**Project/Activity Number:** NC-1203

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

**Period Covered:** 2018-2019

**Date of This Report:** October 4, 2019

**Annual Meeting Date(s):** September 21-22, 2019

**Participants:** Cahoon, Edgar - University of Nebraska (UNL); Clemente, Thomas - UNL; Durrett, Timothy - KSU; Hoffmann-Benning, Susanne - Michigan State University; Kosma, Dylan - University of Nevada Reno (UNR); Lee, Young-Jin - Iowa State University; Louis, Joe - University of Nebraska-Lincoln; Minton, Ernie - Kansas State University (KSU); Roston, Rebecca - UNL; Schrick, Kathrin - KSU; Thelen, Jay - University of Missouri - Columbia; Welti, Ruth - KSU; Om Parkash Dhankher- University of Massachusetts Amherst.

**Brief summary of minutes of annual meeting**: The 2019 NC-1203 meeting was held September 21-22 in Lincoln, Nebraska at the University of Nebraska. The meeting was opened by representatives of the hosts: Jonathan Markham, Rebecca Roston, Ed Cahoon, Julie Stone, Joe Louis, and Tom Clemente, and included remarks from KSU Interim Dean and Multistate Project Administrative Advisor Ernie Minton. A time of introductions was followed by presentations and discussions for each of the individual project aims. These were led by Jonathan Markham, Rebecca Roston, and Ed Cahoon; accomplishments for each of the aims are summarized below. This was followed by a discussion of future plans for each of the aims and the timing of the 2020 annual meeting to be held in Manhattan, Kansas. The 2021 meeting will be held at the Danforth Center and hosted by Doug Allen and Sam Wang. Susanne Hoffmann-Benning was elected as secretary and Michigan State University was chosen as the venue for the 2022 meeting. The project renewal was discussed. Ernie Minton indicated the current project runs until September 30, 2021 but a renewal should be submitted in the fall of 2020. It was agreed that Jonathan Markham would oversee the rewrite, Ruth Welti would address Aim 1, Rebecca Roston Aim 2, and Tim Durrett Aim 3 specifically. The ongoing movement of NIFA to Kansas City was also discussed.

**NC-1203 2018-2019 Accomplishments.**

Activities and accomplishments related to each of the project’s three objectives are described below, based on talks by each member and other information presented at the meeting. At the end of each presentation, group members engaged in active discussion on the challenges of each aim as well as alternative  approaches to overcome these problems and to meet the remaining goals. Members also considered opportunities to work together on joint publications, either individual papers or a special issue on lipids in an appropriate journal.

Objective 1: Improve and extend methods for lipid characterization and measurement.

The Welti group has worked on making all the components available for direct-infusion multiple reaction monitoring (MRM) mass spectrometry using a Sciex 6500+ triple quadrupole MS. The components include a rapid extraction method, standards available at cost, all parameters for MRM (and the data acquisition method downloadable from the web), and a complete data processing method that employs Sciex software and the online site LipidomeDB Data Calculation Environment.  They have written a paper pointing users to all components. They are also updating Lipidome DB Data Calculation Environment to support collision induced fragment analysis by time-of-flight mass spectrometry; this technique can be used for fatty acid analysis at the chemical formula level. Ruth Welti, Tim Durrett, and Jonathan Markham are beginning a project to develop a lipid mass map (visualization of fragments vs. intact ion m/z) which will be used to analyze data from plants across the plant kingdom.  The data will be stored in a database of complex lipid compositions. In addition, Jonathan Markham presented a cross disciplinary platform to measure lipids and model with flux balance analysis with a goal of establishing steps controlling flux through metabolism. He described challenges including a need for multiplexing and faster mass spectrometry, and that some lipid classes were more problematic than others (e.g. phosphatidic acid). In the future his group will attempt to leverage in-source ozonolysis and will create a searchable database for retention times.

The Lee group is also interested in mass spectrometry methods and continues to develop MALDI matrices for the visualization and localization of lipids with screening of  metal target deposition as a MALDI matrix. Gold doped with sodium dramatically improves TAG signals but suppress phospholipids and remains a candidate of interest. Lee and Durrett submitted a NIFA proposal to study the spatiotemporal development of acetyl-TAG in genetically engineered camelina.

Others are using radiolabels as well as microscopy, FRET, and mass spectrometry to interrogate lipid metabolism. Work in the Hoffmann-Benning lab demonstrated that 14C-labeled PA moves in the phloem from source to sink, but it remains to be confirmed whether the labeled mobile compound is still PA and whether PLAFP affects its movement and is a work in progress. Currently the lab is working on FRET experiments to establish PLAFP interactions and whether it moves with the lipid in vivo. In this context the lab has generated constructs using a light-inducible promoter - PLAFP - YFP fusion for inducible and local expression of the gene. A small non-mobile protein and FT are used as negative and positive controls. Movement of the protein can be monitored using confocal microscopy and Western blotting. Movement of lipid will be monitored using mass spectrometry. Constructs are now ready for testing in protoplasts and plants. An NSF EAGER grant is funding this work.

Pertaining to enhanced data analysis, the Wurtele group developed a semi-automated mechanism for upload of data that is connected to Araport and is awaiting data sets from participants for the Plant/Eukaryotic and Microbial Systems Resource (PMR; http://metnetdb.org/PMR/). It is recognized that the analysis of complex lipid data is challenging and impedes progress related to methods being developed as part of this grant.

Objective 2: Lipid-related metabolism and traits relevant for crop improvement

The Cahoon lab described an interest in looking at the extremes of fatty acid biosynthesis including medium chain fatty acid levels in Cuphea and very long chain fatty acids in various Brassicaceae. For the medium chain fatty acids, it may be a question of acyl-ACPs that are present in vivo. Since all fatty acid synthesis genes are expressed, it may be that there are structural or stoichiometric differences. The same may also be true in specialty fatty acids such as petroselinic acid production. Cahoon emphasized that gaps in our knowledge include understanding the stoichiometry of Type II fatty acid synthase components and our ability to routine measure acyl-ACP pools in plant tissues.

Welti, Durrett, and Schrick are working together to characterize the function of several  plant genes that were identified in a lipid profiling screen of Arabidopsis T-DNA mutants as having complex lipid profiles that are significantly different from wild-type plants. One gene is involved in fatty acid desaturation, one is involved cutin metabolism, and one seems to be involved in lysophospholipid acylation. Welti’s lab is also working to identify genes involved in lipid metabolism that have natural variation affecting the activities of associated gene products. Particularly, they are interested in natural variation that results in altered enzyme activities during response to wounding and mild freezing stress.  Some of the lipid analyses have been done in collaboration with Abe Koo. Koo also described the 2018 participants of NSF outreach program Sci-LiFT had conducted no-choice insect feeding assays on lines overexpressing WRI1 and/or those reduced in ADP-glucose pyrophosporylase generated by Sanjaya and Christoph Benning. The Lewis lab also reported on inset feeding by examining sorghum defense response to sugarcane aphids. They have screened NAM parent lines to identify resistance and are exploring the role of lignin, surface waxes, and other lipids in plant defense. The transcriptional regulation of potato tuber wound healing, in terms of the biosynthesis and deposition of the lipidic polymer suberin, is a topic being explored in the Kosma lab. Kosma is continuing characterize potato cultivars that differ in storage life and tuber wound suberin deposition. Kosma has determined that two different transcription factors, StMYB74 and StMYB102, are sufficient for the production of suberin and are likely one component of the differential wound healing capacity of potato cultivars that differ in storage life. Kosma’s group has further conducted a large comparative RNA-seq experiment on 2 potato cultivars that differ in storage life and wound suberin deposition (Snowden deposits more suberin during wound healing and has a longer storage life whereas Atlantic deposits less wound suberin and has a poor storage life). Initial observations confirm that Snowden has higher expression of suberin biosynthetic genes during the course of wound healing. Kosma has generated community resources in the form of a potato wound healing-specific coexpression network that will be released to the public in the next year.

Pertaining to abiotic stresses, the Kutty (Narayanan) lab, in collaboration with Welti, showed that wheat decreases the unsaturation levels of plastidic and extra-plastidic glycerolipids of leaf and/or pollen in order to adapt to heat stress. Lipid analysis of leaves of multiple soy lines under heat stress are underway. The Schrick group has been studying the role of lipids in signaling through identification of START-domain transcription factors and their role in DNA binding, gene regulation and development of trichomes and other features of the epidermis. Phospholipids have been identified as putative binding partners of these transcription factors. One of the family members has been identified to bind a phosphate starvation response element to negatively regulate several phospholipid catabolism genes.

The Hoffmann-Benning lab recently identified a class of novel lipid-binding proteins in phloem sap that provides motivation for a number of studies. Overexpression causes larger plants with bigger vascular bundles. In addition, the phloem lipid profile is changed. Putative interacting proteins have been identified and are investigated for their role in lipid-signaling and would be important to composition and yield.

Overall enhancements in lipid production were also described by the Thelen group. In particular, strategies for improving acetyl-CoA carboxylase performance including BADC repression, alpha-CT overexpression, and CTI repression. Like the BADCs, the CTIs are a previously uncharacterized gene family discovered by the Thelen lab to be associated with the heteromeric ACCase.  The CTIs are envelope membrane proteins that anchor ACCase through direct interaction with the alpha-CT subunits. They are using multiple approaches to examine structure and derive the mechanisms that can lead to higher oil in oilseed species including Arabidopsis, Camelina, and soybean. Some of these lines will be analyzed through an NSF-Plant Genome Research project with Koo and Allen. This project will also develop a website dedicated to the study of oil production in plants called “Fat Plants” available at fatplants.net.  The Allen lab is working on related projects including the analysis of repartitioning of carbon from starch to oil in trangenic tobacco plants that produce high levels of lipids, the analysis of fluxes in Chlamydomonas grown auto- or mixotrophically, and an in-silique method for isotopically labeling camelina that will be used with transgenic lines obtained from Thelen and Koo labs. The Allen lab continues to work on methods for using 13C analysis with high resolution mass spectrometry to analyze fluxes through lipid metabolism and methods to quantify acyl-acyl carrier proteins. A project with the Durrett lab includes investigations in soybean metabolism to understand the partitioning of oil late in development into other storage reserves. The Durrett group has also been exploring the role of the lipase PLIP1 in supplying FA for the synthesis of TAG in developing Arabidopsis seeds. PLIP1 is upregulated in dgat1 seed. Double mutant *plip1 dgat1* seed are green and fail to germinate.  Ongoing work is testing the hypothesis that PLIP1 provides a PUFA-substrate pool necessary for the function of PDAT1. Research from Dhankher group studied the comparative transcriptome and metabolome analysis suggesting bottlenecks that limit seed and oil yields in transgenic Camelina sativa expressing Arabidopsis diacylglycerol acyltransferase 1 (DGAT1) and yeast glycerol‑3‑phosphate dehydrogenase (GPD1). They concluded that TAG production is limited by (1) utilization of fixed carbon from the source tissues supported by the increase in glycolysis pathway metabolites and decreased transcript levels of transcription factors controlling fatty acids synthesis; (2) TAG accumulation is limited by the activity of lipases/hydrolases that hydrolyze TAG pool supported by the increase in free fatty acids and monoacylglycerols.

Research from the Wang group has focused on lipid-protein interactions with cytosolic proteins, some of which are transcription factors, with interesting functions. In addition, the group has been investigating how post-translational modifications on lipid metabolic enzymes that allow attachment to the membrane, and how the stability of these interactions is important for their function.

Objective 3: Develop crops with improved yield and functionality

The Durrett group has been studying the germination of seeds that produce high levels of  acetyl-TAGs. Synthetic biology approaches are being used to alter the timing of EfDAcT expression to increase levels of accumulation in seeds; other projects aim to incorporate other fatty acids (e.g. ricinoleic acid) into acetyl-TAG molecules to further alter properties.

The Clemente group reported on a number of genetic modifications to alter the levels of storage proteins in soybeans, alter sulfur amino acid production, change sucrose levels, and examine QTLs for high protein. Roston, Markham, and Clemente continue to work on cold tolerance in important crops including wheat and sorghum through the expression of transcription factors and examinations in sphingolipid content. The Roston lab has identified important lipid changes cycle in response to cold and current efforts from multiple labs that appeared to be conflicting are now hypothesized to be in congruence. Multiple species have similar cycles in lipid abundance; cold tolerant species deviate from non-tolerant species at key times. They also showed that lipid responses to freezing tolerance are greatly increased in ancient lineages and suggested the hypothesis that severe stress responses may be derived from a stronger ancestral response to damage of any type. The Wurtele group explored the relationship between QQS, carbon and nitrogen partitioning, and defense in a series of Arabidopsis mutants affecting starch metabolism. Results indicate QQS and NF-YC4 can increase protein and improve defensive traits in crops. Using a mapping population of a cross between two potato cultivars that differ in terms of wound suberin deposition and storage life, Kosma’s collaborators, Dave Douches and Ray Hammerschmidt of Michigan State University, have identified 2 QTLs potentially related to better wound suberin deposition. Several of the progeny of the cross were shown to have high wound suberin deposition than either parent. These lines are being evaluated for disease resistance and storage life.  The Dhankher group identified and overexpressed a bifunctional wax synthase/acyl-CoA:diacylglycerol acyltransferase (WSD1) gene, which plays a critical role in wax ester synthesis in Arabidopsis stem and leaf tissues. Gas chromatography and electron microscopy analyses of WSD1 transgenic Arabidopsis seedlings showed higher deposition of epicuticular wax crystals and increased leaf and stem wax loading in WSD1 transgenic lines as compared to wildtype (WT) plants. Transgenic plants showed strong tolerance to drought and salinity. Their results clearly show that the manipulation of cuticular waxes will be advantageous for enhancing plant productivity under changing climate. The Clemente lab reported on progress made in complex metabolic engineering to improve soybean oil composition for food and feed applications. These included the development of a high melting point soybean oil containing elevated content of palmitic acid, stearic acid, and oleic acid. The oil is a semi-solid at room temperature and is now being evaluated for animal fat-type functionality by industry. Also reported were efforts to assemble multi-gene expression cassettes in collaboration with the Cahoon lab to generate soybean oil with omega-3, astaxanthin, and high vitamin E antioxidant content for use as a sustainable aquaculture feedstock. Clemente also discussed the need for low-cost processing technology that can be deployed in rural settings to enable commercialization of high value/low acreage specialty compositional traits in soybeans and other crops. It was also noted that the Clemente and Cahoon labs have been building a cataloged repository of genetic elements (e.g., promoters, 3’UTRs) that have been domesticated for use in GoldenBraid modular gene assembly that are available for the Multistate group to facilitate metabolic engineering and crop improvement efforts. One theme that arose from several talks was reduced seed performance associated with some of the engineered oil traits and the need for a coordinated effort to understand the metabolic basis for these phenotypes.

**Impact Statements.**

The LIPIDS of Crops Multi-state research project have 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. The NC-1203 group has interacted collaboratively to achieve project milestones during the 2018-2019 year as indicated by milestones and 22 publications, 3 software tools, and 3 patents listed below and standards and protocols that have been shared amongst participants. Future work will focus on completing the remaining and future milestones:

**Milestones:**

2017

*Optimize extraction methods for crop plants, develop and exchange among groups*

There are two methods for crop plants from the group (Welti). Roston is doing a comparison among several methods.

*Lipid standards made available for sharing*

A subset of these is now available through the KLRC (Welti)

*Develop rapid lipidomic methods for leaves of camelina, sorghum and soy*

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

*Survey of lipid molecular species to be analyzed in comprehensive analysis*

Welti and Markham are collaborating to complete the survey

*Automated data upload to PMR database*

Partially accomplished - a semi-automatic system with processing times of less than a day has been implemented (Wurtele)

*Identification of camelina mutants with altered leaf and seed composition*

Accomplished (Cahoon, Durrett)

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

Accomplished (Clemente)

2018

*Methods for unprecedentedly comprehensive lipidomic analysis of soy, camelina, and sorghum leaf and seed*

Welti and Markham are collaborating to develop the analyses. More comprehensive, Lipid Atlas for Arabidopsis is underway in Welti lab.

*Visualization of lipids in crop plant tissues and localization in plant tissues of lipids not previously visualized*

Accomplished (Schrick, Lee, Hoffmann-Benning, Durrett)

*Web-based statistical analysis of metabolomics data with transcriptomics data available in PMR*

Accomplished

*PMR is linked to other databases*

We have created a new software, MetaOmGraph (MOG), for interactive exploratory analyze or “big” metabolomics, proteomics and transcriptomic data (Wurtele)

*Development of a shared database with information about lipid-regulated genes in crop plants*

Done for camelina at Michigan State and will be available publically soon (Dhankher)

*Mapping of genetic lesions affecting camelina mutants with altered leaf and seed lipid composition*

Accomplished (Cahoon, Durrett)

*Development of camelina lines with modified oil composition*

Accomplished (Cahoon, Durrett)

*Develop a strategy for upregulating expression of selected oil body coat proteins*

Oleosin in sorghum (Clemente)

Data involved in herbivory of the lines (Koo)

2019 goals

*Large scale growth of camelina lines with modified seed oil composition*

Accomplished (Wang, Cahoon)

*Generate a transgenic crop line*

Accomplished (Clemente, Wang)

*Laboratory test for improved resistance to stress*

Overexpression of PLAFP increases drought tolerance in Arabidopsis (Hoffmann-Benning); experiments to examine role in soybean are planned.

Increase in cold tolerance post manipulation of sphingolipid metabolism in sorghum and soybean demonstrated (Markham)

Evaluated ICE1 overexpression sorghum for cold tolerance improvement (Roston and Clemente)

Evaluation of heat tolerance of soy lines and their lipid metabolism underway (Narayanan)

Overexpression of WSD1 Increases drought, salinity and ABA tolerance in Arabidopsis (Dhankher)

2020 goals

*Extraction and property analysis of modified seed oil*

Accomplished (Durrett, Clemente, Cahoon)

*Information obtained about the physical and chemical properties of one or more modified seed oils.*

Accomplished (Durrett, T. Wang)

*Prepare transgenic soybeans overexpressing selected oil body coat proteins; evaluate changes in lipid composition.*

Group member left the group and goal directions were changed to:

*Engineering ACCase to increase oil content in soybean, camelina and Arabidopsis*

Accomplished (Thelen, Koo, and Clemente)

Generate transgenic lines expressing epitope tagged ribosomal subunit driven by seed specific promoter in the existing ACCase engineered lines to use for TRAPseq analysis (Koo)

2021 goals:

*Field trials for improved resistance to one or more stress.*

Aphid field trials (Clemente, Louis)

Camelina field trials (Dhankher)

**Publications**

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**Software**

1. MetaOmGraph.(PMR, http://metnetdb.org/PMR/ ) Java software enabling users to input and evaluate big data sets. v2019

2. Plant, Eukaryotic and Microbial Metabolomics Systems Resource (PMR, http://metnetdb.org/PMR/ ) A public database for metabolomics data and associated transcriptomics and most recently MS-imaging data from multiple species.

3. LipidomeDB Data Calculation Environment (lipidome.bcf.ku.edu:8080/Lipidomics/) An online site for data processing for direct-infusion mass spectral data.

**Patents**

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