Genetic Regulation of Awn Development in *Brachypodium distachyon*

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Awns in grasses

- ~12,000 different species of grasses
- Awns are hair-like appendage extended from a larger structure and a characteristic of many species of grasses. [2]
- Awns can have many different important functions, but it is unclear how awns form genetically. [3]
- Awn development is disrupted in awnless1 (*awl1*) mutants
- *awl1* plants have a deletion upstream of *DROOPING LEAF (DRL)* ortholog that includes conserved non-coding sequences (CNS)
- 3 most deeply conserved regions are identified

Using Golden Gate Cloning to make constructs

CRISPR will be used to recognize and cut out the 3 identified CNS regions. The constructs will be made using Golden Gate Cloning in the process shown above. The constructs will be transformed into *Agrobacterium*, which can transfer the CRISPR DNA constructs into *B. distachyon*.

CRISPR Constructs

- **A**
  - CNS_1-2_FINAL 16241 bp
- **B**
  - CNS_3-4_FINAL 16241 bp
- **C**
  - CNS_5-7_FINAL 16241 bp
- **D**
  - CNS_1-7_FINAL 16241 bp

Sequencing show good constructs

- **A**
  - Alignment of sequenced spacer 3-4 construct. (B) Alignment of sequenced spacer 5-7 construct. Grey boxes indicate a match between the nucleotides, confirming that the constructs are correctly assembled and contain the desired spacers.

Future Directions

- Assemble rest of CRISPR constructs.
- Spacers 1-2
- Spacers 1-7
- Determine the expression level of *BdDRL* after heterozygous *awl1* plants have been treated with CRISPR constructs.

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References