

Project/Activity Number: NC-1203

Project/Activity Title: LIPIDS of Crops Annual Meeting

Period Covered: 2020-2021

Date of This Report:

Annual Meeting Date(s): August 27, 2021

Participants: Allen, Doug - Danforth Center/USDA; Bates, Philip - Washington State University (WSU); Cahoon, Edgar - University of Nebraska (UNL); Clemente, Thomas - UNL; Dhankher, Om Parkash - University of Massachusetts Amherst; Durrett, Timothy - Kansas State University (KSU); Hoffmann-Benning, Susanne - Michigan State University; Koo, Abraham - MU; Kosma, Dylan - University of Nevada Reno (UNR); Lee, Young-Jin - Iowa State University (ISU); Louis, Joe - University of Nebraska- Lincoln; Minton, Ernie - KSU; Narayanan, Sruthi - Clemson University; Nikolau, Basil - ISU; Roston, Rebecca - UNL; Schrick, Kathrin - KSU; Stone, Julie - UNL; Thelen, Jay - University of Missouri - Columbia; Wang, Xuemin (Sam) - Danforth Center/UMSL; Welti, Ruth - KSU; Yandea-Nelson, Marna

Brief summary minutes of annual meeting: The 2021 NC-1203 meeting was held virtually on August 27. The meeting was opened by the host, Doug Allen and included remarks from Multistate Project Administrative Advisor Ernie Minton. Group members provided updates on experiments and collaborations. The renewal was submitted in fall 2020 and was accepted. The 2022 meeting will be held at Michigan State and hosted by Susanne Hoffmann-Benning. The 2023 meeting will be at University of Nebraska-Lincoln, hosted by Tom Clemente. The 2024 meeting will be hosted by Dylan Kosma at University of Nevada-Reno.

NC-1203 2016-2021 Accomplishments.

Activities and accomplishments related to each of the project's three objectives are described below. In addition, the report communicates on the collaborative efforts among the LIPIDS members, including outcomes of these collaborations in the form of publications and/or grant activities.

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

The Welti group has made all the reagents and methods available for relatively rapid (8 min/sample) direct- infusion multiple reaction monitoring (MRM) mass spectrometry using a Sciex 6500+ triple quadrupole MS (Song et al., 2020). This deliverable includes a rapid extraction method (Shiva et al., 2018), which was independently tested by Roston's group (Mahboub et al., 2020), standards (available at cost), all parameters for MRM, including a data acquisition protocol, downloadable from the web (https://www.k-state.edu/lipid/analytical_laboratory/analysis_components/index.html), along with a complete data processing pipeline, that employs Sciex software and the online site LipidomeDB Data Calculation Environment. The Lipidome DB Data Calculation Environment site has been updated twice: once to support lipids analysis by multiple reaction monitoring (Fruehan et al., 2018; Song et al., 2020), and once to support collision induced fragment analysis by time-of-flight mass spectrometry. This latter technique can be used for fatty acid analysis at the chemical

formula level (Tamura et al., 2020). Markham's group developed a cross disciplinary platform to measure lipids and model, with flux balance analysis, permitting the establishment of the blueprints outlining the steps controlling flux through metabolism.

The Lee group is developing on-tissue chemical derivatization techniques to enhance the visualization and localization of low-abundance metabolites and lipids. This technique has successfully been employed, in conjunction with three derivatization methods, for primary amine, carboxylic and carbonyl functional groups that resulted in the visualization of over 600 new metabolites in maize leaf and root tissue cross sections (Objective 2). Enrichment of Arabidopsis, oxylipins, fatty acid derived signaling molecules associated with plant immune responses, in chloroplasts of FERONIA mutant, was detected employing this approach which was recently communicated (Hansen et al 2019). A collaborative effort between team members Durrett and Lee resulted in a funded USDA NIFA project to study the spatiotemporal localization of acetyl-TAG in camelina carrying a novel transgenic allele. Moreover, the Lee group archived the last ten years' of MS imaging data at METASPACE repository (<https://metaspace2020.eu/>) for public access.

The Schrick lab developed a fluorescent labeling approach to study *in situ* localization of choline phospholipids in plants (Paper et al., 2018). Data suggest choline phospholipids accumulate preferentially in guard cells. In collaboration with the Lee group they were successful in MS imaging of phosphatidylcholines in the epidermis layer of Arabidopsis leaves.

Other LIPIDS team members are using radiolabel reagents, as well as fluorescence resonance energy transfer (FRET) microscopy, and mass spectrometry tools to interrogate lipid metabolism. For example, the Hoffmann-Benning group developed an approach to reproducibly collect, purify and analyze lipids from phloem exudates (Hoffmann- Benning, 2021). They are currently assembling a set of plant usable light-switch elements (PULSE) constructs (red light induced; blue/white light repressed) which can be controlled both spatially and temporally, and allow for monitoring of lipid-binding proteins and their movement. Proof-of-concept of the PULSE constructs has been successfully shown in protoplasts and tobacco model systems. The PULSE constructs have been introduced into Arabidopsis and down-stream characterizations will be initiated shortly. The Hoffman-Benning group is also working on a FRET platform to determine location of protein-lipid interactions and translocation of the complex *in planta*. An NSF EAGER grant is funding to support this latter effort.

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

This component of the LIPIDS program targets two aspects of crop production i) the role of lipids in response to environmental factors, that can be translated to genetic approaches to protect yield and ii) the improvement of lipid profile and/or content in crop plants, and implementing what is learned from these activities to design genetic strategies to improve quality of output traits:

The three KSU groups, Welti, Durrett, and Schrick are collaborating on functional characterization of several plant genes that were identified in a lipid profiling screen of Arabidopsis T-DNA mutants in which the complex lipid profiles are significantly different from wild-type plants. The genes identified from this screen have annotated functions in fatty acid desaturation, cutin metabolism, and transacylase activities.

Another collaborative effort among the Welti, Schrick, and Wang groups resulted in a communication that described the changes which occur in Arabidopsis lipid composition and metabolism under heat stress (Shiva et al., 2020). While a collaboration between the Welti and Koo groups, has ongoing efforts seeking to gain insight on the genetic variation and the impact on the phenotypic outcomes of genes involved in lipid metabolism. Particularly, they are looking at variation that impacts enzyme kinetics during response to wounding and mild freezing stress.

The Louis lab screened a maize NAM population to identify resistance towards insect predation, exploring the role of lignin, surface waxes, and other lipids in the plant defense responses upon an insect challenge. The outcome of this effort found that 12-oxo-phytodienoic acid (OPDA), an intermediate in the jasmonic acid (JA) biosynthesis pathway, promotes heightened maize resistance against corn leaf aphids, independent of the JA pathway.

More recently, Kosma has collaborated with John Cushman (non-member, UNR) to identify and characterize *Camelina* mutants with reduced seed mucilage content that may improve the efficacy of seed processing for oil (Lohaus et al., 2020) and with Isabel Molina (non-member) to provide an extensive characterization of the cuticular waxes, cutin, and suberin of *Camelina* (Razeq et al., 2021). Kosma continues to collaborate on understanding mechanisms for the regulated biosynthesis of cuticular lipids (We et al., 2021).

Kosma's main focus, however, targets understanding the gene regulatory networks controlling the wound suberin deposition with an emphasis on suberin function in potato tuber wound healing given the significant postharvest losses attributed to poor wound healing experienced by the potato industry (~33% *per annum*). Kosma has successfully identified 5 transcription factors in potato, 2 of which were described in a recent publication (Wahrenburg et al., 2021) and 4 in Arabidopsis that regulate different aspects of suberin deposition. Using an RNAseq approach, Kosma has generated community resources in the form of an eFP browser that permits exploration of global changes in the transcriptome of potato cultivars that differ in their capacity to deposit wound suberin and storage life and allows exploration of coexpression networks. Kosma has successfully used CRISPR-Cas9 to edit 3 transcription factors that regulate wound suberin biosynthesis in potato. Haplotype sequences for these 3 transcription factors were obtained using long-range PCR and PacBio SMRT sequencing from the Atlantic cultivar. Sequencing of CRISPR-Cas9 edited lines revealed substantial differences in the editing of different haplotypes. In some cases, multiple haplotypes were edited and in some cases not. Kosma is also working with Dr. Won Yim (non-member) on developing phased genomes for 4 commercial cultivars of chipping potatoes. 2 cultivars genome sequences are ~80% resolved.

Cahoon and Kosma have worked with Lucas Busta to identify genes and corresponding enzymes in the falcarin biosynthetic pathway of carrots (Busta et al., 2018). Falcarins are lipid-derived metabolites known to function in plant defense against pathogens, to have anticancer activities, and one falcarin, falcarindiol, is known to be a major component of the bitter taste of carrots. Cahoon and Kosma recently received a USDA-NIFA grant to continue their study of the falcarin biosynthetic pathway.

Yandeu-Nelson group is the newest member of LIPIDS of Crops joining in 2021. The team, in collaboration with the Nikolau's group, is dissecting the metabolic and genetic networks underlying cuticular lipid deposition on various plant tissues, with an emphasis in maize silks and seedling organs. Using genetic statistical association approaches the team has identified metabolite-transcript correlations that highlight new gene candidates, with annotated functions in

transcriptional regulation, fatty acid elongation, wax biosynthesis, transport, sphingolipid biosynthesis, oxylipin metabolism, and galactolipids synthesis. Her group is now characterizing these candidate genes via reverse genetic and heterologous expression approaches. Moreover, the team is exploring genotype x environment interactions that impact cuticular lipid accumulation, and will test the impacts of specific environmental parameters on cuticle composition under controlled growth conditions. Additionally, the Nikolau group is integrating synthetic biology strategies to reconstitute the maize cuticular lipid pathway either in a single cell platform (i.e., yeast) (Campbell et al 2019) or in the model genetic plant species, *Arabidopsis* (Alexander et al 2020). Furthermore, in collaboration with the Lee-group, Nikolau has molecularly imaged the *in planta* deposition of cuticular lipids at a high spatial resolution (~50- μ m resolution) on the leaf surfaces of maize leaves (Duenas, et al 2017) or on *Arabidopsis* leaves that are transgenically expressing maize cuticular lipid genes (Alexander et al. 2021).

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 wheat exposed to heat stress (Narayanan et al., 2016a, 2016b, 2018). Similar lipid changes are observed in heat stress adaptation in wheat, leaves and pollen, though the lipidomes have inherently distinct compositions. This collaboration also investigated lipid metabolic changes in response to heat stress in soybean (Narayanan et al., 2020). Here 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. Decreases in unsaturation were also found to be associated with heat tolerance in peanut (Zoong Lwe et al., 2021) and *Brassica carinata* (Zoong Lwe et al., 2021). With funding from the USDA-NIFA, Narayanan and Welti will characterize 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 identify molecular markers associated with heat-induced lipid metabolic changes.

The Roston lab is also investigating aspects of protection of yield under abiotic stress. These efforts are searching for the core responses of membranes to temperature, specifically cold stress. Progress in our understanding of the role of cytosolic pH in severe cold sensing (Barnes et al. 2019), and in comparing available lipid abundance changes in related sets of species in response to cold (Yan et al., 2019, Kenchanmane et al., 2018, Zhang et al., 2017) has been accomplished. The Roston and Schrick labs started a new collaboration to investigate the roles of sterols in cold tolerance, and current data suggests that some sterol mutants have reduced freezing tolerance.

The Schrick group has also been studying the role of lipids in signaling through identification of steroidogenic acute regulatory protein (StAR)-related lipid transfer (START)-domain transcription factors. In plants START domains are found in master regulators of cell developmental fate. Here her group is looking at their role in DNA binding, gene regulation and development of trichomes and other features of the epidermis. Lysophospholipids were identified as binding partners of these START- domain transcription factors. One family member was shown to bind a phosphate starvation response element to control the expression of several

phospholipid catabolism genes. Additionally, her group has been investigating signaling functions of other lipid metabolites including sterol glucosides (Pook et al., 2017) and flavonoids (Velazhahan et al., 2020).

Along similar lines, the Hoffmann-Benning lab identified a class of novel lipid-binding proteins (LBPs) in phloem sap with possible signaling function. Expression of genes for several of these proteins increases in response to abiotic stress. Overexpression of one LBP causes larger plants with bigger vascular bundles and a modified phloem lipid. Initial experiments also suggest increased drought tolerance (Barbaglia et al., 2016). Homologues of this LBP gene in soybean and tomato are also induced by ABA, suggesting that this protein has a broader function in plants. Computational analysis and molecular simulation modeling suggested interaction sites with phosphatidic acid, a plant lipid known to be associated with stress signaling. The lab has mutagenized the respective putative phosphatidic interaction sites and is currently investigating the effect on protein-lipid interaction, subcellular localization and functional complementation of *plafp* knock-down mutants.

Research from the Wang group has focused on lipid signaling and regulation of lipid metabolism in plant growth, development, and stress responses, resulting in the identification of lipid-interacting transcription factors (Cai et al., 2019, 2020; Hoffmann-Benning, 2020).

As communicated above, the Cahoon and Kosma labs have an ongoing collaborative effort to describe the genetic and evolutionary basis for a divergence in cuticular wax chemistry between sorghum and maize. Combining chemical analyses, heterologous expression studies, and comparative genomics approaches, they found that: (i) sorghum and maize leaf waxes are similar at the juvenile stage but, after the juvenile-to-adult transition, sorghum leaf waxes are rich in triterpenoids that are absent from maize, (ii) biosynthesis of the majority of sorghum leaf triterpenoids is mediated by a gene that maize and sorghum both inherited from a common ancestor but that is only functionally maintained in sorghum, and (iii) sorghum leaf triterpenoids accumulate in a spatial pattern that was previously shown to strengthen the cuticle and decrease water loss at high temperatures. The translational outcome of these findings that is being pursued is designing and introducing into maize the genetic variation that potentially could lead towards the resurrection of a cuticular triterpenoid-synthesizing gene in maize to create a more heat-tolerant water barrier on the plant's leaf surfaces. To this end, working with the Clemente group and with Lucas Busta's laboratory (U. Minnesota-Duluth) the functional sorghum allele is being introduced into maize in an attempt to generate a sorghum-like wax profile to test the hypothesis that wax triterpenoids are associated with improved water-use efficiency in sorghum.

The Thelen group is developing genetic strategies leading towards the improvement of lipid content in oil seeds, while mitigating reduction in other seed reserves. The research targets approaches to improve acetyl-CoA carboxylase performance, including biotin/lipoyl attachment domain containing (BADC) protein repression, alpha-carboxyltransferase subunit of ACCase (alpha-CT) overexpression, and carboxyltransferase interactors (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 (Ye et al 2020). The CTIs are envelope membrane

proteins that anchor ACCase through direct interaction with the alpha-CT subunits. The group is using multiple approaches to examine structure and mechanistic outcomes of these interactions and translating the learned information for higher seed oil content with a focus on Arabidopsis, Camelina, and soybean. A subset of these large-scale biological findings will be analyzed through an NSF-Plant Genome Research project with Koo, Allen, and Bates groups. This project developed a website dedicated to the study of oil production in plants called “Fat Plants” available at fatplants.net.

The Koo lab has developed camelina events overexpressing pea alpha-CT and RNAi events of *C. sativa* BADCs. Increased seed oil content in these events have been observed, which the Bates lab demonstrated increased rates of fatty acid synthesis in these events. Additionally, events have also been developed that carry genetic elements that will permit data to be captured through Translating Ribosome Affinity Purification RNAseq (TRAPseq) analyses. The Koo lab established and refined technical conditions (Kimberlin et al., 2021) and subsequently carried out RNAseq and TRAPseq analyses on developing seeds of WT and high oil events of Arabidopsis and camelina.

Increases of oil in leaf tissue has been achieved through various metabolic pathway perturbation approaches in, Arabidopsis, tobacco and crop plants. The Koo lab has found positive correlation between increased oil in vegetative tissues and susceptibility of the plants to insect herbivory. Moreover, the group performed lipidomics and fatty acid profile analyses on leaf tissues from some high vegetative oil Arabidopsis events in collaboration with the Welti and Allen labs.

The Allen lab also completed and published an acyl carrier protein (ACP) quantification method (Nam et al. 2020) which has been leveraged to analyze plant seeds and other tissues obtained in collaboration with the Koo, Thelen, and Cahoon groups. Results from these investigations are preliminary and work is ongoing. A further description of the methods was published in 2021 (Jenkins et al. 2021) and several projects are building off the method, though the pandemic has hindered some progress and skill transfer of methods within the lab.

The Durrett and Allen labs have been collaborating to introduce genetic variation into soybeans targeting altered lipid levels by reducing the expression of lipases that are upregulated late in seed development. Work over the past several years focused on obtaining transformants and growing to homozygosity with phenotypic measurements of biomass components including lipids. Biomass from available mutants in selected gene call regions identified in a fast neutron population was assessed throughout development. These results have been communicated (Kambhampati et al. 2019). Identifying correlations between gene expression and changes in lipids, proteins and oligosaccharides is an avenue that can translate towards specific carbon partitioning outcomes in seeds. Recently, results with isotopic labeling experiments described the changes in metabolism over seed development (Kambhampati et al. 2021). Additional studies on central metabolism within a collaboration between the Allen and Clemente labs is nearing publication on the impact of enhanced malic enzyme levels in soybeans. This collaboration on carbon partitioning and flux will continue with crossing of these lines and others to improve

soybean composition. Studies on soybeans will continue in the new cycle of the LIPIDS of Crops program, including further assessment of transgenics aimed at producing high value soybeans.

The Durrett group has also investigated the role of the plastid lipase PLIP1 in supplying FA for the synthesis of TAG in developing Arabidopsis seeds. *PLIP1* is upregulated in *dgat1* seed. Double mutant *plip1 dgat1* seeds are green and fail to germinate (Aulakh and Durrett, 2019). Ongoing work is testing the hypothesis that PLIP1 provides a PUFA-substrate pool necessary for the function of PDAT1.

Research from Dhankher group uses comparative transcriptome and metabolome analysis seeking to uncover bottlenecks that limit seed and oil yields in transgenic *Camelina sativa* expressing the Arabidopsis diacylglycerol acyltransferase 1 (DGAT1) and yeast glycerol-3-phosphate dehydrogenase (GPD1). Their results show 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 (Abdullah et al., 2018). Further characterization of genes, controlling these bottlenecks is in progress. Recently, Dhankher group has ectopically expressed PDCT1 and MGAT1 gene in camelina and the derived events show increased seed and oil yields, most likely due to the increased metabolic carbon flux towards TAG synthesis and accumulation in seeds. Flux analysis on these biologicals is ongoing in collaboration with Yair Shachar-Hill lab at MSU.

The Bates lab joined in 2019. The group's work targets the analysis of lipid fluxes in *Physaria fendleri*, a burgeoning crop species that naturally accumulates unusual fatty acids with valuable industrial applications. Progress to date indicates that *P. fendleri* utilizes TAG remodeling to modify the seed oil fatty acid composition after initial synthesis (Bates & Bhandari, 2021). Ongoing efforts involves characterizing the enzymes involved in TAG remodeling and determining their value for engineering seed oil compositions, in addition the Bate's team is in collaboration with Thelen, Allen, and Koo groups on investigating the control of fatty acids synthesis.

Objective 3: Develop crops with improved yield and functionality

Acetyl-TAGs are unusual TAG molecules that possess reduced viscosity due to the presence of an sn-3 acetate group. By expressing a high activity DAG acetyltransferase, the Durrett group developed camelina events that produced seeds accumulating over 90 mol% acetyl-TAG with minimal effect on seed size, weight and fatty acid content (Alkotami et al, 2021). To further lower the viscosity of seed oil, the Durrett group, in collaboration with the Cahoon group, generated camelina plants capable of synthesizing acetyl-TAG containing medium-chain fatty acids (MCFA). Field trials of the best transgenic events enabled viscosity testing of the acetyl-TAG derived from these lines (Bansal et al, 2018). In addition, the Cahoon and Durrett groups screened a camelina EMS mutant population and identified lines with altered seed fatty acid composition. Sequencing of candidate genes revealed causative mutations in homeologs of FAB1, FAD2, FAD3 and FAE1. Crossing to generate a *fae1c/fad2a/fae1a/fad3a* quadruple mutant resulted in a mid-oleic oils with nearly 40% oleic acid and reduced very long-chain ($\leq C20$) fatty acid content.

The resulting mid-oleic acid oil had improved oxidative stability due to reductions in polyunsaturated fatty acid content, increasing its utility for biofuels and other applications (Neumann et al, 2021).

In *Arabidopsis* 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 based on recent findings from her group. Multiple plants have similar cycles in lipid abundance; cold tolerant species deviate from non-tolerant species at key times. The group also showed that lipid responses to freezing tolerance are greatly increased in ancient plant lineages, which led them to suggest that severe stress responses may be derived from a stronger ancestral response to damage, of any type.

The Wurtele group continues to investigate the relationship between the Qua-Quine-Starch allele (QQS), and carbon and nitrogen partitioning, and along with defense, in a series of *Arabidopsis* mutants affecting starch metabolism. Results indicate QQS and the transcription factor, NF-YC4, can increase protein and improve defensive traits in crops.

As mentioned above Kosma is investigating methods for improving potato tuber storage life through better wound healing. The group is now well positioned to apply gene-editing approaches towards trait improvement. Kosma is working with collaborators, Dave Douches and Ray Hammerschmidt (MSU), to identify two QTLs potentially related to better wound suberin deposition.

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 events as compared to wildtype (WT) plants (Abdullah et al., 2021). Transgenic plants showed strong tolerance to drought and salinity. These results from *Arabidopsis* were successfully translated into *Camelina* and the resulting *Camelina* events also showed strong drought tolerance and produced better seed yield under water deficiency stress. The results clearly show that the manipulation of cuticular waxes will be advantageous for enhancing plant productivity in changing climates. Dhankher group also characterized a novel stress-associated protein 13 (SAP13) from *Arabidopsis* and the overexpression of SAP13 provided strong tolerance to drought and heavy metals stress (Dixit et al., 2018). They have now transferred the *Arabidopsis* SAP13 gene in *Camelina sativa* and *Brassica juncea* to increase productivity of oil seed crops under abiotic stress conditions. The derived transgenic *B. juncea* and *Camelina* events are currently under investigation.

The Clemente lab investigated soybean harboring a transgenic allele that expressed the barley homogentisate geranylgeranyl transferase (HGGT) that leads to the synthesis of very high level of tocotrienol in the seed oil (Konda et al., 2020). In collaboration with the Cahoon lab, they assembled multi-gene expression cassettes to generate soybean oil with omega-3, astaxanthin, and high vitamin E antioxidant content for use as a sustainable aquaculture feedstock. These events have advanced to field evaluation in Mead, NE in 2019 and 2020 and new events are being generated with up to 13-GoldenBraid assembled transgene cassettes per T-DNA element. These genetic designs target both higher seed oil content, in addition to aquaculture oil-quality output

traits. This collaboration resulted in the generation of engineered soybean germplasm with 8- to 10-fold increases in total vitamin E tocopherols, primarily as tocotrienols, by seed-specific expression of a barley homogentisate geranylgeranyl transferase (*HGGT*). This trait was introgressed into soybean events engineered for oils enriched in the polyunsaturated fatty acids gamma-linolenic, alpha linolenic, and stearidonic acids. Oil extracted from *HGGT*-expressing lines had ≥ 6 -fold increase in free radical scavenging activity compared to controls. However, the oxidative stability index of oil from vitamin E-enhanced lines was $\sim 15\%$ lower than that of oil from non-engineered seeds and nearly the same or modestly increased in oil from the GLA, ALA and SDA backgrounds relative to controls. These findings show that soybean is an effective platform for producing high levels of free-radical scavenging vitamin E antioxidants, but this trait may have negative effects on oxidative stability of conventional oil or only modest improvement of the oxidative stability of PUFA-enhanced oil. Field trials were conducted in the summer of 2020 of engineered camelina events at the Eastern Nebraska Research and Extension Center in Mead, NE. Lines engineered for seed oils enriched in oleic acid, palmitic acid, myristic acid, lauric acid, decanoic acid, and astaxanthin were included in a ~ 1.9 acre planting. Seeds were harvested in July, 2020. Oils from lines with modified fatty acid composition will be used for development of new bio-based jet fuel conversion, and the astaxanthin-enriched oil will be used for development of methods for separation of carotenoids from vegetable oils. Clemente reported at the 2021 meeting that EPA and DHA production in seeds of engineered soybean events is approaching commercial relevance, and the next step is to conduct aquaculture feeding trials with extracted oil from these seeds. These events are currently being bulked by field production at ENREC in Mead, NE. Research is also ongoing to modify the protein meal quantity and quality for applications such as meat substitutes.

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 and are available for the Multistate group to facilitate metabolic engineering and crop improvement efforts. Their collaboration has also been extended to the engineering of vegetative oil production in sorghum stems. GoldenBraid assembly of modular gene-expression elements has been used to develop multiple vectors that target enhanced vegetative oil production and the production of oils with higher value fatty acid structures. These vectors have been used for generation of engineered sorghum lines, many of which are now being subjected to the design-build-test-learn cycle to obtain iterative improvements in the targeted oil traits. Utilizing the *Agrobacterium*-mediated sorghum transformation platform developed by the research team (Guo et al., 2015) they continue to pursue genetic approaches to added valuable co-products to the biomass to complement the inherent attributes to further improve this C4 feedstock contribution to the bioeconomy through targeting both input traits (Scully et al., 2018) and output traits (Kempinski *et al.*, 2019). The Clemente and Cahoon labs reported at the 2021 meeting the generation of sorghum lines with $>2\%$ triacylglycerol (TAG) in leaves and stems in greenhouse-grown lines. TAG levels of up to 5% in leaves have been observed during ongoing field studies in 2021. Studies are planned to cross TAG-producing lines in grain sorghum backgrounds into sweet sorghum to test the influence of sucrose levels on TAG accumulation.

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 NC- 1203 group has interacted collaboratively to achieve project milestones as indicated by “milestones”, 73 publications, 4 software tools, and 7 patents listed below and standards and protocols that have been shared amongst participants.

Milestones:

2017

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

Two methods developed for crop plants from the group (Welti). Roston did a comparison among several methods.

- Lipid standards made available for sharing

Phospholipid and galactolipid standards are 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

Accomplished for camelina at Michigan State and will be available publicly at this weblink: <http://camregbase.org/> (Dhankher)

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

Accomplished (Cahoon, Durrett)

- Development of camelina events 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 events

Accomplished (Clemente, Wang)

- Laboratory test for improved resistance to stress

Overexpression of PLAFP increases drought tolerance in Arabidopsis; similar stress response in tomato and soybean (Hoffmann-Benning);
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 events and their lipid metabolism (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)

Accomplished (Dhankher)

WSD1 was overexpressed into camelina to increase drought, salinity and ABA tolerance (Dhankher).

2021 goals:

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

Aphid field trials (Clemente, Louis)

Conduct polysomal RNAseq analysis on high oil lines (Koo)
Study abiotic and biotic stress tolerance of high oil lines (Koo)

Publications

Abdullah HM, Rodriguez J, Salacup JM, Castañeda IS, Schnell DJ, Pareek A, and Dhankher OP. (2021). Increased Cuticle Waxes by Overexpression of Wax Synthase, WSD1, Improves Tolerance Towards Osmotic Stresses in Plants. *Int. J. Molecular Sciences* 22(10): 5173. <https://doi.org/10.3390/ijms22105173>

Abdullah HM, Chhikara S, Akbari P, Schnell DJ, Pareek A, Dhankher OP. (2018). Comparative transcriptome and metabolome analysis revealed the bottlenecks for increasing the seed and oil yields in transgenic *Camelina sativa* expressing diacylglycerol acyltransferase 1 and glycerol-3-phosphate dehydrogenase. *Biotechnology for Biofuels*, 11: 335. doi: 10.1186/s13068-018-1326-2.

Ahsan N, Wilson RS, Thelen JJ. (2018) Absolute Quantitation of Plant Proteins. *Curr Protoc Plant Biol.* 3:1-13.

Alexander, L.E., Gilbertson, J.S., Xie, B., Song, Z., Nikolau, B.J. 2021. High spatial resolution imaging of the dynamics of cuticular lipid deposition during Arabidopsis flower development. *Plant Direct* DOI: 10.1002/pld3.322.

Alexander, L.E., Okazaki, Y., Schelling, M.A., Davis, A., Zheng, X., Rizhsky, L., et al 2020. Maize glossy2 and glossy2-like genes have overlapping and distinct functions in cuticular lipid deposition. *Plant Physiol.* 183:840-853.

Alkotami L, Kornacki C, Campbell S, McIntosh G, Wilson C, Tran TNT, Durrett TP. (2021) Expression of a high activity diacylglycerol acetyltransferase results in enhanced synthesis of acetyl-TAG in camelina seed oil. *Plant J.* 106: 953-964.

Augustin JM, Brock JR, Augustin MM, Wellinghoff RL, Shipp M, Higashi Y, Kumssa TT, Cahoon EB, Kutchan TM (2019) Field performance of terpene-producing *Camelina sativa*. *Industrial Crops and Products* 136: 50-58.

Aulakh K, Durrett TP (2019) The plastid lipase PLIP1 is critical for seed viability in diacylglycerol acyltransferase 1 mutant seed. *Plant Physiology* 180: 1962-1974. doi: 10.1104/pp.19.00600

Aznar-Moreno JA, Durrett TP (2017) Simultaneous targeting of multiple gene homeologs to alter seed oil production in *Camelina sativa*. *Plant and Cell Physiology* 58: 1260 – 1267

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

Bairagi, S., R. Perrin, L. Fulginiti, T. Clemente, C. Hungate, Economic feasibility of high omega-3 soybean oil for deep-water mariculture diets. *Aquaculture Economics & Management* 21, 452-469 (2017).

Barbaglia, A.M., Tamot, B., Greeve, V., and Hoffmann-Benning, S. (2016) Phloem proteomics reveals new lipid-binding proteins with a putative role in lipid-mediated signaling. *Frontiers in*

Plant Science/Plant Physiology; Research topic: How plants deal with stress: exploration through proteome investigation; *Front. Plant Sci.* 7:563. doi: 10.3389/fpls.2016.00563

Barnes AC, Elowsky CG, Roston RL (2019) An Arabidopsis protoplast isolation method reduces cytosolic acidification and activation of the chloroplast stress sensor SENSITIVE TO FREEZING 2. *Plant signaling & behavior.* 14:9, DOI: 10.1080/15592324.2019.1629270

Bates PD (2021) The plant lipid metabolic network for assembly of diverse triacylglycerol molecular species. In *Advances in Botanical Research.* Academic Press (in press)

Bhandari S, Bates PD (2021) Triacylglycerol remodeling in *Physaria fendleri* indicates oil accumulation is dynamic and not a metabolic endpoint. *Plant Physiology* <https://doi.org/10.1093/plphys/kiab294>

Busta L, Yim W, LaBrant EW, Wang P, Grimes L, Malyszka K, Cushman JC, Santos P, Kosma DK, Cahoon EB (2018) Identification of genes encoding enzymes catalyzing the early steps of carrot polyacetylene biosynthesis. *Plant Physiology* 178: 1507-1521.

Brands M, Cahoon EB, Dörmann P (2020) Palmitvaccenic acid (Δ 11-cis-hexadecenoic acid) is synthesized by an OLE1-like desaturase in the arbuscular mycorrhiza fungus *Rhizophagus irregularis*. *Biochemistry* 59: 1163-1172

Busta L, Schmitz EA, Kosma DK, Schnable JC, Cahoon EB (2021) A co-opted steroid synthesis gene, maintained in sorghum but not maize, is associated with a divergence in leaf wax chemistry. *Proceedings of the National Academy of Sciences, USA* 118 e2022982118

Cahoon EB, Li-Beisson Y (2020) Plant unusual fatty acids: learning from the less common. *Curr Opin Plant Biol* 55: 66-73.

Cahoon RE, Gonzalez-Solis A, Markham JE, Cahoon EB (2021) Mass spectrometry-based profiling of plant sphingolipids from typical and aberrant metabolism. In D Bartels, P Dormann, eds, *Methods in Plant Lipids* Springer

Cai G, Kim SC, Li J, Zhou Y, Wang X. Transcriptional Regulation of Lipid Catabolism during Seedling Establishment. *Mol Plant.* 2020 Jul 6;13(7):984-1000. doi: 10.1016/j.molp.2020.04.007. Epub 2020 Apr 22. PubMed PMID: 32334070.

Cai G, Fan C, Liu S, Yang Q, Liu D, Wu J, Li J, Zhou Y, Guo L, Wang X. Nonspecific phospholipase C6 increases seed oil production in oilseed Brassicaceae plants. *New Phytol.* 2020 May;226(4):1055- 1073. doi: 10.1111/nph.16473. Epub 2020 Mar 16. PubMed PMID: 32176333.

Campbell, A.A., Stenback, K.E., Flyckt, K., Hoang, T., Perera, M.A.D., Nikolau, B.J. 2019. A single-cell platform for reconstituting and characterizing fatty acid elongase component enzymes. *PLoS One* e0213620 DOI: 10.1371/journal.pone.0213620

Chen K, Alexander LE, Mahgoub U, Okazaki Y, Higashi Y, Takano K, Loneman D, Dennison TS, Lopez M, Claussen R, Peddicord L, Saito K, Lauter N, Dorman KS, Nikolau BJ, Yandea-

Nelson MD (2021) Dynamic product-precursor relationships underlie cuticular lipid accumulation on maize silks, bioRxiv, 2021.03.31.437946.

Chopra R, Johnson EB, Emenecker R, Cahoon EB, Lyons J, Kliebenstein DJ, Daniels E, Dorn KM, Esfahanian M, Folstad N, Frels K, McGinn M, Ott M, Gallaher C, Altendorf K, Berroyer A, Ismail B, Anderson JA, Wyse DL, Ulmasov T, Sedbrook JC, Marks MD (2020) Identification and stacking of crucial traits required for the domestication of pennycress. *Nature Food* 1: 84-91

Davis JA, Pares RB, Bernstein T, McDowell SC, Brown E, Stubrich J, Rosenberg A, Cahoon EB, Cahoon RE, Poulsen LR (2020) The lipid flippases ALA4 and ALA5 play critical roles in cell expansion and plant growth. *Plant Physiology* 182: 2111-2125

Dixit A, Tomar P, Vaine E., Abdullah HM, Hazen S, Dhankher OP. A stress-Associated protein, AtSAP13, from *Arabidopsis thaliana* provides tolerance to multiple abiotic stresses. *Plant, cell & environment* 41 (5): 1171-1185 (2018). <https://doi.org/10.1111/pce.13103>.

Du ZY, Hoffmann-Benning S, Wang S, Yin L, Zienkiewicz K, Zienkiewicz A, (2021) "Lipid metabolism in development and environmental stress tolerance for engineering agronomic traits" *Frontiers in Plant Science* 12: 739786 <https://doi.org/10.3389/fpls.2021.739786>

Dueñas ME, Lee Y, 2019, Towards Mass Spectrometry Imaging in the Metabolomics Scale: Increasing Metabolic Coverage through Multiple On-Tissue Chemical Modifications, *Frontiers in Plant Science*, 10, 860, DOI: 10.3389/fpls.2019.00860.

Dueñas M.E., Klein, A.T., Alexander, L.E., Yandea-Nelson, M.D., Nikolau, B.J., Lee, Y.J. 2017. High spatial resolution mass spectrometry imaging reveals the genetically programmed developmental modification of the distribution of thylakoid membrane lipids among individual cells of maize leaf. *Plant J.* 89:825-838.

Fernández-Milmanda, G. L., Crocco, C. D., Reichelt, M., Mazza, C. A., Köllner, T. G., Zhang, T., Cargnel, M.D., Lichy, M.Z., Fiorucci, A.-S., Fankhauser, C., Koo, A.J., Austin, A.T., Gershenzon, J., Ballaré, C.L. 2020. A light-dependent molecular link between competition cues and defense responses in plants. *Nat. Plants* 6:223-230.

Fruehan C, Johnson D, Welti R. 2018. LipidomeDB Data Calculation Environment has been updated to process direct-infusion multiple reaction monitoring data. *Lipids* 53, 1019-1020. doi: 10.1002/lipd.12111.

Gonzalez-Solis A, Han G, Gan L, Li Y, Markham JE, Cahoon RE, Dunn TM, Cahoon EB (2020) Unregulated Sphingolipid Biosynthesis in Gene-Edited *Arabidopsis ORM* Mutants Results in Nonviable Seeds with Strongly Reduced Oil Content. *Plant Cell* 32: 2474-2490.

Guo, X., Z. Ge, S. J. Sato, T. E. Clemente, Sorghum (*Sorghum bicolor*). *Methods Mol Biol* 1223, 181- 188 (2015).

Hagely, K., Konda, A. R., Kim, J. H., Cahoon, E. B., & Bilyeu, K. (2021). Molecular-assisted breeding for soybean with high oleic/low linolenic acid and elevated vitamin E in the seed oil. *Molecular Breeding*, 41(1), 1-13.

Hansen R, Guo H, Yin Y, Lee Y, 2019, FERONIA Mutation Induces High Levels of Chloroplast-Localized Arabidopsides which are involved in Root Growth, *Plant J.*, 97, 341-351, DOI: 10.1111/tpj.14123.

Hur M, Ware R, Park J, McKenna A, Rodgers R, Nikolau B, Wurtele E, Marshall A. 2018. Statistically significant differences in composition of petroleum crude oils revealed by volcano plots generated from ultrahigh resolution fourier transform ion cyclotron resonance mass spectra. *Energy and Fuels*. *Fuels* 32:1026–1212 DOI: 10.1021/acs.energyfuels.7b03061

Hoffmann-Benning, S (2020). Beyond membranes: The evolution of plant lipid signaling. *Molecular Plant*; 13, 952-954. doi: 10.1016/j.molp.2020.06.004

Hoffmann-Benning S (2021) Collection and analysis of phloem lipids. *Plant Lipids: Methods and Protocols*, *Methods in Molecular Biology*, vol. 2295, Chapter 19, p.351-361. D. Bartels and P. Dörmann (eds.), Springer Science+Business Media, LLC, part of Springer Nature 2021. https://doi.org/10.1007/978-1-0716-1362-7_19.

Jenkins LM, Nam JW, Evans BS, Allen DK (2021) Quantification of Acyl-Acyl Carrier Proteins for Fatty Acid Synthesis using LC-MS/MS, in *Plant Lipids: Methods and Protocols*, *Methods in Molecular Biology*, Eds. Dorothea Bartels & Peter Dormann, vol. 2295, doi.org/10.1007/978-1-0716-1362-7_13.

Kambhampati S, Aznar-Moreno JA, Bailey SR, Arp JA, Chu KL, Bilyeu KD, Durrett TP, Allen DK (2021). Temporal changes in metabolism late in seed development affect biomass composition. *Plant Physiology* 186: 874-890.

Kambhampati, S., Aznar-Moreno, J.A., Hostetler, C., Caso, T., Bailey, S.R., Hubbard, A.H., Durrett, T.P., Allen, D.K. 2020. On the inverse correlation of protein and oil: Examining the effects of altered central carbon metabolism on seed composition using soybean fast neutron mutants. *Metabolites* 10(1): 18; doi:10.3390/metabo10010018.

Kambhampati S, Li J, Evans BS, Allen DK. 2019. Accurate and efficient amino acid analysis for protein quantification using hydrophilic interaction chromatography coupled tandem mass spectrometry. *Plant Methods* 15:46-58

Kempinski, C., Z. Jiang, G. Zinck, S. J. Sato, Z. Ge, T. E. Clemente, J. Chappell, Engineering linear, branched-chain triterpene metabolism in monocots. *Plant biotechnology journal* 17, 373-385 (2019).

Kenchanmane Raju SK, Barnes AC, Schnable JC, Roston RL (2018) Low-temperature tolerance in land plants: Are transcript and membrane responses conserved? *Plant Science*. 276, 73-86. <https://doi.org/10.1016/j.plantsci.2018.08.002>

Kim S, Nusinow DA, Sorkin M, Pruneda-Paz J, Wang X. 2019. Interaction and Regulation Between Lipid Mediator Phosphatidic Acid and Circadian Clock Regulators. *Plant Cell* 31:399-416.

Kimberlin A, Holtsclaw RE, Koo AJ. (2021) Differential regulation of the ribosomal association of mRNA transcripts in an Arabidopsis mutant defective in jasmonate-dependent wound response. *Front. Plant Sci.* 12:637959.

Koenig A, Benning C, Hoffmann-Benning, S. 2020. Lipid trafficking and signaling in plants. Invited review for “Lipid Signaling and Metabolism” (Elsevier), James Ntambi ed.; Chapter 2, p. 23-44

Koenig AM, Hoffmann-Benning, S (2020) The interplay of phloem-mobile signals in plant development and stress response. *Bioscience Reports* 40: BSR20193329 <https://doi.org/10.1042/BSR20193329>.

Konda A, Nazarens TJ, Nguyen H, Yang J, Gelli M, Swenson S, Shipp JM, Schmidt MA, Cahoon RE, Ciftci ON, Zhang C, Clemente TE, Cahoon EB (2019) Metabolic engineering of soybean seeds for enhanced vitamin E tocopherol content and effects on oil antioxidant properties in polyunsaturated fatty acid-rich germplasm. *Metabolic Engineering* 57:63-73.

Li J, Wang X. 2019. Phospholipase D and phosphatidic acid in plant immunity. *Plant Science* 279:45-50

Li S, Jia S, Hou L, Nguyen H, Sato S, Holding D, Cahoon E, Zhang C, Clemente T, Yu B (2019) Mapping of transgenic alleles in soybean using a Nanopore-based sequencing strategy. *Journal of Experimental Botany* 70: 3825-3833.

Li-Beisson Y, Thelen JJ, Fedosejevs E, Harwood JL. (2019) The lipid biochemistry of eukaryotic algae. *Prog Lipid Res.* 74:31-68.

Lichy, M.D., M. Z., Fiorucci, A. S., Fankhauser, C., Koo, A. J., Austin, A. T., Gershenzon, J., and Ballaré, C. L. (2020) A light-dependent molecular link between competition cues and defense responses in plants. *Nat Plants* 6, 223-230

Mahboub S, Shomo ZD, Register RM, Albusharif M, Roston RL (2021) Three Methods to Extract Membrane Glycerolipids: Comparing Sensitivity to Lipase Degradation and Yield. *Methods Molecular Biology* 2295: 15-27 doi: 10.1007/978-1-0716-1362-7_2

McClain S, Stevenson SE, Brownie C, Herouet-Guicheney C, Herman RA, Ladics GS, Privalle L, Ward JM, Doerr N, Thelen JJ. (2018) Variation in Seed Allergen Content from three Varieties of Soybean Cultivated in Nine Different Locations in Iowa, Illinois, and Indiana. *Front Plant Sci.* 9:1025.

McGinn M, Phippen WB, Chopra R, Bansal S, Jarvis BA, Phippen ME, Dorn KM, Esfahanian M, Nazarens TJ, Cahoon EB, Durrett TP, Marks MD, Sedbrook JC (2019) Molecular tools enabling pennycress (*Thlaspi arvense*) as a model plant and oilseed cash cover crop. *Plant Biotechnol J* 17: 776-788

Moissejev G, Park K, Cui A, Freitas D, Rajagopal D, Konda AR, Martin-Olenski M, Mcham M, Liu K, Du Q, Schnable JC, Moriyama EN, Cahoon EB, Zhang C (2020) RGPDB: database of root-associated genes and promoters in maize, soybean, and sorghum. Database 2020

Msanne J, Kim H, Cahoon EB (2020) Biotechnology tools and applications for development of oilseed crops with healthy vegetable oils. *Biochimie* 178: 4-14.

Mukherjee, T., Lerma-Reyes, R., Thompson, K.A., Schrick, K. 2019. Making glue from seeds and gums: Working with plant-based polymers to introduce students to plant biochemistry. *Biochem Mol Biol Educ* 47:468-475. DOI:10.1002/bmb.21252

Nam, J.W., Jenkins, L.M, Li, J., Evans, B.S., Jaworski, J.G., Allen, D.K. 2020. A General Method for Quantification and Discovery of Acyl Groups Attached to Acyl Carrier Proteins in Fatty Acid Metabolism using LC-MS/MS. *Plant Cell* 32:820-832.

Narayanan S, Prasad PVV, Welti R. (2018). Alterations in wheat pollen lipidome during high day and night temperature stress. *Plant Cell Environ.* 41, 1749-1761. doi: 10.1111/pce.13156.

Narayanan S, Zoong Lwe ZS., Gandhi N, Welti R, Fallen B, Smith JR, Rustgi S. (2020) Comparative lipidomic analysis reveals heat stress responses of two soybean genotypes differing in temperature sensitivity. *Plants* 9: 457.

Neumann NG, Nazareus TJ, Aznar-Moreno JA, Rodriguez-Aponte SA, Veintidos VAM, Comai L, Durrett TP, Cahoon EB (2021) Generation of camelina mid-oleic acid seed oil by identification and stacking of fatty acid biosynthetic mutants. *Industrial Crops and Products* 159: 113074.

O'Neill K, Lee Y, 2020, Visualizing Genotypic and Developmental Differences of Free Amino Acids in Maize Roots with Mass Spectrometry Imaging, *Frontiers in Plant Science*, 639. DOI:10.3389/fpls.2020.00639.

Ortiz R, Geleta M, Gustafsson C, Lager I, Hofvander P, Lofstedt C, Cahoon EB, Minina E, Bozhkov P, Stymne S (2020) Oil crops for the future. *Curr Opin Plant Biol* 56: 181-189

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

Park, H., S. Weier, F. Razvi, P. A. Pena, N. A. Sims, J. Lowell, C. Hungate, K. Kissinger, G. Key, P. Fraser, J. A. Napier, E. B. Cahoon, T. E. Clemente, Towards the development of a sustainable soya bean- based feedstock for aquaculture. *Plant biotechnology journal* **15**, 227-236 (2017).

Park, K., Sanjaya, S. A., Quach, T., & Cahoon, E. B. (2021). Toward sustainable production of value-added bioenergy and industrial oils in oilseed and biomass feedstocks. *GCB Bioenergy*, 00, 1– 14. <https://doi.org/10.1111/gcbb.12883>

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

Qi M, Zheng W, Zhao X, Hohenstein J, Kandel Y, O'Conner S, Wang Y, Du C, Nettleton D, MacIntosh GC, Tylka GL, Wurtele ES, Whitham SA, Li L. 2018. QQS orphan gene and its

interactor NF-YC4 reduce susceptibility to pathogens and pests. *Plant Biotechnology*. DOI:10.1111/pbi.12961. PMID:29878511

Romsdahl TB, Kambhampati S, Koley S, Yadav UP, Alonso AP, Allen DK, Chapman KD (2021) Analyzing Mass Spectrometry Imaging Data of ¹³C-labeled Phospholipids in *Camelina sativa* and *Thlaspi arvense* (Pennycress) Embryos. 11 148. doi.org/10.3390/metabo11030148

Romsdahl T, Shirani A, Minto RE, Zhang C, Cahoon EB, Chapman KD, Berman D (2019) Nature-guided synthesis of advanced bio-lubricants. *Scientific Reports* 9: 11711

Scully, E.D., T. Gries, N. A. Palmer, G. Sarath, D. L. Funnell-Harris, L. Baird, P. Twigg, J. Seravalli, T. E. Clemente, S. E. Sattler, Overexpression of SbMyb60 in *Sorghum bicolor* impacts both primary and secondary metabolism. *The New Phytologist* 217, 82-104 (2018).

Segura, R. R., Quach, T., Gomes-Neto, J. C., Xian, Y., Pena, P. A., Weier, S., Pellizzon, M. A., Kittana, H., Cody, L. A., Geis, A. L., Heck, K., Schmaltz, R. J., Bindels, L. B., Cahoon, E. B., Benson, A. K., Clemente, T. E., Ramer-Tait, A. E., Stearidonic-enriched soybean oil modulates obesity, glucose metabolism, and fatty acid profiles independently of *Akkermansia muciniphila*. *Mol. Nutr. Food Res.* 2020, 64, 2000162.

Shirani A, Joy T, Lager I, Yilmaz JL, Wang H-L, Jeppson S, Cahoon EB, Chapman K, Stymne S, Berman D (2020) Lubrication characteristics of wax esters from oils produced by a genetically-enhanced oilseed crop. *Tribology International* 146: 106234

Singh U, Hur M, Dorman K, Wurtele E. 2019. MetaOmGraph: a workbench for interactive exploratory data analysis of large expression datasets. bioRxiv. 1:698969.

Shiva S, Samarakoon T, Lowe KA, Roach C, Vu HS, Colter M, Porras H, Hwang C, Roth MR, Tamura P, Li M, Schrick K, Shah J, Wang X, Wang H, Welti R (2020) Leaf lipid alterations in response to heat stress of *Arabidopsis*. *Plants* 9: 845.

Song Y, Vu HS, Shiva S, Fruehan C, Roth MR, Tamura P, and Welti R. (2020) A lipidomic approach to identify cold-induced changes in *Arabidopsis* membrane lipid composition. Editors: D. Hinch and E. Zuther. *Methods Mol Biol.* 2156: 187-202.

Tamura P, Fruehan C, Johnson DA, Hinkes P, Williams TD, Welti R. (2021) Fatty acid composition by total-acyl-lipid--collision induced dissociation--time-of-flight (TAL-CID-TOF) mass spectrometry. Editors: D. Bartels and P. Doermann. *Methods Mol Biol.* 2195, 117-133.

Varsani S, Grover S, Zhou S, Koch KG, Huang PC, Kolomiets M, Williams WP, Heng-Moss T, Sarath G, Luthe DS, Jander G and Louis J (2019). 12-Oxo-phytodienoic acid acts as a regulator of maize defense against corn leaf aphid. *Plant Physiology*, 179: 1402-1415.

Velazhahan V, Glaza P, Herrera AI, Prakash O, Zolkiewski M, Geisbrecht BV, Schrick K (2020). Dietary flavonoid fisetin binds human SUMO1 and blocks sumoylation of p53. *PLOS One* 15:e0234468.

Viswanathan MB, Park K, Cheng M-H, Cahoon EB, Dweikat I, Clemente T, Singh V (2020) Variability in structural carbohydrates, lipid composition, and cellulosic sugar production from industrial hemp varieties. *Industrial Crops and Products* 157: 112906

Vogel PA, Bayon de Noyer S, Park H, Nguyen H, Hou L, Changa T, Khang HL, Ciftci ON, Wang T, Cahoon EB, Clemente TE (2019) Expression of the Arabidopsis WRINKLED 1 transcription factor leads to higher accumulation of palmitate in soybean seed. *Plant Biotechnology Journal* 17: 1369- 1379.

Wang, K., Durrett, T.P. and Benning, C. 2019. Functional Diversity of Glycerolipid Acylhydrolases in Plant Metabolism and Physiology. *Progress in Lipid Research*. (in press) <https://doi.org/10.1016/j.plipres.2019.100987>

Wang P, Chai YN, Roston R, Dayan FE, Schachtman DP. (2021) The Sorghum bicolor root exudate sorgoleone shapes bacterial communities and delays network formation. *mSystems*. 6(2):e00749-20. doi: 10.1128/mSystems.00749-20.

Wilson RS, Thelen JJ. (2018) In vivo quantitative monitoring of subunit stoichiometry for metabolic complexes. *J Proteome Res*. 17:1773-1783.

Xie L, Cahoon E, Zhang Y, Ciftci ON (2019) Extraction of astaxanthin from engineered *Camelina sativa* seed using ethanol-modified supercritical carbon dioxide. *Journal of Supercritical Fluids* 143: 171-178.

Yan L, Kenchanmane Raju SK, Lai X, Zhang Y, Dai X, Rodriguez O, Mahboub S, Roston RL, and Schnable JC (2019) Parallels between natural selection in the cold-adapted crop-wild relative *Tripsacum dactyloides* and artificial selection in temperate adapted maize. *Plant Journal*. doi:10.1111/tbj.14376.

Yang F, Kimberlin AN, Elowsky CG, Liu Y, Gonzalez-Solis A, Cahoon EB, Alfano JR (2019) A plant immune receptor degraded by selective autophagy. *Molecular Plant* 12:113-123

Ye Y, Fulcher YG, Sliman DJ 2nd, Day MT, Schroeder MJ, Koppiseti RK, Bates PD, Thelen JJ, Van Doren SR. (2020) The BADC and BCCP subunits of chloroplast acetyl-CoA carboxylase sense the pH changes of the light-dark cycle. *J Biol Chem*. 295:9901-9916.

Ye Y, Nikovics K, To A, Lepiniec L, Fedosejevs ET, Van Doren SR, Baud S, Thelen JJ (2020) Docking of acetyl-CoA carboxylase to the plastid envelope membrane attenuates fatty acid production in plants. *Nature Communications*. 11:6191.

Yurchenko O, Kimberlin A, Mehling M, Koo AJ, Chapman KD, et al. 2018. Response of high leaf-oil Arabidopsis thaliana plant lines to biotic or abiotic stress. *Plant Sig. Behav*. 13: e1464361

Zhang N, Rao RSP, Salvato F, Havelund JF, Møller IM, Thelen JJ, Xu D. (2018) MU-LOC: A machine- learning method for predicting mitochondrially localized proteins in plants. *Front Plant Sci*. 9:634.

Zhang, Q., Wijk, R., Zarza, X., Shahbaz, M., Vermeer, J.E.M., Guardia, A., Scuffi, D., García-Mata, C., Van den Ende, W., Hoffmann-Benning, S., Haring, M., Laxalt, A.M., and Munnik, T. 2018. Knock-down of Arabidopsis PLC5 reduces primary root growth and secondary root formation while overexpression improves drought tolerance and causes stunted root hair growth" *Plant Cell Physiol.* doi: 10.1093/pcp/pcy120

Zhang Y, Ngu DW, Carvalho D, Liang Z, Qiu Y, Roston RL, Schnable JC (2017) Differentially regulated orthologs in sorghum and the subgenomes of maize. *The Plant Cell.* 29, 1938-1951. doi: 10.1105/tpc.17.00354

Zhou X, Chen X, Du Z, Zhang Y, Zhang W, Kong X, Thelen JJ, Chen C, Chen M. (2019) Terpenoid esters are the major constituents from leaf lipid droplets of *Camellia sinensis*. *Front Plant Sci.* 26;10:179.

Zoong Lwe, Z.S., Welti, R., Anco, D., Naveed, D., Rustgi, S., Narayanan, S. 2021. Heat stress elicits remodeling in the anther lipidome of peanut. *Sci Rep.* 10, 22163. doi: 10.1038/s41598-020-78695-3.

Zoong Lwe Z., Sah S., Persaud L., Li J., Gao W., Reddy K.R., Narayanan S. 2021. Alterations in the leaf lipidome of *Brassica carinata* under high temperature stress. *BMC Plant Biol.* In Press.

Software

1. MetaOmGraph.(PMR, <http://metnetdb.org/PMR/>) Java software enabling users to input and evaluate big data sets. v2019
2. LipidomeDB Data Calculation Environment (lipidome.bcf.ku.edu:8080/Lipidomics/) An online site for data processing for direct-infusion mass spectral data.
- 3.Database of root-associated genes and promoters from maize, sorghum, and soybean, <http://sysbio.unl.edu/RGPDB/>

Patents

Cahoon EB, Iskandarov U, Collins-Silva J, Kim HJ (2019) Novel acyltransferases and methods of using. US 10,280,431 B2.

Clemente TE, Park H, Cahoon EB, Nguyen H (2018) Method for the production of high saturated, low polyunsaturated soybean oil. US Patent # US10,100,325B2.

Durrett, T., Tran, T.N.T. and Kornacki, C (2018) Improved enzymes for the synthesis of acetyltriacylglycerols. PCT Application PCT/US2018/029590

Dhankher O.P. and Abdullah, H. (2018). Transgenic Plants having Increased Wax Synthase for Drought and Salt Tolerance. PCT/US2016/193008.

Thelen JJ, Salie MJ (2016) Increasing plant oil content by altering a negative regulator of acetyl-CoA carboxylase. PCT/US2016/041386. Licensed to Yield10 Bioscience.

Thelen JJ (2017) Increasing plant oil content by improving activity of acetyl-CoA carboxylase. PCT/US17/40851. Licensed to Yield10 Bioscience.

Thelen JJ, Ye Y (2018) Increasing seed oil content by manipulating carboxyltransferase interactor protein expression. Disclosure filed. Licensed to Yield10 Bioscience.