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PROJECT 1 ABSTRACT
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The goal of this project is to continue our effort in developing barley genetic engineering platform for the USWBSI barley community to employ transgene approach and the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated 9 nuclease) technology to discover genes involved in *Fusarium* head blight (FHB) susceptibility and to engineer FHB resistance in barley. We have used CRISPR-editing to knock-out Arabidopsis *AtEIN2* (encoding ethylene insensitive 2) and *At2OGO* genes [encoding 2-oxoglutarate Fe(II)-dependent oxygenase] and shown the mutant Arabidopsis plants are resistant to *Fusarium graminearum* (*Fg*). We have complement-transformed the *AtEIN2*-knock out (KO) and *At2OGO*-KO plants with the barley *HvEIN2* and *Hv2OGO* cDNAs and shown the complemented Arabidopsis-KO mutant plants regain FHB susceptibility, indicating *HvEIN2* and *Hv2OGO* are involved in barley-*Fg* interaction and knocking-out these genes will lead to FHB resistance in barley. Another identified FHB susceptibility gene is the *HSK* (*homoserine kinase*). We have developed our barley CRISPR-editing platform and produced a few *Hv2OGO* barley (cv. Conlon) mutants.

Our specific objectives for this project are: (1) Optimization of barley transformation system, (2) Production of *HvEIN2-, HvHSK*- and *Hv2OGO*-edited Conlon and Genesis plants and evaluation of mutant plants' resistance to FHB, and (3) Production of *HvUGT* promoter-edited Morex mutant plants and evaluation of mutant plants' UGT level in relationship to FHB resistance.

The objectives proposed above address the FY20-21 Research Priorities #1, "Identify native and induced wheat and barley gene variants that improve FHB resistance and/or reduce DON accumulation" and #3, "Utilize new technologies to develop effective FHB resistance and/or reduced DON accumulation". Barley *HvEIN2, HvHSK* and *Hv2OGO* genes will be mutated by our CRISPR-editing platform, the interaction between barley plant and *Fg* will be disrupted, leading to FHB resistance and DON reduction. Editing barley *HvUGT* promoter will allow us to study the *HvUGT* gene expression kinetics and its role in FHB resistance.

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 barley research community to edit any barley gene for FHB resistance as well as other barley research areas. Demonstration of the efficacy of this approach will provide a new and complementary approach for manipulating genomes of grain crops.