



INTERNATIONAL CAMELINA CONFERENCE 2024

# Inaugural International Camelina Conference

July 19-20, 2024

Hosted by the University of Nebraska-Lincoln  
Lincoln, Nebraska USA



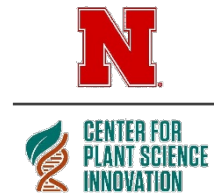
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# ICC 2024 Speaker Bios and Abstracts

## Session 1: Policy and Life Cycle Assessment

### Steve Csonka

Commercial Aviation Alternative Fuels Initiative

Csonka Aviation Consultancy LLC

Steve Csonka is a commercial aviation professional with 38 years of broad, strategic airline, aviation OEM, and SAF experience. Over his career, Csonka has been a strong industry advocate who has worked in roles that involved developing pragmatic solutions to the challenges of aviation growth. Such engagement led him to accepting the current CAAFI executive director role in 2012. CAAFI – the Commercial Aviation Alternative Fuels Initiative – is an industry partnership fostering the development and commercialization of sustainable aviation fuels. CAAFI engages in public-private partnership activities designed to convene the group of participants needed to stand up an entirely new industrial sector.

Steve also continues to serve in leadership, steering committee, consultancy, and BOD roles with multiple aviation industry organizations in areas of applying technology and business concepts to enable commercial progression with SAF, both through the work of CAAFI, as well as independent activities through his consultancy.

“Tangible Aviation Market Opportunity for Sustainable Feedstocks”

Synopsis:

Csonka will provide an overview of sustainable aviation fuel (SAF) industry efforts and progress. CAAFI is a public-private partnership between the aviation industry and the U.S. government founded in 2006. The organization’s role is to foster the development and commercialization of sustainable aviation fuels. Steve will discuss progress on SAF, focus on near term challenges in meeting the 2030 goals of the U.S. Sustainable Aviation Fuel Grand Challenge (3 billion gallons per unit domestic neat SAF blending component production), and review the opportunities for oilseed development and use.

### Edmund K. Mupondwa

Minister of Agriculture and Agri-Food, Canada

Edmund K. Mupondwa is a project leader and research scientist of bioproducts and bioprocesses at the Saskatoon Research and Development Centre's Science and Technology Branch, part of the Minister of Agriculture and Agri-Food in Canada. He also is an adjunct professor in Department of Chemical and Biological Engineering at University of Saskatchewan, Saskatoon, Canada.



“Integration of Camelina (*Camelina sativa* L. Crantz) into a biorefinery concept to advance circular economy: Technoeconomic analysis and life cycle assessment”

Synopsis:

Camelina (*Camelina sativa* L. Crantz) is emerging as an important sustainable dedicated industrial oilseed crop for clean energy, bioproducts, and other non-food applications. However, it is important to evaluate the sustainability of producing and valorizing camelina within the context of a circular economy. This paper provides an integrative technoeconomic analysis and life cycle assessment to link camelina to corresponding conversion pathways, including least-cost integration into the various conversion pathways for bioproduct production.

## Additional Speakers and Presentations

Marisol Berti: "Factors affecting carbon intensity in Camelina production"

Amanda Parsons DeRosier: "The Intermediate Oilseed Crop Alliance: working to expand incentives and recognition of intermediate crops"

## Factors affecting carbon intensity in camelina production

Marisol T. Berti<sup>1\*</sup> and Clara Lizarazo-Torres<sup>2</sup>

<sup>1</sup>North Dakota State University, Fargo, ND, USA

<sup>2</sup>NESTE Corporation, Porvoo, Finland

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Camelina (*Camelina sativa* (L.) Crantz) is a low input oilseed with potential in the U.S. and other countries. There is a big push to bump up camelina acreage to supply renewable biofuels feedstocks). Although, camelina does require much fewer external inputs than other oilseeds, such as rapeseed (*Brassica napus* L.), it also yields about half of other commonly grown oilseed crops. The objective was to determine the carbon intensity (CI) of camelina with varying rates of nitrogen fertilizer (0, 20, 40, 60 and 80 kg/ha). This experiment was planted in Fargo and Prosper, ND on May 22, 2023. The design was a randomized complete block with four replicates. Carbon intensity was estimated using a life cycle assessment (LCA) methodology from 'cradle to farm gate'. Seed yield was 1432 kg/ha at the highest N rate of 80 kg N/ha in Fargo. In Prosper, seed yield (1531 kg/ha) was the highest at the 50 kg N/ha rate. Carbon intensity of seed ranged between 23 and 76 g CO<sub>2</sub> eq./MJ. Lowest CI was with a N rate of 40 kg N/ha at Prosper. Carbon intensity decreased as N rates increased until a rate of 51 kg N/ha for the combined CIs across both locations ( $r^2= 0.95$ ). This indicates that the increase in seed yield by increasing the N rate can offset the increase in CI due to N fertilizer and N<sub>2</sub>O field emissions. Camelina aboveground residue after harvest was 4-5 Mg/ha and had 43.8% C and 0.92% N concentration. Carbon contribution to the soil was only 1.5-2.0 Mg/ha/year. Camelina can be a low CI crop but it depends mainly on seed yield obtained and N fertilizer rate applied.

*Funded by NESTE Corporation*

# Session 2: Camelina Genetics and Genomics

## Isobel Parkin

Agriculture and Agri-Food Canada

Isobel Parkin works as a research scientist for Agriculture and Agri-Food Canada in Saskatoon on the Canadian prairies. Her work focuses on genetics and genomics of Brassicaceae species, studying the evolution of genome structure in these complex polyploid species, and more recently targeting the genetic mechanisms that control chromosome pairing in allopolyploids. She has developed a number of community resources including genome reference sequences, such as that for *Camelina sativa*, and marker platforms for Brassica species, such as the Illumina Brassica 60K array.

Synopsis:

The close relatives of the hexaploid *Camelina sativa* vary in ploidy level, from diploid to tetraploid to hexaploid, providing a useful platform for studying genome evolution. Analyses of genome assemblies across the ploidy range, including five distinct *Camelina* species, provides insights into the evolutionary path to the crop; uncovers the level of gene diversity within the species, and the role of gene expression dominance and repeat diversification in shaping the modern genome of *C. sativa*.

## Additional Speakers and Presentations

Samuel Decker: "Identification of QTLs and Candidate Genes for Camelina Nitrogen Use Efficiency and Oilseed Traits Using a High-quality Genome"

Christina Eynck: "Unraveling flowering behaviour in *Camelina sativa*"

Heike Sederoff: "Circular RNAs in *Camelina sativa* - a new regulator of gene expression"

## Unraveling flowering behaviour in *Camelina sativa*

**Christina Eynck<sup>1</sup>, Vicky Roslinsky<sup>1</sup>, Raju Chaudhary<sup>2</sup>, Isobel A.P. Parkin<sup>1</sup>**

<sup>1</sup>*Agriculture and Agri-Food Canada – Saskatoon Research and Development Centre, Saskatoon, SK, Canada*

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An increasing interest in winter camelina (*Camelina sativa*) as an environmentally friendly feedstock crop for biofuels with low carbon intensity has resulted in the initiation of several winter camelina breeding programs in North America, including at AAFC-SRDC. In contrast to spring types, winter camelina plants do not flower readily, but require an extended period of cold temperatures, also known as vernalization, to trigger the transition from vegetative to generative growth. With a germplasm pool that is much smaller than that of spring-type camelina, winter camelina is often crossed with spring types in the AAFC breeding program in order to introgress desirable traits from spring-type cultivars into winter-type material. This necessitates the ability to be able to track the vernalization requirement trait in segregating breeding populations. To this end, an F<sub>2</sub> population was developed by crossing spring-type cultivar SES0787LS (Cypress) and winter-type cultivar Joelle and subsequent selfing. Days to flowering (DTF) were assessed under greenhouse conditions without vernalization. A detailed genetic map for this population was generated utilizing a genotyping-by-sequencing approach and a quantitative trait loci (QTL) analysis was completed. This analysis identified two major QTL regions located on chromosomes 8 and 13. These regions both contain a flowering locus C (FLC) gene; a gene known to have a significant effect on flowering time in many species. Sequence analysis in combination with previously published research resulted in the development of Kompetitive Allele-Specific PCR (KASP)-based molecular markers suitable for routine screening of *C. sativa* germplasm for the presence of the winter and spring FLC alleles, respectively. Initial validation of these markers was completed utilizing F<sub>2</sub> populations grown in the field in Saskatoon, Canada. Further analysis of a diverse germplasm panel of *C. sativa* spring and winter-type lines, used to validate the broad suitability of the markers, suggests that there is greater than expected variability in the FLC alleles and that segregation may still exist within many of the publicly available winter camelina accessions and cultivars.

## **Circular RNAs in *Camelina sativa* - a new regulator of gene expression.**

Delecia Utley<sup>1\*</sup>, Brianne Edwards<sup>1\*</sup>, Asa Budnick<sup>1</sup>, Erich Grotewold<sup>2</sup>, [Heike Sederoff<sup>1#</sup>](#)

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<sup>1</sup>Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC

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Circular RNAs (circRNAs) are closed-loop RNAs due to their covalent bond between their 3' and 5' ends, rendering them resistant to exonucleases and thus more stable compared to linear RNAs. Identification of circRNAs and distinction from its cognate linear RNA is only possible by sequencing the back splice junction (BSJ) that is unique to the circRNA. CircRNAs have been identified in many eukaryotic organisms and are involved in transcriptional and translational regulation of their cognate RNAs by increasing transcription rates, RNA stability and alternative splicing.

We have identified circRNAs from *Camelina sativa* that are involved in the regulation of germination, light response, and lipid metabolism. We sequenced light-grown and etiolated seedlings after 5 or 7 days post-germination and identified a total of 3,447 circRNAs from 2,763 genes. Because camelina is an allohexaploid, we analyzed homeolog specificity of circRNAs and were able to show that circRNAs can originate either from just one, two, or from all three homeologs in the three subgenomes. CircRNA generation correlates with increased alternative splice versions of their cognate genes in camelina. A network analysis shows the interactions of select miRNA:circRNA:mRNAs for regulation of transcript stabilities when circRNA can act as a miRNA sponge. Several key lipid metabolism genes can generate circRNA and we confirmed the presence of KASII circRNA as a true circRNA. CircRNAs identified in this study are available through CamRegBase <https://camregbase.org/>.

Acknowledgements: We thank Oliver Tessmer2 for adding circRNAs to the CamRegBase.

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## Session 3: End-Use Opportunities and Novel Approaches

### Johnathan Napier

Rothamsted Research

Originally from County Down, Northern Ireland, Johnathan obtained his B.Sc. in Agricultural Sciences from the University of Nottingham, followed by a Ph.D. in plant biochemistry from King's College, London. He carried out postdoctoral research at the University of Cambridge, then taking up a position at Long Ashton Research Station in Bristol. His research group relocated to Rothamsted Research in 2003 where he is currently science director. Johnathan is also an affiliated lecturer at the University of Cambridge and honorary professor at the Institute of Aquaculture, University of Stirling. He has published over 200 peer-reviewed papers and is the inventor on multiple granted patents relating to the biotechnology of plant oils. He was awarded his D.Sc. from the University of Nottingham in 2006 for outstanding contributions to the field of plant lipid research. He is passionate about the importance of public engagement in gaining social license for new technologies and is a regular contributor to discussions about GM food and agriculture.

#### *The Rothamsted Omega-3 Project – a success built on Camelina*

##### Synopsis:

For over two decades, his research group has been evaluating the production of omega-3 fish oils in transgenic plants, to provide a sustainable source of these important nutrients independent of oceanic sources. Attempts to metabolically engineer plants with the biosynthetic pathway for these fatty acids has ultimately led to the production of a transgenic oilseed crop (*Camelina sativa*) which contains over 20% EPA+DHA in its seed oil. This omega-3 trait represents the most complex plant metabolic engineering trait to attempt the transition from a research phase into development, regulatory approval and commercialization, and all of these represent activities beyond the normal scope of academic research. Given the pressing need for answers to the global challenges facing humanity, increased focus and effort could be placed on translation and Napier's talk will consider how this can be achieved without comprising curiosity-driven research. Specifically, there are lessons from an omega-3 project which might yield useful learnings. The fact that it takes 25 years to get to a point where impact is only now likely to be realized also confirms the need for realistic estimates in the time required for basic research to deliver societal benefits.

### Anna Kate Shoveller

University of Guelph

Kate Shoveller received her Ph.D. from the University of Alberta and is currently a professor in the Department of Animal Biosciences, University of Guelph. She was employed by Procter & Gamble and Mars Pet Care where she added to the knowledge of dog and cat nutrition through investigation in the areas of energy metabolism and nutrient budgets of dogs and cats. Shoveller currently teaches Companion and Equine nutrition and runs an active comparative nutrition research group. Her current research focuses on amino acids, fatty acids and energy metabolism in dogs, cats, and horses. She has published over 150 peer-reviewed papers, contributed to multiple book chapters, and applied for multiple patents. She has been awarded over \$8 million in grants and contracts since joining Guelph seven years ago. Her impact was most recently recognized in 2024 when she was named one of the University of Guelph's Research Leadership Chairs.



#### Synopsis:

This talk will describe the differences and similarities among plant-based oils (camelina, flax, and canola) for the diets of dogs and horses on: gross health parameters, serum fatty acid profiles, subjective and objective metrics of skin and coat health, and skin and systemic inflammatory markers. Furthermore, in the case of horses, the inflammatory response will be described in horses fed camelina, flax, or canola. In addition, considerations for camelina oil as a source of essential fatty acids for dog and horse foods will be discussed.

### **Additional Speakers and Presentations**

Edgar Cahoon: "Co-Optimization of Camelina Oil Quality and Conversion Technologies for Sustainable Aviation Fuel"

Peter Eastmond: "Production of the structured triacylglycerol 1,3-olein-2-palmitin in Camelina sativa seeds"

Peter Dörmann: "Ablation of glucosinolate accumulation in Camelina sativa by targeted mutagenesis of genes encoding the glucosinolate transporters GTR1 and GTR2 and regulators of glucosinolate biosynthesis MYB28 and MYB29"

# Production of the structured triacylglycerol 1,3-olein-2-palmitin in *Camelina sativa* seeds

Guillaume Menard, Harrie van Erp, Peter J. Eastmond\*

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In human milk around half the calories are provided as fat and the triacylglycerol (TG) molecules have a distinctive stereoisomeric structure that assists nutrient absorption in the infant gut. Palmitic acid (16:0) is esterified to the middle (sn-2 or  $\beta$ ) position on the glycerol backbone in human milk fat and oleic acid (18:1) predominantly to the outer positions. However, the fat used in most infant formulas is derived from plants, which esterify 16:0 almost exclusively to the outer positions. We have previously showed that the metabolism of the model oilseed *Arabidopsis thaliana* can be altered via genetic engineering to incorporate 16:0 into the middle position of TG and ultimately to produce 1,3-olein-2-palmitin (OPO) as the main molecular species (van Erp et al., 2019; 2021), as is the case in human milk fat. Here we will present our recent progress towards engineering *Camelina sativa* to produce OPO in seeds. The strategy involves heterologous expression of 16:0-specific fatty acyl-acyl carrier protein thioesterase and lysophosphatidic acid acyltransferase (LPAT) isoforms, in combination with suppression of endogenous *LPAT2*, *PHOSPHATIDYLCHOLINE: DIACYLGLYCEROL CHOLINEPHOSPHOTRANSFERASE*, *FATTY ACID DESATURASE 2 & 3* and *FATTY ACID ELONGASE1* genes. Our data suggest that *C. sativa* can be engineered to produce TG that containing OPO and therefore could potentially serve as a source of structured fat ingredient for use in infant formulas.

van Erp et al. (2019) Engineering the stereoisomeric structure of seed oil to mimic human milk fat. PNAS 116: 20947–20952

van Erp et al. (2021) Production of the infant formula ingredient 1,3-olein-2-palmitin in *Arabidopsis thaliana* seeds. Metabolic Engineering 67: 67-74

Funded by UK Biotechnology and Biological Sciences Research Council grant BB/X010988/1.

# **Ablation of glucosinolate accumulation in *Camelina sativa* by targeted mutagenesis of genes encoding the glucosinolate transporters GTR1 and GTR2 and regulators of glucosinolate biosynthesis MYB28 and MYB29**

**Georg Hölzl<sup>1</sup>, Barno Ruzimurodovna Rezaeva<sup>2</sup>, Jochen Kumlehn<sup>2</sup> and Peter Dörmann<sup>1,\*</sup>**

<sup>1</sup>*Institute of Molecular Physiology and Biotechnology of Plants, University of Bonn, Bonn, Germany*

<sup>2</sup>*Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Plant Reproductive Biology, Seeland-Gatersleben, Germany*

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*Camelina sativa* is an oil crop with low input costs and resistance to abiotic and biotic stresses. The presence of glucosinolates, plant metabolites with adverse health effects, restricts the use of camelina seeds for human and animal nutrition. The synthesis and transport of glucosinolates has been extensively studied in the model plant *Arabidopsis thaliana*. Glucosinolate synthesis in vegetative tissues is under control of members of the MYB family of transcription factors. Glucosinolates are then transported into the seed embryos by glucosinolate transporters of the GTR family. Camelina contains three aliphatic glucosinolates, glucoarabin (9-methyl-sulfinyl-nonyl-glucosinolate, GL9), glucoamelinin (10-methyl-sulfinyl-decyl-glucosinolate, GL10) and gluconesliapaniculatin (11-methyl-sulfinyl-undecyl-glucosinolate, GL11). Because camelina harbors a hexaploid genome, up to three homeologs are found for each gene present in the *Arabidopsis* (diploid) genome. Cas9 endonuclease-based targeted mutagenesis of the three homeologs of each of the glucosinolate transporters *CsGTR1* and *CsGTR2* caused a strong decrease in glucosinolate amounts, highlighting the power of this approach for inactivating multiple genes in a hexaploid crop. The low amounts of glucosinolates still found in the mutant embryos are in line with the scenario that glucosinolates were partially synthesized in the embryos. Mutagenesis of the three homeologs each encoding the transcription factors *CsMYB28* and *CsMYB29* resulted in the complete loss of glucosinolates in the seeds, representing the first glucosinolate-free *Brassicaceae* crop. The oil and protein contents and the fatty acid composition of the *csgtr1csgtr2* and *csmyb28csmyb29* mutant seeds were not affected. The decrease and elimination of glucosinolates improves the quality of the oil and press cake of camelina, which thus complies with international standards regulating glucosinolate levels for human consumption and animal feeding.

*Funding source: Bundesministerium für Bildung und Forschung (grant #, BMBF 031B0343, CamPro)*

## Session 4: From the Bench to the Field

### Russ Gesch

U.S. Department of Agriculture-Agricultural Research Service North Central Soil Conservation Research Laboratory

Russ Gesch is a research plant physiologist at the USDA-Agricultural Research Service's North Central Soil Conservation Research Laboratory in Morris, Minnesota. His research program focuses on the physiology and agronomy of new and alternative oilseed crops with the goal of increasing the efficiency and productivity of cropping systems in the Midwestern U.S. while diversifying agricultural systems and protecting the environment. Gesch is currently developing best management practices for double- and relay-cropping winter camelina and pennycress with traditional Midwest crops to provide crop diversity and new economic opportunities for rural Americans.



“Fitting camelina into agricultural systems for sustainable production”

Synopsis:

As demand for biofuels and bioproducts increases, so does the need for oilseed feedstock supply for these products. Large-scale camelina production requires fitting it into farmers' rotations to minimize disruption of conventional food, feed, and fiber crop production. Camelina can be grown as a winter crop between traditional summer annuals to sustainably intensify production. Such systems allow multiple crops to be produced per year, thus providing additional income and environmental benefits. Successes and challenges of double and relay cropping winter camelina with soybean and other short-season summer annual crops in the upper Midwest will be discussed.

### Additional Speakers and Presentations

Oakhairibho Okooboh: "Engineering Camelina sativa for improved productivity and abiotic stress tolerance"

Sara Berzuini (virtual): "Evaluation of a camelina-soybean double cropping system in a Mediterranean climate as an innovative strategy for sustainable intensification"

Chengci Chen: "Variation of Camelina Genotypes in Canopy Area, Biomass, Seed Yield, and Oil Content under Low and High Nitrogen Environments"

# Engineering *Camelina sativa* for improved productivity and abiotic stress tolerance

**Gloria O. Okooboh<sup>1</sup>, Ilka Haferkamp<sup>1</sup>, Marzieh Valifard<sup>1</sup>, Ivo Feussner<sup>2</sup>, Amelie Kelly<sup>2</sup>, Thilo Rühle<sup>3</sup>, Dario Leister<sup>3</sup> and Ekkehard H. Neuhaus<sup>1\*</sup>**

<sup>1</sup>*University of Kaiserslautern, Kaiserslautern, Germany*

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*Camelina* (*Camelina sativa*) is currently attracting growing research interest as an oil-seed crop due to its distinct agronomic characteristics. This study aims to produce genetically modified *Camelina* plants with enhanced productivity and tolerance to environmental stresses. Two groups of genetically modified *Camelina* plants were created by introducing the vacuolar sugar transporter gene, TST1, from sugar beet and the plastocyanin gene, PETE2, from *Arabidopsis* into *Camelina* wild type plants. The investigation involved analysing seedlings and mature plants grown under standard, water-deficient, and salt-stressed conditions. Our findings indicate that the expression of *BvTST1* led to increased sugar export from leaves, resulting in improved seed properties such as higher total seed yield and increased lipid content. Moreover, the transgenic plants exhibited enhanced root architecture, particularly under water-deficient conditions, contributing to improved performance and higher seed yield during drought. Similarly, the heterologous expression of *AtPETE2* enhanced photosynthetic activity, accelerating plant growth and boosting seed yield under standard conditions. Furthermore, the expression of *Camelina* PETE2 was induced by salt stress. Plants with *AtPETE2* expression displayed superior root properties to wild-type plants, leading to larger seeds and increased seed yield under prolonged salt stress. These results demonstrate that increasing TST1 activity and plastocyanin levels in *Camelina* can enhance photosynthesis, productivity, and stress tolerance. In conclusion, this study suggests that enhancing vacuolar TST1 activity or plastocyanin levels can optimize yield in oil-seed crops, particularly under stress conditions, providing a potential strategy for improving drought and salt stress resilience in agriculture.

*Funded by Deutsche Forschungsgemeinschaft (DFG) and the Federal State of Rhineland BioComp.*

## Effect of different harvesting strategies on the yield and qualitative performances of camelina

S. Berzuini<sup>1</sup>, F. Zanetti<sup>1\*</sup>, S. Bergonzoli<sup>2</sup>, L. Pari<sup>2</sup>, A. Monti<sup>1</sup>

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*Camelina sativa* (L.) Crantz is becoming an interesting crop in agricultural and industrial sectors due to its adaptability to different climatic and soil conditions and to the wide possibility of exploitation of its seeds. Seed oil content can reach up to 45% while protein content can vary from 26 to 30%. Moreover, the oil composition is peculiar, resulting in high content of polyunsaturated fatty acids that could be integrated both in human and animal diets. This crop has a short growing cycle and can be harvested 210 days after sowing if seeded in autumn, while when sowing is performed in spring, cycle can shorten to 80-90 days. A three-year trial was set up in Bologna (Italy) to identify innovative harvesting strategies to shorten the growing cycle without negatively affecting seed yield and quality. The experimental layout was CRB with 4 replicates. Camelina var. Alba (provided by CCE, España) was sown in October by means of a mechanical cereal seeder. The tested factors were: i) conventional harvesting at full maturity with a combine harvester when camelina seed moisture was ~10% (H1), ii) cutting and swathing when camelina seed moisture was ~40% and then threshing after one week (when seed moisture was 6-8%) (H2). The cropping cycle was reduced of at least 7 days in H2 compared to H1. And during the period from swathing until harvesting seed moisture decreased from 40% to 6.4%. Camelina seed yield did not vary significantly among treatments, with a mean value of 1.33 Mg DM ha<sup>-1</sup>. Seed oil content did not significantly decrease when seeds were harvested earlier (39.08% and 39.16% in H2 and H1, respectively). In conclusion, we can confirm that anticipating camelina harvest does not influence any of the yield parameters, allowing a reduction in the growing cycle and thus the earlier establishment of a second summer crop at the end of May instead of mid-June.

Funding: 4CE-MED Project funded by the European Union's PRIMA, under grant agreement No. 1911.

# Session 5: Metabolism and Plasticity (Resilience and Metabolism)

## James V. Anderson

U.S. Department of Agriculture-Agricultural Research Service

James V. Anderson is a research chemist and lead scientist with the U.S. Department of Agriculture-Agricultural Research Service's Edward T. Schafer Agricultural Research Center in Fargo, North Dakota. He also holds an adjunct professor appointment and is a member of the affiliate graduate faculty at North Dakota State University in Fargo. His current research program focuses on genetic, molecular, and physiological processes involved in abiotic stress tolerance and flowering in Brassicaceae oilseed crops, small grain cereal crops, and cover crops and forages suitable for colder climates and soils. The objective of the research is to improve sustainable agricultural intensification practices that also provide beneficial ecosystem services. He is a Fellow of the Weed Science Society of America and past president of the Association for the Advancement of Industrial Crops.

### Synopsis:

Food security is important for social wellbeing. Integrating *Camelina sativa* into cropping systems as a feedstock for renewable biofuels should avoid impacting food production. Winter camelina biotypes can be intercropped with food commodities to intensify crop production without impacting food security. Growing camelina on marginal lands is another approach to increasing production. Because carbon intensity is associated with yield, identifying camelina germplasm that yields well under abiotic stress or on marginal lands is critical. This presentation covers the importance of screening camelina germplasm for tolerance to temperature extremes, saline soils, and dehydration that also retain high seed and oil yields.

## Claudia Jonak

AIT Austrian Institute of Technology



Claudia Jonak is an expert in genetic, biochemical, molecular, and physiological adaptation strategies of plants to adverse environmental conditions (abiotic and biotic). Her research focuses on signal transduction towards the coordinated regulation of cellular metabolism and chromatin responses under stress. She is thematic coordinator and senior scientist at the AIT Austrian Institute of Technology. Prior to her current position, she held the role of research group leader at the Gregor Mendel Institute of the Austrian Academy of Sciences and at the University of Vienna. As an EMBO postdoctoral fellow, she worked on shoot development at INRA-Versailles in France. She is the coordinator of the EU-funded project UNTWIST, which aims to unravel the stress response mechanisms of the climate-resilient crop *Camelina sativa* and to implement successful stress adaptation strategies in new agronomic solutions.



#### Synopsis:

Climatic variability and extreme weather events are increasingly impacting crop yield and value. In her talk, “Uncovering camelina’s stress tolerance mechanisms,” Jonak will present the interdisciplinary approach of the UNTWIST consortium to investigate the multi-layered responses of a collection of genetically diverse camelina genotypes to drought and heat stress. This in-depth systems-based approach includes controlled stress experiments and field trials to improve understanding of the complex interactions between genotype, environment, and stress. The data form the basis for the development of mechanistic and predictive models as well as robust markers for crop performance in variable environments.

### Additional Speakers and Presentations

Xiao-Hong Yu: "Creating yellow-seed *Camelina sativa* with enhanced oil accumulation by CRISPR-mediated disruption of Transparent Testa 8"

Ivo Feussner: "Reducing the sinapine levels of *Camelina sativa* seeds through targeted genome editing of REF1"

Federica Zanetti: "Past and ongoing *Camelina* projects: The EU perspective"

# Creating yellow-seed *Camelina sativa* with enhanced oil accumulation by CRISPR-mediated disruption of *Transparent Testa 8*

**Xiao-Hong Yu<sup>1,2\*</sup>, Yuanheng Cai<sup>1,2, †</sup>, Yuanxue Liang<sup>1, †</sup>, Hai Shi<sup>1</sup>, Jodie Cui<sup>1,2</sup>, Shreyas Prakash<sup>1</sup>, Jianhui Zhang<sup>3</sup>, Sanket Anaokar<sup>1</sup>, Jin Chai<sup>1</sup>, Jorg Schwender<sup>1</sup>, Chaofu Lu<sup>3</sup>, and John Shanklin<sup>1\*</sup>**

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*Camelina* (*Camelina sativa* L.), a hexaploid member of the Brassicaceae family is an emerging oilseed crop being developed to meet the increasing demand for plant oils as biofuel feedstocks. In other Brassicas, high oil content can be associated with a yellow seed phenotype, which is unknown for camelina. We sought to create yellow seed camelina using CRISPR/Cas9 technology to disrupt its Transparent Testa 8 (TT8) transcription factor genes and to evaluate the resulting seed phenotype. We identified three *TT8* genes, one in each of the three camelina subgenomes, and obtained independent *CsTT8* lines containing frameshift edits. Disruption of *TT8* caused seed coat color to change from brown to yellow reflecting their reduced flavonoid accumulation of up to 44%, and the loss of a well-organized seed coat mucilage layer. Transcriptomic analysis of *CsTT8*-edited seeds revealed significantly increased expression of the lipid-related transcription factors *LEC1*, *LEC2*, *FUS3*, and *WRI1* and their downstream fatty acid synthesis-related targets. These changes caused metabolic remodeling with increased fatty acid synthesis rates and corresponding increases in total fatty acid (TFA) accumulation from 32.4% to as high as 38.0% of seed weight, and TAG yield by more than 21% without significant changes in starch or protein levels compared to parental line. These data highlight the effectiveness of CRISPR in creating novel enhanced-oil germplasm in camelina. The resulting lines may directly contribute to future net-zero carbon energy production or be combined with other traits to produce desired lipid-derived bioproducts at high yields.

# Poster Abstracts

## Viewing Guide

Topic Area	Board Assignments
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Exploring End-use Opportunities	D-H
Genetics and Genomics	I-T
Resilience and Metabolism	U-Y
Other Posters	C1, C2, W2, B2

## Presenters

### 1. Agronomy

Noemi  
Meghna  
Aritz

Codina Pascual  
Malik  
Royo-Esnal

## Camelina seed weight as affected by temperature

Noemí Codina-Pascual<sup>1</sup>, Federica Zanetti<sup>2</sup>, Ana Marjanović-Jeromela<sup>3</sup>, Barbara Alberghini<sup>2</sup>,  
Dragana Rajković<sup>3</sup>, Aritz Royo-Esnaol<sup>1</sup>

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One of the main effects of climate change is the temperature increase. In some regions, this is accompanied by severe drought periods, like in Lleida (NE Spain), where crop choice should be oriented by water availability. Camelina (*Camelina sativa* L. Crantz) is not an exception and, even though water supply could be managed by external irrigation, little can be done against rising temperatures. In the present study, we evaluated the effect of temperature during camelina late growth stages on seed yield. A multi-location experiment was set at Lleida (Spain), Bologna (Italy), and Novi Sad (Serbia) comparing four different camelina varieties (*Alba*, *Calena*, *Joelle* and *Sonny*) during 2020-22, in two sowing dates, autumn and winter. Camelina phenological development was surveyed along the growing seasons and across locations, and at harvest the productive parameter analysed was 1000-seed weight. Different temperature parameters were analysed: averages of the mean daily temperature ( $T_m$ ) and daily maximum temperatures ( $T_{max}$ ), growing degree days (GDD) and hydrothermal time (HTT) from 50% flowering to seed maturity. Pearson correlation coefficients were used to analyse the relationship between 1000-seed weight and the above-mentioned parameters. The most significant correlation was obtained with  $T_{max}$  and resulted negative. The results showed that an increase of  $T_{max}$  from 21°C to 30°C from 50% flowering to seed maturity can explain up to 21% of seed weight reduction (from approximately 1.20 g to 0.93 g), as 1000-seed weight decreased by 2.6% for every rising °C. A similar but less accurate negative correlation was obtained with  $T_m$ , and it resulted positive with GDD, while HTT did not affect camelina 1000-seed weight. These results suggest that early sowing should be planned to advance the harvest date and to avoid too high temperatures at the end of the crop cycle.

*Funding: Operation Group Projects 56-21-039-2019 of the Catalan Government*

## Yield10 Herbicide Tolerant Camelina

**Meghna R. Malik<sup>1</sup>, Jihong Tang<sup>2</sup>, Nirmala Sharma<sup>1</sup>, Venkatesh Bollina<sup>2</sup>, Lauren Rakochy<sup>1</sup>, Julian Heath<sup>1</sup>, Kristi D. Snell<sup>2</sup>**

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Camelina sativa has the potential to become a major crop in North America and serve as a new crop platform for biofuel feedstocks and novel seed products like omega-3 oils. There is limited broadleaf weed control available for large acreage production of Camelina which can negatively impact crop establishment and yield. Camelina is also highly sensitive to several classes of herbicide residues in soil. Yield10 has developed herbicide tolerant spring and winter Camelina varieties with i) over-the-top weed control with glufosinate (HT), and ii) tolerance to Group 2 herbicide residues in soil persisting from use on prior crops and over-the-top weed control with glufosinate (HT-stack). Field trials were conducted over two spring and one winter season in North America to identify elite events with spray tolerance to glufosinate products and tolerance to residues of imidazolinones (IMI) and sulfonylurea (SU). Lead and back-up commercial-quality spring varieties that showed good tolerance to herbicides and no reduction in seed yield and oil content were selected from the field tests. Yield10's robust weed control package will enable grower adoption of the crop and planting on large acreage to produce feedstocks for biofuel and omega-3 oil for the aquafeed and nutrition markets.

# Optimizing the yield of camelina in a semiarid climate: effect of flood irrigation

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It is generally said that camelina (*Camelina sativa* Crantz) is a drought tolerant crop. But drought tolerance is not the same as drought resistance, and camelina is also affected by hydric stress periods. The Central Ebro Basin, where Lleida is situated in North-eastern Spain, is characterized by semi-arid and sub-humid climates. In dryland fields of this region, the most productive crops are winter cereals, and the scarce alternatives to them has led to consider camelina as a viable crop to implement in rotation in these systems. In this Mediterranean climate, the average yields are 1500 kg ha<sup>-1</sup>, but are limited by irregular precipitations. In experiments conducted in 2020-21, with 213 mm of evenly distributed precipitation throughout the crop cycle, the average yields increased to 1875 kg ha<sup>-1</sup>. However, in 2021-22, with only 107 mm accumulated mainly in autumn, average yields decreased to 530 kg ha<sup>-1</sup>. Because these drought periods are becoming more frequent, in November 2022, we established an experiment under flood irrigation with three camelina varieties (*Calena*, *CO46* and *GP204*), in Lleida area. Winter and spring 2023 resulted extremely dry, accounting only 50 mm from January to May. For this reason, on 29 March 2023 it was applied a support irrigation of 100 mm, at flowering stage of camelina. The crop was harvested on 5 June 2023, and yields varied from 2700 kg ha<sup>-1</sup> to 3100 kg ha<sup>-1</sup>. These results open the possibility of growing camelina in irrigated fields with a better and economically stable production. Winter cereals, when they are grown as main crops or in double cropping with summer crops, need 2-3 irrigation for optimized yields. In this situation, camelina may be used as alternative crop with a lower water support. This is crucial in the climate change scenario where shortage of irrigation water is becoming general.

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## 2. Exploring End-use Opportunities

Ed	Cahoon
Alisha	Fnu
Senorpe	Hiablie
Duane	Johnson
Hyojin	Kim
Pawan	Kumar
Cory	Nykiforuk

# Co-Optimization of Camelina Oil Quality and Conversion Technologies for Sustainable Aviation Fuel

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Toward meeting the sustainable aviation fuel (SAF) Grand Challenge, we describe the co-optimization and integration of oilseed feedstock biodesign and catalytic upgrades for SAF production with reduced cost and energy inputs. Current hydroprocessing processes entail high energy and catalyst inputs to hydrogenate and “crack” fatty acid-rich feedstocks to Jet A kerosene. Here, we used the oilseed crop camelina (*Camelina sativa*) to develop a biodesigned portfolio of seed oils enriched in saturated, medium-chain fatty acids for maximizing SAF production using biphasic tandem catalytic process (biTCP) that uses mild conditions for fatty acid-to-alkane conversion. Using these feedstocks, we maximized SAF yields using the “one-pot” biTCP over a carbon/titania composite-supported Ru catalyst. Biodesigned camelina oil with elevated 14-carbon fatty acids obtained from field-grown camelina had the highest C8-C22 alkane yields: ~77 wt% at 200°C or 96% of the maximum theoretical yield (80 wt%). The highest yield of jet-fuel-range hydrocarbons (C8-C16) reached ~43 wt% at 220°C. Using this information, we iteratively developed camelina with oils containing wider mixtures of saturated medium-chain fatty acids for biTCP to produce SAF that closely mimics JetA composition.

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## **Developing Camelina Oil in EV Lithium Battery Insulation Applications**

**Electric vehicles have a significant disadvantage to liquid-fueled vehicles because of limited range, recharge speed, operating temperatures, and a tendency to combust because of their lithium batteries. Current technologies predominantly use a radiator system which depends on ethylene glycol as a coolant. The coolant is then cooled and returned to the battery unit and recycled continuously. Over-charging, frequent charging, deep discharging and quick charging can all lead to a “thermal runaway” reaction, resulting in fires and explosions.**

**Camelina offers significant advantages including a very high flash and smoke point, well above a temperature instigating a thermal runaway. Second, camelina can act as a liquid thermal transferal agent when batteries are warm and a crystalline thermal blanket when batteries are cold. Batteries will only charge when environmental temperatures are 0C to 50C. In a sealed battery container flooded with camelia oil, battery temperatures will be minimized at -15C and as the container is flooded, air is excluded and a thermal runaway is not possible. The flash point of camelina is 183 C, far above the initiation of a thermal runaway of 100 C.**

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

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Astaxanthin (3,3'-dihydroxy- $\beta$ ,  $\beta'$ -carotene-4,4'-dione) is a red lipophilic pigment derived from  $\beta$ -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  $\beta$ -ring 4-dehydrogenase (CBFD2) and carotenoid 4-hydroxy- $\beta$ -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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### 3. Genetics and Genomics

Sania Zafar	Awan
Rostislav	Blume
Rostislav	Blume
Jordan	Brock
Yen On	Chan
Wilson	Craine
Sabin	Dahal
Samuel	Decker
Abdul	Ghani
Erich	Grotewald
Megan	Letourneau
Chunyang	Lu
Louise	Michaelson
Emily	Pawlowski
Joaquin	Salas
Pallav	Singh
Manish	Sridhar
Jinita	Sthapit
Bijorn	Usadel
Dexter	White
Tingyuan	Xiao
Maneesh	Lingwan
Mark	Smith
Monica	Venegas-Calern

## From Camelina model to crop: enhancing medium chain fatty acids accumulation in seed oil for sustainable biojet fuel

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*Camelina sativa* has received renewed interest in recent decades for development of biotechnological approaches in oilseed-based biofuel research, especially for biojet fuel production. One of the advantages of camelina for genomics and translational research is its close phylogenetic relation to the well-established model plant *Arabidopsis thaliana*. However, *C. sativa* is an allohexaploid ( $2n=40$ ), which may hinder its use in biotechnology and synthetic biology research platform, especially for gene editing studies. On the other hand, a recently described diploid species *C. neglecta* ( $2n=12$ ) possess extremely high genome collinearity with *C. sativa* and amenability to simple transformation. Here, we show that it is possible to use *C. neglecta* as model for testing of phenotypic performance and transformation suitability of constructs designed for manipulating medium-chain fatty acids accumulation in *C. sativa*. In this study, we aimed to enhance the value of camelina seed oil for bio-jet fuel by introduction of seed-specific transgenes for a specialized a FatB thioesterase (*CvFatB1*), a lysophosphatidic acid acyltransferase (*CvLPAT2*) and diacylglycerol acyltransferase (*CpDGAT1*) from *Cuphea* species to produce oils enriched in medium-chain fatty acids (C10, C12, C14), readily convertible to bio-jet fuel. It has been shown that *C. neglecta* T<sub>3</sub> homozygous plants demonstrate the desired changes in accumulation of C10:0, which is naturally is not present in seed lipids of any *Camelina* species. Further, the same transgenes were overexpressed in *C. sativa* plants, followed by introduction RNAi-cassette in T<sub>6</sub> homozygous progeny, designed for silencing of genes for acyl-acyl carrier protein synthases. Such an approach allowed achieving accumulation of up to 30% of medium-chain fatty acids in camelina seed oil. Based on our findings, *C. neglecta* appears to be a good model for *Camelina* functional genomics and biotechnological research, particularly for studies that are complicated by gene redundancy.

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# Population structure of hexaploid *Camelina microcarpa* cytotypes in Ukraine, a hotspot of the species genetic diversity

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False flax, or gold-of-pleasure (*Camelina sativa* (L.) Crantz), is a cutting-edge biofuel oilseed crop, which has gained increasing attention due to a number of beneficial traits, including desirable fatty acid content, reduced vegetation cycle, high genetic transformation amenability, etc. However, cultivated *C. sativa* demonstrates restricted genetic variation across the existing cultivars, which significantly limits breeding potential of this crop. At the moment, little-pod false flax (*C. microcarpa* Andr. ex DC.), the closest non-domesticated relative of camelina, is viewed as a promising candidate for boosting genetic polymorphism of existing *C. sativa* germplasm. Recent studies, suggest that Eastern Europe, particularly Ukraine, appears to be the hotspot of the genetic diversity of hexaploid *Camelina* species. Therefore, the aim of this study was to assess genetic diversity and population structure of *C. microcarpa* in Ukraine and adjacent areas to uncover the distribution of cytotypes, which may be potentially used as wild germplasm donors for breeding.

For such investigation 105 *Camelina* sp. accession were investigated, using combination of TBP/cTBP- (length polymorphism of 1<sup>st</sup> and 2<sup>nd</sup> introns  $\beta$ -tubulin genes) and SSR-markers, capable of analyzing partially degraded DNA material from old herbaria specimens. Whole genome searches within *C. sativa* DH55 assembly revealed the presence of 34 homeologous  $\beta$ -tubulin genes, evenly allocated within almost each of chromosomes from different subgenomes. TBP genotyping technique, implying analysis of these distinct loci, allowed dissecting *C. microcarpa* accessions of different ploidy and cytotype identity. Population analysis revealed the presence of three distinct populations (Central European, North and South Ukrainian) of the most widespread *C. microcarpa* (cyto)Type 2 ( $2n=38$ ). At the same time, area of distribution (cyto)Type 1 *C. microcarpa* ( $2n=40$ ) seems to be restricted to Caucasian region. Clarified population structure and level of genetic polymorphism of *C. microcarpa* provide insights about the usage of crop wild relative germplasm for *C. sativa* improvement.

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# The evolutionary history of *Camelina* and new insights for breeding

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Research interest in the allohexaploid oilseed crop *Camelina sativa* (Brassicaceae) is rapidly increasing. Unfortunately, 20th century germplasm bottlenecks have contributed to a depletion of genetic diversity in the crop, which has hampered breeding progress. We found two distinct cytotypes of the wild pre-domesticated *C. microcarpa*, with one being  $2n=6x=40$  (Type I) and the other  $2n=6x=38$  (Type II). A chloroplast phylogeny from whole chloroplast genomes of 84 individuals indicated a recent shared ancestry involving a common tetraploid progenitor. Divergence time estimates for polyploid lineages closely related to *C. sativa* (*C. intermedia*  $2n=4x=26$  and both *C. microcarpa* cytotypes) suggest a common chloroplast ancestor around ~65 kya. Despite sharing a very recent common chloroplast ancestor, genetic evidence shows that the two cytotypes of *C. microcarpa* have different diploid ancestors in their most recent whole-genome duplication events. Leveraging PacBio HiFi long-read sequencing technology, a new reference genome of *C. sativa* ‘Suneson’ was generated to allow for better genomic inferences for crop improvement. Remapping of whole-genome resequencing data of over 200 lines of *C. sativa* was used to provide new insights on the population structure in the crop and to identify populations and individuals with high value for breeding programs. The discovery of new species and populations of wild *Camelina*, and the characterization of their relationships enables the theoretical framework for the resynthesis of new lines of the allohexaploid to incorporate novel genetic diversity.

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# Role of environment in establishment of subgenome dominance in *Camelina*

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Whole genome duplications (WGD) are prevalent throughout Angiosperm evolution and contribute to shaping adaptive traits in plants. Gene expression is biased in many studied polyploids such that one subgenome maintains a higher global expression level over other(s). However, little is known about how this phenotype is affected, and potentially established, by environmental conditions experienced by recently formed polyploids. Although expression-level dominance has been characterized in many allopolyploid plant species, it is generally described from plants growing in uniform conditions, which is not representative of the expression landscape of plants growing in the wild or in different climates. Parental diploid species adapted to different environmental conditions may contribute differentially to the inherited expression profile in allopolyploids. However, it remains unclear whether subgenome expression shifts toward the more favored subgenome(s) relative to environmental conditions. *Camelina sativa* is a low-input oilseed crop suitable for food and biofuel production on otherwise unsuitable agricultural land. Its favorable seed fatty acid composition and quantity is amenable to aviation biofuel and represents a tractable, low-emission alternative for aviation. The crop has recent hybrid origins from two extant diploid species, *C. hispida* and *C. neglecta*, with a third subgenome derived from a taxon somewhat closely related to *C. neglecta*. As an allohexaploid, *C. sativa*'s genome contains the full genomic complements (subgenomes) from these diploid progenitors and transcribes their genes to perform necessary biological functions with the *C. hispida* subgenome showing expression-level dominance over the other two subgenomes. Growth trials in controlled environmental conditions were conducted to characterize the effect of growing temperature on subgenome dominance and to determine whether these changes correspond to transcriptional changes in the diploid parents. Further understanding the magnitude of this effect, and the potential mechanisms underlying it may lead to a more complete understanding of the establishment of subgenome dominance.

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# GENOME WIDE ASSOCIATION STUDY TO IDENTIFY SIGNIFICANT MARKER-TRAIT ASSOCIATIONS FOR IMPORTANT AGRONOMIC AND SEED QUALITY TRAITS OF CAMELINA

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## ABSTRACT

Camelina (*Camelina sativa*) is an ancient oilseed crop experiencing a recent renaissance as a potential alternative oilseed crop for renewable biofuel production. However, current cultivars are considered quite primitive and can be significantly improved through efforts of modern plant breeding and biotechnology. Minimal work has been done to date to identify and map genetic control of important agronomic and seed quality traits of camelina. This study engineered a novel field trial approach that utilized the paper-pot transplant method to evaluate replicated single plants instead of plots, which greatly reduced the resources (field space, human labor) required to execute a field trial. Important agronomic (days to flower and plant height) and seed quality traits (total oil content, fatty acid composition, seed size and mass) were evaluated in a spring camelina diversity panel. This was the first field trial that evaluated the spring diversity panel for these important agronomic traits and provided important insights into natural phenotypic diversity. The spring camelina diversity was previously genotyped via genotype by sequencing (GBS) to generate 6,192 single nucleotide polymorphism (SNP) markers, and the marker dataset was made publicly available for use in genome wide association studies (GWAS). Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK) was the GWAS method utilized to identify significant SNP marker-trait associations for almost every phenotypic trait of interest. In total, there were 83 total significant SNPs across all traits of interest, with 61 of those SNPs being unique to only one trait. This study demonstrated that replicated single plants, rather than small plots, are a viable approach to field trials, and improved our understanding of phenotypic diversity and genetic control of important agronomic and seed quality traits in camelina. This knowledge will facilitate future camelina genetic research and allow breeders to accelerate improvement of current camelina cultivars.



# Identification of QTLs and Candidate Genes for Camelina Nitrogen Use Efficiency and Oilseed Traits Using a High-quality Genome

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*Camelina sativa* is an oilseed crop with potential for bioenergy production and as a multi-purpose crop. Significant limitations exist that prevent camelina from being efficient bioenergy feedstock and incorporated into modern agricultural production systems, primarily for its low nitrogen use efficiency and low seed and oil yields. To better characterize this species and improve agronomic traits for sustainable production, genomic resources, especially high-quality genomes, are needed for the analysis of variation in the pathways responsible for such traits as seed size, oil content, fatty acid synthesis, and their responses to nitrogen fertilization. In this study, a chromosome-level camelina genome was assembled and large numbers of high-quality molecular markers (SNPs and InDels) were developed from previously re-sequenced camelina populations. A camelina diversity panel was grown in Sidney, Montana, under high and low nitrogen soils. Great variation was observed in many traits related to oil yield and nitrogen use efficiency, and QTLs for several of those traits were identified. Haplotype assemblies along with transcriptome analyses are used to identify candidate genes for future studies. Together these genomic resources should provide powerful tools to understand camelina genetics and physiology, and ultimately develop next generation camelina varieties.

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# Transcriptional regulation of lipid metabolism for enhanced seed oil yield and quality in *Camelina sativa*

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The widespread adoption of *Camelina sativa* as an industrial oilseed crop largely depends on increasing yield, which is in part constrained by inadequate knowledge of gene regulatory networks (GRN) controlling lipid metabolism. To better understand and identify the transcription factors (TFs) associated with the regulation of lipid metabolism genes, we analyzed the expression of 5,590 transcription factors and 2,765 lipid-related genes (LRGs) from >130 RNA sequencing samples<sup>1,2</sup>. We identified 350 TFs highly co-expressed with lipid-related genes (LRGs). These TFs were ranked based on their expression levels in seeds and the number of co-expressed LRGs, of which the top 22 TFs were selected for further characterization<sup>2</sup>. Potential lipid metabolism target genes for 16 TFs were identified by DNA affinity purification-sequencing (DAP-seq), and results validated by co-expression analyses<sup>2</sup>. We also performed the Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq) in developing and mature seed embryos to capture the genome-wide landscape of TF binding sites. The analysis revealed the accessible chromatin regions (ACRs) in genes related to lipid metabolism, seed development, and maturation. These results and their corresponding expression data are currently being analyzed to identify ACRs associated with subgenome dominance and expression bias of homeolog genes and integrated with binding site information acquired from DAP-seq. Further, we investigated the effect best TF candidates on the biosynthesis of seed oil by ectopic overexpressing from a seed-specific promoter. T<sub>3</sub> homozygous plants for each TF were generated, and seed oils as Fatty Acid Methyl Esters (FAMES) were estimated using Gas Chromatography Flame Ionization Detection (GC-FID). Two TFs significantly impacted seed oil accumulation and fatty acid composition. These two TF lines were subsequently subjected to a detailed functional and metabolic characterization.

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## Food for the future: Transforming *Camelina sativa* into a climate stress-tolerant crop

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The consequences of climate change have resulted in over 20% of global cultivated lands to be salinized, leading to all-time high levels of agricultural losses and food insecurity. With saline soils projected to affect 50% of arable land by 2050, developing novel agronomy practices is imperative. A promising option for adapting to saline soils is the genetic transformation of economically relevant crops. *Camelina sativa* (Camelina), is a robust oilseed crop of the *Brassicaceae* family with a unique oil profile containing essential omega-3s and antioxidants. Given its potential for use as an oil supplement in human and livestock diets, Camelina is a prime candidate for optimization in saline soils. Recent work has shown that exapted transposable elements (ETEs), may serve important abiotic stress-related functions within their host, including under salt stress. Accordingly, our work aims to develop salt-tolerant Camelina lines by 1) selecting salt-tolerant ETEs using bioinformatics and phenomics; 2) transforming Camelina using an *Agrobacterium*-mediated transformation protocol; and 3) evaluating the transgenic lines' performance and potential for further applications. We have successfully generated over 70 transgenic lines expressing 5 unique ETEs. 30% of tested lines are showing increased tolerance to salt measured by survival, photosynthetic health, and seed yield. Promising lines are being molecularly characterized to investigate their mechanism of tolerance and evaluate their viability. Furthermore, our lab developed an integrated web portal (SeedML), where Camelina seeds can be evaluated for salt-sensitivity using visible and fluorescent images. This non-destructive portal uses machine learning to characterize seed quality and accurately identify seeds grown in salt. This accessible phenomics tool has significant implications in both the agricultural research and food manufacturing domains. Our work will ultimately produce a complete phenotypic profile of Camelina, while developing a protocol and phenomics approaches that may be adapted to other abiotic stresses associated with climate change.

# Heat Stress during Reproductive Stages Reduces Camelina Seed Productivity and Changes Seed Composition

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Improving tolerance to high temperatures is essential for camelina agronomic sustainability. Camelina plants of two genotypes were exposed to a transient heat stress during the reproductive stages. Four cohorts of pods along the main stem, which were at different stages from fully developed pods, young pods, open flowers and flowering buds at the time of heat treatment, were examined for morphological and seed quality traits at maturity. Our results suggest that heat stress effects are developmental stage specific. Heat stress significantly reduced fertility during flowering, reduced pod and seed sizes, and inhibited storage product accumulation during seed filling which resulted in smaller and lighter seeds. Oil content decreased while protein content increased in seeds from heat treated plants. In addition, fatty acid composition was altered with the reduction of  $\alpha$ -linolenic acid and concomitantly increased linoleic acid being the most significantly affected. To elucidate the genetic response to high temperatures, we used a combinatorial approach to identifying candidate genes associated with heat stress by quantitative trait locus (QTL) mapping and comparative transcriptome profiling. A population of recombinant inbred lines (RILs) was grown in a controlled growth chamber under the high temperature regimes as stated above. The QTL analysis identified several regions with co-located traits related to seed production. Two RILs with contrasting phenotypic responses to heat stress were chosen for gene expression profiling via RNA sequencing. Multiple pathways and genes were found to be strongly affected by heat stress, and many genes expressed differently between the two RILs. Several genes identified within the QTL regions were considered strong candidates that may control heat tolerance during reproduction in camelina. These studies provide resources for future studies that may assist in improving the heat-tolerance of camelina.

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# Interrogating *Camelina sativa* seed developmental transcriptomic data sets to decipher the impact of Omega-3 fatty acid metabolic engineering.

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This study presents a comparative analysis of two RNA-seq experiments aimed at elucidating the seed transcriptional dynamics between *C. sativa* wildtype (WT) and lines engineered (using seven desaturase and elongase elements) to produce novel long chain polyunsaturated fatty acids e.g., docosahexaenoic acid (C22:6 n-3). Specifically, we investigated transgene expression and identified significant transcript changes that could impact product characteristics and oil yield. Differential expression analysis was conducted across a range of seven developmental time points in two separate experiments with and without the seed coat. Our findings reveal several key insights:

**Lipid metabolism:** Trends in transcripts involved in lipid biosynthesis were more pronounced in later sampling stages, although early indications of these trends were evident.

**Proteolysis Transcript Regulation:** Early upregulation of proteolysis transcripts was identified, suggesting potential regulatory mechanisms.

**Impact on Lipid Biosynthetic Network:** Surprising effects on the wider lipid biosynthetic network were observed including changes in transcription factors, indicating complex regulatory interactions.

Overall, our study provides valuable insights into the transcriptional regulation of DHA1 *Camelina* lines compared to WT counterparts, such as a reduction in seed storage transcripts in the transgenic lines as the seed matures. This highlights the importance of considering other transcriptomic effects driven by the transgene expression. This data has utility in understanding metabolic pathways and optimizing desired traits.

## **Development of tools and community resources for *Camelina sativa***

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For *Camelina sativa* to become a viable and widely used oilseed crop, it is essential to develop community-accessible molecular and computational resources that will accelerate research and decrease the barriers for other researchers to investigate this Brassica. We describe here the development of CamRegBase (<https://camregbase.org/>) as a publicly available knowledgebase that provides information on Camelina gene expression, regulatory proteins including transcription factors (TFs) and co-regulators, and burgeoning protein-DNA interactions that constitute the fundamentals of gene regulatory networks. In addition, we have initiated the development of the Camelina TF open reading frame (ORF) collection (TFome) by initially cloning ~900 TF ORFs in recombination-ready vectors that can easily be transferred to bacteria, yeast, or plant expression vectors for easy analysis. Information on these clones is currently provided through CamRegBase, and they will soon be available at the Arabidopsis Biological Resource Center. Further, we are developing a collection of experimentally validated promoters with specific spatial/temporal expression patterns that will be a valuable resource to the Camelina community for targeted metabolic engineering. We have identified 14 genes that exhibit specific vegetative and reproductive tissues and stress response expression patterns, and we are cloning their promoter sequences. The correct annotation for these clones benefited from the sequencing and assembly by our team of the Suneson variety, and this new version of the genome will also be made available through CamRegBase. Given that CamRegBase is a community resource, we expect that researchers around the world will contribute with published or unpublished datasets, making it even more useful to the community.

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# Genetic Research on Camelina: CRISPR/Cas9-mediated gene editing to enhance seed oil in cover crops

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Seed oils produced by cover crops offer a financial incentive for cover-cropping, and the value can be augmented by gene editing. The predominant constituent of seed oils, triacylglycerols (TAGs), can be altered by editing genes involved in lipid metabolic pathways. *Camelina sativa*, a promising cover crop from the Brassicaceae family, is amenable to established methods of plant genetic modification. Four key enzyme-encoding genes were identified in *C. sativa* from well-characterized orthologs in *Arabidopsis thaliana* and targeted for knockout using the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats, CRISPR-associated enzyme 9) system. We hope to increase overall seed-oil content in mature seeds by individually knocking out two lipases, Sugar-dependent 1 (SDP1) and Oil-body lipase 1 (OBL1), which break down TAGs in late-stage seed development. We seek to individually knockout two enzymes that directly synthesize TAGs, Diacylglycerol O-acyltransferase 1 (DGAT1) and Phospholipid:diacylglycerol acyltransferase 1 (PDAT1), which will generate platform crop lines into which transgenic, TAG-synthesizing enzymes that produce specialty oils can be introduced. *C. sativa* presents a unique challenge for gene knockout due to its hexaploidy genome. The CRISPR/Cas9 system must generate a deleterious mutation in all six copies of each gene to achieve a full knockout. To facilitate screening for genetic mutations, CRISPR/Cas9 cloning constructs were designed with three guide RNAs (gRNAs) spaced >100 bp apart to direct Cas9 to cut DNA in multiple locations within the same gene. The purpose was to generate large DNA deletions in each gene for quick detection by PCR amplification and gel electrophoresis, which increases sample throughput and facilitates genetic screening. This research characterizes the efficiency of this method, and it shows preliminary results, providing a valuable tool for researchers working with CRISPR/Cas9 and other hexaploid crops, such as *Triticum aestivum* L. (wheat).

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## Identification of Seed Specific Promoters with A Range of Expression Strengths

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The relative expression levels of genes involved in primary or secondary metabolism can dramatically affect product profile and titer. To augment the suite of very strong seed specific promoters (*e.g.*, napin, glycinin) currently used for oil seed engineering, we have mined existing gene expression databases to identify 10 seed specific genes expressed at levels 0.1x and 0.01x relative to the strong seed specific promoters in these species (*e.g.*, napin orthologs). The promoter and terminator regions of these genes have been cloned to be compatible with Golden Braid 2.0 DNA assembly platform. We will evaluate the strength of this panel of promoters (including existing strong promoters) using by expressing *CpFatB2* sufficient for 14:0 synthesis and *EfDAcT* producing acetyl-TAG in camelina and pennycress. Quantifying the transgene's expression at four different stages of seed development of homozygous T3 plants with single transgene insertions using quantitative reverse transcription PCR (RT-qPCR) and measuring MCFA and acetyl-TAG accumulation in mature seed from these plants. As seed specific promoters typically function effectively across different species especially in Brassicaceae, we will initially only isolate camelina promoters but opportunistically extend to pennycress if warranted.

#### 4. Other Posters

Kaitlin  
Ana Paula  
Laura  
Thiya

Bachle  
Canu  
Marek  
Mukherjee

US National Plant Germplasm System *Camelina* germplasm collection: a public resource

**Laura F. Marek<sup>1\*</sup>**

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The USDA National Plant Germplasm System (NPGS) maintains and distributes samples (most commonly seeds) of more than 16,700 different crop and crop wild relative species for research and educational uses around the world. The NPGS holdings include a collection of 116 accessions of seven species in the *Camelina* genus. Most of the accessions are *C. sativa* (47) and its closest wild relative *C. microcarpa* (46). The camelina collection also contains the only publicly available sample of the recently described species *C. neglecta* discovered during a flow cytometry examination of the collection (reported in 2019, PhytoKeys). In addition, the NPGS is custodian of seven samples, all *C. sativa*, which are still governed by intellectual property right restrictions. One of these will be available in 2027 after its crop science registry expires; the other six are under plant variety protection status (one has an accepted certificate, five are pending) and not available publicly until 2043 or later. The Ames NPGS location manages and maintains the camelina collection. Regenerations ensure that seed is available for distribution and take place in caged plots in the winter greenhouse. Active distribution lots are kept in Ames; back up samples are held by the NPGS location in Ft Collins, CO and 50% of the accessions are also backed up in the global seed storage vault in Svalbard Norway. Almost 7000 camelina samples have been distributed in the United States and around the world since 1993.

## 5. Resilience and Metabolism

Doug	Allen
Phil	Bates
Demian	Dlagic
Jiahong	Zhou
Sommath	Koley
Rossella	Mastroberardino
Octavia	Roman
Maneesh	Lingwan
Matthew	Garneau
Xiao-Hong	Yu

# Does fatty acid turnover occur concurrently with plant lipid synthesis?

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Plant lipid turnover is an essential process for germinating seedlings, and occurs in senescing tissues, but has not been characterized in developing seeds. We observed simultaneous lipid biosynthesis and turnover at multiple stages of seed-filling in *Camelina*. Our results indicate an active carnitine shuttle and non-intuitive oxidation in mitochondria at later seed-filling stages when peroxisomal oxidation is inactive. Importantly, the different subcellular locations may signify the relevance of co-products including metabolic water for seed germination or reductant and energy for seed-filling. Tests in canola and *Arabidopsis* seeds, and engineered high-oil tobacco leaves and *camelina* seeds indicate the observation is not the exception but the rule and may partially explain underperforming engineered lines of the past.

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## Utilization of triacylglycerol remodeling to engineer increased hydroxy- and polyunsaturated containing fatty acids in *Camelina sativa* seed oils

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Seed oil accumulation does not have to be a metabolic endpoint but can be a dynamic metabolic system where the oil fatty acid composition can be changed after oil initial synthesis. Triacylglycerol (TAG) remodeling is defined as the partial degradation of TAG to diacylglycerol (DAG) and subsequent resynthesis with different fatty acids. We previously utilized isotopic tracing to demonstrate that TAG remodeling is the major mechanism by which the *Brassicaceae* species *Physaria fendleri* accumulates TAG molecular species containing two hydroxyl-containing lesquerolic acids in the *sn*-1,3 positions of TAG. Our recent results reveal the enzymatic mechanism of TAG remodeling. *P. fendleri* TAG biosynthesis that starts with the use of phosphatidylcholine-derived DAG containing common fatty acids. The *sn*-1,2-DAG specific *Pfe*DGAT1 initially produces TAG containing one *sn*-3 lesquerolic acid. Subsequently a novel endoplasmic reticulum localized lipase (*Pfe*TAGL1) that interacts with *Pfe*DGAT1 removes the common fatty acid from the TAG *sn*-1 position. The removed fatty acid re-enters acyl editing for further modification (e.g. hydroxylation, desaturation, elongation, etc.). Finally, the *sn*-2,3-DAG selective *Pfe*DGAT2 adds the second lesquerolic acid to the *sn*-1 position, reproducing TAG with a new fatty acid composition. Engineering the *Physaria* TAG remodeling enzymes into *Camelina sativa* previously engineered to accumulate hydroxy fatty acids both increased the hydroxy fatty acid content, and alleviated the reduced oil phenotype of unusual fatty acid engineering. Engineering TAG remodeling into wild-type *Camelina sativa* greatly increased the omega-3/omega-6 ratio by increasing 18:3 while decreasing 18:2 and saturated fatty acids, without affecting total oil levels. Thus, TAG remodeling is a new pathway of TAG biosynthesis and a new bioengineering tool to produce designer seed oil fatty acid compositions.

## Nutrient Availability and Nitrogen Remobilization Efficiency Influence Seed Yield in Camelina

Demian Dlakic, Andreas Fischer

*Camelina sativa* (L.) Crantz (camelina) is an emerging biofuel crop in the Northern Great Plains. Camelina is an oilseed crop that is drought and cold tolerant, and has a short growth cycle that allows it to be grown in rotation with wheat. These traits make camelina well suited to the environment and cropping systems of the Northern Great Plains. One of the largest barriers to increased camelina acreage is the cost of fertilizer, which often accounts for 70% or higher of total input costs. Nitrogen use of variety 'Suneson' was analyzed by growing plants hydroponically, on either 6.5 mM (HN) or 0.65 mM nitrogen (LN). For both HN and LN, nitrogen was either removed from the nutrient solution at the onset of flowering or continued until plant maturity (four treatments). Nitrogen remobilization efficiency varied with nitrogen treatments. Nitrogen removal at anthesis led to efficient remobilization from aboveground tissues to seeds, with N concentrations decreasing from ~4% to ~0.5% of tissue dry mass. N remobilization also occurred under HN – continued, but >2% remained at maturity, leading to substantial loss with plant residue. In all treatments except HN-continued, the largest fraction of plant N is in seeds at maturity, demonstrating efficient nitrogen remobilization. In addition, all seeds contained approximately 3% nitrogen, which may represent the minimal value for seed viability. These results suggest that optimal nitrogen use is achieved when fertilization occurs at high levels early in the growth cycle. Three varieties from field studies were also examined under the same conditions, and there is considerable variation in nitrogen use between different lines. Transcriptomic analysis will be used to identify genes controlling nitrogen remobilization efficiency under these four different treatments, initiating the functional dissection of this economically important trait.

# Metabolic coordination between reproductive components for seed filling in the oilseed *Camelina sativa*

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The reproductive architecture of oilseed plants is elaborate, with the seeds surrounded by silique walls. The nutrient exchange between reproductive components to support seed metabolism remains unclear. In *Camelina sativa*, siliques protrude above the canopy, are exposed to direct sunlight, and remain a deep green color throughout seed filling. Compared to available green leaves at the time of seed filling, silique walls have a lower surface area and stomata density, but have a higher chlorophyll content, suggesting a unique photosynthetic role. Complementary studies, including metabolite quantification, isotope labeling experiments, microscopy, and a multi-tissue flux map, revealed that silique walls photosynthetically account for 33-45% of total seed carbon. A fluorescent dye applied to the surface of the silique indicated movement to encapsulated seeds without translocation to other siliques, implying that silique walls only contribute locally. The supply from the phloem, which was metabolized in the silique wall, and the photoassimilates of the silique wall were transported to seeds in the form of sucrose, glucose, and glutamine. Approximately 2/3rds of carbon imported by seeds was converted into biomass, and 1/4th of CO<sub>2</sub> produced by seeds was recaptured inside the silique. The supply of carbon from siliques and leaves and synergy between silique tissues results in a latent capacity to produce more seed biomass and may account for limited aborted camelina seed phenotypes and plant performance in dynamic climates. The studies shed light on systems-level coordination between multiple plant structures, and highlight their significant influence on oilseed yield.

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# **SIMPEL 2.0: Automating Untargeted Isotope Labeling Analysis and Pathway Elucidation**

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Lipids fill crucial biochemical roles as signaling molecules and establishing membranes that define organelles. Additionally, lipids are the most energy dense storage reserve with twice the energy content of carbohydrates and valued for many food and biotechnological applications. Understanding the dynamics and fluxes of lipid metabolism in plants is crucial to support engineering efforts that enable production of biofuels, renewable feed stocks to supplant petroleum, and vegetable oils for food applications. Isotopes are commonly used to deduce fluxes through metabolism; however, despite significant advances in stable isotope labeling techniques, data analysis is laborious, and if performed manually, prone to error, due to the number of peaks created when isotopes are incorporated into molecules. Traditional metabolomics data analysis methods with untargeted workflows cannot handle isotopes and do not provide any additional information on metabolic pathways. SIMPEL, a computational tool designed to streamline the analysis of stable isotope labeling data in an untargeted manner, addresses these shortcomings. SIMPEL automates isotopologue binning, ensuring accurate identification and characterization of labeled compounds within high-resolution mass spectrometry (HRMS) datasets. By integrating preprocessed HRMS data and extracting labeling information from all identified compounds, the tool considers the unique properties and characteristics of isotopologue distributions and automatically generates a suite of plots to help with interpretation of complex data. SIMPEL can offer valuable insights into context-specific lipid metabolism in plants but is also being employed to investigate other challenging areas in metabolism, including plant -microbe interactions, nutrient exchange with the environment and photosynthesis. The tools offer an unbiased and rigorous approach which will advance isotope labeling studies and our understanding of metabolism.

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