Nebraska Soybean Board
FINAL Research Report Form

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1/3/2019

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 curator of a national database of State, Region, and USA Soy checkoff funded projects.

Project # and Title: #1716, Improvement of Soybean Germplasm for Aquaculture Feed

Principal Investigator: Edgar Cahoon

Co-PI's & Institutions: Co-PI : Tom Clemente
Institution: University of Nebraska-Lincoln

Project Date (Including Extension): 10/01/2015 ***to*** 09/30/2018 ***(example: mm/dd/yyyy to mm/dd/yyyy)***

Total Budget for Project: $ 205,824.00

1. Briefly State the Rational for the Research:

The research addressed the Nebraska Soybean Board FY16-18 focus area of germplasm improvement for oil composition and yield. The research was 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. However, the current soybean-based feed lacks sufficient levels of the fish oil EPA omega-3 fatty acid 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 for fish flesh pigmentation, particularly for farm-raised salmon. In addition, oils with enhanced omega-3 very-long-polyunsaturated fatty acid content are prone to oxidation, which limits the shelf life of fish due to the creation of off-flavors and odors. The goal of this research was to develop soybean germplasm with oil quality traits optimized for high value soy-based aquaculture feeds, one of the most significant new markets for Nebraska soybean producers.

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

The research addressed 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. 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 research addressed 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.

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

The project involved the assembly of large gene constructs using emerging techniques of synthetic biology to generate soybean germplasm with fish oil functionality (EPA/DHA), carotenoid pigments (astaxanthin), and enhanced vitamin E antioxidants (tocotrienols) for a complete aquaculture oil feed ration. These constructs included five transgenes for EPA, seven transgenes for DHA, two transgenes for astaxanthin, and one transgene for vitamin E antioxidants. Constructs were introduced into soybean variety Thorne using Agrobacterium-based transformation at the Plant Transformation Core Research Facility (PTCRF) at UNL. Resulting soybean lines were tested by gas chromatography and high performance liquid chromatography for the desired oil traits and by molecular genetic techniques to confirm that transgenes were introduced and expressed in seeds. Trait-positive lines were evaluated in the greenhouse facility at the Beadle Center at UNL and taken to homozygosity. Seeds from homozygous lines were tested in the biotech field facility at the Eastern Nebraska Research and Extension Center (NREC) in Mead, NE in 2017 and 2018.

More specifically, to produce omega-3 EPA, five transgenes (Ostreococcus tauri Δ6-desaturase, Physcomitrella patens Δ6-elongase, Thraustochytrium sp. Δ5-desaturase, Phytophthora sojae Δ12-desaturase, Phytophthora infestans ω3-desaturase) were synthesized with barley HGGT, which is involved in the production of vitamin E. Adonis aestivalis β-ring oxygenase (keto2) and astaxanthin synthase (HBFD1) were used for astaxanthin production. Keto2 and HBFD1 were cloned into pBinGlyBar1 as named pKeto plasmid and then the pKeto plasmid was combined with the synthesized EPA and bHGGT genes and an 8-transgene expression plasmid was constructed under seed-specific promoters as named pPTN1331. The pPTN1331 plasmid was transformed into soybean. Five independent lines 1079-3, 1079-5, 1079-6, 1079-8, and 1079-9 that produce EPA, high vitamin E, and astaxanthin were generated, but 1079-3 and 1079-5 showed the segregated the traits in their progenies. T1 and T2 plants of 1079-3, 1079-6, 1079-8, and 1079-9 were grown in green house and in field at ENREC in Mead, NE in 2017 and 2018. The presence and copy number of all transgenes in these lines were confirmed by Southern blot analysis and the expression of transgenes were confirmed by RT-PCR, northern blot, and droplet digital PCR. T1 seeds in green house and T2 seeds in the field were analyzed to confirm three traits, omega-3 EPA, vitamin E (tocotrienols) antioxidant, and astaxanthin by gas-chromatograph (GC) and high performance liquid chromatography (HPLC) analyses. To produce a second fish oil component DHA (docosahexaenoic acid) in soybean, Ostreococcus tauri elongase 5 (OtElo5) and desaturase 4 (OtD4) genes were assembled under control of promoters that confer seed expression. These were combined with EPA and vitamin E biosynthetic genes. The new plasmid for a second fish oil component was tested in a model plant to more quickly confirm that the genes function as expected. Additional putative promoters and terminators for soybean Late Embryogenesis Abundant (LEA) genes were isolated for use in seed quality improvement research. These elements were linked to a reporter gene (GUS) that allows visual assessment of gene expression to determine which promoters are best-suited for late stage-seed-specific expression of our transgenes. The expression of the GUS gene was examined in T2 developing seeds of model plants transformed with LEA promoter:GUS gene. The results showed that the visual signal from expression of the GUS gene became progressively stronger as seeds matured and should be a viable new promoter for soybean. To generate new soybean lines that minimize negative effects of astaxanthin production on seed performance, a new expression vector was assembled with the Adonis astaxanthin transgenes (keto2 and HBFD1) and maize phytoene synthase gene (ZmPhy) under control of promoters for LEA genes. Soybean transformation experiments were initiated with this expression vector to produce astaxanthin at later stages of seed development in order to reduce negative effects on seed embryos. The expression vector was tested in a model plant to more quickly confirm that the genes function as expected and found to produce astaxanthin. This indicated that the putative promoters in the new expression vector are functional in the seeds. A second vector without the ZmPhy gene was constructed for astaxanthin production in soybean seeds.

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

Objective 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.
-Deliverables : DNA plasmids, T3 seeds of 3 independent lines which produce omega-3 fatty acid (EPA), astaxanthin, and vitamin E.
We successfully constructed a single plasmid which included the 8 genes for omega-3 fatty acid, astaxanthin, and high vitamin E antioxidant traits. And then we generated Nebraska soybean germplasm producing omega-3 fatty acid (EPA), astaxanthin, and vitamin E. And we generated 3 new vectors to improve seed quality traits.
- Significance: 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 generated soybean germplasm with three aquaculture oil traits that could not be achieved by conventional breeding. In three year, we generated T3 soybean in field which stably produced omega-3 EPA, astaxanthin, and enhanced vitamin E antioxidants. To our knowledge, this is an unprecedented achievement for soybean biotechnology, i.e. to stack eight transgenes in one line and obtain three commercially relevant traits in <3 years.

Objective 2. Improvement of omega-3 EPA content in Nebraska soybean germplasm.
-Deliverables : T3 seeds of independent lines (1079-3, 1079-6, 1079-8, 1079-9) which produced EPA and EPA precursors. New plasmid to produce EPA/ DHA and vitamin E.
- Significance: Our new aquaculture feed soybean germplasm produced EPA (2.5-5.8 mol%) and arachidonic acid (14-17 mol%). Total non-traditional soy fatty acids which were only found in our new aquaculture feed soybean germplasm was up to 38 mol%. These results indicated that our approach is effective for producing EPA. The levels of EPA are close to commercial viability but require optimization of this strategy to consistently achieve our target of 8% to 10% EPA in soybean oil.

Objective 3. Improvement of astaxanthin pigment levels in Nebraska soybean germplasm.
-Deliverables : T3 seeds of independent lines (1079-3, 1079-6, 1079-8, 1079-9) which produce astaxanthin. Two new gene expression constructs were generated to minimize agronomic drag on seeds.
-Significance: Red seeds of 1079-3, 1079-5, 1079-6, 1079-8, 1079-9 lines produced two astaxanthin forms astaxanthin 1 (52-154 μg/g) and astaxanthin 2 (29-75 μg/g). Total astaxanthin pigment concentrations were detected as high as 210 μg/g. The research demonstrated the ability to produce astaxanthin at commercially relevant levels in soybeans.

Objective 4: Evaluation of new oil traits in aquaculture feeding studies.
-Deliverables: Aquaculture feeding studies from prior engineered soybean lines have been coordinated and conducted by Co-PI Tom Clemente and results are provided in his reports. Our latest EPA/astaxanthin/enhanced vitamin E antioxidant lines are also too early in the development queue for sufficient amounts of soybeans for use in feeding studies. We have undertaken additional studies to address this Objective. These have included expanding our fatty acid target from EPA only to EPA and DHA production to generate lines with oils that more closely match fish oil. This has been accomplished by including two additional transgenes into our gene stacks: Ostreococcus tauri elongase 5 (OtElo5) and desaturase 4 (OtD4) genes. The construct has been completed and is in the queue for soybean transformation. This component of the project and feeding studies will extend into our new funding cycle.
-Significance: We are on target for generating sufficient amounts of seeds for feeding trials and research is underway to expand the oil functionality to more closely match fish oil.

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

Impacts

-The research showed 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. Conventional plant breeding and biotechnology approaches typically target one or only a small number of traits at a single time for crop improvement. In contrast, this research was pursued using emerging synthetic biology methodologies for rapidly constructing large (>25 kb) gene expression cassettes for diverse trait genes into soybean genome in a single genetic transformation experiment.

-The research resulted in the production of EPA, astaxanthin, and enhanced vitamin E antioxidants in a single seed by introduction of eight transgenes for complex metabolic pathways. Astaxanthin and vitamin E levels are likely at commercial viability, and EPA levels are approaching commercial viability. To our knowledge, this is an unprecedented achievement for soybean biotechnology, i.e. to stack eight transgenes in one line and obtain three commercially relevant, complex traits in <3 years.

-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 compared to conventional breeding.

-We anticipate that the research conducted in this project will pave the way for increased use of soybean 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.

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

Journal article: 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 (2017) Towards the development of a sustainable soya bean-based feedstock for aquaculture. Plant Biotechnology Journal 15:227-236.

Conference oral presentation: “Development of soybean-based feedstock for aquaculture”, SOY2016 Molecular and Cellular Biology of the Soybean, 16th Biennial Conference, August 9, 2016, Columbus, OH.

Conference oral presentation: “Application of synthetic biology to enhance genetic variation for improved plant quality and performance”, Phytochemical Society of North America Annual Meeting, August 8, 2017, Columbia, MO.

Conference oral presentation: “New oil traits for soybean as a sustainable and high quality feedstock for aquaculture”, 17th Biennial Conference on the Molecular and Cellular Biology of the Soybean, Soy2018, August 26-29, 2018, University of Georgia, Athens, GA.

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 (jmonaghan2@unl.edu) based on the reporting schedule given to you. If you have any questions, please call the ARD at 2-2045 or Victor Bohuslavsky at the Nebraska Soybean Board Office at (402) 432-5720.

Nebraska Soybean Board

FINAL Research Report (Technical Report)

Project # and Title: #1716, Improvement of Soybean Germplasm for Aquaculture Feed

Principal Investigator: Edgar Cahoon

Co-PI & Institution: Tom Clemente, University of Nebraska-Lincoln

Project Date (Including Extension): 10/01/2015-09/30/2018

1. Briefly State the Rational for the Research

The research addressed the Nebraska Soybean Board FY16-18 focus area of germplasm improvement for oil composition and yield. The research was 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. However, the current soybean-based feed lacks sufficient levels of the fish oil EPA omega-3 fatty acid 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 for fish flesh pigmentation, particularly for farm-raised salmon. In addition, oils with enhanced omega-3 very-long-polyunsaturated fatty acid content are prone to oxidation, which limits the shelf life of fish due to the creation of off-flavors and odors. The goal of this research was to develop soybean germplasm with oil quality traits optimized for high value soy-based aquaculture feeds, one of the most significant new markets for Nebraska soybean producers.

2. Research Objectives

The research addressed 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.

3. The major findings of the research or impacts of the educational activity.

1. T_3 generation of soybean seeds that contain omega-3 EPA, astaxanthin, and enhanced vitamin E antioxidants were harvested in field experiments at ENREC in Mead, NE in 2018.

2. Assessment of transgene expression revealed that all introduced genes were expressed but also indicated low expression from one of the promoters that likely limits total EPA production. This finding provides a major clue or optimization of the EPA trait.
3. T_0 plants containing MetA, MetB, MetC, and MetE transgenes to increase methionine content of soybean seed were generated and are growing in greenhouse.
4. Putative promoters and terminators of late embryogenesis abundant (LEA) genes fused with GUS reporter gene in model plants showed that the visual signal from expression of the GUS gene became progressively stronger as seeds matured, suggesting that these promoters are useful for future efforts to engineer aquaculture and other seed quality traits in soybean.
5. Insertion of 8 genes to produce EPA, DHA, and tocotrienols in plant genome was confirmed by gDNA PCR in the model. New vector for a second fish oil component works in plant.
6. The production of astaxanthin using a new expression vector with the adonis astaxanthin transgenes (keto2 and HBFD1) and maize phytoene synthase gene (ZmPhy) under LEA promoters was confirmed in T_2 developing seeds in model plant. This indicates that the putative LEA promoters are functional in the seeds.

Seeds from the T3 generation of lines that produce EPA, high vitamin E, and astaxanthin (1079-3, 1079-6, 1079-8, and 1079-9) were planted and harvested in the biotech field at Eastern Nebraska Research and Extension Center (ENREC) in Mead, NE (Figure 1A). Developing seeds of the T3 generation in the field were collected to confirm the expressions of transgenes and to measure concentrations of the hormone abscisic acid (ABA) that is important for seed germination. (Figure 1B).

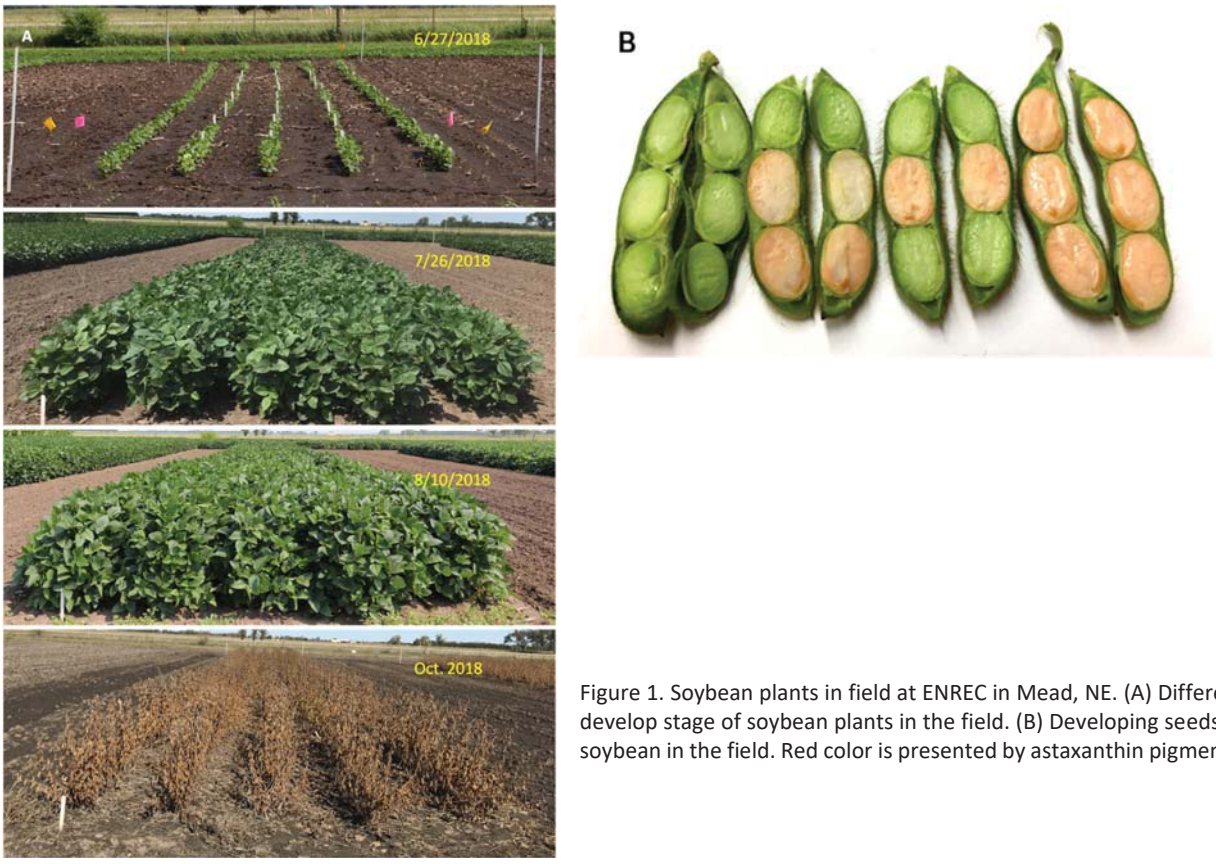

Figure 1. Soybean plants in field at ENREC in Mead, NE. (A) Different develop stage of soybean plants in the field. (B) Developing seeds of soybean in the field. Red color is presented by astaxanthin pigments.

The presence and copy number of transgenes in these lines were confirmed by Southern blot analysis in T1 generation (Fig. 2).

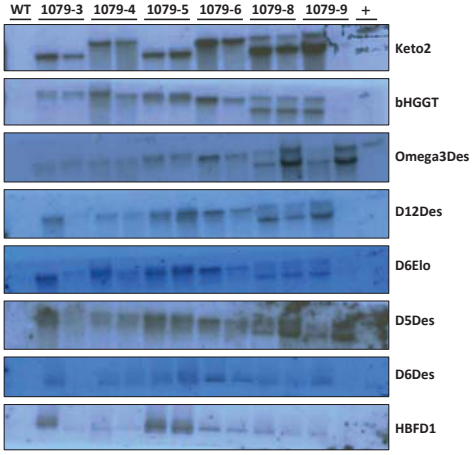

Figure 2. Transgenic allele integration analysis via southern blot of soybean plants carrying pPTN1331. Left to right: WT: Thorne, six soybean events (two plants each), and positive control. Each southern blot was hybridized with a fragment amplified via PCR from Keto2 (524 bp), bHGGT (538 bp), Omega3Des (548 bp), D12Des (553 bp), D6Elo (526 bp), D5Des (518 bp), D6Des (515 bp), or HBFD1 (593 bp). All plants were genotyped via PCR prior to southern blots.

The expression of each transgene integrated into the soybean genome was analyzed by RT-PCR, and all genes used for multi-gene stacking were expressed in soybean seeds from different lines (Fig. 3).

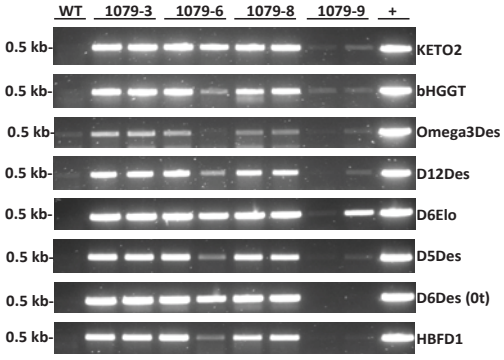

Figure 3. Gene expression analysis via RT-PCR of soybean seeds carrying pPTN1331. Left to right: WT: Thorne, and four independent events (two plants each), and positive control (pPTN1331). Keto2 (524 bp), bHGGT (538 bp), Omega3Des (548 bp), D12Des (553 bp), D6Elo (526 bp), D5Des (518 bp), D6Des (515 bp), and HBFD1 (593 bp).

Droplet digital PCR was performed to confirm the expression of transgenes for omega-3 EPA, astaxanthin, and enhanced vitamin E antioxidants in developing seeds in the field (Figure 4). The expression levels of omega-3 desaturase and Δ12-desaturase were relatively low compared to the expression of other transgenes. This explains that high dihomo-γ-linolenic acid (20:3 Δ8, 11, 14) and arachidonic acid (20:4 Δ5, 8, 11, 14) in transgenic seeds It also indicated that the activity of napin promoter is not efficient in soybean.

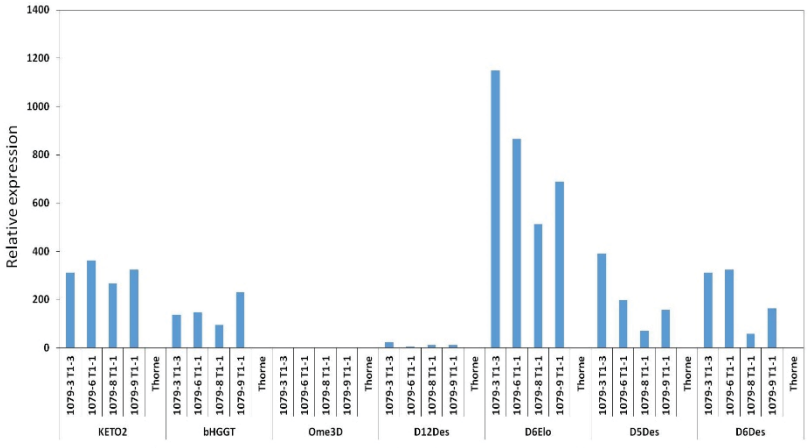

Figure 4. Seed at R5/R6 growth stage selected based on the color. Expression quantification was performed using ddPCR, and normalization was referred to an endogenous gene ATP-binding cassette transporter (Libault, Marc, et al. The Plant Genome1.1 (2008): 44-54). The expression was relatively compared to the reference gene. Seed samples were from three different T2 plants for each event.

All three traits, omega-3 EPA, vitamin E (tocotrienols) antioxidant, and astaxanthin, were stably transmitted to the T_3 generation in lines from the greenhouse and field. T_2 red seeds of lines 1079-3, 1079-5, 1079-6, 1079-8, 1079-9 produced EPA (2.5-5.8 mol%) and its precursors (27-33 mol%). (Figure. 5)

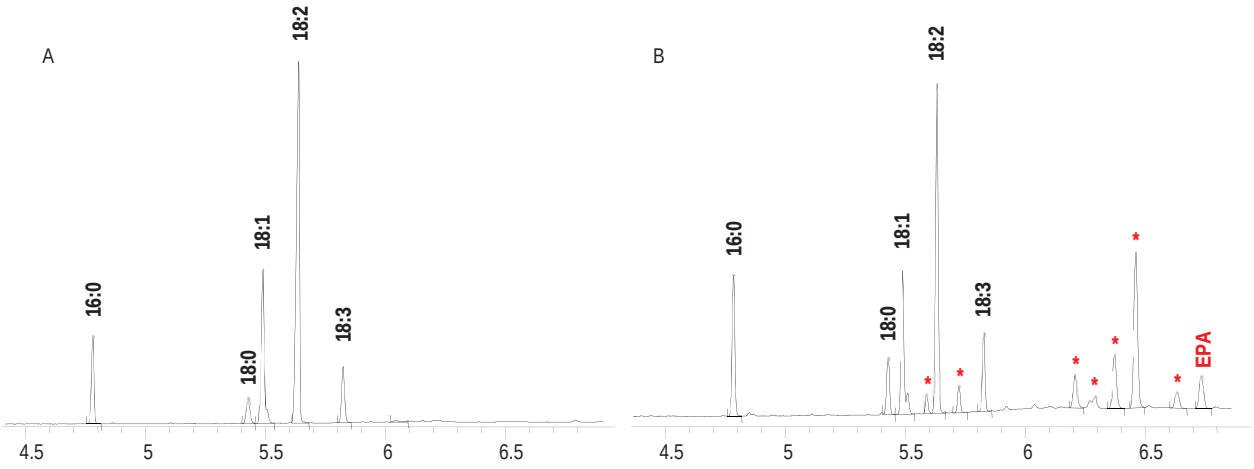

Figure 5. GC chromatogram of fatty acid analysis of transgenic soybean carrying pPTN1331. (A) Wild type soybean seed as negative control. (B) T_2 generation of soybean seed from field. Asterisks indicate the precursors of EPA.

T_2 red seeds of lines 1079-3, 1079-5, 1079-6, 1079-8, 1079-9 produced tocotrienols (530-830 µg/g). (Figure 6)

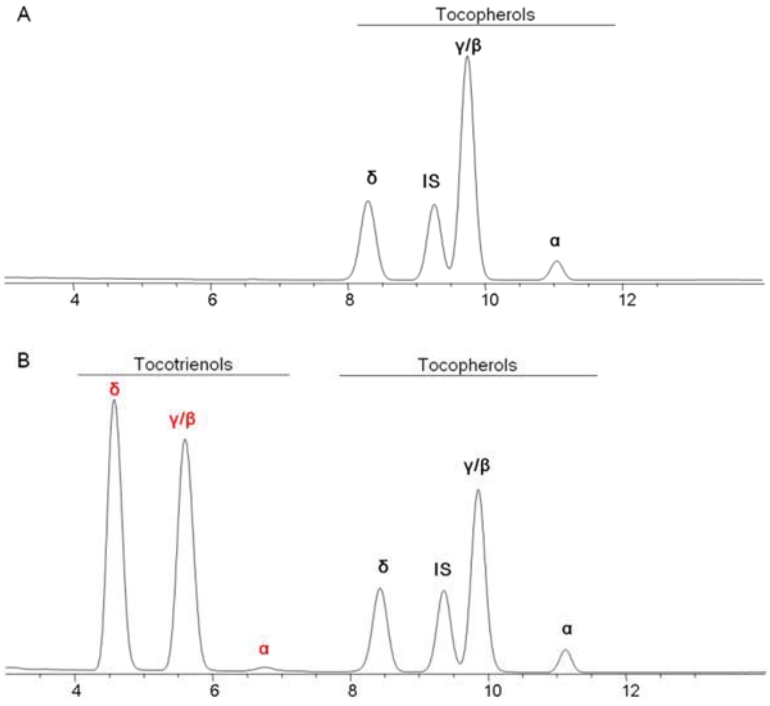

Figure 6. HPLC chromatogram of Vitamin E analysis of transgenic soybean carrying pPTN1331. (A) Wild type soybean seed as negative control. (B) T_2 generation of soybean seed from field. IS indicates tocol as an internal standard for quantification of vitamin E. Engineered soybeans in (B) accumulate high levels of the tocotrienol form of vitamin E.

T_2 red seeds of lines 1079-3, 1079-5, 1079-6, 1079-8, 1079-9 produced two astaxanthin forms astaxanthin 1 (52-154 µg/g) and astaxanthin 2 (29-75 µg/g). Total astaxanthin pigment concentrations as high as 210 µg/g. (Figure7).

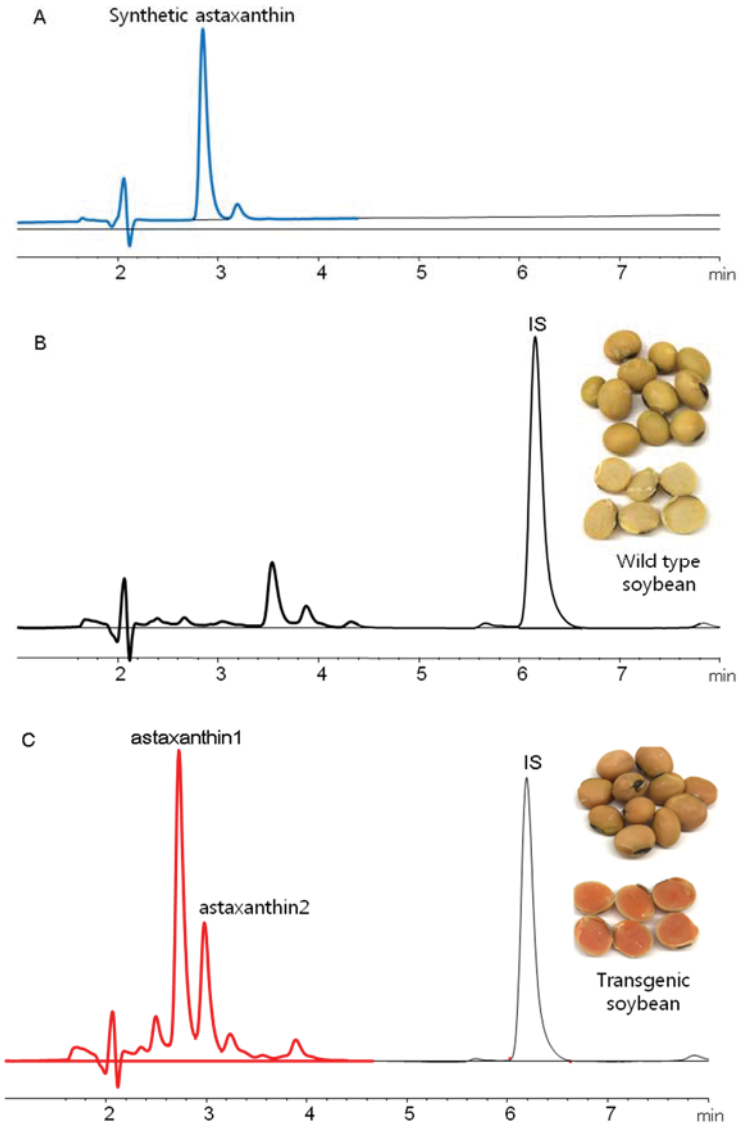

Figure 7. HPLC chromatogram of astaxanthin analysis of T_2 transgenic soybean carrying pPTN1331. (A) Synthetic astaxanthin as standard. (B) Wild type soybean seed as a negative control. (C) T_2 generation of soybean seed from field. IS indicates 8'-apo-beta-Carotenol as an internal standard for quantification of astaxanthin.

To determine whether the vitamin E trait is able to increase astaxanthin content of seeds, our top lines for astaxanthin, line 948-1 (19059 and 19060), were crossed with a high vitamin E line (488-3). The contents of vitamin E and astaxanthin in F2 seeds were analyzed by HPLC. F2 seeds segregated into 4 types that produce tocotrienols and astaxanthin, only astaxanthin, only vitamin E (tocotrienols), or nothing. Astaxanthin-producing F2 red seeds produced two astaxanthin forms astaxanthin 1 (37-66 µg/g) and astaxanthin 2 (27-47µg/g). Total astaxanthin pigments were as high as 113 µg/g in F2 seeds. The amount of tocotrienols in F2 seeds were 283-1411 µg/g. The vitamin E trait did not effect to the amount of astaxanthin, but vitamin E was reduced when astaxanthin was co-expressed.

To generate new soybean lines that minimize negative effects of astaxanthin production on seed performance, a new expression vector was assembled with the Adonis astaxanthin transgenes (keto2 and HBFD1) and maize phytoene synthase gene (ZmPhy) under control of promoters for Late Embryogenesis Abundant (or LEA) genes. Soybean transformation experiments was initiated with this expression vector to initiate astaxanthin production at later stages of seed development to reduce negative effects on seed embryos. The expression vector was tested in model plant to more quickly confirm that the genes function as expected. T2 developing seeds from model plant were analyzed using HPLC to detect the astaxanthin. Astaxanthin was successfully produced in model plant. This indicates that the putative promoters in the new expression vector are functional in the seeds.

A second vector without the ZmPhy gene was constructed and transformed into model plant to test whether the ZmPhy is required for astaxanthin production in soybean seeds.

To produce a second fish oil component DHA (docosahexaenoic acid) in soybean, *Ostreococcus tauri* elongase 5 (OtElo5) and desaturase 4 (OtD4) genes were assembled under control of promoters that confer seed expression. These were combined with EPA and vitamin E biosynthetic transgenes. Soybean transformation experiments are underway with the expression vector for DHA and vitamin E production. The expression vector is also being tested in model plant to more quickly confirm that the genes function as expected. Seven T1 plants of model plant have been generated and the insertion of transgenes were confirmed by gDNA PCR.

Additional putative promoters and terminators for soybean LEA genes were isolated for use in seed quality improvement research. These elements were linked to a reporter gene (GUS) that allows visual assessment of gene expression to determine which promoters are best-suited for late stage-seed-specific expression of our transgenes. The expression of the GUS gene was examined in T2 developing seeds of model plants transformed with LEA promoter:GUS gene. The results showed that the visual signal from expression of the GUS gene became progressively stronger as seeds matured, suggesting that these promoters will be useful for future efforts to engineer aquaculture and other seed quality traits in soybean. Engineered seeds from T3 plants were planted to investigate in detail the LEA promoter activities in different tissues.

Impacts

-The research showed 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. Conventional plant breeding and biotechnology approaches typically target one or only a small number of traits at a single time for crop improvement. In contrast, this research was pursued using emerging synthetic biology methodologies for rapidly constructing large (>25 kb) gene expression cassettes for diverse trait genes into soybean genome in a single genetic transformation experiment.

-The research resulted in the production of EPA, astaxanthin, and enhanced vitamin E antioxidants in a single seed by introduction of eight transgenes for complex metabolic pathways. Astaxanthin and vitamin E levels are likely at commercial viability, and EPA levels are approaching commercial viability. To our knowledge, this is an unprecedented achievement for soybean biotechnology, i.e. to stack eight transgenes in one line and obtain three commercially relevant, complex traits in <3 years.

-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 compared to conventional breeding.

-We anticipate that the research conducted in this project will pave the way for increased use of soybean 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.