There is a great need for the development of alternative and sustainable fuel sources, and switchgrass is poised to become one of the first dedicated biomass crops due to its inherently high yield, C4 metabolism, and perennial nature. Because it is difficult to fix beneficial alleles within outcrossing switchgrass populations, we have initiated a biotechnology-based approach for inbred production via doubled haploids through a process referred to as centromere-mediated genome elimination (Ravi et al., 2010). For this purpose we are using TALENs to target cleavage and repair of several loci encoding CenH3 histones involved in centromere function with the goal of generating whole or partial loss of the functional alleles. Six TALEN pairs were designed to knock out endogenous switchgrass genes PviCENH3-1 and PviCENH3-2. Another 6 pairs of TALEN were designed to knock out the CENH3 and COMT genes of the model grass Brachypodium distachyon. Activity of each TALEN pair was first tested using a yeast-based cleavage assay and then used to create Agrobacterium transformation vectors for stable plant transformation. To express both left and right TALENs in planta, coding regions were separated by a T2A translational skipping sequence and expressed under control of a single maize ubiquitin promoter.

Transgenic T0 lines were regenerated from embryogenic calli and a T7 endonuclease assay was conducted to screen juvenile and flag leaves of these plants for mutations of the target loci. Evidence of somatic mutation at the target loci of these T0 lines will be presented.

Since biotechnologically improved switchgrass will ultimately be grown in open fields, they must be designed to minimize the potential impact of the transgenes on non-target ecosystems. Thus we are implementing a strategy designed to minimize transgene flow from the genetically engineered crop by ablating the transgenic pollen, thus reducing the transmission of transgenes in the environment. We are using the model perennial grass Brachypodium sylvaticum to evaluate the utility of novel transformation constructs to block pollen-mediated transgene flow. We have generated transgenic plants that express barnase under the control of four rice-derived pollen-specific promoters (PS1, PS2, PS3 and OsGEX2). Multiple independent transgenic lines for each construct have been produced and are being evaluated by pollen staining and genetic segregation analyses. Alexander’s staining revealed that, relative to wildtype plants, >50% of the pollen collected from the hemizygous T0 containment lines was dead or severely deformed. Analysis of the selfed T1 progeny showed that transgene heritability was 1:1, consistent with the expected segregation frequency for a single locus male lethal transgene, supporting the conclusion that successful ablation of transgenic pollen was achieved in these Brachypodium sylvaticum transgenic plants. Future work is focused on transferring these functionally validated transgene containment constructs into switchgrass.