Genetic engineering approaches to breeding sterility and reduce invasiveness

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Project Objective

Develop biotechnology methods that will lead to sterile or highly infertile cultivars of invasive or potentially invasive nursery crops

Approaches

- Use genetic engineering to test different approaches for creating seedless and/or pollen-less plants.
- Develop methods that will lead to the more efficient means for creation of genetically engineered plants.
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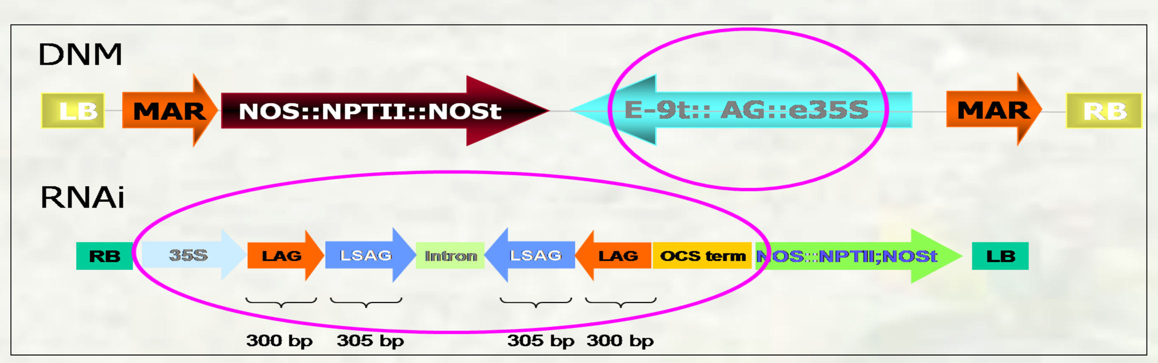
Background

We are seeking improvements in capability for genetic engineering of the diverse plant species used in ornamental horticulture and floriculture, and testing of new and potentially broadly effective approaches for inducing sterility. We have focused on the ornamental taxa sweetgum and poplar as test species. A major field trial has been produced, and a number of transgenic approaches are under study in the laboratory that show promise.



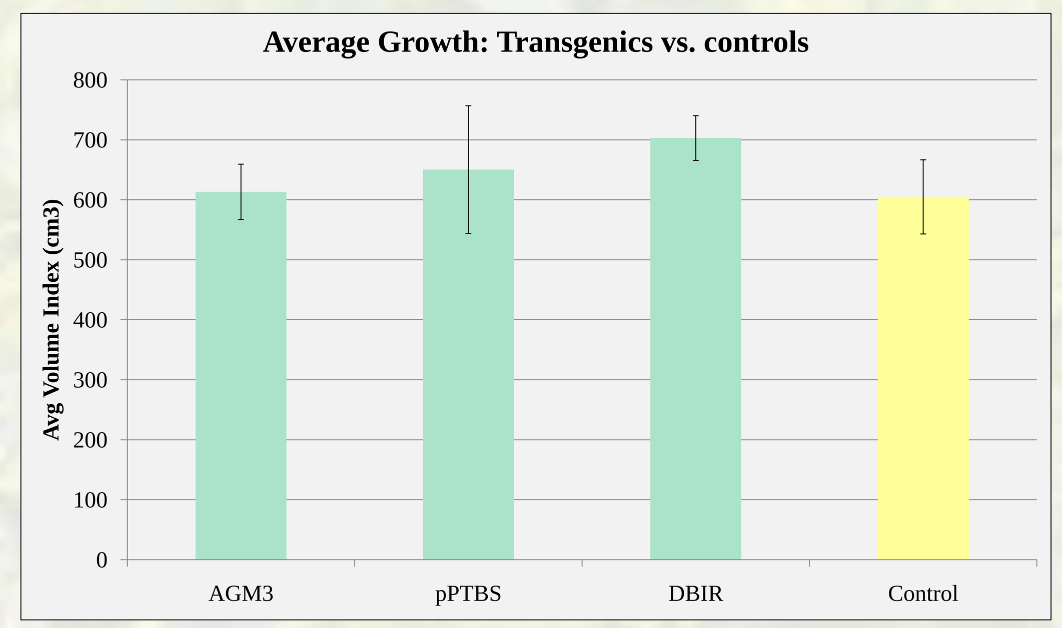
Sterility gene constructs used for sweetgum transformation. *LAG* and *LSAG* are homologs of the Arabidopsis *AGAMOUS* gene. A dominant negative protein (DNM) toward AGAMOUS, and an RNAi-form that targets both its *AGAMOUS* genes, are being studied.

Table 1: Summary of field trial planted June 2007

Construct	Promoter/Genes	Type of construct	Events	Ramets	Trees
pDBIR	*35S::LAG/LSAG-IR*	RNAi	33	4	132
pPTBS	*PTD::BARNASE::G7 35SBP::BARSTAR:: E9, MARs*	Ablation	5	4	20
AGM3	*En35S::AG-M3::E9, MARs*	DNM	20	4	80
Controls				12	12
Borders					84
Total			**58**		**328**

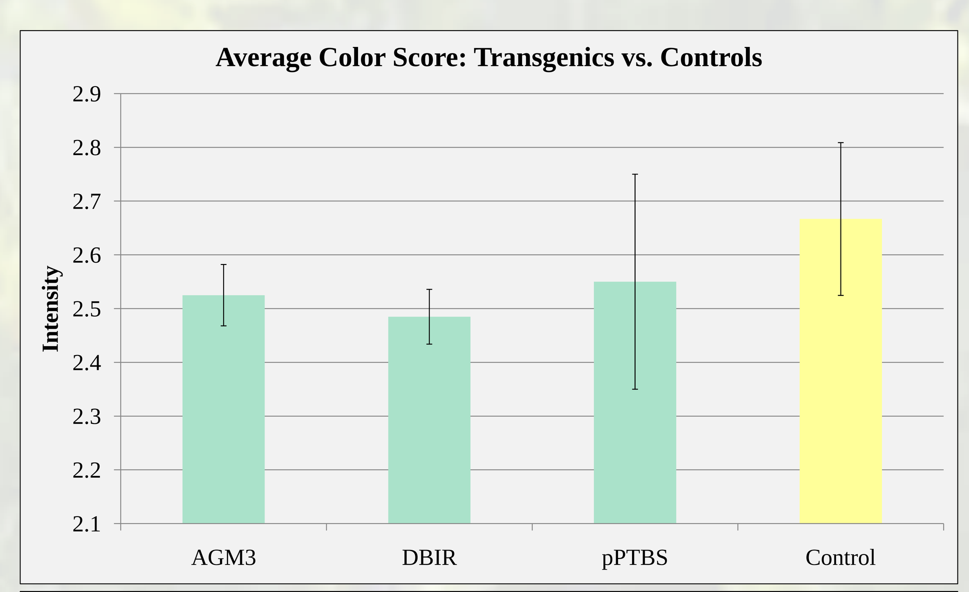
Sweetgum transgenic plantation showing onset of fall color in September 2009

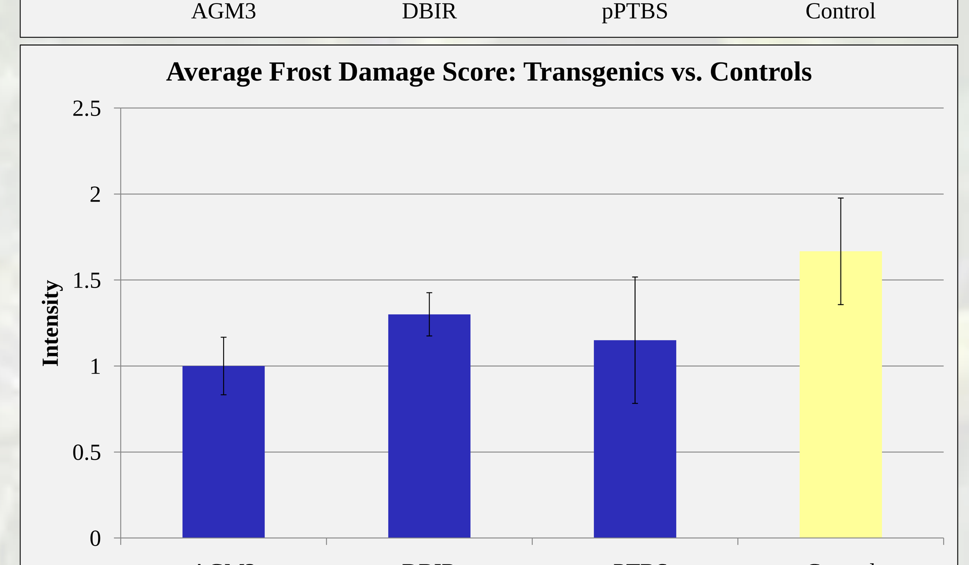
View of the sweetgum field trial and nearby landscape in western Oregon



Normal growth rate and survival in transgenic sweetgum trees in the field. The mean size of trees (November 2008) was not-significantly different between the sterility gene types or the non-transgenic controls after two years in the field. Bars are standard error.s

The transgenic sweetgum trees continued to show strong ornamental color in fall, as expected for this variety (*Worplesdon*).

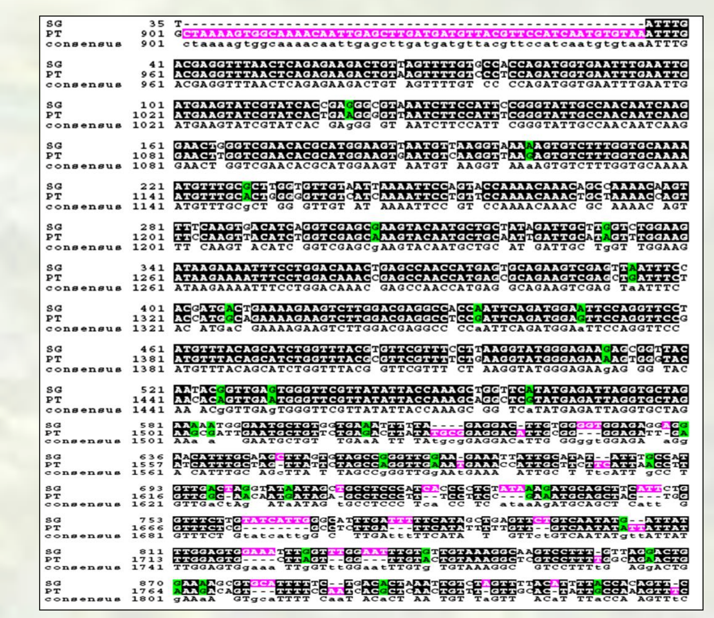




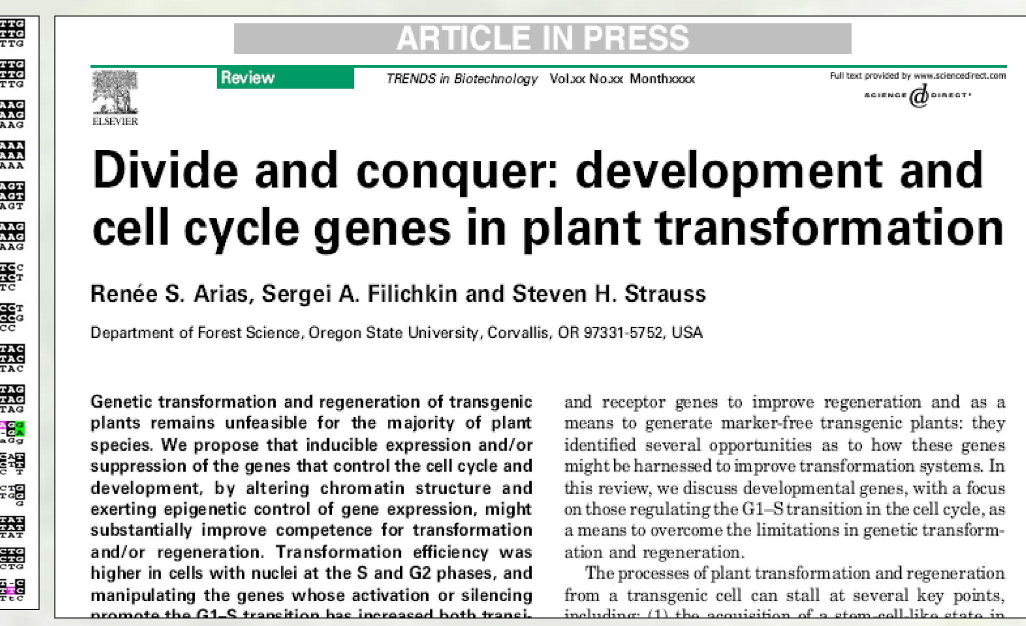
The transgenic types and controls were not significantly different in their fall coloring or sensitivity to an early spring frost in 2008. Higher values indicate greater color intensity and more frost damage to growing tips.

Accomplishments

1. Improvements in rate and efficiency of recovery of genetically engineered plant tissues.
2. Produced and studied in the field 58 types of genetically engineered sweetgum trees for three years.
3. Collection of tissues from all of the field plants for gene expression analysis completed and gene suppression studies are underway using RT-PCR.
4. Produced new forms of gene constructs for interfering proteins for the floral regulatory protein LEAFY, inserted them into poplar, and are stimulating the rapid flowering of poplars with the *FT* gene to speed analysis of results.



Use of 5' RACE to amplify a housekeeping genes to use as an internal control for RT-PCR. Shown is a partial DNA alignment of *P. trichocarpa* and Sweetgum *CAC* genes.



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Divide and conquer: development and cell cycle genes in plant transformation

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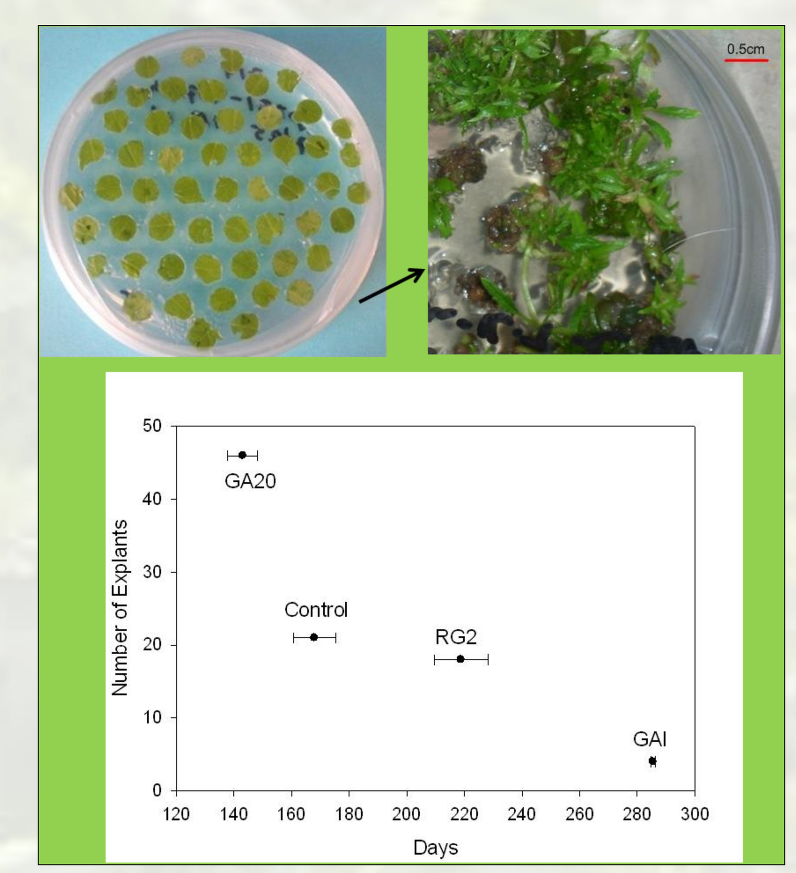
Genetic transformation and regeneration of transgenic plants remains unfeasible for the majority of plant species. We propose that inducible expression and/or suppression of the genes that control the cell cycle and development, by altering chromatin structure and exerting epigenetic control of gene expression, might substantially improve competence for transformation and/or regeneration. Transformation efficiency was higher in cells with nuclei at the S and G2 phases, and manipulating the genes whose activation or silencing

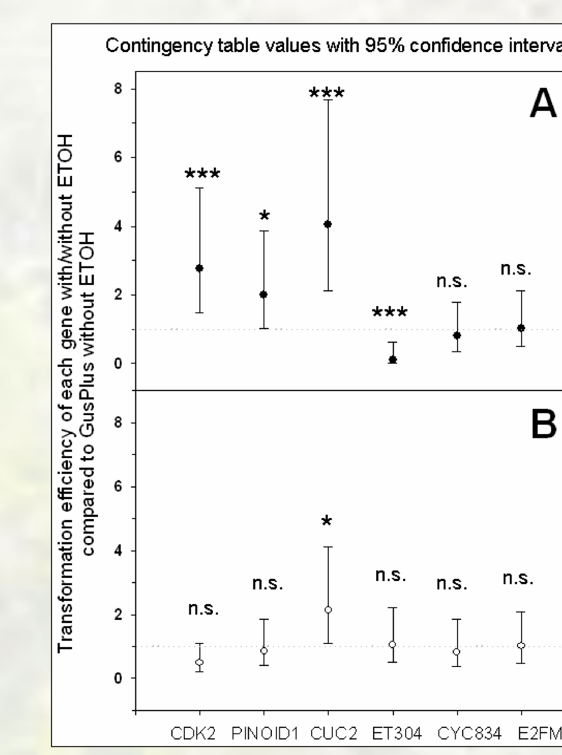
and receptor genes to improve regeneration and as a means to generate marker-free transgenic plants: they identified several opportunities as to how these genes might be harnessed to improve transformation systems. In this review, we discuss developmental genes, with a focus on those regulating the G1–S transition in the cell cycle, as a means to overcome the limitations in genetic transformation and regeneration.

The processes of plant transformation and regeneration from a transgenic cell can stall at several key points,

Review article that we published in *Trends in Biotechnology.* It identifies our major strategy for improving transformation.



Genes that improve transformation efficiency. The *GA20-oxidase* cisgene from poplar improved the rate and frequency of transgenic shoot regeneration in poplar (left). The *CDK2* and *CUC2* genes from poplar improved rate of transformation when induced via an alchol-inducible promoter (right).



Alcohol-induction of candidate shoot regeneration genes: (A) with induction; (B) without induction.

An *FT*-gene induced flower on a 2 month-old poplar. To speed assessment of sterility we have been using a heat-inducible version of the *Flowering Locus T (FT)* gene in poplar. This allows rapid initial assessment of sterility genes prior to field trials to reduce research costs and delays.

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