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:	N/A
USDA-ARS Agreement Title:	Down with DON: Stable Expression of Proven Genes in a Marker-free Background.
FY15 USDA-ARS Award Amount:	$ 5,000

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Down with DON: Stable Expression of Proven Genes in a Marker-free Background.	$ 5,000
	FY15 Total ARS Award Amount	**$ 5,000**

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

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

   Genetic engineering can create valuable germplasm for genetic investigations of the host-pathogen interaction and for breeding programs. This technology is limited by our ability to produce plants with single-copy transgene insertions, to maintain robust and heritable expression of the transgene, and by transgene linkage to undesirable sequences derived from bacterial cloning vectors. Tools to solve these problems are needed, and our research is directed at developing and deploying these tools in barley.

   We are using two methods for improving transgene delivery. One is direct *Ds* delivery, which involves delivery of transgenes as synthetic *Ds* transposons. This method involves the transposition, or movement, of the transgene of interest from the original locus created by *Agrobactericum*-mediated transformation, to a new location. After segregation, this produces barley plants with single-copy transgenes free of the vector DNA and undesirable transgene arrangements that typically exist at the original locus. Transposed loci typically have high and heritable levels of transgene expression. Application of this system is simple, requiring only conversion of a transgene to a synthetic *Ds* transposon via attachment of short sequences to each end of the transgene. These terminal sequences are recognized by a transposase enzyme, which is introduced via hybridization with the primary transgenic plant (produced via *Agrobacterium*-mediated transformation). Progeny are then screened for single-copy, vector-free transgene loci that have transposed from the original, complex locus, and segregated away from the transposase to produce a plant with a relocated, stable, and useful transgene insertion.

   The second method we are developing is recombinase mediated cassette exchange (RMCE), also known as site-specific recombination. This method involves the production of Founder lines that contain a TAG locus possessing selectable markers bordered by specific recombination sites. Suitable Founder lines have single-copy TAG loci in areas supporting good transgene expression. We have engineered TAG loci as synthetic *Ds* transposons to efficiently produce multiple novel Founder lines from a single transformation event. Transgenes of interest can then be incorporated into this locus by introducing an EXCH vector possessing the desired transgene bordered by recombinase recognition sites that interact specifically with the TAG recombination sites. The result is the exchange of the selectable markers for the transgene of interest, and a plant with a single copy of the desired transgene. This system has additional steps relative to direct *Ds* delivery, but once Founder lines are created there is a distinct advantage: any given Founder line can be used repeatedly, enabling the analysis of different transgenes without expression variability caused by variability in the sites of insertion. Furthermore, multiple transgenes can be stacked at the TAG locus.

   The goal of our research is to use both of these methods to introduce transgenes conferring FHB resistance. Our choice of transgenes has evolved with our understanding of RNA interference, and our approach will involve interfering with fungal development and myocotoxin production by suppressing the expression of key fungal genes via *in planta* production of double-stranded RNA that targets key gene transcripts for degradation.

2. List the most important accomplishments and their impact (i.e. how are they being used) to minimize the threat of Fusarium Head Blight or to reduce mycotoxins. Complete both sections; repeat sections for each major accomplishment:

1) Major activities:
The *Ds*-TAG-*Ds* vector for RMCE (site-specific recombination) into Golden Promise and Conlon barley plants have previously been made. Efforts in the Bregitzer lab for multiple transgenic events have been produced in both cultivars, with different TAG platforms introduced encoding selectable markers. Currently these lines are under investigation for intact T-DNA, AC transposase movement and genomic location.

2) Specific objectives:
Development of the EXCH vector possessing the desired transgene bordered by recombinase recognition sites that interact specifically with the TAG recombination sites both with and without the potential FHB disease resistance gene included.

3) Significant results:
Thomson lab is established a protocol for RMCE testing in dicots (Arabidopsis, tobacco and citrus) with DNA exchange rates ranging from 10 to 35%. Currently this technology is being adapted for monocot (barley) species utilization. Anti-FHB gene are being incorporated into these RMCE test systems for immediate use in disease testing

4) Key outcomes or other achievements:
Development of Founder lines and transpose-mediated transposition there of will be used for insertion of anti-FHB transgenes into appropriate locations. The first step of the RMCE (site-specific recombination) process has been shown possible and it application in barley will enable multiple grain crops access to targeted integration technology. Introduction into Conlon is especially desirable because of its direct relevance to the North American malting and brewing industry.

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

None.

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

None.

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?**
   None.
   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?**
   None.
   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?**
   None.
   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?**
   None.
   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.
Nothing to Report