USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Final Performance Report
Due date: July 31, 2018

Cover Page

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Fiscal Year:	2017
USDA-ARS Agreement ID:	59-0206-7-153
USDA-ARS Agreement Title:	Genetic and Molecular Characterization of New Sources of FHB Resistance in Wheat.
FY17 USDA-ARS Award Amount:	$ 98,490
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
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Recipient Identifying Number or Account Number:	FAR0028058
Project/Grant Reporting Period:	5/1/17 - 4/30/18
Reporting Period End Date:	4/30/18

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
DUR-CP	Identify and Map Novel QTL for FHB Resistance Introduced into Durum Wheat.	$ 29,691
VDHR-SPR	Identification and Deployment of Novel FHB Resistance QTL in Spring Wheat.	$ 37,791
VDHR-SPR	Sequencing the 5AL Genomic Region with a Major FHB Resistance QTL in PI 277012.	$ 31,008
	FY17 Total ARS Award Amount	$ 98,490

Shaobin Zhong

7-24-2018

Principal Investigator Date

* 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: *Identify and Map Novel QTL for FHB Resistance Introduced into Durum Wheat.*

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

The major goal of this project is to identify and map QTL for FHB resistance introgressed from tetraploid and hexaploid wheat accessions into adapted durum wheat cultivars. The specific objectives were to 1) Develop a genetic linkage map using a mapping population derived from the cross between durum wheat cultivar Joppa and introgression line 10Ae564; 2) Phenotype FHB resistance and morphological traits of the mapping population from the Joppa/10Ae564 cross; 3) Identify DNA markers linked to QTL for FHB resistance in Joppa and 10Ae564; 4) Transfer and pyramid the FHB resistance QTL into adapted durum wheat cultivars.

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

1) Major activities

A. We evaluated some of the selected recombinant inbred lines (F2:6) derived from the cross between Divide (durum wheat cultivar susceptible to FHB) and PI 277012 (hexaploid wheat line with a high level of FHB resistance) in the Fargo FHB nursery in the summer of 2017.

B. We selected some recombinant inbred lines (RIL) (F2:8) derived from the cross between 10Ae564 and Joppa based on genotypes of DNA markers closely linked to FHB resistance and phenotyped them in the Fargo FHB nursery in the summer of 2017.

C. We crossed Joppa and Divide with durum wheat lines containing FHB resistance derived from PI 277012 in order to pre-breed FHB resistant durum wheat germplasm.

2) Specific objectives

A. Verify the PI 277012 FHB resistance QTL initially transgressed into durum wheat cultivars.

B. Transfer and pyramid the FHB resistance QTL into adapted durum wheat cultivars.

3) Significant results

A. Four RILs from the cross between Divide and PI 277012 exhibited a high level of FHB resistance in the Fargo FHB nursery, with mean disease severity ranging from 7.54 to 24.40%. These durum wheat materials will be used as FHB resistance sources for durum wheat.

B. Six RILs derived from the cross between 10Ae564 and Joppa also showed a high level of FHB resistance, with mean disease severity ranging from 17.6 to 31.6%. These durum wheat lines contains the major QTL (*Qfhb.NDWP-5A*) from 10Ae564 with resistance source coming from PI 277012 and minor QTL (*Qfhb.NDWP-2A and Qfhb.NDWP-7A*) existing in durum cultivars.

C. Progenies from the crosses between FHB resistance introgression durum lines and Joppa as well as Divide were produced and they will be subjected to marker-assisted selection and phenotyping to obtain FHB resistant durum wheat lines with agronomic traits similar to the commercially grown durum cultivars.

4) Key outcomes or other achievements
   A. The RILs with accumulation of existing and new FHB resistance QTL are useful sources of FHB resistance for pre-breeding FHB-resistant durum wheat germplasm.
   B. The progeny from the pre-breeding process will be directly used in durum breeding programs.

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

One Ph.D. students is working on this project and has been trained for FHB phenotyping.

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

Materials have been provided to durum breeders for making crosses. One paper has been published in the peer-reviewed journal.

Project 2: *Identification and Deployment of Novel FHB Resistance QTL in Spring Wheat.*

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

The major goal of this project is to identify and map novel QTL for FHB resistance in PI 277012 and PI 185843. The specific objectives are to: 1) Introgress and pyramid the two major QTL for FHB resistance derived from PI 277012 into adapted spring wheat varieties through backcrosses and marker assisted selection; 2) Identify novel QTL for FHB resistance in PI 185843 by genotyping and phenotyping a population consisting of 200 recombinant inbred lines from the cross between PI 185843 and Wheaton; 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? *Address items 1-4) below for each goal or objective.*

1) Major activities

A. We phenotyped 200 recombinant inbred lines (RILs) derived from the Wheaton/PI 185843 for FHB reaction in two inoculation experiments, one in the 2017 Fargo FHB nursery and one in the greenhouse. DON data were also collected from the greenhouse inoculation experiment.
B. We pre-screened ethyl methanesulfonate (EMS) mutants (M_2) of PI 277012 for FHB reactions in the 2017 Fargo FHB nursery.
C. We sequenced the genome of PI 277012 using the BGISEQ-500 sequencing technology.
D. We continued to develop PCR-based SNP for the 5AL QTL in PI 277012 using the reference genome sequence of Chinese Spring.

2) Specific objectives

A. Map QTL for FHB resistance in PI 185843.
B. Identify FHB susceptible EMS mutants for future validation of candidate genes for the FHB resistance QTL in PI 277012.
C. Develop additional PCR-based SNP markers for map-based cloning of the 5AL FHB resistance QTL in PI 277012.

3) Significant results

A. RILs of the Wheaton/PI 185843 population segregated in FHB severity in the two inoculation experiments, with disease severity ranging from 10.6% to 100%. DON contents are being tested.
B. EMS mutants with more susceptibility to FHB compared to PI 277012 have been identified and are being confirmed in the greenhouse inoculation experiments.
C. 10× genome coverage (160 Gb) of sequences were obtained from PI 277012 using the BGISEQ-500 sequencing platform and A total of 1,048,575 SNPs on chromosome 5A were identified by compared to the Chinese Spring 5A sequence.
D. Additional DNA markers have been developed for the 5AL QTL region and the QTL was narrowed down to a smaller genomic interval of the 5AL chromosome.

4) Key outcomes or other achievements
   A. The EMS mutants and the genome sequence data will facilitate the cloning of the FHB resistance QTL in PI 277012.
   B. The additional DNA markers are very useful not only for marker-assisted selection of the PI 277012 FHB resistance, but also for the map-based cloning of the FHB resistance QTL in this wheat line.

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

Two Ph.D. students are working on this project and received one-on-one training for marker development and QTL mapping. A postdoctoral research associate was working on the project and received one on one training for FHB phenotyping and marker development.

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

The SNP markers associated with the 5AL QTL for FHB resistance in PI 277012 have been provided to other labs for marker-assisted selection. The results have been presented in professional conferences.

Project 3: *Sequencing the 5AL Genomic Region with a Major FHB Resistance QTL in PI 277012.*

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

Our overall goal is to sequence the 1.2 Mb 5AL region containing the QTL for map-based cloning of the FHB resistance gene in the wheat line PI 277012. The specific objectives of this project are 1) Construct a non-gridded BAC library with genomic DNA of PI 277012, 2) Screen the BAC library with DNA markers flanking and within the FHB resistance QTL on 5AL, and 3) Sequence the BACs and build a contig to cover the QTL.

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

1) Major activities
   A. We constructed a non-gridded BAC library using high molecular weight DNA of PI 277012 and made BAC pools in 12 × 96 well plates.
   B. We prepared BAC DNA samples from all the BAC pools, which are stored in 12 × 96 well plates corresponding to the BAC clone plates. Each well has 100ul of DNA with a concentration at more than 100ng/ul.
   C. We also made SuperPools and PlateRowPools of BAC DNA. Each SuperPool consists of ONE corresponding ROW of 12-wells from a 96well plate of Pooled BAC clones. Each PlateRowPool was made by combining the DNA samples from one row of 8-wells from a 96-well plate.
   D. We used PCR to screen the BAC SuperPools and PlateRowPools with DNA markers mapped around the 5AL QTL region.

2) Specific objectives
   A. Construct a non-gridded BAC library with genomic DNA of PI 277012.
   B. Identify BAC clones with DNA markers flanking and within the FHB resistance QTL on 5AL.
   C. Sequence the BAC clones and build a contig for cloning the FHB resistance QTL.

3) Significant results
   A. A non-gridded BAC library has been constructed, which consists of 1,152 pools distributed in 12 × 96 well plates with each pool containing ~300 BAC clones. The total number of BAC clones was calculated to be 34,560 and the mean insert size of the BAC clones was estimated to be 110 kb.
   B. This BAC library resource has 2.26× coverage of the 16,800 Gbp haploid wheat genome.
   C. Using 18 DNA markers mapped at the 5AL QTL region to screen the BAC library showed that 16 markers were positive, indicating BAC clones containing these markers are present in the library.

4) Key outcomes or other achievements
   A. The non-gridded BAC library constructed for PI 277012 are very useful for identification of BACs to cover the target region for gene cloning.
   B. The BAC clones will be sequenced and used to identify candidate genes for the FHB resistance QTL in PI 277012.

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

Nothing to report

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

Nothing to report

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY17 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 FY17 award period?**

   If yes, how many? No

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY17 award period?**

   If yes, how many? Yes, two graduate students earned their Ph.D. degrees.

3. **Have any post docs who worked for you during the FY17 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**

   If yes, how many? No

4. **Have any post docs who worked for you during the FY17 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**

   If yes, how many? No

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 FY17 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations. *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 FY17-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY17 grant. Only include citations for publications submitted or presentations given during your award period (5/1/17 - 4/30/18). 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.

Journal publications.

Wu, D., Wan, J., Lu, J., Wang, X., **Zhong, S.**, Schwarz, P., Chen, B., Rao, J. 2018. Chitosan coatings on lecithin stabilized emulsions inhibit mycotoxin production by *Fusarium* pathogens. Food Control 92: 276-285
Status: Published
Acknowledgement of Federal Support: NO

Zhao, M., Leng, Y., Chao, S., Xu, S. S., and *__Zhong, S__. 2018. Molecular mapping of QTL for FHB resistance introgressed into durum wheat. Theor. Applied. Genet. Jun 4. doi: 10.1007/s00122-018-3124-4. [Epub ahead of print]
Status: Published
Acknowledgement of Federal Support: YES

Wan, J., **Zhong, S**., Schwarz, P., Chen, B., and Rao, J. 2018. Influence of oil phase composition on antifungal and mycotoxin inhibitory activity of clove oil nanoemulsions. Food & Function 9: 2872-2882.
Status: Published
Acknowledgement of Federal Support: NO

Zhao, M., Wang, G., Leng, Y., Wanjugi, H., Xi, P., Grosz, M. D., Pitkin, J., Mergoum, M., and *__Zhong, S__. 2018. Molecular mapping of Fusarium head blight resistance in ND2710. Phytopathology 108:972-979. doi: 10.1094/PHYTO-12-17-0392-R.
Status: Published
Acknowledgement of Federal Support: YES

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

None

Other publications, conference papers and presentations.

Wan, J., **Zhong, S**., Schwarz, P., and Rao, J. 2017. Application of nanoencapsulated clove oils to enhance antifungal activities and inhibit mycotoxin production in vitro in *Fusarium graminearum*. Proceedings of the 2017 National Fusarium Head Blight Forum, Dec 3-5, 2017, Milwaukee, WI. P39. (Poster #39)
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: No (Poster) No (Abstract)

Zhang, Q., Faris, J. D., Chao, S., Friesen, T. L., **Zhong, S.,** Cai, C., Elias, M., and Xu, S. S. 2017. Identification and Molecular Mapping of Quantitative Trait Loci for Resistance to Fusarium Head Blight in Cultivated Emmer PI 272527. Proceedings of the 2017 National Fusarium Head Blight Forum, Dec 3-5, 2017, Milwaukee, WI. P97. (Poster #55)
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Poster) Yes (Abstract)

Zhao, M., Leng, Y., Liu, Y., Xi, P., Li, J., Wang, R., Long, Y., Chao, S., Xu, S. S., and **Zhong, S**. 2017. Fine Mapping of a Novel Major QTL for Fusarium Head Blight Resistance in the Wheat Line PI 277012. Proceedings of the 2017 National Fusarium Head Blight Forum, Dec 3-5, 2017, Milwaukee, WI. P98. (Poster #56)
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Poster) Yes (Abstract)

Poudel, B., and **Zhong, S.** 2017. Evaluation of Fusarium graminearum isolates collected from North Dakota for fungicide sensitivity, spore production and pathogenicity. North Central Division Meeting (oral presentation)
Status: Abstract Published and Oral Presentation Presented
Acknowledgement of Federal Support: YES (Oral presentation) Yes (Abstract)