

**Nebraska Soybean Board**  
**FINAL Research Report Form**



1/2/2019

**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:** #1728: Soybean Phytophthora root rot disease control by identifying protein interactions of virulence effectors in Phytophthora Sojae to soybean proteins

**Principal Investigator:** Chi Zhang

**Co-PI's & Institutions:** University of Nebraska - Lincoln

**Project Date (Including Extension):** 10/1/2016 to 9/30/2018 (example: mm/dd/yyyy to mm/dd/yyyy)

**Total Budget for Project:** \$107,700

**1. Briefly State the Rational for the Research:**

After identification of potential virulence effectors in Phytophthora Sojae (P. Sojae), the causal agent of Soybean Phytophthora root rot disease, we conducted research to discover protein-protein interactions between P. Sojae virulence effectors and proteins in soybean to understand how virulence effectors cause the disease. P. Sojae secretes virulence effectors as major 'weapons' to attack the target host organisms, and understanding the biological functions of virulence effector is the key step to reveal the mechanism of oomycete pathogenicity to hosts and control the disease. We were conducting the so-far blank research area of protein-protein interactions between P. Sojae and soybean by developing a statistical model based on protein-domain interaction prediction. As a result, the mechanism under which P. Sojae interacts with and infects soybean can be elucidated, which may directly lead to feasible and efficient methods to prevent soybean from the Phytophthora root rot disease.

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

Objective One: Identify the protein physical interactions between putative virulence effectors in P. Sojae and proteins in soybeans based on a statistical model training with the know protein domain interaction data, which can confirm the computational discovered virulence effectors and discover novel innate immune components in soybean.

Objective Two: Systematically discover the pathogenic target genes that are enriched in the plant pathways, which can reveal the mechanism of P. Sojae's pathogenicity and be used to develop control methods for Phytophthora root rot.

**Nebraska Soybean Board  
FINAL Research Report Form**

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

Not applicable

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

The goal of our project is to discover the mechanism under which *P. Sojae* interacts with and infects soybean, and the knowledge of interaction mechanism between *P. Sojae* and soybean may directly lead to feasible and efficient methods to prevent soybean from the Phytophthora root rot disease. The interactions between *P. Sojae* and soybean are executed by protein interactions, especially by interactions between *P. Sojae* virulence effectors and soybean proteins. First, we developed a novel computational method to identify the interactions between virulence factor *P. Sojae* and soybean proteins based on the protein-domain interaction network and Bayesian statistics. By screening entire sets of proteins in *P. Sojae* and soybean, we analyzed >80,000 proteins and > 220,000 protein domains. To improve the accuracy, a directed graph model was employed by this model, and pathogenic and host protein domains were distinguished in the network, which is novel in this type of algorithms. Based on our designed statistical network model, we identified the protein interaction networks between the pathogen and soybean. After our algorithm identified the protein-protein interaction information, we could confirm the predicted virulence effectors and understand the functions of these virulence effectors. The model predicted the interactions between 596 *P. sojae* virulence effectors and 18142 soybean proteins. Based the cross-validation evaluation, this new improvement can make the prediction accuracy better than other existing methods, such as methods based on sequence similarity, position weighted matrix (PWM), and hidden Markov model (HMM). Among those predict interactions, many of them are related to the disease.

**Nebraska Soybean Board**  
**FINAL Research Report Form**

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

For example, we found virulence effector Avh105, which cause cell death, interacts with Glyma03g14740, Omega peptidase activity in ER, and Glyma03g29230, ATP transporter. For Objective 2, we found that most *P. sojae* virulence factors are structural constituent of ribosomes (Gene ontology annotation term GO:0003735) and structural molecule activity (Gene ontology annotation term GO:0005198). The results indicate that *P. sojae* pathogens interfere the genetic information flowing and functions of virulence factor are related to cell signals and DNA binding. PI Zhang's Lab and Co-PI Alfano's are working together to design experiments to validate these discoveries.

With our developed computational methods, my lab also collaborated with Drs. Tom Clemente and Bin Yu to analyzed the nanopore sequencing data to identify the insertion position of transgenes in soybean.

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

1. H. Wang, Y. Dou, Chi Zhang, B. Yu, Y. Liu, T. Heng-Moss, G. Lu, M. Wachholtz, J. Bradshaw, P. Twigg, G Sarath. Insect and plant-derived miRNAs in greenbug (*Schizaphis graminum*) and yellow sugarcane aphid (*Sipha flava*) revealed by deep sequencing. *Genes* (2016); 599:68-7.
  2. Y. Dou, S. Li, W. Yang, K. Liu, Q. Du, G. Ren, B. Yu, Chi Zhang. Genome-wide Discovery of Circular RNAs in the Leaf and Seedling Tissues of *Arabidopsis thaliana*. *Current Genomics* (2017); 18(4):360-365.
  3. S. Li, K. Liu, S. Zhang, X. Wang, K. Rogers, G. Ren, Chi Zhang, B. Yu. STV1, a ribosomal protein, binds primary microRNA transcripts to promote their interaction with the processing complex in *Arabidopsis*. *Proc Natl Acad Sci USA* (2017); 114(6):1424-1429.
  4. L. Virilouvet, T.J. Avenson, Q. Du, Chi Zhang, N. Liu, T.J. Avenson, M. Fromm, Z. Avramova, S.E. Russo. Dehydration stress memory: Gene networks linked to physiological responses during repeated stresses of *Zea mays*. *Frontier in Plant Science* (2018); Volume 9, Article number 1058.
  5. R.K. Ramamurthy, Q. Xiang, E. Hsieh, K. Liu, Chi Zhang, B.M. Waters. New aspects of iron-copper crosstalk uncovered by transcriptomic characterization of Col-0 and the copper uptake mutant spl7 in *Arabidopsis thaliana*. *Metallomics* (2018); 10:1824-1840.
  6. S. Li, S. Jia, L. Hou, H. Nguyen, S. Sato, D. Holding, E.B. Cahoon, Chi Zhang, T. Clemente, B. Yu. Mapping of transgenic alleles in plants using a Nanopore-based sequencing strategy. *Plant Biotechnology J* (2018) (submitted).
  7. T. Lu, J. Alfano, Chi Zhang. Pathogen-host protein-protein interaction of *Phytophthora* based on directional domain-domain interaction. *BMC System Biology* (2018) (submitted).
- Conference presentations: IEEE International Conference (eit2017), Next Generation Dx Summit 2017, ISCB annual meeting 2018, Next-generation sequencing workshop, UNL, 2018, and PAG XXVII Jan 2019.
- A web page to show relevant information: <http://sysbio.unl.edu/resources>

**Note:** The above boxes will automatically accommodate 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](mailto: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.