

Project/Activity Number: NC-1203

Project/Activity Title: LIPIDS of Crops Annual Meeting

Period Covered: 2017-2018

Date of This Report: October 22, 2018

Annual Meeting Date(s): September 15-16, 2018

Participants: Allen, Douglas - Danforth Center/USDA-ARS; Cahoon, Edgar - University of Nebraska (UNL); Clemente, Thomas - UNL; Hoffman-Benning, Susanne - Michigan State University; Koo, Abe - University of Missouri-Columbia (UMC); Kosma, Dylan - University of Nevada; Lee, Young-Jin - Iowa State University; Minton, Ernie - Kansas State University (KSU); Narayanan, Sruthi - Clemson University; Parkash, Om - University of Massachusetts - Amherst; Roston, Rebecca - UNL; Schrick, Kathrin - KSU; Stone, Julie - UNL; Thelen, Jay - UMC; Wang, Xuemin - Danforth Center/University of Missouri - St. Louis; Welti, Ruth - KSU.

Brief summary of minutes of annual meeting: The 2018 NC-1203 meeting was held September 15-16 in Columbia, Missouri at the University of Missouri. The meeting was opened by the Chair and the host, Jay Thelen, followed by 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 Ruth Welti, Jay Thelen and Ed Cahoon; accomplishments for each of the aims are summarized below. Dylan Kosma then provided a brief overview of his work on identification of transcriptional regulators of wound suberin deposition to introduce his research program to the group. This was followed by discussion of future plans for each of the aims and the timing of the 2019 annual meeting to be held in Lincoln, Nebraska. Douglas Allen was elected as secretary and the Danforth Center was chosen as the venue for the 2021 meeting.

NC-1203 2017-2018 Accomplishments.

Activities and accomplishments related to each of the project's three objectives are described below. These were presented at the meeting by Ruth Welti (Objective 1), Jay Thelen (Objective 2) and Ed Cahoon (Objective 3). 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.

A new Sciex 6500+ triple quadrupole MS with differential ion mobility spectrometry, funded by an NSF MRI grant is installed at the Kansas Lipidomics Research Center (KLRC) and is functional; Ruth Welti and Tim Durrett have been trained on the instrument. The Welti lab has developed and published an abbreviated lipid extraction method, tested on Arabidopsis and sorghum leaves. The group has also reduced the time required for direct-infusion MRM analysis of ~370 lipids from 30 min to ~9 min. Polar lipid internal standard mixes are now available through the KLRC.

The Markham group has used scheduled MRMs to increase the number of analyses/time during chromatography and is working on intact ion/fragment lipidome map using

accurate mass and chromatography. They are incorporating LC and prediction of retention times into their analytical strategy. Novel ceramide standards have been synthesized. The Lee group has been developing new MALDI matrices for the visualisation and localisation of lipids. They screened various metal target deposition as a MALDI matrix, and found gold doped with sodium dramatically improves TAG signals but suppress phospholipids. Lee and Durrett submitted a NIFA proposal to study the spatiotemporal development of acetyl-TAG in genetically engineered camelina.

The Schrick lab developed and published a fluorescent labeling approach to determine the subcellular localization of choline phospholipids in plants. Data suggest choline phospholipids accumulate preferentially in guard cells. Schrick and Lee were successful in MS imaging of phosphatidylcholine (PC) in the epidermis of *Arabidopsis* leaves. Schrick received a Big 12 Faculty Fellowship to support this collaborative research.

Work in the Hoffman-Benning lab demonstrated that ¹⁴C-labeled PA moves in the phloem from source to sink, but need to confirm whether the labeled mobile compound is still PA and whether PLAFP affects its movement. They are currently designing a FRET experiment to determine whether PLAFP interacts or moves with the lipid in vivo. An NSF EAGER grant has been awarded for this work.

Wurtele is currently waiting for data sets from participants for the Plant/Eukaryotic and Microbial Systems Resource (PMR; <http://metnetdb.org/PMR/>). She has developed a semi-automated mechanism for upload of data that is connected to Araport. Welti reported that the LipidomeDB Data Calculation Environment will be updated from Sept. 19-21, 2018.

The update includes improved ability to edit compounds and fragments, and, most importantly, ability to process MRM data with or without differential ion mobility. Processing includes isotopic deconvolution and normalization to internal standards.

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

The Cahoon group has isolated a new DGAT1-type enzyme from *Cuphea avigera*, with improved specificity for 10:0-CoA. The Cahoon group has also demonstrated that oil from Chinese violet cress has two novel C24 dihydroxy fatty acids which gives it superior performance as a metal-on-metal lubricant. They are determining the oil structure with Kent Chapman (University of North Texas) and colleague in materials engineering and are working to improve the plant as a potential new oilseed.

A class of novel lipid-binding proteins in phloem sap has been identified by the Hoffman-Benning lab. Overexpression causes larger plants with bigger vascular bundles.

In collaboration with John Dyer (USDA-ARS), the Koo group demonstrated that plants engineered to contain higher leaf oil content were differentially susceptible to pest attack depending on the protein that was up-regulated for oil body production.

Dylan Kosma reported the identification of 2 transcription factors in potato that appear to regulate all steps of wound suberin deposition. The same TFs also regulate other signaling and metabolic aspects of plant wound responses.

The Kutty lab showed that wheat decreases the unsaturation levels of plastidic and extraplastidic glycerolipids of leaf and/or pollen in order to adapt to heat stress.

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. They have identified the nuclear localization signal for these transcription factors.

A family of negative regulators to ACCase called the BADCs was discovered by the Thelen group. These proteins resemble the BCCP subunit to ACCase. Knocking out increases oil content in Arabidopsis and the effect is currently being tested in camelina and soybean. The Thelen group also demonstrated that the alpha-carboxyltransferase subunit to ACCase is less abundant in vivo and therefore limits fatty acid synthesis; in collaboration with Koo, overexpression increases oil content in Arabidopsis and camelina.

Doug Allen reported that tobacco engineered to have 30% leaf oil because there is no transient starch. In collaboration with Durrett, he has identified soybean fast neutron mutant lines to reduce oligosaccharides and increase oil.

Research from the Wang group has explored lipid-protein interaction 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.

Rebecca Roston reported that lipid changes in response to cold are equivalent to those observed in response to the diurnal cycle.

Aim3: Develop crops with improved yield and functionality

Through co-expression of AtDGAT1 and GPD1, the Parkash group has increased the oil and seed yield of camelina. They have also identified WRI1, MGAT1, PDCT1 and SDP1 genes as key genes for further improving TAG synthesis and accumulation and are currently overexpressing these genes individually and in combination in camelina.

Allen and Wang coexpressed two Arabidopsis phospholipase genes Dz1 and Dz2 in *Camelina sativa* to test how the conversion of PC to DAG impacts TAG levels in seeds.

The Allen group has developed a new method developed to look at absolute protein quantities using LC-MS/MS and a full set of C13 amino acid standards. This approach compares well to Kjeldahl and other traditional methods.

The Durrett group has developed transgenic camelina lines that produce over 90% acetyl-TAG. These lines have been crossed with lines producing medium-chain fatty acids or ricinoleic acid to further alter the properties of acetyl-TAGs. In collaboration with Toni Wang, the viscosity and cold-temperature properties of blends of acetyl-TAG and regular TAG were quantified.

CRISPR genome editing was used by the Clemente group to modify sorghum K1C genes to create variants with reduced karfirin levels and improved protein quality and digestibility. In collaboration with Cahoon, Clemente enhanced aquaculture feed quality of soybean with EPA, Vitamin E and astaxanthin. However, increased astaxanthin results in decreased seed oil.

Roston and Clemente have collaborated to improve cold tolerance in wheat and sorghum through the expression of transcription factors. Work by Clemente and Markham to modify sphingolipid content has led to improved cold temperature tolerance in sorghum.

Sam Wang has been working to increase abiotic resistance in Arabidopsis and to some extent in rice.

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 potential of QQS and NF-YC4 to increase protein and improve defensive traits in crops.

Impact Statements.

The LIPIDS of Crops Multi-state research project 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. The members of NC-1203 have enjoyed another productive year of research and we have achieved almost all the projected milestones for 2017-2018 (see milestone progress below). The combined output of the group is reflected in 10 publications (see publication listing below). 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)

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

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

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

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

Partially-accomplished (Wurtele)

PMR is linked to other databases

Partially-accomplished (Wurtele)

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

Done for camelina at Michigan State (Parkash)

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 consumption 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 one or more stresses

Evaluate efficacy of oil body protein strategy in transgenic Arabidopsis thaliana

2020 goals

Extraction and property analysis of modified seed oil

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.

Field trials for improved resistance to one or more stresses.

Publications

Bansal S, Kim H-J, Na G, Hamilton ME, Cahoon EB, Lu C, Durrett TP. (2018) Towards the synthetic design of camelina oil enriched in tailored acetyl-triacylglycerols with medium-chain fatty acids. *Journal of Experimental Botany* 69:4395

Belayneh HD, Wehling RL, Cahoon EB, Ciftci ON (2018) Lipid composition and emulsifying properties of *Camelina sativa* seed lecithin. *Food Chemistry* 242:139

Chikara S, Abdullah H, Akbari P, Ablordeppey K, Parkash Dhankher O. (2018) Combined expression of GPD1 and DGAT1 increased oil and seed yield in *Camelina sativa*. *Plant Biotechnology* 16:1034

Li X, Teitgen AM, Shirani A, Ling J, Busta L, Cahoon RE, Zhang W, Li Z, Chapman KD, Berman D, Zhang C, Minto RE, Cahoon EB (2018) Discontinuous fatty acid elongation yields hydroxylated seed oil with improved function. *Nature Plants* 4:711

Narayanan S, Prasad PVV, Welti R. (2018) Alterations in wheat pollen lipidome during high day and night temperature stress. *Plant, Cell and Environment* 41:1749

Paper JM, Mukherjee T, Schrick K. (2018) Bioorthogonal click chemistry for fluorescence imaging of choline phospholipids in plants. *Plant Methods* 14:31

Raju SKK, Barnes A, Schnable JC, Roston RL. (2018) "Low-temperature tolerance in land plants: Are transcript and membrane responses conserved?" *Plant Science* doi: 10.1016/j.plantsci.2018.08.002

Shiva S, Enniful R, Roth MR, Tamura P, Jagadish K, Welti R. (2018) An efficient modified method for plant leaf lipid extraction results in improved recovery of phosphatidic acid. *Plant Methods* 14:1

Yu XH, Cahoon RE, Horn PJ, Shi H, Prakash RR, Cai Y, Hearney M, Chapman KD, Cahoon EB, Schwender J, Shanklin J (2018) Identification of bottlenecks in the accumulation of cyclic fatty acids in camelina seed oil. *Plant Biotechnology Journal* 16: 1926-1938

Yurchenko O, Kimberlin A, Mehling M, Koo AJ, Chapman KD, Mullen RT, Dyer JM. (2018) Response of high leaf-oil *Arabidopsis thaliana* plant lines to biotic or abiotic stress. *Plant Signaling and Behavior* 13: e1464361.