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Comparative Study of the Soluble Storage Proteins in *Gossypium hirsutum* L. Germplasm through Electrophoresis

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ABSTRACT

Electrophoretic profiles of crude proteins extracts from seeds of eight cotton cultivars (two locals and six exotics) and their 56 F_2 progenies were compared. The electrophoretic patterns between F_2 progenies of eight pairs of reciprocal crosses, were different from one another, but were identical with those of their respective maternal parents. While Poly Acrylamide Gel Electrophoretic (PAGE) analysis confirmed the hybridization of the crosses and morphological differences of the parents as their protein profiles were also different from each other. Cluster analysis also confirmed our results of PAGE Analysis, as all of the 64 accessions falls into eight groups due to the differences in the density or sharpness of their protein bands.

Key Words: Cotton; *G. hirsutum L.*; Soluble Storage Proteins; PAGE analysis; Cluster analysis

INTRODUCTION

The systematic study to know the genetic architecture of the cultivars and their progenies, mainly based on morphology, has been improved by the incorporation of biochemical analysis, especially of proteins. One of the biochemical methods more extensively used to determine the success of hybridization has been the Polyacrylamide Gel Electrophoresis (PAGE) analysis of the proteins found in seed and storage organs. These proteins are physiologically stable and easy to handle (Ladizinsky & Hymowitz, 1979). Seed proteins have received extensive attention for the determination of success of hybridization; for example (Dhaliwal, 1977) reported that the electrophoretic patterns within each of three pairs of reciprocal crosses in wheat, were different from one another but were identical with those of their respective maternal parents. The expression of paternal genome is presumably determined by dosage and genetic affinity of the maternal and paternal genome in the hybrid endosperm. Payne *et al* (1981) found no recombinant in the sample of 100 single grains of wheat from each of five different crosses of the type (F_1 of variety A x variety B) x variety C and as expected with a frequency ratio close to 1:1. Khan (1991) found considerable differences in protein banding patterns of nine varieties of American upland and American-Egyptian cotton. Ahmad and Slinkard (1992) studied total seed storage proteins of the cultivated Chick pea *C. arietinum L.* and eight other wild annual *Cicer* species by SDS Polyacrylamide gel electrophoresis. The resultant dendrogram generally agreed with the limited data already available on interspecific relationships in *Cicer* based on morphological characteristics, cross ability, genome pairing in hybrids, karyotype and isozyme analysis. Goyal (1993) investigate protein variation among cultivars of cotton using PAGE and found that cultivars could be identified at 14% acrylamide monomer concentration with distinct and potential results. Tayyar and Waines (1996) determined the pattern of genetic diversity within and among the species of *Cicer* by starch gel electrophoresis. They carried out UPGMA cluster analysis and the results revealed four genetic groups and this species grouping agrees partially with those obtained from cross ability and cytogenetic study.

The proteins of cotton seed can be divided into two groups, water soluble and water insoluble. The water soluble proteins are relatively high in electrophoretic mobility and low in molecular weight (Martinez, 1964). The water insoluble or storage proteins are few in number, relatively low in electrophoretic mobility, and have high molecular weights (Martinez *et al.*, 1965). For this study, band patterns were obtained of the water soluble proteins on polyacrylamide gels.

MATERIALS AND METHODS

Seed material. The seeds used in this study for protein extraction were collected from eight cultivars and their 56 F_2 progenies derived by diallel crossing. The cultivars were denoted by these numbers and are followed throughout this research paper: (i) Laokra 5.5, (ii) DPL 7340-424, (iii) Fregobract, (iv) Glandless 4195-220, (v) SA 100, (vi) Stoneville 857, (vii) AC-134, and (viii) S-12

Table I. Cluster Membership of Accessions

Case	8	7	6	5	4	3	2
1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1
3	2	2	2	2	1	1	1
4	3	3	3	3	2	2	2
5	3	3	3	3	2	2	2
6	4	4	4	4	3	3	3
7	4	4	4	4	3	3	2
8	2	2	2	2	1	1	1
9	1	1	1	1	1	1	1
10	2	2	2	2	1	1	1
11	3	3	3	3	2	2	2
12	3	3	3	3	2	2	2
13	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1
15	2	2	2	2	1	1	1
16	2	2	2	2	1	1	1
17	3	3	3	3	2	2	2
18	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1
20	4	4	4	4	3	3	2
21	1	1	1	1	1	1	1
22	2	2	2	2	1	1	1
23	2	2	2	2	1	1	1
24	2	2	2	2	1	1	1
25	2	2	2	2	1	1	1
26	1	1	1	1	1	1	1
27	4	4	4	4	3	3	2
28	5	5	4	4	3	3	2
29	2	2	2	2	1	1	1
30	3	3	3	3	2	2	2
31	1	1	1	1	1	1	1
32	3	3	3	3	2	2	2
33	3	3	3	3	2	2	2
34	4	4	4	4	3	3	2
35	6	3	3	3	2	2	2
36	3	3	3	3	2	2	2
37	3	3	3	3	2	2	2
38	3	3	3	3	2	2	2
39	3	3	3	3	2	2	2
40	1	1	1	1	1	1	1
41	3	3	3	3	2	2	2
42	1	1	1	1	1	1	1
43	2	2	2	2	1	1	1
44	4	4	4	4	3	3	2
45	4	4	4	4	3	3	2
46	6	3	3	3	2	2	2
47	1	1	1	1	1	1	1
48	4	4	4	4	3	3	2
49	4	4	4	4	3	3	2
50	2	2	2	2	1	1	1
51	4	4	4	4	3	3	2
52	2	2	2	2	1	1	1
53	3	3	3	3	2	2	2
54	4	4	4	4	3	3	2
55	6	3	3	3	2	2	2
56	4	4	4	4	3	3	2
57	2	2	2	2	1	1	1
58	2	2	2	2	1	1	1
59	2	2	2	2	1	1	1
60	7	6	5	5	4	2	2
61	2	2	2	2	1	1	1
62	2	2	2	2	1	1	1
63	8	7	6	2	1	1	1
64	2	2	2	2	1	1	1

Protein extraction. Ten seeds of each accession were dehulled. The kernels were then ground with a mortar and pestle to produce fine flour in liquid nitrogen. Flour (0.4 g) was suspended in 6 mL of reagent grade water. The suspension was agitated for 30 min in a shaker and the suspension was centrifuged at 10,000 RPM (12,000 g) in a refrigerated centrifuge for 15 min at 10°C. The supernatant was then filtered through No. 5A filter paper. The crude protein extract thus obtained was stored in micro centrifuge tubes under refrigeration until its use.

Electrophoresis. The disc electrophoresis system described by Davis (1964) including resolving gel and stacking gel was followed with a little modification as reported by Khan (1991). The gels were prepared as follows:

Resolving gel. Tris-HCI buffer stock solution, pH 8.9, acrylamide stock solution and reagent grade water were mixed in an Erlenmeyer flask with a side arm. After adding ammonium per sulfate solution to this mixture, it was swirled gently to avoid the formation 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.

Stacking gel. Tris-HCI stock solution, pH 6.7, was mixed with acrylamide stock solution and reagent grade water in an Erlenmeyer flask with a sidearm. The solution was degassed thoroughly with vacuum. Ammonium per sulfate solution was added while swirling gently to ensure proper mixing. A comb was inserted into each gel mould and immediately the gel solution was gently overlaid with reagent grade water and was then left at room temperature exposed to fluorescent light for 1 h to polymerize.

Application of samples. 30 µL of each protein sample was loaded into the wells.

Gel electrophoresis. After application of the 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 two gels (10 wells each) during each run. Current was kept constant at 40 milliamperes. Each run took from 5-6 h to complete when dye front was 1 cm from lower end of gels. Temperature of the buffer and gels was kept constant at 10°C, with an LKB 2219 Multi Temp II Thermostatic Circulating Liquid Cooler.

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. Fixing and staining time was 1 h.

De-Staining. Gels were destined in the first destining solution containing 200 mL 95% ethanol and 300 mL 5% acetic acid for 30 min. The final destining was done in the second destaining solution containing 150 mL 95% ethanol and 350 mL of 5% acetic acid for 12 h or overnight.

Photographs. Photographs of the gels were taken after de-staining. Gels were being laid directly on to an illuminator

with an opal white screen (avoiding trapped air bubbles) and kept wet during photography by addition of 7% acetic acid with a Cannon camera.

Analysis of protein bands. The numbers of protein bands revealed by the gels were recorded as present or absent. For analysis, each band was assigned a value of zero (0) when absent, or scored 1 to 4 depending upon their density and sharpness; 1= large band to 4= minor band, when present.

Cluster analysis was performed to provide a statistical basis to establish the number of cluster represented by the 64 genotypes. A clustering procedure (hierarchical cluster analysis) was performed using the un weighted pair group mean with arithmetical averages (UPGMA) method of (Sneth & Sokal, 1973), using computer programme of SPSS/PC+. The output of this analysis was used to derive a dendrogram using PROC TREE, which showed the phylogenetic relationships among all the genotypes.

RESULTS AND DISCUSSION

The seed protein profiles for each sample of cotton (*G. hirsutum L.*) genotypes were arranged in a matrix represting 21 different relative mobility (Rm) values of bands. Each band had been numbered according to its position relative to the origin. The photographs of selected gels were presented in Fig. 1-4. A dendrogram of clustering patterns of each sample based on coefficients of similarity were given in Fig. 5. Cluster membership of cases using average linkage (between groups) was given in Table I.

Comparison of different samples of parents, their direct and reciprocal crosses of the same species of cotton *G. hirsutum L.* in F_2 generation showed that there were significant differences between the parents, and their crosses, for protein bands. The results obtained were in many cases parallel to the morphological differences observed in the field and afterwards in diallel analysis. The results obtained from the present protein analysis confirmed that there were some differences among the parents, and in few cases between direct and reciprocal crosses. These differences may be attributed to maternal or cytoplasmic effects (Dhaliwal, 1977). None of the 64 genotypes studied had identical profiles. These results got full support from (McNeal *et al.*, 1968) who got similar types of results in F_2 generation of wheat.

Construction of a phylogenetic tree. A dendrogram constructed on the basis of genetic distances showed phylogenetic relationships among the 64 genotypes of *G. hirsutum L.* Fig. 5.

Cluster analysis showed clear cut separation of different genotypes at unit 1, but converge into following eight clusters at unit 10 depending upon their similarities (Fig. 5).

The I cluster comprised of 13 cotton genotypes, mostly originating from the parent

Stoneville 857 (parent 6) of USA origin and AC 134 (parent 7) of Pakistan origin.

Fig. 1. PAGE of seed storage proteins in *G. hirsutum* L.

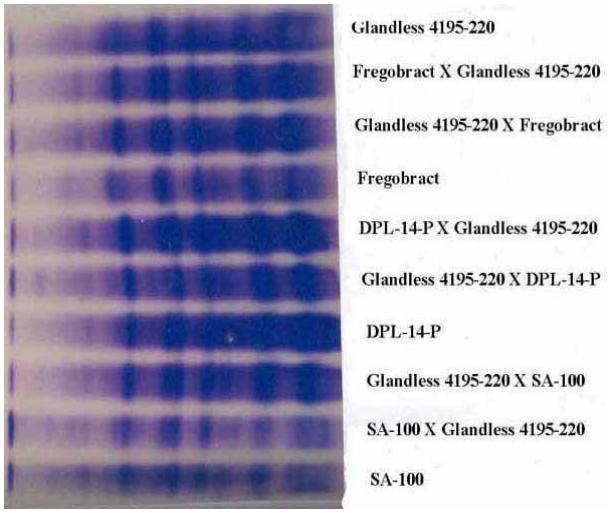


Fig. 2. Seed proteins pattern obtained by PAGE.

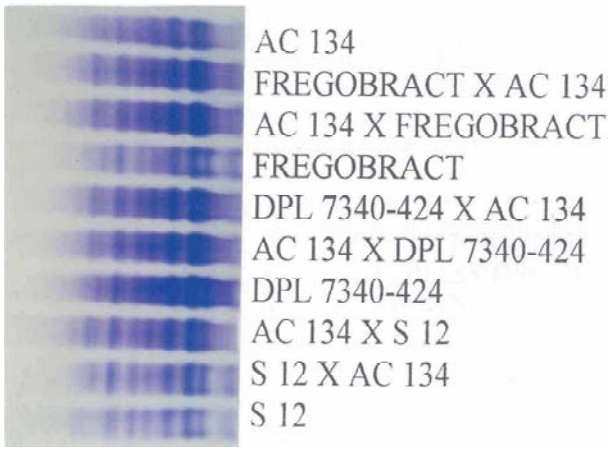
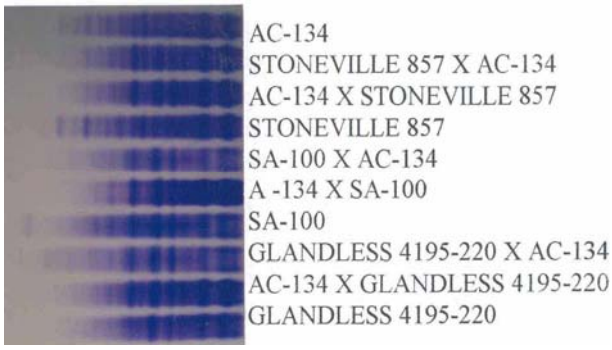


Fig. 3. Seed proteins pattern obtained by PAGE.

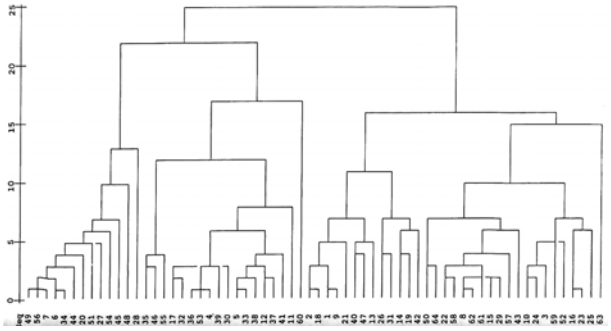


Three genotypes come together to form cluster II, they are the progeny of Glandless 4195-220 with Parent 6 and Parent 7 both in the cluster I. While Parent 7 also combine with SA100 to form the third genotype to come into this cluster.

The III cluster consisted of 14 genotypes and consisted

Fig. 4. Seed proteins pattern obtained by PAGE analysis

Fig. 5. A dendrogram showing genetic relatedness among accessions of *G. hirsutum* L. by the use of UPGMA cluster analysis



of parent Glandless 4195-220 and parent SA100 and their crosses with different other 6 parents.

Parent DPL 7340-424 and Laokra 5.5 with their reciprocal crosses along with 4 other genotype comprised the IV cluster.

Cluster V consisted of 5 genotype, mainly of parent SA100 origin. While 11 cross mainly of parent S12 origin comprised the cluster VI.

In cluster VII, 8 genotypes arising from parent Fregobract come together, while one genotype cross of S12 and Stoneville 857 from cluster VIII.

These VIII clusters, which formed at unit 10, continue to merge with each other as we moved upward on the scale. So at the top i.e. unit 25; the cluster of unit 22 which was comprised of 31 genotypes combined with the already existing cluster of unit 17 consisted of 33 genotypes, making total 64 genotypes. Thus confirming our results of hybridization of 8 X 8 diallel cross of *G. hirsutum L*. origin.

This uniformity of seed protein profile agrees with the findings of Rakhmankulov *et al* (1990), Khan *et al* (1991) and Goyal (1993). Cotton is often cross pollinated crop if environment is suitable otherwise it is a self pollinating plant; there is often minimal intra and inter population variation (Crawford, 1990) and populations may be monomorphic at almost all loci over their geographic ranges.

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