Varietal diversity in cotton based on seed protein profile extracted at high temperature[1]

Diversidade varietal em algodoeiro baseada em perfis protéicos de proteínas extraídas a altas temperaturas

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ABSTRACT

Heat-extracted proteins were used for the first time in this study as biochemical markers to assess genetic diversity in cotton plants. Seeds of eleven cotton varieties were grown in the experimental area of the Department of Agriculture at the Universidade Federal de Lavras. The proteins were analyzed in the Laboratory of Biotechnology and Seed Analysis. Samples of two thousand seeds of Precoce (Epamig-5), Delta Opal, Deltapine, IAC-20, IAC-21, IAC-22, ITA-90, ITA-96, Precoce Alva, Precoce Liça and Redenção were ground and used for protein extraction. Electrophoresis was carried out in SDS-PAGE polyacrylamide gel at 8% (gel separator) and 6% (gel concentrator) and the gels stained with Comassie Blue 0.05% and silver stain. The polymorphism in the protein electrophoretic profiles enabled to classify the varieties in three clusters, according to size and number of bands. The varieties with greater similarity based on heat-extracted proteins profiles were Precoce Alva and Precoce Liça, which are semigametic materials. The heat-extracted proteins seem to be promissory markers for clustering and differentiating genetically close materials.

Index terms: Semigametic varieties, cotton protein, genetic diversity.

RESUMO

Proteínas extraídas pelo calor foram usadas pela primeira vez como marcadores bioquímicos para acessar a diversidade genética em variedades de algodoeiro. Sementes de onze variedades de algodoeiro foram cultivadas em área experimental do Departamento de Agricultura da Universidade Federal de Lavras. As proteínas foram analisadas no Laboratório de Biotecnologia e Análise de Sementes. Amostras de duzentas sementes das variedades Precoce (Epamig-5), Delta Opal, Deltapine, IAC-20, IAC-21, IAC-22, ITA-90, ITA-96, Precoce Alva, Precoce Liça e Redenção foram plantadas e usadas para extração de proteínas. A eletroforese foi conduzida em gel de poliacrilamida a 8 % SDS-PAGE (gel separador) e 6% (gel concentrador) e os géis corados com Azul de Comassie 0,05% e coloração com prata. O polimorfismo nos perfis eletroforéticos de proteína permitiram agrupar as cultivares em três grupos, de acordo com o tamanho e número de bandas. As variedades com maior similaridade pelas proteínas extraídas pelo calor, foram Precoce Alva e Precoce Liça, que são materiais semigaméticos. As proteínas extraídas pelo calor se apresentaram como marcadores promissores para agrupar e diferenciar materiais geneticamente próximos.

Termos para indexação: Variedades semigaméticas, proteínas de algodão, diversidade genética.

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Introduction

There is an increasing need to develop and/or adapt methods to characterize and differentiate cultivars with purposes for protection and registration of breed material.

In Brazil, the Cultivar Protection Law sanctioned on April 25 in 1997, opened a new perspective and interest in protecting and releasing genetic materials. For a cultivar to be registered and protected, it must present the distinction, uniformity and stability criteria (Bayle, 1983).

Morphological and physiological descriptors have been used frequently to register and protect cultivars. However, these are limited not only because they require following inspections during the whole crop cycle, but also because they require large areas for plantation and in certain situations they are unstable for distinction once they are based on the phenotype (Brasil, 1997).

Studies with descriptors have been carried out to distinguish close genotypes (Imolesi et al., 2001; Vieira et al., 2001), and the biochemical markers of proteins with seeds have been used for cultivars certification (ISTA - International Seed Testing Association, 1996; AOSA - Association of Official Seed Analysis, 1991). Some markers, such as hordeins in barley, the secalins in rye, glutelins in wheat, avenin in oats, zeins in maize, lectins and vicilins in *Phaseolus*, legumins in *Pisum sativum*, glycinin in soybean, have aided researchers in marker assisted selection and purity tests (Vieira et al., 2001). However, biochemical markers occasionally are not necessarily very informative because of the low polymorphism in materials with a narrow genetic base.

A group of robust proteins has been described, the hydrophilic proteins, which are extracted under high temperatures in water (Kiegel and Galili, 1994). The conserved nature of these proteins indicated that they might be promising in cultivar identification and certification.

This category of proteins has been quoted in cottonseeds as playing a role in the preservation of cell membranes (Baker et al., 1988). However there is no information in the literature on their polymorphism for cultivar characterization purposes. Thus the present investigation had the purpose to assess the genetic diversity of cotton cultivars by using of heat-extracted proteins as biochemical markers.

Material and Methods

The study was carried out in the Biotechnology and Seed analysis Laboratory at the Department of Agriculture at the Universidade Federal de Lavras, Lavras-MG (Brazil).

The varieties (Table 1) were grown in the experimental area, in plots of 10 x 10 m, which allowed production under the same cultivation conditions to obtain seeds with the same physiological quality. They were harvested manually and dried in the sun until they reached 13% of moisture content.

Proteins were extracted from a sample of two thousand seeds of each cultivar, which were homogenized and triturated in a grinder under refrigeration at 4°C (Tecnal Grinder – Model TE 631). The methodology utilized followed the protocol described by Blackman et al. (1991). A hundred mg of powder obtained was transferred to 1.5 ml micro tubes with 1 ml extraction buffer (500 Mm Tris-HCl pH 7.5; 500 Mm NaCl; 5 Mm $MgCl_2$, 1 Mm phenylmethylsulfonyl fluoride - PMSF). This mixture was homogenized using vortex for approximately 60 seconds, and then centrifuged at 16,000 xg for 30 minutes at 4°C. The supernatant was incubated in a water bath at 85°C for 15 minutes and again centrifuged as stated previously. The supernatant was placed in micro tubes and the precipitate discarded. Protein concentration was determined, using methodology proposed by Bradford (1976) and all the samples were equivalently diluted to a concentration of 4 mg/ml. Aliquots of 10 μl of the protein extracts were removed and 40 μl of sample buffer were added (2.5 ml glycerol, 0.46 g SDS; 20 mg bromophenol blue and the volume completed to 20 ml with buffer Tris-HCl pH 7.5). The tubes were placed for five minutes in boiling water. Seventy microliters of extracted solution were applied to the polyacrylamide gel SDS-PAGE at 8% (gel separator) and 6% (concentrating gel). The gel buffer/electrode system used was Tris-glycine + dodecil sodium sulfate (SDS) pH 8.9. The electrophoreses running was performed at 150 V and the gels stained with Silver and Comassie Blue at 0.05% according to Alfenas et al. (1992) for 12 hours and bleached in Acetic solution 10%, after with 5% Ethanol and last 85% water. For silver staining the gel was rinsed in 50% methanol for five minutes, and washed for 15 minutes with ultra pure water and placed in Ditiotreitol solution (33 μl DTT 1 M + 250 ml of water). It was kept in $AgNO_3$ solution (1.5 ml of

$AgNO_3$ 20% + 250 ml of water) for 15 minutes and rinsed three times in ultra pure water and the following reagent added three times for color development (9g of Na_2CO_3 + 150 μl of formaldeide and 300 ml of water). At the final, the gel was rinsed in 45% citric acid solution.

For estimating the molecular weights, a standard aliquot of proteins was applied in each gel, Bio-Rad Catalog 161-0344, Control 86787 containing miosin-213.000 Da, Beta galactosidase-135.000 Da, Bovine Albumin Serum 85.000 Da, Carbonic Anidrase - 44.100 Da, Soybean Tripsin inhibitor 32.400 Da, Lisozyme 18.500 Da and Aprotinine 8.000 Da.

Table 1 - Origin and agronomic characteristics of cotton varieties. UFLA, Lavras - MG, Brazil. 2001.

Cultivars	Origin	Agronomic traits
Precoce (Epamig 5)	GH 1175-5, Stoneville 213, C-25-1-80	Precocity (quick fructification)
Delta Opal	Australian introduction	Virus Resistant
Delta Pine - Acala 90	North American Introduction	Ramulose resistant
IAC-20	IAC-17, Tamcot SP-37	Precocity yield, multiple resistance to diseases and nematodes and high fiber percentage
IAC-21	Stoneville 2B, Delfos, IAC-12	Precocity, multiple resistance to diseases and nematodes
IAC-22	IAC-20, GH 1197-5	High yield and precocity
CNPA Itamarati - 90	Delta Pine AC-90	High yield and resistance to ramulose
CNPA Itamarati - 96	DPL, Auburn 56, M. D. Beja, Epamig-3	High yield and resistance to ramulose and virus
Precoce Liça	Semigametic	Semigametic
Precoce Alva	Semigametic	Semigametic
Redenção (Epamig –4)	Auburn 56, DACRM3, IAC-17	High yield, resistance to fusariose and high fiber percentage

Source: Cavalleri and Gridi-Papp (1993). Table adapted by Napoleão Esberard de Macedo Beltão (1999) with modifications.

Results and Discussion

The zymogram shows the cotton cultivars variation in the electrophoresis profiles of heat-extracted proteins (Figure 1). These variations occurred in the number of bands.

The cultivars generally were set into three large sub-groups, with small variations in a same subgroup, and this classification was based on the presence or absence of bands in electrophoreses profiles (Figure 1).

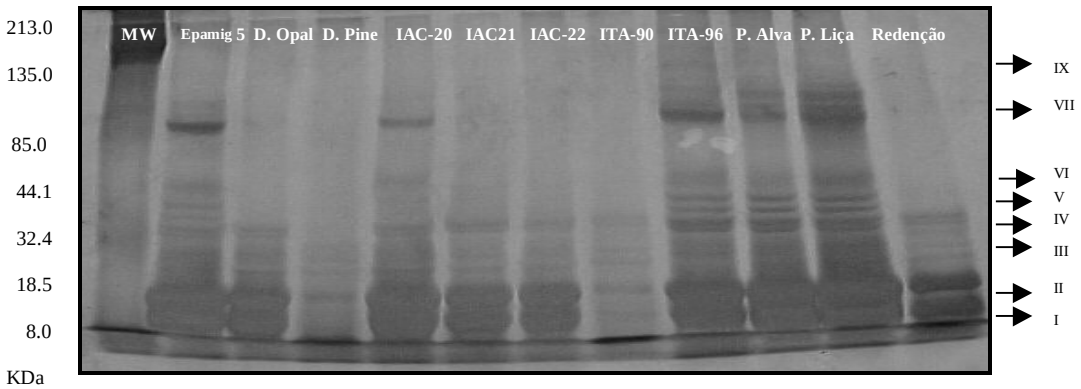

Figure 1 – Polyacrilamide gel of heat-extracted proteins of cottonseeds cultivars. UFLA, Lavras - MG, 2001.

The first large subgroup included the Precoce - Alva, Precoce - Liça, ITA-96 and Precoce (Epamig-5) cultivars (Table 2). Within this sub-group, the cultivars Precoce - Alva and Precoce - Liça presented greatest similarity. Both presented bands I, II, III IV and V quite clearly. It is pointed out that two single semigametic materials are being dealt with, which corroborate this marker as fairly promising for clustering and differentiating genetically close materials. In spite of the high similarity between both materials, this type of marker was efficient in differentiating cotton cultivars. ITA-96 and Precoce (Epamig-5) were clustered in this large subgroup, being similar to each other, and to the above-mentioned cultivars. However, Table 2 shows that none of them was similar for this marker. The main difference of ITA-96 from the other cultivars in this subgroup was the presence of the IX band. The Precoce (Epamig 5) cultivar was the most divergent in this group and did not present the bands VIII and IX. Within this group it was the cultivar that presented earliness as main agronomic characteristic, with quick fructification.

The cultivars Delta, Opal, IAC-20, IAC-21 and Redenção were set in a second subgroup, and Delta Pine and ITA-90 set in a third subgroup.

Among the cultivars in the second subgroup, Delta Opal and IAC-21 were the most similar, as both presented bands I, II and III.

No difference could be detected by this marker between the Delta Opal and IAC-22, or between the Redenção and IAC-21, although Delta Opal has the main agronomic characteristic of long fiber and IAC-22 and 21, precocity, and all have multiple resistances to diseases.

The cultivars in the third subgroup, Delta Pine and ITA-90, are both resistant to ramulose disease, but were completely different for the heat extracted proteins, presenting very weak bands I and II, they did not differ for this trait. It is important to point out that ITA-90 is derived from direct selection on Deltapine (Beltrão, 1999) and does not have common ancestors with the others.

This marker was polymorphic for these cultivars. However, for this biochemical marker to be suggested as a descriptor, investigation must be made on a greater diversity of genotypes, which must be cultivated under different environmental conditions to assess the attributes of homogeneity, distinguish ability and stability.

Table 2 – Protein subunits detected by electrophoresis of heat extracted proteins of cotton varieties. UFLA, Lavras-MG, 2001.

Varieties	Bands (presence)	Sub group
Precoce- Epamig-5	I, II, III, IV, V, VII	1
Delta Opal	I, II, III	2
Delta Pine	I, II	3
IAC-20	I, II, III, VII	2
IAC-21	I, II, III	2
IAC-22	I, II, III	2
ITA-90	I, II	3
ITA-96	I, II, III, IV, V, VI, VII, VIII, IX	1
Precoce Alva	I, II, III, IV, V, VI, VII, VIII	1
Precoce Liça	I, II, III, IV, V, VI, VII, VIII	1
Redenção	I, II, III	2

Conclusions

Precoce Alva and Precoce Liça cotton varieties present higher similarity using heat extracted proteins.

The heat extracted proteins are promissory markers to cluster cotton materials used in this study.

The cultivar ITA-90 is the most divergent material (49% of similarity) detected by molecular markers of heat extracted proteins.

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