

FY21 Performance Progress Report**Due date:** July 26, 2022**Cover Page**

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Phone:	605-688-5743
Fiscal Year:	2021
USDA-ARS Agreement ID:	59-0206-1-199
USDA-ARS Agreement Title:	Improving FHB Resistance in Barley
FY20 USDA-ARS Award Amount:	\$96,899
Recipient Organization:	South Dakota State University Department of Biology and Microbiology 252 McFadden Biostress Laboratory, 1390 College Avenue Brookings, SD 57007
DUNS Number:	929929743
EIN:	46-6000364
Recipient Identifying Number or Account Number, if any:	
Project/Grant Period:	6/1/21 - 5/31/23
Reporting Period End Date:	5/31/2022

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
FY21 Total ARS Award Amount		\$96,899

I am submitting this report as an: Annual Report Final 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 15, 2022

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

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 plasmid constructs for barley transformation; (2) transformed ~2000 (70 plates) barley embryos of Gold Promise (GP) by agrobacterium-mediation and ~1000 (36 plates) GP embryos and ~700 (22 plates) Excelsior Gold (EG; an elite two-row barley cultivar from the Cornell University) embryos by Biolistic-bombardment; and (3) screening of 247 T₀ and 384 plants of 6 T₁ families for transgenes.

Objective 2. (1) Preparation of conidia spores from *F. graminearum* GZ3639; (2) establishment of detached leaf assay method in barley; and (3) conduction of detached leaf assays on 39 T₀ and 147 plants of 5 T₁ families.

Objective 3. (1) Development of 12 primers (six from *mlo* sites and six from *Fhb7* sites) for 18 combinations to detect targeted insertions of *Fhb7* in the *mlo* locus by amplifying the *Fhb7*-*mlo* junctions and (2) screening 39 T₀ plants for targeted insertion.

b) What were the significant results?

Objective 1. (1) Two plasmid constructs were developed: an all-in-one construct including CRISPR/Cas9 and *Fhb7* donor for *Agrobacterium*-mediated transformation and an optimized CRISPR/Cas construct for bombardment together with 5'-phosphorylated and phosphorothioate linkage -protected PCR product of *Fhb7* gene. (2) *Agrobacterium*-mediated transformation produced 247 seedlings based on Hygromycin selection, and PCR screening of these seedlings identified 39 transgenic T₀ plants. From the Biolistic bombardment transformation, 28 GP calluses and 7 EG calluses are under regeneration. (3) Of the 384 seedlings screened, a total of 284 seedlings were found carrying the transgene, and the *Fhb7* transgene is segregating in the T₁ populations screened into 3 (present):1 (absent), indicative of single copy of transgene insertion.

Objective 2. (1) Detached leaf assay of wild-type GP side-by-side with Chinese Spring (CS) (FHB susceptible) and RWG52, which carries *Fhb7* in the CS background, showed 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) Detached leaf assay in T₁ transgenic seedlings showed that most of the seedlings had the level-1 reaction, but a small number of seedlings had the level-0 reaction, suggesting that Fhb7 functions in barley in suppressing the growth of *Fusarium graminearum*. The variation in reaction types of the T₁ seedling to *F. graminearum* is possibly related to the Fhb7 expression level or transgene dosage.

Objective 3. Of the 18 primer combinations tested at different annealing temperatures on both orientations, nine primer combinations gave clear background on the wild-type GP. These primers were used to screen the 39 T₀ transgenic plants for targeted insertion of Fhb7 in the mlo locus, but no positive amplifications were detected in the T₀ population.

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 sophomore undergraduate majored in biology, has been trained in molecular biology by PCR amplification and sequence analysis of the *Fhb7* coding region from the *Thinopyrum elongatum* population. **Mohd Kyum**, a graduate research assistant majored in biology, has been 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. **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 the TRSC project have been presented in the GDER mid-year meeting and *Fusarium* Forum 2021.

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 FY21 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?

- Yes, I've included the citation reference in listing(s) below.
 No, I have nothing to report.

Journal publications as a result of FY21 grant 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 FY21 grant 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 FY21 grant award

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

- Wanlong Li. Using wheat genes to improve barley FHB resistance. GDER mid-year meeting. May 27, 2021
- Wanlong Li, Yanhang Zhang, Nicholas Santantonio, Mark E. Sorrells, Brian Steffenson, Steven S. Xu. Using wheat genes to improve barley FHB resistance. *Proceedings of the 2021 National Fusarium Head Blight Forum*; Virtual. December 6-7, 2021. Retrieved from: <https://scabusa.org/forum/2021/2021NFHBForumProceedings.pdf>