* (L.) Dunal and *Withania coagulans* Dunal are found in India. In modern era, to protect a new variety under the Plant Varieties and Farmers Rights (PVP and FR Act in, 2001) DUS characterization of new variety is very important. Under this act new variety is compared with other known variety on the basis of a set of appropriate traits and this information is required at the time of release of new variety (Yadav et al. 2013). Therefore, keeping the importance of above facts, the present study was undertaken to characterize the ashwagandha HWS 8-18 on the basis of quantitative and qualitative morphological characters and to identify distinctness among the newly developed genotypes.

Materials and Methods

In this experimental study, Ashwagandha (*Withania somnifera*) genotype HWS 8-18 was selected from germplasm of Medicinal and Aromatic plant section of Chaudhary Charan Singh Haryana Agricultural University, Hisar. The genotype was evaluated for morphological characters during late *Kharif* 2018 and 2019. For this study, five randomly selected plants of Ashwagandha were taken. Data was recorded on the

all the morphological characters. All the characters were observed at specified stage of crop growth, when the character under study had full expression. The characters, namely, growth habit, growth pattern, plant pigmentation, leaf texture, leaf color, leaf polishing, flower color, at time of flowering. At harvesting and threshing stage, observations on seed color, seed shape were recorded. For quantitative characters, days to 50% flowering and days to maturity were recorded as number of days from sowing to 50% plants initiated flowering or showed ripe berry at the relevant stage of genotype, respectively. The data on remaining characters i.e. plant height (cm), number of branches per plant, number of berries per plant, berries yield per plant (g), number of seeds per berries, seed yield per plant (g) and biological yield per plant (g) were recorded at the time of harvesting and threshing. The mean values of three replications were calculated and classified into different groups according to germplasm catalog developed by International Board for Plant Genetic Resources, Rome, Italy.

For molecular divergence study, genomic DNA of Ashwagandha was extracted from young leaves using CTAB (Cetyltrimethyl Ammonium Bromide) method with modifications. The quality of DNA was checked by using 0.8% agarose gel in electrophoresis (Parita et al. 2018). A set of three SSR markers was used to study the genetic divergence in ashwagandha genotypes at the molecular level. SSR is a co-dominant marker and it is distributed through the genome, variable, easily scorable, highly reproducible, multiallelic, abundant and in nature that's why it is a good choice for molecular characterization. It has been used in many researches those are based on diversity. Less numbers of SSR markers can be provide better genetic diversity spectrum because of its multi allelic and high polymorphic nature (Shah et al. 2013). All three primers used in this study possessed good transferability in *Withania somnifera* and hence, they were used in present study for diversity analysis. The three primers used in this study are (i) CAMS 340, (ii) CAMS 351 and (iii) CAMS 376 (Table 1).

Results and Discussion

Agro-morphological characters

In the present study, ashwagandha genotype HWS 8-18 was evaluated for its morphological traits. Plant height was one of the most variable characters in ashwagandha. On the basis of this character, genotype, HWS 8-18 was grouped into the intermediate category having about 63.99 cm tall. In ashwagandha, three types of growth habits are found i.e. erect, semi-erect and spreading type. Out of these, the genotype, HWS 8-18 exhibited semi-erect growth habit. On the basis of number of branches per plant genotype was also grouped into the intermediate. The shape of leaves of genotype HWS 8-18 was lanceolate type, and color of foliage leaves was dark green (Table 2). The dark green color of leaves is responsible for harnessing maximum sum light for photosynthesis as compared to light green and pale green. The fruits of ashwagandha are known as berry; generally three types of berry color are reported in literature red, orange and light green. The genotype HWS 8-18 have orange yellow berry colour (Fig. 1). Its plant starts flowering in about 82-93 days. The variety HWS 8-18 takes about 163-171 days to mature and falls in Medium maturity group. Similar findings were also reported by Yadav et al. (2013) in Indian mustard.

Yield and its component characters

The ashwagandha genotype HWS 8-18 has been found superior in dry root yield compared to ASW-1 (best check) 10.78% at National level and 40.99% higher in the Haryana State (Table 3). The mean root yield of HWS 8-18 was 610.87 kg/ha compared to 551.43 kg/ha of ASW-1 (best check) recorded at National level. The genotype HWS 8-18 has given numerically high seed yield (579.41 kg/ha) and at par with best check, AWS-1 (541.05 kg/ha). Further, this DUS Study can be play an important role in maintain of genetic purity of a genotypes as well as in characterization of genotype. Above findings were also supported by Kumar et al. (2007), Joshi et al. (2015), Srivastava et al. (2018) and Shahaji et al. (2020).

DUS characterization of new variety is very important plant breeding program in the era of IPR (Intellectual Property Rights). It provides guarantee of distinct, uniform and stable which has been specified by the breeder. It is very important component of Plant Varieties and Farmers' Rights Act (2001) because it provides basic information of variety which helps in protection (Yadav et al. 2013).

Molecular characterization

With help of SSR markers, the promising genotype HWS 8-18 was also compared with other varieties/ genotypes. It was found unique and different from all other genotypes/varieties. The detailed results on gene distribution among the genotypes/varieties are presented in Table 4. The genotype HWS 8-18 is differentiated on the basis of presence of CAMS 34 primer 50kb band which was absent in HWS-205, HWS-205 and HWS 12-12. In addition to this, it was differentiated from HWS 1203 and JA-20 due to absent of CAMS 34 primer 400kb band, as this was present in HWS 8-18. Parita et al. (2018) also worked on Ashwagandha by using SSR makers.

Conclusions

On the bases of above study on ashwagandha genotype, it can be concluded that HWS 8-18 has been found superior in dry root yield compared to ASW-1 (best check) 10.78% at National level and 40.99% higher in the Haryana State. The mean seed yield of HWS 8-18 was 610.87 kg/ha compared to 551.43 kg/ha of ASW-1 (best check) recorded at National level while at Hisar the mean seed yield was found to be 767 kg/ha as against ASW-1 (544 kg/ha). The genotype HWS 8-18 has given numerically high seed yield (579.41 kg/ha) and at par with best check, AWS-1 (541.05 kg/ha). Lanceolate, smooth, medium dark green leaves, orange yellow berry colour and semi-erect are the marker characters for the HWS 8-18 genotype. On the bases of morphological traits and molecular characterization it can be concluded that HWS 8-18 is superior genotype.

Table 1. List of SSR primers used in the present study.

No	Primer Name	Primer Sequence	% GC Content	Tm (°C)
1	CAMS-340	F: TTTATGCCCATTCACAAAATAA R:GGACGAATTCACCGAGTGC	41.00	66.0
2	CAMS-351	F: CGCATGAAGCAAATGTACCA R: ACCTGCAGTTTGTTGTTGGA	45.00	50.0
3	CAMS-376	F: GGTGCTGGCATAGATGAACA R: TATGTCTGGCTTGGTGCTGA	50.00	69.0

F: Forward primer; R: Reverse primer; Tm: Melting temperature

Table 2. Plant description of HWS 8-18 variety of Ashwagandha.

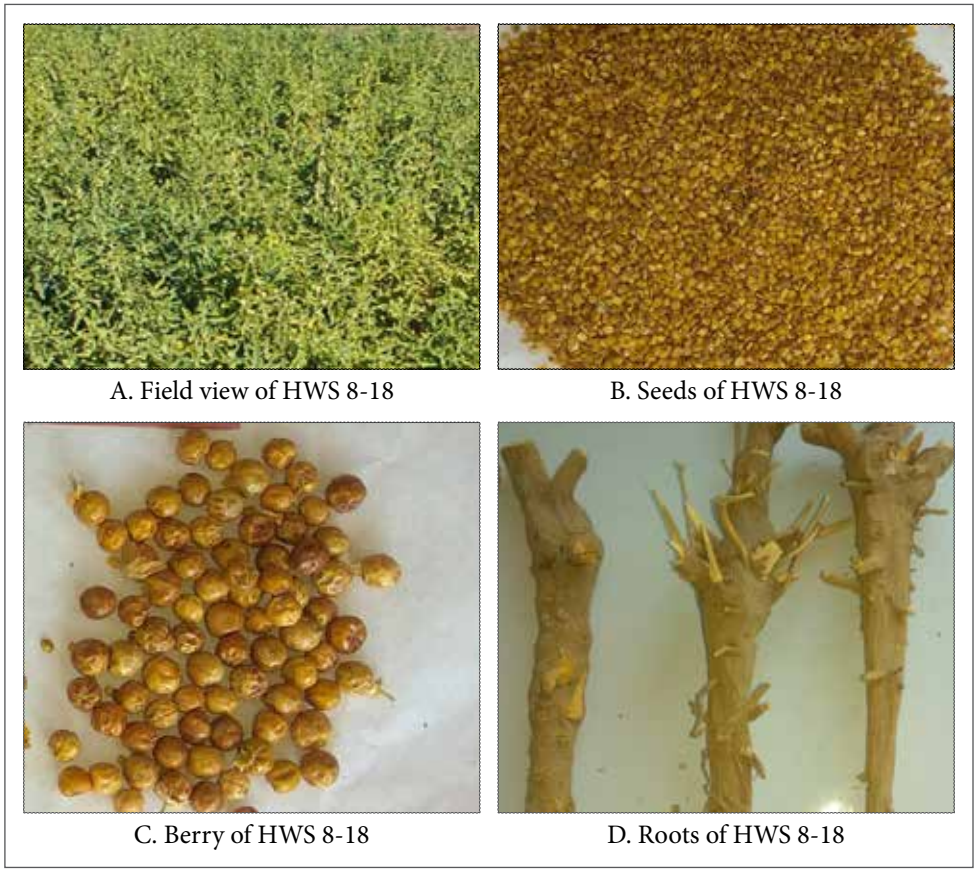
Morphological DUS Characters of HWS 8-18	Lenceolate, smooth, dark green leaves, yellow-orange berry colour and semi-eract plant type
Mean dry root yield (kg/ha)	610.87
Mean seed yield (kg/ha)	579.41
Mean plant height (cm)	63.99
Days to Flowering	87.56
Maturity days	167.45
Root length (cm)	20.59
Root diameter at collar region (mm)	11.89
Number of root branches/plant	2.54
Dry plant yield (kg/ha)	3265.54
Number of stem branches/plant	3.80
Withaferin-A (%)	0.42
Withanolide-A (%)	0.59
12-deoxy-withastramanolide (%)	0.27

Table 3. Dry root yield data of coordinated varietal trials.

Particulars	Year of Testing	No. of Trials	HWS 8-18	HWS 12-12	Check Variety AWS-1	Check Variety JA 20
Mean dry root yield (kg/ha)	2015-16	7	647.29	620.08	637.93	500.02
	2016-17	9	606.78	565.00	525.44	481.33
	2017-18	9	578.55	590.57	490.91	441.92
	Mean		**610.87**	**591.88**	**551.43**	**474.42**
Percent increase over best check	2015-16		1.47	-2.80		
	2016-17		15.48	7.53		
	2017-18		17.85	20.30		
	Mean		**10.78%**	**7.34%**		

Table 4. Profile of the gene distribution in entries and checks using SSR.

No	Genotype\Marker	CAMS 340			CAMS 351			CAMS 376			
	Allele Size	50kb	200kb	400kb	50kb	130 kb	420kb	130kb	170kb	200kb	350kb
1	HWS 8-18	+	+	+	-	-	+	-	-	-	-
2	HWS-205	-	+	+	-	-	+	-	+	-	-
3	HWS-222	-	+	+	-	-	+	-	+	-	-
4	HWS 1203	+	-	-	-	-	-	+	-	-	-
5	HWS 12-12	-	+	+	-	-	+	-	+	+	+
6	JA - 20	+	-	-	+	+	-	-	+	+	+



A. Field view of HWS 8-18 B. Seeds of HWS 8-18

C. Berry of HWS 8-18 D. Roots of HWS 8-18

Figure 1. Ashwagandha HWS 8-18 (A) Field view (B) Seed (C) Berry and (D) Roots. (Original)

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