ISOZYME VARIATION IN SEVERAL SPECIES OF OAKS

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ABSTRACT

This investigation analyzed the isozyme patterns from bud and acorn cotyledon tissues of one or more seed sources of northern red oak, black oak, white oak, swamp white oak, bur oak and chinkapin oak. Isozyme patterns were determined for leucine aminopeptidase, alcohol dehydrogenase, acid phosphatase and glutamic-oxalacetic transaminase.

Most isozyme bands were found to be common to two or more of the species investigated. This isozyme similarity appears to reflect their close phylogentic relationship. However, the individuals of some species exhibited distinctive phenotypes and they could be readily distinguised from other species.

INTRODUCTION

It is well known that the oaks comprise a taxonomically complex and often confusing group of species in the genus Quercus. There are over 70 species native to the United States and over 50 reach tree size (Sudworth 1927). Although some species possess unique and easily recognized morphological and anatomical traits, many others are difficult to differentiate because of the plasticity of many morphological features. Taxonomy is further complicated by apparent hybridization among many species and over 60 hybrids have been reported (Fernald 1950).

Quantitative biochemical markers, such as isozymes, have recently been utilizedin oaks to distinguish between species and also geographic seed sources within a species. Olsson (1975) recovered 11 peroxidase isozymes from leaves of Q. petraea and Q. robur in Sweden. Specific isozyme patterns were associated with each species and some population of Q. petraea showed evidence of introgression with Q. robur. Houston (1978) found that each of six different geographic seed sources of Q. rubra could be distinguished on the basis of leaf peroxidases.

The purpose of this investigation was to determine the suitability of different tissues for the isolation and recovery of oak enzymes and secondly, to determine if specific isozyme band patterns were present that could be utilized as markers to distinguish between species.

Six species were included in this survey, four of which are representatives of the subgenus Leucobalanus. These are: white oak, Q. alba; swamp white oak, Q. bicolor; bur oak, Q. macrocarpa and Chinkapin oak,Q. muehlenbergii. Also investigated were two species of the subgenus Erythrobalanus, which included black oak, Q. velutina and northern red oak, Q. rubra.

Isozyme patterns were determined from buds and acorn cotyledons by starch gel electrophoresis for leucine aminopeptidase, alcohol dehydrogenase, acid phosphate, and glutamic-oxalacetic transaminase.

METHODS AND MATERIALS

Acorns and/or branches with buds were collected from four to 16 trees of each species. Buds and acorns of all species were collected near Fort Wayne, Indiana, and several black and white oaks acorns were also collected near South Bend, Indiana. From two to four branches of several red oak seed sources were collected from Michigan State University's W.K. Kellogg Forest near Agusta, Michigan. Branches were stored in plastic bags in the refrigerator. Black and red oak acorns were stratified in moist sand at 5°C at least 60 days prior to germination. Acorns of the white subgroup were either germinated immediately or stored in moist sand at 5°C. Whenever possible cotyledons of germinated acorns were selected for analysis; however, in some cases, non-germinated red and black oak acorns were used.

From 0.8 to 1.5 grams of cotyledon or two to four buds, with scales removed, were ground by hand with two to five drops of homogenizing buffer and 0.3 to 1.0 grams of insoluble polyvinylpolypyrrolidone. The homogenizing buffer was 0.1 M tris-HCl, pH 8.0, containing: 6 mM ascorbate, 6 mM cysteine, 0.5 M sucrose and 1% V/V tween 80. The macerated plant fluids were absorbed on thick paper wicks and subjected to starch gel electrophoresis. The apparatus used was similar to that described by Conkle (1972). The gel and electrode buffers, composed of lithium borate and tris-citrate, are described by Scandalious (1969). Each gel was cut into four slices prior to staining.

Gels were stained for leucine aminopeptidase (LAP) , acid phosphatase (ACP), and alcohol dehydrogenase (ADH), as described by Scandalious (1969). Glutamate-oxalacetate transaminase (GOT) was stained according to procedures described by Brewbaker et al (1968). Diagrams were made of each gel and band positions are expressed as relative mobility; RM values are calculated as the distance the band migrated from the origin, divided by the distance the lithium borate front had moved from the origin toward the anode.

RESULTS

The physiological status of the cytoledon and bud tissue was found to vary from tree to tree resulting in variable isozyme resolution. The buds of some trees regularly produced moderate to dark stained-bands while other individuals were light-stained or lacked enzyme activity altogether. Extracts from cotyledons generally produced darker bands and more variable phenotypes as expected from open pollincated seed. In most samples identical isozyme mobilities were recovered in bud and cotyledon tissues.

Alcohol Dehydrogenase

Acorn cotyledon tissue generally produced much greater ADH activity and gels were stained in one to two hours. Overnight staining was necessary to resolve the isozymes of bud tissue and about 20 percent of the sample were not legible. Examination of the ADH phenotypes in Figure 1, show that the principle phenotype recovered in white and swamp white oak was a single band at RM .36. This phenotype was also recovered in a few chinkapin and bur oaks. The rest of the white and swamp oak phenotypes were distinctive for these species since they were not found in other members of the subgroup. In chinkapin oak RM .36 was usually associated with a slower band at RM .34 and in some trees also a third band at RM .38. These phenotypes appear to be unique to chinkapin oak. In most bur oaks a distinctive double banded phenotype at RM .40 and .36 was recovered.

All black and and almost one-half of the red oaks exhibited a single band at RM .40. Although this phenotype distinguishes the red oak subgroup from the white oaks, it does not discriminate between red and black oaks. The remaining double and triple banded phenotypes of red oak appear to be characteristic for this specie.

Three phenotypes were recovered from a survey of several red oak seed sources. Trees from Iowa and Missouri exhibited a single fast band at RM .40; a Minnesota source was double banded at RM .40 and .38 and a slower-double banded phenotype at RM .38 and .36 was recovered from New York, Michigan, Tennessee, and Maine seed sources. One of the New York trees was also found to contain this slower double-banded phenotype.

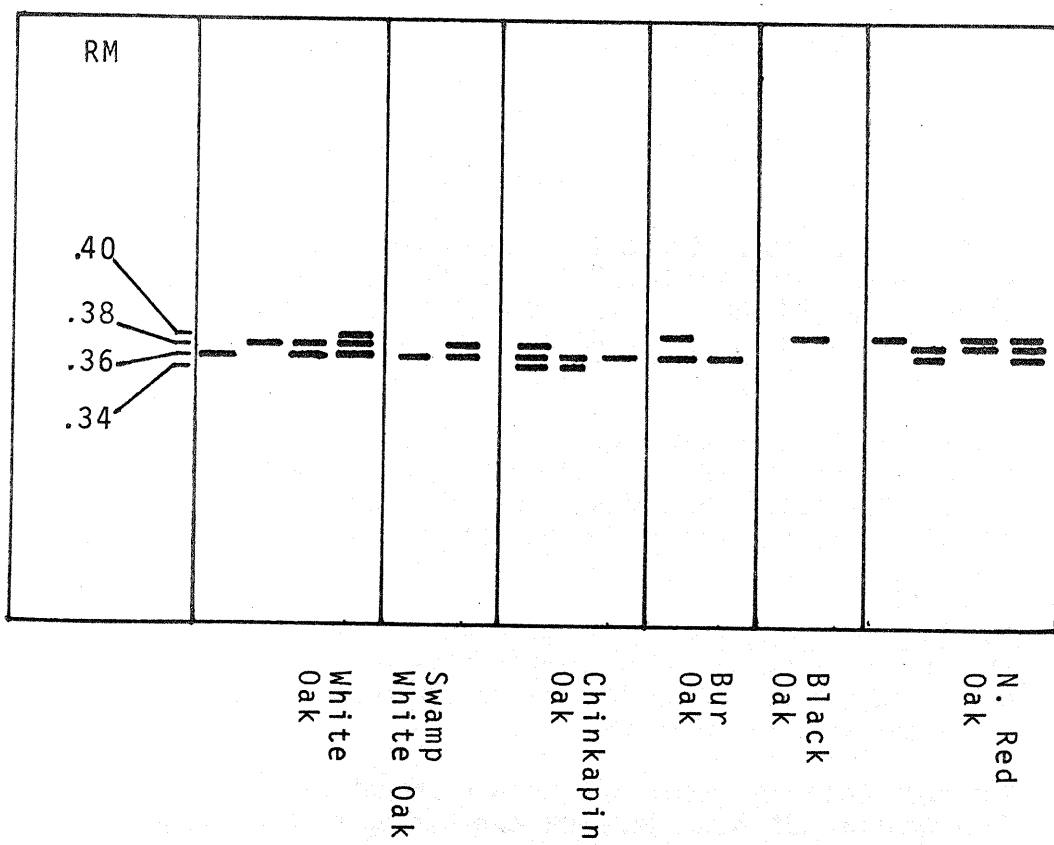

Figure 1. Comparison of Alcohol Dehydrogenase Phenotypes Recovered from Bud and Cotyledon Tissues of Several Species of Oaks.

In summary, ADH phenotypes may be of limited value as genetic markers since the species within each subgroup share several identical bands and have one or more phenotypes in common. However, several phenotypes may be species specific, in particular, those exhibiting the slow band recovered only in chinkapin oak.

Leucine Aminopeptidase

Two zones of activity were recovered for LAP isozymes. The upper zone exhibited dark-stained bands of six different mobilities. However, these bands are difficult to differentiate because they are often 1 to 2 mm wide and only 1 mm or less apart (Figure 2). Approximately 20 percent of the phenotypes in the upper zone could not be determined because of their small differences in mobility. In the lower zone 3 band mobilities were observed which were generally light-stained. Buds and cotyledons stained equally well and produced bands of identical mobilities.

In the faster zone, the most common phenotype of white oak was a single band at RM .50. This phenotype and the double banded type (RM .50 and .48) were also recovered in bur oak. The third white oak band at RM .48 was also observed in chinkapin oak.

The bands of swamp white oak and chinkapin oak exhibit identical mobilities and 2 of their 4 phenotypes were common to both species. The presence of the slowest band, at RM .46, distinguishes these species from white oak and bur oak.

In the lower zone the slower band at RM .38 was recovered in bur, swamp white and chinkapin oaks but not in white oak. One bur oak exhibited the faster band at RM .40 characteristic of white oak.

The most commonly recovered red oak phenotype was a single fast band at RM .49. Red oaks exhibiting this phenotype can be distinguished from black oaks which form slower bands at RM .48 and .46. The band at RM .46 was also recovered in red oak. In the lower zone the majority of trees exhibited a band at RM .41 which was characteristic of the red oak subgroup.

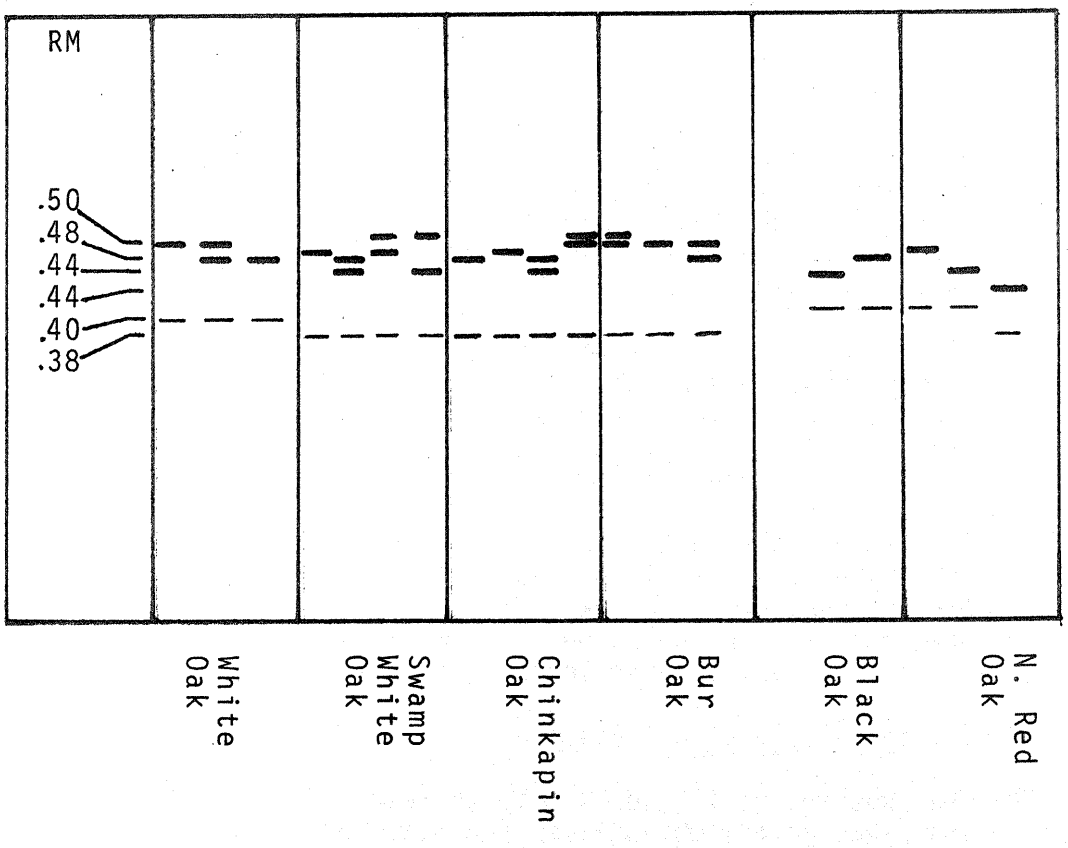

Figure 2. Comparison of Leucine Aminopeptidase Phenotypes Recovered from Bud Tissue of Several Species of Oaks.

LAP isozymes may be useful in distinguishing between some geographic seed sources of red oak. Trees exhibiting the fast band were recovered from Iowa, Michigan Tennessee, Maine, Missouri, and Minnesota sources; the intermediate band from Missouri and New York sources and the slowest band was recovered from a single tree of a New York seed source.

Acid Phosphatase

Acid phosphatase isozymes were recovered from both bud and cotyledon tissues. The latter tissue generally produced much darker stained bands. Each species produced 1 to 5 major bands in the lower portion of the gel and 2 to 4 light-stained bands above (Figure 3). For the major bands, identical mobilities were recovered from both tissues.

All white, swamp white oak and a large portion of bur oaks exhibited a single-banded phenotype at RM .29. Swamp white oak and bur oak also displayed two identical double-banded phenotypes. All chinkapin oak phenotypes, in this study, exhibited a slow band at RM .24. This band can be used to distinguish chinkapin oak from white and swamp white oaks but not from bur oak. Several phenotypes were found to be common to both bur and chinkapin oaks.

All red and black oaks were found to have identical single banded phenotypes at RM .12 or a double band at RM .12 and .16. On the basis of ACP phenotypes, the red and black oaks are easily distinguished from the white oak subgroup but not from each other.

Thus within the white oak subgroup, chinkapin oak exhibited phenotypes that distinguished it from white and swamp white oaks but not from bur oak. White, bur and swamp white oaks have one or more phenotypes in common and therefore cannot be totally separated on the basis of ACP isozymes.

Glutamic-Oxalacetic Transaminase

The isozymes of GOT could only be resolved from bud tissue. The bands were light-stained and some samples were incompletely resolved. The bands from cotyledons were smeared and impossible to differentiate.

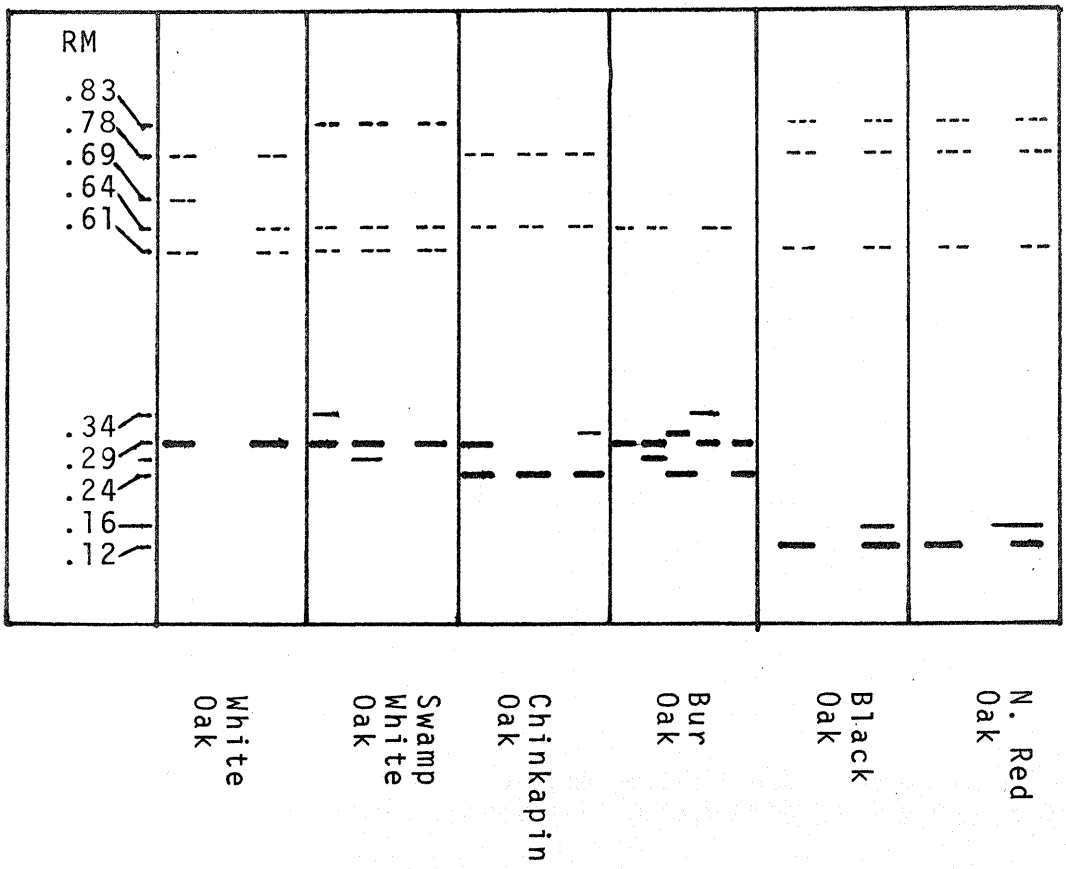

Figure 3. Comparison of Acid Phosphatase Phenotypes Recovered from Bud and Cotyledon Tissues of Several Species of Oaks.

The most common mobility observed was a band at RM .28 (Figure 4). All species except swamp white oak contained this band. A fast band at RM .34 was also common to white, swamp white, and bur oaks but not recovered in chinkapin, black or red oaks. A unique slower triple-banded phenotype was recovered in one chinkapin oak which makes this phenotype readily distinguishable from all other species. Compared to the white oak subgroup, red and black oaks were less variable exhibiting a single band at RM .28.

GOT appears to have little diagnostic value because of its low variability. Only those chinkapin oaks which possess the slow band at RM .24 are readily distinguishable from the other species.

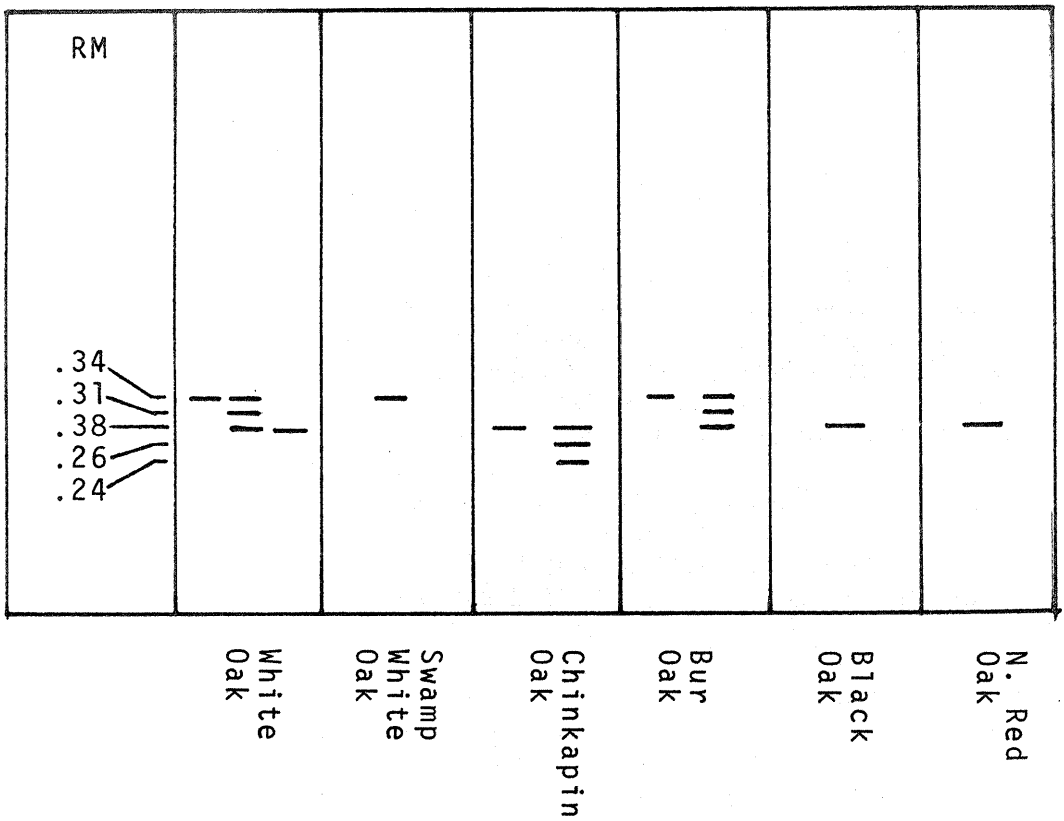

Figure 4. Comparison of Glutamate-Oxalacetic Transaminase Phenotypes Recovered from Bud Tissue of Several Species of Oaks.

DISCUSSION

Seed cotyledon tissue generally produced good quality isozyme bands except for GOT. The phenotypes were more variable than bud tissue, perhaps as a result of crossing with several male parents. The isozyme bands of bud tissue were lighter and some samples lacked bands altogether. However, there are two advantages in using vegetative tissues such as buds: (1) the morphology and isozyme phenotype of a single individual can be compared; (2) it is available on trees of all ages throughout much of the year. A combined analysis of these tissues may prove useful in advancing our understanding of pouplation structure, gene flow and genetic introgression in the oaks.

Many of the isozyme bands and phenotypes observed in this study were common to two or more species. This isozyme similarity appears to reflect their close phylogentic relationship. The genetic similarity of the oaks is demonstrated in part by their ability to hybridize. Bur oak has been reported to hybridize with white and swamp white oaks and apparently white oak hybridizes with swamp white and chinkapin oaks. Black and red oaks have also been reported to hybridize (Fernald 1950). This compatability may explain in part the larger variability of seed phenotypes as compared to bud phenotypes recovered in this study.

The isozymes investigated appear to be much less variable than peroxidases studied by Olsson (1975) and Houston (1978). However, other phenotypes undoubtedly exist but were not recovered because of the limited sample size.

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