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BIOCHEMICAL PARAMETERS OF SEEDS OF PARENTS AND HYBRID OF COTTON

CHITRA SHUKLA AND O.P. SAXENA*

Seed Technology Laboratory, Botany Department, Unversity School of Sciences,
Gujarat University, Ahmedabad - 380 009, Gujarat

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SUMMARY

The seed protein fractions, their profiles through electrophoresis, protease activity and enzyme proteins were examined in hybrid and its parents in cotton. The amounts of protein fractions were intermediate in hybrid to its parent except prolamin and total proteins which showed maximum and minimum values respectively. The total proteins in the seeds were maximum and prolamins were minimum. For protein profiles, no band was observed in the prolamin-like substance fraction. In other fractions, bands differed in their intensities. Qualitative analysis through electrophoresis in terms of presence and absence and differences in the intensities of the bands in different profiles of storage proteins proved to be an important parameter to differentiate parents and hybrid of cotton.

Key words : **Albumin, cotton, globulin, glutelin, prolamin, protease.**

INTRODUCTION

The ability to distinguish between and identify cultivars of species is a fundamental to the operation of seed testing and to modern crop production. Earlier, distinctness, uniformity and stability of any cultivar have relied on morphological methods, which are influenced by environmental conditions (Goodrich *et al.* 1985). The need, therefore, for new tool was desparate. Biochemical methods enable more precise, rapid and cost effective identification method. These too have some disadvantages, viz. they are profoundly influenced by tissue specificity and developmental stage. This disadvantage can be overcome by using the electrophoretic markers of conservative proteins, e.g. seed storage proteins. The advent of the electrophoresis as an analytical tool provide an indirect method for genome probing by exposing structural variations in enzymes or other proteins (Paulis and Wall 1977, Cooke 1984, Gilliland 1989). The seed proteins differ in chemical composition and properties from those found in other plant tissues and vary according to species. Hence, quantitative and qualitative studies were undertaken to differntiate seeds of parents and hybrid of cotton based on biochemical parameters.

MATERIALS AND METHODS

Uniform, sound, well filled, seeds of parents: [(G.Cot-10 (male) and G.Cot-100 (female)] and hybrid (H-6) of cotton *(Gossypium hirsutum* L.) were used for the extraction of different fractions of proteins for electrophoresis studies. The total proteins and protein fractions were extracted, in different solvent system based on their solubility following the method of Landry and Moureaux (1970). Albumins and globulins extracted in water and dilute salt solutions respectively, prolamins and prolamin-like substances extracted in aqueous alcohol, glutelin-like substances and glutelins extracted by weakly acidic or alkaline or dilute SDS solution and residues. The total protein and protein fraction profiles were examined using the method of Laemmeli (1970)

* Corresponding author

and Weber *et al*. (1972), using SDS-PAGE, on 10% running gel (Cooke 1983). The soluble protein bands were stained with coomassie brilliant blue R – 250 and Rm values were calculated.

The mean of five runs were computed to calculate the Rm values. The bands were traced on the graph paper through visual recording and the banding patterns were drawn. The presence or absence of bands, differences in band size and intensity of band were used to differentiate the parents and hybrids of cotton. The quantitative estimation of proteins was conducted by the method of Lowry *et al*. (1951). Protease activity was determined as cytoplasmic and wall bound fraction seperately. The protease activity was analyzed by Penner and Ashton, (1967) method, modified by Cruz *et al*. (1970).

RESULTS AND DISCUSSION

Seed storage proteins are proteins which are synthesized only in seed (in cotyledon or in endosperm) and not in other tissues, during seed development and accumulate to levels which substitute a large proportion of the total seed protein and hydrolysed during germination to provide nutrients for the developing seedlings (Boulter 1981, Higgins 1984, Muntz 1998). They lack any other functional activity besides storage.

The quantitative analysis of seeds of cotton as shown in the fig. 1, showed that the amount of total proteins followed by glutelin-like substances, glutelins, albumin and globulins, residues, prolamin-like substances and prolamins, in decreasing order. The hybrid showed minimum amount of total proteins as compared to their parents while for the other protein fractions hybrid was medium to maximum. No definite pattern regarding the amounts of proteins and protein fraction in the seeds of parents and hybrid of cotton was obtained. Dure (1975) also carried out extensive biochemical studies of seed development in cotton. The accumulation of metabolites was found to be profoundly influenced by tissue specificity and developmental stage of the seed.

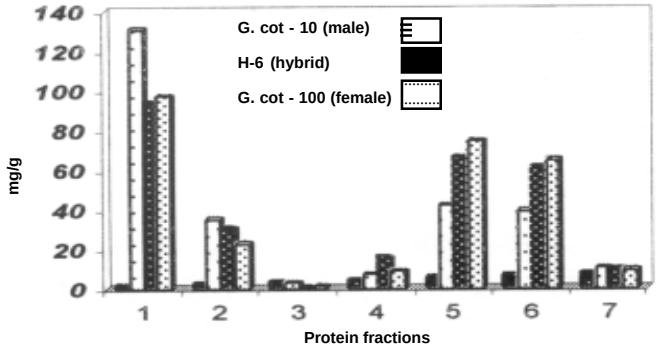

Fig. 1. Seed protein fractions in parents and hybrid of cotton
1: Total seed protein, **2:** Albumin and globulins, **3:** Prolamin, **4:** Prolamins like substances, **5:** Glutelin like substances, **6:** Glutelins and **7:** Residues

The disadvantages of biochemical methods being profoundly influenced by tissue specificity and developmental stage was overcome by using the electrophoretic markers of conservative proteins, e.g. seed storage proteins. Electrophoresis of protein profiles, and storage proteins provide firm basis to differentiate parents and hybrid of cotton and provide methods for genome probing by exposing structural variations in enzymes or other protein genome (Cooke 1984, Gilliland 1989).

The protein and protein fractions profiles showed that bands were generally common to parents and hybrid but the intensity of staining in the bands were different and showed marked differences between parents and hybrid. The total seed protein profile showed a band at Rm value 0.474 unique to hybrid, while band at Rm value 0.448 was common to both the parents and absent in hybrid. Two bands at Rm 0.846 and 0.91 were common to parents and hybrid (Table-1).

Table 1. Total seed protein profiles (Rm values) of parents and hybrid of cotton

Sl. no. band	G.Cot-10 (male)	H-6 (hybrid)	G.Cot-100 (female)
1	0.448	-	0.4487
2	-	0.474	-
3	0.846	0.846	0.846
4	0.91	0.91	0.91

Albumins and globulins profile showed three bands at Rm 0.428, 0.545 and 0.6897 common to parents and hybrid, while band at Rm 0.448 was common to hybrid and its female parent G.Cot-100. G.Cot-10 showed two

polymorphic bands at Rm 0.46 and 0.793 while hybrid and female parent showed one polymorphic band each at Rm 0.779 and 0.7586 respectively (Table-2).

Table 2. Albumin and globulins profiles (Rm values) of parents and hybrid of cotton

Sl. no. band	G.Cot-10 (male)	H-6 (hybrid)	G.Cot-100 (female)
1	0.428	0.428	0.428
2	-	0.448	0.448
3	0.46	-	-
4	0.545	0.545	0.545
5	0.6897	0.6897	0.6897
6	-	-	0.7586
7	-	0.779	-
8	0.793	-	-

Prolamin profile of parents and hybrid showed only one polymorphic band at Rm 0.75, whereas two bands at Rm 0.82 and 0.857 were common to parents and hybrid (Table 3).

Table 3. Prolamin profiles (Rm values) of parents and hybrid of cotton

Sl. no. band	G.Cot-10 (male)	H-6 (hybrid)	G.Cot-100 (female)
1	0.75	-	-
2	0.82	0.82	0.82
3	0.857	0.857	0.857

No band was observed in prolamin-like substances profile as evident from the amount of its fraction in the quantitative analysis, as it showed minimum amounts. Thus, the qualitative and quantitative analysis can be related.

The glutelin like fraction showed one polymorphic band observed in female parent at Rm.0.86 and one band at Rm.0.88 was common to hybrid and male parent. Rest of the three bands were common to parents and hybrid (Table 4).

Table 4. Glutelin-like fraction profiles (Rm values) of parents and hybrid of cotton

Sl. no. band	G.Cot-10 (male)	H-6 (hybrid)	G.Cot-100 (female)
1	0.48	0.48	0.48
2	0.52	0.52	0.52
3	-	-	0.86
4	0.88	0.88	-
5	0.92	0.92	0.92

Similar to glutelin-like fraction, glutelin fraction also showed one polymorphic band at Rm 0.55 in male parent, while one common to hybrid and female at Rm 0.57, and two bands common to both parents and hybrid (Table 5).

Table 5. Gluteliin profiles (Rm values) of parents and hybrid of cotton

Sl. no. band	G.Cot-10 (male)	H-6 (hybrid)	G.Cot-100 (female)
1	0.55	-	-
2	-	0.57	0.57
3	0.893	0.893	0.893
4	0.91	0.91	0.91

The extensive protein polymorphism allows impressive levels of differences between varieties through electrophoresis. Seed protein patterns obtained by electrophoresis has been successfully used to resolve taxonomic and evolutionary problems of several crop species (Ladizinsky and Hymowitz 1979) and are considered particularly reliable, as seed storage proteins are largely independent of environmental factors. The soluble protein and its fractions obtained by acrylamide gel electrophoresis has been used for solving various taxonomic problems of different species of *Brassica* (Raab *et al.* 1992) and cultivars identified on the basis of water soluble proteins by polyacrylamide porosity gradient.

Protease activity (cytoplamic) in hybrid H-6 was medium and maximum in seed and cotyledon fraction

while minimum in seedling and embryo. In wall bound fraction hybrid H-6 showed minimum activity in seed and seedling while medium and maximum in cotyledon and embryo respectively (Fig. 2).

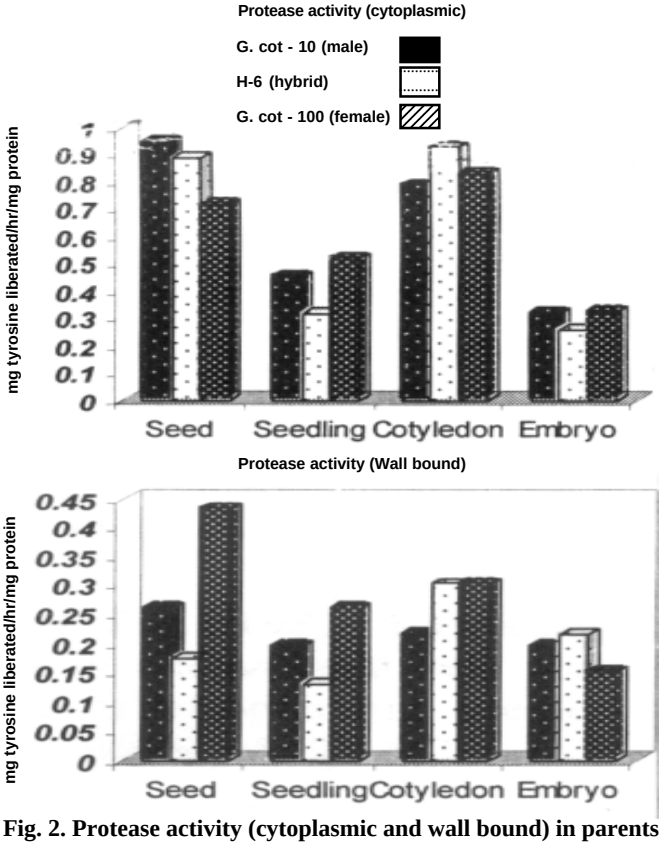

Fig. 2. Protease activity (cytoplasmic and wall bound) in parents and hybrid of cotton

Like quantitative analysis of the total protein and protein fractions no definite pattern was obtained regarding the enzyme activity in the parents and hybrid of cotton, and thus alone cannot be considered to differentiate parents and hybrid of cotton. Since, the constituents of seeds are determined genetically, biochemical markers (enzyme study and proteins quantitative analysis), prove to be a very important tool to distinguish between parents and hybrids of cotton, but the relative amounts of these constituents are sometimes dependent on the environmental factors, in addition, a large number of secondary plant products may be present in the seeds.

Storage protein profiles through electrophoresis provides very good means for cultivar identification and also electrophoretic analysis of seed storage proteins is widely recognized and accpeted as a technique for cultivar identification in the breeding species. This technique might be used on large scale to include the largely out breeding species. Thus it may be concluded that, biochemical methods, enzyme activities along with electrophoretic analysis of seed protein can be used as an important tool to distinguish parents and hybrids of cotton.

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