

formation. Genetic analysis showed the variants to be co-dominant alleles of a single locus designated Ep-1.

Quantitative assays for LAP and endopeptidase activity show the highest levels for both occur in the developing kernel and in the scutella and embryo of the germinating seedling.

We are presently attempting to co-purify the aminopeptidases and the endopeptidase from the inbred line W64A, which possesses the fast endopeptidase variant. One-day imbibed seed are used. Three LAP bands are present in the crude extract (LAP-A, LAP-B and LAP-C, with respect to decreasing anodal migration at pH 7.0). Both LAP and the endopeptidase precipitate at 40-55% saturation with ammonium sulfate and elute from a G-100 Sephadex column in the same volume. The enzymes bind to PE-52 Whatman cellulose at pH 7.5 and are eluted with a linear KCl gradient. An activity peak containing LAP-C elutes before a peak of activity containing LAP-A and LAP-B (as indicated from electrophoresis of the fractions). The endopeptidase peak is intermediate. Homogeneity has not been achieved yet as protein OD280 peaks do not correspond with enzymatic peaks. The endopeptidase shows activation upon addition of ammonium sulfate to 40% saturation. Activation results in activities of approximately 250% of the level of activity in the crude extract. LAP does not show activation.

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3. Diaphorase isozyme patterns in the immature endosperm of *Zea mays* kernels.

Diaphorase (E.C. 1.6.99.-) is a low molecular weight, flavin-containing enzyme which is involved in electron transport in the oxidation of NADH. As well as being found in the free enzyme, diaphorase is also associated with a number of substrate inducible, multisubunit enzymes, such as nitrate reductase and sulfite reductase. In these multisubunit enzymes, the diaphorase active site has been shown to be distinct from the other activities of the enzymes [Losada, M. et al. (1968) Prog. Photosyn. Res. Proc. Int. Congr. 3, 1504-9].

It is conceivable that the diaphorase gene also codes for the subunit which contains this activity in other enzymes. The zymogram

technique can be used to test this hypothesis. Genetically determined electrophoretic variants of diaphorase must be found. Alterations in the charge properties of this enzyme could also lead to alterations in the electrophoretic mobility of those multisubunit enzymes which utilize the diaphorase subunit in their function. If this hypothesis can be demonstrated, then diaphorase becomes an interesting enzyme to study in terms of the regulation of enzyme synthesis.

The endosperm of several inbred lines of corn was tested for diaphorase isozymes. Three phenotypes were observed (Figure 1). The F_1 hybrids between inbred lines with different phenotypes show a 3-banded pattern. Genetic analysis is now warranted to determine whether these phenotypes represent the expression of two genetic loci with codominant alleles at one locus, and a possible null allele at a second locus. It is also possible that the two diaphorase isozymes found in the inbred lines represent duplicated loci, and in one line of corn, this duplication has not taken place.

ANODE

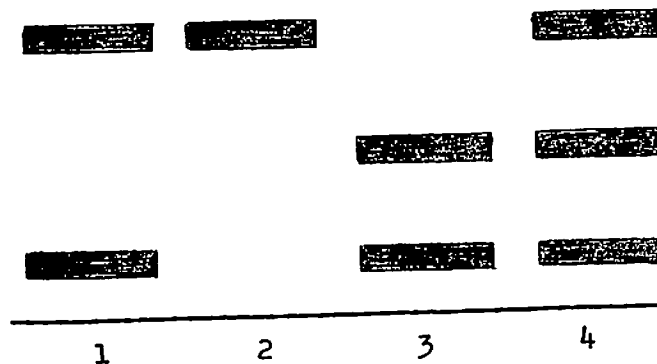


Figure 1. Diaphorase isozyme patterns found in the liquid endosperm of immature maize kernels.

1. Phenotype for inbred lines W64A, 67-2, 77, 67-75, T_4 , 81, 66-37, V_4 .
2. Phenotype for inbred line 38-11.
3. Phenotype for inbred lines T_{21} , 8, 78, T_{20} .
4. Isozyme pattern for the F_1 hybrid from the cross $T_{21} \times T_4$.

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 Ep₁ locus coding for the EP-1 isozymes was found to be completely linked to a locus determining a yellow or white endosperm, probably the Y₁ locus on chromosome 6. (Y₁ = yellow; y₁ = 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 Y₁ marker, confirms the close linkage between Ep₁ and Y₁, but the linkage is not complete. From a cross population of about 300 individuals, at least three kernels with white endosperm (y₁) were found having the EP-1A component. In addition, one of the examined y₁-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 Ep₁ 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 DA and 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.