

USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Final Performance Report
Due date: July 31, 2018

Cover Page

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Fiscal Year:	2017
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Down with DON: Stable Expression of Proven Genes in a Marker-free Background.
FY17 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 RNAi Constructs in a Marker-free Plant.	\$ 5,000
FY17 Total ARS Award Amount		\$ 5,000

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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 RNAi Constructs in a Marker-free Plant.

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

The goal is to reduce FHB and DON in *F. graminearum* (*Fg*)-infected transgenic barley grain via expression of double-stranded (ds) RNA that has homology to key *Fg* genes for mycotoxin synthesis and/or pathogenicity. Expression of targeted genes will be suppressed via RNA interference (RNAi). Although the intended phenotype is FHB resistance, the focus of this proposal is on the development and demonstration of two improved methods of introducing transgenes: direct transposition-mediated (*Ds*) delivery and recombinase-mediated cassette exchange (RMCE). Transgenes will be delivered first via *Agrobacterium* followed by secondary delivery via transposition. Transposition is enabled by flanking transgenes with short, maize-derived *Dissociation* (*Ds*) terminal sequences which interact with *Ac transposase* (*AcT*). *AcT* will be introduced via hybridization with *AcT*-expressing plants. Transposition will deliver single-copy, *Ds*-flanked transgenes to favorable locations, and de-link them from vector backbone and selectable markers, enabling production of transgenic plants without vector backbone or marker genes. Transgenes will be either a *Ds*-flanked dsRNA-producing cassette or *Ds*-flanked TAG site. The TAG site includes sequences that enable RMCE. RMCE requires an extra step: after transposition of TAG sites, EXCH vectors carrying dsRNA transgenes will be introduced and incorporated into TAG sites via site-specific recombination.

Important elements of this proposal include: 1) delivery to useful cultivars (Conlon, Pinnacle); and 2) testing RNAi vectors in *F. graminearum* (*Fg*), prior to their introduction into plants, to enable rapid screening and optimization of potential RNAi vectors. We have secured ARS funding of ~90% of the salary of a post-doctoral researcher to support this aspect of our proposed research. Our objectives are to:

1. Construct a) *Ds*, b) RMCE, and c) EXCH barley backbone vectors (a and b completed; c in progress).
2. Construct fungal RNAi vectors targeting *TRI5*, *TRI6*, & *LAEA*, and test them in *Fg* (in progress).
3. Introduce dsRNA sequences effective against *Fg* into barley *Ds* and EXCH vectors (in progress).
4. Produce transgenic Conlon plants with *Ds*-bordered *Ds*-vectors or TAG sites (in progress).
5. Initiate transposition of *Ds*-bordered sequences by crossing to *AcT* plants (in progress).
6. Select plants with *Ds*-vectors or TAG sites segregated from *AcT* and the original insertion site.
7. For RMCE only: Introduce EXCH vectors carrying antifungal transgenes that will be incorporated into TAG sites via site-specific recombination.
8. Characterize transgene expression, FHB severity/DON, plant performance, and develop resistant lines.

2. What was accomplished under these goals? *Address items 1-4) below for each goal or objective.*

1) major activities

Constructed TAG and EXCH barley backbone vectors.

2) specific objectives

To construct TAG and EXCH barley backbone vectors for RMCE testing.

3) significant results

TAG and EXCH vectors were constructed and delivered to Dr. Bregitzer for RMCE testing in Barley.

4) key outcomes or other achievements

TAG and EXCH vectors were constructed and delivered to Dr. Bregitzer for testing in Barley.

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

None

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

RMCE technology has been published and is available for use in other plant systems.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY17 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 FY17 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 FY17 award period? No**

If yes, how many?

- 3. Have any post docs who worked for you during the FY17 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 FY17 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 FY17 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

Instructions: Refer to the FY17-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY17 grant. Only include citations for publications submitted or presentations given during your award period. If you did not have any publications or presentations, state ‘Nothing to Report’ directly above the Journal publications section.

NOTE: Directly below each reference/citation, you must indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in publication/presentation.

Nothing to report.

Journal publications.

Books or other non-periodical, one-time publications.

Other publications, conference papers and presentations.