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Protein profiling of Different cotton (*Gossypium hirsutum)* Germplasm

H. R. Ramani*[1], Shobha Singh[2], B. D. Solanki3, D. H. Patel[1], B. G. Solanki[1]
[1]Main Cotton Research Station, Navsari Agriculture University, Surat-395007
[2]Shree Ramakrishna institute of computer education and applied sciences, Veer Narmad Sout Gujarat University, Surat,
[3] Departments of Biochemistry, Saurashtra University, Rajkot, Gujarat
*Email:hiteshramani2007@gmail.com

ABSTRACT
The present investigation was carried out at Main Cotton Research Station, Navsari Agricultural University, Athwa farm, Surat during 2016. 12 genotypes were selected for protein study. In leaf proteins profiling showed different banding pattern and different molecular weight and different R_f values of 12 genotypes of were observed. The un-weighted paired group method with arithmetic averages (UPGMA) exhibited 2 clusters which were further grouped into distinct subgroups. Study revealed that Gossypium hirsutum *varieties have different banding pattern, different molecular weight and different morphology but these 12 varieties were closely related genotypes. Based on SDS-PAGE data 4 varieties C-1579, Acala, Pentense Na-4 chines, Bar-12/13 were related to Cluster I and rest 8 varieties EC-12062, IAN-1327, ISC-75-1-12, C-1998, 0821-B-411-7, Demeter, ISC-67-413, C-1622 were related to Cluster II, similarity range from 0.41 to 0.90. Based on NATIVE-PAGE data cluster I with 1 variety C-1579 of cotton and cluster II with 2 groups of 11 varieties Pentense Na-4 Chines, ISC-67-413, ISC-75-1-12, Bar-12/13, EC-12062, Demeter111(1), C-1998, C-1622, IAN-1327, 0821-B-4-11-7 and Acala, similarity range from 0.45 to 1.00.*
KEYWORDS: *Cotton, Germplasm, Protein Profiling, SDS PAGE, Native PAGE*

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INTRODUCTION

Cotton belongs to the genus *Gossypium* under tribe *Gossypieae* of family *Malvaceae*. The name of the genus is derived from the Arabic word goz, which refers to a soft substance [1]. The origin of the genus *Gossypium* is dated to around 5-10 million years ago. The genus *Gossypium* to which cultivated cottons belong, contains about 45 diploid (2n = 2x = 26) and five allotetraploid (2n = 4x = 52) species, all of which are basically tropical perennials. Two of the diploids, *G. arboretum* L. and *G. herbaceum* L. and tetraploid species are *G. hirsutum*, *G. tomentosum, G. mustelinum, G. barbadense*, and *G. darwinii*. New species continue to be discovered [2].

G.hirsutum known as upland cotton, native to Central America, Mexico, the Caribbean and southern Florida (90% of world production); *G.barbadense* known as extra-long staple cotton, native to tropical South America (8% of world production); *G.arboreum* known as tree cotton, native to India and Pakistan (less than 2%); *G.herbaceum* known as Levant cotton, native to southern Africa and the Arabian Peninsula (less than 2). India is the pioneer country in the world for development of cotton hybrid for commercial cultivation [3]. In broader spectrum, the genotypes from various sources differed in grouping and it was difficult to establish relationship between origin and cluster pattern. The protein banding data were investigated in relation to agronomic traits that indicated influence of polymorphic bands on quantitative traits. Particular clusters were better for specific traits that are suggested to utilize in crop improvement program.

MATERIALS AND METHODS

The experimental material (Leaves) 12 varieties of *Gossypium hirsutum* of were obtained from Main Cotton Research Station, Navsari Agricultural University, Athwa Farm, Surat.

Table 1: Germplasm used in the study

Sr. No.	Germplasm	Special Characters
1	C-1579	Compact & Sympodial
2	Pentense Na-4 Chines	Semi & Small Okhra Leaf
3	Bar-12/13	Thick, Smooth & Less Hairy Leaf
4	EC-12062(511-2339-1688-1637)	Broad Leaf
5	ISC-67-413	Monopodial & Open Plant Type
6	ISC-75-1-12	Small Leaf
7	IAN-1327	Red Thick Leaf & Red Flower
8	0821-B-4-11-7	Full Okhra Leaf
9	C-1998	Stay Green Type
10	C-1622	Small Leaf & Medium Ball size
11	Acala-15170X BJA-592-SP-2	Small Ball Size
12	Demeter 111(1)	Big Ball Size

Protein profiling

Extraction of Protein from Sample

Leaves were thoroughly washed with distilled water. From each variety 400 mg leaves were ground with chilled pestle and mortar in 5ml of phosphate buffer. The ground samples were immediately transferred to 2ml eppendorf tubes. All 12 samples had centrifuged at 13000 rpm for 15 minutes. Clear supernatant were transferred in another clean eppendorf tubes which were used for protein SDS-PAGE analysis and NATIVE PAGE analysis.

Analysis of protein profile by SDS-PAGE

Protein profiles analysis elucidated by method of [4] and [5]. Protein bands in gel were analysed using SERVA SDS PAGE protein marker 6.5KDa to 200 KDa. The SDS-PAGE gel process was carried out according to the modified method of Laemmli [5]. This discontinuous gel system used a 12% resolving gel and 6% stacking gel. After the electrophoresis separation, the gel was stained with Coomassie blue G-250 and analyzed.

Analysis of protein profile by NATIVE-PAGE

NATIVE- PAGE analysis elucidated by method of [6]. Protein bands in gel were analysed using BSA protein marker 1mg/ml which Molecular weight is 69 KDa. The Native-PAGE gel process was carried out according to the modified method of [7]. This discontinuous gel system used a 12% resolving gel and 6% stacking gel. After the electrophoresis separation, the gel was stained with Coomassie blue G-250 and analyzed

Analysis of protein bands of SDS-PAGE and NATIVE-PAGE

Molecular weight and R_f were calculated by help of AlphaEaseFC 4.0. To avoid taxonomic weighing, the intensity of bands was not taken into consideration, only the presence of the bands was taken as indicative. Presence and absence of the bands were entered in a binary data matrix where each band was assigned a value of zero (0) when absent, or scored 1 when present. These data matrix were then entered into NTSYS-PC [8] software for cluster analysis which provided a statistical basis to establish the number of cluster represented by the 12 genotypes. A clustering procedure (hierarchical cluster analysis) was performed using the unweighted pair group mean with arithmetical averages (UPGMA) method of [9], using computer programme of NTSYS-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

SDS PAGE:

In the present study an attempt has been made to give a blue print of the genetic diversity of indigenous genotypes of cotton through SDS-PAGE technique. The leaf protein fragments exhibited (Fig.1) appreciable polymorphism amongst genotypes used for the study. The varieties which were indistinguishable on the basis of simple identifiable morphological traits like growth habit; ball size, leaf types and colour etc. could be distinguished on the basis of their electrophoresis patterns, got separated which was not possible by morphological markers. Variability in intensity was observed in some bands that indicated the quantity of protein peptides accumulating at a particular molecular weight.

The banding patterns were characterized by 8 clear distinct zones viz., A=Myosin (200kDa), B=beta-Galactosidase (116kDa), C=Albumin bovine (67kDa), D=Ovalbumin (45kDa), E=Carbonanhydrase (45kDa), F=Trypsin inhibitor(21kDa), G=Lysozym(14.3kDa), H=Aprotinin (6.5kDa). Zone A (200 kDa)

was nearest and H (around 6.5kDa) was farthest from the origin *i.e.* the point of protein sample application. The protein migrated from cathode to anode passing through separating gel. Fig. 1 represents the banding pattern of protein peptides and the diagrammatic representation has been depicted in Zymogram (Fig.2) in *Gossypium hirsutum*. In total, 64 protein subunits were observed and out of these 7 were polymorphic. The protein bands were stacked according to their molecular weight *i.e.* high molecular weight protein were located in upper region and low molecular weight proteins in the middle to lower regions of the gel, respectively.

R_f value and Molecular Weight

The leaves protein profiles for e ach sample of cotton (*G. hirsutum L.*) genotypes were arranged in a matrix representing different retardation factor (R_f) values of bands and different molecular weight. Genotype 1 (C-1579) had 4 bands detected with R_f value were 0.482, 0.512, 0.538, 0.904 and molecular weight were 40.69kD a, 35.62kDa, 31.61kDa and 6.00kDa respectively. Genotype 2 (Pentense Na-4 Chines) had 4 bands detected with R_f value were 0.472, 0.500, 0.535, 0.567 and molecular weight were 42.34kDa, 37.57kDa, 32.03kDa and 27.67 kDa respectively. Genotype 3 (Bar-12/13) had 4 bands detected with R_f value were 0.482, 0.520, 0.558, 0.611 and molecular weight were 40.69kD a, 34.23kDa, 28.80kDa and 22.67kDa respectively. Genotype 4 (EC-12062(511-233 9-1688-1637)) had 7 bands detected with R_f value were 0.480, 0.512, 0.547, 0.579, 0.833, 8 98, 0.956 and molecular weight were 41.23 kDa, 35.62kDa, 30.37kDa, 26.24kDa, 8.25kDa, 6 .16kDa, and 4.72kDa respectively. Genotype 5 (ISC-67-413) had 9 bands detected with R_f value were 0.482, 0.523, 0.573, 0.637, 0.699, 0.795, 0.848, 0.886, 0.944 and molecular weight were 40.69kDa, 33.78kDa, 26.95kDa, 20.11kDa, 15.21kDa, 9.81kDa, 7.72kDa, 6.50kDa and 4.98kDa respectively. Genotype 6 (ISC-75-1-12) had 6 bands detected with R_f value were 0.474, 0.503, 0.564, 0.804, 0.839, 0.904 and molecular weight were 42.34kDa, 37.07kDa, 28.04kDa, 9.43kDa, 8.03kDa and 4.85kDa respectively. Genotype 7 (IAN-1327) had 6 bands detected with R_f value were 0.484, 0.529, 0.851, 0.901, 0.956, 0.984 and molecular weight were 40.15kDa, 32.89kDa, 7.62kDa, 6.08kDa, 4.72kDa and 4.25kDa respectively. Genotype 8 (0821-B-4-11-7) had 3 bands detected with R_f value were 0.848, 0.930, 0.968 and molecular weight were 7.72kDa, 5.32kDa and 4.48kDa respectively. Genotype 9 (C-1998) had 5 bands detected with R_f value were 0.456, 0.506, 0.863, 0.947, 0.985 and molecular weight were 45.86kDa, 36.58kDa, 7.23kDa, 4.91kDa and 4.13kDa respectively. Genotype 10 (C-1622) had 10 bands detected with R_f value were 0.471, 0.512, 0.544, 0.573, 0.594, 0.807, 0.863, 0.895, 0.939, 0.959 and molecular weight were 42.91kDa, 35.62kDa, 30.78kDa, 26.95kDa, 24.55kDa, 9.30kDa, 7.23kDa, 6.24kDa, 5.11kDa and 4.66kDa respectively. Genotype 11 (Acala-15170X BJA-592-SP-2) had 2 bands detected with R_f value were 0.459, 0.953 and molecular weight were 45.25kDa and 4.79kDa respectively. Genotype 12 (Demeter 111(1)) had 4 bands detected with R_f value were 0.477, 0.822, 0.959, 0.971 and molecular weight were 41.78kDa, 8.70kDa, 4.66kDa and 4.42kDa respectively.

Cluster Analysis based on SDS-PAGE data

A dendrogram of clustering patterns of each sample based on coefficients of similarity were given in Table 1. The dendogram was constructed using UPGMA based on Jaccard's similarity coefficient through NTSYSpc-2.02i software for SDS-PAGE data of 12 cotton genotypes (Fig. 3). The genotypes were grouped into two main clusters. Cluster-I and cluster-II shared 41 % similarity. The cluster-I consisted of two subclusters A1 and A2 with 53% similarity. Subcluster A1 further divided into A1 (a) and A1 (b). Subcluster A1 (a) consisted of 2 genotypes C-1579 and Acala with more than 78% similarity, while subcluster A1 (b) consisted of only one genotype Pentense Na-4 chines. Subcluster A2 consisted of one genotype Bar-12/13 similarity of more than 90% similarity. However Cluster-II consisted of two subclusters B1 and B2. Subcluster B1 further divided into B1 (a) and B1 (b). Subcluster B1 (a) consisted of 4 genotypes EC-12062, IAN-1327, ISC-75-1-12 and C-1998 with similarity more than 90% similarity, while subcluster B1 (b) consisted of two genotype 0821-B-4-11-7 and Demeter111(1) with more than 90% similarity. Subcluster B2 contained 2 genotypes ISC-67-413 and C-1622 The phylogenetic evolutionary tree developed from the analysis indicated that most of the cotton varieties relatively close and small clusters were distributed within two broad cluster as can be seen in the phenogram obtained in cluster I with 2 groups of 4 varieties of cotton and cluster II with 2 groups of 8 varieties. However, *Gossypium hirsutum* varieties have different banding pattern, different molecular weight and different morphology but these 12 varieties were closely related genotypes.

NATIVE-PAGE

Native-PAGE represents the banding pattern (Fig.4) of protein peptides and the diagrammatic representation has been depicted in Zymogram (Fig.5). Bovine Serum Albumin was used as marker, molecular weight 66.2kDa nearest and farthest around 29.00 kDa. In total, 43 protein subunits were observed and out of these 10 were polymorphic. Variability in intensity was observed in some bands that indicated the quantity of protein peptides cumulating at a particular molecular weight. The proteins bands were stacked according to their molecular weight, shape and charge.

R_f value and Molecular Weight

Genotype 1 (C-1579) had 3 bands detected with R_f value were 0.272, 0.277, 0.388 and molecular weight were 40.15kDa, 25.56kDa and 12.16kDa respectively. Genotype 2 (Pentense Na-4 Chines) had 3 bands detected with R_f value were 0.260, 0.316, 0.434 and molecular weight were 42.09kDa, 33.90kDa and 21.38kDa respectively. Genotype 3 (Bar-12/13) had 3 bands detected with R_f value were 0.235, 0.519, 0.670 and molecular weight were 46.24kDa, 15.38kDa and 8.58kDa respectively. Genotype 4 (EC-12062(511-2339-1688-1637)) had 3 bands detected with R_f value were 0.245, 0.388, 0.553 and molecular weight were 44.53kDa, 25.56kDa and 13.48kDa respectively. Genotype 5 (ISC-67-413) had 4 bands detected with R_f value were 0.206, 0.362, 0.505, 0.670 and molecular weight were 51.77kDa, 28.35kDa, 16.27kDa and 8.58kDa respectively. Genotype 6 (ISC-75-1-12) had 4 bands detected with R_f value were 0.250, 0.306, 0.381, 0.646 and molecular weight were 43.70kDa, 35.20kDa, 26.29kDa and 9.43kDa respectively. Genotype 7 (IAN-1327) had 4 bands detected with R_f value were 0.337, 0.461, 0.580, 0.706 and molecular weight were 31.15kDa, 19.28kDa, 12.15kDa and 7.45kDa respectively. Genotype 8 (0821-B-4-11-7) had 5 bands detected with R_f value were 0.257, 0.335, 0.498, 0.714, 0.883 and molecular weight were 42.49kDa, 31.44kDa, 16.74kDa, 7.25kDa and 3.75kDa respectively. Genotype 9 (C-1998) had 3 bands detected with R_f value were 0.289, 0.357, 0.575 and molecular weight were 37.59kDa, 28.89kDa and 12.39kDa respectively. Genotype 10 (C-1622) had 4 bands detected with R_f value were 0.345, 0.408, 0.575, 0.847 and molecular weight were 30.28kDa, 23.71kDa, 12.39kDa and 4.32kDa respectively. Genotype 11 (Acala-15170X BJA-592-SP-2) had 5 bands detected with R_f value were 0.180, 0.245, 0.335, 0.459, 0.641 and molecular weight were 57.41kDa, 44.53kDa, 31.44kDa, 19.46kDa and 9.61kDa respectively. Genotype 12 (Demeter 111(1)) had 3 bands detected with R_f value were 0.194, 0.481, 0.760 and molecular weight were 54.26kDa, 17.88kDa and 6.06kDa respectively.

Cluster analysis based on NATIVE-PAGE

A dendrogram of clustering patterns of each sample based on coefficients of similarity were given in Table 2. The dendogram was constructed using UPGMA based on Jaccard's similarity coefficient through NTSYSpc-2.02i software for NATIVE-PAGE data of 12 cotton genotypes (Fig. 6). The genotypes were grouped into two main clusters. Cluster-I and cluster-II shared 45 % similarity. The cluster-I consisted only one genotype C-1579. However Cluster-II consisted of two subclusters A1 and A2 with more than 59% similarity. Subcluster A1 further divided into A1 (a) and A1 (b) with more than 73% similarity. Subcluster A1 (a) consisted of 6 genotypes, which were Pentense Na-4 Chines, ISC-67-413, ISC-75-1-12, Bar-12/13, EC-12062, Demeter111(1) with similarity 100% similarity, while subcluster A1 (b) consisted of two genotype C-1998 and C-1622 and with 100% similarity. Subcluster A2 consisted 3 genotypes IAN-1327, 0821-B-4-11-7 and Acala with 100% similarity. The phylogenetic evolutionary tree developed from the analysis indicated that most of the cotton varieties did not form distinct clusters; rather, the relatively close and small clusters of varieties were distributed within 2 broad cluster as can be seen in the phenogram obtained in cluster I with 1 variety of cotton and cluster II with 2 groups of 11 varieties genes for those characters in genotypes. However, *Gossypium hirsutum* varieties have different banding pattern, different molecular weight by use of NATIVE-PAGE and genotype different morphology, 12 varieties were similar.

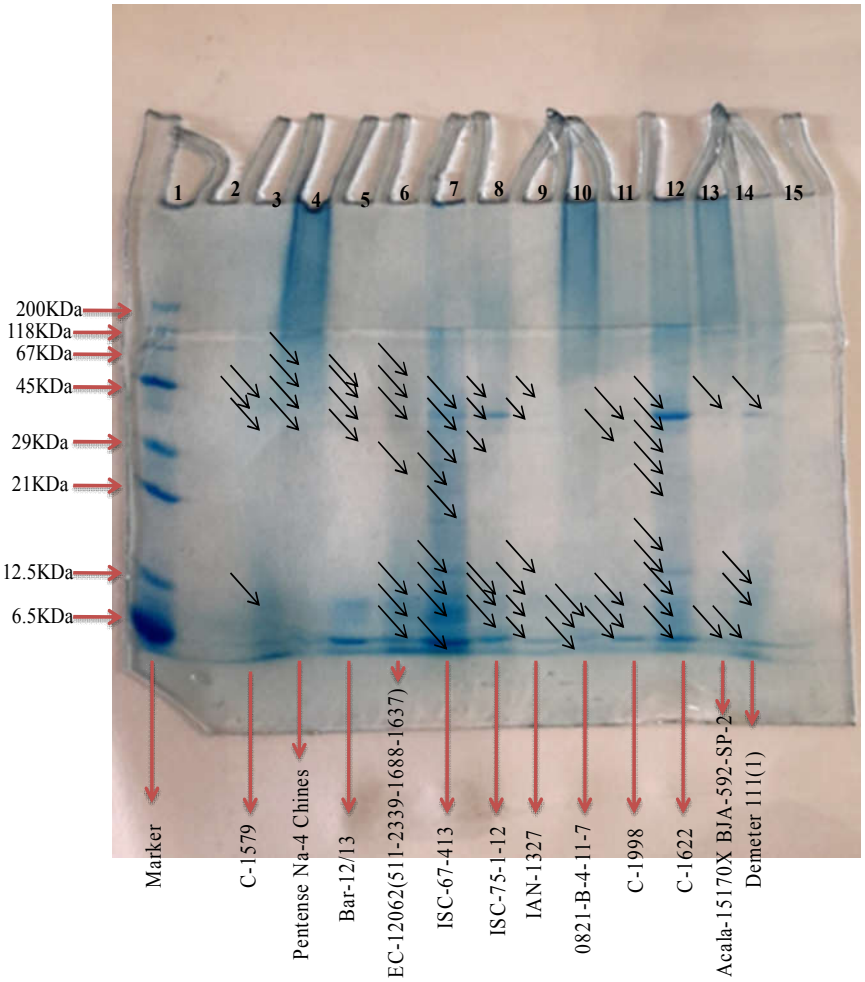

Fig. 1: SDS-PAGE protein-profile of *Gossypium hirsutum* (L.) the arrows represent polymorphic bands. Molecular marker used in this gel was Unstained SDS-PAGE Protein marker

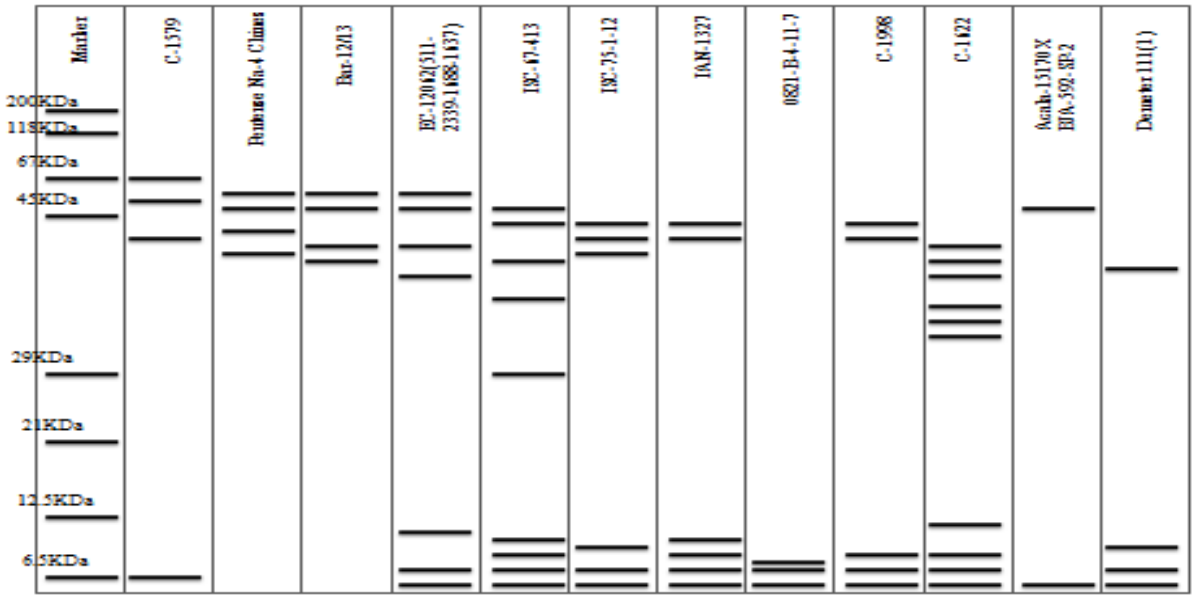

Fig. 2: Zymogram (Leaf protein profiles as resolved through SDS-PAGE)

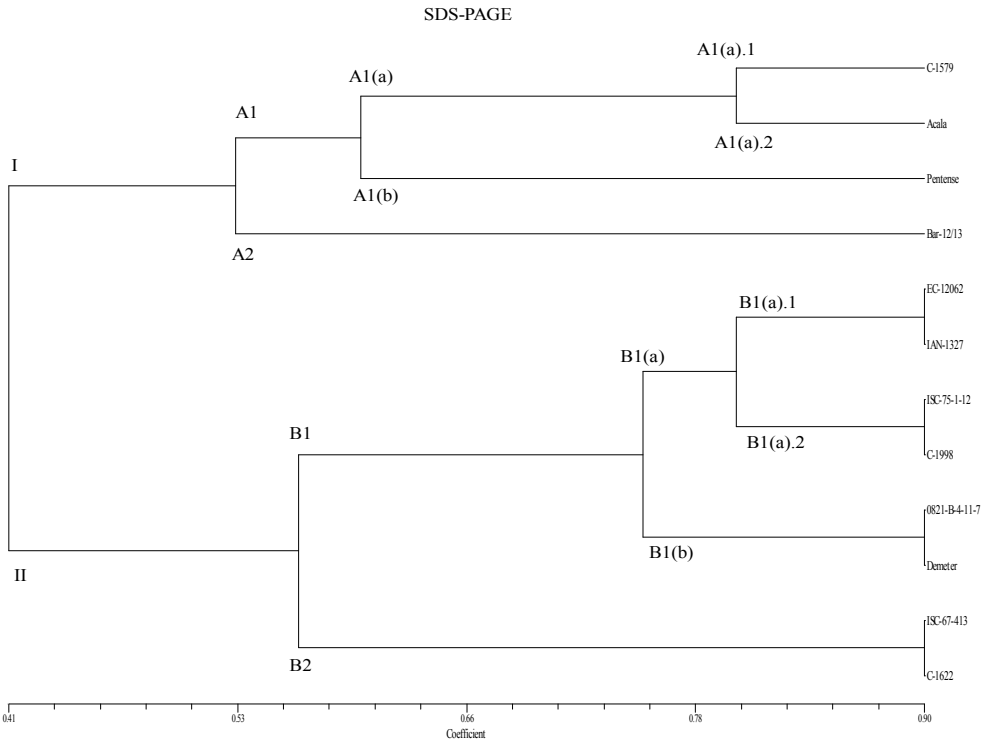

Fig 3: Dendrogram depicting the genetic relationship among 12 cotton genotypes based on protein profiles through SDS-PAGE.

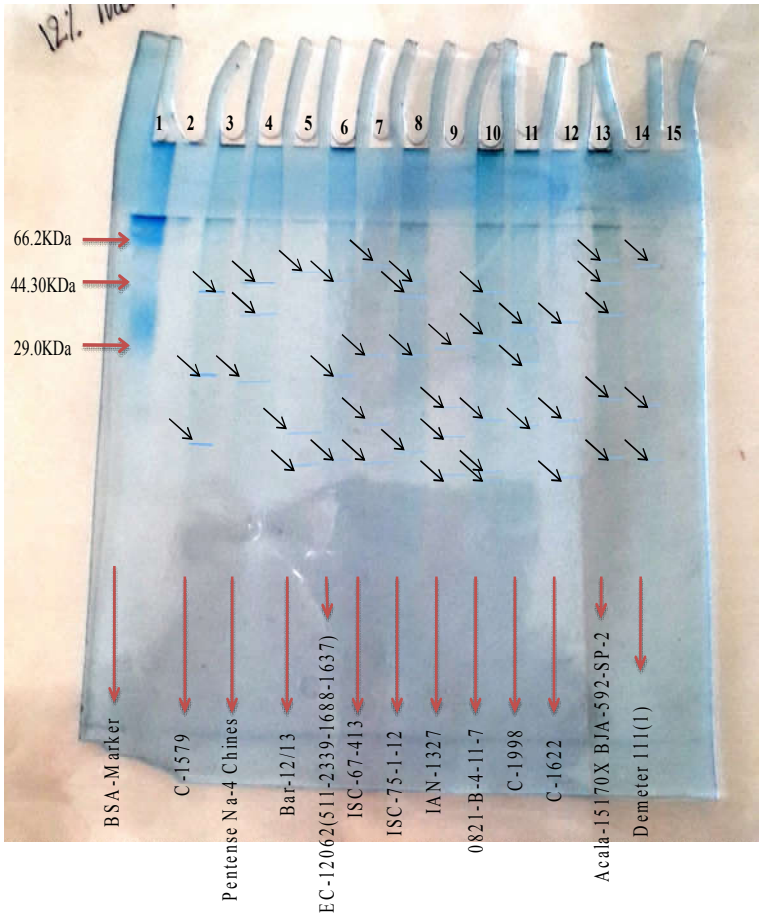

Fig 4: NATIVE-PAGE protein-profile of *Gossypium hirsutum* (L.) the arrows represent polymorphic bands. Molecular marker used in this gel was Bovine Serum Albumin

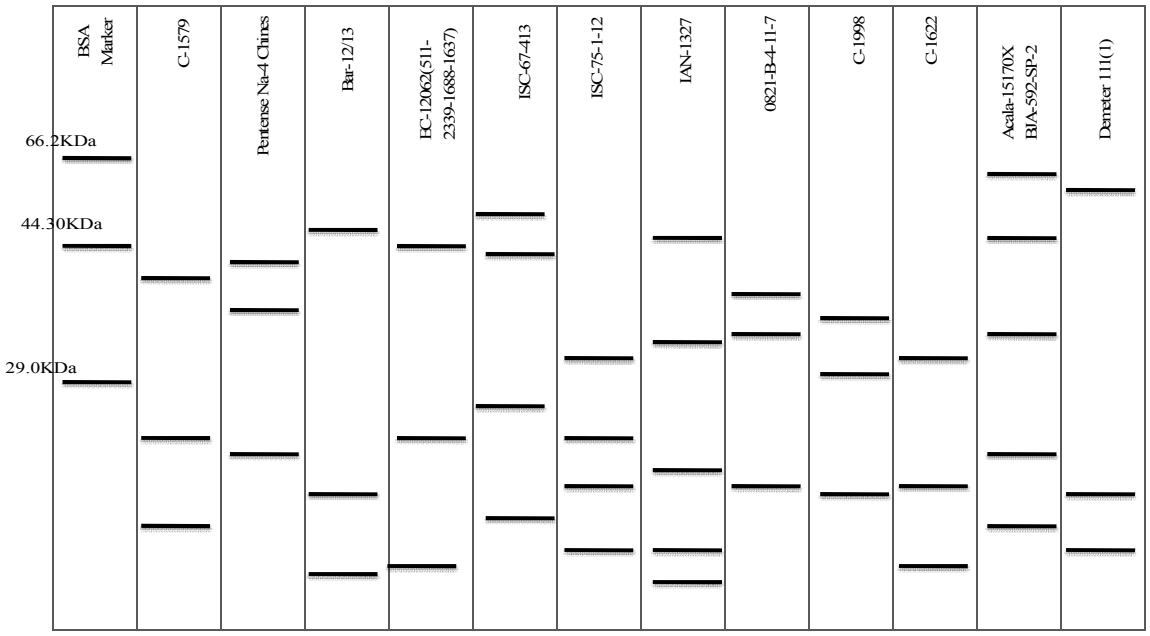

Fig 5: Zymogram (Leaf protein profiles as resolved through Native-PAGE)

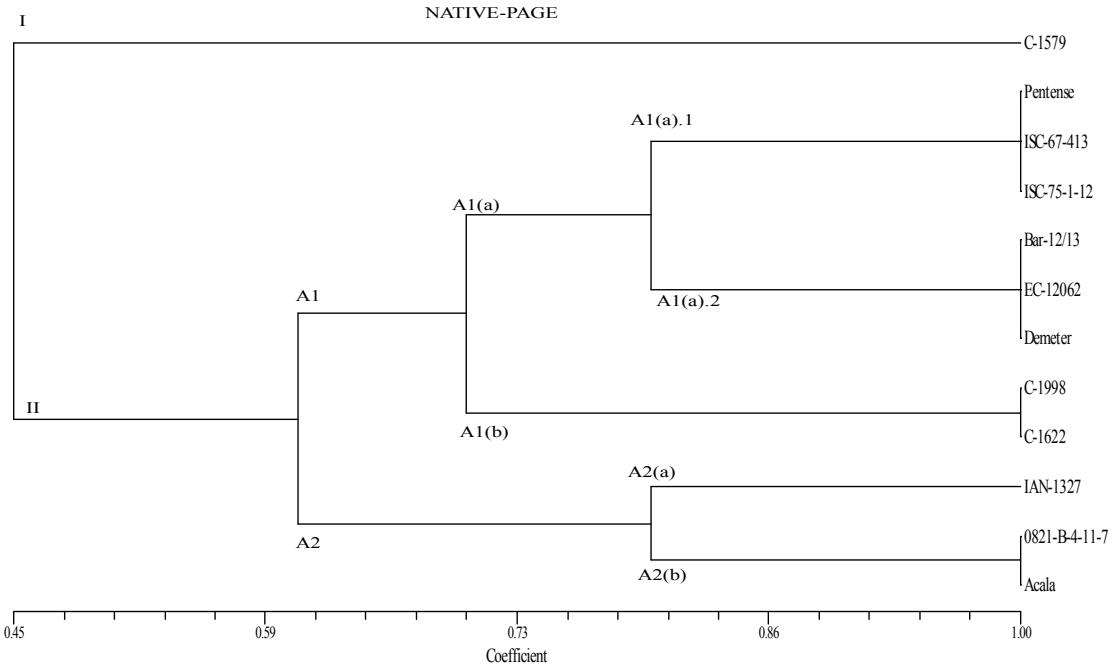

Fig 6: Dendrogram depicting the genetic relationship among 12 cotton genotypes based on protein profiles through NATIVE-PAGE

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