Biocontainment in Perennial Grasses: Transgenic Pollen Ablation in *Brachypodium sylvaticum* and *Panicum virgatum*.

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Project Goal:
Develop a gene containment system to minimize transgene flow from transgenic switchgrass.

Although numerous characteristics make switchgrass a desirable biofuels crop, it is also a wind-pollinated species native to the region where it will be grown. Agricultural cultivation of transgenic switchgrass cultivars with improved traits raises the possibility that engineered traits may move into natural populations with potential unintended environmental impacts. Thus, a means to control transgene escape to wild plant populations is needed prior to commercial release. We are developing a transgene containment system for perennial grasses to address this concern. The ability of novel transformation constructs to block transmission of transgenes via pollen is being evaluated in the model perennial grass *Brachypodium sylvaticum* and switchgrass. Using *Agrobacterium*-mediated transformation, we generated transgenic plants that express Barnase (a toxic ribonuclease) under the control of four rice pollen-specific promoters (*PS1*, *PS2*, *PS3* and *OsGEX2*). Independent transgenic lines for each construct are being evaluated by pollen staining and/or genetic segregation analyses. Alexander's staining revealed that, relative to wildtype plants, >50% of the pollen collected from the T_0 transgenic plants was dead or deformed. Analysis of selfed T_1 *B. sylvaticum* progeny showed that transgene heritability was 1:1, consistent with the expected segregation frequency for a male lethal gene, supporting the conclusion that successful ablation of transgenic pollen was achieved in these transgenic plants. We are currently characterizing multiple independent transgenic switchgrass plants carrying the ablation constructs for their effectiveness at mediating transgene containment. Whether the biocontainment constructs alter plant morphology or biomass yield is also being examined.

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