ELECTROPHORETIC ANALYSIS OF SEED PROTEINS OF VARIOUS COTTON VARIETIES OF DIFFERENT GEOGRAPHIC AREAS

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Nine varieties of American Upland and American-Egyptian cotton, *viz.* TM1, UKA B1-(72)049, Bulgaria 996, Coker 100 WILT, Acala 4-42 WR, Deltapine 16, Gregg 35 x L, Pima 55 and Sea Island Seaberry, from different geographic areas, were tested for their genetic variation through polyacrylamide gel electrophoresis of their seed proteins. Considerable variation in their protein banding patterns were observed.

INTRODUCTION

Polyacrylamide gel electrophoresis of seed proteins offers a biochemical approach to understand the evolutionary aspects of plant species. The background and theory of polyacrylamide gel electrophoresis were thoroughly discussed by Ornstein (1964), and the method and application of the technique to analyse and compare human serum protein were presented by Davis (1964). Amino acid changes within a protein, due to mutational changes, can result in altered protein migration rates when the proteins are compared in the matrix system of polyacrylamide. Therefore, since species differ genetically at many loci, the individuality of each plant species can usually be expressed according to its protein banding pattern. Steward *et al.* (1965), Boulter *et al.* (1966) and Sastry and Virupaksha (1967) modified the polyacrylamide gel electrophoretic technique so as to detect protein changes in developing and in differentiating seedlings and to examine protein content of seeds.

The electrophoretic work of many researchers elucidated or confirmed taxonomic relations between species obtained previously by well known classical methods. Vaughan *et al.* (1966) with brassica and Johnson and Hall (1965) and Johnson *et al.* (1967) with *Triticum*, were able to confirm relationships of species as well as genomes. Larson (1967) applied this technique to separate 61 soybean varieties into two main groups on the basis of their seed protein content. Cherry *et al.* (1969) performed polyacrylamide gel electrophoresis on seed proteins of 26 species and 10 varieties of the genus *Gossypium* and compared the protein banding patterns within and between the six genomes, A, B, C, D, E and F and of different allotetraploid forms.

In this paper, efforts are made to determine genetic variability as measured by protein banding patterns through electrophoretic analysis of seed protein of 9 American upland and American-Egyptian cotton varieties of different geographic areas.

MATERIALS AND METHODS

The study was conducted at the Southern Crops Research Laboratory, Crop Germplasm Research Unit, College Station, Texas, USA during February, 1989.

Nine varieties of American Upland and American-Egyptian cotton (Table 1) were analysed for their seed protein banding pat-

terns through polyacrylamide gel electrophoresis techniques. One, 'TM1' out of these nine was used as a standard. This variety is a homozygous and true representative of *Gossypium hirsutum* species. This has been developed in Texas by continuous selfing and thus is called as TM1, i.e. Texas Marker and is used as standard in such studies.

Solutions used and preparation of protein samples:

1. Stock solutions

a. Tris-glycine electrode buffer, pH 8.33; b. Electrode buffer; c. Tris-HCl buffer, pH 8.9; d. Tris-HCl buffer, pH 6.7; e. Acrylamide solution; f. Ammonium persulfate solution; g. Bromophenol blue solution; h. 40% sucrose solution; i. Stain concentrate; j. 10% acetic acid; l. 95% Ethanol; m. Sample buffer.

Solutions (a) to (h) were stored in the refrigerator. Solutions (a) to (e) were used within 2 weeks, and solution (f) was used within 4 days.

Working solutions: These solutions were used immediately after preparation:

a. Resolving gel solution (for two 1.5 mm, 16 x 18 cm gels).
b. Stacking gel solution (for two 1.5 mm gels).

Table 1. Cotton varieties alongwith their geographic areas

Varieties	Geographic area
TM1	Texas
UKA B1-(72)049	Africa
Bulgaria 996	Bulgaria
Coker 100 WILT	East U.S.
Acala 4-42 WR	California
Deltapine 16	Texas
Gregg 35 XL	High plains
Pima S5*	Arizona, N. Mexico
Sea Island Seaberry*	Coastal Islands of South Carolina and Georgia

* American-Egyptian cotton varieties

Protein extraction: Ten seeds of each variety were delinted. The kernels were then ground with a mortar and pestle to produce a fine flour. Flour (0.4 g) was suspended in 6 ml of reagent grade water. The suspension was agitated for 30 minutes in shaker; after that the suspension was centrifuged at 10,000 RPM (12,000 g) in a refrigerated centrifuge for 30 minutes at 10 °C. The supernatant was filtered through a No. 5 A filter paper. The crude protein extract thus obtained was stored in microcentrifuge tubes under refrigeration until its use.

Protein sample: Protein samples were prepared by mixing the following components in microcentrifuge tubes:

a. 400 μl crude protein extract. b. 200 μl solution (m). c. 100 μl solution (h). d. 20 μl solution (g).

Procedure: The disc electrophoresis system described by Davis (1964) including resolving gel and stacking gel was followed with a little modification. The gels were prepared as follows:

1. Resolving gel: The Tris-HCl buffer stock solution, pH 8.9, acrylamide stock solution and reagent grade water mixed in an erlenmeyer flask with a side arm. After adding the ammonium persulphate solution to the mixture, it was swirled gently to avoid the formulation of air bubbles, and it was degassed. After this, the solution was immediately pipetted into the prepared gel moulds to a height of 12 cm and carefully overlaid with reagent grade water. The gel was left undisturbed for one hour at room temperature in light to polymerize.

Before preparing the stacking gel the overlaid solution was poured off. Any left-over solution was carefully absorbed by using a folded piece of filter paper without disturbing the gel surface. It is important that the surface of the resolving gel is dry, in order to obtain satisfactory polymerization at the resolving gel interface.

2. Stacking gel: The Tris-HCl stock solution, pH 6.7, was mixed with acrylamide stock solution and reagent grade water in an erlenmeyer flask with a side arm. The solution was degassed thoroughly with vacuum. The ammonium persulphate solution was then added while swirling gently to ensure proper mixing. The comb was inserted into each gel mould and immediately the gel solution was added until it was 2 mm from the top of the mould. The open gel surface was gently overlaid with reagent grade water and was then left at room temperature exposed to fluorescent light for one hour to polymerize.

3. Application of protein samples to the wells: 100 μl of each protein sample was applied to the wells. The same 9 samples were used in the 9 wells of 10 in each gel.

4. Gel electrophoresis: After the application of protein samples in the wells, the process of electrophoresis was conducted using an LKB 2001-001 vertical Electrophoresis Unit with LKB 2197 Constant Power Supply Unit. The chamber accommodated 2 gels having 10 wells each. The run took 6 hours to complete when dye front was 1 cm from lower end of the gels. Temperature of the buffer and gels was kept constant at 10 °C, with an LKB 2219 Multi Temp II Thermostatic Circulating Liquid Cooler.

5. Fixing and staining: Immediately upon completion of the electrophoresis, the gels were removed and immersed in the staining solution containing 100 ml of 10% acetic acid and 100 ml of stain concentrate (solution i). Fixing and staining time was one hour.

6. Destaining: Gels were destained in the first destaining solution containing 200 ml 95% ethanol and 300 ml 5% acetic acid for 30 minutes. The final destaining was done in the second destaining solution containing 150 ml 50% ethanol and 350 ml of 5% acetic acid for an overnight.

7. Drying: After destaining, the gels were soaked in a solution containing 4% glycerol and 40% ethanol for one hour. This was done to prevent the gels from cracking. The gels were then placed on wet cellophane papers supported on glass plates. Another wet cellophane paper was placed over each gel taking care that no air bubble was trapped. The cellophane sheets were then wrapped around the glass plates to hold the gels flat during the drying period. The gels were kept in open to dry for about 24 hours.

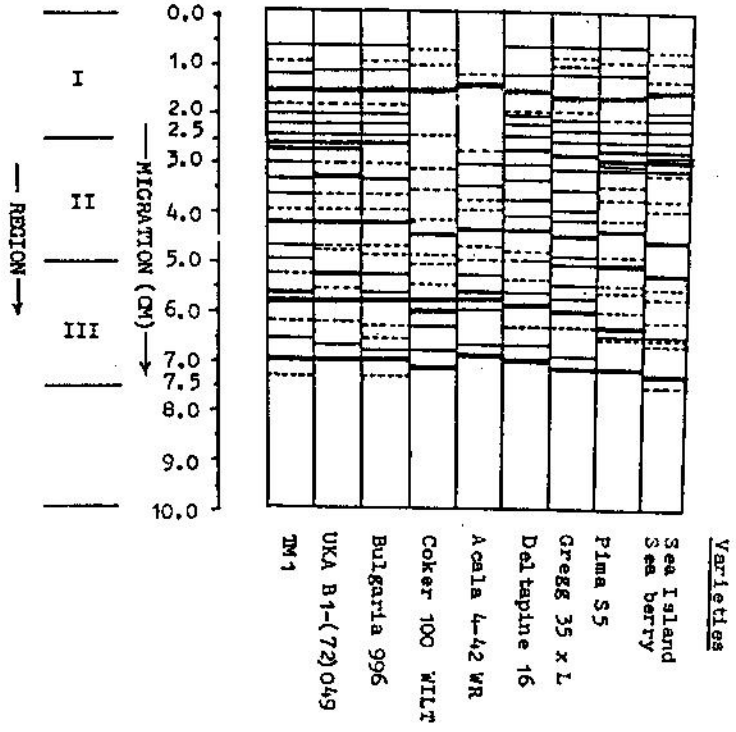
From the electrophoretograms, diagrams were drawn as in Fig. 1. To make the comparison easier, the diagrams showing migration of protein bands were divided into 3 regions measured in centimeters as follows:

Region	Scale
I	0.0-2.5 cm
II	2.6-5.0 cm
III	5.1-7.5 cm

The gels revealed a maximum of 25 protein bands, as in Sea Island Seaberry that varied among the varieties tested. The bands were coded as 1 (major), 2 (minor) and 3 (very minor) depending upon their density and sharpness.

RESULTS AND DISCUSSION

Although a full detail of position of the bands alongwith their distance of movement measured in cm; in the diagrams is pre-



Key:

= Major band
= Diffused major band
= Minor band
= Very minor band

Fig. 1.Diagrammatic acrylamide gel electrophoretic seed protein spectra of nine cotton varities.

sented in Fig. 1 and Table 2, a few considerable differences and similarities are discussed hereunder.

On an average a viable protein banding pattern in all the varieties was observed and all the varieties differed from TM1 in that respect.

Region I (0.0-2.5 cm): The region I of the diagram showed almost similar banding pattern in all the varieties with the exception of Coker 100 WILT, Acala, 4-42 WR and Sea Island Seaberry which indicated a considerable difference of protein bands in this region. A minor band observed in all the varieties at 0.7 cm was altogether absent in these three varieties. Similarly, a diffused major band with a negligible variation in the distance was present in all the varieties at 1.6 cm. A very minor protein present in TM1, UKA B1-(72)049, and Bulgaria 996 at 1.9 cm was missing in all others. Minor bands at 2.1, 2.3 and 2.5 cm were observed in TM1, UKAB1-(72)049, Bulgaria 996 and Deltapine 16 which were missing in all others where similar proteins with a little difference were found to be present at the above mentioned positions of region I except a very minor band at 2.5 cm in Coker 100 WILT.

Region II (2.6-5.0 cm): A minor band was observed at 2.6 cm in Gregg 35 x L, Pima S5, and Sea Islands Seaberry which was absent in all others. At 2.7 cm a major band was only seen in TM1, UKA B1-(72)049 and Bulgaria 996. A similar protein was shown by TM1, UKA B1-(72)049, Deltapine 16, Pima S5 and Sea Island Seaberry at 2.8 cm whereas nothing was observed in others except in Acala 4-42 WR where a very minor band could be seen at this position (Fig. 1). All the varieties showed no protein at 2.9 cm except a major band in Gregg 35 x L and a minor in Sea Island Seaberry. At 3.0 cm, only two varieties i.e. Pima S5 and Sea Island Seaberry showed a major band while this position was found vacant in all others. Whereas considerable variation of minor and very minor bands from 3.1 cm to 4.2 cm in all the varieties could be seen in Fig. 1/Table 2. Only one out of 9 varieties i.e. UKA B1-(72)049 indicated a major band at 3.4 cm. The varieties TM1, UKA B1-(72)049 and Bulgaria 996 had a major band at 4.3 cm; Acala 4-42 WR, Deltapine 16 and Pima S5 at 4.4 cm; Coker 100 WILT and Gregg 35 x L at 4.5 cm and Sea Island Seaberry at the position of 4.6 cm. Variation in the existence of minor and very minor bands was also observed at the distance from 4.7 cm to 5.0 cm in the region II (Table 2).

Region III (5.1-7.5 cm): A considerable variation in protein banding pattern could be seen in this region, for example, a major band is visible at 5.1 cm in Pima S5 whereas there is nothing at this position in all other varieties except a minor in Gregg 35 x L and a very minor in Coker 100 WILT. Similarly, a major band was observed in UKA B1-(72)049 and Sea Island Seaberry at the position of 5.3 cm and in TM1 and Acala 4-42 WR at 5.7 cm. Existence or absence of minor and very minor proteins can be observed from 5.1 to 5.7 cm in Fig. 1/Table 2. A minor band in Gregg 35 x L is visible at 5.8 cm which is represented as a very minor protein in Sea Island Seaberry only and is absent in all others. Five out of nine i.e. TM1, UKA B1-(72)049, Bulgaria 996, Coker 100 WILT and Acala 4-42 WR showed a major protein and nothing by other four at the position of 5.9 cm. Only one i.e. Deltapine 16 indicated a major band at 6.0 cm and nothing in other varieties. Similarly, Coker 100 WILT and Gregg 35 x L had a major protein at the position of 6.1 cm, Pima S5 at 6.3 and Acala 4-42 WR at 6.9 cm. At the distance of 7.0 cm major bands could be observed in TM1, UKA B1-(72)049, Bulgaria 996 and Deltapine 16; similarly, at 7.2 cm in Coker 100 WILT, Gregg 35 x L and Pima S5. A

Table 2. Protein bands, coded as 1 (major), 2 (minor) and 3 (very minor) in different cotton varieties alongwith their migrated distance in cms. within the polyacrylamide gel through electrophoresis

TM1		UKA BI-(72)049		Bulgaria 996		Coker 100 WILT		Acala 4-42 WR		Deltapine 16		Gregg 35 x L		Pima S5		Sea Island Seaberry	
Dista-ance	Band	Dista-ance	Band	Dista-ance	Band	Dista-ance	Band	Dista-ance	Band	Dista-ance	Band	Dista-ance	Band	Dista-ance	Band	Dista-ance	Band
0.7	2	0.7	2	0.7	2	0.8	3	1.3	3	0.7	2	2	0.7	2	0.8	3	
1.0	3	1.2	2	1.0	3	1.1	3	1.5	1	1.3	2	0.9	3	1.0	3	1.0	3
1.3	2	1.6	1	1.2	2	1.6	1	2.8	3	1.6	1	1.1	3	1.3	2	1.4	3
1.6	1	1.9	3	1.6	1	2.5	3	3.1	2	2.0	3	1.3	2	1.7	1	1.6	3
1.9	3	2.1	2	1.9	3	3.2	3	3.5	2	2.1	2	1.7	1	2.2	3	2.0	2
2.1	2	2.3	2	2.1	2	3.6	3	3.8	3	2.3	2	2.0	3	2.4	2	2.2	2
2.3	2	2.5	2	2.3	2	4.2	3	4.0	3	2.5	2	2.2	2	2.6	2	2.4	2
2.5	2	2.7	1	2.5	2	4.5	1	4.4	1	2.8	1	2.4	2	2.8	1	2.6	2
2.7	1	2.8	1	2.7	1	4.9	3	4.7	3	3.1	2	2.6	2	3.0	1	2.8	1
2.8	1	3.1	3	3.1	3	5.1	3	5.0	3	3.4	2	2.9	1	3.1	2	2.9	3
3.1	2	3.4	1	3.4	2	5.5	3	5.3	2	3.8	2	3.2	2	3.2	2	3.0	1
3.4	2	3.7	3	3.7	3	5.9	1	5.7	1	4.1	2	3.6	2	3.5	3	3.2	2
3.7	2	4.0	3	4.0	3	6.1	1	5.9	1	4.4	1	4.0	2	3.8	3	3.3	3
4.0	3	4.3	1	4.3	1	6.3	2	6.1	2	4.8	3	4.2	2	4.2	3	3.8	3
4.3	1	4.7	3	4.7	3	6.8	2	6.7	2	5.0	3	4.5	1	4.4	1	4.0	3
4.7	2	4.8	3	4.9	3	7.2	1	6.9	1	5.4	3	4.9	2	4.9	3	4.6	1
5.0	2	5.3	1	5.3	2				5.7	2	5.1	2	5.1	1	5.3	1	
5.3	3	5.6	3	5.7	2				6.0	1	5.5	2	5.5	3	5.6	3	
5.7	1	5.9	1	5.9	1				6.3	3	5.8	2	5.7	3	5.8	3	
5.9	1	6.2	3	6.3	3				6.7	2	6.1	1	6.1	3	6.2	3	
6.2	3	6.7	2	6.6	3				7.0	1	6.3	3	6.3	1	6.5	2	
												6.9	2	6.5	2	6.6	3
6.6	2	7.0	1	6.8	2							7.2	1	6.6	3	6.7	3
7.0	1			7.0	1									7.2	1	7.3	1
7.3	3			7.3	3											7.5	3

variable existence of minor and very minor proteins could also be observed from 5.8 to 7.5 cm in all the varieties.

Since, the protein is a direct product of the gene, therefore, the variation observed in the protein bands of the varieties reported herein indicated their genetic variation. These genetic differences might have arisen as a result of natural and experimental evolution of these varieties under different geographical regions *vis-a-vis* their adaptation to the specific environments. The present studies also indicated a considerable departure from TM1, the true representative of *Gossypium hirsutum* L., showing thereby the individuality of each variety as expressed according to its protein banding pattern. Inter and intra-specific variation according to protein banding pattern as observed through polyacrylamide gel electrophoretic technique has also been reported by Steward *et al.* (1965), Boulter *et al.* (1966), Sastry and Virupaksha (1967), cherry *et al.* (1969) and Khan and Kohel (1989) in various plant species including that of *Gossypium*.

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