Transgene Containment in Perennial Grasses: Ablation of Transgenic Pollen in *Brachypodium sylvaticum* and *Panicum virgatum*.

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

Interest in the use of perennial grasses, especially those utilizing C_4 metabolism, for biofuel production is increasing because of the low input cost and long-term land use benefits afforded by these plants relative to their annual counterparts. Efforts reliant on biotechnology for the improvement of stress tolerance and biomass production in species like switchgrass (*Panicum virgatum*) must consider that it is an obligate outcrossing, wind-pollinated species native to North America. Thus, a means to control transgene escape to wild plant populations is needed. We are developing a transgene containment system for perennial grasses to address this concern. We are using the model perennial grass *Brachypodium sylvaticum* and switchgrass to evaluate the utility of novel transformation constructs to block transmission of transgenes via pollen. Using *Agrobacterium*-mediated transformation, we generated transgenic plants that express *barnase* under the control of four rice pollen-specific promoters (*PS1*, *PS2*, *PS3* and *OsGEX2*). Multiple independent transgenic lines for each construct have been evaluated by pollen staining and genetic segregation analyses. Alexander's staining revealed that, relative to wildtype plants, >50% of the pollen collected from putative containment T_0 *B. sylvaticum* lines was dead or severely deformed. Analysis of selfed T_1 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 *Brachypodium sylvaticum* transgenic plants. Initial work with *PS2-barnase* transgenic switchgrass suggests that approximately 50% of the pollen produced is inviable. We have generated numerous independent transgenic switchgrass plants with each of the four pollen ablation constructs and will be examining their effectiveness at mediating transgene containment.