Understanding an Agriculturally Important Grass Trait With CRISPR

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Grass Physiology and Awns

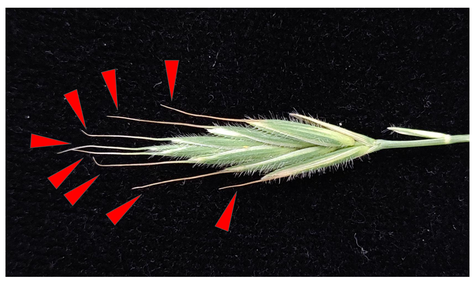
- Plant physiology, structure, and yield are determined by development, and ultimately genetics.
- Awns are grass organs that appear as thin, hairlike structures at the end of each seed.
- Awn morphology and function are diverse.

- Breeding for the lack of awns has led to easier harvesting and increased yield in the most commonly grown domesticated rice. [1]
- Eliminating awns in non-crop grasses could inhibit the weeds' ability to disperse and germinate in fields where they are unwanted.

Rice seeds with and without awns. [1]

Brachypodium distachyon Awns

- The Bartlett lab researches awn development in the model grass species *B. distachyon*.
- Determining the genes that regulate awns in *B. distachyon* could be agriculturally significant for closely related crops or weeds.



B. distachyon awns, indicated by red arrows.

Awn Genetics in a Related Species

- The barley mutant "*lks2"* has awns that are 50% shorter. [2]
- The *lks2* gene, involved in awn elongation, is in a family of transcription factor genes called "*SHORT INTERNODES"* (*SHI*).

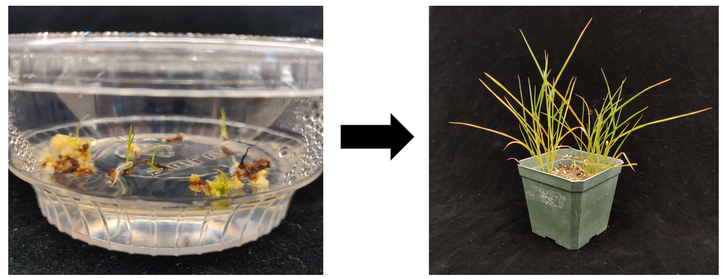
- *B. distachyon* has three *SHI* family genes. Is there a shared function of their *SHI* genes and *lks2* to regulate awn development?
- A triple mutant in *B. distachyon* with altered copies of each *SHI* gene may have a mutant awn phenotype. If so, the *SHI* genes must be essential for normal awn development.
- CRISPR-Cas9 genome editing could be used to create a *SHI* triple mutant.

WT *lks2* [2]

B. distachyon CRISPR-Mediated Mutagenesis

- One advantage of CRISPR-Cas9 mutagenesis is that the transgenic (foreign) DNA can be bred out of the mutant line to create non-GMO crops with targeted mutations that are agriculturally beneficial.
- To make mutations using CRISPR-Cas9 genome editing, target sequences are combined into a DNA plasmid that has the CRISPR-Cas9 protein.
- The process of assembling a final DNA vector takes several steps, and ends with the plasmid being inserted into *Agrobacterium tumefaciens*.
- Wild-type plant tissue undergoes an *Agrobacterium*-mediated transformation, and is then regenerated on plant media and in soil.

Transformed *B. distachyon* plantlets on media.



Transformed *B. distachyon* plants in soil.

Progress on *SHI* Genes

- The CRISPR-Cas9 protein is directed by a short sequence of RNA, and makes a double-strand break in the genomic DNA.
- The mutations caused by CRISPR-Cas9 gene editing result from the DNA being urgently repaired after a double-strand break.
- A previous transformation was done on the three *SHI* genes in *B. distachyon* with one target sequence per gene, but there were no meaningful mutations.
- The plasmid I am assembling uses two target sequences per *SHI* gene that are around 200 base pairs apart.
- These targets together should form dropout mutations in the genes' coding sequences.

Acknowledgements

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References

1. Luo, J., H. Liu, T. Zhou, B. Gu, X. Huang, Y. Shangguan, J. Zhu, Y. Li, Y. Zhao, Y. Wang, Q. Zhao, A. Wang, Z. Wang, T. Sang, Z. Wang, and B. Han. 2013. *An-1* Encodes a Basic Helix-Loop-Helix Protein That Regulates Awn Development, Grain Size, and Grain Number in Rice. *Plant Cell* 25:3360–3376.
2. Yuo, T., Y. Yamashita, H. Kanamori, T. Matsumoto, U. Lundqvist, K. Sato, M. Ichii, S. A. Jobling, and S. Taketa. 2012. A *SHORT INTERNODES (SHI)* Family Transcription Factor Gene Regulates Awn Elongation and Pistil Morphology in Barley. J*ournal of Experimental Botany* 63:5223–5232.