

Genetic Regulation of Awn Development in *Brachypodium distachyon*

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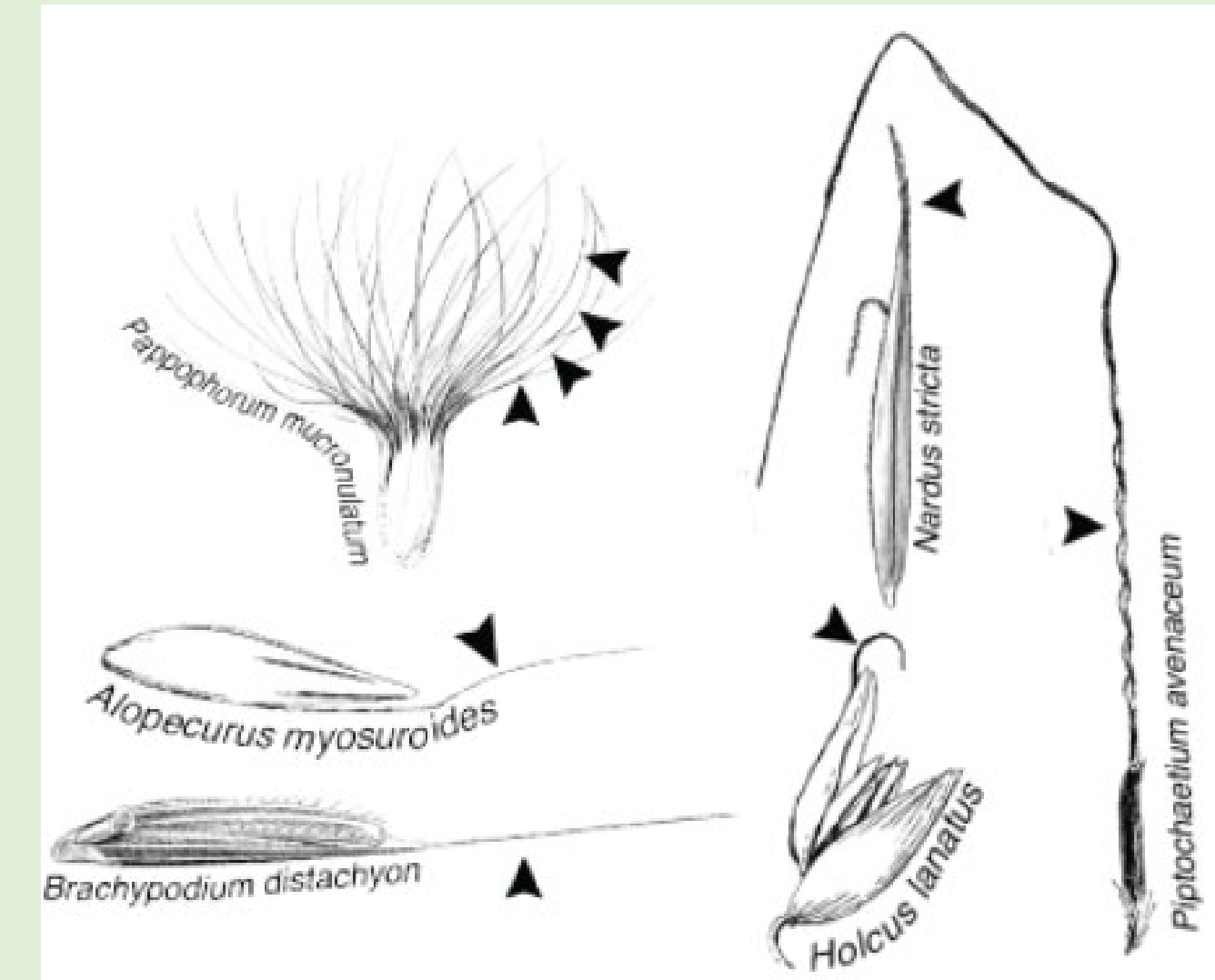
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United States Department of Agriculture
National Institute of Food and Agriculture

Awns in grasses

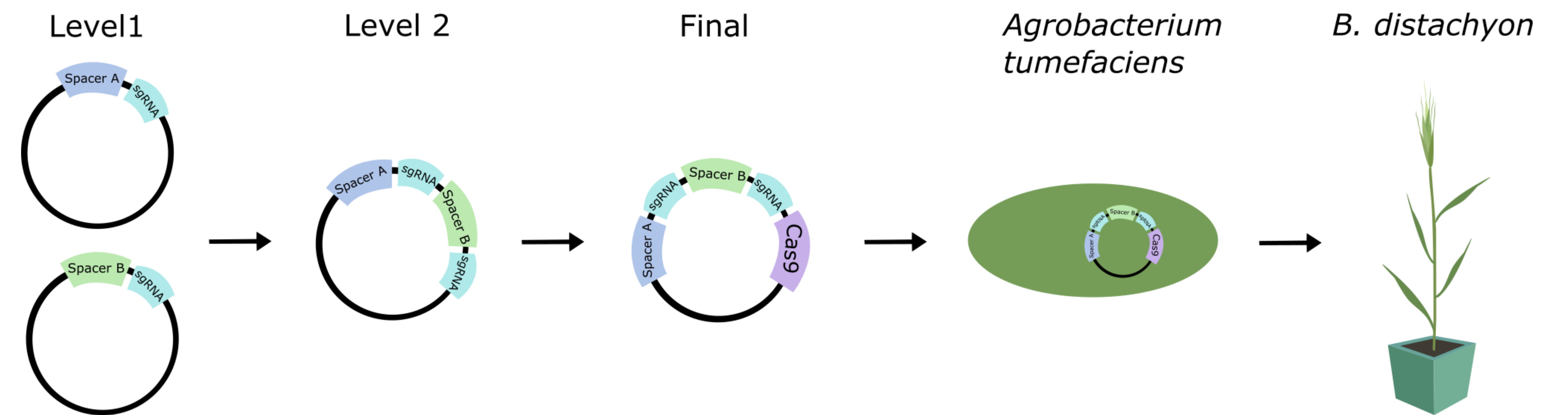
- ~12,000 different species of grasses
- Awns are hair-like appendage extended from a larger structure and a characteristic of many species of grasses. [2]
- Awns can have many different important functions, but it is **unclear how awns form genetically**. [3]
- Awn development is disrupted in *awnless1* (*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



Different species of grasses with awns (arrows). [1]

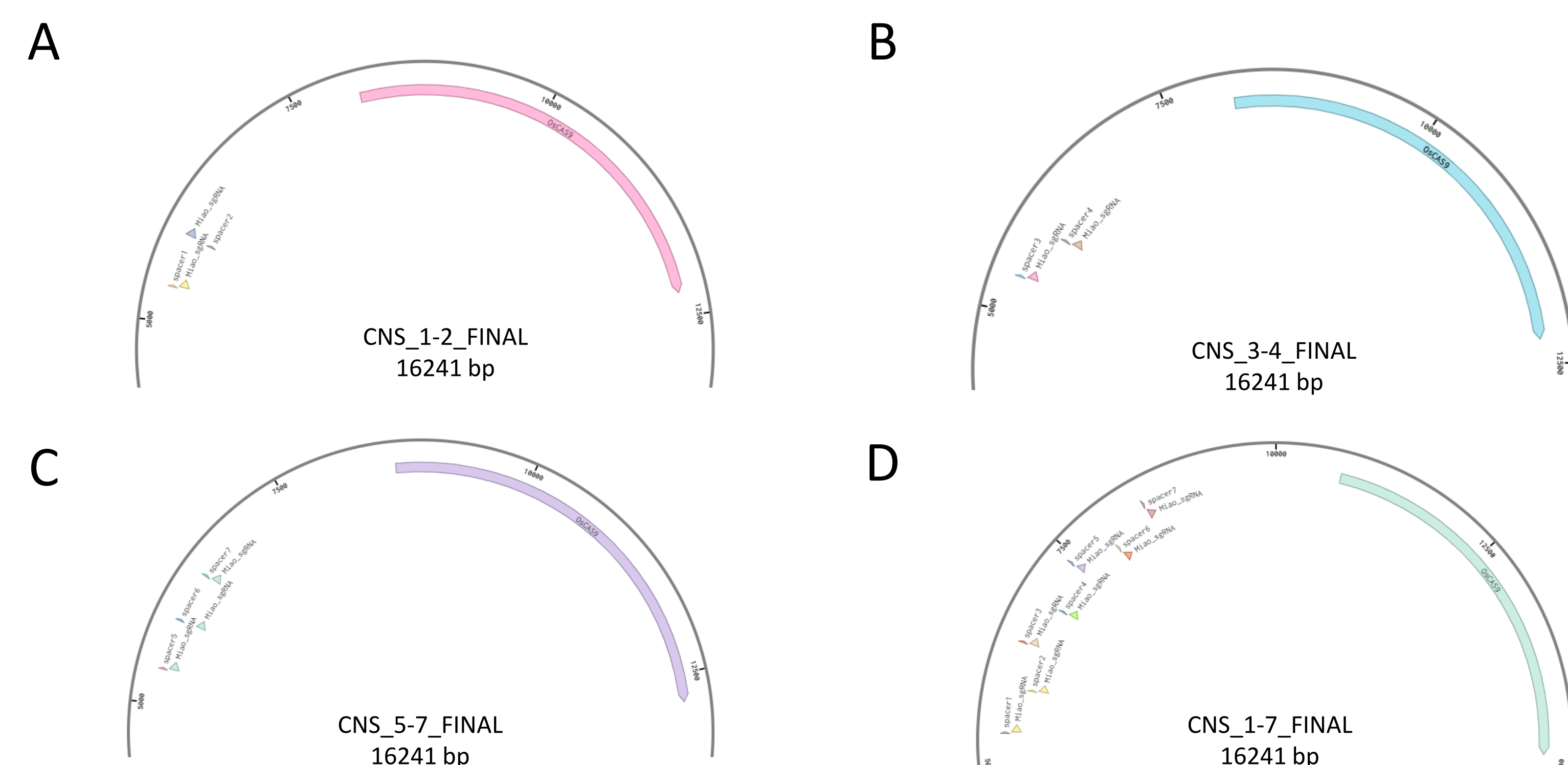


Using Golden Gate Cloning to make constructs



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



Spacers are complimentary DNA sequences that are used to recognize the target sequence. The 4 final constructs will have the following combination of spacers: (A) spacer 1-2, (B) spacer 3-4, (C) spacers 5-7, and (D) spacers 1-7.

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.

Acknowledgement

The author would like to thank the members of the Bartlett Lab and the CAFE Summer Scholar Program for their support.

References

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- Teisher, J. K., McKain, M. R., Schaal, B. A., & Kellogg, E. A. (2017). Polyphyly of Arundinoideae (Poaceae) and evolution of the twisted geniculate lemma awn. *Annals of Botany*, 120(5), 725-738.