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 will impact soybean production.

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

Project # and Title: #1716, Improvement of Soybean Germplasm for Aquaculture Feed **Principal Investigator:** Ed Cahoon & Tom Clemente

Year of Multi Year: 2 of 3 (example: Year 1 of 3, Year 2 of 2)

1. What was the focus of the research project?

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?

Summary of the major findings of FY22 research activities are:

*Seeds from DHA/vitamin E soybeans contained up to 7.6% EPA and 3.8% DHA and displayed normal germination and normal seed shape. The seeds were sown in the greenhouse to generate homozygous lines and to bulk seeds for 2023 field plots.

*Seed oil content was restored to wild-type levels in lEPA/astaxanthin/vitamin E soybean lines by crossing with high oil soybean germplasm. F5 seeds from crosses exhibited restored seed oil content (up to 23 % DW). F5 seeds were sown in the greenhouse to generate homozygous lines and to bulk seeds for 2023 field plots.

*F4 seed from crosses between EPA/astaxanthin/vitamin E soybeans and a high C18 soybean line were successfully germinated in the 2022 field plots. Seeds (F5) from field-grown crosses exhibited increased EPA level (up to 11% of total fatty acid). F5 seeds were sown in the greenhouse to generate a homozygous line and to bulk seeds for 2023 field plots.

*T1 seeds from new EPA soybeans exhibited impressive EPA level, which accumulated 10.2 % of total fatty acid in the best line. T1 plants are now growing in the greenhouse.

*T1 seeds from new DHA/vitamin E soybeans accumulated 5.5 % EPA and 2.5% DHA per total fatty acid in the best line. T1 plants are now growing in the greenhouse.

*T1 seeds from new astaxanthin soybeans displayed red/orange color. We found that the main red pigment of carotenoids were ester forms of ketocarotenoids. T1 plants are now growing in the greenhouse.

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 increase 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.

(a) Farmers

Ed Cahoon was interviewed on the September 17, 2022 US Farm Report, aired on RFD TV. In this interview, Nebraska Soybean Board-funded research and it implications for farmers were discussed. Link: https://www.youtube.com/watch?v=cAy5NecWmLA.

Tom Clemente presented the collaborative findings of this project to farmers, researchers, and industry representatives at the 2022 Soybean Research Forum and Think Tank, August 3-9, 2022, Indianapolis, IN

(b) Public researchers

Ed Cahoon presented project findings in a virtual oral presentation at the 2nd World Congress on Oleo Science (WCOS 2022), "Development of oilseed aquaculture feedstocks: synthetic biology approaches for productions of oils with high value carotenoids", authors: Edgar Cahoon, Hyojin Kim, Truyen Quach, Kiyoul Park, Tom Elmo Clemente, September 2, 2022

Postdoctoral researcher Hyojin Kim presented project findings in an oral presentation at the 2022 Annual Meeting of the Phytochemical Society of North America, "Metabolic engineering of oilseeds for sustainable astaxanthin production", July 26, 2022, Blacksburg, VA

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 Research and Extension Experiences for Undergraduates (REEU), Cahoon (Principal Investigator), Clemente (Investigator) 12/23/2021-12/22/2026, $742,000, "Expanding Opportunities in Agricultural Sciences: Crop-to-Food Innovation". Five year, 10-week summer program to provide undergraduate students research experiences and leadership training in agricultural STEM. Cahoon summer student Gannon Cole (West Virginia State University) conducted research on astaxanthin production in soybean, and Clemente summer student Deuris Pena (Bloomfield College) conducted research on "aqua-soy".

Please submit 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 Agricultural Research Division (402) 472-7082.

Please check your information before submitting the form. Submit by Email

Updated 2021

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: #1716, Improvement of Soybean Germplasm for Aquaculture Feed

Contractor & Principal Investigator: University of Nebraska-Lincoln; PIs: Ed Cahoon & Tom Clemente

Year 2 **of 3 research project**

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:

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2. Optimizing production of astaxanthin in soybean seeds.
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2. What are the major findings of the research?

Summary of the major findings of FY22 research activities are:

• Seeds from DHA/vitamin E soybeans contained up to 7.6% EPA and 3.8% DHA and displayed normal germination and normal seed shape. The seeds were sown in the greenhouse to generate homozygous lines and to bulk seeds for 2023 field plots.

• Seed oil content was restored to wild-type levels in IEPA/astaxanthin/vitamin E soybean lines by crossing with high oil soybean germplasm. F5 seeds from crosses exhibited restored seed oil content (up to 23 % DW). F5 seeds were sown in the greenhouse to generate homozygous lines and to bulk seeds for 2023 field plots.

• F4 seed from crosses between EPA/astaxanthin/vitamin E soybeans and a high C18 soybean line were successfully germinated in the 2022 field plots. Seeds (F5) from field-grown crosses exhibited increased EPA level (up to 11% of total fatty acid). F5 seeds were sown in the greenhouse to generate a homozygous line and to bulk seeds for 2023 field plots.

• T1 seeds from new EPA soybeans exhibited impressive EPA level, which accumulated 10.2 % of total fatty acid in the best line. T1 plants are now growing in the greenhouse.

• T1 seeds from new DHA/vitamin E soybeans accumulated 5.5 % EPA and 2.5% DHA per total fatty acid in the best line. T1 plants are now growing in the greenhouse.

• T1 seeds from new astaxanthin soybeans displayed red/orange color. We found that the main red pigment of carotenoids were ester forms of ketocarotenoid. T1 plants are now growing in the greenhouse.

1. pPTN1558 soybean lines with DHA and Vitamin E in seeds

For the 2021 field growing season, Transgenic T3 seeds from two pPTN1558 events (1300-11 and 1300-38) were harvested. Transgenic T3 seeds harvested from four T2 pPTN1558 plants (T1-8 T2-4, T1-8 T2-5, T1-8 T2-6 and T1-8 T2-7) of 1300-11 event or from five T2 pPTN1558 plants (T1-7 T2-2, T1-7 T2-3, T1-7 T2-4, T1-7 T2-5 and T1-7 T2-6) of 1300-38 event were analyzed their fatty acid profile. Transgenic T3 seeds were chipped and analyzed DHA level through GC analysis. The best line produced 1% of EPA and 1% of DHA in T3 seed from 1300-11 event and 5% of EPA and 2.4% of DHA in T3 seed from 1300-38 event (Figure 1).

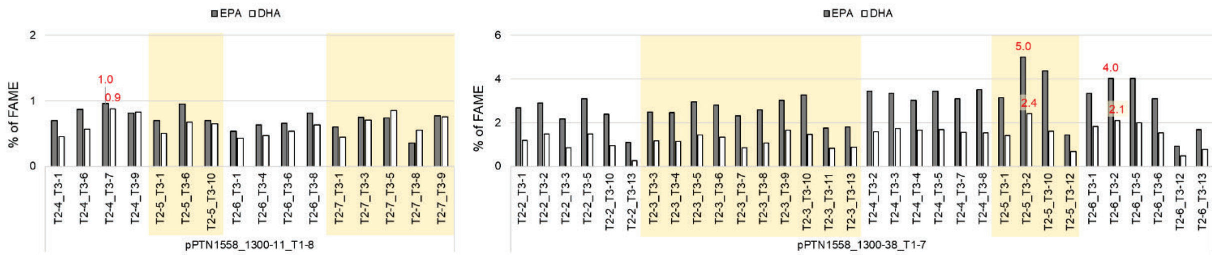

Figure 1 EPA and DHA level in T3 seeds from pPTN1558 transgenic events (1300-11 and 1300-38).

In addition, we explored additional pPTN1558 events, which are 1300-40 and 1300-41 events. Transgenic T2 seeds from three events including 1300-38 event were chipped and analyzed EPA and DHA level. The nine T2 seeds from 1300-38 T1-7 line were accumulated average 5.1 % of EPA and 3.0 % of DHA in total fatty acyl methyl esters (FAME). And total sixty-three T2 seeds from 1300-40 T1-4 line were accumulated average 5.3 % of EPA and 3.7 % of DHA in total FAME. In case of forty-eight T2 seeds from 1300-41 T1-2 line, EPA and DHA level were average 5.3 % and 3.5 % of total FAME (Figure 2). All chipped seeds were tried to germinate. Two T2 seeds of 1300-38 T1-7 line, four T2 seeds of 1300-40 T1-4 line, and four T2 seeds of 1300-41 T1-2 line were successfully germinated and T2 plants were grown in the greenhouse (red boxes in Figure 2).

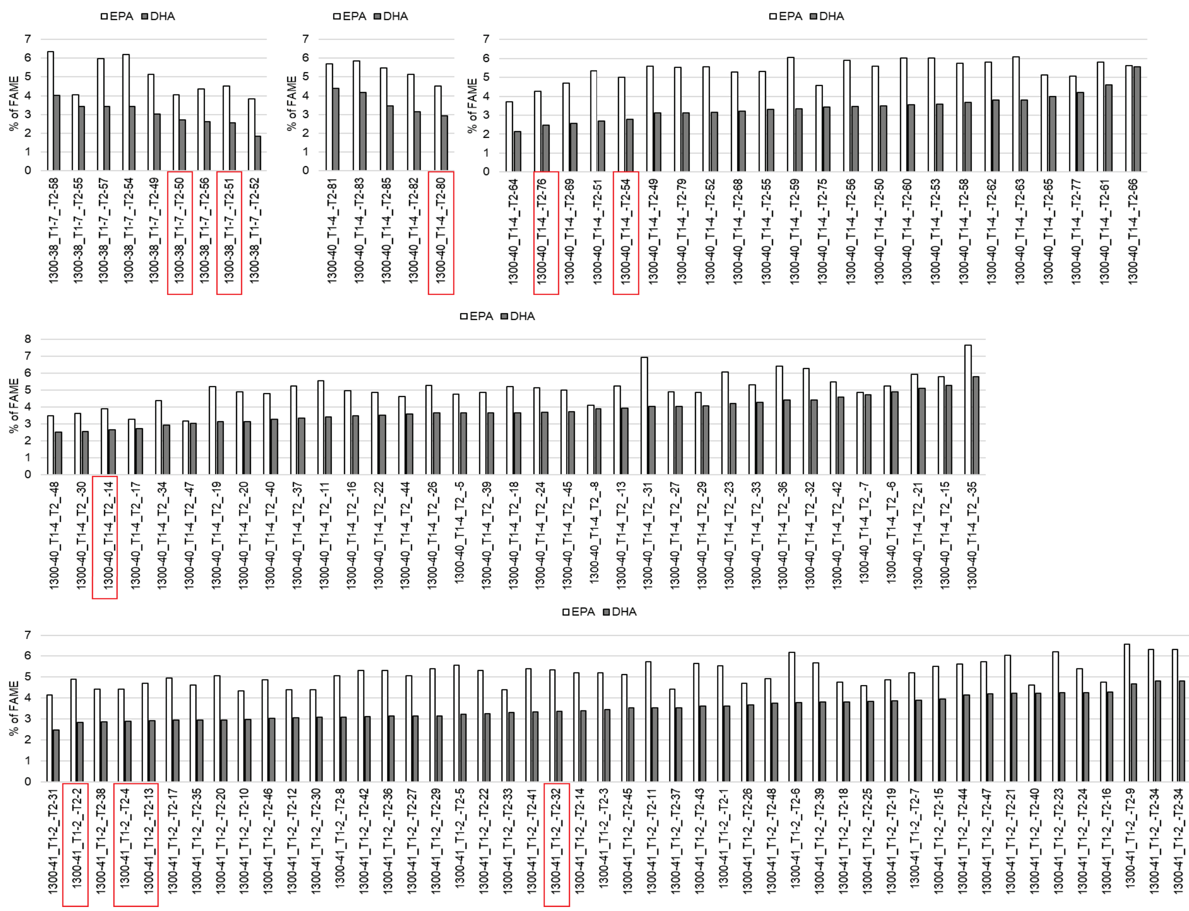

Figure 2 EPA and DHA level in T2 seeds from three pPTN1558 events (1300-38, 1300-40 and 1300-41). Red boxes mean transgenic plant which has grown in the greenhouse.

Then, we harvested T3 seeds from each plants of three pPTN1558 events. For 1300-38 events, among total forty T3 seeds, tweeny-seven T3 seeds were transgenic seeds. And EPA and DHA level in T3 seeds ranged from 3.0 % to 5.2 % of total FAME and from 1.2% to 2.5 % of total FAME, respectively. Among total forty T3 seeds in 1300-40 T1-4 progeny, twenty-nine T3 seeds were transgenic seeds. The EPA and DHA maximum level in T3 seeds were 7.6 % and 3.8 % of total FAME, respectively. Total fifty T3 seeds from 1300-41 T1-2 progeny were analyzed and thirty-nine T3 seeds produced EPA and DHA. And maximum level of EPA and DHA was 7.1 % and 3.2 % of total FAME (Figure 3). Finally, we selected 1300-38 T1-7 T2-50, 1300-40 T1-4 T2-54, and 1300-41 T1-2 T2-13 as lead lines.

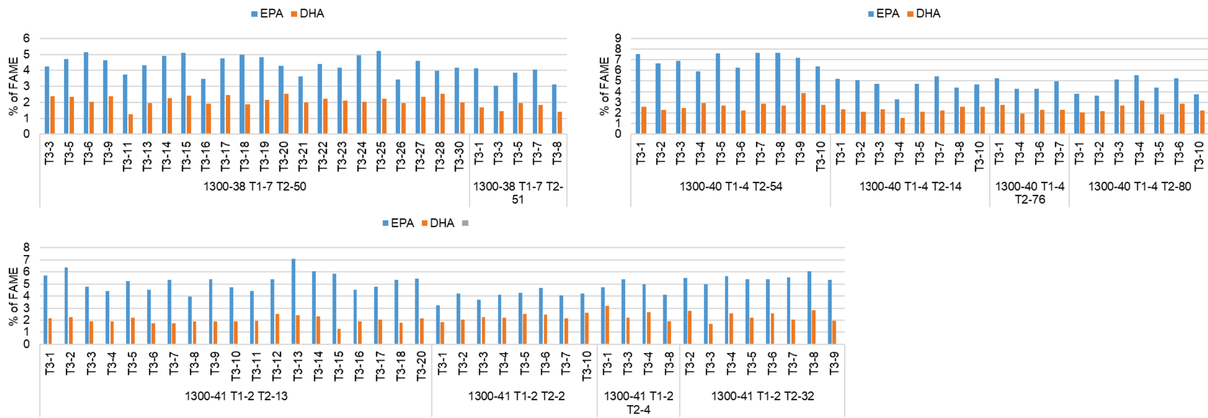

Figure 3 EPA and DHA level in T3 seeds from three pPTN1558 events (1300-38, 1300-40 and 1300-41).

Next, T3 seeds from three lead lines will be planted and T3 plants will be growing to generate a homozygous line. T-DNA copy number will be investigated through southern blot analysis.

2. Generation of high EPA content soybean line by crossing events.

1) Crossing events between pPTN1331 (EPA+Astaxanthin+Vitamin E) and pPTN1314 (KASII+AtWRI1)

The crosses between transgenic soybean with high oil trait (pPTN1314) and pPTN1331 were grown in the greenhouse. F4 seeds from crosses (F3 plants) were harvested. The EPA level in red seeds from two individual crosses showed maximum 2.3% and 2.1% per total fatty acid, respectively, which were similar level with parent line (pPTN1331) (Figure 4A). Moreover, the oil content was increased compared to pPTN1331 parent (parent: 14 % per dry weight vs. crosses: average 18.4 % per dry weight) (Figure 4B).

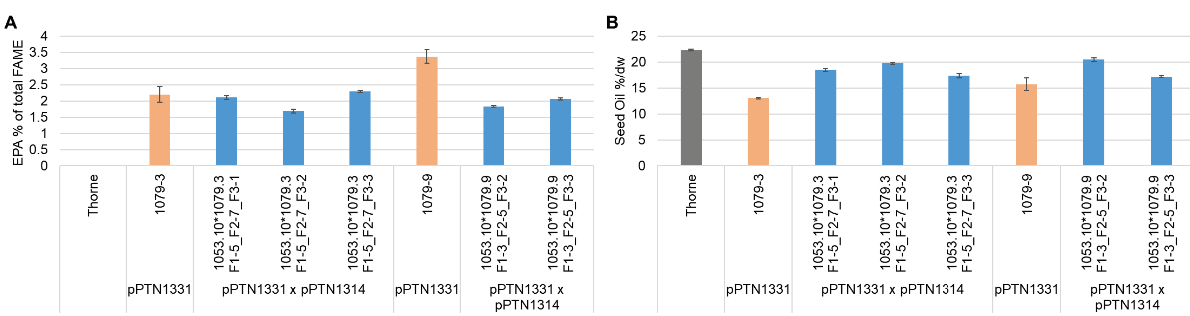

Figure 4 EPA level and total seed oil content in F4 seeds of wild type (Thorne), pPTN1331 line, and crosses between pPTN1331 and pPTN1314.

The ketocarotenoids including astaxanthin produced in red seeds from two individual F3 crosses plants showed 11.0 μg/g and 2.1 μg/g, respectively, which is 1.2-fold change and 10-fold change decrease compared to pPTN1331 parent (Figure 5A). On the other hand, pPTN1331 parent line, 1079.3, defected tocotrienol biosynthesis but pPTN1331 parent line, 1079.9, produced tocotrienol. Then, the red seeds from the crosses with pPTN1331_1079.9 analyzed vitamin E tocotrienol content. The content in red seeds from two plants of crosses showed 1,732 µg/g and 1,432 µg/g (Figure 5B).

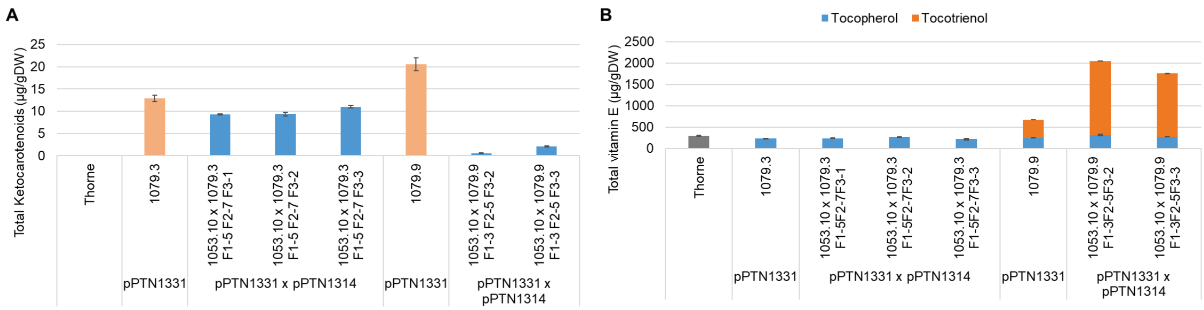

Figure 5 The contents of ketocarotenoids and vitamin E in F4 seeds of wild type (Thorne), pPTN1331 line, and crosses between pPTN1331 and pPTN1314.

For the bulk harvesting of seeds, two lines, 1053-10 x 1079-3 F1-5 F2-7 F3-2 and 1053-10 x 1079-9 F1-3 F2-5 F3-1, were selected and their progenies were grown in the greenhouse. F5 seeds from crosses were harvested. EPA level in seeds of two lead events of pPTN1314 (1053-10) x pPTN1331 (1079-3) crosses were 1.5 % and 2.5 % of total fatty acid (Figure 6A). Total seed oil content in two lead events of pPTN1314 (1053-10) x pPTN1331 (1079-3) crosses were 23.3 % DW and 23.2 % DW, which are not significantly different from wild type value (Figure 6B).

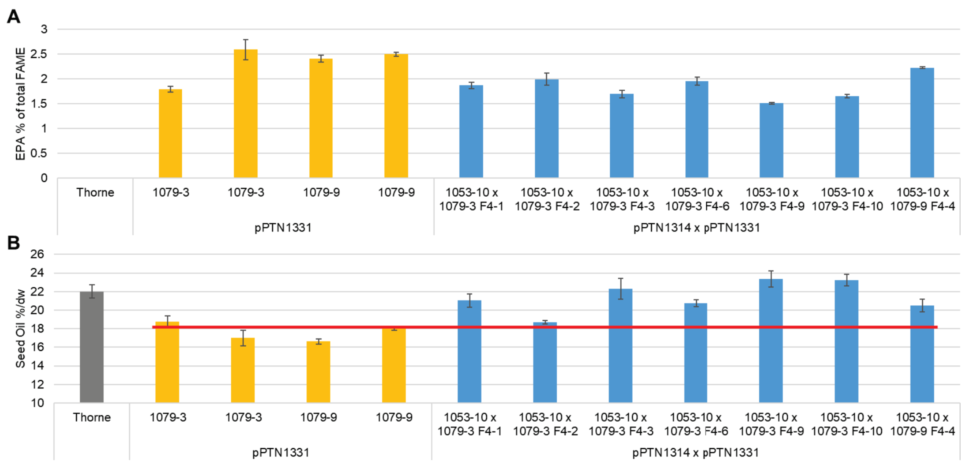

Figure 6 EPA level (A) and seed oil content (B) in F5 seeds of crosses between pPTN1314 and pPTN1331. Red line means average value of pPTN1331 line.

Next, the astaxanthin and vitamin E value will be analyzed in the F5 seeds of crosses. And homozygous lines of two lead events will be generated.

2) Crossing events between pPTN1331 (EPA + Astaxanthin + Vitamin E) and pPTN1248 (AtWRI1 + AtDGAT1)

The crosses between high oil trait soybean line [pPTN1248 (AtDGAT1 and AtWRI1)] and pPTN1331 were grown in greenhouse. F4 seeds from six F3 plants of crosses were harvested. The red seeds from crosses produced EPA ranged from 2.1% to 3.6% per total fatty acid (Figure 7A). And total oil content and wrinkled seed shape were partially rescued in red seeds from crosses compared to pPTN1331 parents (oil content; parent: 14% per dry weight vs. crosses: average 18% per dry weight) (Figure 7B).

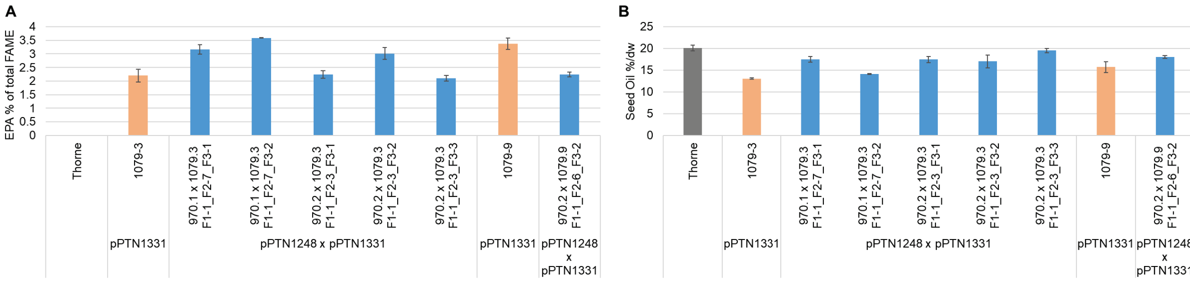

Figure 7 EPA level (A) and seed oil content (B) in F4 seeds of crosses between pPTN1248 and pPTN1331.

The ketocarotenoids including astaxanthin were accumulated in red seeds from crosses. The best line contained 30 µg/g ketocarotenoids (including astaxanthin) (Figure 8A). The red seeds from only one F3 plants of crosses between pPTN1331 (1079.9) and pPTN1248 produced 1429 µg/g tocotrienol (parent: 429 µg/g) (Figure 8B).

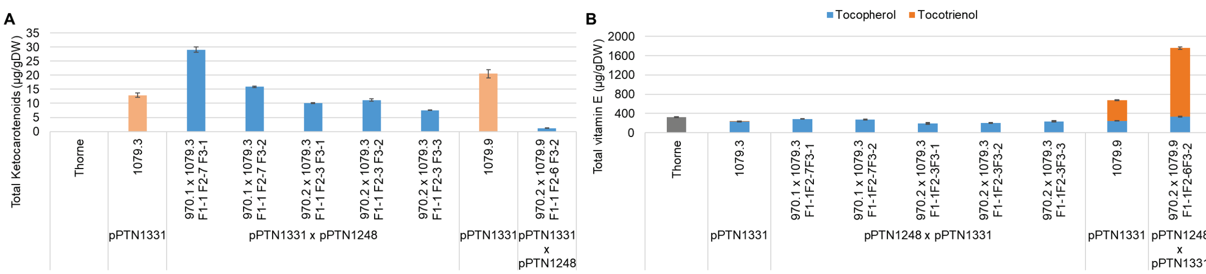

Figure 8 Ketocarotenoid (A) and vitamin E (B) level in F4 seeds of crosses between pPTN1248 and pPTN1331.

For the bulk harvesting seed of best lines, we selected two lines, 970-1 x 1079-3 F1-1 F2-7 F3-4 and 970-2 x 1079-3 F1-1 F2-3 F3-1. Their progenies were grown in the greenhouse and F5 seeds were harvested. EPA level in seeds of two lead plants of pPTN1248 (970-2) x pPTN1331 (1079-3) crosses was 2.0 % and 1.9 % of total fatty acid, which is similar value to pPTN1331 parent line (Figure 9A). And total seed oil content in two lead plants of pPTN1248 (970-2) x pPTN1331 (1079-3) crosses was 21.5 % and 21.2 %, which are similar level to wild type (Figure 9B).

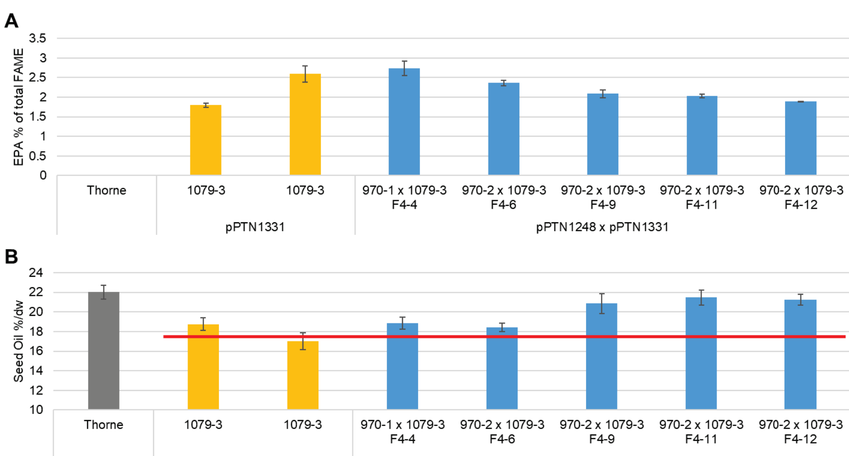

Figure 9 EPA level and seed oil content in F5 seeds of crosses between pPTN1248 and pPTN1331. Red line means average value of pPTN1331 line.

Next, the astaxanthin and vitamin E value will be analyzed in the F5 seeds of crosses. Homozygous lines of two lead events will be generated.

3) Crossing events between pPTN1331 and a high linolenic acid (ALA) line

F3 plants (tweeny-five plants from two crossing events between ALA and pPTN1331) were grown in the greenhouse and F4 seeds were harvested. EPA levels ranged from 4.4% to 17.3% of total FAME (Figure 10A). And total seed oil ranged 15.2% to 23% per seed weight (Figure 10B). The EPA and seed oil level in F4 seeds of crosses were impressive compared to the parent pPTN1331.

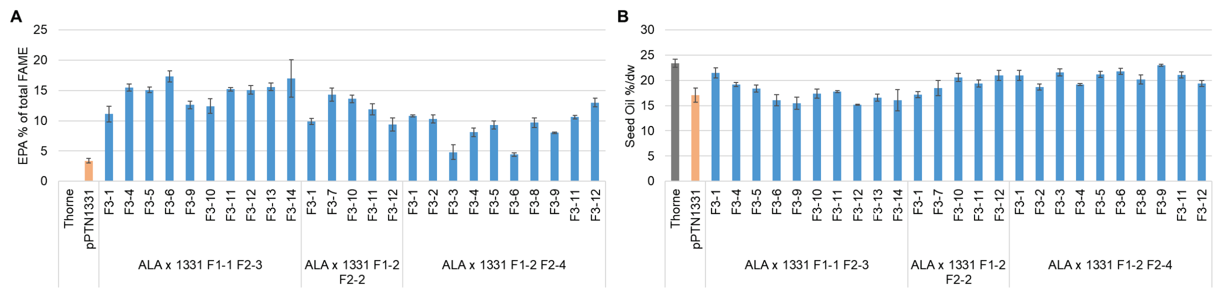

Figure 10 EPA level and total seed oil in wild type seed (Thorne), pPTN1331 seed, and F4 seeds of crosses between ALA and pPTN1331.

Then, we conducted HPLC analysis to measure astaxanthin and vitamin E content. Ketocarotenoid (including astaxanthin) and tocotrienol content ranged from 24.4 µg/g DW to 40.9 µg/g dry weight and 338 µg/g dry weight to 420 µg/g dry weight, respectively, in F4 seeds (Figure 11).

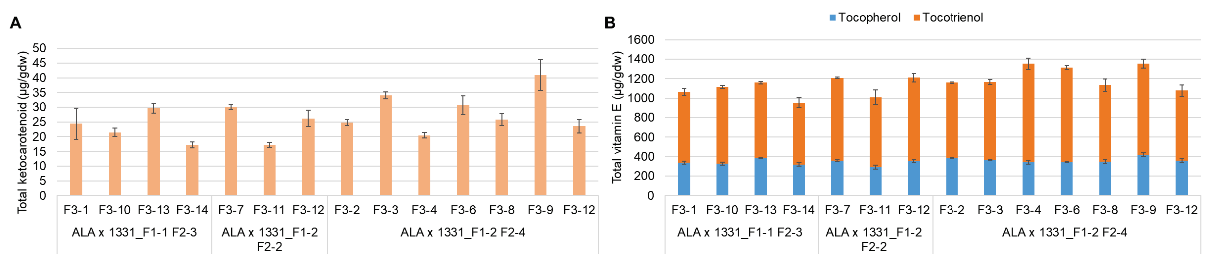

Figure 11 Ketocarotenoid and vitamin E level in F4 seeds of crosses between ALA and pPTN1331

We advanced F4 seeds showing high oil content (>18% of seed dry weight) along with EPA production (more than 8 % of total fatty acid) in the field at ENREC near Mead, NE (Figure 12).

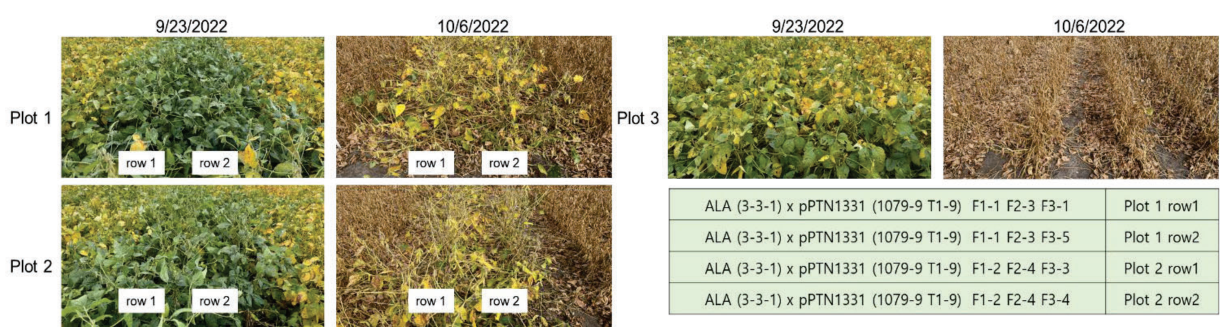

Figure 12 F4 plants of crosses between ALA and pPTN1331 were grown in the field at ENREC near Mead, NE. Plot 3, wild type, Thorne.

To isolate transgenic plant containing ALA trait and pPTN1331 trait, we extracted genomic DNA from the leaf disk of F4 plants from crosses between ALA (high ALA line) and pPTN1331. Then, we found that *delta-15* *desaturase* gene for ALA cassette and *delta-6 elongase* gene for pPTN1331 cassette were successfully amplified using DNA based PCR except one plant, ALA x pPTN131 F1-2 F2-4 F3-4 F4-3 (Figure 13).

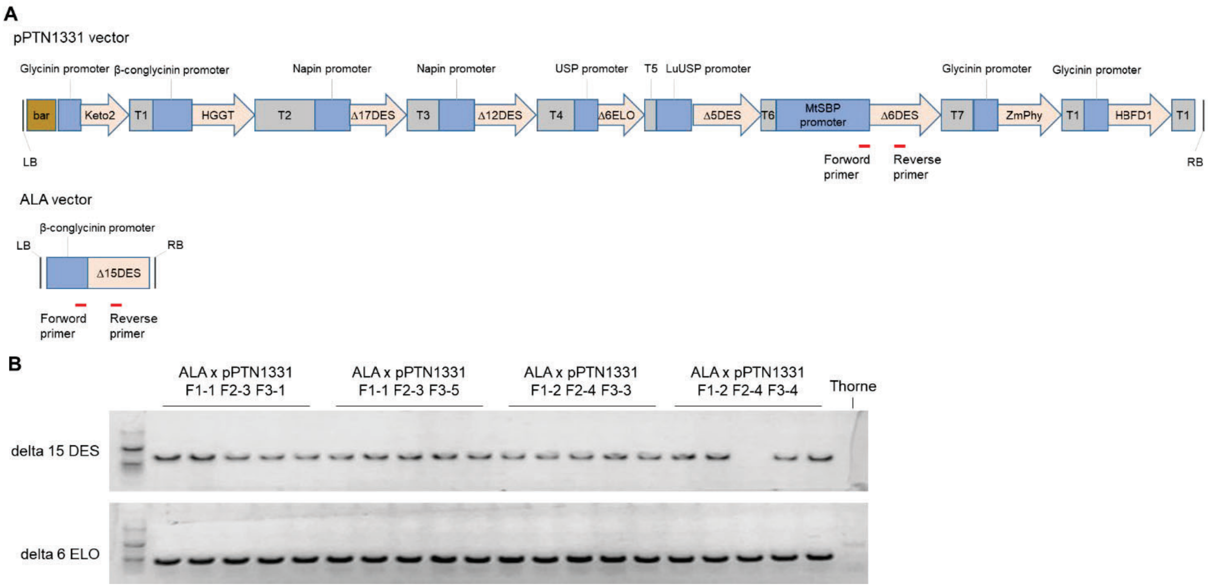

Figure 13 Schematic diagram of pPTN1331 and ALA vector map (A), and DNA-based PCR result (B). Red lines indicate primer position on the vector map.

Even the F5 seeds of crosses were harvested in not quite fully hydrated because of early frost this year (Oct 9, 2022), we found the seed quality of F5 seeds looked good, round and not wrinkled (Figure 14).

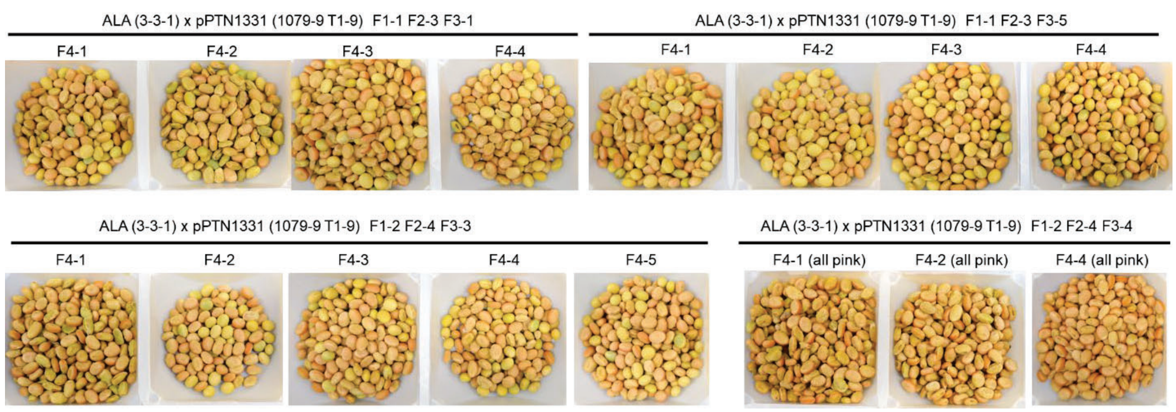

Figure 14 F5 seeds of crosses between ALA and pPTN1331 grown in the field at 2022.

Then, we analyzed EPA level and total oil content through GC analysis. EPA level of F5 seeds shown round pink ranged from 9.1 to 11.3 % of total fatty acid (Figure 15A). And total oil contents were not fully restored from the F5 seeds of crosses to wild type level (17 %/dw in crosses vs. 23 %/dw in Thorne) (Figure 15B).

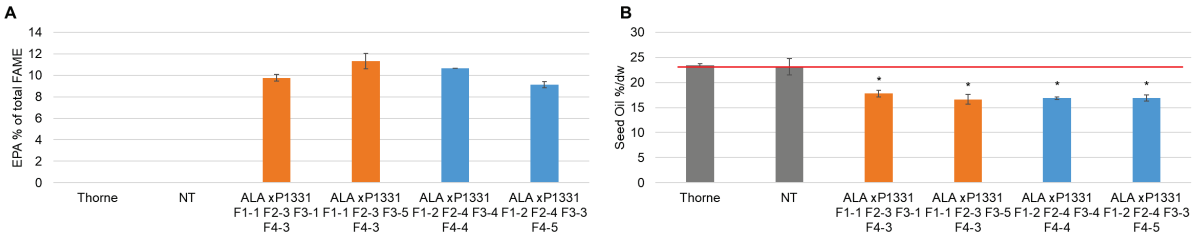

Figure 15 EPA level (A) and seed oil content (B) in F5 seeds of crosses between ALA and pPTN1331. Red line means average of wild type value. Student t-test; *, p<0.01

Next, we will generate a homozygous line for crosses and investigate the transcript level of genes on the ALA cassette and pPTN1331 cassette. Then, the seeds of lead plants will be bulk harvested for large-scale field testing in 2023.

3. pPTN1615 and pPTN1642 for improvement of EPA/DHA production

1) pPTN1615 with enhanced EPA trait

Soybean transformations with pPTN1615 (EPA production) were initiated. The transgenic plants were tested the expression of herbicide resistance gene (bar) by applying the leaf paint assay in T0 plant. To date, we have obtained four transformation events, 1357-1, 1358-2, 1366-1, and 1366-2. T1 seeds from four pPTN1615 events were harvested and chipped for FAME analysis. Only 1357-1 event showed EPA production in seeds to amounts ranging from 6.3 % to 10.2% of total FAME (Figure 16). Twelve T1 seeds were tried to germinate and nine T1 seedlings are growing in the greenhouse.

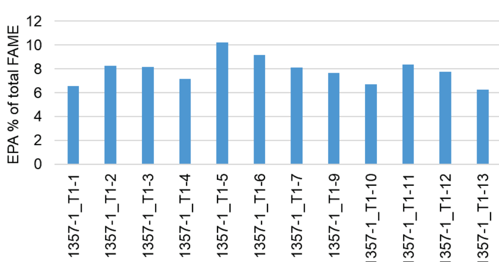

Figure 16 EPA level in T1 seeds of pPTN1615.

In addition, nine T1 seeds from 1357-1 and 1358-2 events were planted without GC analysis and T1 plants are growing in the greenhouse. When we conducted DNA-based PCR analysis using specific primers for pPTN1615 trait, we found four plants from only 1357-1 event contain foreign genes.

Next, we will harvest T2 seeds from thirteen T1 plants and analyze EPA level.

2) pPTN1642 with enhanced EPA/DHA and vitamin E traits

Soybean transformations with pPTN1642 (vitamin E and EPA/DHA production) were initiated. The transgenic plants were tested the expression of bar gene by applying the leaf paint assay. To date, we have obtained ten transformation events, 1356-1a, 13561b, 1356-1c, 1356-1d, 1356-1e, 1356-1f, 1356-1g, 1367-1, 1367-2, and 1379-1. Seven to ten T1 seed from ten events were chipped and analyzed EPA and DHA level. From GC analysis, the seed from six events contains EPA and DHA level, which are 1356-1b, 1356-1c, 1356-1f, 1356-1g, 1367-1 and 1367-2 events. EPA level in T1 seeds from ten pPTN1642 events ranged from 1.9 % to 5.5 % of total FAME. DHA level of those ranged from 1.6% to 2.7% (Figure 17). The chipped seeds were planted and the T1 plants are growing in the greenhouse.

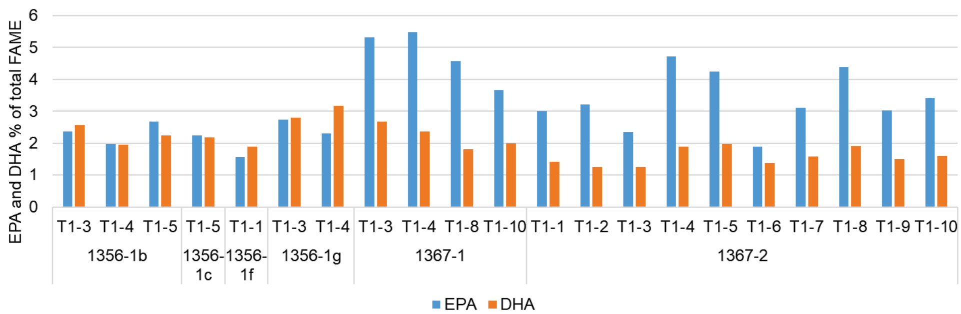

Figure 17 EPA and DHA level in T1 seeds of pPTN1642 events.

Next, we will harvest T2 seeds and analyzed the EPA and DHA level. Then, we will generate homozygous lines.

4. pPTN1660 soybean lines with astaxanthin in seeds

Soybean transformations with pPTN1660 (astaxanthin production) were initiated. To date, among 13 pPTN1660 events, we generated five positive events showing red/orange seed, which are 1372-5, 1372-7, 1372-8, 1374-3 and 1374-6 events. Red/orange T1 seeds were sown and T1 plants are growing in the greenhouse.

To investigate the astaxanthin quality in the pPTN1660 soybean, one T1 seed from 1372-5 event were chopped and extracted carotenoids for TLC analysis. From TLC result, we found that the most pigment showing red/orange were accumulated as ester forms in the seeds (Figure 18).

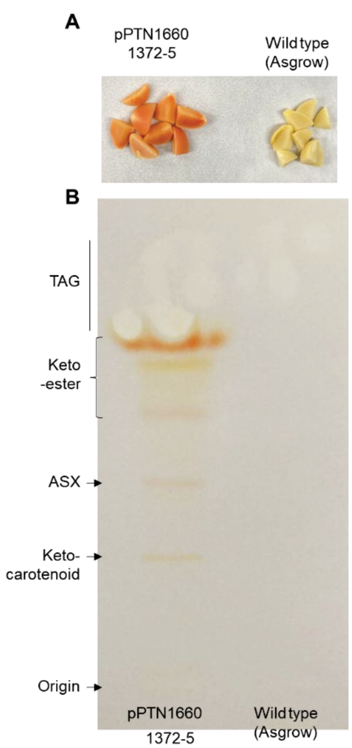

Figure 18 The chopped T1 seed (A) and TLC analysis (B) of pPTN1660 1372-5 event and wild type

Next, we will harvest T2 seed and analyze the ketocarotenoid level through TLC analysis and HPLC analysis.

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. To date, these techniques have delivered soybean lines expressing eight transgenes that produce three high value aquaculture oil traits: fish oil EPA, astaxanthin, and high vitamin E antioxidants. These traits cannot be produced from conventional breeding. Also we have applied this technology to produce seeds with increased methionine content for improved meal quality. 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 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 increase 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.

(a) Farmers

Ed Cahoon was interviewed on the September 17, 2022 US Farm Report, aired on RFD TV. In this interview, Nebraska Soybean Board-funded research and it implications for farmers were discussed. Link: https://www.youtube.com/watch?v=cAy5NecWmLA.

Tom Clemente presented the collaborative findings of this project to farmers, researchers, and industry representatives at the 2022 Soybean Research Forum and Think Tank, August 3-9, 2022, Indianapolis, IN

(b) Public researchers

Ed Cahoon presented project findings in a virtual oral presentation at the 2nd World Congress on Oleo Science (WCOS 2022), "Development of oilseed aquaculture feedstocks: synthetic biology approaches for productions of oils with high value carotenoids", authors: Edgar Cahoon, Hyojin Kim, Truyen Quach, Kiyoul Park, Tom Elmo Clemente, September 2, 2022

Postdoctoral researcher Hyojin Kim presented project findings in an oral presentation at the 2022 Annual Meeting of the Phytochemical Society of North America, "Metabolic engineering of oilseeds for sustainable astaxanthin production", July 26, 2022, Blacksburg, VA

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.) Leveraged funding from NE Soybean Checkoff:

USDA-NIFA Research and Extension Experiences for Undergraduates (REEU), Cahoon (Principal Investigator), Clemente (Investigator) 12/23/2021-12/22/2026, $742,000, "Expanding Opportunities in Agricultural Sciences: Crop-to-Food Innovation". Five year, 10-week summer program to provide undergraduate students research experiences and leadership training in agricultural STEM. Cahoon summer student Gannon Cole (West Virginia

State University) conducted research on astaxanthin production in soybean, and Clemente summer student Deuris Pena (Bloomfield College) conducted research on "aqua-soy".