vacuolar pH is also very low and this plus the nucleases quickly denature and degrade DNA. Vacuolar membranes are likely to be disrupted before cells are broken and Tris and/or other buffers may not penetrate rapidly enough to offset acidity or nucleases. Stern (5) suggested that carrying out the entire isolation at high pH would effectively neutralize nucleases. Thus it is possible that by removing the nuclei from the presence of cytoplasmic degradative enzymes (especially hydrolytic enzymes released from broken vacuoles) prior to lysis, additional protection is afforded the DNA. These enzymes are probably the major cause of poor results in DNA extraction from plants.

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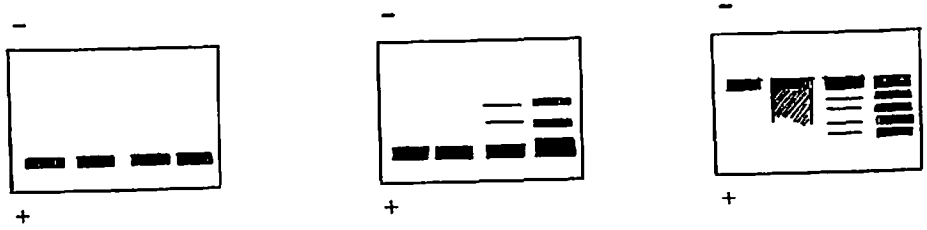
1. Isozyme pattern of catalase in the developing maize endosperm.

It is known that in maize endosperm the enzyme catalase is controlled by the $\underline{Ct_1}$ locus (Scandalios, J.G. 1969). Furthermore, a second locus, $\underline{Ct_2}$, has been detected in the scutella acting to control catalase synthesis. The products of the $\underline{Ct_2}$ and $\underline{Ct_1}$ loci tend to aggregate and, as a result, at a certain stage of seed germination a catalase pattern emerges consisting of 5 isozymes. In this paper we report the isozyme pattern of catalase in the developing endosperm. The study was carried out on 32 inbred lines of maize and 22 interline hybrids. The endosperm was fixed in solid carbonic acid at 13, 16, 19 and 25 days after pollination.

Electrophoretic analysis of endosperm isozymes was carried out according to standard procedures (Poulik, M.D., 1957 and Scandalios, J.G., 1969).

The 32 lines studied were assigned to 3 groups which we have designated: 1-catalase, 3-catalase and 5-catalase. 1-catalase (12 lines) were those in which only one electrophoretic catalase variant was revealed from days 13 to 25 after pollination. 3-catalase (15 lines) were those in which one electrophoretic catalase variant was detected on day 13 and two additional isozymes were established in the developing endosperm. They stain less intensely than the first isozyme. 5-catalase (5 lines) were those in which one electrophoretic catalase variant was found in the endosperm on day 13, and on day 16 a new band was observed which on day 19 displayed five distinct isozymes (Fig. 1).

Fig. 1



Based on these findings, it is considered that in some lines there functions a second locus in the endosperm controlling catalase synthesis. Just as in scutella, there seems to be no hindrance to the aggregation of the subunits of the two loci so that some lines produce 5 isozymes on the zymogram. We cannot attribute this observation to heterozygosity for the $\underline{Ct}_1$ locus, since, in this case, the zymogram shows 4 isozymes with a characteristic distribution of staining intensity of each zone which reflects the double dosage effect of the maternal allele (Scandalios, J.G., 1969); moreover, in the segregating corn-cob half of the seeds would have a single zone of catalase activity. In 5-catalase lines all the seeds exhibited a specific pattern with the electrophoretically slowest isozyme staining most intensely. The other 4 isozymes stained equally well. The

existence of 5-catalase and 3-catalase lines may be explained, on the one hand, by the different activity of the second locus in various lines (i.e., the different amounts of gene product per time unit). It may be suggested, on the other hand, that subunits and catalase molecules differ in the rates of their synthesis or degradation. For this reason, we found no products of the second locus in the developing endosperm in 12 lines studied, in 15 lines the relative contributions of the products of the two loci were such that only three isozymes were distinguished on the zymogram and in 5 lines the proportions of the two types of subunits were more favorable permitting resolution of all 5 isozymes. Scandalios has described a maize line containing 3 catalase isozymes and believes that they are controlled by a sixth allele of the $\underline{Ct}_1$ locus referred to as V'. In the light of our observation, it may be assumed that in this line a second catalase locus is active.

1-catalase, 3-catalase and 5-catalase lines were used in crosses and backcrosses. In these hybrids, the pattern of isozyme catalase was studied in the developing endosperm. Hybrids between lines with the same catalase pattern (same number of isozymes and similar electrophoretic mobility) gave a pattern identical with that observed in these lines. When the crosses involved lines differing in the electrophoretic variant of catalase, a hybrid pattern occurred consisting of 4 isozymes with the distribution frequency of isozyme staining reflecting the effect of double dosage of the maternal allele. In crosses between the 1-catalase line with the 3-catalase and 5-catalase lines, the results depended on the type of cross. When 3-catalase or 5-catalase lines were used as paternal lines, the hybrid pattern of catalase was unaffected. In backcrosses, where the 3-catalase or 5-catalase lines were used as the maternal plants, the pattern deviated from the one usually observed: the bounding lines of the isozymes were blurred and some bands frequently merged into one spot. This is indirect support of the idea that additional isozymes altering this pattern are the product of a second functioning locus.

Thus, evidence has been obtained corroborating a two-loci system which controls catalase synthesis in the developing endosperm of maize.

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