Chemistry of Natural Compounds, Vol. 31, No. 3, 1995

INVESTIGATION OF THE ELECTROPHORETIC COMPOSITION OF THE PROTEINS OF COTTONPLANT POLLEN GRAINS

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UDC 547.962:633.511

We have studied the proteins of the pollen and ovules of the cotton plant. No differences were observed in the electrophoregrams of the proteins from Gossypium hirsutum *L. and from* G. barbadense *L. In the spectrum of the pollen proteins, the main components quantitatively were polypeptides with molecular masses of 34, 44, 47, and 52 kDa. The species* G. raimondii *Ulbr. differed considerably, with the main polypeptides quantitatively having molecular masses of 27, 36, 46, and 58 kDa. In representatives of genomic group C, interspecies differences were observed in the protein spectra of the pollen. No appreciable differences were observed spectrally in the proteins of the cottonplant ovules.*

The biological characteristics of pollen are determined to a considerable degree by the enormous number of genes and are expressed by the fairly large spectrum of the final product of their expression — proteins. Proteins in plants play a decisive role in the interaction of the pollen with a stigma of the pistil. In an investigation of pollen and stigma differing with respect to the genes for sterility of the forms of a number of plants, an absence of individual fractions of glycoproteins in the cells of these organs was detected. In the coat of pollen grains of *Brassica* two main groups of polypeptides specifically interacting with the S-glycoproteins of the stigma have been identified and partially characterized [1]. The localization of the incompatibility proteins in the generative organs of petunia has been shown by immunochemical analysis [2]. The amount and some properties of nucleic acids in the pollen and ovules of the cotton plant before and after fertilization have been studied [3], but until recently no information has been found in the literature on the protein composition of the generative organs.

We have investigated the proteins of the pollen and ovules of heterogenomic groups of cotton plants.

Figure 1 shows electrophoregrams of the proteins of the pollen of some diploid and tetraploid species of cotton plant. Almost no differences were detected between *G. hirsutum* L. and *G. barbadense* L. from the electrophoretic spectra of the pollens. They contained polypeptides with molecular masses of from 10 to 90 kDa.

The main polypeptides quantitatively had molecular masses of 34, 44, 47, and 52 kDa. The same polypeptides are the main ones in the majority of diploid species of genomic group D, although there are slight differences in their quantitative ratios.

The species *G. raimondii* Ulbr. differs strikingly. It is characterized by the presence of protein components that are absent from the other diploid species. For example, polypeptides with molecular masses of 27, 36, 46, and 58 kDa are the main ones in *G. raimondii* Ulbr. but are either totally absent from the other species or are present as minor components. It is interesting to note that the phenomenon of the multicomponent nature of the electrophoretic spectrum of the proteins in *G. raimondii* Ulbr. as compared with other diploid and tetraploid species had been detected previously in an investigation of the electrophoretic spectra of the proteins of the seeds [4].

According to the literature, *G. raimondii* Ulbr. can be freely crossed with other species of genomic group D [5]. It may therefore be assumed that the differences detected in the protein spectra of the pollens are not factors determining the fertility or sterility of the plant. Differences in the electrophoretic composition of the proteins of the pollen of diploid species can be used in a study of the origin of amphidiploid species.

Biolog Scientific Production Combine, Academy of Sciences of the Republic of Uzbekistan. Institute of Genetics, Academy of Sciences of the Republic of Uzbekistan, Tashkent ul. F. Khodzhaeva, 28. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 454-456, May-June, 1995. Original article submitted September 13, 1994.

0009-3130/95/3103-0376$12.50

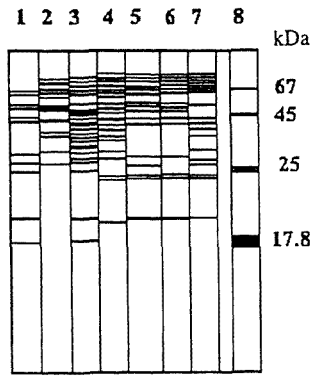

Fig. 1. Electrophoregrams of the proteins of the pollen of heterogenomic species of cotton plants in PAAG/SDS: 1) *G. thurberii*; 2) *G. herbaceum*; 3) *G. raimondii*; 4) *G. mexicanum*; 5) *G. hirsutum* L. (Tashkent-1); 6) *G. hirsutum* L. (108−F); 7) *G. barbadense* L. (C−6037); 8) marker proteins.

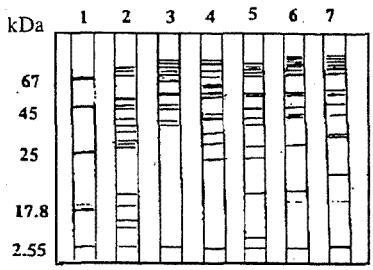

Fig. 2

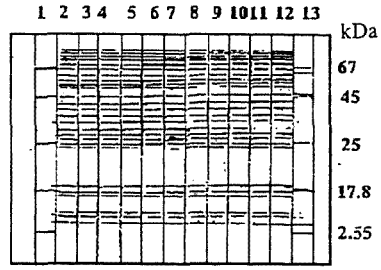

Fig. 3

Fig. 2. Electrophoregrams of the proteins of the pollen of heterogenomic seeds of cotton plant in PAAG/SDS: 1) marker proteins; 2) *G. sturtianum* var *sturtianum* Willis; 3) *G. sturtianum* var *nendewarense* Fryx.; 4) *G. australe* F. Muell; 5) *G. nelsone* Fryx.; 6) *G. bickii* Prokh.; 7) *G. aridum*.

Fig. 3. Electrophoregrams of the proteins of the ovules of various species of cotton plant in PAAG/SDS: 1) marker proteins; 2) *G. arboreum*; 3) *G. herbaceum;* 4) *G. harknessi*; 5) *G. sturtianum* var. *nandewarense* Fryx.; 6) *G. hirsutum* L. (108-F); 7) *G. hirsutum* L. (Krasnolistnaya Akala); 8) *G. barbadense* L. (S-6035); 9) *G. nelsonii* Fryx; 10) *G. australe* F. Muell; 11) *G. sturtianum* var. *sturtianum* Willis; 12) kenaf; 13) marker proteins.

The species *G. sturtianum* var. *nanderwarense* Fryx. differs from other species by the presence of a polypeptide with a molecular mass of 40 kDa, while the species *G. australe* F. Muell has only three quantitatively main peptides, the others being minor (Fig. 2).

The ovules of the cotton flowers showed no interspecies and intervariety differences whatever in the electrophoretic spectra of the proteins by visual analysis of the electrophoregrams. They also contained polypeptides with molecular masses of from 10 to 80 kDa, and many polypeptides with the same moleular masses were detected in the pollen and ovules (Fig. 3). However, in the qualitative respect polypeptides with molecular masses of less than 25 kDa were represented as the main ones in terms of the number of components, while polypeptides with molecular masses of from 25 to 80 kDa in the ovules were distributed more or less uniformly.

The pollens of various species of cotton plants differ in their protein compositions, which is reflected in their electrophoretic spectra. The electrophoretic spectra of the proteins of the ovules, which are similar for different species of cotton plant, show the improbability of their participation in the determination of the selective fertility or sterility of the plants. Similar results may apparently be expected in an investigation of the protein compositions of the stigmas and the styles.

EXPERIMENTAL

We investigated the flowers of diploid species of cotton plants of genomic groups A, C, and D from the collection material of INÉR — *G. arborreum, G. herbaceum, G. thyrberii, G. raimondii* Ulbr., *G. harknessi, G. nelsonii* Fryx., *G. australe* F. Muell, *G. sturtianum* var. *sturtianum* Willis, *G. sturtianum* var *nendewarense* Fryx., *G. bickii* Prokh, *G. aridum* — and the tetraploid species — *G. hirsutum* L. (varieties 108−F, Tashkent-1, and Krasnolistnaya Akala) and *G. barbadense* L. (variety S-6035).

For analysis we took 1 mg of freshly gathered pollen, and added 200 μl of a buffer with the composition 0.0625 M Tris-HCl, pH 6.8; 0.01% Bromophenol Blue; 5% 2-mercaptoethanol, 10% glycerol, and 2% sodium dodecyl sulfate (SDS). The mixture was boiled for 2 min and was centrifuged at 5000 rpm. The supernatant was deposited in wells in 10% polyacrylamide gel (PAAG) with SDS. Electrophoresis was conducted under standard conditions by Laemmli's procedure [6]. After electrophoresis, the gel was fixed and was stained in a 0.1% solution of Coomassie R-250 in ethanol−acetic acid−water (25:5:70). The excess of dye was washed out with 7% acetic acid. As protein markers we used bovine albumin (67 kDa), egg albumin (45 kDa), chymotrypsin (25 kDa), myoglobin (17.8 kDa), and cytochrome C (12.3 kDa).

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