J. Phytol. Res. 20(2): 189-192, 2007

SEED PROTEIN PROFILES IN *WITHANIA SOMNIFERA* (L.) DUN. PLANT TYPES

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Seed protein content and electrophoretic banding pattern of seed protein using SDS-PAGE has been observed in *Withania somnifera* (L.) Dun. plant types (control and 9 induced macromutants). *Thick* stem I, *bushy*, *broad leaf* and *late flowering* mutants had relatively higher protein contents. Gross similarities and differences in electrophoretic banding pattern have been noted among the genotypes. Specific protein bands have been detected in different mutants. Results obtained have been discussed.

Keywords: Macromutants; Protein profile; SDS-PAGE; *Withania somnifera*.

Introduction

Identification of germplasm diversity based on standard morphological markers has proved to be inadequate because of the wide spectrum of phenotypic variation and their interaction with environment[1]. In such instances electrophoretic patterns of seed can be used effectively to decipher interrelationship between/among genotypes and to screen protein markers for identification[2-4]. Electrophoresis technique is widely used because of its reliability, rapidity and cost effectiveness. With this view the present investigation has been undertaken for 'protein fingerprinting' of the plant types of *Withania somnifera* (L.) Dun. (control and 9 induced macromutants[5]), a medicinal crop plant of the family Solanaceae following electrophoretic banding (SDS-PAGE) polymorphism.

Materials and Methods

Seed protein content of the genotypes (control and 9 macromutants) was estimated following Lowry *et al.*[6]. Protein fractionations were done by the method of Osborne[7]. To study protein polymorphism in *W. somnifera* plant types (control; mutants: *dwarf, lax branching, thick stem* I *and* II, *bushy, broad leaf, ovate leaf, early flowering and late flowering*), one dimensional SDS-PAGE (10% separating gel and 4.5% stacking gel) was carried out following Laemmli[8] in a vertical gel system. For the purpose, total protein was extracted in 0.2M Tris-HCl buffer (pH = 8.5), suspended overnight (0-4°C) and centrifuged at 15000 rpm (- 4°C) for 30 minutes. The protein samples along with sample buffer containing bromophenol blue were hydrolysed in boiling water (1 – 2 mins.), cooled and loaded in lanes with micropipettes (8 µl/lane). A protein molecular weight marker (GENEI, Bangalore, Cat. No. PMW – M) was also incorporated into the gel (as marker lane) as reference to detect molecular weights of bands. The gel was run at 30mA (3 mA/lane) for 2 hours, stained in Coomasie Brilliant Blue R250 overnight, destained and stored in 7% acetic acid.

Gel preparation was analyzed in a gel documentation unit (Ultra Lum, USA) using the software Total Lab. Bands were detected and molecular weights and pixel peak (based on area, volume and intensity of the bands) of each band were computed.

Results and Discussion

Seed protein content (%) in control was noted to be 12.00 and it was 11.25 in *dwarf*, 10.75 in *lax branching*, 20.50 in *thick stem* I, 10.75 in *thick stem* II, 15.25 in *bushy*, 18.00 in *broad leaf*, 10.00 in *ovate leaf*, 10.75 in *early flowering* and 18.23 in *late flowering*.

The protein banding pattern of all genotypes (Fig. 1) were compared and results are presented in Table 1. Diverse banding pattern were evidenced in all the genotypes. R_m values ranged from 0.163 to 0.945 indicating wider range of variability in protein band expression. Molecular weights of the bands varied from 13.2 kD to 104.1 kD. Among the plant types, total number of bands ranged between 17 to 39. Number of polypeptide bands detected were 30 in control, 17 in *dwarf*, 23 in *lax branching*, 37 in *thick stem* I, 32 in *thick stem* II, 30 in *bushy*, 30 in *broad leaf*, 34 in *ovate leaf*, 39 in *early flowering* and 36 in *late flowering*. The protein band number 4, 6, 12, 15, 22, 24, 31 and 34 to 40 were universally present in all genotypes, which indicated that the genes controlling the expression of these bands appeared to behave as a single block. Polypeptide band number 19 and 23 were exclusively absent in control and *dwarf* respectively; while, 17 was specific for *early flowering*

Table 1. SDS-PAGE banding pattern of seed storage protein in control and macromutants of *W. somnifera*.

Band number	R_m value	MW (kD)	Plant types									
			Control	*Dwarf*	*Lax branching*	*Thick stem I*	*Thick stem II*	*Bushy*	*Broad leaf*	*Ovate leaf*	*Early flowering.*	*Late* Flowering
1	0.163	104.1	-	-	-	+	-	-	-	+	+	+
2	0.202	92.8	-	-	-	+	-	-	-	+	+	+
3	0.220	87.4	-	-	-	+	-	-	-	-	+	-
4	0.232	83.9	+	+	+	+	+	+	+	+	+	+
5	0.258	76.3	+	+	+	+	+	+	-	+	+	+
6	0.270	73.1	+	+	+	+	+	+	+	+	+	+
7	0.297	66.7	-	-	-	+	+	-	-	+	+	+
8	0.309	64.1	-	-	-	+	+	+	-	+	+	+
9	0.339	58.5	+	-	-	+	+	+	-	+	+	+
10	0.369	53.9	+	-	-	+	+	+	+	+	+	+
11	0.384	51.9	-	-	-	+	-	-	+	+	+	-
12	0.402	49.7	+	+	+	+	+	+	+	+	+	+
13	0.414	48.4	+	-	-	+	-	-	+	-	+	+
14	0.426	47.1	+	-	-	+	+	+	+	+	+	+
15	0.453	44.4	+	+	+	+	+	+	+	+	+	+
16	0.483	41.6	+	-	-	+	+	+	+	+	+	+
17	0.516	38.6	-	-	-	-	-	-	-	-	+	-
18	0.546	36.0	+	-	-	+	-	+	+	+	+	+
19	0.573	33.8	-	+	+	+	+	+	+	+	+	+
20	0.582	33.1	+	-	-	+	+	+	+	+	+	+

Continued.....

Table 1. continued...

Band number	R_m value	MW (kD)	Plant types									
			Control	*Dwarf*	*Lax branching*	*Thick stem I*	*Thick stem II*	*Bushy*	*Broad leaf*	*Ovate leaf*	*Early flowering*	*Late* Flowering
21	0.591	32.4	+	-	-	+	+	+	+	+	+	+
22	0.600	31.7	+	+	+	+	+	+	+	+	+	+
23	0.633	29.4	+	-	+	+	+	+	+	+	+	+
24	0.648	28.4	+	+	+	+	+	+	+	+	+	+
25	0.678	26.6	+	-	+	+	+	+	-	+	+	+
26	0.690	25.9	+	-	+	+	+	+	+	+	+	+
27	0.699	25.4	+	-	+	+	+	+	+	+	+	+
28	0.717	24.5	+	-	+	+	+	+	+	+	+	+
29	0.729	23.9	+	-	+	+	+	+	+	+	+	+
30	0.735	23.6	-	-	-	+	+	-	-	-	-	-
31	0.756	22.6	+	+	+	+	+	+	+	+	+	+
32	0.789	21.1	+	-	-	-	+	-	+	-	+	+
33	0.816	19.8	-	+	+	-	-	-	+	-	+	+
34	0.834	18.9	+	+	+	+	+	+	+	+	+	+
35	0.843	18.4	+	+	+	+	+	+	+	+	+	+
36	0.864	17.4	+	+	+	+	+	+	+	+	+	+
37	0.882	16.4	+	+	+	+	+	+	+	+	+	+
38	0.912	14.9	+	+	+	+	+	+	+	+	+	+
39	0.921	14.4	+	+	+	+	+	+	+	+	+	+
40	0.945	13.2	+	+	+	+	+	+	+	+	+	+
Total			30	17	23	37	32	30	30	34	39	36

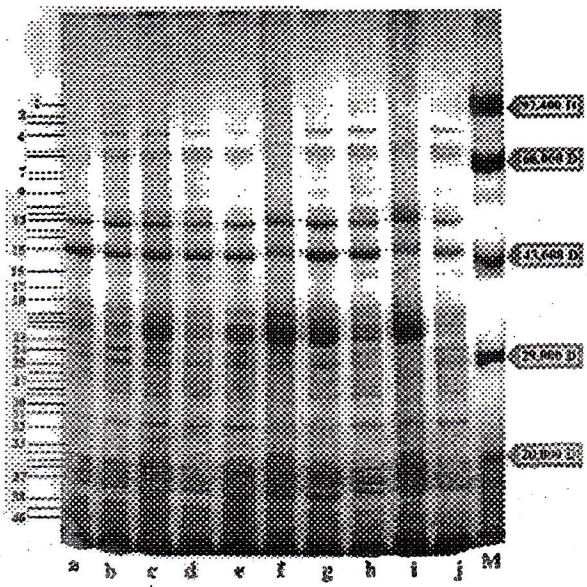

Fig. 1 (a – j, M). Protein profiles in *Withania* plant types (control and mutants) from SDS – PAGE. (a) Control (b) *Late flowering* (c) *Early flowering* (d) *Ovate leaf* (e) *Broad leaf* (f) *Dwarf* (g) *Thick stem* II (h) *Thick stem* I (i) *Lax branching* (j) *Bushy* (M) Marker.

mutant. *Dwarf* mutant lacked the expression of band nos. 1, 2, 3, 7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 20, 21, 23, 25, 26, 27, 28, 29, 30 and 32. Band nos. 1 and 2 were only present in *thick stem* I, *ovate leaf, early flowering* and *late flowering* mutants while band no. 3 was detected from *thick stem* I and *early flowering* mutants. Band no. 5 showed its expression in all genotypes excepting *broad leaf* mutant. Polypeptide band nos. 1, 2, 3, 7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 20 and 21 were absent in *lax branching* mutant. Band no. 11 has been characteristic of *thick stem* I, *ovate leaf, broad leaf* and *early flowering* while band no 13 was present only in control, *thick stem* I, *broad leaf, early flowering* and *late flowering* plant types. Polypeptide band 30 has been specific to *thick stem* I and II mutant. Band 32 is represented in all genotypes except in *bushy, lax branching, thick stem* I, *dwarf* and *ovate leaf* mutants. *Bushy, thick stem* I and II and *ovate leaf* also lacked the expression of band no. 33.

The polypeptide bands were of very high (>70.0 kD: 2 to 6), high (40.0 – 70.0 kD: 2 to 10), medium (25.0 – 39.9 kD: 3 to 11) and low (<25.0 kD: 9 to 12) molecular weights. *Thick stem* I and *early flowering* mutants had the maximum number of very high to high molecular weight bands; while, *dwarf* mutant demonstrated the minimum number of such bands. Based on pixel intensity, the bands were classified into faint (< 80 pixel; number : 3 to 13), medium (80-100 pixel; number : 2 to 18) and intense (>100 pixel; number : 4 to 22) and the genotypes were characterized as follows : 6 F + 2 M + 22 I in control, 3 F + 1 M + 13 I in *dwarf*, 3 F + 9 M + 11 I in *lax branching*, 13 F + 14 M + 10 I in *thick stem* I, 8 F + 6 M + 18 I in *thick stem* II, 8 F + 18 M + 4 I in *bushy*, 6 F + 6 M + 18 I in *broad leaf*, 12 F + 11 M + 11 I in *ovate leaf*, 9 F + 11 M + 19 I in *early flowering* and 8 F + 11 M + 17 I in *late flowering*.

Thus, electrophoretic characterization of seed protein of the mutant plant types compared to control may be used as an additional parameter in better understanding of genetic variations occurring among them. Such variations can be utilized in breeding programme for improvement. Further, this kind of study could lead to the detection of genotype specific band(s) which may be used as reliable seed protein marker.

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