

Electrophoretic Identification of Loblolly Pine-Shortleaf Pine Hybrids

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Abstract

Starch-gel electrophoresis was used to screen loblolly pine and shortleaf pine trees for isoenzymes that would be useful species markers when putative hybrids of the two species are tested for hybrid confirmation. IDH was found to be a monomorphic, monomeric enzyme in both species and differed between species only by migration distance. The apparent species' markers were tested on known loblolly-shortleaf hybrids and found to be accurate and reliable for identifying hybrid trees. Putative hybrid trees previously selected on the basis of cone and needle morphology were then tested with the system to assay their true genetic nature.

Key words: Isocitric dehydrogenase, fusiform rust, littleleaf disease.

Areas where the range of loblolly pine (*Pinus taeda* L.) and shortleaf pine (*Pinus echinata* MILL.) overlap often contain trees which have the physical appearance of hybrids (HARE and SWITZER, 1969; ZOBEL, 1953). Cone characteristics and needle lengths of these putative hybrids are typically intermediate of both parents' traits (LITTLE and RICHTER, 1965) and they are often the only vigorously growing trees in stands that contain some form of disease. Upper Piedmont sites with heavy clay soils often contain loblolly pine stands that are severely affected with fusiform rust (*Cronartium fusiforme*). In areas where loblolly pine grows in conjunction with normally fusiform resistant shortleaf pine, it is possible that the apparently "resistant" loblolly pine trees are actually hybrids (FLORENCE, 1973; HENRY and BERCAW, 1956). Likewise, shortleaf stands that are suffering from littleleaf disease (*Phytophthora cinnamomi*) and are growing in the vicinity of loblolly pine trees often contain apparently healthy shortleaf pine trees which may also be hybrids.

The true genetic nature of these putative hybrids has been investigated by measuring several morphological features such as needle length and cone size (SCHOENIKE, personal communication). Often the putative hybrid trees are found to be intermediate of loblolly pine and shortleaf pine but the genotypic variability of both of the parent species is often compounded by environmental factors such as disease stress which results in such a large degree of phenotypic variability that positive classification is all but impossible.

Isoenzyme analysis offers a potential identification technique that could be used with a high degree of accuracy if species marker genes are available. This has been demonstrated in larch (*Larix* spp.) with the shikimate dehydrogenase (SKDH) isoenzymes (BERGMANN and RUETZ, 1987). If a reliable marker is found in each species' megagametophytes, the first generation hybrids will be identifiable by their production of seeds whose megagametophytes have a 50:50 ratio of parental markers. The purpose of this study was to survey a group of enzyme systems in both

loblolly pine and shortleaf pine and to try to identify a usable marker in each species.

Methods

Twenty shortleaf pine trees and 29 loblolly pine trees were selected for use as a base population. Seeds were collected from trees growing in apparently pure species stands in the Georgia Piedmont, the South Carolina Piedmont, and the South Carolina Coastal Plain. Seeds were also collected from 24 artificially produced loblolly-shortleaf hybrid trees and 8 putative hybrid trees. The artificial hybrid seed was provided to us by the U.S. Forest Service from a hybrid study planted near Dry Branch, Georgia. The putative hybrid trees were growing in littleleaf infected stands of shortleaf pine in the vicinity of one of the "pure" parental shortleaf stands near Clemson, South Carolina. They were designated "putative hybrids" based on needle length and cone size as part of a littleleaf disease resistance study (R. E. SCHOENIKE, personal communication).

Seeds were soaked for three hours at room temperature in a 3% hydrogen peroxide solution, then soaked for three days in 40° F. distilled water, and then placed on moist paper towels to stratify at 40° F. for 28 days. After stratification, the seeds were germinated under artificial light at 72° F. Immediately upon radicle extension, the seeds were placed on moist filter paper in a covered culture dish and stored at 40° F. until they were processed for electrophoresis.

Ten germinated seeds from each tree were used in the analysis. A sample size of ten seeds was used because the probability of randomly selecting ten homozygous megagametophytes from a heterozygous individual is $< .002$. Excised megagametophytes were placed in a few drops of .2M phosphate buffer and ground into a paste with a round-tipped glass rod. Individual seed extracts were drawn onto small paper wicks and then inserted into starch gel slabs (per CONKLE *et al.*, 1982). Each seed's extract was tested on CONKLE'S A, B, and D systems which allowed for a survey of twenty enzyme systems.

Results and Discussion

Only isocitric dehydrogenase, (IDH), assayed on CONKLE'S D system, proved to be a useful marker. IDH produced a monomorphic, single band at 17 mm migration distance for all tested shortleaf pine seeds, including those collected from trees growing in the vicinity of the "putative hybrids". A monomorphic, single band at 22 mm migration distance was produced from all tested loblolly pine seeds (Fig. 1).

The existence of such an apparently simple species marker allowed for easy identification of first generation hybrids. All of the known artificially produced hybrid test seeds contained both parental bands, as expected, and the

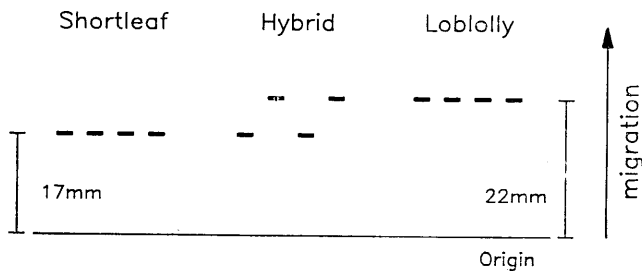


Figure 1. — Illustration of IDH banding patterns in four megagametophytes from a shortleaf pine tree, a hybrid pine tree and a loblolly pine tree.

pooled results from all of the known hybrid seed lots produced an exact 50:50 ratio of parental bands. The 8 putative hybrid trees from the littleleaf infected shortleaf stand were classified by this technique as 6 loblolly-shortleaf hybrid trees, 1 loblolly pine tree and 1 shortleaf pine tree.

Processing single seed samples allowed for only 30 seeds per gel slab and limited us to testing only 3 trees per gel. Large testing programs would require a great many gels using this procedure which may result in a prohibitively high cost in terms of time or money. If a simple, single-banded, monomeric enzyme system, such as IDH, is being used it may be feasible to pool all ten megagametophytes from a single test tree and to use the pooled material as a single tissue sample. This pooling should result in a single-banded sample if the tree is homozygous for either parental band and a double-banded sample if the tree is a hybrid. The ratio of the two parental isoenzyme types in the ten seed sample will determine the relative darkness of the two bands on the gel. We tested this pooling technique on seeds from our known hybrid trees with complete success. All of the tested known hybrid trees produced two distinguishable bands. We then tested different mixtures of the two parent species megagametophytes to determine if both isoenzyme types would still be identifiable when the parent ratio was as high as 9:1. We found IDH activity to be sufficiently strong in the two tested pine species to produce both band types even when the ratios were skewed to this maximum point for a ten seed sample.

Electrophoretic assaying of IDH isoenzymes should be highly accurate for identifying first generation loblolly-shortleaf hybrid trees. However, identification of hybrid trees of more advanced generations may not be as reliable. It is reasonable to assume that natural back-crossing will occur between the first generation hybrids and one of the two parent species. Mendel's principle of segregation would allow for only one half of the progeny to contain both of the IDH bands if the segregation is unrestricted. Likewise, progeny produced as a result of crossing between two

first generation hybrid trees should be 50% heterozygous and 25% homozygous for each of the two parent types. Deviations from these expected segregation ratios is possible if the IDH gene is linked with a trait that increase the probability of individual tree survival in areas where either fusiform rust disease or littleleaf disease is a problem.

When this test is applied to new situations, a large portion of the local parental populations of both species should be tested for IDH homozygosity to minimize the probability of missing a low frequency IDH allele which may cause misclassification problems of both parent trees and hybrids. The possibility of misclassification must be considered even if no low frequency alleles are found. The probability of such a misclassification will undoubtedly be small, but nonetheless must be taken into account when trees are assigned a "hybrid" designation. Use of this test in conjunction with other forms of hybrid identification such as index calculations should increase the accuracy and reliability of the testing program.

Conclusion

An accurate and precise system for differentiating between first generation loblolly-shortleaf hybrids and the two parent species can be developed with simple starch gel electrophoresis. Trees in the South Carolina and Georgia portions of the two parent species ranges were distinguishable by differing IDH bands when either ten single seed samples or a single ten seed pooled sample was used. Use of this type of biochemical system eliminates many of the problems inherent in a taxonomic classification based on quantitative, morphological traits such as needle length or cone size that are subject to environmental modification.

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Stability of Loblolly Pine Families in the Southeastern U. S.

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Abstract

The response of 43 open-pollinated loblolly pine (*Pinus taeda* L.) families from different first-generation seed orchards in the southeastern U. S. were evaluated for 8-year height, DBH, volume and percent fusiform rust (*Cronartium quercuum* (BERK.) MIYABE ex SHIRAI f. sp. *fusiforme*)