USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY19 Performance Report
Due date: July 24, 2020

Cover Page

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Fiscal Year:	2019
USDA-ARS Agreement ID:	59-0206-7-153
USDA-ARS Agreement Title:	Genetic and Molecular Characterization of New Sources of FHB Resistance in Wheat
FY19 USDA-ARS Award Amount:	$ 99,142
Recipient Organization:	North Dakota State University Office of Grant & Contract Accouting NDSU Dept 3130, PO Box 6050 Fargo, ND 58108-0650
DUNS Number:	80-388-2299
EIN:	45-6002439
Recipient Identifying Number or Account Number:	FAR0028058
Project/Grant Reporting Period:	5/1/19 - 4/30/20
Reporting Period End Date:	4/30/2020

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	$ 41,821
VDHR-SPR	Genetic and Molecular Characterization of Novel FHB Resistance QTL in Spring Wheat	$ 57,321
	FY19 Total ARS Award Amount	**$ 99,142**

Shaobin Zhong

July 21, 2020

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

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Project 1: *Identify and Map Novel QTL for FHB Resistance in Durum Wheat*

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 items a-b) below.)*

a) What were the major activities?

We evaluated ~200 RILs (F2:7) derived from the cross between Divide and PI 254188 for FHB resistance in two more greenhouse seasons and one field experiment. We genotyped the RIL population using the genotyping by sequencing (GBS) approach. We constructed a genetic linkage map for the Divide/PI 254188 population with the SNP markers generated from the GBS genotyping. We conducted QTL analysis using the constructed linkage genetic map and the phenotype data from one greenhouse experiment. We also raised another RIL population from the cross between Joppa and one FHB resistant line with the resistance derived from PI 254188.

b) What were the significant results?

The RIL mapping population derived from the Divide/PI 254188 cross segregated for FHB resistance in the greenhouse evaluation; Some RILs had a high level of FHB resistance similar to the resistant parent PI 254188 while some other RILs were very susceptible. GBS of the RIL mapping population and the parents generated over 8,000 SNP markers, and after filtration of low-quality ones, 4,192 SNP markers were used to construct a genetic linkage map, which consisted of 16 linkage groups corresponding to 14 chromosomes of the durum wheat genome. The total map size was 2653.47 cM. A major QTL for FHB resistance from PI 254188 was detected on chromosome 2A based on data from one greenhouse inoculation experiment.

c) List key outcomes or other achievements.

(1) A large number of SNP markers were generated for the RIL population derived from the Divide/PI 254188 cross.
(2) A genetic linkage map with 4,192 SNP markers have been developed.

(2) FHB phenotyping data were collected from three greenhouse inoculation experiments and one field experiment.
(3) One major QTL for FHB resistance was detected on chromosome 2A in the RIL population based on phenotype data from one greenhouse experiment.

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

Yes, the COVID-10 pandemic caused the university to partially close the campus and the research activities had to be reduced to the minimum. The FHB phenotyping in greenhouse was impacted because access to greenhouse is limited and number of people working in the same room is limited to one for keeping social distancing.

4. 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 FHB phenotyping, QTL mapping and marker development. The PI and the participants of this project have attended three seminars and two conferences.

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

Nothing to report

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Project 2: *Genetic and Molecular Characterization of Novel FHB Resistance QTL in Spring Wheat*

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

Our overall goal is to characterize novel FHB resistance quantitative trait loci (QTL) at both genetic and molecular levels in spring wheat. The specific objectives of this project are to:

1) Clone and characterize the major FHB resistance QTL on chromosome 5AL in wheat line PI 277012;
2) Identify novel QTL for FHB resistance in PI 185843, a Brazil wheat cultivar with a higher level of FHB resistance;
3) Develop user-friendly DNA markers for the novel QTLs and deploy them in selection of FHB resistance in wheat breeding programs.

2. What was accomplished under these goals or objectives? *(For each major goal/objective, address items a-b) below.)*

a) What were the major activities?

(1) To clone the major QTL on 5AL for FHB resistance in the wheat line PI 277012, we collected 5A chromosomes from both Grandin and PI 277012 by chromosome sorting technology in collaboration with Dr. Jaroslav Dolezel at the Institute of Experimental Botany, Czeck Republic. We extracted DNA samples from the sorted 5A chromosomes from the two wheat genotypes and generated paired-end sequence reads from them. We assembled the sequence reads into a 5A scaffold using the Chinese Spring 5A pseudomolecule as reference. We annotated the genes on a 1.3 Mb region containing the target FHB resistance QTL and identified genes showing polymorphisms between Grandin and PI 277012. We also screened EMS mutants generated from PI 277012 in the greenhouse.

(2) To map the QTL in PI 185843, we finished the genotyping and genotyping of 200 RILs derived from the Wheaton/PI 185843 cross, and conducted QTL analysis.

(3) To develop user-friendly DNA markers for the novel QTLs for FHB resistance, we identified additional SNP markers for the 5AL QTL region. These SNP markers were converted into PCR-based markers for fine mapping and introgression of the 5AL QTL.

b) What were the significant results?

(1) The 5A chromosomes were collected from both PI 277012 and Grandin using the chromosome sorting technology developed by Dr. Jaroslav Dolezel's group, and the purity of the PI 277012 5A chromosomes was 93.8% and 87.6% for the Grandin 5A chromosomes. A total of 122.3 and 206.4 million paired-end reads (250 bp and 150

pb) were generated for the Grandin 5A chromosome, which were combined to be assembled into an 872.38 Mb scaffold. A total of 313.6 million paired-end reads (150 pb) were generated for the PI 277012 5A chromosome, which were assembled into a 933.66 Mb scaffold. Analysis of sequences in the region flanked by the marker 2375 and 2620 flanking the QTL detected 237 and 289 genes from PI 277012 and Grandin, respectively. Comparative genomic analysis identified 71 genes with polymorphism between the two genotypes. These genes will be further characterized for marker development and candidate gene identification for the QTL. Screening approximately 400 M2 progenies from M1 plants from PI 277012 in greenhouse identified several mutants that were much more susceptible to FHB compared to the parent PI 277012.

(2) Genetics analysis identified four QTLs (genes) for type II FHB resistance on chromosome 2A, 3B, and 4D, respectively. The largest effect QTL was mapped on chromosome 4D and explained 15.8% of the phenotypic variation. QTLs detected on chromosome 2A and 3B are different from previously reported QTLs and may be novel.

(3) Fifteen additional PCR-based SNP markers were developed in the 5AL QTL region of PI 277012 and have used in selection of the QTL in the process of introgression of the FHB resistance into adapted wheat cultivars.

c) List key outcomes or other achievements.

(1) Genome sequences of chromosome 5A were generated from the resistant PI 277012 and susceptible Grandin. The 5A genome assemblies are very useful for identification of candidate genes for the 5AL QTL.

(2) More EMS mutants susceptible to FHB were identified from PI 277012, which will be useful for validation of the candidate genes for the QTL.

(3) Novel QTL were identified from PI 185843, which will be used to develop FHB resistant wheat varieties.

(4) The PCR-based SNP markers developed will facilitate the quick transfer of the FHB resistance into adapted wheat breeding lines.

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

Yes, the COVID-10 pandemic caused the university to partially close the campus and the research activities had to be reduced to the minimum. The FHB phenotyping in greenhouse was impacted because access to greenhouse is limited and number of people working in the same room is limited to one for keeping social distancing.

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

The project provided one research associate and three Ph.D. students with training on QTL mapping and marker development as well as map-based gene cloning. The PI and the participants of this project have attended three seminars and two conferences.

5. 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.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY19 award period (5/1/19 - 4/30/20). 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 FY19 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 FY19 award period?**

   Yes

   If yes, how many? One

3. **Have any post docs who worked for you during the FY19 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 FY19 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 FY19 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

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Publications, Conference Papers, and Presentations

Instructions: Refer to the FY19-FPR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY19 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (5/1/19 - 4/30/20**) 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.

Journal publications.

Wan, J., Jin, Z., Zhong, S., Schwarz, P., Chen, B., and Rao, J. 2019. Clove oil-in-water nanoemulsion mitigates growth of *Fusarium graminearum* and trichothecene mycotoxin production during the malting of Fusarium infected barley. Food Chemistry (in press)
Status: Published
Acknowledgement of Federal Support: YES

Liu, Y., Evan, S., Fiedler, J. D., Hegstad, J. B., Green, A., Mergoum, M., **Zhong, S.**, Li, X. 2019. Genetic mapping and prediction analysis of FHB resistance in a hard red spring wheat breeding population. Front. Plant Sci. 10:1007. https://doi.org/10.3389/fpls.2019.01007.
Status: Published
Acknowledgement of Federal Support: YES

Wu, D., Lu, J., **Zhong, S.**, Schwarz, P., Chen, B., Rao, J. 2019. Physical stability, antifungal and mycotoxin inhibitory activities of lecithin stabilized cinnamon oil emulsions in the presence of chitosan. Food & Function 10:2817-2827.
Status: Published
Acknowledgement of Federal Support: YES

Wan, J., **Zhong, S.**, Schwarz, P., Chen, B., and Rao, J. 2019. Enhancement of antifungal and mycotoxin inhibitory activities of food-grade thyme oil nanoemulsions with natural emulsifiers. Food Control 106: 106709.
Status: Published
Acknowledgement of Federal Support: YES

Wan, J., **Zhong, S.**, Schwarz, P., Chen, B., and Rao, J. 2019. Physical properties, antifungal and mycotoxin inhibitory activities of five essential oil nanoemulsions: impact of oil compositions and processing parameters. Food Chemistry 291:199-206.
Status: Published
Acknowledgement of Federal Support: YES

Szabo-Hever, A., Zhang, Q., Friesen, T. L., Zhong, S., Elias, E. M., Cai, X., Jin, Y., Chao, S., and Xu, S. S. 2018. Genetic diversity and resistance to Fusarium head blight in synthetic hexaploid wheat derived from *Aegilops tauschii* and diverse *Triticum turgidum* subspecies. Front. Plant Sci. 9:1829. doi: 10.3389/fpls.2018.01829.
Status: Published
Acknowledgement of Federal Support: YES

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

None

Other publications, conference papers and presentations.

Poudel, B., Puri, K.D., Leng, Y., Mullins, J., Karmacharya A., Liu, Y., Hegstad, J., Li, X., and Zhong, S. 2019. Molecular mapping of quantitative trait loci for Fusarium head blight resistance in the Brazilian spring wheat cultivar ‘Surpresa’. In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 110-115.), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract and Manuscript Published and Poster Presented
Acknowledgement of Federal Support: YES (Manuscript and Poster)

Ren, S., Leng, Y., Zhang, W., Talukder, Z., Zhong, S., Fiedler, J., Qi, L., and Cai, X. 2019. Molecular mapping of hexaploid wheat-derived Fusarium head blight resistance in durum wheat. In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum, Milwaukee* (p.117), WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)