USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY07 Final Performance Report (approx. May 07 – April 08)
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Cover Page

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Fiscal Year:	2007
USDA-ARS Agreement ID:	59-0790-4-091
USDA-ARS Agreement Title:	Breeding and Development of DNA Markers for Fusarium Head Blight Resistance in Wheat.
FY07 ARS Award Amount:	$ 219,091

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
HGR	Characterization and Pre-Breeding of Novel Fusarium Head Blight Resistance Sources.	$54,974
HGG	Construction and Utilization of a Pooled BAC Library of Sumai 3.	$ 29,775
HGG	QTL Mapping of Wheat Fusarium Head Blight Resistance in the Japanese Landrace PI 81791.	$ 50,732
VDUN	Breeding Fusarium Head Blight Resistant Spring Wheat.	$ 83,610
	Total Award Amount	**$ 219,091**

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Principal Investigator Date

* CBCC – Chemical, Biological & Cultural Control
EEDF – Etiology, Epidemiology & Disease Forecasting
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GET – Genetic Engineering & Transformation
HGR – Host Genetics Resources
HGG – Host Genetics & Genomics
IIR – Integrated/Interdisciplinary Research
PGG – Pathogen Genetics & Genomics
VDUN – Variety Development & Uniform Nurseries

Project 1: *Characterization and Pre-Breeding of Novel Fusarium Head Blight Resistance Sources.*

1. What major problem or issue is being resolved and how are you resolving it?

Fusarium head blight resistant spring wheat germplasm has been identified by vigorous screening of the worldwide collections in the National Small Grains Collection. The utilization of the selections is not satisfactory due to the lack of adequate information on their novelty, and the low direct breeding value of the unadapted germplasm. This project confronts the issue of characterizing the resistance, introgression of the resistance into elite germplam, and finding additional new sources of resistance in spring wheat. We use FHB nurseries to select for FHB resistance and adaptability in spring wheat germplasm introduction and pre-breeding populations. Resistant selections made based on three year replicated FHB screening nurseries were evaluated for point-inoculation in the greenhouse, and genotyped with DNA markers linked to FHB resistant QTLs. The most resistant selections are distributed to breeders.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

Accomplishment: A total of 25 Elite year 2, 21 Elite year 1, 15 advanced plant introductions, 15 selected Abura/Wheaton RIL lines, 74 Wheaton/Tokai 66 RILs; 114 PI 345731/Wheaton RILs; 130 Wheaton/16-52-9 RILs; 138 Wheaton/PI 185380 (Prodigio Italiano) RILs, and 112 Wheaton/PI 285933 (Chudoskaja) RILs were evaluated for FHB resistance in 2007 FHB nurseries at Crookston and/or St. Paul. The Crookston nursery had high disease levels and we were able to make selections within and among the most promising populations. Due to low disease levels in the St. Paul nursery, presumably due to unseasonably high temperatures, we did not get useful, discriminating data. Based on this data, we planted 318 RILs from 5 different populations, and 4 selected RILs (Wheaton/Tokai 66 RIL57, Wheaton/16-52-9 RIL53, Wheaton/Abura RIL64, Wheaton/Abura RIL83) in our 2008 Crookson FHB nursery. Six F_3 populations were also planted in 2008 for generation advancement as we continue to cross the best resistance sources with adapted spring wheat lines. These six populations are: Wheaton/PI 19766, Wheaton/PI 62083, Wheaton/PI 69261, RB07/PI 19766, RB07/PI 62083, and RB07/PI 69261.

Impact: We have continued our characterization of potential new FHB resources and screening of the most promising materials that were crossed to regionally adapted HRS lines. This makes the FHB resistance genes contained in these materials more accessible to the breeding community because they are contained in improved genetic backgrounds. Furthermore, we have prioritized our pre-breeding efforts to focus on materials that do not contain *Fhb1* based on diagnostic markers. This allows us to focus on germplasm more likely to contain novel resistance genes.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?: All germplasm and pre-breeding materials are available upon request. RIL populations developed as part of this project are good candidates for QTL mapping populations. Our Wheaton/PI 81791 population (Project 3 of this report), resulted from this project. After

summarizing multiple years of data for these materials, the results will be published, similar what is shown in Crop Sci. 48:223–235 which summarizes earlier work on this project.

Accomplishment: Five lines, resulting from this project were entered into the 2007 Uniform Regional Scab Nursery. All five were from the cross Wheaton/Abura. Two of the RILs, namely #64 and #83 had the lowest overall DON among the 48 entries in the test.

Impact: Abura is a good source of FHB resistance, but is not well adapted to the HRS wheat region of the U.S. We have incorporated high levels of FHB resistance from this source into a more adapted HRS background by crossing it with Wheaton. The high levels of resistance in progeny from this cross indicate that high levels of FHB resistance can be recovered from single crosses involving a highly susceptible parent.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:
The regional scab nursery provides an effective means of sharing germplasm. Therefore, other regional breeding programs growing this nursery have had these materials and opportunity to cross with them since April, 2007.

Project 2: *Construction and Utilization of a Pooled BAC Library of Sumai 3.*

1. What major problem or issue is being resolved and how are you resolving it?

Bacterial Artificial Chromosome (BAC) libraries are an essential tool for gene cloning and sequence analysis. There are no BAC libraries of wheat available in the U.S. and none of the few existing libraries worldwide were constructed using a known FHB resistance source. The main purpose of this grant it to develop genomics resources that will be useful in our cloning of *Fhb1*, the major QTL for Fusarium head blight resistance in wheat. A BAC library of Sumai 3, the donor of *Fhb1* and at least two other important FHB resistance QTL is being constructed. This library may be useful to other groups attempting to clone other FHB resistance from Sumai 3.

The objectives of the grant are to:

1) Construct a pooled BAC library of Sumai 3 for the wheat community.
2) Construct a Sumai 3 BAC contig spanning *Fhb1*.
3) Develop user-friendly markers for *Fhb1* and provide the markers to the Regional Genotyping Centers.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

Accomplishment: Objective 3 has been completed. Dr. Sixin Liu, postdoctoral research associate, has worked with Shiaoman Chao at the Fargo Genotyping Center in designing and testing new markers for *Fhb1* that are more amenable to the equipment used at the Genotyping Centers. We have developed a robust, co-dominant marker, named UMN10 that can be used to screen for *Fhb1*.

Impact: This marker is more diagnostic and easier to use compared to previously developed markers. This helps breeders select for the exact genotype at the *Fhb1* locus.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?: The Fargo, Manhattan, and Raleigh Genotyping Centers and several wheat breeders in the U.S. have all been using marker UMN10 since December 2007. Wheat breeders can now more efficiently breed for resistance to FHB using this improved marker.

Accomplishment: At the current stage the Sumai 3 BAC library consists of 460,800 clones with the average insert size 120 kb. This number of clones corresponds to 3.5x coverage of the hexaploid wheat genome. All BAC clones are arrayed into 384-well microtiter plates (a total of 1,200 plates) containing LB-based freezing media suitable for long-term storage of bacterial clones at -80C. The original copy of the library is currently being stored in -80C freezer in the Department of Plant Pathology, KSU.

Impact: None realized as yet as the BAC library is still in development. We are using FY08 funding to complete the library and begin screening clones from the library this fall and plan to sequence one or more of the BAC clones containing *Fhb1* within 6 months.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:

There are currently no BAC libraries of hexaploid wheat available in the US. Therefore, Sumai 3 BAC library is important addition to genomic resources available to US wheat researchers. Recent studies of wheat genome evolution demonstrated significant amount of structural variation among different wheat lines. This variation results in absence of perfect synteny between wheat accessions and precludes utilization of available BAC libraries for positional cloning of all wheat genes. Therefore, Sumai 3 BAC library will provide additional resource for positional cloning of agronomically important genes in wheat.

Project 3: *QTL Mapping of Wheat Fusarium Head Blight Resistance in the Japanese Landrace PI 81791.*

1. What major problem or issue is being resolved and how are you resolving it?

We are trying to improve FHB resistance in wheat by incorporating new resistance genes. The resistant wheat landrace, PI 81791, was identified as having high levels of FHB resistance and not containing *Fhb1* as part of another USWBSI-funded project (the progenitor of FY07 Project 1). A mapping population of 150 RILs was constructed by crossing PI 81791 with the agronomically adapted line, Wheaton. Phenotypic evaluation in four different field environments and two greenhouse environments are being performed to evaluate the resistance to *Fusarium graminearum*. These 150 RILs will also be genotyped using SSR markers. Using both the phenotypic and genotypic data, QTL involved in resistance to scab will be identified and mapped. Identification and mapping of these QTL will allow for the resistance to be capitalized upon for use in breeding programs and, potentially, for the eventual release of lines containing this resistance to growers. Furthermore, markers will be assessed for their ability to be used diagnostically in order to identify the resistance QTL in existing and future lines. Additionally, the phenotypic screens will be used to identify RILs that are both resistant and agronomically adapted for use as parents in our breeding program.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

A.) <u>Accomplishment:</u> Completion of disease screenings. Type II resistance screening in the greenhouse has been completed for both the fall 2007 and spring 2008 seasons. Type I and II resistance screening in the field was completed for the summer 2007 season at both St. Paul and Crookston, MN locations. This population is being grown in 2008 FHB nurseries at both locations.

<u>Impact:</u> The greenhouse and field resistance scores will be used as phenotypic data for QTL mapping of the Wheaton/PI 81791 mapping population. Seed from heads harvested from the field screenings are being used to assess visually scabby kernels (VSK) and determine DON content for each of the RILs and parents. Additionally, the scores are being used to identify resistant lines that are also agronomically adapted for use as parents in our breeding program.

<u>As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:</u>

The data collected during the 2007 and 2008 greenhouse seasons and the 2007 field season, as well as the data that will be collected upon the completion of the 2008 field season, has provided a means to more accurately identify the QTL involved in scab resistance from the PI 81791 resistance source. Additionally, the field and greenhouse screenings provide data to identify new potential parents for breeding programs that have shown consistent resistance and agronomic adaptation.

B.) **Accomplishment:** Development of QTL mapping tools. The Wheaton and PI 81791 screening with approximately 880 SSR markers was done at the USDA-ARS Fargo Genotyping Lab. The data generated from that screen resulted in the identification of 519 that are informative (i.e., polymorphic or dominant). DNA has also been isolated from all 150 individuals comprising the Wheaton/PI 81791 RIL mapping population, as well as the parents of this population.

Impact: The DNA will be used for mapping the 519 polymorphic markers available for the population. A majority of these markers will be run by a Ph.D. student on the Anderson project at the USDA-ARS Fargo Genotyping Lab from mid to late August. The markers and DNA will be used to identify the QTL involved with scab resistance donated by PI 81791 via QTL mapping procedures.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?: The polymorphic SSR markers will allow for the identification and locating of resistance QTL. The SSR markers may also be useful for future comparative and association mapping work, as they are being consistently used on several different wheat mapping populations for various traits. Also, once QTL are identified, some of the SSR markers can potentially be used as diagnostic markers. These diagnostic markers may be useful across different populations in identifying scab resistant individuals with resistance originating from PI 81791.

Project 4: *Breeding Fusarium Head Blight Resistant Spring Wheat.*

1. What major problem or issue is being resolved and how are you resolving it?

Wheat varieties with greater resistance to *Fusarium* head blight (FHB) would make a substantial contribution to reducing the losses from this devastating disease. The main objective of this project is to develop Fusarium head blight resistant wheat germplasm and varieties adapted for commercial production in Minnesota and the surrounding region and characterize the level of FHB resistance of all wheat varieties grown in the region.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

Accomplishment: The new variety 'Tom' was released in 2008. Named to honor Tom Anderson, 'Tom' has moderate resistance to FHB and has high grain yields. The overall performance of this variety it similar to 'Freyr', the most popular variety among Minnesota wheat growers in 2007. However, Tom also has moderate resistance to stem rust race Ug99. This provides an additional safeguard in case this new stem rust race reaches the U.S. in the next few years.

Impact: Tom is rated as a 4 for FHB resistance on our 1-9 scale in which 1 is the highly resistant and 9 highly susceptible. Currently, only six other wheat varieties in the region have a scab rating this good. Unfortunately, 4 of these 6 varieties have lower than average yields and are not likely to be popular with growers. Tom's moderate FHB resistance can help reduce losses to this disease.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?: The release of 'Tom' gives wheat growers in the region an additional option when selecting for varieties that provide high yield and FHB resistance. It is vitally important that FHB resistant varieties also have high yields and acceptable agronomic characteristics and end-use quality. Tom is one of only two varieties (Freyr is the other) that combines a moderate level of FHB resistance (4 or lower on our 1-9 scale) and above-average yield among the 20 varieties for which we have at least 3 years of Minnesota yield data.

Accomplishment: Five experimental lines were entered in the 2007 Uniform Regional Scab Nursery. These lines were identified in previous testing as having improved levels of FHB resistance and two of them were among the best performers in the nursery. MN02222-1, a repeat entry from the 2006 nursery had the 3rd lowest DON level among the 48 entries in the trial and was also in the top 10 for lowest disease index and VSK.

Impact: These lines combine FHB resistance from different sources and are candidates for germplasm release. These lines are available and have been requested by other wheat breeders in the region for use as crossing parents.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?: These materials contain unique combinations of FHB resistance genes and in many cases the resistance levels are superior to the resistant checks used in the nursery. Increasingly, the other important characteristics desired of crossing parents are improving as well (e.g. shorter height, greater straw strength, better leaf rust resistance, better grain yield and quality).

Accomplishment: Scab nurseries were established at 3 field sites in 2007. A total of 1,700 genotypes were evaluated in 5,000 total rows among the locations. Dry, hot weather resulted in low FHB at the Morris and St. Paul locations. The Crookston FHB screening nursery was excellent, similar to 2006, and provided highly discriminatory data. As a result of this nursery and results from previous years, the FHB resistance data of 25 spring wheat cultivars was assessed and reported to growers via print media and field day presentations.

Impact: Good field screening nurseries are needed to maintain progress in breeding for FHB resistance. FHB remains a potentially devastating disease in the region as severe damage was inflicted in 2005. Our FHB resistance ratings are an important part of growers' decision regarding which variety they will grow.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before: The U of MN FHB variety ratings are based on data from about six FHB inoculated, mist-irrigated nurseries (fewer than six is possible if the nurseries were highly discriminatory). In most cases, we have ratings of new varieties published by December of the year preceding the sale of certified seed to growers.

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Peer-Reviewed Articles

Anderson, J.A. 2007. Marker-assisted selection for Fusarium head blight resistance in wheat. Intl. J. Food Microbiol. 119:51-53.

Anderson, J.A, S. Chao, and S. Liu. 2007. Molecular breeding using a major QTL for Fusarium head blight resistance in wheat. Crop Sci. 47:S-112-S-119.

Abstracts/Proceedings

Anderson, J.A. 2007. Use of MAS for FHB Resistance: Is it working for Wheat Breeders? *In* S.M. Canty, A. Clark, D. Ellis, and D. Van Sanford (eds), Proceedings of the National Fusarium Head Blight Forum; Dec 2-4, 2007; Kansas City, MO. Okemos, MI: ASAP Printing, Inc. pp.

Reports

Anderson, J., J. Wiersma, J. Kolmer, and R. Dill-Macky. 2007. Spring Wheat. *In* Preliminary Report 24; 2006 Wheat, Barley and Oat Variety Performance in Minnesota, Preliminary Report, Edited by Jochum Wiersma.

Anderson, J.A., G.L. Linkert, and J.J. Wiersma. 2007. Hard Red Spring Wheat. *In* Minnesota Varietal Trials Results, University of Minnesota Extension Service.