On the Cover: The cover image illustrates the use of laser-ablation electrospray ionization (LAESI)-mass spectrometry metabolite imaging to localize metabolites in the flower petals of a *Phalaenopsis* spp. orchid. Although it is becoming increasingly evident that plant metabolites, including specialized metabolites, are not uniformly distributed across tissues and organs, the full extent of this heterogeneity is still poorly known, except when such metabolites are visible to the naked eye. Etalo et al. (pp. 1424–1435) have assessed the value of using LAESI-mass spectrometry metabolite imaging to provide us with deeper insights into the spatial localization of metabolites in plant leaves and flowers. Exploiting first the visible phenotype of anthocyanins to match the localization of these colored cyanidin derivatives with metabolite maps obtained from the LAESI output (left and middle inset), they then localize and map the distribution on nonvisible metabolites such as phenolic compounds (right inset) in *Phalaenopsis* spp. petals. The results indicate that although some phenolic compounds colocalize with anthocyanins in the purple areas, others were restricted to the nonpigmented areas of the petals. Cover image credits: Desalegn Etalo (The Netherlands Institute of Ecology, Wageningen, The Netherlands).

FOCUS ON METABOLISM

EDITORIAL

Focus Issue on Metabolism: Metabolites, Metabolites Everywhere. Alisdair R. Fernie and Eran Pichersky

BREAKTHROUGH TECHNOLOGIES


Laser-ablation electrospray ionization mass spectrometry imaging of living plant tissues gives deeper insights into the heterogenic and dynamic nature of metabolite accumulation in plant organs.

UPDATES

Proteins of Unknown Biochemical Function: A Persistent Problem and a Roadmap to Help Overcome It. Thomas D. Niehaus, Antje M.K. Thamm, Valérie de Crécy-Lagard, and Andrew D. Hanson

The functions of over 60% of plant enzymes are unknown; nearly one-half of these proteins have microbial homologs, enabling the use of cross-kingdom comparative genomics to predict their functions.


Analyzing metabolic network flux and recent findings in plants highlights future challenges.

Natural Variation of Plant Metabolism: Genetic Mechanisms, Interpretive Caveats, and Evolutionary and Mechanistic Insights. Nicole E. Soltis and Daniel J. Kliebenstein

Ecological, evolutionary, and mechanistic insights arise from the study of natural variation in plant metabolism.

Posttranslational Protein Modifications in Plant Metabolism. Giulia Friso and Klaas J. van Wijk

Posttranslational modifications impose multiple levels of control across plant metabolism.
Altering of Plant Primary Metabolism in Response to Insect Herbivory. *Shaoqun Zhou, Yann-Ru Lou, Vered Tzin, and Georg Jander*

The species and feeding habits of insect herbivores influence the changes in photosynthesis, carbon allocation, and nitrogen utilization that are observed in host plants.

Integrative Approaches to Enhance Understanding of Plant Metabolic Pathway Structure and Regulation. *Takayuki Tohge, Federico Scossa, and Alisdair R. Fernie*

Metabolomics-based integrative approaches provide better understanding of gene function, pathway structure, and regulation.

Something Old, Something New: Conserved Enzymes and the Evolution of Novelty in Plant Specialized Metabolism. *Gaurav D. Moghe and Robert L. Last*

Specialized metabolic pathways have evolved by recruitment of duplicated genes encoding enzymes of core metabolism and divergence of the encoded activity, by alteration of biochemical regulation, and by changes in gene expression patterns.

Discovering New Biology through Sequencing of RNA. *Andreas P. M. Weber*

A brief historical perspective informs general guidelines for planning and conducting RNA-Seq projects.

A Revolution in Plant Metabolism: Genome-Enabled Pathway Discovery. *Jeongwoon Kim and C. Robin Buell*

Genome sequencing and genomic technologies facilitate discovery and understanding of plant metabolic pathways.

**RESEARCH ARTICLES**

Integrated Transcriptomic and Metabolomic Characterization of the Low-Carbon Response Using an ndhR Mutant of *Synechocystis* sp. PCC 6803. *Stephan Klähn, Isabel Orf, Doreen Schwarz, Jasper K.F. Matthiessen, Joachim Kopka, Wolfgang R. Hess, and Martin Hagemann*

Acclimation to changing CO₂ levels by *Synechocystis* sp. PCC 6803 includes coordinated changes in gene expression and metabolism, whereby small RNAs and the transcriptional regulator protein NdhR perform distinct regulatory functions.

[OPEN] Loss of FERULATE 5-HYDROXYLASE Leads to Mediator-Dependent Inhibition of Soluble Phenylpropanoid Biosynthesis in Arabidopsis. *Nickolas A. Anderson, Nicholas D. Bonawitz, Kayleigh Nuffeler, and Clint Chapple*

The synthesis of specialized metabolites is actively repressed in a Mediator-dependent manner in an Arabidopsis phenylpropanoid pathway mutant.

[OPEN] Different Reactive Oxygen Species Scavenging Properties of Flavonoids Determine Their Abilities to Extend the Shelf Life of Tomato. *Yang Zhang, Rosalba De Stefano, Marie Robine, Eugenio Butelli, Katharina Bulling, Lionel Hill, Martin Rejzek, Cathie Martin, and Henk-jan Schoonbeek*

The structures of flavonoids influence their ROS-scavenging abilities and extend the shelf life of tomato fruit through effects on both overripening and susceptibility to *Botrytis cinerea.*
Regulation of Carotenoid Biosynthesis by Shade Relies on Specific Subsets of Antagonistic Transcription Factors and Cofactors. Jordi Bou-Torrent, Gabriela Toledo-Ortiz, Miriam Ortiz-Alcaide, Nicolas Cifuentes-Esquivel, Karen J. Halliday, Jaime F. Martinez-Garcı ´a, and Manuel Rodrıguez-Concepcion

Carotenoid biosynthesis is regulated by plant proximity (shade) signals through specific sets of positive and negative transcriptional regulators targeting the first gene of the pathway.

Comprehensive Assessment of Transcriptional Regulation Facilitates Metabolic Engineering of Isoprenoid Accumulation in Arabidopsis. Iris Lange, Brenton C. Poirier, Blake K. Herron, and Bernd Markus Lange

A population of Arabidopsis lines with modulated levels of expression of each gene involved in isoprenoid precursor pathways was used to develop engineering strategies for increasing sterols.

Functional Divergence of Diterpene Syntheses in the Medicinal Plant Salvia miltiorrhiza. Guanghong Cui, Lixin Duan, Baolong Jin, Jun Qian, Zheyong Xue, Guoan Shen, John Hugh Snyder, Jingyuan Song, Shilin Chen, Luqi Huang, Reuben J. Peters, and Xiaoquan Qi

Positive selection and the divergent evolution by exon/intron patterns driving the fast divergence of copalyl diphosphate synthases underlie the biosynthesis of specialized diterpenes.


A plastidial glycolytic enzyme modulates the metabolism of carbon and nitrogen in nonphotosynthetic cells and affects plant organ development.

Molecular Genetic Analysis of Glucan Branching Enzymes from Plants and Bacteria in Arabidopsis Reveals Marked Differences in Their Functions and Capacity to Mediate Starch Granule Formation. Kuan-Jen Lu, Sebastian Streb, Florence Meier, Barbara Pfister, and Samuel C. Zeeman

Expressing glucan branching enzymes from different plants and bacteria in branching enzyme-deficient Arabidopsis plants reveals marked differences in their specificity, yielding unique starch structures.

Acylphloroglucinol Biosynthesis in Strawberry Fruit. Chuankui Song, Ludwig Ring, Thomas Hoffmann, Fong-Chin Huang, Janet Slovin, and Wilfried Schwab

The synthesis of pharmaceutically active acylphloroglucinols during strawberry fruit ripening is catalyzed by dual-function chalcone synthases/calamene synthases.


A diel genome-scale model of leaf metabolism highlights the inherent flexibility in meeting the demands for daytime carbon storage and light-dependent energy balancing.

Identification, Functional Characterization, and Evolution of Terpene Synthases from a Basal Dicot. Mosaab Yahyaa, Yuki Matsuoka, Wolfgang Brandt, Adi Doron-Faigerboim, Einat Bar, Alan McClain, Rachel Davidovich-Rikanati, Efrain Lewinsohn, Eran Pichersky, and Muwafaq Ibdah

Sesquiterpene synthases for 1,8-cineole, cadinene, and geranylinalool appear early in angiosperm lineage.

Noninvasive technologies and modeling demonstrate how the metabolism of different grain organs adjusts to fluctuating environments.


Posttranscriptional regulation shifts the metabolic flux of flavonoids toward coumarin and general phenylpropanoids.


A transcriptomic and metabolomic profiling time course of maize foliar responses to aphid feeding identifies genes for the synthesis of benzoxazinoids, terpenes, and other induced defense metabolites.

Glutathionylation and Reduction of Methacrolein in Tomato Plants Account for Its Absorption from the Vapor Phase. Shoko Muramoto, Yayoi Matsubara, Cynthia Mugo Mwenda, Takao Koeduka, Takuya Sakami, Akira Tani, and Kenji Matsui

Formation of adducts with glutathione and reduction facilitate the absorption of a reactive volatile chemical from the atmosphere in tomato plants.

Changes in Whole-Plant Metabolism during the Grain-Filling Stage in Sorghum Grown under Elevated CO₂ and Drought. Amanda P. De Souza, Jean-Christophe Cocuron, Ana Carolina Garcia, Ana Paula Alonso, and Marcos S. Buckeridge

Elevated carbon dioxide reduces the effects of drought on grain filling.

Thioredoxin f1 and NADPH-Dependent Thioredoxin Reductase C Have Overlapping Functions in Regulating Photosynthetic Metabolism and Plant Growth in Response to Varying Light Conditions. Ina Thormählen, Tobias Meitzel, Julia Grosman, Alexandra Bianca Oechsner, Edda von Roepenack-Lahaye, Belén Naranjo, Francisco J. Cejudo, and Peter Geigenberger

A previously unrecognized interaction between light and NADPH-dependent thiol-redox systems affects photosynthetic metabolism and growth in response to varying light conditions.

Integrated Analysis of Engineered Carbon Limitation in a Quadruple CO₂/HCO₃⁻ Uptake Mutant of Synechocystis sp. PCC 6803. Isabel Orf, Stephan Klühn, Doreen Schwarz, Marcus Frank, Wolfgang R. Hess, Martin Hagemann, and Joachim Kopka

Engineered carbon limitation after deletion of four Ci-uptake systems in Synechocystis sp. PCC 6803 is compensated by an extensive phenocopy of wild-type acclimation to low CO₂ and multilayered Ci regulation.


Proteins annotated as subunits of branched-chain ketoacid dehydrogenase contribute to the regulation of isoleucine, leucine, and valine homeostasis.

Isopropylmalate synthase is a variant Leu biosynthetic enzyme that is dedicated to tomato trichome acylsucrose biosynthesis.

Ectopic Expression of WRINKLED1 Affects Fatty Acid Homeostasis in Brachypodium distachyon Vegetative Tissues. Yang Yang, Jacob Munz, Cynthia Cass, Agnieszka Zienkiewicz, Que Kong, Wei Ma, Sanjaya, John Sedbrook, and Christoph Benning

Unlike Arabidopsis, ectopic expression of the transcription factor WRINKLED1 involved in oil accumulation causes cell death in Brachypodium distachyon due to the distinct fatty acid metabolism of this grass.

REGULAR ISSUE

ON THE INSIDE

Peter V. Minorsky

RESEARCH ARTICLES

BIOCHEMISTRY AND METABOLISM

Temperature Responses of C4 Photosynthesis: Biochemical Analysis of Rubisco, Phosphoenolpyruvate Carboxylase, and Carbonic Anhydrase in Setaria viridis. Ryan A. Boyd, Anthony Gandin, and Asaph B. Cousins

The temperature responses of carbonic anhydrase, phosphoenolpyruvate carboxylase, and Rubisco have implications for modeling C4 photosynthesis.

The Arabidopsis Transcription Factor MYB112 Promotes Anthocyanin Formation during Salinity and under High Light Stress. Magda E. Lotkowska, Takayuki Tohge, Alisdair R. Fernie, Gang-Ping Xue, Salma Balazadeh, and Bernd Mueller-Roeber

The transcription factor MYB112 in Arabidopsis promotes anthocyanin formation under conditions of salinity and high light stress.


Following cleavage of chloroplast transit peptides by stromal processing peptidase, additional processing may occur, avoiding unstable or otherwise unfavorable N-terminal residues.


An anthocyanin acyltransferase with an unusual substrate flexibility is truncated in cv Pinot Noir, a grapevine cultivar lacking acylated red pigments.
CELL BIOLOGY

Exocyst-Positive Organelles and Autophagosomes Are Distinct Organelles in Plants. Youshun Lin, Yu Ding, Juan Wang, Jinbo Shen, Chun Hong Kung, Xiaohong Zhuang, Yong Cui, Zhao Yin, Yiji Xia, Hongxuan Lin, David G. Robinson, and Liwen Jiang

Exocyst-positive organelles are distinct from autophagosomes under normal growth conditions but overlap in the vacuole after autophagic induction.


Protein interactions for two plasmodesmata-localized reticulon proteins suggest that these proteins, in addition to a role in endoplasmic reticulum modeling, may play important roles in linking the endoplasmic reticulum and plasma membrane.


Mutations of myosin motors lead to reduced organelle movements, to altered actin organization, to slower pollen tube growth, and ultimately to reduced fertilization success.

Phosphatidylinositol 3-Phosphate 5-Kinase, FAB1/PIKfyve Kinase Mediates Endosome Maturation to Establish Endosome-Cortical Microtubule Interaction in Arabidopsis. Tomoko Hirano, Teun Munnik, and Masa H. Sato

Phosphatidylinositol 3-phosphate 5-kinase is essential for the endosome maturation and cortical microtubule associations for PIN protein trafficking in young root cortical and stele cells.

Phytophthora infestans RXLR Effector AVR1 Interacts with Exocyst Component Sec5 to Manipulate Plant Immunity. Yu Du, Mohamed H. Mpina, Paul R.J. Birch, Klaas Bouwmeester, and Francine Govers

A Phytophthora infestans RXLR effector alters basal defense by interacting with an exocyst protein associated with vesicle tethering and secretion.

ECOPHYSIOLOGY AND SUSTAINABILITY

The Nitrate-Inducible NAC Transcription Factor TaNAC2-5A Controls Nitrate Response and Increases Wheat Yield. Xue He, Baoguan Qu, Wenjing Li, Xueqiang Zhao, Wan Teng, Wenjing Ma, Yongzhe Ren, Bin Li, Zhensheng Li, and Yiping Tong

A nitrate-inducible transcription factor affects nitrate responses that enable wheat to yield more with less fertilizer.


Mn toxicity results from apoplastic accumulation in soybean leaves, while tolerance occurs through vacuolar Mn(II) sequestration in two lupin species and oxidation to Mn(III) in trichomes of sunflower.

Phenotypic Plasticity Conditions the Response of Soybean Seed Yield to Elevated Atmospheric CO₂ Concentration. Etsushi Kumagai, Naohiro Aoki, Yusuke Masuya, and Hiroyuki Shimono

Soybean cultivars with high phenotypic plasticity can increase seed yield under elevated CO₂ and show higher biomass and pod number per plant at low plant density.
Light-Induced Indeterminacy Alters Shade-Avoiding Tomato Leaf Morphology. Daniel H. Chitwood, Ravi Kumar, Aashish Ranjan, Julie M. Pelletier, Brad T. Townsley, Yasunori Ichihashi, Ciera C. Martinez, Kristina Zumstein, John J. Harada, Julin N. Maloof, and Neelima R. Sinha

Shade avoidance increases indeterminacy in the initiating leaf primordium, increasing leaf complexity and serration through a heteroblastic-independent mechanism.

Aspen Tension Wood Fibers Contain β-(1→4)-Galactans and Acidic Arabinogalactans Retained by Cellulose Microfibrils in Gelatinous Walls. Tatyana Gorshkova, Natalia Mokshina, Tatyana Chernova, Nadezhda Ibragimova, Vadim Salnikov, Polina Mikshina, Theodora Tryfona, Alicja Banasiak, Peter Immerzeel, Paul Dupree, and Ewa J. Mellerowicz

Entrapment of pectic galactan and acidic arabinogalactan II within cellulose fibrils is a distinctive feature of aspen tension wood gelatinous fibers with contractile properties.

Two ATP Binding Cassette G Transporters, Rice ATP Binding Cassette G26 and ATP Binding Cassette G15, Collaboratively Regulate Rice Male Reproduction. Guochao Zhao, Jianxin Shi, Wanqi Liang, Feiyang Xue, Qian Luo, Lu Zhu, Guorun Qu, Mingjiao Chen, Lukas Schreiber, and Dabing Zhang

Two ATP binding cassette G transporters play a collaborative role in transferring lipidic molecules from tapetal cells for the development of anther cuticle and pollen exine.


Stress-inducible linker histone variant is required for adaptive response of Arabidopsis to complex environmental stress.

JACALIN-LECTIN LIKE1 Regulates the Nuclear Accumulation of GLYCINE-RICH RNA-BINDING PROTEIN7, Influencing the RNA Processing of FLOWERING LOCUS C Antisense Transcripts and Flowering Time in Arabidopsis. Jun Xiao, Chunhua Li, Shujuan Xu, Lijing Xing, Yunjuan Xu, and Kang Chong

A Jacalin-lectin manipulates an RNA binding protein in shaping plant development through regulation of pre-mRNA processing of the key flowering repressor genes.

Epigenetic Mutation of RAV6 Affects Leaf Angle and Seed Size in Rice. Xiangqian Zhang, Jing Sun, Xiaofeng Cao, and Xianwei Song

Epigenetic modification of a B3 transcription factor alters rice leaf angle via modulation of brassinosteroid homeostasis.

Plastid Genotyping Reveals the Uniformity of Cytoplasmic Male Sterile-T Maize Cytoplasms. Massimo Bosacchi, Csanad Gurdon, and Pal Maliga

Independently isolated cytoplasmic male-sterile maize shows a uniformity that indicates a single origin and strict maternal cotransmission of plastids and mitochondria to progeny.
Distinct Regulatory Changes Underlying Differential Expression of TEOSINTE BRANCHED-CYCLOIDEA-PROLIFERATING CELL FACTOR Genes Associated with Petal Variations in Zygomorphic Flowers of Petrocosmea spp. of the Family Gesneriaceae. Xia Yang, Xiao-Ge Zhao, Chao-Qun Li, Jing Liu, Zhi-Jing Qiu, Yang Dong, and Yin-Zheng Wang

Complex and distinct regulatory changes contribute to expression differentiation of CYC- and CIN-like TCP genes and morphological divergence of petals in zygomorphic flowers of Petrocosmea spp.

Map-Based Cloning of Seed Dormancy1-2 Identified a Gibberellin Synthesis Gene Regulating the Development of Endosperm-Imposed Dormancy in Rice. Heng Ye, Jiuhuan Feng, Lihua Zhang, Jinfeng Zhang, Muhamad S. Mispan, Zhuanqin Cao, Donn H. Beighley, Jianchang Yang, and Xing-You Gu

Natural variants in a gibberelin synthesis gene affect the development of primary dormancy by regulating seed morphogenesis and maturation programs to influence germination capability at harvest.


Two homeobox domain proteins maintain meristem activity essential for flowering by repressing an organ boundary gene module.

Floral Induction in Arabidopsis by FLOWERING LOCUS T Requires Direct Repression of BLADE-ON-PETIOLE Genes by the Homeodomain Protein PENNYWISE. Fernando Andrés, Maida Romera-Branchat, Rafael Martínez-Gallegos, Vipul Patel, Korbinian Schneeberger, Seonghoe Jang, Janine Altmüller, Peter Nürnberg, and George Coupland

Direct repression of lateral organ boundary genes in the shoot meristem is required for flowering induction.

Functional Analysis of the Arabidopsis TETRASPININ Gene Family in Plant Growth and Development. Feng Wang, Antonella Muto, Jan Van de Velde, Pia Neyt, Kristiina Himanen, Klaas Vandepoele, and Mieke Van Lijsebettens

Genes coding for TETRASPININ membrane proteins have divergent, overlapping, and redundant functions in plant growth and development.

MEMBRANES, TRANSPORT, AND BIOENERGETICS

Grapevine and Arabidopsis Cation-Chloride Cotransporters Localize to the Golgi and Trans-Golgi Network and Indirectly Influence Long-Distance Ion Transport and Plant Salt Tolerance. Sam W. Henderson, Stefanie Wege, Jiwen Qiu, Deidre H. Blackmore, Amanda R. Walker, Stephen D. Tyerman, Rob R. Walker, and Matthew Gilliham

A protein from grapevine that transports sodium, potassium, and chloride ions across endomembranes is important for normal growth and salt tolerance.

SIGNALING AND RESPONSE

Maize Homologs of Hydroxycinnamoyltransferase, a Key Enzyme in Lignin Biosynthesis, Bind the Nucleotide Binding Leucine-Rich Repeat Rp1 Proteins to Modulate the Defense Response. Guan-Feng Wang, Yijian He, Renee Strauch, Bade A. Olukolu, Dahlia Nielsen, Xu Li, and Peter J. Balint-Kurti

Homologs of hydroxycinnamoyltransferase, involved in lignin biosynthesis, interact directly with leucine-rich receptor proteins to suppress the hypersensitive response.
Axial and Radial Oxylipin Transport. Debora Gasperini, Adeline Chauvin, Ivan F. Acosta, Andrzej Kurenda, Stéphanie Stolz, Aurore Chételat, Jean-Luc Wolfender, and Edward E. Farmer

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Sphingolipids play a key role in plant defense towards different lifestyle pathogens by modulating cell death, ROS accumulation, and jasmonate signaling pathway. 2255

The Rice E3-Ubiquitin Ligase HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE1 Modulates the Expression of ROOT MEANDER CURLING, a Gene Involved in Root Mechano-sensing, through the Interaction with Two ETHYLENE-RESPONSE FACTOR Transcription Factors. Tiago F. Lourenço, Tánhia S. Serra, André M. Cordeiro, Sarah J. Swanson, Simon Gilroy, Nelson J.M. Saibo, and M. Margarida Oliveira

Rice root curling, a response to a mechanical barrier that involves the plant hormone jasmonic acid, is modulated by an E3-ubiquitin ligase. 2275

Gibberellic Acid-Stimulated Arabidopsis Serves as an Integrator of Gibberellin, Abscisic Acid, and Glucose Signaling during Seed Germination in Arabidopsis. Chunmei Zhong, Hao Xu, Siting Ye, Shiyi Wang, Lingfei Li, Shengchun Zhang, and Xiaojing Wang

A cell wall-localized protein stimulates the embryonic axis elongation and seed germination, and modulates the cross talk between gibberellins, abscisic acid, and glucose signaling pathways. 2288

Opposing Effects on Two Phases of Defense Responses from Concerted Actions of HEAT SHOCK COGNATE70 and BONZAI1 in Arabidopsis. Mingyue Gou, Zemin Zhang, Ning Zhang, Quansheng Huang, Jacqueline Monaghan, Huijuan Yang, Zhengjing Shi, Cyril Zipfel, and Jian Hua

Antagonized functions between a heat shock and calcium-binding protein affects both preinvasion and postinvasion phases of defense responses. 2304

9-Lipoxygenase-Derived Oxylipins Activate Brassinosteroid Signaling to Promote Cell Wall-Based Defense and Limit Pathogen Infection. Ruth Marcos, Yovanny Izquierdo, Tamara Vellosillo, Satish Kulasekaran, Tomás Cascón, Mats Hamberg, and Carmen Castresana

Lipid signals from the 9-lipoxygenase pathway induce cell wall-based defense by activation of Brassinosteroids, a hormone controlling cell wall repair. 2324
CORRECTIONS

Evolution of a Double Amino Acid Substitution in the 5-Enolpyruvylshikimate-3-Phosphate Synthase in *Eleusine indica* Conferring High-Level Glyphosate Resistance.  Yu Q., Jalaludin A., Han H., Chen M., Sammons R.D., and Powles S.B.  

Root Hair Development in the Grasses: What We Already Know and What We Still Need to Know.  Marzec M., Melzer M., and Szarejko I.  

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