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

Cover Page

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Fiscal Year:	FY12
USDA-ARS Agreement ID:	NA
USDA-ARS Agreement Title:	Transgenic Barley for FHB Resistance.
FY12 USDA-ARS Award Amount:	$ 58,051

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Field Tests of Transgenic Barley Lines.	$ 6,790
BAR-CP	High Efficiency Method for Generating FHB-Resistant Barley: Removing Bottlenecks in the Pipeline for Deploying FHB Resistance Genes.	$ 8,465
BAR-CP	Backcrossing of Promising Transgenes into Cultivars/breeding Lines with FHB Resistance.	$ 12,660
GDER	Development and Testing of Improved Enzymes for Transgenic Control of FHB.	$ 15,403
EC-HQ	Accelerating Transformation Efforts for Barley.	$ 14,733
	Total ARS Award Amount	**$ 58,051**

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Principal Investigator Date

* 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: *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?**

   Transgenic approaches have the potential to increase resistance to FHB and reduce DON in barley. We are conducting replicated field trials of transgenic barley lines developed by multiple labs supported by the USWBSI. Collaborator lines are in the cultivar Golden Promise, so we crossed their lines three times to the adapted cultivar Conlon and developed homozygous lines for field testing in 2012.

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 2012 North Dakota Transgenic Field Trials included 16 transgenic lines, null segregants for four of the lines, plus the susceptible check Conlon, and the resistant checks Quest and CI4196. Disease and toxin levels were moderate, with little difference between the checks. Direct comparisons of the transgenic-null pairs for thionin (transformed by R. Skadsen) showed no effect from the transgene. One of the gastrodianin lines (transformed by T. Abebe) showed approximately half the FHB and DON levels that were found in the corresponding null segregant line, while the other gastrodianin line showed no effect on FHB and DON.

   Impact:

   These transgenic FHB field trials provide accurate assessment of transgene effects on FHB and DON levels in infected barley. 2012 results indicate that thionin does not reduce disease and toxin levels but another year of testing is needed on lines expressing gastrodianin.

Project 2: *High Efficiency Method for Generating FHB-Resistant Barley: Removing Bottlenecks in the Pipeline for Deploying FHB Resistance Genes.*

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

   Multiple candidate genes that may reduce FHB and/or DON have been identified through USWBSI-funded research, using Physcomitrella and VIGS in wheat. We are using transgenic approaches to insert and express these genes in barley. Homozygous lines are being developed and will be tested in the ND and MN transgenic FHB nurseries.

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:

   Received and began transformation efforts with six gene constructs developed by M. Lawton's laboratory, constructs containing genes that were identified by overexpression or silencing in Physcomitrella. Continued transformation efforts with four constructs containing genes identified through virus-induced gene silencing in wheat developed by S. Scofield. The first two lines with TaERF1 were developed in Golden Promise and are being crossed to Conlon for field tests. One of the Golden Promise lines was planted in the 2013 field to see if any FHB results can be obtained. In addition, one line with TaBAK1 and three lines with TaBRI1 are undergoing progeny analysis for transgene segregation. All lines in Golden Promise will be crossed at least 3 times to Conlon, while lines in Conlon will be advanced to develop homozygous lines for field tests.

   Impact:

   This project is creating transgenic barley lines that will be field tested to determine whether the transgenes reduce FHB and DON.

Project 3: *Backcrossing of Promising Transgenes into Cultivars/breeding Lines with FHB Resistance.*

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

   Several transgenic lines (321 and 823) have shown reduced DON in field trials compared to their non-transgenic parent Conlon, while barley breeders have started releasing new cultivars that show reduced FHB and DON compared to previous cultivars. The goal of this project is to transfer the transgenes from 321 and 823 into Quest and ND20448 and field test to determine whether the resistance/low DON from the transgenes is additive to the resistance incorporated through the breeding programs.

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:

   At this time, we have completed three crosses between Quest and the transgenics and between ND20448 and 321. The ND20448 by 823 crosses are one generation behind. Continued crossing over the next year will provide homozygous lines that will be tested in 2014 transgenic field trials.

   Impact:

   In progress, no impact yet.

Project 4: *Development and Testing of Improved Enzymes for Transgenic Control of FHB.*

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

   Research has shown that Tri101 from *F. graminearum* could be altered to improve activity and stability. Collaborator Ivan Rayment has provided us with constructs containing the wild-type and improved *Tri101* genes. We are inserting the genes into barley to test effects on DON contamination in FHB-infected barley.

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:

   Six lines were identified as carrying the transgenes. Leaf tissue from these lines was sent to Dr. Ivan Rayment for protein analysis.

   Impact:

   None yet.

Project 5: *Accelerating Transformation Efforts for Barley.*

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

   Barley transformation efforts are time consuming and labor intensive. Additional personnel can help increase the number of constructs that we can insert into barley each year. The goal of this funding is to increase personnel working on barley transformation.

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:

   Unfortunately because of ARS hiring restrictions, the transformation technician position was open from July 2012 until the end of February 2013, which slowed research. Since the position was filled in late February, the technician has been trained in barley tissue culture and transformation techniques. She is now bombarding at least 360 callus pieces each week, using 2 different constructs each week. We expect this number to double this summer to increase throughput.

   Impact:

   Funding from the USWBSI was critical for student salaries that kept the project going during the time we lacked a transformation technician.

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.

1. Dahleen, L.S., R. Brueggeman, T. Abebe, and R. Skadsen. 2012. 2012 North Dakota transgenic barley FHB nursery report. In: S. Canty, A. Clark, A. Anderson-Scully, and D. Van Sanford (eds.). Proceedings of the 2012 National Fusarium Head Blight Forum. (p.132) East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative.

2. Koeritz, E.J., A.M. Elakkad, L.S. Dahleen, R. Skadsen, T. Abebe, J. Shah, V.J. Nalam, G. Klossner, N. Tumer, R. Di, G.J. Muehlbauer, X. Li, S. Shin, and R. Dill-Macky. 2012. Testing transgenic spring wheat and barley lines for reaction to Fusarium head blight: 2102 field nursery report. In: S. Canty, A. Clark, A. Anderson-Scully, and D. Van Sanford (eds.). Proceedings of the 2012 National Fusarium Head Blight Forum. (p.140-141) East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative.