Project Abstract

Project Title:	Mitigate Fusarium head blight in wheat by knockdown of defense repressors	
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The salicylic acid (SA) receptor NPR1 has an important role in controlling *Fusarium graminearum* infection in Arabidopsis and wheat. NPR3 and NPR4 are SA-binding proteins that repress NPR1 signaling to function as 'susceptibility' genes. SA binding inhibits NPR3/NPR4 defense suppression function. We hypothesize that knockdown of *WhNPR3* and *WhNPR4*, the *NPR3* and *NPR4* orthologs in wheat, respectively, will stabilize wheat NPR1 to promote stronger defenses and resistance to FHB. The goals of this project build upon the success of our ongoing USWBSI-supported project to knock down susceptibility gene function for mitigating FHB.

Project Objectives:

1. Develop RNAi lines to reduce *WhNPR3* and *WhNPR4* expression
2. Identify mutations in *WhNPR3* and *WhNPR4*
3. Characterize response to *F. graminearum* infection in *WhNPR3* and *WhNPR4* knockdown lines.

These objectives are relevant to the FY22 priorities of GDER to (i) Identify native and induced wheat and barley gene variants that improve FHB resistance and/or reduce DON accumulation, and (ii) validate candidate wheat and barley genes for resistance or susceptibility to FHB and/or reduced DON accumulation.

Expected Outcomes: (Objective 1) RNAi constructs to knockdown *WhNPR3* and *WhNPR4* expression will be generated and wheat lines silenced for their expression identified; (Objective 2) Wheat mutants at *WhNPR3* and *WhNPR4* loci will be identified and markers that distinguish the mutant from the wildtype allele will be generated and used to identify homozygous mutant lines. (Objective 3) Knockdown of *WhNPR3* and *WhNPR4* is expected to reduce limit fungal growth, FHB severity, and DON accumulation by promoting robust activation of plant defenses.

Recombinant RNAi constructs targeting *WhNPR3* and *WhNPR4* will be generated and provided to the wheat transformation lab at Kansas State University for transgenic wheat generation. The efficacy of RNAi in knocking down *WhNPR3*/*WhNPR4* expression will be evaluated by qRT-PCR. TILLING lines with mutations in *WhNPR3* and *WhNPR4* will be obtained from University of California-Davis and SeedStor. Markers that distinguish the wildtype from mutant alleles will be generated to facilitate identification of homozygous mutants and lines with mutations at more than one homeolog. The effect of *WhNPR3/ WhNPR4* knockdown on fungal growth, FHB severity, mycotoxin accumulation, and activation of defenses will be tested. Past success with these approaches will facilitate the timely accomplishment of project goals.