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PROJECT 2 ABSTRACT
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The goal of this project is to engineer barley (*Hordeum vulgare, Hv*) plants (cv. Conlon) using CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated 9 nuclease) technology to disrupt genes that condition susceptibility to *Fusarium graminearum* (*Fg*) infection. CRISPR-editing with transiently-expressing gRNA/Cas9 DNA offers the advantage of multigene-targeting and producing transgene-free, gene-edited and FHB resistant barley plants. We propose to initially CRISPR-edit barley genes encoding homoserine kinase (*HvHSK*), 2-oxoglutarate Fe(II)-dependent oxygenase (*Hv2OGO*) and ethylene insensitive 2 (*HvEIN2*) using the transient and integrating vectors. Arabidopsis *dmr1* and *dmr6* mutant plants with *AtHSK* and *At2OGO* knocked-out have been shown to be *Fg* resistant. RNAi-*TaEIN2* knocking-down wheat plants are *Fg* resistant. Choosing these known *Fg* targets will allow us to test the efficacy and efficiency of the CRISPR technology in barley.

Our specific objectives for this 2-year project are: (1) Construction of barley *HvHSK, Hv2OGO* and *HvEIN2* transient and integrating CRISPR-editing vectors; (2) Production of *HvHSK-, Hv2OGO-* and *HvEIN2*-edited barley plants (cv. Conlon). (3) Evaluation of *HvHSK, Hv2OGO* and *HvEIN2* mutant barley plants for FHB resistance.

These objectives address the following FY18-19 Research Priorities: #1. Identify native wheat and barley gene variants that improve FHB resistance and/or reduce DON accumulation: editing barley *HvHSK, Hv2OGO* and *HvEIN2* genes will test their role in FHB susceptibility; #3. Develop effective FHB resistance and/or reduced DON accumulation through transgenic strategies: barley *HvHSK, Hv2OGO* and *HvEIN2* genes will be precisely mutated by CRISPR-editing technology, the interaction between barley and *Fg* will be disrupted, leading to FHB resistance and DON reduction; and #4. Incorporate new technologies for the generation of gene edited or transgenic wheat and/or barley: barley genes will be CRISPR-edited by the gRNA/Cas9 transient or integrating vectors leading to transgene-free and gene-edited barley plants.

The outcome of this project will be FHB resistant barley plants, and a powerful barley CRISPR-gene editing platform that can be used and shared by the USWBSI community to edit any barley gene. Demonstration of the efficacy of CRISPR-editing approach will provide a new and complementary approach for manipulating genomes of grain crops.