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SDS-PAGE CHARACTERIZATION OF INTRA AND INTERSPECIFIC COTTON HYBRIDS

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*F*our Cotton hybrids, two each intra specific (h x h) viz., NHH-44 and PHULE-492 and inter specific (hxb) DCH-32 and PHULE - 388 and their parents were characterized by SDS-PAGE. Variation in number and staining intensity of bands was observed among the different genotypes. The banding pattern of all the four hybrids and their parents was quite distinguishable. The number and intensity of individually stained bands were useful for variety / hybrid identification. The intra specific hybrids and their female parents and inter specific hybrids and their male parents possessed equal number of bands.

Keywords : Cotton hybrid; Inter specific; Intra specific; SDS-PAGE.

Introduction

The development of number of cotton hybrids having good productivity under the average management led to the use and ultimately large-scale production of quality seed in public and private sector. Hand emasculation and pollination are the main constraints in producing hybrid cottonseed, which results in shortage of hybrid seed. This leads to use of low quality seeds and spurious seeds and F_2 seeds are sold by some unscrupulous seed dealers. These facts raised many issues regarding maintenance of genetic purity and the minimum seed standards. In India genetic purity, testing is mandatory for certified seeds. The seed certification agencies follow the field plot technique (GOT) for testing the genetic purity. Genetic purity is tested in field plot technique at flowering stage[1]. This is time consuming, laborious, expensive, tedious and cumbersome. The test has to be done outside the seed laboratory, adequate space must be available[2]. Consequently, greater impetus has been given to laboratory techniqe that can augment the present field test procedure[3]. New biochemical technique of analysis of seed protein using polyacrylamide gel electrophoresis (PAGE) showed reliability, rapidity and saving of cost to determine the genetic purity of cultivars[4-6] In the present study sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) as given by Dadlani and Vavier[7] was used for identification of cotton hybdrids and their parents. Recent upto date review[8] on gel electrophoresis of seed protein indicate that SDS-PAGE offers a biochemical approach to the evolutionary aspect of plant speciation, 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. This technique has showed its significant potential in determining genetic purity of cotton hybrids in laboratory conditon. Looking to the significance of this technieque, a detailed review of the literature is made with view to suggest future line of work in to deploy it more successfully in genetic purity test and supply of quality seeds to the cotton growers. This will curb the tendency of selling spurious seed by the seed dealers.

Materials and Methods

The experimental mateial was consisted of two intra *G. hirsutum* hybrids and two inter specific *G. hirsutum* x *G. barabadense* hybrids and parents of the respective hybrids (Table 1) Parental lines of the hybrids were selfed during summer, 1999. Hybrid seed

was produced by hand emasculation and pollination on the selfed lines.

Extraction of protein

Matured and well developed seeds were selected and decorticated, decorticated single seed material was ground in a small mortar and pastel and defatted in using defatting solvent mixture. The defatted single seed meals were transferred to 1.5 ml eppendorf tubes and 0.3 ml working protein solution was added to each tube. The sample was left for two hrs at room temperature and then kept in refrigerator over night. Then the samples were heated for 10 min in boiling water bath and cooled. The samples were centrifuged at 1500 g for 10 min and clear supernatant was used for electrophoresis. The electrophoresis was carried out by using ATTO AE 6210 slab gel cast with ATTO AE-6220 dual slab chamber unit. After completion of electrophoresis and staining, the band intensity was assessed visually and recorded as dense, medium, light and weak. The relative mobility value for each band was calculated by the following formula.

$$RM=\frac{\text{Distance migrated by protein band from origin (cm)}}{\text{Distance migrated by tracking dye (cm)}}$$

Results and Discussion

The electrophoretic pattern of seed protein is presented in Tables 2, 3 and Fig. 1. The banding patterns of any one cultivars were identical from the other comparing the electrophoregrams of the soluble proteins, the variation in the number and stainning intensity of bands was observed among the hybrids and their parents. The number of bands in different genotypes ranged between 12 to 15 and 41 bands were resolved. Some banding patterns were variety specific while the others were common[9].

INTRA HIRSUTUM HYBRIDS

The hybrid NHH-44 and male parent AC-738 exhibited 14 bands and its female parent BN-1 had 13 bands. The hybrid could be distinguished from its female parent BN-1 by qualitative differences in band located at RM 0.44 and presence of specific bands of RM 0.47, 0.80 and 0.97 and male parent AC-738 by presence of specific band at RM 0.47 and absence of 2 band at RM 0.49 and 0.79. Similar trend of banding pattern was observed by Nerkar and Rao[10] for this hybrid and reported that the hybrids were easily identified because of electrophoresis of soluble proteins.

The hybrid PHULE-492 and its male parent RHC-004 possessed 12 bands and female parent RHC-003 possessed 14 bands. The hybrid PHULE-492 could be identified from its female parent RHC-003 quantitatively by bands at RM 0.39 and 0.49 and by presence of bands at RM 0.28, 0.44, 0.54 and 0.90 and male parent RHC-004 by presence of bands at RM 0.26, 0.57 and 0.62

Table 1. Hybrids and their parents used for SDS PAGE

Sr. No.	Variety/Hybrid	Species
1.	BN-1	*G. hirsutum*
2.	AC-738	*G. hirsutum*
3.	NHH-44 (BN-1 x AC-738)	*G. hirsutum*
4.	DS-28	*G. hirsutum*
5.	SB-425 YF	*G. hirsutum*
6.	DCH-32 (DS-28 x SB-425 YE)	*G. hirsutum x G. barbadense*
7.	RHC-003	*G. hirsutum*
8.	RHC-004	*G. hirsutum*
9.	RHH-0492 (RHC-003 x RHC-004)	*G. hirsutum*
10.	RHC-006	*G. hirsutum*
11.	RHCb-001	*G. Barbadense*
12.	PHULE-388 (RHC-006 x RHCb-001)	*G. hirsutum x G. barbadense*

Table 2. RM values of different protein bands from each cotton genotypes observed in electrophoresis.

	BN-1	AC-738	NHH-44	RHC-003	RHC-004	RHC-492	DS-28	SB-425 4f	DCH-32	RHC-006	RHCb-001	RHB-0388
1	0.27	0.28	0.28	0.28	0.18	0.26	0.27	0.26	0.26	0.28	0.28	0.27
2	0.33	0.34	0.34	0.34	0.34	0.33	0.36	0.36	0.36	0.33	0.33	0.33
3	0.44	0.40	0.40	0.39	0.40	0.39	0.40	0.40	0.39	0.40	0.39	0.39
4	0.48	0.44	0.44	0.44	0.44	0.40	0.44	0.44	0.44	0.44	0.44	0.44
5	0.54	0.49	0.47	0.49	0.49	0.49	0.48	0.49	0.48	0.49	0.49	0.52
6	0.57	0.54	0.59	0.54	0.59	0.57	0.63	0.52	0.52	0.57	0.58	0.57
7	0.61	0.59	0.64	0.59	0.63	0.62	0.65	0.58	0.59	0.63	0.62	0.61
8	0.64	0.64	0.67	0.64	0.67	0.67	0.69	0.63	0.63	0.67	0.70	0.67
9	0.70	0.67	0.72	0.67	0.70	0.69	0.73	0.67	0.67	0.70	0.79	0.69
10	0.77	0.72	0.80	0.71	0.85	0.85	0.80	0.70	0.71	0.77	0.87	0.77
11	0.85	0.79	0.87	0.77	0.87	0.87	0.87	0.73	0.79	0.87	0.89	0.87
12	0.90	0.87	0.90	0.87	0.92	0.92	0.92	0.80	0.87	0.89	0.93	0.89
13	0.96	0.92	0.92	0.89	-	-	0.93	0.88	0.90	0.93	-	0.93
14	-	0.97	0.97	0.90	-	-	0.95	0.92	0.93	-	-	-
15	-	-	-	-	-	-	-	0.95	-	-	-	-

Table 3. The characteristics of banding patterns of cotton genotypes.

Sr.No.	Genotype		Banding
1.	BN-	P	3, 16, 19, 26, 30, 33, 40
		A	4, 8, 11, 18, 24, 32, 34, 37, 41
2.	AC-738	P	13, 15, 31
		A	11, 32, 36
3.	NHH-44	P	11, 32
		A	12, 13, 15, 16, 26, 30, 35, 40
4.	RHC-003	P	4, 10, 15, 22, 27, 30, 35, 36
		A	2, 16, 20, 25, 37
5.	RHC-004	P	1, 10, 18, 26
		A	24, 16, 20, 25
6.	PHULE-492	P	2, 16, 20
		A	1, 4, 10, 15, 18, 21, 27, 30
7.	DS-28	P	9, 23, 25
		A	2, 8, 14, 18, 27, 31, 36
8.	SB-425 YF	P	9, 12, 18, 27, 31, 36, 38
		A	8, 12, 18, 27, 31, 36, 38
9.	DCH-32	P	8, 18, 27, 31, 36, 38
		A	9, 23, 25, 26, 29, 32, 37, 39
10.	RHC-006	P	4, 5, 21
		A	3, 14, 19, 25
11.	RHCb-001	P	13, 17, 20, 31
		A	14, 16, 19, 24, 25
12.	PHULE-388	P	14, 19, 25
		A	9, 13, 21, 26, 31

P - Present, A- Absent

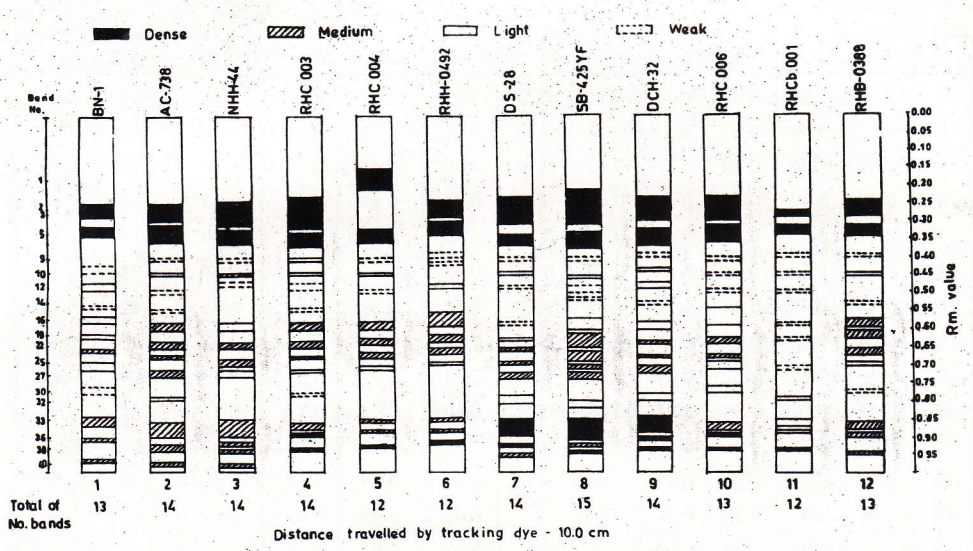
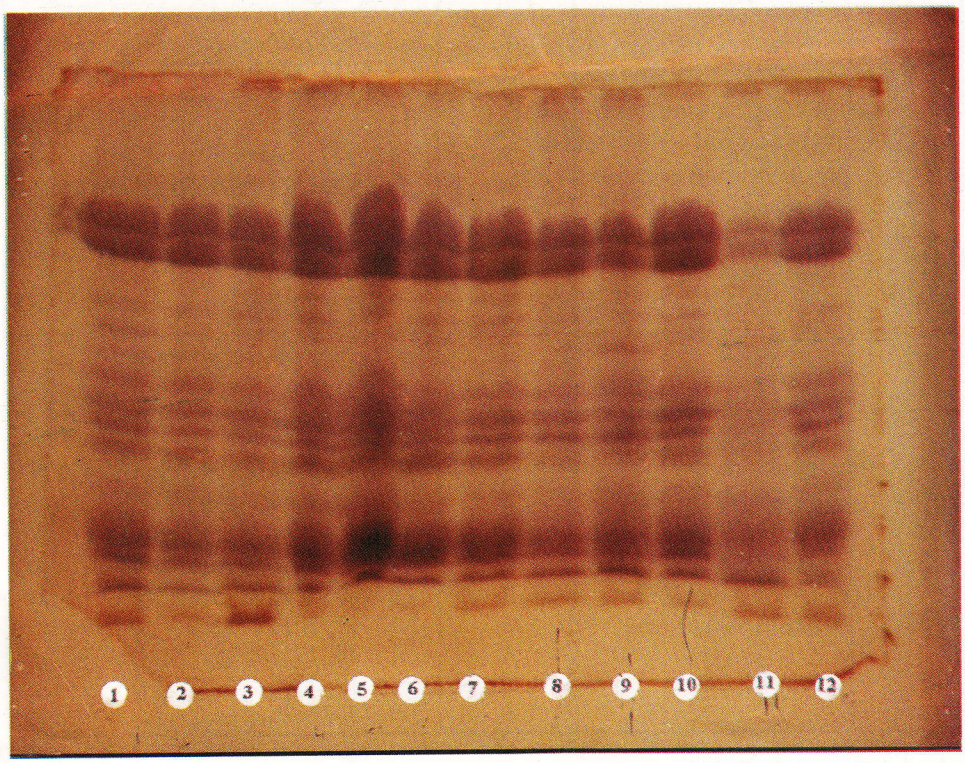


Fig. 1 Electrophoregrams of cotton genotypes



and absence of specific bands at RM 0.18 and 0.44.

INTER SPECIFIC HYBRIDS

The hybrid DCH-32 and its female parent possessed 14 bands each and male parent had 15 bands. The hybrid DCH-32 could be distinguished from the female parent DS-28 by quantitative differences in two bands at RM value 0.48 and 0.63 and by presence of five bands at RM 0.39, 0.59, 0.71, 0.77 and 0.89 and from male parent SB-425 YF by presence of specific bands of RM 0.39, 0.59, 0.71, 0.90 and 0.93.

The hybrid PHULE-388 and its female parent RHC-006 exhibited 13 bands and its male parent RHCb-001 possessed 12 bands. The hybrid PHULE-388 could be distinguished from its female parent by quantitative differences in 3 bands at RM 0.57, 0.77 and 0.89 and by presence of 2 bands at RM 0.52 and 0.61 and absence of bands at RM 0.40 and 0.63 and from male parent by presence of bands at RM 0.52, 0.66, 0.69 and 0.77 and absence of bands at RM 0.49, 0.70 and 0.79.

Earlier, several workers had identified the cotton genotypes by using soluble seed protein electrophoresis[4,5,11].

The banding pattern of soluble proteins, of all the four hyrids was quite distinguishable (quantitatively and qualitatively) from the respective parents. Comparison of banding pattern (band intensity) revealed similarity of hybrids to their respective parents. Some banding patterns were variety specific while the others were common to other varieties[9]. The intra specific hybrids NHH-44 and PHULE-492 and their female parent had equal number of bands, while in inter specific hybrids DCH-32 and PHULE-388 and their male parent possessed euqal number of bands. The banding pattern of all the four hybrids showed appearance of additional bands or disappearance of bands as was found in their respective parents. This clearly indicated the differences in protein expression based on band number and relative dye binding ability.

It is apparent that cotton genotypes could be identified by the presence or absence of bands and intensities of bands that could be used as markers. Earlier, Nerkar and Rao[10] have also demonstrated that cotton could be uniquely identified by using Sodium dodecylsulphate polyacryl amide gel electrophoresis (SDS-PAGE).

As reported by Narojji *et al.*[6], seed protein polymorphism serves as a quick, reliable method of determining the genetic purity of cultivars and hybrid seeds could be distinguished from selfed seeds of female parent.

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