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

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

Please check/fill in appropriate box: ☐ ***Continuation research project***
X ***Year 1 of 3 research project (for example: Year 1 of 2)***

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

The proposed research addresses the need for development of a complete soybean-based aquaculture feed. The current soybean-based feed lacks sufficient levels of EPA omega-3 fatty acids and other oil-based feed components. Because of these deficiencies, soybean-based aquaculture feed currently requires supplementation with fish oil and high-priced astaxanthin pigments, particularly for farm-raised salmon. In addition, oils with enhanced omega-3 fatty acid content are prone to oxidation, which limits the shelf life of fish due to the development of off-flavors and odors. The proposed research will address these deficiencies by:

1. Applying emerging synthetic biology techniques to rapidly stack or combine omega-3 fatty acid, astaxanthin, and high vitamin E antioxidant traits into Nebraska soybean germplasm for optimized aquaculture feed.
2. Improvement of omega-3 EPA content in Nebraska soybean germplasm.
3. Improvement of astaxanthin pigment levels in Nebraska soybean germplasm.
4. Evaluation of new oil traits in aquaculture feeding studies.

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

Summary of the major findings to date:

1. A gene expression vector containing nine genes for multiple aquaculture-related oil traits (omega 3 EPA, astaxanthin, and vitamin E antioxidants) was successfully assembled.
2. It is possible to introduce a single, large T-DNA (25.5 kb) including nine genes into the soybean genome.
3. Soybean seeds were generated that contain omega-3 EPA, astaxanthin, and enhanced vitamin E antioxidants in ~1 year. To our knowledge, the stacking of so many genes and obtaining three quality traits in this short time frame is unprecedented.
4. Soybean seeds with up to 8% omega-3 EPA, 1,000 μg/g vitamin E, and 150 μg/g astaxanthin have been generated.

The emerging technology of synthetic biology was applied for the stacking of multiple oil traits in one loci to produce omega-3 EPA, astaxanthin, and vitamin E (tocotrienols) antioxidant. This is an unprecedented target for soybean seed quality improvement involving the introduction of 9 genes in a 25.5 kb.p T-DNA . 15 independent transformation events (T_0) have been generated and seeds have been recovered (Events 1079-1, 1079-2, 1079-3, 1079-4, 1079-5, 1079-6, 1079-8, 1079-9, 1079-10, 1080-1,1095-1, 1095-2, 1095-3, 1095-4 and 1099-2). PCR-based analysis of genomic DNA isolated from these events has confirmed the presence of all of the nine transgenes. This result demonstrated that we had successfully achieved one of our targets of introducing the nine gene 25.5 kb.p T-DNA into soybean. Seeds from all but one of these transformation events (1079-1) has an altered fatty acid profile, including EPA production, and orangered pigmentation (Fig. 1-6). To further analysis T_1 seeds were generated from the events showing the target traits. Among them, 1079-2 (T1-3), 1079-3 (T1-3), 1079-5 (T1-2), 1079-6 (T1-1), 1079-8 (T1-2), and 1079-9 (T1-2) were analyzed up to now. 1079-6 (T1-1), 1079-8 (T1-2), and 1079-9 (T1-2) segregated as red and yellow seeds. Red seeds of these lines produced EPA (2-5 mol%) and its precursors (28-34 mol%), tocotrienols (420-843 µg/g), and astaxanthin. Yellow seed from these lines showed no production of EPA, tocotrienols, and astaxanthin. This indicates that progeny of 1079-6, 1079-8, and 1079-9 segregated into non-transgenic and transgenic seeds. And the production of EPA, astaxanthin, and tocotrienols in single seed indicates that tansgenic progeny includes all cassette of 9 genes. The seeds of 1079-3 (T1-3) segregated as red and yellow seeds. Red seeds of 1079-3 (T1-3) showed EPA (1-8 mol%) and EPA precursors (25-33 mol%) but no tocotrienols production. Yellow seeds of 1079-3 (T1-3) showed mixed phenotype. 80% of yellow seed produced low level of EPA (1.4-1.6 mol%) and its precursors (22-25 mol%). Tocotrienols were produced in yellow seeds (989 µg/g) of 1079-3 (T1-3). The seeds of 1079-5 (T1-2) segregated as red and yellow seeds. Red seeds of 1079-5 (T1-2) showed EPA (2.7-5.0 mol%) and EPA precursors (30-38 mol%) and tocotrienols production 430-584 µg/g). Yellow seeds of 1079-5 T1-2) also showed EPA and tocotrienols production. This indicates that the target T-DNA including 9 genes was not stable in these lines and segregated into different loci into soybean genome.

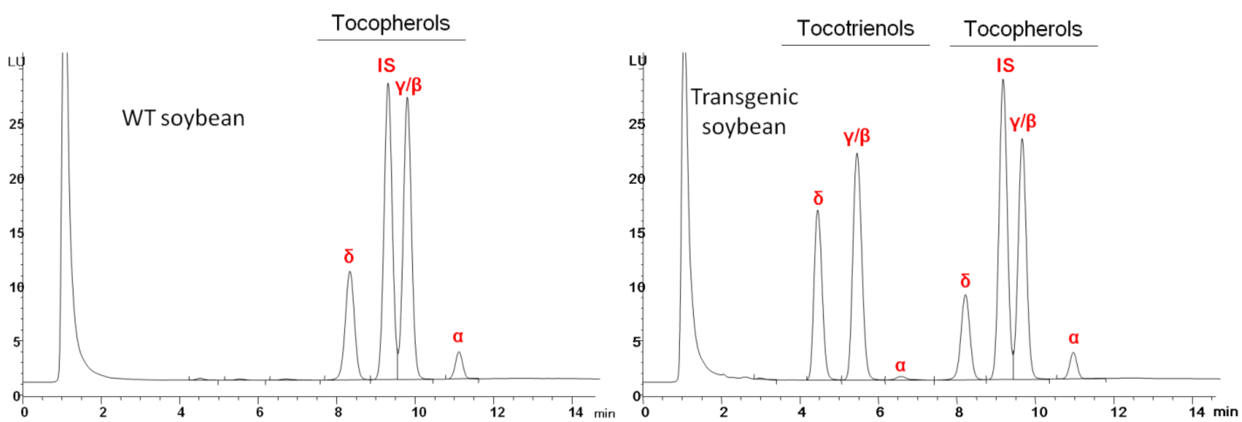

Figure 1. HPLC chromatogram of Vitamin E analysis of soybean.

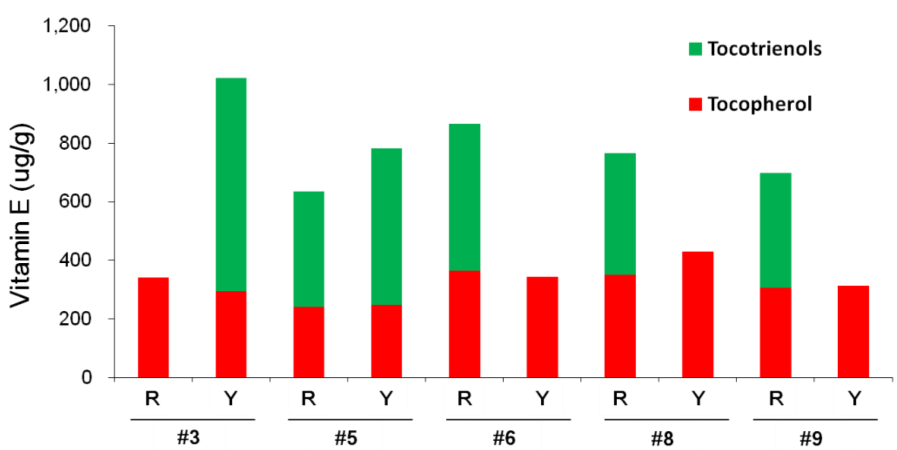

Figure 2. Tocopherol and tocotrienol contents of transgenic soybeans. R and Y indicate red seed and yellow seed, respectively. Three biological replicates were used for red seeds.

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.

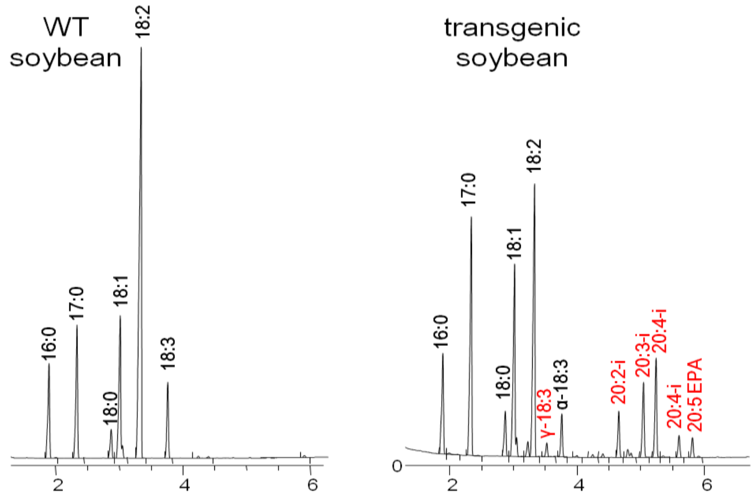

Figure 3. GC chromatogram of fatty acid analysis of soybean.

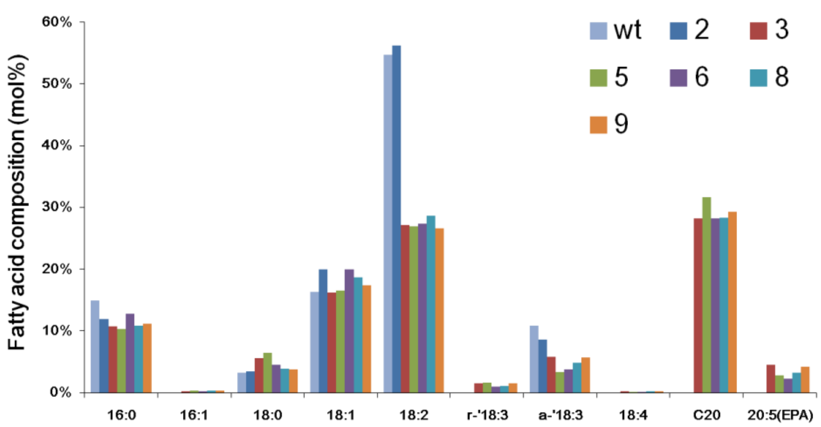

Figure 4. Fatty acid composition of transgenic soybeans.

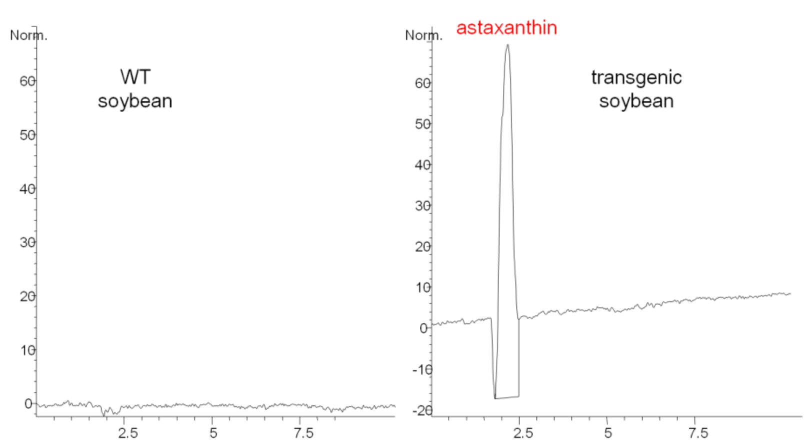

Figure 5. HPLC chromatogram of astaxanthin analysis of soybean.

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

Figure 6. Wild type and astaxanthin-producing soybean seeds.

Impacts

Our results to date demonstrate that new synthetic biology techniques are capable of delivering large numbers of transgenes to soybean to rapidly develop high-value seed quality traits. In one year, we have generated soybean germplasm with three aquaculture oil traits that could not be achieved by conventional breeding. The findings to date are not only significant for soybean improvement for aquaculture feed but also pave the way for adopting synthetic biology approaches to target both output traits (e.g., increased yield) with seed quality traits for rapid improvement.

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 addresses the Nebraska Soybean Board FY17 focus area of germplasm improvement for composition and yield. The project is aimed at enhancing the value of Nebraska soybeans and increasing the use of soybeans for the expanding aquaculture feed market. 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. Meeting this demand requires development of sustainable aquaculture feed sources, which can be met in large part by soybean-based feed. This research project will increase the amount of soybeans used in aquaculture feed by addressing deficiencies in the oil component of soybeans in current soy-based aquaculture feed rations. This is expected to translate into increased demand and expanded markets for Nebraska soybeans. The project builds on successes of the investigators in engineering improved compositional traits in soybean seeds, including enhancements in vitamin E antioxidant and omega-3 fatty acid content. The project also shows the power of synthetic biology technology to deliver large gene stacks to soybean that should provide a route for more rapid improvement of soybean germplasm for Nebraska and US farmers.

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 and a conference presentation.

Publications:

Park H, Weier S, Razvi F, Peña P, Sims N, Lowell J, Hungate C, Kissinger K, Key G, Fraser P, Napier JA, Cahoon EB, Clemente T (2016) Towards the development of a sustainable soya bean-based feedstock for aquaculture. *Plant Biotechnology Journal* In press.

Conference presentation:

Cahoon EB (presenter), Clemente TE. SOY2016 Molecular and Cellular Biology of the Soybean 16th Biennial Conference, Columbus, OH, "Development of Soybean-Based Feedstock for Aquaculture", August 9, 2016.

Tom Clemente has also provided a commentary about this work in an online forum of Forbes: http://www.forbes.com/sites/gmoanswers/2016/10/31/building-better-bean-story/#1623972f1392

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.)

Current leveraged funding from NE Soybean Checkoff:

USDA-AFRI Cahoon (PI) 02/01/2015-01/31/2018 $490,000
Overcoming Metabolic Bottlenecks for Enhanced Vitamin E Production in Crop