USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY12 Final Performance Report
July 16, 2013

Cover Page

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Fiscal Year:	FY12
USDA-ARS Agreement ID:	59-0790-8-060
USDA-ARS Agreement Title:	Engineering Fusarium Head Blight Resistance and Plant Defense Signaling.
FY12 USDA-ARS Award Amount:	$ 62,374

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Targeting Host Defense Mechanism for Enhancing FHB Resistance in Wheat.	$ 62,374
	Total ARS Award Amount	**$ 62,374**

July 2, 2013

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: *Targeting Host Defense Mechanism for Enhancing FHB Resistance in Wheat.*

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

Genetic and molecular approaches were undertaken to identify genes involved in plant resistance and susceptibility to *F. graminearum*. Furthermore, these genes/mechanisms were genetically targeted for enhancing resistance in wheat against FHB. Genetic engineering of these genes/mechanisms provides an alternative approach to develop wheat and barley germplasms with heightened resistance to FHB. For example, ectopic expression of the *NPR1* gene, a regulator of systemic acquired resistance in plants, enhanced FHB resistance in wheat under greenhouse and field conditions. In addition, our results indicate that non-host resistance mechanisms triggered by microbe-associated molecular patterns (MAMPs) as well as factors that contribute to host susceptibility can also be targeted for enhancing FHB resistance. Novel genes and chimeras that are not in the partially resistant germplasms can be introduced into wheat and barley, thus adding to the repertoire of genes that can be utilized in FHB resistance breeding programs.

The specific objectives of this FY12 project were:

1. Characterize FHB resistance and mycotoxin accumulation in wheat plants expressing At*PAD4*, At*WRKY18* and in plants co-expressing At*NPR1* and At*PAD4*.
2. Target non-host resistance mechanism for enhancing FHB resistance.
3. Characterize FHB resistance and mycotoxin accumulation in lipoxygenase-silenced wheat lines.
4. Target expression of a Ca^{2+}-binding protein-encoding gene associated with diterpenoid signaling for promoting FHB resistance in wheat

Progress:

- Two transgenic wheat lines (cv Bobwhite) expressing At*PAD4* and two lines expressing At*WRKY18* from the maize *Ubi* promoter have been evaluated for gene expression, FHB resistance in the greenhouse and in field trials. The impact of these transgenes on mycotoxin content was also evaluated. Both constructs significantly enhanced FHB resistance in greenhouse experiments. However, in field experiments these constructs did not show significant enhancement of FHB resistance, nor did they reduce toxin content.
- Four independent transgenic wheat lines that contain a construct for expressing the flg22-responsive *WRKY29* gene have been identified and seeds harvested. These are currently being evaluated for gene expression and resistance to FHB.
- Two separate constructs for expressing the flg22 peptide in Arabidopsis were generated and used to generate transgenic Arabidopsis plants. Two transgenic lines for each construct were propagated further to get homozygous plants. These constructs express the transgene and have been tested for resistance to *F. graminearum*. All flg22 expressing lines showed high level of resistance to *F. graminearum* infection, thus complementing results from pharmacological studies, which indicated that flg22 and the corresponding non-host resistance mechanism provides a novel strategy for controlling *F. graminearum* infection.

- Two separate constructs for expressing the flg22 peptide in wheat were generated and have been used for biolistic transformation of wheat. Four transgenic wheat plants that contain the flg22 construct has been identified. Two of these are close to setting seeds and should be available within the next few months for further studies. Two others plants are in soil and three additional plantlets are at different stages of tissue culture.
- Progeny of lipoxygenase-silenced lines that showed enhanced FHB resistance in the previous generations have been propagated and evaluated for FHB severity. These silenced lines continue to exhibit enhanced FHB resistance.

We have implemented approaches to rapidly translate candidate gene discovery in model plants to assessing the activity of candidate genes in conferring enhanced FHB resistance in wheat.

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):

a. **Accomplishment**: Two strategies have been utilized to target MAMP-triggered non-host resistance mechanisms for enhancing FHB resistance in wheat. The first approach involves expression of the flg22 peptide in wheat to activate non-host mechanism in response to fungal infection, while the second approach involves promoting expression of a transcription factor (WRKY29) that functions downstream of flg22 perception in promoting non-host resistance mechanisms. The flg22 peptide application enhanced resistance against *F. graminearum* in Arabidopsis and wheat. Furthermore, two rounds of experiments with *in planta* expression of the flg22 peptide in Arabidopsis indicate high level of resistance *to F. graminearum* infection. Preliminary experiments with one *WRKY29* expressing wheat line showed promise in enhancing FHB resistance in greenhouse studies.
Impact: Stimulation of MAMP-triggered non-host defenses for enhancing FHB resistance could provide an alternative strategy that complements existing approaches to control a broad-spectrum of diseases in wheat.

b. **Accomplishment:** We have identified a gene involved in oxylipin metabolism that is targeted by *F. graminearum* for promoting infection in Arabidopsis and wheat. When expression of this 9-LOX is disrupted or silenced in Arabidopsis and wheat, it results in enhanced resistance against *F. graminearum*. This increase in resistance is likely due to the faster activation of defenses in the 9-LOX-silenced lines.
Impact: The wheat 9-LOX-silenced RNAi-silenced lines are being utilized to further characterize the impact of these genes/processes on FHB resistance. Lines exhibiting enhanced resistance will provide novel germplasms for breeding FHB resistance.

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.

Publications

Peer reviewed

Shah, J. and Zeier J. (2013) Long-distance communication and signal amplification in systemic acquired resistance. Frontiers in Plant Sci 4:30. doi: 10.3389/fpls.2013.00030.

Book Chapter

Shah, J. and Chaturvedi, R. (2013) Long-distance signalling in systemic acquired resistance. In *Signaling and Communication in Plants*: *Long-Distance Systemic Signaling and Communication in Plants* (Baluška, F. ed), Springer Verlag, Berlin Heidelberg, pp 1-21.

Proceedings

Shah,J., Nalam, V., Klossner, G., Alam, S. T., Sarowar, S.,Lee, H., McAfee, D., and Trick, H. (2012). "Targeting Defense Regulatory Genes and Host Susceptibility Factors for Enhancing Fusarium Head Blight Resistance in Wheat." In: S. Canty, A. Clark, A. Anderson-Scully and D. Van Sanford (Eds.), *Proceedings of the 2012 National Fusarium Head Blight Forum (* pp.149). East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative.

Koeritz, E.J., Elakkad, A.M., Dahleen, L.S., Skadsen, R., Abebe, T., Shah, J., Nalam, V.J., Klossner, G., Tumer, N., Di, R., Muehlbauer, G.J., Li, X., Shin, S.and Dill-Macky, R. (2012). "Testing Transgenic Spring Wheat and Barley Lines for Reaction to Fusarium Head Blight: 2012 Field Nursery Report**."** In: S. Canty, A. Clark, A. Anderson-Scully and D. Van Sanford (Eds.), *Proceedings of the 2012 National Fusarium Head Blight Forum* (pp.140). East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab I Initiative.

Presentations at Meetings/Conferences

Title: Exploiting non-host resistance to engineer disease resistance in plants (Oral presentation)
Authors: Syeda Alam, Sujon Sarowar and Jyoti Shah
Graduate Day, Dept Biological Sciences, University North Texas, April 20, 2013

Title: Abietane diterpenoid in plant defense signaling (Oral invited presentation)
Authors: Shah, J., : Chaturvedi, R., Venables, B., Petros, R.A.
Conference: Plant Biology 2012 Annual meeting of the American Society of Plant Biologists, Austin, TX, July 20-24, 2012.

Title: Dehydroabietinal signaling in plant defense against pathogen (Poster presentation)
Authors: Zulkarnain Chowdhury, Ratnesh Chaturvedi, Barney Venables, Robby Petros and Jyoti Shah. Poster presentation at the Graduate Day, Dept Biological Sciences, University North Texas, April 20, 2013

Title: Dehydroabietinal signaling in plant defense against pathogen (Poster presentation)

Authors: Zulkarnain Chowdhury, Ratnesh Chaturvedi, Barney Venables, Robby Petros and Jyoti Shah
Conference: Southern Section of American Society of Plant Biologists annual meeting, Little Rock, AK. April 6-8, 2013

Title: An abietane diterpenoid is a potent inducer of systemic acquired resistance in plants (Poster presentation)
Authors: Chaturvedi, R., Venables, B., Petros, R.A., Amruthesh, K. N., and Shah, J.
Conference: 30th New Phytologist Symposium: Immunomodulation by plant-associated organisms, Stanford Sierra Conference Centre, Fallen Leaf Lake, CA, USA, 16-19 September 2012

Title: Abietane diterpenoid: a signaling function in plant defense (Poster presentation).
Authors: Chaturvedi, R., Venables, B., Petros, R.A., and Shah, J.
Conference: American Society of Plant Biologist's annual meeting 'Plant Biology 2012', Austin, TX; July 20-24, 2012.