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

FY21 FINAL Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-0-162
USDA-ARS Agreement Title:	Genetic Characterization and Integrated Deployment of FHB Resistance in Wheat
Principle Investigator (PI):	Shaobin Zhong
Institution:	North Dakota State University
Institution UEI:	EZ4WPGRE1RD5
Fiscal Year:	2021
FY21 USDA-ARS Award Amount:	$172,978
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Period of Performance:	5/15/21 - 5/14/23
Reporting Period End Date:	5/14/2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
DUR-CP	Identify and Map Novel QTL for FHB Resistance in Durum Wheat	$45,071
DUR-CP	Expand FHB Screening Capacity for Durum Wheat	$24,225
VDHR-SPR	Genetic Characterization and Integrated Deployment of FHB Resistance in Spring Wheat	$69,767
VDHR-SPR	Increase Capacity of Two Coordinated FHB Nurseries for Spring Wheat	$33,915
	FY21 Total ARS Award Amount	$172,978

I am submitting this report as a: ☒ 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.

Shaobin Zhong — Principal Investigator Signature

07/24/2023 — 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: Identify and Map Novel QTL for FHB Resistance in Durum Wheat

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

The major goal of this project was to identify, map, and deploy QTLs for FHB resistance in the emmer wheat (*Triticum turgidum* L. subsp. *dicoccum*) line PI 254188. Therefore, the specific objectives are:

1) Develop a mapping population with recombinant inbred lines (RILs) derived from the cross between Divide and PI 254188;
2) Phenotype FHB resistance and morphological traits of the mapping population from the Divide/PI 254188 cross in greenhouse and field;
3) Construct a genetic linkage map of the population using 90k-SNP chips;
4) Identify DNA markers linked to QTL for FHB resistance in PI 254188;
5) Transfer and pyramid the FHB resistance QTL into adapted durum wheat cultivars.

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?

- We generated additional DNA markers for the major QTL on chromosome 2A identified in the mapping population derived from the cross between Divide and PI 254188.

- We evaluated a mapping population consisting of 200 RILs (F2:7) derived from the cross between Joppa and one FHB resistant durum wheat line (LPA-4), which was selected from the Lebsock/PI254188/Alkabo cross, in the greenhouse and field experiments.

- We continued to conduct backcrosses and marker-assisted selection to develop a near-isogenic line containing Fhb1 in the genetic background of the durum wheat cultivar ND Riveland.

b) What were the significant results?

- We developed additional 22 STARP markers in the 2A QTL region with FHB resistance in the mapping population derived from the cross between Divide and PI 254188.

- The FHB severity ranged from 14.3% to 87.44% in the greenhouse experiment and 8.3% to 80.0% in the field experiment for the mapping population derived from the cross between Joppa and LPA-4.

- A near-isogenic line with Fhb1 was developed in the genetic background of ND Riveland.

c) List key outcomes or other achievements.

- The additional STARP markers developed for the 2A QTL will be used for marker-assisted selection during introgression of the FHB resistance into other durum wheat cultivars.

- The FHB phenotyping data collected from both greenhouse and field inoculation experiments for the mapping population derived from the cross between Joppa and LPA-4 will be used for fine mapping of the QTL for FHB resistance in the population.

- The near-isogenic line with Fhb1 in the genetic background of durum wheat cultivar ND Rivaland will be used as FHB resistance source for durum wheat breeding programs.

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

One research associate and two Ph.D. students were involved in FHB phenotyping, QTL mapping, marker development and near-isogenic line creation. The PI and the participants of this project have attended two conferences.

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

Some of the research results were presented in conference presentations and peer-reviewed articles published in scientific journals.

Project 2: Expand FHB Screening Capacity for Durum Wheat

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

The major goal and objective of this research project is to expand the FHB nursery at Fargo location and establish a new FHB nursery at Langdon location to accommodate and screen more advanced breeding lines, mapping populations, and introgression germplasm from durum wheat researchers working in the DUR-CP.

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?

- In the summer season of 2022, we planted 3,360 hill plots of durum wheat materials submitted by durum wheat researchers (1660 hills from Dr. Shaobin Zhong, 1200 hills from Dr. Steven Xu, and 500 hills from Dr. Xuehui Li) at the Fargo location. These durum wheat materials included advanced breeding lines, mapping populations, introgression germplasm, and recurrently selected populations.
- One mapping population consisting of 200 recombinant inbred lines (600 hill plots) and one EMS mutant population of 500 lines from Kronos (1000 hill plots) were inoculated by the point inoculation method and the remaining durum materials were inoculated by the Fusarium-infested corn inoculum spread on the field in mid-June. Overhead misting systems were used for disease development for both inoculation methods.
- A new FHB nursery was established at Langdon for FHB screening in collaboration with Dr. Venkata Chapara at the NDSU Langdon Research Station. In the summer season of 2022, we planted and evaluated a mapping population (660 hill plots) and a recurrently selected population (480 hill plots) for FHB. Fusarium-infested corn inoculum and overhead misting systems were used for disease infection and development At this location.

b) What were the significant results?

- For both Fargo and Langdon locations, high quality FHB data were collected and they are valuable for germplasm development and QTL mapping of FHB resistance in durum wheat.

c) List key outcomes or other achievements.

- The FHB nursery at the Fargo location provided larger capacity for screening of durum wheat materials from PIs involved in the USWBSI program. The FHB nursery at Langdon provided another location for FHB evaluation.

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

- The FHB nurseries provided trainings to four Ph.D. students in FHB phenotyping.

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

Nothing to report.

Project 3: Genetic Characterization and Integrated Deployment of FHB Resistance in Spring Wheat

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

Our overall goal is to clone and characterize a major QTL for FHB resistance in PI 277012 and quickly deploy it along with another major FHB resistance QTL Fhb1 in the four spring wheat breeding programs of the Spring Wheat Coordinated Project (SPR-CP). The specific objectives of this project are:

1) Clone and characterize the major FHB resistance QTL Qfhb.rwg-5A.2 in the wheat line PI 277012.
2) Quickly introgress Qfhb.rwg-5A.2 and Fhb1 into elite spring wheat cultivars and breeding lines by backcrossing, marker-assisted selection and speed breeding approach.
3) Determine the expression of Qfhb.rwg-5A.2 and its interaction with Fhb1 in different genetic backgrounds.

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?

- Sequenced whole genome of the wheat line PI 277012 using the PacBio HiFi sequencing technology
- Identified contigs covering the 5AL QTL (*Qfhb.rwg5A.2*) in the PI 277012 genome.
- Developed additional DNA markers for *Qfhb.rwg5A.2* in PI 277012.
- Flow-sorted and sequenced chromosome 5A of GP112 (a FHB resistant RIL derived from the cross between Grandin and PI 277012) and five EMS mutants of GP112.
- Conducted MutChromSeq analysis to identify candidate genes at *Qfhb.rwg-5A.2*.
- Performed backcrossing and marker-assisted selection for introgression of Qfhb.rwg-5A.2 and Fhb1 into elite spring wheat cultivars or breeding lines of the four spring wheat breeding programs (NDSU, University of Minnesota, SDSU, and WSU).

b) What were the significant results?

- HiFi reads totaling 201.41 Gb (equivalent to ~13x genome coverage) were generated for PI 277012 from eight SMRT cells. The sequence data were used to assemble PI 277012 genome, producing 5,827 contigs with N50=9.1 Mb covering 14.43 Gb of the wheat genome.

- One contig (~ 9.8 Mb) containing *Qfhb.rwg-5A.2* was identified from the genome assembly of PI 277012 sequenced with the PacBio HiFi sequencing technology.

- Mapping of Illumina sequencing reads from flow-sorted chromosome 5A of Grandin to the 9.8 Mb contig with *Qfhb.rwg-5A.2* identified 23,299 putative SNPs, and 88 of the SNPs were mapped at the *Qfhb.rwg-5A.2* region and were further converted into semi-thermal asymmetric reverse PCR (STARP) markers.

- 12 FHB susceptible EMS mutants were identified by screening M2 individuals derived from M1 plants of GP112 and GP226, which are FHB resistant RILs derived from the cross between Grandin and PI 277012.

- Five FHB susceptible EMS mutants derived from GP112 along with the wildtype GP112 were sent to Dr. Dolezel Jaroslav's Lab at Institute of Experimental Botany, Czech Republic, for chromosome 5A sorting and sequencing. MutChromSeq analysis identified candidate genes for *Qfhb.rwg5A.2*.

- By backcrossing and marker-assisted selection, *Qfhb.rwg-5A.2* and *Fhb1* were integressed into six spring wheat cultivars (Glenn, ND VitPro, Linkert, Dayn, Surpass, and WA8283).

c) List key outcomes or other achievements.

- High quality whole genome assembly of PI 277012 was generated with the HiFi sequencing technology. This genome resource is useful for cloning FHB resistance QTL and other genes of interest in PI 277012.

- One 9.8 Mb contig covering the target QTL was identified. This facilitated the identification of candidate genes for *Qfhb.rwg5A.2.*

- New PCR-based DNA markers were developed for *Qfhb.rwg5A.2.* These markers will be useful for transferring the two QTL into other wheat varieties by marker-assisted selection.

- Additional FHB susceptible EMS mutants were identified from RILs with *Qfhb.rwg5A.2* derived from PI 277012.

- Chromosome 5A-specific sequences were obtained by flow-sorting and Illumina sequencing from GP112 and its EMS mutants. Candidate genes for *Qfhb.rwg5A.2* were identified through the MutChromSeq analysis.

- The two major FHB resistance QTL (*Qfhb.rwg-5A.2* and *Fhb1*) were introduced into six spring wheat cultivars and breeding lines by backcrossing and marker-assisted selection, and near-isogenic lines have been developed.

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

The project provided trainings to one research associate and two Ph.D. students on genome analysis, QTL mapping and marker development as well as map-based gene cloning. The PI and the participants of this project attended two conferences.

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

The FHB resistant wheat lines developed and DNA markers associated with the FHB resistance have been provided to and used by other wheat researchers and breeders. Some of the research results were presented in conference presentations and peer-reviewed articled published in scientific journals.

Project 4: Increase Capacity of Two Coordinated FHB Nurseries for Spring Wheat

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

The major goal and objective are to expand the FHB nursery at Fargo location and establish a new nursery at Langdon location to accommodate and screen more advanced breeding lines, mapping populations, and introgression germplasm from NDSU spring wheat breeding program and other researchers who are working in the spring wheat CP.

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?

- In the summer season of 2022, we planted a total of 5,580 hill plots of spring wheat materials from multiple researchers working in the spring wheat CP (2,620 hills from Dr. Shaobin Zhong, 1,000 hills from Dr. Steven Xu, 1,360 hills from Dr. Andrew Green, and 600 hills from Dr. Jason Fiedler) in the FHB nursery at Fargo location. These materials included advanced breeding lines, mapping populations, introgression germplasm, and commercially grown wheat varieties (for variety trials). Among them, 2,415 hill plots were point-inoculated at anthesis and the rest of hill plots were inoculated with Fusarium-infested corn kernels. Overhead misting systems were used for disease development for both inoculation methods.

- We also established a disease nursery at Langdon for FHB screening of spring wheat materials in collaboration with Dr. Venkata Chapara at the NDSU Langdon Research Station. In the summer season of 2022, we planted a mapping population (660 hill plots) and spring wheat materials (480 hill plots) derived from various crosses and backcrosses for introgression of FHB resistance. Fusarium-infested corn kernels were spread on the field as inoculum and overhead misting systems were used for disease infection and development at the Langdon location.

b) What were the significant results?

- FHB developed well in both locations and high-quality disease data were collected.

c) List key outcomes or other achievements.

- The FHB data are very useful for variety and germplasm development, QTL mapping of FHB resistance, and development of a guide for farmers to select varieties for planting.

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

The FHB nurseries provided trainings to one research associate and four Ph.D. students in FHB phenotyping.

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

Nothing to report

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 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).

Poudel, B., Mullins, J., Puri, K.D., Leng, Y., Karmacharya A., Liu, Y., Hegstad, J., Li, X., and **Zhong, S**. 2022. Molecular mapping of quantitative trait loci for Fusarium head blight resistance in the Brazilian spring wheat cultivar 'Surpresa'. Front. Plant Sci. 12:778472. https://doi.org/10.3389/fpls.2021.778472.
Status of publication: published. Acknowledgement of federal support: yes.

Books or other non-periodical, one-time publications as a result of FY21 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).

Nothing to report

Other publications, conference papers and presentations as a result of FY21 award

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

Karmacharya, A., Li, D., Leng, Y., Shi, G., Liu, Z., Yang, S., Du, Y., Dai, W and ***Zhong, S**. (2022). Editing Wheat Genome through Wide Hybridization with Maize Expressing Cas9 and Guide RNA. Proceedings of the 2022 National Fusarium Head Blight Forum; Tampa, FL, December 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf
Status of publication: published. Acknowledgement of federal support: yes.

Karmacharya, A., Leng, Y., and ***Zhong, S**. 2022. Targeting wheat TaHRC gene for FHB resistance through haploid induction coupled with CRISPR/Cas9 genome editing. APS Meeting, Pittsburgh, PA. P200. (Poster)
Status of publication: published. Acknowledgement of federal support: yes.

Safar, S., and ***Zhong, S**. 2022. Development and evaluation of near-isogenic spring wheat lines with the FHB resistance gene *Fhb1*. North Central Division Meeting, Lincoln, NE (Poster)
Status of publication: published. Acknowledgement of federal support: yes.