USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY20 Annual Performance Progress Report
Due date: July 29, 2021

Cover Page

Principle Investigator (PI):	Guihua Bai
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Fiscal Year:	2020
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Identification and Deployment of FHB Resistance QTL in US Hard Winter Wheat
FY20 USDA-ARS Award Amount:	$ 130,000
Project/Grant Reporting Period:	5/1/20 - 4/30/21
Reporting Period End Date:	4/30/2021

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
HWW-CP	Identification and Deployment of FHB Resistance QTLs in US Hard Winter Wheat	$ 80,000
GDER	Function Analysis of *FHB1* using BSMV-mediated CRISPR/Cas9 Gene Editing System	$ 50,000
	FY20 Total ARS Award Amount	**$ 130,000**

7/26/2021

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R- Research
S – Service (DON Testing Labs)
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
EC-HQ – Executive Committee-Headquarters
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
SPR – Spring Wheat Region
NWW – Northern Soft Winter Wheat Region
SWW – Southern Soft Red Winter Wheat Region

FY20 Annual Performance Progress Report
PI: Bai, Guihua
USDA-ARS Agreement #: N/A
Reporting Period: 5/1/20 - 4/30/21

Project 1: *Identification and Deployment of FHB Resistance QTLs in US Hard Winter Wheat*

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

1) Map QTLs for type I resistance in Everest
2) Pyramid QTLs on chromosome 5A from PI 277012 and 2DL QTL with Fhb1 in Overland and Everest backgrounds
3) Develop KASP markers for *Fhb7*
4) Pyramid Fhb7 with *Fhb1*

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) The populations EverestFhb1(R)x GarrisonFhb1(S) and EverestFhb1(R)x KS061406LN-47Fhb1-244(S) were genotyped and phenotyped for type I resistance using spraying method in greenhouses and field experiments, but measurable size repeatable QTL was not identified due to that Type I resistance is highly vulnerable to environmental variation.
2) Both 5A and 2DL QTL were pyramided with *Fhb1* in Everest and Overland backgrounds. Selected lines were phenotyped in KSU FHB nursery and most showed high resistance under high FHB pressure this year.
3) Several near-diagnostic markers for *Fhb7* were developed and two of them were validated in different natural populations. Initial data were presented in 2020 FHB Forum and the first draft of the manuscript is completed and now under review by coauthors.
4) Crosses were made between EverestFhb1 and Jimai22Fhb7 and selected progeny were crossed to Everest.

b) What were the significant results?

1) Selected lines with *Fhb1* and 2DL QTL showed high resistance in both greenhouse and field conditions.
2) *Fhb7* markers we developed were highly diagnostic in a US winter wheat panel. The manuscript will be submit to a journal soon.

c) List key outcomes or other achievements.

1) Selected line with *Fhb1* and 2DL QTL showed high resistance in greenhouse field conditions.
2) *Fhb7* markers we developed are highly diagnostic in US winter wheat.
3) We converted MRASeq, a genome-wide genotyping system, from Ion Proton version into Illumina sequencing-based assays. We are further optimizing the high-

throughput genotyping platform for marker-assisted background selection and genomic selection to replace GBS platform.

3. **Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns and/or restrictions, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.**

No

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

Three graduate students (Dr. Umara Rena, Ms. Ruolin Bian and Mr. Yuzhou Xu) have worked on these projects. They learnt high-throughput DNA isolation, PCR, gene cloning and sequencing, FHB inoculation and taking note for disease and other traits, marker and QTL data analysis, poster presentation and writing reports.

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

- Selected lines with high level of FH resistance will be distributed to breeding programs for further evaluation of FHB resistance or used as parents for further crosses later this year.
- One graduate student deposited her thesis in KSU library for public access.
- Several graduate students and post-docs gave presentations in 2020 FHB Forum.
- The first draft of the manuscript on *Fhb7* marker development is completed and now under review by coauthors.

Project 2: *Function Analysis of FHB1 using BSMV-mediated CRISPR/Cas9 Gene Editing System*

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

1) Use gene editing to validate the functions of TaPFT and TaHRC-R.
2) Determine interactions between TaHRC and TaPFT, and TaHRC and other genes.

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) Made a cross between Cas9-overexpressed (Cas9-OE) 'Bobwhite' and 'Ning7840' and selected F2 progenies carrying all three genes (Cas9, TaPFT and TaHRC-R) using gene-specific markers.
2) Optimized BSMV-mediated gene editing system by using floral dip agroinfiltration method and adding RNA mobility sequences to the gRNA in the viral vectors. Using this system
3) Constructed specific gRNAs targeting at both TaPFT and TaHRC-R and delivered them into the Cas9-OE 'Ning7840' lines using the optimized systems, and recovered 10 TaHRC_R-edited and two TaPFT-edited M0 mutants and all the M1 mutant lines will be evaluated for FHB resistance.

Objective 2:

1) Constructed a TaHRC-S bait vector and screened wheat cDNA library using ULTImate Y2H-Gal4 system and identified several TaHRC-S interacting proteins.
2) Confirmed TaCAXIP4 protein as a candidate that interacts with TaHRC-S by co-transformation in yeast and BiFC assay in planta and determined only N-terminus with a nuclear localization signal (NLS) domain of TaHRC-S plays essential role during the interaction with TaCXIP4.
3) Determined that TaHRC_S sequesters TaCAXIP4 to suppress the expression of TaCAX1 to affect calcium signaling transduction during FHB infection using calcium suppression assay.
4) ROS assay determined that TaHRC_S suppresses chitin-triggered plant immune responses during FHB infection by sequestering TaCAXIP4 to maintain FHB susceptibility.
5) The protein-protein assay determined interaction between TaPFT and TaHRC-S in yeast.

b) What were the significant results?

1) Used our optimized BSMV-mediated gene editing system, we successfully edited two *Fhb1* candidate genes (*TaHRC_R* and *TaPFT*) and recovered stable M1 mutant lines of *TaHRC_R* and *TaPFT* from the infected Cas9-OE 'Ning7840' plants.
2) Identified TaCAXIP4 as an interacting protein to *TaHRC-S* and demonstrated that TaCAXIP4 plays a key role in calcium signaling pathway and chitin-triggered plant immune responses during FHB infection.

1. List key outcomes or other achievements.

1) Successfully optimized BSMV-mediated gene-editing system and new system showed improved editing efficiency.
2) Obtained mutant plants with edited TaHRC_R and TaPFT from Fhb1 resistant cultivar 'Ning7840'.
3) Identified TaCAXIP4 protein as a candidate interacting protein with TaHRC-S and demonstrated that TaHRC-S sequesters TaCAXIP4 to inhibit calcium-mediated defense responses and chitin-triggered plant immune responses to maintain FHB susceptibility during FHB infection.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns and/or restrictions, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

No

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

Trained one visiting scientist (Dr. Y. Pang) and one MS student (V with gene editing techniques

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

Co-PI Dr. Hui Chen was invited to give an oral presentation in USWBSI 2020 FHB Forum. Presented two posters in 2020 World Congress on *in vitro* Biology.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY20 award period (5/1/20 - 4/30/21). The term "support" below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student's stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY20 award period?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY20 award period?**
   ☒Yes ☐No
   If yes, how many? 1

3. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

4. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the FY20 award period (5/1/20 - 4/30/21). All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations.

NOTE: Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.

Name of Germplasm/Cultivar	Grain Class	FHB Resistance	FHB Rating (0-9)	Year Released
Nothing to report.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
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Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

Publications, Conference Papers, and Presentations

Instructions: Refer to the PR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY20 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (5/1/20 - 4/30/21**) should be included. If you did not publish/submit or present anything, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each citation, you **must** indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in the publication/ presentation. See example below for a poster presentation with an abstract:

Z.J. Winn, R. Acharya, J. Lyerly, G. Brown-Guedira, C. Cowger, C. Griffey, J. Fitzgerald, R.E. Mason and J.P. Murphy. 2020. "Mapping of Fusarium Head Blight Resistance in NC13-20076 Soft Red Winter Wheat." In: S. Canty, A. Hoffstetter, and R. Dill-Macky (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum (*p. 12.), Virtual; December 7-11. Online: https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

Zheng T., Chen H., Li L., Sun Z., Yuan M., Bai G., Humphreys G., and Li T. (2020) Integration of meta-QTL discovery with omics: towards a molecular breeding platform for improving wheat resistance to Fusarium head blight. The Crop J, https://doi.org/10.1016/j.cj.2020.10.006
Status: Published
Acknowledgement of Federal Support: No.

Books or other non-periodical, one-time publications.

Rana, U. S. 2020. Genetic mapping of quantitative trait loci conditioning Fusarium head blight resistance in hard winter wheat. Ph.D. Thesis, Kansas State University
Status: Published
Acknowledgement of Federal Support: Yes

Other publications, conference papers and presentations.

Leng, Y., Poudel, B., Bernardo, A., Bian, R., Karmacharya, A., Mullins, J., Bai, G., Xu, S.S., and Zhong. S. 2020. Identification and Molecular Mapping of a Major QTL on Chromosome 2A Conferring Resistance to Fusarium Head Blight in Emmer Wheat. Proceedings of the 2020 National Fusarium Head Blight Forum. Virtual, Dec. 7-11, 2020.
Status: Abstract published and poster presented
Acknowledgement of Federal Support: Yes.

Bian, R., Bernardo, A., Amand, P. St., Fritz, A., Rupp, J. and Bai, G. 2020. Mapping Quantitative Trait Loci for Fusarium Head Blight Resistance and Agronomic Traits in a Mutant from a Wheat Cultivar Jagger. Proceedings of the 2020 National Fusarium Head Blight Forum. Virtual, Dec. 7-11, 2020.
Status: Abstract published and poster presented
Acknowledgement of Federal Support: Yes.

Chen, H., Su, Z., Hao G., and Bai G. 2020. Functional Characterization of TaHRC in Regulating FHB Resistance in Wheat. Proceedings of the 2020 National Fusarium Head Blight Forum. Virtual, Dec. 7-11, 2020.
Status: Abstract published and Invited talk)
Acknowledgement of Federal Support: Yes.

Xu, Y., Bernardo, A., Amand, P.St., Fritz, A.,
Zhang G., Rupp, J. and Bai, G. 2020. Characterization of Quantitative Trait Loci (QTLs) for Wheat Resistance to Fusarium Head Blight using a Jagger Mutant. Proceedings of the 2020 National Fusarium Head Blight Forum. Virtual, Dec. 7-11, 2020.
Status: Abstract published and poster presented
Acknowledgement of Federal Support: Yes.

Zhao, L., Ge, W., Lv, Z., Xu, S., Bernardo, A.,
Wang, H., Kong, L. and Bai, G. 2020. Development of Diagnostic Markers for Fhb7. Proceedings of the 2020 National Fusarium Head Blight Forum. Virtual, Dec. 7-11, 2020.
Status: Abstract published and poster presented
Acknowledgement of Federal Support: Yes.

Chen, H., Tian, B., Liu, Y., Trick, H.N. and Bai, G., 2020. “Development of an Efficient Barley Stripe Mosaic Virus-mediated CRISPR/Cas9 System for Gene Editing in Wheat”. In: IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY-ANIMAL (Vol. 56, No. SUPPL 1, pp. 47-48). Virtual; Jun 6-10.
Status: Abstract published and poster presented
Acknowledgement of Federal Support: YES

Tian, B., Chen, H., Bai, G. and Trick, H.N., 2020. "Plant Transformation and Genome Editing Systems for Hexaploid Wheat". In: IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY-ANIMAL (Vol. 56, No. SUPPL 1, pp. 48-49). Virtual; Jun 6-10.

Status: Abstract published and poster presented

Acknowledgement of Federal Support: YES