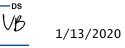# Nebraska Soybean Board
FINAL Research Report Form

1/13/2020

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 National Soybean Checkoff Research Database, (soybeanresearchdata.com).

Project # and Title: Improve soybean resistance to Nematodes with synthetic RNA interference

Principal Investigator: Bin Yu

Co-PI's & Institutions: Dr. Tom Clemente
Center For Plant Science Innovation
Univesity of Nebraska, Lincoln

Project Date (Including Extension): 10/01/2016 **to** 09/30/2019 **(For example: mm/dd/yyyy to mm/dd/yyyy)**

Total Budget for Project: $ 208,800.00

1. Briefly State the Rational for the Research:

As the most destructive pest disease for soybean, SCN cost huge losses every year. However, the limited number of resistant soybean varieties has challenged the management of SCN. This project will generate SCN resistant soybean varieties, and therefore has the potential to help the management of SCN and to benefit soybean production in US including Nebraska. In addition, this project will develop a novel synthetic-RNA interference technology that enables soybean resistance to SCN. This technology will target multiple genes at the same time to enhance RNAi effects on SCN. Furthermore, we will use Polymerase III (Pol III) promoters to eliminate the negative effect of endogenous gene silencing on the application of RNAi. If successful, it will open doors to apply this technology to improve other important soybean traits, such as fungi disease resistance and other insects. Thus this project should have the potential to benefit soybean production more broadly. This project will also study the potential adaptation of SCN to the RNAi from soybean. This will provide intellectual basis to design RNAi against SCN and other insects. In addition, this technology can be combined with existing SCN resistant traits to eliminate the potential adaptation of SCN to resistance. In summary, this project will lead to an environmental friendly and cost efficient way to control SCN disease and therefore benefit the producer.

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

The goals of this research are to develop soybean varieties with high resistance to SCN using synthetic RNAi technology. The specific aims are:
1) Generating SCN resistant variety through synthetic RNAi technology. A vector that harbors four shRNA expression cassettes, each of which will be driven by a different Pol III promoter, will be produced and expressed in soybean. The resistance of transgenic line to SCN will be examined in Dr. Giesler's Laboratory.
2) Analyze the adaption of SCN to the resistance variety expressing shRNAs. We plan to test if SCN can adapt to sRNAi, which will allow SCN to infect soybean expressing shRNAs, and, if so, provide some molecular insight on how this adaptation occurs. Furthermore, we will test if rotation or combination of shRNA lines

Nebraska Soybean Board
FINAL Research Report Form

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

1) Generating SCN resistant variety through synthetic RNAi technology. shRNAi technology is emerging in animals to repress gene expression. This technology takes advantage of RNA polymerase III to express short RNAs that form a short hairpin (shRNA) to produce a singe small RNA. In addition, Pol III promoter subjects less transgene silencing effect, which means stable expression of the shRNA. Therefore, it will produce more sustainable effect. However, this technology is less explored in plants. We will adapt this approach to eliminate the transgene silencing effect. Furthermore, we have isolated four different Pol III promoters, which enable us to produce a synthetic RNAi vector expressing four different shRNAs at the same time.

2). Analyze the adaption of SCN to the resistance variety expressing the synthetic RNAi vector (sRNAi line). When using RNAi technology to improve plants resistance to SCN and other pathogen, a question is if SCN (pathogen) can become adapted to RNAi. We plan to test this possibility, and if so, provide some sight on molecular basis of how this occurs, which will help us to design strategies to overcome it. The eggs survived in the sRNAi lines will be collected and examined for their ability to infect sRNAi lines. If these eggs are adapted to shRNAs, their infection ability will improve from generation to generation. We will further examine if the repression of target RNAs is released in the adapted SCN, which will provide insight how the adaption occurs. We will also test if rotation or combination of natural SCN-resistant line with the shRNA line can reduce this adaptation. Through these studies, we expect to provide basis to better utilize genetic engineering and traditional breeding to improve soybean resistance to SCN and reduce the loss caused by SCN.

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

Objective 1:

We generated vectors that co-expresses four siRNAs targeting four different genes of SCN under the control of four Pol III promoters, respectively. These siRNAs target a Chorismate mutase, which is a critical enzyme for amino acid biogenesis, a ATP synthase subunit, a cysteine protease and a ATP citrate lyase. All these genes have been identified as essential genes for SCN survive. Killing these genes in SCN will limit the growth of SCN. We validated the expression of four siRNAs in a transient system. The transgenic soybean plants were generated in Dr. Clemente's group.

Besides SCN, we generated vectors that co-expresses four siRNAs targeting four different genes of aphids, which also reduces soybean yield. We validated the expression of four siRNAs in a transient system. The transgenic soybean plants were generated in Dr. Clemente's group.

Objective 2, we analyzed the SCN resistance of transgenic plants. Some of them show enhanced resistance, while others are not. We also analyzed the aphids resistance of transgenic plants. Many of them showed improved resistance.

During the process, we think the transgene insertion position may affect the levels of siRNAs. Thus, we developed a method to map the transgene insertion position in soybean genome. Knowing transgene position in genome is important,because the insertion position can affect

2

Nebraska Soybean Board
FINAL Research Report Form

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

the transgene expression levels, and may also affect the function of surrounding genes. Importantly, prior knowledge of map position of a transgenic allele is beneficial when breeding programs begin to introgress the allele into elite germplasms. In soybean, determining the transgenic position has been a challenge because the complexity of its genome. Targeting this gap, we took advantage of a single molecule real-time (SMRT) sequencing technology called Oxford Nanopore Technologies, which can generate long-reading sequence, to develope W pipeline designed for high-throughput mapping of transgenic alleles in plant species. Employing a target enrichment approach using a combination of oligo probes to capture DNA fragments containing the transgenic allele, permitted the rapid identification of map position of a hundred transgenic alleles in a single run. The calculated cost incurred by the procedure to the transgenic allele is estimated to be less than $30 persample, and the results are generated within one week.These results demonstrate that this Nanopore®-based sequencing method is rapid, convenient, reliable, cost-efficient and high-throughput. We expect that this method will benefit the application of trangenic technology in soybean breeding, and can be be easily translated to other crops with complex genomes. Thus, we expect that this research will have a great impact.

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

1, Published manuscripts:
1, Feng, B., Ma, S., Chen, S., Zhu, N., Zhang, S., Yu, B., Yu, Y., Le, B., Chen, X., Dinesh-Kumar, S.P., Shan, L., and He, P. (2016). PARylation of the forkhead-associated domain protein DAWDLE regulates plant immunity. EMBO Rep 17, 1799-1813.
2, Liu, Y., Li, S., Chen, Y., Kimberlin, A.N., Cahoon, E.B., and Yu, B. (2016). snRNA 3' End Processing by a CPSF73-Containing Complex Essential for Development in Arabidopsis. PLoS Biol 14, e1002571.
3, Wang, H., Zhang, C., Dou, Y., Yu, B., Liu, Y., Heng-Moss, T.M., Lu, G., Wachholtz, M., Bradshaw, J.D., Twigg, P., Scully, E., Palmer, N., and Sarath, G.. Insect and plant-derived miRNAs in greenbug (Schizaphis graminum) and yellow sugarcane aphid (Sipha flava) revealed by deep sequencing. Gene 599, 68-77.
4, Wang, X., Wang, Y., Dou, Y., Chen, L., Wang, J., Jiang, N., Guo, C., Yao, Q., Wang, C., Liu, L., Yu, B., Zheng, B., Chekanova, J.A., Ma, J. and Ren, G. (2018) Degradation of unmethylated miRNA/miRNA*s by a DEDDy-type 3' to 5' exoribonuclease Atrimmer 2 in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America, 115, E6659-E6667.
5, Li, S., Xu, R., Li, A., Liu, K., Gu, L., Li, M., Zhang, H., Zhang, Y., Zhuang, S., Wang, Q., Gao, G., Li, N., Zhang, C., Li, Y. and Yu, B. (2018) SMA1, a homolog of the splicing factor Prp28, has a multifaceted role in miRNA biogenesis in Arabidopsis. Nucleic Acids Res. 46:9148-9159
6, Wang, Z., Ma, Z., Castillo-Gonzalez, C., Sun, D., Li, Y., Yu, B., Zhao, B., Li, P. and Zhang, X. (2018) SWI2/SNF2 ATPase CHR2 remodels pri-miRNAs via Serrate to impede miRNA production. Nature, 557, 516-521.
7, Zhang, S., Dou, Y., Li, S., Ren, G., Chevalier, D., Zhang, C. and Yu, B. (2018) DAWDLE Interacts with DICER-LIKE Proteins to Mediate Small RNA Biogenesis. Plant Physiol, 177, 1142-1151.
8, Li S, Liu K, Zhang B, Li, M, Zhang S, Zhang C and Yu B (2017) MAC3A and MAC3B, Two Core Subunits of the MOS4-Associated Complex, Positively Influence miRNA Biogenesis Plant Cell 30:481-494
9, Wang, X., Wang, Y., Dou, Y., Chen, L., Wang, J., Jiang, N., Guo, C., Yao, Q., Wang, C., Liu, L., Yu, B., Zheng, B., Chekanova, J.A., Ma, J. and Ren, G. (2018) Degradation of unmethylated miRNA/miRNA*s by a DEDDy-type 3' to 5' exoribonuclease Atrimmer 2 in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America, 115, E6659-E6667.
10,Li, S., Xu, R., Li, A., Liu, K., Gu, L., Li, M., Zhang, H., Zhang, Y., Zhuang, S., Wang, Q., Gao, G., Li, N., Zhang, C., Li, Y. and Yu, B. (2018) SMA1, a homolog of the splicing factor Prp28, has a multifaceted role in miRNA biogenesis in Arabidopsis. Nucleic Acids Res. 46:9148-9159
11, Wang, Z., Ma, Z., Castillo-Gonzalez, C., Sun, D., Li, Y., Yu, B., Zhao, B., Li, P. and Zhang, X. (2018) SWI2/SNF2 ATPase CHR2 remodels pri-miRNAs via Serrate to impede miRNA production. Nature, 557, 516-521.
12, Zhang, S., Dou, Y., Li, S., Ren, G., Chevalier, D., Zhang, C. and Yu, B. (2018) DAWDLE Interacts with DICER-LIKE Proteins to Mediate Small RNA Biogenesis. Plant Physiol, 177, 1142-1151.
13, Li S, Liu K, Zhang B, Li, M, Zhang S, Zhang C and Yu B (2017) MAC3A and MAC3B, Two Core Subunits of the MOS4-Associated Complex, Positively Influence miRNA Biogenesis 30:481-494
14, Li S, Jia S, Hou L,Nguyen H, Holding H, Cahoon, E, Clemente T and Yu B (2019) Mapping of transgenic alleles in soybean using a Nanopore-based sequencing strategy. J Exp Bot doi: 10.1093/jxb/erz202

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 (jmcmahon10@unl.edu) based on the reporting schedule given to you. If you have any questions, please call Jen McMahon at the ARD at 2-7082.