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Codominant Isoenzymic Alleles as Markers of Genetic Diversity Correlated with Heterosis in Maize *(Zea mays)*

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Summary. The average F1 yields were found to be significantly correlated with the genetic diversity of the parental strains calculated from the zymogram patterns of five enzyme systems in a sample of eight strains and their diallelic crosses.

Despite the relatively small number of loci (eight) studied, there is evidence that these codominant alleles are good markers for genetic heterogeneity that is a source of heterosis.

Much valuable work has been reported showing close relationships between parental genetic diversity and the heterosis observed in their offspring. Among the most recent contributions, for corn, we may mention the work of McDaniel and Sarkissian (1968) on mitochondria, the paper by Lorenz (1972) analysing the free amino-acid pool, and the report presented by Hunter and Kannenberg (1971) on isozymes, which is, in many aspects, comparable to this paper. Those authors found that hybrids from parents with a greater zymogram "diversity index" showed greater heterosis. However, the correlation between the yield and enzymatic diversity was not significant.

Evidence on the molecular level of heterosis, due to single locus overdominance in maize, was presented by Schwartz and Laughner (1969), and Schwartz (1973), for alcohol dehydrogenase. In this example, the hybrid molecule formed, a heterodimer, was better than the molecules produced by the homozygotes in several physicochemical features. Consequently, these are cases of true heterosis, in which the hybrid surpasses both parents, due to allelic interaction at a single locus.

Material and Methods

The strains of corn used in this work have been subjected to at least eight years of selfing and selection for disease resistance, growth habit, number of ears per plant, etc. A summary of character data is presented in Table 1. Yield and the other agricultural characteristics were observed in three different places in the state of Rio Grande do Sul, in the counties of Pelotas, Lajeado, and Passo Fundo. The statistical analysis described by Griffing (1956) has been followed with few modifications.

The buffer systems and the revelation of zymograms are the ones indicated by Brewbaker et al. (1968) for esterases, catalases, acid phosphatases and peroxydases, and by Scandalios (1969) for alcohol dehydrogenase. Horizontal 7% polyacrilamide gel slabs, with a thickness of 2 mm, were used throughout this work.

Three tissues have been studied: coleoptile with plumula and primary roots on the seventh day of germination, under constant illumination at 25° ± 1°C; and the forming endosperm on the fifteenth day.

Results and Discussion

The alleles of eight enzymatic loci are presented in Table 2, according to their distribution in the strains and tissues studied. For each locus we found two alleles in the different strains studied, except for the esterase E4, in which we identified the four alleles studied by Harris (1966). The loci E1 and E3 have been studied in detail by Schwartz (1960) and Macdonald and Brewbaker (1972). The esterase E11 was identified by Heidrich-Sobrinho (1974). The catalase locus was analysed by Scandalios (1969). Among the peroxidases, we studied the Px 11 determined by Heidrich-Sobrinho (1972). The acid phosphatase locus was identified as the Aph 1 studied by Brown and Allard (1969). Finally, the alcohol dehydrogenase Adh 2 is the same as that analysed by Schwartz and Endo (1966). The electrophoretically monomorphic loci are not included in this paper, as they do not contribute to the enzyme diversity index. However, the possibility that the same isoenzyme is produced for longer periods or greater amounts in the hybrids, and for this reason eventually contributes to heterosis, is not discarded. Another possibility is that electrophoretically indistinguishable isoenzymes can be temperature-sensitive mutants, as observed by Bernstein et al. (1973) for xanthine dehydrogenase in the *virilis* group of *Drosophila*, and contribute to heterosis, as discovered for the octanol dehydrogenase locus of *Drosophila pseudoobscura*, studied by Singh, Hubby and Lewontin (1974).

Each allelic difference observed between a pair of strains is counted as a point for the enzymatic diversity

Table 1. Some characteristics of the strains analyzed

Strains	Seed Type	Cycle [a]	Height	Origin
Tuxpan 94.6224	yellow dent	84	tall	Mexico
Tuxpan 1020.17	yellow dent	85	tall	Mexico
T 61.984-1	yellow dent	82	short	USA
NC 83.7628	yellow dent	81	median	USA
F.B. 4.5941	yellow dent	84	median	Rio Grande
Salbert 5392	yellow dent	84	tall	do Sul (Brasil)
SR 201.5232	yellow dent	78	median	do Sul (Brasil)
SR 527.5260	yellow flint	85	short	do Sul (Brasil)

[a] days from planting to flowering (tassel).

Tabel 2. Alleles observed in the eight strains of corn studied

Tissue	Plumula			Roots	Plumula		Endosperm	
Enzyme	Esterase				Peroxidase	Ac. Phosph.	Catalase	Alcohol Dehyd.
Loci / Strain	E_1	E_3	E_{11}	E_4	Px_{11}	Ap_1	Cat_1	Adh_2
Tuxpan 94.6224	F	S	F	C	S	S	F	F
Tuxpan 1020.17	F	S	F	C	S	S	S	F
T 61.984-1	F	S	F	F	S	F	F	F
NC 83.7628	S	S	S	C	S	S	S	S
F.B. 4.5941	S	F	F	E	S	F	S	F
Salbert 5392	F	F	F	E	F	S	S	F
SR 201.5232	S	F	F	D	F	F	S	F
SR 527.5260	F	F	F	D	F	S	F	F

OBS: S = slow allele; F = fast allele; C, D, E, F correspond to the four alleles of E_4 according to Harris nomenclature, from the slower C to the faster F allele.

index and is related to their specific combining ability; the sum of all such indexes in several crosses is the total index to be compared with the general combining ability (Table 3) obtained from the average yield of the crosses of each strain with all the others. The strains NC 83.7628 and Salbert 5392 exhibited the greatest values of general combining capacity and these two strains gave the best results with Tuxpan 94.6224 and SR 527.5260, respectively. These results confirm those of Heidrich-Sobrinho and Ferreira (1972) and Pereira (1974); the lowest yields were produced by the crosses of Tuxpan 94.6224 and Tuxpan 1020-17. Two correlation coefficients were calculated: the first, between the specific combining ability and the enzyme diversity index, gave no significant results ($r = 0.23$, 26df $P < 0.20$); the second, between the general combining ability and the sum of the enzyme diversity index, gave a significant $r = 0.72$, with 6df, and $P < 0.05$. Analysing some of the crosses in Table 3, we can see that, despite the fact that the correlation between genetic diversity and the individual pairs of crosses gave no significant result, divergence between the enzymatic pattern of the NC 83.7628 and all other strains is accompanied by a high combining ability, with the opposite being observed for the cross of Tuxpan 94.6224 and Tuxpan 1020-17 that shows the lowest yield and a single allelic difference. Of course, an isolated instance proves nothing, but this finding corroborates the over-all results observed here. What would the rationale be for these findings?

We take it for granted that the enzymatic diversity index is a measure of the genetic diversity that is essential for heterosis. It is currently being suggested that the codominant allelic variability might contribute to more efficient enzyme activity because a possible diverse intracellular localization. Another important possibility is that protein heterogeneity might allow a better economic balance of the free aminoacid pool, which would not be easily exhausted for high production of a protein at a particular locus since the same functional kind of molecule could be produced by two slightly different sets of aminoacids. This is the most probable basis for the effect of isozyme allelic heterogeneity. Summing up, there are four theoretically possible concurrent sources of heterosis, at the molecular level: the *epistatic* effect when different loci products interact to form dimers and tetramers, exemplified by Cat_1 and Cat_2 (Scandalios et

Table 3. Average yields kg/ha of the specific and general combinations and the enzymatic diversity index

	Tuxpan 1020	T 61.984-1	NC 83.7628	FB 4.5941	Salbert 5392	SR 201.5232	SR 527.5269	General Combining Ability
Tuxpan 94.6224	2887 (1)	5380 (2)	7040 (4)	5078 (5)	6720 (4)	5401 (6)	5741 (3)	5607 (25)
Tuxpan 1020.17	x	5360 (3)	5861 (3)	3667 (4)	6704 (3)	5807 (5)	4913 (4)	5028 (23)
T 61.984-1		x	6067 (6)	6347 (4)	6201 (5)	6293 (5)	6496 (4)	6163 (29)
NC 83.7628			x	6916 (5)	6173 (6)	6473 (6)	6627 (7)	6451 (37)
FB 4.5941				x	6353 (3)	6433 (2)	6207 (5)	5857 (28)
Salbert 5392					x	5153 (3)	7067 (2)	6339 (26)
SR 201.5232						x	6008 (3)	5938 (30)
SR 527.5260							x	6151 (28)

al. 1972); *overdominance*, when a better hybrid enzyme is produced, as in the Adh_1 single gene heterosis (Schwartz 1973); *dominance*, when active alleles cover the null or silent ones, not yet studied in this regard; and the possibility, now shown by our observations, that the *cumulative effect of codominant* isoalleles may contribute to the improvement of the hybrids, as the sample of such loci studied in this paper suggests, when they are taken as markers of genetic diversity.

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