

Electrophoresis Technique for Varietal Identification and Genetic Purity in Hybrid Cotton (*Gossypium hirsutum* L.)

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ABSTRACT Genetic purity is an essential component of seed quality. To ensure the genetic purity of hybrid cotton seeds, seed certification agencies follow the grow out tests which are labour intensive, tedious and time consuming. In post-GATT scenario establishing varietal identity is crucial for protection of plant varieties. In this regard Distinctness, Uniformity and Stability (DUS) tests play an important role and also it has been recommended by the UPOV. Our objective was to identify biochemical markers to establish distinctness and uniformity of cotton hybrids and parental lines and possible application of these markers for genetic purity analysis.

Key Words: Genetic purity, globulin

Cotton is one of the most important commercial crops in India. Hybrid cotton has played an important role in increasing yield potentiality of the crop. In developing a new improved cotton hybrid it is necessary to maintain purity of the seeds. The presence of contaminants above the permissible levels results in significant varietal deterioration. In case of hybrids, maintenance of the genetic purity of the parental lines is as important as that of the hybrid itself. To ensure the genetic purity of hybrid cotton seeds, seed certification agencies follow the grow out tests which are labour intensive, tedious and time consuming. Confirming the genetic purity through a grow-out test is mandatory for the certification of hybrid cotton seed under the Indian seed regulation. In the certified seed of hybrid cotton, genetic purity should be at least 90 per cent.

Under post-GATT scenario, establishment of varietal identity is crucial for protection of plant varieties. In this regard Distinctness, Uniformity and Stability (DUS) tests play an important role and also it has been recommended by the Union

Internationale Pour la Protection des Obtentions Vegetables or simply UPOV [1]. 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 seed [2, 3]. Our objective was to identify biochemical markers to establish distinctiveness and uniformity of cotton hybrids and parental lines and application of those markers for genetic purity test.

MATERIALS AND METHODS

Seeds of cotton hybrids and their parental lines were obtained from the All India Crop Improvement Programme (AICIP) on Cotton, Nagpur. Four hybrids and their parental lines were used in this experiment (Table 1). For genetic purity analysis twenty two commercial lots of hybrid, AAH-1 were received from the National Seed Corporation (NSC), New Delhi. Hybrids and their parental lines were

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Table 1. Cotton hybrids and their parents used in the present study

Hybrid	Female parent	Male parent
TCHB 213	TCH1218	TCB209
AAH1	DS5	HD266
PHH316	PH93	PH325
NHH44	BN-1	ACC738

used to generate reference SDS-PAGE profiles of salt-soluble globulins. Pooled samples of 10 seeds were used SDS-PAGE in 15 per cent acrylamide gel [4]. Seeds were decoated, ground and defatted through repeated extraction in chloroform:methanol:acetone (2:1:1) mix. Defatted seed meals were extracted with tris-HCL buffer (50mM, pH 8.0) containing 5 per cent NaCl and centrifuged at 14000 rpm for 30 mins. The supernatants were cold precipitated at 0°C for 24 hours and the pellet was collected upon centrifugation at 14000 rpm for 30 min. The pellets were dissolved in tris-glycine buffer (25mM, pH 8.3), mixed with equal proportion of Tris-HCl working protein buffer (0.08M, pH 6.8) heated at 100°C for 5 mins. The cooled sample was run in a denaturing 15 per cent polyacrylamide gel. Gels were stained with coomassie blue [5]. Similarity, index and dendogram were constructed by calculating Jacard's similarity coefficient and unweighted pair group method with arithmetic mean (UPGMA) using NTSYS software version 2.0. For genetic purity assessment, SDS-PAGE of salt soluble globulins was performed on 100 single seeds of hybrid cotton. The field grow-out test [6] based on different morphological characters like seed shape, leaf shape, hairiness of the stem etc. of the plant, was done.

RESULT AND DISCUSSION

The assessment of hybrid seed purity requires profiling the parental lines to identify a marker locus, which is polymorphic in these and confirm their appearance in the hybrid. Protein and isozyme markers were used for testing hybridity and genetic purity of commercial seed lots in a number of field crops and vegetables [3, 7, 8]. High

resolution PAGE technique for identification and genetic purity assessment of maize cultivars based on the polymorphism of salt soluble seed proteins are available [9]. SDS-PAGE or alkaline PAGE profiles of tris soluble proteins or salt soluble globulins have been employed by several workers for identification of cotton varieties [2, 8, 10, 11, 12, 13].

Each hybrid showed a distinct banding profile with nine to ten bands (Fig. 1). Hybridity was clearly detectable in TCHB-213 by the presence of complementary bands from its male parent. Hybrid TCB-213 recorded S.I. of 0.70 with both of its parents. Hybrid, NHH-44 was more like its male parent AC738, except for the presence of a major band at Rm 0.57, contributed by the female parent, BN-1. S.I. value of the same with the male and female parents was 0.53 and 0.5, respectively. Hybrid AAH1, having S.I. of 0.59 and 0.53 with its male and female parents was distinguishable by the presence of four bands having Rm values of 0.45, 0.51, 0.60 and 0.74, contributed by its female parent DS5 and five bands having Rm values of 0.39, 0.42, 0.65, 0.78 and 0.80 contributed by its male parent, HD266, respectively, though all the bands (including the minor ones) present in the male and female parents could not be recorded in the hybrid. The banding profile of hybrid PH-93, was quite similar to both the parents. The hybrid was distinguishable from its female parent by the absence of bands with Rm values of 0.36 and 0.39 and from its male parent by the absence of band having Rm of 0.80.

Dendogram constructed based on banding pattern put all the hybrids and their parental lines in four distinct clusters (Fig. 2). All parental lines were distinct from each other. However, the female parents of AAH1 (cluster 2) and TCHB 213 (cluster 4) were similar to that of the respective hybrids. Genetic base of PHH 316 found to be widest in the study (cluster 4).

The uniformity of banding profiles (with respect to all major bands) was checked in 10 single seeds from the authentic seed samples of AAH-1 and its parents. The genetic purity of twenty two commercial lots of hybrid AAH-1 were tested by electrophoresis of 100 single seeds from each lot. Banding pattern of the individual seed was

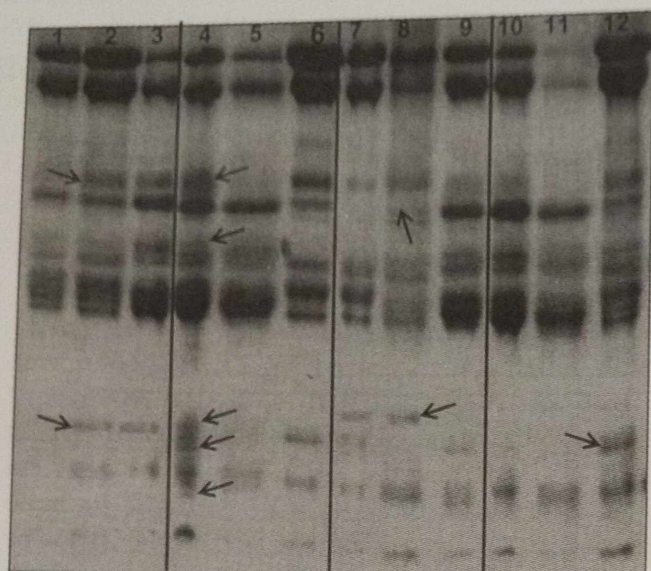


Fig. 1. SDS-PAGE profile of globulin from cotton hybrids and their parental lines. 1. TCH 1218, 2. TCHB 213, 3. TCB 209, 4. PH 93, 5. PHH 316, 6. PH 35, 7. BN 1, 8. NHH 44, 9. ACC 738, 10. HD266, 11. DS5, 12. AAH1

checked with the reference patterns of the hybrid and its female parent. Any other pattern was counted as an off-type. For the purpose of comparison, pooled samples of the hybrid and the

female parent were loaded in the end wells of each gel (Fig. 3).

The genetic purity of the samples based on electrophoretic analysis ranged from 88 to 100 per cent. Only one of the 22 samples was found to be sub-standard on account of its genetic impurity. The assessment of genetic purity based on electrophoresis results correlated well with that based on grow-out test, with a significant positive linear correlation of 0.6* (Table 2).

SDS-PAGE of salt-soluble globulins is more useful than that of Tris-soluble proteins for variety identification [11, 14]. Our result also showed that SDS-PAGE profile of salt soluble globulins provide sharper and more reproducible band resolution than that of total tris-soluble proteins. Though the genetic control of globulin polypeptides in cotton seeds are not well understood, the clear and discrete banding profiles make them ideal markers for the purpose of variety identification. The sequential sampling procedure suggested a reduction in the sample size from 400 to 109 (minimum) for detecting 1.5 per cent off-types [15], provided the number of selfed female seeds/plants

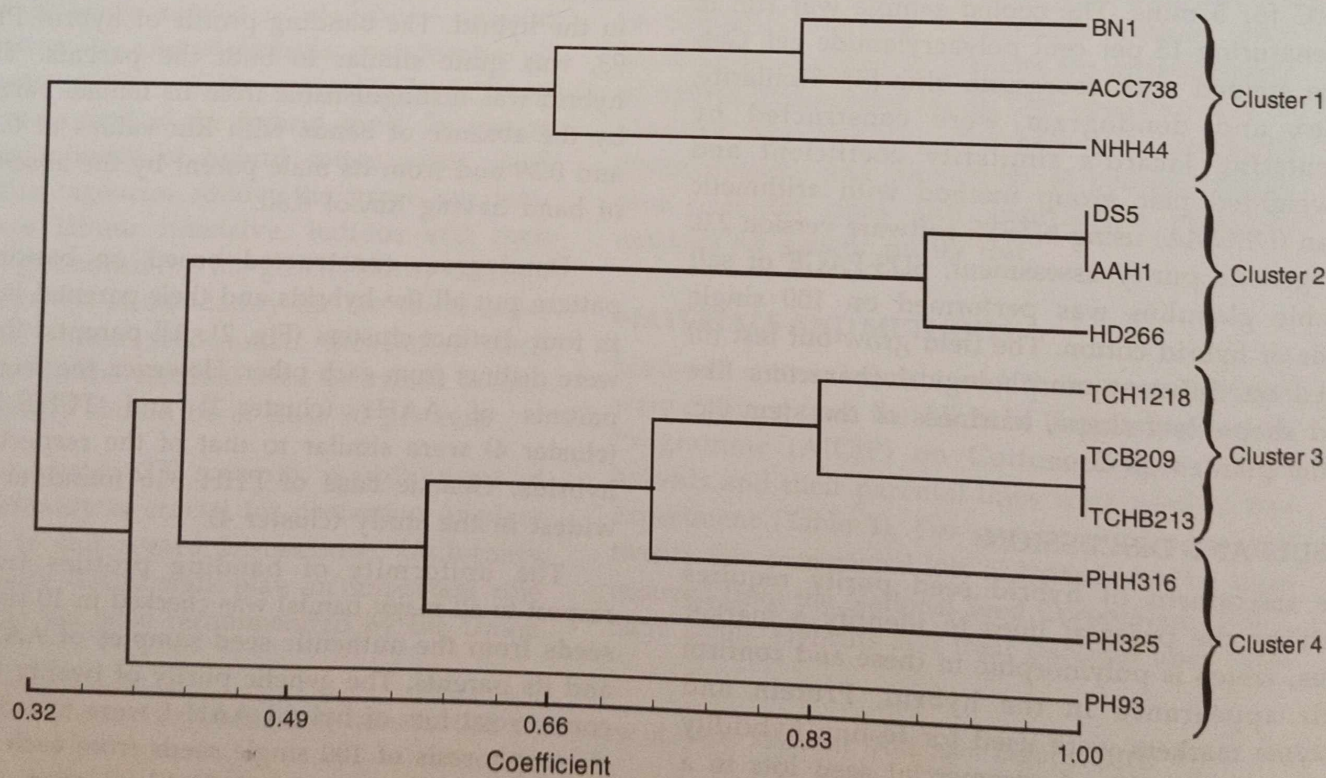


Fig. 2. Dendrogram representing distinctiveness of hybrids

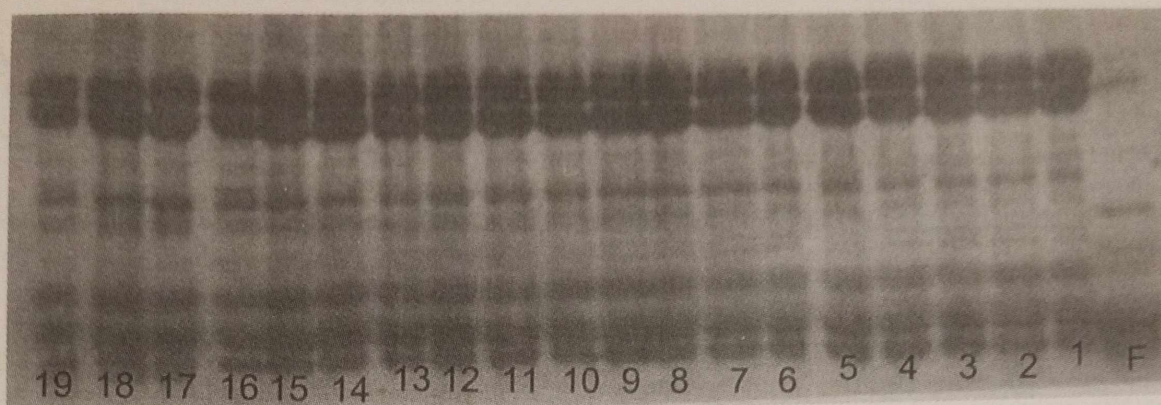


Fig. 3. SDS-PAGE profiles of globulin from single seed cotton hybrid AAH1 for genetic purity analysis

does not exceed 9 out of 109. A significant positive correlation between the field grow-out test and genetic purity tests based on 50, 100, 150 and 200 individual seed analyses [2, 5], suggest a minimum

Table 2. Genetic purity test results according to globulin profile and field grow-out tests of 22 lots of AAH-1

Lot No.	Genetic Purity (%)	
	Electrophoresis	Grow-out
1	92	92
2	100	97
3	100	100
4	100	100
5	100	99
6	100	100
7	100	100
8	100	100
9	100	100
10	98	100
11	99	95
12	90	100
13	99	98
14	99	95
15	90	99
16	98	99
17	95	97
18	88	99
19	98	99
20	94	94
21	99	100
22	99	100

Correlation coefficient of electrophoresis and grow-out data:
0.6*

sample size of 50 seeds for quick and reliable assessment. The minimum number of seeds to be tested for purity assessment depends on the acceptable confidence intervals in each case. It was suggested that testing at least 200 single seeds in order to strike a balance between the accuracy and the time required [16]. In the present study a reliable level of correlation was observed between the field test and electrophoresis analyses of 100 single seeds in 22 commercial seed lots of cotton hybrid AAH-1, having genetic purity of 88 to 100 per cent. This would mean that by running 5 to 6 gels each of 25-20 wells a seed lot can be reliably tested on the basis of 108 to 115 seeds (keeping 2 lanes in each gel for the reference samples of the hybrid and female parent). This would provide a very quick method for internal quality assessment required for decision making, rejecting or accepting a seed lot for procurement or marketing.

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REFERENCES

1. UPOV guidelines for the conduct of tests for distinctness, uniformity and stability, Cotton, (1994), TG/88/6.

2. AGARWAL, P.K., D. SINGH & M. DADLANI (1988). Identification of cotton hybrid seeds using PAGE. *Seed Sci. Technol.*, **16**: 563-569.
3. DADLANI, M., V. VASHIST, D.P. SINGH & A. VARIER (1994). Comparison of field grow-out and electrophoresis methods for testing genetic purity of cotton hybrid seed. *Seed Res.*, **22**(2): 160-162.
4. DADLANI, M. & A. VARIER (1993). Electrophoresis for variety identification. Technical Bulletin. Division of Seed Science and Technology, ICAR, New Delhi.
5. RAO, G. (2000). Biochemical methods for identification and genetic purity testing of cotton hybrids. Ph.D. Thesis submitted to IARI, New Delhi.
6. COOKE, R.J. (1995). Varietal identification of crop plants. In: J. H. Skerritt and Appeals (eds.). *New Diagnostics in crop Sciences*, pp. 33-63. CAB International Wallingford.
7. ORMAN, B., G.D. LAWRENCE, P.M. DOWNES, D.S. PHILLIPS & C.J. RIPERGER (1991). Assessment of maize inbred genetic purity by isozyme electrophoresis. *Seed Sci. Technol.*, **19**: 523-535.
8. KATO, K., Y. AKASHI, A. OKAMOTO, S. KADOTA & M. MASUDA (1998). Isozyme polymorphism in melon (*Cucumins melo* L.) and its application to seed purity and test of F₁ cultivars. *Breeding Sci.*, **48**(3): 237-242.
9. WANG, C., K. BIAN, H.K. ZHANG, Z.M. ZHOU & J.A. WANG (1994). Polyacrylamide gel electrophoresis of salt soluble proteins for maize variety identification and genetic purity assessment. *Seed Sci. Technol.*, **22**(1): 51-57.
10. KAPSE, S.S. & Y.S. NERKAR (1985). Polyacrylamide gel electrophoresis of soluble seed proteins in relation to cultivar identification in cotton. *Seed Sci. Technol.*, **13**: 847-852.
11. RAO, T.N., Y.S. NERKAR & V.D. PATIL (1990). Identification of cultivars of cotton by SDS-PAGE of soluble proteins. *Plant Var. & Seeds.*, **3**: 7-13.
12. ABDEL, T., E.M. FAHMY, M.O. ISMAIL & E.I. DOMYATI (1993). Application of electrophoretic analysis in cultivar identification in cotton. *Ann. Agric. Sci.*, **2**: 489-503.
13. ZHANG, C.Q., Y.P. YIN & R.Q. GAO (1998). Polymorphism of seed protein and identification of cotton cultivar. *Scientia Agri. Sinica.*, **31**(4): 16-19.
14. NERKAR, Y.S. & T.N. RAO (1993). Use of seed protein and enzyme polymorphism in the identification of cultivars of cotton. *Seed Res.*, Special Vol. 1: 375-394.
15. SINGH, D.P. & P.K. AGARWAL (1995). Sequential sampling plan procedure for testing genetic purity. *Seed Sci. Technol.*, **23**: 511-52.
16. ISTA. (1999). International rules for seed testing. *Seed Sci. Technol.*, **27**: 251-270.