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FY 97 Year-End Progress Report (July 96 - June 97)

1. **Molecular Methods for the Production of Sterile Trees (PP97-FS3A)**

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3. **Executive Summary of Work Completed**

The goal of this project is to develop and test genetic constructs that will cause sterility in transformed trees. As noted in our mid-year report, research subsequent to submission of our original proposal showed that gene expression driven from the *Arabidopsis* PAB5 actin promoter was not limited to floral tissues as originally thought. PAB11, another actin promoter that appeared to have the desired stringency, was fused to the β-glucuronidase (GUS) reporter gene, as was a control construct having GUS under control of a nominally constitutive actin promoter (PAB2). These two reporter gene constructs were used to transform embryogenic yellow-poplar cells alone and in combination with the LEAFY and APETALA constructs described in our "Early Flowering" project. Screening of the transformed cell lines showed that GUS activity was high in lines receiving the PAB11/GUS constructs, whereas GUS expression was low in lines containing the PAB2/GUS construct. At first glance this appears to be exactly the opposite outcome from what was expected; however, PAB11 is normally expressed at high levels in developing pollen and embryos and Arabidopsis. Thus, the high level of PAB11 expression in embryogenic cell lines of transgenic yellow-poplar demonstrates at least two significant facts: 1) there is conservation of at least some of the mechanisms regulating gene expression in embryogenic tissues of both yellow-poplar and Arabidopsis, and 2) the cells in our yellow-poplar cultures are truly in an embryogenic state.

The downside of this result is that sterile trees cannot be produced from embryogenic cell lines using genetic constructs controlled by promoters expressed in embryogenic tissues unless a further level of genetic control, i.e. a repressor system, is also used. We are currently evaluating whether or not repressor systems adequate for our needs are available.

We had anticipated this possibility and had obtained the Arabidopsis LEAFY and APETALA promoters from Drs. Wiegel and Yanofsky, respectively. These promoters have already been fused to the DTA cytotoxin gene and placed into expression vectors appropriate for transformation of our embryogenic cultures. Preparation of control plasmids having the GUS gene under control of the LEAFY and APETALA promoters is underway, and as soon as these are available (expected by July1, 1997), all constructs utilizing LEAFY and APETALA promoters will be co-bombarded into embryogenic yellow-poplar cells along with the constructs that are being used to induce early flowering (see year-end report for PP97-FS12). In addition, the TAP29-cytotoxin gene fusion has been subcloned into a vector that can be used for transformation, and this construct will also be co-bombarded along with constructs for early flowering.

A novel means of creating sterile trees was conceived as this project progressed, and efforts to construct vectors appropriate for testing the idea have been initiated. Given that flowering is most

often limited to mature tissues, it is possible that induced juvenility in tissues destined to flower might prevent flowering. To test this hypothesis, a gene whose product synthesizes a potent cytokinin (anti-senescence hormone) is being fused to the LEAFY and APETALA homeotic gene promoters, and the resultant gene fusion will be used to transform early flowering tree lines. We expect these constructs to be ready by the end of July 1997, and they will initially be tested in Arabidopsis.

4. **Deliverables**

Milestones & Dates	Original Proposal	Actual
1. Hardwood cell lines transformed with TAP29 and PAB5 cytotoxic constructs (9/30/96)	Yes	Deferred
2. Hardwood genomes probed for TAP29 and PAB5 homologs (9/30/96)	Yes	Deferred
3. Transformed cell lines transferred to liquid culture (12/31/96)	Yes	Deferred
4. TAP29 and PAB5 homologs identified (12/31/96)	Yes	Deferred
5. Attempts to identify homeotic genes initiated (12/31/96)	Yes	Underway
6. Embryos containing TAP29 and PAB5 constructs germinated and transferred to soil (3/31/97)	Yes	Deferred
7. TAP29 and PAB5 homolog promoters isolated (3/31/97)	Yes	Deferred
8. Homeotic gene fragments isolated and sequenced (3/31/97)	Yes	Underway
9. TAP29 and PAB5 transgenics transferred to greenhouse (6/30/97)	Yes	Deferred
10. Vectors containing cytotoxic gene fused to TAP29 and PAB5 homolog promoters prepared (6/30/97)	Yes	Eliminated
11. Homeotic gene promoter isolation underway (6/30/97)	Yes	Underway
12. Test different actin promoters for flower-specific gene expression	No	**Complete**
13. Test homeobox promoter/cytokinin gene fusion for ability to block florigenesis	No	Underway
14. Prepare vectors having cytotoxin expression controlled by LEAFY and APETALA promoters	No	**Complete**
15. Transform cell lines simultaneously with homeobox promoter/cytotoxin gene and early flowering constructs	No	Underway

5. **Budget**

State Funds	Total FY97 $58,250	12-Month Expended $58,250
Matching Funds	Original Proposal $100,000	Actual $31,168

6. **Additional Information**

The goal of this project is to develop and test genetic constructs that will cause sterility in transformed trees. It is important to note that this project is closely interrelated with a second project, "Molecular Methods for Induction of Early Flowering in Trees" (PP97-FS12), and in fact, it has been proposed that both projects be integrated in FY 98. As a successful outcome to the work in the other project ("Early Flowering") would greatly facilitate this project, efforts to date have focused primarily on the early flowering project.

As noted in the Executive Summary above, the actin promoter (PAB11) that we had hoped to use to ablate developing ovules and pollen in transgenic trees cannot be used for this purpose in transformation systems which rely on embryogenic cell cultures. Further work with the actin promoters is not anticipated at this time as no other actin promoter having activity confined to non-embryonic reproductive tissues has been identified. However, as noted, the LEAFY and APETALA promoters, which we were fortunate to receive from the discoverers (Drs. Yanofsky and Wiegel) prior to publication, should prove even more useful for these purposes. Efforts to isolate the promoters controlling expression of the yellow-poplar LEAFY and APETALA homologs are ongoing, and it is expected that these promoters will be ideally suited for bringing about sterility via ablation of reproductive tissues.

As noted in our previous report, a novel approach to bringing about sterility in transgenic trees was initiated midway through this project. The approach is based on the observation that flowering generally requires that the tissues from which the reproductive structures differentiate must reach a certain maturity before they are competent to initiate this process. Thus, application of cytokinins, a class of phytohormones having anti-senescent properties and often associated with juvenility, can sometimes delay or even prevent flowering. We have obtained a gene encoding an isoprenyladenosine transferase (*ipt*), an enzyme that synthesizes a powerful cytokinin when expressed in plant tissues, and are attempting to place it under the control of the LEAFY and APETALA promoters received from Drs. Weigel and Yanofsky, respectively. Once vectors in which these promoters have been fused to the *ipt* gene are prepared, they will be used to transform *Arabidopsis*. If the resultant transgenic Arabidopsis plants are rendered sterile, the same vectors will be tested in yellow-poplar which flowers precociously.