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

Cover Page

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Fiscal Year:	2017
USDA-ARS Agreement ID:	59-0206-4-019
USDA-ARS Agreement Title:	Breeding and Genomic Selection for Fusarium Head Blight Resistance in Spring Wheat.
FY17 USDA-ARS Award Amount:	$ 195,550
Recipient Organization:	Regents of the University of Minnesota Suite 450 Sponsored FIN RPT-P100100001 Minneapolis, MN 55455-2003
DUNS Number:	555917996
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Project/Grant Reporting Period:	5/13/17 - 5/12/18
Reporting Period End Date:	5/12/2018

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
VDHR-SPR	Breeding Fusarium Head Blight Resistant Spring Wheat.	$ 108,412
VDHR-SPR	Optimization and Establishment of Genomic Selection for FHB Resistance in Wheat.	$ 38,689
VDHR-SPR	Using Targeted Sequencing to Breed for Fusarium Head Blight Resistant Wheat.	$ 48,449
	FY17 Total ARS Award Amount	$ 195,550

07/29/18

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: *Breeding Fusarium Head Blight Resistant Spring Wheat.*

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

The overall goal of this project is to develop spring wheat varieties with improved Fusarium head blight resistance with good adaptation to the North Central region of the U.S. and provide growers with FHB ratings of available varieties. The specific objectives of this research are to:

1) Develop Fusarium head blight resistant wheat germplasm and varieties adapted for commercial production in Minnesota and the surrounding region
2) Characterize the level of FHB resistance of all wheat varieties grown in the region
3) Use FHB markers to characterize potential parental lines and utilize MAS to increase frequency of FHB QTLs in advanced lines
4) Utilize genomic selection to improve the efficiency of identifying FHB susceptible lines.

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

1) major activities – summarized by Objective below

2) specific objectives

Objectives 1-2: Scab nurseries were established at two field sites, Crookston and St. Paul, in 2017. A total of 1,473 genotypes were evaluated in 3,952 total rows at the two locations. We evaluated the FHB reaction of external germplasm from the 2017 Uniform Regional Scab Nursery (31 lines) and 2017 Regional Performance Nursery (31 lines).

Objective 3: Marker-assisted selection was used to characterize parental lines (all done in-house) and Preliminary yield trial candidates (in cooperation with the USDA Fargo Genotyping Lab). We routinely use DNA markers to screen for genes that provide resistance to Fusarium head blight, leaf rust, Ug99 stem rust resistance, tan spot and high molecular weight glutenins that are necessary for good baking quality. Project no. 3 of this report, *Using Targeted Sequencing to Breed for Fusarium Head Blight Resistant Wheat*, was intended to replace gene-specific genotyping of Preliminary yield trial candidates that has routinely been done by USDA-ARS Fargo Genotyping Center. However, because that project failed, the Genotyping Center screened 1,503 pre-yield trial lines with 6 gene-specific DNA markers, generating 9,018 data points. In addition, since Fall 2017 we screened 1,087 individual F_1 plants from topcrosses and backcrosses and 71 parents from Fall 2017 and Spring 2018 crossing blocks for as many as 16 markers in-house, generating a total of 4,589 datapoints.

Objective 4: The genomic selection aspect of this project integrates with my other USWBSI-funded project *Optimization and Establishment of Genomic Selection for FHB Resistance in Wheat.* As part of our breeding efforts we genotyped using GBS 1,551 F_5 lines for FHB severity in our two scab field nurseries. This information, combined with the predictions from genomic selection from a training population of 500 lines that were also phenotyped to include VSK and test weight, and observations from our winter nursery in New Zealand were used to select a set of 353 for entry in to preliminary yield trials in spring 2018.

3) significant results

- Both FHB screening nurseries were excellent, and provided highly discriminatory data. As a result of these nurseries and results from previous years, the FHB resistance of 39 spring wheat cultivars was assessed.
- We used genomic selection at the F_5 stage for FHB to help select lines to advance to preliminary yield trials.

4) key outcomes or other achievements

High yielding wheat varieties with high grain protein content, good straw strength and good scab resistance are in demand by wheat growers because they greatly influence the profitability of wheat production in Minnesota. University of Minnesota developed varieties accounted for an estimated 50.6% of 1.2 million Minnesota wheat acres in 2017 which is the highest proportion in more than 3 decades. Recent releases include 'Linkert' (2013), 'Bolles' (2015), 'Shelly' (2016) and 'Lang-MN' (2017). Germplasm from our breeding program is increasingly being used as parents by private and public breeding programs in the region, too. Our breeding program continues to develop some of the most scab resistant germplasm in the region and this material is used as parents by private and public breeding programs. In addition, we coordinate the testing of 30-40 wheat varieties per year in statewide trials to assess their performance in yield nurseries and reactions to important diseases. This information is critical to growers to make informed choices among varieties.

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

All members of my project, regardless of what species they work on (wheat, intermediate wheatgrass, or field pennycress) help with inoculation and scoring of our FHB nurseries. This provides them with knowledge of the importance of this disease and our screening methodologies.

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

Wheat cultivar performance, including FHB reaction, of 39 spring wheat cultivars was assessed and reported to growers via print media, web-accessible publications, winter meetings, and field day presentations. We routinely enter five lines in the regional FHB nursery and a variety candidate performance nursery. The data of these nurseries is publicly available and other participants in the nursery have access to cross with this germplasm. Variety and germplasm releases are published in the Journal of Plant Registrations. The registration article for 'Bolles' was published during this reporting period and the article for 'Shelly' has been submitted.

Project 2: *Optimization and Establishment of Genomic Selection for FHB Resistance in Wheat.*

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

Our objective was to test the hypotheses that 1) GS models trained using a subset of F_5 lines will have a higher prediction accuracy in predicting F_5 FHB resistance compared to models trained using advanced lines; 2) optimal selection of training populations and marker compositions will increase our FHB GS prediction accuracies and help achieve desired training population sizes.

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

1) major activities

As part of our breeding efforts for FHB resistance, we genotyped 1,551 F_5 lines (17 96-well plates of DNA) using GBS. Of these, 500 F_5 lines were selected as training population and phenotyped for FHB severity in our two scab field nurseries. These 500 lines were also phenotyped for VSK and micro test weight. Predictive ability of genomic selection was increased in this year's training population compared to that in 2016. Using all phenotypic information, genomic selection models were trained and used to select a set of 353 for entry in to preliminary yield trials in spring 2018.

2) specific objectives

- GS models trained using a subset of F_5 lines will have a higher prediction accuracy in predicting F_5 FHB resistance compared to models trained using advanced lines;
- Optimal selection of training populations and marker compositions will increase our FHB GS prediction accuracies and help achieve desired training population sizes.

3) significant results

- Overall, predictive ability was higher for St. Paul traits. Correlation between field trait value and predicted trait value was also higher for St. Paul traits. Correlation values as high as 78% were observed, indicating the robustness of genomic selection.

4) key outcomes or other achievements

Our 2017 genomic selection results were quite promising, and continue to improve. We are also investigating the efficacy of a K-means clustering method in selecting the optimal training population. We are also testing the effect of reducing the training population from 500 to 200 on prediction accuracies. Preliminary analyses for both these studies will be done after phenotyping the 2018 F_5 lines. If successful, these results will have important implications for those breeding for FHB resistance and may allow breeders and pathologists to reduce the size of some selection nurseries and focus on more intensive phenotyping of smaller training populations.

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

All members of my project, regardless of the species they work on (wheat, intermediate wheatgrass, or field pennycress) help with inoculation and scoring of our FHB nurseries. This provides them with knowledge of the importance of this disease and our screening methodologies. Specifically, Prabin Bajgain (postdoc) carried out the phenotypic evaluations, GBS genotyping, and genomic selection work for this project with assistance from Xiaofei Zhang (previous postdoc) and undergraduate researchers. Other graduate students and postdocs on our project and others in our Department have also learned about our experiences with genomic selection.

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

We have discussed this research with many colleagues, including those on the cutting edge of genomic selection research. No formal presentations or publications regarding this work have been made

Project 3: *Using Targeted Sequencing to Breed for Fusarium Head Blight Resistant Wheat.*

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

The main objective of this project is to explore and evaluate a targeted sequencing approach that is expected to reduce the genotyping cost to ~$10/sample for spring wheat breeding efforts.

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

1) major activities - please see below under 'specific objectives'

2) specific objectives

RNA probes (baits) were designed for 5,064 loci, using sequence information obtained from the wheat 9K and 90K iSelect assays as well as GBS sequences obtained from multiple projects conducted by the UMN wheat breeding program. These loci represented all 21 wheat chromosomes with some QTL regions and known sequences of 12 important genes and their alleles that included *Ppd-D1*, *Fhb1*, *Fhb5*, *Rht-B1*, *Rht-D1*, *GPC*, *Lr34*, *Vrn-A1*, *Vrn-B1*, and *Vrn-D1*. Of the 5,064 loci, 2,100 were in the A genome, 2,391 in B genome, and 573 were in D genome. For each candidate locus, two 90 bp long probes were designed. Probes were synthesized by Arbor Biosciences™, formerly *MYcroarray*®.

The 17 DNA plates of F_5 wheat lines (from Project 2) were prepared for sequencing in the following manner: double stranded DNA digestion, ligation, and adapter ligation as per Illumina's instructions. Single-stranded digested DNA sequences were annealed to the RNA probes that represented our 5,064 candidate loci. Captured sequences resulting from the annealing step were sequenced to obtain 145 bp long reads (after trimming the adapter and barcode sequences).

Obtained sequences were filtered for quality (Phred score > 25) then aligned to the loci sequences using the program Burrows-Wheeler Aligner (bwa version 0.7.5a). The aligned reads were searched for polymorphic sites (SNPs) using Samtools version 1.6 and bcftools version 1.6. A blast-search was also conducted by transforming the loci sequences into a local blast database using NCBI Magic-Blast version 1.3.0.

3) significant results

Upon splitting the fastq files by barcodes, approximately 1.5 million reads were obtained per F_5 line, on average. In our experience, this number of reads per line is sufficient to conduct genomic analyses such as GWAS, GS, and QTL mapping. This also implied that,

theoretically, 290 reads per locus per line could be expected. The total number of reads for all F_5 lines would therefore be: 1549 lines x 12 genes/alleles x 290 = 5.4 million. However, the results indicated poor alignment of the sequenced reads to the loci sequences. The number of SNPs with 20% or less missing data from the targeted sequencing project was less than 100 whereas we regularly obtain more than 6,000 SNPs with 20% or less missing data from our GBS pipeline. Specifically, 7,079 SNPs were obtained for the same 2017 F_5 lines from GBS. This indicated that the alignment between the sequenced reads obtained from targeted sequencing and the references (sequences of 5,064 loci) was not good.

To further establish this assumption, every single read obtained from all F_5 lines were blast-searched against the 12 known gene/allele sequences used in bait design. To avoid the loss of partial alignments, partial matching was allowed with no filtering whatsoever. This resulted in 1.25 million reads matching the 12 genes and their alleles, which is less than a fourth of the expected 5.4 million reads. The blast-search also revealed that most alignments were not full length, i.e. 145 bp, as the obtained reads were 145 bp long. The majority (53%) of the reads were shorter than 75 bp and only about 30% of the reads were 100 bp or longer (Figure 1).

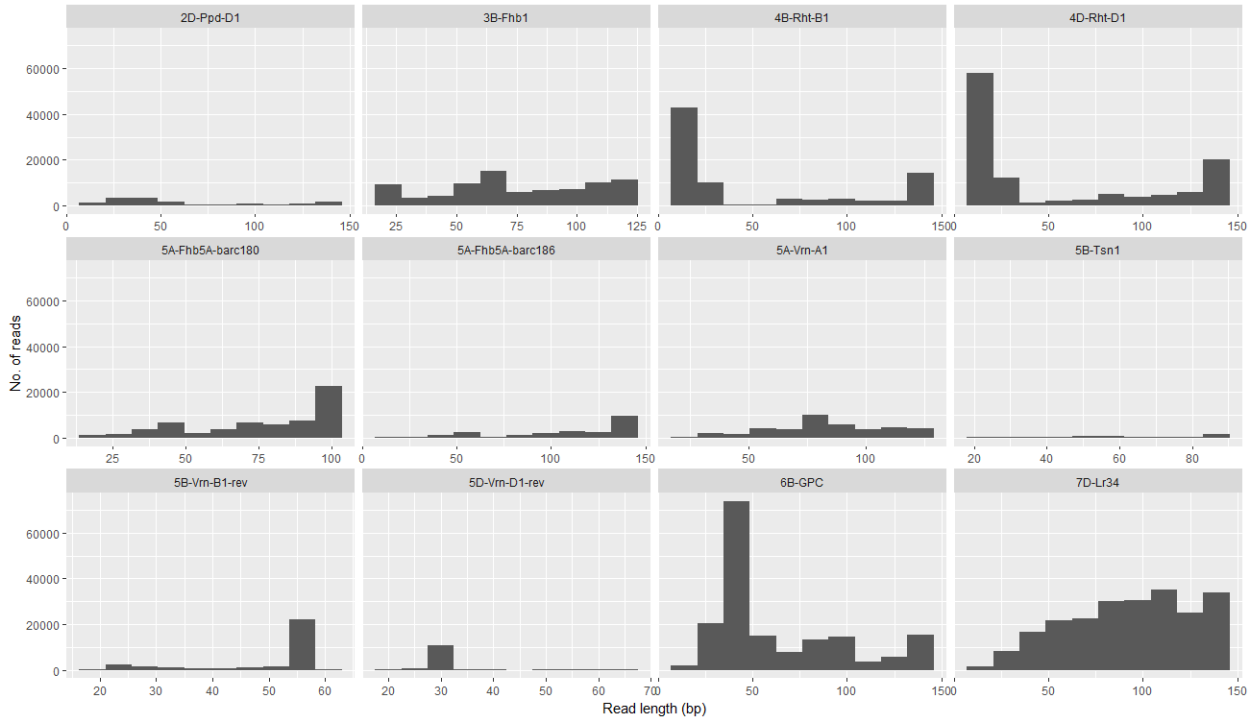

Figure 1: Frequency of read-lengths observed for the 12 wheat genes and their alleles. Majority of the reads were shorter than 75 bp whereas the sequenced read length was 145 bp.

4) key outcomes or other achievements

We learned that a targeted sequencing approach is not the best means to genotype wheat F_5 lines. GBS, despite the problems of missing data and non-uniform sampling of genomic loci, remains the best method to genotype a large panel of breeding materials for GWAS, GS, and other mapping studies. Further modification of experimental protocols for targeted sequencing may improve the success of this method.

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

Postdoc Prabin Bajgain worked with Shiaoman Chao (retired Research Molecular Geneticist, USDA, Fargo) to select candidate loci. Other graduate students and postdocs on our project have also learned about our experiences with targeted sequencing and genomic selection.

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

Targeted sequencing, while not a novel experimental design, was a new methodology for our team. We had the opportunity to learn about the bait design process, selection method of the candidate loci, and the slightly different bioinformatics work needed to obtain the SNP and read alignment metrics. While the results of the project were disappointing, testing the approach led to the understanding that the approach is not suitable for wheat breeding programs, and perhaps improvement of protocols can improve its success.

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?** 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 FY17 award period?** No.

   If yes, how many?

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?** No.

   If yes, how many?

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?** 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 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 (5/13/17 - 5/12/18). 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.

Anderson, J.A., J.J. Wiersma, G.L. Linkert, S. Reynolds, J.A. Kolmer, Y. Jin, M. Rouse, R. Dill-Macky, G.A. Hareland, and J.-B. Ohm. 2018. Registration of 'Bolles' hard red spring wheat with high grain protein concentration and superior baking quality. J. Plant Registrations. doi:10.3198/jpr2017.08.0050crc
Status: Published
Acknowledgement of Federal Support: YES

Haixiao Dong, Rui Wang, Yaping Yuan, James Anderson, Michael O. Pumphrey, Zhiwu Zhang, Jianli Chen. 2018. Evaluation of the Potential for Genomic Selection to Improve Spring Wheat Resistance to Fusarium Head Blight in the Pacific Northwest. Frontiers in Plant Science. doi:10.3389/fpls.2018.00911
Status: Published
Acknowledgement of Federal Support: YES

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

Other publications, conference papers and presentations.

Anderson, J.A. J. Wiersma, R. Dill-Macky, J. Kolmer, M. Rouse, and Y. Jin., M. Smith, and L. Dykes. 2017. Hard Red Spring Wheat. *In* Minnesota Field Crop Trials, University of Minnesota Agricultural Experiment Station.
Status: Published
Acknowledgement of Federal Support: NO