On the Cover: Blooming flowers have fascinated people for thousands of years. Research led to the discovery of major molecular mechanisms regulating flowering time, flower organ, and germline development, as well as fertilization, culminating in the generation of seeds containing a new generation. Depending on developmental and environmental cues, male and female gametophytes are produced in the anther and ovules after meiotic and additional mitotic divisions. Pollination from selfing or mediated by wind or insects then starts a long series of male-female dialogues leading to targeted pollen tube growth to the ovules, delivering immotile sperm cells to the female gametophyte for a double fertilization event that gives rise to the embryo and endosperm. This focus issue highlights the tremendous progress that has been made during the past 10 years to understand flowering and reproduction. The image shows an Amaryllis flower of the cultivar Minerva. Photo taken by Thomas Dresselhaus.

THANK YOU TO REVIEWERS
An acknowledgment of Plant Physiology reviewers.

FOCUS ON FLOWERING AND REPRODUCTION

EDITORIAL
Focus on Flowering and Reproduction. Richard M. Amasino, Alice Y. Cheung, Thomas Dresselhaus, and Cris Kuhlemeier

UPDATES


The circadian clock and light signaling regulate CONSTANS function through intricate mechanisms that reside in phloem companion cells of leaves for controlling photoperiodic flowering in Arabidopsis.


The mechanisms by which the environment regulates flowering time have evolved as crops and wild populations have adapted to diverse climates, and the specific variants involved are increasingly known.

[CLOSED] Winter Memory throughout the Plant Kingdom: Different Paths to Flowering. Frédéric Bouché, Daniel P. Woods, and Richard M. Amasino

Molecular mechanisms contribute to the memory of winter in different plant groups.

[CLOSED] Competence to Flower: Age-Controlled Sensitivity to Environmental Cues. Youbong Hyun, René Richter, and George Coupland

miR156 and SPL transcription factors play various roles in conferring competence to flower in plants.

Continued on next page
Virus-induced flowering combines fundamental research in reproductive biology with efficient tools for manipulating gene expression in nonmodel systems to accelerate discovery and breeding.

The use of new experimental approaches enhances the understanding of floral organogenesis.

Hybridization impacts the evolution of lineages through many mechanisms, including adaptive introgression, transgressive segregation, and hybrid speciation.

Pollen-pistil interactions contribute to mate selection at the postmating, prezygotic level.

Ion homeostasis and signaling are crucial to regulate pollen tube growth and morphogenesis and affect upstream membrane transporters and downstream targets.

Recent studies have elucidated a molecular framework for the attraction of the pollen tube by synergid cells and the control of attraction by female gametes and sensing by the pollen tube.

Intracellular and intercellular mechanisms govern the differentiation of female gametophytic cells.

A comparison of eudicot and monocot model plants explores recent advances and open questions on gene regulatory networks during zygote development, parental influences on early embryogenesis, zygotic genome activation, and cell fate determination.

Recent developments advance our understanding of imprinted gene expression in plants.
RESEARCH REPORT


In Arabidopsis, gamete fusion requires the C2H2 transcription factor SUF4, which regulates the expression of the EGG CELL1 gene family.

RESEARCH ARTICLES


Two glycosyltransferases required for xylan biosynthesis and implicated in AGP galactosylation are involved in pollen primexine wall formation and exine patterning in Arabidopsis.


KNS4/UPEX1 is involved in the synthesis of type II AG of AGPs/pectins that are essential components of pollen grain walls providing the scaffold for exine construction/architecture.

[OPEN] MONENSIN SENSITIVITY1 (MON1)/CALCIUM CAFFEINE ZINC SENSITIVITY1 (CCZ1)-Mediated Rab7 Activation Regulates Tapetal Programmed Cell Death and Pollen Development. Yong Cui, Qiong Zhao, Hong-Tao Xie, Wing Shing Wong, Xiangfeng Wang, Caizi Gao, Yu Ding, Yuqi Tan, Takashi Ueda, Yan Zhang, and Liwen Jiang

MON1/CCZ1-mediated vacuolar transport of tapetum degradation-related cysteine proteases affects tapetal programmed cell death and pollen development in Arabidopsis.

[OPEN] Two Membrane-Anchored Aspartic Proteases Contribute to Pollen and Ovule Development. Hui Gao, Yinghui Zhang, Wanlei Wang, Keke Zhao, Chunmei Liu, Lin Bai, Rui Li, and Yi Guo

Two putative GPI-anchored aspartic proteases, A36 and A39, are involved in pollen and ovule development as well as in directional growth of the pollen tube toward ovules in Arabidopsis.


The cytoplasmic hydroxycinnamoyl-CoA:ω-hydroxy fatty acid transferase DPW2 plays a fundamental role in male reproduction via the biosynthesis of key components of the anther cuticle and pollen wall.


Standing genetic variation for flowering time in a nondomesticated grass encompasses known and novel regulators.

Continued on preceding page

Continued on next page
Genetic Architecture of Flowering-Time Variation in Brachypodium distachyon. 
Daniel P. Woods, Ryland Bednarek, Frédéric Bouché, Sean P. Gordon, John P. Vogel, David F. Garvin, and Richard M. Amasino

QTL associated with VERNALIZATION1/PHYC and VERNALIZATION2 account for much of the natural variation in flowering time between the Brachypodium distachyon lines Bd21 and Bd1-1.

[OPEN] A Conserved cis-Regulatory Module Determines Germline Fate through Activation of the Transcription Factor DUO1 Promoter.  
Benjamin Peters, Jonathan Casey, Jack Aidley, Stuart Zohrab, Michael Borg, David Twell, and Lynette Brownfield

A cis-regulatory module conserved in eudicots directs spatial and temporal control of the transcription factor DUO1 to specify male germline fate.

[OPEN] The Genetic Control of Reproductive Development under High Ambient Temperature.  
Mahwish Ejaz and Maria von Korff

PHOTOPERIOD1 (Ppd-H1) and VERNALIZATION1 (HvVRN1) interact to control reproductive development under high ambient temperature in barley.

[OPEN] IRREGULAR POLLEN EXINE1 Is a Novel Factor in Anther Cuticle and Pollen Exine Formation. 
Xiaoyang Chen, Hua Zhang, Huayue Sun, Hongbing Luo, Li Zhao, Zhaobin Dong, Shuangshuang Yan, Cheng Zhao, Renyi Liu, Chunyan Xu, Song Li, Huabang Chen, and Weiwei Jin

The IPE1 gene, encoding a putative glucose-methanol-choline oxidoreductase targeted to the endoplasmic reticulum, plays an essential role in anther cuticle and pollen exine development in maize.

[OPEN] Two SERK Receptor-Like Kinases Interact with EMS1 to Control Anther Cell Fate Determination. 
Zhiyong Li, Yao Wang, Jian Huang, Nagib Ahsan, Gabriel Biener, Joel Paprocki, Jay J. Thelen, Valerica Raicu, and Dazhong Zhao

The leucine-rich repeat receptor-like kinase SERK1/2 controls anther cell differentiation as a partner of EMS1 in Arabidopsis.

Bing Liu, Nico De Storme, and Danny Geelen

Gibberellin induces non-reductional cell division and diploid pollen formation in Arabidopsis by interfering with DELLA-mediated meiotic cytokinesis.

Jiao Jiao, Akane G. Mizukami, Subramanian Sankaranarayanan, Junichiro Yamaguchi, Kenichiro Itami, and Tetsuya Higashiyama

Structure-activity relationship studies of AMOR disaccharide in Torenia fournieri reveal the various residues that are critical for AMOR activity.

REGULAR ISSUE

ON THE INSIDE

Peter V. Minorsky

Continued on preceding page

Continued on next page
BREAKTHROUGH TECHNOLOGIES

A Tightly Regulated Genetic Selection System with Signaling-Active Alleles of Phytochrome B. Wei Hu and J. Clark Lagarias

Tightly regulated phytochrome B-based cassettes enable facile phenotypic selection of transgenic plants without the use of antibiotics or herbicides.

RESEARCH REPORT


CmOr golden SNP dramatically affects carotenoid content and plastid fate in melon by inhibiting metabolism downstream of β-carotene.

RESEARCH ARTICLES

BIOCHEMISTRY AND METABOLISM

Membrane Proteomic Insights into the Physiology and Taxonomy of an Oleaginous Green Microalga. Adriana Garibay-Hernández, Bronwyn J. Barkla, Rosario Vera-Estrella, Alfredo Martinez, and Omar Pantoja

RNA-Seq-based membrane proteomics provides insights into the biology of the oleaginous microalga Ettlia oleoabundans, allowing the identification of the novel proteins PSBS, MPH1, and RP2-CLC.

Characterization of Class III Peroxidases from Switchgrass. Timothy W. Moural, Kevin M. Lewis, Carlo Barnaba, Fang Zhu, Nathan A Palmer, Gautam Sarath, Erin D. Scully, Jeffrey P. Jones, Scott E. Sattler, and ChulHee Kang

The crystal structure, kinetics assay, molecular docking, and temporal and spatial expression of PviPRX9, as well as its coexpression with other genes, reveals a role of PviPRX9 in lignification.


Respiratory complex I mutants do not properly acclimate to long-day conditions in Arabidopsis, demonstrating the importance of mitochondria for the photoperiod response.

Integrative Approaches for the Identification and Localization of Specialized Metabolites in Tripterygium Roots. B. Markus Lange, Justin T. Fischedick, Malte F. Lange, Narayanan Sridevi, Dunja Šamec, and Brenton C. Poirier

An analysis of the thunder god vine (genus Tripterygium) offers innovative analytical approaches for the identification and localization of specialized metabolites.
Two Trichome Birefringence-Like Proteins Mediate Xylan Acetylation, Which Is Essential for Leaf Blight Resistance in Rice. Yaping Gao, Congwu He, Dongmei Zhang, Xiangling Liu, Zuopeng Xu, Yanbao Tian, Xue-Hui Liu, Shanshan Zang, Markus Pauly, Yihua Zhou, and Baocai Zhang

OsTBL1 and OsTBL2 represent xylan acetyltransferases, which catalyze addition of acetyl groups onto the 2-O and 3-O position of xylosyl residues and is essential for pathogen resistance in rice.

Rice Cellulose SynthaseA8 Plant-Conserved Region Is a Coiled-Coil at the Catalytic Core Entrance. Phillip S. Rushton, Anna T. Olek, Lee Makowski, John Badger, C. Nicklaus Steussy, Nicholas C. Carpita, and Cynthia V. Stauffacher

The crystal structure of a rice CesA8 plant-conserved region (P-CR), solved to 2.4 Å resolution, is docked to a model of the catalytic domain of CesA8 using SAXS molecular envelopes.

Diurnal Solar Energy Conversion and Photoprotection in Rice Canopies. Katherine Meacham, Xavier Sirault, W. Paul Quick, Susanne von Caemmerer, and Robert Furbank

Diurnal photoprotection in Oryza sativa reduces the curvature factor of the relationship between electron transport rate and irradiance, reducing diurnal radiation use efficiency in comparison with steady-state measurements in the same leaves.

A Comprehensively Curated Genome-Scale Two-Cell Model for the Heterocystous Cyanobacterium *Anabaena* sp. PCC 7120. David Malatinszky, Ralf Steuer, and Patrik R. Jones

The genome-scale metabolic model of a nitrogen-fixing filamentous cyanobacterium helps to understand inter- and intra-cellular metabolic interactions and contributes to engineering strategies.

CELL BIOLOGY

Ubiquitin-Proteasome Dependent Regulation of the GOLDEN2-LIKE 1 Transcription Factor in Response to Plastid Signals. Mitsuaki Tokumaru, Fumi Adachi, Makoto Toda, Yasuko Ito-Inaba, Fumiko Yazu, Yoshihiro Hirose, Yoichi Sakakibara, Masahito Suiko, Tomohiro Kakizaki, and Takehito Inaba

Accumulation of the transcription factor GOLDEN2-LIKE 1 in response to plastid signals is regulated by the ubiquitin-proteasome system in Arabidopsis thaliana.

VAMP721 Conformations Unmask an Extended Motif for K+ Channel Binding and Gating Control. Ben Zhang, Rucha Karnik, Sakharam Waghmare, Naomi Donald, and Michael R. Blatt

Channel binding depends on coordination between the two binding sites on VAMP721, consistent with the K+ channels as nucleation points for SNARE complex assembly.

cis-Cinnamic Acid Is a Novel, Natural Auxin Efflux Inhibitor That Promotes Lateral Root Formation. Ward Steenackers, Petr Klíma, Mussa Quareshy, Igor Cesario, Robert P. Kempf, Sander Cornelisse, Pedro Araújo, Tom Viaene, Geert Goeminne, Moritz K. Nowack, Karin Ljung, Jiří Friml, Joshua J. Blakeslee, Ondřej Novík, Eva Zažímalová, Richard Napier, Wout Boerjan, and Bartel Vanholme

The phenylpropanoid cis-cinnamic acid is a natural auxin efflux inhibitor that promotes lateral root formation.

Arabidopsis VPS36, an ESCRT-II component with ubiquitin-binding activity, is critical for MVB-mediated degradation of plasma membrane proteins.

Continued on next page
Phosphorylation of MAP65-1 by Arabidopsis Aurora Kinases Is Required for Efficient Cell Cycle Progression.
Joanna Boruc, Annika K. Weimer, Virginie Stoppin-Mellet, Evelien Mylle, Ken Kosetsu, Cesyen Cedeño, Michel Jaquinod, Maria Njo, Liesbeth De Milde, Peter Tompa, Nathalie Gonzalez, Dirk Inzé, Tom Beeckman, Marylin Vantard, and Daniël Van Damme

Arabidopsis Aurora kinases phosphorylate MAP65-1 at its unfolded tail domain and dynamic switching of its phosphorylation status throughout mitosis is required for proper cell cycle progression.

Homologues of the RPW8 Resistance Protein Are Localized to the Extrahaustorial Membrane that Is Likely Synthesized De Novo. Robert Berkey, Yi Zhang, Xianfeng Ma, Harlan King, Qiong Zhang, Wenming Wang, and Shunyuan Xiao

Localization analyses of RPW8 homologs suggest that extrahaustorial membrane-targeting (EHM) may be an ancestral feature of the RPW8 family and that the EHM induced by powdery mildew is likely synthesized de novo in Arabidopsis.

ECOPHYSIOLOGY AND SUSTAINABILITY

High-Throughput Phenotyping of Maize Leaf Physiological and Biochemical Traits Using Hyperspectral Reflectance. Craig R. Yendrek, Tiago Tomaz, Christopher M. Montes, Youyuan Cao, Alison M. Morse, Patrick J. Brown, Lauren M. McIntyre, Andrew D.B. Leakey, and Elizabeth A. Ainsworth

Partial least-squares regression modeling of leaf reflectance spectra provides a high-throughput and accurate approach to phenotyping photosynthesis and leaf biochemistry in maize.

GENES, DEVELOPMENT, AND EVOLUTION

The Arabidopsis Polycomb Repressive Complex 1 (PRC1) Components AtBMI1A, B, and C Impact Gene Networks throughout All Stages of Plant Development. Wiam Merini, Francisco J. Romero-Campero, Angeles Gomez-Zambrano, Yue Zhou, Franziska Turck, and Myriam Calonje

Genome-wide transcriptomic data in combination with H3K27me3 and protein localization data unveiled the roles of the AtBMI1s and their possible relationship with other PRC1 components.

DNA Topoisomerase Iα Affects the Floral Transition. Ximing Gong, Lisha Shen, Ya Zhi Peng, Yinbo Gan, and Hao Yu

The Arabidopsis DNA topoisomerase Iα modulates the expression of FLC, MAF4, and MAF5 to control flowering time.

Transcriptional Gene Silencing Maintained by OTS1 SUMO Protease Requires a DNA-Dependent Polymerase V-Dependent Pathway. Lei Liu, Xiaoqing Yan, Xiangxiong Kong, Yiqiang Zhao, Zhizhong Gong, Jing Bo Jin, and Yan Guo

SUMOylation is required for the regulation of transcriptional gene silencing, which is dependent on Pol V pathway in Arabidopsis.

Proteomes and Ubiquitylomes Analysis Reveals the Involvement of Ubiquitination in Protein Degradation in Petunias. Jianhang Guo, Juanxu Liu, Qian Wei, Rongmin Wang, Weiyuan Yang, Yueyue Ma, Guoju Chen, and Yixun Yu

The global proteome and ubiquitylome are negatively correlated, with ubiquitination involved in the degradation of proteins during ethylene-mediated corolla senescence in petunias.

Continued on next page
Rice Leaf Angle and Grain Size Are Affected by the OsBUL1 Transcriptional Activator Complex.
Seonghoe Jang, Gynheung An, and Hsing-Yi Li

A complex consisting of an atypical HLH protein, OsBUL1, a protein containing KxDL-motif, LO9-177, and basic HLH protein, OsBC1, positively affects leaf bending and grain size in rice.

Natural Variation of Molecular and Morphological Gibberellin Responses. Youn-Jeong Nam, Dorota Herman, Jonas Blomme, Eunyoung Chae, Mikiko Kojima, Frederik Coppens, Veronique Storme, Twigg Van Dalee, Stijn Dhondt, Hitoshi Sakakibara, Detlef Weigel, Dirk Inzé, and Nathalie Gonzalez

Genetic perturbation of endogenous gibberellin levels shows unexpected flexibility in the wiring of regulatory networks underlying hormone metabolism and signaling.

TATA Box Insertion Provides a Selection Mechanism Underpinning Adaptations to Fe Deficiency. Meiling Zhang, Yuanda Lv, Yi Wang, Jocelyn K. C. Rose, Fei Shen, Zhenyun Han, Xinzong Zhang, Xuefeng Xu, Ting Wu, and Zhenhai Han

TATA-box insertion in the upstream untranslated sequences of genes enhances adaptation to selective pressure.

MEMBRANES, TRANSPORT, AND BIOENERGETICS

Extracellular Vesicles Isolated from the Leaf Apoplast Carry Stress-Response Proteins. Brian D. Rutter and Roger W. Innes

Bacterial infection enhances the production of apoplastic vesicles carrying stress-response proteins in Arabidopsis.

A Palmitic Acid Elongase Affects Eicosapentaenoic Acid and Plastidial Monogalactosyldiacylglycerol Levels in Nannochloropsis. Lina-Juana Dolch, Camille Rak, Giorgio Perin, Guillaume Tourcier, Richard Broughton, Marina Leterrier, Tomas Morosinotto, Frédérique Tellier, Jean-Denis Faure, Denis Falconet, Juliette Jouhet, Olga Sayanova, Frédéric Beaudoin, and Eric Maréchal

A heterokont palmitic acid desaturase initiates the production of eicosapentaenoic acid in the endoplasmic reticulum of Nannochloropsis, which is specifically used for monogalactosyldiacylglycerol assembly in the secondary plastid.

SIGNALING AND RESPONSE

Mitogen-Activated Protein Kinase Phosphatases Affect UV-B-Induced Stomatal Closure via Controlling NO in Guard Cells. Feng-Chen Li, Jing Wang, Mi-Mi Wu, Cai-Ming Fan, Xuan Li, and Jun-Min He

Mitogen-activated protein kinase phosphatases 1 and 6 promote ultraviolet B-induced stomatal closure by modulating hydrogen peroxide-induced nitric oxide production in Arabidopsis guard cells.

A Cysteine-Rich Protein Kinase Associates with a Membrane Immune Complex and the Cysteine Residues Are Required for Cell Death. Koste A. Yadeta, James M. Elmore, Athena Y. Creer, Baomin Feng, Jessica Y. Franco, Jose Sebastian Rufian, Ping He, Brett Phinney, and Gitta Coaker

Arabidopsis CRK28 is synthesized upon immune perception, associates with the FLS2 receptor complex, and silencing multiple related CRKs enhances disease susceptibility.
SAV4 modulates auxin transport through its shootward membrane localization and inhibitory effect on ABCB1-mediated auxin efflux in hypocotyls of Arabidopsis.

The LBD12-1 Transcription Factor Suppresses Apical Meristem Size by Repressing Argonaute 10 Expression.

Rice LBD12-1 functions as a repressor of shoot apical meristem size by down-regulating expression of AGO10 and HD-ZIP III.

Two h-Type Thioredoxins Interact with the E2 Ubiquitin Conjugase PHO2 to Fine-Tune Phosphate Homeostasis in Rice.

Oryza sativa PHO2 interacts with two h-type thioredoxins, which links phosphate homeostasis with redox balance in rice.

Substituted Phthalimide AC94377 Is a Selective Agonist of the Gibberellin Receptor GID1.

AC94377 preferentially binds the GID1 receptor to activate the gibberellin signaling in Arabidopsis.

A Universal Stress Protein Involved in Oxidative Stress Is a Phosphorylation Target for Protein Kinase CIPK6.

Universal stress protein SlRd2 is a Cipk6 target and regulates Cipk6-mediated ROS.

An Ethylene-Induced Regulatory Module Delays Flower Senescence by Regulating Cytokinin Content.

A regulatory module by which ethylene and cytokinin interaction affects rose flower senescence.

A DII Domain-Based Auxin Reporter Uncovers Low Auxin Signaling during Telophase and Early G1.

A new DII reporter indicates a low level of auxin signaling during telophase and G1 in maize.

A metabolic network-guided genome-wide association study of seed free amino acids facilitates the identification of a histidine-specific transporter in Arabidopsis.

Interlinking GWAS of Debranched Starch Structure with Gene Regulatory Networks Uncovers a Regulatory Region for Amylose Synthesis in Rice (Oryza sativa).


The seed-to-seedling transition in Arabidopsis can occur following multiple spatiotemporal patterns of cell expansion, including hypocotyl-based growth promoted by the transcription factor ATHB5.