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Research Category: GDER **Duration of Award:** 1 Year

Project Title: Engineering Gene-for-Gene Resistance to Fusarium Head Blight in Wheat and Barley

PROJECT 1 ABSTRACT

(1 Page Limit)

This proposal focuses on a novel approach for engineering resistance to Fusarium Head Blight (FHB) in wheat and barley. Our strategy represents an extension of our work on the Arabidopsis disease resistance protein, RPS5, which mediates recognition of the cysteine protease, AvrPphB, by sensing cleavage of an Arabidopsis target of AvrPphB, PBS1. We have shown that novel disease resistance traits can be introduced into soybean, which also possesses a resistance protein functionally equivalent to RPS5, and that modification of a soybean PBS1 protein can confer resistance to *Soybean mosaic virus*. These findings provide compelling evidence that synthetic PBS1 decoys can be used to introduce new-to-nature resistance traits in crops.

We now seek funds to extend our work by engineering durable resistance to *F. graminearum (Fg)* in wheat and barley. We have already shown that wheat and barley activate cell death in response to AvrPphB and contain PBS1 homologs that can be cleaved by AvrPphB. We, therefore, predict this strategy can be used to introduce novel resistance against FHB by deploying PBS1 decoys that are targeted by *Fg*-secreted proteases. **This proposal thus addresses the USWBSI GDER goal: utilizing new technologies to develop effective FHB resistance. Additionally, this strategy could be deployed by gene-editing rather than transgenesis, as only seven amino acids of the PBS1 homologs need to be changed.**

Specifically, our project objectives are:

1) Characterize the substrate specificity of the *Fg* effector proteases. Identification of cleavage site sequences for each of the candidate proteases will be determined using a high-throughput, yeast-based system. In parallel, we will also test whether the candidate *Fg* proteases are required for virulence.

2) Generate PBS1-based decoy targets that activate resistance in wheat and barley upon cleavage by *Fg* proteases. Based on the results from objective one, we will generate wheat and barley PBS1 decoy proteins and confirm that they are cleaved by the corresponding protease using a transient expression system in *Nicotiana benthamiana*. In parallel, we will transiently express the PBS1 derivatives and corresponding protease, along with a reporter of cell viability in wheat or barley protoplasts to test whether protease-dependent cleavage activates an immune response *in planta*.

3) Initiate transformation of susceptible wheat and barley varieties with the PBS1 derivatives. Although we are not seeking funds for generation and testing of FHB-resistant wheat and barley lines in this proposal, a positive result from objective two would greatly strengthen our ability to obtain such funds.