Project/Activity Number: NC-1203
Project/Activity Title: LIPIDS of Crops Annual Meeting
Period Covered: 2021-2022
Date of This Report:
Annual Meeting Date(s): September 17, 2022

Meeting Participants: Cahoon, Edgar - University of Nebraska (UNL); Clemente, Thomas - UNL; 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); Schrick, Kathrin - KSU; Thelen, Jay - University of Missouri - Columbia; Welti, Ruth - KSU; Dhankher, Om Parkash - University of Massachusetts Amherst; Koo, Abraham - MU; Narayanan, Sruthi - Clemson University; Wang, Xuemin (Sam) - Donald Danforth Center; Yandeau-Nelson, Marna - Iowa State University; Van Doren, Steven - University of Missouri; Bates, Phil - Washington State University; Tamborindeguy, Cecilia - Texas A&M

Brief summary minutes of annual meeting: The 2022 NC-1203 meeting was held as a hybrid Zoom-in person meeting on September 17 at Michigan State University. The meeting was opened by the host Susanne Hoffmann-Benning. A time of introductions was followed by presentations and discussions for each of the individual project aims. Multistate Project Administrative Advisor Ernie Minton joined later and gave comments about the current status of the group and the need for the group to find another Multistate Project Administrative Advisor. 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 2023 annual meeting hosted by Tom Clemente at the University of Nebraska Lincoln and establishment of future meeting sites; in 2024 the meeting will be held at the University of Nevada Reno (hosted by Dylan Kosma) and in 2025 in Kansas City (alternatively, at Kansas State University) (hosted by Kathrin Schrick).

NC-1203 2021-2022 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/or grant activities.

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

Welti, Markham, and Lee groups, in collaboration with Durrett, are working toward developing a semi-quantitative and semi-targeted catalog of the occurrence and amounts of lipids from Arabidopsis and crop plants. In 2022, they worked on optimizing analysis conditions and standardizing a protocol for comprehensive fatty acid-based negative precursor and positive neutral loss scanning and on incorporation of data from Arabidopsis leaves of varied accessions and camelina seeds into a visual lipidome map.

The Lee group has optimized MALDI-MS imaging conditions to visualize camelina seed lipids. Additionally, an *in vivo* isotope labeling method is currently being developed for MS imaging.

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

Welti, Durrett, and Schrick collaborated on characterization of plant genes identified in a lipid profiling screen of Arabidopsis mutants in which the lipid profiles are significantly different from wild-type plants. One mutant possessed a novel allele of a plastid-localized omega-6 desaturase, consistent with altered ratios of two plastid galactolipids. Heterologous expression of the mutant protein in yeast demonstrated a lack of desaturase activity and a reduction in protein stability (Lusk et al., 2022). Other mutants identified in the lipid profiling screen affect genes with functions in fatty acid desaturation, cutin metabolism, and transacylase activities.

Welti and Durrett collaborated with colleagues in India to characterize acyltransferases of the Himalayan plant, *Buglossoides arvensis*, incorporating polyunsaturated fatty acids into seed triacylglycerols (Parchuri et al., 2022). Welti and Durrett are also characterizing the lipid and gene expression changes in camelina seeds during development and germination.

The Schrick lab demonstrated that a lipid-sensing domain from plant-specific homeodomain leucine-zipper (HD-Zip IV) transcription factors is critical for both protein stability and homodimerization (Mukherjee et al., 2022). The group also showed that although fluorescent protein tags are useful for live imaging and affinity purification, they may lead to enhanced protein stability and changes in agronomically important traits (Subedi and Schrick, 2022). Ongoing work highlights the role of lysophosphatidylcholines in binding to the lipid sensor from a family member that regulates cell-type differentiation of the epidermis.

To address aspects of protection of yield under abiotic stresses, the Narayanan lab, in collaboration with the Welti group, showed that decreases in the unsaturation levels of plastidic and extra-plastidic glycerolipids in leaf and/or pollen is an adaptive outcome in multiple agronomic crops (wheat, soybean, peanut, and *Brassica carinata*) exposed to heat stress (Rustgi et al., 2021; Zoong Lwe et al., 2021). Similar lipid changes are observed in heat stress adaptation in leaves and pollen, though the lipidomes have inherently distinct compositions. In soybean, an observed decrease in levels of lipids containing 18:3 acyl chains (linolenic acid) under heat stress is suggestive of a link with reduced expression of *Fatty Acid Desaturase (FAD) 3A* and *FAD3B* genes. This decrease in 18:3, in heat tolerant genotypes, likely facilitates maintenance of membrane functionality upon exposure to heat. With funding from the USDA-NIFA, Narayanan and Welti are characterizing the heat tolerance of members of a soybean recombinant inbred line population (derived from a genetic cross between a heat-tolerant and a susceptible genotype) and elite soybean genotypes by measuring physiological responses and identifying molecular markers associated with heat-induced lipid metabolic changes. Narayanan lab also investigated lipid alterations under heat stress conditions that might affect seed oil quality. Their recent work found that heat stress during the early flowering stage does not affect seed fatty acid contents in conventional oleic peanut varieties (Kakati et al., 2022). On the other end of the temperature stress spectrum,

the Welti and Wang groups collaborated to identify specific lipid changes associated with plant freezing tolerance (Vu et al., 2022).

The Hoffmann-Benning lab has been studying phloem lipids and lipid-binding proteins with a role in long distance signaling. We have studied their gene expression in response to various abiotic stresses, their localization, and their lipid-binding properties. We have also compared their gene expression to that of lipases that might generate the lipid ligands. We have now successfully established an optogenetics method that allows researchers to induce gene expression in a single leaf and monitor movement of these lipid-binding proteins throughout the plant. Next, we will attempt a FRET approach to monitor protein-lipid interaction during movement. Molecular dynamic simulation has given an indication of the interaction between our protein of interest (PLAFP - Phloem Lipid-associated family protein) and its ligand phosphatidic acid. A combination of mutagenesis, lipid-binding assays and optogenetics / movement studies is currently used to study the importance of specific amino acids on protein-lipid interaction and movement. In the context of this research, the lab has started a collaboration with Dr. Phil Lewis at USDA (Otis Lab) to identify phloem compounds conveying resistance against spotted lanternfly.

The Thelen, Bates, and Koo labs together demonstrated that expression of the pea alpha-CT gene in *Camelina sativa* increases seed oil accumulation (Wang et al., 2022). Lipid flux analysis in these lines demonstrated increased fatty acid biosynthesis that favored triacylglycerol accumulation, but still utilized the membrane lipid phosphatidylcholine as a key intermediate of oil biosynthesis. These data emphasize the overlapping nature of essential membrane lipid biosynthesis with storage oil synthesis. Camelina lines and transgenic Arabidopsis lines overexpressing the same alpha-CT construct, as well as *badc1badc3* double knockout lines, were subject to RNAseq and TRAPseq analysis. The TRAPseq technique complements the proteomics approach to analyze the translational efficiency of ribosome-associated RNAs that are actively engaged in protein translation. The Koo lab in collaboration with Dong Xu at University of Missouri analyzed the data and found several lipid-related genes with significant changes in translational efficiency. Koo lab discovered several pieces of evidence that strongly supported a post-translational mode of regulation for a phospholipase A1 enzyme involved in jasmonic acid (JA) hormone biosynthesis during mechanical stress-elicited JA biosynthesis (Kimberlin et al., 2022). JA is a key lipid-derived hormone controlling many multiple aspects of defense response during plant interaction with insects and fungal pathogens (Koo and Arimura, 2022).

The Louis lab in collaboration with Welti lab are working towards identifying the involvement of host lipids in sorghum defense against sugarcane aphids (SCA), a relatively new and devastating pest of sorghum in the U.S. Previously, we identified varied levels of resistance to SCA in founder lines of the sorghum NAM population compared to RTx430, the common parent used for these NAM lines (Grover et al., 2022). SCA reproduction was low on the inbred line SC265, intermediate on RTx430, and high on SC1345 lines. We utilized electrospray ionization mass spectrometry (ESI-MS) to compare the lipids of SCA resistant, susceptible, and RTx430 sorghum plants in response to SCA feeding. A total of 227 lipids were identified in ESI-MS from SCA infested or uninfested leaves sampled at early and late timepoints in three sorghum genotypes. We are currently working toward identifying the

specific role of sorghum lipid(s) in resistance to aphids and expect to characterize novel aspects of plant lipid metabolism after aphid infestation.

The Kosma and Cahoon labs are working towards identifying the enzymes required for the synthesis of a unique class of lipids with known antimicrobial and anticancer activities, falcarins. Falcarins are a subgroup of polyacetylenic lipids that contain multiple triple bonds and are found in specific, diverse taxa including plants from the Apiaceae (e.g., carrot, celery, parsley), Asteraceae (e.g., sunflowers), and Araliaceae (e.g., ginseng) among others. Kosma and Cahoon recently received funding from NIFA to study the falcarin biosynthetic pathway in carrot and other species (Kosma et al., 2021) and published a review paper on falcarin biosynthesis (Santos et al., 2022). More recently the Cahoon lab developed T_0 transgenic carrot lines overexpressing known and candidate genes from the falcarin biosynthetic pathway. The Kosma lab has developed a semi-*in vitro* hairy root transformation system for characterizing candidate falcarin biosynthesis genes via gain and loss of function approaches. Kosma's group is also developing an extensive tissue and developmental atlas of falcarin accumulation in carrots.

The Cahoon lab, working in collaboration with investigators at Lund University and Swedish University of Agricultural Sciences, developed camelina lines that produce oils enriched in (*Z*)-11-hexadecenoic acid, a sex pheromone precursor in several moth species. These oils provided a feedstock from which the precursor was isolated, purified and transformed into the final pheromone. Trap lures containing this pheromone were assessed for their capacity to manage moth pests in the field. Plant-derived pheromone lures proved equally effective as synthetic pheromone lures in monitoring the diamondback moth, *Plutella xylostella*, in cabbage and disrupting mating of cotton bollworm, *Helicoverpa armigera*, in common bean fields. Our study demonstrated the biological efficacy and economic feasibility of pheromone production in plant factories by metabolic engineering of an oilseed crop (Wang H-L et al., 2022).

The Roston and Schrick labs collaborated to identify that cold tolerance does depend on sterols, and this beginning effort will be followed up. The Roston and Schachtman labs identified that sorghum root exudates include variable amounts of sorgoleone, which changes the ability of sorghum to recruit a strong root microbiome (Wang et al., 2021). The Roston lab is working toward identifying the evolutionary similarities in membrane changes in response to severe low temperature.

The Bates and Allen labs have been studying leaves engineered to produce high levels of lipids. Their work describes the capacity of some plants, such as tobacco, to store starch in leaves non-transiently over the course of development. The sink capacity of these leaves can be converted to store lipid at significant levels which appears to be less successful in plants without the non-transient starch capacity. The work resulted in a publication (Chu et al., 2022) and led to questions about the ability of these plants to subvert abiotic stresses. Given that membrane lipids must maintain a semi-fluid state, the labs are currently investigating the consequences of high lipid production in leaves on temperature stress tolerance.

The Yandeau-Nelson and Nikolau labs collaborated to dissect the genetic networks underlying cuticle development during early seedling establishment in maize. The genetics of cuticle deposition were characterized by integrating metabolomics and transcriptomics data gathered from six different maize seedling organs of four agronomically important maize genotypes. These datasets captured the developmental transition of the seedling from heterotrophic skotomorphogenic growth to autotrophic photomorphogenic growth, which is a transition that is highly vulnerable to environmental stresses. Joint statistical analyses integrated these datasets to reveal gene-to-metabolite relationships in three gene networks connected with the deposition of different fractions of the cuticle: a) cuticular waxes; b) cutin of aerial organs and suberin of roots; and c) both of these fractions. These networks consist of genes that encode known components of the machinery that supports cuticle deposition, demonstrating the utility of this integrated omics approach. Moreover, these gene networks (e.g., transcription factors, post-translational regulators, and phytohormones) reveal three additional metabolic programs that appear to support cuticle deposition, including processes of chloroplast biogenesis, lipid metabolism, and molecular regulation. This lays the groundwork for new targets for modulating properties of this protective barrier. In addition, the Yandeau-Nelson team has probed weather parameters that impact cuticular wax composition on mature maize silks. Using joint statistical analysis of weather data across the growing season and cuticular wax profiles from mature silks for large genetic diversity panels grown in three unique environments, we identified associations between solar radiation patterns at early stages of silk development and precipitation patterns just prior to silk emergence into the external environment that influence cuticular wax compositions of mature silks. This work points to critical windows in development and specific metabolites to target for abiotic stress tolerance.

Dhankher lab characterized a bifunctional wax synthase/acyl-CoA:diacylglycerol acyltransferase (WSD1) gene from Arabidopsis, which plays a critical role in wax ester synthesis in stem and leaf tissues. To enhance plant productivity under adverse conditions, they constitutively over-expressed *AtWSD1* in Arabidopsis and Camelina. The qRT-PCR analysis showed a strong upregulation of WSD1 transcripts by mannitol, sodium chloride (salt), and abscisic acid (ABA) treatments, particularly in Arabidopsis thaliana shoots. Gas chromatography and electron microscopy analyses of Arabidopsis seedlings overexpressing WSD1 showed higher deposition of epicuticular wax crystals and increased leaf and stem wax loading in WSD1 transgenics compared to wild-type plants. WSD1 transgenics exhibited enhanced tolerance to ABA, mannitol, drought and salinity. Furthermore, transgenic plants showed reduced cuticular transpiration rates and cuticle permeability, as well as less chlorophyll leaching compared to the wild type. The knowledge from Arabidopsis was translated to the oilseed crop *Camelina sativa* (L.) Crantz. Similar to Arabidopsis, transgenic Camelina lines overexpressing WSD1 also showed enhanced tolerance to drought stress (Abdullah et al., 2021).

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

Through lipid metabolic flux analysis, the Bates lab has identified a novel triacylglycerol remodeling pathway of seed oil synthesis in burgeoning industrial oil crop *Physaria fendleri* (Bhandari and Bates, 2021). Further characterization of *P. fendleri* lipid metabolic genes has indicated that *PfeSDP1* expression during seed development reduces both total seed oil and

levels of the industrially valuable hydroxylated fatty acid, lesquerolic acid (Azeez et al., 2022).

The Durrett and Allen labs collaborated to alter the partitioning of carbon between oil, oligosaccharides, and protein in soybeans. Specifically, by suppressing the GmSDP1 lipases expressed late in seed development they were able to increase seed oil content and reduce accumulation of unwanted oligosaccharides (Aznar-Moreno et al., 2022).

Acetyl-TAGs are unusual TAG molecules that possess reduced viscosity due to the presence of an sn-3 acetate group. Acetyl-TAGs can be synthesized in transgenic oilseeds by expressing DAG acetyltransferases isolated from species that naturally produce acetyl-TAG. By suppressing or mutating endogenous enzymes that compete for the supply of DAG and acetyl-CoA substrates, the Durrett lab has successfully generated transgenic pennycress capable of accumulating up to 98 mol% acetyl-TAG in seed oil. Characterization of these ultra-high acetyl-TAG lines is ongoing.

Research led by the Cahoon lab discovered that seed oils of many *Thunbergia* species contain up to 92% of the unusual monounsaturated petroselinic acid (18:1Δ6), one of the highest reported levels for a single fatty acid in plants. To elucidate the biosynthetic origin of petroselinic acid, we identified a Δ6-stearoyl-acyl carrier protein (18:0-ACP) desaturase from *Thunbergia laurifolia*, closely related to a previously identified Δ6-palmitoyl-ACP desaturase that produces sapienic acid (16:1Δ6)-rich oils in *Thunbergia alata* seeds. Guided by a *T. laurifolia* desaturase crystal structure obtained in this study, enzyme mutagenesis identified key amino acids for functional divergence of Δ6 desaturases from the archetypal Δ9-18:0-ACP desaturase and mutations that result in nonnative enzyme regiospecificity. Furthermore, the team demonstrated the utility of the *T. laurifolia* desaturase for the production of unusual monounsaturated fatty acids in engineered plant and bacterial hosts. Through stepwise metabolic engineering, the team provided evidence that divergent evolution of high-level petroselinic acid and sapienic acid production arises from biosynthetic and metabolic functional specialization and enhanced expression of specific enzymes to accommodate metabolism of atypical substrates (Gan et al., 2022).

The Clemente lab, working in collaboration with the Ana Alonso lab (University of North Texas) and Leah McHale lab (Ohio State University), evaluated the metabolic and transcriptional responses to *AtWRI1* and *AtDGAT1* expression in soybean seeds. *AtWRI1* is a master regulator of fatty acid (FA) biosynthesis, and *AtDGAT1* encodes an enzyme catalyzing the final and rate-limiting step of triacylglycerol biosynthesis. Expressing these genes in the embryo did not show an increase in total FA content, but did result in changes in the oil and carbohydrate composition. Transcriptomic studies revealed a down-regulation of genes putatively encoding for oil body packaging proteins, and a strong induction of genes annotated as lipases and FA biosynthesis inhibitors. Novel putative AtWRI1 targets, presenting an AW-box in the upstream region of the genes, were identified by comparison with an event that harbored only *AtWRI1*. Lastly, targeted metabolomics analysis showed that carbon from sugar phosphates was used in FA competing pathways, such as starch and cell wall polysaccharides, contributing to the restriction in oil accumulation. These results

allowed the identification of key cellular processes that need to be considered to break the embryo's natural restriction on uncontrolled seed lipid increase (Arias et al., 2022).

The Cahoon and Clemente labs developed camelina and soybeans for aquaculture feed that produce seed oils enriched in the high-value carotenoid, astaxanthin [(3*S*,3′*S*)-3,3′-Dihydroxy-β,β-carotene-4,4′-dione)]. Astaxanthin is a red lipophilic pigment derived from β-carotene and is distinguished by keto groups on each ionone ring ("ketocarotenoid"). Research focused on the discovery of sustainable sources and cost-effective production of natural astaxanthin for use in aquaculture feed and as a natural pigment. Flower petals of Adonis (*Adonis aestivalis*) are one of the few plant sources of this high-value ketocarotenoid. The team focused on the transfer of the Adonis astaxanthin biosynthetic pathway to camelina and soybean seeds for cost-effective, oilseed-based production. Three genes, encoding phytoene synthase (PSY), Adonis β-carotene ketolase (CBFD2) and Adonis β-carotene hydroxylase (HBFD1), were introduced into camelina under the control of seed-specific promoters. The production of ketocarotenoids (~136 μg/g DW) containing astaxanthin (~47 μ/g DW) was obtained in "Asta-camelina" seeds. In addition to the production of astaxanthin, Asta-camelina seeds also produced a novel ketocarotenoid containing one ketone and three hydroxy groups, which is considered an intermediate or derivative in astaxanthin biosynthetic pathways. The astaxanthin-containing oil extracted from Asta-camelina seeds had higher oxidative stability during storage duration than normal camelina seed oil. Astaxanthin biosynthetic genes, *CBFD2* and *HBFD1*, also functioned when transiently expressed in *Nicotiana benthamiana* leaves. Furthermore, it was observed that *N. benthamiana* leaves expressing a combination set of Adonis and bacterial (crtW and crtZ) astaxanthin-biosynthetic genes more effectively converted biosynthetic intermediates to astaxanthin than leaves expressing bacterial genes alone. Overall, results indicated that Adonis genes are a valuable genetic source for astaxanthin metabolic engineering in oilseed crops and for generating vegetable oils with enhanced antioxidant capacity and high-value aquaculture traits.

Through CRISPR-Cas9-based gene editing, the Kosma lab has developed potato lines with enhanced storage life via reduced sprouting (enhanced dormancy) and reduced shrinkage. These lines were developed by targeting transcription factors with a presumed function in regulating the deposition of the lipid-phenolic polymer suberin, which is an integral part of the native skin of potatoes as well as the wound periderm.

Thelen and Van Doren labs are studying the structure-function relationships between catalytic and regulatory subunits of ACCase. Using biophysical techniques, including NMR, MST, and mass spectrometry, we are investigating the biochemical nature and structural basis for the dynamic interactions of the regulatory subunits BADC, CTI, and PII (which collectively now outnumber the catalytic subunits) with their catalytic counterparts. While studies are currently being conducted with recombinant proteins in a pairwise manner, this reductionist vantage point, in conjunction with the development of an expanded ACCase AQUA-MRM assay that includes PII and CTI isoforms, will provide important data (e.g. Kd and Ki values, absolute abundance levels in vivo) to make predictions about ACCase form and function *in situ*.

Dhankher lab previously developed *Camelina sativa* lines, co-expressing AtDGAT1 and yeast GPD1, with more than 60% increase in overall seed and oil yields. They utilized metabolomic and transcriptomic profiling approaches and identified metabolic bottlenecks that control oil production and accumulation in seeds of AtDGAT1+ScGPD1 Camelina lines. Accordingly, they selected several candidate genes/enzymes for metabolic engineering of Camelina. They targeted the overexpression of the Camelina *PDCT* gene, a homolog of the Arabidopsis *Reduced Oleate Desaturation 1* (*ROD1*) gene, which encodes phosphatidylcholine:diacylglycerol cholinephosphotransferase 1 enzyme. PDCT is proposed to act as a gatekeeper responsible for the interconversions of diacylglycerol (DAG) and phosphatidylcholine (PC) pools. To test the hypothesis that increased PDCT activity in developing Camelina seeds would enhance carbon flux toward increased levels of TAG and alter oil composition, Dhankher group overexpressed Cs*PDCT* gene under the control of the seed-specific phaseolin promoter. Transgenic Camelina plants exhibited significant increases in seed mass and seed oil content, higher seed and oil yields per plant and altered polyunsaturated fatty acid (PUFA) content compared to their parental wild-type (WT) plants. Results from [^{14}C] acetate labeling (in collaboration with Yair Shachar-Hill at MSU) of Camelina embryos developing in culture indicated increased rates of fatty acid incorporation into glycerolipids. This resulted in higher total radiolabeled lipid content in the PDCT transgenic lines, particularly in TAG and DAG lipid classes, relative to WT embryos. It was concluded that overexpression of PDCT appears to be a positive strategy to achieve a synergistic effect on flux through the TAG synthesis pathway, thereby increasing oil yields in *C. sativa* (Abdullah et al., 2022, under review).

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 44 publications, 26 grant proposals funded, and 1 patent listed below and standards and protocols that have been shared among participants. Future work will focus on completing the remaining and future milestones:

Milestones:

<u>2022</u>

- *Development of on-tissue chemical derivatization for mass spectrometry imaging.* The Lee group has optimized MALDI-MS imaging conditions to visualize camelina seed

lipids. Additionally, an *in vivo* isotope labeling method is currently being developed for MS imaging.

- *Identification of genes with altered expression in transgenic plants with modified lipid metabolism.* Combined transcriptomic and lipidomic analyses of developing seeds identified differentially expressed genes highly correlated with the accumulation of high levels of acetyl-TAG in transgenic camelina lines. Transcriptomic studies of soybean lines engineered for enhanced fatty acid and TAG flux revealed a down-regulation of genes putatively encoding for oil body packaging proteins, and a strong induction of genes annotated as lipases and FA biosynthesis inhibitors.
- *Quantitative analysis of the role of surface lipids in crop resilience against biotic and/or abiotic stress.* 1) Targeted knockout of a transcription factor implicated to regulate wound suberin deposition led to unexpected phenotypes of delayed tuber sprouting and reduced shrinkage in cold storage. 2) Specific weather parameters at specific stages in maize development were identified that impact cuticular wax composition on maize silks and will ultimately provide metabolite biomarkers for protective capacity against solar radiation and drought stresses.
- *Quantitative analysis of the stress response of phloem lipid-binding proteins.* A heatmap displaying the gene expression of 10 phloem lipid binding proteins as well as several phospholipases in response to multiple abiotic environmental factors has been generated. The gene expression studies together with localization and lipid-binding studies are currently being compiled into a manuscript.
- *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, our 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. The connection to lipid metabolism is being further investigated.
- *Quantification of seed composition in soybean lines engineered for reduced lipid turnover.* RNAi lines for SDP1 in soybean were produced and evaluated resulting in an increase in lipid and decrease in oligosaccharides without negatively impacting protein levels in the seed. The results were published in early 2022.
- *Camelina engineered for reduced lipase activity and overexpression of acyltransferase genes.* CRISPR and RNAi constructs targeting candidate seed lipases have been transformed into camelina. RNAi constructs have been combined with acetyltransferase overexpression and introduced into camelina.
- *Validation of ≥3 candidate genes for enhanced astaxanthin production in model systems.* Camelina and soybean lines were generated that accumulate >100 μg/g astaxanthin per seed weight.
- *Phenotypic and genotypic data for sorghum lines engineered with first iteration of a vegetative oil construct completed.* Sorghum lines developed that accumulate up to 7% DW TAG in leaves and 4% DW TAG in stems.
- *Camelina with >5% higher seed oil content due to overexpression of the least abundant catalytic subunit to ACCase.* Targeted quantitation of the catalytic subunits of the plant heteromeric ACCase showed that the alpha-carboxyltransferase subunit was up to 10-fold lower in absolute abundance than its partner subunit beta-

carboxyltransferase during Arabidopsis seed development. We hypothesized that overexpression of the alpha subunit could enhance ACCase activity, resulting in a greater "push" of carbon into *de novo* fatty acid synthesis and ultimately into storage lipids. Using the pea ortholog and expressing both the full length and catalytic region alone, ACCase specific activity and seed oil content in Arabidopsis were enhanced. The same constructs were expressed in camelina and an increase in seed oil content was observed, supporting the hypothesis that the alpha-carboxyltransferase subunit is an important and perhaps limiting subunit for ACCase activity.

Publications

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Grants awarded

1. PI: Laura Bartley; Co-PI(s): Gary Stacey, Jay Thelen, Dong Xu; Agency: NSF; Program: PGR; Dates: 09/01/2020-08/01/2024; Title: RESEARCH PGR: Genome-enabled characterization of orphan receptor like kinases in plants; Total cost: $2,000,000.

2. PI: Lee, Young-Jin; Agency: NSF; Program: ISO-PGR; Dates: 02/01/2022-01/31/2025; Title: Mass Spectrometry Imaging of in vivo Isotope Labeling; Total cost: $635,498 (direct $466,167).

3. PI: Jiujiu Li; Co-PI(s): Rebecca Roston; Agency: NIH; Program: National Institute of Diabetes & Digestive & Kidney Diseases Dates: 04/01/2022-03/01/2025; Title: Role of chive-derived exosome-like nanoparticles in suppressing inflammation in obesity; Total cost: $1,734,810.

4. PI: T. Durrett, co-PI: D. Allen. Agency: United Soybean Board. 10/01/2021-09/30/2022; Title: Reducing Undesirable Carbohydrates During Seed Development for Enhanced Protein & MEAL; Total cost: $158,810

5. PI: Durrett TP; Co-PI(s): Allen DK, Veena V; Agency: United Soybean Board; Dates: 10/01/2019-09/30/2021; Title: Enhancing Stable Protein Levels in Developing Soybean Seeds; Total cost: $340,672

6. PI: Kathrin Schrick. Agency: NSF-MCB; Dates: 9/15/2016 - 8/30/2023; Title: START Lipid/Sterol Binding Domains in Homeodomain Transcription Factors from Plants; Total cost: $385,150.

7. PI: T. Durrett, co-PIs: D. Allen, V. Veena, S. Kambhampati. Agency: United Soybean Board. Dates: 10/01/2020-09/30/2021; Title: Blocking Production of Non-metabolizable carbohydrates and improving protein amino acid profile in soybeans; Total cost: $157,869

8. PI: Abraham J Koo; Agency: National Science Foundation; Program: IOS; Dates: 01/01/2022-12/01/2024; Title: Regulation of the early steps of wound-activated jasmonate biosynthesis; Total cost: $846,276.

9. PI: Susanne Hoffmann-Benning; Collaborator(s): Phil Lewis; Agency: USDA; Program: APHIS; Dates: 09/30/2022-09/29/2023; Title: Identifying Attractive Components of Phloem Sap for Spotted Lanternfly Control; Total cost: $75,625.

10. PI: Sruthi Narayanan; Co-PI(s): Rustgi S (Clemson University); Collaborator(s): Fallen B (USDA-ARS Raleigh, NC), Smith J (USDA-ARS Stoneville, MS), and Welti R (Kansas State University); Agency: USDA NIFA; Program: AFRI Foundational Program ; Dates: 01/01/2022-12/31/2026; Title: Improving soybean's efficiency for heat tolerance with an integrated metabolic and genetic approach; Total cost: $649,895.

11. PI: Marna Yandeau-Nelson; Co-PI(s): Basil J. Nikolau; Collaborator(s): Erin E. Sparks (University of Delaware); Rajib Saha (University of Nebraska); Agency: National Science Foundation; Program: MCB BIO; Dates: 08/01/2022-07/31/2025; Title: Collaborative Research: PlantSynBio: Deciphering the roles of genetic and biochemical redundancy and pathway regulation via refactoring the protective plant cuticle; Total cost: $2,647,000.

12. PI: Dylan Kosma; Co-PI(s): Won Yim, Edgar Cahoon; Agency: NIFA; Program: AFRI; Dates: 01/15/2021-01/14/2024; Title: Understanding The Biosynthesis Of Health-Promoting Polyacetylenes In Carrot; Total cost: $500,000.

13. PI: Candice Hirsch; Co-PI(s): Marna Yandeau-Nelson; Agency: NSF; Program: PGRP; Dates: 10/01/2016-09/01/2023; Title: ECA-PGR: Dissecting Natural Mechanisms for Genome Content Variation and the Impact on Phenotypic Variation; Total cost: $2,198,800.

14. PI: Jay Thelen; Co-PI(s): Phil Bates, Abe Koo, Doug Allen, Dong Xu; Agency: NSF; Program: PGR; Dates: 11/01/2018-10/01/2022; Title: RESEARCH-PGR: Discovering

new metabolic constraints and regulatory nodes in oilseeds engineered for enhanced fatty acid synthesis and seed oil; Total cost: $3,437,639.

15. PI: Rustgi S. (Clemson University); Co-PI(s): Narayanan S (Clemson University), Kulis M.D., Kim E. (both from The University of North Carolina), Dunne J.C., and Andres R. (both from North Carolina State University); Agency: Foundation for Food and Agriculture Research; Program: Seed the solutions; Dates: 01/01/2022-12/31/2026; Title: Reduced-immunogenicity high-oleic peanuts through applied innovation (genome-editing and breeding); Total cost: $609,816.

16. PI: Ed Cahoon; Co-PI(s): D Allen, P Bates, T Durrett, J Fox, M Gehan, T Joshi, C Lu, M Smanski, J Thelen, R Welti, D Xu; Agency: DOE; Program: Bioenergy Research; Dates: 10/01/2022-09/01/2027; Title: B5: Bigger Better Brassicaceae Biofuels and Bioproducts ; Total cost: $12,800,000.

17. PI: Sruthi Narayanan; Co-PI(s): Agency: National Peanut Board; Dates: 07/01/2022-06/30/2023; Title: Identification of molecular markers for breeding for heat tolerance in peanut; Total cost: $22,494.

18. PI: Fallen B. (USDA-ARS, Raleigh, NC); Co-PI(s): Narayanan S (Clemson University), Mian R (USDA-ARS Raleigh, NC), Walker D (USDA-ARS Raleigh, NC), Lorenz A (University of Minnesota); Agency: United Soybean Board; Program: ; Dates: 10/01/2021-09/30/2022; Title: Developing high protein, low oil content soybeans that provide enhanced nutritional and economic value; Total cost: $184,702.

19. PI: T. Durrett, co-PIs: YJ. Lee, U. Yucel. Agency: USDA-NIFA; Dates: 2020-2023; Title: Optimizing the temporal and spatial synthesis of functional lipids In developing seeds; Total cost:. $500,000.

20. PI: E. Cahoon, co-PIs: A. Ramer-Tait, P. Verlander. Agency: USDA-NIFA Research and Extension Experiences for Undergraduates; Dates: 1/1/2022-12/31/2026. Title: Expanding Opportunities in Agricultural Sciences: Crop-to-Food Innovation; Total cost: $742,000.

21. PI: S. Sanjaya, co-PI: E. Cahoon. Agency: USDA-NIFA; Dates: 3/1/2022-2/28/2025. Title: Storage Compound Biosynthetic Mechanisms and Photosynthetic Processes for Enhancing Crops Yield; Total cost: $150,000.

22. PI: E. Cahoon, co-PI: T. Clemente. Agency: Nebraska Soybean Board; Dates: 10/1/2021-9/30/2022. Title: Development of Improved Soybean Germplasm for Aquaculture Feed; Total cost: $69,000.

23. PIs: E. Cahoon, Y. Blume. Agency: Civilian Research and Development Fund (CRDF) Global; Dates: 10/1/2021-9/30/2022. Title: 2021 US-Ukraine Alternative Energy Competition: Optimization of sorghum as an economically viable advanced biofuel feedstock; Total cost: $15,000.

24. PI: A, Leakey, Investigators: T. Clemente, E. Cahoon. Agency: DOE Bioenergy Research Center; Dates; 11/1/2017-10/30/2022. Title: Center for Advanced Biofuels and Bioproducts Innovation (CABBI); Total cost: $3,800,000 to UNL.

25. PI: Om P. Dhankher, coPIs: B Xing, J. White, V Dhandapani. Agency: NIH RO1 award; Dates: 03/09/2021 - 12/31/2025. Title: A novel strategy for phytoremediation of rrsenic. (We are engineering non-food oil seed crop Crambe abyssinica for arsenic accumulation and detoxification). Total cost: $1,177,305.

26. PI: K. Glowacka, coPIs: R. Roston, N. Buan, J. Stone. Agency: Nebraska Agricultural Experiment Station (NEAES) ; Dates: 10/1/2022- 9/30/2027. Title: Regulation of Photosynthetic Processes (NC-1200); Total cost: $250,000.

Software

None to report.

Patents

1. Berman D, Chapman KD, Romsdahl DB, Minto RE, Zhang C, Cahoon EB (2021) Liquid and semisolid lubricant compositions, methods of making, and uses thereof. US Patent #11,136,525