Project/Activity Number: NC-1203

Project/Activity Title: LIPIDS of Crops Annual Meeting Period Covered: 08/15/2023 - 7/19/2024

Date of This Report: 2024

Annual Meeting Date: July 16th, 2024

# Participants

Allen, Doug - USDA/Donald Danforth Plant Science Center; Cahoon, Edgar - University of Nebraska, Lincoln (UNL); Clemente, Thomas - UNL; Durrett, Timothy - Kansas State University (KSU); Hoffmann-Benning, Susanne - Michigan State University; Huang, Chien-Yu - Louisiana State University; Louis, Joe - UNL; Roston, Rebecca - UNL; Schrick, Kathrin - KSU; Thelen, Jay - University of Missouri (MU), Columbia; Welti, Ruth - KSU; Dhankher, Om Parkash Dhankher - University of Massachusetts, Amherst; Koo, Abraham - MU; Narayanan, Sruthi - Clemson University; Wang, Xuemin (Sam) - Donald Danforth Center; Yandeau-Nelson, Marna - Iowa State University (ISU); Van Doren, Steven - MU; Bates, Phil - Washington State University; Tamborindeguy, Cecilia - Texas A&M;, Scott Peck - MU; Young-Jin Lee - ISU; Adam Yokom - MU;

# Brief Summary of Minutes of Annual Meeting

The 2024 NC-1203 meeting was held as a hybrid in-person and Zoom meeting on July 16, 2024, at the University of Nebraska, Lincoln, to take advantage of the International Symposium on Plant Lipids occurring at the same location. Presentations by the participants occurred throughout the society meeting. We have plans to request a new academic advisor from Kansas State, and proposed addition of several future members. Future meeting sites: In 2025 we plan to hold the annual meeting in Kansas City (alternatively, at Kansas State University) hosted by Kathrin Schrick, in 2026 the meeting will be held at the University of Nevada, Reno (hosted by Dylan Kosma), and in 2027 the meeting will be held in conjunction with the 2027 Gordon Research Conference on Plant Lipids, likely in California, with more than half of attendees planning to attend. Philip Bates will host the 2027 business meeting. Hosting beyond 2026 will depend on renewal of the project. Timothy Durrett volunteered to organize writing of the renewal with Ruth Welti, Susanne Hoffmann-Benning, and Rebecca Roston volunteering to assist.

# NC-1203 2023-2024 Accomplishments

Activities and accomplishments related to each of the project’s three objectives are described below. The report also summarizes the collaborative research efforts of LIPIDS of Crops members including the establishment of new collaborations as well as the outcomes of existing collaborations in the form of publications and grant awards.

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

The **Welti and Durrett groups** (Kansas) collaborated to create a novel semi-targeted mass spectral approach for lipid analysis, capable of yielding over 100,000 mass spectral intensities representing both known and unknown lipids. This method was applied to camelina plants, with the resulting data correlated with transcriptomic analyses by **Trupti Joshi's group** (Missouri). This interdisciplinary effort not only produced valuable insights into lipid metabolism during seed development but also set the stage for future collaborative projects aimed at refining lipid data annotation techniques.

In addition to advancing lipid analysis methods, **Welti’s group** (Kansas) has been instrumental in establishing a global Plant Lipid Interest Group under the International Lipidomics Society. This collaboration involves plant lipidomics labs from Germany, the UK, France, the US, and other countries. The group's first major initiative, a ring trial comparing lipid analyses across different labs, exemplifies the collaborative spirit fostered by this grant. The ongoing work aims to standardize lipidomics practices and make common reference materials available to the global plant lipidomics community, ensuring consistency and reliability in lipid research.

The **Lee group** (Iowa) has also made significant contributions, developing mass spectrometry imaging with isotope labeling to study lipid biosynthesis in various plant species, including duckweed, Arabidopsis, and maize. Their spatiotemporal analysis of lipid labeling patterns has provided new insights into membrane lipid restructuring and biosynthesis, with findings published in multiple papers. These advancements are a direct result of collaborative efforts supported within NC-1203, highlighting its role in pushing the boundaries of lipid characterization and measurement.

# Objective 2: Identify lipid-related mechanisms to increase agricultural resilience

Several labs have focused on the role of lipids in plant signaling and metabolism. The **Hoffmann-Benning lab** (Michigan) developed an innovative optogenetics method to study phloem lipids and lipid-binding proteins, enabling the monitoring of these proteins' movement within plants. This work is being complemented by the **Schrick lab** (Kansas), which has been investigating lipid-binding transcription factors and their role in gene expression regulation. Through collaboration with the **Roeder group** (New York) at Cornell, the **Schrick** lab is exploring how lipids interact with transcription factors to influence plant development. These studies collectively enhance our understanding of lipid-mediated signaling and its impact on plant growth.

Another area of focus has been the relationship between lipid composition and plant resilience to environmental stress. The **Allen** (USDA) **and Bates labs** (Washington), in collaboration with the **Gehan lab** (Missouri) at the Danforth Center, studied how lipid-engineered tobacco plants respond to temperature changes, revealing that increased lipid levels in guard cells can affect stomatal function and leaf temperature regulation. Similarly, the **Roston lab** (Nebraska), in collaboration with the **Welti** (Kansas) and **Durrett labs** (Kansas), has been investigating the role of lipids in cold tolerance, profiling plant responses to low temperatures. These studies highlight the importance of lipid stability and composition in enhancing crop resilience to various environmental challenges.

The metabolic pathways and biosynthesis of lipids in crops have also been a key focus, with the **Bates lab** (Washington) working with USDA scientists to discover a novel metabolic pathway, "triacylglycerol remodeling," in the Brassicaceae species. This pathway influences oil composition in oilseed crops, and their findings are poised to impact agricultural practices. Similarly, the **Yandeau-Nelson and Nikolau teams** (Iowa) have employed synthetic biology approaches to study cuticle synthesis in maize, focusing on how gene mutations affect cuticular wax composition and its protective functions. Their work, in collaboration with **Joe Louis** (Nebraska), is expected to extend to biotic stress resistance, further enhancing crop resilience.

The role of lipids in plant-pathogen interactions has been explored by the **Tamborindeguy lab** (Texas), which has identified bacterial lipoproteins that manipulate plant defenses, setting the stage for better disease management strategies. This theme is echoed in the work of the **Huang lab** (Louisiana), which has been studying the lipid droplets in soybean and fungal pathogens, identifying inhibitors that suppress fungal growth. Both labs are contributing to the development of lipid-based approaches for managing plant diseases.

Finally, the **Welti lab** (Kansas), in collaboration with **Durrett** (Kansas)**, Schrick** (Kansas)**,** and **Trupti Joshi** (Missouri), is advancing our understanding of lipid metabolism by characterizing genes involved in cuticle biosynthesis and sequencing the transcriptome of Chinese elm. Their research is uncovering new insights into lipid functions in plants, with potential applications in crop improvement.

# Objective 3: Develop crops with improved yield and/or functionality

Multiple collaborations originating in the NC1203 have significantly advanced research on improving crop yield and functionality through lipid production. For instance, the **Durrett** lab's work (Kansas) on cloning and characterizing novel seed-specific promoters from camelina, alongside their collaboration with the **Allen** group (USDA), resulted in the generation of soybean lines with improved amino acid compositions, contributing to a better understanding of gene expression's impact on metabolic pathways. Similarly, the Bates lab leveraged triacylglycerol remodeling mechanisms to engineer camelina for enhanced fatty acid compositions, and their findings are now being tested for broader applications.

The **Thelen** lab (Missouri), in collaboration with the **Koo** (Missouri), **Bates** (Washington), and **Allen** (USDA) labs, used global profiling methods to study the metabolic effects of enhancing acetyl-CoA carboxylase activity in Brassicaceae. This collaborative effort uncovered unexpected insights into fatty acid turnover and catabolic pathways, which could inform future strategies for improving oil content in crops.

In another notable partnership, the **Cahoon** lab (Nebraska) teamed up with the Hongfei **Lin** lab at Washington State University to develop biodesigned camelina oils for sustainable aviation fuel (SAF) production. Their work optimized oilseed feedstock for SAF production, achieving high alkane yields that closely mimic Jet A composition, contributing to the SAF Grand Challenge.

Further, the **Dhankher** lab's (Massachusetts) work on engineering camelina for increased oil yield and fatty acid composition, through transcriptomic, metabolomic, and lipidomic approaches, identified key genes responsible for these traits. Their findings have led to significant increases in seed yield and oil content in engineered camelina lines, providing a strong foundation for future crop improvement efforts.

The **Yokom** (Missouri), **Van** **Doren** (Missouri), and **Thelen** (Missouri) labs collaborated to study the structural aspects of acetyl-CoA carboxylase activity in pennycress and other species, focusing on the protein-protein interactions that drive lipid production. Their work has advanced our understanding of the regulation of de novo fatty acid synthesis, offering new targets for crop engineering.

The **Koo** lab's (Missouri) collaboration with the **Welti** (Kansas) and **Allen** (USDA) labs resulted in significant progress in metabolic engineering to increase biomass oil content, with findings under review for publication. Similarly, the **Clemente** (Nebraska) lab's research on the genetic basis of soybean seed protein accumulation led to the identification of gene edits that impact seed protein content and maturity, with ongoing studies aimed at further elucidating these genetic pathways.

In summary, NC-1203 facilitated critical partnerships that advanced the scientific understanding of lipid metabolism and crop yield improvement and laid the groundwork for future research and development efforts, underscoring the impact on the plant lipid field.

# 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. Additionally, LIPIDS of Crops is working to improve crop resilience to environmental stresses, including those associated with climate change. The NC-1203 group has interacted collaboratively to achieve project milestones during the year as indicated by milestones and 40 publications, 10 grant proposals funded, and 1 patents listed below, as well as standards and protocols that have been shared among participants. Future work will focus on completing the remaining and future milestones.

# 2024

* Development of in vivo isotope labeling for mass spectrometry imaging of plant metabolites.
* Analysis of post-translational regulation of  lipid-binding transcription factors during growth and development
* Quantification of protein, lipid, and carbohydrate contents of soybean seed with altered protein amino acid quality.
* Analysis of oil, protein and amino acid content revealed similar oil content between transgenic lines engineered to suppress enzymes associated with cys and met turnover and wild-type control plants, elevated total protein levels (including increased protein-bound cys and met) in transgenic lines, as well as increased free cys and met.
* Wound-healing and lipidomic measurements of engineered potato and camelina lines completed.
* Field trials of DHA-producing soybean lines were conducted.
* Metabolomics of first-iteration of improved camelina astaxanthin lines completed.
* Field trials conducted on second generation of astaxanthin-producing camelina.
* Phenotypic and genotypic measurements of second-generation of sorghum vegetative oil lines completed.
* Single-nuclei sequencing on developing glabra2 (gl2) mutant versus wild-type seed to determine cell- and tissue-specific gene expression differences correlated with higher seed oil production.
* Analysis of the chromatin-remodeling mechanism that lipid-binding HD-Zip IV transcription factors utilize in controlling gene expression.
* Proximity labeling of a lipid sensing HD-Zip IV transcription factor followed by MS-based proteomics to determine the protein complex involved in regulation of gene expression.
* Characterization of the mechanism underlying subcellular localization of transcription factors in response to lipid changes. A predicted nuclear localization signal (NLS) was found to be necessary and sufficient for nuclear localization of two HD-Zip IV transcription factors. While the NLS overlaps with the DNA binding domain, mutant analysis shows that the two functions can be separated. Protein-protein interaction studies and mutant analysis indicate that an alpha importin is required for nuclear import. This work was completed and the paper has been published (Ahmad et al. 2024).

# Publications

# Abdullah, H.M., Pang, N.,  Chilcoat, B., Shachar-Hill, Y., Schnell, D.J., and Dhankher, O.P.. Overexpression of the Phosphatidylcholine: Diacylglycerol Cholinephosphotransferase (PDCT) Gene Increases Carbon Flux Towards Triacylglycerol (TAG) Synthesis in Camelina sativa Seeds. Plant Physiology & Biochemistry, 208: 108470 (2024). https://doi.org/10.1016/j.plaphy.2024.108470

# Alexander, L.E., Winkelman, D., Stenback, K.E., Lane, M., Campbell, K.R., Trost, E., Flyckt, K., Schelling, M.A., Rizhsky, L., Yandeau-Nelson, M.D., Nikolau, B.J. 2024. The impact of GLOSSY2 and GLOSSY2-LIKE BAHD-proteins in affecting the product profile of the maize fatty acid elongase. *Front Plant Sci.* 15: 1403779. doi: 10.3389/fpls.2024.1403779

# Ahmad, B., Lerma-Reyes, R., Mukherjee, T., Nguyen, H.V., Weber, A.L., Cummings, E.E., Schulze, W.X., Comer, J.R., Schrick, K. 2024. Nuclear localization of HD-Zip transcription factor GLABRA2 is driven by Importin alpha. *J. Exp. Bot.* erae326. doi:10.1093/jxb/erae326.

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# Grants awarded

PI: Doug Allen. Co-PI(s): Veena Veena, Timothy. Durrett. Agency: United Soybean Board. Title: Engineering Increased Protein and Oil in Soybeans for Improved Seed Value. Dates 10/1/2023 – 9/30/2024. Total cost: $ 207,183.

PI: Edgar Cahoon; Co-PI: Tom E. Clemente, Nebraska Soybean Board. Title: Biotechnological Development of Optimized Soybean Germplasm for Aquaculture Feedstock. Dates 10/1/2023-9/30/2024. Total cost: $75,000

PI: Edgar Cahoon; Co-PIs: Ruth Welti, Kent Chapman, Marisol Berti, Sanju Sanjaya. USDA-NIFA. Title: 26th International Symposium on Plant Lipids and 1st International Camelina Conference. 3/1/2024-3/28/2025. Total cost: $31,000.

PI: Edgar Cahoon; Co-PIs: Ruth Welti, Kent Chapman, Sanju Sanjaya. NSF. Title: 26th International Symposium on Plant Lipids. 3/1/2024-3/28/2025. Total cost: $21,900.

PI: Edgar Cahoon; Co-PIs: Ruth Welti, Kent Chapman, Sanju Sanjaya. DOE. Title: 26th International Symposium on Plant Lipids. 3/1/2024-3/28/2025. Total cost: $10,000.

PI: Edgar Cahoon; Co-PI: Erich Grotewold. DOE. Title:1st International Camelina Conference. 8/15/2024-8/14/2025. Total cost: $8,000

PI Rebecca Roston. Co-PIs Toshi Obata, James Schnable, Frank Harmon. NSF-PGRP “RESEARCH-PGR: Cycling to low-temperature tolerance” 05/2024 - 04/2027 Total cost: $1,800,000

PI: Steven Van Doren, co-Is: Jay Thelen, Philip Bates. Environmental Molecular Sciences Laboratory and Joint Genome Institute FICUS program. “Structural mechanisms of enzyme regulation to open the tap of plant oil synthesis” 10/2024 – 9/30/2026. In-kind value in 2024: $80,000

PI: Steven Van Doren. University of Missouri Research Council. “Enzyme Controlling Synthesis of Oils and Biofuel in Crops: Validation of Structural Models” 11/2023 - 12/2024 Total cost: $14,993

PI: Steven Van Doren. co-I Adam Yokom. MU CAFNR Joy of Discovery program. “A Braking Mechanism at Initiation of Oil Synthesis by a New Winter Cover Crop” 4/2024 - 3/2026 Total cost: $19,998

# Software

Nothing to report.

# Patents

 Kim H, Park K, Cahoon EB (2023) Methods and compositions for making ketocarotenoids. Application Date 10/09/2023