USDA-ARS | U.S. Wheat and Barley Scab Initiative

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-2-137
USDA-ARS Agreement Title:	Improving Fusarium Head Blight (FHB) Resistance in Barley
Principle Investigator (PI):	Wanlong Li
Institution:	South Dakota State University
Institution UEI:	DNZNC466DGR7
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$96,899
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Period of Performance:	May 1, 2022 – April 30, 2024
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
TSCI	Transfer Fhb7 to Barley Through CRISPR-mediated Targeted Gene Insertion	$96,899
	FY22 Total ARS Award Amount	$96,899

I am submitting this report as an: ☒ Annual Report

I certify to the best of my knowledge and belief that this report is correct and complete for performance of activities for the purposes set forth in the award documents.

Principal Investigator Signature

July 24, 2023
Date Report Submitted

‡ BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
EC-HQ – Executive Committee-Headquarters
FST-R – Food Safety & Toxicology (Research)
FST-S – Food Safety & Toxicology (Service)
GDER – Gene Discovery & Engineering Resistance
HWW-CP – Hard Winter Wheat Coordinated Project
MGMT – FHB Management
MGMT-IM – FHB Management – Integrated Management Coordinated Project
PBG – Pathogen Biology & Genetics
TSCI – Transformational Science
VDHR – Variety Development & Uniform Nurseries
NWW –Northern Soft Winter Wheat Region
SPR – Spring Wheat Region
SWW – Southern Soft Red Winter Wheat Region

Project 1: Transfer Fhb7 to Barley Through CRISPR-mediated Targeted Gene Insertion

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1. **What are the major goals and objectives of the research project?**
   The Objectives of this project are:
   (1) Generate transgenic barley expressing both the CRISPR/Cas9 and the *Fhb7* donor,
   (2) Evaluate the *Fhb7* function in transgenic barley, and
   (3) Screen the transgenic plants for targeted *Fhb7* insertion events.

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?**
   Objective 1. (1) Development of barley transformation system by Biolistic bombardment using an optimized CRIPSR/Cas construct (pWL2168) together with 5'-phosphorylated and phosphorothioate linkage -protected PCR product of the *Fhb7* gene. pWL2168 carries a *SpRy* driven by the *Ubi* promoter, *HygR* fused to *TaWox5*, and sgRNA cassette targeting mlo in the barley genome. (2) Using this system, we have bombarded more than three-thousand embryos of Excelsior Gold (EG), an elite two-rowed malting barley cultivar from Cornell University.

   Objective 2. Continued screening of the 10 more T_1 populations of the Gold Promise (GP) CRIPSR/Fhb7-donor transgenic plants from the first year by detached leaf assay.

   Objective 3. PCR careening of 288 EG regenerated plants for *Fhb7* insertion.

   b) **What were the significant results?**
   Objective 1. (1) A new transformation system developed for targeted insertion: an optimized CRIPSR/Cas construct for bombardment together with 5'-phosphorylated and phosphorothioate linkage -protected PCR product of *Fhb7* gene. An advantage of this system is that *Fhb7* donor DNA is independent of the CRISPR transgene. Thus, the Fhb7 donor/CRISPR ratio can be adjusted and the *Fhb7* insertion can be detected by PCR using *Fhb7*-specific primers. (2) More than three thousand EG embryos were bombarded, from which 302 plants were regenerated.

   Objective 2. (1) As reported in 2021, the detached leaf assay of T_1 plants and wild-type GP was conducted side-by-side with Chinese Spring (CS; FHB susceptible) and RWG52, which carries *Fhb7* in the CS background, showing that 72 hours after inoculation, fungus is growing on CS leaves (level 1) but not RWG52 leaves (level 0) and fungus is growing on the GP leaves and led to chlorosis (yellowing; level 2), indicating that barley is much more susceptible to *F. graminearum* compared to wheat. (2) Assay of Screening >200 plants from 10 more T_1 families showed that a majority of transgenic plants had the level-1 reaction, and a small number of seedlings had the level-0 reaction. Because DON causes leaf photobleaching, the degree of leaf yellowing is an indicator of the presence of DON levels. Thus, our result suggests that Fhb7 functions in barley: degrading DON and suppressing the growth of F. graminearum. The variation in reaction

types of the T_1 seedling is possibly related to the *Fhb7* expression level or transgene dosage. (3) Four T_1 lines were submitted to Steffenson's lab or field test. Late planting due to delay of USDA-APHIS permission and high temperature in the spring, however, may affect the testing results.

Objective 3. (1) Screening the GP populations for *Fhb7* insertion. In the first year, nine primer combinations were selected for amplifying the insertion junctions. The T_1 plants showing Level-1 or -0 reaction were screened, but positive amplification was detected. (2) A population of 288 EG regenerated plants from the bombardments were screened using Fhb7-specific primers, and a putative insertion line (plant #234), was identified very recently. The proximal promoter and coding region are confirmed by PCR sequencing. Based on PCR assay and incomplete sequence of the upstream junction, *Fhb7* was inserted in the last exon of the *Mlo* in the same genomic orientation as the *Mlo* gene, the recipient locus. While screening more populations, we are in the process of validating and characterizing this insertion event using the T_1 populations.

c) List key outcomes or other achievements.
Plasmid constructs and seeds of *Fhb7* transgenic plants are up to request.

3. What opportunities for training and professional development has the project provided?
This project has been providing opportunities for training undergraduate and graduate students and technicians. **Kuol Arop**, a senior undergraduate majoring in biology, was trained in molecular biology by PCR amplification and sequence analysis of the *Fhb7* coding region from the *Thinopyrum elongatum* population. Kuol presented his results in the Undergrad Research Conference at SDSU in April 2023 and won the Outstanding Poster Presentation award. **Mohd Kyum**, a graduate research assistant majoring in biology, was trained in molecular biology including gene editing, plasmid construction and plant genotyping, barley transformation by biolistic bombardment, and plant pathology including preparation of *Fusarium* conidium spores and test of the transgene by detached leaf assays. He left the project for The Sunshine State, Florida, in January 2023, and a postdoc is expected to join the project late next month. **Yanhang Zhang,** a lab technician, was trained in tissue culture and barley transformation**.**

4. How have the results been disseminated to communities of interest?
The results from this TSCI project have been presented in the National Fusarium Head Blight Forum 2022.

Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your FHB work that were a result of funding from your FY22 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period** should be included.

Did you publish/submit or present anything during this award period May 1, 2022 – April 30, 2023?

☒ Yes, I've included the citation reference in listing(s) below.

☐ No, I have nothing to report.

Journal publications as a result of FY22 award

List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Include any peer-reviewed publication in the periodically published proceedings of a scientific society, a conference, or the like.

Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published [include DOI#]; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Books or other non-periodical, one-time publications as a result of FY22 award

Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.

Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (book, thesis, or dissertation, other); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Other publications, conference papers and presentations as a result of FY22 award

Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication.

Li W, Kyum M, Zhang Y, Santantonio N, Sorrells ME, Steffenson B, Xu SS. (2022). Using wheat genes to improve barley FHB resistance. Proceedings of the 2022 National Fusarium Head Blight Forum; Tampa, FL. December 4 – 6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf