USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY18 Performance Report
Due date: July 12, 2019

Cover Page

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Phone:	785-532-1124
Fiscal Year:	2018
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Identification and Deployment of FHB Resistance QTL in US Hard Winter Wheat.
FY18 USDA-ARS Award Amount:	$ 105,000

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Barley Stripe Mosaic Virus-Mediated CRISPR/Cas9 Genome Editing for FHB Resistance Improvement.	$ 30,000
HWW-CP	Identification and Deployment of FHB Resistance QTL in US Hard Winter Wheat.	$ 75,000
	FY18 Total ARS Award Amount	**$ 105,000**

7/1/2019

Principal Investigator Date

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* MGMT – FHB Management
FST – Food Safety & Toxicology
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

Project 1: *Barley Stripe Mosaic Virus-Mediated CRISPR/Cas9 Genome Editing for FHB Resistance Improvement.*

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

Develop a new efficient gene delivery method to accelerate the utilization of genome editing technology in improvement of wheat resistance to FHB in breeding programs

2. What was accomplished under these goals? *Address items 1-4) below for each goal or objective.*

1) major activities

a). We successfully adopted the *Barley Stripe Mosaic Virus* (BSMV)-mediated genome editing system in wheat and used the system to edit a FHB susceptible gene *TaHRC in* Cas9-overexpressing (Cas9-OE) ‘Bobwhite’ transgenic line.

b). We made a cross between a locally adapted cultivar ‘Everest’ and a Cas9-OE ‘Bobwhite’ transgenic line. Its F2 progenies carrying Cas9 gene were selected for further *TaHRC* editing using this system. The selected positive *TaHRC*-edited lines in ‘Everest’ background showed improved FHB resistance.

c). We further crossed a Cas9-OE ‘Bobwhite’ transgenic line to other adapted cultivars including ‘Seneca’, ‘Java’, ‘Ning7840’ and ‘Clark’ and their F2 progenies carrying Cas9 gene will be selected for editing *TaHRC* using this system to validate the system.

2) specific objectives

a). Use gene editing to knock out *HRC* gene in ‘Bobwhite’ to validate function of the gene.

b). Transfer Cas9 gene into other adapted cultivars such as ‘Everest’ and ‘Clark’ to develop a new system for gene editing without direct transformation and limitation by plant genotypes.

3) significant results

a). We successfully recovered stable mutants from the edited ‘Bobwhite’ plants. FHB evaluation of the *TaHRC*-edited mutant plants showed significantly lower FHB severity than non-edited plants.

b). We successfully selected F2 progenies carrying Cas9 gene from the cross between ‘Everest’ and Cas9-OE ‘Bobwhite’ after *TaHRC* gene was edited and recovered three FHB resistant lines from the *TaHRC*-edited ‘Everest’ plants.

4) key outcomes or other achievements

a). We successfully edited *TaHRC* gene in ‘Bobwhite’ and confirmed that knocking out *TaHRC* significantly increased FHB resistance.

b). We successfully developed a new gene editing system without direct wheat transformation process and this system was confirmed to be successful by editing the *TaHRC* gene in ‘Everest’ without direct transformation and obtained resistant plant lines.

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

Trained two visiting scientists with gene editing techniques.

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

a). Presented a poster in 5th Plant Genomics and Gene Editing Congress.

b). Co-PI Dr. Hui Chen was invited to give an oral presentation in USWBSI 2018 FHB Forum and his presentation was highlighted in USWBSI Fusarium Focus, 2019 (P2, Volume 19, Issue 1).

Project 2: *Identification and Deployment of FHB Resistance QTL in US Hard Winter Wheat.*

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

a). Map QTL from moderately resistant cultivar Lyman and CI13227

b). Pyramid QTLs on chromosome 5A from PI 277012 with Fhb1 in Overland and Everest backgrounds

2. What was accomplished under these goals? *Address items 1-4) below for each goal or objective.*

1) major activities

a). Completed data analysis of Lyman/CI13227 F7 RILs, CI13227/Lakin F7 RILs and Lyman/Overley F7 RILs and QTL were identified for type II resistance in those populations

b). For the QTL pyramiding project, 200 Bc_2F_3 lines were phenotyped for one season in both greenhouse and field experiments and they are genotyped with new SNP markers and MRASeq for background analysis.

c). Evaluated Overland/Overley F7 population in both greenhouse and field experiment for type I and Type II resistance and obtained 500 F2 plants for 3 crosses of Garrison-Fhb1 lines x Everest-Fhb1 lines contrasting in type I resistance.

2) specific objectives

a). Identify QTLs in Lyman and CI 13227 and SNP markers linked to these QTLs for marker-assisted selection

b). Pyramid Fhb1 and other QTLs with both major and minor effects using marker-assisted selection and evaluate their individual and combined effects.

3) significant results

a). We identified 3 QTL in CI13227 and three QTL in Lyman. QTLs are different between the two sources. Some markers in the QTL regions are converted into KASP markers for MAS

b). Two 5AS QTLs were successfully pyramided with *Fhb1* in Everest and Overland backgrounds by marker-assisted backcrossing and their phenotypes were evaluated in two greenhouse and two field experiments. Lines with different numbers of QTL were selected.

4) key outcomes or other achievements

a). We determined the QTL in US wheat Lyman and CI13227 and developed KASP markers for these QTL. These KASP markers can be used for MAS in breeding.

b). We selected lines with all three QTLs (Fhb1, 5AS and 5AL) with improved agronomic traits in hard winter wheat backgrounds and these lines can be used as new sources of resistance to improve the level of FHB resistance in hard winter wheat.

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

Five graduate students (Ms. Yaoguang Li, Ms.Umara Rena, Ms.Ruolin Bian, Mr. Abdul Hashimi and Mr. Yuzhou Xu) have worked on these projects. They learnt high-througghput genotyping, FHB inoculation and disease note taking, marker and QTL data analysis, and writing reports.

4. 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
- Two graduate students are writing thesis and they will be deposited in KSU library for public access.
- Several manuscripts have been published.
- The paper on cloning of Fhb1 gene has been published in Nature Genetics and both USDA-ARS and Kansas State University had news release about this breakthrough. Also many other media such as journals published the news on this work.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY18 award period. 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 FY18 award period?**
   No
   If yes, how many?

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY18 award period?**
   Yes.
   If yes, how many?
   Four

3. **Have any post docs who worked for you during the FY18 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   No
   If yes, how many?

4. **Have any post docs who worked for you during the FY18 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   No
   If yes, how many?

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 FY18 award period. 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** (S, MS, MR, R, where R represents your most resistant check)	**FHB Rating** (0-9)	**Year Released**

Add rows if needed.

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

Abbreviations for Grain Classes

Barley - BAR
Durum - DUR
Hard Red Winter - HRW
Hard White Winter - HWW
Hard Red Spring - HRS
Soft Red Winter - SRW
Soft White Winter - SWW

Publications, Conference Papers, and Presentations

Instructions: Refer to the FY18-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY18 grant. Only include citations for publications submitted or presentations given during your award period. If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

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

> Conley, E.J., and J.A. Anderson. 2018. Accuracy of Genome-Wide Prediction for Fusarium Head Blight Associated Traits in a Spring Wheat Breeding Program. In: Proceedings of the XXIV International Plant & Animal Genome Conference, San Diego, CA.
> Status: Abstract Published and Poster Presented
> Acknowledgement of Federal Support: YES (poster), NO (abstract)

Journal publications.

Su, Z., A. Bernardo, B. Tian, H. Chen, S. Wang, H. Ma, S. Cai, D. Liu, D. Zhang, T. Li, H. Trick, P. St. Amand, J. Yu, Z. Zhang, and G. Bai. 2018. A Deletion Mutation in TaHRC Confers Fhb1 Resistance to Fusarium Head Blight in Wheat. Nat. Genet. https://doi.org/10.1038/s41588-019-0425-8.
Status: Published
Acknowledgement of Federal Support: Yes

Su, Z., S. Jin, D. Zhang, and G. Bai. 2018. Development and Validation of Diagnostic Markers for Fhb1 Region, a Major QTL for Fusarium Head Blight Resistance in Wheat. Theor Appl Genet. 131:2371–2380.
Status: Published
Acknowledgement of Federal Support: Yes

Bai, G., Z. Su and J. Cai. 2018. Wheat Resistance to Fusarium Head Blight. Can J. Plant Pathol. 40:336–346.
Status: Published
Acknowledgement of Federal Support: Yes

Zhao, J., Y. Liu, X. Cheng, Y. Pang, J. Li, Z. Su, J. Wu, Q. Yang, G. Bai, and X. Chen. 2018. Development and identification of a dwarf wheat – L. mollis double substitution line with resistance to yellow rust and Fusarium head blight. Crop J. https://doi.org/10.1016/j.cj.2018.11.012
Status: Published
Acknowledgement of Federal Support: Yes

Bakhsh, A., P.S. Baenziger, G. Bai, and W. Berzonsky. 2019. Agronomic performance of hard red winter wheat lines introgressed with the Fhb1 gene. Pak. J. Agri. Sci 56 (3), 623-628

Status: Published

Acknowledgement of Federal Support: Yes

Cai, J., S. Wang, Z. Su, T. Li, X. Zhang, G. Bai. 2019. Meta-analysis of QTLs for Fusarium head blight resistance in Chinese wheat landraces (accepted)

Status: Accepted

Acknowledgement of Federal Support: Yes

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

H Chen, Z Su, B Tian, HN. Trick, G Bai. 2018. CRISPR/Cas9 editing for FHB resistance. USWBSI Fusarium Focus, P2, Volume 19, Issue 1, 2019.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (oral presentation, abstract)

Other publications, conference papers and presentations.

G Bai. 2018. Multiplex restriction amplicon sequencing (MRASeq), a new next generation sequencing-based marker platform for genotyping. P0152. International Plant & Animal Genome XXVI, San Diego, CA, USA.

Status: Published

Acknowledgement of Federal Support: Yes.

Y Xu, A Bernardo, G Bai. 2018. Characterization of Fusarium head blight resistance in a wheat mutant derived from hard winter wheat Jagger using genotyping-by-sequencing (GBS) markers. Proceeding of 2018 National Fusarium Head Blight Forum, P76.

Status: Published

Acknowledgement of Federal Support: Yes.

H Chen, Z Su, B Tian, HN. Trick, G Bai. 2018. CRISPR/Cas9 genome editing technology for FHB resistance improvement in wheat. 2018 National Fusarium Head Blight Forum, P66.

Status: Published

Acknowledgement of Federal Support: Yes.

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P Zhang, C Guo, A Bernardo, H Ma, G Bai. 2018. QTL Mapping for Fusarium Head Blight Resistance in the Wheat population Yangmai 158 x Zhengmai 9023. 2018 National Fusarium Head Blight Forum, P142.
Status: Published
Acknowledgement of Federal Support: Yes.

Y Li, A Bernardo, PSt. Amand, G Bai. 2018. Pyramiding wheat Fusarium head blight resistance genes from different sources using marker-assisted backcrossing and low-cost SNP markers for background selection. 2018 National Fusarium Head Blight Forum, P121
Status: Published
Acknowledgement of Federal Support: Yes.

Umara Sahar Rana and Guihua Bai. 2018. Mapping quantitative trait loci for Fusarium head blight in RIL population derived from hard winter wheat Everest × Overland. 2018 National Fusarium Head Blight Forum, P135.
Status: Published
Acknowledgement of Federal Support: Yes.

B Tian, Y Chen, Z Su, H Chen, G Bai, HN Trick. 2018. Application of CRISPR/Cas9 Genome Editing System for Hexaploid Wheat, In Vitro Cell & Dev Biol-Ani 54, S47
Status: Published

H Chen, B Tian, H Trick, G Bai. 2017. An Efficient *Barley Stripe Mosaic Virus*-mediated CRISPR/Cas9 Genome Editing System in Wheat. 5th Plant Genomics and Gene Editing Congress, Philadelphia, PA, USA.
Status: Published
Acknowledgement of Federal Support: Yes.