USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY13 Final Performance Report
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Cover Page

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Fiscal Year:	FY13
USDA-ARS Agreement ID:	NA
USDA-ARS Agreement Title:	Down with DON: Stable Expression of Proven Genes in a Marker-free Background.
FY13 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
	FY13 Total ARS Award Amount	**$ 5,000**

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

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

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:

Accomplishment: Production of *Ds*-TAG-*Ds* vector for RMCE (site-specific recombination) into Golden Promise and Conlon barley plants. Through the effort of the Bregitzer and Dahleen labs, multiple transgenic events have been produced in both cultivars, with different TAG platforms introduced encoding both screenable and selectable markers.

Impact: These critical first steps of the RMCE (site-specific recombination) process enables transpose-mediated transposition and development of Founder lines that will be used for insertion of anti-FHB transgenes into appropriate locations. Introduction into Conlon is especially desirable because of its relevance to the North American malting and brewing industry.

PI: Thomson, James J.
USDA-ARS Agreement #: NA

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 FY13 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

None.