USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY15 Final Performance Report
Due date: July 15, 2016

Cover Page

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Fiscal Year:	2015
USDA-ARS Agreement ID:	59-0206-2-088
USDA-ARS Agreement Title:	Alien Chromosome Engineering and the Deployment of a Novel Source of Fusarium Head Blight Resistance in Wheat.
FY15 USDA-ARS Award Amount:	$ 63,168
Recipient Organization:	Kansas State University 10 Andrerson Hall Manhattan, KS 66506
DUNS Number:	929773554
EIN:	48-0771751
Recipient Identifying Number or Account Number:	AR9907 / GAPP602517
Project/Grant Reporting Period:	06/25/15-06/24/16
Reporting Period End Date:	06/24/16

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Cloning and Validation of the FHB1 QTL from Sumai3 for Resistance to Wheat Scab.	$ 63,168
	FY15 Total ARS Award Amount	**$ 63,168**

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: *Cloning and Validation of the FHB1 QTL from Sumai3 for Resistance to Wheat Scab.*

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

The overall goal of the proposed research was to clone the *Fhb1* QTL from Sumai 3, which is the most consistently reported source of Type 2 resistance to the devastating Fusarium Head Blight (FHB) disease of wheat. Subsequently validation of the cloned QTL was done using reverse genetics approaches.

2. What was accomplished under these goals?

1) major activities:

The *Fhb1* region of Sumai 3 was sequenced and assembled. Gene annotations were performed on the assembly. Putative candidates were selected based on expression differences between resistant and susceptible near isogenic lines. TILLING was conducted for the selected candidate genes. RNAi was performed on the candidate gene as identified by TILLING. Association mapping across a panel of 40 Chinese landraces was done for characterizing the resistant haplotype.

2) specific objectives:

1. The contiguous sequencing of *Fhb1* region of Sumai3 using Sumai3 BAC library is the first objective. We have obtained ~300 kb of sequence but there are some gaps (>100 kb) which still need to be filled. BAC screening will be done and selected BACs will be sequenced using NGS techniques. The sequences will be annotated for putative candidate genes followed by their validation.
2. From the sequenced portion, we identified three putative candidates based on expression profiling in resistant and susceptible near isogenic lines. Validation of the promising genes will done using reverse genetics approaches of TILLING and RNAi.
3. Association mapping across a panel of several Chinese Landraces with *Fhb1* will be done to find additional alleles, gene features, and conserved domains that may play a role in resistance to FHB.

3) significant results

We identified the gene that controls Fhb1 mediated resistance using expression differences between resistant and susceptible NILs. It was present in resistant NIL but absent in susceptible NIL.Reverse genetics approaches of TILLING and RNAi validated the candidacy of our gene. In the association panel all the resistant landraces were found to contain the candidate gene. One of the susceptible haplotypes had the gene but contained a splice junction aberration, common with one of the TILLING susceptible mutants, confirming that the susceptibility of the landraces of this panel was due to splice junction aberration in our candidate gene. The gene encodes a chimeric lectin containing two agglutinin domains and an ETX/MTX2 domain and we hypothesize it leading to toxicity to the fungus, and thus providing resistance against FHB. Although, more mechanistic studies will be needed to further confirm the exact mode of action of the gene.

4) key outcomes or other achievements

The outcome of the project is the *Fhb1* gene was cloned. Validation using reverse genetics approaches of TILLING and RNAi showed that our candidate gene was necessary for *Fhb1* mediated resistance. Additionally, resequencing of the genes in the association panel showed that all the resistant haplotypes had our gene. One of the susceptible haplotypes which showed the presence of the gene shared a splice junction mutation common with one of our already characterized susceptible mutants.

The specific objective of the proposed research of filling the gap (>100kb) in the final assembly was not met, because of the absence of a corresponding BAC in our BAC library (coverage: ~3x genome equivalent). To find a BAC for every genomic region a higher coverage (upto ~10x genome equivalent) of the BAC library was desirable. However, to search for any genes that might be candidates of Fhb1, we did extensive analysis of the recent NR gene assembly of 3A, 3B, and 3D chromosomes of hexaploid wheat, and 3A and 3B of tetraploid wheat and there were no new potential candidate genes of Fhb1 in the gap region.

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

The Post-Doc (Dr. Nidhi Rawat) working on the project got hands on training in sequencing, sequence assembly and analyses, molecular biology techniques and Fusarium Head Blight screening and scoring. The results of the research were presented in national and international conferences by her (listed under Publications). Dr. Rawat has accepted a faculty position at the University of Maryland.

Two undergraduate students (Kelsey Madden and Reauxqkwuanzyiia C'Lay-Pettis) were hired and trained in DNA extraction, RNA extraction, PCRs, and FHB screening.

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

Talks were delivered reporting the results of the research in national (National Fusarium Head Blight Forum Workshop) and international (Plant and Animal Genome Conference) conferences. Abstracts were published.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY15 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 FY15 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 FY15 award period?** No

   If yes, how many?

3. **Have any post docs who worked for you during the FY15 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?** Yes

   If yes, how many? One

4. **Have any post docs who worked for you during the FY15 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 FY15 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

Refer to the FY15-FPR_Instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY15 grant. If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

Journal publications.

Rawat N, Pumphrey MO, Liu S, Zhang X, Tiwari VK, Ando K, Trick HN, Bockus WW, Akhunov E, Anderson JA, Gill BS. The *Fhb1* gene of wheat encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain.
Status: Under review.
Acknowledgement of Federal Support: YES

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

Nothing to Report

Other publications, conference papers and presentations.

Rawat N, Pumphrey MO, Liu S, Zhang X, Tiwari VK, Trick HN, Bockus WW, Akhunov E, Anderson JA, Gill BS. (2016). Positional cloning of *Fhb1* gene in wheat. 2016. Plant and Animal Genome Conference XXIV. San Diego, CA, USA. Invited talk presented. W976.
Status: Abstract Published and talk delivered.
Acknowledgement of Federal Support: YES

Rawat N, Pumphrey MO, Akhunov E, Anderson JA, Gill BS. (2016).Map-based Cloning Reveals the Origin of *Fhb1* Gene in Wheat. Plant and Animal Genome Conference XXIV. San Diego, CA, USA. Invited talk presented. W786.
Status: Abstract Published and talk delivered.
Acknowledgement of Federal Support: YES

Rawat N. (2015). Map Based Cloning of *Fhb1* Gene in Wheat. 2015 National Fusarium Head Blight Forum. Wheat & Barley Scab Initiative.
Status: Talk delivered
Acknowledgement of Federal Support: YES