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Research Article

Inheritance of growth habit and berry colour in ashwagandha (*Withania somnifera* (L.) Dunal) – A medicinal plant

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Abstract
Genetics studies were studied for the two qualitative character ivz., growth habit (Erect × Procumbent) and for berry (fruit) colour (Yellow × Red) in ashwagandha. The inheritance of the growth habit was controlled by single gene and the procumbent was dominant over the erect type. The gene symbol P for procumbent and p for erect is proposed. Inheritance studies of yellow versus red berry colour indicated that the trait was in control of classical duplicate recessive epitasis and followed the ratio of 9:7 (yellow: red). The berry colour in this cross is controlled by two genes (Y_1 and Y_2) with complementary recessive epitasis and gene symbols are proposed.

Keywords: *Withania somnifera;* growth habit, berry colour, genetics.

Introduction

Ashwagandha [*Withania somnifera (L.)* Duaal] (2n=48) is an erect branching shrub belonging to the family Solanaceae. The cultivated ashwagandha plants attain 30-60 cm height while the wild type reach 90-120 cm, Because of its high medicinal properties and demand this species is under cultivation generally in late *kharif (*August-September sowing) season as rain-fed crop in central India. It is in use as medicinal herb for a very long time and suitable for all age groups and sexes even during pregnancy without any side effects (Sharma *et. al*., 1985). As the plant has high national and international demands it has been brought under commercial cultivation and few varieties have been released (Manivel, 2010 a). These varieties were developed through pure line selection from the local cultivars. Even though species is under the cultivation for more than century, the systematic breeding approaches have been given important only in recent years (Manivel, 2010a and 2010b).

Morphological markers are much useful in maintenance of variety and germplasm. In ashwagandha, as such there were very few markers traits available. Directorate of Medicinal and Aromatic Plants Research (DMAPR) has initiated a systematic breeding for improvement of ashwagandha and in 2009, a new growth habit i.e. procumbent plant (DWS - 6) have been reported (Anonymous, 2009). In contrast to the normal erect growth habit of ashwagandha, DWS-6 had procumbent (spreading on the ground). Berry colour in ashwagandha is an important morphological marker. In ashwagandha, usually the berry colour of the cultivated type is yellow while the wild type has red berries. However, as both wild (perennial) and cultivated (annual) types are easily crossable (Kaul *et al.* 2005) now berries with both colors are available both with annual and perennial growth habits. These traits could be used as potential marker in crop improvement programme. It is essential to know the inheritance pattern of these characters so that these could be used as markers traits and could be incorporated in a desirable genotype. Hence, the present study was undertaken to study the inheritance pattern of two morphological traits i.e. growth habit and colour of berry. To our knowledge there are no studies on genetics of traits in ashwagandha and this is the first report.

Material and Methods

The study was conducted at Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat, India. The experimental field lies in the latitude of 22.5^{o} North and longitude 73.0^{o} East with an average rainfall of 800 mm, minimum and maximum temperatures between 12.7^{o} and 42^{o} C. The ashwagandha pure line DWS-326 has erect growth habit with red color berries was used as female parent crossed with the pure line DWS-6 with procumbent growth habit and yellow color berries. During late *kharif* (2^{nd} fortnight of August – September) 2010, emasculation of erect type was done in the evening (4.00-5.00 pm) of a day before anthesis and the emasculated buds were tagged and bagged. In the next day, pollination was done with

fertile and viable pollen of the male parent in the morning 9:00 to 11:00 am. A total of 25 flowers of female parent were emasculated and pollinated with anthers of male parent. Berry setting was observed in 19 flowers out of which three were damaged by insects and birds. Sixteen berries were harvested and finally 483 well filled hybrid seeds were obtained. During late *kharif* 2011, F_1 hybrid seeds were raised and selfed to obtain F_2 seeds. The seeds of parents, F_1 and F_2s were raised in an unreplicated block with spacing of 45 × 30 cm during late *kharif 2012*. In F_2, there were 289 plants and data were recorded on each plant for growth habit (erect or procumbent) and colour of the berries (yellow or red). The data on growth habit and color of berries obtained in F_2 generation were analyzed using χ^2 test for observed and expected frequencies and the significance was also tested.

Result and Discussion:

This experiment was conducted to study the inheritance of growth habit in *Withania somnifera (*L.) Dunal. Two parents DWS-326 with erect growth habit and DWS - 6 with procumbent (spreading) growth habit (Fig 1.), their F_1 and F_2 generations were used in the study.

Inheritance of growth habit All the F_1 plants were procumbent which indicated that procumbent is dominant over erect growth habit. In F_2 generation, out of 289 plants observed, 203 were procumbent and 86 were erect indicating the Mendelian inheritance pattern. The segregation shows good fit to monohybrid ration of 3:1 which indicates that procumbent growth habit is controlled by single dominant gene and erect growth habit by its recessive allele. The data were confirmed by χ^2 test which shows non-significant chi-square value for growth habit (Table 1). The gene symbols PP for procumbent and pp for erect type plant are proposed (Table 1.).

Inheritance of berry colour Genetics of berry colours (yellow and red) were studied in F_1 and F_2 progenies of the cross, DWS-326 × DWS-6. Appearance of yellow and red coloured berries are 194 and 95 plants, respectively in F_2, fitting with 9:7 ratio suggested that the trait was digenically inherited. Absence of dominant allele only of two genes produced red berry colour (Table 2). Talukdar and Biswas (2007) reported similar type of gene action for green and blackish purple colour of stipules in grass pea.

In the present study, all the F_1 plants had yellow colour berries (Table 2). Out of 289 F_2 plants, 194 had yellow berry while, 95 had red berry. This ratio fitted well with the 9 (yellow): 7 (red) ratio, indicating epistatic gene action. The gene symbols Y (yellow colour of berry) and y (red) are proposed. The berry colour in this cross of ashwagandha is controlled by two genes (Y1 and Y2) with complementary recessive epistasis. The expected ratio have been explained in Table 2 with gene symbols Y_1y_1 and Y_2y_2).

Complementary gene interaction can result when two or more genes code for genes that function at different locations in the same way, so that functional products from all genes involved which needed for production of the final gene product. Results in this study according to the chi-square test fitted the two phenotypic classes. This observed ratios suggest that inheritance of berry colour in ashwagandha is qualitative and influenced by duplicate recessive epistasis.

Thus, the present investigation reveals that in ashwagandha procumbent growth habit is controlled by single dominant gene over erect growth habit, while the berry colour (yellow vs red) was controlled by duplicate recessive epitasis. The simple inheritance pattern of growth habit indicated that this character can be easily incorporated in to a desirable genotype and can also be used as marker in a breeding programme.

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Table 1. Segregation for growth habit and assigned gene symbols for parents, F_1 and F_2 generation for the cross on DWS 326 × DWS 6 for procumbent and erect growth habit of ashwagandha

Generation	Erect	Procumbent	Expected χ^2 ratio	χ^2 value	P value	Gene symbols	Phenotype
P_1 (DWS-326)	41	-	00:01	0	1	PP	Erect
P_2 (DWS-6)	-	23	01:00	0	1	PP	Procumbent
F_1 (DWS-326 × DWS-6)	0	21	00:01	0	1	Pp	Procumbent
F_2	86	203	03:01	3.489	<0.01	PP	Procumbent
						Pp	Procumbent
						pP	Procumbent
						pp	Erect

Table 2. Segregation for berry colour and assigned gene symbols for parents, F_1 and F_2 generation for the cross on DWS 326 × DWS 6 for yellow and red colour berries of ashwagandha

Generation	Yellow berry	Red berry	Expected χ^2 ratio	χ^2 value	P value	Gene symbols	Phenotype
P_1 (DWS-326)	-	41	00:01	0	1	$Y_1Y_1Y_2Y_2$	Yellow
P_2 (DWS-6)	23	-	01:00	0	1	$y_1y_1y_2y_2$	Red
F_1 (DWS-326 × DWS-6)	21	0	01:00	0	1	$Y_1y_1Y_2y_2$	Yellow
F_2	194	95	09:07	13.94	<0.01	Y_1- Y_2-	Yellow
						Y_1Y_1 y_2y_2	Red
						$y_1y_1Y_2Y_2$	Red
						$y_1y_1y_2y_2$	Red

Fig. 1. DWS-6 – a procumbent type plant with yellow colour berries as compared to DWS 326, an erect plant type with red colour berry of Ashwagandha