Project FY24-GD-004: Optimization of Fhb7 to enhance FHB resistance in barley

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1. What are the major goals and objectives of the research project?

Objective 1. Fine-tune Fhb7 expression by inserting it in different locations of a host gene.

2. What was accomplished under these goals or objectives? *(For each major goal/objective, address these three items below.)*

a) What were the major activities?

1. We have synthesized 3 sgRNAs targeting the 5' UTR of the *mlo* gene. We also made a construct for synthesis of one of the sgRNAs by in vitro transcription.
2. We have made two Fhb7 constructs for improving its enzyme activity per an *in vitro* study, i.e., Fhb7-M10 and Fhb7-V29P-M10. The M10 allele carries 20 amino acid substitutions in the middle of the protein. According to the published research (Yang et al 2024, JACS Au 4(2):619–634), the M10 substitution increases enzyme activity over 200 folds, and the V29P substitution increases enzyme activity more than four times. Thus, it is expected that these Fhb7 alleles will significantly enhance FHB resistance in barley.
3. Biolistic transformation of >1,000 Excelsior Gold (EG; an elite two-row barley cultivar from the Cornell University) embryos with the *mlo*-targeting RNP plus Fhb7-M10 donor DNA.

b) What were the significant results?

More than 700 calluses have been generated. So far 28 seedlings have been regenerated. Screening of the first batch of 16 regenerated seedlings detected three *Fhb7*-insertion plants. Based on PCR screening from different portions of Fhb7, partial copies of Fhb7 were inserted.

c) List key outcomes or other achievements.

1. Plasmid constructs are up to request.
2. An improved procedures for Biolistic transformation of immature barley embryos.

3. What opportunities for training and professional development has the project provided?

This project provided **opportunities for training and professional development to a** Ph.D. student and Research scientist. Ph.D. student **Wei Jiang,** previously trained in Oil seeds genetics, worked on molecular biology (cloning), tissue culture, and plant pathology (preparation Fusarium conidia spores and FHB inoculation) in the project. Research assistant **Xi Chen**, previously trained in rice developmental biology, worked on the project in PCR screening of Biolistic transformed plants in the project.

4. How have the results been disseminated to communities of interest?

The results were disseminated conference presentations.

5. What do you plan to do during the next reporting period to accomplish the goals and objectives?

We will continue to transform the constructs and the new donor allele to EG and screening for insertion event.