Abstract

Gene flow into wild and feral populations of forest trees present a significant barrier to field studies and commercial use of exotic and recombinant DNA-modified trees. Both male and female sexual reproduction are significant concerns in most species. To provide asexual containment, we used RNA-interference (RNAi) to suppress the poplar LEAFY (PoLFY) gene, which is essential for development of floral primordia in male and female sexual organs. We transformed this RNAi-PoLFY construct into male and female clones of poplar; here we present results from early-flowering female clone 6K10 (Populus alba). We obtained 15 independent transformed events in clone 6K10 and examined their floral and vegetative traits over 4 seasons of growth in an APHIS-approved field trial. Trees were planted in 2011 and began flowering in 2014. Floral phenotypes were initially assessed through indoor flushing of dormant floral buds followed by observation of field-opened buds. We found that suppression of PoLFY gave rise to complete and stable sexual sterility in the field. Of the 15 RNAi-PoLFY events, 2 had extremely small inflorescences that lacked functional sexual organs and had reduced expression of PoLFY in developing floral buds. The floral phenotype was repeated over two growing seasons, and the trees showed normal survival, seasonal dormancy, vegetative morphology, and growth rate. Suppression or mutation of the LEAFY gene should greatly facilitate field research, regulatory approval, and public acceptance of exotic and recombinant DNA modified forest trees.

Methods

RNAi-PoLFY construct

We created a RNAi construct based on the Populus nigra LEAFY (PoLFY) gene. This construct was used to transform an early-flowering female clone of Populus alba 6K10 (RNAi-PoLFY). Analysis of the LFI coding sequences from both clones showed that they are 98.5% identical across their coding sequences; and 96.6% across the 285 bp region used to generate the inserted repeat. The 6K10 RNAi-PoLFY plants were planted in 2011 as a part of a larger field trial including 3 total poplar clones and 23 total genotype entries. We planted 15 independent 6K10 RNAi-PoLFY events, each represented by 4 genetically identical transgenic ramets (trees), along with 24 non-transgenic 6K10 control trees. Trees were screened yearly for the presence of dormant floral buds, which were larger than vegetative buds.

Indoor bud flush identified two events with small catkins

Dormant buds were collected and screened to identify events with small catkin phenotypes. Samples were imaged at the start of indoor incubation (a,b) and after full catkin emergence (c). (a) Buds from control trees. (b) Buds from RNAi-PoLFY event 194. (c) Buds from event 139-1. Catkins from (d) control and (e) 13 of the RNAi-PoLFY events fully flushed in 3 days (event 194 shown). (f) Catkins from events 17 and 139-1 fully flushed in 7 days (event 139-1 shown). Scale bar, 5 cm.

Field-grown RNAi-PoLFY events had small, late-opening catkins

Catkins from (a–d) control trees and (e–h) RNAi-PoLFY trees with sterile catkins were imaged over time in 2014 until control flowers fully matured and shed cotton (ac). Small RNAi-PoLFY catkins observed on the same date were still encased in bud scales (sc). Catkins were photographed on the dates indicated in the lower right corner. (i) Scoring of floral bud opening over time beginning January 28, 2015 (day 0).

Floral phenotypes were stable across two growing seasons

Non-transgenic control trees formed large catkins with prominent carpels in both 2014 and 2015. RNAi-PoLFY events had sterile catkins with no externally visible carpels in both 2014 and 2015. Images were taken March 26, 2014, and March 26, 2015. Data show that the floral phenotype was stable across flowering seasons.

RNAi-PoLFY catkins were small and lacked stigmas or ovules

We used microscopy and sectioning to obtain detailed views of catkin morphology for normal and tiny catkins. (a) Non-transgenic control catkins with carpels (c) needed inside perianth cups (p) and topped with stigmas (s). (b) Hand-sectioned control carpel with cotton fibers and ovules (ov). (c) RNAi-PoLFY catkins covered by bracts (br) without carpels or ovules (ov). (d) RNAi-PoLFY catkins with carpels (c) and ovules (ov). (e) Hand-sectioned sections of RNAi-PoLFY catkins with carpels and perianth parts (p). Catkins were collected for light microscopy April 9, 2014, control and RNAi-PoLFY catkins were collected for sectioning March 14, 2014. Scale bar, 500 µm.

RNAi-PoLFY trees had normal vegetative growth

Tree Size 2015 (Spatial Adjusted)

We measured tree size at planting and every dormant season. Due to environmental variation across the plantation, tree growth was analyzed with and without spatial adjustment. Adjustment decreases the errors of the measurements. Bars show the adjusted average tree size across events of normal flowering RNAi-PoLFY (green bars) and RNAi-PoLFY trees with sterile catkins (orange bars). Numbers at the base of bars show the number of trees; error bars show error of the means. Stacking trees were not significantly different in size from normal-flowering trees.

RNAi-PoLFY trees had reduced LFY expression in floral buds

We used quantitative real-time PCR to measure relative LFY expression in F1 alfalfa. (a) Analysis of relative PoLFY transcript levels in developing floral buds collected October 5, 2014 showed a significantly lower level of PoLFY in sterile events than control events. (b) Analysis of relative PoLFY levels in young catkins collected March 21, 2014 and mature catkins collected April 2, 2014. Sterile RNAi-PoLFY catkins were collected March 21, 2014. In these samples, sterile catkins have significantly higher levels of PoLFY than control samples. Typically, LFY expression is high in developing floral buds and decreases as flowers mature. Perhaps the high level of LFY in sterile catkins indicates they were stuck at an earlier developmental stage. Representative images of sampled tissues are shown. Bars show standard error, asterisks indicate significant differences from non-transgenic, control samples (P < 0.05).

Summary

• RNAi against LFY was effective for achieving female sterile flowers
• Floral phenotypes were stable across growing seasons
• RNAi-PoLFY trees had normal vegetative phenotypes
• Gene expression levels were associated with floral morphology but were dependent on tissue type and age
• Our results suggest that suppression of LFY is a powerful tool for genetic containment of trees.

Acknowledgements

The project was supported by awards from the USDA National Institute of Food and Agriculture Project (grant 2013-50000-18521-01), National Science Foundation IGERT Center for Advanced Forestry (grant 1544970), USDA 2010-03322-21276, USDA-IA (grant 0836-35674-078), the Frank Schreiber Charitable Foundation, and the USDA Forest Service as part of the USDA-funded NWTC project "Industrial poplar production in the Pacific Northwest: From nutrient demands to a sustainable industrial fiber crop." We thank the researchers involved in the project for their assistance including the USDA-APF-ICTM and the USDA Forest Service for field assistance, Jared Bickel and Dr. Ben Hess for mineral analysis, Dr. Keralynn Henderson for RNA extraction and sequencing, and Dr. Peter Strauss for assistance in the USDA-APF-ICTM lab. We also thank Jamie Pries and Scott Brown for assistance with microarray analysis and Dr. Ken Klocko for assistance with field sites and data collection. We also thank Mhairi Tackett for providing early flowering clone 6K10 for study.