A tapetal ablation transgene induces stable male-sterility and slows field growth in Populus

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Abstract
The field performance of genetic containment technologies, which may be needed for many uses of transgenic trees in forestry, is poorly known. We tested the efficiency of a barnase gene driven by the TA29 tapetum-dominant promoter for influencing growth rate and inducing male-sterility in a field trial of transgenic hybrid poplar (Populus tremula x tremuloides, INRA 353-53). When the stem volume growth of 18 transgenic insertion events with the sterility transgene were compared to non-transgenic controls after two growing seasons, they grew 40% more slowly in stem volume, and all but one transgenic event grew significantly more slowly than the control. In contrast, when the growth of transgenic trees containing four kinds of GUS reporter gene constructs—produced using the same transformation method and poplar clone—were compared to non-transgenic controls, none showed a statistically significant difference in growth after one or three growing seasons in the field. In two years where gross pollen release from catkins was monitored and macroscopically abundant in the control, no pollen could be visibly detected in the transgenic trees, and microscopy suggested the cause was tapetal collapse. In two additional years when viable, well-formed pollen were documented in the control and quantified microscopically, no pollen could be detected in any of the transgenic trees. We conclude that the construct produced extremely high male-sterility that was stable over several years. The promoter and form of barnase tested also caused retarded vegetative growth, though six transgenic events survived and the trees expressing it appeared visibly healthy in the field for 14 growing seasons.

Gene transfer did not hinder growth

Technical advances in gene transfer to trees have been limited by the paucity of effective transgenic trees that are non-sterile and show no deleterious effects on performance. Methods for transgenic trees have been plagued by low transformation efficiencies, low survival rates, and/or the growth of transgenic trees having high levels of sterility. We transformed hybrid poplar (Populus tremula x tremuloides) with a transgenic construct designed to ablate the tapetum, a cellular layer in the anther that produces pollen, using a barnase gene under the control of a tapetum-dominant promoter. The barnase gene causes its target, the endogenous barnase gene, to be expressed at very high levels and causes the tapetum, the pollen-producing layer, to be ablated. In addition, we included four kinds of reporter constructs to document the effectiveness of the barnase gene. We tested the construct in two field trials, each with 18 insertion events, resulting in 36 transgenic trees for each year. The barnase construct was driven by the TA29 tapetum-dominant promoter, a highly active poplar tapetum-promoter, from which the barnase gene is induced in the tapetal cells of the anther. The construct was introduced into one highly fertile transgenic line, threePG, and transformed into ten insertion events in each of two growing seasons. The transgenic and control trees were analyzed for growth and pollen in two growing seasons. The constructs were designated 3A11G, 3A2G, 3PG, and 3SG. The barnase construct was introduced into one fertile line, 3PG. We tested a reporter construct, ACT2::GUS, to verify that the barnase construct was introduced into the genotypes. Three types of reporter constructs were tested: ACT11::GUS, ACT2::GUS, and 35S::GUS. The barnase construct increased the number of events and decreased the basal diameter growth in transgenic trees, and may indicate the stress of transformation did not impede growth.

Catkin collection in the field

Fig. 1. Sterility field study and methods of collection. **Top left**, the two trees growing season after planting. **Note the person (~1.8 m) within the trees. Photo was taken on July 1997.** **Top right**, the trees in fall, after the third growing season after planting. **Photo was taken on November 1998. Bottom left**, collecting catkins with a ladder while using a pole pruner. **Photo was taken on March 2006.** **Bottom right**, collecting catkins by hand with a Swedish ladder while using a pole pruner. **Photo was taken on February 2007.**

Growth rate was slowed in the transgenic trees

Fig. 3. Transgenic trees showed reduced growth when compared to non-transgenic control. The heights and diameters of all the trees were measured in fall 1997, two growing seasons after planting. Each bar identifies an individual event. Control trees are shown in white and the transgenic events in gray. The brackets represent 95% confidence intervals. The stars indicate whether the specific event was significantly different from the control based on a Dunnett’s test (three stars: P<0.001, two stars: P<0.01, and one star: P<0.05, rounded up).

Transgenic catkins were shorter and curved

Fig. 4. Transgenic catkins were not as erect as control catkins. The catkins from the transgenic events were always not as large as the catkins from the controls. They were also always curved.

Anthers showed collapsed tapetum

Fig. 5. Transgenic stomata do not have as much pollen as control. A and B, were from a control tree. C and D, were from transgenic event 12. E and F, from transgenic event 9. G and H, from transgenic event 14. I and J, from transgenic event 8. Photos were taken of freshly collected catkins in March 2009.

Fig. 6. Collapsed tapetum layer and absence of pollen grains in transgenic anthers. In the left column, control anther sac with pollen. In the right column, transgenic anther sac from event 12 with no detectable pollen. Flowers were collected on February 26, 2009. E, epidermis; En, Endothecium; T, Tapetum; PG, Pollen grain; PS, pollen sac.

Transgenic pollen not seen in laboratory

Fig. 7. No signs of pollen in transgenic catkins. A and C, control catkins. B and D, transgenic catkins from events 17 and 14 respectively. A and B, are from 2007. C and D, are from 2009.

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Pollen dehiscence was not observed in transgenic catkins

Fig. 2. Reporter transgenic trees grew as well as non transgenic control. The volume index (height X diameter) of each transgenic construct compared to that of the controls was not significantly different (all Ps >0.05) suggesting that the stress of transformation did not impede growth. The brackets represent the 95% confidence intervals. Transgenic constructs designations: 3PG = PTD::GUS, 3A11 = ACT11::GUS, 3A2 = ACT2::GUS, and 3SG = 35S::GUS.

Fig. 8. Growth rate was slowed in the transgenic trees. A and C, control trees. B and D, transgenic events 17 and 14 respectively. A and B, are from 2007. C and D, are from 2009.