Seed Research, Vol. 35(1): 102-105, 2007

Short Communication

SDS-PAGE of Total Seed Protein in Relation to Cultivar Identification in Desi (*G. arboreum*) and American (*G. hirsutum*) Cotton

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Cotton is an important cash crop playing a key role in the economic and social affairs of India. About 243 lakh bales of cotton are produced in about 89.2 lakh hectares in nine cotton growing states with a productivity of 463 kg/ha [1]. Crop improvement programs in India have generated large number of varieties in the field and vegetable crops in the last 30 years. With the proliferation of newly developed varieties in important cultivated crops, the task of establishing the identity of these varieties and of maintaining their seed lots has become major concern since the variety attains acceptance only when farmers get genetically pure seeds of high standards. Characterization of varieties is, thus of significance, for the purpose of establishment and verification of identity and assessment of varietal purity for seed production and certification.

The descriptors currently available are restricted to plant morphology characters examined in the field. Though morphological description of varieties does not require sophisticated laboratory techniques, the generation of data are time consuming and laborious and the characters are liable to be influenced by a complex genotype x environment interaction. Hence, it is essential to develop alternative methods that are rapid, reliable and less influenced by environment. Electrophoresis of tris-soluble seed proteins and isozymes has been reported to be useful for identification of cotton, hybrids and parental lines [2, 3]. Thus the present investigation was conducted to characterize cotton cultivars on the basis of electrophoresis of total seed proteins.

Sixteen varieties viz. DS-5, HD-107, HD-123, H-777, H-974, H-1098, H-1117, H-1180, HS-6, HS-182, LRK-516, LRA-5166, LH-1556, LH-900, F-1378 and RS-875, thirteen of American (*Gossypium hirsutum*) and three of Desi cotton (*G. arboreum*) were taken for present investigation.

Sample preparation: Total Tris-HCI soluble seed proteins were separated using the method of Dadlani and Varier [4]. Five seeds of each cotton sample 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 0.6 M working protein extraction buffer [4.25ml of stock, (2 g SDS, 10mg Pyronin G, in 10.4 ml of 0.6M Tris-HCI (pH 6.6), 7.9 ml distilled water, 10 ml glycerol) + 0.75 ml of mercaptoethanol and making final volume of 10 ml with distilled water]. The samples were left for 2h at room temperature and then kept in refrigerator overnight. The samples were then kept in a water bath at 40°C for 10 minutes, cooled and centrifuged at 15000 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. 25µl of each sample was loaded in the wells and electrophoresis was carried out initially at 18mA till the samples migrated into the running gel and subsequently at 36mA until the tracking dye reached the bottom of the gel. After

Received June 2006 Revised September 2006 Accepted December 2006

the completion of electrophoresis, the gel was incubated in 15 per cent trichloroacetic acid overnight and stained in 1 per cent commassie brilliant blue for 6-18 h. After proper destaining the gel was photographed. The Rm value of each protein band was calculated by the relationship as follows:

$$Rm = \frac{\text{Distance travelled by protein band}}{\text{Distance travelled by tracking dye}}$$

SDS-PAGE analysis of total seed proteins of 16 cotton varieties revealed a total of 35 bands with Rm value ranging from 0.25 to 0.97(Fig. 1). A wide

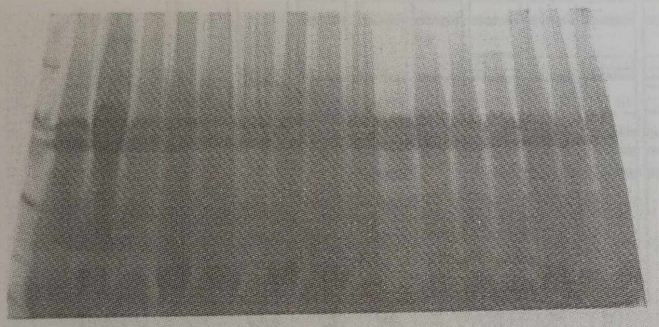

Fig. 1. SDS-PAGE analysis of total seed protein in sixteen cotton varieties: L-R: Protein marker, DS-5, HD-107, HD-123, H-777, H-974, H-1098, LH-900, F-1378, RS-875, H-1117, H-1180, HS-6, HS-182, LRK-516, LRA-5166 and LH-1556

quantitative variation (having different Rm values) was observed in the protein profiles. The *hirsutum* varieties could easily be distinguished with the presence of band no. 29 (Rm 0.84) and *arboreum* varieties could be identified on the basis of presence of band no. 10 (Rm 0.49) and band no. 31 (Rm 0.87) as represented in the electrophoregram (Fig. 2). The protein bands at position no. 8 (Rm 0.43), 9 (Rm 0.47), 25 (Rm 0.72), 26 (Rm 0.75), 33 (Rm 0.92) and 34 (Rm 0.95) were present in all the varieties including both American and Desi cotton. Band no. 32 (Rm 0.89) was absent in *arboreum* varieties whereas in *hirsutum* varieties, it differentiated seven varieties from the rest. LH-900 had a unique band with Rm 0.51 at position no. 13 thus differentiating from the others. Similarly, bands with Rm value 0.25, 0.29 and 0.52 were present only in HD-107 indicating its characteristics feature. Band no. 3 (Rm 0.31) was also present only in two varieties DS-5 and HD-107 out of all varieties studied. Band no. 4 (Rm 0.33) was present in four, two *hirsutum* and two *arboreum* varieties only. Also, band no. 6 and 7 (Rm 0.38 and 0.40) were present only in two varieties i.e. LRA-5166 and LH-1556. Two varieties HS-6 and HS-182 were having equal number of bands with same Rm values; thus, these two could only be distinguished with variability in thickness or intensity of specific bands. Thus, the *hirsutum and arboreum* cotton varieties could be differentiated from each other based on their protein profiles. Based on electrophoregram, flow chart of proteins (Fig. 3) was prepared based on which it was possible to identify each of the genotype individually.

SDS-PAGE of Tris-soluble proteins and salt soluble globulins has been extensively used for plant variety identification in many crops like rice [5], wheat [6], barley [7], maize [8], pearlmillet [9, 10] and castor [11].

In cotton SDS-PAGE of total soluble proteins and globulins has been used by a number of workers for varietal identification and characterization. The invariability in the electrophoretic profiles of total Tris-soluble proteins were studied by Kapse and Nerkar [2] in four intra-*Gossypium hirsutum* hybrids, two *G. hirsutum* x *G. barbedense* hybrids, the parents of respective hybrids and two varieties each of *G. hirsutum* and *G. arboreum* species of cotton. They reported that cultivars could be identified from electrophoregram of soluble proteins of single seed and thus this electrophoretic technique can serve as a supplement, if not a substitute to field test in determining the genetic purity of cotton cultivars. The *arboreum* and *hirsutum* varieties and the "hybrids H4, Godavari and Varalaxmi were easily identified on the basis of globulin fingerprints while the hybrids NHH-44, AHH-468 and DCH-32 were easily identified on the basis of Tris-HCI soluble protein fingerprints. Wang *et al.* [12] also evaluated 73 accessions of *G. herbaceum* by electrophoretic profiles of SDS-PAGE analysis of seed proteins. Abdel *et al.* [13] identified eleven Egyptian cotton cultivars on the basis of electrophoretic profiles of SDS-PAGE analysis of seed proteins. Dadlani *et al.* [14] compared field grow-out test with electrophoresis of seed proteins

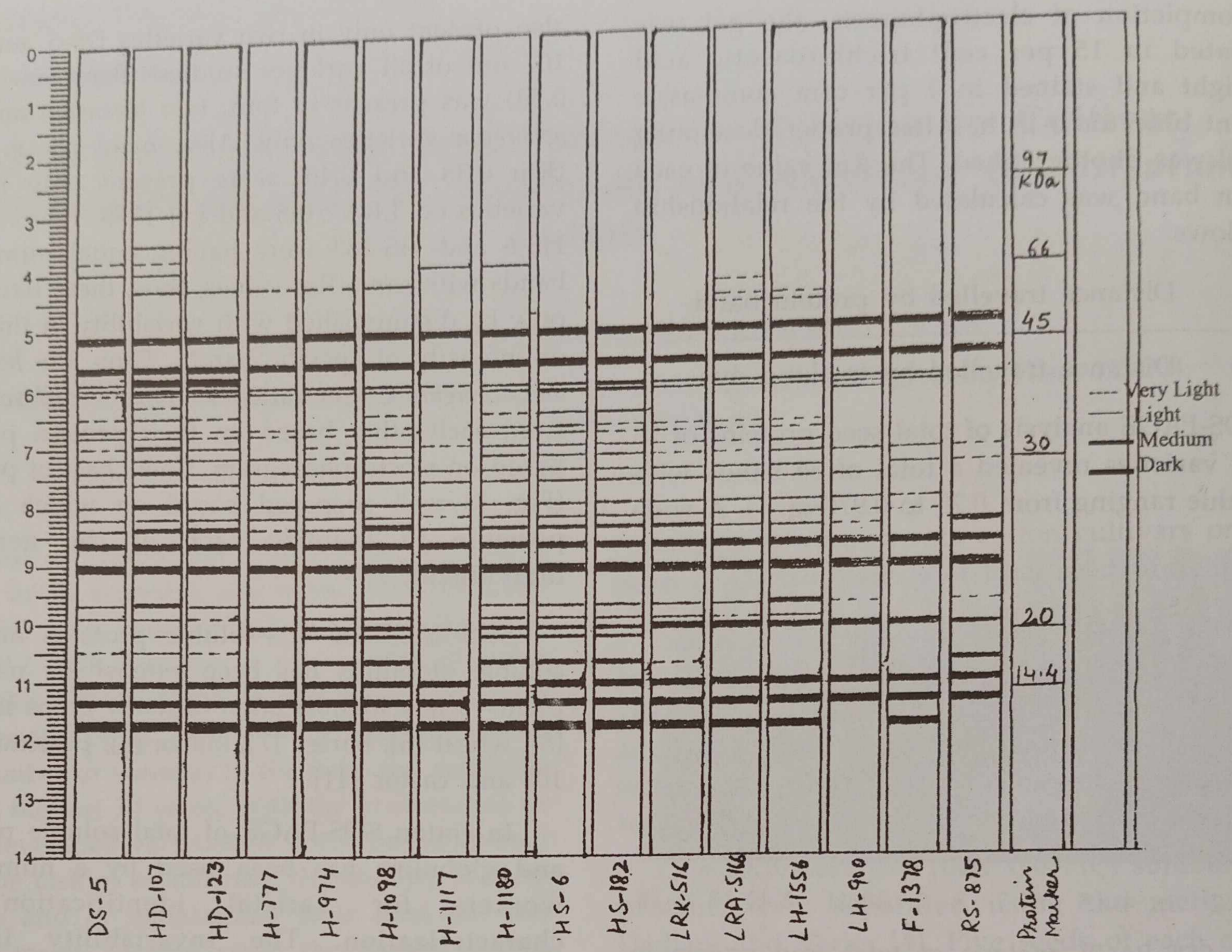

Fig. 2. Electrophoregram of total seed protein profile in cotton varieties of *G. arboreum* and *G. hirsutum*

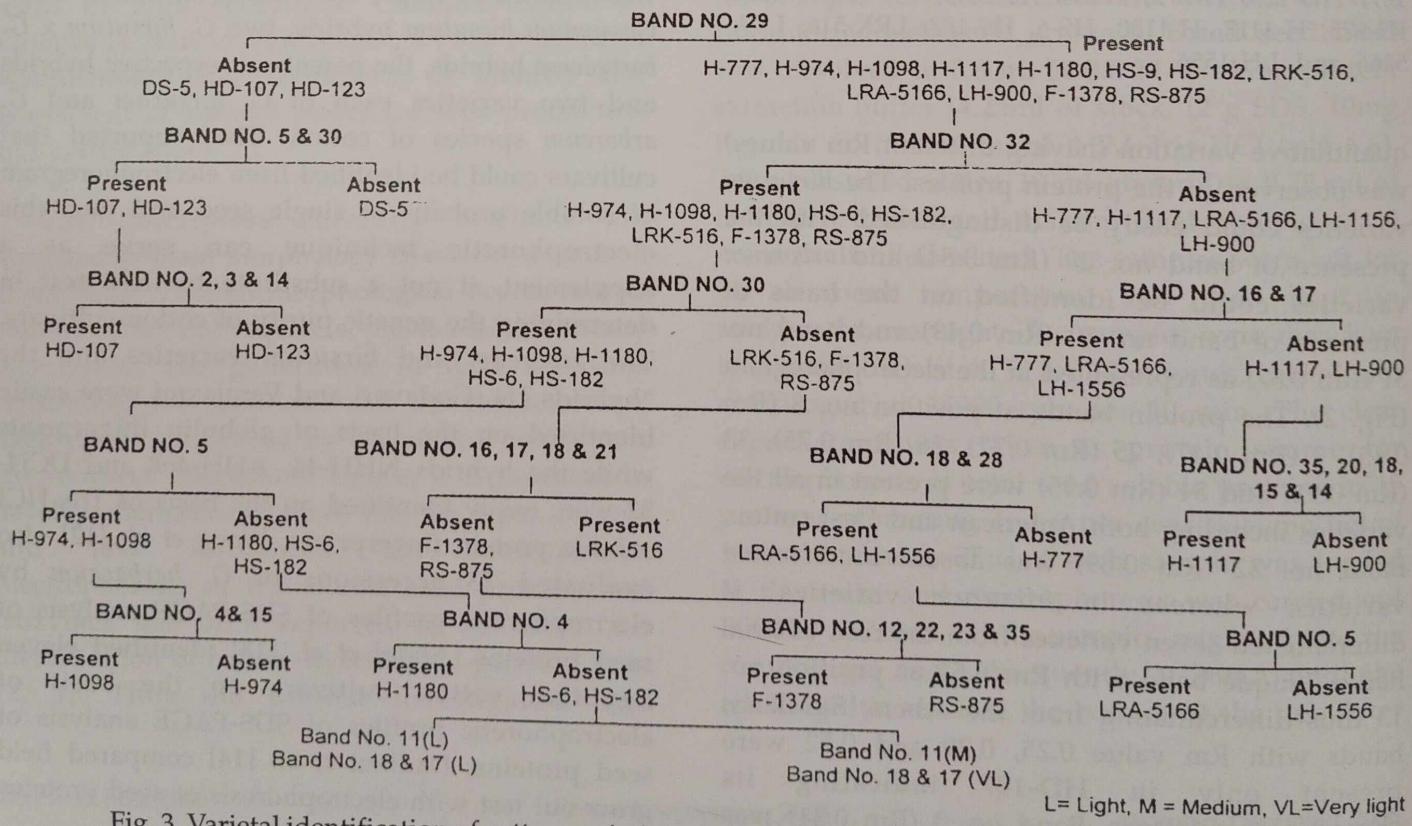

Fig. 3. Varietal identification of cotton varieties on the basis of electrophoresis of total seed protein

for testing the genetic purity of cotton hybrid NHH-44.

Therefore, a comparison of the protein composition of individuals or populations became a comparison of the underlying variation in the gene expression. By considering sufficient protein markers a large portion of the genome can be covered [15]. Thus, the total seed protein profiles can be used further in crop identification and characterization programmes.

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