Proceedings of the Iowa Academy of Science

Volume 77 | Annual Issue Article 8

1970

Esterase Activity in Developing Pods of Soyheans

Richard S. Fawcett

Peter A. Peterson

Esterase Activity in Developing Pods of Soybeans[1]

RICHARD S. FAWCETT* AND PETER A. PETERSON[2]

Abstract. Four stages of soybean pod development of four varieties of *Glycine max* were surveyed for esterase enzymes by starch-gel electrophoresis. Differences in esterase banding patterns occur among the four varieties investigated. Enzyme changes accompanying pod development were observed. Pod collection procedure affected the number of esterase bands visible, with collection in dry ice giving more visible bands than did collection in regular ice.

The separation of enzymes by zone electrophoresis, using a solid supporting medium (Smithies, 1955) in combination with histochemical staining techniques (Hunter and Market, 1957), yields a reproducible pattern (zymogram) of sites of enzyme activity. Enzymes, thus identified electrophoretically, are useful for studying genetic variation within a plant species (West and Garber, 1967; Meisinger, 1967; Scandalios, 1969). Enzyme changes accompanying development have been previously observed (Schwartz, 1962; Steward, Lyndon, and Barber, 1965; Meisinger, 1967[3], Snyder and Kleese, 1968; Scandalios, 1969).

The purpose of this study is to investigate genetic variation of specific esterases as well as changes in the enzymes accompanying developing pods in four varieties of *Glycine max*.

MATERIALS AND METHODS

Seeds from four varieties of *Glycine max*, Ford, Hark, Kazai, and Kimusume, were electrophoretically analyzed for sites of esterase activity by starch-gel electrophoresis. To study the changes in esterase activity during the development of the soybean pod, plantings were made on four different dates in 1968—May 11, May 26, June 15, June 22; these will be referred to as dates 1, 2, 3, and 4, respectively. Comparisons between pods could be made by collecting pods at the same node position on the plants on Aug. 27. Two collecting procedures were used. At the time of collection in the field, some pods were collected and stored in regular ice and, others, in dry ice.

Crude extracts were made by crushing one seed on a 5-mm square of Whatman no. 1 filter paper. Five squares of filter paper saturated with extracts from different seeds were inserted into a horizontal slot made in the center of a starch gel. In this way, valid

*RSF is a NSF URP participant

[1] Journal Paper No. J-6577 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1335.

[3] Meisinger, J. 1967. Esterases in developing soybean pods. Unpublished NSF URP Report—1967.

comparisons of sites of esterase activity could be made. The gels were placed between two electrode trays containing 0.3-M borate buffer (pH 8.5). Current was carried to the gels by strips of filter paper soaked in the borate buffer, with one end in the electrode tray and, the other, over the end of the gel (Fig. 1). A satisfactory separation of esterases was accomplished by using 15 v/cm for 2 hrs. at room temperature.

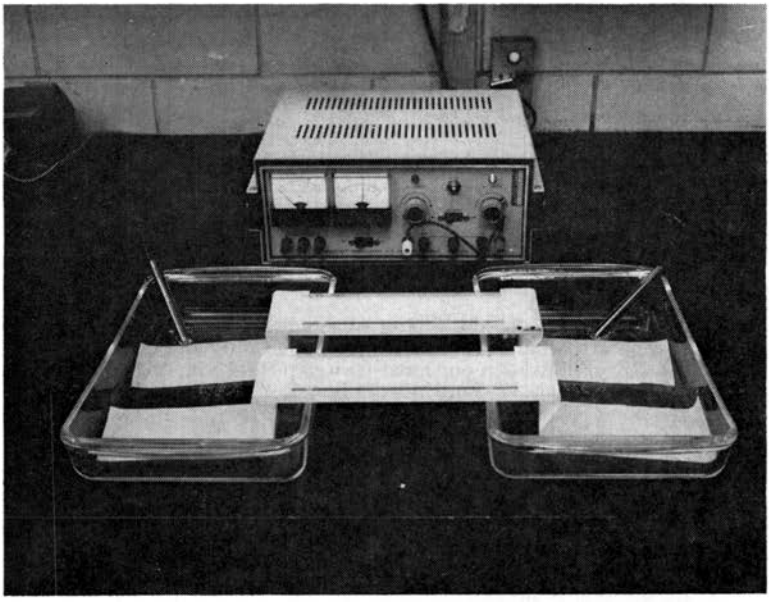

Figure 1. Starch gel electrophoresis apparatus.

Starch gels were prepared by suspending 42 grams of electro starch (Electro-Starch Co., Madison, Wisc.) in 400 ml of 0.03-M borate buffer (pH 8.5). The suspension was heated, evacuated, and poured into molds (200 x 40 x 6 mm).

Developed gels were removed from molds and placed in a staining solution. A solution containing 2 ml of 1% naphthyl acetate (Dajac Laboratories, Philadelphia, Pa.) in 50% acetone, 100 ml of 0.1-M phosphate buffer (pH 6.5), and 75 mg of Fast Red TRN (Dajac Laboratories) in 5 ml of distilled water was used to detect sites of esterase activity. Gels were incubated at 37°C in the reaction mixture until sites were sufficiently dark, then rinsed with distilled water, and recorded. Each of the analyses was subjected to repeated runs.

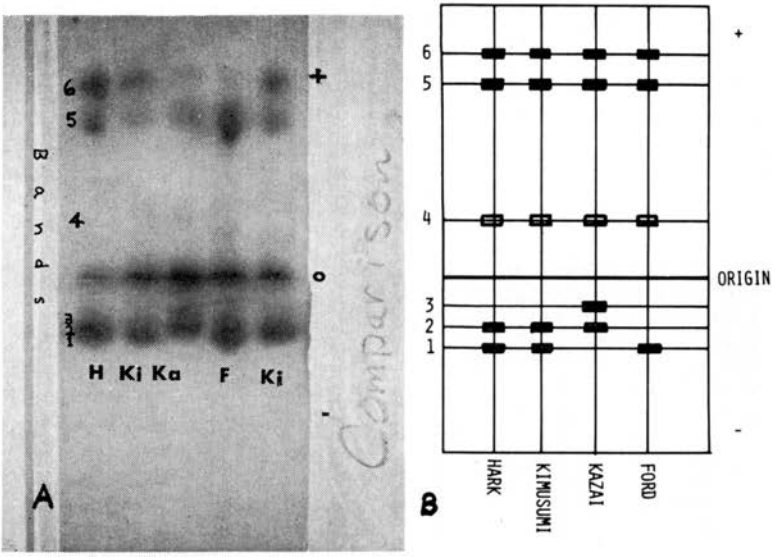

Figure 2. A. Location of the 6 sites of esterase activity in zymogram of mature seeds of Hark, Kimusume, Kazai, and Ford soybeans. Kimusume is represented twice. (H—Hark, Ki—Kimusume, Ka—Kazai, F—Ford, O—Origin). B. Diagramatic representation of sites shown in A.

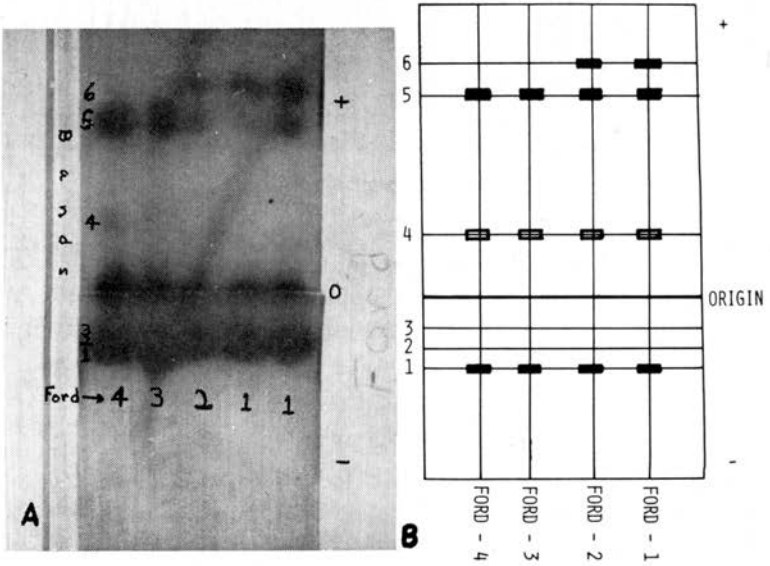

Figure 3. A. Location of sites of esterase activity of four developmental stages of Ford seeds. Ford-4 is the youngest bean while Ford-1 is the most mature. Ford-1 represented twice, O—origin. Long streaks are surface effects. B. Diagramatic representation of sites shown in A.

Results

Considering all varieties tested, there are three anodic and three cathodic sites of esterase activity (Fig. 2). The oldest seeds from all four varieties possessed anodic bands 4, 5, and 6. On the cathode side, Hark and Kimusume had bands 1 and 2. Kazai consistently lacked band 1, but had an extra band, band 3, along with 2. Ford had only band 1.

Differences in banding patterns between plantings are evident in the Ford variety. Band 6 is not present in Ford 3 and 4 (the youngest seed) while it is present in the older seed, Ford 1 and 2 (Fig. 3).

Sample collections in dry ice resulted in more visible bands than did collections made in regular ice. Kazai, Kimusume, and Ford seeds from plantings 3 and 4 (the youngest seeds) lacked band 6 when collections were made in ice. Collections in dry ice gave a faint band 6 in plantings 3 and 4 of Kazai and Kimusume (Fig. 4). Ford, however, consistently lacked band 6 in plantings 3 and 4 under both collecting procedures.

Discussion

There were three esterase banding patterns among the four varieties. Hark and Kimusume were identical, but Kazai and Ford had different banding patterns. Meisinger (1967), using acrylamide-gel electrophoresis, also found differences between varieties of *Glycine max*. West and Garber (1967) used starch-gel electrophoresis to survey the esterases and leucine-amino peptidases in crude extracts from cotyledons of germinating seedlings of 15 species of *Phaseolus*. They found differences in enzymes between species and also within species. Therefore, esterase and leucine-amino-peptidase zymograms seem of taxonomic value if the range of sites of activity are determined.

Differences of banding patterns in different developmental stages of the same variety indicate a correlation between stage of development and enzymes present. The results obtained with Ford soybeans were most conclusive in this respect. Seeds from plantings Ford 3 and 4 lacked band 6. Band 6 appeared as the seeds matured.

These zymograms cannot be evaluated quantitatively because the exact weight of enzymes placed on the filter paper squares was not determined. Even if equal weights of material had been used, the percentages of enzymes would not be constant. Immature seeds have a smaller proportion of carbohydrates and, thus, a larger percentage of enzymes. Enzyme concentrations are therefore less in mature seeds where more carbohydrates appear. Some quantitative inferences can be drawn, however. Examining the zymogram of the

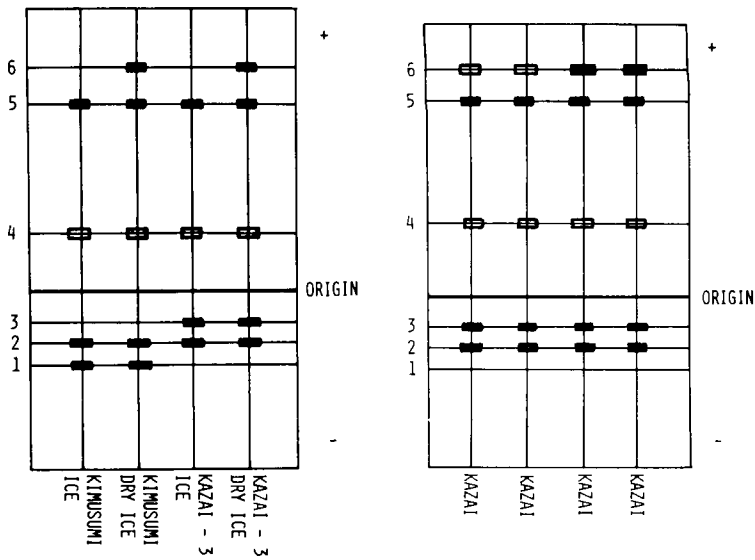

Figure 4. Location of sites of esterase activity in zymogram of Kazai-3 and Kimusume-3 collected in ice and dry ice.

Figure 5. Location of sites of esterase activity in zymogram of four developmental stages of Kazai seeds. Shaded bars represent dark bands and unshaded bars, light bands. Kazai-4 is the youngest seed.

four different maturity samples of Ford, one observes that band 5 on Ford 3 and Ford 4 is very dark, while band 6 is missing (Fig. 3). This supports the assertion that band 6 of Ford 3 and Ford 4 was absent because pod enzyme concentration (as seen in band 5) was clearly sufficient to be recorded. Band 6 of zymograms of Kazai 3 and 4 and Kimusume 3 and 4 was always faint, even when band 5 was dark (Figs. 5, 6). The esterase associated with band 6 appears to have been in lower concentrations in the young seeds.

The differences between the collection in regular ice and dry ice in the appearance of band 6 in the young Kazai and Kimusume pods suggests that this esterase is either unstable or in such small amounts that physiological changes under regular ice conditions obscure its appearance. Its strong appearance in the older seed supports the latter possibility.

Using disc electrophoresis, Meisinger (1967) found esterase banding patterns to differ for different maturities of seeds of the same variety of *Glycine max*. He found that certain bands appeared as seeds matured while others disappeared. In maize, specific alleles were found development dependent. Schwartz (1962) observed that 14-day endosperm of one of the F alleles of the 7.5 esterase pos-

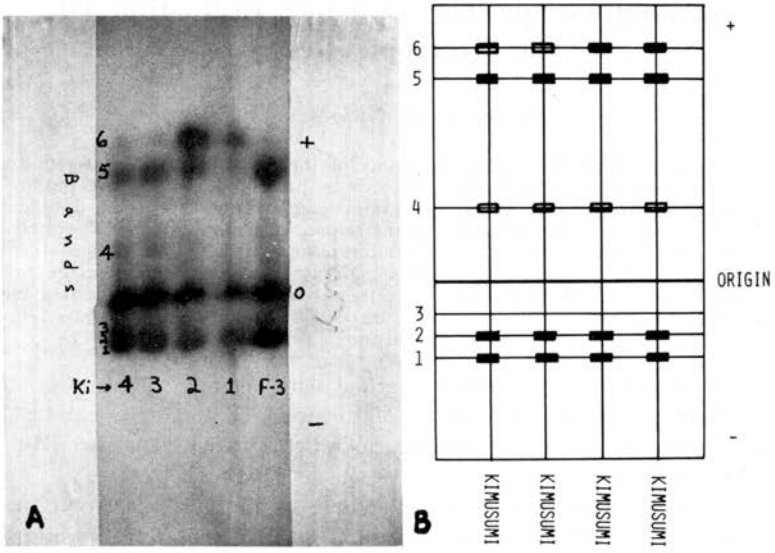

Figure 6. A. Location of sites of esterase activity in zymogram of four developmental stages of Kimusume soybeans. Kimusume-4 is the youngest, while Kimusume-1 is the oldest. Ford-3, on the right is inserted as control (O—origin). B. Diagramatic representation of Kimusume 1-4 in A. Shaded bars represent dark bands and unshaded bars, light bands.

sessed an esterase band that disappeared in the zymograms of 19-day endosperm. Snyder and Kleese (1968) found developmental differences in soluble root proteins in 5-, 7-, 9-, and 15-day-old soybean plants.

Steward, Lyndon and Barber (1965) divided 3-day-old pea-seedling roots into six successive 1-mm segments starting at the tip, and a sample also was taken from the remaining 3-4 cm of the root. Zymogram differences among such samples indicated developmental changes in soluble root proteins.

This study with the developing pods of soybeans represents additional support for the concept that new gene products appear during the development of specific tissue. The genetics of the inheritance of control of esterases is currently under investigation.

Literature Cited

Hunter, R. L. & Market, C. I. 1957. *Science* **125**:1294-1295.
Scandalios, J. G. 1969. *Biochem. Genetics* **3**:37-79.
Schwartz, D. 1962. *Genetics* **47**:1609-1615.
Smithies, O. 1955. *Biochem. J.* **61**:629-641.
Snyder, J. R., & Kleese, R. A. 1968. *J. Heredity* **59**:380-382.
Steward, F. C., Lyndon, R. F., & Barber, J. T. 1965. *Amer. J. Bot.* **52**: 155-164.
West, N. B., & Garber, E. D. 1967. *Canadian J. Gen. Cyt.* **9**:646-655.