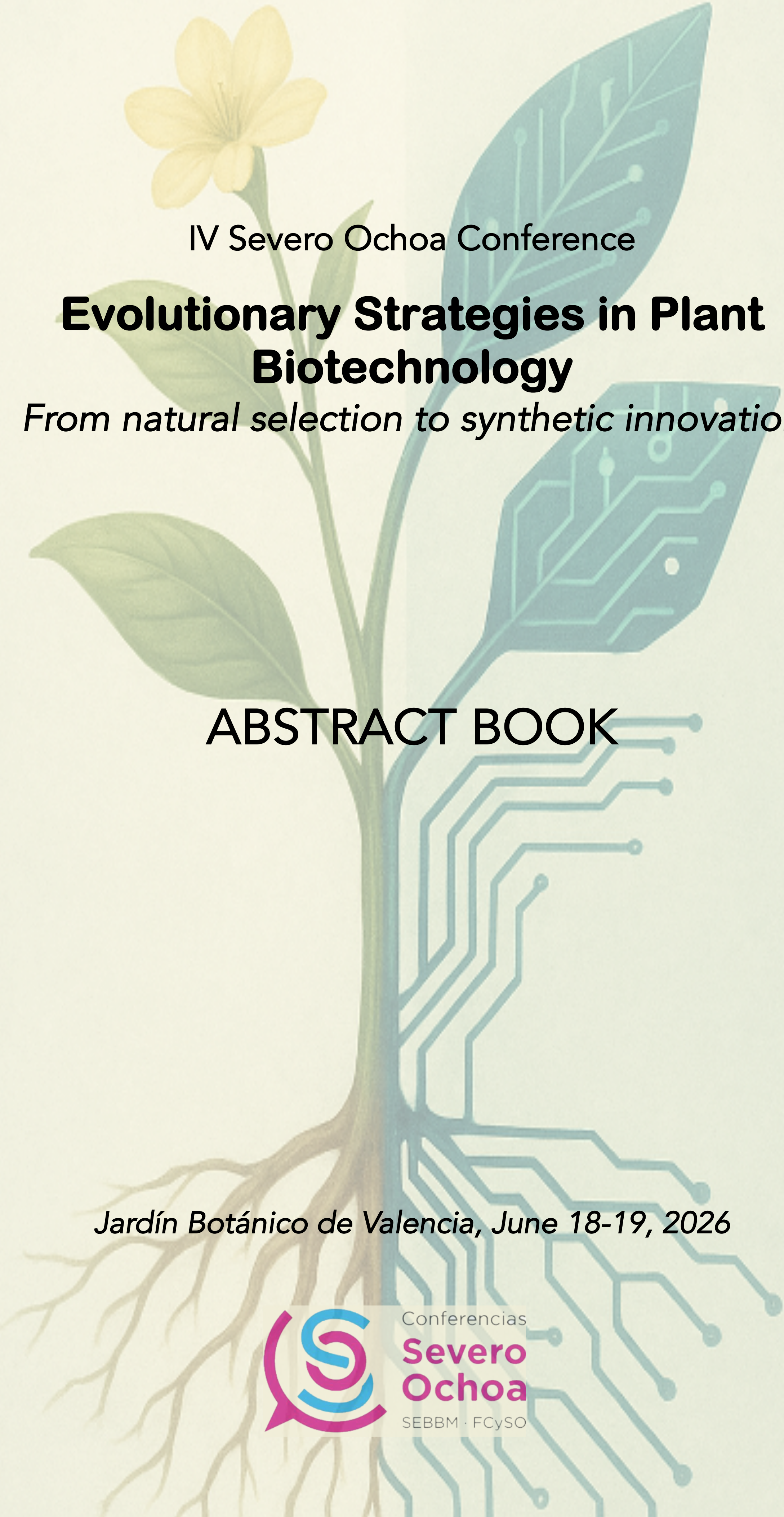

IV Severo Ochoa Conference

Evolutionary Strategies in Plant Biotechnology

From natural selection to synthetic innovation

ABSTRACT BOOK

Jardín Botánico de Valencia, June 18-19, 2026

Conferencias Severo Ochoa
SEBBM · FCySO

IV Severo Ochoa Conference

Evolutionary Strategies in Plant Biotechnology

From natural selection to synthetic innovation

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Historically, Evolution and Biotechnology have been viewed as separate disciplines. However, closer examination reveals a strong potential for synergy. Insights into how metabolic and signaling pathways have evolved in plants can inform both the limitations and possibilities of biotechnological applications. In this context, Synthetic Biology offers a compelling route to bypass evolutionary constraints and develop innovative solutions. We believe that bringing together experts in evolutionary biology and synthetic biology will generate valuable cross-disciplinary dialogue.

Organizers

FUNDACIÓN CARMEN Y SEVERO OCHOA

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Program

Scientific program

Thursday, June 18th, 2026

11:30-13:30	Registration and poster setting
13:30-14:15	Lunch (outdoors, in the Botanic Garden)
14:15-14:30	Welcome address (Organizers, SEBBM, FCSyO)

Session 1: Evolution of Plant Signaling and Bioengineering Tools

(Chair: C. Ferrandiz)

14:30-15:15 Roberto Solano *(CNB, Madrid)*

The "missing hormone" and the evolution of jasmonates in land plants

15:15-16:00 Rubén Garrido-Oter (*Max Planck Institute for Plant Breeding, Cologne*)

Symbiotic and commensal root microbes are key determinants of plant responses to rising CO_2

16:00-16:45 Matias Zurbriggen (*CEPLAS, University of Düsseldorf*)

Plant synthetic and reconstruction biology approaches for the study and control of cellular processes in plants –optogenetics as an enabling technology

16:45-17:15 Coffee break and poster viewing

Session 2: Evolution and Engineering of Metabolism

(Chair: C. Ferrandiz)

17:15-18:00 Alison Smith *(Cambridge University)*

Exploiting B vitamin metabolism for synthetic biology and ecology

18:00–18:45 ***Selected short talks***

Ulrich Eckhard *(IBMB-CSIC, Barcelona)* **From Evolutionary Blueprints to Synthetic Innovation: Engineering Microbial Surface Nanomachines for Targeted Plant Protection**

Armando Albert *(IQF-CSIC, Madrid)* **Evolution-Guided Remodeling of ABA Receptors Enables Engineering of Hormone Perception**

	Maria Lois (*CRAG, Barcelona)* **Evolution of molecular determinants for SUMO-activating enzyme subcellular localization in plants**
18:45-19:30	***Keynote EMBO lecture***
	Ralph Bock (*MPI Mol Plant Physiology, Potsdam)*
	Exploiting horizontal genome transfer in plant breeding and synthetic biology
21:00	Dinner (**Restaurant Contrapunto, Palau de les Arts**)

Friday, June 19th, 2026

Sesión 3: Evolution of Development and Modular Design
(Chair: M. Blázquez)

9:00-9:45	Edwige Moyroud (*SLCU, Cambridge*) ***Blooming across scales: understanding the molecular mechanisms flowering plants use to pattern their petals and their impact on plant fitness*** *Sponsored by The Company of Biologists*
9:45- 10:30	Diego Orzáez *(IBMCP, Valencia)*
	Exploring Novel Plant Traits Beyond Evolutionary Constraints with Bioluminescence as a Probe
10:30-11:15	Selected short talks
	Isabel Monte (*ZMBP, University of Tuebingen*) **Molecular plant-microbe interactions: from evolutionary insights to immune receptor engineering**
	Juan-José Llorens-Gámez *(IBMCP, Valencia)* **CRISPR-Mediated Targeted Gene Replacement at the Arabidopsis thaliana MIR390a Locus Enables Artificial MicroRNA Production and Effective Gene Silencing**
	Monica Meijón *(Universidad de Oviedo)* **From algae to angiosperms: evolutionary strategies of plant adaptation to combined heat and drought stress**
11:15-12:00	Coffee break and poster viewing

Sesión 4: High-precision Plant Biotechnology

(Chair: M. Blázquez)

12:00-12:45	Jake Harris (Cambridge *University)* ***Engineering chromatin states to encode transcriptional immune memory in Arabidopsis***
12:45-13:30	Caixia Gao (*Institute of Genetics and Developmental Biology, Beijing)* ***Precision Genome Editing: From Molecular Mechanisms to Crop Engineering*** *Sponsored by Madeinplant*
13:30 -13:45	Conclusions and Farewell (organizers)
13:45- 15:00	Lunch (outdoors, Botanic Garden)

Invited speakers

T1. The "missing hormone" and the evolution of jasmonates in land plants

Roberto Solano

Department of Plant Molecular Genetics. National Center for Biotechnology (CNB-CSIC). Madrid, Spain

Jasmonates are fatty acid-derived phytohormones structurally similar to metazoan prostaglandins, and essential for plant defense and development.

A. thaliana has been an excellent model system in identifying the bioactive hormone (JA-Ile) and elucidating its signal transduction pathway in eudicots. Nonetheless, information in *A. thaliana* unlikely represents the diversity of this pathway in other plant lineages.

Bryophyte genomes contain conserved sequences for all JA-Ile signaling components, but in contrast to higher plants, lack JA-Ile. I will discuss that despite 450 million years of independent evolution, the JA-Ile co-receptor COI1/JAZ and the rest of the signaling components are functionally conserved between the bryophyte *Marchantia polymorpha* and *A. thaliana*. However, this co-receptor perceives and responds to different ligands in each species. Instead of JA-Ile, the ligand of Marchantia MpCOI1 is the JA-Ile precursor dinor-OPDA. Our analysis of the biosynthetic pathway for dinor-OPDA uncovered an ancient OPR3-independent pathway for JA biosynthesis that is widely distributed from charophycean algae to eudicots. Moreover, we discovered that dinor-OPDA has a COI1-independent function regulating thermotolerance in all land plants. The evolutionary implications of these discoveries and how they inform us for synthetic biology strategies will be discussed during the talk.

T2. Symbiotic and commensal root microbes are key determinants of plant responses to rising CO_2

Rubén Garrido Oter

Max Planck Institute for Plant Breeding, Cologne, Germany

Rising atmospheric CO_2 stimulates plant growth, but this effect is often constrained by nutrient availability. While beneficial soil-derived microbial symbionts such as nitrogen-fixing rhizobia and mycorrhizal fungi are known to mitigate this limitation, the role of plant commensal microbiota remains unclear.

Here, using a gnotobiotic growth system and the model legume *Lotus japonicus,* we show that the magnitude of plant responses to elevated CO_2 is largely explained by the composition of its root-associated microbial community. Axenic plants exhibited limited physiological responses to increasing CO_2 but inoculation with symbiotic and commensal microbes significantly amplified this effect. Commensal bacteria alone, while not increasing overall biomass, shifted plant carbon allocation belowground. Microbial abundances in the root increased with atmospheric CO_2, even when normalized to plant DNA. The strongest plant and microbial responses required the simultaneous presence of rhizobia, arbuscular mycorrhizal fungi, and the commensal bacterial community. These findings demonstrate that multi-partner microbial associations will be critical for maximizing plant growth and carbon sequestration as atmospheric CO_2 concentrations continue to rise.

T3. Plant synthetic and reconstruction biology approaches for the study and control of cellular processes in plants – optogenetics as an enabling technology

Matias Zurbriggen

Institute of Synthetic Biology and CEPLAS, Heinrich-Heine-Universität Düsseldorf, Germany

Our synthetic biology research focuses on applying reconstruction biology approaches in orthogonal cellular systems to the study of plant regulatory and metabolic networks. This approach helps overcoming the experimental constraints posed by the combinatorial genetic complexity and multifactorial dynamic interactions of signaling networks and yields a quantitative understanding of mechanistic and regulatory principles. We combine these strategies with the engineering of molecular photoswitches implementing bacterial and plant photoreceptors with effector output modules mediating the precise control of cellular processes. There is a broad range of optogenetic tools responsive to different wavelengths of the white light spectrum (UV-B, blue, green, red/far-red) and they are designed to control various molecular processes with high precision, quantitative and high spatio-temporal resolution, in a non-invasive way and with minimized toxicity. We implement these molecular tools into microbial, mammalian and plant cells, synthetic organoids and *in vivo* in animals and plants for selectively manipulating signaling networks and metabolic pathways in 2D/3D/4D settings. However, the need of light by plants for growth and development imposes an intrinsic experimental constrain to the use of optogenetic tools under normal plant culture conditions, i.e. cycles of dark/white light. Therefore, molecular engineering strategies have to be employed to overcome this limitation. Selected applications of the synthetic biology approach will be discussed and examples for the light control of CRISPR/Cas9 technologies and the study of signalling networks shown.

T4. Exploiting B vitamin metabolism for synthetic biology and ecology

Alison G. Smith

Department of Plant Sciences, University of Cambridge, UK

B vitamins are complex primary metabolites that are required as essential cofactors for central metabolic pathways. Plants, algae and bacteria make these cofactors *de novo*, but there are some notable exceptions. Many microalgae are auxotrophic for vitamins B12 (cobalamin), B1 (thiamine) or B7 (biotin), with some species requiring two or more of these compounds to grow. Similarly, many bacteria require an exogenous supply of these organic micronutrients. I will highlight some examples and then explain how these apparently deleterious traits may have evolved and what role vitamin auxotrophy might play in aquatic ecosystems. I will then outline how we have exploited the role of B vitamins to generate synthetic gene circuits in microalgae.

T5. Exploiting horizontal genome transfer in plant breeding and synthetic biology

Ralph Bock

Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

Chloroplast genomes can be horizontally exchanged between plant species, a process known from phylogenetic studies as chloroplast capture. We recently discovered that (natural) grafting provides a mechanism by which chloroplast capture can occur. Taking an experimental evolution approach, we demonstrated that chloroplast genomes are horizontally transferred across graft junctions in an asexual process, thus resulting in organelle capture. We now have begun to employ grafting as a tool to produce novel combinations of nuclear and chloroplast genomes by systematically exchanging chloroplasts between species. In my talk, I will describe the generation of a panel of Solanaceous species that harbor alien chloroplast genomes. I will show how horizontal plastid genome transfer can be used to study the interactions between chloroplast and nuclear genomes, and how it can be exploited for plant breeding. Finally, I will show how the horizontal transfer of nuclear genomes leads to the formation of new plant species, and how this phenomenon can be employed to generate new synthetic plant species.

T6. Blooming across scales: understanding the molecular mechanisms flowering plants use to pattern their petals and their impact on plant fitness

Edwige Mouyroud

The Sainsbury Laboratory, University of Cambridge, UK

The colourful motifs on flowering plant petals serve as exceptional models for deciphering how molecular shifts translate across biological scales. These intricate patterns constitute powerful experimental systems for deciphering the processes governing cell behaviour during development and for investigating how changes at the molecular level translate across scales, as ultimately these patterns dictate a plant's ability to navigate biotic interactions with pollinators and withstand abiotic stressors like desiccation and high UV radiation.

In this talk, I will present recent and ongoing research from my group exploring the developmental and evolutionary drivers of petal patterning. Specifically, I will highlight findings identifying small metabolites, such as flavonols, and components of the GABA signaling pathway as potent morphogens and central regulators of cell proliferation and differentiation during flower development. By bridging the gap between molecular regulation and organismal fitness, this work provides a framework for understanding how natural selection shapes floral diversity and how these mechanisms might be leveraged for synthetic innovation in plant biotechnology.

T7 Exploring novel plant traits beyond evolutionary constraints with bioluminescence as a probe

Diego Orzáez

Instituto de Biología Molecular y Celular de Plantas, CSIC-UPV, Valencia, Spain

Plants can be understood as distributed information-processing systems whose complex genetic networks integrate signals to generate anticipatory responses. However, their genetic circuitry is constrained by evolutionary history and is not inherently reprogrammable. We explore how plants can be reprogrammed beyond their evolutionary design space by introducing synthetic gene circuits to enable novel traits, including enhanced adaptive responses and new bioproduction capabilities. To monitor circuit activity, we use autonomous bioluminescence as a real-time, non-invasive and scalable readout.

We will describe two types of synthetic gene circuits developed in our lab. First, conditional amplification systems that couple inducible transcriptional control to viral-derived replicons to enable strong, regulated gene expression for plant-based bioproduction. Second, biosensing sentinel plants, in which pathogen activity is sensed and converted into optical outputs, allowing plants to autonomously signal infection by glowing.

Together, these approaches seek to identify design principles for building robust synthetic gene circuits in plants for sensing and bioproduction applications.

T8. Engineering chromatin states to encode transcriptional immune memory in Arabidopsis

Jake Harris

Department of Plant Sciences, University of Cambridge, UK

Transcriptional memory enables organisms to respond more rapidly to recurrent stress, yet the chromatin features that encode this state remain poorly characterised. Here we define the chromatin architecture associated with pathogen-induced transcriptional memory in *Arabidopsis thaliana*. Memory genes exhibit a characteristic resting-state configuration that includes elevated H3K27me3, reduced H3K36me3, and a subset marked by H3K27me3–H3K4me3 bivalency. Polycomb function is required for memory formation, and priming induces a poised state characterised by increased H3K4me3 and stalled RNA polymerase II. Using locus-specific chromatin engineering, we show that targeted H3K4me3 deposition at the WRKY29 locus is sufficient to establish a synthetic memory-like state, yielding enhanced pathogen resistance that phenocopies natural priming. Single-nucleus transcriptomics further reveals that memory gene activation is concentrated within discrete leaf cell-type populations. Together, these findings identify a chromatin-encoded memory module in plant immunity and provide proof-of-concept that it can be rationally manipulated to enhance disease resistance.

T9. Precision genome editing: from molecular mechanisms to crop engineering

Caixia Gao

Institute of Genetics and Developmental Biology, Beijing

Feeding a growing global population while minimizing the environmental footprint of agriculture is one of the most pressing challenges of the 21st century. Precision genome editing technologies are transforming crop improvement by enabling targeted genetic modifications that range from single-nucleotide substitutions to large-scale chromosome engineering. However, achieving predictable and precise genome editing requires both a deeper mechanistic understanding of editing systems and the development of next-generation technologies.

As genome editing technologies continue to evolve, the key question is no longer simply whether we can edit genomes, but how we can do so precisely and predictably. Addressing this challenge requires answering several fundamental scientific questions. How did CRISPR nucleases evolve, and how can this evolutionary knowledge guide the discovery of new genome-editing tools? What molecular mechanisms determine off-target effects and editing precision? And how can we systematically discover and engineer improved enzymes, such as deaminases, for next-generation genome editors?

In this talk, I will present recent advances in precision genome editing, including the discovery of novel editing systems, the development of improved base editors and prime editing platforms, and new strategies for large-scale genome manipulation. In parallel, approaches integrating in planta directed evolution and AI-guided protein design are accelerating the discovery and optimization of plant genes and genome editing tools. Together, these advances establish an integrated framework for predictive crop engineering and enable the rapid development of improved crop germplasm with enhanced productivity, resilience, and nutritional value.

Selected talks

ST1. From evolutionary blueprints to synthetic innovation: engineering microbial surface nanomachines for targeted plant protection

Eva Estevan Morió[1], Laura Garzón[1], Alea Radcke[1], Emma Bloch[1], Julia Hedengrahn[1], Laia González[1], Oriol Capell Sandin[1], Ariana Ivanić[1], Ruby Van Kessel1, Bryan Hogg[1], Kawtar Ben Larbi[1], Oscar Sánchez Martinez[1], Susana Merino[2], Nicholas Thomson[3], Mark Pallen[3], and Ulrich Eckhard[1,*]

[1]*Synthetic Structural Biology Group, Department of Structural and Molecular Biology, Molecular Biology Institute of Barcelona (IBMB-CSIC), Barcelona, Spain.*
[2]*Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, Barcelona, Spain.*
[3]*Quadram Institute, Norwich Research Park, Norwich, Norfolk, England, UK.*

Bacterial flagella are highly sophisticated organelles that have primarily evolved for motility. However, the discovery of flagellinolysins [1], naturally occurring proteolytic flagellins, provides a compelling evolutionary “proof of concept” for the functionalization of microbial surfaces. Inspired by these natural strategies, our lab employs structure-driven synthetic microbiology and computational protein design to repurpose these ubiquitous appendages into programmable nanomachines for plant biotechnology.

While our previous work established the feasibility of displaying active enzymes and inhibitors on *Escherichia coli*, we are now expanding this platform to well-characterized plant-growth-promoting bacteria, specifically *Pseudomonas protegens*. By leveraging state-of-the-art tools such as AlphaFold3 [2] and RFdiffusion [3], we are engineering these bacteria to display *de novo* designed protein binders and inhibitors that directly target the pathogenicity factors of devastating plant pathogens, such as *Ralstonia solanacearum*.

Likewise, we envision the incorporation of enzymatic domains such as ACC deaminase and phytases that help plants to handle environmental stresses such as drought or phosphate deficiency. In parallel, we are aiming to expand to outer membrane vesicles, inspired by a recently identified family of outer membrane proteins (OMPs) that mirrors the structural- vs-proteolytic duality seen in flagellins.

Importantly, by precisely controlling the display of functional domains on both flagella and OMPs, we aim to transition from “learning from nature” to providing synthetic solutions for sustainable agriculture, with the long-term goal to establish a modular surface-display platform capable of delivering enzymatic cascades and biocontrol agents directly at the site of infection or stress, and ultimately, to enhance crop resilience and food security.

Acknowledgements

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ST2. Evolution-guided remodeling of ABA receptors enables engineering of hormone perception

M. Rivera-Moreno[1], M. Bono[2], Lourdes Infantes[1], Pedro Luis Rodríguez[2] and Armando Albert[1]

[1]*Instituto de Química-Física Blas Cabrera (IQF-CSIC), Madrid (Spain)*
[2]*Instituto de Biología Molecular y Celular de Plantas (IBMCP), Valencia (Spain)*

Drought increasingly limits crop productivity, highlighting the need to understand how plants evolved to sense and respond to water stress. Central to this process is abscisic acid (ABA), perceived by PYR/PYL/RCAR receptors that regulate PP2C phosphatases and downstream stress responses. While this signaling pathway is conserved, how receptor architecture evolved to tune ABA dependence and sensitivity remains unclear.

Here, we combine structural, biochemical, and evolutionary analyses across algal, bryophyte, and angiosperm receptors to define a minimal molecular code underlying ABA perception. We identify a five-residue signature in which three leucines stabilize a closed, ligand-independent conformation characteristic of ancestral receptors, while two interface residues control oligomerization, switching between dimeric and monomeric states and thereby modulating ABA affinity. Structure-guided substitutions interconvert these functional states, recapitulating evolutionary transitions.

Functional analyses reveal that monomeric, high-affinity receptors enable responses to low ABA levels, whereas dimeric, lower-affinity receptors sustain signaling under high ABA conditions, expanding the system's dynamic range. Together, these results establish a structural and evolutionary framework linking receptor architecture to functional adaptation and provide design principles for engineering ABA signaling to enhance drought resilience.

Acknowledgements

Agencia Estatal de Investigación of the Spanish Ministry of Science and Innovation with grant number PID2023-153108OB-I00 funded by MCIN/AEI/10.13039/501100011033 and the ERDF ("A way of making Europe") (A. A.)

Reference

Evolutionary-based Remodeling of ABA Receptors Reveals the Structural Basis of Hormone Perception and Regulation. Maria Rivera-Moreno, Mar Bono, Lourdes Infantes, Pedro L. Rodriguez and Armando Albert, PNAS, 2026 (https://doi.org/10.1073/pnas.2534140123)

ST3. Evolution of molecular determinants for SUMO-activating enzyme subcellular localization in plants

Abraham Más[1], Laura Castaño-Miquel[1], Jordi Pérez-Gil[1], Elena Mellado-Ortega[1,6], Anna Solé[1], Lucia Pirone[1,5], Alex Guillamon[1], Lorenzo Carretero-Paulet[3], Núria Colomé[4], Francesc Canals[4], Lucia Strader[6] and L. Maria Lois[1,2]*

[1]*Center for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, Barcelona, Spain.*
[2]*Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain*
[3]*Department of Biology and Geology. University of Almería. Ctra. Sacramento s/n 04120 Almería, Spain.*
[4]*Vall d´Hebron Institute of Oncology (VHIO), Barcelona, Spain.*
[5]*Present Address: Council for Agricultural Research and Economics, Research*
[6]*Center for Plant Protection and Certification, Rome, Italy. Duke University, Durham, North Carolina, United States*

Post-translational modification by Small Ubiquitin-related Modifier (SUMO) is an essential regulatory mechanism in eukaryotes. In the cell, SUMO conjugates are highly enriched in the nucleus, consistently with a preferential nuclear localization of the SUMOylation machinery components. In this scenario, a major unresolved issue is the mechanism enabling SUMO conjugation of cytosolic targets. We will present the identification of a non-canonical nuclear localization signal (NLS2) in the SUMO activating enzyme subunit SAE2 and evidence for its role in regulating subcellular SUMO conjugation. Our findings point to a possible evolutionary contribution of cytosolic SUMOylation to the emergence of seeds and represents a novel regulatory trait that could be exploited in precision breeding programs to improve seed performance.

Acknowledgements

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ST4. Molecular plant-microbe interactions: from evolutionary insights to immune receptor engineering

Isabel Monte

ZMBP, University of Tuebingen, Germany

Plants and microbes have co-evolved for hundreds of millions of years establishing interactions that range from pathogenic to mutualistic. The characterization of molecular-plant microbe interactions (MPMI) in flowering plants has shaped our understanding of plant immunity. However, we still do not know to which extent immune signaling pathways are conserved or divergent in different land plant lineages. To explore the Evolution of MPMI (EvoMPMI), we have established different pathosystems involving non-seed plants and microbes that associate with both flowering and non-seed plants in nature. Exploiting natural variation and our in-house multiplexed CRISPR pipeline in the liverwort *Marchantia polymorpha*, we identified ecotypes and mutants that exhibit different resistance and susceptibility patterns to pathogenic microbes. Transcriptomic analyses revealed core immune responses that depend on receptor kinases. Through functional and molecular evolution analyses, we discovered that receptor kinases co-receptors underwent domain-specific diversification to regulate immunity or reproduction in land plants. Our results establish the basis for engineering plant immune receptors compatible with lineage-specific signaling components, facilitating immune receptor transfer across large phylogenetic distances to improve plant health.

ST5. CRISPR-mediated targeted gene Replacement at the *Arabidopsis thaliana* MIR390a locus enables artificial microRNA production and effective gene silencing

Juan-José Llorens-Gámez[1], Adriana E. Cisneros[1,2], Alberto Carbonell[1]

[1]*Instituto de Biología Molecular y Celular de Plantas (Consejo Superior de Investigaciones Científicas–Universitat Politècnica de València), 46022 Valencia, Spain*
[2] *Present address: Department of Bioengineering, University of California, San Diego, La Jolla, San Diego, CA, 92093, USA.*

Plant microRNAs (miRNAs) regulate gene expression through sequence-specific RNA degradation. Artificial miRNAs (amiRNAs) are engineered small RNAs designed to selectively silence target transcripts, serving as powerful RNA interference (RNAi) tools[1]. Typically, amiRNAs are expressed from transgenic constructs driven by strong constitutive promoters, which can lead to pleiotropic effects, including cytotoxicity and phenotypic variability. Although CRISPR/Cas technology has been widely used to disrupt endogenous MIRNA loci for loss-of-function studies[2], these loci have not yet been engineered to express amiRNAs.

Here, we edited the Arabidopsis thaliana MIR390a locus using CRISPR/Cas9-mediated targeted gene replacement. This was achieved using an all-in-one construct containing a YAO-promoter-driven Cas9 cassette[3], a single guide RNA targeting the MIR390a locus, and a donor DNA template encoding the corresponding amiRNA/amiRNA* sequences. The native miR390a/miR390a* duplex was replaced with amiR-CH42/amiR-CH42* or amiR-FT/amiR-FT*, targeting CHLORINA 42 (AtCH42) and FLOWERING LOCUS T (AtFT), respectively. Edited amiR-CH42 plants exhibited strong bleaching, while amiR-FT plants displayed significantly delayed flowering. Molecular analyses confirmed high amiRNA accumulation, strong downregulation of target mRNAs and precise editing of the MIR390a locus.

In conclusion, our results demonstrate that endogenous MIRNA loci can be precisely reprogrammed to express amiRNAs for selective gene silencing, providing a novel RNAi strategy for functional genomics and crop biotechnology.

Acknowledgements
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ST6. From algae to angiosperms: evolutionary strategies of plant adaptation to combined heat and drought stress

Mónica Meijón[1,2], Ana Álvarez[1], Sara Guerrero[1], Cristina López-Hidalgo[1], María Jesús Cañal[1,2] and Luis Valledor[1,2]

[1]*Área de Fisiología Vegetal, Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo;* [2]*Instituto de Biotecnología de Asturias, Universidad de Oviedo*

Climate change is increasing the co-occurrence of heatwaves and droughts, posing a major threat to plant survival and ecosystem stability. While physiological responses to these stresses are well documented, their underlying molecular mechanisms remain less understood. Here, we adopt an evolutionary and integrative framework to investigate plant responses to combined heat and drought stress across the green plant lineage. In this study, multi-omics datasets (proteomic, metabolomic, biochemical, and physiological) were integrated across five phylogenetically distant species: *Chlamydomonas reinhardtii*, *Marchantia polymorpha*, *Pinus pinaster*, *Arabidopsis thaliana*, and *Solanum lycopersicum*. The preliminary comparative framework spans key evolutionary transitions, from unicellular algae to early land plants, gymnosperms, and angiosperms. Our results reveal both conserved and lineage-specific stress-response strategies. Core responses include photosynthesis machine preservation, accumulation of osmoprotective metabolites (e.g., sugars, amino acids), and activation of redox and proteostasis networks. However, distinct adaptive strategies emerged: stress avoidance in algae, tolerance in bryophytes, endurance in gymnosperms, and dynamic acclimation or escape strategies in angiosperms. Overall, these patterns suggest an evolutionary shift from generalized metabolic repression toward increasingly refined regulatory mechanisms that balance stress mitigation and growth. This integrative analysis identifies conserved molecular modules and candidate master regulators of stress resilience, providing a foundation for predictive models and the development of climate-resilient crops.

Acknowledgements

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Posters

P1. Studying programmable neotelomere formation in *Physcomitrium patens* at chromosome ends.

Mari-Carmona, C., Petrova, V., Lobkis, E., Rudolf, G., Bacelis Bacchi, G., Zurbriggen M., Urquiza U.

Institute of Synthetic Biology, Heinrich-Heine-Universität, Düsseldorf, Germany;
Cluster of Excellence on Plant Sciences, Heinrich-Heine-Universität, Düsseldorf, Germany.

Telomeres are essential for chromosome stability and genome integrity in all eukaryotes. While telomere maintenance has been extensively studied, the mechanisms underlying de novo telomere establishment at newly generated chromosome ends remain poorly understood in plants. Here, we present the development of a synthetic cassette designed to study programmable neotelomere formation in the moss *Physcomimtrium patens*.

The system is based on a CRISPR-Cas12a-mediated strategy to expose telomeric repeat sequences at defined genomic locations. The synthetic cassette contains telomeric seed repeats flanking Cas12a target sites together with an mVenus fluorescent reporter. Following co-transformation with Cas12a and guide RNAs, targeted cleavage releases the telomeric repeat sequences, thereby generating exposed chromosome ends that may serve as substrates for de novo telomere formation and elongation by endogenous telomerase activity.

Loss of mVenus fluorescence provides a rapid visual readout for successful cassette excision and Cas12a activity. By integrating the construct at chromosome ends, this platform enables the controlled study of telomere seeding and neotelomere establishment in plants. As *P. patens* combines efficient homologous recombination with conserved telomere biology, this system offers a versatile framework to investigate chromosome-end stabilization mechanisms and synthetic telomere engineering in plants.

P2. Next-generation RNAi-based fungicides for crop protection using artificial small RNAs delivered by viral vectors

A.H. Tomassi and A. Carbonell

Instituto de Biología Molecular y Celular de Plantas (IBMCP, Consejo Superior de Investigaciones Científicas – Universitat Politècnica de València), Valencia, Spain.

Plants are severely threatened by fungal pathogens, which cause major crop and post-harvest losses worldwide. Current control strategies rely heavily on chemical fungicides; however, their extensive use under global warming conditions accelerates the emergence of resistant strains and raises environmental concerns. RNA interference (RNAi) has gained considerable attention for antifungal control, particularly through exogenousa pplications of double-stranded RNA. However, Dicer-mediated processing generates heterogeneous small RNA (sRNA) populations, limiting precise control over size and sequence specificity. Artificial sRNAs (art-sRNAs) provide a highly specific alternative but have so far been largely restricted to transgenic systems. Recently, transgene-free expression of art-sRNAs using viral vectors applied as crude extracts has been developed for endogenous gene silencing and antiviral resistance[1-3].

Here, we adapt and validate these methodologies for a new application: antifungal plant protection. Using *Botrytis cinerea* as a model, art-sRNAs targeting *BcDCL1*, *BcDCL2*[4], *BcERG11*[5], and *BcVPS51*[6] were computationally designed based on conserved sequences across 43 natural strains. Artificial microRNA (amiRNA) constructs were generated and initially evaluated by agroinfiltration in *Nicotiana benthamiana*, where no antifungal effect was observed. To address this limitation, we implemented an alternative screening strategy based on the exogenous application of total RNA extracted from plants expressing anti-*BcERG11* amiRNAs. This approach revealed that the mixture of amiRNAs targeting *BcERG11* reduces fungal growth in planta. Ongoing work aims to identify the most effective amiRNAs and incorporate them into viral vectors for GMO-free delivery as a new generation of RNA-based fungicides.

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P3. Evolving specificity: E3 ligases as drivers of stress adaptation.

Damian da Silva[1], Jeremy T. Boyle[2] and Beatriz Orosa-Puente[1,2]

[1]*Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CiQUS), Universidade de Santiago de Compostela, Spain.*
[2]*Institute of Molecular Plant Sciences (IMPS), Schools of Biological Sciences, University of Edinburgh, UK.*

Adaptive responses to environmental and developmental challenges require regulatory systems capable of rapidly and reversibly modulating cellular pathways. In plants, ubiquitin signalling plays a central role in this process, with E3 ubiquitin ligases determining substrate specificity and representing one of the largest and most functionally diverse gene families. The evolutionary versatility of E3 ligases is closely linked to their modular domain architecture. Substrate recognition domains, which typically recognize short linear peptide motifs, often display low sequence conservation and diversify through recurrent duplication, and domain rearrangements. This modularity provides a mechanistic basis for the extensive expansion and functional diversification of E3 families across plant evolution.

Here, we investigated the evolutionary dynamics of E3 ubiquitin ligase repertoires spanning from early diverging land plants to angiosperms, including domesticated species. Using a domain aware comparative framework, we systematically characterized E3 ligases in terms of abundance, subfamily composition, and proportional representation within proteomes. Our analyses reveal widespread expansion and lineage specific diversification of E3 families throughout plant evolution, with distinct subfamilies exhibiting differential retention and amplification patterns across lineages. These trends are consistent with lineage specific selective pressures and the emergence of specialized regulatory functions associated with environmental adaptation and developmental complexity.

Together, these findings support a model in which E3 ligase diversification contributed to the evolutionary rewiring of regulatory networks by expanding substrate recognition capacity and enhancing the adaptive plasticity of ubiquitin mediated signalling in plants.

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P4. The microtubule-associated protein MAP65-1 is involved in the response and acclimation to heat stress.

D. Herencias-Mateos, E. Hoyas-Sánchez, M. Saez-Díaz, J.C. Montesinos

Instituto Universitario de Biotecnología y Biomedicina (BIOTECMED), Departamento de Bioquímica y Biología Molecular, Universitat de València, 46100 Burjassot, Spain.

As global temperatures rise, understanding the mechanisms underlying plant adaptation to heat stress becomes increasingly relevant from both an evolutionary and biotechnological perspective. In *Arabidopsis thaliana* roots, heat stress triggers rapid and reversible depolymerization of cortical microtubules. Pharmacological stabilization with Taxol exacerbates heat-induced root growth inhibition, indicating that microtubule remodeling is an active adaptive strategy rather than a passive consequence of cellular damage.

Heat stress also promotes the formation of spherical cortical structures consistent with liquid-liquid phase separation (LLPS), specific to thermal stress and not induced by drug-mediated microtubule depolymerization. These condensates colocalize with stress granule markers, suggesting that cytoskeletal remodeling and stress granule assembly are functionally coordinated as part of the same adaptive response.

Within this framework, we investigate the role of MAP65-1, a microtubule-bundling protein, in the heat-stress response. MAP65-1 localizes to heat-induced cortical condensates, suggesting it operates within these compartments during stress. The *map65-1-2* loss-of-function mutant shows altered microtubule dynamics and enhanced sensitivity to Taxol during recovery, resulting in greater root growth inhibition than wild-type plants. Importantly, MAP65-1 function appears particularly critical during post-stress recovery, positioning it as a promising target for engineering thermotolerance in crops, where recovery efficiency is as important as the immediate stress response.

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P5. Plasma membrane signaling couples heat perception to microtubule remodeling as an adaptive strategy in *Arabidopsis*

M. Sáez-Díaz[1], E. Hoyas-Sánchez[1], D. Herencias-Mateos[1], J.C. Montesinos[1]*

[1]Instituto Universitario de Biotecnología y Biomedicina (BIOTECMED), Departamento de Bioquímica y Biología Molecular, Universitat de València, 46100 Burjassot, Spain.

The ability of plants to sense and respond to heat stress is a critical evolutionary trait, particularly in the context of climate change. In *Arabidopsis thaliana* root epidermal cells, heat stress triggers a rapid and reversible reorganization of the cortical microtubule cytoskeleton, including depolymerization and inhibition of root growth. This response is an active adaptive process, as pharmacological stabilization of microtubules with Taxol impairs proper acclimation, highlighting the importance of cytoskeletal remodeling as an evolved adaptive strategy.

However, the upstream signaling events that connect heat perception at the plasma membrane to cytoskeletal remodeling remain poorly understood. Using a combination of genetic, pharmacological, and live-cell imaging approaches, our results support a role for phospholipase D**δ** (PLD**δ**) as an upstream regulator that promotes cortical microtubule depolymerization upon heat stress. In parallel, we are characterizing the timing and functional contribution of heat-induced cytosolic calcium increases and their relationship with microtubule reorganization, using calcium reporter lines and specific inhibitors.

Together, these findings support a model in which plasma membrane-associated signaling, involving PLD**δ** and calcium-dependent pathways, couples heat perception to rapid cytoskeletal remodeling. Deciphering these upstream regulatory mechanisms opens new avenues for the rational design of thermotolerant crops through targeted modulation of stress signaling pathways.

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P6. Key root parameters in the development of pepper hybrids as rootstocks under low phosphorus conditions

M. Diez-Diaz[1], MJ. Perez-Cordoba[2], A. de Luis[1], A Rodriguez-Burruezo[2], A. Fita[2]

[1]*Departamento de Biotecnología, Universidad Católica de Valencia San Vicente Mártir, Spain.*
[2]*Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universidad Politécnica de Valencia, Spain.*

Agriculture faces the challenge of increasing crop yields in a complex scenario, with changing environmental conditions but also with possible shortage of raw materials for fertilizers such as phosphate rock. Recently, some pepper (Capsicum annuum L.) genotypes have been identified as having better performance under low phosphorous conditions. In this study, different hybrids bred for better root characteristics and greater P acquisition under conditions of low P supply have been tested as rootstocks on two commercial varieties. The analysis of their performance has been carried out by studying yield, biomass, mineral content, as well as root parameters such as length, weight, diameters and root branching among others. Among the hybrids, we have found good candidates as rootstocks in conditions of low P supply. On the other hand, in some cases we have found some incompatibility, even when using the variety to be grafted as the parent of the hybrids. Root parameters such as branching angle and length of secondary roots are confirmed here as key breeding targets for plant growth under conditions of low phosphorus supply. Therefore, the design of hybrids with these root characteristics is a good option for obtaining rootstocks tolerant to low P input environment.

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P7. Enhanced microRNA accumulation and gene silencing efficiency through optimized precursor base pairing

Juan-Jose Llorens-Gamez, Pedro Jose Garcıa-Cano, Sara Rico-Rodrigo, Lucıa Duyos-Casanova, Sara Toledano-Franco and Alberto Carbonell

Instituto de Biologıa Molecular y Celular de Plantas (Consejo Superior de Investigaciones Cientıficas–Universitat Politecnica de Valencia), 46022, Valencia, Spain

MicroRNAs (miRNAs) are endogenous 21-nucleotide small RNAs that direct sequence-specific silencing of complementary messenger RNAs to regulate a wide range of biological processes. In plants, miRNA precursors are processed from imperfect foldback structures by the RNase III enzyme DICER-LIKE[1], in coordination with accessory proteins. While mismatches flanking the miRNA/miRNA* duplex in endogenous precursors can strongly influence miRNA accumulation[1], their impact has not been thoroughly examined in the context of artificial miRNAs (amiRNAs) used for targeted gene silencing in plants.

Here, using silencing sensor systems in *Nicotiana benthamiana*, we systematically investigated how base pairing at or near DCL1 cleavage sites affects amiRNA production from the recently described minimal *shc* precursor[2]. Independent pairing of naturally mismatched positions revealed that introducing a G–C pair immediately upstream of the mature amiRNA remarkably enhances amiRNA accumulation and silencing efficiency. This effect was further validated in Arabidopsis transgenic lines targeting endogenous genes and confirmed by deep sequencing, which revealed highly accurate processing and predominant release of the intended amiRNAs, supporting the specificity of the approach. Our findings show that a single structural modification in an amiRNA precursor can significantly enhance the efficacy of amiRNA-mediated gene silencing. This optimized amiRNA platform is well suited for large-scale functional genomics screens and should facilitate the development of next-generation crops with enhanced resilience to environmental stresses.

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P8. Symbiosis with *Penicillium* sp. D7 rewires molecular modules to boost plant growth and As(III) stress tolerance.

E.A. Rodríguez-Dobreva[1], J. Vicente-Carbajosa[1,2] and S. Pollmann[1,2]

[1]*Centro de Biotecnología y Genómica de Plantas (UPM-INIA/CSIC), Spain.*
[2]*Departamento de Biotecnología-Biología Vegetal, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Spain*

Arsenic contamination severely compromises plant growth and agricultural productivity, particularly in environments affected by mining or industrial activities. Microbial symbionts adapted to metalliferous soils represent a promising yet underexploited resource for enhancing plant tolerance to As toxicity. Here, we isolated fungal endophytes from plants thriving on As-rich tailings of the Mónica mine (Bustaviejo, Spain) and identified *Penicillium* sp. D7 as an As-tolerant symbiont with pronounced plant growth–promoting effects. When co-cultivated with Arabidopsis, D7 significantly enhanced shoot biomass under As(III) stress and mitigated characteristic toxicity symptoms. Transcriptome profiling revealed that D7 reprograms plant hormone signaling of the host in an organ-specific manner: the fungus suppresses ABA biosynthesis and ABA-responsive stress pathways in roots, while simultaneously inducing auxin biosynthesis and signaling in shoots. Moreover, D7 elicited the coordinated induction of key components of the plant's intrinsic As detoxification machinery, including *ARQ1*, *PCS1*, and the transporters *ABCC1* and *ABCC2*. Functional analyses using the *abcc1 abcc2* and the *cad1-3* mutants demonstrated that vacuolar sequestration of As(III) in roots is essential for D7-mediated stress tolerance, indicating that fungal colonization enhances the host's capacity for intracellular detoxification rather than reducing As uptake. Together, these results demonstrate that D7 reinforces plant resilience to As(III) by simultaneously attenuating stress perception, promoting growth-related hormonal programs, and stimulating vacuolar sequestration of As. This study highlights D7 as a promising tool for supporting vegetation establishment and phytostabilization efforts in As-contaminated environments.

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P9. Evolution of plant adaptation to vegetation proximity: balancing growth and senescence

Alexa Aguilar-Acevedo, Ángela Sanchez-Garcia, and Jaume F Martínez-Garcia

[1] *Institute for Plant Molecular and Cell Biology (IBMCP), CSIC-UPV, 46022 - València, Spain.*

Vegetation proximity can limit the light available for plant photosynthesis. To adapt, plants use two divergent strategies: shade avoidance and shade tolerance. Our research compares two closely related species: *Arabidopsis thaliana*, which avoids shade by elongating its hypocotyls, and *Cardamine hirsuta*, which tolerates it and does not elongate under shade. These differences arise from variations in the activity of components involved in light perception and/or signaling (1). Another key difference between these two strategies is the timing of dark-induced senescence (DIS), a process that occurs significantly faster in *A. thaliana* than in *C. hirsuta*. This suggests that delayed senescence helps *C. hirsuta* to better survive in shaded environments. The molecular mechanisms behind this delay in *C. hirsuta* remain unknown. In *A. thaliana*, DIS is induced by PHYTOCHROME INTERACTING FACTOR proteins and the abscisic acid and ethylene hormones, which activate the expression of the transcription factor *ORESARA1* (*ORE1*) to promote senescence. Our comparative studies reveal differences in *ORE1* expression and in these hormone responses between the two species. These findings highlight how evolution has shaped alternative strategies for plants to balance growth and survival under shade. Our communication will share our latest results.

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P10. DELLA proteins modulate responsive and buffered transcriptional programs in Arabidopsis and rice roots under water limitation

C. Wong, D. Alabadí, M. A. Blázquez, B. Catarino*

Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), 46022-Valencia, Spain

Drought is a major environmental stress that impedes the growth and productivity of crops (1). However, the molecular mechanisms that govern the root responses during water stress remains understudied. The phytohormone Gibberellins (GAs) and its repressors, the DELLA proteins, are key regulators of plant growth (2, 3), but the dynamic role of DELLAs in shaping the transcriptional responses imposed by water limitation, and the extent to which the functions of DELLAs are conserved across angiosperms, are unknown. Here, we examined the regulatory roles of DELLAs in the roots of *Arabidopsis thaliana* and *Oryza sativa* during osmotic stress simulated by polyethylene glycol (PEG). Our phenotypic data demonstrate that loss of functional DELLAs decreases sensitivity to PEG-induced root growth inhibition in both species. Transcriptional profiling further identified two distinct regulatory mechanisms: (i) "WT-responsive" genes, which require DELLAs for proper stress response, and (ii) "DELLA-buffered" genes, whose responsiveness is suppresed by DELLAs. Comparative analyses furthered revealed that although DELLA function is conserved, downstream transcriptional programs are largely species-specific. These findings suggest that DELLAs act as dual-purpose regulators – both enabling specific PEG-induced stress pathways and constraining broader transcriptional changes under PEG-induced water limitation.

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P11. Model-assisted CRISPR editing for precise modification of *cis*-regulatory elements

E.G. Minguet[1, †,*], J.C. Pérez-Martínez[1,†], I. Rodrigo-Puerto[1], M. López-Gisbert[1], J. Muñoz-Bertomeu[1]

[1]*Departament de Biologia Vegetal, Universitat de València, Burjassot, Spain.*
[†]*These autors have contributed equally.* *Corresponding author*

An important source of evolutionary change are mutations affecting gene expression, which are sufficient to cause dramatic phenotypic variation (1). Cis-regulatory elements (CREs) are noncoding DNA sequences with binding sites for transcription factors (TFs) or other regulatory molecules that affect transcription. Mutations in CREs have significantly influenced crop domestication by reshaping the transcriptome (2, 3), with nearly half of domestication-related mutations found in CREs (4); however, the small size of CRE has hampered precise directed mutagenesis to selectively alter TF binding sites.

We propose the use of accurate predictive models of DNA binding and CRISPR-based genome editing as an efficient strategy for targeted CRE modification. As a proof of concept, we used LEAFY, a key regulator of reproductive development (5-7). We identified candidate CREs in several LEAFY target genes and designed guide RNAs for genome editing. We used MORPHEUS (8) to predict LEAFY binding affinity for the edited alleles. Predictions suggested a spectrum of effects, from negligible changes in binding affinity to a near-complete loss of LEAFY recognition at the edited CRE. The possibility of modulating specific regulatory nodes to fine-tune gene expression offers a precise and promising approach to rewire transcriptional regulatory networks.

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P12. A multiplexed visual reporting system for the identification of virus-mediated gene editing in seeds.

Fernando Merwaiss, Verónica Aragonés and José-Antonio Daròs.

Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas – Universitat Politècnica de València, Valencia, Spain

Virus-induced gene editing (VIGE) has emerged as a powerful, tissue-culture-independent alternative for plant genome engineering, offering the potential to bypass time-consuming transformation protocols. The delivery of CRISPR/Cas components via viral vectors directly to the germline is particularly relevant, as it enables the rapid production of edited progeny in a single generation. However, because plant germline cells possess innate mechanisms to restrict viral replication and transmission, obtaining seeds that carry the desired edits remains a significant challenge. This study addressed this limitation by developing a high-throughput visual screening system to identify edited events in Nicotiana benthamiana and Solanum lycopersicum (cv. Micro-Tom). To facilitate the identification of edited progeny without compromising plant fitness, a strategy involving transgenic lines constitutively expressing the fluorescent protein dsRED was implemented. By delivering guide RNAs (gRNAs) targeting the dsRED transgene using viral vectors, we successfully achieved the loss of fluorescence in reproductive tissues. This allowed for the non-destructive, qualitative analysis and pre-selection of edited seeds under a stereomicroscope. Furthermore, a multiplexing strategy was established where a second gRNA, delivered by the same viral vector, targeted a gene of interest. The findings confirmed that the loss of dsRED fluorescence is a highly reliable predictor of concurrent editing at the target locus. This dual-marker approach provides a robust and scalable tool for the rapid identification of edited T1 progeny, which could be used to study how to overcome biological barriers of viral exclusion in the germline.

P13. Functional conservation of FRUITFULL in regulating flowering duration and its potential to enhance yield in legumes.

Irene Martínez-Fernández[1], Chloe Fourquin[1], Ana Berbel[1], Vicente Balanzà[1], Donna Lindsay[2], Tom Warkentin[2], Francisco Madueño[1], Cristina Ferrándiz[1].

[1]*Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas—Universidad Politécnica de Valencia, 46022, Valencia, Spain.*
[2]*Department of Plant Sciences, College of Agriculture and Bio-Resources, University of Saskatchewan, Saskatoon, SK S7N5A8, Canada.*

Legumes are valuable crops with remarkable sustainability traits. Among them, they improve soil fertility through biological nitrogen fixation and are a major source of high-quality plant protein for human consumption, as well as for animal feed. Legumes are also a basic component of the mediterranean diet, and a wealth of landraces are available, which constitute a huge reservoir of biodiversity still to be fully exploited. However, the legumes are not the crop of choice for most farmers because of reasons such as their low and unstable yield. Some factors that limit legume yield are biotic and abiotic stresses or a low effort in plant breeding. Therefore, it is necessary to generate knowledge that could support the development of more resilient varieties. We are studying the function and the biotechnological potential of pea (*Pisum sativum*) *PsFUL* genes, key controllers of the length of the reproductive phase[1]. Loss-of-function mutants in *PsFUL* genes show an extended reproductive phase, with many more flowering nodes produced before inflorescence growth gets arrested, meaning a significant increase in pod/seed production per plant, which leads to up to 60-80% more yield, with no observable changes in seed composition and quality[2].

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P14. Plant BCL-DOMAIN HOMOLOG proteins play a conserved role in SWI/SNF complex stability

Joan Candela-Ferre[1], Jaime Pérez-Alemany[1], Borja Diego-Martin[1], Vijaya Pandey[2], James Wohlschlegel[2], Jorge Lozano-Juste [1], Javier Gallego-Bartolomé[1]

[1]Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), Valencia, Spain. [2]David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA.
Corresponding author: Javier Gallego Bartolomé (jagalbar@ibmcp.upv.es)

SWI/SNF complexes are conserved chromatin remodelers essential for nuclear functions. In plants, BDH proteins were recently identified as shared subunits of all SWI/SNF complexes, playing a key role in chromatin accessibility and development. In this study, we performed a comprehensive characterization of *bdh* mutants, revealing the role of BDH in hypocotyl cell elongation. Additionally, we identified a plant-specific N-terminal domain that facilitates the interaction between BDH and the rest of the complex. Additionally, we uncovered the critical role of the BDH **β**-hairpin domain, which is phylogenetically related to mammalian BCL7 SWI/SNF subunits. While phylogenetic analyses did not identify BDH/BCL7 orthologs in fungi, structure prediction modeling demonstrated strong similarities between the SWI/SNF catalytic modules of plants, animals, and fungi and revealed the yeast Rtt102 protein as a structural homolog of BDH and BCL7. This finding is supported by the ability of Rtt102 to partially rescue the *bdh* mutant. Further experiments revealed that BDH promotes the stability of the ARP4-ARP7 heterodimer, leading to the partial destabilization of ARP4 in the SWI/SNF complexes.

P15. Exploiting circadian timing for crop defense: insights from GIGANTEA function

Rosa Salazar-Vergara^, Ane Umerez-Chotro^, Juan M. Acien-Luque[a], Clara Inés Ortiz-Ramírez, Laetitia Poidevin, María A. Nohales*

Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas - Universidad Politécnica de Valencia, 46022 Valencia, Spain;
[a]*Present address: Departament de Biologia, Bioquímica i Ciències Naturals, Universitat Jaume I, 12071 Castelló de la Plana, Spain*

^ Equal contribution. *Corresponding author: manozaf@ibmcp.upv.es

The circadian clock synchronizes plant physiology with daily environmental cycles, enhancing adaptation and resilience under changing climates (1). Although known to influence stress responses, the molecular links between clock components and immune pathways remain unclear. Here, we identify a direct mechanistic link between the circadian oscillator and plant immunity. We demonstrate that the clock protein GIGANTEA (GI) modulates defense responses against pathogens by acting on the jasmonic acid (JA) signaling pathway. GI physically interacts with key transcriptional regulators in this pathway, regulating their activity and fine-tuning JA-responsive gene expression at dawn. This temporal gating is required to properly phase defense outputs across the day, resulting in enhanced resistance to fungal pathogens during early morning hours.
Our findings reveal a mechanistic basis for the circadian control of biotic stress responses and highlight the adaptive value of temporal regulation in plants. To assess the translational potential of this mechanism, we are investigating its conservation in tomato (*Solanum lycopersicum*). We have established a Virus-Induced Gene Silencing (VIGS) system and generated CRISPR-Cas9 *gi* mutants to dissect *GI* function in this crop. Characterizing GI-dependent regulatory networks will support the development of strategies to exploit circadian regulation for improved crop performance (2,3).

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P16. Harnessing coding-sequence-encoded regulation to engineer spatial gene expression in crops

Raúl Denia-Mondéjar[1], Paula Brunot-Garau[1], Antonio Serrano-Mislata[1], María Dolores Gómez[1], Christian S. Hardtke[2] and Noel Blanco-Touriñán[1,*]

[1]Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas – Universitat Politècnica de València, 46022, Valencia, Spain.
[2]Department of Plant Molecular Biology, Biophore, UNIL-Sorge, University of Lausanne, Lausanne, Switzerland.

Precise quantitative and spatial control of gene expression underpins plant development, yet how such patterns are encoded within gene sequences remains poorly understood. Brassinosteroid (BR) signaling provides a paradigmatic system to address this question, as its tissue-specific manipulation can enhance crop performance while avoiding the detrimental effects of systemic misregulation[1]. In Arabidopsis roots, BR signaling operates in a context-dependent manner, activating distinct programs in different cell types[2]. These responses depend on cell-autonomous perception enabled by ubiquitous expression of the receptor BRI1, but the regulatory basis of this expression pattern is unclear[3,4].

Here, we show that the BRI1 coding sequence itself encodes regulatory information required for its transcription across tissues. Recoding disrupts uniform transcript accumulation under tissue-specific promoters, demonstrating that regulatory elements reside within the gene body. We identify a conserved exonic enhancer that acts in synergy with promoter activity to sustain transcription.

Genome-wide analyses in *Arabidopsis* reveal that exonic enhancers are widespread, non-randomly distributed, and associated with specific transcriptional networks. A meta-analysis integrating these *Arabidopsis* datasets with exonic regulatory activity identified by STARR-seq in rice suggests a certain degree of conservation, although this activity may be more lineage-specific. These findings position coding sequences as an additional regulatory layer that warrants investigation across diverse species, and they provide a conceptual framework for engineering spatial gene expression in crops.

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P17. Environmental control of vascular development in early land plants

Xulia Noya-Falcón#, Paula Brunot-Garau# and Noel Blanco-Touriñán*

Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas – Universitat Politècnica de València, 46022, Valencia, Spain.
#Co-first authors

Wood, the largest terrestrial carbon reservoir, is primarily composed of xylem tissue derived from the (pro)cambium, the precursor of vascular tissues. In angiosperms such as *Arabidopsis thaliana*, xylem development in roots, hypocotyls, stems, and leaf vasculature is highly responsive to environmental cues[1]. For instance, in *Arabidopsis* roots, which branch through lateral root formation, salinity stress can induce discontinuities in the xylem, potentially limiting sodium transport, whereas osmotic stress promotes premature xylem differentiation.
Early land plants exhibited dichotomous root branching[2], meaning that a single root axis splits into two equivalent branches. This type of branching is retained in lycophytes such as *Selaginella moellendorffii* and *Selaginella apoda*, which also possess xylem tissue. However, how environmental factors influence vascular development and dichotomous branching in these species remains largely unknown.
Here, we aim to provide new insights into how environmental regulation and developmental plasticity operate across different root branching systems. Understanding these mechanisms may ultimately inform novel strategies for plant biotechnology.

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P18. Natural variation reveals candidate regulators of dichotomous branching in *Marchantia polymorpha*

Joan Jordà-Segura [1], Maxime Bonhomme [2], Pierre-Marc Delaux [2], Javier Agustí [*1], Miguel A. Blázquez[*1].

[1]Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas - Universitat Politècnica de València, Valencia, Spain.

[2]Laboratoire de Recherche en Sciences Végétales (LRSV), Université de Toulouse, CNRS, UPS, Castanet Tolosan, France.

Maintaining meristematic activity is fundamental for plant development, yet our understanding of this process remains largely shaped by studies in vascular plants. In bryophytes such as *Marchantia polymorpha,* vegetative growth depends on the repeated duplication of apical notches, which drives dichotomous branching of the thallus. However, the cellular and genetic mechanisms controlling the timing of these events remain poorly understood. In this study, we investigate the regulation of the *M. polymorpha* plastochron, defined as the interval between successive apical notch divisions. We show that this trait is highly plastic and responds to environmental cues, including light intensity and temperature. We further demonstrate that natural variation in plastochron length provides an opportunity through genome-wide association studies. This approach is being used to uncover candidate genes controlling dichotomous branching and to place them in relation to the few regulators described so far, including MpACL5 (1) and MpCYP78E1 (2). Together, our results establish the *M. polymorpha* plastochron as an environmentally responsive and genetically structured trait. More broadly, they provide a framework to dissect how meristematic activity is regulated during the vegetative phase of a bryophyte, offering evolutionary insight into the mechanisms that shape plant body architecture across land plants.

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