USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY08 Final Performance Report
No Cost Extension for FY09 and FY10
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Cover Page

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Fiscal Year:	2008
USDA-ARS Agreement ID:	59-0790-6-057
USDA-ARS Agreement Title:	Engineering Barley with Antifungal Gene Gastrodianin to Enhance Resistance to Scab Disease.
FY08 ARS Award Amount:	$ 12,819

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
GDER	Transformation and Field Testing of Transgenic Barley Lines.	$ 12,819
	Total Award Amount	**$ 12,819**

7/14/2011
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: *Transformation and Field Testing of Transgenic Barley Lines.*

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

   Barley has very limited resistance to *Fusarium* head blight (FHB. Quantitative trait loci (QTLs) for FHB resistance reported in barley often map to the location of other QTLs such as heading date, plant height, spike angle, and two-row spike type. Because of the lack of strong resistance, development of FHB resistant barley lines through breeding is slow. Introduction of anti-*Fusarium* genes through genetic engineering can complement breeding efforts to improve resistance to FHB and increase yield and quality of barley. We have developed Golden Promise barley lines expressing antifungal *gastrodianin* gene from a Chinese herb *Gastrodia elata*. Gastrodianin is a 12 kDa, non-agglutinating, monomeric, mannose- and chitin-binding lectin that belongs to the superfamily monocot mannose-specific lectins. *In vivo* studies have established that gastrodianin inhibits the growth of many saprophytic fungi including *F. graminearum*.

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 all three sections (repeat sections for each major accomplishment):**

   Accomplishment:

   Expression of *gastrodianin* was targeted to the husk (lemma and palea) and epidermis of the kernel of Golden Promise using a spike-specific *Lem2* promoter we isolated from Morex barley. Transgenic barley lines were tested for resistance to FHB in a greenhouse screen conducted by Dr. Ruth Dill-Macky at the University of Minnesota, St. Paul, MN. Resistance was assessed using T2 plants from seven transformation events. Non-transformed (wild-type) Golden Promise and transgenic Golden Promise expressing *gfp* only (Lem2Bgfp-GP) were included as negative controls. Conlon (FHB susceptible two-row), M122 (FHB moderately resistant six-row), Stander (moderately susceptible six-row), and Robust (FHB moderately susceptible six-row) were included as checks. Mean FHB severity (average percentage of symptomatic spikelets/spike) for each transgenic line, the negative control expressing gfp only (Lem2Bgfp-GP), and checks was compared with that of the wild type Golden Promise using Student's *t*-test. Transgenic lines 50A4, 50D3, and 51E2 had significantly higher levels of FHB infection. Transgenic lines from event 58 either had similar (58B5) or lower (58D5) FHB severity. Among the checks, only Conlon had significantly higher FHB infection than the wild-type Golden Promise (Table 1).

Table 1. Average FHB severity (% symptomatic spikelets/spike) for transgenic barley lines expressing *gastrodianin*, negative controls, and FHB checks. Values are 6-8 replications with each replication consisting of 3-5 plants.

Plants	**% FHB severity**
A. Check lines	
- Conlon (FHB susceptible, 2-row)	88.9
- Robust (FHB moderately susceptible, 6-row)	69.7
- Stander (FHB moderately susceptible, 6-row)	60.9
- M122 (FHB moderately resistant, 6-row)	74.4
B. Negative controls	
- Golden Promise (wild type)	55.9
- Lem2Bgfp-GP (Golden Promise expressing only *gfp*)	43.5
C. Transgenic lines	
- 48A1	77.7
- 48B3	72.6
- 50A4	81.5*
- 50D3	84.3*
- 51E2	80.3*
- 52D6	77.1
- 52G2	73.2
- 53A1	55.5
- 56A1	61.2
- 56D3	73.6
- 58B5	45.5
- 58D5	24.0

*Exhibits significantly different FHB severity ($p<0.05$) compared with the wild type Golden Promise.

Impact:

Greenhouse evaluation of transgenic plants expressing *gastrodianin* has produced at least one line (58D5) with better resistance to FHB. Field testing of transformants is the best way to accurately determine FHB resistance under natural conditions. However, Golden Promise is not ideal for screening FHB resistance because of the fact that the spike does not emerge fully from the boot. This becomes challenging under field conditions when other stress factors, such as drought, occur. Crossing to an elite variety is necessary to overcome this problem and also remove any unwanted traits including low seed setting, stunted growth and slow maturity of transgenic lines. Dr. Lynn Dahleen, USDA-ARS, Red River Valley Agricultural Research Center, Fargo, ND has crossed the transgenic Golden Promise lines into Conlon (female parent). Field evaluation of the crosses was performed at Aberdeen, ND and Rosemount, MN in 2010. The field data has been inconclusive due to drought. The field test is currently repeated to make sure the resistance observed in greenhouse conditions is real. If the field tests show results comparable to the greenhouse screening, *gastrodianin* will be an excellent gene to combat FHB in barley and other cereals.

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.

Eng-Hwa Ng, Tilahun Abebe, James E. Jurgenson, Ruth Dill-Macky, Lynn Dahleen, and Ronald Skadsen. 2010. Greenhouse evaluation of transgenic barley expressing *gastrodianin* for resistance to Fusarium head blight, National Fusarium Head Blight Forum Proceedings, December 7-9, 2010, Hyatt Regency Milwaukee, WI, p 28-32.

Dill-Macky R, Elakkad AM, Dahleen LS, Skadsen RW, and Abebe T. 2010.Testing transgenic spring barley lines for reaction to Fusarium head blight: 2010 field nursery report. National Fusarium Head Blight Forum Proceedings, , December 7-9, 2010, Hyatt Regency Milwaukee, WI, p 16.

Dahleen LS, Dill-Macky R, Shah J, Muehlbauer G, Skadsen RW, Manoharan M, Tilahun A, Jurgenson J. 2009. Transgenic field trials for fhb resistance and related research in wheat and barley. In: Ouellet T and Leger D (eds). Proceedings of the 6th Canadian Workshop on Fusarium Head Blight, November 1-4, 2009, Ottawa, ON, Canada, p. 38.

Ng EH, Abebe T, Skadsen RW, Jurgenson JE. 2008. Engineering barley with *gastrodianin* for resistance to scab disease. Poster presented at the American Phytopathological Society centennial meeting, July 26-30, 2008, Minneapolis, MN.

Abebe T, Ng EH, Skadsen R and Jurgenson JE. 2008. Progress in engineering barley with *gastrodian* for resistance to scab disease. Presentation at the Barley Coordinated Project Meeting, Ft. Collins, CO, June 3, 2008.

Ng EH, Abebe T, Jurgenson JE and Skadsen RW. 2007. Engineering barley with *gastrodianin* for improved resistance to *Fusarium* head blight. Proceedings of the National Fusarium Head Blight Forum, 2-4 December, 2007, Kansas City, MO, p. 54-57.