# 2024 International Symposium on Plant Lipids

## Addendum
## Poster Abstracts

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# Genomic and transcriptomic insights into adaptation of soybean lipid metabolism genes under environmental stresses

**Wenhao Shen[1], Neeta Lohani[1], Gezahegn Tessema[1], Yong-qiang Charles An[1,2]***

*[1] Donald Danforth Plant Science Center, St. Louis, Missouri, USA*
*[2] US Department of Agriculture, Agricultural Research Service, St. Louis, Missouri, USA*
*[*]Corresponding author: yong-qiang.an@usda.gov*

Soybean [*Glycine max* (L.) Merr.] is one of the most significant crops globally, serving as a primary source of dietary protein and oil in the plant kingdom. Environmental changes pose a significant threat to lipid metabolism, leading to substantial economic losses worldwide. Understanding the genomic diversity and transcriptomic responses of soybean lipid metabolism genes to these diverse environmental changes is critical for soybean improvement. The vast amounts of genome and transcriptome sequencing data generated by the soybean research community provide an opportunity to identify new alleles that may be involved in environmental adaptation and resilience. In this study, a total of 1,402 accessions with whole genome sequence data were selected from the USDA-ARS germplasm resources information network. Significant SNPs and genes associated with adaptation to geographic latitudes were thoroughly investigated for possible correlations with lipid metabolism-related traits such as fatty acid, oil content, and seed composition. Meanwhile, we consolidated and analyzed a total of 7,847 transcriptome sequencing data representing 2,932 distinct biological processes, generating a list of sensitive genes highly responsive to internal factors and external stresses in soybean. Based on these results, the lipid metabolism genes within the genomic regions and the sensitive genes list were identified, clarifying their potential roles in response to adaptation and environmental changes. These new insights into the soybean lipid metabolism genes hold great potential for developing effective strategies to enhance climate change resilience and overall soybean improvement.

# Metabolic Profiling and Genetic Screening of Enzymatic Degradation of Glycosylinositolphosphoceramide

**Toshiki Ishikawa[1,2*], Sho Sanada[1], Yudai Kondo[1], Rina Ichikawa[1], Akane Kadoya[1], Yuta Umemura[3], Hide-Nori Tanaka[4], Minoru Nagano[5], Tamotsu Tanaka[6], Masatoshi Yamaguchi[1], Maki Kawai-Yamada[1]**

*[1]Graduate School of Science and Engineering, Saitama University, Saitama, Japan*
*[2]Green Bioscience Research Area, Strategic Research Center, Saitama University, Saitama, Japan*
*[3]United Graduate School of Agricultural Sciences, Gifu University, Gifu, Japan*
*[4]iGCORE, Gifu University, Gifu, Japan*
*[5]College of Life Sciences, Ritsumeikan University, Shiga, Japan*
*[6]Graduate School of Technology, Industrial and Social Sciences, Tokushima University, Tokushima, Japan*
*[*]Corresponding author: toishika@mail.saitama-u.ac.jp*

Sphingolipids are ubiquitous membrane lipids in all eukaryotes. Complexed sphingolipids not only play a key role for membrane nanodomains but also serve as signaling messengers triggered by enzymatic hydrolysis. Here, we report technical advances in metabolomic profiling of glycosylinositolphosphoceramide (GIPC) and its hydrolytic products occurring in homogenates of plant tissues. A comprehensively targeted LC-MS/MS analysis of hydrophobic and hydrophilic products demonstrated that two phospholipases, PLC and PLD, are responsible for the enzymatic degradation of GIPC. A screening system was established for genes encoding GIPC-hydrolyzing enzymes by the metabolomic profiling coupled with agrobacterium-mediated transient expression. We found two GIPC-PLD branches in a phospholipase family, which seem to have been evolved independently in bryophytes and seed plants. Our findings suggest functional consequences of the GIPC hydrolysis in plants.

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# Biosynthesis of Falcarin-Type Polyacetylenic Lipids

Fan Huang[1], Brenda Trinh[2], Bonnie Lee[2], Miles Mayer[1], Lucas Busta[3], Patrícia Santos[2], Won Cheol Yim[2], Edgar Cahoon[1], Dylan Kosma[2*]

*[1]University of Nebraska-Lincoln, Lincoln, Nebraska, USA*
*[2]University of Nevada, Reno, Nevada, USA*
*[3]University of Minnesota Duluth, Duluth, Minnesota, USA*
*[*]Corresponding author: dkosma@unr.edu*

Falcarins, C17 fatty acid derivatives that contain multiple triple bonds, are believed to have antifungal and anticancer activity. These molecules occur in selected plant families, notably Apiaceae, Asteraceae, and Araliaceae. In Apiaceae, including carrot (*Daucus carota*), specific falcarins, such as falcarinol, falcarindiol, and falcarindiol-3-acetate, have been identified and are typically enriched in the root periderm. Falcarins are synthesized from linoleic acid, with the desaturation to form the Δ12 triple bond catalyzed by specialized Fatty Acid Desaturase 2 (FAD2) variants, known as acetylenases. Previous studies have demonstrated that a functional acetylenase from parsley (*Petroselinum crispum), ELI12* (AAB80679.1), is induced upon fungal elicitation. Despite this, the downstream steps of falcarin biosynthesis remain largely uncharacterized. In this study, we used carrot as a model system to identify enzymes and their corresponding genes associated with falcarin biosynthesis. A revised annotation of the carrot genome was developed and used to identify new candidate genes involved in the falcarin pathway. This revised genomic data facilitated the creation of a detailed “Carrot Falcarin Tissue and Development Atlas,” providing a spatiotemporal map that reveals the developmental dynamics of falcarin accumulation in carrot tissues. In addition, we generated transgenic carrot lines that overexpress genes for variant FAD2s, resulting in elevated concentrations of selected falcarins. We also used CRISPR-Cas9 to knock out the three genes for putative acetylenases that catalyze the first step in falcarin biosynthesis, with the goal of removing falcarins to test their antifungal properties in carrot. Edited lines for each of these genes were obtained. We are currently evaluating these lines to determine their falcarin levels. Overall, this research is setting the stage for the transfer of the falcarin biosynthetic pathway to other plant families to enhance their innate fungal disease resistance for improved agricultural productivity.

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# Elucidating Fatty Acid Biosynthesis and Turnover in Camelina Seeds that Produce Medium Chain-Containing Lipids

**Maneesh Lingwan[1], Somnath Koley[1], Doug K. Allen*[1,2]**

*[1]Donald Danforth Plant Science Center, St. Louis, MO*
*[2]USDA-ARS, St. Louis, MO*
**Corresponding author: doug.allen@usda.gov*

Biofuels that are environmentally friendly, with tailored compositions are needed to meet renewable fuel applications. Nature produces concentrated forms of diverse fatty acids that could meet this demand, if incorporated into crop plants. Previously, genes from Cuphea, that is comprised of fatty acids with a chain length of 90% C8-C12, were engineered into *Camelina sativa,* an emerging biofuel oilseed. A fatty acid thioesterase (CvFatB1), a lysophosphatidic acid acyltransferase (CvLPAAT2), and a diacylglycerol acyltransferase (CpuDGAT1) were incorporated, resulting in fatty acid compositions that contained up to 23% medium chain lengths at mid-development of seeds; however final levels were 13% of the total lipid. We hypothesize that the reduced quantity at maturity, either indicates a lower rate of medium chain biosynthesis in late development compared to other long chain fatty acids, or the plants may turn over some medium chains as a counter mechanism to the engineering efforts. Modified Camelina seeds and wild-type lines were collected over development at seven stages (10, 15, 20, 25, 30, and 35 days after flowering and maturity) and analyzed for fatty acid, protein, central metabolites, acyl carrier protein (ACP), and acyl-CoA content. Quantified ACP levels including fatty acid biosynthetic intermediates of the cycle differed over development and were not intuitive. The results likely reflect the kinetics of the CvFatB1 and the available substrates including nonacylated ACP-SH. ACP analysis combined with central metabolites and short to medium chain acyl-CoAs which can describe the dynamics of medium chain lipid metabolism will be presented.

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# Polyunsaturated fatty acid production from the polar marine microalga *Chlamydomonas* sp. RCC2488 *malina* using potato peel hydrolysates in mixotrophic conditions

**Sha Rucksana Akhter[1], Laura Barth[2], Alfredo Martínez-Jiménez[2], Daniela Morales-Sánchez[2]***

[1]Department of Chemistry, Universita Degli Studi di Bari Aldo Moro, Bari, Italy
[2]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México
*Corresponding author: daniela.morales@ibt.unam.mx

*Chlamydomonas* sp. RCC2488 *malina* is a polar microalga with high PUFA and pigment production under phototrophic conditions. In order to investigate the feasibility of growing under mixotrophic conditions using an organic carbon source, cultivations of *C. malina* with glucose and potato peel hydrolysates (PPH) were performed in flasks at 120 µmol photons $m^{-2}$ $s^{-1}$ of light intensity. The potato peel was subjected to an acid-hydrothermal pretreatment. The product of this was separated in three treatments: PPH1, supernatant + paste; PPH2, only paste previous intensive wash step with water; PPH3, only supernatant. Then, each treatment was subjected to an enzymatic hydrolysis with amylase and amyloglucosidase. Transmembrane glucose transport, growth kinetics, macromolecular composition and lipid types and profile of *C. malina* were determined. The microalga was able to transport glucose at a rate of 0.015 $\mu$mol $g^{-1}$ $min^{-1}$. Highest concentration of reducing sugar (glucose) was found in PPH1 and PPH3. However, *C. malina* was not able to grow on those carbon sources due mainly to the presence of furfural, Hydroxymethylfurfural (HMF) and acetic acid in high concentrations. In contrast, *C. malina* had the highest biomass productivity in glucose and PPH2. The PPHs promoted the lipid accumulation in *C. malina* but with different lipid composition. PPH1 and PPH3 promoted the synthesis of TAG while PPH2 allowed the accumulation of polar lipids with high linoleic and linolenic acid content. The presence of furfural, HMF and acetic acid in the pretreatment of the potato peel inhibited the growth and biomass productivity in *C. malina* but accumulated lipids. Therefore, optimization of the pretreatment and hydrolysis is necessary in order to use potato peel as an efficient carbon source without undesired by-products. Mixotrophic cultivation using *C. malina* is possible but not ideal since the microalga grow better under phototrophic conditions.

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# Recent progress in understanding the role of Sphinganine C4-monooxygenase 1 (SBH1) in *acd5* mediated cell death

Yi-Li Chen, Nan Yao*

*State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Stress Biology, School of Life Sciences, Sun Yat-sen University, Guangzhou, P. R. China*
**Corresponding author: yaonan@mail.sysu.edu.cn*

Sphingolipids, as crucial components of biomembrane structures, play a pivotal role in maintaining membrane stability and are also involved in various biological processes as bioactive molecules. The *acd5* mutant, a ceramide kinase deficient cell death mutant, hyper-accumulates ceramides. The role of ACD5 is further highlighted by its interaction with plant hormones such as salicylic acid and jasmonic acid, which modulate cell death pathways in response to various stimuli. This study identified and obtained multiple *suppressors of acd5* (*safes*) that inhibit the *acd5* cell death phenotype using forward genetics and sphingolipidomic detection. The suppressor lines *safe1-1, safe1-2, and safe1-3* can completely suppress the *acd5*-mediated cell death phenotype. Through BSA sequencing, and genetic complementation experiments, we identified the candidate gene *SBH1* (*SPHINGOID BASE HYDROXYLASE 1*) as the suppressor gene of the *acd5* mutant. SBH1 is one of the key enzymes in the sphingolipid metabolic pathway, mainly functioning in hydroxylating d18:0 LCB into t18:0 LCB. Sphingolipidomics analysis demonstrated that the contents of t18:0 ceramide in *safe1-1*, *safe1-2*, and *safe1-3* were significantly reduced compared to *acd5*. Further *in vitro* and *in vivo* experiments confirmed that t18:0 ceramides were the key sphingolipid component mediated *acd5* cell death.

# Nature-Guided Strategies to Maximize Astaxanthin Production and Purity in Camelina Oil for Aquaculture Feed and High-Value Food Applications

**[1]Hyojin Kim, [1]Kiyoul Park, [1]*Edgar B. Cahoon**

*[1]University of Nebraska-Lincoln, USA*
**Corresponding author: ecahoon2@unl.edu*

Astaxanthin (3,3'-dihydroxy-β, β'-carotene-4,4'-dione) is a red lipophilic pigment derived from β-carotene and is distinguished by keto groups on each ionone ring ("ketocarotenoid"). Recent research has focused on the discovery of sustainable sources and cost-effective production of natural astaxanthin for use in aquaculture feedstocks to confer red color to products such as salmon and shrimp. Flower petals of *Adonis aestivalis* are one of the few plant sources of astaxanthin, which have among the highest astaxanthin concentrations in land plants (~1.5% DW). Our research has focused on the transfer of the *Adonis* astaxanthin biosynthetic pathway to *Camelina sativa* (camelina) seeds for cost-effective and oilseed-based production. In our first prototype, we introduced and expressed transgenes of maize phytoene synthase, *A. aestivalis* carotenoid β-ring 4-dehydrogenase (CBFD2) and carotenoid 4-hydroxy-β-ring 4-dehydrogenase (HBFD1) under seed-specific promoters. While this strategy was effective at generating seeds rich in astaxanthin, the seeds also contained ketocarotenoid intermediates and had delayed germination. To identify additional genes to maximize astaxanthin production and purity, we conducted transcriptome profiling of *A. aestivalis* flower petals. Candidate genes from the transcriptome were initially characterized using *Agrobacterium*-infiltration of *Nicotiana benthamiana* leaves for transient astaxanthin production. To improve quantity and quality of astaxanthin in camelina seed, we used seed-specific expression of transgenes which encode astaxanthin biosynthetic enzymes (CBFD1/2 and HBFD1/2), candidate for astaxanthin esterifying enzyme and maize phytoene synthase. Engineered camelina accumulated the high purity of astaxanthin in seed and displayed normal seed germination and seedling establishment compared to the first prototype. We will describe the use of the top candidate genes for generating high astaxanthin concentrations while maintaining uncompromised seed fitness.

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# ATP-BINDING CASSETTE G23 contributes to the transport of suberin monomers in Arabidopsis seed coats

**Ryeo Jin Kim, Yuyang Zhang, and Mi Chung Suh***

*Department of Life Science, Sogang University, Seoul 04107, Republic of Korea*

**Corresponding author: mcsuh@sogang.ac.kr*

Suberin is an extracellular hydrophobic polymer deposited in seed coats and plays as a barrier to regulate the movement of ions, water and gases and to protect seeds against pathogen attack. However, relatively little is known about molecular mechanisms underlying seed coat suberin deposition. In this study, the *in planta* role of an ATP-BINDING CASSETTE G23 (ABCG23) was investigated in Arabidopsis seed coat. The *ABCG23* transcripts were predominantly expressed in the outer integument I of seed coats and the endodermal cells of roots. The fluorescent signals from *eYFP:ABCG23* construct were observed in the plasma membrane of tobacco epidermal cells. Total suberin monomer loads in *abcg23-1* and *abcg23-2* seeds decreased by approximately 4% and 2% compared to the wild type, respectively, and a decrease in the levels of primary alcohols (C18 and C22), ω-hydroxy fatty acids (C18:1, C22, C24, and 10,16-C16:0), and ferulate was prominent. *abcg23-1* and *abcg23-2* seed coats exhibited reduced autofluorescence under UV light and increased permeability to tetrazolium salts relative to the WT. The ratio of seed germination and seedling establishment of *abcg23-1* and *abcg23-2* was noticeably reduced compared to WT under salt and osmotic stress conditions. Bimolecular fluorescence complementation assay showed homodimeric interaction of ABCG-2, -6, -20, and -23, and heterodimeric interaction between ABCG23 and ABCG-2, -6, or -20. Therefore, our findings provide that the ABCG23 contributes to the transport of suberin monomers in Arabidopsis seed coats.

# Small RNA-mediated regulation of cuticular wax biosynthesis in Arabidopsis

**Quang Ha Dang[1], Ryeo Jin Kim[1], Si-in Yu[1], Christian Møller[2], Byeong-ha Lee[1], Seong Wook Yang[2], and Mi Chung Suh[1*]**

*[1]Department of Life Science, Sogang University, Seoul 04107, Republic of Korea*

*[2]Department of Systems Biology, Institute of Life Science and Biotechnology, Yonsei University, Seoul 120-749, Republic of Korea*

**Corresponding author: mcsuh@sogang.ac.kr*

The aerial surfaces of terrestrial plants are covered with a hydrophobic cuticle composed of cutin polyester and cuticular wax. Cuticular wax biosynthesis is precisely regulated for protecting plants from environmental stress, but its regulatory mechanisms are much less understood. Cuticular waxes are synthesized by alkane-forming pathway and alcohol-forming pathway, which are catalyzed by ECERIFERUM1 (CER1)/CER3/mid-chain alkane hydroxylase1 (MAH1) enzymes and fatty acyl-CoA reductase (FAR3/CER4)/wax ester synthase/diacylglycerol acyltransferase (WSD1) enzymes, respectively. Overexpression of *CER1, CER3, or FAR3* minimized or abolished wax crystal formation and reduced the levels of alkanes or primary alcohols on the Arabidopsis stem surface, consistent with the decreased endogenous and exogenous levels of *CER1*, *CER3* or *FAR3* transcripts. In contrast, increasing the expression of *CER1* and *FAR3* in *rdr6-11* plants, which are deficient in small interfering RNA (siRNA) biosynthesis, significantly enhanced the total wax loads. The levels of *CER1* and *FAR3* siRNA duplexes significantly increased in *CER1OX*/Col-0 and *FAR3OX*/Col-0, respectively, but not in *CER1OX*/*rdr6-11* and *FAR3OX*/*rdr6-11,* respectively, compared to their respective controls. In addition, both Arabidopsis leaves overexpressing *CER1* or *FAR3* and dehydration-treated Arabidopsis leaves exhibited down-regulation of miR156 and miR157 and up-regulation of *SPL9*, which is a target of miR156 and activates *CER1* and *FAR3* expression. The observations revealed that cuticular wax biosynthesis is finely regulated by siRNA- and miRNA-dependent pathways.

**Characterization of Transcription Factors that Putatively Regulate Cuticle Synthesis on Maize Silks**

Full Author List: Madison Lane[1,2], Jingjing Tong [3], Keting Chen[1], Erin E. Sparks [3], Marna D. Yandeau-Nelson [1,2]

[1]Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011

[2]Molecular, Cellular and Developmental Biology Interdepartmental Graduate Program

[3]Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19713

The hydrophobic cuticle, which covers aerial portions of plants, is the first line of defense against environmental stresses, including drought, UV radiation, temperature, and insects and pathogens. This cuticle is comprised of a cutin polyester matrix that is infused with and laid atop by cuticular waxes, comprised of differing combinations of very long chain fatty acids (VLCFAs), hydrocarbons, aldehydes, alcohols, wax esters, ketones and sometimes terpenoids, depending on the species, organ and stage of development. The cuticular waxes on maize silks, which are the stigmatic floral organs that are receptive to pollen, are rich in hydrocarbons, with minor amounts of VLCFAs and trace aldehydes and alcohols that together provide important protection for this tissue during the critical period of pollination.

Cuticle biosynthesis within the epidermal cells is tightly regulated at both the transcriptional and post-translational levels. We have identified candidate transcription factors through multi-omics approaches whose expression levels are associated with cuticle composition on maize silks. The potential functions of these TFs in regulation of cuticle biosynthesis and deposition are being assessed via synthetic biology approaches. We utilize a root protoplast expression system assess how candidate TFs impact the expression of cuticle-related target genes via quantitative-PCR. The utility of this system has been demonstrated by expression of the OCL1 TF that is already known to regulate cuticle synthesis. Increased expression of a known cuticle gene target, *abct,* which encodes ABCT, an ABC transporter that transports cuticle components to the epidermal cell surface, was observed. Characterization of these TFs singly and in combination in protoplasts and in planta will facilitate a better understanding of how the plant cuticle is formed and deposited, and lays the foundation for future applied breeding approaches to generate “designer” protective cuticles.

**Systemic signaling in plants; from chemicals to cuticle**

Huazhen Liu, Keshun Yu, Aardra Kachroo, Pradeep Kachroo

Department of Plant Pathology, University of Kentucky, Lexington, KY 40546

Systemic acquired resistance (SAR) is a form of broad-spectrum resistance induced in response to local infection that protects uninfected parts against subsequent secondary infections. Several diverse chemical signals contributing to SAR have been isolated and characterized, including glycerol-3-phosphate (G3P) and pipecolic acid (Pip), both of which play an important role in human and plant disease physiologies. In addition to these, salicylic acid (SA) also regulates SAR and normal transport of SA and G3P is required for *de novo* biosynthesis of Pip in the distal leaves. Pip is catabolized via multiple pathways, and this in turn regulates vitamin homeostasis. A normal SAR also requires a normal cuticle, which in turn regulates water potential and thereby apoplastic transport of SA. Interestingly, cellular pH also plays an important role in SA transport and this was established using pH sensor lines as well as analysis of mutants affected in pH homeostasis. Both SA and G3P regulate the stability of trans-acting small interfering RNA (tasi-RNA), which function as an early mobile signal in SAR. Conversely, knock-out mutations in tasi-RNA or RNA silencing components required for tasi-RNA biogenesis compromise SAR without altering levels of SA or G3P. Together, these results highlight a novel relationship between plant cuticle, SA, G3P, Pip and RNA-mediated signaling in SAR.

Defining cold tolerance using measurable traits of vegetative stage sorghum.

*FangyiLi[1,2], James C. Schnable[1], RebeccaRoston[2]
**1 Department of Agronomy and Horticulture, University of Nebraska–Lincoln, Lincoln, NE 68588
2 Department of Biochemistry, University of Nebraska–Lincoln, Lincoln, NE 68588

Low temperatures limit the productivity of many important crops domesticated from tropical ancestors, such as maize, rice, and sorghum. To meet the increasing global food demand, we must explore all strategies to improve food production. One such strategy is crop cold tolerance. The first step in engineering cold tolerance is an efficient, accurate screening method. Our current strategy of cold tolerance screening has always focused on the traits during the seedling stage, however, in later vegetative stages the available methods are time intensive and have mixed accuracy. Here we developed a sorghum greenhouse test to measure different biochemical changes of photosynthesis, lipid changes, membrane damage, and metabolite changes, which are involved biological processes hypothesized to contribute to slow growth upon cold exposure during vegetative stages. The measurement of the trait changes would help to understand the cold tolerance better and find the measurable traits able to be applied in cold tolerance screening during sorghum vegetative stages.

# Forward Genetics to Discover Novel Components of Chloroplast Lipid Derived Signaling

Yosia Mugume[2], Ron Cook[2], Jinjie Liu[2,4], Linda Danhof[2], Breana Hagerty[2], Jordyn Flemming[2], Halle Purcell[2], Michael Beecher[2], Nianyuan (Sam) Hu,[2,3], Christoph Benning[1,2,3*]

[1]Department of Biochemistry and Molecular Biology., [2]MSU-DOE Plant Research Laboratory., [3]Department of Plant Biology, [4]Biological Sciences Program, Michigan State University, East Lansing, MI 48824

[*]*Corresponding author: benning@msu.edu*

The Arabidopsis chloroplast lipase PLIP3 releases 18:3 fatty acids that can be converted into 12-oxo-phytodienoic acid (OPDA) which is metabolized to oxylipins such as Jasmonic acid (JA). Overexpression of *PLIP3* resulted in stunted plant growth due to the accumulation of JA. To identify new factors involved in OPDA metabolism and perception of its metabolites, we implemented a forward genetic screen in the background of transgenic *PLIP3*-OX (*PLIP3* overexpression) plants. Five suppressor lines have been isolated, and their likely causal mutations narrowed down by bulk genomic DNA sequencing of mapping populations. For three, the causal mutations appear to be in genes with described functions, *KEG, LOX3*, and *CDK8*. The causative mutations in the fourth and fifth line has not been predictable based on annotation of the possible candidates and awaits further identification. KEG is involved in coregulation of abscisic acid and JA responses, and its mutation likely stabilizes JAZ12 a transcriptional repressor of JA response. The mutation in the lipoxygenase LOX3 slows the conversion of OPDA and provides a first example for a specific *lox3* mutant phenotype. The kinase CDK8 affects transcriptional regulation of JA response genes. Work is currently ongoing to fully characterize these lines and additional suppressor mutants.

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# Proteomic analysis identifies drought-tolerance genes and pathways in cowpea (*Vigna unguiculata L.*)

**Mohit Verma** [1], Xin Ye [2], Nagib Ahsan [3], Omolayo Olorunwa [3], Casey Barickman [3], George Popescu [2, 4], Sorina Popescu [2], and Trupti Joshi [1,5,6]

[1]Department of Biomedical Informatics, Biostatistics and Medical Epidemiology (BBME), University of Missouri, Columbia, MO, 65211, USA
[2]Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology, Mississippi State University, Mississippi State, MS, 39762, USA
[3]Department of Plant and Soil Sciences, Mississippi State University, North Mississippi Research and Extension Center, Verona, MS, 38879, USA
[4]Institute for Genomic, Biocomputing, and Biotechnology, Mississippi State University, Mississippi State, MS, 39762, USA
[5]MU Institute of Data Science and Informatics, University of Missouri, Columbia, Missouri, USA
[6]Christopher S. Bond Life Sciences Center, University of Missouri-Columbia, Columbia, MO, USA

Cowpea (*Vigna unguiculata*) is a vital legume crop grown in semi-arid regions, where drought stress severely affects its productivity. To understand how cowpea responds to drought stress, this study utilized proteomics analysis. Leaf samples were collected for proteomics analysis from three cowpea genotypes, namely Arkansas Blackeye (ARB), X17-111, and Top Pick Cream (TPC), which exhibit varying levels of drought tolerance. These genotypes were exposed to drought stress under different volumetric water content (VWC) treatments. In response to drought stress, a total of 1,085 differentially abundant proteins (DAPs) were identified. Each genotype exhibited unique DAPs, with 154 in ARB, 170 in X17-111, and 242 in TPC. Across the three VWC treatments, 601 unique DAPs were identified, including 175 proteins in the drought-stressed treatment, 281 in the intermediate treatment, and 145 in the well-watered treatment. Functional annotation of the DAPs revealed their involvement in processes such as photosynthesis, oxidative stress, and carbohydrate metabolism. The drought-tolerant genotype showed higher abundance of stress response proteins, including chaperones, lyases, hydrolases, antioxidant enzymes, as well as proteins related to osmotic adjustment and carbon fixation, compared to the other genotypes. Analysis of functional pathways highlighted significant changes in the ribosome pathway across most genotype comparisons, suggesting the importance of translation regulation in drought-resistant cowpea varieties. Notably, the ARB genotype exhibited downregulated DAPs under metabolic pathways during drought stress, while the TPC and X17-111 genotypes showed upregulated DAPs. The X17-111 and ARB genotypes had downregulated DAPs under the spliceosome pathway.

# GLOSSY2 and GLOSSY2-LIKE demonstrate both unique and overlapping functions in maize cuticular wax biosynthesis

**Dirk C. Winkelman[1,2,3], Tim Stanley[2,5], Liza E. Alexander[1,3], Elysse Trost[2,4], Basil J. Nikolau[1,3]; Marna D. Yandeau-Nelson[2,3*]**

[1]Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, Iowa, USA
[2]Department of Genetics, Development and Cell Biology, Iowa State University, Ames, Iowa, USA
[3]Center for Metabolic Biology, Iowa State University, Ames, Iowa, USA
[4]Undergraduate Genetics Major, Iowa State University, Ames, Iowa, USA
[5]Undergraduate Biochemistry Major, Iowa State University, Ames, Iowa, USA

**Marna Yandeau-Nelson: myn@iastate.edu*

The cuticle is a hydrophobic barrier that covers all surfaces of the aerial organs of land plants and provides the first line of defense from biotic and abiotic stresses that negatively impact plant health. The cuticle is composed of solvent-extractable cuticular waxes that are both intercalated within and laid atop an insoluble cutin polyester matrix. Cuticular wax composition varies depending on organ and stage of development, but usually consists of combinations of very long chain fatty acids (VLCFAs) and their derivatives, including hydrocarbons, alcohols, aldehydes, ketones, and wax esters. Classical genetic strategies have identified numerous *glossy* genes required for normal cuticle deposition in maize, and molecular characterization of these genes provides insights on cuticle formation. This study focuses on the maize *Glossy2* (*Gl2*) gene. Although the biochemical function of GL2 remains unclear, homozygous *gl2* mutant seedlings exhibit a glossy phenotype and the cuticular waxes of mutant plants are of shorter chain lengths, presumably due to an alteration of the fatty acid elongase complex (FAE) that produces VLCFAs. The recently identified GL2-paralog, GLOSSY2-LIKE, shares 63% amino acid similarity with GL2. To assess the *in planta* function of *Gl2-like,* six mutant alleles were generated via CRISPR-Cas9 genome editing. Cuticle analyses of single *gl2* and *gl2-like* mutants, as well as *gl2;gl2-like* double mutants demonstrate both overlapping and distinct contributions to FAE across different tissue types. While cuticular wax composition on maize leaves is primarily affected by the *gl2* mutation, a chemotype was only observed on maize silks in the *gl2;gl2-like* double mutant. The functionality of GL2 and GL2-LIKE proteins is also being investigated via a yeast synthetic biology approach, in which the entire yeast FAE pathway is being replaced with maize FAE enzymes. This combination of multidisciplinary strategies is unraveling the roles that *Gl2* and *Gl2-like* serve in maize cuticle biosynthesis.

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