USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY10 Final Performance Report
July 15, 2011

Cover Page

PI:	Michael Lawton
Institution:	Rutgers
Address:	Biotech Center - Cook College 59 Dudley Road New Brunswick, NJ 08901-8520
E-mail:	lawton@aesop.rutgers.edu
Phone:	732-932-8165 ext. 223
Fax:	732-932-6535
Fiscal Year:	FY10
USDA-ARS Agreement ID:	59-0790-6-063
USDA-ARS Agreement Title:	A Rapid Assay System for Transgenes that Confer Resistance to DON and FHB.
FY10 USDA-ARS Award Amount:	$ 57,958

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	A Rapid Assay System for Transgenes that Confer Resistance to DON and FHB.	$ 48,202
BAR-CP	High Efficiency Method for Generating FHB-Resistant Barley: Removing Bottlenecks in the Pipeline for Deploying FHB Resistance Genes.	$ 9,756
	Total ARS Award Amount	**$ 57,958**

7/15/11

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: *A Rapid Assay System for Transgenes that Confer Resistance to DON and FHB.*

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

   Introduction of genes into crop plants by transgenic technologies is a useful approach for assessing genes for their potential to confer FHB resistance. Because transformation of wheat and barley is both time- and resource-consuming, direct screening of large numbers of transgenes genes in these crop plants is not yet practical. The recombinogenic plant *Physcomitrella patens*, provides a rapid and efficient gene assay system for gene function via the creation of gene knockouts or gene overexpression. This rapid assay system has helped identify a number of genes that confer resistance to DON and to FHB. These assays flag genes that can prove useful against FHB in wheat or barley. Together, these assays (in model systems and in crop plants) form a research and development pipeline for gene discovery and for the deployment of anti-FHB genes in wheat and barley. This project addresses GDER Goal 1 to more efficiently identify candidate genes for resistance against FHB and reduced DON accumulation through high-throughput functional screening assays.

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:
   We used the rapid assay model system *Physcomitrella patens* to identify a number of gene effective against FHB. These genes are associated with the response to ER stress and with the broad immune response induced by chitosan. Our data show that knockouts for some, but not all ER stress-related genes, displayed a greater sensitivity to FHB, suggesting the importance of these pathways in the signaling of, or direct response to FHB. Gene knockout of critical components in the chitin signaling pathway largely abolished the ability of chitosan to induce immunity against FHB in Physcomitrella. These studies suggest that both the ER stress response pathway and the innate immunity induced by chitosan contribute the expression of effective resistance against FHB and flag these pathways and these genes for similar manipulation in crop plants. Additional Physcomitrella knockouts (corresponding to genes that alter resistance to tricothecin when knocked out in yeast) are currently being analyzed for altered sensitivity to mycotoxins and will provide an additional source of genes for introduction into wheat and barley. One promising indicator of the likely success of this approach is that Physcomitrella knockouts generated for a tryptophan-rich sensory protein, which was one of the genes flagged in the Tumer lab yeast screen, are markedly less sensitive to cell death signals, suggesting a high degree of functional conservation between yeast and plants. In order to prioritize analysis of yeast genes and to better understand their function, we also performed localization studies of the encoded proteins using GFP fusions.

   Impact:
   Rapid assay systems have a direct impact on the development of strategies for introducing genes into wheat and barley that are effective against FHB. They provide an efficient and

efficient means to screen genes identified in other studies for efficacy against FHB. They also provide the means for *de novo* discovery of genes effective against FHB. We have used both strategies in these studies to define a collection of genes that can be targeted for improvement in crop plants by molecular or marker-assisted methods or through gene transfer. These approaches can also throw light on the cellular and molecular mechanisms through which FHB resistance operates and this can be useful in designing transgenic strategies that deploy multiple genes to confer robust resistance against FHB in the field. These studies address Research Priority #2, to *"develop effective FHB resistance through transgenic strategies."*

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?

These studies address whether genes identified from rapid heterologous assay systems are able to confer resistance to FHB in barley. They constitute the downstream components of a pipeline for generating and assessing transgenic FHB-resistance crops. These studies address Objectives 4 and 6 of the Barley Coordinated Project to develop barley varieties with enhanced resistance to FHB and to evaluate promising transgenes in adapted genetic backgrounds.

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:
We have defined a group of barley homologs of Physcomitrella genes previously shown to be effective against FHB in Physcomitrella. We isolated all of the barley homologs of these genes and characterized those gene family members that are most effective against FHB when expressed in Physcomitrella. The best performing genes were introduced into the pBRACT binary vectors for introduction into barley via Agrobacterium-mediated transformation.

Impact:
The generation of transgenic plants expressing novel genes provides a formal test of the validity of the rapid assay system for selecting and screening genes for introduction into barley. Comparison of gene performance in barley and Physcomitrella will also allow us to evaluate which resistance mechanisms are conserved. The generation of transgenic plants will also provide new germplasm for introduction into regionally adapted varieties of barley.

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.

Publication:

Lawton, M. and Saidasan, H. (2010). Cell wall genomics in the recombinogenic moss Physcomitrella patens. IN: The Routes to Cellulosic Ethanol (M. Buckeridge, Ed.) Springer. NY.