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Immunochemical Prognosis of Heterosis in *Zea mays*

P. DIMITROV, O. NASHKOVA, S. PETKOVA, D. NASHKOV and **E. MARINKOV**

The Genetics Chair, Academy of Agriculture "G. Dimitrov" and the Biology Chair, Institute for Foreign Students, Sofia (Bulgaria)

Summary. Heterosis is a complex biolcgical 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. Antigenie 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 can not 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 mgt/ml. The extracts were lyophilized in order to avoid depositions from denaturized 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 absorbed 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*.

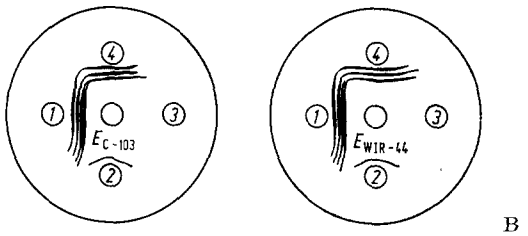
The serum *WIR-44*, absorbed with the heterologous extract *WIR-38*, was marked *AS WIR-44/E WIR-38*.

4. Double diffusion in agar gel. The Ouchterlony method was used, with 1% noble agar "Diphko", in the buffer veronalnatrium 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.



A B

Fig. I-A. Immunodiffusion of extract from seeds of inbred line *C-103*.

1 — against homologous not absorbed immune serum S (C-103);
2 — against homologous serum absorbed with heterologous extract (*AS C-103/E WIR-44*);
3 — against heterologous serum, absorbed with homologous extract (*AS WIR-44/E C-103*);
4 — against heterologous not absorbed serum (*S WIR-44*)

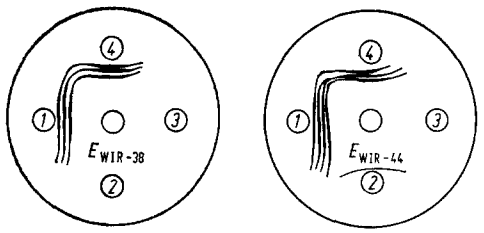
Fig. I-B. Immunodiffusion of extract from seeds of inbred line *WIR-44*.

1 — against homologous not absorbed serum (*S WIR-44*);
2 — against homologous serum, absorbed with heterologous extract (*AS WIR-44/E C-103*);
3 — against heterologous serum, absorbed with homologous extract (*AS C-103/E WIR-44*);
4 — against heterologous not absorbed serum (*S C-103*)

Fig. I-A shows that extract *C-103* gives 4 precipitation arcs with its homologous serum (1), and 3 precipitation arcs with its heterologous serum (4). The same extract (C-103) does not react with its heterologous serum, absorbed with it (3), whereas it gives only one precipitation arc (2) with its homologous serum, absorbed with the heterologous extract.

The results obtained from the experiments with immunodiffusion of extract *WIR-44* (Fig. I-B) are similar. This extract is characterized by the following: it gives 4 precipitation arcs with its not-absorbed serum (1), 3 arcs with the heterologous not-absorbed serum (4), does not react with the heterologous, but absorbed immune serum (3), while giving but one precipitation arc with its homologous serum, absorbed with heterologous extract (2).

The above results show that the extracts from seeds of inbred lines *C-103* and *WIR-44* contain 3 protein fractions each, common to both inbred lines, while the



A B

Fig. II-A. Immunodiffusion of extract from seeds of inbred line *WIR-38*.

1 — against homologous not absorbed immune serum (*S WIR-38*);
2 — against homologous serum absorbed with heterologous extract (*AS WIR-38|E WIR-44*);
3 — against heterologous serum, absorbed with homologous extract (*AS WIR-44|E WIR-38*);
4 — against heterologous not absorbed serum (*S WIR-44*)

Fig. II-B. Immunodiffusion of extract from seeds of inbred line *WIR-44*.

1 — against homologous not absorbed serum (*S WIR-44*);
2 — against homologous serum absorbed with heterologous extract (*AS WIR-44|E WIR-38*);
3 — against heterologous serum, absorbed with homologous extract (*AS WIR-38|E WIR-44*);
4 — against heterologous not absorbed serum (*S WIR-38*)

fourth protein fraction is specific for each separate inbred line.

The case is similar with inbred lines *N-6* and *WIR-44*.

Fig. II-A shows that extract *WIR-38* gives three precipitation arcs with its homologous and heterologous not-absorbed sera (1,4), while not reacting with the absorbed homologous and heterologous immune sera (2,3).

Extract WIR-44 (Fig. II-B) gives 4 precipitation arcs with its homologous not-absorbed serum (1), 3 precipitation arcs with the not-absorbed heterologous serum (4), 1 precipitation arc with the homologous immune serum, absorbed with the heterologous extract (2), and does not react with the absorbed heterologous immune serum (3).

The above data show that the extracts from seeds of the inbred lines *WIR-44* and *WIR-38* contain 3 common protein fractions. The extract from WIR-44 contains, as well as the three common protein fractions, a fourth which can not be found in the extract from seeds of *WIR-38*.

The immunodiffusion of extracts of inbred lines *N-6* and *C-103* is another similar case.

Discussion

The experimental results give information which is discussed with a view to the possible prognosis of the heterosis effect in maize.

The investigations confirm the facts reported earlier by us (1972), pointing to the existence of a fifth protein fraction in the heterotic maize hybrids.

The regularities we observed while conducting the comparative study of parent inbred lines and their hybrids show beyond any doubt that it is possible not only to "find" heterotic inbred lines of maize, but also to determine the direction of crosses in which a heterosis effect might be expected. This can be admitted on the following grounds:

a) The finding of 3 protein fractions common to the inbred lines and also of a fourth one, typical for the heterotic inbred lines, was confirmed. The inbred lines *N-6*, *C-103* and *WIR-44* contain a specific protein fraction. Inbred line *WIR-38 does not* contain any specific protein fraction.

b) The emerging regularity with which, after crossing two inbred lines, each of which is a carrier of a specific protein fraction, there is a heterosis effect in both directions of cross. Example:

C-103 × *WIR-44*	—	heterosis effect
WIR-44 × *C-103*	—	heterosis effect
N-6 × *WIR-44*	—	heterosis effect
WIR-44 × *N-6*	—	heterosis effect

c) When a specific protein fraction is present in one of the inbred lines being crossed, there is a heterosis effect only in the case in which the carrier of the individual antigen is the male parent line. Example:

WIR-38 × *WIR-44* ♂	—	heterosis effect
WIR-44 ♀ × *WIR-38*	—	no heterosis effect
WIR-44 ♀ × *C-103* ♂	—	heterosis effect
WIR-44 ♀ × *WIR-38*	—	no heterosis effect

The presence of a specific (individual) antigen in the heterotic inbred lines of maize allows the problem of prognosing maize heterosis to be discussed with respect to the following questions:

1. Who is the carrier of the specific protein fraction?

Our data show that both inbred lines being crossed could be the carriers of the specific protein fraction. Usually such lines manifest themselves as heterotic in both directions of cross. In some cases, however, the inbred lines (carriers of a specific protein fraction) need an additional description. For instance, there is the different reaction of the inbred line *N-6* (as male parent), which we found to contain a specific protein fraction. A specific protein fraction is also contained in its crossed partner *C-103*. The cross *N-6* × *C-103* shows a heterosis effect, whereas the cross *C-103* × *N-6* does not. This fact needs an explanation, since both crossed lines contain specific protein fractions. Our further investigations throw some light upon this seemingly contradictory phenomenon.

We found the following:

a) When comparing (after Ouchterlony 1958) the inbred lines *WIR-44* and *N-6*, both lines gave a specific protein fraction with their homologous antiserums, absorbed with the heterologous extract.

b) During a similar comparison of the inbred line *N-6* with inbred line *C-103*, both lines *did not give*

specific protein fractions with their homologous antiserums, absorbed with heterologous extract.

c) However, when comparing inbred line *C-103* with inbred line *WIR-44*, both lines gave one specific protein fraction each with their homologous antisera, absorbed with heterologous extract.

The above facts shed light on the nature of the individual factor, carried by the inbred line, effective as the male parent. It is obvious that the inbred lines *C-103* and *WIR-44* show some similarity in the character of their specific antigen, which allows them in all cases (when used as male parents) to give heterotic hybrids. It is apparently possible also to describe the inbred lines, determined previosuly as carriers of specific protein fractions.

2. *Which is the carrier of the information for the synthesis of a specific protein fraction — the nucleus or the cytoplasm?*

We determined that the inbred lines containing specific protein fractions usually gave heterotic hybrids in both directions of cross. It can not be inferred from this, however, whether it is nuclear or cytoplasmic factors which are involved. Our data indicate that when the carrier of a specific protein fraction is one of the inbred lines being crossed, the heterosis effect occurs *only* when the carrier of that individual agent is the line used as a male parent. This is convincing proof that in this case we have nuclear factors coming into play.

3. *Is the doubling of the individual factor in the zygote of any importance concerning the appearance of the heterosis effect?*

The answer to this question is given by the third aspect of our investigations — the comparison of simple (heterotic and nonheterotic) hybrids with backcrosses, where in one case the inbred line carrier of a specific protein fraction is used as male parent, while in the other the line not carrying such fraction is used. When the *nonheterotic simple hybrid* (*WIR-44* × *WIR-38*) was crossed with the line, used in the simple hybrid as male parent, in that case *WIR-38*, which does not carry any specific protein fraction, the backcross *was not* heterotic, except that it showed a slightly expressed dotted arc. When, however, that simple *nonheterotic hybrid* was crossed with the line used in the simple hybrid as a female parent and carrier of a specific protein fraction, the backcross carried a clearly expressed heterosis effect. In the last case (*WIR-44* × *WIR-38* × *WIR-44*) the individual factor carried by the inbred line *WIR-44* is doubled in the zygote from which the backcross originated.

The above regularity is even more clearly expressed when one compares *simple heterotic hybrids* (*N-6* × *C-103*) with the backcross, where, as male parent, the inbred line (*C-103*) was used, which in the simple hybrid was male parent and which contains the specific antigen. In such cases there was a gradual heterosis effect in the simple hybrid (*N-6* × *C-103*) and a strongly expressed one in the backcross ((*N-6* × *C-103*) × *C-103*). The presence of a protein fraction much richer in subfractions in the backcross, compared with the fifth protein fraction in the simple heterotic hybrid, corresponds to the stronger heterosis effect in the backcross of the above type determined by us under field conditions.

It should be pointed out that the inbred line *N-6*, containing a specific antigen, acts as a "weak male parent" in the backcross as well. Apparently that is why the backcross ((*N-6* × *C-103*) × *N-6*) is *nonheterotic*, although it also exibits a weak, dotted arc.

The above information is of value not only for clarifying the importance of the doubled individual factor in the zygote, but also with a view to creating complex heterotic hybrids on scientific grounds.

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The facts and regularities found show the possibility of prognosing maize heterosis. They are also important for the further study of heterosis and its manifestations.

In general, inbred lines, whose seed sera show a precipitation arc with their homologous serum, absorbed with the extract from its partner, produce a heterosis effect. The specific protein fractions, however, need additional study. Their form and localization, shown in Figs. I-A, I-B, II-A and II-B, indicate their high diffusion coefficient, higher than that of rabbit γ globulines, and a molecular weight of about 160,000. It would also be interesting to clarify whether these specific protein fractions, contained in the different inbred lines, differ in their antigenic structure and also whether they show any catalytic activity.

Further investigation would be interesting of *the fifth* protein fraction found in the heterotic hybrids and especially its "thickening", based on the increased number of subfractions contained in it. Comparing such investigations with the conclusions of immunochemistry, according to which a number of organismic proteins are not homogeneous but are a mixture of two or more similar proteins, differing in their aminoacid content and sequence and often in one single aminoacid (Haurowitz 1968), could lead to information concerning the genetic nature of heterosis.

Our investigations continue.

Zusammenfassung

Die vorliegende Untersuchung ist das Ergebnis einer vergleichenden Bearbeitung von Inzuchtlinien und heterotischen Maishybriden; bei einigen von

ihnen wurde ein Unterschied in ihrer Antigen-Struktur festgestellt (Dimitrov et al. 1972).

Verglichen wurden miteinander:

Inzuchtlinien, die nach reziproken Kreuzungen und Rückkreuzungen heterotische Hybriden ergeben;

Inzuchtlinien, die nur in einer Kreuzungsrichtung Heterosis ergeben;

Inzuchtlinien, die Heterosis ergeben, wenn sie als männlicher Kreuzungspartner in Kreuzungen mit verschiedenen anderen Linien (als ♀) verwendet werden;

Inzuchtlinien, die als weiblicher Elter nach Kreuzung mit einigen Inzuchtlinien Heterosis ergeben, mit anderen aber nicht; einfache heterotische sowie nicht-heterotische Hybriden mit Rückkreuzungen, bei denen die F_1-Hybriden (als Mutter) in einigen Fällen mit der mütterlichen, in anderen Fällen mit der väterlichen Linie rückgekreuzt wurden.

Benutzt wurden die Inzuchtlinien N-6, C-103, WIR-44 und WIR-38. Die Untersuchungen wurden in der Weise durchgeführt, daß jeder Extrakt geprüft wurde gegenüber nicht-absorbierten und absorbierten homologen und heterologen Seren.

Das Auftreten von Heterosis wurde unter Feldbedingungen geprüft. Als heterotisch wurden nur diejenigen Kreuzungen bezeichnet, die im Kornertrag den Ertrag jeder einzelnen zur Kreuzung verwendeten Inzuchtlinie übertrafen.

Unsere Untersuchungen bestätigen die bereits früher von uns mitgeteilten Daten über das Vorhandensein von 4 spezifischen Eiweißfraktionen in den Inzuchtlinien und von einer 5. Eiweißfraktion in den heterotischen Hybriden.

In den Inzuchtlinien wurde das Vorhandensein von je 3 diesen Linien gemeinsamen Eiweißfraktionen und einer 4. festgestellt, die für diejenigen Inzuchtlinien spezifisch ist, welche Heterosis ergeben. Die Inzuchtlinien N-6, C-103 und WIR-44 enthalten eine spezifische Eiweißfraktion, die Inzuchtlinie WIR-38 hingegen nicht.

Es wurde festgestellt, daß ein Heterosiseffekt in beiden Kreuzungsrichtungen nur dann beobachtet wird, wenn Inzuchtlinien gekreuzt werden, von denen jede Träger einer spezifischen Eiweißfraktion ist. Wenn nur eine der verwendeten Inzuchtlinien eine spezifische Eiweißfraktion enthält, so wird nach unseren Ergebnissen nur dann ein Heterosiseffekt beobachtet, wenn die Vaterlinie der Träger des individuellen Antigens ist. Dies spricht dafür, daß die verantwortlichen Erbanlagen im Zellkern liegen.

Wir führten einen Vergleich durch zwischen einfach heterotischen sowie nicht-heterotischen Hybriden und Rückkreuzungs-Generationen, für die als Vater in einigen Fällen eine Inzuchtlinie verwendet wurde, welche eine spezifische Eiweißfraktion enthält, im anderen Falle eine Linie, die keine spezifische Fraktion enthält. Dieser Vergleich zeigt, daß die Verdoppelung des individuellen Faktors in der Zygote — dem Ausgangspunkt der Rückkreuzung — zu einem gesteigerten Heterosiseffekt führt, der mit der Erhöhung der Subfraktionszahl der im Extrakt der Rückkreuzungspflanzen enthaltenen Eiweißfraktion verbunden ist.

Die von uns ermittelten Daten und Gesetzmäßigkeiten eröffnen Möglichkeiten zur Vorhersage von Heterosiseffekten bei *Zea mays* und zugleich zur Schaffung von komplexen heterotischen Hybriden auf wissenschaftlicher Grundlage.

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Prof. Peter Dimitrov
16 Aprilov St.
Sofia 4 (Bulgaria)