Abstract

The dispersal of transgenics from genetically engineered plants presents substantial challenges to biotechnology regulatory bodies. Because forest trees are weakly domesticated, have wild relatives, and pollen or seeds can spread widely, they are especially problematic. However, plantation trees are often vegetatively propagated, making fertile pollen unnecessary for commercial use. Thus, genes that induce complete sterility could provide strong and simple mitigation of dispersal, simplifying regulatory decisions. We are studying the efficacy, stability, and ecological impacts of floral developmental genes as tools for mitigating or preventing transgene spread. We established a plantation of transgenic Populus alba containing 19 different constructs that modify the expression of poplar orthologs of conserved floral development genes, including LEAFY (LFY), AGAMOUS (AG), and APETALA2 (AP2). Some constructs are designed to target two or three genes simultaneously. The overarching hypothesis is that we are testing is that suppression of selected floral development genes results in complete sterility could provide strong and simple mitigation of dispersal, simplifying regulatory decisions.

Pt-LFY:RNAi trees had robust vegetative growth

Tree size (calculated as diameter) was measured after three growth cycles. On average, the Pt-LFY:RNAi trees (all T2) were similar in size to the control (CTR) trees. All Pt-LFY:RNAi events grew well, none were significantly smaller than controls. Bars show average tree size by event (number of ramets is shown in the base of the bar). Error bars show standard error across ramets. Tree flowering was scored in 2015. Floral phenotypes (normal flowers, intermediate flowers, tiny flowers) as assessed by field phenotypes, is indicated by bar color (green, blue, or orange). Events with tiny flowers grew very well and were similar in appearance to control trees.

Floral phenotypes were stable across two growing seasons

We used quantitative real-time PCR (QPCR) to measure the relative expression level of the LFY gene in young floral buds and catkins of each event as compared to an ACTIN housekeeping gene. Events 17 and 139-2 have tiny catkins, event 194 has catkins with a normal appearance. Both young and mature control catkins were tested as LFY is predicted to have higher expression in younger catkins. We found that young floral buds events that developed tiny flowers had less LFY expression than floral buds of normal flowered or control catkins. As predicted, the younger control catkins had more LFY expression than the mature control catkins. Event 194, which had normal catkins, also had similar LFY expression as the control samples. Surprisingly, the two events with tiny catkins had much higher expression of LFY than control catkins. We hypothesize that this increased LFY expression may be due to the underdeveloped state of these small catkins. At the time of collection, the young control catkins were much larger and far more developed than the catkins from events 17 and 194. Bars show standard error between biological replicates, asterisks show significant differences (P < 0.05).

Summary

• Trees for all constructs are growing well
• Several RNAi constructs give interesting, potentially sterile phenotypes
• Floral phenotypes are stable across two growing seasons
• Gene expression levels are associated with floral morphology but are dependent on tissue type and age
• Our results suggest that disruption of LFY is a powerful tool for genetic containment of trees

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