CHLOROPLAST DNA VARIATION IN SHORTLEAF, SLASH, LONGLEAF, AND LOBLOLLY PINES

W. L. Nance[1/], C. D. Nelson[2/], D. B. Wagner[3/], T. Lib[4/], R. N. Patel[5/], and D. R.Govindaraju[6/]

Abstract. -- We have identified a chloroplast DNA polymorphism that separates shortleaf, slash, longleaf, and loblolly pines into three groups. Although little intraspecific variation was evident, the marker was polymorphic in slash pine. This marker may be useful for monitoring interspecific hybridization and introgression in the southern pines.

Keywords: Pinus echinata Mill., Pinus palustris Mill., Pinus elliottii Engelm., Pinus taeda L., **RFLP.**

[1/] Project Leader, Genetics and Pathology of Southern Pine Forest Ecosystems, Southern Forest Experiment Station, Gulfport, Mississippi.

[2/] Research Scientist, Genetics and Pathology of Southern Pine Forest Ecosystems, Southern Forest Experiment Station, Gulfport, Mississippi.

[3/] Assistant Professor, Department of Forestry, University of Kentucky, Lexington, Kentucky.

[4/] Graduate Research Assistant, Department of Forestry, University of Kentucky, Lexington, Kentucky.

[5/] Principle Laboratory Technician, Department of Forestry, University of Kentucky, Lexington, Kentucky.

[6/] Research Associate, Department of Forestry, University of Kentucky, Lexington, Kentucky. Present address: Department of Biology, Case Western Reserve University, Cleveland, Ohio.

INTRODUCTION

Recently, chloroplast DNA (cpDNA) has been added to the list of markers that are useful in the investigation of interspecific hybridization in conifer populations, because cpDNA markers often distinguish congeneric species (Wagner et al. 1987, Szmidt et al. 1988, Govindaraju et al. 1989, Wang and Szmidt 1990). The predominantly paternal mode of inheritance of coniferous chloroplast genomes (Ohba et al. 1971, Neale et al. 1986, Szmidt et al. 1987, Wagner et al. 1987, Chesnoy 1987, Neale and Sederoff 1988, Szmidt et al. 1988, Neale and Sederoff 1989, Neale et al. 1989, Stine et al. 1989, Wagner et al. 1989, Stine and Keathley 1990, Neale et al. 1991) may permit novel insights into the mechanisms and consequences of natural hydridization in conifers. Unfortunately however, the potential applications of cpDNA markers have not been fully explored in the economically important pines of the southeastern USA (but see Neale and Sederoff 1989, Ali et al. 1991).

We are interested in using cpDNA as a marker to study hydridization and possible introgression in the southern pines (for example, in longleaf x loblolly pine) and have recently identified a cpDNA polymorphism in shortleaf, slash, longleaf, and loblolly pines. Here we present survey data for this polymorphism.

MATERIAL AND METHODS

We surveyed 195 individuals from a research plantation of the Southwide Southern Pine Seed Source Study (Wakeley 1953) in Pearl River County, Mississippi. This included 45 individuals from 7 populations of shortleaf pine, 32 individuals from 6 populations of slash pine, 40 individuals from 6 populations of longleaf pine, and 78 individuals from 14 populations of loblolly pine.

The methods for total cellular DNA purification, restriction endonuclease digestion, gel electrophoretic fractionation of restriction fragments, and transfer of restriction fragments to Biotrans membranes (blots) have been described previously (Southern 1975, Murray and Thompson 1980, Wagner et al. 1987, Sambrook et al. 89). Chloroplast DNA BamHI fragments on blots were hybridized with a ^{32}P-labeled restriction fragment cloned from the chloroplast genome of lodgepole pine (Pinus contorta Dougl.), and visualized by autoradiography (Southern 1975, Feinberg and Vogelstein 1983, Lidholm et al. 1988).

RESULTS AND DISCUSSION

The cpDNA polymorphism was nearly monomorphic within shortleaf, longleaf, and loblolly pines (Table 1), and the four unusual individuals in these three species may have resulted from interspecific hybridization. In contrast, four genotypes were detected in slash pine, none of which occurred in any sample from the other three species. Four of the six slash pine populations were polymorphic, with three of these populations containing at least three genotypes.

The polymorphism provided a species-specific marker for slash pine in our samples, while the cpDNA distinctions among shortleaf, longleaf, and loblolly pines were less distinct (Table 1). Nonetheless, since cpDNA variant frequencies appear to

distinguish longleaf from both loblolly and slash pines, it is potentially valuable for use in screening non-grass stage phenotypes in longleaf pine nursery beds. However, the actual utility of cpDNA markers in screening material for longleaf pine breeding programs can be determined only by further testing.

Table 1. Chloroplast DNA variant numbers in four pine species.

Chloroplast DNA varianta	Numbers of each variant			
	Shortleaf	Slash	Longleaf	Loblolly
2.8/10.5	42	0	40	1
2.5/4.0	3	0	0	77
2.5/7.9	0	7	0	0
2.5/10.5	0	22	0	0
2.5/4.0/10.5	0	2	0	0
2.5/4.0/7.9	0		0	0
Total	45	32	40	78

[a]Variants are denoted by the sizes (in kilobase pairs, kbp) of variable restriction fragments. The probe was a 6.4 kbp BamHI fragment, extending in the lodgepole pine chloroplast genome from 16S to psbC/psbD (J. Lidholm, pers. comm; Strauss et al. 1988).

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