Project/Activity Number: NC-1203 Project/Activity Title: LIPIDS of Crops Annual Meeting Period Covered: 2019-2020 Date of This Report: Annual Meeting Date(s): September 11-13, 2020

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

Brief summary minutes of annual meeting: The 2020 NC-1203 meeting was held virtually on September 11 through 13. The meeting was opened by representatives of the hosts: Timothy Durrett and Ruth Welti, and included remarks from Multistate Project Administrative Advisor Ernie Minton. Rather than giving detailed updates, the group discussed their research in the context of the upcoming project renewal and the respective proposed experiments and collaborations. The renewal will be submitted in fall 2020. The 2021 meeting will be held at the Danforth Center and hosted by Doug Allen and Sam Wang. The 2022 meeting will be held at Michigan State University and be hosted by Susanne Hoffmann-Benning. Jonathan Markham will oversee the rewrite, Ruth Welti and Young-Jin Lee will address Aim 1, Rebecca Roston and Susanne Hoffmann-Benning Aim 2, and Tim Durrett and Ed Cahoon Aim 3 specifically.

NC-1203 2019-2020 Accomplishments.

Activities and accomplishments related to each of the project's three objectives are described below, Members also considered opportunities to work together on joint publications, either individual papers or a special issue on lipids in an appropriate journal.

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

The Welti group has made all the components available for relatively rapid (8 min/sample) directinfusion multiple reaction monitoring (MRM) mass spectrometry using a Sciex 6500+ triple quadrupole MS (Song et al., 2020). The components include 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 (and data acquisition method downloadable from the web), and a complete data processing method that employs Sciex software and the online site LipidomeDB Data Calculation Environment. They also updated Lipidome DB Data Calculation Environment twice: once of 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 technique can be used for fatty acid analysis at the chemical formula level (Tamura et al., 2020). Jonathan Markham's group developed a cross disciplinary platform to measure lipids and model with flux balance analysis with a goal of establishing 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. In a connection to objective 2, combining three derivatization techniques for primary amine, carboxylic and carbonyl functional groups led to the visualization of over 600 new metabolites in maize leaf and root cross sections. Enrichment of Arabidopsides, well-known oxylipin as a wounding response, was discovered in FERONIA mutation and its localization to chloroplast. Durrett and Lee were recently funded for a NIFA project to study the spatiotemporal development of acetyl-TAG in genetically engineered camelina.

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

Others are using radiolabels as well as microscopy, FRET, and mass spectrometry to interrogate lipid metabolism. The Hoffmann-Benning lab developed a phloem-lipid extraction method (Hoffmann-Benning, 2020) and is currently working on PULSE constructs (red light induced; blue/white light repressed) constructs of lipid-binding protein to control their expression both, spatially and temporally. Proof-of-concept constructs are currently being tested in protoplasts and tobacco. The lab is working on a FRET system to determine location of protein-lipid interaction and movement of the complex. An NSF EAGER grant is funding this work.

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

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

Two aspects of crop improvement are studied in this objective: i) the role of lipids in response to environmental factors and ii) the improvement of lipid profile and/or content in crop plants:

Welti, Durrett, and Schrick are working together to characterize the function of several plant genes that were identified in a lipid profiling screen of Arabidopsis T-DNA mutants as having complex lipid profiles that are significantly different from wild-type plants. One gene is involved in fatty acid desaturation, one is involved cutin metabolism, and one appears to be a transacylase. The Welti, Schrick, and Wang groups recently described the changes that occur in Arabidopsis lipid composition and metabolism in heat stress (Shiva et al., 2020). Welti's lab, in collaboration with the Koo lab, is working to identify genes involved in lipid metabolism that have natural variation affecting the activities of associated gene products. Particularly, they are interested in natural variation that results in altered enzyme activities during response to wounding and mild freezing stress. The Louis lab screened NAM parent lines to identify resistance and are exploring the role of lignin, surface waxes, and other lipids in plant defense. They reported 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.

Dylan Kosma joined the group in 2018. Kosma is characterizing the biosynthesis and transcriptional regulation of lipid-derived compounds that function in plant protection with an emphasis on reducing postharvest crop losses. Kosma has successfully collaborated with Cahoon on characterizing the biosynthesis of potent, lipidic antimicrobial compounds that crop species such as carrot utilize to protect themselves against opportunistic pathogens. Similarly, 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. Kosma's main focus, however, is understanding the gene regulatory networks controlling the deposition of the lipid-phenolic polymer suberin in response to wounding and other stress 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 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. The browser will be available later this year.

Pertaining to abiotic stresses, the Narayanan lab, in collaboration with Welti, showed that wheat decreases the unsaturation levels of plastidic and extra-plastidic glycerolipids of leaf and/or pollen in order to adapt to heat stress (Narayanan et al., 2016a, 2016b, 2018). Similar lipid changes contribute to HT adaptation in both leaves and pollen, though the lipidomes have inherently distinct compositions. They also investigated if lipid metabolic changes mediate heat stress responses in soybean (Narayanan et al., 2020). They found that a decrease in the levels of lipids containing 18:3 acyl chains (linolenic acid) under heat stress in the tolerant genotypes is a likely consequence of reduced expression of *Fatty Acid Desaturase (FAD) 3A* and *FAD3B* genes; and the decrease in 18:3 contributes to tolerant genotypes' maintenance of membrane functionality and heat tolerance.

Also studying temperature stress, the Roston lab has been searching for the core responses of membranes to temperature, specifically cold stress. Progress has been advancing understanding 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). The Roston and Schrick lab started a new collaboration to investigate the roles of sterols in cold tolerance.

The Schrick group has been studying the role of lipids in signaling through identification of STARTdomain transcription factors and their role in DNA binding, gene regulation and development of trichomes and other features of the epidermis. Phospholipids and lysophospholipids were identified as putative binding partners of these transcription factors. One family member has been shown to bind a phosphate starvation response element to control the expression of several phospholipid catabolism genes. Additionally, the Schrick 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 suggest increased drought tolerance (Barbaglia et al., 2016). Homologues in soybean and tomato are also induced by ABA, suggesting that this protein has a universal function in plants. Schrick, Stone and Hoffmann-Benning are discussing a collaboration to elucidate the function of one group of signaling LBPs, Annexins.

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).

The Cahoon and Kosma labs collaborated to describe the genetic and evolutionary basis for a divergence in cuticular wax chemistry between sorghum and maize. Combining chemical analyses heterologous expression, and comparative genomics, we 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. These findings uncovered the possibility for resurrection of a cuticular triterpenoid-synthesizing gene in maize to create a more heat-tolerant water barrier on the plant's leaf surfaces. Cahoon and Clemente are now collaborating with Lucas Busta (U. Minnesota-Duluth) to transfer the functional sorghum gene to maize 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 strategies for improving acetyl-CoA carboxylase performance including BADC repression, alpha-CT overexpression, and CTI repression. Like the BADCs, the CTIs are a previously uncharacterized gene family discovered by the Thelen lab to be associated with the

heteromeric ACCase. The CTIs are envelope membrane proteins that anchor ACCase through direct interaction with the alpha-CT subunits. They are using multiple approaches to examine structure and derive the mechanisms that can lead to higher oil in oilseed species including Arabidopsis, Camelina, and soybean. Some of these lines will be analyzed through an NSF-Plant Genome Research project with Koo and Allen. This project will also develop a website dedicated to the study of oil production in plants called "Fat Plants" available at fatplants.net. The Koo lab has developed Camelina lines overexpressing pea alpha-CT and RNAi lines of *C. sativa* BADCs. Increased seed oil content in these lines have been observed. These lines have also been engineered for Translating Ribosome Affinity Purification RNAseq (TRAPseq) analyses. Koo lab has found positive correlation between increased oil in vegetative tissues and susceptibility of the plants to insect herbivory. Increase of oil in leaf tissue has been achieved through various metabolic pathway engineering. The Allen lab completed and published an 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.

The Durrett and Allen labs have been collaborating to produce soybeans with altered lipid levels by reducing the expression of lipases that are upregulated late in development. Work over the past year focused on obtaining transformants and growing to homozygosity with phenotypic measurements of biomass components including lipid. Biomass from available mutants of a fast neutron population was assessed throughout development (Kambhampati et al. 2019). Changes in lipids, proteins and oligosaccharides may correlate with genes and indicate promising avenues for carbon partitioning in seeds. This work will continue in the new cycle of the Lipids in Crops grant, including further assessment of transgenics aimed at producing high value soybeans.

The Durrett group has 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* seed 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 studied the comparative transcriptome and metabolome analysis suggesting bottlenecks that limit seed and oil yields in transgenic *Camelina sativa* expressing Arabidopsis diacylglycerol acyltransferase 1 (DGAT1) and yeast glycerol-3-phosphate dehydrogenase (GPD1). They concluded that TAG production is limited by (1) utilization of fixed carbon from the source tissues supported by the increase in glycolysis pathway metabolites and decreased transcript levels of transcription factors controlling fatty acids synthesis; (2) TAG accumulation is limited by the activity of lipases/hydrolases that hydrolyze TAG pool supported by the increase in free fatty acids and monoacylglycerols (Abdullah et al., 2018). Further characterization of genes controlling these bottlenecks is in progress.

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 lines that produced seeds containing over 90 mol% acetyl-TAG with minimal effect on seed size, weight and fatty acid content. 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 lines 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.

Roston, Markham, and Clemente continue to work on cold tolerance in important crops including wheat and sorghum through the expression of transcription factors and examinations in glycero and sphingolipid content. The Roston lab has identified important lipid changes cycle in response to cold and current efforts from multiple labs that appeared to be conflicting are now hypothesized to be in congruence. Multiple species have similar cycles in lipid abundance; cold tolerant species deviate from non-tolerant species at key times. They also showed that lipid responses to freezing tolerance are greatly increased in ancient lineages, and suggested the hypothesis that severe stress responses may be derived from a stronger ancestral response to damage of any type. The Wurtele group explored the relationship between QQS, carbon and nitrogen partitioning, and defense in a series of Arabidopsis mutants affecting starch metabolism. Results indicate QQS and NF-YC4 can increase protein and improve defensive traits in crops.

Kosma is investigating methods for improving potato tuber storage life through better wound healing. Kosma has established methods to generate CRISPR/Cas9 edited potatoes and has successfully generated gene-edited potato lines for validating gene function in the tuber wound healing process. Kosma's group is now well positioned to apply gene-editing towards trait improvement. Kosma has worked 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 lines as compared to wildtype (WT) plants. Transgenic plants showed strong tolerance to drought and salinity. 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 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 lines have advanced to field evaluation in Mead, NE in 2019 and 2020 and new lines are being generated with up to 13-GoldenBraid assembled transgenes to also confer higher oil content, in addition to aquaculture oil-quality traits. This collaboration resulted in the generation of engineered soybean germplasm with 8- to 10-fold increases in total vitamin E tocochromanols, primarily as tocotrienols, by seed-specific expression of a barley homogentisate geranylgeranyl transferase (*HGGT*). This trait was introgressed into soybean lines engineered for oils enriched in the polyunsaturated fatty acids gamma-linolenic, alpha linolenic, and stearidonic acids. Oil extracted from *HGGT*-expressing lines had \geq 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 lines 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.

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).

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 during as indicated by milestones and 73 publications, 4 software tools, and 7 patents listed below and standards and protocols that have been shared amongst participants.

Milestones:

<u>2017</u>

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

Two methods developed for crop plants from the group (Welti). Roston is doing 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 stressinduced 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)

<u>2018</u>

• 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 publically soon (Dhankher)

• *Mapping of genetic lesions affecting camelina mutants with altered leaf and seed lipid composition* Accomplished (Cahoon, Durrett)

•Development of camelina lines with modified oil composition

Accomplished (Cahoon, Durrett)

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

Oleosin in sorghum (Clemente)

Data involved in herbivory of the lines (Koo)

2019 goals

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

Accomplished (Wang, Cahoon)

• Generate a transgenic crop line

Accomplished (Clemente, Wang)

• Laboratory test for improved resistance to stress

Overexpression of PLAFP increases drought tolerance in Arabidopsis; 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 lines 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)

2021 goals:

Field trials for improved resistance to one or more stress.

Aphid field trials (Clemente, Louis) ; Camelina field trials (Dhankher)

Conduct polysomal RNAseq analysis on high oil lines (Koo)

Study abiotic and biotic stress tolerance of high oil lines (Koo)

Publications

- 1. 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.
- 2. Ahsan N, Wilson RS, Thelen JJ. (2018) Absolute Quantitation of Plant Proteins. *Curr Protoc Plant Biol.* 3:1-13.
- 3. 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.
- 4. 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
- 5. 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
- 6. 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
- S. Bairagi, 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).
- 8. 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
- 9. 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
- 10. 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.
- 11. Brands M, Cahoon EB, Dörmann P (2020) Palmitvaccenic acid (Δ11-cis-hexadecenoic acid) is synthesized by an OLE1-like desaturase in the arbuscular mycorrhiza fungus Rhizophagus irregularis. Biochemistry 59: 1163-1172
- 12. Cahoon EB, Li-Beisson Y (2020) Plant unusual fatty acids: learning from the less common. Curr Opin Plant Biol 55: 66-73.
- 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.
- 14. 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.

- 15. 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
- 16. 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
- 17. 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.
- 18. 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.
- 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., and Ballaré, C. L. (2020) A light-dependent molecular link between competition cues and defence responses in plants. *Nat Plants* 6, 223-230
- 20. 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.
- 21. 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.
- 22. X. Guo, Z. Ge, S. J. Sato, T. E. Clemente, Sorghum (Sorghum bicolor). *Methods Mol Biol* **1223**, 181-188 (2015).
- 23. 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.
- 24. 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
- 25. 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
- 26. Hoffmann-Benning, S. (2020). In Methods in Molecular Biology: Plant Lipids. Editors Dorothea Bartels and Peter Dörmann. Chapter 2. Accepted.
- 27. 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.
- 28. 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

- 29. C. Kempinski, 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).
- 30. 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
- 31. 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.
- 32. 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
- 33. Koenig AM, Hoffmann-Benning, S (2020) The interplay of phloem-mobile signals in plant development and stress response. Bioscience Reports 40: BSR20193329 <u>https://doi.org/10.1042/BSR20193329</u>.
- 34. Konda A, Nazarenus 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 tocochromanol content and effects on oil antioxidant properties in polyunsaturated fatty acid-rich germplasm. *Metabolic Engineering* 57:63-73.
- 35. Li J, Wang X. 2019. Phospholipase D and phosphatidic acid in plant immunity. Plant Science 279:45-50
- 36. 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.
- 37. Li-Beisson Y, Thelen JJ, Fedosejevs E, Harwood JL. (2019) The lipid biochemistry of eukaryotic algae. *Prog Lipid Res.* 74:31-68.
- 38. Mahboub S, Shomo ZD, Regester RM, Albusharif M, Roston RL (2020) Three methods to extract membrane glycerolipids: comparing sensitivity to lipase degradation and yield. In Methods in Molecular Biology: Plant Lipids. Editors Dorothea Bartels and Peter Dörmann. Chapter 2. Accepted.
- McClain S, Stevenson SE, Brownie C, Herouet-Guicheney C, Herman RA, Ladics GS, Privalle L, Ward JM, Doerrer 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.
- 40. McGinn M, Phippen WB, Chopra R, Bansal S, Jarvis BA, Phippen ME, Dorn KM, Esfahanian M, Nazarenus 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
- 41. Moisseyev 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
- 42. 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

- 43. 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.
- 44. 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.
- 45. 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.
- 46. 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.
- 47. 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
- 48. Paper, J.M., Mukherjee, T., and Schrick, K. 2018. Bioorthogonal click chemistry for fluorescence imaging of choline phospholipids in plants. Plant Methods 14:31.
- 49. H. Park, 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).
- 50. 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.
- 51. 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
- 52. Romsdahl T, Shirani A, Minto RE, Zhang C, Cahoon EB, Chapman KD, Berman D (2019) Natureguided synthesis of advanced bio-lubricants. Scientific Reports 9: 11711
- 53. E. D. Scully, 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).
- 54. 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.
- 55. 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 geneticallyenhanced oilseed crop. Tribology International 146: 106234
- 56. Singh U, Hur M, Dorman K, Wurtele E. 2019. MetaOmGraph: a workbench for interactive exploratory data analysis of large expression datasets. bioRxiv. 1:698969.
- 57. 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.

- 58. 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. Hincha and E. Zuther. Methods Mol Biol. 2156: 187-202.
- 59. Tamura P, Fruehan C, Johnson DA, Hinkes P, Williams TD, Welti R. (2020) 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. *In press.*
- 60. 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.
- 61. 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.
- 62. 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
- 63. 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.
- 64. 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
- 65. Wilson RS, Thelen JJ. (2018) In Vivo Quantitative Monitoring of Subunit Stoichiometry for Metabolic Complexes. *J Proteome Res.* 17:1773-1783.
- 66. 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.
- 67. 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/tpj.14376.
- 68. 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
- 69. Ye Y, Fulcher YG, Sliman DJ 2nd, Day MT, Schroeder MJ, Koppisetti 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.* In press.
- 70. 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.* Revision submitted.
- 71. 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

- 72. 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.
- 73. 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. Knockdown 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
- 74. 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
- 75. 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.

Software

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

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

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

4. Database of root-associated genes and promoters from maize, sorghum, and soybean, <u>http://sysbio.unl.edu/RGPDB/</u>

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 carboxyltransferse interactor protein expression. Disclosure filed. Licensed to Yield10 Bioscience.