Identification of a New Retrotransposable Element in Loblolly Pine

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We initiated a project to locate the genomic position of fusiform rust resistance gene 1 (*Fr1*) in loblolly pine using fluorescent *in situ* hybridization (FISH). Four random amplified polymorphic DNA (RAPD) markers previously found to be tightly linked to *Fr1* were cloned and sequenced, providing a total coverage of about 2 Kb. In order to obtain discernible signal of single-copy sequences using FISH, a minimum of 5 Kb of DNA is required. Therefore, GenomeWalker (Clontech) was used to obtain flanking genome clones and sequences for each of these markers. We were successful in obtaining an additional 2.3 to 3.6 Kb for three of the four markers, totaling 8.7 Kb. Initially, DNA from these three markers was mixed in a single cocktail and used to probe loblolly pine chromosomal spreads. Assuming that each of the markers consisted of single-copy DNA, we expected to observe FISH signals on only a single pair of homologous chromosomes. However, fairly intense FISH signals were observed throughout the entire *Pinus* genome, including loblolly, slash, and longleaf pines. Probing chromosome spreads using DNA clones individually from each marker suggested that two of the three clones contained high-copy DNA. One of the repetitive clones was found to be highly similar to a retrotransposable element in the model angiosperm *Arabidopsis*. Interestingly, this retroelement has not invaded the *Pinus* centromeres or major rDNA sites. More research is underway to study the distribution of this retroelement in various *Pinus* species as well as in closely and distantly related gymnosperms. Work also continues on localization and characterization of the *Fr1* locus in loblolly pine.

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