FY21 Performance Progress Report

Due date: July 26, 2022

Cover Page

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Fiscal Year:	2021
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
Recipient Organization:	USDA-ARS Plant Science and Entomology Research Unit (PSERU) 4008 Throckmorton Hall, 1712 Chaflin Rd. Manhattan, KS 66506
DUNS Number:	N/A
EIN:	N/A
Project/Grant Period:	5/1/21 - 4/30/22
Reporting Period End Date:	4/30/2022

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
FY21 Total ARS Award Amount		$130,000

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.

_____ 7/18/2022 _____
Principal Investigator Signature   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: Identification and Deployment of FHB Resistance QTLs in US Hard Winter Wheat

1. **What are the major goals and objectives of the research project?**
   Map QTLs for type I resistance to FHB from the source of Everest; pyramid *Fhb1* with *Fhb7* and a major QTL from 2DL in US HWW Everest by marker-assisted backcross (MAB); and develop functional markers for *Fhb7* for marker-assisted breeding.

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. Completed the greenhouses and field experiments for type I resistance and data analysis.
      2. 2DL QTL was pyramided with Fhb1 in Everest and Overland backgrounds. Five selected resistant lines were phenotyped for agronomic performances in three Kansas locations.
      3. Two near-diagnostic markers for Fhb7 were developed and the paper was published online
      4. Backcross was made between EverestFhb1/Fhb7F1 and Everest.

   b) **What were the significant results?**
      a). Two selected lines with Fhb1 and 2DL QTL showed acceptable agronomic performance in field conditions.
      b). Fhb7 marker paper has been published
      c). Fhb7 transgenic lines were phenotyped and showed high FHB resistance in greenhouse experiments

   c) **List key outcomes or other achievements.**
      a). Selected lines with Fhb1 and 2DL QTL showed high resistance and some of them show acceptable agronomic performance
      b). Protocol for Fhb7 diagnostic markers has been released through publication in Crop Science
      c). An EMS mutant M2 population of 2000 lines with Fhb7 in Chinese Spring background has been advanced to M4 for future screening of yellow flour gene mutant.
      d). Eight Fhb7 transgenic lines with GST gene were evaluated in two greenhouse cycles for FHB resistance and most of them showed high Fhb7 expression and high FHB resistance.

3. **What opportunities for training and professional development has the project provided?**
   Three graduate students (Mr. Volodymyr Kavetsky, Ms. Ruolin Bian and Mr. Yuzhou Xu) have worked on these projects. They learnt gene cloning and sequencing, Fusarium inoculum culturing and taking note for disease and other traits, marker and QTL data analysis, poster presentation and writing reports.

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

- Selected wheat FHB resistance gene pyramiding lines with high level of FH resistance have been distributed to several breeding programs by request and used as parents for further crosses.
- Several graduate students and post-docs gave presentations in 2021 FHB Forum.
- The several manuscripts on FHB research have been published.

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

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1. **What are the major goals and objectives of the research project?**
   Goal 1: Select wheat edited lines with improved resistance to FHB
   Goal 2: Release to other programs for further breeding activities.

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?**
   For goal 1:
   1. We edited three different sites (one before and one within and one after the nuclear localization signal domain, NLS) of wild-type allele TaHRC_S in a susceptible wheat cultivar 'Bobwhite' using our recently optimized BSMV-mediated gene-editing system.
   2. We identified one mutant each at the three different target sites, respectively, with two insertion mutations and one deletion mutation.
   3. We selected the homozygous M2 mutant plants and inoculated with a conidiaspore suspension of F. *graminearum* (GZ3639) by single spikelet injection at early anthesis in a growth chamber.
   4. We scored the percentage of symptomatic spikelets (PSS) in a spike in the edited mutants and determined reduced FHB susceptibility in the mutants with the disrupted N-terminus is due to abolished TaHRC function and the NLS domain in the N-terminus is critical for regulating FHB susceptibility in wheat.

   For goal 2:
   We successfully identified two TaHRC-edited 'Bobwhite' lines and three TaHRC-edited 'Everest' and evaluated all the edited lines with improved resistance to FHB. The mutants will be released to wheat breeding programs for further breeding activities

   b) **What were the significant results?**
   1. We successfully edited TaHRC_S at the three different target sites and identified two mutant lines with improved FHB resistance.
   2. We successfully determined the NLS domain in the N-terminus of TaHRC_S is critical for regulating FHB susceptibility in wheat.

   c) **List key outcomes or other achievements.**
   1. We successfully confirmed the hypothesis that wheat have FHB susceptible genes.
   2. We provided new strategies for developing new sources of FHB resistance by knocking out susceptible genes in wheat cultivars using CRISRP/Cas9 gene editing.

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

Trained one visiting scientist and one MS student with gene editing techniques.

4. How have the results been disseminated to communities of interest?
  1. Presented two posters in USWBSI 2021 FHB Forum
  2. Two related papers have been published in the high-impacted journals.
  3. HRC-S gene edited Bobwhite seeds were requested by McGill University in Canada for FHB research
  4. We have been contacted by the USDA lab in Albany and a lab in Virginia Tech University for assistance and collaboration in using the technology to edit wheat for different traits.

Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your <u>FHB work</u> that were a result of funding from your FY21 grant award. Only citations for publications <u>published</u> (submitted or accepted) or presentations <u>presented</u> 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).

1. H Chen, Z Su, B Tian, G Hao, HN. Trick and G Bai. (2022) Wild-type allele of TaHRC suppresses calcium-mediated plant immune response by hijacking TaCAXIP4 to trigger FHB susceptibility in wheat. Plant Physiol.
    Status: Accepted
    Acknowledgement of Federal Support: Yes

2. Zhao L, Ge W, Lyu Z, Xu S, Xu Y,Bernardo A, Zhang Q, Xu SS,Wang H, Kong L, Bai G. (2022) Development and validation of diagnostic markers for the wheat Fusarium head blight resistance geneFhb7. Crop Sci. DOI:10.1002/csc2.20754
    Status: Published
    Acknowledgement of Federal Support: Yes

3. Ghimire B, Mergoum M, Martinez-Espinoza AD, Sapkota S, Pradhan S, Babar MA, Dong Y, and Buck JW. (2022) Genetics of Fusarium head blight resistance in soft red winter wheat using a genome-wide association study. DOI: 10.1002/tpg2.20222
    Status: Published
    Acknowledgement of Federal Support: Yes

4. H Chen, Z Su, B Tian, Y Liu, Y Pang, V Kavetskyi, HN. Trick and G Bai. (2022) Development and optimization of a Barley stripe mosaic virus (BSMV)-mediated gene editing system to improve Fusarium head blight (FHB) resistance in wheat. Plant Biotech J. 20:1018-1020. DOI: 10.1111/pbi.13819
    Status: Published
    Acknowledgement of Federal Support: Yes

5. H Li, F Zhang, J Zhao, G Bai, P St. Amand, A Bernardo, Z Ni, Q Sun, Z Su. (2022) Identification of a novel major QTL from Chinese wheat cultivar Ji5265 for Fusarium head blight resistance in greenhouse. Theor Appl Genet. https://doi.org/10.1007/s00122-022-04080-5
    Status: Published
    Acknowledgement of Federal Support: Yes

6. J Zhang, HS Gill, NK Brar, J Halder, S Ali, X Liu, A Bernardo, P St. Amand G Bai, US Gill, B Turnipseed, SK Sehgal. (2022) Genomic prediction of Fusarium head blight resistance in early stages using advanced breeding lines in hard winter wheat. Crop J. https://doi.org/10.1016/j.cj.2022.03.010

   Status: Published
   Acknowledgement of Federal Support: Yes

7. L Zhao, P Su, B Hou, H Wu, Y Fan, W Li, J Zhao, W Ge, S Xu, S Wu, X Ma, A Li, G Bai, H Wang, L Kong. (2022) The black necrotic lesion enhanced Fusarium graminearum resistance in wheat. Front in Genet.

   Status: published
   Acknowledgement of Federal Support: Yes

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.

1. Y Xu, R Bian, Y Dong, L Zhao, Y Xu, A Bernardo, P St. Amand, J Rupp, M Bruce, and G Bai. 2021. Characterization of Quantitative Trait Loci for Resistance to Fusarium Head Blight in a Winter Wheat Population. *Proceedings of the 2021 National Fusarium Head Blight Forum;* Virtual. December 6-7, 2021. Retrieved from: https://scabusa.org/ forum/2021/2021NFHBForumProceedings.pdf

2. SK. Sehgal, J Zhang, HS Gill, NK Brar, J Halder, S Ali, X Liu, A Bernardo, P St Amand, G Bai, B Turnipseed 2021. Breeding for FHB Resistance in Hard Winter Wheat. *Proceedings of the 2021 National Fusarium Head Blight Forum*; Virtual. December 6-7, 2021. Retrieved from: https://scabusa.org/forum/2021/2021NFHBForumProceedings.pdf

3. R Bian, A Bernardo, P St. Amand, G Bai. Quantitative Trait Loci Mapping for Fusarium Head Blight Resistance in a Wheat EMS Mutant from 'Jagger'. *Proceedings of the 2021 National Fusarium Head Blight Forum*; Virtual. December 6-7, 2021. Retrieved from: https:// scabusa.org/forum/2021/2021NFHBForumProceedings.pdf

4. L Zhao, W Ge, Z Lyu, S Xu, Y Xu, A Bernardo, Q Zhang, SS. Xu, H Wang, L Kong, and G Bai. 2021. Development and Validation of Diagnostic Markers for the Wheat Fusarium Head Blight Resistance Gene Fhb7. *Proceedings of the 2021 National Fusarium Head Blight Forum*; Virtual. December 6-7, 2021. Retrieved from: https://scabusa.org/ forum/2021/2021NFHBForumProceedings.pdf

5. V Kavetskyi, H Chen, G Bai, 2021. Using a New Genome Editing System to Validate the Functions of Wheat Candidate Genes of FHB1 in Fusarium Head Blight Resistance. *Proceedings of the 2021 National Fusarium Head Blight Forum*; Virtual. December 6-7, 2021. Retrieved from: https://scabusa.org/forum/2021/2021NFHBForumProceedings.pdf

6. H Chen, V Kavetskyi and G Bai. 2021. Nanoparticle-mediated Genome Editing System for FHB Resistance Improvement in Wheat. *Proceedings of the 2021 National Fusarium Head Blight Forum*; Virtual. December 6-7, 2021. Retrieved from: https://scabusa.org/forum/2021/2021NFHBForumProceedings.pdf