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ELECTROPHORETIC ANALYSIS OF PROTEINS FROM SEEDS OF TRANSFORMED COTTON HYBRIDS

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The need to obtain information about a plant genome, its properties, and the biochemical processes occurring in it makes it necessary to apply biochemical research methods in addition to the classical methods of morphological, physiological, and genetic analysis [1]. Any changes in the living organism are reflected in changes of the protein structures, which in turn are products of genome activity [2].

Herein we report the electrophoretic analysis of the action of xenotypic DNA (*H. syriacus*) on cotton (*G. hirsutum* L.) and the nature of its succession in hybrid generations. The goal of the analysis was to reveal changes in protein synthesis in seeds of hybrids that occur after the genetic transformation.

Distribution spectra of water-soluble proteins (DSWSP) from seeds of hybrids obtained by hybridization of a transformed cotton form designated PG with variety Yulduz (*G. hirsutum* L.) in direct and reverse crossing combinations and their F_1—F_3 generations were investigated. The PG form was obtained by application of a suspension of cotton pollen with DNA isolated from *H. syriacus* pollen.

Ground seeds were defatted with acetone. The water-soluble protein fraction was extracted with distilled water at a 1:10 powder:water ratio over 30 min. The extract was centrifuged at 8000 rpm [3]. Before electrophoresis, the protein extracts were denatured with heat for 3 min and centrifuged at 8000 rpm. Wells were charged with 10 μL each.

Electrophoresis was carried out in 12% PAAG using the standard Laemmli and Favre method [4].

Eight bands of varying color intensity were seen visually in the DSWSP from seeds of the parental form PG (P_1) (Fig. 1a). These included fractions with electrophoretic mobility R_f 0.42, 0.60, and 0.82, which were the most intensely colored and differed significantly in amount of protein compared with the other fractions. The polypeptide fractions with R_f 0.145, 0.21, and 0.29 were moderately colored; with R_f 0.88 and 0.90, weakly colored.

The fraction with R_f 0.90 was characteristic of P_1 and was absent for P_2. The DSWSP from seeds of the second parental form P_2 (Yulduz variety) differed in that it had 16 fractions (Fig. 1b), among which were fractions with R_f 0.04, 0.51, and 0.77 and weakly colored fractions with R_f 0.66, 0.82, 0.98, and 0.99 that occurred only for the second parental form and were absent for the first (Table 1).

Polypeptide fractions with R_f 0.145, 0.21, 0.29, 0.42, 0.60, 0.82, 0.86, and 0.92 that were present in spectra of both parents were classified as the major fractions; fractions with R_f 0.64, 0.82, and 0.92, which were weakly colored, as minor fractions.

From a genetic viewpoint, according to Mendel's law, there should have been unique signatures in the first hybrid generation (F_1). As a rule, this principle should also be applicable to the succession of proteins. This was not observed in the experiment on F_1 hybrids performed by us. The total amount of proteins in seeds of hybrids of the direct crossing combination differed slightly from the reverse.

In seeds of the F_2 generation of direct and reverse crossing combination, the amount of water-soluble proteins was slightly reduced according to the distribution pattern of proteins that was analogous to samples of the parental forms although differences were seen. Thus, minor proteins with R_f 0.08 and 0.49 (Fig. 1e) and those with R_f 0.62, 0.63, and 0.71 (Fig. 1f) have not been observed previously in parental and hybrid samples (Table 2).

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TABLE 1. Electrophoretic Mobility (R_f) of Water-Soluble Proteins from Seeds of Parental Forms

Form	Electrophoretic mobility of fraction															
P_1	-	0.145	0.21	0.29	0.42	-	0.60	-	-	-	0.82	0.88	0.90	-	-	-
P_2	0.04	0.145	0.21	0.29	0.42	0.51	0.60	0.66	0.77	0.77	0.82	-	-	0.92	0.98	0.99

P_1, PG; P_2, Yulduz variety.

TABLE 2. Electrophoreticc Mobility (R_f) of Polypeptides from Seeds of Hybrid Forms (F_1, F_2, F_3)

Form	Electrophoretic mobility of fraction
F_1 (c)	0.10, 0.35, 0.55, 0.65, 0.79, 0.84, 0.88, 0.93, 0.98
F_1 (d)	0.12, 0.29, 0.35, 0.45, 0.55, 0.64, 0.66, 0.79, 0.84, 0.88, 0.93, 0.98
F_2 (e)	0.04, 0.08, 0.14, 0.20, 0.26, 0.28, 0.29, 0.41, 0.50, 0.55, 0.58, 0.62, 0.76, 0.80
F_2 (f)	0.04, 0.08, 0.14, 0.20, 0.25, 0.28, 0.29, 0.41, 0.48, 0.55, 0.58, 0.62, 0.63, 0.72, 0.76, 0.80
F_3 (j)	0.04, 0.14, 0.21, 0.29, 0.42, 0.56, 0.60, 0.69, 0.72, 0.76, 0.82, 0.86, 0.92, 0.98
F_3 (h)	0.04, 0.14, 0.21, 0.29, 0.42, 0.56, 0.60, 0.69, 0.72, 0.76, 0.82, 0.86, 0.92, 0.98

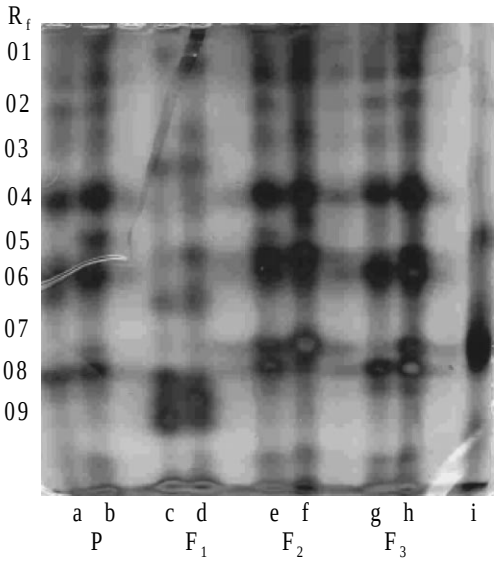

Fig. 1. Electrophoregram of water-soluble proteins from seeds of a transformed form (PG), Yulduz variety, and their hybrids: PG (P_1) (a), Yulduz variety (P_2) (b), F_1 (Yulduz variety × PG) (c), F_1 (PG × Yulduz variety) (d), F_2 (Yulduz variety × PG) (e), F_2 (PG × Yulduz variety) (f), F_3 (Yulduz variety × PG) (g), F_3 (PG × Yulduz variety) (h), and markers (i).

Thus, high-, middle-, and low-molecular-weight proteins were synthesized more extensively in seeds of second and third hybrid generations. They were more varied compared with the spectrum of the parental forms and samples from hybrids of the first generation.

Our results indicate that the biosynthesis was reduced and correlate with data from classical hybridization between distant species of a single family [3]. In this instance morphological analysis of the plants revealed a similarity of the hybrids to the parental forms.

A stabilized accumulation of water-soluble proteins was observed in the F_3 generation. The distribution pattern of polypeptides from hybrid samples was analogous to those of water-soluble proteins from the parental forms.

However, here also there were definite differences. For example, a protein with R_f 0.67 appeared that had not been previously observed for any parent or F_1-F_3 hybrids.

The results suggest that xenotypic genetic information (DNA) can affect biosynthesis and succession of water-soluble proteins in successive hybrid generations if incorporated into a donor organism. However, this effect appears only in seeds of hybrids of the first generation and is reflected in the synthesis of previously absent protein fractions. Changes in the biosynthesis of water-soluble proteins are reversible in nature. Based on the analysis of proteins from seeds of F_2 and F_3 generations, it can be concluded that these changes are not passed on to successive generations because the spectrum of proteins analogous to the distribution pattern of protein fractions from seeds of parental forms is reduced.

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