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Immunoelectrophoretic Studies of Heterosis Effect in *Zea mays*

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Summary. The present report is based on tests using antigenic analysis of inbred lines and their hybrids for the prognosis of heterosis (hybrid vigour) in *Zea mays*.

Inbred lines, whose hybrid vigour has been proved under field conditions, and their hybrids were characterized by the method of Grabar and Williams.

The results of the electrophoretic and immunoelectrophoretic investigations show that the extracts from maize seeds of the inbred lines C-103, N-6 and WF-9 contain four protein fractions, while those of the hybrids N-6 × C-103 and WF-9 × N-6 also contain a fifth fraction.

The immunoelectrophoretic pattern of the extracts from the hybrid seeds N-6 × C-103 shows that, with their homologous serum, they give a precipitation line (arc *X*) which is not obtained by the interaction of the parent line extracts with homologous and heterologous serums.

In this case we probably have a protein synthesis in the hybrid which is caused by the deblocking of some links in the biosynthetic apparatus of the inbred lines.

It was established that the extracts from the hybrid N-6 × C-103 gave a larger number of precipitation arcs with the heterologous serum anti C-103.

The characterization of the antigenic structure of the inbred lines enables one to determine not only the fractions common to them but also the number of those fractions by which they differ from one another. Proceeding in this manner, one could accomplish an immunoelectrophoretic prognosis of heterosis and penetrate deeper into its essence.

Early prognosis of heterosis (hybrid vigour) enables the breeder to avoid the time, room and expense involved in testing a large number of inbred lines and interbreeding them.

Indirect indicators — cytological, embryological, physiological and morphological — have been used for this purpose by many investigators. The conflicting data in the literature show that it is hardly possible to discover the basic mechanism and causes of heterosis by statistical methods only.

Rédei and Li (1969) obtained a model of the biochemical mechanism of heterosis in *Arabidopsis* by inducing auxotrophic mutants, the model being based on the simple and complete domination of two unconnected loci (4, 5, 7, 8, 9, 10, 11).

The present report is based on tests of antigenic analysis for the characterization of inbred lines and their hybrids.

Materials and Methods

1. *Plant Material.* The investigations were carried out with inbred lines of maize, N-6, C-103 and WF-9 whose hybrid vigour and differences between every hybrid and parent were statistically proved under field conditions (Table 1).

2. *Extracts.* The proteins subjected to analysis were extracted from dry maize seeds. The seeds were ground into a fine powder which was extracted by physiological solution in the proportion 2:1 according to the method of Halle (1959). The exctracts were diluted with saline as to obtain a final cone dentraion of 17.5—18 mg/ml, and were kept in lyophilised condition at 4 °C. Before use, they were dissolved in destilled water to the initial volume and concentrated of the proteins.

3. *Immune Sera.* The following immune sera were obtained: anti N-6, anti C-103, anti N-6 × C-103 anti WF-9, anti WF-9 × N-6, anti M-14, anti WF-9 × M-14. Three rabbits of local breed, body weight 3 kg, were used to obtain the above antisera for each inbred line and hybrid. The animals were immunized subcutaneously with 2.5 ml of extract of maize seeds three times a week for a period of 6 weeks. The separate sera were preserved with merthiolat in the proportion 1:10,000 and stored at 4 °C.

4. *Agar Plate Electrophoresis.* The purpose of electrophoresis on agar gel was to determine the electrophoretic properties of the more important protein fractions contained in the extracts and thereby to attempt their classification. The Grabar and Williams method (1955) was used, with agar plates 18 × 13 cm, tension 90 volts and current power 53 ma, for a duration of 4.5 hours. Fixation and staining of the proteins were carried out according to the method of Uriel and Grabar (1956). Amido-black 10 B was used for the determination of simple proteins and Sudan-black for the lipoproteins. The glycoproteins were stained by incubation in Alpha-naphthole and p-phenylenediamine solution after previous oxidation with periodic acid.

The amounts of the separate fractions were determined by the use of a densitometer with integrating arrangement.

5. *Immunoelectrophoresis.* Antigenic analysis by the method of Grabar and Williams was carried out for a more complete characterisation and determination of the specifity of the fractions obtained.

A 0.1 ml mixture of equal parts of the extract under investigation and agar was placed into the central well. After electrophoresis, the immune sera were placed into the lateral wells; the plates were kept in a humid chamber, and the precipitation lines were read several days later.

Results

Agar Electrophoresis. The presence of four protein fractions, designated I, II, III and IV, was established by electrophoresis in the extracts of the pa-

Table 1. *Difference in the yield (heterosis effect) between inbred N-6 and C-103 lines and their hybrids obtained under field conditions**

Comparison between N-6; N-6 × C-103 and C-103

8		10		11	
12	21	148	138	38	15
16	25	120	116	30	36
27	13	148	142	33	
27	8	176	152	30	
14	19	160	144	25	
13	14	160	120	19	
15	10	176	122	17	
12	19	170	144	29	
9	23	128	172	19	
10	19	124	180	41	
25	17	106	128	28	
17	13	152	144	30	
14	13	126	144	22	
27	17	144	108	26	
12	13	132	148	25	
$n = 30$		$n = 30$		$n = 17$	
$\Sigma x = 494$		$\Sigma x = 4272$		$\Sigma x = 463$	
$\Sigma x^2 = 9048$		$\Sigma x^2 =$		$\Sigma x^2 =$	
		$= 620328$		$= 13481$	
$x_8 = 16.46$		$x_{10} = 142.40$		$x_{11} = 27.23$	
$S x_8 = 1.04$		$S x_{10} = 3.71$		$S x_{11} = 1.79$	

$$S_{\bar{x}_8} = \sqrt{\frac{9048 - 494^2/_{30}}{30.29}} = 1.04$$

$$S_{\bar{x}_{10}} = \sqrt{\frac{620328 - 4272^2/_{30}}{30.29}} = 3.71$$

$$S_{\bar{x}_{11}} = \sqrt{\frac{13481 - 463^2/_{17}}{17.16}} = 1.76$$

Comparison between N-6 a. N-6 × C-103

$D = \bar{x}_{10} - \bar{x}_8 = 142.40 - 16.46 = 125.94$ gr.

$Sd = \sqrt{1.04^2 + 3.71^2} = 3.85$

$t_{exp.} = D/Sd = 125.94/3.85 = 32.71$

$FG = /30-1/ + /30-1/ = 58$

$t_{5\%} = 2.00$; $t_{1\%} = 2.66$; $t_{0.1\%} = 3.46$

The difference is very well expressed

Comparison between N-6 × C-103 and C-103

$D = x_{10} - x_{12} = 142.40 - 27.23 = 115.17$ gr.

$Sd = \sqrt{3.71^2 + 1.79^2} = 4.12$

$t_{exp.} = D/Sd = 115.17/4.12 = 27.95$

$FG = /30-1/ + /17-1/ = 29 + 16 = 45$

$t_{5\%} = 2.01$; $t_{1\%} = 2.69$; $t_{0.1\%} = 3.52$

The difference is very well expressed

* 8 = N-6; 10 = N-6 × C-103; 11 = C-103

rents, N-6, C-103 and WF-9. A fifth fraction, II-a, was established in the extracts of the hybrids.

Fraction I of the inbred lines investigated moves towards the anode a distance of 20 mm from the start; fraction II is slightly mobile and remains at the start; fractions III and IV migrate towards the cathode 12 and 37 mm respectively. None of the fractions was stained by Sudan-black and Alpha-naphthole.

The quantitative relationship between the separate fractions is given in table 2.

Table 2. *Percentage content of the separate fractions in maize extracts**

Extracts from seeds of lines and hybrids	Fractions in %				
	I	II	III	IV	II – a
N-6	45.50	14.97	17.86	22.00	—
N-6 × C-103	34.72	19.22	13.38	15.76	16.89
C-103	52.44	22.02	13.82	11.89	—
WF-9	41.30	23.66	19.15	15.85	—
WF-9 X N-6	38.72	12.62	18.16	17.22	13.25
N-6	45.50	14.97	17.86	22.00	—

* The results are the average from 9 experiments.

The table shows that fraction I is present both in the parents and the hybrids. In the inbred lines it represents almost 50% of the entire protein content of the extracts. In the hybrid N-6 × C-103, the quantity of fraction I is much lower, being below that of the parent line having the poorest content. The percentage content of the II, III and IV fractions tends to average that in the two parent lines. Fraction II-a (the fifth fraction) was observed only in the hybrid form. Its electrophoretic mobility was between that of fractions II and III.

Immunoelectrophoresis. The results of the immunoelectrophoretic investigations of the extract from C-103 by homologous anti-C-103 and heterologous serum anti N-6 are illustrated in fig. 1. This figure shows that the protein fractions I, II and III are heterogeneous and made up of 2, 3 and 2 subfractions respectively. Fraction IV is homogeneous. Serum anti N-6 gives the same number of precipitation arcs with identical form and disposition as serum anti C-103 against extract C-103, except that the precipitation line which corresponds to fraction III is stained more intensively by Amido-black, whereas in extract C-103 the precipitation line corresponding to fractions I and III is most strikingly expressed. It is quite possible that the N-6 extract contains other fractions besides those that are common to both N-6 and C-103. The characterisation in this manner of the parent inbred lines may reveal not

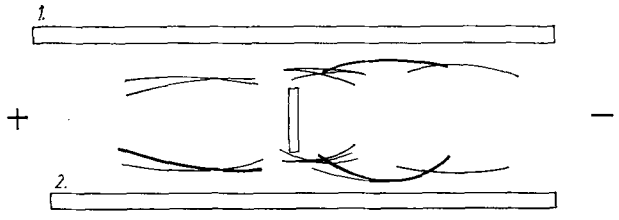

Fig. 1. Immunoelectrophoregram of the extract from C-103
Central well – extract from inbred line C-103
1. Heterologous serum anti N-6
2. Homologous serum anti C-103

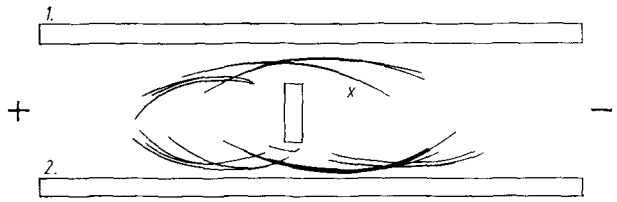

Fig. 2. Immunoelectrophoregram of the extract from N-6 × C-103 Central well — extract from hybrid N-6 × C-103
1. Homologous serum anti N-6 × C-103
2. Heterologous serum anti C-103

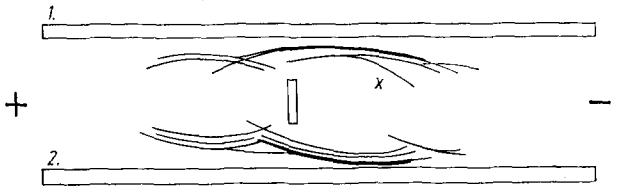

Fig. 3. Immunoelectrophoregram of the extract from N-6 × C-103 Central well — extract from N-6 × C-103
1. Homologous serum anti N-6 × C-103
2. Heterologous serum anti N-6

only their common fractions but also the number of fractions by which they differ from each other. In this way one may accomplish not only the immunoelectrophoretic prognosis of heterosis but also a deeper penetration into its entity.

Fig. 2 represents the immunoelectrophoregram, obtained from the extract of hybrid N-6 × C-103, homologous serum anti N-6 × C-103 and heterologous serum anti C-103. This figure shows that the extract from N-6 × C-103 gives 7 precipitation arcs with the homologous serum and 8 precipitation arcs with the heterologous serum anti C-103.

Fig. 3 represents the immunoelectrophoregram of another series extracted from hybrid N-6 × C-103 against homologous serum anti N-6 × C-103 and heterologous serum anti N-6. The figure illustrates that the hybrid extract gives 7 precipitation lines.

The immunoelectrophoretic patterns of the hybrid extract N-6 × C-103 and the corresponding homologous serum are the same as in fig. 2. The precipitation arc, marked with *X* in the figures, is characteristic for both extract series. This arc begins at the start, advances towards the cathode and sharply deviates from the lateral well, gradually losing its contours. No such precipitation line is observed between extracts of the hybrid and antisera against extracts from C-103 and N-6. Fig. 3 shows that there is a well expressed difference between the reactions of the hybrid with each one of the parents.

Discussion

The combined results of the electrophoretic and immunoelectrophoretic investigations show that the extracts from maize seeds of the C-103, N-6 and WF-9 lines contain 4 protein fractions, indicated as I, II, III and IV from the anode towards the cathode, while in extracts of the hybrids N-6 × C-103 and WF-9 × N-6 there is one additional, fifth, fraction, designated II-a.

None of the these fractions can be stained by Sudanschwarz, showing that they do not belong to the lipo- or the glyco-proteins. Only fraction IV is homogeneous. The other fractions differ in their immunological properties and consist of 2 or 3 subfractions. Thus, the extracts from C-103 and N-6 contain a total of 8 protein subfractions each, and those of the hybrids 7 protein subfractions each. Fraction I is most strikingly expressed both in the parents and in the hybrids, representing about 50% of the total protein content of the extracts. In the hybrids, its quantity is below that of the parent line having the poorest fraction content.

It is characteristic of the immunoelectrophoretic patterns of the extracts from hybrid seeds N-6 × C-103 that, with their homogeneous serum, they give a precipitation line (arc X), which is not observed in the interaction of extracts from the parent line with homologous and heterologous sera. This shows that in the hybrid there is a protein fraction which is not to be found in the seeds of the parent lines. These observations are in agreement with those of Irwin (1951) for the formation of hybrid substances in some hybrid species. In our case, it is more probable that there is synthesis of protein in the hybrid due to the unblocking of some links in the biosynthetic apparatus of the inbred lines. This fraction could be used in genetic analysis to discover the biochemical mechanism of heterosis, based on the simple and complete dominance of unconnected loci.

The experiments carried out indicate that the extracts from the N-6 × C-103 hybrid give a larger number of precipitation arcs with the anti C-103 heterologous serum. A similar phenomenon has also been observed by Nikolaeva et al. (1969). They consider that the immunological spectrum of the protein from the Olev and Vesselovsky potato varieties is greater with the heterologous than with the homologous serum. They have not established the reason for this phenomenon.

Our investigations continue.

Zusammenfassung

Die vorliegende Mitteilung basiert auf Versuchen, die Antigenanalyse von Inzuchtlinien und ihren Hybriden für eine Vorhersage des Auftretens von Heterosis bei *Zea mays* zu verwenden. Inzuchtlinien und ihre Hybriden, deren Heterosis unter Feldbedingungen erwiesen ist, wurden nach der Methode von Grabar und Williams geprüft.

Die elektrophoretischen und immunelektrophoretischen Untersuchungen ergaben, daß die Extrakte von Maiskörnern der Inzuchtlinien C-103, N-6 und WF-9 vier Proteinfraktionen enthalten, während die Extrakte der Hybriden N-6 × C-103 und WF-9 × × N-6 noch eine fünfte Fraktion aufweisen.

Das immunelektrophoretische Muster der Extrakte aus den Hybridkörnern von N-6 × C-103 zeigt, daß mit dem homologen Serum eine Präzipitationslinie, arc X, gebildet wird, die bei Reaktion der Extrakte aus den Elternlinien mit homologen und heterologen Seren nicht auftritt. Wahrscheinlich handelt es sich um eine zusätzliche Synthese von Protein, die auftritt, nachdem die Blockierung einiger Glieder im biosynthetischen Apparat der Inzuchtlinien aufgehoben worden ist.

Es wurde festgestellt, daß die Extrakte der Hybride N-6 × C-103 mit heterologem Serum anti C-103 eine größere Anzahl Präzipitationslinien ergeben.

Die Charakterisierung der Antigenstruktur der Inzuchtlinien ermöglicht nicht nur die Ermittlung von gemeinsamen, sondern auch von unterschiedlichen Fraktionen. Auf diese Weise könnten eine immunelektrophoretische Diagnose hinsichtlich Heterosis erreicht und Möglichkeiten zur eingehenderen Erforschung des Heterosisphänomens erschlossen werden.

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