USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Final Performance Report – NCE for FY18
Due date: July 12, 2019

Cover Page

Principle Investigator (PI):	James Anderson
Institution:	University of Minnesota
E-mail:	ander319@umn.edu
Phone:	612-625-9763
Fiscal Year:	2017 (NCE for FY18)
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-P10010000 Minneapolis, MN 55455-2003
DUNS Number:	555917996
EIN:	41 -6007513
Recipient Identifying Number or Account Number:	CON000000017418
Project/Grant Reporting Period:	5/13/18 - 5/12/19
Reporting Period End Date:	05/12/19

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

(signature)   July 11, 2019

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

FY17 Final Performance Report – NCE for FY18
PI: Anderson, James
USDA-ARS Agreement #: 59-0206-4-019
Reporting Period: 5/13/18 - 5/12/19

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 2018. A total of 1,728 genotypes were evaluated in 1 to 3 replications for a total of 4,486 across the two locations. We evaluated the FHB reaction of external germplasm from the 2018 Uniform Regional Scab Nursery (24 lines) and 2018 Regional Performance Nursery (38 lines). We completed Visual Scabby Kernel (VSK) assessment of all materials from the St. Paul nursery and received DON data from select materials, mostly the most advanced nurseries.

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. The Genotyping Center screened 1,277 pre-yield trial lines with 11 gene-specific DNA markers, generating 14,047 data points. In addition, since Fall 2018 we screened 558 individual F_1 plants from topcrosses and backcrosses and 82 parents from Fall 2018 and Spring 2019 crossing blocks for as many as 22 markers in-house, generating a total of 2,776 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 2,533 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 545 lines that were

(Form – FPR17-18)

also phenotyped to include VSK and test weight, and observations from our winter nursery in New Zealand were used to select a set of 360 for entry in to preliminary yield trials in spring 2019.

3) significant results

- The St. Paul FHB screening nursery was excellent, and provided highly discriminatory data. The Crookston nursery did not receive enough irrigation water and as a result about 50% of the later heading lines largely escaped infection. As a result, only limited severity data was collected and the rows were not harvested for further assessment. From the 2018 St. Paul FHB nursery data and results from previous years, the FHB resistance of 38 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.
- 'MN-Washburn' was released in 2019. It is moderately resistant (4 on 1-9 scale) to FHB and is meant to replace Linkert, the leading HRS variety which is rated as a '5' for FHB.

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 52% of 1.6 million Minnesota wheat acres in 2018 which is the highest proportion in more than 3 decades. Recent releases include 'Linkert' (2013), 'Bolles' (2015), 'Shelly' (2016), 'Lang-MN' (2017), and 'MN-Washburn' (2019). 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 38 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 'Shelly' was published during this reporting period.

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
   In the past year, 2,533 F_5 lines and 45 parental lines were genotyped with the Genotyping by Sequencing method. A Euclidean distance matrix between the F_5 lines was calculated from the genotypic markers. A k-means clustering analysis was then applied to the Euclidean distances to generate partitions that represent the groups/structure present in the F_5 population. Three clusters (K-1, K-2, and K-3) were generated with this algorithm so that the lines within a cluster are more similar to each other than lines belonging to other clusters. A subset of lines proportional to the size of the cluster was selected to serve as the training population to predict the performance of other lines within that cluster. For this study, 200 F_5 lines (95 lines from K-1, 46 lines from K-2 and 59 lines from K-3) were selected to represent a portion of the training population. A supplementary 300 F_5 lines selected based on pedigree relationship, and 45 parental lines were also included to make a training population with a total size of 545 lines. All 545 lines were phenotyped for FHB severity and some post-harvest traits such as visual scabby kernels and micro test weight.

2) specific objectives
   We did a five-folds cross-validation to compare the predictive ability in two scenarios:
   a.   within the entire training population (500 F_5 lines with parents, 545 total)
   b.   within each cluster with parents (n = 140 in cluster one, 91 in cluster two and 104 in cluster three)

2)   significant results
   Predictive abilities ranged from 0.12 to 0.39 in the first scenario (large population) but ranged from 0.17 to 0.60 in the second scenario (smaller populations).

3)   key outcomes or other achievements
   The results from last year's genomic selection study was very encouraging. We are also investigating this method in the 2019 study. If the trend holds, we could significantly reduce the size of the F_5 training population that would be phenotyped. In fact, instead of testing 500 lines in just one replication, we can test 200 lines selected by genotypic profile in two replications to increase the quality of our training data, yet still reduce the number of lines phenotyped.

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) and Emmanuel Adeyemo (graduate student) carried out the phenotypic evaluations, GBS genotyping, and genomic selection work for this project. Other graduate students, 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. Emmanuel Adeyemo (graduate student) presented a poster at the 2018 USWBI meeting in St Louis, MO incorporating these results and a manuscript that summarizes the results is in preparation.

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).
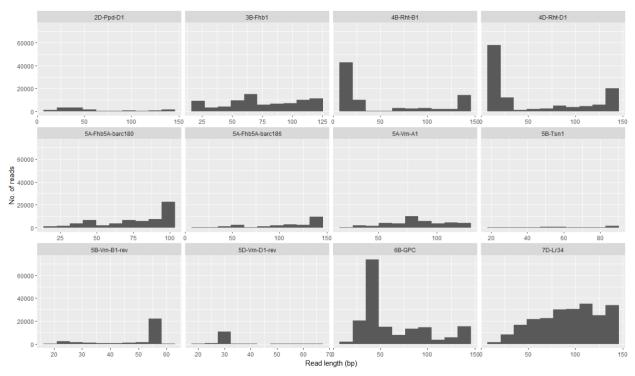

Figure 1: Frequency of read-lengths observed for the 12 wheat genes and their alleles. Majority of the reads were shorter than 75 bp whereas the sequenced read length was 145 bp.

Despite the failures, Jason Fiedler, the new Molecular Geneticist at USDA Fargo, re-analyzed the data in April 2019 by expanding the target region to 1000 bp flanking the SNP loci. The idea behind this was to see if our probes followed by sequencing captured flanking regions. If we could capture additional variants among the lines, that would allow

us to re-evaluate the method and optimize it so that it would still provide meaningful differences among the wheat lines. Dr. Fiedler used only a small subset of the sequence data (96 F_5 lines) for his investigation, which were further analyzed by postdoc Prabin Bajgain. After filtering the sites based on genotype quality (GQ) and depth (DP) of 1, it was found that:

- Of the 12,547 newly discovered SNPs, only 933 have less than 20% missing alleles, our typical cutoff in the GBS pipeline.
- Of the 12,547, only 721 matched with our probe designed alleles. Expanding the reference sequence to a flanking length of 1000 bp was still unable to give us the genotypes of these lines for the 12 known genes.
- Additionally, of the 721 SNPs that had the same alleles as the ones we designed, only 12 have less than 20% missing alleles.
- The median read depth per SNP was approximately 5K, i.e. about 55 reads per SNP per F_5 line. This is very low compared to the theoretical 290 reads per target locus per F_5 line.

The fact that we were able to detect more SNPs by increasing the flanking length of reference probes was quite encouraging. While it would have been good to re-discover the exact SNPs we designed, this data would have still been useful if not for the very low number of markers with low missing data %. The number of markers obtained after stringent filtering is not enough to carry out a sound GS/GWAS work. Alternatively, the reads could be aligned against the whole genome and see if this yields better results. At the very least, we will see where the reads are aligning to and be able to answer oversampling vs random sampling of the sequences.

4) key outcomes or other achievements

We learned that a targeted sequencing approach is not the best means to genotype wheat F_5 lines. This was re-confirmed by the new analysis we carried out earlier this year. GBS, despite the problems of missing data and non-uniform sampling of genomic loci, is the best method to genotype a large panel of breeding materials for GWAS, GS, and other mapping studies. We are currently also exploring the use of amplicon sequencing (or targeted sequencing) to genotype a large breeding population at a low cost.

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. During the past year Prabin Bajgain also worked with Jason Fiedler, the new Research Molecular Geneticist at USDA, Fargo. 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-NCE 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-NCE 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-NCE period?**

    No

    If yes, how many?

3. **Have any post docs who worked for you during the FY17-NCE 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-NCE 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 <u>full or partial</u> support through the USWBSI during the <u>FY17-NCE period</u>. 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
MN-Washburn	HRS	MR	4	2019

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-NCE grant period. Only include citations for publications submitted or presentations given during your award period (5/13/18 - 5/12/19). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

Journal publications.

Anderson, J.A., J.J. Wiersma, S.K. Reynolds, G.L. Linkert, R. Caspers, J.A. Kolmer, Y. Jin, M.N. Rouse, R. Dill-Macky, M.J. Smith, L. Dykes, and J.-B. Ohm. 2019. Registration of 'Shelly' hard red spring wheat. J. Plant Registrations, doi:10.3198/jpr2018.07.0049crc
Status: Published
Acknowledgement of Federal Support: YES

Singh, L., J.A. Anderson, J. Chen, B.S. Gill, V.K. Tiwari, and N. Rawat. 2019. Development and Validation of a Perfect KASP Marker for Fusarium Head Blight Resistance Gene *Fhb1* in Wheat. The Plant Pathology Journal, 35:200-207, https://doi.org/10.5423/PPJ.OA.01.2019.0018.
Status: Published
Acknowledgement of Federal Support: YES

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

None.

Other publications, conference papers and presentations.

Adeyemo, E., P. Bajgain, and J.A. Anderson. 2018. Optimizing Training Population Size to Improve Prediction Accuracy of Fusarium Head Blight Traits in Wheat. In: S. Canty, A. Hofstetter, B. Wiermer, and R. Dill-Macky (Eds.), *Proceedings of the 2018 National Fusarium Head Blight Forum* (p. 99). East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster), NO (abstract)

Qiu, R., C. Yang, M. Ali, J. Anderson, B. Steffenson, P. Marchetto. 2018. Detection of Fusarium head blight in small grains using hyperspectral imaging. In: S. Canty, A. Hofstetter, B. Wiermer, and R. Dill-Macky (Eds.), *Proceedings of the 2018 National Fusarium Head Blight Forum* (p. 32). East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster), YES (abstract)

Singh, L. J. Anderson, B.S. Gill, and N. Rawat. 2018. Expression of phytohormone related wheat defense genes in PFT-mediated resistance against Fusarium graminearum. In: S. Canty, A. Hofstetter, B. Wiermer, and R. Dill-Macky (Eds.), *Proceedings of the 2018 National Fusarium Head Blight Forum* (p. 74). East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster), YES (abstract)