**Project/Activity Number:**

**Project/Activity Title:** LIPIDS of Crops Annual Meeting

**Period Covered:** 2016-2017

**Date of This Report:** September 20, 2017

**Annual Meeting Date(s):** September 15-17, 2017

**Participants**: Cahoon, Ed –University of Nebraska (UNL); Minton, Ernie - Kansas State University (KSU); Welti, Ruth - KSU; Nikolau, Basil – Iowa State University (ISU); Wurtele, Eve – ISU; Allen, Doug – Danforth Center; Thelen, Jay – University of Missouri-Columbia (UMC); Koo, Abe - UMC; Parkash, Om – University of Massachusetts-Amherst; Wang, Tong – ISU; Clemente, Tom – UNL; Durrett, Tim – KSU; Schrick, Kathrin – KSU; Hoffmann-Benning, Susanne – Michigan State University; Narayanan, Sruthi – Clemson University.

**Brief summary of minutes of annual meeting**: Introduction was made by KSU Associate Dean and Multistate Project Administrative Advisor Ernie Minton followed by Chair and host Basil Nikolau. Scientific meeting began with 25 min presentations from Tong, Abe, Susanne, Om, Tom, Kathrin, Jay, Tim, Ruth, Eve, Sam and Doug. Meeting adjourned at 6 pm and continued at 9 am the next day with a scientific talk from Ed followed by a discussion of a potential funding opportunities for the group. Ed, Doug and Basil also presented information pertaining to past, successful center grants and future opportunities. Meeting adjourned with the election of Tim Durrett as secretary and selection of Kansas State University as the venue for the 2020 meeting.

**Accomplishments**

**Short-term outcomes and outputs:** At the 2017 annual meeting the LIPIDS of Crops group members presented original research aimed at enhancing the production of value-added oils and protein meal in crops like soybean and camelina. Multiple ongoing collaborations between members of this group were identified and discussed. Future collaborations were discussed including the chemical and physical analysis of acetylated TAG vegetable oils and sharing of plant transformation constructs, protocols, and technologies. Details for each objective are listed below in activities and milestones.

**Activities and Milestones:** Activities related to each of the three milestones listed in the proposal are discussed below.

**1. Improve and extend methods for lipid characterization and measurement.**

The Welti lab presented new experimental approaches to profile plant lipids. The approach involves different lipid extraction procedures and enhances both lipid coverage and detection sensitivity. Additionally, the Welti lab (Co-PIs Durrett, Schrick, and others) successfully obtained an NSF-MRI grant to purchase a new triple quadrupole mass spectrometer with ion mobility spectrometry to be dedicated for the analysis of plant lipids. A total of 15 labs are allocated for time on this project with the NSF, but an additional 42% time is un-allocated and available for the current crop lipid group to use and further its goals.

The Schrick lab is collaborating with the Young-Jin Lee lab at Iowa State University to develop mass spectrometry imaging of lipids in epidermal cells. Schrick received a Big12 Faculty Fellowship to fund visits to ISU during 2017-18.

**2. Identify and characterize lipid-related metabolism and traits relevant for crop improvement.**

The Koo lab discovered that plants engineered to contain higher leaf oil content were differentially susceptible to pest (larval) attack, depending upon the type of protein up-regulated for oil body production. It was posited that such an observation may suggest a link between biotic stress and reserve deposition in leaves.

Additionally, research was presented that showed novel strategies to increase seed oil content in camelina by engineering the committed step for de novo fatty acid biosynthesis. Two different strategies were presented, affecting two different parts of the enzyme complex acetyl-CoA carboxylase.

The Hoffmann-Benning lab identified a class of novel lipid-binding proteins in phloem sap. The PLAFP proteins preferentially bind phosphatidic acid and when over-expressed results in larger plants and bigger vascular bundles. Their location and expression suggests a role in systemic signaling in response to abiotic environmental factors. The protein – lipid complex appears to contribute to the regulation of root development and vascular system growth.

The Schrick lab further studies the role of lipids in signaling through identification and characterization of START-domain containing transcription factors and their role in DNA binding, gene regulation, and development of trichomes and other features of the epidermis.

The Cahoon and Clemente labs have completed studies involving the use of camelina to test novel genes from Cuphea species associated with the biosynthesis of seed oils rich in medium-chain fatty acids for industrial and bio-jet fuel applications. Seed oils of many Cuphea sp. contain >90% of medium-chain fatty acids, such as decanoic acid (10:0). These seed oils, which are among the most compositionally variant in the plant kingdom, arise from specialized fatty acid biosynthetic enzymes and specialized acyltransferases. These include lysophosphatidic acid acyltransferases (LPAT) and diacylglycerol acyltransferases (DGAT) that are required for successive acylation of medium-chain fatty acids in the sn-2 and sn-3 positions of seed triacylglycerols (TAGs). Here we report the identification of a cDNA for a DGAT1-type enzyme, designated CpuDGAT1, from the transcriptome of C. avigera var pulcherrima developing seeds. Microsomes of camelina seeds engineered for CpuDGAT1 expression displayed DGAT activity with 10:0-CoA and the diacylglycerol didecanoyl, that was approximately 4-fold higher than that in camelina seed microsomes lacking CpuDGAT1. In addition, coexpression in camelina seeds of CpuDGAT1 with a C. viscosissima FatB thioesterase (CvFatB1) that generates 10:0 resulted in TAGs with nearly 15 mol % of 10:0. More strikingly, expression of CpuDGAT1 and CvFatB1 with the previously described CvLPAT2, a 10:0-CoA-specific Cuphea LPAT, increased 10:0 amounts to 25 mol % in camelina seed TAG. These TAGs contained up to 40 mol % 10:0 in the sn-2 position, nearly double the amounts obtained from coexpression of CvFatB1 and CvLPAT2 alone. Although enriched in diacylglycerol, 10:0 was not detected in phosphatidylcholine in these seeds. These findings are consistent with channeling of 10:0 into TAG through the combined activities of specialized LPAT and DGAT activities and demonstrate the biotechnological use of these enzymes to generate 10:0-rich seed oils in crops such as camelina.

**3. Develop crops with improved yield and/or functionality.**

The Clemente lab presented data showing that overexpressing the genes Wri1, DGAT1, and KasII together under seed-specific promoters, raised 16:0 fatty acid concentration but not total seed oil. Transforming soybean with lanceolate leaves allowed more light through to the canopy leaves and thus produced more biomass. The down side to use of this variety of soybean is the potential for lodging due to the tall high achieved with these plants. In the future they are planning a five-gene stack to optimize oil content. In his experience, he has never seen more than a 10% increase in soybean seed oil content no matter what gene or genes he has used for transformation.

Ongoing research to test the chemical properties of oils such as acetylated TAG were discussed as well as the potential for new markets for oils with modified or unusual fatty acids. The Cahoon lab is developing camelina with value-added traits to both oil and protein meal. One success is the down-regulation of storage proteins to allow the production of the high-value protein resilin. Additionally, engineering of soybean and camelina to produce polyunsaturated fatty acids for fish aquaculture was presented. Some of the challenges ahead for the plant synthetic biologist include stabilizing large binary vectors within Agrobacterium. Currently, large constructs (>31 kb) with multiple, stacked gene cassettes undergo rearrangement when transformed into Agrobacterium.

The CRISPR/Cas9 nuclease system is a powerful and flexible tool for genome editing, and novel applications of this system are being developed rapidly. The Cahoon and Clemente labs used CRISPR/Cas9 to target the FAD2 gene in the emerging oil seed plant, Camelina sativa, with the goal of improving seed oil composition. We successfully obtained Camelina seeds in which oleic acid content was increased from 16% to over 50% of the fatty acid composition. These increases were associated with significant decreases in the less desirable polyunsaturated fatty acids, linoleic acid (i.e. a decrease from ~16% to <4%) and linolenic acid (a decrease from ~35% to <10%). These changes result in oils that are superior on multiple levels: they are healthier, more oxidatively stable and better suited for production of certain commercial chemicals, including biofuels. In the allohexaploid, Camelina, guide RNAs were designed that simultaneously targeted all three homoeologous FAD2 genes. This strategy that significantly enhanced oil composition in T3 and T4 generation Camelina seeds was associated with a combination of germ-line mutations and somatic cell mutations in FAD2 genes in each of the three Camelina subgenomes.

A collaboration between the Clemente and Cahoon labs aims to increase the properties of soybean oil for aquaculture feed applications. 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. To address the need for optimized soybean feedstocks for aquaculture feed, the Clemente and Cahoon labs successfully introduced eight transgenes assembled on a single T-DNA to soybean. These transgenes included five genes for production of the major fish oil fatty acid eicosapentaenoic acid (EPA), two genes for the high-value carotenoid astaxantin, and one gene for high vitamin E antioxidants. The insertion of these seed-specific transgenes into the soybean genome was confirmed by Southern blot analyses. Lines with 5% to 13% EPA, >100 µg/g astaxanthin, and ~1,200 µg/g vitamin E tocotrienols were identified. These lines were tested in the UNL biotech field in Mead, NE in 2017.

**Impact statements:** The LIPIDS of Crops Consortium has an overarching goal to increase the value of crop oilseeds by increasing seed oil content, making unusual and economically important fatty acids, finding new markets for existing or future vegetable oils and oilseed crops (e.g. camelina), and also adding value to the defatted meal particularly for niche crops like camelina. Each of these goals has the potential to impact the economy and move towards renewable energy independence. At the present time each member of this consortium has their own independent or occasional collaborative, joint funding from one of the following federal agencies: USDA, DOE, NSF, ARPA-E. Additionally, some members have USB funding as well as local soybean merchandise council funding. There was extensive discussion about how to leverage the diverse research interests and expertise of the group to put forth a successful proposal in response to one or more recent RFPs. This will continue to remain an ongoing discussion as no immediate action was taken towards drawing up a proposal for a specific program.

**Milestones (2017):**

**1.\_Optimized extraction methods for crop plants developed and exchanged among groups**

Welti lab has developed less labor-intensive extraction methods and a paper for Plant Methods is currently in revision.

**2. Lipid internal standard mixes available for sharing**

KLRC has standard mixes of glycerolipids available <http://www.k-state.edu/lipid/analytical_laboratory/prices/index.html>

**3. Rapid lipidomic methods for leaves of sorghum, camelina, and soy**

A method specific to sorghum covering glycerolipids including oxidized lipids and other stress-induced lipids has been developed.

**4. Automated data upload to PMR database**

An automated data upload program has been written and is being tested.

**5. Identification of camelina mutants with altered leaf and seed lipid composition**

The Cahoon and Durrett groups screened a camelina mutant population for lines containing altered profiles of fatty acids or neutral glycerolipid molecular species in mature seeds. Using a candidate gene sequencing strategy, mutations in at least one of the camelina homeologues of *FAD2*, *FAD3*, *FAE1* and *KASII* were identified that were likely responsible for the observed lipid phenotypes in different lines. In addition, both the Cahoon and Durrett groups used CRISPR/Cas-mediated genome editing to induce mutations in key camelina lipid biosynthetic genes and alter the fatty acid composition in seed.

**6. Select, clone, and characterize promoter regions for selected oil body coat proteins**

Clemente lab did this and it is described above.

**Publications:**

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Jose A. Aznar-Moreno and Timothy P. Durrett. (2017) Simultaneous targeting of multiple gene homeologs to alter seed oil production in *Camelina sativa*. Plant and Cell Physiology 58: 1260 - 1267

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.

Pook, V.G., Nair, M., Ryu, K., Arpin, J.C., Schiefelbein, J., Schrick, K., and DeBolt, S. (2017) Positioning of the SCRAMBLED receptor requires UDP-Glc:sterol glucosyltransferase 80B1 in Arabidopsis roots. Sci Rep. 7(1): 5714.

Salie MJ, Zhang N, Lancikova V, Xu D, Thelen JJ. (2016) A family of negative regulators targets the committed step of de novo fatty acid biosynthesis. Plant Cell. 28:2312-2325. Selected by editors for In-brief Highlight.

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Zi-Wei Ye, Shiu-Cheung Lung, Tai-Hua Hu, Qin-Fang Chen, Yung-Lee Suen, Ming-Fu Wang, Susanne Hoffmann-Benning, Edward Yeung, Mee-Len Chye (2016) Arabidopsis acyl-CoA-binding protein ACBP6 localizes in the phloem and affects jasmonate composition. Plant Mol Biol 92(6):717-730.