Lois Ronhovde

Nebraska Soybean Board
FINAL Research/Extension Education Report Form

Note: Submit this report no later than 60 days after the NSB-funded project officially terminates.

This post-project 60-day time-frame will allow the Lead PI/Extension Educator time to complete any final data analysis and a final technical report, plus the drafting of any articles for submission to scientific journals.
This completed report will be provided to the National Soybean Checkoff Research Database: soybeanresearchdata.com.

Project # and Title:

#1734: Using pathogen effectors to improve the soybean immune system

PI / Extension Educator:

Tom Elmo Clemente

Co-PI's / Co-Extension Educator's:

Project Date ***(Including No-Cost Extension)*****:** 10/1/21 **to** 9/30/22

Total Budget for Project: $ 59,000

1. Briefly State the Rationale for the Research.

Pathogens have co-evolved with their host plants. This has led to an "arms race" such that the pathogen acquires the ability to circumvent weak links of the plant's immune system that if disabled will result in disease, and the host subsequently develops strategies to counter the pathogen's ability to induce disease. This proposal seeks to exploit our current understanding underlying how plant hosts developed disease resistance strategies, gleaned from model organisms, and translate these findings to soybean [Glycine max (L.) Merr] with the goal of enhancing resistance to biotic stress in the crop.

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

1. Continue to evaluate soybean events expressing GRP7 RNA-binding protein to monitor for enhanced resistance towards oomycete pathogens.
2. Develop genetic approaches to modulate cellular microtubule network, as a means to improve soybean resistance to pests.
3. Determine if the soybean blue light receptor protein, Phototropin 2, is associated with plant disease resistance in a similar mechanism observed in Arabidopsis.
4. Continue to identify soybean cellular targets of type III effectors injected upon infection by the pathogen P. syringae pv. glycinea, evaluate the importance of these targets to soybean immunity, and explore the extent that these targets can be manipulated to produce soybean plants with improved resistance to biotic stress.

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

Previous studies carried out at UNL revealed that in the host/microbe interaction of the model plant Arabidopsis and the pathogen Pseudomonas syringae, the pathogen injects specific "effector" proteins into the host that target various cellular proteins in the host, to bypass the plant's defense responses. When these host target proteins are over expressed in Arabidopsis, a mitigation in disease induced by this pathogen is observed. To assess if this outcome can be translated to soybean, we introduced transgenic alleles, designed to encode these effector targeted host proteins, into soybean and monitored the derived soybean events for enhancement in biotic stress tolerance, and in some studies, abiotic stress tolerance. In addition we investigated the mechanism by which one specific P. syringae effector designated HopAM1 is able to help the pathogen circumvent the host's defense response.

Laboratory, growth chamber and greenhouse activities were carried out at the George W. Beadle Center on the campus of UNL, while field studies were conducted at UNL's Eastern Nebraska Research, Extension and Education Center.

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

1-Continue to evaluate soybean events expressing GRP7 RNA-binding protein
to monitor for enhanced resistance towards oomycete pathogens: Transgenic soybean events were created that carry a transgenic allele for expression the Arabidopsis GRP7 RNA-bind protein and ectopic expresion of the soybean version of GRP7. Soybean events were characterized, enhanced resistance towards P. syringae (soybean strain), Phytophthora sojae, and cold stress. The outcome revealed a small but statistical reduction in P. syringae infection, no impact on Phytophthora pathogenesis, and a small, and not statistical change in cold stress.

2. Develop genetic approaches to modulate cellular microtubule network, as a means to
improve soybean resistance to pests: Here we introduced transgenic alleles designed to expression both the Arabidopsis and soybean versions of gene referred to as map65. The derived soybean events were characterized for tolerance towards cold stress, P. sojae infection, P. syringae infection, nematode (SCN) and the herbicide oryzalin, whose mode of action is through destabilization of microtubules. The results revealed that these soybean events displayed a significant change in tolerance to bacteria infection (P. syringae), no change in oomycete pathogenesis, statistically improved tolerance to cold, no difference in SCN infection, and a high tolerance to oryzalin applications. In addition, we created transgenic stacks with GRP7 and map65 alleles, and have F4 homozygous lineages. A manuscript on these activities has been drafted with a planned submission by the end of Jan 2023.

3. Determine if the soybean blue light receptor protein, Phototropin 2, is associated with
plant disease resistance in a similar mechanism observed in Arabidopsis:

Transgenic soybean events were created that carry transgenic alleles designed to express a blue light receptor protein, Phototropin 2, another identified pathogen effector target in plant systems. Transgenic soybean events ectopically expressing the soybean Phototropin 2 allele or expressing the Arabidopsis Phototropin 2 allele were characterized for enhanced resistant towards bacterial challenge (P. syringae soybean strain) and changes in photosynthesis. The results over two rounds of studies for each trait evaluation were rather variable, so it was decided to abandon this component of the project.

4. Continue to identify soybean cellular targets of type III effectors injected upon infection by the pathogen P. syringae pv. glycinea, evaluate the importance of these
targets to soybean immunity, and explore the extent that these targets can be manipulated to produce soybean plants with improved resistance to biotic stress.
This component of the project identified a strategy plant pathogens exploit to circumvent the host's immune system. It was found that th effector designated HopAM1 when secreted into plant cells rapidly alters cellular levels of a key metabolite NAD+, which in turn impacts susceptibility, in favor of the pathogen. These findings were reported in the journal New Phytologist and a review of this mechanism of pathogenesis was communicated in the journal Molecular Plant Microbe Interactions, and a third paper in the journal Science. In addition, we confirmed the pathogen effector designated HopAW1 has cysteine protease activity, meaning it can cleave specific cellular proteins, which results in changes in those cleaved proteins function, that translates to favor pathogenesis by the pathogen. Secondly, we identified an additional 11 putative soybean proteins that are potential targets by pathogen effector proteins.

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

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

This program contributed to the communication of two peer reviewed publications:

Eastman, S., T. Smith, M.A. Zaydman, P. Kim, S. Martinez, N. Damaraju, A. DiAntonio, J. Milbrandt, T.E. Clemente, J.R. Alfano, and M. Guo. 2022. A phtobacterial TIR domain effector manipulates NAD+ to promote virulence. New Phytologist 233:890.

Eastman, S., A. Bayless, and M. Guo. 2022. The nucleotide revolution: Immunity at the intersection of Toll/Interleukin-1 receptor domains, nucleotides, and Ca2+. Mol Plant Micro Inter 35:964-976.

Manik, M.K, Y. Shi, S. Li, M. Zaydman, N. Damaraju, S. Eastman, T.G. Smith, W. Gu, V. Masic, T. Mosaiab, J.S. Weagley, S.J. Hancock, E. Vasquez, L. Hartley-Tassell, N. Kargios, N. Maruta, B.Y.J. Lim, H. Burdett, M.J. Landsberg, M.A. Schembri, I. Prokes, L. Song, M. Grant, A. DiAntonio, J.D. Nanson, M. Guo, J. Milbrandt. T.Ve, and B. Kobe. 2022. Cyclic ADP ribose isomers: Production, chemical structures, and immune signaling. Science 377: eadc8969.

Note: 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 the form with the following file name format: #XXX_FINAL_Project Title_LastName

Please submit this completed form with attached files to the Agriculture Research Division, jmcmahon10@unl.edu, based on the reporting schedule given to you.

If you have any questions, please call Jen McMahon at the Agricultural Research Division (402) 472-7082.

Please click to attach technical reports, etc. Attach files Please check your information before submitting the form. Submit by Email

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