Immunochemical Prognosis of Heterosis in *Zea mays*

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Summary. Heterosis is a complex biological phenomenon. Because of the complex interaction and interrelation between “genes — metabolism — environment”, it is hardly possible to expect a clarification of the heterosis phenomenon through simple genetic explanations only (Hagemann et al. 1967).

We have followed an immunochemical aspect and method of research. The antigenic analysis of inbred lines and their hybrids was used for studying heterosis and for investigating the possibilities for its prognosis.

The heterosis effect was proved under field conditions. Our investigations (Dimitrov et al. 1972) show the presence of four protein fractions in the seed extracts of inbred maize lines, while the heterotic hybrids contain a fifth protein fraction. Antigenic analysis by the method of Grabar and Williams was carried out for a more complete characterization and determination of the specificity of the fractions obtained (Grabar and Williams 1955).

The present publication is a result of our research upon maize inbred lines, simple heterotic and nonheterotic hybrids and their backcrosses. A double diffusion in agar gel, according to the Ouchterlony method (Ouchterlony 1958), confirmed the presence of a fifth protein fraction in the heterotic hybrids, which cannot be found in the inbred lines or in the nonheterotic hybrids.

In the inbred lines we found 3 protein fractions common to all of them, and also a fourth (individual antigen), contained only in the inbred lines that produce a heterosis effect when crossed. It was determined that the carrier of the information for the synthesis of the individual antigen is a nuclear factor.

We also determined the conditions under which (after direct and inverse crosses and after crosses in one direction only) heterotic hybrids are obtained. Some backcrosses show a marked heterosis effect connected with the doubling of the factor, carrier of the information for the individual antigen. This fact is important for the scientific verification of the methods for obtaining complex heterotic hybrids.

Our results throw some light upon the genetic nature of the heterosis phenomenon.

The heterosis effect was determined only for inbred lines whose seed extracts have a precipitation arc against their homologous serum, absorbed with the extract of its partner. This allows for the prognosis of heterosis in maize, i.e. for the determination in advance (through the double immunodiffusion of the inbred lines and also of the direction of crosses that produce the heterosis effect.

The present publication is a continuation of our report (Dimitrov et al. 1972) concerning the research on the nature of heterosis in connection with its prognosis. In our investigation we have accepted as heterotic only hybrids whose seed yield exceeds the seed yields of each of the separate inbred lines being crossed.

Materials and Methods

1. Aspect of the investigations. Once determined, the differences in the antigenic structure of the inbred lines and their hybrids led us to comparative investigations, which aimed at the following:

   — Comparison of inbred lines that produce heterosis effect through direct and inverse crosses;

   — Comparison of inbred lines that produce heterosis effect in one direction of hybridization only;

   — Comparison of inbred lines that (as male parents) give a heterosis effect with different inbred lines, the latter used as female parent;

   — Comparison of inbred lines which [used as female parent] give heterosis effect with one and the same line, used as male parent;

   — Comparison of inbred lines, which (as female parent) with inbred line (male parent) give a heterosis effect, while with another inbred line (male parent) do not give such an effect;

   — Comparison of simple heterotic and nonheterotic hybrids as follows:

     with a backcross where the female parent of the simple hybrid has been used as male parent;

     with a backcross in which the male parent of the simple hybrid has been used as male parent.

2. Plant material. The investigation was carried out as follows:

   With inbred lines of maize which produce the heterosis effect in the two directions of crosses:

   I group C-103 × WIR-44 (heterosis effect)

   WIR-44 × C-103 (heterosis effect)

   II group N-6 × WIR-44 (heterosis effect)

   WIR-44 × N-6 (heterosis effect)

   With inbred lines of maize which produce the heterosis effect in one direction of cross only —

   III group WIR-38 × WIR-44 (heterosis effect)

   WIR-44 × WIR-38 (no heterosis effect)

   IV group N-6 × C-103 (heterosis effect)

   C-103 × N-6 (no heterosis effect)

   With backcross hybrids and their simple hybrids, the latter producing no heterosis effect —

   V group WIR-44 × WIR-38 (no heterosis effect)

   (WIR-44 × WIR-38) × WIR-38 (no heterosis effect)

   (WIR-44 × WIR-38) × WIR-44 (strong heterosis effect)

   VI group C-103 × N-6 (no heterosis effect)

   (C-103 × N-6) × C-103 (weak heterosis effect)
With backcross hybrids and their simple hybrids, the latter producing heterosis effect —

VII group  
N-6 ° C-103 (heterosis effect)  
(N-6 × C-103) × N-6 (no heterosis effect)  
(N-6 × C-103) × C-103 (very strong heterosis effect)

3. Extracts and immune sera. The water extracts of maize seeds and the corresponding immune sera were derived according to the methods previously described (Dimitrov et al., 1972). The protein content of the extracts was determined at about 10 mg/ml. The extracts were lyophilized in order to avoid depositions from denatured proteins as well as colouring due to oxidation of some protein contents. In this way the extracts became more stable. Only sera with high precipitation titre were used. The immune sera obtained were adsorbed with the corresponding lyophilized homologous extract, a surplus of the extract (from 80 to 100 mg) was added to 1 ml of serum. The sera were then incubated for 1 hour at 37 °C, left in a refrigerator at +4 °C and finally centrifuged. The overlying fluid (supernatant) was the absorbed serum aimed at. All the immune sera absorbed with the corresponding homologous extract gave no positive reaction according to Ouchterlony (Ouchterlony 1958).

The immune sera were absorbed in a similar fashion with homologous maize extracts as follows:

The serum, derived from rabbits immunized with the extract from seeds of inbred line C-103, was adsorbed with the extract from seeds of inbred line WIR-44. That serum is referred to in our work as absorbed serum C-103 with heterologous extract WIR-44 (AS C-103/E WIR-44).

The serum from rabbits immunized with the extract from seeds of inbred line WIR-44 was absorbed with the extract from seeds of inbred line C-103 and marked, correspondingly, as AS WIR-44/E C-103.

The serum from rabbits immunized with the extract from seeds of inbred line C-103 was absorbed with its homologous extract C-103 and correspondingly marked AS C-103.

The serum from rabbits immunized with the extract from seeds of inbred line WIR-44 was absorbed with its homologous extract WIR-44 and marked AS WIR-44.

The not-absorbed serum, derived from rabbits immunized with the extract from seeds of inbred line C-103, was marked S C-103.

The not-absorbed serum, derived from rabbits immunized with the extract from seeds of inbred line WIR-44 was marked S WIR-44.

Analogous absorptions were also carried out with the immune serum derived from the immunization of rabbits with the extract from seeds of inbred line WIR-38, correspondingly marked S WIR-38.

The immune serum WIR-38 (S WIR-38), absorbed with its homologous extract WIR-38, was marked AS WIR-38.

The serum WIR-38, absorbed with the heterologous extract (WIR-44), was marked AS WIR-38/E WIR-44.

The serum WIR-44, absorbed with the heterologous extract WIR-38, was marked AS WIR-44/E WIR-38.

4. Double diffusion in agar gel. The Ouchterlony method was used, with 1% noble agar "Diphko", in the buffer veronal with pH 8.2, and reservoirs with a diameter of 10 mm at a distance of 5 mm from one another.

Results

The investigations were carried out while each extract was being left to diffuse against the homologous and heterologous not-absorbed serums as well as against the homologous and heterologous absorbed serums.

The results from the investigations conducted with extracts from seeds of inbred lines giving a heterosis effect in both directions of cross are shown in Figs. I-A and I-B, while the results from the lines giving the heterosis effect in one direction only are given in Figs. II-A and II-B.

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