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IMMUNOCHEMICAL PROPERTIES OF THE SPECIES-SPECIFIC PROTEINS OF COTTON SEEDS OF THE SPECIES *Gossypium hirsutum* AND *G. barabadense*

A. M. Érmatov, D. A. Khashimov, and P. Kh. Yuldashev

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As we have reported previously [1], many physicochemical properties of the species-specific proteins H.0.43 and H.0.51 of *G. hirsutum* and B.0.37 and B.0.48 of *G. barbadense* are very close, which indicates their close affinity in the genealogical respect. It was of interest to trace their immunochemical properties and compare them.

In order to obtain antisera to the species-specific proteins of the cotton plant, we immunized rabbits with the H.0.43, H.0.51, B.0.37, and B.0.48 proteins. In each case, 24 mg of protein was dissolved in sterile 0.15 M NaCl solution and was injected subcutaneously in admixture with complete Freund's adjuvant (1:1) [2] once a week for one month. A week after the last injection of the antigen, 5-10 ml of blood was taken from an auricular vein. After 30 min, the blood clot that had formed was eliminated by centrifugation at 3000 rpm for 5 min. The blood serum with the antibodies (antiserum) was lyophilized. In this way we obtained four antisera to the proteins H.0.43, H.0.51, B.0.37, and B.0.48.

The specificities of the antisera obtained were studied by Ouchterlony's double-immunodiffusion method [3, 4], and their titers were determined. Plates were prepared with a 1.5 mm thickness of 1% agar in 0.05 M Tris-HCl buffer, pH 8.3, containing 0.15 M NaCl and 0.5% of the nonionic detergent Triton X-100. Using a template, a central well and, around it, another six wells were cut out. A solution of an antigen (the H.0.43 protein) was poured into the central well, and solution of the antigen to the serum with definite dilutions into the peripheral wells. The optimum concentrations of the solutions of antisera and antigens for obtaining a clear pattern of immunoprecipitation in the agar gel were selected. The titer was determined from the degree of dilution of the antiserum solution at a concentration of antigen in the solution of 0.015-0.020 mg/ml. For the antisera to the H.0.43 and H.0.51 proteins the titer was 1:64, and for the antisera to the B.0.37 and B.0.48 proteins it was 1:128 (Fig. 1).

Analysis of the immunodiffusion results showed that the antisera to the H.0.43 and H.0.51 proteins gave immunoprecipitation reactions not only with the antigens of these proteins but also with the B.0.37 and B.0.48 proteins. The antisera to the B.0.37 and H.0.48 proteins gave immunoprecipitation not only with their own antigens, B.0.37 and H.0.48, but also with the H.0.43 and H.051 proteins.

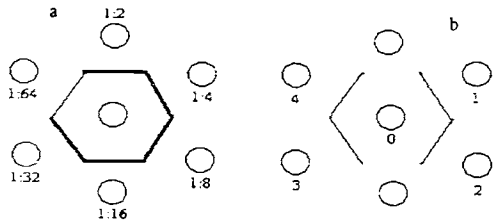

Fig. 1. Ouchterlony immunoprecipitation bands; a) determination of the titer of the antiserum to the H.0.43 protein; b) analysis of the specificity of the antiserum to the H.0.43 protein (0) with the antigens H.0.43 (*1*), H.0.51 (*2*). B.0.37 (*3*), and B.0.48 (*4*).

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 89 14 75. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 113-114, January-February, 1996. Original article submitted July 10, 1995.

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These results show that the determinant sections in these four proteins are identical.

Thus, it has been established that antisera give cross-immunoprecipitation reactions with the proteins studied, and this shows the immunochemical identity of these proteins and the fact that the proteins are in extremely close phylogenetic relationship to one another.

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