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SHORT COMMUNICATION

SEED PROTEIN CHARACTERIZATION FOR MORPHOTYPE IDENTIFICATION IN *WITHANIA SOMNIFERA* (L.) DUNAL

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Characterization of five promising morphotypes, viz. AGB (Ashwagandha germplasm bank) -002, AGB-009, AGB-015, AGB-025 and AGB-030 of *Withania somnifera* have been made on the basis of seed protein profiles. SDS-PAGE of different morphotypes, revealed variation in number, width and intensity of bands. The molecular weight of protein bands by SDS-PAGE obtained varied between 29.0 to 97.5 kD (Kilo-dalton) in seeds of all morphotypes. There was morphotype specificity in electrophoretic pattern of seed protein. The observations revealed that seed protein polymorphism provides a useful information regarding variability among morphotypes under investigation. This offers a useful and rapidly performed adjunct to traditional morphological methods of identification in *Withania somnifera*.

***Key words*: Ashwagandha, electrophoresis, Indian ginseng, protein polymorphism.**

Classical identification of a cultivars is carried out on the basis of morphological markers (grow out test) and / or with the help of certain additional physiological traits. Cultivars identification based on standard morphological markers has proved to be inadequate because of wide range of phenotypic variation and their interaction with environment. In recent years biochemical attributes such as protein markers provide useful evidences which serve as a reliable adjunct to more traditional methods to study variation within cultivars (Goyal *et al*. 1986, Cooke 1995, Rao *et al.* 2001).

Withania somnifera (L.) Dunal (Indian ginseng) commonly known as Ashwagandha or Asgandh is an evergreen, tomentose perennial shrub and most valued medicinal plants in ayurveda and other traditional systems of medicine (Singh and Kumar 1998). Various pharmacological activities associated with this plant attributed to the presence of withanolides and alkaloids (Budhiraja *et al*. 2000). Natural chemo-variability is wide spread in this plant specie, which is not preferred in drug industry in order to maintain uniformity of the pharmaceutical preparations. Owing to this proper cultivar identification to maintain uniformity of the drug is highly desirable. Hence reliable procedures for characterization are required for identification of cultivars in this plant specie. In this study, seed proteins of Ashwagandha (*Withania somnifera*) morphotypes were characterized by analyzing seed protein polymorphism by SDS-PAGE.

Five different accessions of *Withania somnifera* collected from different geographic locations enlisted in Table 1 were grown in uniform field conditions at RRL, Jammu, India (32.43 N 74.54 E). Seed collected from each of the accessions utilized as experimental material. Protein content was estimated following the method of Lowry *et al*. (1951). The colour intensity was measured

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Table 1. Morphometric characteristics of morphotypes used and location of collections.

Accessions	Locations	Latitude	Longitude	Morphometric characteristics
AGB-002	Bikaner-Rajasthan	28.01 N	73.22 E	Perennial, tall (100-110 cm), 4-5 branches, root yield 21-23 g plant^{-1} with red berry
AGB-009	Amritsar-Punjab	31.37 N	74.55 E	Perennial, tall (100-120 cm), 4-5 branches, root yield 15-28 g plant^{-1} with red berry
AGB-015	Gajiabad-Uttar Pradesh	28.40 N	77.28 E	Annual, dwarf (40-50 cm), 2-3 branches, root yield 3-6 g plant^{-1} with orange berry
AGB-025	Neemuch-Madhya Pradesh	24.27 N	74.52 E	Annual, dwarf (35-40 cm), 6-7 branches, root yield 2-6 g plant^{-1} with orange berry
AGB-030	Bhopal-Madhya Pradesh	23.16 N	77.36 E	Perennial, tall (70-75 cm), 2-3 branches, root yield 3-6 g plant^{-1} with red berry

at 750nm. Seed protein polymorphism was studied by one dimensional SDS-PAGE (10 % separating gel and 5 % stacking gel) following the method of Laemmli (1970) in a mini vertical gel system. The soluble protein was extracted by homogenizing the seeds with 0.1M Tris-Hcl buffer pH 7.5, 0.5% β-Mercaptoethanol and 1% Polyvinyl Pyrollidone at 4 ^{0}C in a mortar and pestle. To minimize proteolytic activity a pinch of the protease inhibitor, PMSF (Phenyl methyl sulphonyl fluoride) was added during grinding. The extract was centrifuged at 9500×g and 4 ^{0}C for 20 minutes. The extract of each sample was mixed with an equal volume of sample buffer (0.0625 M Tris-HCl buffer pH 6.8, containing 10 % SDS gels as per method. Standard proteins 3.5 kD to 100 kD were run along with the 10 µl sample. The gel was run at 20.4 mA for 2 hours followed by staining with Coomassie brilliant blue R-250 and destained with repeated washings with mixture of MeOH: ACOH: H_2O (50: 70: 880 v/v/v respectively) till the bands were clearly visible. Relative mobility (Rm) of the protein band was determined.

Different morphotypes recorded variable seed protein content (Table 3). AGB-002 displayed highest protein content. AGB-025 on the other hand recorded minimum seed protein content. Seed protein electrophoregrams were analysed for presence and/ or absence of protein bands. The protein band profile for each was obtained. Protein zymogram of five morphotypes is shown in Fig. 1. The morphotypes were distinguishable from each other by determining the presence and/ or absence of specific protein band/s in their electrophoregrams (Table 2). The seed protein band profiles of all five morphotypes revealed variation in number, width and intensity of bands. This clearly showed a distinguishable pattern for each morphotype. The maximum 12 protein bands were exhibited in different morphotypes, being maximum 12 in AGB-002 and minimum 3 in AGB-030. The accession namely AGB-009 having band number 4 was missing. In AGB-015 band number 3, 4, 5, 6, 7 and in AGB-025, band number 1, 3, 4, 5, 6, 7, 8 were missing. In AGB-030 accession, band number 2, 10 and 12 were present

Table 2. SDS-PAGE banding pattern of seed proteins of five selected accessions of *Withania somnifera* (Presence/Absence of protein band and intensity).

Band No.	Rm value	AGB-002 (12)	AGB-009 (11)	AGB-015 (07)	AGB-025 (04)	AGB-030 (03)	Band intensity	Band width	Molecular weight kD	Grouping of bands
1	0.162	+	+	+	–	–	+++	N	97.5	1
2	0.229	+	+	+	+	+	+++	B	92.5	1
3	0.266	+	+	–	–	–	++	N	88.0	1
4	0.325	+	–	–	–	–	++	N	82.0	1
5	0.488	+	+	–	–	–	+++	B	67.0	2
6	0.525	+	+	–	–	–	++	N	66.5	2
7	0.548	+	+	–	–	–	++	N	60.0	2
8	0.651	+	+	+	–	–	++	N	52.0	3
9	0.666	+	+	+	+	–	+++	B	50.0	3
10	0.740	+	+	+	+	+	+++	B	42.5	3
11	0.792	+	+	+	–	–	+++	B	37.5	4
12	0.888	+	+	+	+	+	+++	N	29.0	4

Where +: Band present, –: Band absent, ++: Faint, +++: Intense, N: Narrow, B: Broad, Rm: Relative mobility

Table 3. Soluble seed protein content and polymorphism of protein bands in selected accessions of *Withania somnifera*.

Morphotypes	Soluble protein (mg g^{-1} seeds)	Total number of bands	Peptide polymorphism of bands	
			Present	Absent
AGB-002	15.2 ± 1.01	12	97.5-29 kD	-
AGB-009	13.5 ± 0.83	11	97.5-29 kD	82.0 kD
AGB-015	8.6 ± 0.31	07	97.5-29 kD	88.0-60.0 kD
AGB-025	7.40 ± 0.65	04	92.5-29 kD	97.5, 88.0-52.0 and 37.5 kD
AGB-030	9.80 ± 0.52	03	92.5-29 kD	97.5, 88.0-50.0 and 37.5 kD

Results expressed are the mean of quadruplicates ± S.E.

while others were missing. Seed protein of accession AGB-002 expressed all the 12 bands.

Band 1, 2, 3 and 4 might be considered representing one protein group and both would likely to have molecular weight ranged between 97.5-82.0 kD. Band 5, 6 and 7 represented second group of protein with varying width and intensity pattern when compared with standard BSA (Bovine serum albumin-66 kD mol. wt.), and these bands 5, 6 and 7 would have molecular weight of 67.0, 66.0 and 60 kD' respectively. Third group comprising 3 bands 8, 9 and 10 of different width having molecular weight would be in the vicinity of 52.0-42.5 kD. The fourth group with two bands 11 and 12 of same

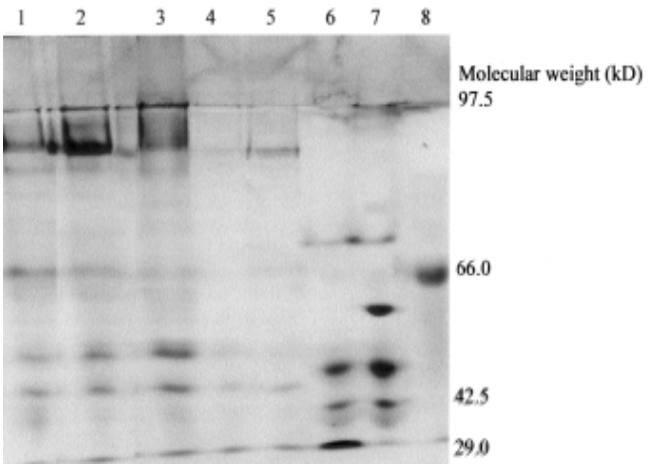

Fig. 1. SDS-PAGE electrophoretic pattern of seed storage proteins of *Withania somnifera* (1. AGB-002; 2. AGB-009; 3. AGB-015; 4. AGB-025; 5. AGB-030; 6. Marker M_1 3.5-43 kD; 7. Marker M_2 14-100 kD; 8. BSA 66 kD)

intensity would likely to represent the molecular weight in the range of 37.5-29.0 kD. The similarities of certain protein bands among accessions obviously were due to the genetic relationships among them. It is evident that seed protein polymorphism provides meaningful information which suggests and indicates existence of genetic variability among morphotypes of *Withania somnifera* studied. Our results are in accordance with the earlier findings of Goyal and Sharma (2003) in Clusterbean varieties. The seed protein polymorphism reported here for the first time in *Withania somnifera* is quick, informative and reliable which could be helpful to characterize Ashwagandha morphotypes.

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