Isozyme Specificity During Germination and Early Growth of Knobcone Pine

M. THOMPSON CONKLE

Made in the United States of America
Reprinted from FOREST SCIENCE
Vol. 17, No. 4, December 1971
pp. 494–498

Isozyme Specificity During Germination and Early Growth of Knobcone Pine

M. THOMPSON CONKLE

Abstract. Five enzyme classes from 11 developmental stages of germinating embryos were separated by starch gel electrophoresis. Alcohol dehydrogenase isozymes found in embryos of dry seed were most active at the time of radicle emergence; activity decreased thereafter, fading below the level of detection when seed coats were shed. Peroxidase isozymes were absent and esterase isozymes were nearly absent from stratified seed, but both increased steadily in number and stainability from time of radicle emergence through epicotyl elongation. Leucine aminopeptidase and catalase isozymes were detectable in all growth stages. In later stages, esterases and leucine aminopeptidases were found primarily in seedling tops, and many catalase and peroxidase isozymes differed between roots and tops. Both the developmental stage of the plant and the tissue assayed determine the presence of specific isozymes. ***Forest Sci. 17: 494–498.***

Additional key words. *Pinus attenuata* Lemm., seed, alcohol dehydrogenase, leucine aminopeptidase, esterase, peroxidase, catalase.

THE GENETIC analysis of isozymes depends on identifying developmental stages and plant parts with high enzyme activity. Currently held hypotheses suggest that enzyme activity arises from *de novo* enzyme synthesis, with activity being lost through enzyme decay (Filner, Wray, and Varner 1969). Scandalios' review (1969) of studies of several higher plants indicates that enzyme patterns change during the development of an organism. Isozyme bands from particular organs shift during development, resulting in patterns specific for the particular organ.

To characterize pine enzymes, a study of knobcone pine (*Pinus attenuata* Lemm.) seed and seedlings was undertaken. Seeds were germinated, and the enzymes from young plants representing growth stages to that of fully expanded cotyledons were examined by the technique of starch gel electrophoresis. The liquid fraction from crushed embryos and seedlings was examined for alcohol dehydrogenase (ADH), leucine aminopeptidase (LAP), esterase (E), peroxidase (P), and catalase (C) activity.

Materials and Methods

Preparation of plant material. Knobcone pine seed from several different two-parent crosses (1967 seed crop) were stratified at 3° C on moist filter paper for 30 days. After stratification the seeds were germinated in the laboratory. A portion was placed on filter paper on glass slants and watered with distilled water. Seed and seedling material from a wide range of growth stages was available for analysis.

These 11 developmental stages were analyzed:

1. Dry seed.
2. Seed stratified.
3. Seed germinating: seed coat cracked but radicle not visible.
4. Radicle exposed about 1 mm beyond the seed coat.
5. Radicle elongating but hypocotyl not visible.
6. Hypocotyl exposed about 1 mm beyond the seed coat.

The author is Geneticist, Pacific Southwest Forest and Range Exp. Sta., Forest Service, U. S. Dep. Agr., Berkeley, Calif. Manuscript received Jan. 28, 1971.

7. Hypocotyl elongating but cotyledons not visible.
8. Cotyledons exposed about 1 mm beyond the seed coat.
9. Cotyledons extending, seed coat attached.
10. Seed coat just shed.
11. Cotyledons horizontal and fully extended; epicotyl visible.

An embryo was separated from its seed coat and megagametophyte, crushed, and the liquid fraction picked up on filter paper wicks. Seedlings in stages 7 through 11 were separated into roots and tops. Tops included cotyledons and epicotyl; hypocotyls were discarded. Roots and tops were crushed separately and the liquid absorbed on paper wicks. A minimum of 20 plants were analyzed in each of the 11 growth stages.

Electrophoresis. Enzymes were separated by starch gel electrophoresis. Gel preparation followed methods described by Kristjansson (1963). Buffer formulas reported by Scandalios (1969) were used. Gel slabs consisting of 12 percent hydrolyzed potato starch were buffered with a tris-citric acid solution at pH 8.3. Each gel, 13 cm wide, accommodated 20 wicks. A solution of lithium hydroxide-boric acid at pH 8.3 served as the electrode buffer. Horizontal gels were supplied 400 V- DC at 100 mA. Wicks were inserted in gel slabs 5 cm from the cathode end. This location was defined as the point of origin. Electric current was applied for two hours until the visible migrating front reached 8 cm beyond the origin.

Electrophoresis of the gels was carried out under refrigeration at about 5° C. Wicks were removed after the first 12 minutes.

Gel slabs were cut horizontally into five slices. Each slice was stained for a specific type of enzyme with the stain formulas of Scandalios (1969). Using both the original gels and photos, I compiled a series of zymograms showing the presence, the relative migration distance, and the intensity of staining of various bands for each enzyme type.

Results

Significant changes related to seedling growth stage and tissue sampled took place in each of the five classes of enzymes studied. Isozymes could be classified by their appearance or loss as plant development progressed (Fig. 1).

Alcohol dehydrogenase. ADH isozymes assayable in the dry seed stained most darkly at growth stage No. 4. Bands persisted in some seedlings up to seed coat drop (stage 10), but no ADH activity could be detected at later stages. Though stainability decreased with growth, no tissue differences were detectable between roots and tops in stages 7, 8, and 9. Both plant parts produced faint bands on the zymograms.

Leucine aminopeptidase. LAP isozymes were the most consistent of the five classes of enzymes assayed. In embryos from dry seed LAP activity produced moderately staining bands on the gel. The same strongly staining bands were produced throughout the remaining growth stages. LAP activity decreased in the roots; and in stage 11, fell below the activity level needed for detection.

Esterase. E isozymes were nearly absent from embryos in early germination stages, but they increased in number and stainability as growth progressed. E bands outnumbered ADH and LAP bands. Like that of LAP isozymes, E activity was much greater in tops than in roots in the later stages. E activity in roots could not be detected in stages 10 and 11. In contrast, seedling tops in stages 10 and 11 were judged to be the most desirable for E analysis.

Peroxidase. P bands, like E bands, increased in number and stainability as germination progressed. But unlike other enzymes studied, numerous darkly staining isozymes migrated toward the cathode. The preponderance of anodally migrating isozymes was from roots. The most advanced germination stages are preferred for the genetic analysis of P isozymes.

Catalase. Some C isozymes were lost as development progressed and others appeared. A pair of bands close to the origin

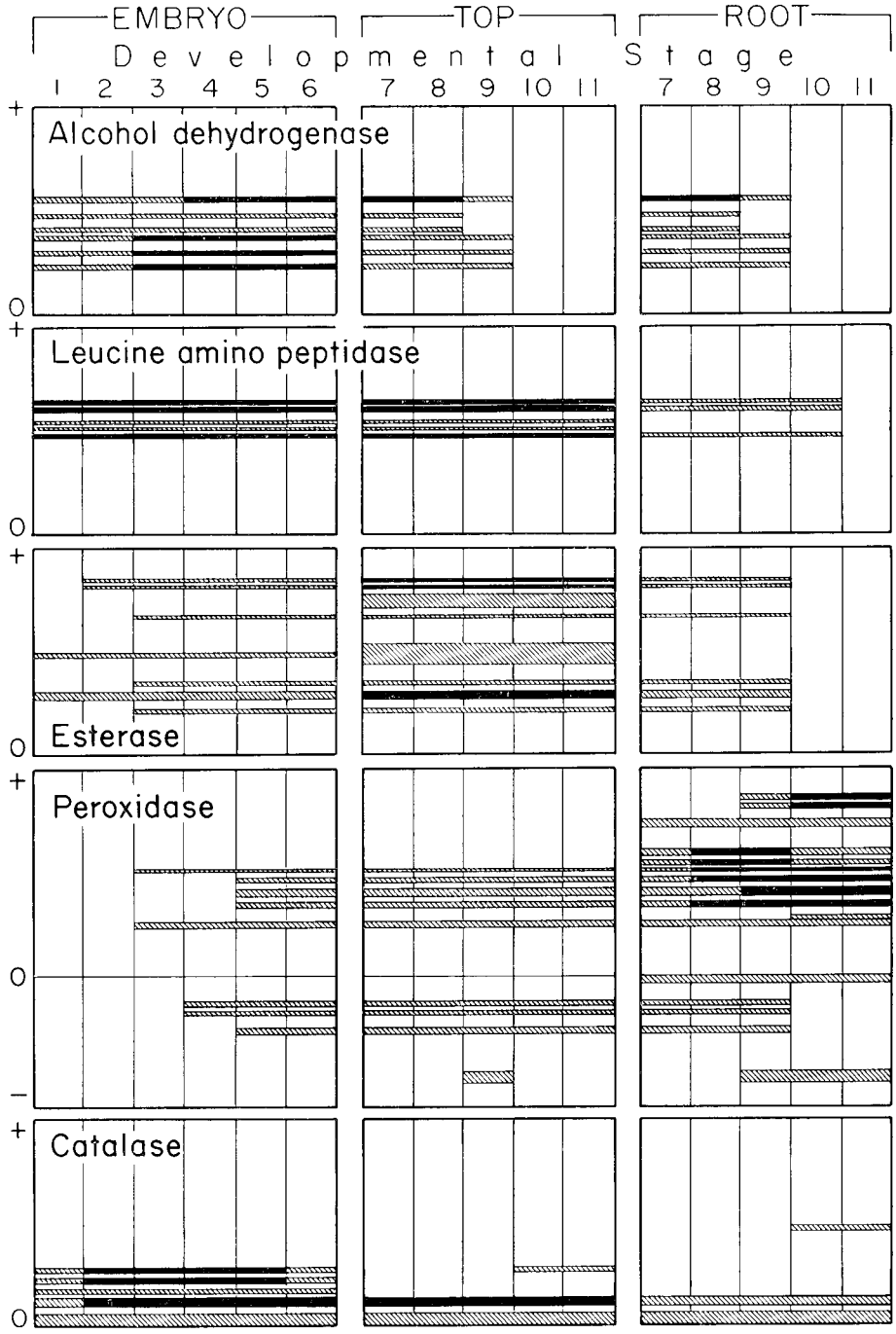

FIGURE 1. *Zymograms for knobcone pine plants in differing stages of germination. See text for a definition of the 11 developmental stages. Plant material was whole embryos for stages 1 through 6, roots and tops for stages 7 through 11. O = origin; + = anode; and − = cathode. The + location corresponds to the 8 cm front, and band positions accurately reflect Rf values. Dark shading indicates dark staining bands, cross hatch shading connotes less than intense staining.*

was consistently present throughout germination and was confined to plant tops in later stages.

Discussion

Knobcone pine embryos and seedlings undergo significant enzymatic changes during germination. The presence of specific isozymes depends on germination stage and tissue sampled. The sequence of enzyme appearance suggests that plant developmental stages are determined by or closely correlated with enzyme changes. Alcohol dehydrogenase, present in the dry seed, reached a peak activity when the radicle was just visible beyond the seed coat. Isozyme bands faded as germination progressed, and all bands were lost by the time cotyledons were fully expanded. These results agree with findings from other studies (Bartels 1960). Barnett and Naylor (1969) reported changes in alcohol dehydrogenase activity in germinating longleaf (*Pinus palustris* Mill.) and slash (*P. elliottii* Engelm.) pine seeds. They found highest activity in embryos at germination. Enzyme activity decreased as the radicle elongated, declining to near zero as the epicotyl developed.

Leucine aminopeptidase was a persistent enzyme during most growth stages. It was detected in all stages from dry seed through epicotyl enlargement. LAP activity faded in roots at the growth stage when the cotyledons began to extend. The persistence of pine LAP during germination agrees with findings for corn (Scandalios 1969).

Both esterases and peroxidases as groups reacted similarly during pine germination. Bands were absent or only weakly detectable in dry seeds. As germination progressed bands appeared and became more darkly stained. Some bands were lost. Differences in isozyme bands become associated with specific organs. Roots and tops have different banding patterns. Similar developmental patterns for E and P have been found in corn (Scandalios 1969, Hamill and Brewbaker 1969), soybeans (Brim, Usanis, and Tester 1969), and barley (Upadhya and Yee 1968).

Pine catalase bands were persistent during early stages of germination, but became organ specific as development progressed. Scandalios (1969) reported similar findings for corn.

The regular sequence of isozyme development sets bounds for the genetic analyses of specific isozymes. A two-stage sampling procedure maximizes the number of isozymes that can be observed in this pine material. Stratified seed should be germinated to the stage where the radicle begins to emerge from the seed coat (stage 4). This stage is desirable for detection of ADH, LAP, and C isozymes. A portion of the plant material should be grown to the stage when cotyledons are fully expanded and epicotyl growth is not yet appreciable (stage 10). This is a prime stage for E and P isozyme detection. The isozymes of both E and P are specific to different plant parts, and the analysis benefits from a separation of tops and roots.

Each isozyme band consists of proteins that are coded for by nuclear genes (DNA). Pine embryos and seedlings, being diploid, produce some bands that are allelic products from heterozygous loci (Conkle 1971). The three slower-migrating ADH bands are known to be polymorphic products from a single locus. The two fast-migrating LAP bands and the two adjacent slower-migrating LAP bands are known to be polymorphic products from two independent loci.

The relation between nuclear DNA and isozymes provides a powerful tool for resolving genotypes. The suggested sampling procedure resolves 6 isozyme bands for ADH, 5 for LAP, eight for E, 16 for P, and 6 for C. This totals 41 individual bands for the test material, despite its restricted parentage. Widening the genetic base should increase the number of bands within the differing enzyme classes. The importance of this genotypic resolution will be obvious to forest geneticists and physiologists who have had a paucity of genetic markers in their plant material.

Literature Cited

BARNETT, J. P., and A. W. NAYLOR. 1969. Alcohol dehydrogenase activity and ethanol utilization in germinating longleaf and slash pine seeds. Forest Sci 15:400–403.

BARTELS, H. 1960. Enzymaktivitäten in Ruhenden und Keinsenden Samen von *Pinus nigra* Arn. Planta 55:573–597.

BRIM, C. A., S. A. USANIS, and C. F. TESTER. 1969. Organ specificity and genotypic differences in isoperoxidases of soybeans. Crop Sci 9:843–845.

CONKLE, M. T. 1971. Inheritance of alcohol dehydrogenase and leucine aminopeptidase isozymes in knobcone pine. Forest Sci 17:190–194.

FILNER, P., J. L. WRAY, and J. E. VARNER. 1969. Enzyme induction in higher plants. Science 165:358–367.

HAMILL, D. E., and J. L. BREWBAKER. 1969. Isoenzyme polymorphism in flowering plants. IV. The peroxidase isoenzymes of maize (*Zea mays*). Physiol Plantarum 22:945–958.

KRISTJANSSON, F. K. 1963. Genetic control of two pre-albumens in pigs. Genetics 48:1059–1063.

SCANDALIOS, J. G. 1969. Genetic control of multiple molecular forms of enzymes in plants: A review. Biochem Genetics 3:37–79.

UPADHYA, M. D., and J. YEE. 1968. Isoenzyme polymorphism in flowering plants. VII. Isoenzyme variations in tissues of barley seedling. Phytochem 7:937–943.