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SDS-PAGE technique - A confirmatory tool for genetic purity assessment in *desi* cotton (*Gossypium arboreum* L.)

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ABSTRACT : To ensure the genetic truth of a cotton variety seed certification agencies follow the grow out tests which are time consuming and laborious. So to meet the objective 20 seed samples of *desi* cotton cv HD 123 were collected from different firms and divided into 2 groups *i.e.* group A (good seed germination, >65%) and group B (marginal seed germination, <65%). To study the protein profile and genetic purity, all the seed lots were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Similarity index of protein pattern were estimated by Nei and Li equation. A total of 11 bands were observed with Rm value ranging from 0.2609 to 0.9217. All bands are present in all seed lots. A wide quantitative variation was observed in Rm values, electrophoretic mobility of protein band, but there is no variation in protein banding pattern in seed lots. These confirm the genetic purity of all seed lots and make SDS-PAGE a confirmatory tool for varietal identification in *desi* cotton.

Key words : *Desi* cotton, genetic purity, SDS-PAGE, seed protein

Crop improvement programs have generated large number of varieties / hybrids of cotton. The *desi* cotton has its regular importance and grown in constant areas of the country. The task of establishing the identity of these crop varieties and of maintaining their genetic purity of seed lots has became major concern, since the variety attains acceptance only when farmers get genetically pure seeds of high standard. Maintenance of genetic purity of seeds is essential to ensure maximum benefits from high yielding varieties. To ensure the genetic purity of cotton variety, seed certification agencies follow grow out tests which is time consuming and laborious method. So, it is essential to develop methods for genetic purity assessment that are rapid, reliable and less influenced by environment. Progress in molecular genetics has provided plant breeders with a rapid and powerful alternative approach of selection which may substitute morphological testing to a great extent. Polymorphic markers based on SDS-PAGE of total tris soluble proteins were reported to be useful to evaluate the genetic purity of hybrid cotton seeds. The uniformity of seed protein profile, its additive nature and negligible effect by environmental conditions or seasonal fluctuations makes it a unique and powerful tool for genetic purity assessment and genotype characterization. Seed protein profile can be used for testing genetic purity of seed lots. Electrophoresis of tris soluble seed proteins has been reported to be useful for genetic purity assessment. Thus, the present investigation was conducted to assess genetic purity of *desi* cotton on the basis of electrophoresis of total seed protein.

MATERIALS AND METHODS

Experimental materials : The present investigation comprised of 20 seed samples of *desi* cotton (*Gossypium arboreum*) cv HD 123 were procured from different sources like Cotton Section, RDS Seed Farm, Director Farm, CCS Haryana Agricultural University, Hisar and different private seed companies. Based on the germination percentage, all seed lots were

divided into 2 group *i.e.* group A with standard germination above 65 per cent (Good quality seed group) and group B with standard germination below 65 per cent (marginal quality seed group), having 10 samples in each group (Table 1).

SDS-PAGE electrophoresis of proteins : The protein profile and total *tris* soluble seed proteins were obtained by SDS-PAGE. Five seeds of each seed lot were decoated, crushed and defatted in 3-4 changes of defatting solvent mixture (2:1:1 chloroform; methanol; acetone). Dried seed meal was dissolved in 0.3 ml of 0.6M working protein extraction buffer [4.25ml of stock, (4g SDS, 10 mg pryronin G in 10.4 ml of 0.6M Tris HCL (pH6.6), 7.9 ml distilled water, 10 ml glycerol) to 0.75 ml of mercaptoethanol and making final volume of 10ml with distilled water]. The samples were left for 2 h at room temperature and then kept in refrigerator overnight and then kept in water bath at 40^0C for 10 min. cooled and centrifuged at 10000 rpm for 10 min. the clear supernatant was taken as protein source for electrophoresis of total seed soluble proteins in 15 per cent separating and 6 per cent stacking gel of 1 mm thickness. 15 ml of each sample was loaded in the wells and electrophoresis was carried out 1.5 mA/well till the samples migrated into running gel and subsequently at 2 mA/well was given until the tracking dye reached the bottom of gel. After the completition of electrophoresis the gel was incubated in 15 per cent trichloroacetic acid for overnight and stained in 1 per cent commassie brilliant blue for 16-18 hr. Destaining was done for 1-2 days with destaining solution (50:70:880 Methnol: acetic acid: water) to clear the gel background. After proper destaining, gels were stored in 7 per cent acetic acid solution. The Rm value of each protein band was calculated by the relationship as follow

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

Similarity index value were calculated based on proportion on common bands by using the Nei and Li equation

$$F = \frac{2Mxy}{Mx + My}$$

Where F is similarity index, Mx is no. of bands in variety 'x', My is no. of bands in variety 'y', and Mxy is the number of bands common to both x and y. F x 100 gives the per cent similarity between 2 variety thus F = 1.0 would mean that patterns in the variety are identical.

Table 1. The detailed list of 20 seed lots of *desi* cotton cv. HD 123

	Genotype/ Variety HD 123						
Lot No	Source	Standard germ-ination (%)	Initial moisture content (%)	Lot No	Source	Standard germ-ination (%)	Initial moisture content (%)
	Group A				**Group B**		
L_1	A one Seed Company,Hisar	76.00	9.48	L_{11}	Director Farm, CCSHAU, Hisar (F/C)	57.67	8.57
L_2	Jai Bharat Seeds, Hisar (Breeder)	73.33	7.50	L_{12}	Unnat Seeds Company,Hisar	56.67	7.24
L_3	Jai Bharat Seeds,Hisar (Foundation)	68.67	7.44	L_{13}	C.R.S. Sirsa	58.00	8.67
L_4	Ashoka Seeds, Hisar	73.33	6.98	L_{14}	Central State Farm,Hisar	60.00	8.61
L_5	Quality Hybrid Seeds,Hisar	72.67	9.20	L_{15}	Shakti Seeds,Hisar	63.00	8.28
L_6	New HSR Beej Company,Hisar	76.00	8.45	L_{16}	CCSHAU,Hisar (Breeder Seed)	60.00	8.74
L_7	IFFCO Seeds,Hisar	68.00	8.09	L_{17}	B.K. Seeds,Hisar	53.33	8.89
L_8	KRIBHCO Seeds,Hisar	71.00	8.01	L_{18}	Director Farm, CCSHAU,Hisar (B/F)	52.67	9.16
L_9	Chaudhary Seeds,Hisar	83.33	7.14	L_{19}	R.D.S. Seed Farm,Hisar (F/C)	53.00	8.80
L_{10}	CCSHAU,Hisar(B/F)	70.00	8.37	L_{20}	R.D.S. Seed Farm, Hisar (B/F)	52.33	9.16

RESULTS AND DISCUSSION

SDS-PAGE or alkaline PAGE profiles of tris soluble proteins or salt soluble globulins have been employed for identification of cotton variety. SDS-PAGE analysis of total tris soluble seed proteins of 20 seed lots revealed a total of 11 bands. The first band was present at a distance of 3.0cm with Rm value 0.2609 where as last band was formed at a distance of 10.6cm with Rm value 0.9217. Rest 9 bands were lied in between at various distances with separate Rm value (Table 2). A wide quantitative variation was observed in Rm values, electrophoretic mobility of protein band, but there is no variation in protein banding pattern in seed lots. Total 11 bands were observed (Plate 1 and 2) and present in each seed lots (Table 3). This indicate 100 per cent similarity in all seed lots, as there is no variation in protein banding pattern in good quality and marginal quality seed group. Similarity index based on relative mobility of protein band indicate close association among different seed lots tested. The similarity index value is 1.0 among the seed lots. The similarity index was calculated in all combination and their values obtained are presented in similarity index table (Table 4). There is no genetically variation between good quality and marginal quality seed group which results no influence of germination potential on genetic quality of seed. No significant changes in banding pattern of total tris soluble seed protein of good and marginal quality seed group were observed. Moreover, neither any additional band appeared nor any band disappeared. Similarly no marked changes

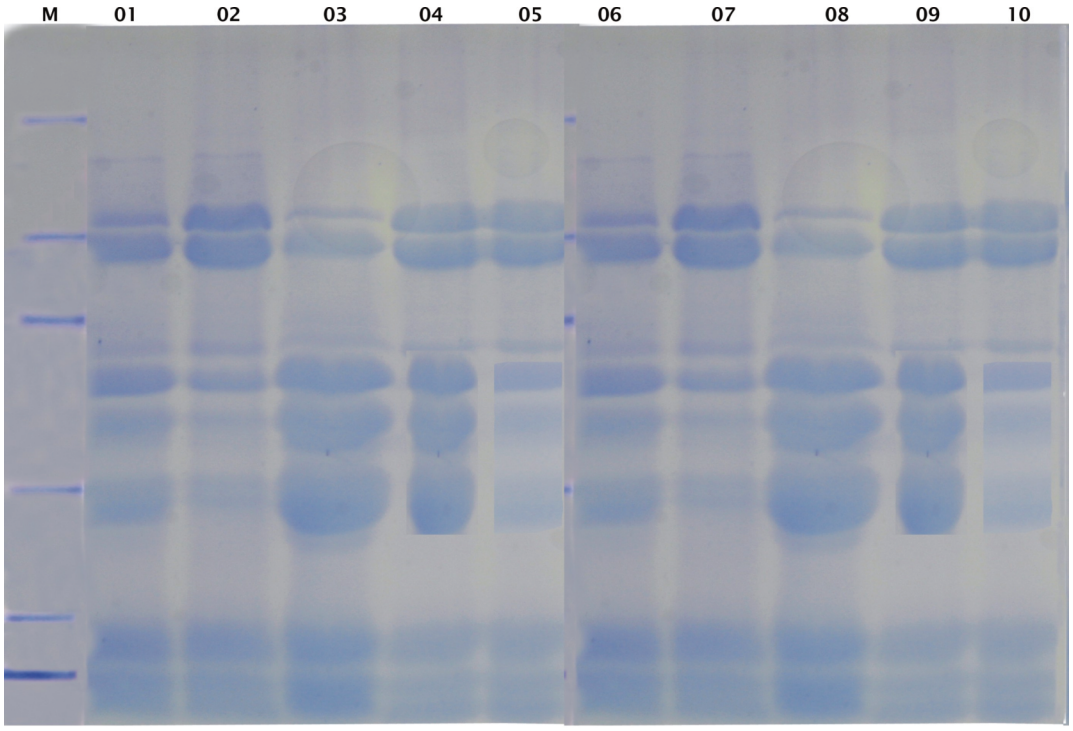
Table 2. Total number of bands, their position and Rm value of tris soluble seed protein of *desi* cotton cv HD 123

Band Number	1	2	3	4	5	6	7	8	9	10	11
Band position	3.0	3.5	4.9	5.4	6.1	6.4	7.0	7.3	9.5	10.0	10.6
Rm value	0.2609	0.3043	0.4261	0.4696	0.5304	0.5565	0.6087	0.6348	0.8261	0.8696	0.9217

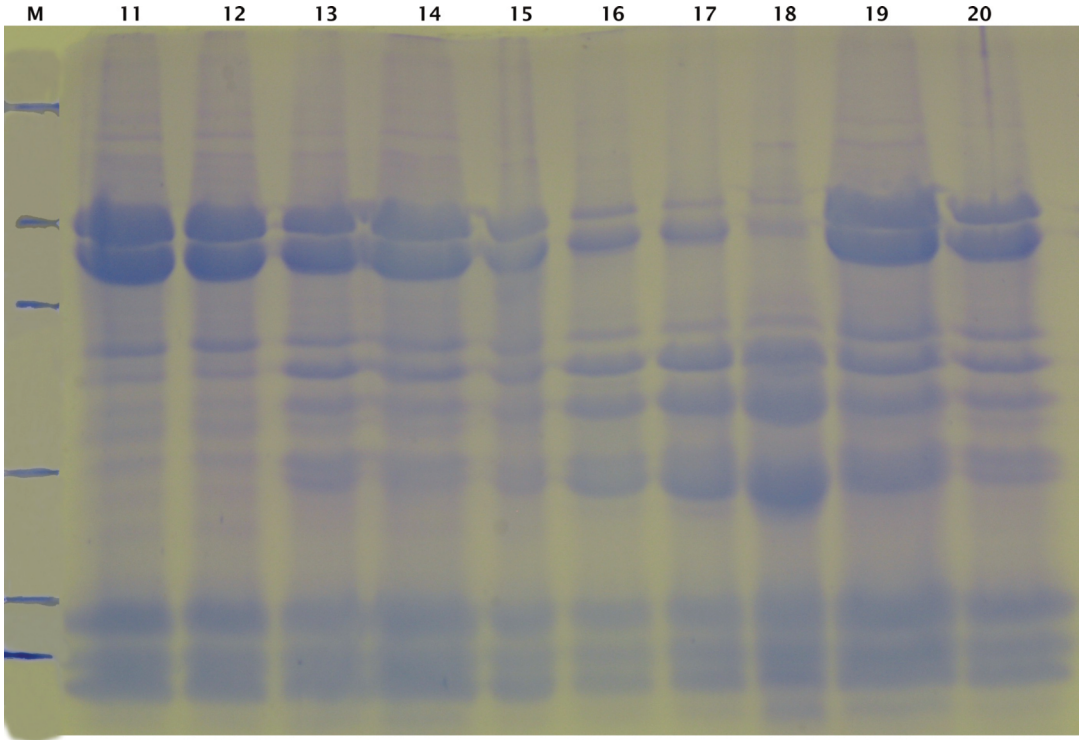
Table 3. Band map of good and marginal quality seed lots of *desi* cotton cv HD 123

Lot/Band Number	1	2	3	4	5	6	7	8	9	10	11
L_1	+	+	+	+	+	+	+	+	+	+	+
L_2	+	+	+	+	+	+	+	+	+	+	+
L_3	+	+	+	+	+	+	+	+	+	+	+
L_4	+	+	+	+	+	+	+	+	+	+	+
L_5	+	+	+	+	+	+	+	+	+	+	+
L_6	+	+	+	+	+	+	+	+	+	+	+
L_7	+	+	+	+	+	+	+	+	+	+	+
L_8	+	+	+	+	+	+	+	+	+	+	+
L_9	+	+	+	+	+	+	+	+	+	+	+
L_{10}	+	+	+	+	+	+	+	+	+	+	+
L_{11}	+	+	+	+	+	+	+	+	+	+	+
L_{12}	+	+	+	+	+	+	+	+	+	+	+
L_{13}	+	+	+	+	+	+	+	+	+	+	+
L_{14}	+	+	+	+	+	+	+	+	+	+	+
L_{15}	+	+	+	+	+	+	+	+	+	+	+
L_{16}	+	+	+	+	+	+	+	+	+	+	+
L_{17}	+	+	+	+	+	+	+	+	+	+	+
L_{18}	+	+	+	+	+	+	+	+	+	+	+
L_{19}	+	+	+	+	+	+	+	+	+	+	+
L_{20}	+	+	+	+	+	+	+	+	+	+	+

L – Lots; B – Band; + indicate presence of band; - indicate absence of band



Electrophoregram of total seed storage proteins in good quality seed lots of *desi* cotton
Plate : 01



Electrophoregram of total seed storage proteins in Marginal good quality seed lots of *desi* cotton
Plate : 02

Table 4. Similarity index of seed storage protein of different seed lots of *desi* cotton cv. HD 123

Lots	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}	L_{11}	L_{12}	L_{13}	L_{14}	L_{15}	L_{16}	L_{17}	L_{18}	L_{19}	L_{20}
L_1	1.0																			
L_2	1.0	1.0																		
L_3	1.0	1.0	1.0																	
L_4	1.0	1.0	1.0	1.0																
L_5	1.0	1.0	1.0	1.0	1.0															
L_6	1.0	1.0	1.0	1.0	1.0	1.0														
L_7	1.0	1.0	1.0	1.0	1.0	1.0	1.0													
L_8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0												
L_9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0											
L_{10}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0										
L_{11}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0									
L_{12}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0								
L_{13}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0							
L_{14}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0						
L_{15}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0					
L_{16}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0				
L_{17}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0			
L_{18}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0		
L_{19}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
L_{20}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

were reported in soluble seed protein profile between natural aged and fresh aged seed of cotton except for faint staining of protein bands. SDS-PAGE of protein was used for testing genetic purity of commercial seed lots in a number of field crops (Mann *et al.,* 2002; Punia *et al.,* 2007 and Rakshit *et al.,* 2007). SDS-PAGE technique would provide a very quick and confirmatory tool for genetic purity assessment required for decision making rejecting or accepting a seed lot of *desi* cotton for procurement or marketing.

REFERENCES

Mann, A., Ram, C. and Singh, R. 2002. Characterization of cotton hybrid and their parental lines using SDS-PAGE. *Seed Tech News*. p. 150

Punia, R.C., Luthara,P., Mann A., and Ram, C. 2007. SDS-PAGE of total seed protein in relation to cultivar identification in *desi* (*G. arboretum*) and American (*G. hirsutum*) cotton. *Seed Res*. **35** : 102-05

Rakshit, A., Vashist, V., Rakshit S. and Dadlani M. 2008. Electrophoresis technique for varietal identification and genetic purity in hybrid cotton (*Gossypium hirsutum* L.) *Seed Res*. **36** : 28-32.

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