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

Cover Page

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Phone:	208-397-4162 x116
Fiscal Year:	2015
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Down with DON: Stable Expression of Proven Genes in a Marker-free Background.
FY15 USDA-ARS Award Amount:	$ 52,200

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Crossing and Field Tests of Transgenic Barley.	$ 27,200
GDER	Down with DON: Stable Expression of Proven Genes in a Marker-free Background.	$ 25,000
	FY15 Total ARS Award Amount	**$ 52,200**

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

FY15 Final Performance Report
PI: Bregitzer, Phil
USDA-ARS Agreement #: N/A

Project 1: *Crossing and Field Tests of Transgenic Barley.*

1. **What are the major goals and objectives of the project?**
   The goal of the research was to develop Fusarium head blight (FHB)-resistant transgenic barley lines in genetic backgrounds adapted to the upper Midwest, and/or introduce these genes from unadapted to adapted background by standard breeding methods.

2. **What was accomplished under these goals?**
   1) major activities
   For FY15, the major activities were to test ten transgenic lines plus five control (non-transgenic) barley lines under field conditions in North Dakota and Minnesota.

   2) specific objectives
   a. measurement of FHB incidence and severity
   b. determination of deoxynivalenal (DON) in harvested grain

   3) significant results
   Reductions of severity and/or DON were apparent in three lines in the Quest background, and in one line in the ND20448 background, each of which contained a thaumatin-like protein (TLP) gene from *Oryza sativa* (Table 1). Lines where no advantage was seen contained these transgenes: snakin antifungal protein from *Solanum tuberosum*, a receptor kinase gene from *Triticum aestivum,* and an RNAi construct targeting *F. graminearum Vel1*.

2014-15 mean results, transgenic field trials

Unmisted nursery				Misted nursery			
Line	Incidence (%)	Severity (%)	DON (ppm)	Line	Incidence (%)	Severity (%)	DON (ppm)
28	53.3	5.03	2.63	28	80.0	10.90	11.64
28-null	61.7	5.63	2.35	28-null	85.0	10.63	9.76
78	61.7	6.25	1.54	78	91.7	13.82	11.06
423	70.0	5.78	2.76	423	80.0	10.75	7.02
83	46.0	4.52	2.93	83	91.7	11.60	9.37
96	58.3	5.55	2.59	96	88.3	12.94	8.96
Conlon	**53.3**	**4.77**	**2.62**	**Conlon**	**90.0**	**10.58**	**7.82**
321Q3	48.3	2.37*	5.12*	321Q3	76.7	8.93	13.00
82Q3-2	48.3	5.30	3.27*	82Q3-2	86.0	11.22	8.99*
82Q3-6	51.7	2.47*	4.32*	82Q3-6	86.7	9.73	18.10
Quest	**50.0**	**4.47**	**7.48**	**Quest**	**78.0**	**9.93**	**16.34**
82ND2-2	53.3	4.20	3.48*	82ND2-2	83.3	11.76	16.19
82ND2-6	60.0	9.32*	8.85	82ND2-6	98.3	24.45*	22.93
ND20448	**53.3**	**4.94**	**7.21**	**ND20448**	**81.7**	**8.17**	**20.27**
Stander	58.3	3.83	8.30	Stander	88.3	16.12	28.66
lsd 0.1	ns	1.98	1.88	lsd 0.1	ns	5.55	5.52

4) key outcomes or other achievements
   a) The TLP gene may have application in creating resistant barley varieties
   b) The development of these lines by the Dahleen lab occurred without the necessary resources to conduct robust analyses for gene expression and protein production. Therefore the negative results for genes other than TLP must be interpreted with caution.
   c) With respect to b), it is imperative that future studies of transgenic plants in the field be conducted only after appropriate molecular characterizations have been made.

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

Nothing to report

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

These results have been reported formally at the 2015 National Fusarium Head Blight Forum in a poster entitled *Reactions of Transgenic Barley Lines to FHB Inoculation in 2014 and 2015 in North Dakota* and in a poster entitled *Testing Transgenic Spring Wheat and Barley Lines for Reaction to Fusarium Head Blight: 2015 Field Nursery Report.*

In addition, detailed discussions concerning improved protocols for future work involving transgenic barley development and testing were held with cooperators that had supplied various transgenic constructs, and other members of the GDER and Barley Coordinated Projects.

Project 2: *Down with DON: Stable Expression of Proven Genes in a Marker-free Background.*

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

To reduce FHB severity and DON via induction of RNAi-based silencing by expression of constructs encoding double-stranded (ds) RNA targeting *Tri5* and *Tri6* (the first step of mycotoxin synthesis, and a regulator of *Tri5*, respectively). There are two components:
i) direct or indirect (via recombinase-mediated cassette exchange, RMCE) delivery of transgenes via transposition to optimize transgene expression; and
ii) rapid vetting of transgene expression cassettes designed to silence key *Fusarium graminearum* (*Fg*) genes via testing them in transgenic *Fg.*

2. What was accomplished under these goals?

1) major activities:

a) Ten independent *Fg* lines produced expressing dsRNA targeting full-length (600 bp) *Tri6* produced (plasmid: pTRM-*Tri6*); six phenotyped for virulence on wheat and barley; for DON production in rice cultures and on wheat; and for *Tri5* expression. Three lines characterized for small (s) RNA profiles to examine RNAi-processing of the construct. Five additional vectors created targeting 150–200 bp of *Tri6* to facilitate gene "stacking" for targeting multiple genes) and introduced into 6–10 lines for each vector; one of each examined for sRNA profiles.

b) Transgenic barley with RMCE/TAG vectors (sequences to enable site-specific recombination) produced in Conlon, Golden Promise, and Pinnacle, respectively: 5, 8, and 1.

c) TAG site insertions in eight transgenic barley lines sequence and mapped. F_1 seed recovered from 65 crosses of TAG lines to *Ac transposase* (*AcT*)-expressing lines (the first step to initiate TAG site transposition and enable isolation of lines with single TAG insertions).

2) specific objectives for FY2015

a) Construct, transform *Fg* with vectors against *Tri5, Tri6*; use *in vitro* assays to assess growth/ DON reduction; construct barley *Ds*/RMCE vectors based on vectors that were effective in *Fg*.

b) Produce transgenic Conlon plants with *Ds*-bordered antifungal transgenes or TAG sites.

c) Initiate transposition of *Ds*-bordered transgenes and TAG sites by crossing to *AcT* plants.

3) significant results: Expression of dsRNA targeting *Tri6* was evident based on novel, *Tri6*-specific sRNAs and reduced Tri5 expression. DON production by pTRM-*Tri6* lines was reduced in rice cultures and wheat spikes; severity was reduced in wheat spikes (single floret inoculations at anthesis; avg. of three assay; vs. wildtype *PH1* and pUCH2-8 selectable-marker-only transgenic lines; Figure 1). Severity was unaffected on barley spikes (whole-spike inoculations at anthesis; one assay) but pTRM-*Tri6* lines were less aggressive: kernels infected with pTRM-*Tri6* lines were heavier, and infection with PH1 or pUCH2-8 lines often resulted in rapid floret death and negligible kernel development (Figure 2).

4) key outcomes or other achievements: To support and expand future FHB-related research:
i) Constructed a pTRM-*Tri6*-based vector for barley and initiated barley transformation.

ii) *Fg* genes potentially contributing to virulence were examined. *FDB2* knock-out and RNAi-silenced *Fg* lines suggested sensitivity to plant-produced BOA; further work is needed to judge utility for RNAi-based downregulation or breeding for increased BOA levels. The necessity for oxalate production by *OAH* for reduction of pH and mycotoxin production during infection via *Fg-OAH* knockout lines did not affect the ability to lower pH or virulence on wheat.

iii) To prevent unwanted mutations during *Fg* transformation and facilitate comparisons among transgenic lines, two vectors were designed to enable integration of transgenes via homologous recombination (HR) into intergenic areas. Lines with integrations of RNAi vectors were created and are being characterized for sRNA profiles and virulence.

iv) Transgenic TAG/*AcT* population development is ahead of schedule; 38 F_2 populations developed and screening for transposed TAG sites has been initiated.

v) Experiments designed to silence specific *Fg* genes via direct exposure to sRNA or dsRNA met with very limited success; thus the focus on the successful alternative, transformation.

vi) Manuscripts in preparation for pTRM-*Tri6* and HR-based transgene expression in *Fg*.

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

PI mentored Dr. Baldwin in the areas of quantitative genetics, breeding, and transgenic plants as germplasm. Dr. Baldwin mentored PI and Ms. Caspersen via his expertise is *Fusarium* genetics. Professional development of PI and Dr. Baldwin via attendance at FHB forums, interactions with colleagues, and individual studies of relevant topic (transformation, *Fusarium*, RNAi, etc.).

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

Via personal communication with other scientists and posters at presentation at the 2015 FHB forum; via personal communication with stakeholders such as AMBA, the Idaho Barley Commission, and local producers; via publication in *Capital Press* (http://www.capitalpress.com/content/jo-gmo-barley-082713) and *The Scientist* (http://www.the-scientist.com/?articles.view/articleNo/40021/ title/Barf-Less-Brews/); and presentation at the Western Barley Growers Association 2014 Convention.

Figure 1

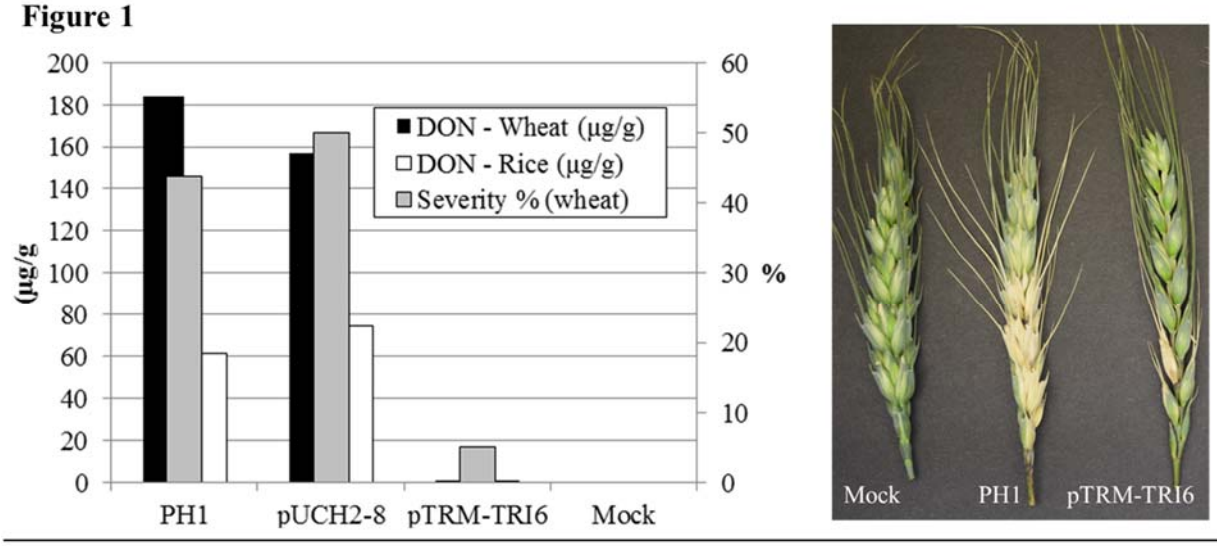
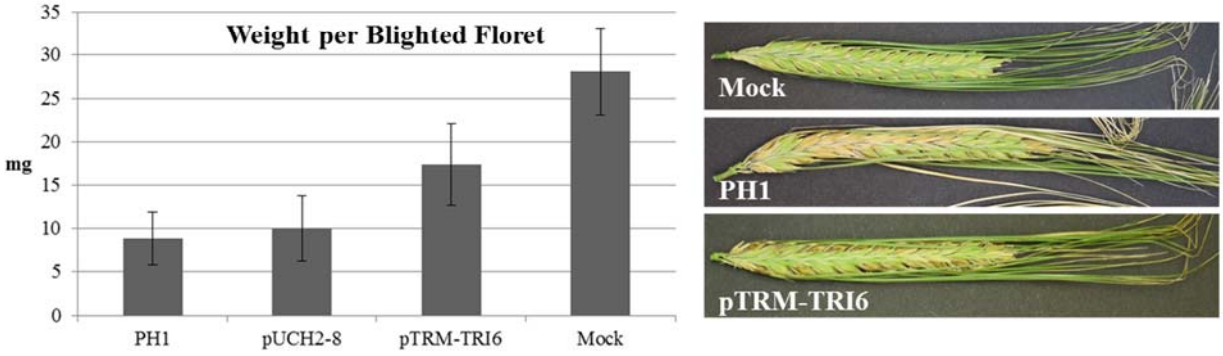


Figure 2



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?** No

   If yes, how many?

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.
Nothing to report

Books or other non-periodical, one-time publications.
Nothing to report

Other publications, conference papers and presentations.

Baldwin T., and Bregitzer, P. 2015. Analysis of the small interfering RNA profiles of randomly inserted pTRM-*Tri6 Fusarium graminearum* mutants and their DON related phenotypes. In: S. Canty, A. Clark, S. Vukasovich, and D. Van Sanford (Eds.) *Proceedings of the 2015 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat and Barley Scab Initiative. P. 43.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: Abstract: Yes; Poster: No

Bregitzer, P., Brueggeman, R., Dahleen, L., and Gross, P. 2015. Reactions of transgenic barley lines to FHB inoculation in 2015 North Dakota field trials. In: S. Canty, A. Clark, S. Vukasovich, and D. Van Sanford (Eds.) *Proceedings of the 2015 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat and Barley Scab Initiative. P. 44.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: No

Dill-Macky, R., Elakkad, A.M., Sha, J., Trick, H.N., Sarowar, S., Alam, S., Dahleen, L.S., Skadsen, R.W., Bregitzer, P.P. 2015. Testing transgenic spring wheat and barley lines for reaction to Fusarium head blight: 2015 report. In: S. Canty, A. Clark, S. Vukasovich, and D. Van Sanford (Eds.) *Proceedings of the 2015 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat and Barley Scab Initiative. P. 45-46.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: Yes (Abstract and Poster)

Bregitzer, P. Transposons, RNAi, and selection: bioinformatics and the quest for better beer and oatmeal. Invited presentation given at the IBEST Bioinformatics Facility, University of Idaho, May 19, Moscow, ID. 2016.
Status: Presented (no abstract)
Acknowledgement of Federal Support: yes