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SEED PROTEIN PROFILE IN SESAME (SESAMUM INDICUM L.) PLANT TYPES

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Electrophoretic banding pattern of seed protein using SDS-PAGE has been observed in sesame (*Sesamum indicum* L.) plant types (control and 9 induced macromutants). Gross similarities and differences in electrophoretic banding pattern have been noted among the genotypes in relation to number of bands (7 to 21, a total of 28 types), molecular weights, band percentage and pixel peak. Specific protein bands have been detected in different mutants.

Keywords : Macromutants; Polymorphism; Protein profile; SDS-PAGE; Sesame.

Introduction

The electrophoretic banding patterns of seed proteins have been effectively used to decipher the similarities and differences between genotypes and to screen protein markers for identification[1-6]. With a view to it, present investigation has been undertaken to characterize the plant types (control and 9 induced macromutants[7-9]) of sesame (*Sesamum indicum* L., an oilseed crop of the family Pedaliaceae) following electrophoretic banding (SDS-PAGE) polymorphism.

Material and Methods

To study protein polymorphism in sesame plant types (control - C; mutants: *non-shattering capsule* - NS, broad *leaf* - BL, *cluster flower* - CF, *dark reddish brown seed-coat* - DRB, *thick leaf* - TL, *globular fruit* - GF, *early flowering* - EF and *late flowering* - LF), one dimensional SDS-PAGE (10.0% separating gel and 4.5% stacking gel) was carried out following Laemmli[10] in a vertical gel system (BIOTECH). For the purpose, total protein was extracted in 0.2M Tris-HCl buffer (pH - 8.5), suspended overnight (0-4°C) and centrifuged at 15,000 rpm (- 4°C) for 30 minutes. The protein samples along with sample buffer containing bromophenol blue were hydrolyzed in boiling water (1 - 2 mins.), cooled and loaded in lanes with micropipette (8 μl / lane). A protein moleular weight marker (GENEI Bangalore, Cat No. PMW-M) was also incorporated into the gel (as marker lane) as reference to detect molecular weights of the bands. The gel was run at 33 mA (3 mA / lane) for 2 hours, stained in Coomassie Brilliant Blue R250 for overnight, destained and stored in 7% acetic acid.

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

Results and Discussion

SDS-PAGE of seed proteins of sesame plant types showed distinct polymorphism in their electrophoretic banding patterns (Fig.1) in relation to band numbers, molecular weights, band percentage and pixel peak (Tables 1-2). A total of 28 bands were detected in the plant types of which control had 15 and the mutants exhibited 7 (LF) to 21 (DB and TL). Mostly the bands were of medium molecular weights (25 Kd to 50 Kd); although, DB, TL and EF showed relatively higher number of bands with high molecular weights (> 50 Kd). Band percentage (NS : 1.4-15.5, BL : 2.7- 15.3, DB : 1.2-12.6, CF : 1.2- 16.3, C : 2.1-12.0, DRB : 1.1-18.3, TL : 1.4-10.7, GF : 2.1-17.0, EF : 0.4-13.9 and LF : 6.2-16.2) and pixel peak (NS : 59.9 - 193.6, BL : 90.2 - 117.7, DB : 51.2 - 149.4, CF : 65.6 - 172.6, C : 59.3 - 165.3, DRB : 113.5 - 181.1, TL : 60.1 - 189.7, GF : 68.7 - 188.5, EF : 67.6 - 182.2 and LF : 74.0 - 175.7) noted for individual band among the genotypes indicated predominant occurrence of thinner and lighter bands; however, DRB showed only medium to dark bands.

Although banding pattern varied, band 17, 25, 26, 27 and 28 were common and 11, 12, 13, 14, 18 and 23 appeared mostly (excepting LF and band 11 in GF) among the genotypes. Band 1, 2 and 8, 16 and 9 were characteristically present in DB, TL, LF and EF respectively; while, 3, 4 and 21 have been common in DB and TL. Band 5 was observed in DB, TL and EF as compared to 10 in DB, CF and GF, while 15 was studied only in BL, DB and CF. Results indicated gross similarities and differences in banding pattern and it seems that LF is characteristically different from the rest. Seed protein expression resulting in the formation of polypeptide bands has been considered to be completely dominant over non-expression[11]. Mutation in regulatory and/or structural genes may lead to failure of protein expression[12]. In the context it can be inferred that presence or absence of

Table 1. Polypeptide banding patterns in sesame plant types.

No. of bands	Rm value	Plant types									
		Non-shattering capsule	*Broad leaf*	*Diffused branching*	*Cluster flower*	Control	*Dark reddish brown seed-coat*	*Thick leaf*	*Globular fruit*	*Early flowering*	*Late flowering*
1	0.08	-	-	+	-	-	-	-	-	-	-
2	0.11	-	-	-	-	-	-	+	-	-	-
3	0.13	-	-	+	-	-	-	+	-	-	-
4	0.16	-	-	+	-	-	-	+	-	-	-
5	0.19	-	-	+	-	-	-	+	-	+	-
6	0.22	+	-	-	-	-	-	+	-	+	-
7	0.25	+	-	+	-	+	-	+	-	+	-
8	0.26	-	-	+	-	-	-	-	-	-	-
9	0.30	-	-	-	-	-	-	-	-	+	-
10	0.36	-	-	+	+	-	-	-	+	-	-
11	0.38	+	+	+	+	+	+	+	-	+	-
12	0.41	+	+	+	+	+	+	+	+	+	-
13	0.43	+	+	+	+	+	+	+	+	+	-
14	0.45	+	+	+	+	+	+	+	+	+	-
15	0.49	-	+	+	+	-	-	-	-	-	-
16	0.59	-	-	-	-	-	-	-	-	-	+
17	0.62	+	+	+	+	+	+	+	+	+	+
18	0.64	+	+	+	+	+	+	+	+	+	-
19	0.69	-	-	-	-	+	-	-	+	+	+
20	0.71	+	-	-	+	+	-	+	+	+	-
21	0.73	-	-	+	-	-	-	+	-	-	-
22	0.74	-	+	+	+	+	-	+	+	+	-
23	0.76	+	+	+	+	+	+	+	+	+	-
24	0.80	-	-	-	-	-	-	+	-	-	-
25	0.90	+	+	+	+	+	+	+	+	+	+
26	0.92	+	+	+	+	+	+	+	+	+	+
27	0.93	+	+	+	+	+	+	+	+	+	+
28	0.98	+	+	+	+	+	+	+	+	+	+
Total no. of bands		**14**	**13**	**21**	**15**	**15**	**11**	**21**	**14**	**18**	**07**

Table 2. Molecular weights, band percentage and pixel peak in different plant types of sesame.

Plant types	Molecular weight (Kd)			Band percentage			Pixel peak		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
	<25.0	25.0-50.0	>50.0	<5.0	5.0-10.0	>100.0	<100.0	100.0-150.0	>150.0
Non-shattering capsule	4	7	3	7	2	5	5	1	8
Broad leaf	4	8	1	4	5	4	3	4	6
Diffused branching	4	9	8	14	5	2	13	8	-
Cluster flower	4	9	2	6	5	4	7	2	6
Control	4	10	1	8	3	4	6	5	4
Dark reddish brown seed-coat	4	6	1	3	5	3	-	3	8
Thick leaf	4	11	7	15	2	4	12	3	6
Globular fruit	4	9	1	7	3	4	8	-	6
Early flowering	4	9	5	11	4	3	10	3	5
Late flowering	4	3	-	-	2	5	3	1	3

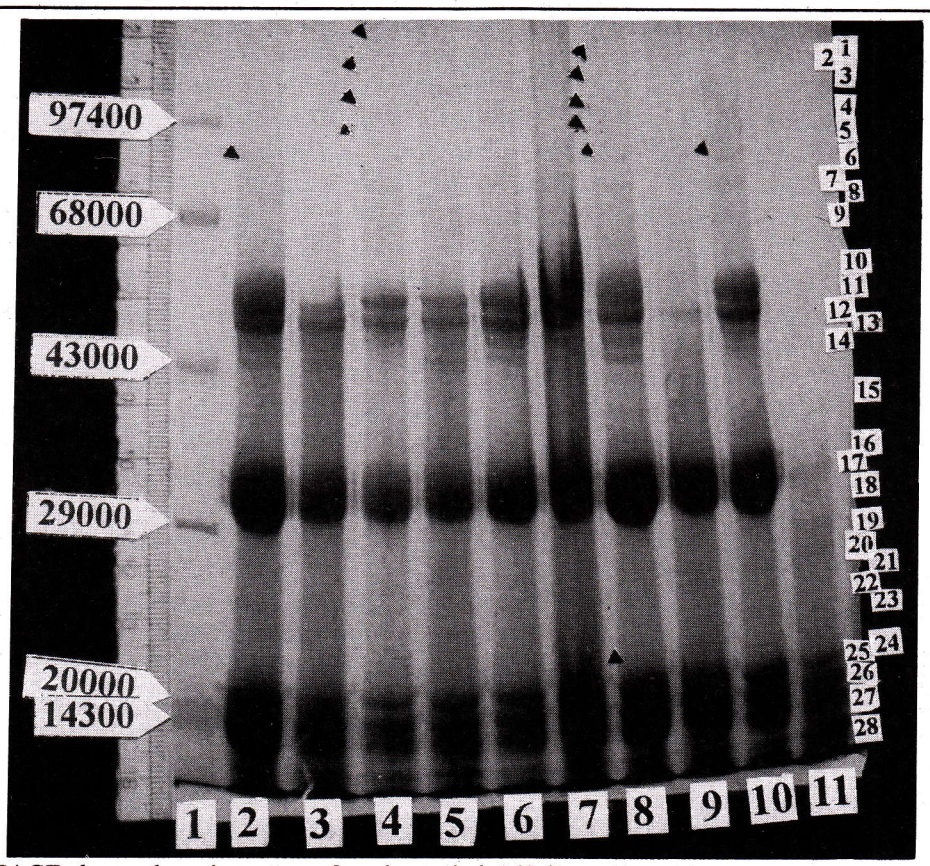

Fig.1. SDS-PAGE electrophoretic pattern of seed protein in NS (Lane 2), BL (L-3), DB (L-4), CF (L-5), C (L-6), DRB (L-7), TL (L-8), GF (L-9), EF (L-10) and LF (L-11). Marker protein with molecular weights in Lane 1. Pin head arrow has been used to mark band 1, 3, 4 and 5 in L-4; 2,3,4,5,6 and 24 in L-8 and 6 in L-2 and 10 for their faintness.

polypeptides in different mutants as compared to control plant type of sesame can be used as reliable marker for their identification and preparation of protein profiles.

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