Before conclusions can be drawn concerning the genetics of diaphorase in maize, it must be demonstrated that the activities measured here represent the single subunit enzyme, and that the alterations in electrophoretic mobility are not the result of association with multisubunit enzymes. This can be verified by determining the molecular weight of these isozymes from tissue extracts using standard techniques such as sucrose gradient centrifugation.

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4. New alleles and chromosome localization of the locus for maize endopeptidase.

In a previous report (Melville and Scandalios, 1972) we described a single form of maize endopeptidase designated EP-1 with two variants A and B. The $\underline{Ep}_1$ locus coding for the EP-1 isozymes was found to be completely linked to a locus determining a yellow or white endosperm, probably the $\underline{Y}_1$ locus on chromosome 6. ($\underline{Y}_1$ = yellow; $\underline{y}_1$ = white). All kernels with yellow endosperm were found to be of type A or AB and all the white of type B.

Further work with maize trisomics for chromosome 6, containing the $\underline{Y}_1$ marker, confirms the close linkage between $\underline{Ep}_1$ and $\underline{Y}_1$, but the linkage is not complete. From a cross population of about 300 individuals, at least three kernels with white endosperm ($\underline{y}_1$) were found having the EP-1A component. In addition, one of the examined $\underline{y}_1$-marker samples was homozygous for the A type.

In the trisomic samples, three new EP-1 variants were found designated C, D and E in order of discovery (Fig. 1) and a non-expressed (null) variant designated O. Formal genetic analyses show that all variants are coded by alleles in the $\underline{Ep}_1$ locus. One of the samples examined was homozygous for the null-allele.

From samples containing kernels trisomic for chromosome 6, several plants were found giving three endopeptidase bands on the zymogram. All the three-banded plants were trisomics, indicating that three different alleles are present on the three replicates of chromosome 6. The phenotypes $\underline{DA}$ and $\underline{AC}$ (Fig. 1), showing a gene-dosage effect in diploid tissues, are caused by two chromosomes with an A allele and one with a D or C allele, respectively.

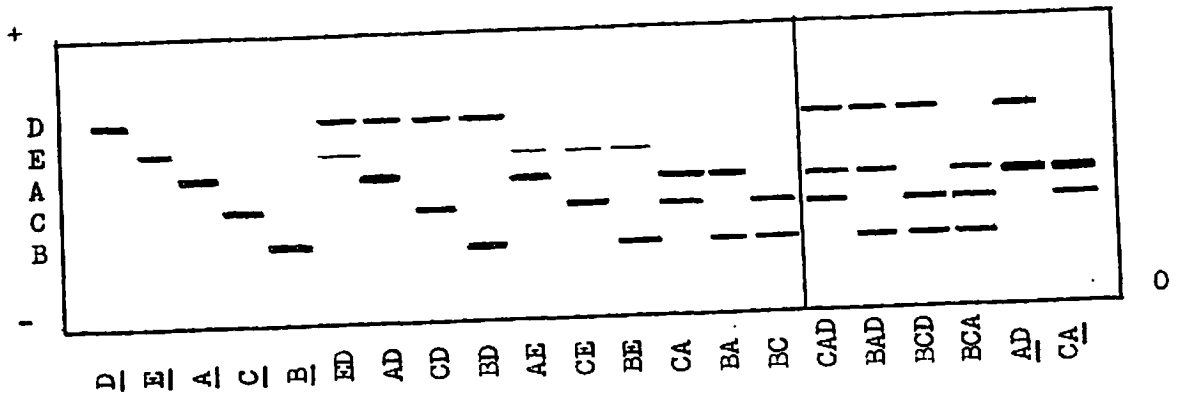

Fig. 1. Electrophoretic patterns of maize endopeptidase. The diagram shows the five diploid homozygotes, the ten corresponding diploid heterozygotes, the four three-banded trisomics and two of the two-banded trisomics with dosage effect. The E-type has not been incorporated in the trisomic samples. The relative strength of the bands is approximately according to the intensity on the zymogram.

Some of the Tri-6 plants were verified as trisomics by chromosome counts but showed two bands without dosage effects. Progeny from such plants pollinated with plants having two other alleles showed several cases of patterns without female parent bands, indicating a null allele on the third chromosome in the trisomic female parent. The genotypes for a typical cross and the progeny were the following:

D/B/O (♀) x A/C (♂) → D/A, D/C, A/B, C/B, A/O, C/O, D/A/B, D/C/B.

The progeny may also contain the genotypes D/A/O, D/C/O, A/B/O, and C/B/O, but chromosome counting is necessary to distinguish them from the respective diploids without the null allele as they are phenotypically identical. The progeny from one of the crosses contained kernels without endopeptidase activity. Both the parents were trisomics of the phenotypes AB or DB without dosage effect indicating the genotypes to be A/B/O and D/B/O, respectively. A small fraction of the progeny will then be of the genotype O/O (null).

Several crosses were made between various EP-1 types of the trisomic plants, and all the four three-banded trisomics shown in Figure 1 were found and several of the two-banded trisomics showing gene-dosage effects in the diploid tissues (scutellum or roots from 5-8 day seedlings). The E-allele is not present in any of the trisomic plants but is found in

homozygous condition as well as heterozygous with any of the other alleles including the null-allele.

Reference:
Melville, J. C. and J. G. Scandalios, 1972, Biochem. Genetics 7:15-31.

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1. Tcms restoring genes in open-pollinated varieties.

Transfer of Texas male-sterile cytoplasm to several varieties was begun several years ago for the dual purpose of obtaining satisfactory restoring inbreds and the possible development of equilibrium populations for use in areas where the use of hybrid seed was not practical. The intent was that cultivators of maize could produce their own seed by harvesting ears from pollen sterile plants that would have been pollinated by plants carrying restorer gene(s) and presumably producing some degree of vigor. For various reasons, the method did not prove to be practical.

The percent fertile plants, following the crosses to Tcms, ranged from 0 in T61Y Syn. to 40.5 in Jellicorse (MGCNL31). Varieties Jellicorse, Rockdale and Salisbury White were considered good potential sources for restoring genes. Ten backcrosses of each variety using bulk pollen were made on sterile plants of the variety before the study was terminated.

Fertile plants in Jellicorse, Neal Paymaster, Teko Yellow, and Potchefstroom Pearl were self pollinated to homozygosity for fertility restoration. Crosses were then made among plants within and between varieties, as well as with restoring inbred T115. Following selfing, all crosses produced only fertile plants and the restoring gene(s) in all varieties are considered to be identical with those in T115.

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