Nebraska Soybean Board
FINAL Research Report Form

DS VB

1/3/2019

Note: Submit this report no later than 90 days after the NSB-funded project officially terminates.
This post-project 90-day time-frame will allow the Lead PI time to complete any final data analysis and a final technical report, plus the drafting of any articles for submission to scientific journals. Note that this completed report will be provided to the curator of a national database of State, Region, and USA Soy checkoff funded projects.

Project # and Title: #1717: Using Pseudomonas Syringae Effectors to Discover and Manipulate Soybean Immunity Components

Principal Investigator: James R Alfano

Co-PI's & Institutions:

Project Date (Including Extension): 10/01/2015 ***to*** 09/30/2018 ***(example: mm/dd/yyyy to mm/dd/yyyy)***

Total Budget for Project: $ 235,523.00

1. Briefly State the Rational for the Research:

The research seeks to identify new components of soybean immunity by identifying which soybean proteins interact with Pseudomonas syringae pv glycinea LN10 type III effector proteins. These effectors are injected into soybean cells by the pathogen's type III protein secretion system. P. syringae effectors are generally known to suppress plant immunity and a number of them have been shown to interact with components of immunity mostly in the model plant Arabidopsis. This project seeks to do these experiments directly with soybean because the resources for soybean have been developed such that there is less need to first work with a model system.

2. Research Objectives (copy from project, but keep in a brief bullet format):

i. Finish the experiments to determine if the soybean GRP7 RNA-binding protein can be manipulated to improve resistance to biotic stress in the field.
ii. Continue to identify Pseudomonas syringae glycinea (Pgl) LN10 type III effectors (T3E) soybean targets using multiple approaches.
iii. Determine if the microtubule network, a target of multiple type III effectors, can be manipulated to improve plant immunity.

3. General Approach Used and (if applicable) the Nebraska Test Locations:

We used molecular microbiology approaches and plant molecular biology approaches to do our research. These include making bacterial and plant mutants, performing protein-protein interaction assays, immunity output assays, and field experiments. The field experiments were done at Mead, Nebraska.

4. Describe: Deliverables & Significance Attained for Each Research Objective:

Alfano, J.R., A. Joe, T.E. Clemente, Z.Q. Fu, M. Guo, B.-R. Jeong, and T. Elthon. 2015. Transgenic soybean plants expressing a soybean homolog of glycine-rich protein 7 (GRP7) and exhibiting improved innate immunity. Patent Number: US 9,169,489.

Objective i. We have seed available from these plants for further experimentation. We have shown that they are more resistant to the bacterial pathogen Pseudomonas syringae in the greenhouse and have a similar yield to wild type soybean in the field. Therefore, the expression of GRP7 does not reduce yield.

We will continue working with these transgenic plants to determine if they are more resistant to other pathogens such as viral, fungal, or oomycete pathogens. Also, we have plans to do additional field experiments where we infect plants in the field.

If the plants show improved resistance we will try to market these as a biotechnology product.

Objective ii. This continues to be a work in progress. We have identified several new targets for P. syringae pv glycinea LN10 type III effectors. These projects take a long time to develop but we have been making consistent progress and it is quite likely that several of these potential targets are new components of soybean immunity.

Objective iii. With funds from the Nebraska Soybean Board, we found that a P. syringae type III effector (HopE1) targets a protein (called MAP65-1) that plays a critical role in the microtubule network. It is clear the P. syringae targets this protein to inactivate the microtubule network. We think that by doing so it inhibits the secretion of immunity-related components to the plant apoplast. We recently received in August 2018 a 3 year $600,000 grant to develop this further. This grant would not have been possible without the support of the Nebraska Soybean Board. Moreover, this project may lead to soybean plants that are more resistant to pathogens.

4. Describe: Deliverables & Significance Attained for Each Research Objective (continued):

5. List where the Project Research Results/Findings were Publicized:

Guo, M., P. Kim, G. Li, C.G. Elowsky, and J.R. Alfano. 2016. A bacterial effector Co-opts calmodulin to target the plant microtubule network. Cell Host & Microbe, 19: 67-78.

October, 2018 International Symposium on Plant Pathogenic Bacteria, Nanjing, China
October, 2018 Huazhong Agriculture University – UNL Joint Symposium on Root Biology & Plant-Microbe Interactions, Wuhan, China
June, 2018 New Frontiers in Plant Biology Workshop, Biotechnology and Plant Genomics Center, Madrid, Spain
October, 2017 Seminar at the South Texas Center for Emerging infectious Diseases, University of Texas, San Antonio, Texas
September, 2017 Departmental Seminar, Department of Botany and Plant Pathology, Purdue University, West Layfayette, Indiana
August, 2017 Departmental Seminar, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China
June, 2017 15th Congress of the Mediterranean Phytopathological Union, Cordoba, Spain
April, 2017 Complex Biosystems Seminar Series, University of Nebraska, Lincoln, Nebraska
January, 2017 Agriculture Builders of Nebraska, Inc. Annual Meeting, Lincoln, Nebraska
March, 2016 Virginia Tech Life Science Seminar, Virginia Tech University, Blacksburg, Virginia
December, 2015 Departmental Seminar, Department of Biological Sciences, University of South Carolina, Columbia, South Carolina

Note: The above boxes will automatically accomodate for your text inputs; HOWEVER, the Final Report comprised of the above listed items must be kept to THREE PAGES. A Technical Report of no more than TEN PAGES (preferably fewer) can be appended to this report.
Submit both reports as a single PDF with this file name format: #XXX > FINAL > Project Title > PI last name

Please email this completed form to the Agriculture Research Division (jmonaghan2@unl.edu) based on the reporting schedule given to you. If you have any questions, please call the ARD at 2-2045 or Victor Bohuslavsky at the Nebraska Soybean Board Office at (402) 432-5720.