TISSUE-SPECIFIC ISOZYME VARIATIONS IN MAIZE

JOHN G. SCANDALIOS*

IN recent years a large number of enzyme polymorphisms have been reported in a great variety of organisms[7]. However, these variations have not been studied with as great an intensity in plants as in animals, although some rather important findings concerning enzyme variations in maize endosperm have been reported recently[2,10].

With the introduction of new and improved methods for the electrophoretic separation, in starch gel, of different molecular forms of enzymes coupled with various enzyme staining methods (the zymogram technique)[4,5], a new impetus has been afforded for isozyme[5] studies in plants. It is well established that different proteins with similar enzymatic activities may exist in the same organism and within the same tissue[6]. The present investigation deals with the distribution, and isozyme variations, of the enzymes leucine-aminopeptidase (LAP), esterase, peroxidase and catalase in various tissues of *Zea mays*.

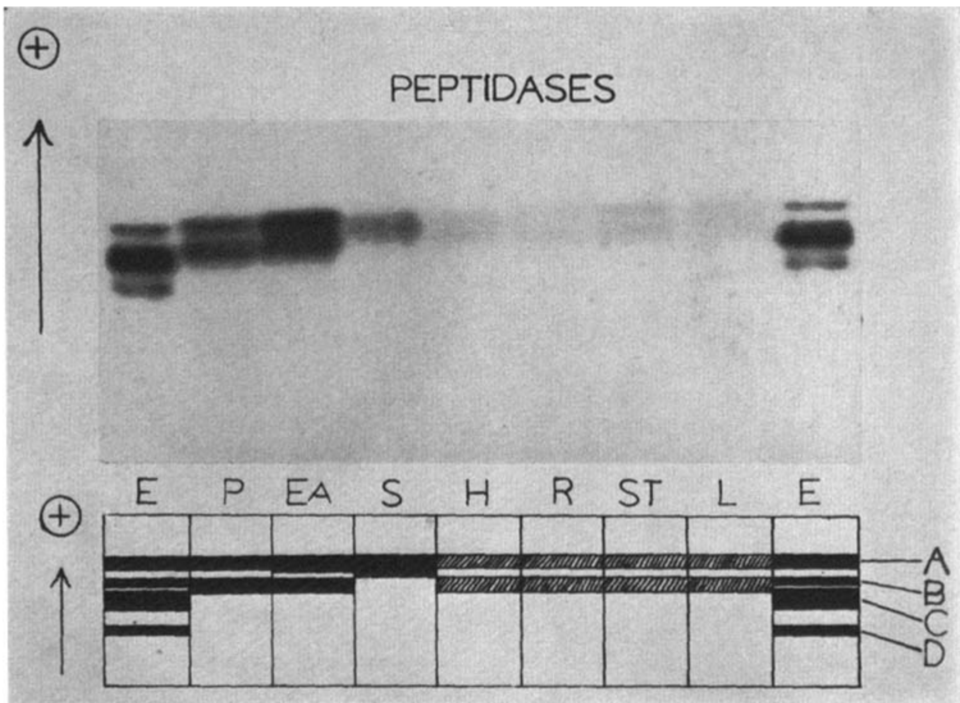
Materials and Methods

One inbred strain of corn was used in this investigation; AA4 (University of Hawaii) which was kindly supplied by Dr. J. L. Brewbaker of the Department of Horticulture, University of Hawaii.

The methods used to extract the liquid content of the various tissues for use in preparing samples for electrophoresis were identical except for the endosperm. Extracts of leaf, stem, root, husk, silks, young immature ears and pollen were prepared from plants in the field about fifty days after planting. Homogenates were prepared from tissues after weighing equal portions from each and adding 0.3 ml of 0.9 percent saline. The homogenates were frozen-thawed five times and then centrifuged. The supernatant solution of each sample was absorbed onto a piece of filter paper (5×5mm) which was inserted into a starch gel. The endosperm samples were prepared from maize ears which were harvested on the fifteenth day after pollination. The liquid endosperm from individual kernels was squeezed onto a piece of filter paper and inserted into a starch gel. The electrophoresis was conducted in a discontinuous buffer system[1] (pH 8.6) until the visible borate front zone had migrated about 7 cm from the sample slot.

For the demonstration of leucine-aminopeptidase the starch gel s rips were incubated for one hour in a solution consisting of 40 mg L-leucyl-β-naphthylamide-HCl, 50 mg Black K salt and 100 ml 0.2 M Tris-maleate buffer, pH 6.0. The esterases were demonstrated by incubating the gels for one hour in 100 ml of 0.1 M phosphate buffer, pH 6.0 containing α-naphthyl acetate as substrate and Fast Blue RR salt as a dye-coupler. Peroxidase activity was revealed by soaking the gels for one minute in a mixture consisting of equal amounts of 1 percent hydrogen peroxide solution and benzidine solution (1 gm benzidine, 9 ml acetic acid, 36 ml water). Visual observation of catalases was made possible by first soaking the gel for one minute in 0.5 percent hydrogen peroxide, washing twice in water and then immersing it in a 1 percent solution of potassium iodide acidified with acetic acid. In all

* Department of Genetics, University of Hawaii, Honolulu, Hawaii. Contribution No. 29 of the Pacific Biomedical Research Center.



LAP PATTERNS

Figure 15

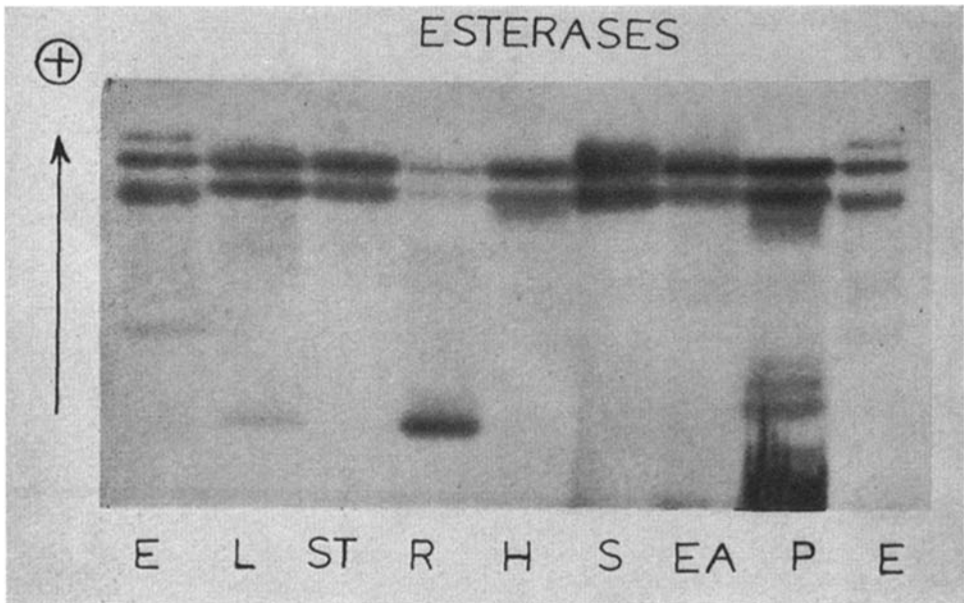
Photograph and schematic drawing showing the LAP patterns in the various tissues. E = endosperm, P = pollen, EA = young ear, S = silks, H = husk, R = root, ST = stem, L = leaf. Letters to the right of the drawing (A,B,C,D,) indicate the four LAP bands. The arrow shows the direction of migration towards the anode.

cases the gels were sliced horizontally after the electrophoresis and before staining.

Results and Discussion

It was found that there is a relatively high degree of homogeneity in the distribution of LAP in the major tissues of corn (Figure 15). It is interesting to note this identity in LAP zones is limited to the A and B bands; the bands C and D are characteristic of the endosperm extracts only. Silk was the only tissue which apparently lacked the B band, and in which the A band appeared more concentrated. The intensity of staining reveals the relative concentrations of the enzyme in the various tissue homogenates.

The esterase zymograms revealed a main fast-migrating zone with little variability among the different tissues (Figure 16). The endosperm had three distinct bands in this zone of which only one (the middle) band was also represented, in varying concentrations in all other tissues tested. There are several slow-migrating zones, but of these, few have a high enough activity to become visible. The roots and leaves have a common slow band, but the concentration difference is a very distinct one. The pollen also shows a very unique pattern of three slow-moving bands which have no corresponding representatives in any of the other tissues. A rather intense enzyme staining was found following these three slow bands of the pollen extract; this



ESTERASE PATTERNS

Figure 16

Photograph of starch gel showing the esterase patterns in the various tissues. The arrow shows the *direction of migration towards the anode.*

might obscure eventual small distinct fractions in that region.

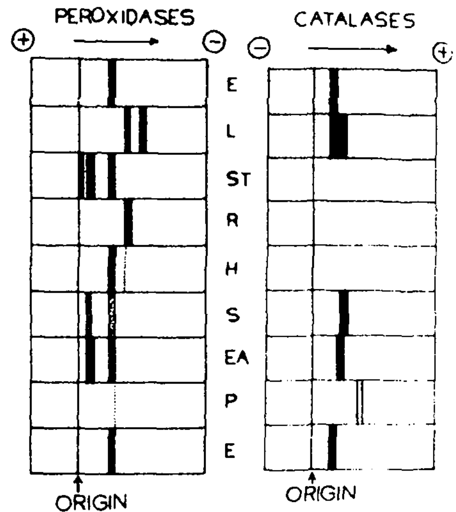
Starch gels stained for peroxidase activity revealed two distinct groups; one group of peroxidases migrated towards the positive pole at pH 8.6 and the other migrated towards the negative pole at the same pH. Only those peroxidases that migrate to the negative pole will be discussed here, since those migrating to the anode blurred upon staining and could not be clearly distinguished. The peroxidases, like the esterases, show quite a large variability within the tissues (Figure 17). The endosperm, stem, husk and young ear have one band which stains with the same intensity; the silks and pollen have the same band but in a less concentrated form. The leaves have a very unique fast-moving component and a slower band which is also represented intensely in roots and very faintly in husk; the roots also show a faster-migrating component, not clearly separated from the slow band of this region. A slow zone is to be found in the homogenates of the stem, silks and young ear with the fast band of the zone being common, in varying concentrations, to all three tissues. A slow intense band in this region is found only in the stem extract not far from the origin.

Catalase activity was found to be completely lacking in the stem, root and husk homogenates, but present in varied patterns and concentrations in the other five tissues (Figure 17). The endosperm shows one very dense band which is also present in the leaf but in a more dilute form. The fast component seen in the leaf is also represented in the same intensity as in the silk homogenate. The young ear shows a distinct component intermediate in migration to the two bands found in the leaf. The fastest band is

found in the pollen extract in a very dilute form. Of the enzymes discussed here the catalases are the slowest anode-migrating enzymes, the esterases the fastest and LAP is intermediate in distance from the origin.

The data accumulated in this study suggest that several enzymes existing in multiple molecular forms are present in the different tissues of maize. The degree of heterogeneity was found to vary considerably with regard to the particular enzyme system and to the specific tissue. In no instance were the different enzyme activities located in the same positions in the zymograms (i.e., no overlapping of band patterns). LAP in known to be distributed widely in both plants and animals. It is a proteolytic enzyme that is probably of considerable importance in protein degradation during growth and development but its specific function in the different tissues is not yet understood. The persistant presence of LAP bands A and B in all the tissues examined at various developmental stages[9] renders this system a rather interesting one for studying the developmental physiology of maize.. In a recent communication from this laboratory[2] the genetic control of band A and D of LAP was discussed in studies with endosperm variations. Band D was recently found to be absent at both very early and very late stages of endosperm development[9], indicating, perhaps, the presence of an inducer-repressor system vulnerable to genetic analysis.

The esterase data present a more heterogeneous picture in that there are several zones of enzyme activity with variants in each zone. The fastest-moving region consists of two bands in all tissues except the triploid endosperm tissue which has three distinct bands of which only one is common to the other tissues. It has been suggested that esterases may be involved in starch synthesis[10]; therefore, this enzyme would be expected to be most active in the endosperm where such activity is greatest. The slower zones exhibit more heterogeneity and it is



PEROXIDASE AND CATALASE PATTERNS

Figure 17

Schematic drawings showing the peroxidase patterns (left) and the catalase patterns (right) in the various tissues. The arrows show the direction of peroxidase migration towards the cathode and catalase migration towards the anode. Note the absence of catalase activity in the stem, root and husk homogenates.

interesting to note that the haploid pollen shows a greater number of isozyme bands than the other tissues, diploid and triploid alike.

Although the role of peroxidases is not clear, it has been speculated that they may play an inhibiting role in plant growth by limiting the amount of the auxin, indoleacetic acid (IAA), through oxidation[8]. Interestingly, the results of this investigation show that peroxidase activity is greatest in the most actively growing tissues (leaves, root, stem, husk, silks and ear) where IAA activity would be expected to be predominant. Since auxin is known to act as a growth inhibitor when it reaches very high concentrations in the plant, it is perhaps safe to assume that these peroxidases may act to destroy excessive amounts of IAA in an attempt to maintain the effective auxin level in the various growing tissues.

The only case in this investigation where enzyme activity appears to be

completely absent from any tissue was that of catalase activity in stem, root and husk homogenates. It was recently found in this laboratory that there are two electrophoretic variants of catalase in maize endosperm under genetic control. The pattern of inheritance suggested that this catalase exists as a tetramer[3]. It would be interesting to determine the mode of inheritance of the catalase variants in the other tissues.

The function of these four enzymes in the differentiation and development of the corn plant is not known at the present time, but as the genetic mechanisms controlling the presence or absence of these enzymes unfold, a better comprehension of their physiological role will result.

Summary

The presence of tissue-specific variants of the enzymes leucine-aminopeptidase, esterase, peroxidase and catalase have been shown to exist in maize by use of the zymogram technique. The data give supporting evidence for the existance of multiple molecular forms of various enzymes within the same organism and within the same tissue. The possible physiological significance of multiple forms of enzymes for tissue function are discussed.

Literature Cited

1. Ashton, G. C. and A. W. H. Braden. Serum β-globulin polymorphisn in mice. *Austr. Jour. Biol. Sci.* 14:248–254. 1961.

2. Beckman, L., J. G. Scandalios and J. L. Brewbaker. Genetics of leucine-aminopeptidase isozymes in maize. *Genetics* 50:899–904. 1964.

3. ———. Catalase hybrid enzymes in maize. *Science* 146: 1174-1175. 1964.

4. Hunter, R. L. and C. L. Markert. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. *Science* 125: 1294-1295. 1957.

5. Markert, C. L. and F. Møller. Multiple forms of enzymes: Tissue, ontogenic and species specific patterns. *Proc. Natl. Acad. Sci.* 45:753-763. 1959.

6. ——— and R. L. Hunter. The distribution of esterases in mouse tissues. *Jour. Histochem. Cytochem.* 7:42-49. 1959.

7. Multiple Molecular Forms of Enzymes. Edited by F. N. Furness. *Ann. N. Y. Acad. Sci.* 94. 1961.

8. McCune, D. C. Multiple peroxidases in corn. *Ann. N. Y. Acad. Sci.* 94: 723-729. 1961.

9. Scandalios, J. G. Unpublished data. 1964.

10. Schwartz, D. Electrophoretic and immunochemical studies with endosperm proteins of maize mutants. *Genetics* 45:1419-1427. 1960.

Wilhelmine E. Key Lectureship Announced

The Council of the American Genetic Association is pleased to announce the second Wilhelmine E. Key Lectureship. Dr. Rollin D. Hotchkiss of the Rockefeller Institute has accepted the Association's invitation to present a lecture on Tuesday evening, August 17, 1965, during the annual AIBS meetings at Urbana, Illinois. Dr. Hotchkiss's lecture is entitled: Portents For a Genetic Engineering.

The lectureship was made possible by a bequest of Wilhelmine E. Key, a New England schoolteacher and long-time member of the American Genetic Association. Miss Key designated that a generous portion of her modest estate be used by the Association to support lectures on the implementation of genetics for human welfare and improvement.

Dr. Sheldon C. Reed, Director of the Dight Institute for Human Genetics, presented the first Key lecture, "The Relationship of Human Welfare to Marriage Selection," at the August 1962 meetings of the American Society of Human Genetics at Corvallis, Oregon.