Nebraska Soybean Board
Year-End Research Findings Report

Please use this form to summarize the practical benefits of your research project and what has been accomplished. Your answers need to convey why the project is important and how the results impact soybean production.

Project Title: #1719, Improving Soybean Immunity through Exploring the Ubiquitination System

Contractor & Principal Investigator: University of Nebraska - Lincoln ARD, Lirong Zeng

Please check/fill in appropriate box: ☐ ***Continuation research project***
■ ***Year 1 of 2 research project (for example: Year 1 of 2)***

1. ***What was the focus of the research project or educational activity?***
   This project focused on the identification of soybean genes that are related to the ubiquitination system (USB) at genome-scale and characterizing some of these genes that are critical for soybean immunity against pathogens such as soybean cyst nematode (SCN) and Pseudomonas syringae pv. *glycinea. The long-term goal of this project is to manipulate the key components of soybean UBS we identified and characterized for the improvement of soybean immunity. Specifically, we aim to 1) identify the Soybean genes encoding components of the soybean ubiquitination system; 2) pinpoint the members of soybean UBS that are involved in host immune signaling; and 3 elucidate the mechanism underlying the regulation of host immune signaling by the soybean UBS-related genes.*

2. ***What are the major findings of the research or impacts of the educational activity?***
   (1) Using bioinformatics tools, we identified two soybean genes encoding ubiquitin E1s, 106 genes encoding UBC domain-containing proteins (including ubiquitin E2s), 145 genes encoding U-box domain-containing E3s, 547 genes encoding putative F-box-containing proteins, and 1045 genes encoding RING finger domain-containing proteins. (2) Based on the published RNA-seq data, we identified approximately 200 ubiquitination-related, differentially regulated genes from their dataset. These ~ 200 UBS related genes were differentially regulated (two folds) either in resistant or susceptible infection in at least one of the time points after the soybean roots are infected by SCN. (3) we performed real-time RT- PCR to confirm the differential expression of selected genes during SCN infection. We confirmed at least five soybean UBS-related gene showed significantly differential expression during SCN infection. (4) we selected and cloned fifteen soybean genes that belong to different categories of the UBS (RING, UBOX, UBC, E1) and express them in bacterial system for recombinant protein purification for checking their in vitro biochemical activity using in vitro ubiquitination assay.

3. ***Briefly summarize, in lay terms, the impact your findings have had, or will have, on improving the productivity of soybeans in Nebraska and the U.S.***
   We revealed that some soybean UBS-related genes showed significant differential expression in resistant and susceptible soybean plants (root) during SCN infection. In depth analysis and characterization of these genes will allow us to better understand the mechanism involved in soybean host immunity against SCN infection and these genes can be utilized by breeding programs to develop soybean verities with improved tolerance to SCN infection.

4. ***Describe how your findings have been (or soon will be) distributed to (a) farmers and (b) public researchers. List specific publications, websites, press releases. etc.***

*****This form must be completed and submitted with the fourth quarter report.***

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We are awaiting the data of biochemical characterization of the recombinant proteins of soybean UBS-related genes for the first manuscript of this project. We expect the first manuscript will be ready at the end of next spring as the appointment for the current postdoc who worked on this project will not be extended for the second year of this project.

5. Did the NE soybean checkoff funding support for your project leverage any additional state or Federal funding support? (Please list sources and dollars approved.)

We are planning to use the data we obtained through the support of NE Soybean check off funding for extramural funding after our first manuscript is published. We sincerely appreciate the NE soybean checkoff funding that allows us to investigate the involvement/regulation of soybean by the UBS, a topic that has not been under-understood so far.

*****This form must be completed and submitted with the fourth quarter report.***