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

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Develop Genomic Resources to Improve Wheat FHB Resistance
Principle Investigator (PI):	Guihua Bai
Institution:	USDA-Agricultural Research Service
Institution UEI:	N/A
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$145,000
PI Mailing Address:	4008 Throckmorton Hall Kansas State University Manhattan KS 66506
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Period of Performance:	May 1, 2022 - April 30, 2023
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Develop a New Transgene Free Editing System for Gene Function Validation and Breeding	$65,000
HWW-CP	Pyramiding and Deployment of Multiple FHB Resistance QTLs in Hard Winter Wheat	$80,000
	FY22 Total ARS Award Amount	$145,000

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.

7/23/2023

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: Develop a New Transgene Free Editing System for Gene Function Validation and Breeding

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

The major goals of the project are to develop an efficient nanoparticle-mediated gene delivery system for genome editing in wheat without gene transformation step, and to use this system to improve FHB resistance and mitigate mycotoxin accumulation in wheat.

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?

- The binding capacity and ratio of 25nm and 10nm NPs to either Cas9/gRNA complexes have been determined by experiments.
- NP-mediated CRISPR/Cas editing for TaHRC was conducted using the regrown seedlings by treating embryos of imbibed dry 'Bobwhite' seeds for protocol optimization.
- A high-throughput NGS-based mutant screening protocol was established for identifying mutations, and evaluating TaHRC-editing efficiency and off-target effects.
- Using optimized protocol of NP-mediated editing, Ning7840 seeds were treated with NP-borne gRNA (4 targets in TaHRC) and Cas9. A total of 1050 plants were generated and screened using NGS and identified 9 plants with putative mutations in the target 4 and these plants will be validated for inheritable mutation in M1 this fall.

b) What were the significant results?

- Identified right type and size of NPs for gene delivery in wheat.
- Validated that NP-mediated gene-editing is possible in a cultivar that is difficult for transformation.
- Optimized the binding capacity and ratio of NPs with Cas9/gRNA complexes and evaluated TaHRC-editing efficiency using a high-throughput screening technology.

c) List key outcomes or other achievements.

- Established right type and size of NP for delivery of NPs with Cas9/gRNA to wheat cells.
- Validated that NP-mediated gene-editing is possible in a cultivar that is difficult for transformation.
- Optimized the NP-mediated gene editing protocol.

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

One MS student (Mr. Vova Kavetskyi), one ARS technician (Dr. Hongliang Wang), and one ARS scientist (Dr. Katie Jordan) were trained in the project the past year.

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

This project is to develop and optimize protocol for NP-mediated gene editing. The technology is novel and not available for generating inheritable mutation in wheat, even in plants. We need to develop, optimize and evaluate every step in the protocol. Now we are in the process to improve editing efficiency. We will publish the results and new protocol in a peer-reviewed journal after another validation trial is successfully completed.

Project 2: Pyramiding and Deployment of Multiple FHB Resistance QTLs in Hard Winter Wheat

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

- Identify Fhb7 donor with malfunctioned PSY-E2 for yellow pigment content.
- Pyramid the major QTL in locally adapted germplasm from HWW-CP breeding programs

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?

- Collected 15 elite breeding lines/cultivars from 7 HWW-CP breeding programs in OK, KS, CO, SD, NE and ND states and USDA.
- Screened the mutant population (2000 lines) using flour color assay and identified 10 lines with light flour color and identified two of them with light flour color and FHB resistance after evaluation of their FHB resistance and rechecked their flour color with large seed samples.
- Make crosses between a light flour *Fhb7* donor line and the Everest line carrying *Fhb1* and 2DL QTL to pyramiding the three genes.
- F1 plants were marker-selected and heterozygous F1 with all three genes were planted for backcrosses.

b) What were the significant results?

- Identified lines with light flour and Fhb7 resistance.
- Obtained F1 seeds from all 15 crosses and BC1F1 from 13 crosses.

c) List key outcomes or other achievements.

- We identified lines with light flour and Fhb7 resistance.
- We obtained F1 seeds from all 15 crosses and BC1F1 from 13 crosses.
- We sequenced the PSY-E2 gene from the 7E segment of Fhb7 donor and the sequence is quite different from the PSY-E2 gene previously reported.
- We repeatedly evaluated the Fhb7 transgenic plants in Fielder background and these lines showed high FHB resistance in greenhouses.

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

One post-doc (Dr. Lanfei Zhao) and two students (Yuzhou Xu and Ruolin Bian) have been trained on cross and marker-assisted backcrossing breeding, and involved in the project the past year.

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

The lines development is in progressing and selected lines carrying all three genes from BC2F3 will be sent to breeders for further breeding process.

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?

x 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).

1. Y Xu, Y Li, R Bian, G Zhang, AK Fritz, Y Dong, L Zhao, Y Xu, N Ghori, A Bernardo, P St. Amand, JL Shoup Rupp, M Bruce, W Wang, E Akhunov, B Carver and G Bai. 2023. Genetic architecture of quantitative trait loci (QTL) for FHB resistance and agronomic traits in a hard winter wheat population. The Crop Journal. Status: Revised manuscript submitted. Acknowledgement of Federal Support: Yes
2. HS Gill, N Brar, J Halder, C Hall, BW Seabourn, YR Chen, P St. Amand, A Bernardo, G Bai, K Glover, B Turnipseed, SK Sehgal. 2023. Multi-trait genomic selection improves the prediction accuracy of end-use quality traits in hard winter wheat. The Plant Genome, https://doi.org/10.1002/tpg2.20331. Status: Published. Acknowledgement of Federal Support: Yes
3. Y Ding, F Zhang, F Sun, J Liu, Z Zhu, X He[1], G Bai, Z Ni, Q Sun, Z Su. 2023. Loss of OsHRC function confers blast resistance without yield penalty in rice. Plant Biotechnology Journal https://doi.org/10.1111/pbi.14061 Status: Published. Acknowledgement of Federal Support: Yes
4. A Szabo-Hever, JS. Sharma, JD Faris, S Zhong, TL Friesen, AJ Green, G Bai, SS Xu. 2023. Identification and mapping of quantitative trait loci for Fusarium head blight resistance in synthetic hexaploid and spring wheat. The Plant Genome.Status: Submitted.Acknowledgement of Federal Support: Yes

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).

Kavetskyi, V. 2023. Using a BSMV mediated genome editing system to validate the function of Fhb1 candidate genes in Fusarium head blight resistance. MS Thesis, Kansas State University. Status: Published Acknowledgment of Federal Support: Yes

Ruolin Bian, 2023. Characterization of QTLs for disease resistance and rolled leaf traits in a mutant derived RIL population, Ph.D. Thesis, Kansas State University. Status: Published. Acknowledgement of Federal Support: Yes

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.

Chen, H., and Bai, G. H. (2022). Develop a transgene-free CRISPR/CAS genome editing system for improvement of FHB resistance in wheat. Proceedings of the 2022 National Fusarium Head Blight Forum. Tampa, FL. Dec. 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf. Status: Published Acknowledgment of Federal Support: Yes

L Tidakbi, G Bai, JL Rupp and KW Jordan. (2022). Haplotype-Informed Prediction of Fusarium Head Blight Resistance in USA Wheat Breeding Programs. Proceedings of the 2022 National Fusarium Head Blight Forum. Tampa, FL. Dec. 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf. Status: Published. Acknowledgment of Federal Support: Yes

Harsimardeep S. Gill, Jinfeng Zhang, Navreet K Brar, Jyotirmoy Halder, Shaukat Ali, Amy Bernardo, Paul St Amand, Guihua Bai, Upinder S. Gill, Brent Turnipseed and Sunish K. Sehgal. (2022). Genomic Prediction to Improve FHB Resistance in Winter Wheat. Proceedings of the 2022 National Fusarium Head Blight Forum. Tampa, FL. Dec. 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf. Status: Published. Acknowledgment of Federal Support: Yes

Agnes Szabo-Hever, Jyoti S. Sharma, Justin D. Faris, Shaobin Zhong, Timothy L. Friesen, Andrew J. Green, Guihua Bai and Steven S. Xu. (2022). Identification and Mapping of Quantitative Trait Loci for Fusarium Head Blight Resistance in Synthetic Hexaploid and Spring Wheat. Proceedings of the 2022 National Fusarium Head Blight Forum. Tampa, FL. Dec. 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf Status: Published. Acknowledgment of Federal Support: Yes