DS VB 11/8/2019

Nebraska Soybean Board

Year-End Summary Research Report Form For Multi-Year Projects

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 ǿʎť impact soybean production.

Note that this form must be submitted with the 4th Quarter Report in all multi-year projects.

Project Title: Improvement of Soybean Germplasm for Aquaculture Feed (#1716)

Principal Investigator: Ed Cahoon (PI), Tom Clemente (co-PI)

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1. What was the focus of the research project or educational activity?

The proposed research addresses the need for soybean germplasm with high-value oil quality traits for aquaculture feed. The current soybean-based aquaculture feedstocks lack EPA and DHA omega-3 fish oil fatty acids and other oil-based feed components. Because of these deficiencies, soybean-based aquaculture feed requires supplementation with fish oil and high-priced astaxanthin flesh pigments, particularly for farm-raised salmon. In addition, oils with enhanced omega-3 fatty acid content are prone to oxidation, which reduces the shelf life of fish due to the development of off-flavors and odors. The proposed research will address these limitations in oil quality for increased use of soybeans for aquaculture feed by:

1. Developing soybean germplasm with oils enriched in EPA and DHA omega-3 fatty acids.
2. Optimizing production of astaxanthin in soybean seeds.
3. Applying emerging synthetic biology techniques to stack EPA/DHA omega-3 fatty acid, astaxanthin, and high vitamin E antioxidant traits into Nebraska soybean germplasm.
4. Conducting physiological and field-evaluation of new aquaculture germplasm to optimize agronomic performance.

2. What are the major findings of the research or impacts of the educational activity?

Summary of the major findings to date:

- Soybean T2 lines (pPTN1398) containing MetA, MetB, MetC and MetE transgenes to increase methionine seed methione content were advanced in the greenhouse and confirmed to express three transgenes.
- Soybean T3 lines (pPTN1331) engineered to produce the high value aquaculture traits omega-3 EPA, astaxanthin, and high vitamin E antioxidants were grown in the 2019 season at Eastern Nebraska Research and Education Center in Mead, NE. Seeds from these lines were confirmed to produce the three aquaculture traits.
- As a clue to development problems of astaxanthin-producing seeds, we found that seeds of a model species engineered to produce astaxanthin had reduced concentrations of the hormone ABA. Experiments are underway to confirm this finding in developing soybean seeds with the EPA, astaxanthin, and high vitamin E trait stack.
- Gene expression constructs were generated for EPA and DHA production with optimized promoters to obtain higher expression of transgenes and higher EPA and DHA yields.
- Soybean T1 seeds (pPTN1451) were obtained for production of EPA and DHA fish oil and high vitamin E. DHA production was confirmed in these seeds. A new expression construct for these aquaculture traits to optimize and increase expression of key transgenes is nearing completion.
- Construction of the new vector containing soybean WRINKLED1 and engineered soybean DGAT1b to increase seed oil content was completed and is now being used to stack with transgenes for the EPA/DHA, astaxanthin, and high vitamin E traits.
- To increase EPA production in seeds, crosses of the pPTN1331 EPA, astaxanthin, and high vitamin E line and a high linolenic acid line were completed. F1 seeds from the crosses were advanced in the greenhouse.
- To increase seed oil content of EPA lines, crosses of the pPTN1331 EPA, astaxanthin, and high vitamin E line with a KASII/Wrinkled1 line as well as with a DGAT1/Wrinkled1 line were completed, and F2 lines were planted in the field in Mead, NE. F3 seeds were harvested for assessment of the traits.

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.

The project has addressed the Nebraska Soybean Board focus area of germplasm improvement for composition and yield. The project has generated germplasm that produces seed oils with the key, high value traits: fish oil EPA, astaxanthin pigment for consumer-desired fish flesh color, and high vitamin E antioxidants to stabilize EPA from production of off-flavors. Nearly 50% of fish that is consumed globally is farm-raised, and this production system is anticipated to expand as world population grows, ocean stocks of fish dwindle, and consumers place more emphasis on fish for healthy diets. Soybean is and will increasingly be a major sustainable source of aquaculture protein and oil feedstocks. Our research will increase the bushel price of soybeans and deliver high value oil traits that will advance the market share of Nebraska and US soybean for the aquaculture feed market.

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.

The findings have been distributed through research publications in FY2019:

Konda A, Nazarenus TJ, Nguyen H, Yang J, Gelli M, Swenson S, Shipp JM, Schmidt MA, Cahoon RE, Ciftci ON, Zhang C, Clemente TE, Cahoon EB (2019) Metabolic engineering of soybean seeds for enhanced vitamin E tocochromanol content and effects on oil antioxidant properties in polyunsaturated fatty acid-rich germplasm. Metabolic Engineering, In Press.

Vogel PA, Bayon de Noyer S, Park H, Nguyen H, Hou L, Changa T, Khang HL, Ciftci ON, Wang T, Cahoon EB, Clemente TE (2019) Expression of the Arabidopsis WRINKLED 1 transcription factor leads to higher accumulation of palmitate in soybean seed. Plant Biotechnology Journal 17: 1369-1379.

Li S, Jia S, Hou L, Nguyen H, Sato S, Holding D, Cahoon E, Zhang C, Clemente T, Yu B (2019) Mapping of transgenic alleles in soybean using a Nanopore-based sequencing strategy. Journal of Experimental Botany 70: 3825-3833.

Findings were also presented to public researchers through a conference oral presentation:
Lipidomics Study and Research Group (GERLI) 15th Lipidomics Meeting, Compiègne, France, “Probing Fatty Acid Natural Diversity for Enhancement of Plant Oils”, September 30, 2019.

5. Did the NE soybean checkoff funding of your project, leverage additional State or Federal funding support? Please list sources and dollars approved.

USDA-NIFA grant, Cahoon (PI) Overcoming metabolic bottlenecks for enhanced vitamin E production in crop plants. 02/01/2015 - 01/31/2019 $490,000

Please e-mail this report to the Agriculture Research Division (jmonagham2@unl.edu).