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Please Note that elements of this report will be reiterated in a combined project report to be submitted by Dr. Jeff Dean of UGA.

FY 97 FINAL REPORT

1. PROJECT TITLE: Genetic Engineering Center of Excellence - Early Flowering (FS-12)

2. PRINCIPAL INVESTIGATOR: Dr. John Cairney

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3. EXECUTIVE SUMMARY OF WORK COMPLETED

- The expression of the LFY gene in Loblolly Pine zygotic embryos has been demonstrated. Expression at this stage of development had not been previously observed in plants.
- Several clones have been generated from Loblolly Pine DNA using LFY primers, these are currently being analyzed.
- Two "Universal Genome Walker" Library has been generated from Loblolly Pine DNA. This will enable us to isolate promoter quickly and will be a resource for our colleagues at UGA
- Two Poster Presentation will be given at International Conferences in the next three months.
- A Post-Doctoral Scientist with several year experience in the molecular biology of woody plants has been recruited.

Introduction and Background

Progress in the genetic improvement of forest trees has traditionally lagged behind that in agronomic species due to the long life cycles and large sizes of forest trees. Not only have these problems dictated the pace of tree improvement for the leading commercial forest species, but they have also limited genetic improvement programs to a handful of North American trees. Tree breeding could be greatly accelerated if trees could be induced to flower while they are still seedlings--that is, within 1 or 2 years following germination. Not only would this dramatically shorten the breeding cycle, but it would also make breeding in the controlled environment of the greenhouse possible. The ability to induce accelerated flowering in forest trees would also provide a critical tool for a related project in which we intend to test strategies for the production of sterile trees (proposal submitted to GCTCPP for FY 1997). Finally, induction of early flowering in forest trees would enable us and other researchers to rapidly characterize the inheritance of inserted DNA in the progeny of transgenic trees, something which has yet to be accomplished, even though such trees have existed since 1989.

Recently, research with a group of flower-meristem-identity genes in the herbaceous model plant, Arabidopsis, has culminated in the production of transgenic Arabidopsis and hybrid aspen plants in which precocious flower development was induced. In one study, hybrid aspen plants transformed with the Arabidospsis LEAFY gene (LFY) under the control of the cauliflower mosaic virus 35S promoter produced flowers when they were only 5 months old. We propose to demonstrate that the strategy used to induce precocious flowering in transgenic Arabidopsis and hybrid aspen (i.e. fusion of a constitutive promoter to genes encoding flower-meristem-identity genes) can be applied to induce precocious flowering in commercially important southeastern hardwoods (yellow-poplar, sweetgum, and cottonwood) and precocious strobilus production in conifers (loblolly pine). Future objectives include transforming these same species with genetic constructs in which inducible promoters have been fused to the Arabidopsis flower- meristem-identity genes, as well as cloning and characterizing one or more flower-meristem-identity genes from tissues of the tree species under study.

The ultimate goal of this research is to generate trees in which the timing of flower production can be controlled, enabling accelerated breeding for operational tree improvement and accelerated testing of sterility strategies. In projects previously supported by the GCTCPP, we have developed the expertise necessary to complete the work proposed here; that is, we have produced transgenic trees expressing foreign genes and have rapidly cloned tree genes using DNA sequences conserved in other organisms.

This proposal represents a new collaboration between researchers at the University of Georgia and the Institute for Paper Science and Technology. A significant component of this collaboration will be to bring together the substantial skills that IPST researchers have demonstrated in somatic embryogenesis and molecular biology of loblolly pine with the expertise demonstrated by UGA researchers in using microparticle bombardment to generate transgenic plants. By performing this work in a collaborative effort, we can assure that techniques for inducing controlled precocious flowering will be developed as rapidly as possible for the premier commercial tree species of the

Southeastern U.S. (loblolly pine). The program described in this proposal will also be tightly integrated with projects to develop techniques for production of sterile hardwood and softwood trees as described in proposals previously submitted by UGA and IPST researchers for consideration by the GCTCPP.

Precocious flowering and sterility represent opposite sides of the same coin, yet they both hold enormous potential value for the forest products industry. Genetic engineering provides the means for rapidly introducing valuable new traits into trees. Controlled precocious flowering would allow us to fix those new traits in the genetic background or move them into new backgrounds through greatly speeded breeding programs. These accelerated breeding programs should enable us to bring loblolly pine breeding on a par with the Eucalyptus breeding programs that have led to such rapid improvements in that species over the past two decades. However, we will be prevented from planting genetically engineered trees widely in the environment if we cannot prevent their outcrossing to surrounding populations of wild, native trees. Thus, the simultaneous pursuit of techniques for inducing precocious flowering and forced sterility through a set of integrated and collaborative programs put forth by UGA and IPST researchers provides the best hope for the rapid development of tools that will be critical for southeastern forestry in the next century.

The Anticipated starting date of this work was July 1996, however, we have until now had difficulty finding a suitable post-doc for this project but recently Dr. Lin Ge has joined our group and we anticipate an acceleration of progress with her appointment. Previous work has been conducted by students in our laboratory at IPST.
Sequencing of the LFY clones isolated earlier has been conducted and has shown these clones to be false positives. We have learned of similar difficulty in obtaining LFY in a number of laboratories. In the absence of material from the developing cones, we are adopting new strategies to clone LFY form Loblolly Pine

1. We are conducting Southern Blots, using an Arabidopsis LFY cDNA to probe DNA, isolated from Loblolly Pine Somatic Embryos. This should indicate that LFY is present on a Restriction fragment of a given size. DNA will be size fractionated on gels and appropriate size fractions will be isolated and sub-genomic libraries will be made. This will allow the isolation of promoter fragments for future expression studies in transgenic plants.
2. We are constructing "Promoter Libraries". This is a technique which uses PCR to generate clones of DNA fragments adjacent to a gene of interest. Genomic DNA was prepared and purified by Cesium Chloride gradient, and digested by Restriction Enzymes, EcoRV, DraI, PvuII, SacI and StuI. Adaptor Primers were then ligated onto the ends. Gene Specific Primers (GSPs) for Leafy have been designed and these are now being used to amplify regions between the LFY coding region and the end of the restriction fragment. Details of the procedure are shown in Figure 1 in the Attachments. We have made a set of five "libraries" from Loblolly Pine DNA extracted from somatic embryos genotype 333 and similarly from genotype 260. These are being employed in the laboratory on various GA consortium supported

projects and are available, as a resource to our colleagues at UGA with whom we are collaborating.

3. Early Stage Somatic Embryo RNA has been probed with Arabidopsis LFY cDNA and signals detected. We are in process of constructing a cDNA library from somatic embryo RNA which will be used to isolated cDNA clones of LFY and other floral homeotic genes.

This project is now closely tied with the groups of Drs. Jeff Dean, Scott Merkle and Sarah Covert at UGA, Athens. Additional work is being conducted at UGA transforming Arabidopsis LFY into tree species and the results will be communicated by Dr. Dean in a separate report.

4. DELIVERABLES

Major Milestones & Dates	Original Proposal	Actual
Isolation of homologs of floral homeotic genes from Loblolly Pine	12/31/'96	Ongoing
Construction of Genomic Library	3/31/97	Completed (as a Promoter library)
Vector Construction and Transformation	12/31/'96	Ongoing

5. BUDGET

State Funds	Total FY 97 $	6-Month Expended $
Matching Funds	Original Proposal $	Actual $

6. Additional Information/Results/Graphics can be attached if available

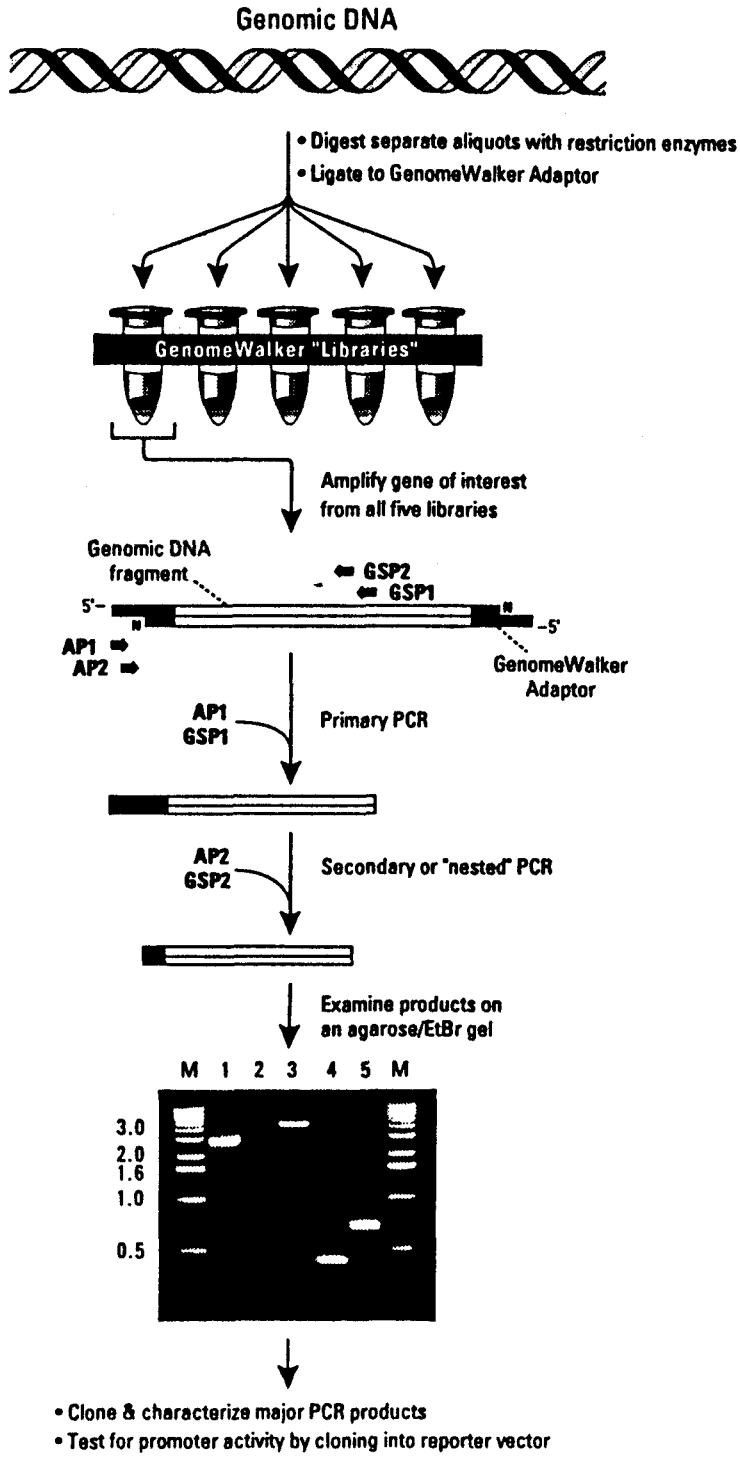

Figure 1. Flow chart of the GenomeWalker protocol. The gel shows the products generated by walking with human GenomeWalker libraries and gene-specific primers derived from the human p53 cDNA sequence. Lane 1: *Eco*R V Library. Lane 2: *Sca* I Library. Lane 3: *Dra* I Library. Lane 4: *Pvu* II Library. Lane 5: *Ssp* I Library. Lane M: DNA size markers. The absence of a major product in the *Sca* I Library (lane 2) is not unusual. In our experience, there is no major band in one or more lanes in approximately half of the GenomeWalker experiments. As explained in the Expected Results and Troubleshooting Guide (Section VI), this is usually because the distance between the primer and the upstream restriction site is greater than the capability of the system. N: Amine group that blocks extension of the 3' end of the adaptor-ligated genomic fragments. GSP: Gene-specific primers.