USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY12 Final Performance Report
July 16, 2013

Cover Page

<table>
<thead>
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<tbody>
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<td>Fiscal Year:</td>
<td>FY12</td>
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<tr>
<td>USDA-ARS Agreement ID:</td>
<td>59-0790-8-071</td>
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<tr>
<td>USDA-ARS Agreement Title:</td>
<td>Management and Resistance Sources for Control of FHB in Barley.</td>
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<tr>
<td>FY12 USDA-ARS Award Amount:</td>
<td>$ 24,458*</td>
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USWBSI Individual Project(s)

<table>
<thead>
<tr>
<th>USWBSI Research Category**</th>
<th>Project Title</th>
<th>ARS Award Amount</th>
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<tbody>
<tr>
<td>BAR-CP</td>
<td>Coordination of NABSEN and Utilizing Historic Data Set for Association Mapping.</td>
<td>$ 20,865</td>
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<tr>
<td>BAR-CP</td>
<td>Field Tests of Transgenic Barley Lines.</td>
<td>$ 3,593</td>
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<td><strong>Total ARS Award Amount</strong></td>
<td></td>
<td><strong>$ 24,458</strong></td>
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</table>

Principal Investigator Date

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* Partial funding for this research is under ARS agreement # 59-0206-9-062
** MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
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: Coordination of NABSEN and Utilizing Historic Data Set for Association Mapping.

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Scab is the most insidious disease of barley and this project addresses the issues of developing genetically resistant varieties of barley that can be grown in combination with fungicide use and cultural practices to mitigate the effect of scab. The objectives of the project are to coordinate the disease screening of elite North American barley germplasm in uniform FHB nurseries in North America and China as well as utilize the historic data for association mapping to possibly identify novel resistance QTL. The advanced barley lines from the NDSU 2-rowed and 6-rowed breeding programs, the Univ. of Minnesota, Busch Ag, and Agriculture and Agri-Food Canada were tested in mist-irrigated nurseries, as well as under normal rainfall conditions. The mist-irrigated nurseries were supplemented with corn inoculum containing *Fusarium graminearum* spores. The FHB nurseries have been continually established and evaluated for the project for more than 12 years and currently is known as the North American Scab Evaluation Nursery (NABSEN). In 2012, we established two mist-irrigated nurseries located in Fargo and Langdon, North Dakota. Other locations established by cooperators included Osnabrock (ND), St. Paul and Crookston (MN), Brandon (Manitoba) and Yancheng (China). All the NABSEN nurseries contained the same set of elite breeding germplasm with putative FHB resistance. The historic NABSEN data was utilized for association mapping analysis, however it was determined that a different analysis strategy must be employed and this process is currently being worked out. The data set is being analyzed using best linear unbiased prediction (BLUP) on the mixed model with multiple error terms. The analysis should allow for the identification of FHB QTL and markers associated with these loci that can possibly be utilized in MASS or genome wide association mapping.

2. List the most important accomplishment and its impact (i.e. how is it being used) to minimize the threat of Fusarium head blight or to reduce mycotoxins. Complete both sections (repeat sections for each major accomplishment):

**Accomplishment (1):**

The nursery contained 50 lines, including 6 resistant and susceptible controls. Short rows with three replications were planted at each location. Heading dates were recorded at Fargo, Langdon, Crookston, St. Paul and Brandon and FHB incidence were recorded at Fargo, Langdon, Osnabrock and Brandon. FHB severity was recorded at Fargo, Langdon, Osnabrock, St. Paul, Crookston and Brandon; all of these locations were under mist irrigation except Osnabrock. DON levels were recorded from all seven locations in U.S. and Manitoba, Canada. Disease levels in 2012 were very low at Crookston and Casselton dryland nursery locations, thus no FHB incidence or severity data were taken from these nurseries. FHB disease levels were highest at Langdon, Brandon Manitoba and Crookston misted locations; Fargo and St. Paul levels were moderate. DON levels were highest at Langdon and the other locations were all moderate. Temperatures were above average and
precipitation below the 30-year average in August. Temperatures were some of the highest in recent years and precipitation the lowest.

Plants were harvested and samples analyzed for DON in Paul Schwarz’s lab at North Dakota State University or measured by ELISA technique at ECORC, Ottawa. DON levels varied much between locations with the dryland locations having the lowest levels with a range from 0.3-2.1 ppm and misted nurseries ranged from 3.7 to 25.8 ppm. The NDSU two-rowed line 2ND29827 had the lowest mist irrigated DON level (3.7 ppm), which was lower than Conlon the two-rowed standard with a mean DON level of 4.3. None of the six-rowed lines under irrigation had lower DON accumulation levels than the six-rowed standard ND20493, which had a mean DON level of 4.7. Seed was also redistributed and sent to Yancheng, China for testing.

**Accomplishment (2):**

Another important accomplishment is the development of a PCR based genotype-by-sequencing platform utilizing Ion Torrent next generation sequence technology that allows the genotyping of barley lines with 384 SNP markers for approximately $2.00/ line. The 384 marker platform was designed based on SNPs from the barley 9k bead express Illumina platform, which will allow the integration of both data sets as well as validation of the GBS data. We have designed the 384-primer set and are planning to genotype all of the historic barley lines that are missing genotype data with this platform for the downstream association mapping.

**Impact:**

Significant progress is being made toward developing FHB resistant barley cultivars. All North American barley breeders have access to the data collected in this project. The breeders are able to use the relative performance data to make decisions about continuing or dropping particular breeding lines. Breeders now have: 1) tests of the resistance stability of their breeding lines across a range of environments and disease pressures; 2) a measure of the resistance in their advanced lines compared to those of the other barley breeders in North America; 3) access to unique germplasm with resistance to FHB and DON accumulation. Four six-rowed and two two-rowed NDSU lines were submitted for AMBA pilot scale evaluation in 2012 that were from the FHB-resistance breeding program and underwent evaluation in the NABSEN. Three new lines from the University of Minnesota breeding program were entered into 2013 industry malt evaluations with lower DON than the variety Quest and line M149, similar to Quest DON levels, is being considered for industry plant-scale brewing evaluations. These lines also underwent evaluation in the NABSEN.

The 13 years of FHB data and SNP genotyping of the NABSEN entries will provide the opportunity to conduct the association mapping analysis to identify additional FHB resistance QTL.
Project 2: Field Tests of Transgenic Barley Lines.

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

   Commercially accepted barley lines grown in the upper Midwest are susceptible to FHB and have DON accumulation levels exceeding those acceptable by the malting industry. Breeding programs have made consistent progress to bring DON levels down and some of these lines have been approved for malting quality. However, DON accumulation may still be above acceptable industry standards in these new lines when environmental conditions are conducive for a major FHB epidemic. Transgenic barley lines with novel or synthetic sources of resistance will allow for a boost in resistance and lower DON accumulation that is not available through endogenous genes currently available from the primary barley germplasm pool.

2. List the most important accomplishment and its impact (i.e. how is it being used) to minimize the threat of Fusarium head blight or to reduce mycotoxins. Complete both sections (repeat sections for each major accomplishment):

   Accomplishment:

   The NDSU barley pathology program is cooperating with Dr. Lynn Dahleen from the USDA-ARS facility in Fargo, to field screen transgenic materials she has developed or has acquired from collaborators. The NDSU barley pathology project planted the material, applied corn based inoculum, set up the misting system and maintained the nursery, all in Langdon, ND. Dr. Dahleen evaluated the nursery for disease and harvested the grain for DON evaluation. See Dr. Dahleen’s project report for the accomplishments she has achieved with this project.

   Impact:

   See Dr. Dahleen’s report on this project.
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.

