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1. Inherited variation of catechol oxidase.

Toru Endo (unpublished) first observed inherited variation of catechol oxidase behavior between inbred maize lines. When extracts of shoots from 3-4 day old seedlings were subjected to starch gel electrophoresis at pH 8.3, and the gels incubated in 0.01 M catechol at pH 4.2 to reveal the enzyme bands, three true breeding classes were observed.

Class I: zymograms showed a cathodally migrating band.

Class II: showed no band of enzyme activity. However, if the seeds were presoaked in 0.01 M maleic hydrazide, the same cathodally migrating band was revealed.

Class III (Null class): no enzyme band could be detected either with or without maleic hydrazide treatment.

The inheritance of enzyme variants.

In the present study a slower moving electrophoretic variant of a Class II line was found in a survey of inbred lines. Genetic studies indicate that the common fast migrating isozyme (F), the variant slow isozyme (S), and the null form (Class III) are specified by different alleles of a gene, designated here as catechol oxidase or Cx.

Using the wx linked translocations, Cx has been located on Chromosome 10. In crosses to the endosperm marker dull (du) a recombination frequency of 0.16% (2/1197) was observed indicating very close linkage of du and Cx.

The difference between Class I and Class II.

Zymograms of extracts from shoots of Class II maize lines only show a catechol oxidase band if the seeds have been presoaked in 0.01 M maleic hydrazide. Concomitant treatment with protein synthesis inhibitors (cycloheximide and chloramphenicol) does not prevent the maleic hydrazide mediated appearance of a band of enzyme activity. This suggests that the appearance of a band after maleic hydrazide treatment does not involve a de novo protein synthesis.

There is evidence suggesting that Class II seedlings contain a "modifier" which rapidly reacts with catechol oxidase during extraction, so that the enzyme no longer migrates in electrophoresis. The existence of a non-migrating enzyme in extracts of untreated Class II seedlings can be demonstrated by electrophoresis on cellulose acetate strips. Zymograms from these strips show a band of catechol oxidase activity remaining at the origin.

The amount of "modifier" present in untreated Class II seedlings is limited, but sufficient to prevent the appearance of the cathodally migrating band. Thus, when shoots from Class II seedlings carrying the $\underline{Cx}^{F}$ allele are homogenized in the presence of increasing concentrations of S enzyme extract (partially purified on a sephadex column), the F enzyme band is eventually revealed in electrophoresis.

We suggest that the difference between Class I and Class II lines is due to the amount of "modifier" present. The amount of "modifier" is sufficient in Class II lines to inhibit the migration of all of the catechol oxidase, but insufficient in Class I, thus allowing the appearance of an enzyme band in zymograms from untreated seedlings. On this basis, the treatment of seeds with maleic hydrazide in some way affects the amount or availability of "modifier" in shoots, so that the migration of enzyme in Class II seedlings is not affected and extracts give rise to a cathodal band in electrophoresis.

Tony Pryor

2. Substrate inhibition of allelic isozymes of alcohol dehydrogenase in maize.

Differences in the pH optima of the enzyme forms specified by the $\underline{Adh}_1^{C(m)}$ and $\underline{Adh}_1^{S}$ alleles were reported previously (Schwartz & Laughner, Maize News Letter 42:83, 1968). Further studies on the kinetic properties of these alcohol dehydrogenase isozymes have revealed striking differences in their inhibition by specific substrates.

Alcohol dehydrogenase (E.C. 1.1.1.1.) catalyzes the following reaction:

$$\text{Ethanol} + \text{NAD} \underset{\text{foward}}{\overset{\text{backward}}{\rightleftharpoons}} \text{Acetaldehyde} + \text{NADH}$$