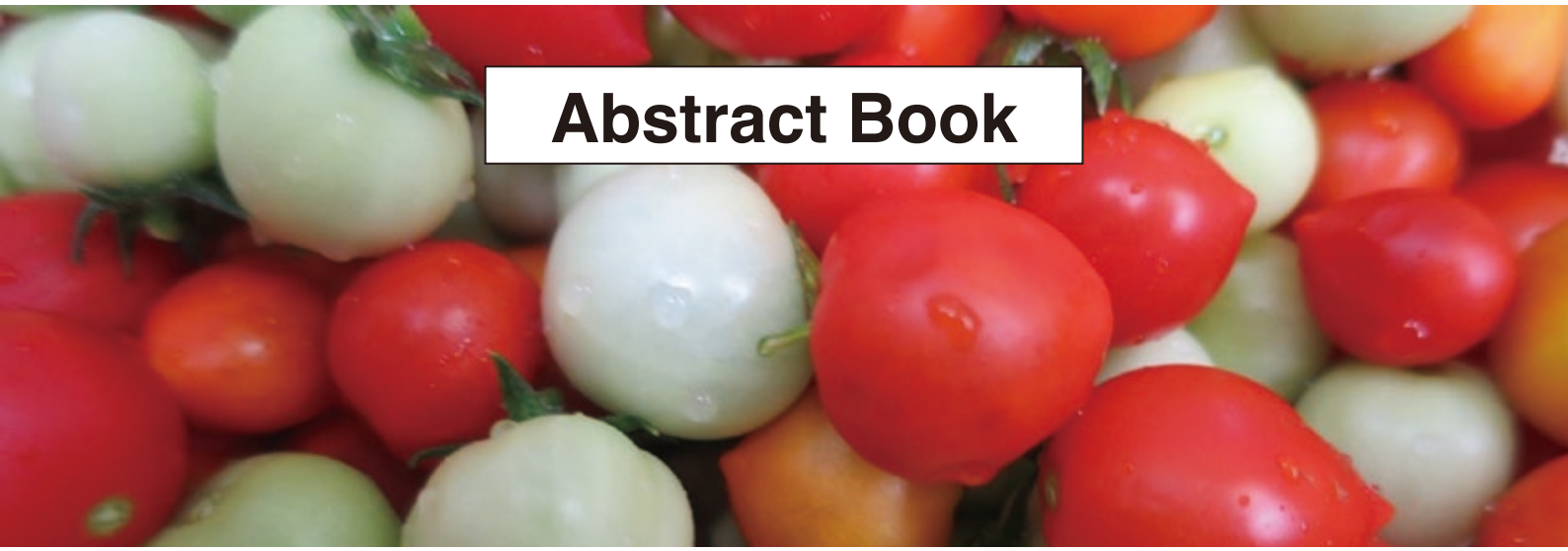
SOL 2024

XIX International Solanaceae Conference

November 25th – 28th, Tsukuba, Japan



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Day 1 (Nov 25th)

10:30- Registration Desk open

12:30-12:45 Opening Remarks
(Hiroshi Ezura)

Session I - Social Implementation of Research Achievements
(Chair: Hiroshi Ezura)

12:45-1:15 INVITED SPEAKER
Gregg A. Howe (Michigan State University, USA)
"Multi-layered mechanisms of insect resistance in tomato."

1:15-1:35 Soon Ju Park (Gyeongsang National University, South Korea)
"Advancing Crop Improvement through Polyploid Genome Engineering."

1:35-1:55 Marietheres Kleuter (Wageningen University and Research, The Netherlands)
"Beyond the fruits: tomato leaves as a future source of proteins."

1:55-2:10 Yoshihiro Okabe (NIPPN CORPORATION, Japan)
"Isolation of a new Pectate Lyase mutant allele and its application to processing tomato breeding for improving fruit shelf-life."

2:10-2:25 Short coffee break

2:25-2:40 Toon Suzuki (University of Tsukuba, Japan)
"Characterization of a genome-edited high-GABA tomato (Solanum lycopersicum) for fruit quality and yield under salt stress cultivation."

2:40-3:00 Hiroshi Ezura (University of Tsukuba, Japan)
"High GABA CRISPR-tomato: Journy from lab to table."

Session II – Biotechnologies
(Chair: Kristina Gruden & Aureliano Bombarely)

3:00-3:20 Jae-Yean Kim (Gyeongsang National University, South Korea)
"Precise Genome Editing: Innovations in Gene Targeting and Prime Editing in Tomato."

3:20-2:40 Hisashi Udagawa (Japan Tobacco INC.)
"Construction of a mutation database by whole genome sequencing for 2,019 ethyl methanesulfonate mutant lines developed from the Japanese tobacco cultivar 'Tsukuba 1'."

3:40-4:00 Kristina Gruden (NATIONAL INSTITUTE OF BIOLOGY, Slovenia)
"Stress Knowledge Map: A knowledge graph resource for improved understanding of plant responses to environment."

4:00-4:15 Short coffee break

Session III – Biodiversity and Evolution
(Chair: Aureliano Bombarely & Kristina Gruden)

4:15-4:55 INVITED SPEAKER
Pieter van't Hof (Universidad San Francisco de Quito, Ecuador)
"Exploring the rhizosphere microbiome of wild tomato species in their center of origin in the Andes and at the Galapagos Islands."

4:55-5:15 Laura Shannon (University of Minnesota, USA)
"How the potato genome was shaped by autotetraploidy: high diversity, many paralogs and low recombination."

5:15-5:35 Giovanni Giuliano (ENEA, Italy)
"Association analyses reveal both anthropic and environmental selective events during eggplant domestication."

5:35-5:55 Luiz Augusto Cauz-Santos (University of Vienna, Austria)
"Genomic insights into drought adaptation in the wild allopolyploid tobaccos (*Nicotiana* sect. *Suaveolentes*)."

5:55-6:15 Aureliano Bombarely (Institute for Plant Molecular and Cellular Biology (IBMCP), Spain)
"Understanding the evolution of the *Suaveolentes* polyploid clade through the study of the *Nicotiana africana* genome"

6:15- Poster Viewing I with Welcome reception

Open until 8:30 p.m.

Day 2 (Nov 26th)

Session IV - Solanaceae Research in Emerging Countries
(Chair: Seung Won Kang)

9:30-9:45 Seung Won Kang (University of Tsukuba, Japan)
"SOLANACEAE for sustainable production in Emerging Countries"

9:45-10:25 INVITED SPEAKER
Ya-Ping Lin (World Vegetable Center, Taiwan)
"The Strategies of Tropical Tomato Breeding in the World Vegetable Center."

10:25-10:45 Yellamaraju Sreelakshmi (University of Hyderabad, India)
"Introgression of a dominant *phototropin1* mutant enhances carotenoids and boosts flavour-related volatiles in genome-edited tomato *RIN* mutants."

10:45-11:00 Short coffee break

11:00-11:20 Pengxiang Fan (Zhejiang University, China)
"Recruitment of an Acyl-CoA Synthase into Branched-Chain Fatty Acid Biosynthesis Drives Metabolic Diversity of Tomato Defensive Acylsugars."

11:20-11:40 Rahul Kumar (University of Hyderabad, India)
"SlMIPS2, a myoinositol phosphate synthase gene, regulates phosphate homeostasis by influencing the SPX-PHR regulatory circuit in tomato seedlings."

11:40-1:30 LUNCH

Session V – Postharvest Biology and Cultivation Technology
(Chair: Mondher Bouzayen)

1:30-1:50 Jieun Seo (Seoul National University, Republic of Korea)
"Indole-3-acetic acid is a potential hormone involved in seed browning in pepper fruit under chilling temperatures."

1:50-2:10 Paula Teper-Bamnolker (Volcani Aro, Islael)
"Utilizing Vacuolar Invertase Mutants in Potato as a Platform for Advanced Genome Editing."

2:10-2:30 Michitaka Notaguchi (Kyoto University, Japan)
"Study on molecular mechanism of *Nicotiana benthamiana* interfamily grafting."

2:30-2:50 Group Photo

2:50-4:20 Poster Viewing II with coffee break

Session VI – Biotic and Abiotic Stresses
(Chair: Lukas Mueller & Mara Ercolano)

4:20-2:40 Dennis Psaroudakis (Forschungszentrum Jülich GmbH, Germany)
"Identification of core, conditional and crosstalk components of tomato heat stress response using integrative transcriptomics and orthology."

4:40-5:00 Leila Asadyar (Queensland University of Technology, Australia)
"Genetic Basis of Divergent Drought Strategies in *Nicotiana benthamiana* Ecotypes."

5:00-5:20 Dani Eshel (ARO, The Volcani Institute, Islael)
"Characterization of an ABA-Independent Drought Tolerance Pathway in Potato."

5:20-5:35 Short coffee break

5:35-5:55 Xin Yan (Seoul National University, Republic of Korea)
"The comparative genomics analysis of tobacco NLRs unveiled complex dynamics that underlie non-host resistance."

5:55-6:15 Hanna McCoy (University of New Brunswick, Canada)
"Bulking Up for Battle: Bulked Segregant Analysis of Colorado Potato Beetle Resistant *Solanum okadae*."

6:15-6:35 Francisco Vázquez Prol (IBMCP (UPV-CSIC), Spain)
"Exploring novel plant-to-plant intercommunication pathways in combined biotic and abiotic stresses"

Announcement from SGN and Breedbase

6:35-7:00 Lukas Mueller (Boyce Thompson Institute, USA)
"SGN update"

Day 3 (Nov 27th)

Session VII – Metabolism, Quality and Nutrition
(Chair: Yellamaraju Sreelakshmi & Giovanni Giuliano)

9:30-10:00 INVITED SPEAKER
Yoshiyuki Tanaka (Kyoto University, Japan)
"Research of vanillin aminotransferase (VAMT), a key enzyme in the capsaicin biosynthesis pathway in chili pepper."

10:00-10:20 Maria Ercolano (University of Naples Federico II, Italy)
"Integrative analysis of sensory attributes, VOC profile and genetic data for enhancing tomato quality."

10:20-10:40 Esteban Burbano Erazo (Instituto de Biología Molecular y Celular de Plantas, Spain)
"Specific geranylgeranyl diphosphate synthase and phytoene synthase combinations control the production of carotenoids and ABA in different tomato tissues."

10:40-11:00 Short coffee break

11:00-11:20 Jacopo Menconi (Sant'Anna School of Advanced Studies, Italy)
"Anthocyanin synthesis in tomato fruit is regulated by a multiprotein network composed of BBX and COP1 proteins converging on HY5."

11:20-11:40 Hiroki Ikeda (Utsunomiya University, Japan)
"Characterization of Tomato Introgression Line IL5-4: A Valuable Resource for High-Brix Breeding and Investigation of Blossom-End Rot Mechanisms."

11:40-12:00 Charles Goulet (Université Laval, Canada)
"Structural Variations in the Phytoene Synthase I Gene: Unraveling the Genetic Basis of Color Diversity in Tomato Fruits."

12:00- LUNCH pickup

Afternoon Excursion bus tour

Night Banquet at Hotel Nikko

Day 4 (Nov 28th)

Session VIII – Vegetative and Reproductive Growth & Development
(Chair: Esther van der Knaap & Zhengguo Li)

9:30-10:05 INVITED SPEAKER
Lazaro Peres (University of Sao Paulo, Brazil)
"Key Steps to Developing Insect-Resistant Tomatoes with natural and induced variations."

10:05-10:25 Esther van der Knaap (University of Georgia, USA)
"Common mechanisms controlling fruit shapes may be mediated by changes in cell wall properties."

10:25-10:45 Julien Pirrello (INP-Toulouse, France)
"ERF.E1, a hypoxia regulated genes triggering ripening."

10:45-11:00 Short coffee break

11:00-11:20 Alexander Goldshmidt (ARO Volcani, Islael)
"*SlKNOXII* and *SlBLH1-like* Genes Control the Coordination Between Fruit Pigmentation and Softening During Ripening."

11:20-11:40 Zhengguo Li (Chongqing University, China)
"GRAS transcription factors regulate fruit development and ripening in tomato."

11:40-12:00 Silvia Manrique (Polytechnic University of Valencia, Spain)
"Unraveling the molecular crosstalk between reproduction and stress in eggplant, a model for andromonoecious Solanaceae."

12:00-1:30 LUNCH

Session IX – Genomics, Genetics and Breeding
(Chair: Helen Tai & Byoung-Cheorl Kang)

1:30-1:50 Ido Nir (ARO - Volcani Institute, Islael)
"Tomato stomatal development: Diverse mechanisms of adaptive flexibility revealed by multi-species analysis."

1:50-2:10 Marcela Martinez-Sanchez (University of Auckland, New Zealand)
"SmuMYB113 is the determinant of fruit color in pepino (Solanum muricatum)."

2:10-2:30 Kenta Shirasawa (Kazusa DNA Research Institute, Japan)
"Genome and phenotype variations in a tomato (*Solanum lycopersicum*) cultivar Micro-Tom."

2:30-2:50 Zahra Zangishei (Heinrich Heine University Düsseldorf, Germany)
"Unraveling the Epipangenome of Solanum pennellii: Insights into the Genetic Variation and Abiotic Stress Responses for Crop Improvement."

2:50-3:10 Peter Waterhouse (Queensland University of Technology, Australia)
"The next step: building a unified Nicotiana benthamiana genomic resource."

3:10-3:30 Fumiya Kondo (Kyoto University, Japan)
"Availability of phenotypic simulation for fruit-related traits in crossing progenies of chili peppers (*Capsicum annuum*) using genomic prediction based solely on parental information."

3:30- Closing Remarks
(Hiroshi Ezura)

Poster Session

(Nov 25th Discussion core time; Odd number 6:30-7:00/Even number 7:00-7:30)

(Nov 26th Discussion core time; Even number 2:50-3:30/Odd number 3:30-4:10)

Poster No.	Presenter	Title
P1	Kouki Nakata	Comparative genomics of two wild tobacco species revealed their same tempo of diploidization
P2	Junya Sorita	Effects of low-concentration ozonated water treatment on the growth of grafted tomato seedlings
P3	Yong-Gen Yin	A Novel Approach to Managing Carbon Partitioning Based on the Visual Analysis of Carbon Flow and Vascular Bundle Networks in Tomato Plants
P4	Koichi Sugimoto	Large dataset of exome sequencing from EMS- and gamma ray-induced tomato mutant lines
P5	KAIXIAN CAI	Altered Photoassimilates Transport in Stem of Starch-Defect Tomato
P6	Karen Gi	Role of the blue light receptor SlFKF1 in tomato fruit coloring
P7	Rita Dublino	Identification of multi-stress response genes: decoding stress resistance in *Solanaceae* crops
P8	Shota Nagai	Fine mapping of *HLA1* locus causing hybrid lethality in interspecific hybrids of *Nicotiana* using bulked segregant RNA-Seq
P9	Ayaka Tabei	Physiological Study on Quantitative Traits Related to Fruit Size Using Introgression Lines of Tomato
P10	Akihito Morimoto	Identification and characterization of a novel leaky *VAMT* allele in chili pepper (*Capsicum chinense*)
P11	Jae-In Chun	Trichome development and terpene accumulation are controlled by the *Hairless-3* gene in tomatoes
P12	Xiaodong Xie	NtbHLH18 is a jasmonate-responsive transcription factor that regulates the biosynthesis of nicotine in tobacco
P13	Seungki Back	Genetic analysis of Three Loci Responsible for Capsaicinoid Biosynthesis Through an EMS-Induced Mutant in *Capsicum annuum* L.
P14	Joung-Ho Lee	Discovering Disease-Resistance Genes via Pan-Genome Analysis in Pepper
P15	Jung-Min Kim	Identification and Functional Validation of Novel Genes Involved in Capsaicinoid Biosynthesis in *Capsicum annuum* L through Transcriptome Analysis
P16	Seong-Min Kim	Identification of genes involved in multi-cellular trichome development in tomato

Poster No.	Presenter	Title
P17	Min-Seok Oh	Identification of Key Genes in the Jasmonate Signaling Pathway for Enhanced Insect Resistance in Tomato
P18	Jeongeun Kim	Molecular Mapping of *cmr2* for breeding CMV-P1 Resistant Pepper Cultivars
P19	Jiyoung Kim	Identification of a trichome development gene important for pest resistance in tomato
P20	Seong-Yeop Kim	The tomato *juhwang* mutant exhibits elevated levels of β-carotene and increased shelf-life
P21	Chen Yu Lin	Application of transcriptome sequencing to identify candidate genes associated with PVMV resistance of tomato.
P22	Sojiro Tsusaka	QTL analysis for capsaicinoid content in Aji pepper (*Capsicum baccatum*)
P23	Ga Hui Kang	Enhancing virus-mediated genome editing for cultivated tomato by low temperature
P24	Jun woo LEE	Genetic Study of Changes in Apical Dominance in Tomato Inflorescences
P25	Soo Jeong Yu	Reassessing the contribution of *TAGL1* and *SPL-CNR* to fruit ripening by CRISPR/Cas9 mutagenesis
P26	Lucas Munnes	The chromosome-scale *Solanum nigrum* genome sheds lights on its origins and on its drought response
P27	Ken-ichi Kurotani	Establishing an analysis platform for *Nicotiana benthamiana* genome and transcriptome
P28	Mingeng Li	Effect of Starch-deficiency on Fruit Metabolism in Tomato
P29	Lei Zhang	A QTL for fine tuning methyl salicylate level in tomato fruits
P30	Fabien Lombardo	Disruption of the *HWS* gene results in enlarged phloem in tomato pedicels, potentially enhancing sugar transport efficiency
P31	Yisen Shi	Single-cell transcriptomic analysis reveals the pivotal effects of protein kinase CPK28 on the hypocotyl development in tomato
P32	Geon Woo Kim	Prediction of Capsaicinoid Content in *Capsicum annuum* Hybrids Using Parental Genotypic Data
P33	Kyeong-seok Lee	Identification of Candidate Genes for Cold Tolerance in Pepper (*Capsicum annuum*)
P34	YUKI OI	Development of molecular markers related to GABA content and fruit weight from introgression lines of *Solanum pennellii*
P35	Keunhwa Kim	Polyploid System as a Tool for Testing the Breeding Potential of Gene Engineering

Poster No.	Presenter	Title
P36	Aijun Zhang	Horizontal transfer of extrachromosomal circular DNAs across grafting junctions in Solanaceae
P37	Citra Bakti	SATREPS Project: Developing a TILLING Platform using Ethyl Methane Sulfonate (EMS) to Induce Anthracnose Resistance in *Capsicum annuum* L. var. Tanjung 2
P38	Ai Nagamine	Genome editing of *DWARF* and *SELF-PRUNING* rapidly confers traits suitable for plant factories while retaining useful traits in tomato
P39	Xuewei Song	Interaction between BZR1 and histone deacetylase SRT1 promotes shoot branching in tomato
P40	Rui Deng	Brassinosteroids Receptor StBRI1 Promotes Tuber Development by Enhancing Plasma Membrane H+-ATPase Activity in Potato
P41	Tzahi Arazi	MADS gatekeepers: An ovule protein complex ensures fertilization-dependent fruit set in tomato
P42	Rika Nakajima	orf137 knockout can partially restore fertility in CMS potato
P43	DAOYUN CHEN	*ERECTA* Modulates Seed Germination and Fruit Development
P44	Yukako Nomura	Spatiotemporal control of jasmonate-mediated fruit set in tomato
P45	Takahiro Maki	A chili pepper mutant *tn-1* produces seedless fruits due to the mutation of *CaCKI1*
P46	Taira Ogawa	Characterization of a locule number mutant in chili pepper (*Capsicum annuum*)
P47	Kentaro Ezura	The Emerging Roles of SlKN5-SlBLH Regulatory Networks on Tomato Fruit Development and Coloration
P48	Nayoung Lee	Characteristics of Florigens and Florigen-Producing Cells under Natural Sunlight-Mimicking Conditions in Plants
P49	Blanca Salazar Sarasua	Parthenocarpy and domestication in tomato
P50	Hiroshi Magome	Reverse genetics using the tobacco mutant library: Carotenoid cleavage dioxygenase 4 mutants and Lycopene epsilon cyclase mutants and their characteristics
P51	Natalia Rodriguez Granados	Standing against floods: Towards the understanding of morphophysiological and molecular responses to waterlogging stress in potato
P52	Shoko Kokubo	The response of lipocalins to phytohormones in tomato

Poster No.	Presenter	Title
P53	Khazar Edrisi Maryan	Transcriptome analysis reveals abiotic stress boosts secondary metabolite production in Capsicum
P54	Mitsuki Aota	Effect of Starch Accumulation on Salt Stress Tolerance in Seedling Growth of Tomato
P55	Lia Ooi	Investigation of the Physiological Effects of Long-term High Temperature and High Humidity Conditions on *Solanum lycopersicum* cv. Micro-Tom
P56	Hermann PRODJINOTO	Physiological and Morphological Adaptations of Tomato Plants to Waterlogging and Oxygen Deficiency
P57	Seyedeh Sara Naseri Rad	Salt-priming induces salt tolerance in young tomato plants
P58	Jo-yi Yen	Characterization of Root System and Architectural Traits for Heat Tolerance in Tomato (Solanum lycopersicum L.) Lines
P59	Ranveer Pratap Singh	Understanding the physiological and genetic basis for Blossom-end rot
P60	Peijian Cao	Genome-wide identification of the N^6-methyladenosine regulatory genes reveals NtFIP37B increases drought resistance of tobacco (*Nicotiana tabacum* L.)
P61	Nozomu Kobayashi	Calcium Localization and Physiological Mechanisms Underlying Blossom-End Rot in Tomatoes and Their Application to Understanding Watercore in Japanese Pears
P62	Marie-Claire Goulet	Cysteine protease inhibitors as triggers of drought tolerance and tuber yield in potato
P63	Farida Damayanti	Principal Component and Cluster Analysis of Heat Tolerance Traits in 13 Tomato Varieties under Heat Stress Conditions in Indonesia
P64	Malgorzata Czernicka	Transcriptomic, proteomic, biochemical, and immunohistochemical analysis revealed the role of stress indicators, hormonal changes, and cell wall remodeling in response to hypoxia stress priming in roots of *Solanum lycopersicum* L.
P65	Zhangjian Hu	Phosphorylation and ubiquitination of SlERF.D2 are integral to SlCPK27-mediated drought tolerance in tomato
P66	jianrong lv	Impact of Carbonic Anhydrase βCA2.2 Phosphorylation Levels on Elevated CO2-Mediated Thermotolerance in Tomato
P67	Huanran Shi	The role of SlCRK2-SlC3H39 in regulation of cold resistance in tomato.
P68	Zelan Huang	miR164a targets NAM3 to enhance thermotolerance in tomato via regulation of HSFA4b-dependent redox homeostasis

Poster No.	Presenter	Title
P69	Ting Yang	NBR1a mediates root-knot nematode resistance by modulating antioxidant system, jasmonic acid and selective autophagy in Solanum lycopersicum
P70	Lijuan Zhu	Role of phospholipase A1 and its product lysophospholipids in tomato tolerance to cold stress
P71	Junesung Lee	Global co-expression network for key factor selection on environmental stress RNA-seq dataset in *Capsicum annuum*
P72	Byoung-Cheorl Kang	Discovering Disease-Resistance Genes via Pan-Genome Analysis in Pepper
P73	Guillermo Merino Martin	Unveiling the effector arsenal of Phytophthora capsici: a transcriptomics journey
P74	Luyang Wang	A *Glutathione S-Transferase* gene confers robust resistance to Fusarium crown and root rot in tomato
P75	Romanos Zois	Ty-genes effectiveness to ToLCNDV and different TYLCV strains
P76	Eom-Ji Oh	Dual functions of bacterial Chp family proteins in virulence and induction of hypersensitive response in *Solanaceae* plants
P77	Guoyun Xu	Metabolic engineering of a 1,8-cineole synthase enhances aphid and root-knot nematode repellence and in transgenic tobacco
P78	Masaaki Osaka	Identification and characterization of *Colletotrichum* species and *Alternaria alternata* associated with fruit rot on processing tomato in Japan.
P79	Yun-che Hsu	Characterization of resistance phenotypes of tomato lines carrying various Ty gene combinations upon exposure to different Tomato yellow leaf curl virus strains
P80	Pichaya T. Cheewapoonphon	PMR4 is a susceptibility gene for soft rot disease in potato
P81	Oscar Witere Mitalo	Screening of chili pepper genotypes with potential resistance to anthracnose caused by various *Colletotrichum* species
P82	Taewon Kim	Structure-guided identification of common host factor targeted by diverse pathogen effectors
P83	Fitri Widiantini	Variance virulence of Colletotrichum spp. isolated from various chili planting locations in Lembang, West Java, Indonesia
P84	Vagner Benedito	Dissecting the Genetic Basis of *Septoria* Leaf Spot (SLS) Resistance in Tomato: Insights from Wild Relatives
P85	Kee Hoon Sohn	Independently evolved NLR protein Rps-amr1 confers recognition of a bacterial protease effector AvrPphB in *Solanum americanum*

Poster No.	Presenter	Title
P86	Ji-Su Kwon	Optimized Bacterial mRNA Enrichment for Dual RNA-Seq to Explore Plant-Bacterial Interaction Dynamics
P87	Ségolène Bressoud	The plastoglobule associated NAD(P)H dehydrogenase C1 (NDC1) is essential for vitamin k1 accumulation and involved in prenyllipid metabolism in tomato (*Solanum lycopersicum*) leaves and fruit.
P88	Miho Ida	Isolation and characterization of linalyl-glycosyltransferases from coffee.
P89	Jianjing Wang	Reconstruction of capsaicin biosynthesis in Nicotiana benthamiana using a newly identified acyl-CoA synthetase
P90	Gianluca Gambacorta	Exploring the Role of Tryptamine and Serotonin in Tomato Reproductive Development
P91	Kyeonglim Min	Investigation of NAC Transcription Factors Involved in Ripening of Pepper (*Capsicum annuum* L.) Fruit
P92	Jiawei Li	The NAC transcription factor, Ripening Accelerator, regulates light stress response in tomato
P93	Yasuhiro Ito	Crossing a CRISPR/Cas9 transgenic tomato plant with a wild-type plant yields diverse mutations in the F1 progeny
P94	Hyeran Kim	Precise Pepper Improvement via RNA-Guided Endonucleases
P95	Seungje Choi	High sugar and high GABA accumulating Tomato cultivar creation by genome editing
P96	Misaki Kobayashi	Development of *in planta* genome editing by transient expression of genome-editing enzymes
P97	Guan Ling Chen	Evaluation of the potential of the VIGE system on petunia.
P98	ShengWen Wang	Evaluation of TSWV-based VIGE system on different pepper lines
P99	Ping-Yen Chiu	Evaluation of the potential of the VIGE system on different tomato lines.
P100	Chien-Hung Chung & Hsin-Mei Ku	The development of virus-induced gene editing system for solanaceae crops.
P101	Dmytro Yevtushenko	Exploring Potato as a Bioreactor for High-Level Accumulation of Bovine Myeloid Antimicrobial Peptide-18 in Tubers.
P102	Marc Simanowitz	Enhancing Xanthophyll Accumulation in Tomato Fruits: Strategies for Improving Nutritional Value and Human Health Benefits
P103	JUI CHIEH LIU	Evaluation the effect of varieties and agrobacterium concentrations on tomato virus-induced gene silencing

Poster No.	Presenter	Title
P104	Ryoichi Yano	CoreNet+, a web application system for trans-omics data mining
P105	Nono Carsono	Optimization of Callus Induction in Six Genotypes of Tomato (*Solanum Lycopersicum*)
P106	Shujia Li	Heat-responsive optimization of carbon partitioning by prime editing enhances fruit yields in tomato
P107	Perla N. de Oliveira	SAMBA, a plant-specific APC/C regulator, is involved in development and fruit metabolites in tomato

Oral Presentations

Day 1 (Nov 25th)

Session I

O-I-1:
Multi-layered mechanisms of insect resistance in tomato

Gregg A. Howe[1,2,3]
[1]Plant Research Laboratory, Michigan State University, USA
[2]Plant Resilience Institute, Michigan State University, USA
[3]Department of Biochemistry and Molecular Biology, Michigan State University, USA

Insect herbivores and their host plants are engaged in a co-evolutionary battle to eat or not be eaten. Interactions between these two groups of organisms—which comprise the most species-rich multicellular lineages on earth—have dramatically shaped organismic diversity in terrestrial ecosystems. Cultivated and wild tomato species comprise an excellent experimental system in which to elucidate fundamental mechanisms of plant defense against insect herbivory. Here, I will highlight our laboratory's progress in understanding constitutive and inducible mechanisms of anti-insect defense in tomato, with emphasis on the role of jasmonate in regulating multiple layers of protection. These findings are now being extended to better understand how warming climate conditions affect crop resilience to insect attack.

O-I-2:
Advancing Crop Improvement through Polyploid Genome Engineering

Eunsong Lee[1], Keunhwa Kim[1], Soon Ju Park[1]
[1]Division of Applied Life Science (BK21 four) and Plant Molecular Biology and Biotechnology Research Center (PMBBRC), Gyeongsang National University, Jinju 52828, Republic of Korea

Recent advances in genome sequencing and editing have enabled polyploid genome engineering, opening new possibilities for genetically modifying complex staple crops. We focused on the hexaploid black nightshade species *Solanum nigrum*, assembling its genome and identifying homoeologous gene sets through comparative analyses with diploid relatives. Using CRISPR-Cas9-mediated mutagenesis, we generated various mutation combinations in homoeologous genes. These mutant genotypes exhibited quantitative phenotypic changes, resulting in a broad-spectrum effect on the quantitative traits of hexaploid *S. nigrum*. Our new system allowed us to explore intermediate inflorescence and floral phenotypes, providing a method to uncover useful quantitative traits. We then applied similar genetic engineering approaches to tomato (*S. lycopersicum*), a diploid crop. By editing the *AN* promoter and crossing these edited genotypes with *an*/+ heterozygotes, we produced hybrids with an expanded range of quantitative traits. This demonstrates that insights from our polyploid engineering systems can be translated to improve agricultural traits in diploid crops, offering a promising approach for crop improvement through single-gene modifications and strategic genetic combinations

O-I-3:
Beyond the fruits: tomato leaves as a future source of proteins

Marietheres Kleuter[1], Yafei Yu[2], Francesco Pancaldi[1], Atze Jan van der Goot[2], Luisa M. Trindade[1]
[1] Plant Breeding, Wageningen University, The Netherlands
[2] Laboratory of Food Process Engineering, Wageningen University, The Netherlands

Research on Solanaceae crops, like tomato, paprika, and eggplant, has set the spotlight on cultivating large, healthy fruits, while the leaves and stems are not further utilized. Yet, the necessary shift towards a circular bioeconomy urges us to rethink this paradigm. Tomato leaves, so far discarded as agricultural waste, have great potential to function as a source of valuable nutrients, such as proteins. In my talk I will unravel promising insights of tomato leaves as a protein source, essential for transitioning towards more plant protein-based diets. In addition, I will share the main bottlenecks and obstacles of this concept. Based on the insights and tools that we developed, this concept is brought a step closer to its application.

Our initial findings revealed that proteins can be extracted from tomato leaves, however, the extraction efficiency is closely tied to leaf age. While young, vegetative leaves from the top have a protein extraction yield of above 50%, lower old leaves around mature fruits combat a yield of less than 3 % (Kleuter et al. 2024; Yu et al. 2023). This led to two central research questions: 1) Which biochemical factors restrict protein extraction yield and 2) where should future breeding efforts focus to maximize these yields?
To address this, we conducted comprehensive biochemical studies investigating the protein extraction yield, protein content and its composition, as well as the cell wall composition across the developmental stages of the tomato plant. To understand the underlying genetics for the aforementioned traits, the research was complemented by transcriptomic data.
We observed that the remarkable decline in protein extraction yield along plant development is not due to a change in total nitrogen content but rather a significant decrease in the protein-to-peptide ratio. We hypothesized that this decline is driven by the onset of leaf senescence, a degenerative process characterized by the degradation and mobilization of cellular components, including proteins. The hypothesis was supported by an increased expression of members of the aspartic, cysteine and serine proteases pointing to extraplastidic protein degradation as the key cause (Kleuter et al. under review). In addition, the biochemical analysis of the cell wall revealed meaningful correlations: a strong negative correlation between protein extraction yield and the contents of arabinose and galacturonic acid, and a positive correlation with galactose content (Kleuter et al. 2024). These findings highlighted the critical role of the pectin network in protein extractability.
As a result, the extracellular protein degradation and the pectin network are stated as biochemical factors that restrict protein extraction and could thus serve as potential breeding targets to boost protein extraction yields from tomato leaves. By following up on these insights, tomato leaves can be transformed from agricultural waste to a valuable protein source. We envision a future with ‘whole biomass crop tomatoes’, where both fruits and leaves are part of a sustainable food supply.

Acknowledgements
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Moreover, I thank my former students Mayra Nagtzaam, Frank Riga, and Lukas Verdegaal for their committed help in various parts of this project.

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O-I-4:

Isolation of a new Pectate Lyase mutant allele and its application to processing tomato breeding for improving fruit shelf-life

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Improving fruit firmness and shelf-life is one of the main targets in breeding programs of processing tomato. It leads to increased yield in the field and manufacturing process and also allows us to harvest efficiently and reduce the labor. The present study was aimed to breed new tomato varieties harboring the traits for extended fruit shelf-life and increased fruit firmness by conventional mutation breeding method. To explore new breeding materials, we focused on Pectate Lyase gene (Solyc03g111690) and screened the mutant alleles from Micro-Tom EMS-mutagenized populations. Six mutant alleles including a nonsense mutation were found by TILLING. Among these, the null mutation allele (*Slpl-1*) was used for further analysis as a candidate allele of breeding program. As expected, *Slpl-1* homozygous line with Micro-Tom genetic background showed extended fruit shelf-life and increased fruit firmness.

Next, we introgressed the *pl-1* mutant allele into our breeding lines (BLs), and at least five times backcrossing was performed to remove undesired mutations. These breeding lines (BC5F2, BC5F3) were used for further evaluation of the field-trial to estimate fruit yield and the percentage of fruit decay. Compared to the control lines, the percentage of standard fruit for the yield was increased at least 15% in the *Slpl-1*-BLs. The number of fruits was increased approximately 10%, whereas the fruit weight per fruit was decreased 4 to 15% in *Slpl-1*-BLs, compared to the control. Regarding the factor related to fruit shelf-life, *Slpl-1*-BLs showed decreased rate of cracking and fruit decay, up to approximately 20% and 5%, respectively.

Taken together, these results indicated that *Slpl-1* would be a useful breeding material for improving fruit shelf-life and fruit firmness by conventional breeding.

Acknowledgements
Tomato seeds of Micro-Tom mutants were provided by University of Tsukuba, Tsukuba Plant Innovation Research Center, through the National Bio-Resource Project (NBRP) of the MEXT/AMED, Japan.

O-I-5:
Characterization of a genome-edited high-GABA tomato (Solanum lycopersicum) for fruit quality and yield under salt stress cultivation

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Gamma-aminobutyric acid (GABA) is mainly synthesized by glutamate decarboxylase (GAD). Plant GAD has an autoinhibitory domain at its C-terminus, which undergoes conformational changes when plants are exposed to environmental stresses, resulting in increased enzyme activity and increased intracellular GABA accumulation. We previously generated a high-GABA tomato variety in which the autoinhibitory domain of *SlGAD3*, a key enzyme for GABA biosynthesis in tomato fruit, was excised using CRISPR-Cas9 technology. In this study, we investigated the salt stress response of the high-GABA tomato variety. We conducted a comparative experiment with the original and high-GABA tomato varieties under normal, salt stress conditions, investigated the fruit quality, yield, and contents of GABA, glutamic acid, sugar, and carotenoids, etc. GABA was further increased in the high-GABA tomato variety under salt stress conditions. In addition, the high-GABA tomato showed a similar increase in sugar content under salt stress conditions as the original variety, whereas fruit yield and fruit weight were higher in the high-GABA tomato than in the original variety. In follow-up experiments, similar trends were observed in other varieties where the same trait was introduced. These results suggest that the high-GABA tomato has better cultivation performance under salt stress conditions compared to the original variety. These results indicate that fruit-specific GABA accumulation may have a stress-reducing effect. It is also found that tomatoes with high fruit-specific GABA accumulation can be useful in salt-affected areas and in the production of high-sugar tomatoes.

Acknowledgements

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O-I-6:
High GABA CRISPR-tomato: Journy from lab to table

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CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats / CRISPR associated proteins 9) technology was awarded the Nobel Prize in 2020, eight years after its initial publication, which represents unprecedented speed. Genome editing (GE) is featured as a technology for rapid plant breeding, and CRISPR/Cas9 technology is attracting attention due to its convenience and editing efficiency. Because of these characteristics, this technology has been actively used in the research and development of Solanaceae crops. On December 11, 2020, notification was given to the Japanese government of the first GE tomato containing a high level of GABA (ɣ-aminobutyric acid), which is well known for its human health-promoting benefits such as lowering blood pressure, reducing a stress, improving sleep quality and so on, developed using this technology. By May 12, 2021, a start-up company launched from University of Tsukuba, Sanatech Seed Co. Ltd. (Currently named Sanatech Life Science Co. Ltd.), had begun providing seedlings to more than 4,000 home gardeners free of charge to monitor the cultivation. Subsequently, on September 15, 2021, online sales of the GE fresh tomato fruits began. This was the first agricultural product in the world to be launched using CRISPR/Cas9 technology. On March, 2023, the GE tomato fruits become available in supermarket stores in Japan. Here, I would like to share the background into how this new development occurred, and how it has been accepted by Japanese consumers as a case study of social implementation of GE Solanaceae crops.

Oral Presentations

Day 1 (Nov 25th)

Session II

O-II-1:
Precise Genome Editing: Innovations in Gene Targeting and Prime Editing in Tomato

Tien Van Vu[1], Ngan Thi Nguyen[1], Minh Huy Vu[1], Jihae Kim[1], Song Young Jong[1], Thu Hoai Nguyen[1], Yeon Woo Sung[1], Jae-Yean Kim[1,2,3,*]

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The quest for precision in plant genome editing has led to advancements in Homology-Directed Repair (HDR) and Prime Editing (PE) technologies, addressing limitations in plant genetic engineering. This talk synthesizes findings from four research efforts, enhancing genome editing efficiency and precision in dicotyledonous plants, especially tomatoes. The first part examines three approaches to improve HDR-based editing in tomatoes: using a transient CRISPR/Cas12a-geminiviral replicon system, modifying the LbCas12a-crRNA system with chemical inhibitors, and redirecting DNA repair pathways with a dominant-negative KU80 mutant (4,5). These methods report up to a 9.84-fold increase in gene targeting efficiency without antibiotic markers, emphasizing the optimization of culture conditions and molecular interventions. The second part highlights a breakthrough in Prime Editing, achieving heritable and precise edits in tomatoes with up to 38.2% efficiency (1-3). Novel combinations of PE components demonstrate its versatility and high specificity. The capability of HDR to repair long DNA targets and the flexibility of PE to introduce various edits without double-stranded breaks mark significant advancements in plant genome editing. These developments open new avenues for precision agriculture and lay the groundwork for addressing complex genetic challenges in plant science and breeding, promising sustainable and efficient crop improvement.

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O-II-2:

Construction of a mutation database by whole genome sequencing for 2,019 ethyl methanesulfonate mutant lines developed from the Japanese tobacco cultivar 'Tsukuba 1'

Authors: Hisashi Udagawa, Takanori Takeuchi, Hiroshi Magome, Yoshimitsu Takakura
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Tobacco (*Nicotiana tabacum* L.), an allotetraploid species of the Solanaceae family, is cultivated in more than 100 countries. It has a large genome (approximately 4.5 Gb) that evolved by interspecific hybridization of ancestral *N. sylvestris* and *N. tomentosiformis*. 'Tsukuba 1' is a Japanese tobacco cultivar with multiple disease resistance that was developed using doubled haploid technology. Previously, we generated an ethyl methanesulfonate (EMS) mutant library using 'Tsukuba 1' to study gene function and to increase beneficial alleles for plant breeding. Generally, screening to find mutant lines with desirable mutations is a time-consuming and laborious process. If genome-wide mutations in each line have already been catalogued in a library, mutations can be detected without the screening step, thus considerably accelerating reverse genetics. Here, we report the whole genome sequencing of 2,019 tobacco EMS mutant lines and the construction of a mutation database. For accurate mutation detection, we first performed *de novo* assembly of the 'Tsukuba 1' genome and predicted the genes. The assembly resulted in 804 scaffolds with N_{50} of 24.7 Mb and total length of 4.06 Gb. Gene prediction using RNA sequencing data from 14 kinds of organ samples identified 65,601 genes, which is comparable to the number of genes predicted in previously reported tobacco genomes. Then, in each of the 2,019 EMS mutant lines, bulked DNA was extracted from leaf samples of eight individuals of the M_2 generation and subjected to 2 × 150 bp whole genome sequencing, which yielded a minimum of 109 Gb (26.8-fold coverage of the 'Tsukuba 1' genome). After detecting mutations in each line, all the detected mutations were catalogued in a database. We found that over 90% of the genes had at least one nonsense mutation. This high degree of saturation of nonsense mutations can be explained by redundancy in gene function. There are two functional copies of most tobacco genes, and therefore the effects of loss-of-function mutations in a single gene are frequently masked by the gene redundancy, allowing deleterious mutations to be retained. Our EMS mutant population and mutation database, which can easily provide loss-of-function alleles for most of the genes *in silico*, are powerful tools for gene function analysis as well as for tobacco molecular breeding.

O-II-3:
Stress Knowledge Map: A knowledge graph resource for improved understanding of plant responses to environment

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Stress Knowledge Map (SKM, https://skm.nib.si) is a publicly available resource containing two complementary knowledge graphs describing current knowledge of molecular interactions in plants: a highly curated model of plant stress signalling (PSS, 543 reactions) and a large comprehensive knowledge network of molecular interactions (CKN, 488,390 interactions). Both were constructed by domain experts through the systematic curation of diverse literature and database resources. SKM provides a single entry point for plant stress response investigations and the related growth trade-offs, through interactive exploration of current knowledge, as well as integration of experimental omics data. PSS also provides qualitative and quantitative models for systems biology, and thus represents a starting point of a plant digital twin. The features of SKM will be presented through case studies, to show how SKM resource can be used for systematic hypothesis generation, design of validation experiments, and to gain new insights into experimental observations in plant biology.

Oral Presentations

Day 1 (Nov 25th)

Session III

O-III-1:
Exploring the rhizosphere microbiome of wild tomato species in their center of origin in the Andes and at the Galapagos Islands.

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In the past decade, it has become evident that the plant microbiome is a key driver of nutrient acquisition, plant growth and tolerance to biotic and abiotic stresses. To date, the assembly of the tomato rhizosphere microbiome has been mostly studied in cultivated varieties and soils subjected to agricultural management practices. However, the microbiome of wild tomato genotypes grown naturally in their native habitats remains largely unexplored. To study the functional potential of the wild tomato rhizosphere microbiome, we sampled 34 populations of modern tomatoes' closest relative *Solanum pimpinellifolium* (*PIMPI*) in three natural habitats in the Andean region of Southern Ecuador, to identify the taxonomic and functional diversity of the wild tomato rhizosphere microbiome in its center of origin. The results showed that, despite genotypic differences between the wild tomato populations, different soil types and soil microbiome compositions, the natural rhizosphere microbiome of the wild tomato plants was strikingly similar in composition across populations. Proteobacteria, in particular taxa classified as Enterobacteriaceae, as well as specific unclassified fungal taxa were highly represented in the rhizosphere of wild PIMPI populations. Metagenomic analyses further suggested that the prevalence of Enterobacteriaceae on wild tomato roots may be explained by several traits. To investigate the impact of the functional soil microbiome and its ability to promote insect tolerance, we sampled native and agricultural soils from this center of origin and performed a controlled greenhouse bioassay to determine the effect of the soil microbiome on tomato leaf tissue damage caused by the invasive sap-sucking insect *Prodiplosis longifila* (Diptera: Cecidomyiidae). We observed no significant differences in insect damage on wild tomato *PIMPI* and cultivated *S. lycopersicum (LYCO)* cv. Moneymaker on either native or agricultural soils. However, when we autoclaved these native and agricultural soils, the leaves of the wild tomato showed significantly higher damage levels compared to leaves of wild tomato grown in both non-sterilized soils. Collectively these results show on the one hand a conserved signature of specific members of the rhizosphere microbiome recruited by wild *PIMPI* populations grown under native conditions in the center of origin soils. On the other hand, it reveals that wild *PIMPI*, in contrast to the cultivated *LYCO* variety Moneymaker, relies on rhizosphere-mediated responses belowground to boost the protection against *P. longifila* attack aboveground. Where wild *PIMPI* populations grow under semi-arid conditions in the Andes, the endemic Galapagos tomato species *S. galapagense* (*GAL*) and *S. cheesmaniae* (*CHS*) have evolved to survive under harsh environmental circumstances in arid, saline, and/or volcanic soils. Although the genetic background responsible for the high tolerance to these stresses has been extensively studied, surprisingly little is known on the taxonomic and functional microbial diversity associated with the endemic Galapagos tomatoes. We explored the microbiome composition of both endemic GAL and CHS species, the cultivated *LYCO* varieties and the invasive native PIMPI as collected on the inhabited islands Isabela, Santa Cruz, Floreana, and San Cristóbal using 16S and ITS metabarcoding. Our study reveals for the first time insights into how the Galápagos

tomatoes recruit microorganisms in their rhizosphere of endemic and introduced tomato species at the Galápagos archipielago and we believe these interactions to be probably linked to the tomato survival to such harsh environmental conditions as can be found at the Galapagos Islands.

Acknowledgements:

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O-III-2:
How the potato genome was shaped by autotetraploidy: high diversity, many paralogs and low recombination

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Diversity panels provide the opportunity to better understand the potato genome. The Potato 2.0 project has developed a panel of 97 dihaploids extracted from commercial tetraploid germplasm and advanced breeding lines, sequenced with ~20X coverage using Illumina short reads. These lines are characterized by high diversity. Our initial results revealed only 12 fixed genes, but 34 genes with 194 alleles, and an average of 27.9 alleles per locus. However, further investigation suggested that much of this diversity stemmed from paralogous sequences rather than true allelic variation. When a more conservative single copy gene set based on synteny analysis was used, per gene haplotype number ranged from 1 to 71 with an average of 12 alleles per locus. However, this conservative data set was limited to the 1,123 genes that are present in single copy in all sequenced potato, which is a small subsample of the genome. In general structural variants are rampant in the potato genome. We found over 92,000 distinct insertions and deletions among the dihaploids. Regardless of data set used, heterozygosity was high, ranging from 0.46% to 0.69% in the original data set and 16.36% to 26.43% in the conservative set. In general we would expect high diversity to translate to high population level recombination measures. However, we found an average genome wide rho (recombination rate per generation times effective population size) of 1.43/1 Kb. This is lower than estimates for wheat, sorghum, barley, and strawberries. We hypothesize that relaxed selection due to autopolyploidy allows for the accumulation of SNPs and paralogs. This is consistent with our observations from genebank data of higher observed and expected heterozygosity in tetraploid potatoes as compared to diploid landraces even when correcting for ploidy in the analysis. Conversely, we hypothesize that recombination in tetraploid potato is reduced as compared to diploid potato in order to stabilize meiosis as observed in synthetic polyploid Arabidopsis, and that reduction in combination with its clonal nature has resulted in low population size adjusted recombination rates in potato. In addition to providing insight into the tetraploid potato genome the findings from this dihaploid diversity panel inform diploid potato breeding starting with dihaploid base populations.

Acknowledgements
This work is part of the Potato 2.0 project and the panel described in this abstract was the work of a large number of scientists including: Dr. Robin Buell, Dr. Jeffrey Endelman, Dr. David Douches, Dr. Ek Han Tan, Dr. Josh Parsons, Dr. Sagar Sathuvalli, Dr. Shelley Jansky, and Dr. Paul Bethke. The project was funded by the Potato 2.0, SCRI from the USDA-NIFA 2019-51181-30021.

O-III-3:
Association analyses reveal both anthropic and environmental selective events during eggplant domestication

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Eggplant (*Solanum melongena*) is one of the four most important Solanaceous crops, widely cultivated and consumed in Asia, the Mediterranean basin, and Southeast Europe. We studied the Genome-wide association (GWA) of historical genebank phenotypic data on a genotyped worldwide collection of 3,449 eggplant accessions. A total of 334 significant associations for key agronomic traits were detected using three genome-wide association methods. Significant correlations were obtained between different types of phenotypic data, some of which were not obvious, such as between fruit size/yield and fruit color components, suggesting simultaneous anthropic selection for genetically unrelated traits. Anthropic selection of traits like leaf prickles, fruit color, and yield, acted on distinct genomic regions in the two domestication centers (South and South-East Asia), further confirming the multiple domestication of eggplant. To discriminate anthropic from environmental selection in domestication centers, we conducted a Genotype-Environment Association (GEA) on a subset of georeferenced accessions from the Indian subcontinent. The population structure in this area revealed four genetic clusters, corresponding to a latitudinal gradient, and environmental factors explained 31% of the population structure when the effect of spatial distances was removed. GEA and outlier association (OA) identified 305 candidate regions under environmental selection, containing genes for abiotic stress responses, plant development, and flowering transition. Finally, in the Indian domestication center anthropic and environmental selection acted largely independently, and on different genomic regions. These data allow a better understanding of the different effects of environmental and anthropic selection during domestication of a crop, and the different world regions where some traits were initially selected by humans.

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O-III-4:
Genomic insights into drought adaptation in the wild allopolyploid tobaccos (*Nicotiana* sect. *Suaveolentes*)

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The structure and function of plant genomes have been marked by a history of repeated cycles of whole genome duplications followed by diploidization. Despite the number of duplications in their ancestry, most extant plants, particularly herbaceous groups, exhibit low chromosome numbers, but the drivers of this descending dysploidy remain little understood. This is the case of *Nicotiana* sect. *Suaveolentes*, a group of wild tobaccos with variable ecologies, including adaptation to deserts, and series of chromosome reductions. Here, we used genomic and transcriptomic data to investigate the evolution of these native tobaccos, which putatively entered the arid Australian Eremaean Zone (EZ) 5 Mya. The original migrants from South America were adapted to mesic areas of Australia and putatively radiated recently in the EZ, including sandy dune fields, after developing drought adaptations. Based on coalescent analyses designed to corroborate timing of the Australian radiation independently, arrival of *Nicotiana* on the continent occurred approximately 6 Mya, and ancestors of the Pilbara (Western Australian) lineages radiated there at the onset of extreme aridity 5 Mya by locally adapting to these various ancient, highly stable habitats. Furthermore, our results indicate that while many populations within the *N.* section *Suaveolentes* show extensive current migration, suggesting genetic interconnectivity, migration rates are very low as a result of high inbreeding. Particularly looking for drought adaptation, we identified genes responding to drought using two species pairs including a species with higher chromosome number that prefers mesic habitats, and a related species with lower chromosome number that is adapted to extreme arid condition. The transcriptomic responses varied significantly between sister species pairs, highlighting different strategies in gene regulation under drought stress, particularly in oxidative stress and phytohormone signaling pathways. This study reinforces the importance of understanding both historical demographic events and current genetic structures to fully evaluate the adaptive capacities of arid-adapted species. Our results provided insights on the contributions of post-WGD processes to evolutionary success and phenotypic novelty, providing new information on how genomic reorganization contributes to adaptation, speciation and adaptive radiation.

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O-III-5:

Understanding the evolution of the *Suaveolentes* polyploid clade through the study of the *Nicotiana africana* genome

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The genetic mechanisms behind the species diversification processes are poorly understood. How changes in the genome drive to phenotypic diversity and adaptation is one of the main questions in evolutionary genomics. *Nicotiana* is an herbaceous plant genus of the Solanaceae family with more than 90 naturally occurring species divided into 13 sections. Five sections contain allopolyploids formed by interspecific hybridization between diploids. The largest section is Suaveolentes with more than 40 species, such as the well-studied *Nicotiana benthamiana*. The origin of this paleopolyploid clade is dated 6 MYA. Suaveolentes is an excellent example of rapid diversification compared with other *Nicotiana* polyploid clades. Our previous analysis revealed an explosion of LTR/Copia elements at the time of the diversification of the clade, shaping the gene space of the most recent *Suaveolentes* species. *N. africana* is the oldest extant *Suaveolentes* species and the only native African *Nicotiana* species. N. africana diverged before the LTR/Copia element explosion that we have detected in other Suaveolentes species. The annotation of the *N. africana* genome identified 61,808 gene models compared with the 58,012 gene models identified in the *N. benthamiana* genome, showing a more advanced process of diploidization in *N. benthamiana* driven by the action of the LTR/Copia elements.

Keywords: Diversification, Genome Evolution, Nicotiana, Polyploidy

Oral Presentations

Day 2 (Nov 26th)

Session IV

O-IV-1:
SOLANACEAE for sustainable production in Emerging Countries

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Emerging country is defined as an economy outside of the world's largest industrial nations that is experiencing rapid growth, meanwhile emerging markets are considered a country with a fast-growing economy that may have some characteristics of a developed country, such as a high GDP or widespread industrialization. But there is no precise definition between the two terms, but emerging markets are commonly and widely used to describe countries with rapid economic growth. Emerging countries include BRICS (Brazil, Russia, India, and China), CIVETS (Colombia, Indonesia, Vietnam, Egypt, Turkey, and South Africa) and other countries (Chile, Czech Republic, Hungary, Malaysia, Mexico, Poland, Saudi Arabia, Thailand, and Peru). Interestingly, many emerging counties are ranked in the top 10 counties producing tomatoes (China, India, Turkey, Egypt, Mexico, Brazil, Nigeria) and chili pepper (China, Mexico, Indonesia, Turkey, India, Nigeria, Egypt, Bangladesh) (FAO https://www.fao.org/faostat/en/#data/QCL). Not all but many of emerging countries are in the Tropical region where about 32% of world population (3 billion people) live and it is estimated that 67% children will live in this region by 2050. Indonesia is located in the Southeastern Asia of Tropical region and one of the representative ASEAN country with large population of about 280 million (the top 4th in the world) and had showed rapid economic growth with average 5% every year except 2020 and 2021 due to COVID-19. Tomato and chili pepper are the most important fruit vegetables in Indonesia and their demand has been rapidly increasing accompanied by huge economic growth. Since 2023, therefore, we have started five years of research project with Universitas Padjadjaran in Indonesia entitled "Breeding Innovation in Chili Pepper and Tomato to Accelerate Sustainable Vegetable Production in Tropical Regions" (https://www.jst.go.jp/global/english/kadai/r0407_indonesia.html). This is a five-year project named SATREPS (Science and Technology Research Partnership for Sustainable Development). As one aspect of "science and technology diplomacy", the Objective of this program is to promote international joint research not only to resolve global issues but also to connect science and technology with diplomacy for the mutual advancement between Japan and counterpart country. To reach out our activities to Tropical regions including our team, we organized this special session by virtue of SOL2024 conference committee and invited researchers from emerging countries such as Brazil, China, India, and Taiwan. This session goes with one invited lecture by Dr. Lin from World Vegetable Center in Taiwan and four oral presentations.

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O-IV-2:
The Strategies of Tropical Tomato Breeding in the World Vegetable Center

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Tomato production in tropical regions faces significant challenges, primarily due to heat and disease stresses. Heat stress negatively affects critical reproductive processes by reducing pollen quantity, pollen viability, flower production, and fruit set, concluding in substantial yield losses. Similarly, diseases such as tomato yellow leaf curl virus and bacterial wilt cause severe damage, potentially leading to complete yield failure. The majority of cultivated tomato varieties lack inherent resilience to heat and disease, making wild species essential sources of genetic resistance. However, the utilization of wild tomatoes in breeding programs is complicated by the need to restore desirable horticultural traits, such as fruit size, which requires considerable effort.

For several decades, the World Vegetable Center (WorldVeg) has prioritized the enhancement of heat and disease tolerance in tomatoes. This objective has been pursued through the strategic crossing of heat-tolerant lines with those exhibiting disease resistance. The progeny of these crosses is advanced through successive generations and subjected to rigorous field evaluations during the spring seasons in Tainan, providing critical insights into their performance under tropical conditions. A comprehensive analysis of the genetic profiles of 300 active WorldVeg breeding lines revealed a broader genetic variation compared to a global collection of cultivars, underscoring the profound impact of sustained, long-term pre-breeding programs on the population structure of cultivated tomatoes. Furthermore, a Multi-parent Advanced Generation InterCross (MAGIC) population was developed by combining disease-resistant and heat-tolerant tomato varieties, facilitating the pyramiding of traits favorable for tropical climates. These foundational materials, along with existing active breeding lines, constitute the initial population for genomic selection efforts aimed at enhancing heat tolerance. Additionally, a transformer-based object detection model was employed for flower and fruit identification, achieving mean Average Precision (mAP) scores of 93.7% and 83.6%, respectively. The integration of this pipeline with an automated image capture device is expected to significantly accelerate and standardize phenotypic evaluations within WorldVeg's tomato breeding programs. Parallel initiatives are also underway to identify functional candidate genes for bacterial wilt and bacterial spot resistance through RNA sequencing. Upon validation, these markers will be incorporated into WorldVeg's tomato breeding programs, thereby expediting the development of disease-resistant varieties on a foundation of heat-tolerant materials.

Acknowledgements
This study was supported by the National Science and Technology Council, Taiwan (MOST 110-2313-B-125-001-MY3). We also thank long-term strategic donors to the World Vegetable Center: from Taiwan, Germany, Thailand, Philippines, South Korea, Japan, UK, USAID and ACIAR.

O-IV-3:
Introgression of a dominant *phototropin1* mutant enhances carotenoids and boosts flavour-related volatiles in genome-edited tomato *RIN* mutants

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The tomato (*Solanum lycopersicum*) ripening inhibitor (*rin*) mutation is known to completely repress fruit ripening. The heterozygous (*RIN*/*rin*) fruits have extended shelf life, ripen normally, but have inferior taste/flavour. Mutants of the tomato ripening inhibitor (*RIN*) gene generated through genome editing had longer shelf life but lower levels of carotenoids. To overrides these negative traits, the genome-edited *RIN* alleles were crossed with a dominant *phototropin1* mutant, which increases carotenoids and volatiles. The resulting double hybrids had longer shelf life, four times more carotenoid levels than the wild-type parent, and were enriched in metabolites and volatiles preferred by consumers. The dominant *phototropin1* mutant gene can be used to improve the carotenoids, taste, and flavour of tomato mutants/cultivars.

Acknowledgements
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O-IV-4:

Recruitment of an Acyl-CoA Synthase into Branched-Chain Fatty Acid Biosynthesis Drives Metabolic Diversity of Tomato Defensive Acylsugars

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Abstract: Branched-chain fatty acids (BCFAs) are key components of bacterial membranes, enabling bacterial environmental adaptation. However, BCFAs are rarely found in plants as they can interfere with membrane integrity. In this study, we performed genetic screening of *Solanum lycopersicum* × *Solanum pennellii* introgression lines and identified a chromosome 2 locus containing a type I glandular trichome-specific, plastid-localized acyl-CoA synthase (GTACS1) involved in the biosynthesis of BCFAs. These BCFAs are subsequently converted to odd-numbered branched-chain acylsugars, which are essential tomato defensive metabolites secreted by trichomes. Through a combination of genetic, isotopic labeling, and biochemical assays, we demonstrated that GTACS1 activates isovaleric acid (iC5) – an abundant branched-chain amino acid breakdown product in glandular trichomes – to form iC5-CoA, which is then channeled into the two-carbon elongation-based fatty acid biosynthetic pathway. We observed an allelic variation in the promoter region of *SlGTACS1* and *SpGTACS1*, resulting in the lack of *SlGTACS1* expression in type I trichomes. Consequently, only IL2-5, which harbors the type I trichome-specific *SpGTACS1*, could produce BCFA-containing acylsugars. Interestingly, *SpGTACS2*, an ACS tandem duplicate located adjacent to *SpGTACS1*, also lacks the ability to generate BCFA-containing acylsugars. This is attributed to a variation in the protein N-terminal region that alters the subcellular localization of SpGTACS2, preventing it from entering the plastid to fulfill its function, despite having similar biochemical activity to SpGTACS1. This study demonstrates how natural variation can recruit a primary metabolism gene into non-canonical fatty acid biosynthesis, ultimately shaping the diversity of Solanaceae trichome defensive metabolites.

O-IV-5:

SlMIPS2, a myoinositol phosphate synthase gene, regulates phosphate homeostasis by influencing the SPX-PHR regulatory circuit in tomato seedlings.

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ABSTRACT

Phosphorus (P) is a quintessential macronutrient plants utilize to support various metabolic processes during growth and development. Recent studies have revealed the pivotal role of inositol hexa-kis/pyrophosphate (InsP6-8), the derivatives of Myo-Inositol (MI), in the regulation of phosphorus starvation responses (PSR) in plants. InsPs are crucial to facilitate the interaction between SYG1/PHO81/XPR1 (SPX) and Phosphate starvation response (PHR) proteins to control PSR. Myo-inositol phosphate synthase (MIPS), an evolutionarily conserved enzyme, catalyzes the first committed step in MI biosynthesis. Although the role of MIPS genes in mediating several biotic and abiotic stresses in plants is well elucidated, its role in phosphate (Pi) deficiency remains largely unexplored. This study demonstrates that out of the five MIPS genes encoded by the tomato genome, only *SlMIPS2* is sharply induced at an early stage of Pi starvation in tomato seedlings. Silencing of *SlMIPS2* led to improved seedling growth with enhanced total soluble Pi and total P levels in the silenced plants compared to their empty vector controls under high Pi availability. *SlMIPS2* silencing also caused a significant reduction in MI and InsP6 content in the tomato seedlings. The depleted InsP6 levels caused degradation of the SlSPX2 protein but stabilized SlPHL1 levels to perturb physical interaction between these proteins. Consequently, SlPHL1 is released to activate PSI genes such as Pi transporters and PSI purple acid phosphatases in the *SlMIPS2*-silenced seedlings, even under high Pi conditions. The results assign a novel role to SlMIPS2 in regulating cellular InsP6 levels and SPX-PHR regulatory transcriptional circuit to control Pi homeostasis in tomato seedlings.

Acknowledgments

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Oral Presentations

Day 2 (Nov 26th)

Session V

O-V-1:
Indole-3-acetic acid is a potential hormone involved in seed browning in pepper fruit under chilling temperatures

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Pepper (*Capsicum annuum* L.) fruit is highly susceptible to temperatures below 10°C. The most representative symptom of chilling injury (CI) in peppers is seed browning. This study uses seed browning as a primary indicator to assess the chilling sensitivity among various pepper genotypes. Two distinct groups of peppers were identified: chilling-sensitive genotypes with severe seed browning and chilling-insensitive genotypes with minimal or no seed browning. A comparative study was conducted on two pepper genotypes with differing chilling sensitivities: the chilling-insensitive 'Takanotsume' and the chilling-sensitive 'Gyeonggiyangpyeong'. Both genotypes were stored at 2°C for 21 days at their green mature stage. 'Takanotsume' exhibited low levels of CI and reactive oxygen species (ROS) production, while 'Gyeonggiyangpyeong' showed high CI and ROS production. A significant increase in indole-3-acetic acid (IAA) content was observed in 'Gyeonggiyangpyeong' but not in 'Takanotsume'. Additionally, 'Gyeonggiyangpyeong' displayed higher expression levels of amidase 1 and auxin response factor 1. Amino acid analysis revealed more significant changes in amino acid composition in 'Gyeonggiyangpyeong' compared to 'Takanotsume' during chilling storage, with increases in alanine, GABA, and glycine. Notably, proline content was significantly lower in 'Gyeonggiyangpyeong' than in 'Takanotsume' prior to chilling treatment. The correlation network analysis indicated a stronger connection between CI levels and IAA-related factors in 'Gyeonggiyangpyeong' compared to 'Takanotsume'. These findings highlight the different responses of the two pepper genotypes to chilling stress, suggesting that IAA may play a crucial role in chilling-induced seed browning in pepper fruit.

Acknowledgements
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O-V-2:
Utilizing Vacuolar Invertase Mutants in Potato as a Platform for Advanced Genome Editing

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In response to the rising global food demand and the challenges posed by climate change, there is an urgent need to develop potato (*Solanum tuberosum* L.) tuber varieties with enhanced stress resilience and improved tuber performance. Cold-induced sweetening (CIS) represents a significant obstacle in potato cultivation, negatively affecting tuber quality by altering carbohydrate composition and disrupting dormancy through metabolic changes that involve starch degradation and sucrose synthesis. The key enzymes α-amylase (AMY) and vacuolar acid invertase (VInv) play crucial roles in this process: AMY hydrolyzes starch into glucose, while VInv converts sucrose into hexoses, leading to increased osmotic concentration in tuber parenchyma, which in turn contributes to undesirable sprouting and browning during frying. In this study, CRISPR-mediated knockout of the *StVInv* gene in potato protoplasts was employed to generate non-transgenic mutants with varying numbers of mutated alleles. These mutants exhibited reduced VInv activity, decreasing CIS and prolonged dormancy (1, 2). Remarkably, the mutated tubers demonstrated significantly lower lipid oxidation and reduced hydrogen peroxide levels under cold stress conditions. Sugar analysis indicated an upregulation of the raffinose family oligosaccharides (RFOs) pathway in the absence of VInv activity (1). Furthermore, double knockout mutants of *stamy23/stvinv* showed not only reduced CIS and extended endodormancy but also increased starch content and yield. These findings underscore the potential of *StVInv* knockout lines to enhance resilience to abiotic stresses, offering a promising platform for further advancements in potato trait improvement.

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(2) Danieli R, Assouline S, Salam BB, Vrobel O, Teper-Bamnolker P, Belausov E, Granot D, Tarkowski P, Eshel D. (2023) Chilling induces sugar and ABA accumulation that antagonistically signals for symplastic connection of dormant potato buds. Plant Cell & Environment. 46:2097-2111.

O-V-3:
Study on molecular mechanism of *Nicotiana benthamiana* interfamily grafting

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Plant grafting has been an important technique in agriculture to propagate fruit trees as clones and to obtain benefits of certain rootstocks. However, graft-incompatibility avoiding cell-cell adhesion has limited the technique. In general, it has been thought that grafting can be done successfully between the same species, genus, and family, but not between different families because of incompatibility. Here, we found that *Nicotiana* species showed an extreme capability of grafting and achieved grafts with a wide range of vascular plants, including eudicots and monocots, where cell walls were apparently digested in a part of graft boundary. To investigate the molecular basis of *Nicotiana* interfamily grafting, we performed time-course transcriptome analysis on graft region. Among upregulated genes during grafting, the cell wall modification related genes were significantly enriched. We then revealed that GH9B3 clade of β-1,4-glucanase gene family was upregulated during only in the case of grafting being successful. When we performed grafting using knocked-down or -out plants of the identified genes, grafting successful rate or post grafting growth decreased. Thus, cell wall digestion by the identified clade of β-1,4-glucanase, which probably target cellulose in cell walls, is crucial to reestablish cell-cell adhesion of the wounded tissues at graft boundary (***Science* 2020**). Tissue adhesion at graft boundary was followed by cell-to-cell connection through secondary plasmodesmata formation (***Plant Cell Physiology* 2021**) as well as tissue differentiation. We then focused on xylem formation that is a key process for establishment of interfamily grafting and conducted comprehensive gene regulatory network analyses with 36 sets of transcritptome data as the input. We found two major gene network modules under the control of *NbVND7*, the master regulator for xylem vessel formation; a module for xylem cell differentiation and a newly identified module centering a *PR* gene. The latter indicates that plant immune response is accompanied with xylem formation, an event to enlarge apoplastic region inside of the tissues. We validated and confirmed the reliability of the network through a functional analysis of *Nicotiana benthamiana XYLEM CYSTEINE PROTEASE* (*NbXCP*) genes, the typical genes found in the network module; *NbXCPs* are important in establishment of interfamily grafting. Since *NbXCPs* play a role in cellular autolysis to accomplish xylem differentiation, we overexpressed *NbXCP* gene under the transcriptional/translational enhanced own promoter and succeeded to fasten the timing of TE formation at the graft region as well as to increase scion growth and fruit size of interfamily grafts (***Horticulture Research* 2023**). These results will be introduced.

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Oral Presentations

Day 2 (Nov 26th)

Session VI

O-VI-1:
Identification of core, conditional and crosstalk components of tomato heat stress response using integrative transcriptomics and orthology

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Heat stress significantly affects agricultural yield and since the frequency and severity of heatwaves is expected to increase due to climate change, this is a growing challenge for global food security. Tomato plants are particularly prone to heat exposure in both the field and the greenhouse which makes increased heat stress resilience a key trait for breeding. Several heat-associated genes have been identified in individual studies but to fully characterize the complex network of actors involved in the heat stress response, quantitatively integrating data from multiple experiments can provide a more complete answer. We have therefore compiled a comprehensive data resource containing both novel and publicly available RNA-seq data on tomato in heat stress spanning multiple tissues, genotypes, and levels and durations of stress exposure. We show that the large majority of the response is specific to the individual study but by intersecting differentially expressed genes across experiments, we can identify a robust core response of 57 genes which encode heat shock proteins, transcriptional regulators, enzymes, transporters and several uncharacterized proteins. The sequence of these core response proteins is strongly conserved across diverse tomato accessions and they disproportionately directly and indirectly interact with proteins experimentally confirmed to affect heat tolerance traits in tomato. 17 of core response genes themselves lie within previously identified genetic loci associated with heat tolerance traits. To understand the relationship of the heat stress response to other abiotic stresses, we applied the same approach to all publicly available tomato RNA-seq data on drought and salt stress. Even though many genes were responsive in individual studies across multiple stresses, the core responses derived in the above manner were mostly stress-specific. Finally, we show that the core responses to these stresses are enriched with evolutionarily ancient genes with orthologs across all domains of life and that the heat core response genes form identifiable co-evolving clusters within the Streptophyta. Our study exemplifies the importance and advantage of using FAIR public data to interpret results of new stress experiments, and provides tools to perform such analyses in a relatively short time. This work is available as a preprint at https://doi.org/10.21203/rs.3.rs-4337825/v1 .

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O-VI-2:
Genetic Basis of Divergent Drought Strategies in *Nicotiana benthamiana* Ecotypes

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Nicotiana benthamiana is an allotetraploid species predominantly distributed in a range of aridic locations in northern Australia. Some ecotypes modulate their growth and physiology to mitigate the effects of drought while others employ a fast life-cycle escape strategy. In response to drought, ecotype NQ, from Northern Queensland, alters its leaf cuticle composition increasing the length of its very long chain alkanes (VLCA) and reducing cuticular permeability to water. Escaper ecotype CA (widely known as LAB) from Central Australia, scarcely alters its cuticular alkane chain length or permeability. Using chromosome level genome assemblies of LAB and NQ, six homoeologous pairs of MYB-like genes (*NbMYB1a* through to *NbMYB6b*) were identified as possible orthologs of wax biosynthesis-regulating 2R-MYBs in Arabidopsis. NbMYBs 1, 2 and 6 up-regulated wax biosynthesis genes, especially *NbCER2* (a key player in VLCA chain extension) in transient leaf expression assays, whereas NbMYBs 3, 4 and 5 generally acted as repressors. The expression levels of *NbMYB1* and *NbMYB2*, and their targets, *NbCER1* and *NbCER2*, all greatly increased upon drought-treatment in NQ but were little-altered in drought-treated CA. NbMYB1 and NbMYB2 sequences cluster with those of drought-induced wax-biosynthesis-activating AtMYB96 and AtMYB94 and pathogen-induced AtMYB30. NbMYB4 and NbMYB5 appear to be the first described R2R3 MYBs playing a role in repressing cuticular wax biosynthesis. The dichotomy of drought response strategy in *N. benthamiana* appears well suited to the original habitats of the ecotypes, and to have evolved in <1MY with the difference in cuticle modification due to both response speed and protein dysfunction.

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O-VI-3:
Characterization of an ABA-Independent Drought Tolerance Pathway in Potato

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Abiotic stresses are major contributors to oxidative stress and physiological damage in plants. In response, plants synthesize sugars and oligosaccharides, with vacuolar acid invertase (VInv) playing a critical role during stress, by rapidly hydrolyzing sucrose into hexoses (1). In this study, we demonstrate that knockout of the *Solanum tuberosum* VInv gene (*stvinv*) results in elevated sucrose and raffinose family oligosaccharides (RFOs) levels, leading to reduced oxidative damage during stress. The *stvinv* mutants exhibit enhanced tolerance to both cold and drought stresses, with notably higher evapotranspiration during drought stress compared to wild-type plants. This suggests a drought tolerance mechanism that is likely ABA-independent and not related to stomatal closure. Transcriptome and metabolome analyses of *stvinv* lines reveal upregulation of genes related to osmoprotection and antioxidation during cold and drought stresses, supporting the ABA-independent mechanism hypothesis. Our research aims to elucidate the molecular pathways by which altered sucrose metabolism enhances drought stress tolerance in potato plants. Specifically, we characterized the differential metabolome of *stvinv* lines under drought stress, identify regulatory mechanisms linking sucrose metabolism to drought tolerance, and explore the role of sucrose accumulation in ROS and ABA signaling and metabolism. Additionally, we investigated whether RFOs contribute to drought tolerance via ABA-independent pathways. Understanding these mechanisms will provide deeper insights into potential drought tolerance strategies that function independently of ABA.

(1) Teper-Bamnolker P, Roitman M, Katar O, Peleg N, Aruchamy K, Suher S, Doron-Faigenboim A, Leibman D, Omid A, Belausov E, Andersson M., Olsson N., Fält A-S, Volpin H., Hofvander P., Gal-On A. and Eshel D. (2023) An alternative pathway to plant cold tolerance in the absence of vacuolar invertase activity. The Plant Journal 113:327-341.

O-VI-4:
The comparative genomics analysis of tobacco NLRs unveiled complex dynamics that underlie non-host resistance

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The tobacco plant (*Nicotiana tabacum*), widely cultivated for its agricultural value, exhibits non-host resistance to several pathogens, including *Phytophthora infestans*, *Phytophthora capsici*, and *Xanthomonas campestris*. This characteristic makes it a valuable resource for resistance breeding. When challenged by non-adapted pathogens, *N. tabacum* triggers a hypersensitive response (HR) through effector-triggered immunity (ETI)-mediated resistance.

To explore the molecular basis of non-host resistance in *N. tabacum* compared to *N. benthamiana*, numerous effectors that induce HR-like cell death in *N. tabacum* were identified. Notably, RxLR effectors from *P. infestans*, such as Avrblb2, the *Xanthomonas* effector AvrBS2, and Avr3a homologs from *P. capsici*, were discovered. Given that the immune receptors Rpi-blb2, R3a, and BS2, which correspond to Avrblb2, Avr3a, and AvrBS2, respectively, have been previously reported, it was hypothesized that homologs of these resistance (R) genes in *N. tabacum* might play a crucial role in defending against microbial infection.

However, results from transient co-expression of AVR/*Nt*NLR pairs in *N. benthamiana* suggest that the sensors *Nt*R3a, *Nt*Rpi-blb2, and *Nt*BS2 might not be directly involved in the non-host resistance of *N. tabacum*. Additionally, the helper NRC4 clade shows divergent evolutionary trajectories between *N. tabacum* and *N. benthamiana*. These findings enhance our understanding of the complex mechanisms underlying non-host resistance in tobacco plants.

O-VI-5:
Bulking Up for Battle: Bulked Segregant Analysis of Colorado Potato Beetle Resistant *Solanum okadae*

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Leptinotarsa decemlineata (Say), the Colorado potato beetle (CPB), continues to pose an immense threat to the potato agricultural industry due to its adaptive nature and the susceptibility of commercial potato varieties. A strategy to improve resistance to the pest involves development of CPB resistant varieties using cross-pollinations with wild relatives of potato. *Solanum okadae* (PI 458367), a wild potato native to Bolivia and Argentina has CPB feeding deterrence. Metabolomic analyses of *S. okadae* clone OKA15 have indicated high production of leaf-specific lactone-containing metabolites not present in CPB susceptible *S. tuberosum*. An inexpensive and widely accessible colorimetric assay to quantify lactone-containing metabolites in leaves was developed and used for phenotyping. A CPB susceptible diploid *S. tuberosum* clone W5281.2 that had low levels of lactones was crossed with CPB resistant OKA15. An F1 clone (W52OKA) from this cross producing a high level of lactones was backcrossed to W5281.2 to generate a genetic mapping population that was phenotyped with the colorimetric assay for lactone-containing metabolites. A bulked segregant analysis (BSA) approach was used for QTL mapping of the lactone-production trait. Initial screening of 40 backcross clones demonstrated a distribution of lactone levels with extremes. A preliminary BSA analysis was done using the Illumina platform to generate sequence reads that were aligned to the OKA15 genome. A pool of seven clones with the highest lactone production and another pool of seven with the lowest was sequenced along with parental clones. The results were promising and phenotyping a larger population with greater variation in lactone production is underway. The colorimetric assay also demonstrated wide ranging variation of lactone production in natural populations of *S. okadae* with few clones showing high levels of lactone-containing metabolites. This colorimetric assay has potential application as a breeding tool for rapid screening of lactone-derived CPB resistance.

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O-VI-6:
Exploring novel plant-to-plant intercommunication pathways in combined biotic and abiotic stresses

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Plants often grow in communities with conspecifics or other species, engaging in complex interplant communication through the emission of volatile organic compounds (VOCs). These VOC signals can be constitutive or induced by environmental stimuli, such as the presence of neighboring plants or pathogen infections. In shade-avoiding species, the detection of competition signals from nearby plants triggers the shade-avoidance syndrome (SAS), which is known to reduce plant defenses against pathogens. To investigate how VOCs mediate plant-to-plant communication under combined stress from vegetation shade and microbial infection, we analyzed the VOC profile of the shade-avoiding species *Solanum lycopersicum* (tomato) infected with *Pseudomonas syringae* pv. tomato DC3000 under simulated proximity shade conditions (low ratio of red to far-red light) using GC-MS.
Our study revealed no early metabolomic response to shade, indicating that tomato plants require prolonged exposure to W+FR (white plus far-red light) to exhibit significant changes. We exposed tomato plants to W+FR for varying durations (0, 2, 5, and 10 days) before infecting them with *P. syringae.* PLS-DA analysis showed distinct metabolic profiles in plants exposed to 2 and 5 days of W+FR, which differed significantly from non-exposed plants, whereas metabolic differences diminished after 10 days, suggesting a stabilization of the metabolism. Furthermore, bacterial counts revealed increased susceptibility to *P. syringae* in plants exposed to W+FR for 2 and 5 days, with this susceptibility reversing after 10 days of exposure. Through metabolic profiling, we identified specific compounds associated with W+FR response, bacterial infection, and metabolites emerging from the combined stress.
Additionally, when tomato plants—both infected and non-infected—were placed in an experimental airflow system, we observed that infected plants emitted unique VOCs not present in control plants. Remarkably, exposure of non-infected plants to the atmosphere of infected plants for 48 hours increased their resistance to *P. syringae*. This study highlights the complex role of VOCs in mediating plant responses to combined stress and suggests potential strategies for enhancing plant resistance through volatile signaling.

Acknowledgements
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Oral Presentations

Day 2 (Nov 26th)

Announcement from SGN and Breedbase

O-S-1:
SGN update

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The Solgenomics Network (SGN, https://solgenomics.net/) is a long-time genomics and phenomics resource dedicated to the reference and non-references species of the Solanaceae. The site aims to provide all Solanaceae genomes in a comparative format along with tools such as BLAST, sequence alignment, comparative maps and genome viewers. Individual plant accessions can be searched and viewed with metadata such as pedigree information, trait ontology-based phenotypes, experiment usage and images. Recently, the database system used for SGN has evolved into a breeding application, allowing entire breeding programs to be run through the system that we now call Breedbase. Breedbase can be used with any crop; to use Breedbase, each breeding program typically requests their own instance of Breedbase and can generate trials with barcoding support, data collection with the Fieldbook and other PhenoApps, manage genotyping data, and analyze data. Built-in analysis tools provide information on stability and heritability of lines, and combining both phenotypic and genotypic data in the system allows analyses such as GWAS and Genomic Selection workflows to be run based on the solGS tool. For further information, see https://solgenomics.net and https://breedbase.org/.

Oral Presentations

Day 3 (Nov 27th)

Session VII

O-VII-1:
Research of vanillin aminotransferase (VAMT), a key enzyme in the capsaicin biosynthesis pathway in chili pepper

Yoshiyuki Tanaka
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The pungent components in the fruits of chili peppers (*Capsicum*) are capsaicinoids. The chemical structure of capsaicinoids comprises an acid amide of vanillylamine with a fatty acid. Capsaicinoids are specifically produced in the placental septum of *Capsicum* fruits, and are not synthesized in other plants. The production of vanillylamine from vanillin is a unique reaction in the capsaicinoid biosynthetic pathway. This reaction is known to be catalyzed by vanillin aminotransferase (VAMT : originally called as pAMT). Here, I will introduce VAMT mutations related to low-pungent analogues biosynthesis, and the evolutionary aspect of VAMT.

A series of our researches has revealed that loss-of-function VAMT mutations leads to the biosynthesis of low-pungency analogues called capsinoids (Fig.1). Capsinoids are structurally similar to capsaicinoids, but have an ester bond instead of the amide bond. Compared with capsaicinoids, capsinoids have similar physiological activity but a much lower pungency. Because of the low-pungency, capsinoids are more attractive ingredients for dietary supplements. In particular, there are various mutant alleles of *VAMT* in *C. chinense*, which are useful for breeding to adjust pungency and capsinoid levels.

In order to understand how VAMT was established in *Capsicum* evolution, we compared genomes of chili pepper and other Solanaceae plants. Phylogenetic analysis indicated that VAMT is a member of the Solanaceae cytoplasmic γ-amino butyrate aminotransferase (GABA-T). Comparative genome analysis suggested that Solanaceae cytoplasmic GABA-Ts including VAMT occurred in a specific Solanaceae genomic region via duplication of a chloroplastic GABA-T ancestor and subsequent loss of the plastid transit signal. A recombinant protein assay demonstrated that VAMT had higher vanillylamine synthase activity than those of other plant GABA-Ts. *VAMT* was expressed exclusively in the placental septum of mature green fruit, whereas tomato orthologs SlGABA-T2/4 exhibit a ubiquitous expression pattern in plants. These findings suggested that both the increased catalytic activity and transcriptional changes in *VAMT* may have contributed to establish vanillylamine synthesis in the capsaicinoid biosynthesis pathway. This study provides insights about how *Capsicum* had established a specific secondary metabolite biosynthesis in the evolution of Solanaceae.

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[2] H. Kusaka, S. Nakasato, K. Sano, K. Kobata, S. Ohno, M. Doi and Y. Tanaka. (2023) An evolutionary view of vanillylamine synthase pAMT, a key enzyme of capsaicinoid biosynthesis pathway in chili pepper. Plant J. 117: 1453–1465.

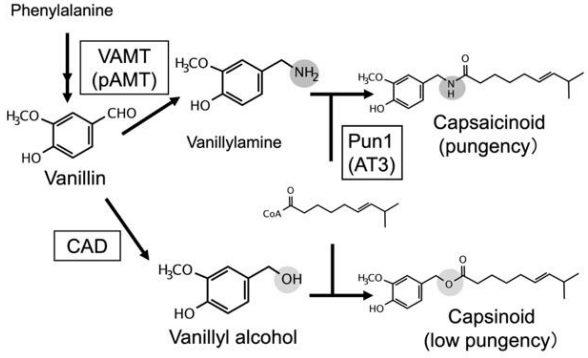

Fig. 1. The overview of biosynthesis pathway of capsaicinoid and low-pungent analogs.
pAMT: putative aminotransferase, VAMT; vanillin aminotransferase, CAD: cinnamyl alcohol dehydrogenase. Mutations in VAMT (pAMT) decrease the content of vanillylamine and capsaicinoid, simultaneously causing the accumulation of low-pungency capsaicinoid analogs, termed capsinoids, instead of capsaicinoids. CAD catalyzes the conversion of vanillin to vanillyl alcohol.

O-VII-2:
Integrative analysis of sensory attributes, VOC profile and genetic data for enhancing tomato quality.

Daniela D'Esposito[1,2], Guadagno Anna[1], Liberata Gualtieri[2], Vittorio Rosanova[1], Francesca Colonna [1], Giuseppe Aprea[3], Paola Ferrante[3], Giovanni Giuliano[3], Sharon Puleo[1], Michelina Ruocco[2], Maurilia Maria Monti[2], Rosella Di Monaco[1], Maria Raffaella Ercolano[1]

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Tomato quality improvement is a significant topic in agricultural research, driven by consumer preferences for taste, texture, and aroma, while maintaining production efficiency. Sensorial attributes such as sweetness, sourness, firmness, smell and juiciness significantly influence consumer satisfaction and marketability. Additionally, volatile organic compounds (VOCs) play a crucial role in defining the aroma and overall sensorial profile of tomatoes. Understanding the interplay between these sensorial attributes and VOCs is essential for improving tomato quality. In this study, an F2 progeny of 196 tomato plants, derived from the self-pollination of an elongated type combination, was evaluated through a comprehensive analysis based on key sensorial attributes and volatile organic compounds (VOCs) profile. From a preliminary sensory analysis conducted on eight tomato varieties, key sensorial attributes essential for the characterization of tomatoes fruits were identified. The main discriminating sensorial attributes were sourness, firmness, sweetness, tomato smell and juiciness. Major outcomes revealed that sourness was positively correlated with firmness and negatively with sweetness, while tomato smell was positively correlated with juiciness. The volatilome profiles showed that there is a variability in tomato fruits coming from the F2 population and interesting VOC emission patterns associated with fruit quality were observed. To further explore the genetic underpinnings of these traits, the DNA was extracted and sequenced by Single Primer Enrichment Technology (SPET). These data were used to perform a Quantitative Trait Loci (QTL) analysis for identifying significant loci linked to sweetness, sourness, firmness, tomato smell and juiciness. Our integrated approach underscores the potential of combining phenotypic and genotypic analyses to advance tomato quality improvement. By providing valuable insights into the genetic basis of key sensory traits and demonstrating the effectiveness of integrating sensory evaluation with VOC analysis. Ultimately, these findings offer a powerful tool for tomato breeding programs aimed at enhancing quality traits, significantly contributing to the advancement of Solanaceae crops.

O-VII-3:
Specific geranylgeranyl diphosphate synthase and phytoene synthase combinations control the production of carotenoids and ABA in different tomato tissues

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Plant carotenoids are plastidial isoprenoids with roles as photoprotectants, pigments, and precursors of bioactive molecules such as the hormones abscisic acid (ABA) and strigolactones. The first and main rate-determining step of the carotenoid biosynthesis pathway is the production of phytoene from geranylgeranyl diphosphate (GGPP), catalyzed by phytoene synthase (PSY). While the GGPP produced by plastidial GGPP synthase (GGPPS) enzymes is also used to produce gibberellins, diterpenes, plastoquinone, phylloquinone, tocopherols, or chlorophylls, direct interaction of GGPPS and PSY enzymes facilitates its channeling to the carotenoid pathway. Unlike the model plant *Arabidopsis thaliana*, the genome of most crops typically harbor multiple copies of differentially expressed genes encoding PSY and GGPPS paralogs. Three plastid-localized GGPPS isoforms (referred to as SlG1 to 3) and three PSY enzymes (PSY1 to 3) are present in tomato (*Solanum lycopersicum*). Our results with CRISPR mutants defective in individual genes suggest a housekeeping role for SlG3 and a helper role for SlG2 in the production of carotenoids for photoprotection in leaves and for pigmentation and ABA synthesis in ripening fruits. Loss of SlG1 function had no impact in carotenoid or ABA levels but led to a reduction in the production of strigolactones in roots. Single CRISPR mutants defective in either PSY1 or PSY2 confirmed that PSY1 is the main isoform for carotenoid and ABA biosynthesis in the fruit pericarp with a minor contribution of PSY2, whereas the production of leaf carotenoids mainly relies on PSY2 with PSY1 contributing when an extra supply is required in response to high light. PSY2 is also associated with ABA synthesis in seeds and salt-stressed roots. PSY3 appears to only function in the root, specifically interacting with SlG1 to produce strigolactones. On the other hand, PSY1 and PSY2 interact with SlG2 but not with SlG1 or SlG3. We have also generated and analyzed different combinations of double mutants to investigate the contribution of specific GGPPS and PSY pairs to the production of carotenoids and ABA in different tissues of the tomato plant. The results provide valuable information to engineer the accumulation of carotenoids in specific tissues without negatively impacting the production of other GGPP-derived products required for normal plant functions.

Acknowledgements

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O-VII-4:
Anthocyanin synthesis in tomato fruit is regulated by a multiprotein network composed of BBX and COP1 proteins converging on HY5

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Anthocyanins, polyphenolic pigments conferring fruit coloration, are beneficial for human health. In the past two decades, several research efforts led to the development of anthocyanin enriched tomato lines representing a novel nutraceutical food. One of these lines, obtained by breeding, was called "SunBlack". The "SunBlack" line synthesizes anthocyanins in the fruit peel under light, producing purple fruits thanks to specific alleles of *myb-atv* and *Aft* genes.

Interestingly, both light and temperature play a crucial role in pigmentation during fruit development. Whereas high light exposure or cool temperatures are required to allow strong purple coloration, shade or high temperatures repress anthocyanin synthesis, potentially affecting the nutraceutical content of the anthocyanin enriched lines and underlying the need to understand how light and temperature regulate this mechanism.

To investigate this photo/thermo-dependent process, we focused on its major players: ELONGATED HYPOCOTYL 5 (HY5), the master-positive regulator, which activates many processes under light, included anthocyanin biosynthesis, and CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), the negative regulator of photomorphogenesis, which tags its targets for degradation under dark. We further characterized the activity of four B-box proteins as HY5 cofactors under light: their expression levels, possible interactions with HY5 and other light signaling proteins, as well as their ability to activate the expression of anthocyanin related genes were analysed. Stable overexpression lines were produced, which showed extremely high pigment contents demonstrating the role of these B-box factors as key regulators of anthocyanin synthesis.

Then, we focused on the molecular mechanisms underlying temperature mediated anthocyanin synthesis. We evaluated COP1 and HY5 levels in fruits under different conditions, as well as the levels of their molecular targets, and showed how COP1 in the nucleus increases with temperature, allowing HY5 degradation, and consequent inhibition of anthocyanin production, even under light. Very useful was the use of the *high pigment 2 (hp2)* mutation, which, by leading to non-functional COP1 complex, confers high pigment accumulation in tomato leaves and fruits even at high temperatures, potentially leading to new breakthrough tomato lines accumulating high levels of anthocyanins even under very warm conditions.

On the whole, our study provides new insights into the complex network of light and temperature crosstalk regulating anthocyanin synthesis in tomato fruits.

O-VII-5:
Characterization of Tomato Introgression Line IL5-4: A Valuable Resource for High-Brix Breeding and Investigation of Blossom-End Rot Mechanisms

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Brix is a crucial indicator of tomato (*Solanum lycopersicum*) quality and enhancing Brix levels is a key breeding objective. The production of high-Brix tomatoes requires genetic and breeding studies on the fruit. During domestication, *S. lycopersicum* lost genetic diversity from its wild relatives, which could be beneficial for breeding purposes. In this study, we analyzed introgression lines (ILs) derived from a cross between the wild relative *Solanum pennellii* and the cultivated tomato *S. lycopersicum* 'M82'. Although numerous genetic and physiological studies have highlighted the advantages of *S. pennellii* ILs, few have focused on their potential for producing high-Brix fruit. Therefore, in this study, we aimed to identify ILs that yield high-Brix fruits, thereby providing valuable genetic and genomic resources for investigating phenotypes derived from the *S. pennellii* genome. Among the lines studied, IL5-4 appears to carry a segment of the *S. pennellii* chromosome on chromosome 5 of 'M82'. Previous research indicated that IL5-4 fruit have higher Brix levels compared to 'M82' fruit. Our findings confirm these observations and reveal dynamic changes in Brix levels during fruit development. Additionally, IL5-4 plants exhibited a higher incidence of blossom-end rot (BER), which is a significant physiological disorder in tomatoes. To understand the underlying physiological mechanisms contributing to this increased incidence of BER, we focused on calcium content, which is often associated with BER. Our results showed that the total and water-soluble calcium contents in the proximal fruit tissue of IL5-4 were significantly lower than those in 'M82', while no differences were found in the distal tissue. These findings suggest that the higher BER incidence in IL5-4 fruits may not be directly related to calcium content in the distal tissue, but rather may be influenced by genetic factors from the *S. pennellii* chromosome. The characterization of IL5-4 in this study demonstrates its potential as a valuable genetic and genomic resource for breeding high-Brix tomatoes and exploring novel mechanisms of BER development.

Acknowledgements
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O-VII-6:
Structural Variations in the Phytoene Synthase I Gene: Unraveling the Genetic Basis of Color Diversity in Tomato Fruits

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Tomato fruits normally accumulate large amounts of the red pigment lycopene in their chromoplasts. However, some tomato cultivars (*Solanum lycopersicum*) display distinct phenotypes, ranging from a pure yellow hue to bicolor fruits with red and yellow sections. In this study, we show that alleles of the phytoene synthase 1 gene (PSY1), the first gene of carotenoid synthesis pathway, are responsible for the yellow, but also the bicolor phenotype. Introgression lines carrying the PSY1 allele from the green-fruited species *S. habrochaites* show reduced expression of the enzyme, resulting in a bicolor phenotype. In contrast, in tomato bicolor cultivars, the same coloration pattern is caused by a 3789 bp-deletion in the promoter region of PSY1. Since this deletion includes part of the 5'UTR region of PSY1, translation efficiency is likely decreased, leading to reduced lycopene accumulation. Furthermore, we identified that the yellow r^y phenotype is caused by a duplication and an inversion affecting PSY1 and its downstream neighbor gene. This genomic rearrangement changes the end of PSY1 amino acid sequence. Under certain conditions, yellow r^y cultivar fruits can still accumulate lycopene near the blossom-end, though to a lesser extent than in bicolor cultivars. In contrast, fruits of the yellow r cultivars never present fleshy red sections due to the insertion of a single long terminal repeat from the Rider transposon in the first exon of PSY1, resulting in a non-functional protein. These results demonstrate how multiple phenotypes can arise from structural variations in a key gene.

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Oral Presentations

Day 4 (Nov 28th)

Session VIII

O-VIII-1:
Key Steps to Developing Insect-Resistant Tomatoes with Natural and Induced Variations

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Obtaining cultivated tomato varieties naturally resistant to insect pests has long been a primary goal for breeders. Arthropod resistance is present in certain wild relatives of the tomato, such as *S. habrochaites*, *S. galapagense*, and *S. pennellii*, which was stored and distributed from bioresource centers. This resistance is based on various metabolites produced primarily in glandular trichomes of types IV and VI. Type IV trichomes produce the sticky compound acyl sugar, while type VI trichomes synthesize plastid-derived methylketones and sesquiterpenes. One of the main challenges in transferring this type of resistance to cultivated tomatoes lies in its inherently polygenic nature, as it requires the integration of complex developmental pathways governing trichome formation and biochemical pathways responsible for producing specific metabolites. Cultivated tomatoes do not develop type IV trichomes in the adult phase, and their type VI trichomes are morphologically distinct from those of wild species, which possess greater storage capacity. Furthermore, cultivated tomatoes do not produce methylketones or plastid-derived sesquiterpenes, and the absence of type IV trichomes limits the high production of acyl sugars. Since direct approaches to introduce insect resistance into cultivated tomatoes have so far been unsuccessful, fundamental research on trichome development and metabolism could help overcome these challenges. To this end, monogenic components of both pathways are being isolated in our lab using the genetic model system Micro-Tom. One advantage of Micro-Tom is that the natural genetic variation discovered in wild species can be combined with the extensive knowledge provided by large mutant collections, such as those available through TOMATOMA, supported by the National BioResource Project (NBRP). We will provide an overview of recent discoveries using these resources and how they can be utilized to inform the key steps necessary for developing insect resistance in cultivated tomatoes.

Acknowledgements
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O-VIII-2:
Common mechanisms controlling fruit shapes may be mediated by changes in cell wall properties.

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Fruit shape variation is abundantly present in horticultural crops. Many of the underlying genes have been cloned in tomato, offering insights into the molecular mechanisms of morphological diversity. Specifically, members of the OFP, TRM and SUN family regulate produce shape variation in tomato and other crops, thereby highlighting the importance of these three families in regulating phenotypic diversity. Despite the knowledge of the genes, mechanistic insights into the function of members of these three gene families are lacking. Our research on the tomato genes *OVATE* and *OFP20* has shown that changes in produce shapes are noticeable early in the development of the flower. Cell counts in ovaries at anthesis implied that changes in cell division patterning may underlie morphological diversity. However, gene expression studies showed that morphological changes are instead associated with cell wall processes and not with changes in cell division patterning.

Acknowledgements
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O-VIII-3:
ERF.E1, a hypoxia regulated genes triggering ripening.

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During ripening phase fruits acquire their sensory qualities and post-harvest potential. The processes that trigger the transition to ripening remain poorly deciphered. While so far, transcriptomic profiling of tomato fruit ripening has mainly focused on the changes occurring in pericarp tissues between Mature Green and Breaker stages, our study addresses the changes between Early Mature Green and Late Mature Green stages. The data show that shift from inability to initiate ripening to capacity to undergo full ripening requires a massive transcriptomic reprogramming that takes place first in the locular tissues before extending to the pericarp. Interestingly, transcriptomic data highlight ERF.E1 as a putative regulator of ripening. The data show the prominent role of ERF.E1 in the promotion of ripening. Interestingly, this ERF is regulated at the transcriptional level, by hypoxia taking place in the gel during ripening. While ERF.E1 overexpressed lines show an early ripening, knock-out line show a delay ripening demonstrating the role of ERF.E1 in ripening onset. Mining of transriptomic data and physiological analysis support the role of this ERF in ethylene production before the beginning of climacteric process. For the first time, this study highlights the role of ERF in ripening triggering in relation with hypoxia event occurring in fruit.

Acknowledgements
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O-VIII-4:

SlKNOXII and *SlBLH1-like* Genes Control the Coordination Between Fruit Pigmentation and Softening During Ripening

Alexandra Keren-Keiserman[1], Iben Sorensen[2], Stephen I. Snyder[2], Etel Motenko[1,3], Lamar Knaan[1], Roni Hasman[1,3,#], Ziva Amsellem[4], Sharon Alkalai-Tuvia[5], Carmit Ziv[5], Jocelyn Rose[2], Alexander Goldshmidt*[1].

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Ripening, defined as a fruit's ability to change pigmentation and soften, is a distinct characteristic of fleshy fruits, which are an essential component of the human diet. This process determines economically significant fruit quality and preservability characteristics and has been extensively studied. These studies have indicated that the progression of the ripening process is tightly coordinated via hormonal and complex transcriptional networks. In our previous research, we found that the four tomato *CLASS-II KNOX* (*TKN-II*) genes are jointly involved in coordinating the ripening progression from internal locular tissue to the external pericarp domain. Recent studies in the tomato cultivar Micro-Tom demonstrated that TKN-II proteins form heterodimers with three fruit-expressed tomato BLH1-like proteins (SLBLHL1). It was also shown that the *triple blhl1* mutant shows fruit coloration phenotypes comparable to those observed in the *tknII3* mutant fruits. Our experiments in a different cultivar, M82, support these observations while also demonstrating that in the *high-order tknII* mutant fruits, the delay of chlorophyll degradation is significantly stronger than in the *triple blhl1* mutants. Moreover, we found that while the delay in pigmentation change of the *high-order tknII* mutants is strongly associated with the inhibition of pericarp softening, the pericarps of the *triple blhl1* mutants become significantly softer than the control with the onset of ripening.These results suggest that *TKN-II* and fruit-expressed *BLHL1* genes are likely involved in controlling the coordination between the two key processes defining fruit ripening: change in fruit pigmentation and progression of pericarp softening.

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O-VIII-5:
GRAS transcription factors regulate fruit development and ripening in tomato

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Abstract
GRAS family proteins are plant-specific transcription factors that play critical roles in plant development, phytohormones signaling transduction and stress responses. There are 53 *GRAS* genes in tomato, and some of them have been functional identified. SlDELLA was an important regulator of gibberellin signaling, the mutant was conferred persistent gibberellin response, which induced parthenocarpy. Overexpression of *SlGRAS7*, *SlGRAS24* or *SlGRAS40* was broken the homeostasis between gibberellin and auxin, a variety of agronomic traits were affected. Down-regulation of *SlGRAS2* inhibited gibberellin biosynthesis and signaling transduction, whereafter inhibited the cell expansion and ovary growth, that cause smaller fruit. Repression of *SlGRAS15* or *SlGRAS26* resulted in the decrease of gibberellin biosynthesis and affected the plant architecture. In *SlGRAS38*-RNAi fruit, the accumulation of lycopene was reduced, and the activities of cell wall degradation enzymes were also reduced, which prolonged the fruit shelf life. Our study identified that SlGRAS4 acts as a new regulator of fruit ripening through regulating ethylene biosynthesis. On the other hand, SlGRAS4 also mediated a new cold response pathway conferring chilling tolerance in tomato fruit independently of the CBF pathway. Furthermore, we uncovered the important role of SlGRAS9 in controlling chlorophyll and carbohydrate accumulation in tomato fruit. Knock-out of *SlGRAS9* exhibited increased cuticle deposition, conferring extended fruit shelf-life and higher tolerance to postharvest fungal infection. Here, we summarized tomato GRAS transcription factors involve in the regulation of fruit development, ripening and senescence, which provides new possibility for breeding strategies aiming to improve qualities of tomato fruit.

Key words: GRAS, transcription factor, development, fruit ripening, tomato (*Solanum lycopersicum* L.).
Acknowledgements
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O-VIII-6:
Unraveling the molecular crosstalk between reproduction and stress in eggplant, a model for andromonoecious Solanaceae

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When plants face environmental stress during the reproductive phase, they have to split their resources between reproduction and stress endurance. By 2050, we expect a rise of mean temperatures of up to 2-3°C in some areas of the world. This will be accompanied by other environmental challenges, like water scarcity and more frequent extreme weather events such as heatwaves and droughts. The increase of stressful events during the reproductive phase might lead to reduced progeny causing a decline in plant populations increasing their risk of extinction, as well as reduced crop yield. Therefore, understanding plant responses to stress during the reproductive phase becomes imperative for preserving biodiversity and agricultural yields.
Several species of the Solanaceae family, including cultivated eggplant (*S. melongena*), exhibit different forms of andromonoecy, a sexual system characterized by the coexistence of hermaphrodite and male flowers in each individual. In some of these species, the strength of andromonoecy has been proposed to be linked with stress and resource allocation but this has never been proved at the molecular level.
Our lab developed an early-flowering, dwarf eggplant variety, ("Micromel,") that facilitates molecular studies. We found that in Micromel plants, male flowers increase in response to several stimulus like the presence of developing fruits and heat stress. Transcriptomic and metabolomic data from pistils and leaves from plants under heat stress revealed metabolic reprogramming and changes in transcriptional and translational activity leading to a "energy saving mode" linked to the SnRK1 kinase system, a key energy sensor that maintains cellular homeostasis under energy deficit. This provides molecular proof of the hypothesis that the increase of andromonoecy is an adaptative response to cope with environmental stress during the reproductive phase and contributes essential information to devise strategies to mitigate the impacts of climate change in domesticates and wild Solanaceae species.

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Oral Presentations

Day 4 (Nov 28th)

Session IX

O-IX-1:
Tomato stomatal development: Diverse mechanisms of adaptive flexibility revealed by multi-species analysis

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Stomata are pores in the plant epidermis that control gas exchange between the plant and atmosphere. In Arabidopsis, stomatal development requires the bHLH transcription factor SPEECHLESS (AtSPCH) and perception of signals from adjacent cells, from other parts of the plant and from the environment. SPCH is thought to be a critical target for environmental inputs into development. Despite the power of Arabidopsis as a model for stomatal development, we found crop plants like tomatoes often lean on different cellular and genetic strategies to achieve optimal stomatal distributions. By making genetically encoded reporters of the stomatal lineage, and long-term confocal microscopy we tracked the developing epidermis of M82 (WT) and mutant tomato seedlings. We found that, like in Arabidopsis, tomato undergoes a series of asymmetric and symmetric cell divisions to produce stomata. However, we found one type of asymmetric division (ACD) was missing in the tomato epidermis, and other ACDs could be used to generate non-stomatal cells. These data suggest differences in ACD strategies that control stomatal production between plant species. Since SPCH serves as the major integrator of environmental information in stomatal development, we targeted the tomato SPCH promoter for CRISPR-based mutagenesis. By screening lines in response to light, temperature, and drought we found putative SPCH *cis*-regulatory elements that indicate complexity in the regulation of developmental flexibility. Taken together these results further our understanding of the species-specific cellular and genetic pathways plants use to adapt to their environment.

O-IX-2:
SmuMYB113 is the determinant of fruit color in pepino (Solanum muricatum)

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Abstract

Pepino (*Solanum muricatum*) is an herbaceous crop phylogenetically related to tomato and potato. Pepino fruit vary in color, size and shape, and are eaten fresh. In this study, we use pepino as a fruit model to understand the transcriptional regulatory mechanisms controlling fruit quality. To identify the key genes involved in anthocyanin biosynthesis in pepino, two genotypes were studied that contrasted in foliar and fruit pigmentation. Anthocyanin profiles were analysed, as well as the expression of genes that encode enzymes for anthocyanin biosynthesis and transcriptional regulators using both RNA-seq and quantitative PCR. The differential expression of the transcription factor genes R2R3 MYB *SmuMYB113* and R3MYB *SmuATV* suggested their association with purple skin and foliage phenotype. Functional analysis of these genes in both tobacco and pepino showed that SmuMYB113 activates anthocyanins, while SmuATV suppresses anthocyanin accumulation. However, despite elevated expression in all tissues, *SmuMYB113* does not significantly elevate flesh pigmentation, suggesting a strong repressive background in fruit flesh tissue. These results will aid understanding of the differential regulation controlling fruit quality aspects between skin and flesh in other fruiting species.

O-IX-3:
Genome and phenotype variations in a tomato (*Solanum lycopersicum*) cultivar Micro-Tom

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Micro-Tom is a miniature tomato cultivar, originally developed in the U.S. for ornamental purposes. It has since become a widely used model for research due to its compact size and short life cycle under laboratory conditions. Our previous study revealed that there are several genetically distinct lines of Micro-Tom; however, variations in their genomes and phenotypes had not been reported. Moreover, the global distribution of these Micro-Tom lines remains unclear. To address these gaps, we collected six Micro-Tom lines from the Tomato Genetics Resource Center (University of California, Davis, USA), INRA (France), Universidade de São Paulo (Brazil), and three Japanese institutes (University of Tsukuba, NARO, and Kazusa DNA Research Institute). The distribution path of the Micro-Tom lines was partially revealed in accordance with the investigation to colleagues in the tomato research community. Comparative phenotypic analysis showed differences in plant height and fruit size among the lines. Additionally, the genome sequence of the Kazusa DNA Research Institute line was determined using short-read technology to identify sequence variants. We also assembled the genome sequence of the University of Tsukuba line, TOMJPF00001, a standard line used in the National BioResource Project, using long-read technology. This analysis revealed a cluster of rDNA genes spanning a 15 Mb region on the short arm of chromosome 2, a region not found in previous tomato genome assemblies, likely due to the limitations of earlier sequencing technologies in handling repetitive DNA. The highly accurate genome information from this study is expected to contribute to the breeding programs for tomato and other vegetables and to further advance genomic and genetic research in tomato.

Acknowledgements
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O-IX-4:
Unraveling the Epipangenome of *Solanum pennellii*: Insights into the Genetic Variation and Abiotic Stress Responses for Crop Improvement

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Food security for a growing population is increasingly threatened by climate extremes, such as rising temperatures and drought. Under future climate change scenarios, crops will face more adverse conditions, exacerbating the challenge of sustaining agricultural productivity. Due to the relatively narrow genetic base of many crops—resulting from domestication and selective breeding efforts—numerous alleles conferring tolerance to abiotic stresses may have been inadvertently lost. To address this, the introgression of wild alleles has become an effective strategy in many breeding programs aimed at improving yield and stress tolerance.

In this context, wild relatives of cultivated crops, particularly those adapted to harsh environments, offer a valuable genetic diversity. The wild tomato species *Solanum pennellii*, known for its remarkable resistance to abiotic stresses, is of particular interest. *S. pennellii*, adapted to arid regions, has been utilized in breeding programs through the development of introgression lines (ILs) and backcrossed inbred lines (BILs), which have helped to identify a wide array of quantitative trait loci (QTL). Most of these QTL have been based on IL/BIL populations derived from accessions LA716 or LA5240.

However, we have observed substantial variation in the abiotic stress responses among the 47 *S. pennellii* accessions available at the Tomato Genetics Resource Center (TGRC) (https://tgrc.ucdavis.edu/). These accessions were subjected to drought stress, revealing marked differences in their responses. To better understand this variation, we generated a *S. pennellii* epipangenome using a hybrid approach with long-read ONT Nanopore and PacBio HiFi data, shedding light on structural variation and DNA methylation patterns.

Next, we employed a holistic approach combining RNA sequencing (RNA-Seq) and metabolomics to analyze the drought stress response of *S. pennellii*. Our study presents the transcriptomic differences in conjunction with physiological and metabolomic data, highlighting accession-specific molecular adaptations that may contribute to drought tolerance in this wild tomato species.

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O-IX-5:
The next step: building a unified Nicotiana benthamiana genomic resource.

Jiyuan An, Aureliano Bombarely Giovanni Giuliano, Li Guo, Feng Li, Michitaka Notaguchi, Ah-Young Shin, Peter Waterhouse, Christopher Winefield.

One near-isogenic line of *Nicotiana benthamiana* is used in laboratories around the world for basic and applied research and there are at least 6 recent chromosome-level sequence assemblies of its genome. Despite large concordance among the assembly sequences, the number of identified genes ranges from ~45-67K. There is also considerable variation in the designated gene and chromosome IDs and the chromosome orientations. Establishing a unified reference genome sequence, chromosome attribution and gene annotation with associated gene expression profiles and epigenetic landscapes, would provide a lingua franca for the global scientific community. This presentation seeks to provide and discuss the way to achieve this aim in a timely and collaborative manner.

O-IX-6:

Availability of phenotypic simulation for fruit-related traits in crossing progenies of chili peppers (*Capsicum annuum*) using genomic prediction based solely on parental information

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Chili peppers (*Capsicum* spp.) are one of the major crops in the Solanaceae family, utilized not only as vegetables but also as spices and ornamental plants. Because of their many uses, various fruit characteristics, including sizes, shapes, and pungency levels, are in demand. However, their genetic improvement is challenging through conventional cross-breeding due to the quantitative traits that make it difficult to predict phenotypes in the progeny. As a breakthrough, we focused on phenotypic simulation techniques in the crossing progenies via genomic prediction (GP) based solely on parental information, performed in several other crops (Iwata et al. 2013; Yamamoto et al. 2021). As a primal trial in chili peppers, we aimed to assess the feasibility for fruit-related traits in F_1 progenies.

In the present study, a total of 291 *C. annuum* accessions were subjected, including two populations: inbred (n = 132) and F_1 accessions (n = 159) derived from 20 inbred accessions. They were cultivated for three years in a farm field at the Faculty of Agriculture, Shinshu University, Japan. We collected data on five traits (fruit length, width, weight, shape index (length/width)). Pungency levels were also evaluated by quantifying capsaicinoid contents in the placental septum via HPLC. A total of 3,194 genome-wide SNPs were obtained by multiplexed inter-simple sequence repeat genotyping by sequencing (MIG-seq). With these data, we performed phenotypic simulations in the F_1 accessions. Briefly, we estimated genotypic data of the F_1 accessions from their parents, and we simulated all traits by inputting them into the optimized GP models (five fruit-related traits: GBLUP-GAUSS; pungency level: GBLUP-RR), based solely on the inbred accessions.

As a result of the phenotypic simulation, we observed strong positive correlations between the simulated and observed phenotypic values for fruit length (r = 0.901), width (r = 0.895), shape index (r = 0.908), weight (r = 0.833), and pericarp thickness (r = 0.865). These results demonstrate that it is possible to rank these phenotypic values among the F_1 progenies with a reasonable degree of accuracy based solely on parental information. Although the degree of dominance effects varied depending on the accessions across all traits (data not shown), these effects did not significantly impact the ranking results. Whereas the pungency level (capsaicinoid) showed lower simulation accuracy (r = 0.652) compared to the other traits. GP accuracies for capsaicinoid content are known to fluctuate depending on whether the marker sets intensively capture the genotypic effects of the major QTLs (Kim et al., 2022), which may have caused difficulty in accurate simulation in this study. Nonetheless, this study provides new empirical insights into the utility of phenotypic simulation via GP in chili pepper breeding, offering valuable information for its application in the field.

Acknowledgment

The authors thank Dr. Motoyuki Ishimori (Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan) for his theoretical and technical advice regarding GP.

Poster Presentations

P1:
Comparative genomics of two wild tobacco species revealed their same tempo of diploidization

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Diploidization is the process by which a polyploid genome converts to a diploid-like state through chromosomal rearrangements (CRs) between sub-genomes leading to chromosome number reduction, and gene copy number reduction. This phenomenon is observed in most plants and characterized by numerous studies. However, detailed investigations into the tempo of diploidization remain scarce, especially at the genome sequence level. If the diploidization tempo is similar across species diverged from a common ancestral polyploid, this would suggest a common regulatory mechanism for the diploidization process.

Three species belonging to genus *Nicotiana* (x = 12), *N. amplexicaulis* (2n = 36), *N. benthamiana* (2n = 38), and *N. tabacum* (2n = 48), were analyzed. The first two species are mesopolyploid wild tobacco plants that have diverged from a common ancestral allotetraploid. *N. tabacum*, a cultivated tobacco plant, is a recently established allotetraploid and used as a control species less progressed to diploidization. The whole genome of *N. amplexicaulis* was *de novo* sequenced using the Oxford Nanopore Technology. Genome sequences of the other two species were obtained from public databases. To the genome sequences of the three species, the genome sequence of *N. sylvestris* (2n = 24), a common sub-genome donor of those species, were mapped using minimap2 v2.24. Structural variations (SVs) were detected using SVIM-sim v1.0.3, to determine the number and genome-wide distribution of CRs in the genomes of the three species. Genome-wide gene copy numbers of the three species were investigated using BUSCO v5.5.0 to confirm single-copy gene proportions.

The newly obtained genome sequence of *N. amplexicaulis* was consisting of 3,383 contigs with N50 of 2.16 Mb. The average coverage of *N. sylvestris* sequences in *N. amplexicaulis*, *N. benthamiana*, and *N. tabacum* genome was 0.196, 0.185, and 0.488, respectively. SVs detected between the genomes of these three allotetraploid species and *N. sylvestris* genome were 154,303, 146,200, and 134,804. Genome-wide mapping of *N. sylvestris* sequences and uniform distribution of SVs were shown in *N. amplexicaulis* and *N. benthamiana*, while distinct regions of high and low mapping coverage and SV density existed in *N. tabacum*. This result indicates that CRs between sub-genomes occur widely and uniformly in *N. amplexicaulis* and *N. benthamiana*, whereas the average density of CRs in *N. tabacum* is lower and unevenly distributed. Single-copy gene proportions were approximately 0.47 in both *N. amplexicaulis* and *N. benthamiana*, compared to 0.21 in *N. tabacum*.

The above findings suggest that the CRs between sub-genomes and gene copy number reduction are equally advanced in the mesopolyploid species, *N. amplexicaulis* and *N. benthamiana*, while being less advanced in the recently established tetraploid, *N. tabacum*. Since a similar diploidization tempo was observed in the two wild tobacco species, there may be a common factor controlling this process. Further elucidation of these factors could enhance our understanding of diploidization mechanisms.

Acknowledgments

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P2:
Effects of low-concentration ozonated water treatment on the growth of grafted tomato seedlings

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Ozone is a reactive oxygen species and strong oxidant. However, it is unstable and easily decomposes into oxygen. Ozonated water (OW) is produced by dissolving gaseous ozone in water or direct water electrolysis. The use of ozone in agriculture is mainly based on its sterilizing power, as in washing harvested products. Previous studies have reported that komatsuna treated with low OW concentrations show growth-promoting effects under low-temperature conditions (Onoue et al., 2018). We have confirmed that OW also promotes tomato-cutting seedlings growth due to the development of adventitious roots. This result shows that OW induces wounding stress tolerance. This study aimed to clarify the effects of OW on tomato grafting with wounding stress. Thus, we investigated the growth of grafted tomato seedlings treated with low OW concentrations before and after grafting.

Tomatoes were cultivated at 25℃ under white LED lamps and 16 h day length. “Suzukoma” and “B-Barrier” were used as scions and rootstocks, respectively. These seedlings were grafted 20 days after sowing. The grafted seedlings were kept in the dark and high-humidity conditions for 2 days. After that, they were acclimatized for 4 days. OW was prepared using a prototype OW sprayer (Tamura TECO). OW treatment was carried out by foliar spraying with low OW concentrations. Foliar spraying was carried out twice, before grafting and once after grafting. Three treatments were included in this study. Demineralized water was sprayed before and after grafting in control and control treatment (CC). OW was sprayed only before grafting in OW and control treatment (OC) and both before and after grafting in OW and OW treatment (OO). Plant growth (fresh weight, dry weight, dry matter rate, stem diameter, main stem length, and number of leaves) was investigated 14 days after grafting.

Results showed that the above-ground dry matter rate significantly increased at the 5% level in OC and 1% level in OO compared to that in CC. The main stem length was significantly reduced at the 5% level in OC compared to that in CC. No significant differences were detected in the other parameters. These results suggested that OW treatment promoted photosynthesis, resulting in compact and vigorous grafted seedlings. The positive effect of OW treatment was observed even when OW was sprayed only before grafting, suggesting that OW can be applied to grafted seedling production. The increase in the above-ground dry matter rate in OW treatment suggested that healing between scion and rootstock may have progressed faster than that in CC. Future research should focus on the timing of graft healing to clarify the effects of OW on grafting and investigate the effect on comprehensive gene expression through RNA sequencing.

Acknowledgements
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Reference
Onoue, M., Tatsuzawa, F., Kanayama, Y., Kato, K., 2018. Promotion of plant growth under low temperature by ozonated water at low concentration in komatsuna (*Brassica rapa* L. *perviridis* Group). Ozone-Sci. Eng. 40 (5), 415-419.

P3:
A Novel Approach to Managing Carbon Partitioning Based on the Visual Analysis of Carbon Flow and Vascular Bundle Networks in Tomato Plants

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The core of crop production is managing the transport and allocation of photoassimilate (carbon), specifically the translocation of carbohydrates from source leaves to sink organs (harvest sites) through sieve elements. The process of carbon translocation requires a thorough understanding of the anatomical structures that form the transport pathways between key organs, connected by sieve-tube networks, together with the spatio-temporal dynamics of carbon flow. We are working on developing a multimodal imaging technique to visualize carbon dynamics and sieve-tube arrangement in the plant body with high spatial and temporal resolution, using radiation imaging technologies, specifically positron-emitting tracer imaging system (PETIS) and X-ray computed tomography (X-ray CT). In this study, we aimed to develop a new method for artificially controlling and optimizing carbon flow, based on visualized images of carbon transport pathways.

The tomato cultivar 'Moneymaker' was cultivated in a plastic pot until the fruit clusters developed three tiers, at which point the apical parts, including the fourth inflorescence and the stem above it, were removed. A leaf above the second fruit cluster was treated with 10 mM $BaCl_2$ from the cut surface as a contrast agent and then imaged using the X-ray CT to visualize the arrangement of vascular bundles in the plant body. Carbon-11 (half-life 20 min) labeled CO_2 ($^{11}CO_2$) was assimilated by the leaf in the same position, and the subsequent translocation of ^{11}C-labeled photoassimilates to the fruits was visualized using the PETIS. Based on these imaging results, we identified the sections of the stem through which the pathway of photoassimilates passed from the fruit clusters toward the root system and made a cut approximately 5 mm wide with a blade. After sealing the incision with parafilm to promote wound healing, the same leaf was used to evaluate alterations in photoassimilate transport using the PETIS.

As a result, the translocation pathway of photoassimilates from the leaf to the roots was successfully targeted and served to alter the carbon flow. Specifically, in contrast to the carbon flow observed before the incision, that translocation pathway stopped at the point of the incision in the stem. The operation reduced the amount of ^{11}C-photoassimilates translocated to the roots and significantly enhanced the rate of translocation and the amount of accumulation in the fruit. After four hours, the amount of ^{11}C-photoassimilates accumulated in the fruit cluster had more than tripled. Our results indicate the potential for purposeful design and management of carbon accumulation in the harvest parts, suggesting that tomato fruits have significant untapped production capacity. In the future, we plan to further develop this new approach to enable precise management of carbon translocation and partitioning.

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P4:
Large dataset of exome sequencing from EMS- and gamma ray-induced tomato mutant lines.

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Tomato (*Solanum lycopersicum*) is one of the widely used model plants in the world following the Arabidopsis and rice, and a dwarf variety, cv. Micro-Tom, has various beneficial traits for genetics/gene discovery, e.g. short life cycle under the artificial light conditions (fluorescent tubes or LEDs), easy transformations, and dense cultivation per area, compared to the other commercial varieties. One of the difficulties of tomato genetic research is few numbers of available mutant lines for genetic interactions and epistatic test, for example. To tackle this difficulty, we developed publicly available mutant collections with the support of the National BioResource Project (NBRP) Japan. NBRP-Tomato collected ca. 2700 Micro-Tom mutants induced by ethyl methanesulfonate (EMS) treatment and gamma-ray irradiation with visible phenotypes, such as leaf shape, flower color, fruit development, and so on. The phenotypic information is open in our mutant database called TOMATOMA (https://tomatoma.nbrp.jp/) and the seeds could be available from the same site.

Here, we collected the DNA sequences of exons (exomes) from ca. 700 individual mutants of NBRP-Tomato. Obtained reads were mapped on the tomato reference genome and SNP information was called. To test the data usefulness for reverse genetics, we extracted the mutation on the previously reported genes involved in trichome development by organizing cytoskeletons. Ten probable mutations (nonsense and frame shift mutations) was found out of fifty-five detected mutations in 5 known genes. To confirm the phenotypic contribution of these mutations, we re-grew the candidate lines and found 8 of 10 lines showed the distorted shape of trichomes as shown in the previous report (Chang et al. 2019 PLOS Genet. e1008438). This result suggests the potential effectiveness of our exome data in tomato genetic analysis. We should also test with nonvisible mutations, such as specialized metabolite contents as a next step. The genotypic information should be going to provide from out TOMATOMA database in near future.

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P5:
Altered Photoassimilates Transport in Stem of Starch-Defect Tomato

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Yield is one of the most important traits in crops. Most of the previous studies on yield have focused mainly on source and/or sink organs such leaf, seed and fruit. Although stem plays a role as long-distance transport pathway between source and sink organs, only few studies have focused on the stem. Our works to date is suggesting that the stem is not merely a transport conduit but takes a role in regulation in assimilates partitioning. In a previous study, we had generated the starch-defect lines and confirmed modified sink strength in the fruits. Since it was expected that assimilate transport property is influenced in those lines, in this study, we analyzed assimilate transport profile by the tracer analyses feeding either ^{11}C- or ^{13}C-labeled carbon dioxide (CO_2) to source leaves of wild-type (WT) and the starch-defect lines. Imaging analysis with Positron-Emitting Tracer Imaging System (PETIS) revealed that photoassimilates transport in the stem was faster in the starch-defect lines than the WT. Additionally, the assimilates were transported mainly toward fruit cluster in the WT, whereas it tended to pass through the node and to flow to the base in the starch-deficient lines. Those results indicate that dynamics of the photoassimilates transport was altered in the starch-defect lines. In order to analyze the comprehensive gene expression profile in these lines, we conducted RNA-seq analyses with the node and peduncle tissues. PCA results showed no significant difference between WT and the starch-defect line was observed in the peduncle tissue, whereas it largely changed between the two lines in the node tissue. We are currently screening differentially expressed genes (DEGs) specific to the node tissues and identified several sugar transporters as candidates function in photoassimilates partitioning in the node tissue.

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P6:
Role of the blue light receptor SlFKF1 in tomato fruit coloring

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As the effect of light on fruit coloring is related to the cultivation, keeping, and improvement of fruit quality during distribution and storage, it is important to clarify this mechanism. There are many findings on the effect of light on fruit coloring during cultivation and post-harvesting, and several reports on the influence of light quality, such as red and blue light. When considering the effect of light quality, it is important to consider the role of photoreceptors. In this study, we focused on the FLAVIN-BINDING, KELCH REPEAT, F-BOX1 (FKF1) which is blue light receptor whose role is relatively unknown. According to previous studies, FKF1 is a blue light receptor, that promotes flowering in *Arabidopsis thaliana* under long-day conditions. In addition, the relationship between FKF1 and CONSTSNS (CO) and FLOWRING LOCUS T (FT), which are involved in photoperiodic flowering has been clarified. However, the role of FKF1 homologs in day-neutral plants, including tomatoes, has not been elucidated. To date, it has been clarified that the FKF1 homolog SlFKF1 in tomatoes affects flowering, and it has been suggested that it may affect fruit coloring. Therefore, in this study, we investigated the role of SlFKF1 expression on fruit coloring in tomatoes. We cultivated tomato cv. Micro-tom with the expression of *SlFKF1* suppressed via RNAi. Cultivation was performed at 25℃ with white LEDs for 16 h, and the effects on vegetative growth, flowering, and fruit growth were evaluated. Moreover, to investigate the relationship between SlFKF1 and fruit coloring, the change in fruit color was measured using a spectrophotometer for 20 days after the breaker stage, and the carotenoid content (lutein, β-carotene, and lycopene) was measured 15 days after the breaker stage by UPLC. In addition, RT-PCR was performed for the carotenoid synthesis-related genes. As a result, delayed flowering and an increase in the number of leaflets per leaf were confirmed as characteristic phenotypes because of *SlFKF1* expression suppression. It is known that the promotion of flowering and the number of leaflets per leaf are related to CO and FT and the expression of their homologs, which suggests that SlFKF1 has a role in regulating flowering and plant morphology through the tomato homologs of CO and FT. In addition, when measuring the fruit color, a difference appeared a few days after the breaker stage between the RNAi line and wild type, thus indicating that SlFKF1 suppression affected the fruit coloring, such as a decrease in the red color of the fruit. Moreover, from the results of UPLC and RT-PCR, lycopene content in the middle of the carotenoid synthesis pathway was decreased in the suppressed expression line, and the expression of most carotenoid synthesis-rerated genes upstream of lycopene, such as phytoene synthase 1 and phytoene desaturase, was downregulated in the suppressed expression line. This suggests that SlFKF1 may affect fruit coloring by acting directly or indirectly on genes related to carotenoid synthesis. These results indicate that SlFKF1 plays a multifaceted role in day-neutral plants and tomatoes, which includes flower formation, leaf morphology, and fruit coloration.

P7:
Identification of multi-stress response genes: decoding stress resistance in *Solanaceae* crops.

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In the context of global climate change, agricultural systems are increasingly challenged by various stresses, which undermine crop productivity and food security. In this scenario, it is essential to identify genes potentially involved in responses to multi-stress. Plant receptor genes are crucial components of the plant response to stresses. Among them, NBS-LRR (nucleotide-binding site leucine-rich repeat) genes form the largest class of R genes that play a critical role in plant defense. This study aims to identify genes involved in the response to various stresses in *Solanaceae* crops, including tomato, potato, eggplant and pepper, with a specific focus on R genes. Twelve publicly available RNA sequencing (RNA-seq) data of resistant and susceptible tomato genotypes exposed to both biotic and abiotic stresses were reanalyzed. Within each experiment, differentially expressed genes (DEGs) showing consistent expression changes during different stresses were identified. Differentially expressed R genes were also re-annotated based on a genome annotation of *S. lycopersicum* (v.3.0), previously performed by our group. This annotation was conducted both manually and through a pipeline that identifies R gene domains. We detected a total of 166 R genes expressed during abiotic stress and 677 R genes during biotic stress. The percentages of R genes identified varied according to different stresses with notable results such as 71.92% and 28.26% R genes out of total DEGs under *Phytophthora infestans* infection and low temperature stress respectively. This study provides a valuable list of genes potentially involved in multi-stress responses in tomato crop. Leveraging the developed pipeline, the research was extended to other Solanaceae crops, incorporating further 80 transcriptomic experiments. The analysis of large datasets can help us lay the foundation for future genetic research aimed at improving crop resilience and developing different varieties with greater resistance to different environmental challenges.

P8:
Fine mapping of *HLA1* locus causing hybrid lethality in interspecific hybrids of *Nicotiana* using bulked segregant RNA-Seq

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Hybrid lethality, a postzygotic mechanism of reproductive isolation, is a phenomenon where F_1 hybrid seedlings undergo genetic death. Hybrid lethality is generally caused by the epistatic interaction between two or more loci. In the genus *Nicotiana*, hybrid lethality occurs in interspecific crosses between the cultivated species *N. tabacum* of the section *Nicotiana* and most wild species of the section *Suaveolentes*. This hybrid lethality is caused by the interaction between the dominant allele *Hla2-1* at the *HLA2* locus in *N. tabacum* and the dominant allele *Hla1-1* at the *HLA1* locus in wild species of the section *Suaveolentes*. *Hla2-1* has been isolated, and has been revealed to be an NBS-LRR resistance gene. In contrast, *HLA1* has been genetically mapped to the linkage map, but its precise location remains unclear, and it has not yet been isolated. Therefore, to identify the precise location of *HLA1*, we performed bulked segregant RNA-Seq (BSR-Seq) using progeny from a triple-cross involving *N. debneyi* carrying the *Hla1-1* allele, *N. fragrans* carrying the recessive allele *Hla1-2* at the *HLA1* locus, and *N. tabacum*. Furthermore, using DNA markers developed based on the SNPs detected by BSR-Seq, we conducted linkage analysis and fine mapping of *HLA1*. Additionally, we analyzed the expression levels of the genes in the identified candidate region. As a result of BSR-Seq, *HLA1* was mapped to three scaffolds, Nbe.v1.1.chr12, Nbe.v1.1.chr15, and Nbe.v1.1.chr19, in the *N. benthamiana* genome. *N. benthamiana* was utilized because it is a closely related species belonging to the same section as *N. debneyi* and *N. fragrans*, and it serves as a model plant with a reference genome sequence available, unlike *N. debneyi* and *N. fragrans*. Linkage analysis revealed that all the markers designed on Nbe.v1.1.chr12, Nbe.v1.1.chr15, and Nbe.v1.1.chr19 were linked to each other in the F_2 segregating population of the *N. debneyi* × *N. fragrans*, and were also linked to *HLA1*. This result indicated that, although *N. debneyi*, *N. fragrans*, and *N. benthamiana* are closely related species within the monophyletic section *Suaveolentes*, significant differences exist in their chromosomal structures, suggesting that large-scale genome rearrangements occurred during the evolutionary history of the section *Suaveolentes*. Additionally, it was found that *HLA1* is located within a 2.8 cM interval between the SNP markers Nbs7_89 and Nbs7_96 on Nbe.v1.1.chr12. Fine mapping revealed that *HLA1* was located between the DNA markers Nb12_540 and Nb125. The distance between these markers corresponds to a 413 kb interval in the genome sequence of *N. benthamiana*, within which 13 genes are located. Finally, gene expression analysis suggested that *Nbe.v1.1.chr12g25860*, *Nbe.v1.1.chr12g25870*, *Nbe.v1.1.chr12g25880*, and *Nbe.v1.1.chr12g25960* are prominent candidates for *HLA1*.

Acknowledgements
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P9:
Physiological Study on Quantitative Traits Related to Fruit Size Using Introgression Lines of Tomato

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Fruit size is a critical factor in determining tomato yield, and improving fruit size is a major goal of breeding programs. In this study, we investigated the fruit size of tomato introgression lines (ILs) in which chromosome 12 of *Solanum lycopersicum* 'M82' was replaced with a chromosome from its wild relative *Solanum pennellii*. We compared fresh weight, dry weight, dry matter ratio, diameter, and fruit height among the ILs (IL12-1, IL12-1-1, IL12-2, IL12-3, and IL12-3-1) and found that only IL12-1-1 showed an increase in all parameters compared to those of 'M82'. A comparison of the cell number per unit area of fruit skin at different developmental stages between 'M82' and IL12-1-1 showed that IL12-1-1 had an increased cell number at 20 days after flowering (DAF). We then measured the concentrations of the phytohormones, auxins and cytokinins, in early stage fruits (10 and 20 DAF). The results showed that IL12-1-1 exhibited a higher auxin concentration at both 10 and 20 DAF compared to that shown by 'M82'. Similarly, cytokinin concentration was higher in IL12-1-1 at 20 DAF. These findings suggest that the increased fruit size in IL12-1-1 is due to the increased number of cells in the fruit skin during the early developmental stages. Subsequently, we analyzed the expression levels of two genes located in the chromosomal region replaced by *S. pennellii* in IL12-1-1: *Solyc12g005250*, which is suspected to be involved in cell division, and *Solyc12g005310*, which is believed to contribute to auxin regulation in fruits at 10 and 20 DAF. The results indicated that *Solyc12g005250* showed higher expression in IL12-1-1 than in 'M82' at both 10 and 20 DAF, whereas *Solyc12g005310* showed higher expression only at 10 DAF. These findings suggest that the increased fruit size in IL12-1-1 plants may be caused by the high expression of either *Solyc12g005250* or *Solyc12g005310*. Based on these results, we are currently exploring *cis*-elements, the regions in promoters where transcription factors bind, using bioinformatic approaches. We are also advancing genome editing techniques that target mutations in the *cis*-elements of *S. pennellii*.

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P10:
Identification and characterization of a novel leaky *VAMT* allele in chili pepper (*Capsicum chinense*)

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Capsaicinoids are the compounds responsible for pungency in chili pepper. They are utilized for food industrial and medical purposes. Despite these benefits, the further utilization of capsaicinoids is limited due to their intense pungency. Capsinoids, low pungent capsaicinoid analogues, have been reported to have similar benefits as well or better utilization as food additives. In general, pungent varieties contain mainly capsaicinoids, with only trace amounts of capsinoids. Our previous analyses of chili pepper genetic resources have shown that the pungent variety 'Charapita' (*Capsicum chinense*) has a unique composition with a higher capsinoid content than other pungent varieties (Tanaka et al. 2009). This study aimed to identify mutation responsible for the high capsinoid content in 'Charapita'.

The QTL analysis was conducted using the F_2 population derived from a cross between 'Red Habanero' (RH) and 'Charapita' (CH). A major QTL affecting the capsinoid content was detected on chromosome 3. By further genetic analysis, the QTL was narrowed down to approximately 400 kb region, which includes *VAMT* (*vanillin aminotransferase*). Previous studies have reported that the loss-of-function mutation of *VAMT* inhibits capsaicinoid production and increases capsinoid content. Allelism test crosses between 'Belize sweet' (loss-of-funciton *vamt*) x CH resulted in F_1 hybrid with high capsinoid content, demonstrating that CH possesses a leaky *VAMT* allele. The sequencing analysis revealed CH-type *VAMT* allele encodes 459 amino acids as RH, but it has a unique amino acid substitution (G → E) due to a SNP in exon 15 (Fig.1). CH-type *VAMT* increased capsinoid content by about 2.5 times compared with RH-type. qRT-PCR demonstrated that there was no difference in the expression levels of *VAMT* or other capsaicin biosynthesis-related genes between RH and CH. The vanillylamine (V-NH_2) synthesis activity was evaluated using crude protein suspensions extracted from placental septum. It showed that the V-NH_2 synthesis activity in CH was less than 1/30 compared with RH. Given that the enzyme activity significantly decreased with no transcriptional change, the amino acid substitution likely reduces VAMT activity, conferring the characteristic composition of capsaicinoids and capsinoids in CH.

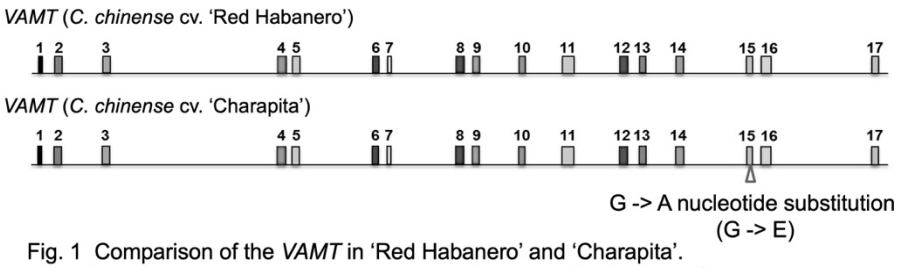

Fig. 1 Comparison of the *VAMT* in 'Red Habanero' and 'Charapita'.
'Charapita' *VAMT* has a single nucleotide substitution in the 15th exon region.
Letters in parenthesis indicate amino acid.

P11:

Trichome development and terpene accumulation are controlled by the *Hairless-3* gene in tomatoes

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Trichomes are specialized epidermal structures that play a vital role in plant defense against environmental stresses. However, the genetic mechanisms governing trichome development in tomatoes are not well understood. This study aimed to identify the genes involved in trichome formation, morphology, and terpene biosynthesis through transcriptomic analysis. We examined leaf morphology and compared the expression of candidate genes related to trichome formation in wild-type (WT) and *hairless-3* (*hl-3*) tomato mutants. The *hl-3* mutants exhibited swollen and distorted trichomes, reduced trichome density (types I and IV), and diminished terpene synthesis relative to WT plants. Gene expression analysis indicated that *Actin-Related Protein Component1* (*ARPC1*) was significantly upregulated in WT compared to the *hl-3* mutant, suggesting its critical role in trichome morphology and density. Moreover, the expression levels of *MYC1* and several terpene synthase genes (*TPS9, 12, 20*), associated with type VI trichome initiation and terpene synthesis, were lower in the *hl-3* mutant than in WT plants. The introduction of WT *ARPC1* into the *hl-3* mutant restored normal trichome structure and density, as well as terpene production. Structural and amino acid sequence analysis revealed a missplicing mutation in *ARPC1* in the *hl-3 mutant*, responsible for the abnormal trichome characteristics and impaired terpene synthesis. In conclusion, this study demonstrates that *ARPC1* is essential for regulating trichome development and terpene biosynthesis in tomatoes.

Acknowledgements

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P12:
NtbHLH18 is a jasmonate-responsive transcription factor that regulates the biosynthesis of nicotine in tobacco

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Nicotine is one of the primary alkaloid metabolites in tobacco and plays a crucial role in the plant's defense against insect pests. The biosynthesis of nicotine is regulated by various complex factors, such as hormonal signals, growth environments, developmental stages, and genetics. In this study, a new transcription factor, NtbHLH18, was identified in tobacco (*Nicotiana tabacum*). Phylogenetic analysis indicates that NtbHLH18 is homologous to Arabidopsis proteins AtbHLH18, AtbHLH19, AtbHLH20, and AtbHLH25, sharing a close evolutionary relationship and belonging to the IVa subfamily of the bHLH protein family. In contrast, previously identified proteins NtMYC2a/b and NbbHLH1/2 belong to the IIIe subfamily of the bHLH protein family. The expression pattern of NtbHLH18 in different tobacco tissues was analyzed using quantitative PCR (qPCR), revealing that *NtbHLH18* is specifically highly expressed in roots. Expression analysis under various hormone treatments showed that the expression of *NtbHLH18* is significantly induced by methyl jasmonate (MeJA), while auxin has a certain inhibitory effect on the expression of *NtbHLH18*, indicating that *NtbHLH18* is a jasmonate-responsive transcription factor. Subcellular localization analysis demonstrates that NtbHLH18 is localized in the nucleus. Utilizing RNA interference (RNAi) technology, we have created transgenic tobacco materials with significantly reduced expression of the *NtbHLH18* gene. Liquid chromatography-tandem mass spectrometry was employed to measure nicotine content in RNAi lines, and it was found that the nicotine content in *NtbHLH18*-RNAi plants was markedly decreased compared to wild-type tobacco. Further gene expression analysis revealed that the expression of nicotine biosynthesis-related genes, such as *PMT1* and *BBL1*, was significantly repressed, suggesting that NtbHLH18 is involved in the nicotine biosynthesis process by targeting the expression of genes involved in nicotine synthesis. This research provides new genetic targets and theoretical foundations for a deeper understanding of nicotine biosynthesis.

P13:
Genetic analysis of Three Loci Responsible for Capsaicinoid Biosynthesis Through an EMS-Induced Mutant in *Capsicum annuum* L.

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Capsaicinoids, unique alkaloids found in peppers (*Capsicum* spp.), are synthesized via the condensation of byproducts from the phenylpropanoid and branched-chain fatty acid pathways, and accumulating in the placenta. In this study, we characterized an allelic ethyl methanesulfonate-induced mutant line with significantly reduced pungency, designated as '1505-5-6i'. This mutant, derived from the pungent Korean landrace 'Yuwolcho', exhibited a lower capsaicinoid content compared to Yuwolcho but still retained small amounts, with an abnormal reduction in capsaicin levels relative to dihydrocapsaicin, as well as novel phenolic compound. A genetic cross between the mutant and a pungent pepper line 'Micro-Pep' suggested that three recessive mutations are responsible for the observed phenotype of '1505-5-6i'. We designated the causal loci as *Pun5.1* (responsible for the reduction in capsaicin content), *Pun5.2* (responsible for the reduction in overall capsaicinoid content), and *Pun5.3* (responsible for the presence of the novel phenolic compound). To identify the genes associated with these loci, we employed a combination of genome-wide polymorphism analysis and transcriptome analysis with bulked-segregant analysis using the Yuwolcho genome as a reference. This approach allowed us to narrow down the locations of *Pun5* genes to a 2-Mb region on chromosome 1 (containing two candidate genes for *Pun5.1*), a 150-200 Mb region on chromosome 2 (containing four candidate genes for *Pun5.2*), and a 262-Mb region on chromosome 9 (containing two candidate genes for *Pun5.3*). Functional analysis of these candidate genes is expected to lead to the identification of causal genes.

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P14:
Discovering Disease-Resistance Genes via Pan-Genome Analysis in Pepper

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Pepper (*Capsicum annuum* L.) is a significant horticultural crop with substantial economic importance. To date, a considerable amount of pepper genome information has been reported, including approximately 30 genome assemblies and three pan-genomes. Nevertheless, the application of these pan-genomes to agricultural studies has been limited due to the lack of reliable re-sequencing and phenotype datasets in pepper. To address these issues, we have updated the pepper pan-genomes by integrating high-quality genomes and reformatting the pan-genome variant information into a graph-based structure. To precisely annotate and compare the sequence variation of pepper disease resistance genes, we employed resistance gene enrichment sequencing (RenSeq) technology, a targeted enrichment sequencing method for nucleotide-binding leucine-rich repeat (NLR) genes. The updated pepper pan-genome analysis catalogued genetic variations across 26 *C. annuum* genomes and identified several candidate genes associated with disease resistance in pepper. Notably, two candidate genes for resistance against root-knot nematodes and ChiVMV were discovered. The candidate genes for root-knot nematode resistance were found on chromosome 9 and characterized by small indels in their exon regions. The candidate gene for ChiVMV resistance was located on chromosome 6 and marked by three SNPs in its exon region. To validate these findings, we cloned the candidate gene for potyvirus resistance and demonstrated a significant interaction between this gene and ChiVMV. These findings are crucial for advancing omics-based studies and disease-resistance breeding programs in pepper.

Acknowledgements
This work was carried out with the support of "Project RS-2023-00231366" Rural Development Administration, Republic of Korea.

P15:

Identification and Functional Validation of Novel Genes Involved in Capsaicinoid Biosynthesis in *Capsicum annuum* L through Transcriptome Analysis

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Capsaicinoids are unique secondary metabolites synthesized exclusively in *Capsicum* species, conferring the characteristic pungency that has made peppers commercially significant as spices. Previous studies have functionally validated several genes—namely, acyltransferase (*Pun1*), putative aminotransferase (*pAMT*), CaMYB31 (*Pun3*), and putative ketoacyl-ACP reductase (*KR*)—as contributors to the capsaicinoid biosynthetic pathway. Despite these advancements, the complete biosynthetic pathway and its regulatory network remain inadequately understood. Moreover, the mechanisms regulating capsaicinoid biosynthesis in the pericarp tissues of specific *Capsicum* species are yet to be elucidated. In this study, transcriptomic data from pepper placenta and pericarp tissues, derived from previous research, were analyzed to identify candidate genes implicated in capsaicinoid biosynthesis. Differentially expressed gene (DEG) analysis and Weighted Correlation Network Analysis (WGCNA) were used to uncover potential candidate genes. By aligning these genes with Quantitative Trait Loci (QTL) positions identified in prior studies, three genes—*Pun1*, *pAMT*, and *G3PAT*—were pinpointed in the placenta transcriptome, and two genes—*KR* and *HDG11L*—were identified in the pericarp transcriptome. To validate the functional roles of these genes, virus-induced gene silencing (VIGS) was utilized. Silencing of *G3PAT* resulted in a significant reduction of capsaicinoid content in the placenta of pepper fruits, accompanied by alterations in the expression of genes within the phenylpropanoid pathway. In addition, we silenced candidate genes identified through transcriptome analysis that did not align with known QTLs, resulting in the identification of five genes potentially associated with capsaicinoid biosynthesis. To achieve functional validation with knock-out mutation of *G3PAT* and five candidate genes, genome editing constructs were developed and applied to *Cas9*-transformed pepper plants. These findings offer insights into the capsaicinoid biosynthetic pathway and contribute to a deeper understanding of its underlying mechanisms.

Acknowledgements
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2021R1A2C2007472)

P16:
Identification of genes involved in multi-cellular trichome development in tomato

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Plants utilize defensive mechanisms against insect pests, with trichomes derived from epidermal cells playing a crucial role in plant defense. These structures hinder insect access and release chemical compounds that exhibit repellent and insecticidal properties. This study focuses on the genetic basis of trichome development in tomato as a potential resource for enhancing insect resistance. Two genes, *Sol20* and *Sol14*, hypothesized to regulate trichome development, were selected for analysis. We generated knockout (ko) mutants using the CRISPR-Cas9 system. Trichome development in *sol20* single ko (sko) and *sol14* sko mutants did not differ from wild-type (WT) plants; however, *sol20/sol14* double ko (dko) mutants exhibited a significant reduction in trichome number and length compared to WT and sko plants. To elucidate the regulatory mechanism of trichome development by *Sol20* and *Sol14*, transcriptomic analysis was performed on *sol20/sol14* dko and WT plants. Differentially expressed genes (DEGs) were extracted between *sol20/sol14* dko and WT plants. Gene ontology (GO) enrichment and KEGG pathway analysis revealed that *Sol20/Sol14* mutations affect various biosynthetic processes and transcription-related activities. Furthermore, we compared the expression profiles of known trichome regulators between *sol20/sol14* dko and WT plants. Future investigations will focus on direct regulatory mechanisms of target genes influenced by *Sol20* and *Sol14*. This research provides a foundation for exploring the potential of trichomes as breeding materials for developing insect-resistant tomato varieties.

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P17:
Identification of Key Genes in the Jasmonate Signaling Pathway for Enhanced Insect Resistance in Tomato

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To combat biotic stresses from insect herbivores and pathogens, plants rely on the jasmonic acid (JA) signaling pathway, which regulates plant immune responses. Activation of this pathway stimulates the production of secondary metabolites like terpenes and acyl-sugars and promotes trichome formation, thereby strengthening physical defenses. In stress-free conditions, JR (Jasmonic Repressor) proteins serve as negative regulators of the JA pathway. Upon stress-induced conversion of JA to JA-Ile, JR proteins degrade, triggering the expression of downstream genes, including transcription factors (TFs) that interact with JR. This study aims to enhance insect resistance in tomatoes by targeting JR proteins. A yeast two-hybrid (Y2H) assay was conducted to investigate interactions between 11 cloned SlJR proteins and potential target genes. The system was validated by confirming interactions between SlJR and known trichome regulators SlMYC1 and Wo, suggesting a role for SlJR proteins in trichome development. To further explore the function of SlJR in trichome formation, SlJR knockout plants were generated using CRISPR-Cas technology. These findings will deepen our understanding of JA-mediated defenses and trichome development, contributing to the development of insect-resistant tomato varieties.

Acknowledgments
This study was supported by grants from the New Breeding Technologies Development Program (RS-2024-00322125) from the Rural Development Administration, Republic of Korea, and by the Basic Science Research Program (NRF2022R1A2C1008643) through the National Research Foundation of Korea funded by the Ministry of Education, Republic of Korea. Also, this work was supported by the BK21 FOUR, Global Smart Farm Educational Research Center, Seoul National University, Seoul, Korea.

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P18:
Molecular Mapping of *cmr2* for breeding CMV-P1 Resistant Pepper Cultivars

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Cucumber mosaic virus (CMV) is one of the most widespread and destructive plant viruses in pepper production, causing mosaic or necrotic symptoms in pepper leaves. Numerous studies have investigated pepper CMV resistance, particularly focusing on the *Cucumber mosaic resistant 1* (*Cmr1*) and *CMV resistance gene 2* (*cmr2*) genes. *Cmr1* confers resistance against the CMV-P0 strain through dominant inheritance, while *cmr2* provides resistance to the evolved CMV strain (CMV-P1) with recessive inheritance. *cmr2* was first identified in *C. annuum* 'Lam32' and initially mapped to 2.3 cM on chromosome 8. However, the precise physical location of the *cmr2* remains uncertain. In this study, we investigated the location of the *cmr2* gene in a *C. annuum* 'Jeju' x 'Lam32' F_2 population using bulked segregant analysis by RNA sequencing (BSR-seq). Our analysis identified 11 single nucleotide polymorphisms (SNPs) located on chromosome 8 that are potentially linked to *cmr2*-mediated resistance. Based on these SNP information, we mapped the genetic position of *cmr2* to 1.7-4 cM on chromosome 8. These newly identified markers are closer to *cmr2* compared to the previously developed 2.3 cM markers, Affy4. Furthermore, our SNP markers demonstrated broader applicability across diverse pepper germplasm compared to Affy4. These findings contribute not only to revealing the precise physical position of *cmr2* but also to the breeding of CMV-P1 resistant pepper cultivars.

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P19:
Identification of a trichome development gene important for pest resistance in tomato

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Abstract
Trichomes are morphologically diverse appendages of the outer epidermis of plants and are found on the surfaces of various species. They play a crucial role in protecting plants from abiotic stresses through their physical structure and secretion of chemical compounds with repellent or insecticidal effects. These defenses help reduce pest damage, which is a significant issue in global tomato cultivation. Tomato production, projected to reach 222 million tons by 2030, has been hindered by pests, causing a yield reduction of approximately 20-30%. This research aims to develop tomatoes with increased pest resistance by modulating trichome development. We selected candidate genes implicated in trichome development based on previous studies and generated *sol12* knock-out (ko) plants using the CRISPR-Cas9 system to investigate its role in trichome formation. Preliminary results suggest differences in trichome numbers between *sol12* ko and WT plants. Future research will focus on phenotypic analysis of T_1 plants to assess changes in trichome development, with the ultimate goal of improving insect resistance in tomato crops.

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P20:

The tomato *juhwang* mutant exhibits elevated levels of β-carotene and increased shelf-life

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Tomato ripening (*Solanum lycopersicum*) is characterized by carotenoid accumulation and fruit softening. This study employed a mutagenesis approach on wild-type tomato (cv. Micro-Tom) using proton treatment at the Korea Atomic Energy Research Institute to identify genetic mutations affecting fruit traits. We characterized a newly identified single-recessive mutant, designated *juhwang* (*jh*), to assess its impact on fruit color and shelf life during ripening. Results indicated that *jh* fruit exhibited a distinct orange color compared to wild-type (WT) fruit. Carotenoid analysis showed a reduction in lycopene and an increase in β-carotene levels in *jh* after the completion of ripening. Furthermore, *jh* demonstrated a longer shelf-life than WT, with no notable developmental differences aside from the orange coloration and enhanced shelf life. Gene expression analysis of carotenoid synthesis, ethylene production, and cell wall softening was conducted using quantitative reverse transcription polymerase chain reaction. Ongoing research involves the identification of the *jh* mutation through map-based cloning. This study highlights the potential of the *jh* mutant as a valuable genetic resource for enhancing fruit quality and shelf-life in tomatoes.

Acknowledgements
This study was supported by grants from the New Breeding Technologies Development Program (RS-2024-00322125) from the Rural Development Administration, Republic of Korea, and by the Basic Science Research Program (NRF2022R1A2C1008643) through the National Research Foundation of Korea funded by the Ministry of Education, Republic of Korea. Also, this work was supported by the BK21 FOUR, Global Smart Farm Educational Research Center, Seoul National University, Seoul, Korea.

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P21:
Application of transcriptome sequencing to identify candidate genes contributing to PVMV resistance in tomato.

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Pepper veinal mottle virus (PVMV), a member of the Genus *Potyvirus*, was first identified infecting tomatoes in Taiwan in 2006, leading to symptoms such as leaf necrosis and stunted growth. The virus, which can be transmitted through contact and by aphids, poses a significant threat to tomatoes, resulting in substantial yield losses. Understanding the genetic mechanisms underlying resistance to PVMV is crucial for developing resistant tomato varieties. Previous studies have shown that the eukaryotic translation initiation factor 4E (eIF4E) is a key component in plant cellular translation and plays a crucial role in *Potyvirus* infection. Mutations in eIF4E can confer resistance to *Potyvirus* by disrupting the virus's ability to hijack the host's translation machinery. In this study, two wild tomatoes, *S. pimpinellifolium* L. and *S. peruvianum* L., were identified as tolerant and immune to PVMV, respectively. To further uncover candidate genes responsible for *Potyvirus* resistance, we conducted a comparative transcriptomics analysis of before and after the inoculation plants of these two accessions. The differentially expressed genes identified in this study will undergo further validation to elucidate theirs role in resistance, offering valuable insights that contrast with known susceptibility mechanisms.

Acknowledgements
This study was supported by Ministry of Agriculture, Taiwan, though the project of Taiwan-Thailand Strategic Research Alliance to Promote Sustainable Tomato Production. We also thank long-term strategic donors to the World Vegetable Center: from Taiwan, Germany, Thailand, Philippines, South Korea, Japan, UK, USAID and ACIAR.

P22:
QTL analysis for capsaicinoid content in Aji pepper (*Capsicum baccatum*)

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Chili peppers (*Capsicum*) are globally important spices and vegetables. There are five domesticated species, and *C. baccatum* is less known than major domesticated species *C. annuum*. But it is known as Aji and consumed in South America. *C. baccatum* includes disease resistance and unique flavor compounds, which are different from *C. annuum*. Therefore, *C. baccatum* has the potential to genetically improve chili peppers worldwide. Our previous research evaluated capsaicinoid content in *C. baccatum* accessions. It revealed that whereas most of the accessions were pungent, one accession ('NP-1') exhibited no capsaicinoid. 'NP-1' will be potential bioresource for sweet pepper breeding in *C. baccatum*. The mechanism underlying non-pungency in *C. baccatum* is less understood. This study aimed to identify genetic loci related to capsaicinoid content in 'NP-1'.

Two *C. baccatum* accessions were used for this study: a non-pungent accession 'NP-1' (capsaicinoid content: 0 μg/gDW) and a pungent accession 'Arivivi' (capsaicinoid content: 3000 μg/gDW). qRT-PCR analysis revealed that transcription factors and structural genes involved in capsaicinoid biosynthesis were highly expressed in 'Arivivi', but most of them were hardly expressed in 'NP-1'. This result suggests that the entire capsaicinoid biosynthesis pathway is slow down due to low expression level of transcription factors, resulting in non-pungency in 'NP-1'. For genetic analysis, F_2 population derived from cross between 'NP-1' and 'Arivivi' was constructed. The F_2 population exhibited continuous distribution in capsaicinoid content, with an average content of 1106 μg/gDW, and a range from 7.9 to 3379 μg/gDW (Fig. 1). A significant QTLs affecting the capsaicinoid content were detected on chromosome 2, 4, and 11 (qCAP2, 4, 11). This result suggests that the non-pungency in 'NP-1' may be caused by the additive effect of multiple QTLs. Among them, qCAP4 had the highest LOD score. The relationship between qCAP4 genotypes and capsaicinoid content was investigated. It showed that the capsaicinoid content in 'Arivivi' homozygote plants were more than twice than that of 'NP-1' homozygote plants (Fig. 2). A known capsaicinoid biosynthetic gene *BCAT1* was located nearby qCAP4. Thus, the relationship between qCAP4 and *BCAT1* were checked by additional DNA makers. The maker analysis confirmed that *BCAT1* was located outside the QTL affecting capsaicinoid content. This result indicates that the causative gene in qCAP4 is not *BCAT1*, and an unknown capsaicinoid biosynthesis gene within the qCAP4 region is involved in controlling capsaicinoid content in *C. baccatum*.

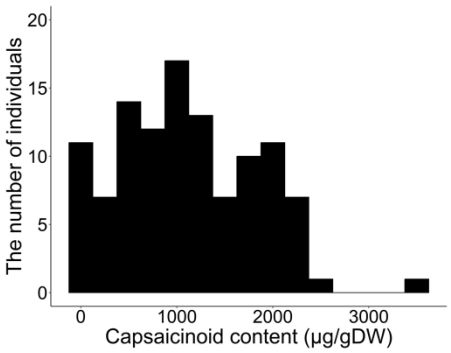

Fig. 1 Frequency distribution of capsaicinoid content in the F_2 population

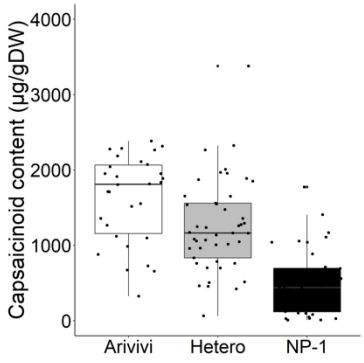

Fig. 2 Relationship between qCAP4 genotypes and capsaicinoid content in the F_2 population

P23:
Enhancing virus-mediated genome editing for cultivated tomato by low temperature

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The CRISPR/Cas system, a powerful gene-editing technology, has significantly advanced plant breeding by enabling precise genetic modifications. Developing simple and highly efficient genome editing tools for crops is essential for their effective implementation in plant breeding. We have enhanced the virus-induced genome editing (VIGE) system for cultivated tomato (*S. lycopersicum* cv. Moneymaker) by optimizing it for low temperature conditions. Using TRV and PVX vectors, we delivered sgRNA targeting *phytoene desaturase* (*SlPDS*), along with mobile RNA elements, such as tFT or $tRNA^{Ileu}$, into Cas9-overexpressing Moneymaker. Our results demonstrated that low temperature incubation improved gene editing efficiency in somatic cells. However, mutant progeny was not obtained from MM-Cas9 plants infected with TRV or PVX. We addressed this challenge through tissue culture methods. By applying low temperature conditions at the initiation stage of tissue culture, we increased the mutation rate of both vectors, resulting in a >70% mutation rate for *SlPDS* in regenerated shoots. The virus-free, gene-edited progenies were successfully obtained. This study offers an efficient strategy to enhance VIGE efficiency in cultivated tomato, accelerating the development of gene-edited lines for tomato breeding.

Acknowledgements
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P24:
Genetic Study of Changes in Apical Dominance in Tomato Inflorescences

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The inflorescence is one of the stems that efficiently arrange flowers to enhance reproductive success in flowering plants. In most wild tomato species, the inflorescence stem sustains the apical dominance to the top of the plant, attracting pollinators but also competing with the apically top position against the sympodial shoot stem. Here, we hypothesize that tomato inflorescences have been domesticated to reduce apical dominance and suppress inflorescence branching, promoting the most optimal fruit production in the plant system.

In this study, we report that enhanced apical dominance of the inflorescence stem was highly associated with delayed maturation of inflorescence meristems and increased inflorescence branches, that observed in mutants showing delayed maturations in the reproductive period. In *sft*, *mc*, and *j* mutants, delayed SAM maturation leads to stronger apical dominance and increased inflorescence branches. Inflorescence branching mutants such as *s*, *an*, and *fa*, which exhibit delayed inflorescence development, also show increased apical dominance of the inflorescence stem. Interestingly, strong apical dominance is associated with elongated peduncles, suggesting delayed maturation may influence apical dominance.

Therefore, we suggest that the maturation of shoot apical meristem (SAM) in the reproductive stem was promoted during the domestication of tomato, resulting in accelerated inflorescence development and flowering after transitional meristem (TM) from vegetative to reproductive of main stem, compared to wild tomato species.

P25:
Reassessing the contribution of *TAGL1* and *SPL-CNR* to fruit ripening by CRISPR/Cas9 mutagenesis

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Fruit ripening in tomato is a complex process regulated by multiple genetic factors, including *TOMATO AGAMOUS LIKE 1* (*TAGL1*) and *SQUAMOSA promoter binding protein-like* (*SPL*)-*CNR*. Using CRISPR/Cas9 mutagenesis, we re-evaluated the roles of these key regulators. *TAGL1*- and *SPL-CNR*-edited lines exhibited delayed ripening, reduced carotenoid accumulation, decreased ethylene biosynthesis, and altered volatile profiles. These mutants also showed downregulation of key genes involved in ripening, including those related to ethylene production, cell wall modification, and carotenoid synthesis. CRISPR/Cas9-edited *SPL-CNR* quantitatively controls tomato fruit ripening in contrast to the *Cnr* mutant. Ripening fruits in the double mutant of *rin* and *tagl1* showed a more extreme phenotype compared to the *rin* mutant. Mutagenesis of *TAGL1* and *SPL-CNR* by CRISPR/Cas9 strengthens their regulatory functions controlling ripening parameters and provides new insights into fruit ripening.

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This work was supported by the New Breeding Technologies Development Program (RS-2024-00322217), Rural Development Administration, and the National Research Foundation of Korea grant funded by the Korea government (MSIT) (2021R1A2C2093789).

P26:
The chromosome-scale *Solanum nigrum* genome sheds lights on its origins and on its drought response

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The black-fruited, hexaploid *Solanum nigrum* (2n= 6x= 72), might have originated from a tetraploid *S. villosum* and diploid *S. americanum*. *S. nigrum* exhibits a broad geographical distribution, encompassing regions across Europe, Asia, Africa, and Australia. There, *S. nigrum* is growing in disturbed areas including urban areas. *S. nigrum* is known to be a medicinal source of polyphenols, alkaloids, and anthocyanins and is sometimes used as a vegetable. However, a complete chromosome-scale genome is missing.

This study presents a *de novo* chromosome-scale genome assembly for *S. nigrum*, generated from Oxford Nanopore (ONT) long-read DNA sequencing and Pore-C data including the analysis of whole DNA methylation patterns. As for tomato and potato, we could observe a relative enrichment of genes towards the chromosome ends whereas repetitive elements were more prevalent toward the middle of the chromosomes which was correlated with CG and CHG methylation contexts.

To understand the evolutionary development of hexaploidy, a comparative analysis between *S. nigrum* and the diploid progenitor species *S. americanum* was carried out by analyzing chromosome triplication, genome synteny, and gene distribution. Subgenome sorting of *S. nigrum* revealed one group of triplicated chromosomes which was structurally more related to *S. americanum* than the other two subgenomes.

To evaluate the potential drought stress tolerance of *S. nigrum*, generation of omics data from plants grown under drought stress has been conducted. Data on the genome of *S. nigrum* and its comparison to progenitor species as well as its response to drought will be presented.

Acknowledgments

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P27:
Establishing an analysis platform for *Nicotiana benthamiana* genome and transcriptome

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Nicotiana benthamiana is widely used as a model plant for dicotyledonous angiosperms. In fact, the strains used in research are subject to plant pathology and plant-microbe interactions. In terms of plant-plant interactions, *N. benthamiana* is one of the plants that exhibit grafting affinity with plants from different families. Thus, *N. benthamiana* is a good model for plant biology, however, *N. benthamiana* has a complex genome structure called allotetraploid, , which has prevented detailed analysis of its genome structure for many years. Using the latest next-generation sequencing technology to decode long sequences, we have revealed a genome structure as 21 scaffolds covering 2.8 Gb, 95.6% of the genome. This resulted in a highly reliable estimate of 57,583 gene annotations. Following our decoding report, several institutes reported the results of their genome analyses. Based on these comparisons, we reconstructed the chromosomes as 19 pairs. Based on the obtained gene annotation information, we performed RNA-seq in various parts, organs, and growth stages of the plant, and constructed a gene expression profile database of *N. benthamiana*. An integrated analysis platform centered on these genome assemblies and gene expression databases was constructed on the web. This is expected to lead to the development of further utilization of *N. benthamiana* for its useful properties and the advancement of genetic analysis of interactions between organisms in plants.

Acknowledgements

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P28:
Effect of Starch-deficiency on Fruit Metabolism in Tomato

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Starch is a major storage carbohydrate in plants. In tomato (*Solanum lycopersicum* L.) fruit, starch accumulates at immature- and mature-green stages with high level and disappeared during fruit ripening. In previous study, we had reported starch gives an impact on fruit sugar contents under salt-stress condition but not in ordinary circumstance. In order to elucidate physiological roles of starch in fruit metabolism, we generated starch-defect lines in which the expression of ADP-glucose pyrophosphorylase (AGPase) genes was suppressed by RNAi.

In the *AGPase*^{RNAi} lines, significant difference was not observed in fruit ripening process whereas fruit firmness reduced compared to the wild-type. Detail characterizations of the *AGPase*^{RNAi} fruit showed i) soluble sugar contents in red-ripe fruit reduced by maximum around 30%, however impact of the starch deficiency was limited, ii) pectin content in red-ripe fruit was decreased by maximum around 60%, iii) thickness of fruit cuticle layer was reduced compared to the WT fruit. Those results suggest starch degradation products arisen during ripening are utilized as substrates for secondary cell wall and cuticle. In order to clarify the effect of starch deficiency on transcription of pectin- and cutin-metabolic enzyme genes, quantitative real-time PCR (qRT-PCR) analyses were conducted with the starch-defect and WT fruit at four developmental stages: immature green, mature green, yellow, and red-ripe. The transcriptional analyses revealed i) the pectin-metabolic pathway is upregulated in its biosynthesis at fruit developing stages and downregulated in its degradation during ripening stages, ii) the cutin biosynthetic pathway is downregulated at fruit developing stages. Those results indicate the pectin metabolic pathway is regulated by substrate (= starch degradation products)-dependent manner whereas the cutin biosynthesis is not affected by the starch degradation products, suggesting the starch deficiency affects pectin and cutin metabolic pathways in different manner.

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P29:
A QTL for fine tuning methyl salicylate level in tomato fruits

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Methyl salicylate (MeSA) is an important signaling molecule within and between plants during pathogen and herbivore attack. The accumulation of MeSA is mainly regulated by a *methyl esterase* locus (*MES*) and *Non-Smoky Glucosyl Transferase 1* (*NSGT1*) and its levels can vary up to 9000-fold in color fruited tomato. *MES* removes the methyl group from MeSA to produce salicylic acid whereas *NSGT1* catalyzes an irreversible glycosylation of MeSA. Tomato with functional alleles of both genes generally present low level of MeSA.

MeSA in tomato is associated with low liking by consumer taste panels. The low level of MeSA in many tomato accessions, except some modern types, is likely due to selection for superior taste. Breeding programs have not typically focused on consumer preferences, potentially leading to inadvertent increases in MeSA in the fruit in modern types. However, the volatile may play an important role in defense responses during fruit growth and/or after harvest. We aim to identify additional MeSA regulators that could function in fine-tuning its levels to achieve the proper balance between the fruit taste and defense.

We mapped a single MeSA QTL on the bottom of chromosome 3 (*MeSA3.1*) in an F_2 mapping population. This locus co-localized with *GWAS* QTLs for several phenylpropanoid volatiles that shared a part of the biosynthesis pathway with MeSA. Further fine-mapping and CRISPR-Cas9 mediated knock-out of a few candidate genes are underway to identify the causal gene.

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P30:
Disruption of the *HWS* gene results in enlarged phloem in tomato pedicels, potentially enhancing sugar transport efficiency

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The *HWS* gene has been previously studied in Arabidopsis, rice, and tomato, where it influences various morphological traits, most notably in leaves and flowers. The morphological changes associated with *hws* mutations are thought to result from an accumulation of specific microRNA species. Here, we demonstrate that mutations in *HWS* also lead to an increased phloem area in tomato pedicels, with the degree of enlargement depending on allele severity.

Phloem exudates from the *hws* mutant showed elevated sucrose levels, suggesting that phloem expansion in the mutant may enhance sugar transport. Scanning Electron Microscopy confirmed that cell size and shape in the phloem remained unchanged in the mutant.

Additionally, mRNA sequencing data from pedicel samples revealed no significant changes in the WUSCHEL-CLAVATA pathway, indicating that microRNA accumulation in *hws* may impact vascular meristem development through an alternate mechanism.

The gene's effect on sugar transport highlights its potential for enhancing crop yield across various plant species.

Acknowledgements
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P31:

Single-cell transcriptomic analysis reveals the pivotal effects of protein kinase CPK28 on the hypocotyl development in tomato

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Abstract: Calcium-dependent protein kinases (CPKs) are plant-specific Ca^{2+} sensors that play important and diverse roles in plant development. Previously, we identified that the tomato *cpk28* seedlings showed stunted hypocotyl growth. According to the results of paraffin-embedded section and the microscopic 3D scanning imaging, we found knocking out *CPK28* mainly affected the xylem development. Recent advances in single-cell technologies enable us to explore the mechanisms of plant gene regulation at single-cell resolution. Therefore, we perform the single-cell RNA sequencing on the hypocotyls from 14 d tomato seedlings of *cpk28* mutants and wild-type (WT) plants. Total about 24,895 protoplasts isolated from WT hypocotyls were unsupervisedly classified into 10 distinct cell clusters, which could be further grouped into 7 cell populations with the assistance of reported cell marker genes. We found that the number of parenchyma cells in the xylem of the *cpk28* mutants was significantly increased. By the analysis of developmental trajectory and transcription factor regulatory network of the cambium cells and xylem cells, we speculated a model for xylem development in hypocotyl growth. In this model, jasmonic acid process and ethylene process played the pivotal roles in CPK28-meidated xylem development, supporting by the differential gene expression of several hormone response-related genes (such like *ERF110*, *ERF071*, *OPR3*, and *AOS1*) in the xylem tissue of *cpk28* mutants and WT plants. Collectively, a model for early development of the vascular system is proposed here, which sheds light on further deciphering mechanism of plant cell differentiation.

Acknowledgements
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P32:

Prediction of Capsaicinoid Content in *Capsicum annuum* Hybrids Using Parental Genotypic Data

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Capsaicinoids are the compounds that are responsible for the characteristic pungency of chili peppers (*Capsicum* spp.). Although several structural and transcription factor genes are known to regulate capsaicinoid content, numerous other genes influencing this trait remain unidentified, thereby complicating efforts to develop pepper cultivars with varying levels of capsaicinoids. Genomic selection (GS) is a powerful tool that employs genome-wide random markers, including those in undiscovered genes, to enhance the efficiency of trait selection. Previous studies on the prediction of capsaicinoid content have primarily utilized homozygous genetic resources, given their fixed genetic background. However, given that the majority of commercially available pepper cultivars are produced as F_1 hybrids, it is of paramount importance to be able to predict the capsaicinoid content that will result from the combination of different homozygous breeding lines. In this study, we employed a series of genomic selection (GS) models to predict the capsaicinoid content of pepper hybrids. These models were trained on genotypic and phenotypic data derived from a core collection of 351 accessions, which exhibited significant genetic diversity. The testing population consisted of 30 hybrids, created through the crossing of commercial elite lines from a seed company. The genotypic information for these hybrids was inferred from the parental genomes, rather than being obtained through sequencing. Based on this inferred data, phenotypic predictions were made. To ascertain the optimal number of single nucleotide polymorphism (SNP) markers and the most suitable population structure for genomic selection (GS), a series of tests were conducted on a range of genome-wide SNP marker sets, with particular consideration given to their call rates and the subgroups present within the species. Ten-fold cross-validation demonstrated that the optimal results were achieved when utilizing 38,632 SNPs with a call rate of 70% and the entire core collection. The models trained under these conditions were subsequently employed to predict capsaicinoid content in the F_1 hybrids, and the accuracy of these predictions was evaluated by calculating the correlation between the predicted values and the actual measurements obtained via HPLC. Of the ten genomic selection (GS) models tested, the reproducing kernel Hilbert space model (RKHS) demonstrated the highest accuracy, with a correlation coefficient of 0.366. Furthermore, when the hybrids were classified into high, medium, and low pungency groups, the model correctly identified approximately 47% of the hybrids. While the current accuracy of phenotypic prediction for F_1 hybrids remains limited, this approach demonstrates the potential for facilitating the efficient development of pepper cultivars with targeted pungency levels by simulating hybrid combinations based on the genomic information of superior parental lines.

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P33:

Identification of Candidate Genes for Cold Tolerance in Pepper (*Capsicum annuum*)

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Cold tolerance is an important agricultural traits that protects the plant species from low-temperature stress, which can negatively impact on crop yield and survival, especially the face of higher incidence of extremes in temperature due to climate change. To identify candidate genes associated with cold tolerance, we carried out a correlation analysis that suggested relatively strong associations of stem color and stem thickness with cold tolerance. Based on these findings, we conducted genome-wide association studies utilizing 225 *Capsicum annuum* accessions derived from a pepper core collection to identify candidate genes associated with cold tolerance and its related traits, stem color and stem thickness. By employing phenotypic data alongside genotyped-by-sequencing data encompassing 73,502 SNPs from the core collection, we identified 17 significant SNPs, which comprised three associated with cold tolerance, eight with stem color, and six with stem thickness. As a result of detecting genes within LD blocks spanning up to 2 Mb range that including significant SNPs, a total of 78 genes were identified within the cold tolerance-associated blocks defined as haplotype blocks containing significant SNPs detected by GWAS, and four genes each were identified within the blocks associated with stem color and stem thickness. Among the gene annotations, *cysteine protease RD21A*, *abscisic acid 8'-hydroxylase 4*, and *MPK homolog NTF3* were notable for being reported to influence abiotic stresses including cold stress. This study narrowed the range of candidate genes associated with cold tolerance and provided valuable insight for future breeding. Such findings can contribute to improved low-temperature stress resistance, thus enhancing productivity and resilience of crops against more frequent temperature fluctuations under climate change.

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P34:

Development of molecular markers related to GABA content and fruit weight from introgression lines of *Solanum pennellii*

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The nutritional content of the fruit is an important target for the development of tomatoes (*Solanum lycopersicum*). For example, gamma-aminobutyric acid (GABA) has attracted the attention of several food companies. Among the tomato introgression lines (ILs), we found one containing part of chromosome three from *Solanum pennellii* with approximately three times the GABA content and half the fruit weight compared to normal varieties. We designed DNA markers in the region where the wild fragment was introduced and performed a genetic mapping experiment using the progeny obtained by crossing the high GABA line with our inbred breeding line. A marker designed near the gene encoding glutamate decarboxylase (GAD) was associated with GABA levels. By narrowing the wild-type fragment region using DNA markers, we developed a line with high GABA levels but no reduction in fruit weight. These markers can be used in practical breeding to develop varieties with high GABA levels.

P35:
Polyploid System as a Tool for Testing the Breeding Potential of Gene Engineering

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Genes linked to flower organ and floral meristem development can be precisely modified to enhance fruit yield in tomatoes. However, mutations in key genes like *ANANTHA* (*AN*) and *FALSIFLORA* (*FA*) often cause deleterious effects, leading to abnormal flower development when knocked out. Heterozygous mutants of these genes do not show significant differences from wild-type plants and fail to exhibit overdominant traits, complicating their use in breeding. To explore their potential for novel breeding applications, we employed a polyploid system using *Solanum nigrum*, a hexaploid species, to edit the *AN* and *FA* genes via CRISPR-Cas9-induced knockout mutations. Homoeologous genes orthologous to tomato *AN* and *FA* were identified, and their functional conservation was examined. This approach allowed for the generation of a series of knockout mutants, which exhibited intermediate traits in inflorescence branching and flower morphology. Lines with reduced functional copies of the *AN* or *FA* genes displayed intermediate phenotypes, including defects in inflorescence structure and flower formation. These findings suggest that modifying key genes, which typically cause harmful mutations in diploid systems, within a polyploid background can facilitate the assessment of their breeding potential, opening new avenues for crop improvement through gene engineering.

Acknowledgements
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P36:
Horizontal transfer of extrachromosomal circular DNAs across grafting junctions in Solanaceae

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The transfer of genetic material between stocks and scions has been studied extensively; however, it remains a mystery and is still underexplored. In this study, we established an intergeneric grafting system using woody goji as the stock and herbaceous tomato as the scion, employing both in vitro and in vivo approaches within the Solanaceae family. We confirmed that multiple nuclear DNA fragments were horizontally transferred from donor goji cells to recipient tomato cells. Notably, recipient tomato tissues containing goji donor fragments at or near the grafting junctions exhibited a perennial anatomical structure, from which we were able to induce regenerated roots or shoots. Furthermore, we determined that the majority of these fragments consisted of extrachromosomal circular DNAs (eccDNAs). These eccDNAs traversed the grafting junctions into recipient tomato cells, persisted in the regenerants derived from them, and were maintained in asexual offspring. Plants with transferred stock-to-scion eccDNAs in regenerated roots or shoots (designated ‘Go-tomato’) were successfully grown perennially and exhibited excellent performance. Our research provides insights into the replication ability and potential function of eccDNAs in regulating the pleiotropic traits of ‘Go-tomato’. The discovery of horizontally transferred mobile eccDNAs offers the first direct evidence of stock-to-scion mobile DNA exchange as vectors beyond chromosomes and organelles, contributing to the understanding of grafting-induced genetic variation and its potential evolutionary and breeding implications.

Acknowledgements
This work was supported by grants from the Zhejiang Provincial ‘Three Rural Nine Parties’ Science and Technology Cooperation Plan Project (2022SNJF027).

P37:

SATREPS Project: Developing a TILLING Platform using Ethyl Methane Sulfonate (EMS) to Induce Anthracnose Resistance in *Capsicum annuum* L. var. Tanjung 2

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Chili pepper (*Capsicum annuum* L.) is one of the important agricultural commodities in Indonesia, yet its production remains insufficient to meet rising demand, and its extremely high price can cause inflation. One of the major constraints in chili pepper production is the widespread anthracnose disease, which is caused by the fungus *Colletotrichum* spp. To address the issue, a research collaboration between the University of Tsukuba and Universitas Padjadjaran under the SATREPS Project aims to develop anthracnose-resistant chili varieties using the TILLING platform. Mutant populations generated through this platform will provide genetic variation to achieve this goal. It comprises the seed multiplication from one plant of Tanjung 2, treatment of seeds with EMS at different concentrations and soaking durations, observation of the M_1 mutant population, mutation screening, and phenotype characterization of mutant plants. This study focuses on the mutation induction of the Tanjung 2 chili pepper variety, a high-yielding, nationally popular cultivar in Indonesia known for its compact architecture, attractive red fruit, and moderate anthracnose tolerance. The first phase of TILLING platform development involves generating a mutant population through ethyl methane sulphonate (EMS) treatment. The experiment tested five EMS concentrations (0%, 0.5%, 1.0%, 1.5%, and 2.0%) and three soaking durations (6, 12, and 24 hours). Results indicated the lethal concentration (LC50) for EMS at 1.99% for 12 hours and 0.82% for 24 hours of soaking, while no LC50 was detected for 6 hours. Phenotypic characterization is ongoing, and further screening of the M2 population for anthracnose resistance-associated mutations will be conducted using high-throughput sequencing techniques.

Keywords: anthracnose resistance, *Colletotrichum*, crop improvement, mutagenesis, plant breeding, high-throughput sequencing

Acknowledgements

This study was supported by the SATREPS Project (University of Tsukuba – Universitas Padjadjaran).

P38:

Genome editing of *DWARF* and *SELF-PRUNING* rapidly confers traits suitable for plant factories while retaining useful traits in tomato

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Plant factories with artificial light are less affected by environmental influences in crop cultivation and are gaining attention as a solution to environmental changes, enabling cultivation in areas unsuitable for farming and promoting sustainable agriculture. Many companies have successfully grown crops like lettuce, herbs, strawberries, and tomatoes in these factories, with the market for leaf lettuce and small leafy vegetables expanding. Since these crops are often grown for fresh consumption, the controlled, clean environment adds value by preventing quality loss from insect damage. If the added crop value offsets cultivation costs, vertical farming in plant factories could become even more commercially viable. For crops to be commercially viable in plant factories, they must meet several conditions. They should have a short plant height to allow multiple tiers, a short cultivation period, and added value, such as being damage-free with minimal losses. If these traits can be introduced into currently unsuitable varieties, new crops for large-scale plant factory production could be developed. The dwarf tomato variety Micro-Tom is widely used in molecular biology research due to its compact growth. Micro-Tom has a short plant height of around 15 cm, with small leaves and fruits, enabling the cultivation of many plants in limited space. It grows well under fluorescent light and fruits in about three months. The dwarfism trait involves three genes: *DWARF* (*D*: Solyc02g089160), *SELF-PRUNING* (*SP*: Solyc06g074350), and *miniature*. Although the *miniature* gene is unidentified, introducing mutations in *DWARF* and *SELF-PRUNING* could make other varieties suitable for plant factory cultivation. In this study, we created a system to rapidly produce crops adapted for plant factory cultivation, by introducing traits suited to plant factories into the GABA-enriched genome-edited tomato variety #87-17, which lacks the self-inhibitory domain of the *SlGAD3* gene, faster than traditional breeding methods. The *DWARF* and *SELF-PRUNING* genes of #87-17 were genome-edited using CRISPR-Cas9, and the dwarfism and fruit traits of the resulting T_1 lines were evaluated while being compared to those of the *d/sp/GAD3* triple mutants created through conventional breeding methods. As a result, the desired traits were obtained in the T_1 genome-edited generation, and the fruit traits were almost the same as those of the original variety. On the other hand, the F_2 hybrids of #87-17 containing the *d* mutation and *sp* mutation and Micro-Tom exhibited dwarfism, but the fruit phenotypes were a mix of the characteristics of both varieties. This demonstrates that genome editing of these two genes using CRISPR-Cas9 can efficiently introduce traits suited for plant factory cultivation while retaining the beneficial traits of the original cultivated varieties.

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P39:
Interaction between BZR1 and histone deacetylase SRT1 promotes shoot branching in tomato

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Shoot branching is a critical determinant of plant architecture that impacts crop yields. The growth of lateral buds is regulated by brassinosteroid (BR), which serves as an integrator of both hormone networks and environmental cues. However, the way in which epigenetic modifications influence shoot branching is not yet fully understood. Here we found that the promotion of shoot branching by BR is accompanied by a decrease in histone H3K9 acetylation level. Furthermore, we identified SRT1, the only histone deacetylase that interacts with BZR1, and confirmed its positive regulation in shoot branching. Additionally, SRT1 does not influence the content and signaling of BR, yet the accumulation of the SRT1 protein is promoted by BR. Genetic and molecular experiments confirmed that the core branching inhibitor gene, *BRC1*, is interdependently regulated by SRT1 and BZR1. ChIP-qPCR and qRT-PCR revealed down-regulation of *BRC1* accompanied by an increase in H3K9 acetylation in its promoter. In conclusion, SRT1 and BZR1 synergistically regulate tomato shoot branching by inhibiting *BRC1* expression through decreasing the level of H3K9 acetylation in its promoter.

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P40:

Brassinosteroids Receptor StBRI1 Promotes Tuber Development by Enhancing Plasma Membrane H^+-ATPase Activity in Potato

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The BRASSINOSTEROID-INSENSITIVE 1 (BRI1) as receptor of brassinosteroids (BRs) plays a critical role in plant growth and development. Although much is known about how BR signaling regulates growth and development in many crop species, the role of StBRI1 in regulating tuber development is not well understood. A series of comprehensive genetic and biochemical methods were applied in this investigation. It was recovered that StBRI1 and PHA2, the plasma membrane (PM)-localized proton ATPase, play important roles in potato (*Solanum tuberosum*) tuber development. Their overexpression led to a 22% and 25% increase in tuber yield per plant, respectively. Consistent with the genetic evidence, the interaction analysis *in vivo* with the double transgenic lines, and the determination of PM H^+-ATPase activity, indicate that StBRI1 interacts with the PHA2 C-terminus and restrains the intramolecular interaction of the PHA2 C-terminus with the PHA2 central loop to attenuate the autoinhibition of PM H^+-ATPase activity, resulting in an increase in activity of PHA2. Furthermore, it is shown the extent of PM H^+-ATPase autoinhibition involving in a series of phosphorylation-dependent mechanisms is correlated with phosphorylation of the penultimate Thr-951 residue. These results suggest that StBRI1 phosphorylates PHA2 and enhances its activity, which subsequently promotes tuber development. A novel StBRI1-PHA2 module involved in regulating tuber development has been uncovered, and a prospective strategy has emerged for improving tuberous crop growth and increasing yield via the cell surface-based BR signaling pathway, BR-StBRI1-PM H^+-ATPase.

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P41:
MADS gatekeepers: An ovule protein complex ensures fertilization-dependent fruit set in tomato

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To avoid the production of seedless fruits, which is counterproductive from the plant's perspective, the ovary halts its growth shortly before anthesis, entering a state of arrest, until ovule fertilization resumes ovary growth i.e. fruit set. However, little is known about the mechanism of ovary arrest, compared with the mechanism of fruit set, which has been extensively investigated. We have found that in tomatoes, ovaries of *AGAMOUS-LIKE6* (*SlAGL6*) loss-of-function mutant (*slagl6*$^{CR\text{-}sg1}$) develop normally until anthesis but, contrary to undergoing arrest, continue to grow and set normal yet seedless fruits independent-of-fertilization (parthenocarpic fruits), indicating that the SlAGL6 MADS-box protein is pivotal in maintaining ovary arrest. Importantly, the parthenocarpic phenotype of *slagl6*$^{CR\text{-}sg1}$ can be eliminated by expression of *SlAGL6* specifically within the ovule suggesting that the SlAGL6 protein operates within the ovules of the arrested ovary. Transcriptome analysis of isolated wild-type and *slagl6*$^{CR\text{-}sg1}$ ovules suggests that SlAGL6 prevents parthenocarpy by regulating the transcription of a set of genes involved in the fertilization-induced reprogramming of ovules that underlies fruit set. The *AGL6* gene encodes a type II MADS-box protein suggesting that it exerts its functions by complexing with other MADS-box proteins. Screening a Yeast Two-Hybrid library of arrested ovaries with the full-length SlAGL6 protein identified eight MADS-box transcription factors as candidate interactors (CIs). BiFC and FLIM-FRET analyses confirmed that SlAGL6 interacts *in planta* with the MADS-box proteins CI-2 and CI-3, both individually and in a SlAGL6-CI-2-CI-3 complex. Similar to SlAGL6, the expression of CI-2 and CI-3 peaks in the ovules of the arrested ovary and decreases in the set fruit. Moreover, genetic interaction studies demonstrated that SlAGL6, CI-2, and CI-3 synergistically function as suppressors of parthenocarpy. Collectively, our results suggest that SlAGL6, CI-2 and CI-3 are components of a protein complex which acts from the ovules to maintain ovary arrest and prevent parthenocarpy.

P42:
orf137 knockout can partially restore fertility in CMS potato

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Tetraploid potatoes are a vital crop cultivated globally. Due to their reliance on vegetative reproduction, outbreaks of disease or pest infestation often result in widespread damage. There are growing concerns regarding the potential decline in potato production caused by the increased prevalence of diseases, pests, and environmental stressors linked to climate change, such as global warming. Addressing these challenges requires the rapid development of cultivars with enhanced resistance to diseases and environmental stressors. However, many potato cultivars suffer from cytoplasmic male sterility (CMS), a condition caused by a CMS-associated gene in the mitochondrial genome, which presents a significant barrier to crossbreeding efforts.

CMS potatoes are classified into three types (W/α, T/β, and W/γ) based on gene polymorphisms in the mitochondria. We focused on T/β because it is the most widely cultivated type in Japan (e.g., Irish Cobbler, May Queen). Previous research from our laboratory identified that CMS in tomatoes leads to abnormal pollen tube elongation, with the *orf137* gene inducing male sterility (Kuwabara et al., 2022). We discovered that T/β also contains *orf137* and employed mitoTALEN (mitochondrially targeted transcription activator-like effector nucleases) to knock out *orf137*.

The *orf137*-knockout potato line was generated using the Agrobacterium-mediated transformation method. PCR analysis confirmed the successful knockout of *orf137*. The *orf137*-knockout potato lines were grown and flowered in a netted chamber. Pollen from the blooming flowers was collected and stained using Alexander staining, which resulted in successful staining (unlike in the wild type, where pollen is not stained). Self-pollination of the *orf137*-knockout potato lines produced fruit. Unfortunately, weather issues led to the plants dying before the fruit could fully ripen. Nevertheless, our results demonstrate that the *orf137* knockout can partially restore fertility in CMS potatoes.

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P43:
ERECTA Modulates Seed Germination and Fruit Development via Auxin Signaling in Tomato

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The improvement of tomato (Solanum lycopersicum) fruit quality through breeding prioritizes the selection of desirable tastes and characteristics and enhanced disease resistance and yield. Seed germination is a crucial initial step in the plant life cycle, directly impacting crop productivity and yield. Abscisic acid (ABA) is a well-known phytohormone that inhibits seed germination, and auxin enhances the inhibition of seed germination by ABA. ERECTA (ER), a receptor-like kinase (RLK) family protein, is known for its involvement in various developmental processes. In our investigation of a Micro-Tom EMS mutant designated as a knock-out mutant of *sler*, which affected traits such as fruit development, seed number, and seed germination. Our research indicates that *sler* inhibits seed germination via enhanced ABA signaling by auxin, as evidenced by elevated auxin levels and alterations in the expression of *ABSCISIC ACID INSENSITIVE 3* (*ABI3*) and *ABI5* in *sler* seeds compared to the wild type (WT). Furthermore, we observed an increase in auxin content in the *sler* ovary and changes in the expression of auxin biosynthesis rate-limiting genes *YUCCA flavin monooxygenases 1* (*YUC1*), *YUC4*, *YUC5*, and *YUC6*, as well as auxin response genes *AUXIN RESPONSE FACTOR 5* (*ARF5*) and *ARF7*, implying that *SlER* regulates fruit development through auxin signaling. Our study revealed a new mechanism by which *SlER* affects auxin biosynthesis and response pathways. The study suggested that the knock-out mutant of *SlER* can seriously reduce seed germination rate. Screening for relatively mild knock-down mutants could be more suitable for tomato breeding.

P44:
Spatiotemporal control of jasmonate-mediated fruit set in tomato

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Tomato (*Solanum lycopersicum*) production depends on the efficiency of fruit set, the transition of ovary into fruit. Pollination is normally required for fruit set but it is negatively impacted by adverse conditions such as high temperature. Parthenocarpy, the pollination-independent fruit set, is an attractive trait for horticultural crops, although the detailed molecular mechanism remains elusive. We isolated the parthenocarpic mutant, *Sldad1* (*Solanum lycopersicum defective in anther dehiscence1*), from 'Micro-Tom' EMS mutagenesis library. The causative gene, *SlDAD1*, encodes an enzyme involved in jasmonate (JA) biosynthesis. We previously reported that *SlDAD1* transcript was exclusively observed in the filament before flowering, whereas JA accumulated not only in the filament but also in the ovary before flowering, especially in the ovule. However, how JA regulates tomato fruit set is poorly understood.

Here, we investigated the regulatory networks involved in tomato fruit set mediated by JA localization in the ovule. We obtained the transcriptomes of wild-type (WT) and *Sldad1* ovaries during early fruit development and analyzed the expression patterns, focusing on genes expressed in the ovule. Among these genes, one of the significantly downregulated genes in *Sldad1* ovaries encodes the ethylene biosynthesis enzyme SlACO4. The gene expression pattern showed high level in WT ovaries both before flowering and at anthesis, similar to the changes in JA levels. Moreover, we found the transcription factor gene *SlMYB21*, which is expressed in ovules, showed the similar expression pattern to *SlACO4*. To comprehensively identify the SlMYB21 binding region in the tomato genome, we performed DNA affinity purification sequencing (DAP-seq). This analysis revealed that strong SlMYB21 binding peaks located in the promoter region of *SlACO4*. Additionally, the production of ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) was significantly reduced in *Sldad1* ovaries compared to WT at anthesis. Notably, *SlACO4* knockout mutants generated using the CRISPR/Cas9 system exhibited parthenocarpy. These results suggest that JA localization in the ovule positively regulates ethylene biosynthesis through the transcriptional control of SlMYB21, leading to the suppression of tomato fruit set. Further work will be needed to be clarify the regulatory networks between phytohormones.

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P45:
A chili pepper mutant *tn-1* produces seedless fruits due to the mutation of *CaCKI1*

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Seedless fruits are favorable because seed is troublesome waste for consumers and processing industries. However, knowledge and resources for developing seedless chili peppers is still limited. Our previous study isolated a chili pepper mutant, *tn-1*, that stably bears seedless fruits. In this study, we aimed to elucidate the mechanism underlying seedlessness in *tn-1*. Pollen germination and pollen tube elongation were normal, however, observation of ovules by confocal laser scanning microscopy revealed the defective embryo sac development in *tn-1*. The *tn-1* locus was delimited to a 313 kb region on the chromosome 12 by marker analysis. Combining with further mapping-by-sequencing approach, CA12g21620, encoding a histidine kinase, was identified as a candidate gene. Phylogenetic analysis of histidine kinases revealed that CA12g21620 is a homolog of *Arabidopsis CKI1* (*Cytokinin Independent 1*), which is known as the key gene for the female gametophyte development, and CA12g21620 was named as *CaCKI1*. The *tn-1* type allele has a 3 bp insertion in the 6th exon leading to single lysine (K) residue insertion into the Receiver (REC) domain of CaCKI1. REC domain is important to interact with downstream proteins in the phosphorylation relay. In addition, the amino acid sequence around single Lysine insertion is widely conserved among CKI1 orthologs in various plants. These results suggest that the insertion of one K residue may disturb the function of CaCKI1 protein and inhibit normal development of the female gametophyte, resulting in seedless fruit in *tn-1*. Furthermore, we found that decreased expression of *CaCKI1* by virus-induced gene silencing reduced the percentage of normally developed female gametophyte in chili pepper. This study demonstrates the important role of *CaCKI1* in seed development in chili pepper and the possible utilization to develop seedless cultivars by introducing its mutation.

P46:
Characterization of a locule number mutant in chili pepper (*Capsicum annuum*)

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Chili pepper is an important vegetable crop and an ornamental plant, which harbors the diversity of fruit shapes and colors. The mechanism to determine fruit shape of chili peppers are fully unknown. A novel fruit shape mutant was isolated in EMS mutagenesis population derived from the ornamental chili pepper, and named as *malformed fruit-2* (*maf-2*). The objective of this study is to investigate the phenotypes and identify the candidate gene of *maf-2* mutant.

Phenotyping revealed that the fruit shape was significantly changed in *maf-2* mutant compared with wild-type (WT). The fruit width was increased, whereas fruit length was reduced in *maf-2* mutant. Interestingly, the petal number and locule number were obviously increased in *maf-2* mutant. The floral bud meristems of WT and *maf-2* were fixed with FAA and paraffin sections were prepared. In the *maf-2* mutant, the floral bud meristem was enlarged about 1.5 times compared with than in the wild type (Fig.1). Bulk-segregant analysis combined with genome resequencing showed that a C-to-T single nucleotide substitution, which located within the coding region of CA04g21950, was a candidate SNP for the *maf-2* mutant phenotype. CA04g21950 encoded the *CaCLV1* in chili pepper, and this substitution changed the 683th-codon into a stop codon in *maf-2* mutant, and the kinase domain was absent in CaCLV1^{maf-2} (Fig.2). Co-segregation between the SNP within *CaCLV1* coding region and the malformed fruit phenotype was confirmed in the WT×*maf-2* F_2 population. The CLAVATA (CLV)-WUSCHEL (WUS) pathway plays a conserved role in coordinating stem cell proliferation with differentiation. RT-qPCR showed that the expression levels of genes related to CLV-WUS pathway were changed in *maf-2* mutant. This *maf-2* mutant may provide opportunity to study how CLV-WUS pathway determines fruit shape of chili pepper, and will be new resource to diverse the fruit shape.

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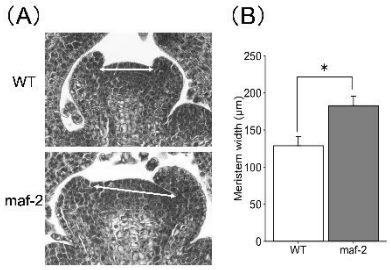

Fig. 1. Comparison of floral bud meristem between wild type and *maf-2* mutants
(A) Histological observation of floral bud meristem. The double arrows indicate the stem cell regions (B) Comparison of floral bud meristem size. Significant difference at the 5% level (t-test).

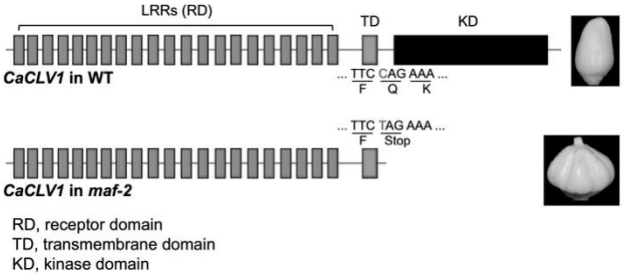

Fig. 2. A single nucleotide substitution (C-to-T) leads *CaCLV1* to produce a truncated protein in *maf-2* mutant.

P47:

The Emerging Roles of SlKN5-SlBLH Regulatory Networks on Tomato Fruit Development and Coloration

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The KNOTTED1-like homeodomain (KNOX) and BEL1-like homeodomain (BLH) proteins, members of the TALE superfamily of homeodomain transcription factors, play crucial roles in plant morphogenesis and environmental responses. The protein-protein interaction between KNOX and BLH proteins influences their binding affinity to target DNA sequences, which is critical for regulating downstream genes. Our high-throughput protoplast two-hybrid (P2H) assay revealed the interaction network among eight KNOX and fourteen BLH proteins of tomato (*Solanum lycopersicum*), identifying SlKN5 as a key player in fruit development. The KNOX protein family is divided into two classes, class I (KNOX I) and class II (KNOX II), both of which are essential for regulating plant organ differentiation. While tomato KNOX I proteins are known to stimulate chloroplast development in fruit, affecting fruit coloration, the role of KNOX II proteins in this process remains unclear. We employed the CRISPR/Cas9 system to specifically knock out the *SlKN5* gene, a highly expressed KNOX II member in fruit. These mutants exhibited increased leaf complexity, a phenotype commonly associated with reduced KNOX II activity, as well as enhanced chloroplast and chlorophyll accumulation in the smaller cells of young, unripe fruit. Transcriptome analyses indicate that SlKN5 suppresses the transcription of genes involved in chloroplast biogenesis, chlorophyll biosynthesis, and gibberellin catabolism. Furthermore, P2H assays revealed that SlKN5 physically interacts with three transcriptional repressors from the BLH1 clade of the BLH protein family—SlBLH4, SlBLH5, and SlBLH7—with SlBLH7 showing the strongest interaction. The knockout of these *SlBLH* genes via the CRISPR/Cas9 system confirmed their overlapping roles in suppressing chloroplast biogenesis, chlorophyll biosynthesis, and lycopene cyclization. Transient assays further demonstrate that the SlKN5-SlBLH7 interaction enhances the binding capacity to regulatory regions of key chloroplast- and chlorophyll-related genes. Collectively, our findings elucidate that the KNOX II SlKN5-SlBLH regulatory modules contribute to fine-tuning the color transition from immature green fruit to mature red fruit.

Acknowledgments
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P48:
Characteristics of Florigens and Florigen-Producing Cells under Natural Sunlight-Mimicking Conditions in Plants

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Plants determine the timing of flowering in response to various environmental cues, including seasonal changes. Floral induction is primarily controlled by the expression of florigens, such as FLOWERING LOCUS T (FT) and its orthologs. Based on their response to photoperiod, plants are classified into three groups: long-day plants, short-day plants, and day-neutral plants. Generally, the expression levels of FT orthologs are closely associated with the initiation of flowering.

In long day plants like Arabidopsis, FT is highly expressed at the end of the day only under long-day conditions. Conversely, in short-day plants, high levels of FT ortholog expression occur under short-day conditions. Recently, we identified that expression profile of Arabidopsis FT (AtFT) under natural sunlight differs from that observed under laboratory white light conditions. However, it has not yet been thoroughly examined whether expression profiles of other FT orthologs similarly respond to different light qualities. To address this question, we analyzed publicly available expression data and identified that some FT orthologs displayed expression profiles similar to AtFT from plants grown under natural sunlight-mimicking conditions.

AtFT is expressed in unique phloem companion cells. However, it remains elusive which genes are co-expressed with FT and whether they also function in flowering. To answer this, we performed tissue-specific translatome analysis, Translating Ribosome Affinity Purification (TRAP)-seq in Arabidopsis. FT-producing cells expressed different types of genes compared to mesophyll cells and epidermal cells, while they expressed genes in a similar way observed in phloem companion cells. However, not all the genes were overlapped between FT-producing cells and phloem companion cells. We further investigated the differentially expressed genes, and identified that a gene encoding a small protein, FPF1-LIKE PROTEIN 1 (FLP1) is required for flowering under sunlight-mimicking conditions, independently of FT. FLP1 is conserved across different plant species. Our data suggest that florigen expression is finely regulated by light quality, highlighting potential multilayered regulatory mechanisms for floral regulation that could be targeted to optimize crop yields.

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P49:
Parthenocarpy and domestication in tomato

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The huge diversity of cultivated tomatoes is the result of a long process of domestication followed by intensive breeding. Breeding efforts have been focused on increasing fruit size and on the diversification of fruit phenotypes. The formation of seedless (parthenocarpic) fruits in tomato plants is an interesting trait for growers, providing a mechanism to overcome fertilization failure under unfavorable environmental conditions. Early anther or pollen ablation is an effective strategy to promote parthenocarpy in tomato plants and was proven to be effective in several tomato cultivars. Whether this is an ancestral trait or was acquired during domestication and breeding is unknown. In this study, we evaluated the formation of parthenocarpic fruits in the cultivated tomato and the wild relative Solanum pimpinellifolium through the generation of malesterile mutants. Only cultivated tomatoes, but not Solanum pimpinellifolium plants, produced seedless fruits. Expression analyses showed that parthenocarpy correlates with the activation of fertilization-independent gibberellin biosynthesis in the ovaries. When compared with wild relatives, modern tomato cultivars present small deletions in the promoters of these genes that could account for the differences in gene expression that ultimately trigger parthenocarpy. Our results suggest that seedless fruit production was actively repressed in the absence of pollination in the ancestral tomato lineages.

Acknowledgements
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P50:

Reverse genetics using the tobacco mutant library: Carotenoid cleavage dioxygenase 4 mutants and Lycopene epsilon cyclase mutants and their characteristics

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The reverse genetics approach is powerful way of gene functional analysis in plants, while its usage is still limited due to gene duplication in high polyploidy plants. The *Nicotiana tabacum* (Tobacco) is allotetraploid and its genome is likely composed of two genomes (S- and T-genome) derived from *N. sylvestris* and *N. tomentosiformis*. We have developed a tobacco mutant library using chemical mutagenesis (Udagawa *et al.* present in this conference). In this study, the capability of this mutant library was assessed using carotenoid phenotypes as a case study. Isolation of single mutants using this mutant library and following making multiple mutants by crossing, we obtained carotenoid mutants of *Carotenoid cleavage dioxygenase4* (*CCD4*) and *Lycopene epsilon cyclase* (*LCY-ε*) genes and analyzed their characteristics. CCD4 is involved in the decomposition of carotenoids and resultant production of apocarotenoids, such as β-ionone derived from β-carotene in other plant species. *N. tabacum* has three *CCD4* genes (*CCD4-S*, *CCD4-T1* and *CCD4-T2*). Mature (senescent) leaves of *ccd4* double-mutant plants showed a stronger yellow color, and those of triple-mutant plants showed a pronounced yellow color. Carotenoid analysis of the leaves from the mutants showed that lutein and β-carotene increased in line with the degree of color change compared to wild type. Analysis of apocarotenoids in flue-cured leaves of the multiple-mutant plants showed that many compounds, including megastigmatrienones, were decreased in comparison to wild type, whereas intriguingly β-ionone and dihydroactinidiolide were increased. LCY-ε is known to be located at one of the two branches of the carotenoid biosynthetic pathway, and its dysfunction has a major impact on carotenoid composition in other plant species. *N. tabacum* has two *LCY-ε* genes (*LCY-ε-S* and *LCY-ε-T*). The *lcy-ε* single and double mutants showed no apparent phenotypic alterations both green and flue-cured leaves compared to the wild type. Apocarotenoid analysis of flue-cured leaves showed that some of the compounds, such as β-cyclocitral and β-damascenone specifically increased in the double mutants. These results indicate that our tobacco mutant library can be used to produce tobacco with altered carotenoid and apocarotenoid profiles by reverse genetics approach.

P51:

Standing against floods: Towards the understanding of morpho-physiological and molecular responses to waterlogging stress in potato

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Global warming has increased the frequency and intensity of floods. Flooding represents almost two-thirds of the agricultural damage worldwide, and is a major concern for potato farmers. Despite the importance of potato as a staple food crop and its well-established high sensitivity to flooding, molecular and physiological level responses to this stress remains largely unexplored. Our main goal is to address this knowledge gap. In this talk I will focus on 1) potato morphophysiological responses to waterlogging (i.e. root flooding) and underlying genetic variation, 2) dynamics of transcriptional, organ-specific responses to waterlogging, 3) Investigation of how key waterlogging cues are sensed and translated for mediating acclimation and recovery responses. Our work forms the basis for further elaboration of the molecular pathways mediating flood sensing and signaling, and paves the way for identifying tolerance traits and mechanisms to improve resilience of this vital food crop.

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P52:
The response of lipocalins to phytohormones in tomato

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Lipocalins are a widely conserved family of proteins, including invertebrates, vertebrates, insects and plants, whose structure consists of α-helices and β-barrels and hydrophobic proline-rich HPR motifs that anchor to the plasma membrane. Therefore, the roles of lipocalins include transport of hydrophobic small molecules, signal transduction, and stress responses such as high and low temperature. Lipocalin is highly expressed under the low temperature stress and promote the plasma membrane integrity and confer stress tolerance in rice. We have analyzed the plastid proteome of two tomato cultivars with different fruit colors and 'Micro-Tom' fruits at different stages and the proteome data revealed that temperature-inducible lipocalin (TIL1 and TIL2) was the different mass and the isoelectric point of peptide spots in each tomato cultivars. To further understand the role of the lipocalins in tomato, we made the transgenic tomatoes that over-expressed *SlTIL1*, *SlTIL2*, and *SlCHL* and tomatoes in which *SlTIL1*, *SlTIL2*, and *SlCHL1* were silenced using virus induced gene silencing (VIGS), and then we observed the relationship between lipocalins and reactive oxygen species (ROS), which are closely related to photosynthesis. All over-expressed lipocalin plants were significantly scavenged ROS compared to WT under heat stress, while all suppressed lipocalin plants weren't able to scavenge ROS. This result indicates that lipocalin contributes to ROS scavenging.

Moreover, *cis*-element analysis of the promoter region of lipocalins revealed to include many *cis*-elements for light response, ethylene response, ABA response, and various stress responses. In this study, we investigated the expression of lipocalin genes under treatment of ABA, ethylene and JA to analyze the relationship between phytohormones and lipocalins. These results showed that mRNA of *TIL1* was accumulated in the young leaves under ethylene treatment from 2 hours, and *TIL1* and *TIL2* was highly expressed in the leaves with ABA at 24 hours. TILs are involved in stress tolerances, such as those involving the response of ABA and ethylene, in leaves. The expression of *TIL1* increased during fruit maturation from the orange fruit stage to the red fruit stage. In the case of the fruit with ABA treatment, the expression of *TIL1* quickly increased at the yellow fruit stage and also the expression of *ACO1* and *ACS2*, ethylene biosynthesis genes, was increased at the yellow fruit stage before the ripening. These expression responses were the cause of the enhanced ethylene synthesis by ABA treatment. On the other hand, photosynthetic activity was reduced as carotenoid synthesis was promoted by the ethylene synthesis, resulting in the accumulation of excess light energy and the generation of large amounts of ROS. TIL1 was highly expressed to scavenge this increased ROS. Therefore, these results suggest that lipocalin contributes not only to the stress response but also to the early process of fruit ripening.

We also thank Prof. Ma Gang of Shizuoka University's faculty of Agriculture for doing something useful.

P53:

Transcriptome analysis reveals abiotic stress boosts secondary metabolite production in Capsicum

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Peppers (*Capsicum* spp.) belong to the *Solanaceae* family and are rich source of bioactive compounds (secondary metabolites, SM) such as phenols, carotenoids, flavonoids, and capsaicinoids which have been successfully exploited for food and pharmaceuticals industry. The horticultural production of *Capsicum annum* generates large quantities of residual biomass which could be considered for exploitation of bioactive compounds. Accumulation of SM in plants often occurs in response to various stresses. Therefore, applying abiotic stress could be used as a strategy for increasing the production of desirable SMs. Here, we examined the effect of moderate cold stress, high nutrition (HN: 5 fold Hoagland solution) and the combination thereof on four different Capsicum varieties including *C. chinense* (CAP1035), *C. annuum* var. glabriusculum (CAP1639 and CAP539) and *C. annuum* var. annuum (CAP1434) by RNAseq analysis.
Distinct stress responses were observed in the transcriptome analysis. In CAP1035, cold and the combined HN-cold stress triggered the activation of flavonoid biosynthesis genes, such as phenylalanine ammonia-lyase, chalcone synthase, chalcone isomerase, and flavonoid 3'-monooxygenase. Conversely, in CAP1639, the flavonoid biosynthesis pathways exhibited upregulation exclusively under HN-cold stress condition. CAP539 demonstrated an upregulation of genes related to both flavonoid and terpenoid biosynthesis, such as carotenoid cleavage dioxygenase 1-like, vetispiradiene synthase 2-like, and 5-epiaristolochene synthase-like isoform X2, in response to HN and combined HN-cold stresses. Additionally, in CAP1434 genes involved in secondary metabolism included not only flavonoids and terpenoids, but also alkaloids, highlighting a diverse metabolic response to stress conditions. In total, the combined HN-cold stress had higher impact on gene expression changes in secondary metabolism pathways than individual stresses in all species.
In addition, several transcription factors (TFs) from bZIP, bHLH, MYB, WRKY and NAC families, which are known to be responsible for the regulation of SM biosynthesis in various plant species, were induced under the stress conditions. Promoter analysis of differentially expressed flavonoid biosynthesis genes revealed the presence of binding sites of the induced TFs and also of hormone-related *cis*-elements (such as abscisic acid, jasmonic acid and ethylene). Furthermore, promoter analysis of these TF genes indicated also the presence of several hormone-related *cis*-elements. An *in silico* protein-protein interaction network analysis showed interaction of upregulated flavonoid biosynthesis genes and some differentially expressed TFs. Therefore, the stress-induced expression of flavonoid-related genes might be regulated by an intricate network of various hormones and transcription factors. These results provide insights into the effect of stress conditions on SM pathways highlighting the possibility of increased SM production in Capsicum under abiotic stress treatments through hormonal and transcriptional regulatory networks. This findings could be applied to develop strategies to enhance the plant SM contents in the residual leaf biomass of Capsicum species to benefit the value added in food and medicine industry.

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P54:

Effect of Starch Accumulation on Salt Stress Tolerance in Seedling Growth of Tomato

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In past decades, salt accumulation in farmland and groundwater has emerged as one of global problem. Therefore, importance of crop development with salt stress tolerance has been growing for sustainable agricultural production. We have been studying the sugar accumulation in tomato (*Solanum lycopersicum* L.) fruits enhanced by salt stress, and reported the enhanced starch biosynthesis by salt stress is essential for this phenomenon. Recently, the relationship between starch accumulation and salt stress tolerance has been suggested in plants such common reed and wild azuki beans, however its mechanism(s) remains to be unclear. In this study, we report effect of endogenous starch accumulation on salt stress tolerance in tomato plant.

As plant materials, seedlings of starch-defect or over-accumulating tomato lines in which either ADP-glucose pyrophosphorylase (AGPase) gene, which is a regulatory enzyme for starch biosynthesis, are suppressed or overexpressed were submitted. Salt stress was treated by adding NaCl to the hydroponic culture solution with 100 ~ 150 mM concentration. Seedling growth was significantly suppressed in the starch-defect lines compared to the wild type (WT). Tracer analysis feeding radio-labeled ^{22}Na Cl revealed that Na^+ uptake into the seedlings of the starch-defect lines tended to be faster and higher compared to the WT. On the other hand, although a slight reduction in salt stress-induced growth inhibition, no significant increase in salt stress tolerance was observed in the starch over-accumulating lines. These results suggest that endogenous starch take some role(s) in suppression of Na^+ uptake into cells. The gene expression of Na^+/H^+ antiporter *SOS1* in leaves decreased in the starch-deficient lines compared to the WT, suggesting endogenous starch is involving in emission of ^{22}Na through the regulation of *SOS1* expression under the salt stress.

P55:
Investigation of the Physiological Effects of Long-term High Temperature and High Humidity Conditions on *Solanum lycopersicum* cv. Micro-Tom

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Solanaceous plants are one of the major agricultural crops with significant economic importance. Global warming is impacting growth and yield of solanaceous plants, such as tomatoes. It is known that short-term high temperature imposes significant impact on flower morphology resulting in yield reduction of tomatoes, but the impacts of long-term high humidity and high temperature on tomatoes are not well-studied. As climate change is predicted to result in more frequent high-temperature and high-humidity days, it is important to investigate this aspect for development of adaptation or mitigation strategies in sustaining food security in the face of climate change. Here, we investigated the physiological effects of continuous high day-time temperature (35° C), and high humidity (80% RH) on tomatoes using model plant *Solanum lycopersicum* cv. Micro-Tom in environment-controlled chambers, aiming to provide more insights on potential impacts of long-term high temperature high humidity (HTHH) conditions on the growth and development of tomatoes. The impacts of HTHH are observed in term of growth rate, flowering time, flower morphology, number of flowers per inflorescence, whole plant physiology, fresh and dry weight of shoot and root, root length, observation of signs of senescence and so on. More attentions were given on investigating the HTHH effects on flower physiology such as ovary height, style and anther lengths, pollen germination rate, as those factors are directly related to fertilization rate and fruit yield. It was observed that Micro-Tom plants exposed to long-term HTHH flowered and senesced earlier as compared to plants grown in the 25° C control chamber with the same %RH. HTHH-challenged Micro-Tom also showed smaller, odd-shaped flowers, low pollen production and pollen germination rate, with a higher rate of floral fasciation. Interestingly, plants grown in the control chamber shown an enclosed canopy with leaves growing closer to each other, while plants in HTHH chamber have wider space in between leaves with retarded growth of lateral shoots. The underlying mechanisms for the observed physiological changes are under investigation.

P56:
Physiological and Morphological Adaptations of Tomato Plants to Waterlogging and Oxygen Deficiency

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Global warming is intensifying heavy rainfall, leading to floods across 188 countries and causing US$ 7.8 billion in agricultural damages, making flooding the second most destructive agricultural disaster after drought, according to the FAO. It is increasingly evident that flood tolerance in plants results from a combination of structural and metabolic traits, with the development of adventitious roots, aerenchyma, and the ability to withstand post-anoxic damage recognized as key indicators of tolerance. However, tomato plants are particularly sensitive to flooding, posing significant challenges. We hypothesize that tomato introgression lines IL11-4 and IL8-1, which exhibit varying levels of drought response, will show distinct physiological and morphological responses to waterlogging compared to the mother plant M82, due to their differential ABA-mediated stress responses. To test this hypothesis, we conducted experiments under oxygen deficiency (achieved using nitrogen gas) and waterlogging conditions, focusing on selected IL lines with different water balance and drought response regulation. Our results revealed that oxygen deficiency significantly increases soil pH while decreasing its redox potential, leading to a proton transport imbalance that reduces mineral absorption and transpiration rates. Waterlogging, resulted with similar reduced transpiration rates, shoot length, root length, and the root-to-shoot ratio. It also significantly increased adventitious root formation, with varying effects across different tomato varieties. M82 consistently demonstrated low sensitivity to flooding as it reduces transpiration in lower percentage than IL 8-1 and IL11-4, despite the fact that these lines significantly produced more lateral roots than M82 at the soil-air surface. Flooding significantly affects more introgression lines than their mother plant M82, with IL 8-1 showing the highest flood sensitivity than IL11-4. Interestingly, although flooding induced adventitious root production, especially in sensitive varieties and at the soil surface, this response did not promptly restore normal transpiration rates. While adventitious roots are one of the main morphological adaptations for coping with waterlogging stress, this adaptation was not verified for M82 introgression lines, suggesting that ABA regulation might play a critical role in plant adaptation to flooding. For future research, utilizing specific tomato introgression lines (ILs) to investigate how genetic differences influence plant resilience under waterlogging could provide new insights for breeding strategies.

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P57:
Salt-priming induces salt tolerance in young tomato plants

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Crops are increasingly exposed to a broad range of stresses that require strategies for improving tolerance. Besides breeding techniques, priming is a mechanism that induces plant tolerance against abiotic and biotic stresses without the need for chemical intervention. To determine the benefits of abiotic stress priming on young tomato plants as a crop protection method in tomato production, young tomato plants were subjected to salt stress to induce priming and after a following 5 days or 10 days lasting recovery phase plants were exposed to a repeated salt stress treatment.
Plants' defense responses in each phase of experiment were analyzed by image-based phenotyping, quantification of secondary metabolites and gene expression analysis.
Young tomato plants had reduced size and relative growth rate due to the priming stress-treatments, but salt-primed plants established a significantly higher relative growth rate after the second salt stress treatments. Quantification of secondary metabolites revealed that salt-priming induced a higher content of total phenolics and anthocyanins in leaves after subsequent salt stress. Accumulation of these metabolites in salt-stressed plants was accompanied by higher expression of dihydroflavonol 4-reductase (DFR) gene in leaves. RNA-sequencing revealed significant differences in transcription of genes in primed compared to unprimed plants during subsequent salt stress treatment. In response to salt, primed plants versus unprimed plants showed overrepresentation of early upregulated genes in functional groups for example related to phytohormone, chromatin organization, cell division, and in plant secondary metabolism related to phenolics/flavonoid biosynthesis.
The applied salt-priming improved growth and enhanced metabolic stress responses by inducing corresponding genes like under salt stress. By further understanding the underlying mechanisms and the durability of the priming memory, priming might serve as a plant protection method in tomato production in future.

P58:
Characterization of Root System and Architectural Traits for Heat Tolerance in Tomato (Solanum lycopersicum L.) Lines

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Abstract

Global climate change poses significant challenges to tomato breeding, especially by impacting crop production. Extreme temperatures lead to reduced yields, lower fruit quality, and shorter shelf life, highlighting the urgent need to develop stress-resilient tomato varieties. However, breeding efforts predominantly focused on above-ground trait, such as flowering, fruit set, size, and yield.

The root system is fundamental to plant growth and plays a critical role in plant performance under stress. This study investigates the effects of heat stress on root development in tomato lines from WorldVeg Heat Tolerance MAGIC population program. This diverse panel of tomato lines was evaluated under control and heat stress conditions in controlled environment. Key root traits—such as biomass, length, surface area, and main root axis length—were measured at the flowering, fruiting, maturity, and harvest stages. Through detailed phenotypic analyses, we sought to uncover unique traits associated with heat tolerance correlated to the root system performance.

Our results showed that heat-tolerant tomato lines exhibited compact surface area and root volume under heat conditions. Significant differences were found in root orientation, depth and total root length between the parent and the progeny group. Additionally, heat-tolerant plants displayed compact canopy architectures and other parameters indicating reducing water loss through transpiration. These findings offer key insights into heat tolerance in tomatoes, marking a crucial step in understanding the root system's role. By identifying traits linked to heat-tolerant plants, we can better understand how they enhance above-ground performance and inform breeding strategies for heat-resistant varieties.

P59:
Understanding the physiological and genetic basis for Blossom-end rot

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Blossom-end rot (BER) is a physiological disorder in tomato which results in huge economic losses. It initiates in young fruit, mostly around 7-10 days post anthesis (DPA) as a water-soaked area in the distal pericarp of the fruit. Calcium deficiency at the distal end of the fruit is generally associated with BER incidence. In this study, we investigated the physiological and genetic basis of BER. The plant material for this study has been created by crossing a resistant (BGV007900) and susceptible (BGV007936) parent followed by generating an F_2 segregating population. In this population a QTL-sequencing approach was used, to identify 3 different QTLs associated with BER: *BER3.2*, *BER4.1*, and *BER11.2*. Near Isogenic Lines (NILs) segregating only for QTL *BER11.2* was developed with a size of 1.13Mbp. This QTL contains 141 genes within the interval including *FASCIATED* (*fas*) which is known to control the locule number and affects fruit weight. These lines were used for characterization of calcium distribution and xylem functionality. Calcium concentration was determined at 7, 10 and 14 DPA in three tissue types: proximal pericarp, distal pericarp, and inner tissues between the resistant and susceptible NILs. The concentration of calcium in the inner tissues (comprising of placenta, columella and immature seeds) displayed a significantly higher calcium concentration in the resistant NILs at 10 and 14 DPA. Analysis of functional xylem at 14 DPA using a water-soluble dye indicated that fruit from the resistant NILs displayed a significantly higher number of functional xylems per cross-sectional area in distal pericarp, placenta and columella compared to susceptible NILs. These results suggest that a higher number of functional xylems in the resistant NILs may lead to increased calcium translocation at the distal end of the fruit. During fine-mapping, an additional QTL *BER11.1* was identified close to *BER11.2*. NILs were developed harboring both QTLs (*BER11.1 & BER11.2*), with 372 genes within the interval spanning 3.07 Mbp. RNA-sequencing was performed on 7 and 10 DPA fruit using the distal pericarp and distal inner tissues of resistant and susceptible NILs harboring *BER11.1* and *BER11.2*. Downstream bioinformatic analyses identified 53 and 17 differentially expressed genes (DEGs) at distal inner tissue at 7 and 10 DPA respectively, while 40 and 59 DEGs at distal pericarp at 7 and 10 DPA respectively between the two lines. Of these, based on gene function, further candidates will be short-listed for functional validation.

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P60:

Genome-wide identification of the *N*^{6}-methyladenosine regulatory genes reveals *NtFIP37B* increases drought resistance of tobacco (*Nicotiana tabacum* L.)

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N^6-methyladenosine (m^{6}A) is one of the common internal RNA modifications found in eukaryotes. The m^{6}A modification can regulate various biological processes in organisms through the modulation of alternative splicing, alternative polyadenylation, folding, translation, localization, transport, and decay of multiple types of RNA, without altering the nucleotide sequence. The three components involved in m^{6}A modification, namely writer, eraser, and reader, mediate the abundance of RNA m^{6}A modification through complex collaborative actions. Currently, research on m^{6}A regulatory genes in plants is still in its infancy. In this study, we identified 52 candidate m^{6}A regulatory genes in common tobacco (*Nicotiana tabacum* L.). Gene structure, conserved domains, and motif analysis showed structural and functional diversity among different subgroups of tobacco m^{6}A regulatory genes. The amplification of m^{6}A regulatory genes were mainly driven by polyploidization and dispersed duplication, and duplicated genes evolved through purified selection. Based on the potential regulatory network and expression pattern analysis of m^{6}A regulatory genes, a significant number of m^{6}A regulatory genes might play important roles in growth, development, and stress response processes. Furthermore, we have confirmed the critical role of *NtFIP37B*, an m^{6}A writer gene in tobacco, in enhancing drought resistance. This study provides useful information for better understanding the evolution of m^{6}A regulatory genes and the role of m^{6}A modification in tobacco stress response, and lays the foundation for further elucidating the function of m^{6}A regulatory genes in tobacco.

Acknowledgements
This study was supported by Natural Science Foundation of HeNan (232300420220) and Beijing Life Science Academy [BLSA: 2023000CC0110].

P61:

Calcium Localization and Physiological Mechanisms Underlying Blossom-End Rot in Tomatoes and Their Application to Understanding Watercore in Japanese Pears

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Physiological disorders present a critical challenge in crop cultivation. Blossom-end rot (BER) is a significant physiological disorder that affects fruits, including tomatoes. The stability of the cell membranes in fruit cells is significantly influenced by Ca^{2+} localized in the apoplast, suggesting that apoplastic Ca^{2+} levels may be linked to the occurrence of BER. In the present study, we aimed to elucidate the physiological mechanisms of BER in IL5-4 tomato plants. IL5-4 is a tomato line that carries a chromosome segment from *Solanum pennellii* on chromosome 5 of *Solanum lycopersicum* 'M82'. Despite sufficient Ca^{2+} levels in the fruit, IL5-4 exhibits a higher incidence of BER compared to 'M82'. Through bioimaging, we quantified the apoplastic Ca^{2+} and discovered that the concentration of Ca^{2+} in the apoplast of IL5-4 was lower than that in 'M82' at 15 days after flowering (DAF). IL5-4 fruits exhibit larger size and faster growth compared to 'M82' during 10–15 DAF. Our findings suggest that the localization of Ca^{2+} and the high demand for Ca^{2+} due to rapid fruit growth in IL5-4 result in reduced apoplastic Ca^{2+} concentrations, leading to decreased membrane stability and a higher incidence of BER. Additionally, transcriptome analysis (RNA sequencing) of 'M82' and IL5-4 fruits at 15 DAF revealed that 60 genes exhibited tissue-specific expression in the distal part of IL5-4 fruit, compared to the proximal and distal parts of 'M82' and the proximal part of IL5-4. Notably, *Solyc05g054250*, located on chromosome 5, was highly expressed in the distal part of IL5-4 fruit. Conversely, the expression of certain Ca^{2+}-ATPases, which are involved in increasing apoplastic Ca^{2+} levels and maintaining cell membrane stability, was lower in IL5-4 compared to 'M82'. Building on these findings, we are currently applying our research methods to investigate physiological disorders, known as watercore disorders, in Japanese pears. Similar to BER in tomatoes, watercore in Japanese pears is believed to be caused by a deficiency of Ca^{2+} within the fruit. However, the precise physiological mechanisms watercore remain unclear. Therefore, we utilized bioimaging techniques established in our research on tomatoes to explore and clarify watercore causes in Japanese pears.

Acknowledgements

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P62:
Cysteine protease inhibitors as triggers of drought tolerance and tuber yield in potato

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Current climate change scenarios predict an increased incidence of drought episodes likely to affect potato crops worldwide. Potato exhibits a low-density, shallow root system that makes it particularly vulnerable to water shortage and any successful attempt to implement drought tolerance in cultivated potato varieties is potentially relevant from an agronomic standpoint. In this study, we assessed the potential of cysteine protease inhibitors (CYS PIs) to promote drought tolerance in CYS PI-expressing potato lines by induction of stress-related pleiotropy. Up to now, CYS PIs have been mostly considered as biotechnological tools to engineer pest or pathogen resistance in crops, but several recent studies have also revealed a possible link between abiotic stress tolerance and these regulatory proteins in leaf tissue. CYS PI-expressing plantlets grown on culture medium containing the drought mimic polyethylene glycol (PEG) exhibited an elevated root-to-shoot ratio, an indicator of drought tolerance in potato. A similar conclusion could be drawn with greenhouse-grown acclimated plants, confirming a relative root growth-promoting effect for the recombinant inhibitor upon water deficit. Transgenic potato lines also showed a high tuber yield compared to the control line under both limiting and non-limiting water regimes, suggesting an improved efficiency of the primary metabolism and the avoidance of a growth/stress response tradeoff in the modified lines. Accordingly, CYS PI expression was associated with a stress response-oriented proteome in leaves likely explained by pleiotropic effects of the recombinant inhibitors driving the constitutive expression of stress-related proteins and the upregulation of primary metabolism-associated proteins. Overall, these data suggest the potential of CYS PIs as molecular triggers of tuber biomass production and drought resilience in potato. Complementary studies will be warranted to assess tuber yield of the transgenic lines under different water regimes in field conditions.

Acknowledgements
This work was supported by a Discovery grant from the Natural Science and Engineering Research Council of Canada.

P63:

Principal Component and Cluster Analysis of Heat Tolerance Traits in 13 Tomato Varieties under Heat Stress Conditions in Indonesia

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Tomato (*Solanum lycopersicum* L.) is a significant horticultural commodity in Indonesia, highly valued for its antioxidant and vitamin content. However, heat stress, particularly during the dry season, negatively impacts tomato production in greenhouse environments, where temperatures can reach up to 45°C and substantially reducing plant productivity. This research aimed to evaluate 13 tomato varieties with diverse genetic backgrounds for heat tolerance traits under greenhouse conditions. The study was conducted from July to November 2023 at the greenhouse facilities of Bale Tatanen Padjadjaran, Faculty of Agriculture, Universitas Padjadjaran, Indonesia. The experiment was arranged in a randomized complete block design with three replications. Data were analyzed using variance analysis, the Scott-Knott test, principal component analysis (PCA), and cluster analysis. The results showed significant differences among the 13 genotypes in most heat stress-related traits observed, including leaf, flower, fruit, and fruit quality traits. The varieties 'Black Cherry' and 'Yellow Pear' exhibited the highest fruit set (tolerant), while 'Golden Sunray,' 'Golden Jubilee,' and 'Beef Steak' had the lowest fruit set (very susceptible). Most Indonesian varieties were categorized as susceptible to moderately tolerant to heat stress. PCA revealed that the first two components (PC1 and PC2) explained 57.44% of the total variation across 33 traits. PC1 accounted for 35.87% of the variation, primarily influenced by flower, fruit, and fruit quality traits, while PC2 explained 21.50% of the variation, mostly due to leaf and fruit quality traits. Cluster analysis grouped the 13 varieties into three clusters: Cluster 1 (C1) included seven varieties, Cluster 2 (C2) three varieties, and Cluster 3 (C3) three varieties. These findings provide insights into the mechanisms of heat tolerance in different tomato varieties and offer valuable information for selecting parental lines based on key heat tolerance traits for tomato breeding in Indonesia.

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P64:

Transcriptomic, proteomic, biochemical, and immunohistochemical analysis revealed the role of stress indicators, hormonal changes, and cell wall remodeling in response to hypoxia stress priming in roots of *Solanum lycopersicum* L.

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Waterlogging caused oxygen deprivation stress that negatively impacts the growth and yield of tomato. The priming treatment can be solution for plant adaptation to stress. Our studies have shown that under first (1xH) and second (2xH) hypoxia stress, the protein profile shifts of tomato roots in two accessions, waterlogging sensitive (WL-S) PZ-215 and waterlogging tolerant (WL-T) POL 7/15. Accumulation of alcohol dehydrogenase (ADH2) – marker of hypoxia, decreased after priming treatment for PZ-215, which was consistent for POL 7/15. Based on RNA-seq data, the hypoxia-specific genes annotated to various biological pathways were selected. The effect of hypoxia on the changes of content of jasmonic acid (JA), its methyl derivative (JAMe), and 1-aminocyclopropane-1-carboxylate **(**ACC) in tap and lateral roots was determined. The second stress treatment effected decreasing of JA level for both of accessions. The high level of ACC, the precursor of ethylene, especially after the 2xH may indicate that ethylene is a crucial factor in the adaptation to the low oxygen environment for sensitive as well tolerant access. The content of the stress related indicator, malondialdehyde (MDA) in lateral roots depends on tomato accessions. Furthermore, the accumulation of proline and proline-betaine after first hypoxia stress exposure, important organic osmolytes and abiotic stress markers, may indicate an increase in the activity of enzymes involved in the oxidative defense system. The modification of cell wall structure and composition in response to environmental factors is one of the key elements of the plant defense system. Oxygen deficiency caused changes in xyloglucan distribution after both hypoxia treatments, particularly in the zone of lateral root formation. To determine the content of xyloglucans in lateral roots, immune-dot blot assays were performed using the LM15, LM24, and LM25 epitopes. To sum up, our findings reveal that the hypoxia stress and priming in tomato roots is accompanied by transcriptomic, proteomic and physiological responses that involve the expression of genes, changes of hormones levels and remodeling of the cell wall with xyloglucans. These modifications may improve the hypoxia acclimation in root tissues. The data on the plant response to the priming effect can be useful in the selection of new tomato varieties that are more resistant to the stress conditions.

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P65:
Phosphorylation and ubiquitination of SlERF.D2 are integral to SlCPK27-mediated drought tolerance in tomato

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Abstract：Plants inevitably encounter a diverse array of constantly changing environmental stresses, and drought stands out as one of the most serious threats for plants. Calcium Dependent Protein Kinases (CPKs) play pivotal roles in plant drought tolerance, especially in ABA response. We previously found that tomato CPK27 conferred drought tolerance by positively transmitting ABA signal. Here, we uncover that CPK27 interacts with and phosphorylates ERF.D2, an Ethylene Response Factor, to promote its degradation in a ABA-dependent manner. Furthermore, we identified a U-box protein, PUB22, that ubiquitinated ERF.D2 to promote its degradation via 26s proteasome, which was enhanced by CPK27-mediated phosphorylation. As a transcription factor, ERF.D2 could bind to the promoter of jasmonic acids (JA) biosynthesis genes, *AOC* and *OPR3*, to repress their expression under normal conditions, preventing JA excessive accumulation. These findings demonstrate that CPK27 and PUB22 function as positive drought stress regulators, to fine-tune ERF.D2-mediated JA homeostasis in response to drought stress in tomato plants.

P66:

Impact of Carbonic Anhydrase βCA2.2 Phosphorylation Levels on Elevated CO_2-Mediated Thermotolerance in Tomato

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The intensification of the greenhouse effect leads to rising temperatures and increased CO_2 concentrations. In recent years, an increasing amount of literature has suggested that elevated CO_2 can mitigate the negative effects of heat stress, but the underlying mechanisms remain largely unknown. In this study, we verified that increasing CO_2 concentrations enhanced tomato thermotolerance and confirmed that the primary carbonic anhydrase involved in this process was βCA2.2. Through immunoprecipitation mass spectrometry, we identified the interacting protein phytosulfokine receptor 1 (PSKR1) of βCA2.2, and demonstrated that their interaction strength increased with the addition of HCO_3^-. Additionally, we showed through hybridization that PSKR1 functions upstream of βCA2.2. Our findings revealed that the phosphorylation levels of βCA2.2 changed under high-temperature and elevated CO_2 conditions. As a receptor-like protein kinase, PSKR1 was able to phosphorylate βCA2.2, and a mutation at ser231 that blocked phosphorylation, impaired βCA2.2-mediated thermotolerance. Global transcriptome analysis and phosphatidic acid (PA) content measurement indicated that βCA2.2 may influence thermotolerance by affecting the phospholipid signaling pathway. These results highlight the crucial role of the PSKR1-βCA2.2–PA signaling pathway in elevated CO_2-induced thermotolerance in tomato. Thus, this study enhances our understanding of the mechanisms underlying the stress response to elevated CO_2 and may provide valuable insights for breeding heat-tolerant plants in the face of climate change.

P67:
The role of SlCRK2-SlC3H39 in regulation of cold resistance in tomato.

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In this study, we found that SlCRK2 (Calcium Dependent protein kinase related 2) negatively regulates tomato cold resistance. SlCRK2 interacts with another negative cold resistance regulator-SlC3H39, a CCCH-type transcription factor, by Y2H, BiFC, GST-pull down. Phosphorylation assays in vitro demenstrate that SlCRK2 phosphorylates SlC3H39 at a tyrosine site. Besides, we found that myristoylation of SlCRK2 at its N-terminal inhibits interaction between C3H39. Thus, our next goal is to investigate the role of myristoylation regulation towards tomato cold stress resistance.

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P68:

miR164a targets NAM3 to enhance thermotolerance in tomato via regulation of HSFA4b-dependent redox homeostasis

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Abstract

Extreme weather events, including high temperatures, frequently occur and adversely affect crop growth, posing significant challenges to global agriculture. MicroRNAs (miRNAs) play integral roles in regulating plant growth and responses to various stresses. In this study, we revealed that miRNA164a/b in tomato (Solanum lycopersicum) serves as a pivotal element that exhibits a rapid positive response to heat stress (HS) among multiple miRNAs, while its target NAM3 shows an opposite complementary response. MiR164a/b-5p-deficient mutant and NAM3-overexpressing plants resulted in increased sensitivity to HS, whereas mutants with reduced NAM3 levels exhibited enhanced thermotolerance. Importantly, HS-induced reactive oxygen species (ROS) accumulation and antioxidant enzyme activities were positively regulated by miR164 and negatively by NAM3, respectively. Furthermore, we demonstrated that NAM3 transcriptionally activate the expression of HSFA4b, and silencing HSFA4b improves tomato thermotolerance. HSFA4b represses the expression of the antioxidant gene APX1 and the heat shock protein gene HSP90, disrupting redox homeostasis and exacerbating oxidative stress. Our findings unveil a pivotal regulatory pathway governed by the miR164-NAM3 module that confers thermotolerance in tomato via its influence on ROS-related and HSP pathways. These findings provide valuable insights into the molecular mechanisms that underpin tomato thermotolerance, which is crucial for advancing sustainable agricultural practices, particularly in the face of the challenges presented by global climate change.

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P69:

NBR1a mediates root-knot nematode resistance by modulating antioxidant system, jasmonic acid and selective autophagy in Solanum lycopersicum

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Root-knot nematodes (RKNs; Meloidogyne incognita) are recognized as the most destructive plant pathogenic nematodes, posing a significant threat to global food security. Selective autophagy, a conserved process for degrading and recycling cellular components, plays an essential role in the growth, development, and immune response of eukaryotes. The established role of selective autophagy receptors in plant responses to diverse stresses is well recognized, however, their precise functions in plant defense against RKN infection remain largely unexplored. This study investigates the response of nine selective autophagy receptors in tomato (Solanum lycopersicum) to RKN infection. Notably, the relative expression of NBR1a, PUX7a and ATI3d showed significant induction. NBR1a-silenced plants and nbr1a mutants both exhibited heightened sensitivity to RKN compared to the pTRV control and wild-type (WT) plants, while silencing of PUX7a and ATI3d genes did not exhibit increased sensitivity to RKN compared to the pTRV control plants. Furthermore, antioxidant enzyme activity, jasmonic acid (JA), as well as the number of autophagosomes and autophagic vesicles in root tip cells, did not exhibit significant induction in the nbr1a mutant upon RKN infection, in contrast to WT plants. These findings strongly suggest that the selective autophagy receptor NBR1a enhances tomato resistance against RKNs by modulating the antioxidant system, JA and selective autophagy.

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P70:
Role of phospholipase A1 and its product lysophospholipids in tomato tolerance to cold stress

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Cold stress poses a significant challenge to the growth, productivity and quality of tomato. It has been apparent that membranes and their lipid composition play a crucial role in plant cold tolerance. In this study, we performed a metabolomic analysis of cold-sensitive cultivated tomato (*Solanum lycopersicum*) and cold-tolerant wild tomato (*S. habrochaites*). Results showed that among 22 kinds of lysophospholipids identified, 18 kinds of lysophospholipids accumulated after 24h of low temperature treatment in cultivated tomato. The main lysophospholipids accumulated in cultivated tomato were LPC (16:0), LPE (16:0), and LPC (18:2). However, the accumulation of these lysophospholipids in cold tolerant varieties was significantly reduced. By further analyzing the RNA-sequencing data, we found that the transcripts of *PLA1-PLIP2* was significantly induced in cultivated tomato. Under the same time, the expression of this gene in cold-tolerant tomatoes was much lower than that in cold-sensitive tomato. In vitro experiments confirmed that the protein encoded by this gene could catalyze the production of lysophospholipids. Based on the above results, we proposed that *PLA1-PLIP2* plays an important role in membrane lipid remodeling, which in turn affects the cold tolerance of tomato.

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P71:
Global co-expression network for key factor selection on environmental stress RNA-seq dataset in *Capsicum annuum*

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Environmental stresses significantly affect plant growth, development, and productivity. Therefore, a deeper understanding of the underlying stress responses at the molecular level is needed. In this study, to identify critical genetic factors associated with environmental stress responses, the entire 737.3 Gb clean RNA-seq dataset across abiotic, biotic stress, and phytohormone conditions in *Capsicum annuum* was used to perform individual differentially expressed gene analysis and to construct gene co-expression networks for each stress condition. Subsequently, gene networks were reconstructed around transcription factors to identify critical factors involved in the stress responses, including the NLR gene family, previously implicated in resistance. The abiotic and biotic stress networks comprise 233 and 597 hubs respectively, with 10 and 89 NLRs. Each gene within the NLR groups in the network exhibited substantial expression to particular stresses. The integrated analysis strategy of the transcriptome network revealed potential key genes for complex environmental conditions. Together, this could provide important clues to uncover novel key factors using high-throughput transcriptome data in other species as well as plants.

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P72:
Discovering Disease-Resistance Genes via Pan-Genome Analysis in Pepper

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Pepper (*Capsicum annuum* L.) is a significant horticultural crop with substantial economic importance. To date, a considerable amount of pepper genome information has been reported, including approximately 30 genome assemblies and three pan-genomes. Nevertheless, the application of these pan-genomes to agricultural studies has been limited due to the lack of reliable re-sequencing and phenotype datasets in pepper. To address these issues, we have updated the pepper pan-genomes by integrating high-quality genomes and reformatting the pan-genome variant information into a graph-based structure. To precisely annotate and compare the sequence variation of pepper disease resistance genes, we employed resistance gene enrichment sequencing (RenSeq) technology, a targeted enrichment sequencing method for nucleotide-binding leucine-rich repeat (NLR) genes. The updated pepper pan-genome analysis catalogued genetic variations across 26 *C. annuum* genomes and identified several candidate genes associated with disease resistance in pepper. Notably, two candidate genes for resistance against root-knot nematodes and ChiVMV were discovered. The candidate genes for root-knot nematode resistance were found on chromosome 9 and characterized by small indels in their exon regions. The candidate gene for ChiVMV resistance was located on chromosome 6 and marked by three SNPs in its exon region. To validate these findings, we cloned the candidate gene for potyvirus resistance and demonstrated a significant interaction between this gene and ChiVMV. These findings are crucial for advancing omics-based studies and disease-resistance breeding programs in pepper.

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P73:
Unveiling the effector arsenal of Phytophthora capsici: a transcriptomics journey

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Pepper (*Capsicum annuum*) cultivation holds significant economic importance globally; however, its productivity faces substantial threats from plant pathogens, notably *Phytophthora capsici*. This oomycete presents a challenge due to its diverse genetic makeup, high capacity for adaptation to its plant host, and possession of a remarkable set of effectors used to infect host plants. To address this challenge, we conducted a comprehensive transcriptomics analysis to decipher the molecular arsenal of *P. capsici*. Using a time-course RNA sequencing approach, we sampled RNA from infected pepper roots across all infection stages, from zoospore to sporulation. Focusing on the biotrophic phase of infection, we identified 2,187 differentially expressed genes, pinpointing potential effector candidates. Subsequently, by utilizing various criteria such as expression profiles, secretion patterns, structural features, and biochemical properties, we selected 150 promising effector gene candidates. These candidates will be validated through PVX-mediated expression in planta to assess their potential in triggering a hypersensitive response (HR) in resistant pepper accessions. Our study sheds light on the interplay between *P. capsici* and pepper, deepening our understanding of effector biology in plant pathogens. These results lay the groundwork for future research aimed at mitigating the impact of this notorious plant pathogen.

P74:

A *Glutathione S-Transferase* gene confers robust resistance to Fusarium crown and root rot in tomato

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Fusarium crown and root rot (FCRR), caused by fungal pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Forl*), is one of the most devastating diseases threatening tomato production. An oxetane-containing diterpene named FCRR-toxin has been reported as a potential toxin in *Forl* pathogenesis. The most effective way to control FCRR is cultivating resistant cultivars. According to previous studies, a single dominant locus named *Frl* provides resistance to FCRR in tomatoes. This resistance locus was introgressed from the Peruvian tomato, located on chromosome 9. To date, *Frl* has not been cloned, and the molecular basis of this durable resistance of *Frl* against *Forl* remains unknown. In this study, we found the key biosynthesis gene of FCRR-toxin is *TPS1*. The production of FCRR-toxin was totally lost in *Δtps1* mutants. Decreased pathogenicity in *Δtps1* mutants indicates that FCRR-toxin is required for *Forl* full virulence. To determine the candidate gene underlying the *Frl* locus, we assembled a genome for the FCRR resistance cultivar Momor and identified *Frl* by map-based cloning. *Frl* encodes a Glutathione S-Transferase. Frl confers FCRR resistance by detoxifying FCRR-toxin through the conjugation of a GSH unit onto the oxetane group of FCRR-toxin. Expression of *Frl* in other tomato cultivars and *N. benthamiana* enhances the resistance to *Forl*, providing a solution for FCRR resistance breeding.

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P75:
Title: Ty-genes effectiveness to ToLCNDV and different TYLCV strains

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Tomato yellow leaf curl disease (TYLCD), caused by begomoviruses such as the monopartite Tomato yellow leaf curl virus (TYLCV) and the bipartite Tomato leaf curl New Delhi virus (ToLCNDV), poses a significant threat to global tomato (*Solanum lycopersicum*) production, leading to substantial economic losses. Resistance to TYLCD has been identified in various wild *Solanum* species, resulting in the discovery of six TYLCV resistance genes (*Ty-1* to *Ty-6*).In this study, agro-infectious clones of the ToLCNDV-Jessore strain, along with TYLCV strains TYLCV-IL, TYLCSV, and their natural recombinant TYLC-IS76, were used to challenge tomato lines carrying different *Ty*-genes. Symptom severity was assessed 30 days post-inoculation (DPI), and viral titter was quantified using qPCR.
Our findings revealed that the TYLCSV strain induces significantly milder symptoms compared to the other TYLCV strains, regardless the presence or absence of *Ty*-genes. *Ty-1* plants exhibited no symptoms following TYLCSV inoculation and only mild leaf yellowing when infected with TYLCV-IL and TYLC-IS76. Notably, the viral titter of TYLCSV in *Ty-1* plants was significantly reduced compared to TYLCV-IL and TYLC-IS76, which both exhibited high viral titter. These results contrast with previous reports suggesting that TYLCV-IS76 can overcome *Ty-1* resistance more effectively than TYLCV-IL. In *Ty-2* plants, no symptoms were observed with any of the TYLCV strains. Although TYLCV-IL and TYLCV-IS76 showed detectable but low viral titter, the TYLCSV strain displayed high viral titters, indicating that the *Ty-2* gene is compromised by TYLCSV, consistent with findings from other studies. Both *ty-5* and *Ty-6* plants were symptomless when infected with TYLCSV but exhibited clear symptoms upon infection with TYLCV-IS76 and TYLCV-IL strains. However, these symptoms were milder than those in the susceptible Moneymaker cultivar. High viral titters of all TYLCV strains were observed in both *ty-5* and *Ty-6* plants, indicating that these genes confer tolerance rather than resistance to TYLCV. *Ty-1* plants were symptomless upon ToLCNDV infection and exhibited reduced viral titters compared to susceptible plants, suggesting that *Ty-1* is able to halt ToLCNDV symptoms and proliferation. In contrast, the rest *Ty*-gene lines displayed severe symptoms and high viral titters, similar to the susceptible Moneymaker plants, suggesting that these genes are ineffective against ToLCNDV. Our ongoing research focuses on evaluating the effectiveness of combining different *Ty*-genes to enhance resistance against both ToLCNDV and TYLCV strains.

Acknowledgements
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P76:
Dual functions of bacterial Chp family proteins in virulence and induction of hypersensitive response in *Solanaceae* plants

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Two bacterial species, *Clavibacter capsici* and *C. michiganensis*, cause bacterial canker disease in *Solanaceae* plants such as pepper and tomato, respectively. They use putative serine proteases as key virulence factors. Chp family proteins are putative serine proteases, and Pat-1_{Cm} and $ChpG_{Cc}$ in this protein family are critical for the virulence of these bacteria in host plants. Previously, we showed that Pat-1_{Cm} can induce the hypersensitive response (HR) to nonhost plants. In this study, we determined the HR-inducing ability of $ChpG_{Cc}$ and other Chp family proteins. To confirm their HR induction ability, the signal peptide of nine Chp family proteins was removed, and they were fused to the tobacco PR1b signal sequence to ensure that their mature forms are secreted and localized in the apoplast of plant cells by *Agrobacterium*-mediated transient assay in *Nicotiana tabacum*, *N. benthamiana*, tomato, and eggplant. Two *Clavibacter* species had four common Chp family proteins, including ChpC, ChpE, ChpG, and Pat-1. In contrast, ChpF was found only in *C. michiganensis*. Among these Chp proteins, ChpG and Pat-1 induced an HR on *N. tabacum* leaves, regardless of the bacterial species, while neither of the two ChpC proteins induced HR in nonhost plants. In addition, none of the Chp family proteins could elicit an HR on the leaves of *N. benthamiana*, tomato, and eggplant. Furthermore, the $ChpG_{Cc}$ alanine substitution mutants, $ChpG_{Cc}$-S231A and $ChpG_{Cc}$-C187A, lost their ability to induce HR. For the virulence assay, the same amino acids were substituted with alanine (*chpG*$_{Cc}$-S231A and *chpG*$_{Cc}$-C187A) and cloned into the *Clavibacter* expression vector. These two substitution mutants were unable to cause disease in the host plant *Capsicum annuum*. Our results indicate that $ChpG_{Cc}$ is involved in both the virulence and HR induction of *C. capsici* in *Solanaceae* plants, similar to Pat-1_{Cm}, and $ChpG_{Cm}$ and that Ser231 and Cys187 are essential amino acid residues for $ChpG_{Cc}$ function.

Acknowledgements
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P77:

Metabolic engineering of a 1,8-cineole synthase enhances aphid and root-knot nematode repellence and in transgenic tobacco

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Abstract:

Root-knot nematodes (RKNs) and green peach aphids (*Myzus persicae*) are significant agricultural pests that threaten global crop production. Traditional chemical control methods have detrimental effects on the environment and human health, prompting a search for sustainable alternatives. This study explores the potential of metabolic engineering of the *Salvia officinalis* 1,8-cineole synthase (*CINS*) gene in transgenic tobacco plants to enhance resistance against these pests.

We demonstrate that overexpression of the *CINS* gene in tobacco plants (*CINS*-OE) results in the increased emission of root volatiles, including 1,8-cineole, which plays a key role in repelling *Meloidogyne incognita* (*M. incognita*), a major root-knot nematode. The *CINS*-OE plants exhibited a deterrent effect on infective second-stage juveniles (J2s) of *M. incognita*, leading to reduced gall numbers and egg mass counts compared to wild type (WT) plants. In vitro assays confirmed that a mixture of commercially available volatile organic compounds (VOCs) had a significant deterrent effect on infective J2s. Furthermore, our findings reveal that *CINS*-OE tobacco plants also exhibit repellent properties against the green peach aphid. Overexpression of *CINS* led to an increase in trichome density and the emission of 1,8-cineole at levels sufficient to deter aphids without affecting their development or fecundity. Y-tube olfactometer assays and free-choice assays provided evidence of the repellent effect of *CINS*-OE plants on aphids.

In conclusion, the metabolic engineering of the *CINS* gene in tobacco plants offers a viable and sustainable approach to enhance resistance against both root-knot nematodes and aphids. By enhancing the emission of root volatiles, particularly 1,8-cineole, these transgenic plants provide a promising alternative for pest management in agriculture.

Acknowledgements

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P78:
Identification and characterization of *Colletotrichum* species and A*lternaria alternata* associated with fruit rot on processing tomato in Japan.

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Tomato anthracnose is one of the most important diseases of processing tomato in Japan. Anthracnose is a frequent problem in the latter part of the growing season on ripening tomato fruit. The disease results in a fruit rot which reduces the quality and yield of tomatoes. Especially, the diseased fruits are the cause of fruit cracking at the time of transport to plant, resulting in rotting and discarding. However, the species that cause tomato anthracnose and the mechanism of infection have not been clarified. In this study, we isolated and identified from diseased fruit rot in the field in Japan. Molecular phylogenetic and morphological analyses revealed that six isolates belonged to *C. cliviicola*, *C. fioriniae* and *C. coccodes*, were different of *Colletotrichum* species. When these isolated strains were inoculated into tomato fruits, they were pathogenic to its. In addition, *Alternaria alternata* were isolated from diseased of fruit rot, and their pathogenicity to processing tomato was also confirmed. These results suggest that the fruit rot is caused by a variety of *Colletotrichum* species and *Alternaria alternata* pathogens.

P79:
Characterization of resistance phenotypes of tomato lines carrying various Ty gene combinations upon exposure to different Tomato yellow leaf curl virus strains

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Tomato yellow leaf curl diseases (TYLCD), caused by begomoviruses, is a major constraint for tomato production in tropical and subtropical regions. Symptoms of the disease include leaf curling, yellowing, mottling, and reduced plant size. In severe cases, plants exhibit stunted growth with shortened internodes and may experience complete yield loss, particularly if infections occur early in plant growth stages. In the absence of resistant tomato varieties, farmers often resort to frequent insecticide applications to control whiteflies in open field production. The rapid emergence, worldwide spread, and evolution of begomoviruses pose a significant limitation for using resistance genes to control the disease. At least 70 different tomato-infecting begomovirus species, including monopartite and bipartite forms, have been found infecting the tomato crop in various regions. Some begomoviruses exhibit exceptional capacity to spread beyond their original geographic region, often displacing or recombining with local begomoviruses and leading to sudden increases in TYLCD incidence and severity. Five major genes providing resistance to TYLCD (Ty genes) are available in tomato, offering the opportunity to pyramid multiple Ty genes in a single tomato variety, which is probably the most effective means of achieving stronger and longer-lasting resistance. The effectiveness of several Ty resistance genes, both individually and in various combinations, was evaluated against selected aggressive begomoviruses through multilocation trials and at disease hotspots. Trials with 11 tomato lines homozygous for selected Ty genes and gene combinations were conducted across different regions and growing conditions in collaboration with partners. Overall, lines homozygous for one or two Ty genes exhibited reduced TYLCD symptoms compared to lines lacking Ty genes, and lines containing markers TY1, TY3, and TY3a, in various combinations, showed significant resistance and reduced spread of the disease in the field. Collaborative research with the University of Valencia (Spain) demonstrated that the TY2 gene confers resistance to the TYLCV-IS76 viral strain, consistent with previous reports. Moreover, tomato lines containing only the ty5 and TY6 genes exhibited tolerance to TYLCV-IS76, similar to lines containing TY1/3. In trials conducted in Varanasi, India, different combinations of Ty genes showed varying protective effects. In lines combining TY3a with TY2 adding ty5 enhanced resistance, while adding ty5 to lines carrying TY1/TY3 and TY2 had no significant effect. Based on the observation that pyramiding different TY genes can increase resistance, it is proposed to develop lines that incorporate the TY6 marker in addition to other resistance gene combinations. Adding TY6 will allow assessing the contribution of this gene towards enhancing resistance against TYLCV strains.

P80:

PMR4 is a susceptibility gene for soft rot disease in potato

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Bacterial soft rot affects a large number of economically important plant species, and is characterized by the breakdown of plant tissue into a soft, watery mass. In potato, soft rot is predominantly caused by *Pectobacteriaceae* including *Pectobacterium* and *Dickeya* species. Infections can lead to significant losses in storage, during cropping, in downgrading and rejection of seed lots. In order to diminish the susceptibility to soft rot diseases, we set out to identify and eliminate susceptibility (*S*) genes. Seventeen *S* genes from *Arabidopsis* were selected for targeting the potato orthologs via RNAi in transgenic Desiree. Plants in which *Powdery Mildew Resistance 4* (*PMR4*) was targeted, showed strongly reduced lesions in leaves upon inoculation with *Pectobacterium* and *Dickeya* species. This suggested that potato *PMR4* could serve as a *S* gene in potato. To confirm this hypothesis, we generated *PMR4* CRISPR/Cas9 mutants in potato variety Desiree. After sequencing of transformants, we identified different mutant alleles in the different events. One of the mutants had mutations in all four alleles resulting in frameshifts or large deletions. This mutant showed reduced lesion sizes as compared to non-transgenic Desiree after leaf inoculations. Also, after stem inoculations, smaller lesions were observed. After root inoculation, a delay in colonization was found. In addition, we found 3-4 fold induced expression of *Pathogenesis Related Protein 1* (*PR1*) in the stem and leaves of the mutant, suggesting a role of salicylic acid signaling in the reduced susceptibility. We are currently further investigating the reduced susceptibility through transcriptome sequencing of root, stem, and leaves of non-transgenic Desiree and the PMR4 mutant. Furthermore we compared the susceptibility levels of Desiree and the PMR4 mutant for other relevant diseases in potato. We found that the mutant showed less or equal suscptibility than Desiree, showing that *PMR4* mutants can provide a valuable contribution to sustainability goals in agriculture. However, since the *PMR4* mutants were generated using GM technology, they cannot be used in agriculture in many parts of the world. Therefore, we also screened classical breeding material for *PMR4* alleles with potential loss off function. Results of *PMR4* as a susceptibility gene for soft rot will be presented and the strategies for resistance breeding in potato will be discussed.

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P81:
Screening of chili pepper genotypes with potential resistance to anthracnose caused by various *Colletotrichum* species

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Chili pepper (*Capsicum* spp., Family: Solanaceae) is one of the important fruit vegetables, being ranked as the fourth major crop in the world. However, its productivity is largely affected by various diseases including those caused by viruses (cucumber mosaic virus; CMV, chili leaf curl virus; CLCV, pepper gemini viruses, etc.), *Colletotrichum* species (anthracnose), and *Pseudomonas* species (bacterial wilt). *Colletotrichum* spp. are ranked among the top ten major fungal pathogens in the world and the anthracnose disease they cause accounts for 10–80% yield losses in the chili industry. Conventional anthracnose control methods such as fungicide application are not always effective and often come along with environmental concerns. Therefore, the present study aims to screen anthracnose-resistant chili pepper germplasms which can be potentially used as a breeding material in the future. We have thus far obtained 30 different *Colletotrichum* strains of various species (*C. gloeosporioides, C. higginsianum, C. scovillei, C. aenigma, C. fructicola, C. karsti, C. sojae* and *C. tropicale*), which have been previously shown to cause anthracnose in chili peppers and other economically important crops. Additionally, we have collected 62 different chili pepper varieties originated from different regions in Japan and across the world and comprising all the five *Capsicum* species. (*C. annuum*: 45, *C. frutescens*: 12, *C. chinense*: 3, *C. pubescens*: 1, *C. baccatum*: 1). At the moment, we are carrying out pathogenicity tests on both fruits and leaves to determine the resistance/susceptibility levels of these chili pepper genotypes against the *Colletotrichum* isolates. Seedling assays are being carried out on 6-week old seedlings by spraying with $\geq 5x10^5$ conidia/mL fungal inoculum, while fruit assays are performed on matured green to red ripe fruits by injecting with 5 uL of the same fungal inoculum concentration. Our preliminary results indicate that strain 243181 (isolated from cacao) has virulence on the leaves of most of the tested chili pepper varieties. This strain can also cause anthracnose symptoms on fruits of various chili peppers. Interestingly, there are some chili pepper germplasms (e.g. NV157) that show moderate susceptibility against strain 243181 and high resistance to strain, 243178. In the future, we hope to select several chili pepper genotypes showing resistance against certain *Colletotrichum* species for further analysis of the genetic mechanisms involved.

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P82:
Structure-guided identification of common host factor targeted by diverse pathogen effectors

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Effectors secreted by phytopathogens, including bacteria, fungi, and oomycetes, play a crucial role in manipulating host immune responses. Due to the evolutionary divergence of pathogen species and the rapid evolution of effectors, the functional relationship between effectors may be concealed at the sequence level. Using ColabFold, we predicted the structural models of 3,346 cytoplasmic effector protein candidates from nine fungal, four chromista, and three bacterial pathogens infecting tomato (*Solanum lycopersicum*). We performed all-by-all structure alignment and structural similarity-based clustering of effectors with Foldseek, identifying 376 non-singleton structural clusters. We identified 98 sequence-unrelated structurally similar (SUSS) effector clusters, possessing 26.5% of the total clusters, by combining structural analysis with sequence alignment using MMseqs2. Among the SUSS clusters, five clusters comprised effectors from bacteria, fungi, and oomycetes, while 22 clusters consisted of effectors from two different kingdoms. Protein-protein interaction assays showed that XopAU, XopAU-like oomycete effectors (XOE) 1, XOE2, and XopAU-like fungal effector (XFE) 1 interacted with SlMKK2, a known host target of XopAU. In addition to screen host factors via computational analysis, we predicted structural model of binders docking with both XopAU and XopAU-like effectors *in silico* using RFdiffusion. The protein complex models of 98 binder-like tomato proteins (BLPs) with XopAU, XOE1, and XFE1 were predicted using AlphaFold-Multimer, identifying 17, 4, and 8 models with ipTM score greater than 0.6, respectively. *In silico* interactions between BLPs and effectors were experimentally validated with yeast-two hybrid assay. Our structure-based study demonstrates that the rapid advancement of AI-based protein structure prediction can be serve as a powerful strategy for unveiling host-pathogen interactions.

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P83:
Variance virulence of *Colletotrichum* spp. isolated from various chili planting locations in Lembang, West Java, Indonesia

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Colletotrichum spp., the causal agent of anthracnose in a wide range of plant species, is considered one of the most important pathogenic fungi in agriculture. Its infection on chili fruits (*Capsicum* spp.) can result in significant yield losses. The virulence of *Colletotrichum* spp. is influenced by various agronomic practices, including the use of different plant varieties and fungicide applications. This study aimed to assess the virulence levels of *Colletotrichum* spp. isolates obtained from chili plants grown in the Lembang region, West Java, as one of the chilies plantation centers in Indonesia. The methodology involved collecting chili fruits exhibiting anthracnose symptoms, followed by the isolation of *Colletotrichum* spp. and subsequent virulence testing on chili fruits of three different varieties commonly grown in the area. Additionally, enzymatic activity assays were performed for each *Colletotrichum* spp. isolate. The results demonstrated significant variation in the virulence among *Colletotrichum* spp. isolates, with the largest lesion area induced by the isolate collected from Kayuambon Village (isolate KYA2). All isolates were found to produce extracellular enzymes associated with pathogenicity, with activity levels varying across the isolates.

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P84:

Dissecting the Genetic Basis of *Septoria* Leaf Spot (SLS) Resistance in Tomato: Insights from Wild Relatives

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Septoria leaf spot (SLS), caused by the fungal pathogen *Septoria lycopersici*, represents a significant challenge to tomato production, especially under humid and rainy conditions. To address this, we aim to introduce durable genetic resistance from wild tomato species into cultivated varieties and to unravel the genetic mechanisms underlying this resistance. Our research identified robust SLS resistance in *S. peruvianum* and *S. arcanum* accessions. Using in vitro ovule culture, we developed five F1 interspecific hybrids between these wild species and cultivated tomatoes, with Hybrid-4 (H4) demonstrating the strongest resistance. H4 has been advanced to the BC5 generation through accelerated backcrossing, combining greenhouse cycles and annual field trials in an organic cropping system. In parallel, we are conducting quantitative trait locus (QTL) analysis using Composite Interval Mapping (CIM) to dissect the genetic components of SLS resistance. CAPS marker evaluation of a segregating population (n=250) suggests an oligogenic inheritance, with three loci implicated. Ongoing work focuses on refining these QTLs and confirming their role in resistance. Our research produces highly resistant tomato lines and generates genetic tools and molecular markers for breeders. Additionally, this work serves as a model for exploring resistance mechanisms against *Septoria* species across other crops, providing insights that may benefit a wide range of agricultural and forestry systems.

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P85:

Independently evolved NLR protein Rps-amr1 confers recognition of a bacterial protease effector AvrPphB in *Solanum americanum*

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The *Pseudomonas syringae* cysteine protease AvrPphB cleavages the AtPBS1 protein into two fragments which leads to an activation of *RPS5*-mediated immune responses in *Arabidopsis thaliana*. In order to examine if AvrPphB triggers immunity in Solanaceae plants, we tested *Solanum americanum* accessions for AvrPphB-induced hypersensitive response. Using Next Generation Sequencing based BSA (Bulk Segregant Analysis), we identified three candidate NLR (NB-LRR R gene) genes at the lower arm of chromosome 1. Various functional analyses revealed that one of the genes in this locus confers AvrPphB recognition. Thus, this gene was designated as *Resistance to P. syringae–in solanum Americanum1* (*Rps-amr1*). Our study shows that independently evolved NLR gene in wild Solanum species can recognize AvrPphB that was previously shown to trigger immunity in Arabidopsis and barley.

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P86:
Optimized Bacterial mRNA Enrichment for Dual RNA-Seq to Explore Plant-Bacterial Interaction Dynamics

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Abstract

Dual RNA sequencing is a valuable tool for understanding plant-microbe interactions, but technical challenges arise, particularly in bacterial transcriptomics, due to the abundance of ribosomal RNA (rRNA), which limits transcript coverage. To improve bacterial mRNA sequencing, this study modified a strand-specific dual RNA-Seq method to enrich bacterial mRNA in plant samples infected by bacteria. The approach involved separating plant mRNA via poly-A selection and removing rRNA, followed by strand-specific RNA-Seq library preparation. The efficiency of this enriched method was evaluated against a conventional RNA-Seq method in various plant-bacterial interactions, including host and non-host resistance with pathogenic bacteria and beneficial rhizosphere bacteria in pepper and tomato plants. Across all interactions, the enriched method showed improved mapping efficiency despite lower read counts, for instance, in the interaction with *Xanthomonas campestris pv. Vesicatoria* race 3 (Xcv3), the enriched method increased mapping to the Xcv3 genome by 1.45-fold and to the coding sequences (CDS) by 1.49-fold. Additionally, the enriched method identified more differentially expressed genes (DEGs) than the conventional method, especially during the early stages of Xcv3 infection in peppers. Gene Ontology analysis revealed enrichment in functions like proteolysis, kinase activity, and heme binding. This improved RNA-seq method offers a cost-effective approach to enrich bacterial mRNA, providing deeper insights into plant-bacterial transcriptomic interactions.

Acknowledgements

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P87:
The plastoglobule associated NAD(P)H dehydrogenase C1 (NDC1) is essential for vitamin k1 accumulation and involved in prenyllipid metabolism in tomato (*Solanum lycopersicum*) leaves and fruit.

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University of Neuchâtel

Plastoglobules (PG) are dynamic plastid-associated lipid droplets that are involved in neutral lipid and isoprenoid storage and metabolism. Recent studies have shown that PG play active roles and possess their own proteome and metabolome. Metabolic enzymes associated with PG contribute to prenylquinone synthesis and metabolism (phylloquinone (vitamin k_1), plastoquinone (PQ-9), plastochromanol (PC-8), tocopherol (vitamin E)). Plastoquinone and phylloquinone participate in the photosynthetic electron transport chain and, together with tocopherol, also play important roles as lipid antioxidants. NAD(P)H dehydrogenase C1 (NDC1), a type II NAD(P)H dehydrogenase associated with PG, has been found to be involved in the last step of phylloquinone biosynthesis and, in a separate role, to control the overall redox state of plastoquinone. In this study, we disrupted the NDC1 gene of *Solanum lycopersicum* (Solyc03g043750 / Sl-NDC1) using CRISPR-Cas9 to investigate its role in chloroplast and chromoplast PG of tomato. We showed that NDC1 is required for vitamin k_1 accumulation in both plastid types, as the *ndc1* mutant accumulates very low levels of phylloquinone and higher levels of its latest precursor, demethyl phylloquinone. We also showed that chromanol levels are altered in the *ndc1* mutant as we measured a PC-8 reduction and a PQ-9 increase in both leaves and fruits. This study and the interesting properties of NDC1 may inform new approaches to tomato fruit nutrient improvement and fortification.

P88:
Isolation and characterization of linalyl-glycosyltransferases from coffee.

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【Introduction】

The aroma is principal to decide the value of coffee beans. Linalool, one of the volatile terpene compounds, is constitutive in the coffee aroma. Since terpenoids are generally accumulated as glycosides in plants, the UDP-glycosyltransferases (UGTs) catalyze the glycosylation. To reveal the accumulation mechanism of those volatile compounds in coffee, the functional analysis of the *UGT* genes that work terpenoids such as linalool was performed. We expect to develop coffees with a rich aroma using this information. In this study, we identified those *UGT* genes from *Coffea arabica* and analyzed them with recombinant enzymes. Here, we report the isolation and characterization of *UGT* genes with activity towards linalool.

【Methods】

UGT85K11, which catalyzes the glycosylation of terpenoids, was isolated from *Camellia sinensis*[1]. We performed *in silico* screening of the genes from *C. arabica* based on the nucleotide sequence of *UGT85K11*. Then, five candidate genes were identified and analyzed. After sequence analysis, those recombinant enzymes were produced using a pET-system. Those enzyme activities were measured by radioisotope-mediated TLC assay and LC-MS/MS analysis.

【Results】

Four genes (*UGT85A192*, *UGT85AF14*, *UGT85A193* and *UGT85A195*) were isolated from young leaves, and *UGT85A194* was isolated from flower buds. UGT85A192, UGT85A193 and UGT85A194 can catalyze glycosylation of linalool, citronellol, perillyl alcohol, terpineol, and geraniol. UGT85A194 also showed activity in the glycosylation of menthol. Compared with the results of the TLC assay of UGT85A192, UGT85A193 and UGT85A194, a clear signal of the linalool glucoside was observed in UGT85A192. The apparent K_m values for linalool of UGT85A192 and UGT85A193 were 39.07 μM and 293.4 μM, and it was evident that UGT85A192 has a higher affinity for linalool than UGT85A193. In the LC-MS/MS analysis of UGT85A192 reactant using linalool as a substrate, fragment ions at *m/z* 361, 315, and 151 were detected.

【Conclusion】

We identified five genes, *UGT85A192*, *UGT85AF14*, *UGT85A193*, *UGT85A194* and *UGT85A195*, showed about 60% amino acid identities with UGT85K11. In functional analysis using radioisotopes, UGT85A192 showed critical activity against linalool, which had a higher affinity for its substrate than that of UGT85A193 and UGT85A84[2] from *Osmanthus fragrans*. The LC-MS/MS analysis also revealed that UGT85A192 mainly produces linalyl glucoside.

1) S. Ohgami *et al*., *Plant Physiol.* 168 (2015) 464–477
2) R. Zheng *et al*., *Front. Plant Sci.* 10 (2019) 01376

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P89:
Reconstruction of capsaicin biosynthesis in *Nicotiana benthamiana* using a newly identified acyl-CoA synthetase

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Capsaicin, a pungent alkaloid unique to *Capsicum* genus of the Solanaceae family, serves not only as a key food flavorant but also possesses significant medicinal properties. The biosynthesis of capsaicin involves the condensation of vanillylamine and 8-methyl-6-nonenoyl-CoA by a capsaicin synthase, CaPun1. However, the key genes that convert 8-methyl-6-nonenoic acid into the corresponding CoA ester, a crucial substrate for capsaicin synthesis, remain unknown. In this study, we identified a capsaicin biosynthesis-related acyl-CoA synthetase (*CaCACS*) in *Capsicum annuum* through spatio-temporal transcriptome analyses correlated with capsaicinoid levels. Biochemical characterization of recombinant CaCACS from *Escherichia coli* confirmed its role in catalyzing the formation of 8-methyl-6-nonenoyl-CoA from 8-methyl-6-nonenoic acid and Coenzyme A. To reconstruct capsaicin biosynthesis in a non-pungent Solanaceae species, we transiently expressed *CaPun1* and *CaCACS* in *Nicotiana benthamiana*, followed by precursors feeding. Capsaicin production was successfully achieved in *N. benthamiana*, but only when both substrates and CaCACS were present, suggesting that *N. benthamiana* lacks the CACS function and does not produce the capsaicin precursors 8-methyl-6-nonenoic acid and vanillylamine. Our findings provide insights into the evolution of pungency traits within the Solanaceae family and demonstrate the potential for metabolic engineering of capsaicin production in non-pungent plants.

P90:
Exploring the Role of Tryptamine and Serotonin in Tomato Reproductive Development

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Tryptamine (TAM) and serotonin (SER) are two tryptophan-derived compounds that belong to a widespread class of bioactive molecules known as indolamines or indole alkaloids. In plants, TAM and SER are mainly known for being intermediates in the biosynthesis of melatonin, a well-studied molecule involved in important biological processes such as biotic and abiotic stress responses, ROS scavenging, embryo development, and plant morphogenesis. Although TAM and SER have been detected at very high concentrations (μg/g of fresh weight) in the edible fruits and seeds of numerous plant species, their biological functions in reproductive organs remain unclear, and their metabolic pathways still need to be fully elucidated.
In plants, the biosynthesis of TAM and SER typically involves consecutive decarboxylation and hydroxylation reactions of tryptophan, catalyzed by tryptophan decarboxylase (TDC) and tryptamine 5-hydroxylase (T5H) enzymes, respectively. Our recent research has focused on the functional characterization of a three-member *TDC* gene family and a single *T5H* gene involved in TAM and SER biosynthesis in the model species Solanum lycopersicum. Our findings support a model wherein SlTDC1 promotes TAM accumulation in fruits, SlTDC2 mediates TAM production in aerial vegetative organs, SlTDC3 drives TAM synthesis in roots and seeds, and SlT5H is responsible for the conversion of TAM to SER in the entire plant (Commisso et al., 2022). Our current research aims to unravel the biological function of these two indolamines in various organs and tissues of the tomato plant. We implemented a metabolic engineering approach, using both traditional transgenesis and CRISPR/Cas9-mediated gene knockout, to generate different tomato genotypes characterized by altered levels of TAM and SER.
Phenotypic characterization of *SlTDC1*-overexpressing lines and *SlTDC1*-knockout mutants revealed significant and consistent changes in the number and dimensions of ripe fruits compared to the wild-type genotype. Furthermore, seeds of *sldtc1* lines showed an altered seed coat pigmentation and a consistent reduction of germination capacity. Overall, these findings suggest a potential role of TAM and SER in reproductive development.

P91:
Investigation of NAC Transcription Factors Involved in Ripening of Pepper (*Capsicum annuum* L.) Fruit

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Pepper (*Capsicum annuum* L.) is harvested at either mature- (full green) or ripen-stage (full red), and fruit quality such as color, texture, and nutritive values is various depending on fruit developmental stages. Unveiling a molecular mechanism of pepper fruit ripening is necessary to control fruit quality and to reduce postharvest loss. In this study, NAC transcription factors (TFs) involved in pepper fruit ripening were identified, and their molecular relationships were analyzed. Among 104 NAC TFs previously identified, *CaNAC14*, *45*, *84*, and *92* were selected as candidate regulators for the ripening with phylogenetic analysis. In phylogenetic tree, the 4 *NAC* genes were closely related to NAC TFs playing a role in ripening or senescence in other crop species. Expressions of the 4 genes also increased at the ripening stage or induced by abscisic acid (ABA), the major regulator of non-climacteric fruit ripening, meaning their substantial role in the ripening. To investigate their downstream target genes, dual luciferase reporter assay was performed. Of the 4 genes, CaNAC84 enhanced the promoter activity of *CaPSY1* and *CaSGR*, the carotenoid biosynthesis and chlorophyll degradation genes, respectively, and CaNAC92 only increased that of *CaSGR*. Furthermore, yeast-two-hybrid and bimolecular-fluorescence complementation assays showed CaNAC92 interacted with CaPYL12, the ABA receptor, in a nucleus with an ABA-dependent manner. These results indicated that CaNAC84 and CaNAC92 may play a crucial role during pepper fruit ripening by regulating expressions of genes related to fruit color and by interplaying with ABA signaling. These findings revealed molecular mechanisms regulating the ripening process and provided genetic information to improve the quality of pepper fruit.

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P92:
The NAC transcription factor, *Ripening Accelerator*, regulates light stress response in tomato

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The NAC transcription factor family is one of the largest, playing crucial roles in various physiological processes, including stress responses, flowering, fruit ripening, and senescence. In tomato gene expression profiling, the candidate gene *Ripening Accelerator* (*RAR*) stands out for its notably higher expression levels compared to other NAC family genes. In this study, RNA interference (RNAi) was employed to repress *RAR* expression and examine its role in regulating light stress response in tomato. The *RAR* RNAi lines exhibited a shortened life cycle, including accelerated rate of aging, shortened fruit ripening period and reduced storage longevity under light stress. The expression of ethylene biosynthesis genes (*ACS2* and *ACO1*) and key transcription factors of ripening (*RIN*, *NOR*) was upregulated in *RAR* RNAi lines. Moreover, *RAR* expression was upregulated in the fruits of the *nor* and *rin* mutants, indicating potential interregulation between *RAR*, *NOR*, and *RIN*. The results of transcriptional activity analysis showed that *RAR* can activate the transcription of *ACS2*, indicating that *RAR* is an indirect ethylene regulator in response to light stress. We speculated that *RAR* is an intermediary, which can achieve the regulatory purpose by combining positive or negative regulatory factors in different environments. Taken together, our data show that *RAR* acts a positive transcriptional regulator of light stress response, by controlling the expression of ethylene biosynthesis genes.

P93:
Crossing a CRISPR/Cas9 transgenic tomato plant with a wild-type plant yields diverse mutations in the F_1 progeny

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Generating CRISPR/Cas9-mediated mutants in tomato (*Solanum lycopersicum* L.) involves screening shoots regenerated from cultured cells transformed with a T-DNA harboring sequences encoding Cas9 and single guide RNAs (sgRNAs). Production of transformants can be inconsistent and obtaining transformants in large numbers may be difficult, resulting in a limited variety of mutations. Here, I report a method for generating various types of mutations from one transgenic plant harboring the CRISPR/Cas9 system. In this method, a wild-type plant was crossed with a T0 biallelic mutant expressing two sgRNAs targeting the *RIPENING INHIBITOR* (*RIN*) gene, and the resulting F1 seedlings were classified using a kanamycin resistance marker on the T-DNA. Genotyping of the RIN locus revealed that kanamycin-sensitive F_1 seedlings, which carried no T-DNA, always harbored the wild-type allele and a mutant allele from the transgenic parent. Kanamycin-resistant F_1 seedlings, which do carry the T-DNA, harbored a variety of novel mutant alleles, but not the wild-type allele, suggesting that it was mutated during crossing. The novel mutations included one-base insertions or short deletions at each target site, or large deletions across the two target sites. This method was also successfully applied to produce mutations in *Geranylgeranyl pyrophosphate synthase 2* (*GGPS2*). Because this method involves crossing rather than transformation, it can be readily scaled up to produce numerous novel mutations, even in plant species or cultivars for which transformation is inefficient. Therefore, when initial transgene experiments fail to induce the desired mutation, this method provides additional opportunities for generating mutants.

Reference
Yasuhiro Ito: Crossing a CRISPR/Cas9 transgenic tomato plant with a wild-type plant yields diverse mutations in the F1 progeny. *Frontiers in Plant Science* 15: doi.org/10.3389/fpls.2024.1447773 (2024)

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P94:
Precise Pepper Improvement via RNA-Guided Endonucleases

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Precise crop improvement is essential for enhancing global food security and advancing human nutrition. Modern molecular breeding techniques, alongside traditional breeding programs, have significantly increased crop yield and quality. The advent of CRISPR technology has further revolutionized plant genome modification, enabling more precise editing of genetic material. Notable successes in genome-edited crops include the development of high oleic soybeans, powdery mildew-resistant wheat, and brown-free mushrooms. A landmark achievement was Japan's introduction of the first CRISPR-enabled GABA-enriched tomato to local markets in May 2021. Despite these advancements, the application of precise genome editing in recalcitrant species remains a challenge, particularly regarding efficient plant regeneration. This presentation will highlight the achievements and limitations of current genome editing techniques and discuss recent progress in pepper genome editing, exploring potential applications and future directions for enhancing crop resilience and nutritional value.

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P95:
High sugar and high GABA Tomato accumulating cultivar creation by genome editing

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Currently, various techniques are being developed in breeding. Among these, genome editing technology is one that has been developed in recent years. Genome editing is the process of inducing a DNA double-strand break (DSB) at an arbitrary location on the genome using an artificial nuclease that recognizes and cleaves the target sequence, and then making use of repair errors in the cut DNA to generate mutations. Genome editing has the advantage of being able to edit various genes at the same time. In this study, we developed tomato cultivar with high sugar and high GABA content, with the aim of adding value to tomatoes, which are widely cultivated around the world.
The targeted genes were *SlESK* (Eskimo) and *SlGAD3*. *SlESK* is a homolog of Arabidopsis *ESK*, and there are three homologues in tomato. The studies in Arabidopsis have reported that *esk* mutants are more resistant to low-temperature stress due to improved accumulation of soluble sugars. The *SlGAD3* gene is one of the genes encoding glutamate decarboxylase (GAD, GABA synthase) and contains an autoinhibitory domain at the C-terminus. In this study, we succeeded in obtaining tomatoes with mutations in three *SlESK* genes and one with a mutation in *SlGAD3*. Comparing the obtained tomato lines with the wild type, the wild type had a sugar content (Brix) about 4.26%, while the mutant had about 7.9%. Regarding GABA content, accumulation was observed to be about 5 times higher in the mutants than in the wild type. On the other hand, a delay in plant growth was observed in the genome-edited plant.
To explain why the sugar contents increased in the *slesk1-3* mutants, we examined MDA (Malondialdehyde) content. After the cell membrane is damaged, the cell membrane will undergo a peroxidation reaction. The final product of this reaction is MDA. Therefore, MDA is an important indicator of membrane system damage and cellular metabolism deterioration. The MDA was about 5 times higher in the mutants than that in the WT.
In conclusion, we had gotten the high-sugar and high-GABA mutants by editing the *SlESK1-3* and *SlGAD3*. However, the mutants have stressed phenotype identical to *atesk* and the yield was decreased.

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P96:
Development of *in planta* genome editing by transient expression of genome-editing enzymes

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Genome editing is a technique for introducing mutations into target genes by inducing double-strand breaks in DNA sequences using artificial nucleases. Genome editing allows the introduction of mutations in targeted sequences without altering other elite traits, thus reducing the amount of labor required for mutant selection and backcrossing. Therefore, breeding using genome editing is expected to enable the development of varieties in a shorter time than mutation breeding or crossbreeding. There are two main steps that are important in genome editing technology in plants. Transformation by stable expression, in which nucleic acids encoding genome-editing enzymes are introduced into plant cells, and the regeneration of plant individuals from cells harboring the mutation by genome-editing enzymes through tissue culture. The efficiency of transformation and regeneration by tissue culture vary across plant species and are low in some practical crop species, limiting the plant species to which conventional genome editing can be adapted. *In planta* methods have been developed to exclude the need for tissue culture. But there are few reports on methods that do not require the need for stable expression at the same time. Therefore, the aim of this study is to develop a new genome editing method that does not require transformation and tissue culture by combining the *in planta* method with transient expression of genome editing tools instead of stable expression. The 'Tsukuba system', a high-level transient expression system in plant was used for transient expression. Cas9, gRNAs, and isopentenyl transferase, a type of DRs that are factors involved in mitotic tissue induction, were transiently expressed by agroinfiltration in the stem tissue cut surfaces of tomato. As a result, new chimeric cells containing a mixture of cells with or without mutations introduced at or near the target sequence were successfully obtained. To improve the efficiency of genome editing, the concentration of *Agrobacterium* with vectors expressing Cas9 and gRNA was changed to OD_{600}=0.8, 1.0, 1.2, and 1.6. The most successful concentration was OD_{600}=1.2, and the efficiency to obtain mutations was 27.3%. In addition, the number of shoots regenerated and the indel mutation rate tended to increase when tomatoes after infection were treated with high concentration of liquid fertilizer and with heat shock at 37°C. In this study, the *in planta* method with transient of genome editing tools and induction of meristematic tissue have enabled the introduction of genome-edited mutations in T_0 tomato

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P97:
Evaluation of the potential of the VIGE system on petunia.

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Petunia (*Petunia hybrida*) is a commercially horticultural crop worldwide. Virus-induced gene editing (VIGE) performs plant gene editing through modified viral vectors. Tomato spotted wilt virus (TSWV) has a wide host range and the TSWV-based VIGE system carrying CRISPR/Cas reagents has been shown to successfully gene editing in several plant species. This study intends to explore the potential application of TSWV-based VIGE on petunia. The systemic infection of TSWV-based VIGE vectors through agro-infection will be evaluated. The expression of GFP and Cas proteins on petunia plants will be investigated.

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This study was supported by the grant for Hsin-Mei Ku "The development of TRV-VIGE (virus-induced genome editing) system targeting TCTP (translationally controlled tumor protein) to generate broad-spectrum resistance against potyviruses in crops" (NSTC 112-2313-B-005-035-MY3).

P98:
Evaluation of TSWV-based VIGE system on different pepper lines

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Pepper (*Capsicum annuum*) is a popular vegetable in the worldwide markets. An efficient tool for the functional analysis of pepper genes is of great interest for pepper breeding. Virus-induced gene editing (VIGE) has become a popular tool for plant gene editing in recent years. Tomato spotted wilt virus (TSWV)-based VIGE system carrying CRISPR/Cas reagents has been developed in pepper. This study intends to explore the potential application of TSWV-based VIGE on different pepper lines. Agro-infection of TSWV-based VIGE vectors will be conducted and the systemic infection and the expression of the GFP and Cas proteins on different lines will be investigated.

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This study was supported by the grant for Hsin-Mei Ku "The development of TRV-VIGE (virus-induced genome editing) system targeting TCTP (translationally controlled tumor protein) to generate broad-spectrum resistance against potyviruses in crops" (NSTC 112-2313-B-005-035-MY3).

P99:
Evaluation of the potential of the VIGE system on different tomato lines.

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Tomato (*Solanum lycopersicum*) is a commercially important crop worldwide. An efficient tool for the functional genomics of tomato is in great demand. Virus-induced gene editing (VIGE) has become a popular tool for plant gene editing. The TSWV-based VIGE system carrying CRISPR/Cas reagents has been developed in tomato. This study intends to explore the potential of TSWV-based VIGE on different tomato lines. The systemic infection of TSWV-based VIGE vectors through agro-infection and the expression of GFP and Cas proteins on different tomato lines will be discussed.

Acknowledgments
This study was supported by the grant for Hsin-Mei Ku "The development of TRV-VIGE (virus-induced genome editing) system targeting TCTP (translationally controlled tumor protein) to generate broad-spectrum resistance against potyviruses in crops" (NSTC 112-2313-B-005-035-MY3).

P100:
The development of virus-induced gene editing system for solanaceae crops.

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VIGE (virus-induced genome editing) is a genome editing strategy in which sgRNA carried by a virus vector and agroinfiltration into a transgenic plant expressing Cas9. VIGE provides an efficient way to edit several different gRNA-targeting regions in an efficient way which would be especially beneficial to functional characterize a protein with multiple domains. TRV-VIGE has been shown to be able to produced edited progeny successfully through seed transmission and bypass tissue culture process which make it an efficient and valuable tool for crop improvement. Phytoene desaturase (*PDS*) gene and TCTP (translationally controlled tumor protein) are the targets of VIGE and editing both genes of tobacco (*Nicotiana benthamiana*) has been identified in this study.

Acknowledgements
This study was supported by the grant for Hsin-Mei Ku “The development of TRV-VIGE (virus-induced genome editing) system targeting TCTP (translationally controlled tumor protein) to generate broad-spectrum resistance against potyviruses in crops” (NSTC 112-2313-B-005-035-MY3).

P101:
Exploring Potato as a Bioreactor for High-Level Accumulation of Bovine Myeloid Antimicrobial Peptide-18 in Tubers.

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The potato is the most important non-cereal food crop widely grown throughout the world. Its high tuber biomass yield makes it suitable for the molecular farming of recombinant therapeutics. As specialized storage organs, tubers are evolutionary adapted for the accumulation of large quantities of protein(s). Targeting recombinant proteins to potato tubers enhances protein stability, similar to that of endosperm-specific protein accumulation in cereal seeds. We have previously shown that the promoter of luminal binding protein from Douglas-fir (*PmBiPPro1* promoter), particularly its full-length version *PmBiPPro1-1*, exhibits high transcriptional activity in microtubers generated in vitro. This prompted us to evaluate the suitability of this promoter for targeted expression of host defense peptides (HDPs) in potato tubers. The aim of the present study was to express a modified bovine myeloid antimicrobial peptide, BMAP-18, in transgenic potato and to evaluate the potential use of tubers for high-level accumulation of therapeutic HDPs. BMAP-18 is a truncated form of BMAP-27, which belongs to the cathelicidin family of HDPs known for their potent antimicrobial properties against bacterial species pathogenic to humans. In contrast to parental BMAP-27, BMAP-18 has low cytotoxicity to human erythrocytes and neutrophils while maintaining strong antimicrobial activity. Additionally, BMAP-18 has strong anti-parasitic activity against trypanosomes and *Leishmania* in vitro, which makes this HDP a promising candidate for therapy of trypanosome-infected hosts, such as cattle, fish and humans. Here, a plant-optimized nucleotide sequence encoding BMAP-18 was transcriptionally fused to the *PmBiPPro1-1* promoter and introduced into potato plants (cultivar Desiree) via *Agrobacterium*-mediated transformation. The presence of the *BMAP-18* transgene in plants regenerated on selection medium was confirmed by PCR, whereas transgene copy-numbers were determined through qPCR. All transgenic lines were transferred to a greenhouse and grown to maturity to generate tubers. Most plants retained normal morphology of potato plants. Experiments to verify the presence and functionality of the plant-produced BMAP-18 peptide are currently underway.

P102:
Enhancing Xanthophyll Accumulation in Tomato Fruits: Strategies for Improving Nutritional Value and Human Health Benefits

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Xanthophylls have essential roles in plant biology. They contribute to the formation and function of photosynthetic complexes in leaves, giving color to reproductive organs, flowers, and fruits. Some xanthophylls have specific health benefits for humans, such as β-cryptoxanthin, lutein, zeaxanthin, and astaxanthin. As a result, significant efforts have been made to increase the content of these xanthophylls in crop plants, aiming to improve human nutrition. Our research has focused on developing strategies, including classical genetic methods and genetic engineering techniques, to increase the production of valuable xanthophylls in tomato fruits. We aim to enhance their biosynthesis and accumulation within chromoplasts. Previously, we developed a non-transgenic tomato line called 'Xantomato,' which accumulated non-esterified zeaxanthin in the fruit, reaching concentrations exceeding 50 μg/g fresh weight, constituting around 50 percent of total carotenoids. One challenge in achieving even higher zeaxanthin levels is how it is stored in the cells. Research has shown that esterifying carotenoids can help increase their accumulation and stability within chromoplasts. The enzyme PALE YELLOW PETAL 1 (PYP1) has been found to increase the levels of xanthophyll esters in flowers. To investigate its impact on fruits, we developed transgenic tomato lines from the M82 variety that over-express the flower-specific SlPYP1 gene in the fruit. While these transgenic fruits showed a significant increase in overall carotenoid content compared to the control variety M82, they lacked xanthophyll esters due to the predominant accumulation of carotenes. We are using this approach to investigate the effects of PYP1 in zeaxanthin-accumulating tomato lines to unravel its potential to enhance xanthophyll accumulation. Another bottleneck in β-xanthophylls accumulation in fruits is the rate of β-carotene hydroxylation. We used a transgenic approach to overcome this hurdle by expressing the CYP97A gene, encoding a p450 β-carotene hydroxylase, in β-carotene accumulating fruit. This led to a significant accumulation of zeaxanthin and β-cryptoxanthin in the transgenic fruits.

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P103:
Evaluation the effect of varieties and agrobacterium concentrations on tomato virus-induced gene silencing

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Tomato (*Solanum lycopersicum*) is a commercially important crop worldwide. With tomato whole genome sequencing available, it is urgent to identify gene functions for tomato. Virus-induced gene silencing (VIGS) provides an alternative approach for tomato functional genomics. Tobacco rattle virus (TRV) has wide host range including Solanaceae crops and has been modified and popular for VIGS. Phytoene desaturase (*PDS*) gene are commonly used as reporter for evaluating the VIGS efficiency. This study intends to explore the effect of different Agrobacterium concentrations and different tomato varieties including M82, Micro-Tom, Ta209, CLN1558A, and Nongyou 301 on VIGS. The silencing frequency and silencing effectiveness were investigated at 21 dpi. The optimal tomato varieties and agrobacterium concentrations for VIGS have been identified in this study.

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P104:
CoreNet+, a web application system for trans-omics data mining

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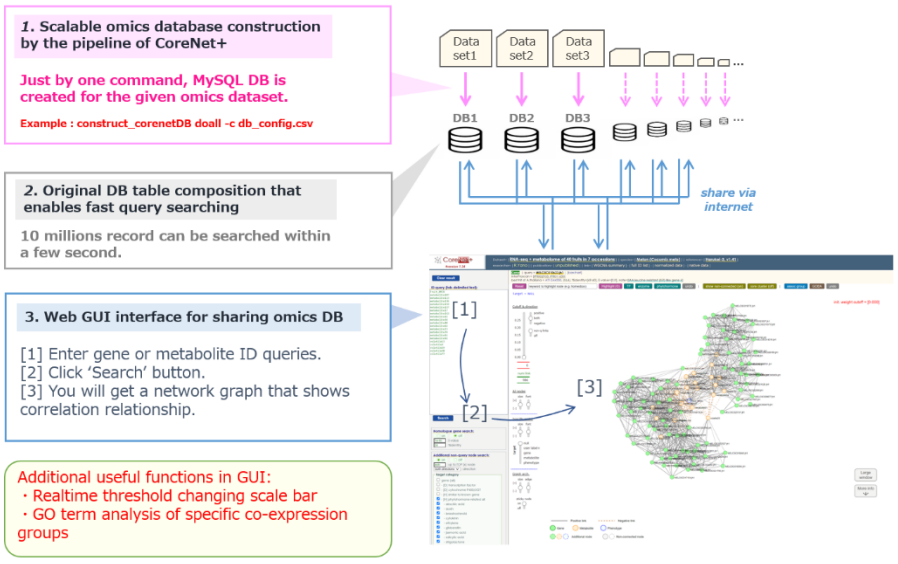
With the advance of omics analysis technologies, a tons of omics datasets have been published so far. They include transcriptome (RNA-seq), metabolome, phenome, microbiome, and so on. There has been an increasing demand of managing such huge omics data to use them for data sharing and mining. For this purpose, web applications are convenient because worldwide researchers can access to datasets via internet. However, although useful web-based databases and omics applications are provided by several institutes, it is almost impossible for researchers outside the institute to add their own omics datasets to the database partly because they do not have permission to perform data processing in the web server. In this study, a new web application designated “CoreNet+” was developed to promote omics-based data mining and research. The CoreNet+ software package is composed of largely two functional programs; one is the “DB construction pipeline” and another is a suite of web scripts that provides graphic user interface (GUI). In the former, all analyses including functional gene annotation and co-expression are automatically performed by just one command on Linux machine. It is also possible to upload datasets and operate the command using web browser. This pipeline generates one MySQL database for the given dataset, and one MySQL (one URL) is assigned to each dataset. Hence, database can be extended in a scalable manner. Once MySQL DB is constructed, researchers are able to access to the dataset with web browser. In this presentation, I’d like to introduce some examples of CoreNet+.



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P105:
Optimization of Callus Induction in Six Genotypes of Tomato (*Solanum Lycopersicum)*

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In vitro culture plays essential role in supporting the genetic engineering of tomato. Optimizing in *in vitro* culture for various cultivars will support the success of genetic engineering and other biotechnological approaches. This study evaluated the effects of genotype and plant growth regulators (PGRs) on callus induction from cotyledon explants. The explants were cultured on Murashige and Skoog (MS) medium with varying PGR combinations and concentrations. A significant genotype × medium interaction was observed in the induction of cream-greenish callus capable of producing shoot primordia. Genotype responded differently to the PGR treatments, ranked from most to least responsive as follows: Yellow Pear, Beef Steak, Intan, Red Pear, Ratna, and Black Cherry. Yellow Pear, Beef Steak, and Intan achieved higher callus induction rates (59.4%, 56.3%, and 43.8%, respectively) compared to other genotypes. Yellow Pear also showed the highest percentage of cream-greenish callus (53.1%) on media containing 0.1 mg/L IAA and 1 mg/L BAP. These findings suggest that genotype-specific optimization of regeneration conditions is crucial for improving regeneration efficiency in tomato.

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P106:
Heat-responsive optimization of carbon partitioning by prime editing enhances fruit yields in tomato

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Global warming has led to an increased frequency of extreme high temperatures, resulting in heat stress that disrupts the balance between carbon sink and source activities and contributes to reduced crop yields. Enhancing the utilization efficiency of sucrose, the primary transportable carbon assimilate, in sinks such as seeds, flowers, and fruits could promote their development and improve crop yields under diverse environments. However, conventional genetic engineering targeting genes involved in sucrose conversion increases stress tolerance but often accompanied by developmental defects, thus hampering productivity in challenging environmental conditions. In this study, we employed enhanced prime editing tools to incorporate a cis-element into the promoter of a tomato cell wall invertase gene (CWIN). Cas9-free progeny of CWIN promoter-edited plants exhibited a spatio-temporal increase in its expression compared to wild type, thus resulting in remarkably increased productivity under normal condition and reduced yield loss under heat stress. Collectively, we optimized heat-responsive carbon partition by prime editing-mediated cis-element knock-in to enhance fruit yield in tomatoes. This strategy holds potential for application in various crops to develop climate-resilient crops.

P107:
SAMBA, a plant-specific APC/C regulator, is involved in development and fruit metabolites in tomato

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The Anaphase-Promoting Complex/Cyclosome (APC/C) is an E3 ubiquitin ligase involved in ubiquitin-dependent proteolysis of key cell cycle regulators by the 26S proteasome. The spatio-temporal degradation of mitotic cyclins and securins ensures the correct onset of the cell cycle phases and exit from the cell division program, respectively. The identification of genes encoding APC/C subunits in *Arabidopsis thaliana* suggests that the complex has other specific functions during plant development, such as embryogenesis, gametogenesis, in growth regulation, hormone signaling and symbiotic interactions. One of these proteins, SAMBA, proved to be a highly promising candidate in Arabidopsis in terms of increasing productivity, through production of larger leaves, roots and seeds. Here, we investigated the role of *SAMBA* in tomato plants. Tomato is an excellent model for investigating fleshy fruit development due to its brief life cycle, economic importance, and extensive genomic resources. SISAMBA-EGFP was ubiquitously detected in the cytoplasm and nuclei, confirming the in silico analyses. *SlSAMBA* expression was observed in different parts of the pistil, including the ovary and stigma, but not in the style. In the androecium, *SlSAMBA* expression occurred in both the anther and pollen grains. In later stages of fruit development, the highest *SlSAMBA* expression occurred at 10 DPA, a phase known for intense cell division, and decreased afterward until the ripening stage, where the expression was restricted to the septum and columella. Thus, to gain more insight into the role of SAMBA, we used CRISPR/Cas9 technology to characterize sequence-specific mutations at *Solanum lycopersicum* (tomato) cv. Micro-tom in the *SAMBA* gene. Surprisingly, our results revealed reduced plant size, altered fruit morphology, and compromised seed set in *samba* mutants. Additionally, metabolomic analysis revealed altered flavonoid profiles, with smaller fruits being sweeter. Thereby, overall, our results unravel the important insights highlight the critical role of SAMBA in tomato fruit development and will hopefully contribute to future breeding programs directed towards improved nutritional quality in Solanaceae fruit crops.

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Participants list

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NBRP National BioResource Project

TOMATO National BioResource Project

SATREPS Science and Technology Research Partnership for Sustainable Development Program

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