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The 9th Asian-Oceanian Symposium on Plant Lipids

October 10(Tue)-13(Fri), 2023

Saint Ignatius House (Bldg. #11), Sogang University, Seoul, Korea

Organized by

ASPL Korean Local Committee

Co-organized by

한국식물생명공학회 The Korean Society for Plant Biotechnology

서강대학교 SOGANG UNIVERSITY

BK21 FOUR 서강대학교 생명과학과 스트레스 대응 생체분자기능 교육연구팀

BK21 FOUR IT-Bio융합시스템농업교육연구단 Center for IT-Bio Convergence System Agriculture

선도연구센터 (SRC) 식물생체리듬연구선 Plant Biological Rhythm Research Ce

RCPP Research Center for Plant Plasticity 식물 가소성 연구센터

NBTC 차세대농작물신육종 기술개발사업단 New Breeding Technology Center

부산대학교 PUSAN NATIONAL UNIVERSITY 생명산업융합연구원

부산대학교 PUSAN NATIONAL UNIVERSITY 장수해양바이오혁신인력 양성교육연구단

The 9th Asian-Oceanian Symposium on Plant Lipids

October 10-13, Saint Ignatius House (Bldg. #11), Sogang University, Seoul, Korea

Organizers	
SUH, Mi Chung (Chairperson)	Sogang University, Seoul
KIM, Hyun Uk (Chairperson)	Sejong University, Seoul
Local Organizing Committee	
KIM, Sang Gyu	KAIST, Daejeon
KIM, Yu-Jin	Pusan National University, Miryang
LEE, Kyeong-Ryeol	RDA, Jeonju
LEE, Ok Ran	Chonnam National University, Gwangju
LEE, Saet Buyl	RDA, Jeonju
LEE, Youngsook	POSTECH, Pohang
LEE, Yuree	Seoul National University, Seoul
LIM, Gah-Hyun	Pusan National University, Pusan
RYU, Stephen Beungtae	KRIBB, Ochang
SEO, Pil Joon	Seoul National University, Seoul
YAMAOKA, Yasuyo	Catholic University, Bucheon

Welcome to the 9th Asian-Oceanian Symposium on Plant Lipids

Dear Colleagues,

On behalf of all organizing committee members, we would like to welcome all of you to the 9th Asian-Oceanian Symposium on Plant Lipids (ASPL2023). We are delighted to host all of you to in-person conference after the long COVID-19 pandemic.

This is the second organization of ASPL in Korea, following the 5th ASPL held in Gwangju 2013. Since the 1st ASPL was held in Tokyo 2005, we are sure you agree with us that our community has been well established and advanced friendly, mutually supportive, and fruitfully.

Lipids are one of essential biological molecules in plants. The study of plant lipids has evolved from classical biochemical approaches to more advanced molecular, transcriptomic, functional genomic, lipidomic, and imaging techniques, which have unveiled a plethora of novel lipid functions in various aspects of plant biology. In the program of ASPL2023, we focus on the recent advances in plant lipid biology including novel functions and functional mechanisms of enzymes, the regulation of metabolic pathways, the roles of lipids and secondary metabolites in physiology, development, and defense against biotic and abiotic stresses, re-designing of metabolic pathways and its application, and development of industrially valuable oil crops. The knowledge gained from these studies can lead to innovative strategies to address challenges related to food production, crop yields, and nutritional and industrial needs under climate change.

Last but foremost, we would like to thank local organizing committee members, ASPL board members, co-organizers, and all the sponsors. Their generous supports have played a pivotal role in making the ASPL2023 possible.

We hope that all attendees will enjoy lectures, poster presentations, and meaningful discussions that will lead to new insights and collaborations at the ASPL2023, Seoul.

Warmest wishes,

MiChungSuh 김 현 욱

Mi Chung Suh and Hyun Uk Kim

Chairpersons of the 9th Asian-Oceanian organizing committee

The 9th ASPL Board Members

CHECHETKIN, Ivan	Kazan Institute of Biochemistry and Biophysica, Russia
CHYE, Mee-Len	University of Hong Kong, Hong Kong
GUO, Liang	Hua Zhong Agriculture University, China
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OHTA, Hiroyuki	Tokyo Institute of Technology, Japan
SANINA, Nina	Far Eastern Federal University, Russia
SINGH, Surinder	CSIRO Agriculture & Food, Australia
SUH, Mi Chung	Sogang University, Korea
WADA, Hajime	The University of Tokyo, Japan
WENK, Markus R	National University of Singapore, Singapore
YANG, Chunhong	Institute of Botany CAS, China
ZHOU, Xue-Rong	CSIRO Agriculture & Food, Australia

History of Asian-Oceanian Symposium on Plant Lipids

	Year	Date	Place	Chair
1st	2005	Nov 25 – 27	Tokyo, Japan	WADA, Hajime
2nd	2007	Nov 30 – Dec 2	Tokyo, Japan	NISHIDA, Ikuo
3rd	2009	Nov 27 – 29	Yokohama, Japan	OHTA, Hiroyuki
4th	2011	Dec 2 – 4	HongKong	CHYE, Mee-Len
5th	2013	Nov 29 – Dec 1	Gwangju, Korea	HAN, Oksoo
6th	2015	Dec 2 – 4	Singapore	WENK, Markus R
7th	2017	Nov 29 – Dec 2	Taipei, Taiwan	NAKAMURA, Yuki
8th	2019	Nov 19 – 22	Canberra, Australia	ZHOU, Xue-Rong, SINGH, Surinder
9th	2023	Oct 10 – 13	Seoul, Korea	SUH, Mi Chung, KIM, Hyun Uk

Cooperation

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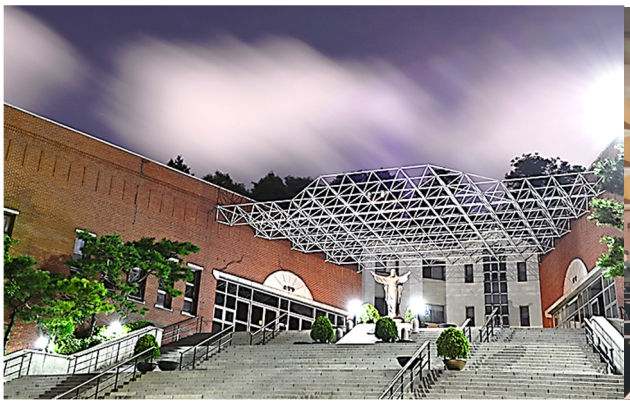
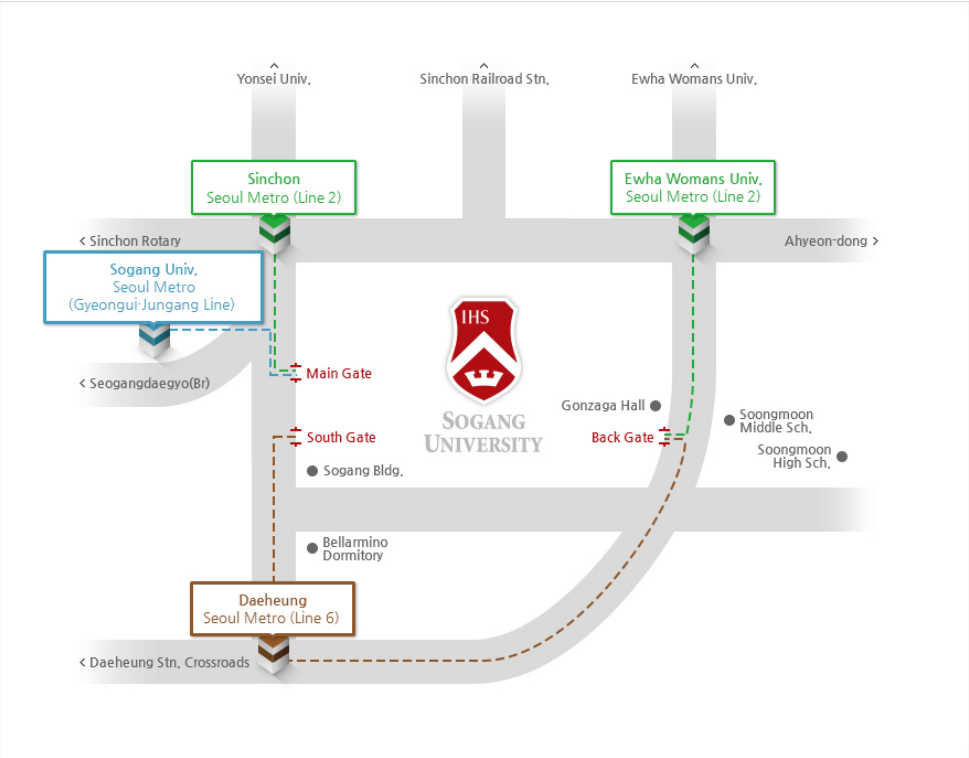
General Information

Venue

Saint Ignatius House (Bldg. #11), Sogang University Campus, Seoul, Korea





From Incheon International Airport (ICN)

By Limousine Bus

1. Right outside the arrival floor of the airport, find the airport bus stop 5B or 12A.
2. Take the Airport Limousine Bus heading to “Sinchon Station” at the bus stop.
3. After getting off at “Sinchon Station” from the bus, walk straight from Exit No. 6 for about 8 minutes and you will see the front gate of Sogang University.

By Airport Railroad Express Train (AREX)

1. From the arrival floor of the airport, go down to B1 floor.
2. Follow the signs to Transportation Center and you will find the subway station to take the airport train AREX.
3. Take the airport train AREX heading to “Gong Deok Station”.
4. Once you arrive at “Gong Deok Station,” walk up the stairway and find a taxi.
5. Ask the taxi driver to drop you off at Sogang University.

From Gimpo International Airport (GMP)

By Subway

1. Take the subway Line No. 9 from “Gimpo International Airport Station”.
2. Transfer to the subway Line No. 2 at “Dangsan Station” and get off at “Sinchon Station”.
3. Take Exit No. 6 and walk straight for about 8 minutes and you will see the front gate of Sogang University

Oral Presentation

Please check the **length of oral presentation** in program table.

The authors of each presentation are asked to bring **PowerPoint** slides.
Presentation PPT: **16:9 ratio** is recommended.

We will prepare a laptop computer for presentation and speakers are encouraged to use the conference computer for presentation to avoid wasting time switching between personal laptops. To avoid software compatibility problems (MS PowerPoint), speakers are advised to save their PowerPoint presentation on a USB memory stick, and bring a backup PDF version of your presentation.

*It is not recommended to bring your own laptop computer (especially MacBook) unless your presentation requires any special software and/or hardware.

Files should be uploaded to the local PCs in the session room during the breaks between the sessions. Speakers should arrive in the session room 10 minutes BEFORE the start of their sessions to report to the session chair.

Poster Presentation

Put-up Date: From 16:00 P.M. October 10 (Tuesday), 2023

Presentation Time: **Odd Number** 16:00 P.M. to 17:15 P.M., October 11 (Wednesday), 2023
Even Number 17:15 P.M. to 18:30 P.M., October 11 (Wednesday), 2023

Take-down Time: Before 12:00 P.M. October 13 (Friday), 2023

Each poster should indicate the poster title, authors, and affiliation and must fit within a **0.9m (Width) x 1.2m (Length)** space.

The poster board is self-standing.

Please check **each poster's presentation code** in program table and each poster's presentation code will be shown on the board.

Outstanding Poster Award Ceremony

Date and Time: October 13 (Fri), 12:10

Session Chair Guideline

Please be present in your assigned room at least 10 minutes before the start of your session to introduce yourself to the speakers in your session.

As participants may have planned to attend specific presentations, please kindly keep the presentation as scheduled. Each speaker shall be advised of his responsibility to stay on schedule at the beginning of the session.

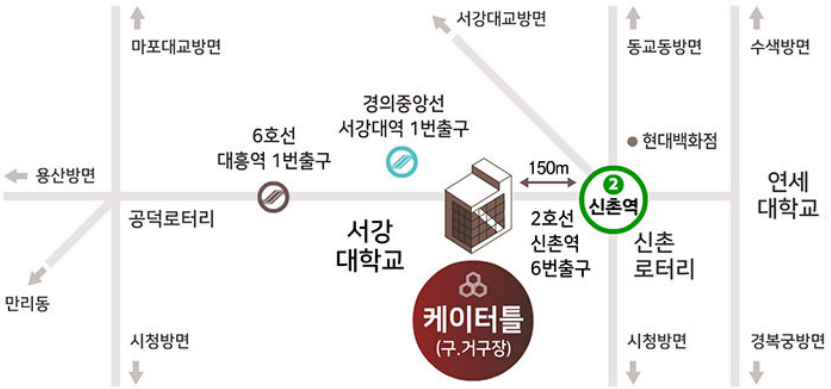
At the beginning of the session, ask participants to turn off their cell phones.
Inform that photograph is not permitted to be taken within the session rooms.

Reception and Poster Put-Up

Place: Conference Venue, #11, Saint Ignatius House, Sogang University Campus
Data and time: October 10 (Tuesday), 16:00~19:00
Catering: Offering 17 kinds of finger foods

Banquet

Place: K-Turtle: Traditional Korean Style (Hanjeongsik)
Data and time: October 12 (Thursday), 19:00~22:00



Lunch

Lunch is provided by the host for 3 days
Place: K-Turtle: Traditional Korean Style

Day 2, October 11 (Wed) : Galbitang (Short Rib Soup)
Day 3, October 12 (Thr) : Bibimbap
Day 4, Octover 13 (Fri) : Samgyetang (Ginseng Chicken Soup)

Excursion

Date and Time: October 13 (Fri), 14:00 ~ 21:00

Changdeokgung Palace Tour
Sogang University Pick up (14:00) ▶ Changdeok Palace (14:30~16:00) ▶ Insadong (16:10~18:30) ▶ Han River Cruise (19:30~20:40) ▶ Drop off at Hotel (Gongdeok station, 21:00)

Korean Folk Village Tour
Sogang University Pick up (14:00) ▶ Korean Folk Village (4.5 h) ▶ Drop off at Hotel (Gongdeok station, 21:00)

The 9th Asian-Oceanian Symposium on Plant Lipids

October 10-13

Saint Ignatius House (Bldg. #11), Sogang University, Seoul, Korea

Program

Day 1 (Tuesday 10, October 2023)

Saint Ignatius House (Bldg.#11)

Registration [16:00-19:00] with Catering Finger Foods & Coffee &Tea

Poster Put-Up and Discussion [16:00-19:00] with Catering Finger Foods

Day 2 (Wednesday 11, October 2023)

8:50-9:00 Opening Remark & Welcome Message

Session 1 (09:00-10:40) Phospholipid and Galactolipids

(Chair: AWAI, Koichiro_Shizuoka U.)

Co-organized by 부산대학교 생명산업융합연구원

S1-1 09:00~09:30 (30 min) NISHIDA, Ikuo (Saitama U, Japan) Differential responses of male and female gametophytes to the genetic disruption of the CDP-choline pathway to phosphatidylcholine biosynthesis in *Arabidopsis thaliana*

S1-2 09:30~09:55 (25 min) NAKAMURA, Yuki (RIKEN, Japan) ER-chloroplast contact in regulating ER glycerolipid biosynthesis

S1-3 09:55~10:20 (25 min) WADA, Hajime (U. of Tokyo, Japan) Deacylation of lipids accelerates the recovery process of photo-damaged photosystem II complex

S1-4 10:20~10:40 (20 min) YAO, Hong-Yan (Fudan U, China) *Arabidopsis* Sec14 proteins (SFH5 and SFH7) mediate inter-organelle transport of phosphatidic acid and regulate chloroplast development

Coffee Break (10:40-11:00)

Session 2 (11:00-12:40): Sphingolipids and Extracellular Lipids

(Chair: LI-BEISSON,Yonghua_CEA)

Co-organized by

S2-1 11:00~11:30 (30 min) LEE, Youngsook **(**POSTECH, Korea) The lipid research at Plant Cell Biology Lab of POSTECH

S2-2 11:30~11:55 (25 min) CAHOON, Edgar (UNL, USA) The long and short of sphingolipid homeostatic regulation in plants

S2-3 11:55~12:20 (25 min) FRANKE, Rochus (U. of Bonn, Germany) Crosstalks supporting and limiting surface lipid deposition - small steps towards the structure of the suberin polyester

S2-4 12:20~12:40 (20 min) LU, Shiyou (Hubei U, China) Fine-tuning KCS3 and KCS12 activity in *Arabidopsis* is essential for sustaining cuticle integrity

Lunch (12:40-14:20)

Session 3 (14:20-16:00) Plant Lipids in Response to Biotic and Abiotic Stresses

(Chair: CHYE, Mee Len_U of Hong Kong)

Co-organized by 부산대학교 PUSAN NATIONAL UNIVERSITY 장수해양바이오혁신인력양성교육연구단

S3-1 14:20~14:50 (30 min) KACHROO, Pradeep (U of Kentucky, USA) Systemic signaling in plants; from cuticle to RNA

S3-2 14:50~15:15 (25 min) MONGRAND Sébastien (CNRS, France) Role of lipids in the reconstitution of plant plasma membrane nanodomains: the case of group 1 REMORIN, a protein involved in plant immunity

S3-3 15:15~15:40 (25 min) SHIMOJIMA, Mie (TIT, Japan) Membrane lipid remodeling under phosphate starvation and low-temperature conditions in *Marchantia polymorpha*

S3-4 15:40~16:00 (20 min) PARK, Hee Jin (CNU, Korea) Lipid modification of SOS3 ensures floral transition under saline stress

Poster presentation [odd number] (16:00-17:15), [even number] (17:15-18:30) with Coffee & Tea

Day 3 (Thursday 12, October 2023)

Session 4 (08:45-10:10): Microbial, Algae, and Early Vascular Plant Lipids

(Chair: SHIMOJIMA, Mie_Tokyo Tech,)

Co-organized by 식물생체리듬연구센터 Plant Biological Rhythm Research Center

S4-1 08:45~09:10 (25 min) LI-BEISSON, Yonghua (CEA, France) Recent progress on our understanding of algal lipid droplet: knowns and unknowns

S4-2 09:10~09:30 (20 min) YAMAOKA, Yasuyo (Catholic U, Korea) Adapting to stress: unraveling lipid metabolism regulation in microalgae Chlamydomonas and aquatic plant duckweeds

S4-3 09:30~09:50 (20 min) KONG, Fantao (DUT, China) Enhanced triacylglycerol accumulation through genetic engineering lipid transporters and regulators in microalgae under standard growth conditions

S4-4 09:50~10:10 (20 min) ZHOU, Xue-Rong (CSIRO, Australia) Variants of *Ostreococcus tauri* Δ6-desaturase revealed the important regions toward activity

Coffee Break (10:10-10:30)

Session 5 (10:30-12:00) Lipase and Oxylipins

(**Chair: DÖRMANN, Peter_U of Bonn**)

Co-organized by 식물생체리듬연구센터 Plant Biological Rhythm Research Center

S5-1 10:30~10:55 (25 min) LEE, Ok Ran (CNU, Korea) Phospholipase-mediated *in planta* haploid inducer, the cornerstone technology in plant breeding

S5-2 10:55~11:20 (25 min) MANO, Jun'ichi (Yamaguchi U, Japan) Acrolein and related *α,β*-unsaturated carbonyls can regulate stomata aperture by modulating both abscisic acid and blue light signaling pathways

S5-3 11:20~11:40 (20 min) LUNG, Shiu-Cheung (U of Hong Kong, Hong Kong) Type-1 lipooxygenase dissociates from Class II acyl-CoA-binding proteins to trigger salt-stress response in soybean

S5-4 11:40~12:00 (20 min) FAN, Ruyi (HAU, China) Insights into the mechanism of phospholipid hydrolysis by plant non-specific phospholipase C

Lunch (12:00-13:50) and Board Member Meeting for ASPL

Session 6 (13:50-15:30) Lipids in Plant Development

(Chair: NAKAMURA,Yuki_RIKEN)

Co-organized by RCPP

S6-1 13:50~14:20 (30 min) AHN, Ji Hoon (Korea U, Korea) Florigen and membrane phospholipids: how they regulate temperature-responsive flowering

S6-2 14:20~14:45 (25 min) LEE, Yuree (SNU, Korea) Deciphering cellular reprogramming for surface barrier restoration in *Arabidopsis*

S6-3 14:45~15:10 (25 min) KIM, Sangchul (U of Missouri-St. Louis & DDPSC, USA) Reciprocal regulation between circadian clock and lipid metabolism in plants

S6-4 15:10~15:30 (20 min) KIM, Yu-Jin **(**PNU, Korea) Role of lipids on rice male reproductive development

Coffee Break (15:30-15:50)

Session 7 (15:50-17:10) Secondary Metabolites and their Application

(Chair: LEE, Ok Ran _CNU)

Co-organized by RCPP

S7-1 15:50~16:15 (25 min) DÖRMANN, Peter (U of Bonn, Germany) Genetic variability of carotenoid (provitamin A) and tocochromanol (vitamin E) metabolism in African oil palm (*Elaeis guineensis* Jacq.)

S7-2 16:15~16:35 (20 min) KIM, Sang-Gyu (KAIST, Korea) Ecological function of inducible lignin in stem

S7-3 16:35~16:55 (20 min) KIM, Hyojin (UNL, USA) Learning from nature for strategies to maximize production and purity of the high value carotenoid astaxanthin in oilseeds

S7-4 16:55~17:10 (15 min) LEE, Saet Buyl (RDA, Korea) Engineering for flavonoid production in *Nicotiana benthamiana* using synthetic biology

Session 8 (17:10-18:30) Multi-Omics and Oil Crops

(Chair: ZHOU, Xue-Rong_CSIRO)

Co-organized by BK21 FOUR IT-Bio융합시스템농업교육연구단 Center for IT-Bio Convergence System Agriculture

S8-1 17:10-17:35 (25 min) GUO, Liang (HAU, China) Mining the seed oil content-related genes by multi-omics analysis in rapeseed

S8-2 17:35~17:55 (20 min) TAN, Xiao-Li (Jiangsu U, China) A new cold tolerant germination rapeseed germplasm is associated with seed fatty acid profile

S8-3 17:55~18:15 (20 min) LEE, Kyeong-Ryeol (RDA, Korea) Effect of diacylglycerol acyltransferases on the seed oil content and acyl preference in C*amelina sativa*

S8-4 18:15~18:30 (15 min) LU, Shaoping (HAU, China) Regulation of seed oil accumulation by lncRNAs in *Brassica napus*

Banquet (19:00 ~ 22:00)

Day 4 (Friday 13, October 2023)

Session 9 (08:45-10:20) Fatty acids, Lipid Droplets and Oils

(Chair: CHEN, Grace_USDA)

Co-organized by NBC 차세대농작물신육종 기술개발사업단 New Breeding Technology Center

S9-1 08:45~09:10 (25 min) BEISSON, Fred (CNRS, France) Photoconversion of fatty acids to hydrocarbons in algae

S9-2 09:10~09:35 (25 min) BATES, Phil (WSU, USA) Dynamic seed oil assembly: acyl and stereochemical selective enzymes remodel triacylglycerol fatty acid composition after initial oil synthesis

S9-3 09:35~10:00 (25 min) MA, Wei (NTU, Singapore) New perspectives on the growing complexity of WRINKLED1 in plant lipid metabolism

S9-4 10:00~10:20 (20 min) RUAN, Chengjiang (DMU, China) Full transcriptome sequencing screening key regulation factor regulating lipid biosynthesis in developing seeds of yellowhorn (*Xanthoceras sorbifolium*)

Coffee Break (10:20-10:40)

Session 10 (10:40-12:10) Lipid Metabolism and Biotechnology

(Chair: CAHOON, Edgar_UNL)

Co-organized by NBC 차세대농작물신육종 기술개발사업단 New Breeding Technology Center

S10-1 10:40~11:05 (25 min) CHEN, Grace (USDA, USA) Biotechnology for Industrial materials production: current progress on improving hydroxy fatty acid content in Lesquerella (*Physaria fendleri*)

S10-2 11:05~11:25 (20 min) ABDUL MASANI, Mat Yunus (ABBC, Malaysia) The current progress of CRISPR/CAS9 technology development in oil palm

S10-3 11:25~11:40 (15 min) XIONG, Yao (HAU, China) Overexpression of CIDEs improves seed oil content of *Brassica napus*

S10-4 11:40~12:10 (30 min) SINGH, Surinder (CSIRO, Australia) Update on the development of plant-based long-chain omega-3 oils

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Closing Remark and Poster Award Ceremony (12:10 ~ 12:50)

Poster Take-Down Before 12:00

Lunch (12:50-14:00)

Excursion (14:00 ~21:00)

Poster Presentations

Session 1: Phospholipid and Galactolipids

P1	YOSHIHARA, A.	Plastid anionic lipids PG and SQDG are important for etioplast development and de-etiolation of *Arabidopsis thaliana*
P2	JIMBO, H.	Turnover of PG and MGDG in PSII repair

Session2: Sphingolipids and Extracellular Lipids

P3	SUZUKI, Y.	Glycosphingolipids are essential for plant development but not for cell proliferation in *Arabidopsis thaliana*
P4	ROH, Y.M.	Investigating the role of sphingolipids in ER stress response of *Chlamydomonas reinhardtii*
P5	TOKIMIZU, H.	Sphingolipid profiles in the leaves of several plants of Amaranthaceae
P6	ISHIKAWA, T.	Sphingolipid ceramide unsaturation in plants: gene evolution, analytical chemistry, and biological functions
P7	KIM, R.J.	ATP-BINDING CASSETTE G23 is required for suberin deposition in *Arabidopsis* seed coats
P8	KIM, R.J.	Arabidopsis 3-ketoacyl-CoA synthase 17 produces tetracosanoic acids required for synthesizing seed coat suberin
P9	CHOI, J.	Identification of a GPI-anchored lipid transfer protein involved in suberization in *Arabidopsis* seedling roots
P10	KIM, H.J.	Protein-protein interactions between fatty acid elongase complex proteins and cuticular wax biosynthetic enzymes
P11	DANG, Q.H.	Regulation of cuticular wax biosynthesis in *Arabidopsis* via CER1 or FAR3-mediated post-transcriptional gene silencing

Session 3: Plant Lipids in Response to Biotic and Abiotic Stresses

P12	KIBA, A.	Inositol phospholipid turnover negatively regulates hypersensitive immune response in *Nicotiana benthamiana*
P13	YU, L.	A chloroplast diacylglycerol lipase modulates glycerolipid pathway balance in *Arabidopsis* in response to environmental stresses
P14	YAO, X.	The function of BnaGDSL in leaf cutin deposition and its influence on drought resistance of *Brassica napus*
P15	ZAKIROVA, R.	Growth-stimulating and anti-stress properties of lipids from *Zygophyllum oxianum* boriss. seeds under salt stress conditions
P16	LEE, J.M.	Deciphering the roles of WOUND ASSOCIATED FACTORs in wound healing processes of *Arabidopsis* Leaves
P17	NGO, H.A.	Non-specific phospholipase C3 is involved in endoplasmic reticulum stress tolerance in *Arabidopsis*

Session 4: Microbial, Algae, and Early Vascular Plant Lipids

P18	KURIMA, K.	Chain length of fatty acids affects photoinhibition of PSII in cyanobacteria
P19	KAWAI-YAMADA M.	Molecular analysis of NAD kinase genes in oleaginous algae *Nannochloropsis oceanica*
P20	KIM, S.K.	Screening of Chlorella mutant strains with high lipid contents for the development of microalgal-based meat alternatives
P21	AHN, J.	Uncovering the role of MYB1 transcription factor under stress conditions in *Chlamydomonas reinhardtii*
P22	JE, S.J.	Sterol biosynthesis contributes to brefeldin-A-induced endoplasmic reticulum stress resistance in *Chlamydomonas reinhardtii*

Session 5: Lipase and Oxylipins

P23	NOH, G.Y.	Functional studies of patatin-like phospholipase in rice male reproduction
P24	PARK, C.W.	Patatin-related phospholipase, pPLAIIIs influence the recovery of defects in rice pollen development
P25	JANG, J.H	Development of *ZmPHOSPHOLIPASE A1* homolog-mediated *in vivo* haploid induction system in tomato and soybean
P26	NAKAYAMA, K	Ca^{2+}-induced activation of lipoxygenase 2 accounts for green leaf volatile-burst in *Arabidopsis* leaves
P27	NAGATA,Y.,	Characterization of enzyme system responsible for the production of mushroom volatile compound, 1-octen-3-ol

Session 6: Lipids in Plant Development

P28	JEONG, S.J.	Function of seed-specific transcription factor ARR13 and ARR21 in the cytokinin signaling pathway
P29	HAN, M.S.	Identifying molecular mechanisms of protective layer formation after floral organ abscission in *Arabidopsis*
P30	KIM, K.Y.	Understanding cuticular adaptation to the water contact surface in *Spirodela polyrhiza*
P31	KIM, E.B.	Impact of Indole-3-acetic acid (IAA) signaling on membrane lipid remodeling in *Arabidopsis thaliana*
P32	SON, Y.J.	Pollen specific receptor kinases are required for pollen tube germination in rice

Session 7: Secondary Metabolites and Its Application

P33	SAKURAI, C.	Improved method for analyzing reactive carbonyl species from small amounts of plant material

P34	KIYOSUE, M.	FATTY ACID DESATURASE5 deficientry suppresses the generation of reactive carbonyl species in *Arabidopsis* crumpled leaf mutant
P35	HADA, A.	Alliin is an excellent scavenger of acrolein, a reactive carbonyl species derived from lipid peroxides
P36	NAKASUGA, R.	Lipid peroxide-derived reactive carbonyl species are common endogenous substrates of glutathione transferase Tau isozymes
P37	NTORURU, J. M.	Linalool exposure enhanced defense against a herbivore and caused accumulation of linalyl diglycoside
P38	KIM, Y.H.	Two MYC2 transcription factors positively regulate ginsenoside biosynthesis in *Panax ginseng*
P39	LEE, S.H.	Genome editing for manipulating glucosinolates in *Brassica rapa*
P40	CHUNG, S.J.	JA-dependent pith lignification in *Nicotiana attenuata* against the stem-boring herbivore
P41	Lee, S.	READRetro: AI-assisted prediction for natural product biosynthesis

Session 8: Multi-Omics and Oil Crops

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Oral Presentations

MEMO

MEMO

S1-1

Differential responses of male and female gametophytes to the genetic disruption of the CDP-choline pathway to phosphatidylcholine biosynthesis in *Arabidopsis thaliana*

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Plant gametogenesis includes not only mitosis but also other cellular processes that are also required for the offspring's survival. Thus, gametogenesis may be a checkpoint process so that lethal mutations can be eliminated before fertilization.

Phosphatidylcholine (PC) biosynthesis is important for the growth and development of plants. However, little is known about if the failure of PC biosynthesis is lethal or the defects in PC biosynthesis can be eliminated before fertilization.

In *Arabidopsis thaliana*, PC biosynthesis is regulated by *CCT1* and *CCT2* genes encoding CTP:phosphorylcholine cytidylyltransferase. Using T-DNA-tagged mutants of *cct1-3*, *cct2-3* and *cct2-5*, we herein showed that no *cct1-3 cct2-3* or *cct1-3 cct2-5* seedings were found among the F3 progeny. Reciprocal crosses of *cct2-3/CCT2* (or *cct2-5/CCT2*) plants in the *cct1-3* background revealed that *cct2-3* and *cct2-5* are not transmissible via *cct1-3* male gametophytes. In contrast, *cct2-3* and *cct2-5* were partly transmissible via *cct1-3* female gametophytes. Alexander's tests for the viability of pollen grains from *qrt1-1 cct1-3 cct2-5/CCT2* plants revealed that *qrt1-1 cct1-3 cct2-5* pollen grains are all alive. However, none of the *qrt1-1 cct1-3 cct2-5* pollen grains could genminate in vitro. Most of mutant pollen grains subjected to in vitro germination conditions lost their ultrafine-fine cellular structues and those with less severe phenotype contained autophagic bodies, suggesting that transmission of *cct1-3 cct2-5* via male gametophytes is prohibited by negation of pollen germination. On the other hand, female gametophytes strategically remains a chance, if any, to rescue the genetic background of *cct1-3 cct2-5* ovules by fertilization with normal pollen.

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MEMO

S1-2

ER-chloroplast contact in regulating ER glycerolipid biosynthesis

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In ER-localized glycerolipid biosynthesis pathway, phosphatidic acid phosphatase catalyzes a crucial reaction step to produce phospholipids, glycoglycerolipids and triacylglycerol. In *Arabidopsis*, we found that a pair of lipid phosphate phosphatases (LPPs) with differential subcellular localizations are required for ER glycerolipid metabolism. LPPα2 and LPPε1, which encode phosphatidic acid phosphatase activity, were differentially localized at ER and chloroplast outer envelopes despite their similar tissue expression pattern. Although no mutant phenotype was observed in single knockout mutants, genetic suppression of these *LPP*s affected pollen growth and ER phospholipid biosynthesis in mature siliques and seeds with compromised triacylglycerol biosynthesis. Although chloroplast-localized, LPPε1 was localized close to the ER and ER-localized LPPα2. This proximal localization is functionally relevant, because overexpression of chloroplastic LPPε1 enhanced ER phospholipid and triacylglycerol biosynthesis similar to the effect of LPPα2 overexpression in mature siliques and seeds. We suggest that ER glycerolipid metabolism requires a chloroplast-localized enzyme in *Arabidopsis*, highlighting the importance of possible ER-chloroplast contact in glycerolipid homeostasis.

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MEMO

S1-3

Deacylation of lipids accelerates the recovery process of photo-damaged photosystem II complex

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Photosystem II (PSII) complex in thylakoid membrane is an efficient energy converter that oxidizes water molecules by using light energy to supply electrons to the following components involved in photosynthetic-electron transport. PSII complex contains many lipid molecules that are essential for the function and maintenance of PSII. Under strong light conditions, PSII complex is dynamically modified during the repair process; however, the molecular mechanism of the dynamic changes in the PSII structure is still unclear. Recently, we identified two genes for lipases, LipA and Pla2, which hydrolyze fatty acids at the *sn*-1 position of MGDG and at the *sn*-2 position of PG, respectively, in the genome of the cyanobacterium *Synechocystis* sp. PCC 6803 and constructed the *lipA* and *pla2* mutants of *Synechocystis*. With the mutants we studied roles of turnover of MGDG and PG in the repair process of PSII complex damaged under strong light. The obtained results indicated that photodamaged PSII dimer is monomerized by the action of LipA, which degrades MGDG molecules in PSII dimer, then PG molecules in the resultant monomer is digested by Pla2 to facilitate degradation and synthesis of D1 that is required for recovery of photodamaged PSII. These findings demonstrate that the turnover of MGDG and PG plays important roles in the recovery process of PSII damaged under high light condition.

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MEMO

S1-4

Arabidopsis Sec14 proteins (SFH5 and SFH7) mediate inter-organelle transport of phosphatidic acid and regulate chloroplast development

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Lipids establish the specialized thylakoid membrane of chloroplast in eukaryotic photosynthetic organisms, while the molecular basis of lipid transfer from other organelles to chloroplast remains further elucidation. Here we revealed the structural basis of *Arabidopsis* Sec14 homology proteins AtSFH5 and AtSFH7 in transferring phosphatidic acid (PA) from endoplasmic reticulum (ER) to chloroplast, and whose function in regulating the lipid composition of chloroplast and thylakoid development. AtSFH5 and AtSFH7 localized at both ER and chloroplast, whose deficiency resulted in abnormal chloroplast structure and decreased thickness of stacked thylakoid membranes. We demonstrated that AtSFH5, but not yeast and human Sec14 proteins, could specifically recognize and transfer PA *in vitro*. Crystal structures of AtSFH5-Sec14 domain in complex with L-α-PA and DPPA (1,2-dipalmitoyl-sn-glycero-3-phosphate) revealed that two PA ligands nestled in the central cavity with different configurations, elucidating the specific binding mode of PA to AtSFH5, different from the reported PE/PC/PI binding modes. Quantitative lipidomic analysis of chloroplast lipids showed that PA and monogalactosyldiacylglycerol (MGDG), particularly the C18 fatty acids at *sn*-2 position in MGDG were significantly decreased, indicating a disrupted ER-to-plastid (chloroplast) lipid transfer, under deficiency of AtSFH5 and AtSFH7. Our studies identified the role and elucidated the structural basis of plant SFH proteins in transferring PA between organelles, and suggested a model for ER-chloroplast inter-organelle phospholipids transport from inherent ER to chloroplast derived from endosymbiosis of a cyanobacterium, providing a new mechanism involved in the adaptive evolution of cellular plastids.

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MEMO

S2-1

The lipid research at plant cell biology lab of POSTECH

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In my lab, we continuously had some lipid-related projects. At the beginning it was about signaling lipids such as phosphatidylinositols. Later it was on ABC transporters that are involved in oil synthesis, or in transport of lipidic molecules, which coat the surface of various tissues and organs of plants. For example, we found that *Arabidopsis* ABCA9 is important for triacylglycerol synthesis in ER. We also found that *Arabidopsis* ABCG5 was important for coating the surface of cotyledons, protecting them against water-sogging of seedlings. Most recently, we studied microalgal oil, and found in Chlamydomonas a homolog of AtABCA9, which is important for lipid synthesis in Chlamydomonas.

We had great adventures discovering new knowledge in this field. I thank many wonderful collaborators who helped us.

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MEMO

S2-2

The long and short of sphingolipid homeostatic regulation in plants

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Regulation of sphingolipid homeostasis in plants is critical for maintaining sufficient glycosphingolipid amounts for growth while limiting accumulation of biosynthetic intermediates that trigger programmed cell death, until needed for microbial pathogen defense. Central to sphingolipid homeostatic regulation is the ORM protein, which functions as a negative regulator of serine palmitoyltransferase (SPT), the first step in long-chain base synthesis. We have shown that gene-edited *Arabidopsis* mutants lacking ORMs have non-viable seeds that hyperaccumulate ceramides from the loss of SPT regulation. ORMs reversibly control SPT biosynthetic flux in response to intracellular sphingolipid concentrations: SPT-repression in response to excess intracellular sphingolipids and SPT de-repression when sphingolipids are required for growth. Plants have the additional need to accumulate the apoptotic-inducers ceramides and long-chain bases to rapidly induce programmed cell death for the hypersensitive response for bacterial and fungal pathogen defense. The mechanism for reversible ORM regulation of SPT has been unclear. Recent evidence from the cryo-EM structure of the *Arabidopsis* SPT complex revealed that ceramides bind ORMs to repress SPT activity. Most effective for SPT repression are ceramides with trihydroxy long-chain bases that are typically paired with very-long chain fatty acids, derived from LOH1 and LOH3 ceramide synthase activity. These ceramides are essential for cell growth. In contrast, ceramides with dihydroxy long-chain bases, which are typically bound to shorter chain C16 fatty acids via LOH2 ceramide synthase activity and do not support growth, were less effective in ORM-mediated SPT repression. These and recent findings will be discussed in the context of a role for LOH2 ceramide synthase in overriding SPT regulation to mount pathogen defense.

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MEMO

S2-3

Crosstalks supporting and limiting surface lipid deposition - small steps towards the structure of the suberin polyester

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Suberin is a heterogenous polyester, built of long-chain and very-long-chain fatty acid derivatives and minor amounts of phenolic constituents such as ferulic acid. Suberin is precisely deposited between the primary cell wall and the plasma membrane. There it appears in a highly organized lamellar structure and acts as a diffusion barrier. This barrier contributes to the protective function of periderms and is required to control the uptake and loss of water and nutrients in roots. What determines structure and the position of suberin in the cell wall is greatly unknown.

Mass spectrometry based linkage type analyses in suberin biosynthetic mutants highlighted low abundant ω-hydroxyacid ferulate ester (>4%) as potential structural suberin components. Investigating suberin plasticity in subsequent molecular genetic approaches uncovered that depletions in alkyl ferulate ester content or crosslinking delay the development of suberized barriers in roots and seeds. Moreover, the ultrastructural analysis revealed that the precise subcellular deposition of suberin is disturbed indicating that ferulate cross-linking is involved in the accurate anchoring of the lipid polymer in the cell wall. Furthermore, suberized tissues affected in ferulate ester crosslinking exhibit a higher permeability for water, solutes and organic compounds. Together, the deposition of the lipid polyester suberin is delayed and forms a less efficient barrier without coordinated supply of phenolics.

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MEMO

S2-4

Fine-tuning KCS3 and KCS12 activity in *Arabidopsis* is essential for sustaining cuticle integrity

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Cuticle consisting of wax and cutin is required for plant survival under flexible conditions. -ketoacyl-CoA synthases KCSs usually act as a component of fatty acid elongation (FAE) complex participating in the production of very long chain fatty acids (VLCFAs), providing precursors for the synthesis of various lipids including wax. However, our recent study showed that KCS3 and KCS12 act as negative regulators of wax biosynthesis instead. In this study, we found that different from KCS3 OE lines, KCS12 OE lines displayed floral organ fusion phenotype owing to disturbed cuticle biosynthesis. This intrigued us to compare the functions of KCS3 and KCS12 in cuticle formation. Our results showed that KCS3 mutation caused greater impacts on wax production whereas KCS12 mutation exerted severer effects on cutin synthesis. Simultaneous mutation of KCS12 and KCS3 significantly increased the amounts of wax and cutin, moreover, the increasing rate exceeds that of either parent, suggesting that KCS12 and KCS3 play additive roles during cuticle biosynthesis. The cuticle permeability of double mutants was greatly enhanced as compared with the single mutant, thus increasing plant susceptibility to drought stress and causing floral organ fusion. Taken together, our study confirmed the regulatory roles of KCS3 and KCS12 in cuticle biogenesis and identified that maintaining the KCS3 and KCS12 at certain levels is essential for forming a functional cuticle layer.

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MEMO

S3-1

Systemic signaling in plants; from cuticle to RNA

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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), which also serves as a precursor for glycerolipids. A normal SAR also requires a normal cuticle, which in turn regulates water potential and thereby apoplastic transport of salicylic acid (SA). 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 and RNA-mediated signaling in SAR.

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MEMO

S3-2

Role of lipids in the reconstitution of plant plasma membrane nanodomains: the case of group 1 REMORIN, a protein involved in plant immunity

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The plasma membrane forms a selective barrier between the inside and the outside the cell. It is a highly organized proteo-lipidic matrix, subdivided into membrane domains necessary to fulfill its physiological functions, particularly for signal transduction. In this work, we used a range of biophysical methods to describe how a specific set of plasma membrane lipids (i.e. lecithins, particularly phosphoinositides, and sterols) and the plant protein Remorin display a complex set of interactions and create Remorin-enriched nanodomains in the plane of the lipid bilayer. Remorins are a family of multigenic phosphoproteins of the plasma membrane, involved in biotic and abiotic plant interaction mechanisms, partnering in molecular signaling cascades. Signaling activity of remorins depends on their phosphorylation states and subsequent clustering into nano-sized membrane domains. The presence of a coiled-coil domain and a C-terminal domain is crucial to anchor remorins to negatively charged membrane domains, however the exact role of the N-terminal intrinsically disordered domain (IDD) on protein clustering and lipid interactions is largely unknown. Here we combine chemical biology and imaging approaches to study the partitioning of group 1 remorin into anionic model membranes mimicking the inner leaflet of the plant plasma membrane. Using reconstituted membranes containing a mix of saturated and unsaturated PhosphatidylCholine (PC), PhosphatidylInositol Phosphates (PIPs), and sterol, we investigate the clustering of remorins to the membrane and monitor the formation of nano-sized membrane domains. REM1.3 promoted membrane nanodomain organization on the exposed external leaflet of both spherical lipid vesicles and flat supported lipid bilayers. Our results reveal that REM1.3 drives a mechanism allowing lipid reorganization, leading to the formation of remorin-enriched nanodomains. Phosphorylation of the N-terminal IDD by the calcium protein kinase CPK3 influences this clustering and can lead to the formation of smaller and more disperse domains. Our work reveals the phosphate-dependent involvement of the N-terminal IDD in the remorin-membrane interaction process by driving structural rearrangements at lipid-water interfaces.

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MEMO

S3-3

Membrane lipid remodeling under phosphate starvation and low-temperature conditions in *Marchantia polymorpha*

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Under inorganic phosphate (Pi) starvation, phospholipids are degraded and galactolipids compensate for the absence of phospholipids in the membranes. This lipid remodeling system is widely conserved in seed plants, but it remained unclarified how the system evolved during terrestrialization of plants. In *Arabidopsis*, phosphatidic acid phosphohydrolase (PAH) and non-specific phospholipase C 5 (NPC5) are involved in phospholipid degradation during Pi starvation, although NPC5 contributes lower than PAH. In a non-vascular plant *Marchantia polymorpha*, however, we found that PAH is not mainly involved in the lipid remodeling. To clarify the role of three Marchantia NPC homologs (MpNPCa, MpNPCb, MpNPCc), we produced knock-out mutants of each NPC. Under Pi starvation, the phenotype and lipid compositions were comparable among wild type (WT) and the mutants. We also compared them under low-temperature conditions because expression of *NPCb* was suggested to be associated with low-temperature stress. In WT, the molar ratio of phospholipids containing high level of C20:5 (EPA) increased with decreasing that of plastid glycolipids, and the amount of EPA in phospholipids also increased under low temperature. The lipid composition of the NPCb mutant was similar to that of the WT, but the proportion of EPA in phospholipids was found to be slightly lower than the WT. These results indicated that the NPCs are not phospholipases involved in the lipid remodeling in Marchantia, as in *Arabidopsis*, but also suggested that NPCb might be slightly involved in phospholipid degradation under low temperature.

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MEMO

S3-4

Lipid modification of SOS3 ensures floral transition under saline stress

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Lipid modifications of proteins play crucial roles in various biological processes. N-myristoylation of SOS3 is normally located inside the protein. However, upon salt-induced calcium binding, it becomes exposed. The activated SOS3 then interacts with SOS2, a kinase, and together they form the SOS2-SOS3 complex. This complex translocates to the plasma membrane where SOS1, a sodium transporter, is located. Once SOS1 is phosphorylated and activated, it facilitates the efflux of sodium ions out of cells to reduce cytosolic sodium levels. High salt conditions delay the flowering process by degrading GIGANTEA (GI) protein, a key regulator of photoperiodic-dependent flowering. Here, I present that S-acylation (also known as palmitoylation) of SOS3 enables its interaction with GI in the nucleus, protecting GI proteins from salt-induced and proteasome-mediated degradation. This additional regulatory layer, facilitated by the lipid modification of SOS3, ensure the floral transition despite the delay caused by high salt conditions.

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MEMO

S4-1

Recent progress on our understanding of algal lipid droplet: knowns and unknowns

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Lipid droplets (LDs) are ubiquitous and specialized organelles in eukaryotic cells. Consisting of a triacylglycerol core surrounded by a monolayer of membrane lipids, LDs are decorated with proteins and have myriad functions, from carbon/energy storage to membrane lipid remodeling and signal transduction. The biogenesis and turnover of LDs are therefore tightly coordinated with cellular metabolic needs in a fluctuating environment. This is particularly true for algae where they have to adapt their metabolism to face dynamic environmental conditions constantly. In this talk, I discuss current findings on LD dynamics and their function during acclimation to environment, with a particular focus on LD proteins and their physiological functions in the model microalga *Chlamydomonas reinhardtii*.

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MEMO

S4-2

Adapting to stress: unraveling lipid metabolism regulation in microalgae chlamydomonas and aquatic plant duckweeds

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Microalgae are promising candidates for sustainable biofuel production due to their ability to accumulate high levels of oil under stress conditions. However, the underlying regulatory pathways governing lipid metabolism in response to stress remain incompletely understood. This study aimed to understand the stress response mechanism and identify key regulators of lipid metabolism under stress conditions in microalgae.

The MYB-type transcription factor MYB1 was highly induced under various stress conditions in the green microalga *Chlamydomonas reinhardtii*. Through analysis of *myb1* mutants, we observed reduced total fatty acids and storage lipids compared to the parental strain upon nitrogen depletion. This identifies MYB1 as a crucial positive regulator of lipid accumulation in *C. reinhardtii* during nitrogen depletion stress.

Additionally, we explored the response to endoplasmic reticulum (ER) stress, induced by the accumulation of misfolded proteins. The Chlamydomonas CrIRE1/CrbZIP1 pathway emerged as a key player, stimulating the unfolded protein response (UPR) and triggering diacylglyceryltrimethylhomoserine (DGTS) and pinolenic acid (18:3Δ5,9,12) synthesis in response to ER stress. Recent studies also highlighted its regulatory role in sterols and sphingolipids, emphasizing its significance as an essential component of the ER stress response in microalgae.

Furthermore, lipid metabolomics data of duckweeds, known for rapid growth and high biomass production, will be presented. This investigation elucidates lipid alterations enabling duckweeds to overcome various stress conditions. These findings promise to contribute to our understanding of lipid metabolism regulation in microalgae and duckweeds, with implications for sustainable biofuel and bioproduct production.

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MEMO

S4-3

Enhanced triacylglycerol accumulation trough genetic engineering lipid transporters and regulators in microalgae under standard growth conditions

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Microalgae are promising platform for biofuel production. Lipid transporters and Transcription factors (TFs) play important role in lipid metabolism in microalgae. We previously indentified and characterized novel lipid transporter ABCA2 and transcription factor MYB1, which are the key regulators of lipid metabolism in green microalga *Chlamydomonas* under nitrogen (N) depletion conditions. However, the functions of these lipid regulators on lipid metabolism in microalgae under standard growth conditions remain poorly understood.

Recently, we examined the effects of lipid transporters and TF overexpression on lipid metabolism and physiological changes in *Chlamydomonas*. Under standard growth conditions, our results showed that the co-expression of chloroplast and ER-localized lipid transporters (*FAX1/FAX2/ABCA2*-OE) has an additive efect on enhancing triacylglycerols (TAGs), total fatty acids (tFA) and membrane lipid accumulation. Moreover, *FAX1/FAX2/ABCA2*-OE accelerates the polyunsaturated FA remobilization from membrane lipids to TAG by fine-tuning the key genes in lipid metabolism under standard growth conditions. *FAX1/FAX2/ABCA2*-OE shows better traits for lipid accumulation than the parental line and previously reported individual FA transporter-OE. In addition, we found that TF *MYB1* overexpressiong transformants increased both lipid and starch contents without compromising growth, and enhanced biomass production under standard growth conditions. To the best of our knowledge, this is the first report about employing TF engineering to synergistically increased lipid, starch and biomass in microalgae. Take together, our study provides potential useful strategies to increase the production of FA-derived energy-rich and value-added compounds in microalgae.

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MEMO

S4-4

Variants of *Ostreococcus tauri* Δ6-desaturase revealed the important regions toward activity

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Fatty acid Δ6-desaturases belong to a family of membrane-bound front-end desaturases, which catalyse double bond formation at specific positions near the fatty acid carboxyl end. Δ6-Desaturases introduce a double bond at the Δ6-position of polyunsaturated fatty acids including ω6 linoleic acid and ω3 linolenic acid, producing gamma-linolenic acid and stearidonic acid, respectively. Front-end desaturases contain a highly conserved HPGG motif in the heme-binding region of a N-terminal cytochrome b_5 domain, and three histidine boxes (His Box). We further explored the effect of other regions of *Ostreococcus tauri* Δ6-desaturase on enzyme activity, by rationally designing 30 different variants. These variants included deletions at N-terminus or C-terminus, multiple amino acid replacement, or single substitutions of highly conserved amino acids outside the HPGG motif and His boxes for alanines. Wild type and variant Δ6-desaturases were transiently expressed in *Nicotiana benthamiana* leaf to characterise their Δ6-desaturation activity on endogenous omega-3 and omega-6 fatty acid substrates. Compared to WT Δ6-desaturase, more than half of the variants had reduced or no activity on both omega-3 and omega-6 substrates. Surprisingly, many single substitutions of highly conserved amino acids did not affect activity. In contrast, a variant with 41 non-conserved amino acid residue replacement with alanine show no activity. Some variants with single amino acid substitutions showed reduced activity, indicating the WT residues at those positions were important for Δ6-desaturase activity. In addition to the linear sequence, secondary structure regions formed by residues from non-linear linkages also play a significant role.

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MEMO

S5-1

Phospholipase-mediated *in planta* haploid inducer, the cornerstone technology in plant breeding

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Doubled haploid technology is a potent method for generating pure homozygous lines within two generations through haploid induction and chromosome doubling. However, traditional *in vitro* haploid induction systems have limitations, being labor-intensive and genotype-dependent. To overcome these limitations, an innovative *in planta* haploid induction system was developed, utilizing the sperm-specific phospholipase A, MATRILINEAL/NOT LIKE DAD/ZmPHOSPHOLIPASE-A1 (MTL/NLD/ZmPLA1). Mutations in MTL/NLD/ZmPLA1 induce maternal haploid embryos in monocots, such as rice, wheat, and foxtail millet, but their potential in dicots remains unexplored. Our study aims to identify novel MTL/NLD/ZmPLA1 homologs involved in haploid induction in *Arabidopsis* and rice. In *Arabidopsis*, the *AtpPLAIIγ* gene was expressed in the gynoecium, particularly in the funiculus and ovule during flower stages 13 and 14. Loss-of-function mutations in *AtpPLAIIγ* trigger maternal haploid embryos when used as the female parent, with an average rate of 1.07%. An intriguing observation in the funiculus of *AtpPLAIIγ* edited lines reveals mis-localization of PIN1 and PIN3, key auxin transporters, hinting at a potential mechanism contributing to haploid induction. In rice, the homologous gene *OspPLAIIη/OsMATL2*, expressed in pollen, enables haploid induction through CRISPR-Cas9-mediated editing in *japonica* rice, with an average efficiency of 6.34%. Our findings demonstrate the potential of genetically manipulating *AtpPLAIIγ* and *OspPLAIIη/OsMATL2* to activate the haploid induction system, resulting in viable haploid seeds in both monocot and dicot crops. This technology holds significant promise for enhancing efficiency of crop breeding programs across diverse plant species.

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MEMO

S5-2

Acrolein and related α,β-unsaturated carbonyls can regulate stomata aperture by modulating both abscisic acid and blue light signaling pathways

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Oxidation of membrane lipids by reactive oxygen species (ROS) leads to the formation of various oxylipin carbonyls. Those oxylipin carbonyls that have the α,β-unsaturated bond, such as acrolein and 4-hydroxy-(*E*)-2-nonenal, are potent electrophiles and collectively designated as reactive carbonyl species (RCS). RCS can mediate oxidative stimuli to proteins and exert various biological activities as signals and injurying agents. For plants, we have demonstrated the involvement of RCS in the tissue injury under salt stress and aluminium stress, programmed cell death during senescence, and the auxin signaling for lateral root formation.

Abscisic acid (ABA) signaling in guard cells leading to stomata closure involves ROS as an intermediate signal. We verified the possibility that RCS mediate the signal for stomata closure. Application of ABA to *Arabidopsis* leaves caused significant increase in the RCS levels. When the carbonyl scavenger dipeptide carnosine was added, the RCS increases were suppressed and the stomata closure was inhibited. Exogenously added acrolein activated the inward Ca^{2+} channel and promoted stomata closure. Thus, RCS mediate ABA signal for stomata closure.

We then tested the effect of RCS on the blue light (BL) signal pathway for stomata opening. It was found that the addition of RCS (acrolein and HNE) inhibited stomata opening and H^+ pumping in response to BL. The target site(s) of RCS was confined to the plasma membrane H^+-ATPase or its activation/deactivation machinery.

These results indicate that RCS have critical roles in driving stomata closure by accelerating ABA signal and inhibiting BL signal in guard cells. Possible mechanisms are discussed.

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MEMO

S5-3

Type-1 lipooxygenase dissociates from class II acyl-coA-binding proteins to trigger salt-stress response in soybean

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Phosphatidic acid (PA) is produced during salt-stress signaling. In *Arabidopsis*, PA generation from membrance phospholipids is facilitated by Class II acyl-CoA-binding proteins (ACBPs). Besides the conservation of an acyl-CoA-binding domain, Class II ACBPs are characterized by the presence of *C*-terminal ankyrin repeats for mediating protein–protein interactions. This mechanistic study aimed to unravel the cooperative role of Class II GmACBPs (GmACBP3 and GmACBP4) with protein partners in triggering salt-stress response in soybean (*Glycine max*) roots. GST:GmACBP4 pull-down assays identified type-1 lipoxygenase (VLXB) as a protein interactor. Bimolecular fluorescence complementation and Strep-Tactin pull-down assays indicated that Class II GmACBPs, when bound with specific (C18:2 and C18:3) acyl-CoAs, sequestered VLXB at the endoplasmic reticulum. Lipidomic analysis showed that salt-treated roots were enriched in C32:0-PA as a salt-stress signal. The competition of PA with acyl-CoAs for binding with Class II GmACBPs weakened the GmACBP–VLXB interaction. Moreover, (q)RT-PCR and immunblot analyses indicated that salt treatment induced alternative splicing of *GmACBPs* to produce protein variants incapable of VLXB interaction. Together, we demonstrate a dual mechanism that triggers the dissociation of VLXB from Class II GmACBPs for the generation of oxylipin signals in the salinity response.

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MEMO

S5-4

Insights into the mechanism of phospholipid hydrolysis by plant non-specific phospholipase C

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Non-specific phospholipase C (NPC) hydrolyzes major membrane phospholipid, such as glycosyl inositol phosphoryl ceramide (GIPC), phosphatidylcholine (PC), mediates the remodeling of membrane lipids, recovers and utilizes inorganic phosphorus. Despite extensive studies on NPCs reveal their fundamental roles in plant growth and development, the mechanistic understanding of phospholipid-hydrolyzing by NPCs, remains largely unknown. And, among various classes of phospholipases (including PLA1, PLA2, PLD, PI-PLC, and NPC), eukaryotic NPC is the only one whose structure and working mechanism have remained uncharacterized. In this work, we report the crystal structure of *Arabidopsis* NPC4 at a resolution of 2.1 Å. NPC4 is divided into a phosphoesterase domain (PD) and a C-terminal domain (CTD), and is structurally distinct from other characterized phospholipases. The previously uncharacterized CTD is indispensable for the full activity of NPC4. Mechanistically, CTD contributes NPC4 activity mainly via CTDα1-PD interaction, which ultimately stabilizes the catalytic pocket in PD. Furthermore, we identified the phosphothreonine replacing T158 in the catalytic pocket, suggesting a phosphoenzyme intermediate. Together with a series of structure-guided biochemical studies, our work elucidates the structural basis and provides molecular mechanism of phospholipid hydrolysis by NPC4. Through Macromolecular docking and molecular dynamics simulation, we further found that a variety of phospholipid molecules can be combined into the catalytic pocket of NPC4, thus confirming that NPC4 harbors promiscuous activities. This study adds new insights into the members of phospholipase family, and can provide an important reference for genetic improvement of efficient use of phosphorus in plants and crops.

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MEMO

S6-1

Florigen and membrane phospholipids: how they regulate temperature-responsive flowering

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Flowering in plants, a crucial lifecycle event, is modulated by temperature via altering florigen activity. We showed that the mobile florigen FLOWERING LOCUS T (FT) in *Arabidopsis* thaliana interacts with negatively charged phospholipid, phosphatidylglycerol (PG), in cellular membranes and binds lipid bilayer. Disrupting PG biosynthesis in phloem companion cells induces temperature-insensitive early flowering, implicating membrane sequestration as a key regulatory mechanism in temperature-responsive flowering. Lower temperatures enhance FT sequestration in the cellular membrane of the companion cell, decreasing soluble FT levels, thus delaying flowering. In contrast, a mutant in PHOSPHATIDYLGLYCEROLPHOSPHATE SYNTHASE 1 accumulates increased soluble FT even at low temperatures, exhibiting reduced temperature sensitivity. We identified a crucial 7-amino acid motif in the C-terminus of FT for FT–PG interaction and FT activity. We also found that FT interacts with THYLAKOID FORMATION 1 (THF1), a chloroplast-localized, transmembrane protein, located in the outer chloroplast envelope. A lesion in THF1 causes temperature-insensitive early flowering, suggesting its primary role in the leaf vasculature to regulate flowering time. A coiled-coil domain of THF1, which is necessary for FT interaction, is critical for its function. Furthermore, we found that a mutation in cis-PRENYLTRANSFERASE 7, which causes the cellular membrane more fluidic and affects the biophysical properties of the thylakoid membranes by restricting membrane fluidity, influences FT distribution in the cellular membrane and its transport from the leaf to the shoot apical meristem. Our findings suggest that the membrane fluidity and the interaction with membrane phospholipid are important for florigen sequestration and temperature-responsive flowering in *Arabidopsis*.

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MEMO

S6-2

Deciphering cellular reprogramming for surface barrier restoration in *Arabidopsis*

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Cuticular wax envelops the outermost layer of plant leaves, providing effective protection against various biotic and abiotic stresses. However, this protective barrier can be compromised by mechanical stress or herbivore attacks, and in some instances, it is deliberately breached during programmed organ shedding processes. While the signaling pathways involved in mechanical wounds and abscission have been extensively studied, the molecular mechanisms underlying the restoration of a damaged surface barrier remain largely unknown. In this study, we aimed to unravel the cellular reprogramming events that occur within internal tissues during the regeneration of the cuticle layer after abscission. To achieve this, we employed a combination of pharmacological approaches and forward and reverse genetic screenings. Through these methods, we successfully identified critical molecular regulators, including phytohormones and transcriptional networks, which play essential roles in the formation of the protective cuticle layer. We are currently engaged in characterizing the detailed pathway associated with these regulators. Additionally, to better understand the differences in the restoration process between programmed and accidental occurrences, we examined the healing process following a mechanical wound. These comparative analyses will provide a broader understanding of surface barrier restoration mechanisms.

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MEMO

S6-3

Reciprocal regulation between circadian clock and lipid metabolism in plants

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The circadian clock and metabolism are interconnected. The clock modulates the daily abundance of cellular factors involved in metabolism while metabolic activities feedback to influence circadian rhythms. However, the precise mechanism by which the clock and lipid metabolism interplay is not well understood in plants. Here, we show that the central glycerolipid metabolite phosphatidic acid (PA) interacts with and modulates the function of the core clock transcription factors LATE ELONGATED HYPOCOTYL (LHY) and CIRCADIAN CLOCK ASSOCIATED1 (CCA1) in *Arabidopsis*. PA reduced the ability of LHY/CCA1 to bind the promoter of their target gene *TIMING OF CAB EXPRESSION1*. Increased PA accumulation and inhibition of PA-producing enzymes had opposite effects on circadian clock outputs. Reciprocally, LHY and CCA1 impact the accumulation of membrane and storage lipids in *Arabidopsis*. Diurnal change in levels of several membrane phospholipid species, including PA, observed in wild-type (WT) was lost in *LHY* and *CCA1* double knockout mutant (*lhycca1*). Triacylglycerol (TAG) accumulation and the expression of genes involved in fatty acid synthesis, including the one encoding β-ketoacyl-ACP synthase III (KASIII), were increased in developing seeds of *LHY*- or *CCA1*-overexpressing plant and decreased in those of *lhycca1* compared to WT. LHY and CCA1 directly bound and activated the promoter of *KASIII* and the binding was inhibited by PA, a metabolic precursor of TAG. Based on the data, we propose that the physical interaction of PA with the clock regulators may function as a cellular conduit to integrate the circadian clock with lipid metabolism in plants.

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MEMO

S6-4

Role of lipids on rice male reproductive development

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Pollen is the male gametophyte of flowering plants and is important for grain production in cereal crops. To produce fertile pollen grain, cell division and pollen wall synthesis are required. From meiosis when the callose surrounding the microspore is degraded by callases secreted from the tapetum anther wall, pollen wall starts to synthesize. Lipids and their derivatives, including fatty acids, waxes, and phospholipids are important components of the pollen wall. We found that Poaceae-specific EXINE PATTERN DESIGNER 1 (EPAD1), lipid transfer protein, function for primxine integrity. In vitro assays indicate that EPAD1 can bind phospholipids, suggesting that plasma membrane lipids bound by EPAD1 may be involved in recruiting and arranging regulatory proteins in the primexine to drive correct exine deposition. In addition, we will present about recent findings that function of patatin-like phospholipases related to meiotic chromosome division, as well as pollen tube growth. Understanding genetic and biochemical mechanisms of lipids underlying pollen development will contribute to male fertility control, which is important for crop breeding.

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MEMO

S7-1

Genetic variability of carotenoid (provitamin A) and tocochromanol (vitamin E) metabolism in African Oil Palm (*Elaeis guineensis* Jacq.)

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The African Oil Palm (*Elaeis guineensis*) which originates from West Africa is the largest oil crop in the world. The crude palm oil (CPO) is extracted from the mesocarp of the fruits. It is rich in saturated fatty acids, carotenoids (provitamin A) and tocochromanols (vitamin E), but it oxidizes rapidly and gets rancid. We screened ~1500 mostly wild palm trees from Ghana and Cameroon for alterations in the contents of carotenoids and tocochromanols. We identified >28 trees that produce mesocarp with highly different composition in carotenoids or tocochromanols. For some lines, we identified the molecular basis of the alterations because they carry polymorphisms in specific biosynthetic genes. These trees will be used for breeding lines with improved vitamin contents and oxidative stability. In proof of principle experiment, a wild oil palm tree (C59) from Cameroon was characterized whose mesocarp completely lacks α-tocopherol and α-tocotrienol and instead accumulates the respective γ forms, suggesting that the activity of γ-tocopherol methyltransferase (VTE4) was affected. Sequencing of the VTE4 locus in plant C59 identified a G/C polymorphism that causes the exchange of a highly conserved tryptophan at position 290 with serine. The W290S exchange renders the VTE4 enzyme inactive, as shown after heterologous expression. The oxidative stability of carotenes in the mesocarp of palm C59 was enhanced compared with control accessions. Therefore, the introduction of the high γ-tocotrienol trait into elite breeding lines represents a strategy to extend the shelf life of CPO.

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MEMO

S7-2

Ecological function of inducible lignin in stem

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Plants have developed tissue-specific defense strategies in response to various herbivores with different feeding habits. Although defense responses to leaf-chewing insects have been well studied, little is known about stem-specific responses, particularly in the pith, to stem-boring herbivores. To understand the stem-specific defense, we first conducted a comparative transcriptomic analysis of the wild tobacco *Nicotiana attenuata* before and after attack by the leaf-chewing herbivore *Manduca sexta* and the stem-borer *Trichobaris mucorea*. When the stem-boring herbivore attacked, lignin-associated genes were upregulated specifically in the inner parenchymal cells of the stem, the pith; lignin also highly accumulated in the attacked pith. Silencing the lignin biosynthetic gene cinnamyl alcohol dehydrogenase enhanced the performance of the stem-boring herbivore but had no effect on the growth of the leaf-chewing herbivore. We also examined how JA signaling activates pith-specific lignification in *N. attenuata*. These results suggest that lignin provides a stem-specific inducible barrier, protecting plants against stem-boring insects.

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MEMO

S7-3

Learning from nature for strategies to maximize production and purity of the high value carotenoid astaxanthin in oilseeds

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Ketocarotenoids, including astaxanthin, are red lipophilic pigments derived from the oxygenation of β-carotene ionone rings. These carotenoids have exceptional antioxidant capacity and high commercial value for natural pigment applications, especially for aquaculture feedstocks to confer red color to products such as salmon and shrimp. Ketocarotenoid biosynthetic pathways occur in only selected bacterial, algal, fungal, and plant species, which provide gene sources for ketocarotenoid biotechnological production. We examined the efficacy of ketocarotenoid production in oilseeds using camelina (*Camelina sativa*) and soybean (*Glycine max*) as production platforms and genes expressed in flowers of *Adonis aestivalis*, which have among the highest astaxanthin concentrations in land plants (~1.5% DW). For our first prototype, we used seed-specific expression of transgenes maize phytoene synthase and *A. aestivalis* carotenoid β-ring 4-dehydrogenase (CBFD2) and carotenoid 4-hydroxy-β-ring 4-dehydrogenase (HBFD1) to produce high-levels of β-carotene and to modify its ionone rings to contain the 4-OH and keto groups found in astaxanthin. 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 astaxamthin production and purity, we conducted transcriptome profiling of *A. aestivalis* flower petals. Candidate genes from the transcriptome were initially characterized using Agrobacterium-infiltration of *Nicoticana benthamiana* leaves for transient astaxanthin production. We will describe the use of the top candidate genes for generating high astaxanthin concentrations while maintaining uncompromised seed fitness.

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MEMO

S7-4

Engineering for flavonoid production in *Nicotiana benthamiana* using synthetic biology

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Flavonoids are one of the most abundant plant secondary metabolites with diverse biological activities such as antioxidant, anti-inflammatory, and anti-cancer. Flavonoids having C6-C3-C6 ring structure have been widely used in various industrial fields including pharmaceuticals and foods. However, some flavonoids are present at low concentrations in plants, and chemical synthesis is difficult due to structural complexity. To overcome these difficulties, efforts have been made to increase the production of flavonoids through efficient technologies. Transient gene expression system in tobacco (*Nicotiana benthamiana*) has been widely used to produce target compounds in plants. Here, we produced flavonoid compounds, including apigenin, luteolin, and chrysoeriol, in tobacco leaves using synthetic biology techniques. The target flavonoid metabolic pathway was simplified and gene combinations were transiently expressed in plants. We also examined the optimal conditions for enhancing flavonoid production in plants. This system can be used for production of flavonoids and their derivatives in plants within a short period without providing substrates.

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MEMO

S8-1

Mining the seed oil content-related genes by multi-omics analysis in rapeseed

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Rapeseed produces approximately 15% of the edible oil globally. Seed oil content (SOC) is an important trait which has great phenotypic variation in rapeseed. In this study, we comprehensively investigated the genes regulating the SOC in rapeseed by multi-omics approaches including genome-wide association analysis studies (GWAS), transcriptome-wide association analysis studies (TWAS), mGWAS, eGWAS and co-expression analysis. GWAS identified 27 reliable loci controlling SOC in eight environments. TWAS identified 692 genes and four gene modules significantly associated with SOC. Furthermore, 2,172 metabolites in mature seeds were quantified by LC-MS/MS, in which 131 marker metabolites were identified to be correlated with SOC. These 131 metabolites were selected for further mGWAS analysis and a few mQTLs were identified to impact the SOC. Based on the results of multi-omics analysis, we selected a few candidate genes for functional study. Nine candidate genes were shown to significantly impact the SOC in rapeseed. We also dissected the molecular and biochemical mechanism of these validated SOC-controlling genes. We comprehensively dissected the genetic and molecular mechanisms of SOC in rapeseed by multi-omics analysis using genomic, transcriptomic and metabolomic data. These works provide important resources for breeding rapeseed cultivars with high SOC.

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MEMO

S8-2

A new cold tolerant germination rapeseed germplasm is associated with seed fatty acid profile

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Winter oilseed (*Brassica napus* L.) is the main planted specie in China and largely grown under an intensive rice-oilseed rape cropping system along Yangtze River. Due to the delay of rice harvesting, gemination and growth of rape seedlings are adversely affected by cold temperature, seriously reducing the rapeseed production. We identified a new rapeseed germplasm named as gec (germination under cold), which can germinate under low temperature, among a total of 658 rapeseed varieties. The gec germplasm accumulates less reactive oxygen species (ROS), including H2O2 and $O2^{-}$, accompanied by more accumulation of SOD and POD, which can remove H2O2 and $O2^{-}$ respectively. Excessive ROS can damage cell membrane and cause ion leakage. The less ion leakage of the gec germplasm after cold stress is consistent with the lower ROS abundance and higher accumulation of SOD and POD. Genetics analysis indicating that the cold tolerant phenotype is controlled by two recessive loci. Interestingly, the unsaturated fatty acid content is more while saturated fatty acid abundance is less in the gec germplasm compared to wild-type. Transcriptome and quantitative PCR analysis showed that cold resistant genes such as DREB1B and DREB1C and JA marker genes MYC2 and VSP2, which is a key cold-defensive hormone, are expressed more in cold-tolerant plants . Therefore, a new germplasm which can germinate under cold is identified and the genes controlling the cold resistance and the mechanism is being explored.

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MEMO

S8-3

Effect of diacylglycerol acyltransferases on the seed oil content and acyl preference in *Camelina sativa*

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Triacylglycerol (TAG) is a storage oil of plant seeds or fruits that provides energy for seed germination and seedling development. TAG is synthesized by DIACYLGLYCEROL ACYLTRANSFERASES (DGAT) 1 and 2 through the endoplasmic reticulum (ER) pathway, and DGAT3 through the cytosolic pathway. In this study, we confirmed the molecular and functional characterization of *CsDGAT1*, *CsDGAT2*, and *CsDGAT3* from *Camelina sativa*. The *CsDGAT*s showed a seed-specific expression, and the transcript levels of *CsDGAT3*s were higher than those of *CsDGAT1* in developing seeds of camelina. We also confirmed that CsDGAT1-GFP and CsDGAT2-GFP were localized to the ER, whereas CsDGAT3-GFP was localized to the cytosol by transient expression in *Nicotiana benthamiana* leaves. *Csdgat*-edited camelina plants were generated using CRISPR/Cas9. Seed fatty acid levels of *csdgat3*-edited camelina were 19% lower and their C18:3 and C20:1 levels were reduced as compared to WT. *Csdgat1*-edited camelina showed decreased C18:1 and C18:2 contents and increased C18:3 content compared to that of WT. Most of the complete *csdgat2*-edited camelina lines had a fatty acid composition similar to WT. However, EDD2#9-2 and EDD2#30-5 showed increased C18:1 and C18:2 content and decreased C18:3 content. Taken together, CsDGAT3 contributes to the accumulation of TAG and prefers the acylation of C18:3. CsDGAT1 prefers C18:1 and C18:2 as acyl donors. Meanwhile, CsDGAT2 may prefer C18:3 similar to CsDGAT3. These findings can be applied to the development of oilseed plants with enhanced seed storage oils containing tailored fatty acid composition.

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MEMO

S8-4

Regulation of seed oil accumulation by lncRNAs in *Brassica napus*

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Studies have indicated that long non-coding RNAs (lncRNAs) play important regulatory roles in many biological processes. However, the regulation of seed oil biosynthesis by lncRNAs remains largely unknown. Here, we comprehensively identified and characterized the lncRNAs from seeds in three developing stages in two accessions of *Brassica napus* (*B. napus*), ZS11 (high oil content) and WH5557 (low oil content). Finally, 8094 expressed lncRNAs were identified. LncRNAs *MSTRG.22563* and *MSTRG.86004* were predicted to be related to seed oil accumulation. Experimental results show that the seed oil content is decreased by 3.1 - 3.9 % in *MSTRG.22563* overexpression plants, while increased about 2 % in *MSTRG.86004*, compared to WT. In *MSTRG.22563* transgenic seeds, most genes related to lipid metabolism had much lower expression, and the content of some metabolites in the processes of respiration and TCA (tricarboxylic acid) cycle was reduced. In *MSTRG.86004* transgenic seeds, the expression of genes involved in fatty acid synthesis and seed embryonic development (e.g. *LEC1*) was increased, but genes related to TAG assembly was decreased. These results suggest that *MSTRG.22563* impacts seed oil content by affecting the respiration and TCA cycle, while *MSTRG.86004* plays a role in prolonging the seed developmental time to increase seed oil accumulation.

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MEMO

S9-1

Photoconversion of fatty acids to hydrocarbons in algae

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Fatty acid photodecarboxylase (FAP) is an algal-specific flavoprotein that we originally isolated from the green microalga Chlorella variabilis NC64A (1). FAP converts fatty acids into hydrocarbons using light (EC 4.1.1.106). Alongside DNA photolyases and the light-dependent protochlophyllide oxidoreductase, FAP is thus one of the only photoenzymes identified in nature to date. From an applied point of view, FAP from Chlorella variabilis (CvFAP) is an interesting redox-neutral, light-dependent catalyst for the production of n-alka(e)nes and other specialty chemicals.

In this presentation, I will review the studies we have carried out in recent years on the photoenzymatic activity of CvFAP, its use in biotransformation and the possible physiological roles of FAPs in algae (2-6). New work on FAPs and the diversity of fatty acid-derived hydrocarbons found in algae will also be discussed.

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MEMO

S9-2

Dynamic seed oil assembly: acyl and stereochemical selective enzymes remodel triacylglycerol fatty acid composition after initial oil synthesis

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Two major pathways for production of diacylglycerol (DAG), the immediate precursor to triacylglycerol (TAG), have been identified in plants: de novo DAG synthesis, and conversion of the membrane lipid phosphatidylcholine (PC) to DAG, with each pathway producing distinct TAG compositions. However, neither pathway fits with previous biochemical and transcriptomic results from developing Physaria fendleri seeds for accumulation of TAG containing >60% lesquerolic acid (an unusual 20 carbon hydroxylated fatty acid) which accumulates at only the sn-1 and sn-3 positions of TAG. Isotopic tracing of developing P. fendleri seed lipid metabolism identified that PC-derived DAG is utilized to initially produce TAG with only one lesquerolic acid at sn-3. Subsequently a non-hydroxylated fatty acid is removed from the TAG sn-1 position (transiently reproducing an sn-2,3 DAG) and a second lesquerolic acid is incorporated. Thus, a dynamic TAG remodeling process involving anabolic and catabolic reactions controls the final TAG fatty acid composition. The control of TAG remodeling involves two separate diacylglycerol acyltransferases with selectivity for sn-1,2-DAG or sn-2,3-DAG which interact with a novel acyl selective TAG lipase that localizes to the endoplasmic reticulum-lipid droplet junction. Finally, we demonstrate that the bioengineering of TAG remodeling can lead to both higher seed oil and increased levels of unusual fatty acids in model species and crop plants.

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MEMO

S9-3

New perspectives on the growing complexity of WRINKLED1 in plant lipid metabolism

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Plant oils have significant importance in both human diets and as renewable resources for diverse industrial applications. WRINKLED1 (WRI1) is a pivotal transcriptional regulator governing plant oil biosynthesis. However, the precise molecular mechanism of WRI1 still remains unclear. In my presentation, I will discuss the advancement we have made in unraveling the intricate regulatory mechanisms of WRI1. Our recent research has provided valuable insights into several critical aspects of WRI1 regulation, encompassing functional domains and motifs, interacting regulators, and post-translational modifications like phosphorylation. In particular, we have identified three intrinsically-disordered regions (IDRs) within WRI1, with the C-terminal IDR3, playing a pivotal role in mediating WRI1 stability. We have achieved a high-resolution crystal structure of the WRI1-DNA complex, revealing essential residues crucial for WRI1's DNA binding capabilities. Alongside these findings, we have also made significant discoveries regarding novel WRI1-interacting regulators, including 14-3-3s (a family of phosphopeptide-binding proteins), as well as TCP4, bZIP52, BBX32 transcriptional regulators. These regulators exert effects on the activity of WRI1, thereby impacting the production of plant oils. The insights gained from our studies on WRI1 significantly enhance our understanding of its complex regulatory mechanism, providing valuable guidance for improving strategies to utilize WRI1 in the development of oil crops.

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MEMO

S9-4

Full transcriptome sequencing screening key regulation factor regulating lipid biosynthesis in developing seeds of yellowhorn (*Xanthoceras sorbifolium*)

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Background: Yellowhorn (*Xanthoceras sorbifolium*) seeds can have as high as 67% oil content and are especially rich in oleic acid, linoleic acid, and nervonic acid. Exploration of the lipid biosynthesis regulatory network is essential for increasing the yellowhorn oil content. Long non-coding RNAs (lncRNAs) play important roles in various plant biological processes; however, there is no report on the identification of lncRNAs involved in yellowhorn seed development and lipid biosynthesis affecting oil production.

Methods: The whole transcriptome sequencing of yellowhorn seeds at four developmental stages were done for screening key regulation factor regulating lipid biosynthesis in developing seeds of yellowhorn.

Key findings and conclusions: A total of 16,920 putative lncRNAs were identified, in which 325 lncRNAs were revealed to trans-regulate 58 key genes in fatty acid (FA) and triacylglycerol (TAG) biosynthesis pathways. Of these, ECR-2–LNC_009778 was found to be involved in nervonic acid biosynthesis and DGAT-1–LNC_009778 was beneficial to TAG accumulation. sRNA-seq was performed, and 55 microRNAs (miRNAs) were found to target 26 genes involved in FA and TAG biosynthesis; miR396a-4 targets FAD2, affecting linoleic acid biosynthesis, and miR156f-5p targets PDAT-2, contributing to TAG accumulation. Interestingly, 30 lncRNA–miRNA–gene modules involved in FA and TAG biosynthesis were identified, in which the KCS11-1–miR156g-2–LNC_000849 module was found to participate in nervonic acid synthesis, and the DGAT-2–miR172j–LNC_005874 module was assumed to contribute to the accumulation of TAG. Our results constitute the first comprehensive identification of lncRNAs in developing seeds of yellowhorn and serve as a new theoretical reference for improving oil content in the future.

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MEMO

S10-1

Biotechnology for industrial materials production: current progreass on improving hydroxy fatty acid content in Lesquerella (*Physaria fendleri*)

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The conventional source of hydroxy fatty acid (HFA) is castor (Ricinus communis) oil which contains 90% ricinoleic acid (18:1OH) of total fatty acids in seed. HFA and its derivatives are used as raw materials for numerous industrial products, such as lubricants, plasticizers and surfactants. The production of castor oil, however, is hampered by the presence of the toxin ricin and hyperallergic 2S albumins. Lesquerella does not have such biologically toxic compounds and also contains a major HFA, lesquerolic acid (20:1OH), at 55-60% of seed oil. Therefore, lesquerella is being developed as a new industrial oilseed crop in the US. Synthesis of 20:1OH is through elongation of 18:1OH, and the step is regulated largely by gene transcription of an elongase, PfKCS3. By silencing PfKCS3, transgenic lesquerella increased 18:1OH content from ~3% to ~27%. It is known that most of the HFAs in lesquerella are located only at sn-1 and sn-3 positions of triacylglycerols (TAG). To improve HFA levels in lesquerella seeds, castor lysophosphatidic acid acyltransferase gene 2 (RcLPAT2) have been introduced into lesquerella. The resulted transgenic lesquerella seeds increase 18:1OH content at the sn-2 position of TAG from 2% to 17%, and consequently, oil accumulates more TAGs with all three sn positions occupied by HFA. The results enhance our understanding of plant lipid metabolism and provide invaluable guidance for future research, not only for enhancing HFA content in lesquerella, but also for HFA production in other oilseeds.

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MEMO

S10-2

The current progress of CRISPR/CAS9 technology development in oil palm

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Recent genome editing technology marked a new era in assisting conventional breeding for oil palm improvements. The remarkable technology allows targeted traits to be obtained more precisely and faster than conventional breeding. Advancements in oil palm genetic engineering tools, such as vector construction for assembling genes of the targeted traits, transformation methods for introducing genes of interest into target tissues, selection and regeneration of transformed tissues, and availability of the genomic sequence, enable us to apply the genome editing technology for oil palm improvement. Recently, the genome editing tool, specifically a CRISPR/Cas9 system, has been developed for modification of the oil palm genome. Due to its diploid genome, a multiplex CRISPR/Cas9 genome editing tool is a vital system to be developed for editing multiple genes in oil palm. Currently, the oil palm *phytoene desaturase* (*EgPDS*) gene has been used as a model gene to quickly define the efficient parameters influencing the multiplex CRISPR/Cas9 since its mutation could give rise to the albino phenotype. The positive results from CRISPR/Cas9 targeting *EgPDS* genes have led to the initiation of CRISPR/Cas9 for editing valuable target traits such as oil palm *palmitoyl-ACP thioesterase* (*EgPAT*) and *oleoyl-CoA desaturase* (*EgFAD2*) genes for increasing oleic acid content, oil palm *gibberellin acid 20 oxidase* (Eg*GA20ox)* genes for reducing tree height increment and oil palm *virescens* (*EgVIR*) gene for generating virescens fruits type. Overall, this presentation aims to provide the current progress of CRISPR/Cas9 genome editing research in oil palm.

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MEMO

S10-3

Overexpression of CIDEs improves seed oil content of *Brassica napus*

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Cell death-Inducing DNA Fragmentation Factor Alpha (DFFA)-like Effector (CIDE) is a class of mammalian protein associated with obesity, insulin resistance, cardiovascular disease and other ailment. CIDEs are involved in regulating the size and morphology of the lipid droplet (LD) when they are located on the surface of the lipid droplet. There are three *CIDEs* including *CIDEA, CIDEB* and *CIDEC* in animal. However, no homologous gene of *CIDE* has been found in plants. Here, we transiently expressed *CIDEA*, *CIDEB* and *CIDEC* in the leaves of tobacco, respectively. The results show that the number of LDs is 20-fold higher and the size of LDs is two-fold larger in transformed leaves than that of non-transformed leaves. The content of total fatty acid is almost increased by 100%. The content of phosphatidic acid (PA) is more than 2-fold and the content of phosphatidylcholine (PC) is significantly decreased in transformed leaves compared to non-transformed leaves. The content of triacylglycerol (TAG) in transformed leaves is 2-3 folds higher compared to non-transformed leaves. Meanwhile, *CIDEA*, *CIDEB* and *CIDEC* are specifically expressed in the seeds of *Brassica napus,* respectively. The oil content of transgenic seeds is increased by at least 7.5% compared to the wild type, and the data show that the other agronomic traits of transgenic plants is not altered. Taken together, our study provides a new idea for improving seed oil content accumulation in *Brassica napus*.

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MEMO

S10-4

Update on the development of plant-based long-chain omega-3 oils

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Omega-3 long chain polyunsaturated fatty acids (ω3 LC-PUFA), in particular EPA (eicosapentaenoic acid, 20:5ω3) and DHA (docosahexaenoic acid, 22:6ω3), play a critical physiological role in health, and they are nutritionally important for both humans and animals. Dominant resources of ω3 LC-PUFAs especially from wild-caught marine fish are declining. Other factors such as heavy metal contamination in marine fish and vegetarians not being able to eat fish, lead to insufficient intake of ω3 LC-PUFA in many countries. These have resulted in a widely recognised need for new sustainable and vegetarian sources of ω3 LC-PUFA. We have developed oilseed crops producing ω3 LC-PUFAs. Genetic engineering of ω3 LC-PUFAs into oilseed crops involved microalgae survey, gene discovery, multiple-gene ω3 LC-PUFA synthesis pathway design, crop transformation and elite event selection resulted in a canola crop with fish oil-like levels of DHA. The canola crop rich in DHA oil is approved by multiple regulators in Australia, USA and Canada for large scale cultivation and the DHA-rich oil is approved for food and feed applications. First commercial sale of Aquaterra®oil for use in aquafeeds occurred in 2020. DHA-rich canola is now also available for human nutrition markets as Nutriterra®. Other plant-based long-chain omega-3 oils with different fatty acid profiles are also being developed. These genetically engineered oilseed crops can and will help meet the increasing market demand for ω3 LC-PUFAs in aquaculture and human nutrition. The land-based source of ω3 LC-PUFAs offers a safe, cost effective, scalable and sustainable solution, which can have critical and positive health, economic and environmental impacts.

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MEMO

Poster Presentations

MEMO

P1

Plastid anionic lipids PG and SQDG are important for etioplast development and de-etiolation of *Arabidopsis thaliana*

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Cotyledon cells of dark-germinated angiosperms have chloroplast precursors, etioplasts, which develop 3D lattice membrane structures called prolamellar bodies (PLBs). PLBs contain the chlorophyll intermediate protochlorophyllide (Pchlide) forming a ternary complex with NADPH and light-dependent NADPH-Pchlide oxidoreductase (LPOR). During de-etiolation process under continuous illumination, PLB lattice structures collapse into the lamellar structures of the thylakoid membrane, and etioplasts differentiate into chloroplasts. Two anionic lipids, phosphatidylglycerol (PG) and sulfoquinovosyldiacylglycerol (SQDG), account for ~20% of the total amount of lipids in PLB and thylakoid membranes. However, the importance of PG and SQDG for processes of etioplast development and de-etiolation is still unknown. To reveal their roles in these processes, we characterized etiolated and de-etiolated *Arabidopsis* mutants which are deficient in the biosynthesis of PG and/or SQDG. A partial deficiency in PG biosynthesis (*pgp1-1*) loosened the lattice structure of PLBs in etioplasts and caused a faster disassembly of PLBs during de-etiolation. In addition to the aberrant membrane organization, *pgp1-1* mutation impaired the Pchlide biosynthesis in etioplasts, leading to a significant decrease in Pchlide content. The *pgp1-1* also inhibited the accumulation of chlorophyll during de-etiolation, indicating that PG is required for the chlorophyll biosynthesis pathway. Although a complete lack of SQDG biosynthesis (*sqd1*) did not notably affect both etioplast development and de-etiolation, the *sqd1 pgp1-1* double mutation caused strong impairments in these processes, suggesting an auxiliary role of SQDG that partially complement PG roles. These data demonstrate the pleiotropic roles of anionic lipids in etioplast development and de-etiolation processes.

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P2

Turnover of PG and MGDG in PSII repair

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Thylakoid membrane consists of four main classes of glycerolipids: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG), and phosphatidylglycerol (PG) as membrane lipids. Crystallographic analysis of photosystem II (PSII) dimer from *Thermosynechococcus vulcanus* shows that PSII contains 20 lipid molecules per reaction center. PSII is particularly sensitive to strong light, and light damaged PSII is rapidly repaired. Repair of PSII requires dissociation of damaged PSII dimer and following dissociation of CP43 protein from the racrtion center. In the present study, we examined the role of lipid turnover in the dissociation of PSII complexes and repair of PSII in *Synechocystis* sp. PCC 6803. In mutants lacking a galactolipase A1 (named LipA) or phosphoalipase A2 (PLA2), the repair of PSII was suppressed by slower degradation of D1, a reaction center of PSII. A recombinant LipA protein reduced MGDG content in PSII dimers and decomposed PSII dimer into monomers, while PLA2 reduced PG content in PSII monomer and decomposed PSII monomer into CP43 and RC47 (CP43-less monomer). Therefore, the deacylation of MGDG and PG by the LipA and PLA2, respectively, enhances PSII repair and D1 degradation via disassembly of PSII dimer through RC47 under strong light.

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P3

Glycosphingolipids are essential for plant development but not for cell proliferation in *Arabidopsis thaliana*

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Glycosylinositolphoshoceramide (GIPC) is the most abundant phytosphingolipid and indispensable for plant development. In addition, GIPC is an attractive resource for ceramide production for skincare and cosmetics industry. However, the complex heads of GIPC are thought to inhibit its digestion and absorption in human body. We have attempted genetic/metabolic suppression of GIPC biosynthesis to accumulate its substrates free ceramide or intermediates with shorter head groups in plant tissues.

According to previous works, we focused on the cell culture system to avoid the lethality of GIPC-deficient mutant. When explants of *Arabidopsis gmt1* mutant lacking the hexose residue of GIPC were cultured in callus-inducing medium, callus formation was similarly observed in the cotyledons and hypocotyls. On the other hand, callus was formed at a significantly higher rate in *gmt1* roots than wild type roots. *gmt1* callus normally proliferated but no shoot tissues were formed when caltured in shoot-inducing medium. These results indicate that GIPC is not essential for proliferation of plant cells, suggesting abnormal ceramides/intermediates can be produced using the cultured cells. In addition, this study found a new phenotype of *gmt1* in root. We are evaluating the root phenotype involved in the enhanced callus formation.

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P4

Investigating the role of sphingolipids in ER stress response of *Chlamydomonas reinhardtii*

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The endoplasmic reticulum (ER) is an important intracellular compartment in eukaryotic cells. ER stress is caused by the stress-induced accumulation of unfolded proteins in the ER. Previous studies have shown that lipid metabolism was altered in many types of cells exposed to ER stress. Sphingolipids, a class of bioactive lipids present in cell membranes, play pivotal roles in diverse signaling pathways in development and stress responses, including ER stress response in animals or yeast. However, the roles of sphingolipids in microalgae have not been elucidated.

In this study, we investigated the impact of ER stress on sphingolipid accumulation in a model microalgae Chlamydomonas reinhardtii. Interestingly, we found that ER stress triggered the accumulation of ceramide in mutants of ER stress sensor IRE1. Contrarily, glycosyl-inositolphosphoceramide (GIPC) accumulated in Chlamydomonas parental strain CC4533 but not in *ire1* mutants. Furthermore, the phenotypes of Chlamydomonas mutants that are defective in sphingolipid metabolism under ER stress conditions will be discussed in my presentation. Our study might be able to shed light on the role of sphingolipids in microalgae's response to ER stress.

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P5

Sphingolipid profiles in the leaves of several plants of Amaranthaceae

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In plants, the variety and structure of sphingolipid long-chain bases (LCBs) in glucosylceramides (GlcCers) are more complicated than those in free ceramides (Cers) and glycosylinositolphosphoryl ceramides (GIPCs). One possibility to explain this complexity in the structure of sphingolipid LCBs of GlcCers is elevated amounts of 8-*cis* sphingolipid LCBs in GlcCers compared with 8-*tarns* forms. Nine forms of sphingoid LCBs in plant GlcCers are assigned structurally to the 3 types of analogs: (1) 4-saturated dihydroxy analogs comprising sphinganine (dihydrosphingosine, d18:0), 8-*trans*-sphingenine [d18:1 (8t)], and 8-*cis*-sphingenine [d18:1 (8c)]; (2) 4-*trans*-unsaturated dihydroxy analogs comprising 4-*trans*-sphingenine [sphingosine, d18:1 (4t)], 4-*trans*, 8-*trans*-sphingadienine [d18:2 (4t, 8t)], and 4-*trans*, 8-*cis*-sphingadienine [d18:2 (4t, 8c)]; and (3) 4-hydroxy analogs comprising 4-hydroxysphinganine (phytosphingosine, t18:0), 4-hydroxy-8-*trans*-sphingenine [t18:1 (8t)], and 4-hydroxy-8-*cis*-sphingenine [t18:1 (8c)]. We have previously reported the sphingoid LCB composition of GlcCers in the leaves of Amaranthaceae such as spinach, beet and a halophytic plants, *Salicornia europaea.* Here, we analyzed GIPCs, GlcCers and free Cers in the leaves of several plants of Amaranthaceae by LC-MS/MS. The GlcCer species containing d18:1 (8t) with 2-hydroxy palmitic acid [d18:1 (8t)-16h:0] and t18:1 (8t) with 2-hydroxy lignoceric acid [t18:1 (8t)-24h:0] were major components in *Celosia argentea*. Whereas the Cer species containing t18:1 (8t) with lignoceric acid [t18:1 (8t)-c24:0] was a major component in *Celosia argentea*. Sphingolipid profiles obtained from Amaranthaceae will be discussed in comparison with those in other plant families.

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P6

Sphingolipid ceramide unsaturation in plants: gene evolution, analytical chemistry, and biological functions

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Ceramides are the hydrophobic backbone of sphingolipids. Even though ceramides are conserved in all eukaryotes, the chemical structures are highly diversified among organisms. Unique ceramide modifications are developed in plants, implying plant-specific functions of ceramides. We here report the genetic, analytical and biological aspects of plant ceramide unsaturation. One of the plant-unique ceramide structures is the Δ8 double bond of long-chain base (LCB). Although not only plant but some aquatic eukaryotes have the conserved LCB Δ8 desaturase SLD, only plants contain both *Z* and *E* isomers at various ratio. First, we established isomer-selective methods based on LC-MS/MS detection. Chemical derivatization and a cholesterol-immobilized column were highly effective for separating LCB and glucosylceramide isomers. Second, we characterized the stereo-specificity of various plant SLDs using yeast heterologous expression system, showing the phylogenetic distribution of the enzyme properties in land plants. We also found that amino acid substitutions in the substrate pocket of SLDs govern the evolutionary changes of the *Z*/*E* preference. Third, we characterized plants with genetic modifications of ceramide desaturases, demonstrating two basic functions of unsaturated ceramides. One is *Z*-unsaturated glucosylceramide-dependent Al^{3+} tolerance, which was evidenced using rice plants with modified ceramide unsaturation. Another function is cold tolerance of *Arabidopsis*. We found that an *Arabidopsis* double mutant lacking SLD1 and ADS2, a very-long chain fatty acyl CoA ω9 desaturase, displays severe cold-sensitive phenotype even in non-freezing temperatures. These insights indicate that along the land plant evolution, ceramide unsaturations have been developed for stress tolerance via plasma membrane integrity and fluidity.

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P7

ATP-BINDING CASSETTE G23 is required for suberin deposition in *Arabidopsis* seed coats

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Suberin is an extracellular hydrophobic polymer deposited in seed coats and plays as a barrier to regulate the internal ions flux and water, protect against pathogen attack, and limit desiccation. However, relatively little is known about molecular mechanisms underlying suberin deposition in seed coats. In this study, the *in planta* role of an ATP-binding cassette transporter G protein 23 (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 detected in the plasma membrane of tobacco epidermal cells. Total suberin monomer loads in *abcg23-1* and *abcg23-2* seeds decreased by approximately 20% and 10% compared to the wild type (WT) respectively, and a significant decrease in the levels of C24 ω-hydroxy fatty acids, C24 α, ω-dicarboxylic acids, C16 fatty acids, C22 α, ω-alkane diols, 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* were noticeably reduced compared to WT under salt and osmotic stress conditions. Bimolecular fluorescence complementation assay showed homodimeric interaction of ABCG23. Therefore, our findings provide that the ABCG23 contributes to the export of suberin monomers in *Arabidopsis* seed coats.

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P8

Arabidopsis 3-ketoacyl-CoA synthase 17 produces tetracosanoic acids required for synthesizing seed coat suberin

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Very long-chain fatty acids (VLCFAs) are precursors for the synthesis of membrane lipids, cuticular waxes, suberins, and storage oils in plants. The 3-ketoacyl CoA synthase (KCS) catalyzes the condensation of C2 units from malonyl-CoA to acyl-CoA, the first rate-limiting step in VLCFA synthesis. In this study, we revealed that KCS17 catalyzes the elongation of C22 to C24 VLCFAs required for synthesizing seed coat suberin. Histochemical analysis of *Arabidopsis thaliana* expressing *GUS* under the control of the *KCS17* promoter revealed predominant *GUS* expression in seed coats, petals, and premature pollens. The expression of *KCS17:eYFP* driven by the *KCS17* promoter was observed in the outer integument1 of *Arabidopsis* seed coats. The KCS17:eYFP signal was detected in the endoplasmic reticulum of tobacco epidermal cells. The levels of C22 VLCFAs and their derivatives, primary alcohols, α,ω-alkane diols, ω-hydroxy fatty acids, and α,ω-dicarboxylic acids increased by approximately 2-fold, but C24 VLCFAs, ω-hydroxy fatty acids, and α,ω-dicarboxylic acid levels were reduced by half in *kcs17-1* and *kcs17-2* seed coats relative to wild-type. KCS17 formed homo- and hetero-interactions with KCR1, PAS2, and ECR but not with PAS1. Therefore, KCS17-mediated VLCFA synthesis is required for suberin layer formation in *Arabidopsis* seed coats.

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P9

Identification of a GPI-anchored lipid transfer protein involved in suberization in *Arabidopsis* seedling roots

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Plant germination starts with the emergence of radicle from the seed. The radicle develops into a primary root which supports a seedling to develop by water and nutrient absorption. Suberin, a complex polyester composed of various fatty acids, alcohols, phenolic compounds and glycerol, is known to be accumulated in the roots and the seed coats, where its hydrophobic barrier controls water and ion tranport and protects the plant from abiotic and biotic stresses. Here, we investigated suberization process in *Arabidopsis* seedling roots and characterized a GPI-anchored lipid transfer protein involved in suberin deposition in this stage of development. RT-qPCR analysis and GUS and eYFP assays under the control of LTPG promoter revealed that *LTPG* is expressed in the root endodermis of young seedlings, which are suberin accumulation sites. Subcellular localization in *Nicotiana benthamiana* epidermal cells showed that LTPG is localized in the plasma membrane. Two alleles of *ltpg* mutant lines were generated using CRISPR-Cas9 method for further analysis, and were named *ltpg-crp1* and *-crp2*. Suberin monomer analysis with 4-day-old-root showed overall decrease in the amount of each suberin monomers in *ltpg-crp*s compared to the wild type, suggesting that LTPG is involved in suberin transport of young seedling roots. This study expands our understanding about the suberization in *Arabidopsis* seedling roots.

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P10

Protein-protein interactions between fatty acid elongase complex proteins and cuticular wax biosynthetic enzymes

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Fatty acid elongase (FAE) is a complex composed of multiple proteins that catalyze the production of very-long-chain fatty acids (VLCFAs, ≥ C20), which serve as precursors for the synthesis of phospholipids, sphingolipids, cuticular waxes, suberin, and storage oils. In particular, VLCFAs are further modified to cuticular wax components by alkane- or alcohol-forming pathways which are mediated by ECERIFERUM1 (CER1)/CER3/and mid-chain alkane hydroxylase1 (MAH1) enzymes and FATTY ACID REDUCTASE3 (FAR3)/wax ester synthase/diacylglycerol acyltransferase (WSD1) enzymes, respectively. Kim et al. (2022) reported the quaternary structure of FAE complexes, which are crucial for VLCFA synthesis. In this study, we investigated the protein-protein interactions (PPI) between wax biosynthetic enzymes (WBE) and PPI between FAE complex proteins and WBE by bimolecular fluorescence complementation (BiFC) and Split-Ubiquitin Yeast Two-Hybrid (SUY2H) assays. In summary, 1) homo-interactions of CER3, CER1, and WSD1 were detected in the ER by BiFC and SUY2H assays and homo-interaction of MAH1 and FAR3 was only detected in BiFC and SUY2H assays, respectively. 2) Hetero-interactions of all WBEs except FAR3 were detected by BiFC assay, but hetero-interactions between CER3 and CER1, WSD1, or FAR3 and between CER1 and FAR3 were only observed by SUY2H assays. 3) In BiFC assay, WBEs except FAR3 exhibited hetero-interactions with FAE complex proteins (KCS6, KCR1, PAS2, ECR, CER2, and CER2-like2), and only MAH1 formed hetero-interactions with PAS1. In SUY2H assay, CER3 interacts with FAE complex proteins (PAS1, KCS6, KCR1, PAS2, and ECR). WSD1 also showed hetero-interactions with PAS1, PAS2, and ECR. In this presentation, the significance of interactions between alkane- and alcohol-forming pathways and between WBEs and FAE core complexes, which facilitate metabolic channeling will be discussed.

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P11

Regulation of cuticular wax biosynthesis in *Arabidopsis* via *CER1* or *FAR3*-mediated post-transcriptional gene silencing

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The aerial surface of land plants is covered with a hydrophobic cuticle, which is composed of cuticular waxes and cutin polyesters and acts as the first barrier against biotic and abiotic stresses. Cuticular wax biosynthesis is precisely regulated for optimal growth and development and 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 mediated by ECERIFERUM1 (CER1)/CER3/and mid-chain alkane hydroxylase1 (MAH1) enzymes and FATTY ACID REDUCTASE3 (FAR3)/wax ester synthase/diacylglycerol acyltransferase (WSD1) enzymes, respectively. Interestingly, overexpression of *CER1, CER3, or FAR3* in *Arabidopsis* wild type exhibited no or a few wax crystals on the surface of stems, but no alterations in cuticular wax deposition were observed in transgenic stems overexpressing *MAH1* or *WSD1*. Based on the previous report that *CER3* expression is controlled by the post-transcriptional gene silencing (PTGS), we investigated the regulation of cuticular wax biosynthesis *via CER1*- or *FAR3*-mediated PTGS in *Arabidopsis*. The levels of alkanes and primary alcohols were significantly reduced in *CER1*OX/Col-0 and *FAR3*OX/Col-0 compared to wild type (Col-0), respectively. However, no noticeable alterations in the formation of epicuticular wax crystals and wax composition and amounts were detected between *rdr6-11* mutant, which is defective in the conversion of single-stranded RNA into double-stranded RNA, and *CER1*- or *FAR3*-overexpressing *rdr6-11* lines. Small RNAs generated from *CER1* and *FAR3* cleavage were detected in *CER1OX*/Col-0 and *FAR3OX*/Col-0, but not in *CER1OX*/*rdr6-11*and *FAR3OX*/*rdr6-11,* respectively. These findings indicate that alkane- and alcohol-forming pathways in *Arabidopsis* wax biosynthesis are regulated *via* the PTGS of *CER1* and *FAR3*.

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P12

Inositol phospholipid turnover negatively regulates hypersensitive immune response in *Nicotiana benthamiana*

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Inositol phospholipids play important roles in regulation of plant immune responses. We previously identified seven class of phosphatidylinositol specific phospholipase C (PI-PLC : NbPLC1~7). We used virus-induced gene silencing (VIGS) for functional analysis of PLCs. Among the seven class of NbPLCs, *NbPLC1, 3, 4*-VIGS plants showed accerelated hypersensitive response (HR) challenged with incompatible *Ralstonia solanacearum* 8107. The accerelated HR cell death was accompanied with hyper-activation of salicylic acid (SA), jasmonic acid (JA), reactive oxygen species (ROS) and MAP kinase signaling. PI-PLCs hydrolyze a phosphatidylinositol 4,5-bisphosphate (PIP_2) generating secondary messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). Further analyze the regulatory mechanisms of HR cell death, we identified the gene encoding ITPK4 (NbITPK4) that was reportedly to phospholylate IP_3 and convert to IP_4. Induction of the HR cell death was accelerated in *NbITPK4*-VIGS plants. During the HR cell death accerelation, over production of ROS and hyper-activation of MAP kinases were observed in *NbITPK4*-VIGS plants. The HR cell death acceleration by *NbITPK4*-silencing was compromised by the concomitant suppression of *NbrbohB* encoding ROS generating enzyme. Co-suppression of *NbITPK4* with *NbCoi1* encoding JA receptor or *NbMEK2* encoding MAP kinase kinase partially restored the accerelated HR cell death. These resuls suggested that inositol phospholipid turnover may negatively regulate HR cell death through complex signalings including MAP kinase-, ROS and JA-dependent pathways.

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P13

A chloroplast diacylglycerol lipase modulates glycerolipid pathway balance in *Arabidopsis* in response to environmental stresses

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Abstract: Two parallel pathways compartmentalized in the chloroplast and the endoplasmic reticulum (ER) contribute to thylakoid lipid synthesis in plants, but how these two pathways are coordinated during thylakoid biogenesis and remodeling remain unknown. We report here the molecular characterization of a homologous *ADIPOSE TRIGLYCERIDE LIPASE-LIKE* gene, previously referred to as *ATGLL*. The *ATGLL* gene is ubiquitously expressed throughout development and rapidly upregulated in response to a wide range of environmental cues. We show that ATGLL is a chloroplast non-regioselective lipase with a hydrolytic activity preferentially towards 16:0 of diacylglycerol (DAG). Comprehensive lipid profiling and radiotracer labeling studies revealed negative correlation of *ATGLL* expression and the relative contribution of the chloroplast lipid pathway to thylakoid lipid biosynthesis. Additionally, we show that genetic manipulation of *ATGLL* expression resulted in altered responses to heat stress and dark-induced senescence. We propose that ATGLL, through affecting the level of prokaryotic DAG in the chloroplast, plays an important role in balancing the two glycerolipid pathways in plants, and is important for overall plant fitness under stress conditions.

Keywords: *Arabidopsis thaliana*, Lipid Remodeling, Diacylglycerol Lipase, Galactolipid, Phospholipid, Triacylglycerol.

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P14

The function of BnaGDSL in leaf cutin deposition and its influence on drought resistance of *Brassica napus*

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The leaf cuticle of plant epidermal cells is able to help plants resist water loss and cope with environmental stress. In recent years, much progress has been made in the key metabolic steps of cuticle formation, however, the mechanism of cutin synthesis and deposition is largely unknown. *BnaGDSL* encodes a putative GDSL lipase and our results showed that it was localized at cuticle and active in hydrolyzing ester bonds. Moreover, *bnagdsl* mutants were found to exhibit reduced drought resisitance with thinner cuticle and cell wall. We also observed that the *BnaGDSL* mutation resulted in increased stomatal density and index. Therefore, BnaGDSL may affect leaf water retention by controlling cutin deposition and stomatal development. In order to analyze the BnaGDSL function in cutin deposition, the cutin component contents in leaf cuticle werer measured. We found that the contents of some cutin componets including hydroxy fatty acids and fatty acids was significantly decreased in the *bnagdsl* mutants. Further studies were needed to determine the substrate specificity of BnaGDSL to explore the its role in cuticle development and influence on drought resistance of *Brassica napus*. These work provides genetic resources and theoretical basis for breeding drought-resistant varieties of *Brassica napus*.

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P15

Growth-stimulating and anti-stress properties of lipids from *Zygophyllum oxianum* Boriss. seeds under salt stress sonditions

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The role of lipid components in plant cells as protectors against stress factors is well-known. Changes in membrane lipids in response to salinity have been observed in various plant species, including halophytes and glycophytes. Lipid messenger molecules play vital roles in the adaptive mechanisms that enable plants to withstand salt stress. This study aimed to investigate the lipids from Zygophyllum oxianum Boriss. seeds (belonging to the family Zygophyllaceae) and their impact on the growth of wheat shoots under saline conditions, as well as their effect on the lipid composition of wheat seedlings.

The analysis of seedlings from untreated and treated wheat seeds grown under saline conditions revealed differences in the fatty acid composition. Treated seeds displayed the presence of polyunsaturated eicosatrienoic 20:3 and arachidonic 20:4 acids, which are known for their increased biological activity. Arachidonic acid, a rare component in higher plants, has been found to act as an effective plant growth regulator, inducing systemic resistance to abiotic and biotic damaging factors and diseases. Additionally, arachidonic acid improves plant generative capacity and promotes rapid recovery after damage by hail or pests, stimulating continued vegetation for restoring physiological balance.

The observed growth-stimulating and anti-stress properties of neutral substances in Zygophyllum oxianum seeds can be attributed to their high content of phytosterols and triterpenols, as well as the presence of provitamin A (carotenoids) and the isoprenoid hydrocarbon squalene. Phytosterols offer a wide range of biological activities, including improved cell membrane stability, fungicidal, antiparasitic, antibacterial, and antioxidant properties. Triterpenoids play a crucial role in cell functions as biotransformers, enhancing the absorption and digestibility of active ingredients with low toxicity and antimicrobial activity. The presence of endogenous unsaturated fatty acids, particularly arachidonic acid, significantly influences plant gene expression and metabolism, contributing to systemic resistance against stress factors and diseases. These compounds' presence in Z. oxianum seeds elucidate their growth-stimulating and anti-stress properties, as observed in this study.

In conclusion, the study highlights the growth-stimulating and anti-stress potential of neutral substances found in Zygophyllum oxianum seeds, which can positively impact the growth and development of seedlings under normal and saline conditions. The presence of arachidonic acid, a rare component in plant lipids, demonstrates its role as an effective plant growth regulator and inducer of systemic resistance. Additionally, the high content of phytosterols and triterpenols in the seeds further contribute to their beneficial properties, including improved cell membrane stability and antimicrobial activity. Understanding these lipid components' effects on plant growth and stress responses opens up possibilities for potential applications in agriculture and biotechnology.

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P16

Deciphering the Roles of WOUND ASSOCIATED FACTORs in wound healing processes of *Arabidopsis* Leaves

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Mechanical wounds are often caused by environmental or biological factors, which affects plant metabolic processes. Exposure of internal tissues by wounds not only causes water and ion leakage, but also make plants vulnerable to bacterial infection. Thus, wound healing is critical for plant survival. Although major signaling pathways after wounds have been elucidated, little is known about how local cell wall remodeling occurs to seal the exposed area. To understand local signaling networks triggered by wounds, we performed transcriptome analysis at multiple time points after mechanical wounding in 4-week-old *Arabidopsis* leaves. The transcriptome dynamics at the local wound site were dissected into short-term (hours) and long-term (days) ranges, with a specific focus on hormonal changes, transcription factor networks, and cell wall remodeling components. Based on RNA-seq analysis, we identified candidate genes, designated as WOUND ASSOCIATED FACTORs (WAFs), implicated in of wound healing modulation. Subsequently, we assessed the phenotypes of *wafs* mutants to elucidate their contribution to the formation of wound-induced barriers. Recent progress will be presented, which will provide new insights into the spatiotemporal wound healing processes.

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P17

Non-specific phospholipase C3 is involved in endoplasmic reticulum stress tolerance in *Arabidopsis*

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Non-specific phospholipase C (NPC) is an emerging family of lipolytic enzyme unique in plants and bacteria, playing crucial roles in growth and stress response. Among 6 copies of NPC isoforms in *Arabidopsis*, role of NPC3 remains elusive to date. Here, we show that NPC3 is a functional non-specific phospholipase C, which is involved in the tolerance to tunicamycin (TM)-induced ER stress through phosphocholine (PCho), a reaction product of NPC3. The npc3 mutant showed reduced sensitivity to TM treatment. Recombinant NPC3 protein possessed significant phospholipase C activity to hydrolyze phosphatidylcholine (PC). The hyposensitivity of *npc3* to TM treatment was complemented by exogenous PCho, suggesting that NPC3-catalyzed PCho production is involved in TM-induced ER stress tolerance. NPC3 was localized at the ER and predominantly expressed in root, which was further induced by TM-induced ER stress. Intriguingly, the *npc3* mutant reduced PCho content significantly in ER-stressed shoot. These results suggest that ER stress induces NPC3 to produce PCho and cope with TM-induced ER stress tolerance.

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P18

Chain length of fatty acids affects photoinhibition of PSII in cyanobacteria

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Light is essential for driving photosystem II (PSII), however, excess light damages PSII. Damaged PSII is recovered by a repair cycle depending on the *de novo* synthesis of proteins. When the degree of photodamage exceeds the repair, photoinhibition of PSII becomes apparent. A palmitic acid at the *sn*-2 position in cyanobacterial phosphatidylglycerol (PG) is crucial for PSII activity. Most of cyanobacteria species including *Synechocystis* sp. PCC 6803 (*Synechocystis*), have C-16 fatty acids at the *sn*-2 position of glycerolipids, while *Cyanothece* sp. PCC 8801 (*Cyanothece*) has C-14 fatty acids. However, the importance of chain length of fatty acid in photoinhibition of PSII remains unclear. Under strong light, *Cyanothece* had a higher PSII repair activity than *Synechocystis*. A mutant of *Synechocystis* expressing a T-1274 gene for lysophosphatidic acid acyltransferase from *Cyanothece* had an increased content of C-14 fatty acids and promoted PSII repair under strong light. In addition, the amount of singlet oxygen ($^1O_2$) that inhibits *de novo* synthesis of proteins decreased in the mutant cells by the alteration of energy transfer between photosystems. These results suggest that C-14 fatty acids promote PSII repair by suppressing the production of $^1O_2$.

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P19

Molecular analysis of NAD kinase genes in oleaginous algae *Nannochloropsis oceanica*

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The microalgae *Nannochloropsis* is considered as one of the promising species for biofuel production due to its ability to generate triacylglycerol (TAG) in cells. TAGs are synthesized from fatty acids derived from photosynthetically fixed carbon. We focused on NAD(P)(H) metabolism, which is playing crucial role in cellular energy metabolism and proliferation. NAD kinase (NADK) is an enzyme that catalyzes the phosphorylation of NAD^+ to NADP. Higher plants, such as *Arabidopsis*, have three types of NADKs (NADK1-3) with different intracellular localizations. *Nannochloropsis* is secondary symbiotic algae, and metabolic pathway for NAD(P)(H) production remains unclear. To clarify the biological properties of NADKs in *Nannochloropsis*, we conducted gene identification, expression analysis, and NAD(P)(H) measurements. First, we identified two NADK genes (*NoNADKa* and *NoNADKb*) from *N. oceanica*. NoNADKa showed the highest similarity to *Arabidopsis* NADK3, which is reported to be a peroxisome-localizing enzyme. However, NoNADKa lacked a peroxisome-targeting sequence at its C-terminus. On the other hand, NoNADKb showed high similarity to *Arabidopsis* NADK2, which localizes in the chloroplast. Under nitrogen-deficient conditions, *N. oceanica* activates the TAG synthesis pathway. Expression analysis demonstrated that *NoNADKa* and *NoNADKb* showed different expression patterns, suggesting different roles in stress-related responses. Furthermore, NAD(P)(H) measurements showed a transient increase (-24 h) in the total NAD(P)(H) contents, which subsequently decreased after 48 h of nitrogen depletion. Additionally, the redox ratio (NADPH/NADP) showed an increase during nitrogen deficiency (-144 hours). These data indicate that nitrogen deficiency induces extensive metabolic changes involving NAD(P)(H) metabolism in *N. oceanica*.

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P20

Screening of *Chlorella* mutant strains with high lipid contents for the development of microalgal-based meat alternatives

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Microalgae are a promising feedstock for biofuel production and plant-based meat alternatives. Above all, Chlorella is known for its high growth rates, ability to use carbon dioxide as a carbon source, and high lipid and protein content. These characteristics make it an attractive candidate for the production of biofuels and food products. In this research, we attempt to isolate Chlorella strains with an enhance the production of lipid moiety. In order to select the Chlorella parental strain with the best biomass, the growth of 20 UTEX Chlorella strains was compared. We further compared the carbon source and nitrogen source that provided better biomass and lipid content. Next, we performed the high-throughput screening and selection of high-lipid Chlorella mutants using a Percoll-based density gradient. Currently, we isolated several chlorella mutants with high lipid content. We will introduce the characteristics of our chlorella mutants in this presentation.

Keywords: Chlorella vurgaris, microalgal-based meat alternatives, mutant screening.

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P21

Uncovering the role of MYB1 transcription factor under stress conditions in *Chlamydomonas reinhardtii*

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Microalgae have emerged as promising biofuel feedstocks due to their capacity to accumulate high levels of oil under stress conditions. However, the precise biosynthetic pathways responsible for lipid production remain incompletely understood. The previous our study demonstrated that the gene encoding the R2R3-type MYB family transcription factor MYB1 in *Chlamydomonas reinhardtii* is highly induced under stress conditions. Two *myb1* mutants exhibited reduced levels of total fatty acids and storage lipids compared to the parental strain specifically during nitrogen (N) depletion.

In this study, we examined the impact of MYB1 on growth, viability, and lipid metabolism in *myb1* mutants subjected to under various stress conditions, including ER stress, salt stress, ROS stress and osmotic stress. Our research for the comprehensive understanding of MYB1 regulatory mechanisms under diverse stress conditions in Chlamydomonas not only deepens our insight into the lipid biosynthesis pathways but also provides valuable knowledge for engineering microalgae strains with enhanced lipid productivity for sustainable biofuel production.

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P22

Sterol biosynthesis contributes to brefeldin-A-induced endoplasmic reticulum stress resistance in *Chlamydomonas reinhardtii*

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The endoplasmic reticulum (ER) stress response is an evolutionarily conserved mechanism in most eukaryotes, while sterols in the phospholipid bilayer play a crucial role in controlling membrane fluidity and homeostasis. Despite the significance of both ER stress response and sterols in maintaining ER homeostasis, their relationship remains to be explored.

Our investigation focused on Chlamydomonas strain CC-4533 and revealed that under ER stress, free sterol biosynthesis increased, except in the mutants of the ER stress sensor IRE1. Transcript analysis unveiled the regulatory role of the IRE1/bZIP1 pathway in inducing the expression of ERG5, which encodes C-22 sterol desaturase, during ER stress in Chlamydomonas. Through the isolation of three erg5 mutant alleles, we observed a defect in the synthesis of Chlamydomonas' sterol end products, ergosterol and 7-dehydroporiferasterol. Furthermore, these *erg5* mutants exhibited increased sensitivity to ER stress induced by brefeldin A (BFA), an inhibitor of ER-Golgi trafficking, whereas other ER stress inducers had no such effect. Intriguingly, the sterol biosynthesis inhibitors fenpropimorph (Fp) and fenhexamid (Fh), which impede the upstream steps of the ERG5 enzyme in sterol biosynthesis, rescued the hypersensitivity to BFA in CC-4533 cells. Collectively, our findings support the conclusion that the accumulation of intermediates in the sterol biosynthetic pathway influences ER stress in a complex manner. This study highlights the significance and intricacy of regulating sterol biosynthesis during the ER stress response in microalgae.

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P23

Functional studies of patatin-like phospholipase in rice male reproduction

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Pollen tube transfers two sperms into female gametophytes for double fertilization. Pollen-specific patatin-like phospholipase A (pPLA) was reported to mediate maternal haploid induction. Breeding using haploids has the advantage of being able to rapidly fix useful traits through chromosome doubling. However, functional studies on pPLA in rice (*Oryza sativa*) is not much. OsMATL (OspPLAIIβ) found in *indica* subspecies has been reported to induce maternal haploid, although the function was not confirmed in *japonica* rice. We found another pPLA gene, OsMATL2 (OspPLAIIη), induces maternal haploid in *japonica* rice, examined by CRISPR-Cas9 generated homozygous mutant. Through gene expression and protein localization analysis, we confirmed that OsMATL and OsMATL2 express highly in pollen and localize in sperm cell. In order to identify the mechanisms of pPLA-mediated haploid induction, it is necessary to establish a system that can observe lipids in pollen and pollen tube. We generated transgenic rice of lipid markers that combine a lipid binding domain and fluorescent protein, to examine the intracellular location and pattern of phoshpolipids. PI3P is mainly associated with intracellular compartments, and PI(4,5)P_2 is localized in plasma membrane. PI4P and PA were localized to the plasma membrane and endomembrane. Finding lipids that interact with pPLA genes and analyzing lipid changes in pollen and pollen tube of mutant line through lipid markers will be useful in identifying the HI mechanism.

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P24

Patatin-related phospholipase, pPLAIIIs influence the recovery of defects in rice pollen development

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Patatin-related phospholipase (pPLAs) constitute a major enzyme family in plants, catalyzing fatty acid release from glycerolipids. They are classified into three subfamilies: pPLAI, pPLAII, and pPLAIII. pPLAIII enzymes, lacking a canonical catalytic serine motif, play essential roles in plant development, impacting lignin content and modifying cell wall structures (Jang et al., 2020, Jang et al., 2021). Moreover, they are involved in hormone-mediated organ development, exhibiting various phenotypes in mutants. These enzymes are also implicated in signal transduction, membrane remodeling, lipid metabolism, and play a crucial role in pollen development stages (Li et al., 2020). Further research is needed to gain a comprehensive understanding of specific pPLAIII functions. In *Oryza sativa*, *csm*-knockout plants showed defects during meiosis, leading to the failure to produce normal tetrads and exhibiting a reduced seed fertility ratio. However, when some of the putative *OspPLAIII* genes, namely *OspPLAIIIδ*, *OspPLAIIIγ*, and *OspPLAIIIε*, were simultaneously knocked out together with the *csm* gene, it was observed that the normal tetrads were formed, leading to an increase in seed fertility ratio. This result indicates that OspPLAIIIs could play a role in pollen development stages, especially during meiosis stages.

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P25

Development of *ZmPHOSPHOLIPASE A1* homolog-mediated *in vivo* haploid induction system in tomato and soybean

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As the world's population continues to grow, the global demand for food is steadily increasing, making the enhancement of crop productivity through advanced breeding methods a critical concern. Recently, extensive research has focused on double haploid (DH) technology, which shows great promise for efficient crop breeding. DH technology is considered the most rapid method for fixing genetic traits from either the maternal or paternal line, significantly increasing the efficienty of time required to produce homozygous lines compared to traditional breeding techniques. Among the haploid inducer genes, the sperm-specific *ZmPHOSPHOLIPASE A* (MTL/NLD/ZmPLA1) gene has demonstrated *in vivo* haploid induction capabilities in numerous monocots when mutated. Additionally, recent report suggests that the loss-of-function of the gynoecium-expressed *AtpPLAIIγ* gene triggers maternal haploid induction in the dicot *Arabidopsis*. Building upon this knowledge, our study aimed to develop a haploid induction system in economically important crops such as tomato and soybean. Utilizing the CRISPR-Cas9 system, we successfully edited the *SolycpPLAII* gene in the Sweet-100 tomato cultivar. The resulting mutants exhibited smaller fruit and seed size, as well as lower seed fertility. In soybean, we employed the Kwangan cultivar for *GmpPLAII* gene editing. The loss-of-function mutation in *GmpPLAII* did not affect seed fertility but led to a modulation of seed fatty acid contents. In future work, we will focus on investigating the haploid-inducing function in the mutants through DNA content and karyotype analysis. These findings will provide valuable insights into the development of improved crop breeding strategies.

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P26

Ca^{2+}-induced activation of lipoxygenase 2 accounts for green leaf volatile-burst in *Arabidopsis* leaves

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Green leaf volatiles (GLVs) consist of six-carbon volatile compounds such as (*Z*)-3-hexenol. GLVs are responsible for defense against herbivores and pathogens. The amounts of GLVs in intact plant tissues are low; however, when plant tissues would be damaged, GLVs are formed within minutes (GLV-burst). Lipoxygenase (LOX), more specifically, LOX2 in *Arabidopsis*, is an enzyme catalyzing dioxygenation reaction on lipids/fatty acids to form lipid-hydroperoxides, essential intermediates to form GLVs. When *Arabidopsis* leaves were disrupted in the presence of Ca^{2+}-chelating reagents, the GLV-burst was efficiently suppressed. Whenever GLV-burst suppression was observed, the production of lipid/fatty acid hydroperoxides was also suppressed; thus, it is clear that LOX plays a central role in controlling the GLV-burst. In order to get insight into the mechanism of Ca^{2+}-activation of AtLOX2, *E. coli*-expressed recombinant AtLOX2 was prepared. It showed little activity against linolenic acid in the absence of Ca^{2+}, but the activity appeared immediately upon the addition of Ca^{2+}. The Ca^{2+} concentration for half-maximal activation was ca. 0.6 mM. With animal LOXs, Ca^{2+} binds the N-terminal PLAT domain. After Ca^{2+}-binding, the animal LOXs showed higher affinity to the membranes where their substrates are abundant. The structural model of AtLOX2 made with AlphaFold2 showed that AtLOX also has the amino residues that might bind Ca^{2+}. Accordingly, we hypothesize that the binding of Ca^{2+} to the PLAT domain induces a conformational change in AtLOX2, resulting in more efficient access of AtLOX2 to its substrates, which facilitates the activation of AtLOX2 by Ca^{2+}.

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P27

Characterization of enzyme system responsible for the production of mushroom volatile compound, 1-octen-3-ol

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1-Octen-3-ol is a volatile oxylipin found ubiquitously in Basidiomycota and Ascomycota. 1-Octen-3-ol is also found in plants, such as those belonging to Fabaceae and Lamiaceae. It is also known that exposing 1-octen-3-ol induces defense response in plants; therefore, it was assumed that 1-octen-3-ol is an airborne signal compound involved in plant-fungi interactions. The biosynthetic pathway forming 1-octen-3-ol from linoleic acid via linoleic acid 10(*S*)-hydroperoxide was proposed 40 years ago in mushrooms, yet the enzymes involved had not been identified. Therefore, the purpose of this study is revealing the entire biosynthetic pathway of 1-octen-3-ol. We show that the dioxygenase 1 (Ccdox1) in the mushroom *Coprinopsis cinerea*, which contains an N-terminal cyclooxygenase-like heme peroxidase domain and a C-terminal cytochrome P450-related domain is responsible for dioxygenation of linoleic acid to form 10(*S*)-hydroperoxide. We demonstrated that disruption of the *Ccdox1* gene suppressed 1-octen-3-ol synthesis, although linoleic acid 10(*S*)-hydroperoxide was still efficiently converted into 1-octen-3-ol in the knock-out mutant strain. Therefore, there must be another enzyme that accounts for the cleavage of linoleic acid 10(*S*)-hydroperoxide into 1-octen-3-ol. Preliminary experiment indicated that the cleaving enzyme subsides in microsome membrane, and now we are trying to purify the enzyme. Molecular identification of the cleaving enzyme and subsequent comparison with a plant counterpart, i.e., fatty acid hydroperoxide lyase to form green leaf volatiles in plants, should provide new perspectives on how organisms in different kingdom (fungi and plant) come to use fatty acid-derived volatile compounds in their interactions with the ecosystems that surround them.

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P28

Function of seed-specific transcription factor ARR13 and ARR21 in the cytokinin signaling pathway

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Type-B *ARABIDOPSIS* RESPONSE REGULATORS 13 (ARR13) and ARR21 are transcription factors (TF) whose function is regulating various genes. They function to regulate cytokinin-responsive genes in the final step of the cytokinin signaling pathway. However, the specific roles of ARR13 and ARR21, as well as the downstream genes in the cytokinin signaling pathway, have not been completely identified. In this study, we hypothesize that seed-specific TF ARR13 and ARR21 can affect in seed through cytokinin stimulation. Due to high level of protein sequence redundancy between ARR13 and ARR21, we investigate these two TFs together. As a result of analyzing tissue-specific expression patterns using reverse transcription-quantitative polymerase chain reaction (RT-qPCR), the expression of ARR13 and ARR21 is the most in developing seeds than in other plant tissues. When comparing the expression of ARR13 and ARR21, the expression of ARR21 was significantly higher than expression of ARR13. To reveal the function of ARR13 and ARR21, We made two mutants for each line using the clustered regularly interspaced short palindromic repeats-CRISPR-associated protein 9 (CRISPR-Cas9) system: arr13 single mutants, arr21 single mutants, and arr13arr21 double mutants. Our final objective is to identify the downstream genes of ARR13 and ARR21 in the seed cytokinin signaling pathway.

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P29

Identifying molecular mechanisms of protective layer formation after floral organ abscission in *Arabidopsis*

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Abscission, which refers to the process of shedding unnecessary organs, is essential for plants to adapt to environmental changes and ensure sustainability. While the molecular mechanisms of the abscission process have been extensively studied in the floral organ abscission of *Arabidopsis*, our understanding remains fragmented, particularly regarding the transition of abscission zone cells into epidermal cells and the subsequent cuticle formation after abscission.

To address this knowledge gap, we conducted transcriptome analysis of three mutants (bop1/2, hae/hsl2, and nev) with impaired abscission and subsequent deficiencies in protective layer formation. By comparing transcriptome profiles between wild type and mutants, we identified candidate genes, including transcription factors, signaling components, and cell wall remodeling enzymes involved in cuticle biosynthesis.

Through further experiments investigating spatial expression patterns and validating knockout phenotypes, we successfully identified mutants, referred to as decs, which exhibited deficiencies in cuticle formation. Our ongoing research aims to elucidate the regulatory mechanisms of DECs in cuticle formation and investigate how their spatiotemporal expression is influenced by external environmental cues.

These investigations will provide valuable insights into the mechanisms of cuticle formation and the interplay between environmental perception and gene activation during this process.

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P30

Understanding Cuticular Adaptation to the Water Contact Surface in *Spirodela polyrhiza*

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Cuticles, composed of a covalently linked cutin polyester and waxes, from a hydrophobic barrier on plant shoot surfaces, providing protection against desiccation and pathogen invasion. The structure and composition of cuticle are complex and can vary significantly depending on the development stages and environmental factors. However, the precise relationship between structural properties and biological functions remains elusive, and the underlying mechanisms driving this dynamic phenomenon are not well understood. In this study, we aimed to address these gaps by analyzing the cuticle of both the upper and lower surfaces of fronds from *Spirodela polyrhiza*, commonly known as duckweed, an aquatic plant species that floats on water surfaces. By comparing the cuticle structures between the adaxial (air-exposed) and abaxial (submerged in water) sides of the fronds, we sought to gain valuable insights into the distinct properties of the cuticle layer in response to environmental stimuli. To examine the ultrastructure of the cuticle layers, we used a transmission electron microscope and discovered a notable difference in cutin density between the adaxial and abaxial fronds. The cutin density in the abaxial frond appeared paler but thicker compared to the adaxial frond. Gas chromatography analysis was performed on the adaxial and abaxial epidermis to analyze the cutin polyester monomers. RNA-seq analysis of both adaxial and abaxial sides revealed that lipid metabolic process gene expression was enriched in the adaxial side of the frond. These studies will contribute to understand the difference of cuticles from the adaptation to the water contact surface.

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P31

Impact of Indole-3-Acetic Acid (IAA) signaling on membrane lipid remodeling in *Arabidopsis thaliana*

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Indole-3-acetic acid (IAA), the main auxin in land plants, has profound effects on plant growth and development. As an important phytohormone that has long been discovered, numerous studies have been conducted using auxin. Despite extensive research on auxin's effects, the mechanisms underlying its involvement in membrane lipid remodeling remain poorly understood. In this study, we revealed how IAA changes lipid composition and which lipid biosynthesis genes are involved in the model land plant *Arabidopsis* thaliana. IAA is known to inhibit root growth and enhance shoot growth. According to the gas chromatograph analysis, an increase of palmitic acid 16:0 and a decrease of α-linolenic acid 18:3 were detected under the treatment of 0.01 μM or 0.1 μM IAA. *Arabidopsis* mutants of one of the auxin transporters also showed a similar fatty acid phenotype. In addition, the expression level of a gene encoding endoplasmic reticulum fatty acid desaturase was increased. On the other hand, plastidial fatty acid desaturase was not increased in *Arabidopsis* grown under the treatment of IAA. The current study uncovered the impact of IAA signaling on the fatty acid profile in *Arabidopsis*.

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P32

Pollen specific receptor kinases are required for pollen tube germination in rice

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Pollen hydration, germination and tube growth are important processes for successful fertilization of flowering plants. These processes involves a complex signaling pathway and molecular regulators are less known in crop plants. Previous studies in dicots such as *Arabidopsis* and Tomato have shown that Pollen Receptor Kinases (PRKs) play a role in pollen tube growth. Here, we first found five PRKs in rice (*Oryza sativa*). These PRKs have conserved domains including leusine rich repeat domain, kinase domain. Like most AtPRKs, PRK3 have polybasic domain in the juxtamembrane and this domain may help PRK3 bind to anionic phospholipids. We confirmed pollen preferred expression of PRKs by qRT-PCR. By transient expression of tobacco epidermal leaves, we confirmed their localization into plasma membrane. To find out functions of PRKs, we produced the triple knock out line (*prk1/2/3*) using multiple gRNA of CRISPR/Cas9 system. The *prk1/2/3* showed the male sterile phenotype with normal vegetative growth and floret formation. Reduced seed fertility was due to defects in pollen hydration and germination with low reactive oxygen species (ROS) accumulation. We are conducting more detailed studies of relationships between PRKs and their interacting candidates such as cysteine rich peptide, anioninc phospholipids to reveal the mechanism of their role and polar distribution. Our findings suggest that rice PRKs redundantly play a role in ROS signaling for pollen hydration and germination.

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P33

Improved method for analyzing reactive carbonyl species from small amounts of plant material

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Reactive carbonyl species (RCS), α,β-unsaturated carbonyls such as acrolein and HNE, are produced from lipid peroxides. RCS are important as a signal of stress response in plants. For determination of RCS content in plants, aldehydes are extracted from plants, derivatized to hydrazones and separated and quantified by HPLC. In this method, the amount of plant sample required per measurement is about 100 mg, which corresponds to, for example, 100 individuals of 5-day-old *Arabidopsis* thaliana. This requirement significantly limits the number of conditions and repetitions in one experiment. In this study, to reduce the amount of sample for a measurement, we rearranged the procedure after derivatization, and we achieved the reduction of the sample amount per measurement to 1/4 of the conventional method. Here we present actual examples of aldehyde analysis using the improved method.

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P34

FATTY ACID DESATURASE5 deficientry suppresses the generation of reactive carbonyl species in *Arabidopsis* crumpled leaf mutant

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Arabidopsis CRUMPLED LEAF deficiency mutant crl develops localized cell death (LCD), a type of programmed cell death, in leaves. LCD process is thought to be initiated by oxidative signal from the chloroplast because the deficiency of the CRL protein, a regulator of the outer envelope protein translocator complex, impairs chloroplast development and division. Recently, Li et al. (2020) found that the deficiency of *FAD5* suppressed the LCD in *crl*. FAD5 is the chloroplast-localized fatty acid desaturase 5, which catalyzes the conversion of palmitic acid (16:0) to palmitoleic acid (16:1) specifically at the *sn*-2 position of monogalactosyl diacylglycerol (MGDG). This suggests that certain C16 unsaturated fatty acids in MGDG or their metabolites are the chloroplast signal to initiate LCD. In this study, we hypothesized that the signal mediator is reactive carbonyl species (RCS), the lipid peroxide-derived α,β-unsaturated carbonyls. Carbonyl analysis of leaves revealed that the levels of RCS such as acrolein and HNE in *crl* mutant were significantly higher than those in wild type (Col-0) and *fad5* single mutant. Interestingly, the RCS levels in *crl fad5* double mutant were not higher than those in the wild type. Thus the high leaf RCS levels strongly correlated with the development of LCD. Possible mechanisms are discussed.

Reference: Li et al. (2020) Plant Cell 32, 3240-3255

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P35

Alliin is an excellent scavenger of acrolein, a reactive carbonyl species derived from lipid peroxides

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Reactive carbonyl species (RCS), the α,β-unsaturated aldehydes and ketones derived from lipid peroxides, are generated under oxidative stress in plants and play vital roles in determining cell fate as signaling and damaging molecules. In this study, we aimed at discovering plant compounds that can scavenge RCS. We employed acrolein (Acr) as a typical RCS and determined the Acr-consuming capacity of 80% ethanol extract from plant tissues. Among the 46 angiosperm species tested, the highest capacites were found in various species of diverse families, e.g. garlic, spinach, avocado, broccoli and lotus. We purified the active ingredient from garlic cloves through two steps of hydrophilic chromatography, and identified it as *S*-allyl-cysteine sulfoxide (alliin), an amino acid derivative typical of *Allium* species. The Acr-scavenging rate of authentic alliin was comparable to that of carnosine, an RCS-scavenging dipeptide in musles, slower than that of Cys, and faster than those of amino acids such as His and Lys and polyphenols such as epigallocatechin gallate and resveratrol. Product analysis with liquid chromatography tandem-mass spectrometry showed that alliin, isoalliin and methiin can bind two Acr molecules. Reaction mechanism is discussed.

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P36

Lipid peroxide-derived reactive carbonyl species are common endogenous substrates of glutathione transferase Tau isozymes

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Reactive oxygen species (ROS) are generated when plants are exposed to environmental stresses. ROS oxidize biomembranes to produce lipid peroxides, which are degraded to produce α,β-unsaturated carbonyls (reactive carbonyl species: RCS) such as acrolein and 4-hydroxynonenal. Due to their high electrophilicity, RCS cause cytotoxicity by modifying proteins and nucleic acids. Plants have several types of enzymes with substrate specificity for RCS, such as aldehyde reductase, aldehyde dehydrogenase and 2-alkenal reductase, but their *K*m values are considerably higher than the intracellular concentrations of RCS in plant.

We have found that 12 out of 23 *Arabidopsis* GST Tau class isozymes (GSTUs) have RCS scavenging activity. Among them, GSTU19 had a high activity for acrolein (50.7 nkatal/mg) and GSTU17 a high activity for HNE (39.0 nkatal/mg). The GST isozymes have different specificity for individual RCS.

In this study we investigated whether GSTs from plants other than *A. thaliana* also act as RCS scavenging enzymes or not. cDNA of GSTUs from *Thellungiella halophila* (*Brassicaceae*) and *Lotus japonicus* (*Fabaceae*) were obtained and expressed in *Escherichia coli*. Activity assay using 4 different RCS as substrates showed that two GSTUs in *T. halophila* and three GSTUs in *L. japonicus* had RCS scavenging activity. In particular, one isozyme from *T. halophila* was highly active for HNE at 72.2 nkatal/mg, and another from *L. japonicus* for acrolein at 21.4 nkatal/mg. These results show that GST tau isozymes are common RSC scavenging enzymes in angiosperms.

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P37

Linalool exposure enhanced defense against a herbivore and caused accumulation of linalyl diglycoside

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Previously we found that exposing tomato plants to (Z)-3-hexenol led to the accumulation of (Z)-3-hexenyl β-vicianoside concomitant with higher defense against a herbivore. This prompted us to investigate the effects of volatile exposure on the defense in crop plants. In this study, we treated soybean plants with a vapor of linalool which is a common monoterpene alcohol found widely in plants. Soybean plants exposed to linalool acquired higher defense against the herbivores, Spodoptera litura (common cutworm). LC-MS analysis of aroma glycosides with the exposed plants showed that they had a higher amount of linalyl diglycoside. We purified linalyl diglycoside from soybean leaves and its structure was elucidated by 1H- , 13C-NMR, and high-resolution mass spectrometry, and subsequently, it was identified as linalyl β-vicianoside.

When phytohormone (jasmonic acid, oxophytodienoic acid, and salicylic acid) levels in the exposed soybean plants were estimated, no significant increase was observed, from which it was suggested that the higher defense found in exposed plants was independent of jasmonic acid- and/or salicylic acid-dependent signaling pathway. We also confirmed that treating the soybean plants with methyl jasmonate resulted in little change in the amount of linalyl - vicianoside. In summary, it was assumed that intake and glycosidation of exogenously supplied linalool and subsequent accumulation of its vicianoside at least partly account for the higher defense of the treated plants against the herbivore. Treating crop plants with naturally occurring volatile compounds might be a useful and ecological-friendly strategy to enhance their defense mechanism in an agricultural field.

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P38

Two MYC2 Transcription Factors Positvely Regulate Ginsenoside Biosynthesis in *Panax ginseng*

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Panax ginseng is one of the most valuable medicinal crops that utilized in many countries worldwide due to its remarkable biological and pharmacological activities attributed to its own triterpenoid saponins known as ginsenosides. Ginseng has unique biosynthetic pathway responsible for the production of ginsenosides, derived from isoprenoid pathway. Among the phytohormones, jasmonic acid (JA) plays crucial role in enhancing ginsenoside accumulation in ginseng. MYC2 transcription factor serves as main key regulator of gene expression through JA-signaling pathway in many plants, which has not been discovered in ginseng. We identified two MYC2 transcription factors in ginseng genome, namely PgMYC2a and PgMYC2b, respectively. The expression patterns of *PgMYC2a* and *PgMYC2b* were examined in various ginseng tissues, and their expression level was increased by JA. Likewise with other transcription factors, in subcellular localization assay using tobacco leaves, the transient expression of *PgMYC2a* and *PgMYC2b* was detected in the nucleus of tobacco cells. To further investigate their roles related with ginsenosides, we successfully generated each of *PgMYC2a* and *PgMYC2b*-overexpressed ginseng callus lines and confirmed that the overexpression of two transcription factors induced the expression of ginsenoside biosynthetic genes. Furthermore, ginsenoside contents of the overexpression lines were significantly higher than control, demonstrating that PgMYC2a and PgMYC2b can be positive regulator of ginsenoside biosynthesis. Through our study, these newly identified transcription factors are anticipated eagerly to provide advanced aspects for understanding ginsenoside biosynthesis in ginseng.

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P39

Genome editing for manipulating glucosinolates in *Brassica rapa*

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The glucosinolate biosynthetic pathway has been well-characterized in the model plant *Arabidopsis thaliana*. Glucosinolates are the key specialized metabolites synthesized through three major pathways: aliphatic glucosinolates from methionine, indolic glucosinolates from tryptophan, and aromatic glucosinolates from phenylalanine. Notably, the Chinese cabbage *Brassica rapa* exhibits a remarkable capacity to generate substantial amounts of both aliphatic and indolic glucosinolates. Putative biosynthetic genes of glucosinolates in *B. rapa* have been proposed based on their sequence similarity with *A. thaliana* biosynthetic genes. However, the functional validation of candidate genes remains constrained. In this study, we have focused on generating insertion or deletion mutations on two BCAT4 genes responsible for catalyzing the initial step in aliphatic glucosinolate biosynthesis, three CYP79B2 genes catalyzing the initial phase of indolic glucosinolate biosynthesis, and two CYP81F4 genes catalyzing the final phase of indolic glucosinolate biosynthesis. Guide RNAs targeting multigene family were designed, and *Agrobacterium*-mediated transformation was performed. The amount of major glucosinolates in gene-edited lines was measured using a LC-MS/MS instrument. In addition, we have developed virus-induced gene editing system to manipulate target genes in non-model plants.

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P40

JA-dependent pith lignification in *Nicotiana attenuata* against the stem-boring herbivore

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The stem, a pivotal organ for plant survival and reproduction, facilitates metabolite transport and supports reproductive structures. Despite its significance, limited research has explored stem defense mechanisms against stem-boring insect herbivores due to their cryptic life history. We previously reported a systemic lignification response in stem-borer-attacked wild tobacco (*Nicotiana attenuata*) pith, which is reducing the performance of stem-boring weevil larvae (*Trichobaris mucorea*). This defensive lignification is triggered by the phytohormone called jasmonic acids (JAs). However, the intricate molecular mechanisms linking JAs to defensive lignification remain elusive. Here, we focused on MYC orthologs, the JA-responsive transcription factors, to unveil the mechanism driving pith lignification against stem-boring herbivores. We generated single and double mutants of MYC paralogs using the virus-induced gene-editing system based on the CRISPR-Cas system. We performed comparative lignin quantification and staining, transcriptome analysis, and larval bioassays in multiple *Namyc* single and double mutants. The *T. mucorea*-infested *Namyc2/3* pith was not lignified, and *Namyc2/3*-fed larvae were significantly heavier than WT- or single mutant-fed larvae. Also, RNA-seq between control and attacked pith among single and double mutants revealed that lignification-associated genes were not induced in the infested *Namyc2/3* lines. To support that NaMYC2 and NaMYC3 redundantly upregulate JAs-dependent lignification in the *T. mucorea*-infested pith, we additionally performed promoter transactivation assays with MYC2 and MYC3 on promoters of lignification-associated genes. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) using lines overexpressing the epitope-tagged MYC2 or MYC3 will be performed and elucidate their direct targets even more in detail. These results suggest that two JA-dependent transcription factors, NaMYC2 and NaMYC3, are the missing link between the JA signaling pathway and the induced lignification in pith.

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READRetro: AI-assisted prediction for natural product biosynthesis

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Elucidating the biosynthetic pathways of natural products has been a major focus of biochemistry and pharmacy. However, predicting the whole pathways from target molecules to metabolic building blocks remains a challenge. Here we propose READRetro as a practical bio-retrosynthesis tool for proposing the biosynthetic pathways of natural products. READRetro effectively resolves the tradeoff between generalizability and memorability in bio-retrosynthesis by implementing two separate modules; each module is responsible for either generalizability or memorability. Specifically, READRetro utilizes a rule-based retriever for memorability and an ensemble of two dual-representation-based deep learning models for generalizability. Through extensive experiments, READRetro was demonstrated to outperform existing models by a large margin in terms of both generalizability and memorability. READRetro was also capable of proposing the known pathways of complex plant secondary metabolites such as monoterpene indole alkaloids and the unknown pathway of menisdaurilide, demonstrating its applicability in the real-world bio-retrosynthesis of natural products. A website (https://readretro.net) and open-source code have been provided for READRetro, a practical tool with state-of-the-art performance for natural product biosynthesis research.

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P42

Comprehensive metabolomic and lipidomic alterations in response to heat stress during seed germination and seedling growth of *Arabidopsis*

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Temperature affects seed germination and seedling growth, which is a critical and complex stage in plant life cycle. However, comprehensive metabolic basis on temperature implicating seed germination and seedling growth remains less known.

Here, we applied the high-throughput untargeted metabolomic and advanced shotgun lipidomic approaches to profile the *Arabidopsis* 182 metabolites and 149 lipids under moderate (22°C, 28°C) and extreme high (34°C, 40°C) temperatures.

Our results showed that metabolism related to organic acids/derivates and amines was enriched at moderate temperatures, which involved many cellular responses towards tricarboxylic acid cycle (TCA), carbohydrates and amino acids metabolism, peptide biosynthesis, phenylpropanoid biosynthesis and indole 3-acetate (IAA) biosynthetic pathway. Whereas, under extreme high temperatures, no seed germination was detected, but 148 out of 182 metabolites were highly enriched, including galactose metabolism, fatty acid degradation, tryptophan/phenylalanine metabolism, and shikimic acid-mediated pathways (especially alkaloids metabolism and glucosinolate/flavone/flavonol biosynthesis.) Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) gradually increased from moderate to extreme high temperatures; while phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylglycerol (PG), monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG) decreased.

Under moderate temperatures, we found that TCA, disaccharides, nucleotides, polypeptides, SQDG and the biosynthesis of fatty acids and glucobrassicin-mediated IAA decreased at 28°C, while amino acids, trisaccharides, PE, PC, PA, PS, MGDG, DGDG and diacylglycerol (DAG) increased.

Taking together, our results provided a comprehensive metabolites phenotype, revealed the characteristics of metabolites necessary for seed germination and/or seedling growth under different temperatures, and provided insights into different metabolic regulations of metabolites and lipid homeostasis for seed germination and seedling growth.

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P43

Purification of avocado ingredients that scavenge acrolein

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Reactive oxygen species (ROS) are constantly generated in vivo, and when some of them oxidize unsaturated fatty acids in membrane lipids, lipid peroxide is formed. Some of the lipid peroxides break down to form electrophilic α,β-unsaturated aldehydes and ketones called reactive carbonyl species (RCS), which are responsible for cell-damaging stress disorders.

In order to discover substances that scavenge RCS, we evaluated the acrolein (ACR) scavenging capacity of plant extracts and found the highest ACR scavenging capacity in avocado pulp and garlic. In this study, we aimed to identify the ACR scavengers' compounds in avocado pulp. Extracts obtained from avocado pulp were purified by four steps of chromatography. LC-MS/MS analysis of the purified fraction showed several substances as ACR scavengers. These substances were searched in databases such as AraCyc, KEGG and Plant Cyc, and one was presumed to be glutamine. LC-MS/MS analysis of the glutamine sample showed that the MS/MS and retention times were consistent, leading to the conclusion that one of the ACR scavengers in avocado is glutamine. Since there are other ACR scavenger candidates, we plan to purify and identify them in the future.

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P44

Effects of disruption of ABC transporter genes on metabolites associated with oil bodies in *Marchantia polymorpha* L.

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The liverwort *Marchantia polymorpha* possesses oil bodies in idioblastic oil body cells scattered in its thallus. Specific sesquiterpenes and bisbibenzyls are accumulated in oil bodies. Therefore, a specialized system for the biosynthesis and accumulation of these defense compounds in oil bodies has been implied. As biosynthesis of defense chemicals likely proceeds in the cytoplasm, the chemicals should be transported from the cytoplasm into oil bodies across oil body membranes; however, the mechanism of transportation has not been fully elucidated. It has been reported that ABC-transporter type Gs (ABCGs) are involved in the transportation of defense chemicals across membranes. One of ABCGs in *M. polymorpha*, namely, Mp*ABCG1* is specifically expressed in the oil body cells and localizes to the oil body membrane. When the gene function of Mp*ABCG1* was knocked-out, the amounts of sesquiterpenes and bisbibenzyls in the thallus were substantially reduced compared to those of the wild type strains. To examine the effect of gene disruption on the formation of oil bodies, the oil body membrane marker was introduced in the Mp*abcg1* mutants, and the number of oil bodies in the resulting transformants was compared to that expressed in the wild type strains. The number of oil bodies detected with a fluorescent microscope showed no significant difference per fixed area during the growth of the thallus, but a decreasing trend was observed in the Mp*abcg1* knockout, implying that the accumulation of defense chemicals by MpABCG1 controls the development of the oil bodies.

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P45

Flavanone 3'-hydroxylase determines the Typical Flavonoid Profile of Purple Chinese cabbage

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To elucidate the flavonoid biosynthetic pathway in Chinese cabbage (*B. rapa* L. subsp. pekinensis), we analyzed flavonoid contents in two varieties of Chinese cabbage with normal green (5546) and purple (8267) leaves. The 8267 variety accumulates significantly higher levels of quercetin, isorhamnetin, and cyanidin than the 5546 variety, indicating that 3'-dihydroxylated flavonoids are more prevalent in the purple than in the green variety. Gene expression analysis showed that the expression patterns of most phenylpropanoid pathway genes did not correspond to the flavonoid accumulation patterns in 5546 and 8267 varieties, except for BrPAL1.2. In particular, the flavanone 3'-hydroxylase BrF3'H (Bra009312) is expressed almost exclusively in 8267. We isolated the coding sequences of BrF3'H from the two varieties and found that both sequences encode identical amino acid sequences and are highly conserved with F3'H genes from other species. An in vitro enzymatic assay demonstrated that the recombinant BrF3'H protein catalyzes the 3'-hydroxylation of a wide range of 4'-hydroxylated flavonoid substrates. Kinetic analysis showed that kaempferol is the most preferred substrate and dihydrokaempferol (DHK) is the poorest substrate for recombinant BrF3'H among those tested. Transient expression of BrF3'H in *Nicotiana benthamiana* followed by infiltration of naringenin and DHK as substrates resulted in eriodictyol and quercetin production in the infiltrated leaves, demonstrating the functionality of BrF3'H in planta. Our study provides insight into the molecular mechanism underlying purple coloration in Chinese cabbage.

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Identification of High Oleic Acid Natural Mutant *hoa* and CRISPR-mediated *BnaFAD2/FAE1* Creating High Oleic Acid Germplasm in *B.napus*

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Rapeseed (*Brassica napus* L.) is one of the largest oil crops in the world. Rapeseed oil is essential for human daily life. The improvement of the oleic acid content and quality of rapeseed has become an important breeding goal by changing its fatty acid composition.

In this study, a rapeseed mutant with an oleic acid content of up to 79% was selected from a natural population of *B.napus* and was named *hoa* (high oleic acid). After hybridization, the F1 and F2 populations were obtained, and through fatty acid content measurement and genetic analysis of the F2 population, we speculate that the high oleic acid trait is a quantitative genetic trait controlled by an allele. We found that there is a 336bp deletion in the intron of the 5'-UTR region of the *BnaFAD2* gene and the change of an amino acid at position 20. At the same time, transcriptome sequencing showed that fatty acid metabolism, fatty acid biosynthesis, and unsaturated fat acid synthesis pathways were enriched in *hoa*.

Furthermore, we utilized the CRISPR/Cas9 system to simultaneously edited the genes *BnaFAD2.A05*, *BnaFAD2.C05*, *BnaFAE1.A08*, and *BnaFAE1.C03*, and we selected plants with oleic acid content of up to 89%. We successfully obtained new germplasm of high oleic acid rapeseed, providing new ideas for the goal of high oleic acid breeding in rapeseed.

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P47

Multiple mutations of the FIBRILLIN family in the chloroplast plastoglobules of *Arabidopsis*

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Plastoglobules (PGs) are spherical lipoproteins found in all types of plastids. The lipids in chloroplast PGs mainly consist of prenylquinone (PQ) and triacylglycerol (TAG). PGs play crucial roles in synthesizing, storing, and transporting isoprenoids and neutral lipids. They are also dynamically involved in responding to both abiotic and biotic stresses. Fibrillins (FBNs) are lipid-associated proteins present in chloroplasts. They act as structural proteins in thylakoids, stroma, and PGs. In the *Arabidopsis* genome, there are 14 FBN gene families, and the FBNs present in PGs are particularly essential for the formation and maintenance of these PGs. In fact, seven FBNs are responsible for about 70% of the PG protein content. Previous studies of FBNs present in PGs have shown that FBN1a and FBN1b interact with each other to establish the PG structure. And FBN2 is associated with the accumulation of prenyl lipids in PG under high light stress, and also contributes to the accumulation of metabolites associated with JA-induced senescence, such as phytyl esters and TAG. To further understand the functions of FBNs in PGs, a study subjected the seven FBN genes present in PGs to multiple mutations using CRISPR/Cas9 technology. This approach allowed researchers to identify and characterize the specific roles of FBNs in PGs and various cellular processes related to lipid metabolism and stress responses in chloroplasts.

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P48

Unraveling the transcription factors regulating triacylglycerol biosynthesis through the regulation of LEAFY COTYLEDON 2

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Triacylglycerol (TAG), used as an energy source for seed germination, is accumulated during seed development. LEAFY COTYLEDON 2 (LEC2) controls various transcription factors (TFs) for TAG biosynthesis as a master regulator. However, the TFs regulated by LEC2 have been little studied. Here, we identified new seed-specific TFs upregulated by LEC2. Each 25 TFs overexpression in *Nicotiana benthamiana* leaves by Agrobacterium infiltration showed various changes in TAG accumulation. As a result, five TFs overexpression accumulated TAG contents as high as LEC2 overexpression. They were transactivated directly or indirectly by LEC2 in transcriptional activity assay. One of them is AINTEGUMENTA-LIKE 6 (AIL6), a member of APETALA2/ETHYLENE RESPONSE FACOTR (AP2/ERF) domain TF family. The overexpression of AIL6 in *Arabidopsis* increased total fatty acid (FA) content in seed compared to the WT. Their FA composition showed an increase of 16:0, 18:0, 18:1, and 18:2 FAs and decrease of 18:3 and 20:1 FAs compared to the WT. Furthermore, the various fatty acid and TAG biosynthesis genes were highly expressed in AIL6 overexpression line. Therefore, our results suggest that AIL6 may act as a positive regulator for TAG biosynthesis and this study will be very useful for improving seed oil content and specific fatty acid synthesis.

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ERF55, an AP2/ERF transcription factor, regulates seed triacylglycerol content in *Arabidopsis thaliana*

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ERF55, a member of the ERF-group I of the AP2/ERF family, is known to be involved in plant growth, development, responses to biotic and abiotic stresses, as well as hormone signal transduction. In this study, we investigated the role of the transcription factor ERF55 in seed triacylglycerol (TAG) synthesis. We first examined the subcellular localization of ERF55 and confirmed that it localizes to the nucleus through colocalization studies with DAPI staining. We then conducted GUS staining and RT-qPCR analysis to determine the tissue-specific expression pattern of ERF55. The results revealed that ERF55 is expressed in the anthers of flowers and seed embryos in *Arabidopsis*. To understand the role of ERF55 in TAG biosynthesis, we generated transgenic *Arabidopsis* lines using CRISPR/Cas9 technology to create knockout mutants for ERF55. While the seed size of the *erf55* mutants remained similar to the wild type (WT), there was a slight reduction in seed weight. Importantly, the seed TAG content in the *erf55* mutants was significantly decreased compared to the WT. Additionally, there were notable changes in the TAG composition, with reductions in 18:1 and 20:1 fatty acids and an increase in 18:2 levels. Taken together, these findings strongly indicate that ERF55 plays a crucial role in regulating TAG synthesis in *Arabidopsis* seeds

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Enhancing seed fatty acid content through CRISPR-Cas9-mediated patatin-related phospholipase A *pPLAII* gene editing in *Arabidopsis* and camelina

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Camelina (*Camelina sativa*), recognized for its unique fatty acid profile enriched with polyunsaturated fatty acids, holds significant promise as an oilseed crop. The utilization of camelina offers several advantageous characteristics, including a short growth cycle, inherent resistance to pathogens and pests, and reduced agricultural input demands. These qualities make camelina an attractive choice for sustainable oil production. Patatin-related phospholipase As (pPLAs) play a pivotal role in plant lipid metabolism. For instance, the overexpression of castor *pPLAIIIβ* facilitates the removal of hydroxy fatty acids from phosphatidylcholine, while overexpression of *Arabidopsis pPLAIIIδ* increases seed oil content with long-chain fatty acids. Despite these discoveries, the functional role of the *pPLAII* genes in regulating fatty acid composition remains unexplored. In our study, we found that loss-of-function mutations in the *pPLAII* gene reduced seed weight by 20% but increased total fatty acid content by 30% in *Arabidopsis*. Building on these findings, our future research aims to investigate the function of the *pPLAII* gene in camelina through identify its ortholog, gene editing, and explore its impact on camelina seed fatty acid composition. This investigation is of utmost significance as it deepens our understanding of the genetic mechanisms governing fatty acid synthesis and accumulation in oil crops. The outcomes of this study could offer valuable insights for tailoring camelina seed fatty acid profiles to meet specific industrial needs, thereby enhancing the utility of this versatile oil crop.

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Polyketide Synthase-Like Functionality Acquired by Plant Fatty Acid Elongase

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Fatty acid elongation in the plastid and ER involves four sequenctial reactions that extends fatty acids by two-carbons with each cycle. Polyketide synthases found in many microbial species use analogous enzymatic reactions to elongate fatty acids but can skip steps in the elongation cycle ("discontinuous elongation") to generate novel in-chain hydroxy and keto groups.We recently discovered that a specialized fatty acid elongase 1 (FAE1) enzyme is responsible for a novel discontinuous fatty acid elongation pathway for C24 di-hydroxy fatty acid synthesis in the seed of *Orychophragmus violaceus*, a China-native Brassicaceae. Here, we describe the discovery of seed oils with keto hydroxy fatty acids and a variation in discontinuous fatty acid elongation in seeds of the closely related species *O. limprichtianus*. The structure of these fatty acids were identified by gas chromatograph-mass spectrometry to be 7-(O)-18-(OH)-24:1Δ15, 9-(O)-20-(OH)-26:1Δ17, and 11-(O)-22-(OH)-28:1Δ19. We further show that introduction of a structurally divergent fatty acid elongase OlFAE1-2 is sufficient to confer production of the dihydroxy fatty acids such as a Nebraskanic acid (7,18-(OH)2-24:1Δ15) when expressed with fatty acid hydroxylase (*OvFAD2-2*) for production of ricinoleic acid in Arabidopsis seed. Moreover, we found that introduction of a structurally divergent fatty acid reductase OlKCR1-1, which is expressed in developing *O. limprichtianus* seed, along with *OlFAE1-2* and *OvFAD2-2* in Arabidosis occurs accumulation of 7-(O)-18-(OH)-24:1Δ15 in the seed oil. Our study explains that 3-keto C20 hydroxy fatty acids synthesized by a special elongation module act as precursors, causing additional C2 extension through other elongation module in developing *O. limprichtianus* seed. These and recent findings highlight the plasticity of plant fatty acid elongase to genrate polyketide synthase-like products and the possibility of enginnering plant fatty acid elongase to generate novel oil functionalities.

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P52

The roles of two pairs of *FAD3* in tetraploid perilla evolution: A key factor in high α-linolenic acid content

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Perilla (*Perilla frutescens* (L.) var. *frutescens*) is an allotetraploid oil crop and has high oil contents containing 64% α-linolenic acid (18:3) and 14% linoleic acid (18:2) in seeds. Two mutant lines (DY-1-19 and NC-110-18) generated by gamma-ray irradiation, showed a high content of 18:2 fatty acid compared to wild type in seeds. The changes in fatty acid composition might be related to the loss of function of FAD3 which synthesizes the 18:3 fatty acid in plants. DY-1-19 significantly increased the 18:2 content, surpassing 27%, due to a 4-base pair deletion in the *PfrFAD3b*. Similarly, NC-110-18 showed an increase of over 15% in 18:2 content resulting from a large deletion in the *PfrFAD3a*. To validate the functional role of these genes in 18:3 synthesis, *PfrFAD3a*, and *PfrFAD3b* were transformed respectively into *Arabidopsis fad3-2* mutants. As a result, both *PfrFAD3a* and *PfrFAD3b* restored 18:3 content in seeds. Additionally, simultaneous knockout of *PfrFAD3a* and *PfrFAD3b* using CRISPR/Cas9 resulted in an increase of 18:2 content up to 75% and coincided with a decrease of 18:3 content up to 0.3% in seeds. This suggests that the duplicated *FAD3* in perilla, arising from the evolution from diploid to tetraploid, plays a crucial role in 18:3 synthesis in perilla seeds. In other words, the duplication of seed-specific *FAD3*, encoding the final enzyme in 18:3 synthesis, likely contributes to the observed high content of 18:3 fatty acid in perilla.

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P53

Exchanging FAE1 with PfKCS18 in transgenic *Arabidopsis* increases hydroxy fatty acids

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Castor (*Ricinus communis L.*) seeds contain unusual fatty acid, hydroxy fatty acid (HFA), used as a chemical feedstock. Castor cultivation is limited by the potent toxin ricin in its seeds and other poor agronomic traits, so it is advantageous to develop a suitable HFA-producing crop. In our previous result, we designed a transformation construct that allowed the co-expression of five essential castor genes (named *pCam5*) involved in HFA biosynthesis, including *FAH12*, *DGAT2*, *PDAT1-2*, *PDCT*, and *LPCAT*. Transgenic *Arabidopsis pCam5* lines produced HFA counting for 25% in seed oil. In this study, we introduced a Lesquerella elongase (*PfKCS18*), which specifically elongates 18:1-OH to 20:1-OH, into *pCam5* and *pCam5-atfae1*. As a result, the averages of total HFA significantly increased to 31.5–32.5% in *pCam5+PfKCS18-atfae1* compared with 25.2–26.3% in *pCam5+PfKCS18*. Among transgenic plants, *pCam5+PfKCS18-atfae1* accumulated HFA to the highest at 73.8 μg/mg, followed by *pCam5* and *pCam5+PfKCS18* at significantly lower levels at 46.3–48.1 μg/mg. The seed size was no different among wild type, *pCam5+PfKCS18*, and *pCam5+PfKCS18-atfae1*, but *pCam5* was reduced. In terms of plant phenotype, the *pCam5+PfKCS18-atfae1* line showed a similar leave phenotype with WT, but the *pCam5+PfKCS18* line showed a small and curling leave. Our results provide not only insights for future research uncovering mechanisms of HFA synthesis in seed but also metabolic engineering strategies for generating safe HFA-producing crops.

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High oleic and low saturated soybean development via multi-gene editing of *FAD2* and *FATB*

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CRISPR/Cas9 is a general technique to knock out a gene of interest. However, when targeting a multi-gene family or multiple genes, it is necessary to construct a multiple single guide RNAs (sgRNAs) vector to knock out. In this research, the newly modified Golden Gate cloning method was used to generate multiple sgRNAs-Cas9 vector. Using this vector, *Fatty Acid Desaturase 2 (FAD2*) and *Fatty Acyl-ACP Thioesterase B* (*FATB*) in soybean were targeted for a simultaneous knockout. In each generation, Next-Generation Sequencing for indel investigation and gas chromatography for fatty acid analysis was done. The T1 seed showed maximum 84% increased oleic acid (18:1) and 11% decreased saturated fatty acid (SFA; 16:0, 18:0). However in T2 seed, the general amount of fatty acid has decreased including WT due to vegetation environment but most of T2 plant's indels were fixed. The last harvested seed was T3 and it showed maximum 87% increased oleic acid and 7% decreased saturated fatty acid. By comparing various indel type, we found that *FAD2s/FATBs* edited lines has larger oleic acid content about 6~10% and smaller SFA amount about 3~5% than *FAD2* edited lines. We will compare more indel types and investigate the detailed contribution of each gene.

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Author Index

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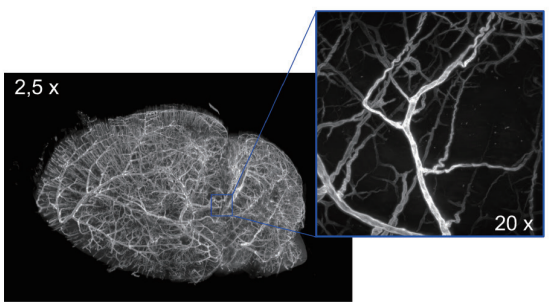
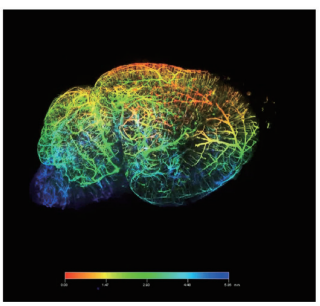
Light sheet fluorescence microscopy for Multiview imaging of living and cleared specimens.



Vasculature mapping of whole mouse brain, cleared using iDISCO+ protocol, equilibrated in ethyl cinnamate.
Image volume is 13.1 × 13.1 × 6 mm at a pixel resolution of 1.83 × 1.83 × 6.77 µm.
Acquired in 40 minutes in 4×4 tiles, 866 z-sections. Sample courtesy of E. Diel, D. Richardson. Harvard University



C57 BL6J mouse perfused with PBS, CellTracker™ CM-DiI Dye, and
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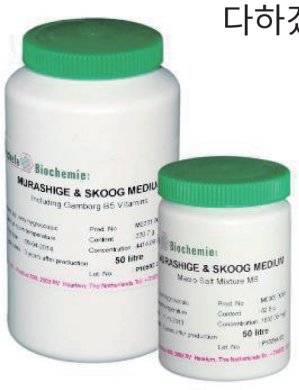

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파이젠은 생물정보 분석 전문회사로서 DNA 정보(Information)를 지식(Knowledge)으로 전환하여 유전체 정보의 새로운 가치를 창출하고자 합니다. 파이젠은 전문적인 생물 정보 분석 경험을 바탕으로 고객의 요구에 부합한 다양한 Omics 정보 통합 분석 서비스를 제공합니다.

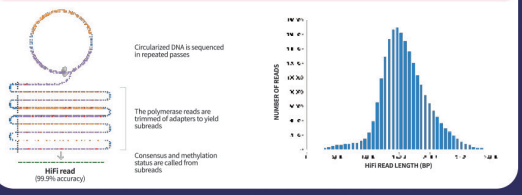
주요 분석 품목 | Long Read Sequencing / Genome / Transcriptome / Metagenome / Big data / Machine Learning

PacBio HiFi Sequencing

Revio System

+ 저렴한 가격으로 고품질 대용량 HiFi 데이터 생산 가능

Read Length	Read Accuracy	1 Cell Output	Sequel IIe System 대비
15 ~ 20 kb	Q31 (99.9%)	~ 90 Gb	>15X Performance ↑



+ HiFi 데이터 기반 High-Level 유전체 연구 가능

표준 유전체 완성 (Telomere to Telomere) | Pan-genome 분석

파이젠 창립 10주년 이벤트

고객님들의 성원에 감사드리며,
HiFi Sequencing + De Novo Assembly 를
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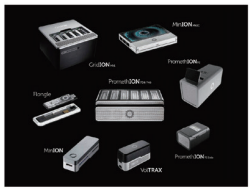


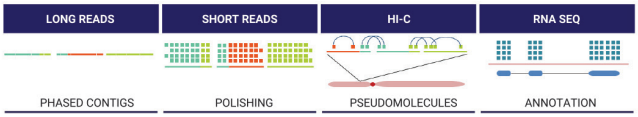
Library Type	Application
Ligation (DNA)	*De Novo* Assembly, Structural Variation
Direct RNA	Alternative Splicing, Gene Prediction
16S Barcoding	Metagenome with Full 16S Sequence

Applications of Long Read Sequencing

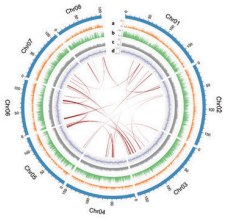
Microbiology | Environmental research | Microbiome | Basic genome research | Human genetics | Cancer research

Clinical research | Plant research | Transcriptome analysis | Population-scale genomics | Animal research

De Novo Genome Assembly



Pan-Genome / Comparative Genome Analysis



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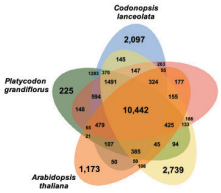

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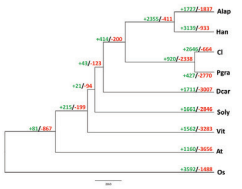

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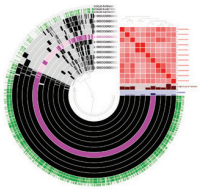
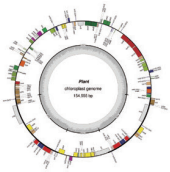


Pan-genome 분석

Organellar Genome Analysis



식물 엽록체 (100~200kb)

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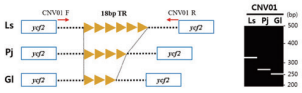

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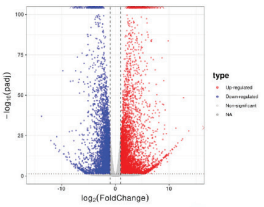
Transcriptome Analysis



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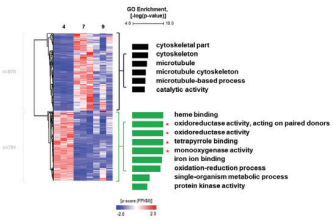

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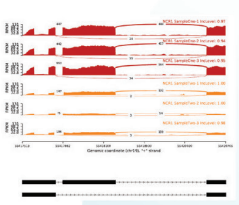

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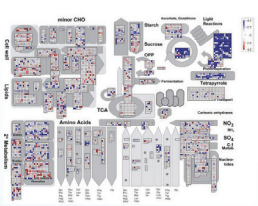

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