

## **Genome Editing of CENH3 in Switchgrass and Brachypodium: A Histone Variant Essential for Centromere Specification**

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### **Project Goals:**

**Develop a CENH3-based method for creating doubled haploid grasses and demonstrate it in switchgrass.**

### **Background**

Self-incompatibility and outcrossing behavior in switchgrass prevent effective hybrid development and fixation of gene combinations. This problem is being addressed through biotechnological approaches enabling creation of haploid inducer lines use with doubled haploid breeding strategies. In order to engineer haploid inducer lines in grasses via centromere-mediated genome elimination, we are introducing mutations into both switchgrass and Brachypodium CenH3 genes by genome editing techniques. These mutations could then be rescued or complemented with CenH3 variants similar to those known to induce uniparental genome elimination (1). These as well as genome editing of the COMT gene of Brachypodium that is involved in monolignol synthesis are underway. In order to determine if genome doubling techniques are efficient a population of neo-octaploid `Liberty` switchgrass was also created.

Transformations of switchgrass and Brachypodium with genome editing constructs using both TALEN and CRISPR/Cas9 systems have been conducted and switchgrass lines are being crossed with wild type plants to segregate away the transgene from the desired mutation. With these constructs, target-endonuclease activity was validated either by employing heterologous yeast system, or a GUS reporter gene containing target sequences that interrupted the ORF. GUS activity was detected in onion epidermis after cobombardment of the reporter gene and the binary plant transformation vector. Activity in yeast was detected using a  $\beta$ -galactosidase reporter gene. These assays indicated that target-endonuclease activity could restore an active ORF and that the CRISPR binary vectors were functional at least a transient plant-assay system.

### **Brachypodium CenH3 TILLING mutants:**

Working with a Brachypodium TILLING resources at INRA, France and a screening center managed there, efforts were made at mutation screening within the CenH3 gene. Ten lines were isolated, all with point mutations in CenH3 that have now been validated by Sanger sequencing. One of these families (5173) contains a predicted Ser to Phe change at position 82 and has reduced stature and fertility. We are now backcrossing this line to WT Bd21-3 to reduce background mutations and analyze further. Other online Brachypodium resources including T-DNA insertion populations and mutant resequencing projects were screened as well but did not yield mutations in CenH3.

### **Brachypodium TALEN line A/B 1 is active against its BdiCenH3 target**

We have focused most of our efforts so far on a Brachypodium line that contains a very active TALEN T-DNA as judged by pooled sequencing approaches as well as high resolution melt analysis, and Sanger sequencing. High resolution melt analysis has proved as efficient in our hands as gel-based mutation screening and we have screened for T-DNA presence using *FokI* and *HptII*-specific primers. We have not isolated any mutations in *CenH3* in the absence of the TALEN T-DNA. Our hypothesis now is that most mutants in BdiCenH3 are embryo-lethal and we have since retransformed this line with altered versions of CenH3 in an effort to obtain viable knockout/substitution lines.

### **Switchgrass neo-octoploid lines**

Seedling treatment to induce polyploidy could be effective for breeding purposes where genetic exchange between different cytotypes is desired. In this study the cultivar 'Liberty' was chosen because it is of recent hybrid upland/lowland origin, has superior yields, and is adapted to hardiness zones that are considered to be suitable for upland cultivars which are frequently octaploid.

Initial lines were largely cytochimeras containing 4x and 8x sectors (2). Individual octaploid tillers were identified by flow cytometry and allowed to intercross to generate 100% octaploid individuals. Seed harvests indicated that seed derived from octaploid sectors was 19% larger compared to tetraploid sectors and that fertility in these sectors was significantly lower. Non-chimeric octaploid progeny of the treated individuals were confirmed to have approximately twice the number of chromosomes of tetraploid lines although there was high variability in these counts. Induced octaploids had larger pollen and leaf cell size. Guard cell density was found to be significantly lower in induced octaploid compared to tetraploid individuals. An earlier publication found no substantial differences in guard cell density in some naturally occurring octaploid and tetraploid switchgrass populations. Our results showing significant differences should have implications with respect to gas exchange rates and photosynthesis.

This population has been clonally propagated and sent to ARS collaborators at the University of Lincoln, Nebraska. Intercrosses with other octaploid populations are being conducted there as is further phenotypic analysis. One potential benefit is that the larger seed size will improve seedling vigor and stand establishment, as these traits are strongly correlated in switchgrass and other perennial grasses.

### **References**

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2. Yoon S, Aucar S, Hernlem BJ, et al (2016) Generation of Octaploid Switchgrass by Seedling Treatment with Mitotic Inhibitors. *Bioenerg Res* 1–9. doi: 10.1007/s12155-016-9795-2

*This material is based upon work supported by the US Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0008797. This work was also supported by the United States Department of Agriculture, Agricultural Research Service (USDA-ARS) Current Research Information System (CRIS) 2030-21000-023.*