At the Forefront of Plant Research 2019

BARCELONA, MAY 6-8, 2019

ABSTRACT BOOK

Scientific & Organizing Comittee

CRAG
CENTRE FOR RESEARCH IN AGRICULTURAL GENOMICS

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CONFERENCE ORGANIZATION

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WELCOME

At the Forefront of Plant Research 2019

BARCELONA, MAY 6-8, 2019

Plants are the basis of human society -food, feed, fiber, fuel, medicine-; our life depends on plants in a myriad of ways, and addressing pressing current and future societal challenges (the ever increasing demand on agricultural products and natural resources at a global scale, or food security and quality in a changing environment) will crucially depend on plant research at multiple levels. The past twenty years have witnessed a revolution in our understanding of plant biology. The aim of this conference is to present the current forefront of plant research and to reflect on the prospects and challenges for today's fundamental science and tomorrow's agriculture.

This workshop follows on the footsteps of previous meetings organized by CRAG (From model systems to crops: challenges for a new era in plan biology; Barcelona 2014) and VIB (At the forefront of Plant Research; Ghent; 2017)

We are glad to welcome you to this exciting conference in the lively city of Barcelona.

The Organizing Comittee

PROGRAM

Monday May 6th

MORNING SESSION

08:30 - 09:30	**Registration**
09:30 - 09:45	**Welcome from the organizers**
09:45 - 10:30	**Xuemei Chen.** (Chair) - UC Riverside, USA **TREX-2 and a nuclear pore protein in microRNA biogenesis in Arabidopsis**
10:30 - 11:15	**Franziska Turck.** Max Planck Institute for Plant Breeding Research (MPIPZ). Germany **A composite cis-regulatory code underpins epigenetic gene repression in plants**
11:15 - 11:45	**Coffee**
11:45 - 12:30	**Asaph Aharoni.** Weizmann Institute of Science, Israel **Hijacking GAMEs: Evolution and Domestication of the Alkaloids Pathway in the Genus Solanum**
12:30 - 13:15	**Paloma Mas.** Centre for Research in Agricultural Genomics (CRAG), Spain **Cellular and molecular mechanisms of circadian clock function in Arabidopsis thaliana**
13.15 - 14:30	**Lunch / Posters**

AFTERNOON SESSION

14:30 - 15.15	**Magnus Nordborg.** (Chair) - Gregor Mendel Institute of Molecular Plant Biology (GMI), Austria **The genetics of epigenetics**
15:15 - 16:00	**Siobhan Brady.** UC Davis, USA **Tomato Roots and their Cell Types – Turning Things Inside Out!**
16:00 - 16:30	**Coffee**
16:30 - 17:15	**Henrik Jönsson.** Sainsbury Laboratory at Cambridge University (SLCU), UK **How many stem cells can you fit in a plant shoot?**
17:15 - 18:00	**Natalia Dudareva.** Purdue University, USA **Phenylalanine Biosynthetic Network: Past, Present and Future**
18:00 - 18:45	**Holger Puchta.** Karlsruher Institut für Technologie, Germany **Using CRISPR/Cas in plants: From gene editing to gene targeting and genome engineering**

Tuesday May 7th

MORNING SESSION

09:00 - 09:45 **Zachary Lippman.** (Chair) - Cold Spring Harbor Laboratory, USA
Cryptic variation and epistasis in flower production and crop improvement

09:45 - 10:30 **Claudia Köhler.** Swedish University of Agricultural Sciences (SLU), Sweden
Domestication of transposable elements for plant reproduction

10:30 - 11:00 **Coffee**

11:00 - 11:45 **Björn Hamberger.** Michigan State University, USA
Plant diterpene metabolism: newly evolved strategies for discovery in the mint family and engineering of production

11:45 - 12:30 **Salomé Prat.** National Centre for Biotechnolgy (CNB), Spain
FLOWERING-LOCUS T: more than flowering inducing signals

12:30 - 13:15 **Jane Parker.** Max Planck Institute for Plant Breeding Research (MPIPZ), Germany
Analysis of NLR immunity signalling across plant species

13:15 - 14:30 **Lunch / Posters**

AFTERNOON SESSION

14:30 - 15:15 **Marja Timmermans.** (Chair) - Center for Plant Molecular Biology (ZMBP), Germany
Making a flat leaf: Pre-patterning, morphogenic small RNAs, and growth

15:15 - 16:00 **Yves Van de Peer.** Vlaams Instituut voor Biotechnologie (VIB), Belgium.
(Plant) Life with more than one genome

16:00 - 16:30 **Coffee**

16:30 - 17:15 **Li Li.** Cornell University, USA.
Mechanistic insights into carotenoid accumulation in plants

17:15 - 18:00 **Yrjö Helariutta**
Sainsbury Laboratory at Cambridge University (SLCU), UK
Integration of hormonal and transcriptional control during vascular development

Wednesday May 8th

MORNING SESSION

09:00 - 09:45 **Julia Bailey-Serres.** (Chair) – UC Riverside, USA
Mixed message: Making, holding, decoding and destroying mRNAs for stress resilience

09:45 - 10:30 **Doris Wagner.** University of Pennsylvania, USA
(Re)programming cell identity and function in response to developmental and environmental cues

10:30 - 11:00 **Coffee**

11:00 - 11:45 **Kenneth Birnbaum.** New York University (NYU), USA
Systemic approaches to dissect cell-cell signalling

11:45 - 12:30 **Veronica Grieneisen.** John Innes Centre (JIC), UK
Developmental Homeostasis: unearthing the multiscale mechanisms underlying optimal nutrient uptake in roots

12:30 - 13:15 **Uta Paszkowsky.** University of Cambridge, UK
Molecular genetics of arbuscular mycorrhizal symbiosis in cereals

13:15 - 13:25 **Closing remarks & Farewell**

INVITED SPEAKERS

Asaph Aharoni
Weizmann Institute of Science. Israel

Julia Bailey-Serres
UC Riverside. USA

Kenneth Birnbaum
New York University (NYU). USA

Siobhan Brady
UC Davis. USA

Xuemei Chen
UC Riverside. USA

Natalia Dudareva
Purdue University. USA

Veronica Grieneisen
John Innes Centre (JIC). UK

Björn Hamberger
Michigan State University. USA

Yrjö Helariutta
Sainsbury Laboratory at Cambridge University (SLCU). UK

Henrik Jönsson
Sainsbury Laboratory at Cambridge University (SLCU). UK

Claudia Köhler
Swedish University of Agricultural Sciences (SLU). Sweden

Li Li
Cornell University. USA

Zachary Lippman
Cold Spring Harbor Laboratory. USA

Paloma Mas
Centre for Research in Agricultural Genomics (CRAG). Spain

Magnus Nordborg
Gregor Mendel Institute of Molecular Plant Biology (GMI). Austria

Jane Parker
Max Planck Institute for Plant Breeding Research (MPIPZ). Germany

Uta Paszkowsky
University of Cambridge. UK

Salomé Prat
National Centre for Biotechnolgy (CNB). Spain

Holger Puchta
Karlsruher Institut für Technologie. Germany

Marja Timmermans
Center for Plant Molecular Biology (ZMBP). Germany

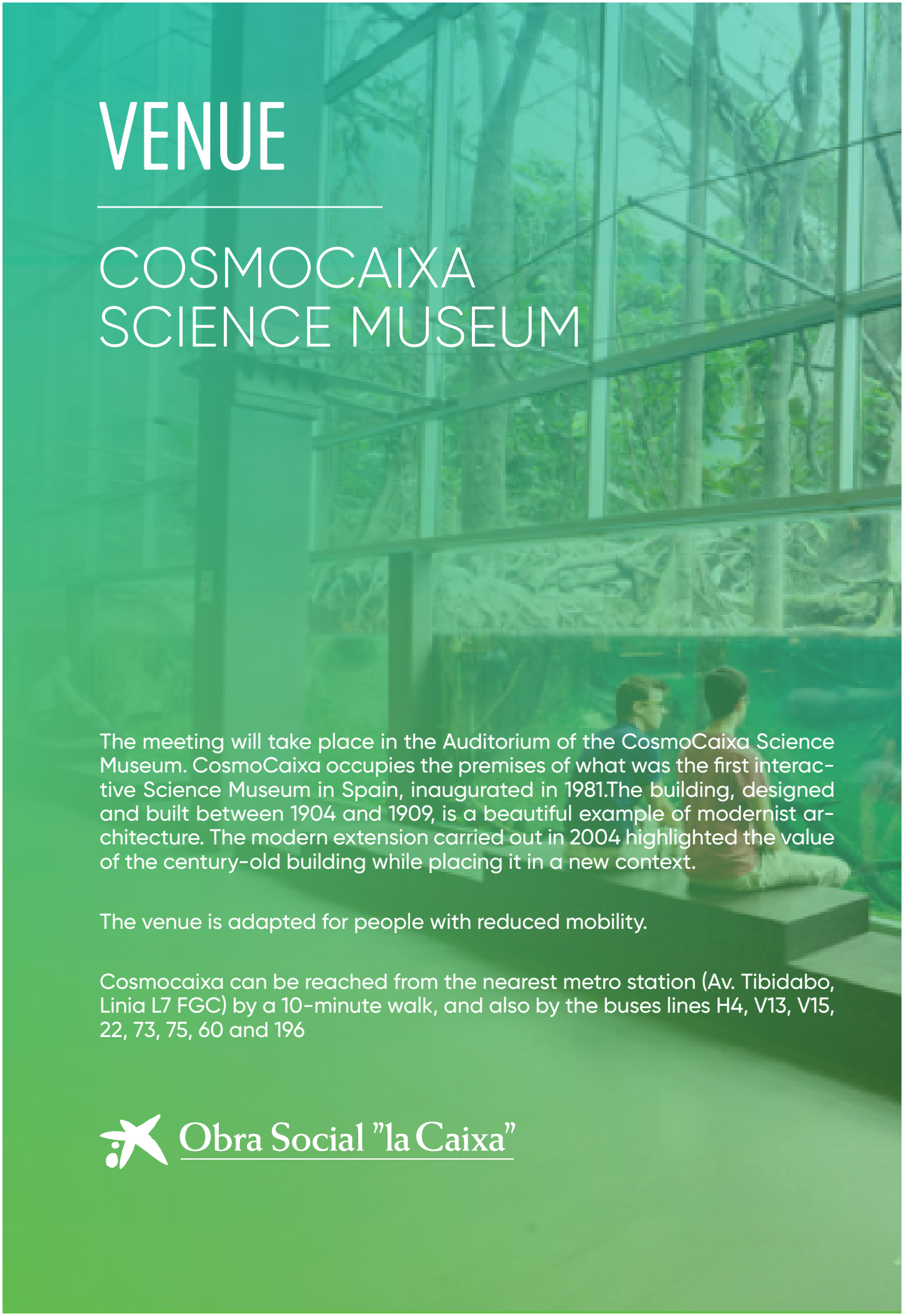
Franziska Turck
Max Planck Institute for Plant Breeding Research (MPIPZ). Germany

Yves Van de Peer
Vlaams Instituut voor Biotechnologie (VIB). Belgium

Doris Wagner
University of Pennsylvania (UPenn). USA

VENUE

COSMOCAIXA SCIENCE MUSEUM



The meeting will take place in the Auditorium of the CosmoCaixa Science Museum. CosmoCaixa occupies the premises of what was the first interactive Science Museum in Spain, inaugurated in 1981.The building, designed and built between 1904 and 1909, is a beautiful example of modernist architecture. The modern extension carried out in 2004 highlighted the value of the century-old building while placing it in a new context.

The venue is adapted for people with reduced mobility.

Cosmocaixa can be reached from the nearest metro station (Av. Tibidabo, Linia L7 FGC) by a 10-minute walk, and also by the buses lines H4, V13, V15, 22, 73, 75, 60 and 196

Obra Social "la Caixa"

TALK ABSTRACTS

Hijacking GAMEs: Evolution and Domestication of the Alkaloids Pathway in the Genus Solanum

Speaker: Asaph Aharoni
Weizmann Institute of Science. Israel

Asaph Aharoni
Department of Plant & Environmental Sciences, Faculty of Biochemistry, Weizmann Institute of Science, P.O.B. 26, Rehovot, 7610001, Israel

The Metabolomes of plant are notorious for their size and structural diversity. Secondary metabolites represent a large portion of this metabolic repertoire counting thousands in an individual plant. Generating such chemical complexity in secondary metabolism requires continues evolution of genes encoding proteins producing novel metabolites with selective advantage in a particular environmental niche. Genes with new function in secondary metabolism could arise from other genes of secondary metabolism, through gene duplication or directly through allelic variation. Yet, likely often, they arise following duplication of genes involved in primary metabolites formed across all species. In the past years we discovered a series of enzymes, regulatory and signalling proteins as well as transporters involved in steroidal GlycoAlkaloids MEtabolism (GAME) in the genus Solanum. This class of secondary metabolites represents potent defence molecules with notorious anti-nutritional activity towards humans (e.g. α-Solanine in potato). In the presentation, I will portray several different molecular mechanisms wherein genes of core, primary metabolic pathways (e.g. membrane sterols metabolism and the GABA shunt) were 'hijacked', providing a template for the evolution of new enzymatic functions in glycoalkaloid metabolism. This will be complemented by examples of chemical diversity formed following neofunctionalization of genes derived from highly related, secondary metabolism genes. Selected alleles involved in generating secondary metabolites having beneficial attributes to mankind where part of the domestication of Solanum crop plants (e.g. tomato and potato) cultivated today. The case of steroidal glycoalkaloids is most likely a common evolutionary strategy of plants by which the chemical diversity of secondary metabolism has been endlessly revised.

Mixed message: Making, holding, decoding and destroying mRNAs for stress resilience

Speaker: Julia Bailey-Serres
UC Riverside. USA

Julia Bailey-Serres, Thanin Chantarachot, Travis Lee and Maureen Hummel
Center for Plant Cell Biology, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521 USA

Plant survival in a dynamic environment requires timely and tempered responses to abiotic and biotic stress to maintain growth and fecundity. The transcriptome, assayed by polyA+ mRNA-seq, provides information about steady state mRNA dynamics including alternative splicing, but yields limited information on transcriptional priming and the regulated processes of translation, degradation or and sequestration. We applied genome-scale technologies [chromatin-immunopurification (ChIP), Isolation of Nuclei TAgged in specific Cell Types (INTACT), Assay for Transposase Accessible Chromatin (ATAC), mRNP complex immunopurification (RIP) and Translating Ribosome Affinity Purification (TRAP)] coupled with sequencing to decipher interconnections in the gene regulatory hierarchy in response to changes in in oxygen availability. This uncovered rapid and distinct modulation of transcription, nuclear export, turnover and translation of mRNAs associated with stress resilience and growth. We resolved that, under hypoxia, many transcripts associated with growth continue to be synthesized but are retained in the nucleus until reoxygenation. To better understand the role of mRNA decay in transcriptome dynamics and homeostasis, we studied the Arabidopsis orthologs of yeast and metazoan DEAD-box RNA helicase (RH) DHH1/DDX6 proteins, confirming their association with the multi-subunit decapping complex that initiates 5′-to-3′ decay. These nucleocytoplasmic proteins are indispensable for developmental maturation and promote turnover of a subset of mRNAs. An integrated analysis of mRNA decay kinetics using transcriptome and translatome profiling unveiled that RH deficiency perturbs the growth-stress homeostasis. Our work defines the plant DDH1/DDX6 orthologs as a variable component of the 5′-to-3′ RNA decay machinery that are needed for the high-flux decay of stress-associated transcripts under control growth conditions. Multiple tiers of post-transcriptional regulatory control contribute to priming and executing a stress response as well as subsequent recovery. Funded by US NSF (MCB-1716913).

Systemic approaches to dissect cell-cell signaling

Speaker: Kenneth Birnbaum
New York University (NYU), USA

Kenneth Birnbaum
New York University (NYU)

One of the promises of new single-cell RNA seq technology is the potential to "democratize" tool building in new species; that is, to rapidly characterize the anatomy of species that don't have extensive resources of fluorescent markers and other tools. In particular, maize has a different root anatomy than Arabidopsis and many features are important for crop performance, such as many cortical layers that serve the plant for symbiosis or drought protection. We used a new dye sorting technique to provide a pooled-cell tissue-specific profile of the maize root. We then used single-cell RNA-seq for about 6,000 cells to reconstruct the root cell-by-cell using the tissue profiles as a scaffold. The results provided an extremely fine-scale map of the maize root. We showed we could use this scaffold to "hop" to Setaria, another monocot species. I will provide some details on expression anomalies that indicate changes in classic pathways that enable monocots to specify extra cortex layers.

Tomato roots and their cell types – turning things inside out!

Speaker: Siobhan Brady
UC Davis. USA

Concepcion Manzano[1,2], Kaisa Kajala[1,3], Alex Canto-Pastor[1], Lidor Shaar-Moshe[1], G. Alex Mason[1], Mona Gouran[1], Robertas Ursache[3], Carlos del Pozo[2], Niko Geldner[4], Siobhan M. Brady[1]
[1] Department of Plant Biology and Genome Center, University of California – Davis, USA
[2] Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (Madrid) Spain.
[3] Utrecht University, Utrecht, Netherlands
[4] University of Lausanne, Switzerland

The exodermis cell type is present within most angiosperms but is understudied due to its absence in Arabidopsis thaliana. Exodermis cells are able to form a barrier which is thought to be analogous to the Casparian strip in the root endodermis. We describe the morphology and the timing of the formation of this barrier in the exodermis relative to the endodermis, the ability of the exodermis to act as a functional barrier, and the composition of this barrier. Cell type-specific molecular profiling and gene reporters are used to assess the degree to which the endodermis development and Casparian strip regulatory module has been co-opted for acquisition of exodermis identity. We propose a novel regulatory pathway for exodermis specification, differentiation and suberization.

TREX-2 and a nuclear pore protein in microRNA biogenesis in Arabidopsis

Speaker: Xuemei Chen
UC Riverside. USA

Bailong Zhang[1], Chenjiang You[1,2], and Xuemei Chen[1]
[1] University of California, Riverside, CA 92521, USA
[2] Shenzhen University, Guangdong Province, China

The biogenesis of microRNAs (miRNAs) in plants is a multistep process that entails transcription of MIR genes, processing of microRNA precursors, loading of miRNAs into ARGONAUTE1 (AGO1), and nuclear export of miRNA-AGO1 complexes. Whether or how these steps are coordinated is unknown. We show that the TREX-2 complex is required for miRNA biogenesis in Arabidopsis, and that TREX-2 promotes and probably coordinates the transcription, processing, and nuclear export steps in miRNA biogenesis. A nuclear pore protein that interacts with TREX-2 influences the loading of miRNAs into AGO1. In mutants of TREX-2, endogenous siRNAs from transposable elements show enhanced loading into AGO1. Therefore, the coordination of the major steps in miRNA biogenesis ensures the proper partitioning of AGO1 in binding miRNAs vs. siRNAs.

Phenylalanine Biosynthetic Network: Past, Present and Future

Speaker: Natalia Dudareva
Purdue University. USA

Natalia Dudareva
Department of Biochemistry, Purdue University, West Lafayette, IN 47907, USA

Plants have a high demand for the aromatic amino acids L-phenylalanine (Phe), L-tyrosine, and L-tryptophan, as they serve as precursors for the formation of proteins and numerous aromatic primary and secondary metabolites. Phe is a common precursor of >8000 phenolic compounds, which constitute approximately 30-45% of plant organic matter. In plants Phe biosynthesis occurs via two alternative pathways, both requiring conversion of chorismate, the final product of the shikimate pathway, to prephenate by chorismate mutase (CM). Using a functional genomics approach and petunia flowers, which emit high levels of Phe-derived volatiles as a model system, we have identified genes encoding proteins involved in the arogenate and phenylpyruvate Phe biosynthetic pathways as well as in Phe export from plastids. We have shown that, while plants predominantly synthesize Phe in plastids via the arogenate pathway, the microbial-like phenylpyruvate pathway also contributes to Phe formation. By combining reverse genetic and metabolic flux analysis, we have elucidated the structure, molecular players and subcellular localization of the phenylpyruvate pathway. We showed that the cytosolic CM is responsible for directing the carbon flux towards cytosolic Phe production and an alternative transcription start site of a known plastidial enzyme produces a functional cytosolic prephenate dehydratase that catalyzes the conversation of prephenate to phenylpyruvate, the intermediate step between CM and phenylpyruvate aminotransferase. Obtained results complete elucidation of Phe biosynthesis via phenylpyruvate in plants, showing that the entire pathway is localized in the cytosol and it branches from the arogenate pathway at chrorismate instead of prephenate as previously thought. This presentation will also discuss (i) an interconnection between aromatic amino acid catabolism and biosynthesis; (ii) plasticity and complex regulation of Phe biosynthetic pathways, and (iii) why plants contain a functional phenylpyruvate route and its role in planta.

Developmental Homeostasis: unearthing the fast dynamics underlying root growth and optimal nutrient uptake

Speaker: Veronica Grieneisen
John Innes Centre (JIC). UK

Verônica A. Grieneisen
Affiliation: School of Biosciences, Cardiff University, Wales, UK.

We analyze the developing root as a system in which information flows are coordinated through tissue polarity and relayed via phytohormones and nutrients to achieve higher level robustness as well as plasticity. Combining different molecular-genetic interferences and imaging, we have elucidated an important mechanism by which root meristem size can be sustained or quickly altered by the coordination of phytohormones. Moreover, by adopting a morphoengineering view on plant-nutrient uptake, we could find universal dynamical constraints that operate in transport systems of polarized tissues, to avoid traffic-jam phenomenon of flows. I will discuss how such mechanisms underlying developmental robustness can also explain fast time-scale adaptations, and the importance of considering intrinsic instabilities when striving to make plants more efficient in their nutrient uptake capacity.

Plant diterpene metabolism: newly evolved strategies for discovery in the mint family and engineering of production

Speaker: Björn Hamberger
Michigan State University. USA

Björn Hamberger
Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan, USA.

The mint family (Lamiaceae) represents an incredibly rich resource of structurally diverse diterpenoids defining their value in culinary, flavor and fragrance to pharmaceutical applications. With the increasing availability of deep genomics resources, such as the recently sequenced 48 transcriptomes in Lamicaeae[§], we needed to adapt our strategy for diterpenoid pathway discovery. A focus on species with candidate genes recognized, yet, without reported diterpenoids highlights the potential of a transcriptome-guided approach.[1] Among the novel diterpene synthases, we identified one enzyme from common bugle, catalyzing the first step towards insect-antifeedant compounds. In Marjoram, the discovery of an unexpected enzyme guided the discovery of novel specialized diterpenes in that species. Similarly, the recent discovery of a possible connection between subcellular features and terpenoid accumulation has inspired adjustments in the functional expression and reconstruction of heterologous pathways. This includes a strategy to co-engineer terpene biosynthetic pathways and formation of lipid droplets in plant cells.[2] Examples of these cases will be highlighted which represent new tools in our biotechnological approaches toward engineering of terpenoid production.

[1] Johnson et al., (2018) A database-driven approach identifies additional diterpene synthase activities in the mint family (Lamiaceae). JBC doi.org/10.1074/jbc.ra118.006025

[2] Sadre et al., (2019) Cytosolic lipid droplets as engineered organelles for production and accumulation of terpenoid biomaterials in leaves. Nat Comm doi.org/10.1038/s41467-019-08515-4

[§] http://mints.plantbiology.msu.edu/

Integration of hormonal and transcriptional control during vascular development

Speaker: Yrjö Helariutta
Sainsbury Laboratory at Cambridge University (SLCU). UK

Ykä Helariutta
Sainsbury Laboratory Cambridge University
Bateman Street, Cambridge CB2 1LR

Vascular plants have a long-distance transport system consisting of two tissue types, phloem and xylem. During root primary development, xylem is specified early as an axis of vessel element cell files, whereas phloem is established through a set of asymmetric cell divisions also contributing to the intervening procambial tissue (Mähönen et al. 2000 Genes Dev). We have recently been able to determinate how the key hormonal (auxin, cytokinins) and transcriptional cues (class III HD-ZIP genes, PEAR genes) are integrated to specify the primary vascular pattern (Miyashima et al., 2019). This highlights early phloem as an important organizer. Subsequently, we are investigating the interaction of phloem with the flanking vascular tissues at a single-cell resolution.

How many stem cells can you fit in a plant shoot?

Speaker: Henrik Jönsson
Sainsbury Laboratory at Cambridge University (SLCU). UK

Henrik Jönsson
Sainsbury Laboratory at Cambridge University (SLCU)

The continued growth of the aboveground part of a plant is dependent on stem cells located in the shoot apical meristem. While it has been suggested the number of stem cells stay relatively constant throughout the life of a plant, I will discuss how this depends on the size of the meristem and the plant and how this is influenced by environmental factors. I propose that an integrated analysis of the system taking genetic, hormonal and mechanical factors is necessary to gain an understanding of the growth dynamics. Finally I will discuss how a detailed and scaled up analysis of cell lineages and gene expression may be used to better understand growth and morphogenesis by improving our hypothesis-driven computational modelling approach.

Domestication of transposable elements for plant reproduction

Speaker: Claudia Köhler
Swedish University of Agricultural Sciences (SLU). Sweden

Rita A. Batista, Jordi Moreno-Romero, Joram van Boven, Yichun Qiu, Juan Santos-González, Duarte D. Figueiredo, Claudia Köhler
Department of Plant Biology, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Uppsala, Sweden

In flowering plants, type I MADS-box transcription factors are associated with reproductive development and many are active in the endosperm, a nutritive tissue supporting the embryo. Deregulation of the encoding genes has been frequently linked to failure of endosperm development and seed inviability. We found that the imprinted Arabidopsis thaliana MADS-box transcription factor PHERES1 has a central role in endosperm development as a master regulator of imprinted gene expression, especially of paternally expressed genes, which have been previously implicated in endosperm development. Control of imprinted gene expression by PHERES1 is mediated by parental asymmetry of epigenetic modifications in PHERES1 DNA-binding sites, conferring different accessibilities to maternal and paternal alleles. Importantly, the DNA-binding motifs used by PHERES1 to access gene promoters are carried by RC/Helitron transposable elements, providing an example of molecular domestication of these elements. Thus, transposable elements are intrinsically linked to imprinting and endosperm development, not only by enforcing specific epigenetic landscapes, but also by serving as important sources of cis-regulatory elements.

Mechanistic insights into carotenoid accumulation in plants

Speaker: Li Li
Cornell University. USA

Li Li
Robert W. Holley Center for Agriculture and Health, USDA-ARS, Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA)

Carotenoids are a diverse group of isoprenoid pigments synthesized de novo in all photosynthetic organisms. They are essential for photosynthesis in plants, and serve as antioxidants and precursors for vitamin A synthesis in human diets. The pivotal roles of carotenoids to plants and humans have prompted significant efforts toward understanding of carotenoid metabolism and the intriguing regulatory mechanisms underlying carotenoid accumulation in plants. Carotenoid accumulation is a dynamic process, which is determined by biosynthesis capacity, degradation rate, and stable storage in plastids. Therefore, regulation of each activity affects the final carotenoid level in plant tissues. Phytoene synthase (PSY) is the major rate-limiting enzyme of carotenogenesis. As a specialist enzyme, PSY is subjected to regulation at multifaceted levels and by various factors. We discover post-translational regulation of PSY being an important mechanism by which carotenoid biosynthesis is controlled in plants. Our recent study via 3D protein structure modeling also identifies the key amino acid residues responsible for PSYactivity. While biosynthesis capacity defines the pool size of carotenoids in plants, we show that stable storage of the synthesized products in plastids is critically important for carotenoid accumulation. ORANGE (OR) represents a bona fide regulator of chromoplasts, the plastids massively accumulated carotenoids. Through investigation of its roles in carotenoid accumulation, we recently further demonstrate that regulation of chromoplast number and size to alter plastid sink strength profoundly affects the final carotenoid content in plants. Global food security to feed the growing population necessitates not only the increased crop yield, but also more nutritious foods. Our studies also provide novel genetic tools and strategies for effective development of carotenoid enriched crops with enhanced nutritional quality.

Cryptic variation and epistasis in flower production and crop improvement

Speaker: Zachary Lippman, Ph.D.
Cold Spring Harbor Laboratory. USA

Zachary Lippman
Cold Spring Harbor Laboratory, NY USA

Strong convictions often emerge when debating the significance of epistasis in plant evolution, domestication and breeding. I will share a remarkable multi-faceted case of epistasis in tomato that captures meristem development, flower production, gene family evolution, cryptic mutations, structural variation, dosage, selection, mechanized agriculture, and yield. What we have learned from the sum, or rather the interaction, of all of the above is guiding our current and future efforts in using genome editing to unveil and harness mechanisms of epistasis and quantitative variation for both basic biology and crop improvement.

Cellular and molecular mechanisms of circadian clock function in *Arabidopsis thaliana*

Speaker: Paloma Mas
Centre for Research in Agricultural Genomics (CRAG). Spain

Paloma Mas [1,2]
[1] Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, 08193 Barcelona, Spain.
[2] Consejo Superior de Investigaciones Científicas (CSIC), 08028 Barcelona, Spain.

The circadian clock is a timing mechanism able to coordinate the rhythms of multiple biological processes. In plants, the circadian function is critical for proper fitness and survival. Knowing how the circadian system works provides an efficient tool to understand the temporal compartmentalization of plant physiology, development and metabolism in synch with the daily and seasonal environmental changes. In our lab, we have recently discovered the molecular mechanism controlling the rhythms of transcript initiation and elongation as well as the rhythms in nascent RNAs. The mechanism relies of a multifunctional clock protein complex that recruits the RNA Polymerase II and the transcript elongation FACT complex to rhythmically co-occupy clock target loci. Our findings explain how genome readout of environmental information ultimately results in rhythmic changes of gene expression. We have also recently found that the circadian clock, through the function of the clock component TOC1 (TIMING OF CAB EXPRESSION1/PSEUDO RESPONSE REGULATOR1), drives the speed of the cell cycle in Arabidopsis. By regulating the DNA pre-replicative machinery, the circadian clock modulates cell division during proliferation and somatic ploidy during differentiation and thus controls plant growth in resonance with the environment.

Epigenetic variation in *Arabidopsis thaliana*

Speaker: Magnus Nordborg
Gregor Mendel Institute of Molecular Plant Biology (GMI). Austria

Magnus Nordborg
Gregor Mendel Institute of Molecular Plant Biology (GMI), Austria

Epigenetics continues to fascinate, especially the notion that it blurs the line between "nature and nurture" and could make Lamarckian adaptation via the inheritance of acquired characteristics possible. That this is in principle possible is clear: in the model plant *Arabidopsis thaliana* (thale cress), experimentally induced DNA methylation variation can be inherited and affect important traits. The question is whether this is important in nature. Recent studies of A. thaliana have revealed a pattern of correlation between levels of methylation and climate variables that strongly suggests that methylation is important in adaptation. However, somewhat paradoxically, the experiments also showed that much of the variation for this epigenetic trait appears to have a genetic rather than an epigenetic basis. This suggest that epigenetics may indeed be important for adaptation, but as part of a genetic mechanism that is currently not understood. Genome-wide association studies revealed a striking genetic architecture of methylation variation, involving major-effect polymorphisms in many genes involved in silencing, and this can be utilized to determine whether the global pattern of methylation variation has a genetic or an epigenetic cause, and to elucidate the ultimate cause of the global pattern of variation: natural selection.

Analysis of NLR immunity signalling across plant species

Speaker: Jane Parker
Max Planck Institute for Plant Breeding Research (MPIPZ). Germany

Jane Parker
Max-Planck Institute for Plant Breeding Research, Dept. Plant-Microbe Interactions, Cologne, Germany; parker@mpipz.mpg.de

Mechanisms of surveillance by plants and counter-surveillance by microbes provide a fascinating framework for deciphering host-pathogen coevolution and identifying disease resistance signalling nodes. We're studying host recognition of biotrophic pathogens and the processes by which intracellular (NLR) immune receptors, sensing pathogen interference with host cells, transmit recognition to downstream resistance pathways. Using molecular genetic, transcriptomic and protein structural approaches in Arabidopsis we've identified various NLR-induced defense pathways (sectors) that contribute to immunity and we're interrogating how these pathways operate within the broader environmental stress response network. Arabidopsis also serves as a springboard for exploring stress network properties in other plant species with different NLR receptor repertoires. I'll describe progress in analysis of NLR activation and signaling and convergence on the transcriptional machinery to reprogram cells for resistance. From these studies, we're getting new insights to plant immunity network resilience against microbial disease.

Molecular genetics of arbuscular mycorrhizal symbiosis in cereals

Speaker: Uta Paszkowsky
University of Cambridge. UK

Uta Paszkowski
University of Cambridge, Downing Street, Cambridge CB2 3EA, United Kingdom

The arbuscular mycorrhizal (AM) symbiosis is a fascinating mutualistic interaction between roots of most land plants and fungi of the phylum of the Glomeromycota. The development of this life-long alliance starts with reciprocal recognition in the rhizosphere, reprogramming both symbionts for the anticipated association. The interaction proceeds towards extensive root colonization which culminates in the formation of fungal feeding structures, the arbuscules, inside root cortex cells. As the arbuscule develops, the plant cell dramatically increases membrane biogenesis to envelope the growing hyphal structure. Thereby a hugely enlarged intracellular surface area is created between the two organisms, appearing ideally adapted for the exchange of signals and nutrients.
The nature and complexity of the establishment of AM symbioses must be the result of a well-orchestrated exchange of molecular signals between the plant and the fungus. The nature of some of the signals has been discovered in recent years, providing a first insight into the type of chemical language spoken between the two symbiotic partners. My group has taken molecular genetics and lately advanced imaging approaches to elucidate the molecular mechanisms underpinning this apparently harmonious symbiosis. I will introduce some of our recent observations which have led us to propose fundamentally new communication mechanisms operating during this intimate plant-fungal partnership.

FLOWERING-LOCUS T: more than flowering inducing signals

Speaker: Salomé Prat
National Centre for Biotechnolgy (CNB). Spain

Eduard Cruz-Oró, Evyatar Steiner and Salomé Prat
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Members of the FT protein family were originally identified as long distance signals for flowering transition. Expression of these genes is activated in the leaves in a day length dependent manner, and the protein is transported via the phloem to the shoot apical meristem to the induction of floral transition. Homologues of Arabidopsis FT were also found to have a prevalent role in the control of bud dormancy in trees, whereas in potato they modulate the formation of underground storage organs or tubers. In Solanaceae, the FT family has undergone preferential diversification and in addition to homologues with a role in floral induction, it includes members with a positive (SP6A) and a negative role (SP5G) in tuberization transition. The SP6A mobile tuberization signal has been established to bind 14-3-3 proteins and the bZIP FDL1 factor in underground stolons to form a tuberization activator complex (TAC) similar to the flowering FAC complex described for AtFT and Hd3a. Notably, the potato TAC complex activates expression of the MACROCALIX AP1 and the MADS-box FUL genes, which indicates that it regulates the same downstream targets as seen for the Arabidopsis and rice proteins in the shoot apical meristem. The function of AP1 and FUL in storage fate differentiation will be discussed, as well as the nature of the undifferentiated cells in underground stolons that initiate this secondary growth developmental process.

Using CRISPR/Cas in plants: From gene editing to gene targeting and genome engineering

Speaker: Holger Puchta
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Botanical Institute, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

By applying the CRISPR/Cas system for double strand break (DSB) induction, the knockout of genes by non-homologous end-joining (NHEJ) has become routine for plants. Several years ago we were able to demonstrate Streptococcus pyogenes (Spy)Cas9 nuclease induced, heritable targeted mutagenesis in Arabidopsis thaliana. Later on, we applied Cas9 of Staphylococcus aureus (SauCas9), obtaining higher efficiencies. In contrast, gene targeting (GT), the programmed change of genomic sequences by homologous recombination (HR) is still a major challenge. We previously developed an in planta GT strategy in which the repair template is stably integrated into the plant genome and excised at the same time as DSB induction occurs in the target locus. Using SpCas9, we obtained seeds harboring GT events, although at low frequency. Recently, by using SauCas9 under the control of an egg-cell specific promotor we could enhance in planta GT frequencies by one order of magnitude, in comparison to our earlier approaches with SpyCas9. We got a targeting efficiency of up to 5% for specific T2 lines. Moreover, we have to take the next step from gene to genome engineering. Future efforts should aim to establish technologies for the restructuring of chromosomes and the breaking of genetic linkages. As a first step, we developed an efficient procedure for chromosomal inversions. Using the Cas9 nuclease from S. aureus (SaCas9), we were able to obtain scarless heritable inversions with high efficiency in the model plant Arabidopsis thaliana, at different genomic loci and at intervals between 3 and 18 kb, in the percentage range, in the T1 generation. By screening individual lines, inversion frequencies of up to the 10% range were found in the T2. Using our approach in crop plants, it should be possible to reverse natural inversions and induce artificial ones to break or fix linkages between traits at will.

Making a flat leaf: Pre-patterning, morphogenic small RNAs, and growth

Speaker: Marja Timmermans
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Development of flat leaf architecture posses an unusual and mechanistically challenging problem; namely, how to create a stable adaxial-abaxial (top-bottom) boundary within the plane of a long and wide, but shallow, structure. The acquisition and maintenance of adaxial-abaxial polarity involves an intricate gene regulatory network with several highly conserved transcription factors that promote either adaxial or abaxial fate at its core. These are expressed in complementary domains delineating the top and bottom side of the initiating organ, respectively. The positional information needed to define these domains is provided in part by small RNAs that, reminiscent to classical morphogens, generate sharply defined domains of target gene expression through an intrinsic and direct threshold-based readout of their mobility gradients. While the polarity network is sufficient to cleanly separate regions of adaxial and abaxial identity, our most recent findings from mathematical modeling predict that additional inputs are needed to maintain a robust, uniformly positioned developmental boundary. Interestingly, anisotropic growth is one such input. The maintenance of a stable adaxial-abaxial boundary during primordium growth may thus rely on the anisotropic nature of that growth.

A composite cis-regulatory code underpins epigenetic gene repression in plants

Speaker: Franziska Turck
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Polycomb group repressive protein complexes (PRCs) are essential for transcriptional regulation in higher eukaryotes; they act through the establishment of repressive epigenetic marks at specific chromatin regions. A key research questions remains how PRC complexes find their target regions. In Drosophila, PRC2, responsible for establishing H3K27me3, is recruited via several well described motifs present in Polycomb response elements (PREs). In mammals, PREs and associated motifs are less obvious, while PRC recruitment through long non-coding RNAs has gathered much attention. In plants, until recently, PRC recruitment by cis-elements or non-coding RNA was not described with the exception of a few case studies.
I will present evidence for general mechanisms of PRC1 and PRC2 recruitment by B3 domain transcription factors and TELOMERE REPEAT BINDING FACTORs (TRBs), respectively. RY-motifs and teloboxes, the cognate elements of these DNA-binding protein classes, distribute along H3K27me3 marked regions with a high likelihood of presence in gene bodies. Approximately a fifth of PcG target genes depends on the joint action of TRBs and PRC2 for H3K27me3 coverage and transcriptional repression, these genes are particularly enriched in teloboxes but relatively depleted in RY-motifs. In contrast, genes enriched for RY-motifs but depleted for teloboxes gain H3K27me3 in trb mutants suggesting that these motifs compete for enzymatic activities that stabilize H3K27me3. In conclusion, plant PREs appear to be discontinuous and composed of a set of motifs that act in partial redundancy allowing for a concerted regulation of distinct groups of Polycomb target genes.

(Plant) Life with more than one genome

Speaker: Yves Van de Peer
Vlaams Instituut voor Biotechnologie (VIB). Belgium

Yves Van de Peer
Department of Plant Biotechnology and Bioinformatics and Center for Plant Systems Biology (VIB), Ghent University, Belgium

Thousands of species are currently polyploid, and contain multiple copies of their genome. On the other hand, the long-term establishment of organisms that have undergone ancient whole genome duplications (WGDs) has been exceedingly rare. The apparent paucity of ancient genome duplications and the existence of so many species that are currently polyploid provides a fascinating paradox. Interestingly, many ancient WGDs seem to have been established at very specific times in evolution, for instance during major ecological upheavals and periods of extinction. Our work has shown that WGDs observed for many different plant lineages seem to have coincided with the most recent major mass extinction, i.e. the K/Pg extinction, 66 million years ago. I will put forward different hypotheses of why polyploids, compared to their diploid progenitors, might have had some selective advantage that might explain their survival at times of extinction or environmental turmoil. Also, I will discuss how WGD events might lead to an increase in biological complexity. WGDs copy entire pathways or networks, and as such create the unique situation in which such duplicated pathways or networks could evolve novel functionality through the coordinated sub- or neofunctionalization of its constituent genes.

(Re)programming cell identity and function in response to intrinsic and extrinsic cues

Speaker: Doris Wagner
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Doris Wagner and Yang Zhu
University of Pennsylvania, Philadelphia, PA, USA

Plant development and survival is tuned in response to endogenous and environmental cues in the context of chromatin. All living plant cells can be triggered to de-differentiate, to assume a different identity and function, or to form an entire new organism. My lab is particularly interested in the series of events that lead from cue perception to the transcriptional, epigenetic and cellular reprogramming during the switch to formation of flowers in the inflorescence. The timing of this developmental switch not only determines the species-specific inflorescence architecture, but is also critical for reproductive fitness and yield. I will discuss new mechanistic insight into the question how seasonal information impacts onset of flower formation and sculpts inflorescence architecture.

POSTER ABSTRACTS

01

A bioinformatic approach to reveal plant growth-promoting potential of bioinoculants, such as Kosakonia radicincitans

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Bioinoculants promise to have a high impact on plant growth improvement and stress tolerance, and are considered to support a sustainable agriculture. The identification of plant growth-promoting bacteria (PGPBs) and their underlying metabolic key mechanisms are challenging problems that require large-scale screening experiments. Advanced bioinformatics approaches for e.g. transcriptomics and metagenomics will help to uncover interactions between bacteria and host plants leading to beneficial co-existence.
Here we present a promising strategy for predicting the PGP potential of bacteria by applying a new hierarchical functional classification of PGP traits (PGPTs). It can be used to analyze the presence/absence pattern and frequency of PGPTs in genomes or metagenomes. Our studies find increased copy numbers of genes coding for PGP traits in the highly effective bioinoculant strain K. radicincitans DSM16656T compared to related species across genera. We have also explored additional gene sets on the chromosome for bacterial competition and host interaction, like tailocins and type VI secretion systems. Notably, our analysis suggests that the Kosakonia strain harbours genes on its plasmid to exploit further plant-derived metabolites, that improves its life cycle within the host.
Moreover, the implementation of this new functional classification in the MEGAN6 software allows an easy abundance estimation of PGP gene/protein families in crop or in agricultural soil metagenomes with and without bioinoculant application, and thus facilitates the exploration of the predominant microbial potential under distinct environmental conditions. The term PGPT or its subterms, comprising one ore more protein families, can also be used for gene set enrichment analyses of RNA-seq expression profiles to investigate active metabolic pathways.
We hope that the new hierarchical functional classification for PGPTs will support research on beneficial crop-microbe interactions and on the use of bioinoculants in agriculture. Rather, we see applcations in risk management by differentiating PGPBs from virulent strains.

02

GMO-free RNAi in plants

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In plants, RNA interference (RNAi) is triggered by the presence of double stranded RNAs (dsRNAs) which are processed by DICER LIKE endonucleases (DCLs) into 21-24 nucleotide short interfering RNAs (siRNAs) that recognize complementary transcripts for degradation. Given its tremendous potential in crop protection, RNAi has been conventionally triggered by transgenes expressing dsRNAs targeting selected plant and/or pathogen targets. Yet, the employment of transgenes and genetically modified organisms (GMOs) has raised considerable public and scientific concerns and their future implementation in modern crop protection platforms is dubious. In this line, exogenous application of RNA molecules having the potential to trigger RNAi is a promising alternative to transgenic crops. We have recently developed methods based on high pressure spraying and trunk injection for exogenous delivery of RNA molecules with the capacity to trigger RNAi in herbaceous (Nicotiana benthamiana) and woody plants (apple and grapevine), respectively (Dalakouras et al. 2018; Dalakouras et al. 2016). Depending on the method of application, the RNA molecule may be delivered in the symplast or the apoplast, a choice that greatly influences the efficiency of RNAi.

04

The discovery of a novel link between TORC1 signaling and translation initiation in plants

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The Target of Rapamycin (TOR) kinase is a conserved central coordinator of transcription and translation for metabolic reprogramming upon the perception of nutrient and energy signals across eukaryotes. Genetic research in plants indeed shows that overexpression of TOR and other components of the TORC1 signaling pathway increases growth whereas suppression of the TORC1 pathway using mutants, RNAi or chemical inhibition results in developmental defects. The molecular mechanisms by which nutritional cues promote plant growth, remains to date largely unknown. The research group lead by Prof. Geert De Jaeger recently published a comprehensive TORC1 signaling network for plants based on the integration of a dynamic sucrose- and TOR-dependent phosphoproteomics screen with an extensive protein complex analysis in Arabidopsis cell suspension cultures (Van Leene et. al., 2019, Nature Plants). This resource paper represents a dataset of known and novel TORC1 targets in plants, and provides insight into putative direct TORC1 targets, thereby contributing to a better molecular understanding of TORC1-mediated signal transduction.

Here we focus on a plant-specific association between the TORC1 complex and the eukaryotic initiation factor 2 and 2B (eIF2 and eIF2B), and TOR-dependent phosphorylation of the eIF2Bδ1 subunit. In yeast and animals, eIF2B interacts with and acts as a guanine exchange factor for eIF2, resulting in an active GTP-bound eIF2 complex which initiates translation. Phosphorylation of the eIF2α subunit by the stress-induced GCN2 kinase stabilizes the eIF2-eIF2B interaction, thereby inhibiting translation initiation. In plants, it remains unclear if eIF2B maintains its GEF activity, and the phosphorylation status of eIF2α is independent of TOR activity, pointing to alternative regulatory mechanisms to fine-tune the rate-limiting step in protein synthesis. So far, RNAi of several eIF2B subunits result in severe growth defects suggesting an important role in plant development. Our goal is to explain the unique, plant-specific regulatory link between TORC1 and these translation initiation factors using the inducible RNAi mutants, and study the effect on protein synthesis by shotgun proteomics and ribo-seq experiments.

05

Unraveling the role of the cysteine protease AtMC3 in plant development

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Programmed cell death (PCD), an intracellular program for cells to die in an controlled manner, plays a fundamental role in various biological processes, including growth and development, in almost all eukaryotes. In plants, PCD is essential for many development processes and also plays a key role in defense against pathogens and abiotic stress. Plant metacaspases belong to a protease family and harbor structural similarities to animal caspases. They have been shown to be involved in different types of PCD.
In plants, formation of the vascular tissue involves PCD in the case of xylem and in case of phloem cells, a terminal differentiation program takes place that leaves the cells enucleated and heavily depending on the neighboring companion cells for survival. The proteases involved in phloem terminal differentiation are poorly defined. AtMC3 shows an expression pattern mostly restricted to the companion cells and AtMC3 mutants are impaired in their development and have a high degree of sterility, which indicates that this protease might be a key player in development. Severe phenotype has been also observed in the meristematic activity in the root. In our work, we are trying to deciphering the role of this protease.
To functionally address the question we are trying to create knock out mutants and overexpressing lines of AtMC3 in order to define the complete expression pattern during the plant life. Furthermore we will use proteomic techniques to detect interactors and natural proteolytic substrates of AtMC3, as it could be part of a regulatory cascade in vascular development.

06

Regulation of vitamin C biosynthesis in higher plants

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Ascorbate, also known as vitamin C, plays fundamental roles in biotic and abiotic stress resistance in plants. In green tissues, ascorbate is mainly synthesized through the Smirnoff-Wheeler pathway. Increasing ascorbate in breeding programs is important to enhance food quality but also to increase resistance to expected environmental challenges due to global warming. To achieve this is necessary a thorough understanding of the regulation of biosynthetic pathway of ascorbate. Although it is known that VTC2 (GDP-L-Galactose Phosphorylase) is the bottleneck of the pathway, little information is available on the regulation of the different biosynthetic enzymes at the biochemical and cellular level. We have generated a number of molecular tools that is allowing us to obtain detailed information about the protein regulation, localization and interaction among different biosynthetic components. We are also investigating their role of the different enzymes in ascorbate levels using a heterologous system such as Nicotiana bethamiana.
This research was supported by a grant from the Spanish Ministerio de Educación, Cultura y Deporte para la formación del Profesorado Universitario (FPU014/01974), as well as by the Ministerio de Economía, Industria y Competitividad (cofinanced by the European Regional Development Fund; grant no. BIO2016-81957-REDT and BIO2017-82609-R). We also acknowledge the support by the Plan Propio from University of Malaga, Campus de Excelencia Internacional de Andalucía.

07

Dynamic control of enhancer activity drives stage-specific gene expression during flower morphogenesis

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Enhancers are critical for developmental stage-specific gene expression, but their dynamic regulation in plants remains poorly understood. Here we compare genome-wide localization of H3K27ac, chromatin accessibility and transcriptomic changes during flower development in Arabidopsis. H3K27ac prevalently marks promoter-proximal regions, suggesting that H3K27ac is not a hallmark for enhancers in Arabidopsis. We provide computational and experimental evidence to confirm that distal DNase I hypersensitive sites are predictive of enhancers. The predicted enhancers are highly stage-specific across flower development, significantly associated with SNPs for flowering-related phenotypes, and conserved across crucifer species. Through the integration of genome-wide transcription factor (TF) binding datasets, we find that floral master regulators and stage-specific TFs are largely enriched at developmentally dynamic enhancers. Finally, we show that enhancer clusters and intronic enhancers significantly associate with stage-specific gene regulation by floral master TFs. Our study provides insights into the functional flexibility of enhancers during plant development, as well as hints to annotate plant enhancers.

08

Localization analysis of VPS41, a protein that confers resistance to cucumber mosaic virus

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Cucumber mosaic virus (CMV) is one of the plant viruses with the broadest host range. In Cucumis melo L., the Spanish cultivar Piel de Sapo (PS) is susceptible to all CMV strains, producing a systemic infection. However, the Korean cultivar Songwhan Charmi (SC) encodes one gene, cmv1, which confers resistance only to CMV strains from subgroup II (SG II), but not to subgroup I (SG I) strains. In the lines containing cmv1, the SG I strain FNY is able to reach the phloem and develop a systemic infection while SG II strain LS can replicate, and move cell to cell but it is restricted in the bundle sheath cells (BS) and does not reach the phloem. Recently, it has been discovered that the viral virulence factor that communicates with cmv1 is the Movement Protein (MP) and that cmv1 encodes a Vacuolar Protein Sorting 41 (CmVPS41), a protein involved in intracellular trafficking to the vacuole.

We have examined the relationship between CmVPS41 and the viral MP by studying their cellular localization. CmVPS41 from PS (susceptible) and SC (resistant) genotypes show significant differences in their localization pattern, with structures such as nuclear speckles, membrane dots and transvacuolar bridges in CmVPS41PS, whereas in CmVPS41SC there are much fewer. These CmVPS41 characteristic structures co-localize with the late endosome. CmVPS41 from the exotic resistant melon accessions I136 and C32, harbouring a causal mutation for resistance, show a pattern similar to that of CmVPS41SC. However, CmVPS41I180, without causal mutation, shows an intermediate pattern between PS and SC. Finally, the presence of MP from CMV-FNY produces changes in CmVPS41SC expression, which becomes similar to the susceptible CmVPS41PS localization pattern. Therefore, this suggests that those structures could be involved in susceptibility to CMV.

09

ETHQV8.1, a new player in melon fruit ripening

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Fruit ripening is an essential physiological process in plant development, and has an important impact in fruit quality and post-harvest storage. Generally, fleshy fruits are classified in climacteric fruits, when a peak of ethylene and respiration occurs at the onset of ripening; or non-climacteric, when ripening is not related to an increase of autocatalytic ethylene. Nevertheless, the genetic control of ripening is not completely understood. In the last years, melon (Cucumis melo L) has been proposed as a model to study ripening due to the coexistence of climacteric and non-climacteric varieties within the species.

Using a RIL population funded by a cross between the cultivars "Vedrantais" (cantalupensis, highly climacteric) x "Piel de Sapo" (inodorous, non-climacteric), we identified several QTLs controlling different climacteric ripening-related traits, such as ethylene production, earliness of climacteric ripening, aroma production or abscission layer formation. A major QTL in chromosome 8, named ETHQV8.1, was detected for almost all the tested traits related to climacteric ripening. Using the same parental cultivars, we developed two introgression lines (ILs) carrying reciprocal introgressions covering the region of the QTL. Using these ILs, we narrowed down the interval of the QTL to a 154 kb region, containing 14 annotated genes. The non-climacteric allele in a climacteric background delayed and decreased the production of ethylene. Moreover, introgressing the climacteric allele in a non-climacteric background caused a weak climacteric behavior, with a low amount of ethylene production and other ethylene-related traits such as aroma production.

With this work, we present a new QTL involved in climacteric ripening in chromosome 8, evidencing also the existence of other QTLs controlling ripening behavior in the RIL population.

10

MyROOT: A method and software for the semi-automatic measurement of primary root length in Arabidopsis seedlings

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Root analysis is essential for both academic and agricultural research. Despite the great advances in root phenotyping and imaging, calculating root length is still performed manually and involves considerable amounts of labor and time. To overcome these limitations, we have developed MyROOT, a software for the semi-automatic quantification of root growth of seedlings growing directly in agar plates. Our method automatically determines the scale from the image of the plate, and subsequently measures the root length of the individual plants. To this aim, MyROOT combines a bottom-up root tracking approach with a hypocotyl detection algorithm. At the same time as providing accurate root measurements, MyROOT also significantly minimizes the user intervention required during the process. Using Arabidopsis, we tested MyROOT with seedlings from different growth stages and experimental conditions. Upon comparing the data obtained using this software with that of manual root measurements, we found a high correlation between both methods (R2 = 0.997). When compared to previous developed softwares with similar features (BRAT and EZ-Rhizo), MyROOT offers an improved accuracy in root length measurements. Thus, MyROOT will be of great aid to the plant science community by permitting high-throughput root length measurements while saving on both labor and time.

11

Fine mapping of major genes identified in peach x almond crosses

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Peach (Prunus persica) is an economically important fruit crop and one of the model species of the Rosaceae family. Peach breeding programs include different objectives as prolonged shelf life, fruit quality and disease resistance. One of the main limitations is the low levels of genetic variability, hence it is important to find new sources of variability. One of the ways to do so would be the introgression of valuable alleles from its closely related and cross-compatible species such as almond (P. dulcis) or other wild species (P. davidiana, P. cersasifera, P. mira) of peach. From the interspecific cross beween 'Texas' almond x 'Earlygold' peach, eleven major genes have been identified and mapped. Two of them, almond fruit type (Alf) and juiciness (Jui), define the main differences between almond and peach fruits. Other interesting genes were blood flesh (DBF2), powdery mildew resistance (Vr3) and flower type (Sh). They have been mapped to genomic regions covering between 200kb to 1.6 Mb. Currently we are fine mapping them saturating target regions with more markers developed from the resequences of the parental lines and identifying new recombinant individuals. The aim is to reduce the target genomic regions and the list of candidate genes for each gene to facilitate functional validation.

12

Clonal diversity and fine-scale genetic structure of Ilex aquifolium L. (Aquifoliaceae)

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Ilex aquifolium L. (Aquifoliaceae) is small tree native of European and Mediterranean forests characterized by its resprouting capability from stem and from root. In the present study we evaluated the clonal diversity and fine-scale spatial genetic structure of Ilex aquifolium in a sub-Mediterranean mixed forest of Central Spain. Using seven microsatellite loci we assessed the contribution of clonal reproduction, fifty years after the cessation of permanent cattle presence in this forest. The results showed a lower genetic diversity compared with other studies with absence of endogamy and clonal diversity in the same levels as other clonal species. Clones were spatially aggregated in round-shaped groups and stems were separated distances up 10.78 meters. Dense aggregations of stems were in some cases compounded by various genotypes. The results also showed seed recruitment reflected in small unique and isolated stems. There was evidence of fine-scale spatial genetic structure at close distance ranges being stronger in individuals of greater dbh.

13

Unraveling the mechanism of TTL genes in cellulose biosynthesis

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As sessile organisms, plants require mechanisms to sense and respond to the challenging environment, that encompass both biotic and abiotic factors that results in differential development. In these conditions is essential to balance growth and stress responses. As cell walls shape plant growth, this differential growth response cause alterations to the plant cell wall where cellulose is the major component. Therefore, understanding the mechanisms that regulate cellulose biosynthesis is essential to develop strategies to improve plant production. In Arabidopsis, the TETRATRICOPEPTIDE THIOREDOXIN-LIKE(TTL) gene family is composed by four members (TTL1 to TTL4) and mutations in TTL1,TTL3, and TTL4 genes cause reduced growth under salt and osmotic stress due to defects in plant cell wall integrity. We observe association of TTL3 with most core components in traducing BR signalling, such as LRR-RLK BRI1 or GSK3 BIN2 that modulate cellulose biosynthesis through phosphorylating cellulose synthases (CesA). Here, we show that ttlmutants present defects in the plant cell wall, particularly in Isoxaben, salt or sucrose stress. Spinning disk microscopy in etiolated hypocotyls reveals that, TTL proteins are responsible for the cellulose synthase complex (CSC) stability in plasma membrane (PM) upon sucrose stress. Moreover, TTL3 associates with LRR-RLKs that have been shown to be important for cellulose biosynthesis such as FEI1 in the FEI1/FEI2/SOS5 pathway. We aim to investigate the mechanisms by which TTL proteins regulate CesA stability in PM under stress, using a combination of genetics, biochemical, and molecular and cell biology approaches.

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14

Steryl ester biosynthesis in tomato: Effect of phospholipid:sterol acyltransferase inactivation on plant growth and development.

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Steryl esters (SE) serve as a storage pool of sterols that helps to maintain free sterol (FS) homeostasis in cell membranes throughout plant growth and development, and is involved in the recycling of FS and fatty acids released from disorganized cell membranes in senescing tissues (Bouvier-Navé et al., 2010). SE are synthesized by sterol acyltransferases, a class of enzymes that catalyze the transfer of fatty acid groups to the free hydroxyl group at C3 position of the sterol backbone. These enzymes are categorized into acyl-CoA:sterol acyltransferases (ASAT) and phospholipid:sterol acyltransferases (PSAT) depending on whether the fatty acyl donor is an acyl-CoA or a phospolipid. We have very recently cloned and functionally characterized the tomato (S. lycopersicum var. MicroTom) PSAT1 and ASAT1 enzymes. PSAT1 has a strong substrate preference for the major plant sterol end products, namely b-sitosterol, estigmasterol and campesterol, whereas ASAT1 preferentially esterifies cycloartenol, the first cyclic intermediate of the sterol biosynthesis pathway (Lara et al., 2018). To get insight into the biological role of SE metabolism in tomato, we used the CRISPR/Cas9-induced genome editing technology to generate tomato psat1 knockout mutant plants. Experimental strategies based on restriction fragment length polymorphism (RFLP) and DNA sequencing of the SlPSAT1 gene target sequence were used to identify homozygous mutant lines carrying mutations that lead to the inactivation of SlPSAT1. Preliminary analysis of these mutants revealed that complete loss of function of SlPSAT1 results in a drastic decrease of SE content in seeds concomitant to an increase in FS compared to wild type seeds, severe alterations of seed germination and the early stages of vegetative development, as well as a mild dwarf phenotype and alterations in leaf morphology. Work is in progress to assess the effect of PSAT1 inactivation on fruit development and its agronomic traits, and to elucidate the molecular bases of the observed phenotypes.

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15

Metabolic changes through a bacterial enzyme Maleate Isomerase in transgenic Tomato plants reveal the role of organic acids in fruit quality and crop growth

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Organic acids are of fundamental importance in all plant species. They have been clearly described to have roles as important as photosynthate, energy production, carbon storage, biosynthesis of amino acids, stomatal conductance, and plant-microbe interactions (REF). In addition to these varied roles, organic acids are important for taste, being responsible for sourness and contributing to the flavour. Acidity is also one of the main ripening indices that determines the harvest date of fruits. But, in addition, in the fruit of tomato has shown that the levels of this acid are related to the maintenance of the quality of the fruit in post-harvest [1,2].

Understanding the mechanistic basic of ripening regulation and postharvest has been the focus of industry. In particular, tomato (Solanum lycopersicum) fruit has special importance since it is one of the most important horticultural crops worldwide (http://faostat3.fao.org/home/E). Also, tomato has emerged as the pre-eminent experimental model for studying fleshy fruit, including the developmental control of ripening and ethylene synthesis and perception[3,4] .

In this study, we investigated the photosynthesis and primary metabolome, of leaves and fruits jointly with ripening-related gene expression of fruit from transgenic tomato plants overexpressing a bacterial maleate isomerase gene to better understand the factors that influence the concentration of two important acids, fumarate and malate, in fruit and plant.

In the transgenic plants we observed dwarf phenotype, flowering delay, and alteration in postharvest life. Furthermore, metabolomics analysis allowed us to assess the changes of amino acids, sugars and organic acids during fruit ripening and leaf development of the transgenic plants indicating a pivotal role of malate and fumarate, as regulatory metabolites.

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RNA Processing Bodies shape Cauliflower Mosaic Virus Infection

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Plants can rapidly alter mRNA homeostasis and translation when adapting to sudden biotic and abiotic stresses. Processing bodies (PBs) are cytoplasmic ribonucleo-protein granules involved in various mRNA quality control processes. The multifunctional nature of these foci is highlighted by the enrichment of diverse RNA decay associated proteins, including deadenylases, exonucleases, microRNA-mediated silencing factors, nonsense mediated decay proteins and the mRNA decapping machinery. PBs have been shown to be targeted by various viruses in the mammalian field and several components were found to serve pro- or antiviral purposes functions in addition to being direct targets of virus manipulation. Whether PBs have a similar importance during plant virus infection has remained largely elusive. We found that upon Cauliflower Mosaic Virus (CaMV; family Caulimoviridae) infection, the number of PBs was drastically increased. CaMV is a plant pararetrovirus with a dsDNA genome that infects Arabidopsis thaliana. In plant mutants lacking specific PB components, virus accumulation was decreased and disease was attenuated when compared to wild type. Intriguingly, the CaMV P6 protein was found to co-localize with the PB marker Decapping protein 1 (DCP1). During viral infection, the P6 protein accumulates in large cytoplasmic aggregates termed viral factories. These factories are considered sites of viral replication, translation and virion assembly. We observed that PBs cluster together with these aggregates. Future work will elucidate the underlying mechanism by which PBs support or restrict CaMV infection, possibly involving regulation of viral RNA functions.
The role of PBs in mRNA degradation and storage and their interplay with other cellular compartments is still poorly understood. Gaining insight into how viruses utilize these foci for their own benefit will not only improve our knowledge of plant-virus interactions, but also shed light on the canonical functions of plant PBs.

17

Transcriptional profiles of 375 individual meristems by single cell genomics technology capture divergent and unexpected expression dynamics

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The transition-to-flowering of shoot apical meristems (SAM) is a tightly regulated developmental process. The molecular programs that drive this process, however, are still largely unknown. In the tomato SAM, which is comprised of 1000-1500 cells, the shift from a vegetative to reproductive growth is accompanied by only mild and gradual morphological changes. In this study, we adapted sensitive single cell protocols to characterize transcriptomes of single meristems at high temporal resolution, focusing on a 3-days developmental window along the transition-to-flowering axis. We utilized barcoded RT-oligos to scale up the number of samples, and aggregated several libraries from each meristem in order to gain coverage depth. Surprisingly, data from WT meristems identified small cohort of genes (including the transition markers PUCHI and LOB30) to be either missing or highly expressed in individual SAMs, suggesting the existence of a switch-like regulatory mechanism. Comparison to pooled SAMs validated similar averaged expression trends, but found transcriptional programs activated throughout the process to display variable dynamics. We also identified surprising inter-meristem variability orthogonal to the developmental stage, suggesting the existence of additional coordinated programs within the SAM. Re-examination of the process under perturbed conditions or genetic backgrounds may highlight the temporal patterns necessary for flowering. In conclusion, we developed a new experimental pipeline to capture transcriptional dynamics of a process at high temporal resolution, which can be implemented in a wide range of developmental and physiological contexts.

18

Examine the role of benzoxazinoids in plant defense mechanisms and its synergistic effect coupled with insect pathogens against biotic stresses

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Plants in nature are continuously challenged by insect herbivores. To deter their attackers, minimize pest damage, and preserve their fitness, plants produce specialized defense metabolites. In agriculture, some of these second metabolites have been undertaking to use as biopesticides like terpenes or neem oil. In cereal, economically important crops, the main defense metabolites are benzoxazinoids (BX(.

Although some of the biopesticides are very successful, their abilities are limited due to the environment affect and there on active limitation. In our research, we would like to increase the biopesticide effect combining insect pathogens and secondary metabolite application. This bioassay will be practice on wheat plants that accumulate different amount of BX due to Virus-induced gene silencing system (VIGS). After accruing the mutation, we will apply on the wheat plant the bird cherry oat aphids (Rhopalosiphum padi) and different insect pathogen. This will allow us to detect synergism action with the pathogen and the BX levels and measure the changes in the aphid reproduction.

This research will add to the fundamental knowledge about benzoxazinoid role in metabolic defense mechanisms. It will also open anew possibility for biocontrol that can combine insect pathogen and second metabolite to increase the effectivity of insect control.

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Characterization of AtDGK2 in relation to Contact Site

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Contact Sites are evolutionarily conserved cellular microdomains where two membranes of different organelles are very close (typically 10-30 nm) without fusion. Contact sites between the endoplasmic reticulum (ER) and the plasma membrane (ER-PM CS) have been described to play important roles in specialized metabolic functions such as ER-PM communication, lipid homeostasis, and Ca2+ influx. Our group has identified AtDGK2 (Diacylglycerol kinase 2, At5g63770) as an interactor of Synaptotagmin1 (SYT1, At2g20990), which is protein located at ER-PM CS.
Diacylglycerol kinases are proteins that phosphorylate DAG (diacylglycerol) to produce phosphatidic acid (PA), both important signalling molecules. In response to a stress stimulus phospholipase C (PLC) is activated at the plasma membrane to hydrolyse PIP(4,5)P2 or PI4P in order to generate DAG and PI3 or PI2 respectively. DAG molecules, in turn, can be phosphorylated by DGKs. Of the seven AtDGKs encoded in Arabidopsis thaliana genome, only AtDGK1 and AtDGK2 have a transmembrane domain that anchors them to the endoplasmic reticulum while the rest are cytoplasmic. AtDGK2 is induced by exposure to low temperature, pointing to a role of this protein in cold responses. Using confocal microscopy we have analysed the subcellular localization of these two proteins and investigated their interaction with SYT1 using co-immunoprecipitation studies. We are generating over-expression lines, and determined that dgk2 and dgk2/syt1 show reduced freezing tolerance than wild type plants. Additionally, we report that DGK1 is a recessive lethal gene. Our studies suggest that DAGK1 and DGK2 act in concert with SYT1 to regulate the production of PS at ER-PM CS and highlight the importance of these proteins for the correct response to stress tolerance.

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Will Die Slowly family in Arabidopsis development and responses to osmotic stress and their interaction with mitogen-activated protein kinase pathway

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Will Die Slowly (WDS) gene family was identified from genomic analysis and was suggested to have anti-cell death and anti-senescence functions by phenotypic analysis of single or double mutants. wds1 plants started to show signs of leaf senescence sooner than the wild type. RNA integrity was fully maintained during the early stage of SA-induced cell death. The lack of remarkable differences between the phenotypes of WT, wds1, wds2 and wds1/wds2 plants indicates that WDS1 and WDS2 do not mediate cell death pathways that are responsible for shaping the architecture of the Arabidopsis plant. The expression level modulated at different developmental stages, reaching a peak when 50% flowers have opened. WDS1 OE lines showed enhanced tolerance to osmotic stress. WDS1 is predicted to be located in the cytoplasm, to contain three possible ERK docing sites, to interact with a protein that appears to function in the repression of genes that may induce apoptosis via a mitochondrium-medated pathway and also a rpotein that seems to be part of an ubiquitin ligase complex.

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Aroma map in European woodland strawberry

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Woodland strawberry (Fragaria vesca, 2x) is the diploid closest ancestor of the cultivated strawberry (Fragaria ´ annassa, 8x) and the model species for genetic studies in the Fragaria genus. It is naturally distributed all across Europe and it is appreciated for their delicate aroma and flavor. Aiming to describe the genetic and organoleptic diversity of European woodland strawberry and decipher the genetic control of its characteristic volatile compounds, we have sequenced and metabolically-phenotyped a diverse collection of 199 geographically distant European accessions. The metabolic profiling of the lines includes a set of 100 unambiguosly identified volatiles.

This study has revealed genetic and metabolic differences between subpopulations with different geographical origin. In addition, Genome Wide Association Analysis points to several candidate genetic regions controlling the accumulation of volatiles compounds sharing common biosynthetic pathways. Specifically, we have detected SNPs associated to the accumulation of methyl ketones and their corresponding alcohols mapping to a small region of chromosome 4 with a reduced set of candidate genes.

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Design of multiplexing CRISPR/Cas9 constructs for plant genome editing using the GoldenBraid DNA assembly standard

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Due to the huge potential of CRISPR/Cas9 for synthetic biology and genome engineering, many plant researchers are adopting this technology in their laboratories. CRISPR/Cas9 allows multiplexing of guide RNAs (gRNAs), therefore targeting several loci in the genome simultaneously. However, making DNA constructs for this purpose is not always straightforward for first-time users. We developed a set of plasmids and a set of software tools for facilitating the assembly of multiplex CRISPR/Cas9 constructs using the GoldenBraid (GB) DNA assembly system.

As an example, we targeted the best six candidate genes encoding for the N-methyl putrescine oxidase 1 (MPO1) in Nicotiana tabacum cv. K326 using eleven gRNAs. MPO1 is an enzyme that supplies the pyrrolidine moiety of nicotine and is dispensable on the biosynthesis of other alkaloids, such as anatabine, that do not contain pyrrolidine moiety. Tobacco transformation with a plasmid including a 6X and a 5X polycistronic gRNAs along with the Cas9 using Agrobacterium rhizogenes resulted in hairy roots with a variety of profiles of edited genes. Most of the roots showed increased levels of anatabine and reduced levels of nicotine reaching maximum levels of 18 mg of anatabine per gram of dry matter, 300 times higher than in wild type.

GoldenBraid facilitated the assembly of a 11X multiplex CRISPR/Cas9 construct that lead to hairy roots with multiple profiles of edited genes and different nicotine to anatabine ratios. Correlation of the phenotype and genotype data helped on the identification of the MPO1 genes playing a key role on the alkaloids biosynthesis in tobacco.

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The scope of microRNAs expression changes during chilling stress in soybean

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Soybean is an annual legume grown for its edible seeds. Exceptional nutritional value of soybean, with high protein (40%) and oil (20%) contents make it one of the staple crops. Political situation in EU greatly hampers the cultivation of GM crops, meanwhile extensive meat production enforces the need for substantial import of soy meal mainly from Argentina. Additionally, in Polish climate soybean is exposed to chilling stress, which impairs yield quality. Thus, in order to provide a sustainable source of soybean we must turn to other solutions, namely development of chilling resistant cultivars. We aimed to decipher the role of miRNAs and their target genes in plant chilling stress response, by determining the changes in their expression levels. Diverse soybean cultivars were employed for the comprehensive investigation of stress response molecular basis. Small RNAs were isolated from explants of soybean cultivated in stress and control conditions. Harvested samples consisted of roots and leaflets from seedlings and trifoliates of plants at vegetative growth stage (V1). MicroRNAs associated with chilling stress response, chosen based on the review of the literature, were used in the initial analysis of expression levels. Furthermore, gene ontology analysis offered candidates for target genes of studied miRNAs. Confirmation of the differential expression of miRNAs was performed using high throughput sequencing of 72 small RNA libraries (accounting for three biological replicates). Additionally, degradome sequencing was conducted in order to analyze the RNA degradation patterns and confirm the activity of miRNAs.
The work is supported by a grant no. UMO-2014/15/B/NZ9/02312 from National Science Centre, Poland and the Ministry of Science and Higher Education of the Republic of Poland by the KNOW program.

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The polyamine transcriptome in Arabidopsis reveals H2O2, EDS1, NPR1 and salicylic acid - dependent gene expression sectors related with stress.

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A common metabolic hallmark of plant stress is the accumulation of certain polyamines. This accumulation has been associated with enhanced tolerance to different types of abiotic and biotic stresses. Despite this body of evidence, the signaling pathways by which polyamines exert their functions remain illdefined.

Here, we have analyzed by RNAseq the gene expression changes triggered by natural and synthetic polyamines in Arabidopsis thaliana. For this, we have treated wild-type Arabidopsis seedlings with 100 µM putrescine, spermidine, spermine, thermospermine, cadaverine or the synthetic polyamine 1,7-diaminoheptane. Our results indicate that most transcriptional responses are conserved between polyamines, although they exhibit quantitative differences. Analysis of the transcriptional responses to exogenously supplied putrescine in the presence of the hydrogen peroxide scavenger DMTU (dimethylthiourea) identified the ROS-dependency of such responses. This analysis has been complemented with the identification of EDS1, NPR1 and salicylic acid - dependent gene expression sectors in response to putrescine, the polyamine which exhibits the highest increases in most types of abiotic and biotic stresses. Our results help in the identification of components involved in the polyamine signaling pathway in plants.

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Alterations in the stomatal abundance affect the physiological performance of plants grown under optimal and supra-optimal temperatures

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Stomata are dynamic valves that regulate gas exchange between leaves and the atmosphere. Stomata number, size and distribution determine the maximum potential for gas exchange, influencing transpiration, photosynthesis and plant fitness under different environmental conditions. In Arabidopsis, stomatal density (SD) is a complex character determined by a gene network that regulates stomatal development during leaf growth and in response to environmental cues. Using mutants for a panel of these genes, we examined the effect of alteration of SD on indicators of plant fitness under optimal growth temperature. High SD and aberrant stomatal spacing patterns negatively affect plant fitness (in terms of PSII efficiency and non-photochemical quenching), even under no water shortage. On the other hand, a lower SD does not seem to have a significant impact on physiological performance. We also evaluated the capacity of selected genotypes to adapt to supraoptimal growth temperatures. Under no water restrictions, increased SD could alleviate the inhibition of photosynthesis caused by stomatal closure. Moreover, decreased SD, when accompanied by certain compensatory mechanisms, could also provide some advantages over the wild type.

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Recreation of allopolyploids by horizontal genome transfer to gain insights in speciation and genome evolution

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Allopolyploidization, the merger of two genomes of different species, is known to be a driver of speciation and a major source of evolutionary novelty. Allopolyploidization has contributed greatly to the rise and the astonishing diversification of angiosperm plants including modern crops. Despite its significance in plant evolution and crop domestication, the mechanisms of polyploid formation, their subsequent evolution and adaptation to the environment are still poorly understood. It is generally believed that allopolyploids arose by hybridization between species. In fact, several polyploid plants have been recreated by crossing their known progenitors. However, this path is restricted to sexually compatible plants. We have recently discovered that grafting, a commonly occurring physical interaction between plants in nature, can lead to nuclear genome transfer, thus providing an alternative asexual allopolyploidization mechanism. We now want to recreate allotetraploid species and produce new synthetic allopolyploids in the genus Nicotiana using grafting-mediated horizontal genome transfer. This will enable us to characterize the early genomic, transcriptomic and metabolomic changes in newly evolved allopolyploids. To facilitate efficient selection for horizontal genome transfer, we have established transformation protocols for a panel of diploid Nicotiana species. Progress with the generation of novel polyploid Nicotiana species will be reported.

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The CRISPR/Cas9 system is an efficient tool to silence microRNAs (miRNAs) in rice plants

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MiRNAs are small non-coding RNAs of 21-24 nucleotides in length that regulate gene expression at post-transcriptional level by triggering cleavage or translational inhibition of target mRNAs. Plant miRNAs have been described to regulate multiple biological processes, including developmental processes and adaptation to environmental stresses. The functional characterization of a miRNA of interest requires the use of gain- and loss-of function approaches. However, because of the small size of MIR genes, identifying mutant alleles for miRNAs in insertional mutant collections is unlikely. Alternative techniques have been developed to study miRNA function, such as interference with miRNA activity through target mimicry. Nowadays, the CRISPR/Cas9 (clustered regulatory interspaced short palindromic repeat-associated nuclease 9) technology opens new possibilities to knock-out expression of MIR genes, an aspect that still remains poorly explored. Among many other applications, the CRISPR/Cas9 system allows the functional analysis of individual miRNA family members. In this work, a two single-guide RNA (sgRNA) CRISPR/Cas9 system has been successfully used to create mutations in the rice MIR399f and MIR827 genes, which are known to be key players in the phosphate starvation response in Arabidopsis. The sgRNAs were designed to target regions within miRNA precursor. Mutations induced by CRISPR/Cas9 in 46% of T0 rice plants included 87 or 67 nucleotides deletions and 25 nucleotides insertions, which can disrupt miRNA precursor structures, hence miRNA function. MiR399- and/or miR827-mediated alterations in phosphate content might be an important factor in plant performance and productivity. Results obtained in this study demonstrated that using the CRISPR/Cas9 technology has important applications for targeted mutagenesis of MIR genes in rice, a species of evident agronomical interest.

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Global proliferative arrest control in monocarpic plants: searching for new members of the FUL/AP2 pathway

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A large proportion of economically important crops belong to the group of monocarpic plants. In monocarpic plants, after the production of a certain number of fruits, the activity of the shoot apical meristem (SAM) and of all the meristems of the secondary axes stops in a coordinated way, a phenomenon called Global Proliferative Arrest (GPA). In studies carried out in the model plant Arabidopsis thaliana, some factors have been shown to affect the moment in which GPA occurs. The most important factor seems to be the production of seeds, as sterile mutants or those with reduced fertility produce many more flowers than fertile ones. On the other hand, there is evidence that there is also a genetic control of GPA dependent on the age of the plant mediated, at least in part, by the antagonistic action of FRUITFULL and APETALA2-like genes in the SAM. We intend to investigate which are the genetic bases that determine how long the plants are able to produce flowers and fruits, in order to identify which are the genes with the potential to increase the productivity of the crops through their genetic modification. Our goal is to delay the proliferative arrest of the meristems, and therefore, to obtain plants with a longer life cycle that produce more fruit and/or seeds. Previous work in our laboratory has identified some genes working upstream and downstream the FUL/AP2 module that could be related with GPA control. Currently we are characterizing the potential role of these genes in this pathway by mutant phenotypic characterization, expression analyses and determination of regulatory interactions among them to confirm their relationship with the FUL/AP2 pathway and to assess their biotechnological potential to design new strategies for GPA control.

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Plants as factories of antimicrobial peptides

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The increase in multidrug resistance bacterial and fungal infections has become a major concern to human health and global food security. Antimicrobial peptides (AMPs) might contribute to alleviate the problem of resistance and the shortage of effective compounds because of their potent and durable activity against a broad spectrum of pathogens. However, their commercial exploitation is limited today by the low yield from natural source purification, by the high cost of their chemical synthesis, and by the difficulties to produce them through biotechnology. Here, we present the development of a plant-based platform for fast and high efficient production of AMPs. The system uses a viral vector for transient and high expression of AMP genes in Nicotiana benthamiana leaves. We also show that the AMP targeted accumulation to cellular apoplasts reduces their host toxicity and allows high production levels, as well as it facilitates downstream purification. Using this plant production platform, we produce different types of AMPs, including the highly active bactericide cecropin A, the cell penetrating PAF102 antifungal peptide and the cysteine-rich antifungal AfpB peptides. Our results show that yield is highly dependent on the AMP host toxicity and stability, but transient expression seems the best option for producing these bioactive peptides. We also demonstrate that AMPs produced in plants are fully active against target pathogens, thus supporting the idea that plants can assist the AMP biotechnological production to bring them to the market for multiple applications.

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Exploring the role of brassinosteroids in the primary root growth and development of the primary embryonic root of Sorghum bicolor

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Brassinosteroids (BRs) are steroid hormones essential for plant growth and development as well as for the adaptation to drought. A wealth of studies report the importance of BRs in superior plants1, mostly Arabidopsis. How BRs modulate root formation and adaptation to abiotic stress in monocot cereals awaits to be understood.
In this study, we explore the role of BRs in root growth and development in cereal Sorghum bicolor. We focus on the analysis of embryonic roots by implementing a set of in vitro and microscopy techniques, such as mPS-PI and EdU staining2. Our analysis reveals the organization of the primary root in how different cell types at the root apex behave in response to BRs and abiotic stress3. In addition, we used a mutant collection of Sorghum bicolor4 to identify a number of mutants in BR signalling components. Mutant analysis will be key to begin to decipher the role of BRs in sorghum primary root growing in normal and stress conditions, and will be instrumental for their future improvement5.

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Epigenomic control during the plant-nematode interaction in Arabidopsis

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Plant-parasitic nematodes affect all major crops causing high agricultural loss worldwide (Nicol et al., 2011). Within them, the root-knot nematodes (RKN), Meloidogynespp., are sedentary obligate parasites that feed from their host plants to complete their life cycles (Bird, 1962; Escobar et al., 2015). RKNs form galls in the roots where they induce specialized feeding cells, named giant cells (GCs) by the action of nematode effectors in the vascular tissues (Escobar et al., 2015). The global gene repression observed in Arabidopsis galls at early stages of development, that include plant defense genes (Barcala et al., 2010) is at least, partially regulated by small RNAs (sRNAs), such as miR390, miR172, and miR159 in Arabidopsis that repress ARF3, TOE1 and MYB33, respectively (Cabrera et al., 2016; Diaz-Manzano et al., 2018; Medina et al., 2017).Itis well documented that RNA directed methylation (RdDM) represses plant defense-related genes in biotrophic plant–pathogen interactions, such as bacteria and fungi (Yu et al., 2013; Dowen et al., 2012; López et al., 2011), yet,knowledge of DNA methylation pathways responding to nematode infections is still very scarce (Hewezy et al., 2017).

Our holistic analysisof RNA and DNA extracted simultaneously from the same biological replicates of galls and control root tissue (Da Silva et al.,2019; submitted) showed that at early infections times, the general gene repression observed, correlates with the increase of rasiRNAs preferentially located in pericentromeric region (Ruiz-Ferreret al.,2018) and a hypermethylation of DNA in galls compared to control roots. Consequently, retrotransposon (COPIA, GYPSY, LINE and SINE) superfamilies were drastically repressed (Ruiz-Ferrer et al.,2018). Infection tests with M. javanicain Arabidopsis mutants defective in key functions from RdDM pathways revealed that the concerted action of different epigenetic regulatory mechanisms (canonical and non-canonical RdDM), mediate alteration of gene expressionafter M. javanicainfection.

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Diversifying biosynthesis and storage capacity of carotenoids in plants

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Many specialized plant metabolites, including terpenoids, are valued for their function as nutrients, pharmaceuticals and fragrances. Specialized terpenoids are often biosynthesized by the plants in very small amounts, in specific tissues and/or only under certain (a)biotic stress conditions. To reach the full potential of these metabolites it is essential to develop methods to boost their biosynthesis. However, modifying a plant to produce any metabolite in large amounts can have toxic effects on the plant. This is especially true for metabolites whose production or/and accumulation can interfere with the metabolic homeostasis of the plant. Hence the capacity of the plants to store these valuable metabolites may need to be increased simultaneously.
The focus of the presented research is on terpenoid metabolites derived from C20 geranylgeranyl diphosphate (GGPP), e.g. the C40 tetraterpenes such as the health-promoting carotenoid pigments. The biosynthesis of these tetraterpenoids includes the production of GGPP in the plastids followed by further downstream steps also in the plastid. Previous results have shown that plant cells can be engineered to produce carotenoids in the cytosol by using a virus to transfer bacterial carotenoid genes. However, high carotenoid levels caused necrosis in the leaves, which was speculated to arise because of the absence of proper storage structures (e.g. vesicles or lipid bodies). In this project, the supply of GGPP in the cytosol is increased by simultaneous expression of a GGPP synthase and the key step in the MVA pathway, HMGR. As a proof of concept, two carotenoids, phytoene and lycopene are studied, as well as two systems of potential storage structure for carotenoids. The first system is lipid bodies produced during ER-membrane proliferation and the second system is production of oil bodies normally produced in seeds. These lipid/oil bodies may provide a proper storage compartment for extraplastidial carotenoids.

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‘Able to talk again?’ - A screen of gun1 suppressors to identify factors in chloroplast retrograde signaling.

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Plant development is influenced by multiple internal and external factors. Among these, light is of utmost importance due to its function as source of energy and also as an information signal. After the first light exposure, seedlings undergo deetiolation, whereby development switches from skotomorphogenic to photomorphogenic, characterized by the opening and expansion of the cotyledons and chloroplast development to support autotrophic growth. This process is accompanied by a massive reprogramming of the transcriptome. However, an excess of light can cause chloroplast damage, triggering a retrograde signal (RS) to the nucleus that suppresses photomorphogenesis by inhibiting the switch in gene expression that takes place during deetiolation. In this process, the chloroplast-localized protein GENOMES UNCOUPLED1 (GUN1) is a key factor mediating the RS, as gun1 mutants do not show the suppression of photomorphogenesis after chloroplast damage. However, how GUN1 mediates the RS transmitted to the nucleus remains unclear. We are performing a gain-of-function approach using the FOX (Full-length cDNA Over-eXpressing) gene hunting system (a library of about 10.000 independent cDNAs expressed under the 35S promoter) to identify suppressors of the gun1 mutant phenotype. These suppressors will potentially correspond to factors downstream of GUN1 in the RS pathway that are able to restore the communication between the chloroplast and the nucleus in the gun1 mutant.

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Chloroplast retrograde signaling and regulation of rice seedling development

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Retrograde signaling from chloroplast to nucleus has been reported to be critical to adjust chloroplast biogenesisand modulate growth and development in Arabidopsis1. GENOMES UNCOUPLED 1 (GUN1), a chloroplast-localized pentatricopeptide-repeatprotein, integrates multiple signals derived from plastid and takes an importantrole in retrograde signaling communication2. Furthermore, GUN1-mediated retrograde signaling pathwaysregulate early seedling photomorphogenesis under excessive light condition by suppressing GOLDEN2-LIKE 1(GLK1) expression1. In contrast, whether chloroplast retrograde signaling regulates seedling photomorphogenesis in monocotyledonsis less studied.
In rice, many pentatricopeptiderepeatproteins have been found to be required for chloroplast development, seedling growth and abiotic stress response3,4. In addition, OsGLK1 has been described to regulatechloroplast development under the control of light and phytohormones5. These results suggest that GUN and GLK may regulate chloroplast development by a mechanism conserved in Oryza sativaand Arabidopsis. Our experimental system and recent data on the role of retrograde signaling in light-regulated rice developmentwill be discussed.

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Development of Molecular Markers for Fruit Skin Color in Japanese Plum (Prunus salicina Lindl.)

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Japanese plum is a diploid fruit tree species, member of the Rosaceae family, generated by hybridization of Prunus salicina with diverse Prunus species. Cultivars show great variability for fruit skin and flesh color, which are both major objectives in plum breeding. Subsequently, molecular markers for early selection of these traits in breeding programs are highly desirable. Despite candidate genes for fruit color have been identified in several Rosaceae species, no markers have been described for Japanese plum yet. In Rosaceae family, MYB10 transcription factor has been described as the main gene determining anthocyanin pigment accumulation, which is responsible for red, purple and black coloration. In order to design a useful marker for marker-assisted selection (MAS), we have explored the variability of the MYB10 gene group in Japanese plum and its association with fruit color. Primers designed in peach (Prunus persica) conserved MYB10 domains were used to genotype a collection of P. salicina cultivars and several progenies. Allele cloning identified 12 MYB10 amplicons. Homology and segregation analysis in progenies allowed assigning some of them to five loci, homologous to the three MYB10 genes located in peach LG3, suggesting the duplication of the MYB10.1 peach gene. Whole genome-resequencing with Illumina technology of two varieties with contrasting phenotypes allowed full gene cloning and detection of polymorphisms. Our data identified one dominant allele associated with anthocyanin accumulation in the skin and two other recessive alleles associate with yellow/green skin phenotypes. Current work is focusing in the design of a marker specifically targeting the associated alleles. This marker will be validated in a wider germplasm collection of commercial varieties and segregating progenies. Future assembly of long-range reads of a red variety will give more insight into the complexity of the region, and will serve for other P.salicina genomic studies.

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Transcriptomic analysis of the interaction geminivirus-tom

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Geminiviridae family is one of the main families of plant pathogenic viruses with large relevance as they cause great losses worldwide in commercial crops and crops destined to food production. Geminiviruses present a little single-stranded DNA genome and a capsid composed of two twin icosahedral parts. Tomato Yellow Leaf Curl Virus (TYLCV) belongs to the Begomovirus genus and is transmitted by the whitefly Bemisia tabaci. With only 6 viral proteins, this geminivirus must create a proper environment for viral replication, transcription and propagation. Behind the apparent simplicity of geminiviruses lies a complex network of molecular interactions with their host and even their natural vector, which induces a wide variety of transcriptional, post-transcriptional and chromatinic changes in both the plant and the geminivirus. In order to study these changes and decipher the effects of the transmission vector on the infection, we carried out a global approximation of the TYLCV-tomato interaction to generate integrated single-base resolution maps by NGS (next-generation sequencing) of the transcriptome, smallRNAome and methylome of the pathogen and the host.

Tomato plants (Moneymaker) were infected with TYLCV under controlled conditions of light and temperature using Agrobacterium tumefaciens or its natural vector. Apical tissue from these plants was collected at different time points (2, 7, 14 and 21 days after inoculation), and three biological replicas were generated for each treatment and time. Total RNA and DNA was extracted and analysed by RNA-Seq, smallRNA-Seq and Bisulfite-Seq. The transcriptome of the tomato-TYLCV interaction will be presented and discussed.

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Alternative splicing of PIN auxin efflux carriers

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Majority of Arabidopsis genes are alternatively spliced. However, role of alternative splicing (AS) in plant development is poorly understood. We have chosen two closely related auxin efflux carriers, PIN4 and PIN7, as they both undergo the same type of AS resulting in two transcripts and serve as an important factors crucial for auxin-mediated plant morphogenesis.
These mRNAs (termed a and b) are conserved within rosales and show comparable levels in available profiles. PIN7 isoforms derived from their cDNAs show similar auxin transport properties in BY-2 cell culture system. While PIN7a cDNA is able to complement almost all mutant phenotypes PIN7b rescued mutant phenotypes in lesser extent. Utilizing confocal microscopy, known drugs interfering with subcellular dynamics of PIN proteins and FRAP, we show that the two isoforms show different intracellular dynamic and likely employ different vesicle trafficking pathways.
We also designed system for visualization of PIN7a and b expression at the tissue level. This system revealed differential levels of PIN7a and PIN7b in the root pericycle and in the hypocotyl epidermis during apical hook formation. Together with differences in complementation tests that suggests different function of PIN7 isoforms within developmental processes.
In addition, we developed a genetic screen which aims to find upstream factors required for regulation of the AS event described.

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The SALT-HYPERSENSIVITY (SHE) gene is required for salt tolerance in tomato via regulating long-distance Na+ transport

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Abiotic stresses, especially salinity and drought, have a huge effect on agriculture by reducing crop growth and productivity worldwide. Hence, the development of crop varieties able to maintain optimal growth and yield under abiotic stress conditions is a critical issue. With the aim of better understanding the molecular mechanism underlying abiotic stress tolerance in plants, we performed an in vitro phenotypic screening assay to identify mutants with altered salt stress responses in a T-DNA mutant collection of tomato (Solanum lycopersicum), a crop species of the greatest socio-economic importance. As a result, we have identified two new recessive mutants showing a salt-sensitive phenotype, named salt-hypersensivity1 (she1) and she2. Subsequently, salt sensitivity of the selected mutant lines was corroborated in vivo by growing them under control and salt stress conditions in greenhouse. Molecular analyses indicated that the she phenotypes are not associated with a T-DNA insertion, suggesting that somaclonal variation during tissue culture was responsible for the mutant phenotypes. To identify the mutations that underlies the she1 and she2 loci, we performed mapping-by-sequencing on F2 populations derived from the cross between a mutant plant and the wild tomato S. pimpinellifolium accession (LA1589). Allele frequency and SNP variant analyses revealed two different protein-truncating mutations in the same gene, which codes for a respiratory burst oxidase. The allelic nature of she1 and she2 mutations was further confirmed by a genetic complementation test.

The Na+ toxicity triggered swelling and chlorosis in the she leaves from the first day of salt stress treatment, and finally caused plant death after several days of stress. A significant higher Na+ accumulation was observed in she leaves under salt stress, which was not associated with a lower retention capacity of Na+ in roots. Furthermore, reciprocal grafting experiments revealed that the salt-sensitive phenotype of she plants is driven by roots. Thus, the she mutant shoot grafted on the wild-type root exhibited normal growth and a rate of leaf Na+ accumulation similar to that found in wild-type plants. Class I HIGH-AFFINITY POTASSIUM TRANSPORTERS (HKT1) proteins are critical determinants of Na+ unloading from root to shoot through xylem. Gene expression analysis revealed that tomato HKT1-like genes (SlHKT1;1 and SlHKT1;2) were down-regulated in she mutants compared with wild-type plants, both under control and salt stress conditions. Hence, results suggested that SHE participates in tomato salt-stress tolerance mechanisms by regulating long-distance Na+ transport from root to shoot through SlHKT1 activity modulation.

Genome-wide analysis of DICER-LIKE1 substrates

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Dicer RNase-III endonucleases process long double-stranded RNA (dsRNA) into small-RNA (sRNA) duplexes bearing 2-nucleotide (nt) 3′-overhangs and 5′-monophosphates. Both metazoan and plant DICER-LIKE (DCL) proteins display a multi-domain organization including DExD-box, helicase-C, PAZ, RNase-III, and dsRBD domains. Arabidopsis genome encodes 4 different DCLs genes. While DCL1 process imperfect stem-loops to release discrete 21-nt microRNAs (miRNAs), Arabidopsis DCL2, DCL3, and DCL4 process long, near-perfect dsRNA substrates into populations of 22-, 24-, and 21-nt small interfering RNAs (siRNAs), respectively. Although plants DCLs have central roles in the biogenesis and functions of sRNAs their dsRNA substrates have never been directly isolated, instead, they were indirectly inferred by mapping sRNA sequencing data to the corresponding genomic regions-of-origin. To address these key issues, we have introduced point-mutations in the RNAseIII domains of full length DCL1 to produce catalytically deficient-proteins and, hence, biochemically stalled complexes facilitating the systematic analysis of DCL1 substrates. Upon formaldehyde crosslinking in vivo, dcl1-7 mutant plants with Wild-Type (WT) or catalytically inactive (Ci) versions of DCL1 protein, were used to perform RNA-immunoprecipitation (RIP). DCL1-Ci RIP showed a higher stabilization of RNA substrates complex than DCL1-WT RIP. RNA sequencing analysis of DCL1-Ci/dcl1-7 RIP allowed the discovery of novel DCL1 substrates in addition to the near-entirety known miRNAs precursors (20% of total IPed regions) described in the literature. These include intergenic regions (24%), protein-coding gene mRNAs (25%) and transposable element (TE) (26%). Aside significant number of new miRNAs detected on intergenic regions, we were able to establish two novel functions of DCL1: (i) TE silencing through the production of 24nt sRNA and (ii) regulation of protein-coding genes by interaction with hairpins located in the 5′-UTRs.

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Dynamics of rna silencing suppression upon pathogen infections

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RNA silencing is an antiviral defence mechanism in plants and animals. In order to evade that layer of defence, viruses produce counteracting effectors, known as silencing suppressors. It has been recently found that pathogens other than viruses, such as bacteria and oomycetes, also produce silencing suppressors as part of their infection strategy. Despite of that evolutionary convergent strategy, the ways those different classes of pathogens infect and spread in the host are diverse. Those dissimilarities might encompass a differential regulation of the production of their specific silencing suppressors and the host cells target of their action. The intracellular presence of silencing suppressors does not only disrupt a host´s defence system but interferes with other endogenous processes orchestrated by this universal gene regulatory system. Of especial interest is their impact on micro RNA-mediated gene regulation. Plant miRNAs tend to regulate the expression of genes with pivotal roles in plant development and stress responses. Since the repertoire of miRNAs and their targets is cell-type specific, the molecular events conducting to effector-mediated transcriptional reprogramming in plant cells might differ. Establishing the dynamics of pathogen-triggered miRNA dysfunction is a first key step to broaden our knowledge about the subsequent molecular events led by pathogen-deployed silencing suppressors. To reveal at which point of the infections and in which cell-types different pathogens interfere with miRNA function, we have combined fluorescently-labeled pathogens and plants bearing a sensor of miRNA activity.
Results from experiments with Plump Pox Virus and the bacterium Pseudomonas syringae will be discussed.

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Dehiscence zone as a developmental timer for explosive seed dispersal in Cardamine hirsuta

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Adaptations for dispersal are ubiquitous in nature and fruits play an important role in the seed dispersal of flowering plants. Seed dispersal occurs via a process called pod shatter in both explosive fruit of Cardamine hirsute and the non-explosive fruit of Arabidopsis thaliana, and relies on the precise patterning of fruit tissues during development. The dehiscence takes place by physical separation of specialized tissues at the valve margin in both species. In A. thaliana, this occurs as the fruit dries out, separating the valve from the replum and exposing seeds for dispersal. In contrast to this, seed dispersal by explosive pod shatter occurs before the fruit dries out in C. hirsute where established pre-tension and accumulation of elastic energy drive the explosive coiling of the vales.

This raises the hypothesis that the developmental control of dehiscence zone formation may trigger the process of explosive seed dispersal in C. hirsute. To test this hypothesis, we have performed comprehensive genetic analysis of fruit patterning genetic network in C. hirsute. Mutation in valve margin regulator INDEHISCENT (IND) abolishes dehiscence zone formation. In chind mutants, the energy required for explosion is trapped due to inability of valve tissue physically separate from the replum. We also found that in C.hirsuta the function of FRUITFULL (FUL) is conserved. FUL specified valve tissue by repression of valve margin genetic network and ful mutants exhibit homeotic conversion of valve into valve margin. However, unlike A. thaliana ful, where the ectopic valve margin identity consists mostly of lignified cells, the ectopic valve margin identity in C. hirsuta ful consists mostly of separation layer cells. Collectively, these results suggest that the genetic network controlling valve margin identity in C. hirsuta shows conservation with A. thaliana but has been re-wired to set separation layer as predominant valve margin fate.

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Light-up aptamers as a tool for RNA imaging in plant cells

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After DNA, the RNA is the key biomolecule of the life. In plants like in animals its function includes interpretation of genetic information, structural support for molecular machines, regulation and silencing of gene expression, stress response, and many others. Monitoring in vivo is a first step in order to elucidate the molecular function in a specific cell; then, the ability to acquire complete spatial-temporal image of RNA synthesis, transport and processing in living cells is critical to understand molecular functions and patterns. RNA light-up aptamers can be used to detect and image RNA or specific metabolites, they are short RNA sequences that fold itself into specific shapes, bind a dye and confers fluorescence needed for imaging fragment of interest. This practical tool has been successfully used in bacterial, mammalian cells, and other models to in vivo monitoring mRNAs. Recently, our research group probe the useful of light-up aptamers in the chloroplast of Chlamydomonas reinhardtii, encoding RNA light up termed Spinach, transcriptionally fused to the aphA-6 gene and after incubation with 3,5-difluoro-4- hydroxybenzylidene (DFHBI) the transplantomic lines expressing aphA6/Spinach mRNA were observed, this evidence motivates our work approach to development of similar biomarkers in model plant Arabidopsis thaliana. Here, using a binary vector carrying light-up aptamers BabySpinach, Broccoli and Mango we can detected the mRNA of uidA gene (encodes GUS protein). After fluorescence microscopy analysis we conclude that these aptamers are a platform capable to observe the processing of different RNAs and we suggest that using light up aptamers in plants could be a tool development of biosensors in plant synthetic biology.

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Gene targeted insertion of the AP-MS tag in plant cells through a combined approach using CRISPR/Cas9

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One of the most advanced methods to investigate protein complexes is Affinity Purification of tagged proteins in combination with mass spectrometry of the purified complexes (AP-MS). In plants, protein tagging relies on expression of ectopic DNA. Interference of the native, untagged protein with tagged complex formation is decreased either by overexpressing the tagged bait protein, or by complementing null mutants for the bait. However, overexpression can lead to false positive results and complementation is not always possible. This is true in many crop plants, but also for essential genes where mutations would be lethal.
To address these problems we have developed an approach for AP-tag insertion through in planta gene targeting. So far, gene targeting through homologous recombination (HR) has been extremely inefficient in plants. It has been shown that the presence of double stranded breaks (DSB) can greatly increase the efficiency of HR. With the discovery of the CRISPR/Cas9 system (and TALENs before that) researchers acquired a tool to induce targeted DSBs.
Here, we show an approach based on a combination of targeted DSBs, homology arms and viral elements to achieve targeted tag insertion via HR. As proof of concept, Cas9 was delivered to Arabidopsis cell cultures via Agrobacterium mediated transfer, together with a gene specific gRNA and a donor template consisting of a GFP tag flanked by two sequences that are homologous to the 3'-part of the targeted gene. Cas9 induced a DSB in the targeted gene, which was subsequently repaired via homology directed repair, leading to targeted insertion of the AP tag.
Several small molecules and treatments have been shown to potentially increase HR efficiency. Using our Arabidopsis cell culture system and the in planta gene targeting approach, we are now setting up an assay to analyze the effect of different small molecules on HR efficiency.

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Is there a future for Gregor Mendel's constant hybrids in plant breeding?

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It is now clear that Gregor Mendel made a distinction between two types of hybrids: 1. Variable hybrids, like the garden pea (Pisum) and 2. Constant hybrids like hawkweed (Hieracium) (van Dijk and Ellis 2016). Variable hybrids produced offspring that differed from the mother hybrids. In contrast, constant hybrids produced offspring that were identical to the mother hybrids. Today it is known that constant hybrids reproduce by apomixis, i.e. the formation of asexual seeds. Apomixis does not occur in major crops. Genetic studies in natural apomicts demonstrate that apomixis can be transmitted by pollen grains in crosses with sexual relatives and that apomixis is controlled by a few dominant major loci. Attempts are underway to identify natural apomixis genes. Using this information it may be possible to introduce apomixis into sexual crops. Theoretically this would make one-step fixation of agriculturally important, but genetically complex traits possible (Van Dijk et al. 2016). At the moment we investigate proof of concept for a commercial trait. The opportunities of apomixis in future plant breeding will be discussed.

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The genetics of pollinator-mediated speciation

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The recruitment of animals to perform pollination services is a major innovation in angiosperm evolution. Animal-mediated pollination, however, is exquisitely vulnerable to fluctuations in pollinator availability, a problem that has become more urgent due to climate change. Plants can evolve to attract a new pollinator, a phenomenon that has happened repeatedly in many taxa. We use a combination of genetics, genomics, biophysics and behavioral ecology to study the molecular basis of such shifts in pollination syndromes in the genus Petunia (Solanaceae). Petunia comprises species that differ in color, scent, nectar and morphology, and that are adapted to pollination by bees, nocturnal hawkmoths and hummingbirds. We found that the genetic architecture of shifts in pollination syndromes is surprisingly simple. Even the modification of single genes can strongly affect pollinator preference and thereby cause reproductive isolation. Knowledge of the functional polymorphisms underlying shifts in pollinator preference can give insight in the process of speciation.
Sheehan et al. (2016) Nature Genet. 48: 159-166; Esfeld et al. (2018) Curr. Biol. 28: 3776-3786.

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DELLA proteins acted as transcriptional hubs in the ancestor of all land plants

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DELLA proteins are transcriptional regulators which have been shown to modulate the activity of over 150 transcription factors (TF) in Arabidopsis, involved in multiple physiological and developmental processes. DELLAs are proposed to transduce environmental information into pre-existing transcriptional circuits because their stability is regulated by gibberellins (GAs), whose homeostasis largely depends on environmental cues. The ability of GAs to promote DELLA degradation has been associated to the emergence of vascular plants. However, DELLA proteins are present also in early-diverging land plants, indicating that some of their functions might predate GA signaling. Previous in silico network analysis is suggestive of gradual acquisition, since DELLA emergence increased the connectivity between putative targets, and it was further increased when they became GA signaling elements. To investigate whether ancestral DELLA proteins act as transcriptional hubs, or this feature was gradually acquired during land plant evolution, we have analyzed DELLA structure and performed targeted DELLA-TF interactome studies in several species, combined with complementation analyses in dellaKO mutants. Our results indicate that DELLA's interactome was established early during land plant evolution, given that most of DELLA-TF interactions are conserved, while some particular interactions may have appeared and evolved by different protein-protein interaction co-evolutionary mechanisms. In summary, we propose that DELLA proteins already behaved as hubs in transcriptional networks in the ancestor of all land plants, while the strict conservation until present time provides a measure of the high biological relevance of DELLA's molecular promiscuity for plant survival.

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The polyamine putrescine contributes to H2O2 and RbohD/F-dependent positive feedback loop in Arabidopsis PAMP-triggered immunity

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Polyamines have been involved in defense against pathogenic microorganisms in plants. However, the role of putrescine during plant defense has remained elusive. In this work, we have studied the implication of polyamines during pathogen-associated molecular pattern (PAMP) – triggered immunity (PTI) in the model species Arabidopsis thaliana. Our data indicate that polyamines, and particularly putrescine (Put), accumulate in response to nonpathogenic P. syringae pv. tomato DC3000 hrcC and in response to the purified PAMP flagellin22. Exogenously supplied Put to Arabidopsis seedlings induces defense responses compatible with PTI activation, such as callose deposition and transcriptional up-regulation of several PTI marker genes. Consistent with this, we show that Put primes for resistance against pathogenic bacteria. Through chemical and genetic approaches, we find that PTI-related transcriptional responses induced by Put are hydrogen peroxide and NADPH oxidase (RbohD and RbohF) dependent, thus suggesting that apoplastic ROS mediates Put signaling. Overall, we find that Put amplifies PTI responses through ROS production leading to enhanced disease resistance against bacterial pathogens.

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tRNA modification t6A and its role in plant development

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RNA modifications are essential players of many biological processes, representing a rapidly developing research field. Our project focuses on the role of tRNA modification threonylcarbamoyladenosine (t6A) which is present at postition A37 of tRNA molecule and conserved virtually in all organisms. t6A was proposed to play a role in the translation efficiency, however, despite high effort, the deeper understanding of its function, in particular in multicellular organisms, is still incomplete. We are characterizing Arabidopsis thaliana homologs of t6A forming enzymes. We isolated candidate mutants with the abolished t6A formation and we show that t6A biosynthesis genes are essential for the earliest steps of plant morphogenesis, including gametophyte development. We also examined subcellular localization of t6A biosynthesis proteins and reveal that different steps of t6A biosynthesis take place in different compartments of plant cell, such as nucleus, plastids and mitochondria. We have also used proteomics approach aiming to identify novel components required for t6A formation in plants.

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A virus-targeted plant receptor-like kinase promotes cell-to-cell spread of RNAi

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RNA interference (RNAi) in plants can move from cell to cell, allowing for systemic spread of an anti-viral immune response. How this cell-to-cell spread of silencing is regulated is currently unknown. Here, we describe that the C4 protein from Tomato yellow leaf curl virus can inhibit the intercellular spread of RNAi. Using this viral protein as a probe, we have identified the receptor-like kinase (RLK) BARELY ANY MERISTEM 1 (BAM1) as a positive regulator of the cell-to-cell movement of RNAi, and determined that BAM1 and its closest homologue, BAM2, play a redundant role in this process. C4 interacts with the intracellular domain of BAM1 and BAM2 at the plasma membrane and plasmodesmata, the cytoplasmic connections between plant cells, interfering with the function of these RLKs in the cell-to-cell spread of RNAi. Our results identify BAM1 as an element required for the cell-to-cell spread of RNAi and highlight that signalling components have been co-opted to play multiple functions in plants.

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Artificial evolution identifies NB-LRR intragenic mutations suppressing immune related HI in Arabidopsis and their effect on disease resistance to local Hpa isolates

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The Arabidopsis thaliana accession Landsberg (Ler), originary from Gorzów Wielkopolski (Poland) triggers immune-related hybrid incompatibility with central Asian accessions (Kashmir-2 or Kondara). The Ler incompatible locus maps to a cluster of TIR-NB-LRR genes (RPP1-like) that, in combination with Kas-2 or Kond SRF3 alleles, induce EDS1 and SA-dependent hybrid necrosis. The RPP1-like locus has been reported to be involved in the recognition of certain effectors from the natural pathogen Hyaloperonospora arabidopsidis. Incompatible hybrids that severely affect growth and reproduction are unlikely to be frequent in nature unless transient environmental conditions enable their growth and reproduction. Adaptive mutations may also be acquired by hybrids that suppress incompatible epistasis before mating. However, such mutations cannot be identified due to the lack of a phenotypic trait in the respective parental lineages. Here, we have made use of artificial evolution to identify, by next-generation sequencing, intragenic mutations to the RPP1-like Ler locus that suppress Ler/Kas-2 incompatibility. We identify complex additive and epistatic interactions within the RPP1-like Ler locus contributing to incompatibility. We also evaluate their contribution to disease resistance to a local pathogenic oomycete as potential evolutionary driving force for the occurrence of incompatible RPP1-like alleles in nature.

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Differential contributions among IAA metabolic circuitry to auxin homeostasis

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Most developmental processes during the plant life cycle are tightly dependent on the homeostasis of the main auxin, indole-3-acetic acid (IAA). Spatiotemporally regulated IAA gradients act as morphogenic signals that shape the plant and determines the timing of developmental events. IAA homeostasis is regulated by a fine balance between transport and metabolism. To date, several proposed routes in the IAA metabolic pathways remain to be elucidated.

To better understand the metabolic pathways involved in IAA homeostasis, we have performed a screening of Arabidopsis mutants based on high-throughput IAA metabolite profiling by LC MS/MS[(1)] coupled to macro-confocal screening of mutagenized IAA-reporter lines, which led to the identification of 49 lines with significantly altered IAA metabolite profiles. These lines might be instrumental to resolve uncharacterized steps of the IAA metabolic pathways.

IAA is metabolically inactivated mainly by (1) conjugation to amino acids (IAA-aa) by members of the GH3 amido synthetases[(2)]; (2) oxidation to 2-oxoindole-3-acetic acid (oxIAA) by DIOXYGENASE FOR AUXIN OXIDATION (DAO) enzymes[(3)]; and (3) conjugation to sugars as glucose (IAA-glc) by members of the UDP-glucosyltransferases (UGT) superfamily[(2)]. We have previously shown that the GH3 and DAO pathways redundantly cooperate to maintain IAA homeostasis. The role of IAA-glc is, however, not yet well defined. UGT84B1 conjugates glucose to IAA in vitro, but it is expressed in Arabidopsis reproductive tissues. The high IAA-glc levels in Arabidopsis roots suggest the existence of other uncharacterized IAA UGTs.

To determine the differential contribution of the pathways for IAA conjugation and degradation, we are using CRISPR-Cas9 to knock-out all auxin inactivation pathways and their combinations. IAA metabolite profiling, gene expression studies, reporter assays, root phenotyping, and modelling approaches will be performed on these lines to predict the impact of IAA inactivation pathways on auxin homeostasis and root architecture.

[(1)] Pencik, et al., 2018; [(2)]Ljung (2013); [(3)]Porco et al., 2016

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Combinatorial design of modular and programmable transcriptional regulators in plants

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Synthetic Biology (SynBio) aims at rewiring plant metabolic and developmental programs with orthogonal regulatory circuits. This endeavour requires new molecular tools able to interact with endogenous factors in a potent yet at the same time highly specific manner. A promising new class of SynBio tools that could play this function are the synthetic transcriptional activators based on CRISPR/Cas9 architecture, which combine autonomous activation domains (ADs) capable of recruiting the cells transcription machinery, with the easily customizable DNA-binding activity of nuclease-inactivated Cas9 protein (dCas9), creating so-called Programmable Transcriptional Activators (PTAs). In search for optimized dCas9-PTAs we performed a combinatorial analysis with seven different ADs arranged in four different protein/RNA architectures. This analysis resulted in the selection of a new dCas9-PTA with improved features as compared with previously reported activators. The new synthetic riboprotein, named dCasEV2.1, combines EDLL and VPR ADs using a multiplexable mutated version (v2.1) of the previously described aptamer-containing guide RNA2.0. We show here that dCasEV2.1 is a strong and wide spectrum activator, displaying variable activation levels depending on the basal activity of the target promoter. Maximum activation rates reaching up to 10000 fold were observed when targeting the NbDFR gene. Most remarkably, RNAseq analysis of dCasEV2.1-transformed N. benthamiana leaves revealed that the topmost activation capacity of dCasEV2.1 on target genes is accompanied with strict genome-wide specificity, making dCasEV2.1 an attractive tool for rewiring plant metabolism and regulatory networks.

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Plant phenotyping by multiple imaging sensors and machine learning

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The combination of a collection of optical sensors can give a more comprehensive description of plant performance, for example about the alteration in plant metabolism upon pathogen infection (1). Thus, resistance or susceptibility of different plant genotypes to specific pathogens or strains could be analysed in a non-destructive quantitative way. In the same way, primary disease foci and areas with different disease severity could be identified. Moreover, it has been suggested by several authors that the combination of several imaging techniques could allow obtaining disease signatures for specific pathogens or other stress condition.
Our recent research has been focused in the detection of pathogen infected plants by chlorophyll fluorescence (Chl-FI), multicolour fluorescence (MCFI), multispectral reflectance and thermography. These techniques provide information about photosynthetic performance, secondary metabolism, as well as leaf transpiration, respectively. The data obtained were analysed by machine learning tools, such as classifiers that look for patterns of parameters from the data provided, and classify new data according to those patterns. This approach allowed the detection of viral, fungal and bacterial infections at lab and field scale (2,3,4).

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Metabolite profiling of postharvest senescence in different strawberry cultivars

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The cultivated strawberry (Fragaria x ananassa) is the berry most consumed worldwide, being well appreciated for its flavour and nutritional characteristics. However, strawberries possess a very short postharvest shelf-life due to their high respiration rate and their susceptibility to water loss, mechanical damage and fungi deterioration (Feliziani and Romanazzi, 2016). Extension of fruit shelf-life is a major economic goal, and measures are commercially taken to delay senescence, including the use of low temperature storage alone or in combination with controlled atmosphere (Pedreschi and Lurie, 2015). To improve our understanding of the molecular and biochemical mechanisms underlying the deterioration of fruit quality attributes during senescence, we realized a metabolite profiling of five commercial strawberry cultivars under different postharvest treatments. Ripe fruits were harvested and kept at 4ºC during three, six and ten days in ambient, CO2-enriched and O3-enriched atmospheres. We used a combination of gas chromatography-mass spectrometry (GC-TOF-MS), ultra-performance liquid chromatography-Orbitrap mass/mass spectrometry (UPLC-Orbitrap-MS/MS) and headspace solid phase micro extraction (HS-SPME) coupled with GC-MS to identify and semi-quantify 49 primary metabolites (sugars, amino and organic acids), 132 polar secondary metabolites (mainly polyphenols) and 70 volatile compounds. Multivariate statistical approaches were used to characterize the variation in metabolite content during the strawberry fruit postharvest life and to identify the biochemical pathways which are most affected in the senescence processes. Preliminary analysis pointed out that changes in primary metabolism were possibly related to responses to abiotic stress. In addition, postharvest was most drastically affecting strawberry volatile profile, even if this impact seemed to be treatment-dependent, suggesting a role for ozone atmosphere in preventing 'off aroma' formation. Network-based methods are ongoing, and will highlight the regulatory factors and molecular mechanisms underlying strawberry fruit senescence.

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Delving into the ancient stem cell niche 55

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Plant growth and development is sustained by a pool of undifferentiated and pluripotent stem cells. These cells have the ability to self-renew and give rise to all type of tissues. Genes in the WUSCHEL-RELATED HOMEOBOX(WOX) family constitute the master regulators that maintain a stable stem cell population in superior plants. The WOX family consists of three clades: the ancient clade, present in the earliest diverging green plants; the intermediate clade that emerged in vascular plants; and the WUSCHEL(WUS) clade, which appears specifically in ferns and seed plants. Therefore, the complexity of WOX protein family has increased during plant evolution coupled with a tighter regulation and organization of stem cells. To date, the major studies to understand stem cell regulation were carried out in angiosperms models. However, the knowledge about stem cell control in early divergent land plants is still limited. Focusing our efforts in this direction may help to clarify how the activity and regulation pathway of WOXs members evolved. In this sense, the bryophyte Marchantia polymorpha constitute a suitable model due to its critical evolutionary position and genome simplicity. Here, we identified one WOX protein(MpWOX) closely related with members from the WOX ancient clade. Overexpressor, amiRNA and reporter lines of MpWOX were generated in Marchantia and a phenotypic analysis of these lines in meristematic and stem cell function is undergoing. In addition, we are carrying a complementation of Arabidopsis thaliana wox mutants to investigate the ancestral role of these proteins. Our preliminary results indicate a possible conservation of the WOX function on stem cells maintenance.

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CRISPR/Cas9-mediated editing of the TM6 MADS-box gene in the octoploid strawberry (Fragaria x ananassa)

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The B-class of MADS-box transcription factors has been studied in many plant species, but remain functionally uncharacterized in the Rosaceae family. APETALA3 (AP3), a member of this class, controls the identity of petals and stamens in Arabidopsis thaliana. In this work, we identified two members of the AP3 lineage in the cultivated strawberry (Fragaria × ananassa): FaAP3 and FaTM6. Interestingly, FaTM6, and not FaAP3, shows an expression pattern equivalent to that of AP3 in Arabidopsis. Genome editing using Cluster Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system is becoming a robust tool for targeted and stable mutagenesis of DNA. However, whether it can be efficiently used in an octoploid species such as F. × ananassa is not known. In our study, we report the application of the CRISPR/Cas9 in F. × ananassa to characterize the function of FaTM6 in flower development. An exhaustive analysis by high-throughput sequencing of the FaTM6 locus spanning the target sites showed a high efficiency genome editing already in the T0 generation. The phenotypic characterization of the mutant lines indicates that FaTM6 plays a key role in petal and especially in anther development in strawberry. Our results validate the CRISPR/Cas9 strategy for gene functional analysis in an octoploid species such as F. × ananassa, and offer new opportunities for engineering strawberry to improve traits of interest in breeding programs.

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Understanding the combined visual and chemical signals governing plant-pollinator interactions in desert communities

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Pollination is a key factor in the reproductive success of angiosperms. Understanding the mechanisms underlying successful pollination services by animals is particularly important in areas in which pollinators are a limiting factor, e.g. deserts and due to the current decline in pollinators and changing climate.
The key for effective pollination is ensuring constancy in pollinator visitation of conspecific flowers. This is achieved by a suite of floral phenotypes (architecture/color/scent) aimed at foraging pollinators that use these signals to distinguish between rewards given by each plant species.
Current models predict the divergence of floral phenotypes in cases of species isolation or reducing competition in coexisting, closely-related lineages. Conversely, coexisting species benefit from a shared synergistic advertisement towards the same guild. Therefore, it is important to understand the model of floral phenotype variance in wild populations by meticulously measuring these signals and determining the contribution of forces that shape these pollination syndromes. Using the Brassicaceae family as model group, our overall goal was to evaluate the contribution of various factors (be it genotype, pollinator identity or environment) in shaping the pollination syndromes of desert plant communities.
We performed a comprehensive characterization of pollinator-attracting traits in 17 Brassicaceae species found in the Negev desert, Israel, yielding high-throughput data sets. To this end we have: 1) elucidated diurnal/nocturnal floral scent profiles by measuring volatile emissions, 2) measured the content of floral pigment and petal reflectance spectra, 3) generated individual insect vision models for the flowers and 4) quantified the floral architecture of our accessions. The aforementioned floral phenotypes were associated to the different factors that are known to shape pollination syndromes, for which, among others, we have generated a phylogenetic tree of all 17 species and constructed a database representing the species distribution and environmental conditionds within habitats. Network analysis between traits and the degree of contribution of the aforementioned factors are presented. This is the first work in the field of pollination-ecology that details several floral phenotypes and association studies thereof at such a wide scope.

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Impaired sterol glycosides biosynthesis induces resistance to Botrytis cinerea in the Arabidopsis mutant ugt80A2B1

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Free and glycosylated sterols are structural components of cell membranes that play a key role in regulating their biophysical properties and consequently different PM-associated processes like plant adaptation to biotic and abiotic stress, signalling or transport (Griebel and Zeier, 2010; Posé et al, 2009; Gamir et al, 2016). However, the specific role of glycosylated sterols in these processes is far from being understood. Arabidopsis contains two UDP-glucose sterol glycosyltransferases (UGT80A2 and UGT80B1) that catalyse the glycosylation of the hydroxyl group at C-3 position of free sterols to produce steryl glucosides (SG). To gain insight about the role of glycosylated sterols in the plant response to biotic stress, we investigated the response of the Arabidopsis double knockout mutant ugt80A2B1, impaired in SG biosynthesis (DeBolt et al. 2009), against the infection with the necrotrophic fungus Botrytis cinerea. The ugt80A2B1 mutant exhibits enhanced resistance against B. cinerea when compared to wild-type plants, and the resistance phenotype correlates with increased levels of jasmonic acid (JA) and enhanced expression of the JA-responsive genes PDF1.2 and PR4. Moreover, upon B. cinerea infection the ugt80A2B1 mutant also accumulates higher levels of camalexin than wild type plants. Consistent with this observation, the expression of several genes related to camalexin biosynthesis and that of the camalexin synthesis regulator genes AtMYB51 and AtWRKY33 is higher in the mutant than in wt plants after infection. Altogether, the results of this study show that glycosylated sterols play an important role in the regulation of Arabidopsis response to B. cinerea infection and suggest that this occurs through a signalling pathway involving the hormone JA and camalexin, the main Arabidopsis phytoalexin. The possible role of glucosinolates, secondary metabolites involved in plants defense, in this resistance response will also be discussed.

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Understanding the function of VEG1 in the identity of the secondary inflorescence meristems

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The genetic control of inflorescence architecture is a key aspect for plant developmental biology, for biotechnology and for breeding strategies, being directly related to crop yield and yield stability. Grain legumes possess a typical compound inflorescence, more complex than those of species like Arabidopsis, where flowers are not formed in the main stem, but in lateral secondary inflorescences (I2). Recently, our group has produced relevant contributions to genetics of legume inflorescence development that allowed us to propose a genetic model that explains how meristems of the compound legume inflorescence are specified.
Thanks to this previous works we know that the transcription factor VEGETATIVE1 (VEG1) directs the formation of the I2 meristems at the apex of the legume compound inflorescence. Nevertheless, still many questions need to be answered, such as: how does VEG1 work in order to specify the formation of the I2? What genes mediate VEG1 function?
In order to identify transcriptional targets of VEG1 we have performed RNA-seq comparing the transcriptome of inflorescence apices of pea wild type and mutants affected in the specification of the I2 meristems, including veg1, vegetative2 (veg2) and proliferating inflorescence meristem (pim). We have identified a number of genes which show differential expression between the mutants, which represent possible targets of VEG1.
On the other hand, we try to use a method recently developed in Arabidopsis, TARGET (Transient Assay Reporting Genome-wide Effects of Transcription Factors) in order to identify direct targets of VEG1, by combining the use of protoplast transfection and an activable form of VEG1 (VEG1-GR). We are working to set up a protocol to perform this assay in pea. Considering that there is not a reproducible method to obtain transgenic plants in pea, this assay could be a powerful technique with multiple applications in this legume.

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Polyamines are metabolic targets of effector triggered immunity (ETI) in Arabidopsis thaliana

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A crucial functionality of the plant immune system is to recognize pathogens and trigger a defense response. Plants apply multi-level recognition systems to be protected against pathogen attack. Effector-Triggered Immunity (ETI) is a robust and long-lasting defense response that is activated upon recognition of pathogen effectors by Resistance proteins. The Arabidopsis RPM1 protein recognizes AvrRpm1 type III effectors from the bacteria Pseudomonas syringae. ETI involves extensive transcriptional and metabolic reprogramming leading to cell death and ultimately, pathogen growth inhibition. Polyamines (PAs) have been involved in the defense response. However, the defense pathways involved in PA responses to pathogen recognition have not been addressed in depth. PAs are small polycationic compounds present in all living organisms. Putrescine (Put), spermidine, spermine and thermospermine are the most abundant PAs in Arabidopsis and their increase have been associated with enhanced tolerance to different types of stresses. In this study, the variation of PA levels has been analyzed during ETI triggered by Pst DC3000 AvrRpm1 inoculation in Arabidopsis thaliana wild-type and loss-of-function mutants: eds1, pad4, sid2, npr1 and rpm1 . Our results indicate that PAs, and particularly Put, are metabolic targets of ETI triggered by AvrRPM1 recognition, and identify defense signaling components required for such responses.

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Geminivirus replication protein decreases pcna sumoylation at two acceptor sites

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Geminiviruses are plant viruses with circular, single-stranded DNA (ssDNA) genomes that infect a broad range of plants causing substantial crop diseases worldwide. They replicate in nuclei of infected cells by using host DNA replication machinery and an essential protein encoded in their genome designated Rep (replication-associated protein). This multifunctional protein induces the accumulation of the host factors involved in replication and it is capable of interacting with a lot of plant proteins including PCNA (Proliferating Cell Nuclear Antigen), a processivity factor that coordinates a wide range of processes involved in maintenance, duplication and transmission of the genome, and the sumoylation enzime that conjugates SUMO to target proteins (SUMO-conjugating enzyme- SCE). PCNA modification by SUMO and also ubiquitin, has long been known to be of key importance for determining how DNA damage is processed by the replisome and for maintenance of overall genome integrity. In yeast, PCNA sumoylation has been associated to DNA repair involving homologous recombination (HR). Previously, we reported that Rep ectopic expression does not result in broad changes in the sumoylation pattern of plant cells, but it modifies the sumoylation state of selected host proteins. In this work, we show, using a reconsituted sumoylation system in Escherichia coli, that tomato PCNA is sumoylated at two residues, K254 and K164, and that co-expression of the Rep protein suppresses PCNA sumoylation at these lysines. Finally, we confirm that PCNA is sumoylated and that Rep also interferes with PCNA sumoylation in planta.
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Looking for candidate genes for some pleasant key aroma compounds in F1 strawberry population

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Cultivated strawberries (Fragaria x ananassa) are appreciated for their taste and aroma. Although their octoploid nature (2n = 8x = 56), several efforts have been done to characterize and identify the 19 key volatile compounds responsible of this characteristic aroma. In order to deeply study it, a F1 population of 63 progenies from FC50 and FD54 cross were phenotype in 6 harvests during 3 years (2016-18). Fruits were harvest at maturation stage and volatile composition was determined by GC-MS. A SNPs genetic map of this population was constructed by genotyping 55 individuals with IStraw35k array. The GC-MS of these 19 key compounds permit to do a QTLs analysis for volatiles. In total, we found 64 QTLs but only 14 were stable during 3 years. From linalool and nerolidol, there is a common QTL in the beginning of LG3B covering a region of 15cM. Recent access to octoploid strawberry genome, it shows that this region has 725 genes including FaNES1, nerolidol synthase 1, which could be a candidate gene. Furthermore, there is another QTL for methyl hexanoate and ethyl hexanoate in the end of LG4B covering a region of 7cM with a total of 263 genes including several candidate genes. There are others QTLs for these compounds that are not in common. Some transcript experiments will be done soon.

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Roundup® resistance trait traced in genomes of maize inbred lines

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Glyphosate is an active compound of a systemic, nonselective and most widely used herbicide in the world - Roundup®. It causes broader range of physiological alterations than previously assumed and some plants gain higher level of resistance without the need to use genetic engineering methods. The holistic understanding of Roundup® mechanism of action is of great importance since it has been shown that glyphosate affects the growth of plants not only by inhibiting EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) but also through altering several crucial physiological processes (e.g., photosynthesis, carbon metabolism, mineral nutrition, oxidative events).

To study the genetic variations between genomes of plants that are naturally tolerant of and sensitive to Roundup®, we used two Zea mays L. lines traditionally bred in Poland. To overcome the complexity of the maize genome two sequencing technologies (Illumina and SMRT PacBio) were employed. Corrected PacBio reads were used to identify the genome structure variations (SV), and single nucleotide polymorphism and insertions-deletions (indels) were revealed using Illumina short reads.

We identified 11 thousand structural variants, 4 million SNPs and approximately 800 thousand indels differentiating the two genomes. Detailed analyses allowed us to identify 20 variations within the EPSPS gene, but all of them were predicted to have moderate or unknown effects on gene expression. Other genes of the shikimate pathway encoding bifunctional 3-dehydroquinate dehydratase/shikimate dehydrogenase and chorismate synthase were altered by variants predicted to have a high impact on gene expression. Additionally, high-impact variants located within the genes involved in the active transport of glyphosate through the cell membrane encoding phosphate transporters as well as multidrug and toxic compound extrusion have been identified.

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A set of PLETHORA transcription factors maintain undifferentiated state in the vascular cambium

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The Vascular Cambium - a secondary meristem in plants produces secondary xylem (wood) and secondary phloem. Meristematic activity in vascular cambium ensures the production of phloem and xylem, which are essential for transportation of nutrients and water. Thus understanding the molecular mechanism behind the maintenance of meristematic state of vascular cambium and its development becomes essential. The PLETHORA (PLT) transcription factors are the central regulators of the primary meristems. Recent research works on PLT/ AINTEGUMENTA (AIL) and/ or AINTEGUMENTA (ANT) genes provide the insight of stem cell maintenance in plant primary meristems and their role in phyllotaxis and rhizotaxis. However, their functional role in a secondary meristem is largely unknown and it needs to be elucidated. Therefore, we studied whether the PLT/AIL factors have a function also in the vascular cambium. In our work, we observed that several PLT/ AIL family members are expressed in cambium, and when we generated mutant combinations from the cambium-expressed PLT/AILs, we found defects in vascular patterning and cambial cell maintenance in a few double and triple mutant combinations. In addition to that overexpression of PLT/AILs inhibit the differentiation of the cambial cells to xylem and promote ectopic cell proliferation. We aim to further specify the role of PLT/AILs in cambial maintenance, as well as how they interact with other known cambial regulators. Such knowledge will significantly contribute to the improvement of the quality, biomass of the forest industry and bioenergy.

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Revealing the molecular mechanisms underlying peridermis suberization in Arabidopsis and Cork Oak

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Peridermis is a protective tissue composed of three different cell types: the phellogen, phelo-derm, and phellem cells. During peridermis development, phellem cells undergo secondary cell wall thickening by suberin deposition, forming a compact barrier shielding plant organs from the environment. Suberization of phellem cells was shown to be a tightly controlled process regulated by endogenous and exogenous factors, however, its development and regulatory mechanisms are still poorly known. For a better understanding of the suberization process during peridermis development we used the model Arabidopsis thaliana, which is known to undergo secondary growth in stem, root and hypocotyl, and share the same suberization pathways as other plant models. To follow up the suberization process we performed a detailed chronolo-gical study, analysing anatomically the activation of specific markers involved in the suberin biosynthetic pathway (FAR4 and GPAT5 genes) and suberin deposition (using specific stainings), after the onset of secondary growth in roots. We demonstrated that first cells undergoing pe-ridermis differentiation were visible at the root-hypocotyl junction zone, early at 8 days after germination, with specific activation of suberin biosynthesis genes in pericycle daughter cells, followed by suberin deposition. During the subsequent days, pericycle divisions for peridermis tissue are gradually intensified and more cells undergo suberization, resulting in fissures and the peeling-off of the epidermis and cortex layers. To identify tissue-specific molecular regulators during peridermis differentiation, a transcriptomic experiment targeting the peridermis suberi-zing cells is being performed, using TRAP-Seq technology. Furthermore, this study was exten-ded to an economically important tree, cork oak (Quercus suber), where the application of a combination of heat and drought stress, at the onset of secondary growth, showed to affect peridermis development and suberization. By integrating the results from both plant systems we will deepen our knowledge on cork development and on the impact of environmental stresses on this economically important plant-derived material.

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Generating Brassica with supernumerary trichomes

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An estimated 42% of global crop production is lost every year due to issues such as pests, weeds and disease. To counteract this huge amounts of pesticide are used every year which results in many unwanted side effects such as reduction of biodiversity, contamination of soil and water and potential risk to human health. Some pests have developed resistance to certain pesticides in addition to the use of some pesticides being restricted or banned by environmental agencies. Protection of crops is becoming increasingly difficult so novel strategies are necessary to guarantee crop security in the future. One solution is the use of new crop cultivars with increased resistance to pests and disease that give stable yield and need reduced quantities of pesticides. Here, based on work carried out by previous members of our lab in Arabidopsis thaliana, we aim to generate new cultivars of Brassica crops with improved pest resistance. Previous work in our group has looked at the role of key regulatory genes of Arabidopsis flower development - flowers produce much of the food that humans and livestock consume and they have been identified as key targets for crop improvement.

Trichomes are small hairs which are typically found on the leaves and stems of plants. Due to their function in the protection of plants against pests, trichomes are being looked at as a possible mean of crop protection. Work carried out by previous lab members showed that the C function gene AGAMOUS (AG) acts to repress trichome formation on floral organs. Additional work showed that when AG function was knocked down in combination with loss of function of the known trichome repressors TRIPTYCHON (TRY) and CAPRICE (CPC), trichomes formed on reproductive floral organs.

We aim to test the potential of floral trichomes for crop protection by generating Brassica rapa and Brassica oleracea plants with supernumerary trichomes by recapitulating the work carried out in Arabidopsis. If successful, we will apply this approach in Brassica napus which is an oilseed crop of global importance and is derived from an interspecific cross between B. rapa and B. oleracea.

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A network of splicing factors interact with the ASCO long non-coding RNA to modulate transcriptomic diversity in Arabidopsis

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Alternative splicing (AS) is one of the mayor sources of transcriptome and proteome diversity of higher organisms. Long noncoding RNAs (lncRNAs) have emerged as regulators of AS in a wide range of molecular mechanisms, including the interaction with splicing factors. In Arabidopsis, the ALTERNATIVE SPLICING COMPETITOR (ASCO) lncRNA is recognized by the AS factors NSRa and b, regulating auxin-driven lateral root formation. Here, we analyse the effect of the knock-down of ASCO at genome-wide level and found that only a minor subset of genes overlapped with the AS defects of the nsra/b double mutant. In particular, a high number of deregulated and alternatively spliced genes of ASCO knockdown plants were related to the response to flagellin and biotic stress. In agreement, ASCO-deregulated plants are more sensitive to flagellin, exhibiting a significant arrest of primary root development. Furthermore, we demonstrated that ASCO is recognized also by PRP8a, a key component of the spliceosome. ASCO deregulation impairs PRP8a recognition of flagellin-related transcripts, indicating that the function of ASCO on AS involves the interaction with multiple splicing factors. Our results hint the existence of a dynamic network between lncRNAs and splicing factors that modulate the transcriptome diversity during development, conditioning the response to environmental cues.

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Characterization of the molecular and signaling events regulating the proliferative arrest in Arabidopsis thaliana

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Many annual and some perennial plant species show a monocarpic lifetime pattern in which the plant senesces and dies after a single reproductive cycle. In monocarpic plants, such as Arabidopsis thaliana, after the production of a certain number of fruits, all reproductive meristem activity arrests coordinately and flower production ceases. This process is termed Global Proliferative Arrest (GPA) and is a fundamental evolutionary adaptation that ensures nutrient availability and redistribution for the production of seeds. Moreover, from an agricultural point of view, GPA is of particular interest because it significantly affects fruit production, being therefore an important target for crop breeding. Recent findings have provided valuable information about genes associated to GPA in the model species Arabidopsis. However, the specific regulatory mechanisms that coordinate GPA are still poorly understood. This study is focused on the characterization of the cellular and molecular events that control GPA with high spatial-temporal resolution by using confocal live imaging. In particular, we have monitored the expression pattern of different meristem activity regulators and known GPA-related genes in inflorescence meristems at different time points close to and during the GPA process. In addition, we have tracked several hormone sensors to gain new insights into the signaling pathways regulating this process. Our results suggest significant changes in CLAVATA3, WUSCHEL and FRUITFULL expression patterns as well as in auxin and cytokinin levels during GPA.

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Regulation of MIR166 in the specification of adaxial-abaxial leaf polarity

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Specification of adaxial-abaxial polarity is essential for the formation of flat leaves optimized for photosynthesis, which forms a major innovation in land plant evolution. The clean separation of adaxial and abaxial domains relies on an intricate gene regulatory network comprised of sets of antagonistic Transcription Factors (TFs) that promote either adaxial or abaxial cell fate, and mobile small RNAs that provide the needed positional information. Particularly the miR165/166 directed regulation of HD-ZIPIII TFs forms a key node in the polarity network. Using live cell imaging we show that MIR166A is expressed on the abaxial side of growing primordia, as well as in boundary regions between older primordia and the meristem, from which new primordia initiate. Clonal analysis reveals this latter expression of MIR166A to predetermine abaxial cell fate in the incipient primordium. What factors determine the spatio-temporal expression of MIR166A during adaxial-abaxial polarity establishment is not known. To understand this, we performed a genome wide screen for TFs regulating MIR166A expression, the results of which will be presented.

The microRNA miR7695 is involved in the rice immune response to pathogen infection

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MicroRNAs (miRNAs) are short regulatory non-coding RNAs that guide gene silencing by triggering sequence-specific cleavage or translational repression of target transcripts. In plants, miRNAs emerged as versatile regulators of gene expression in developmental processes and adaptive responses to environmental stresses, including pathogen infection. Certain plant miRNAs have been shown to function in cross-kingdom regulation of gene expression (i.e. miR159 and miR166 can move from cotton plants to the fungal pathogen Verticillium dahliae for specific silencing of pathogen virulence genes). In rice, the fungus Magnaporthe oryzae is the causal agent of the rice blast disease, one of the most devastating fungal diseases of cultivated rice worldwide. Although a plethora of rice miRNAs have been shown to be regulated during M. oryzae infection, their biological function remains largely unknown. Here, we report that a miRNA, miR7695, contributes to rice immunity. MiR7695 targets an alternatively spliced transcript of the Natural resistance-associated macrophage protein 6 (OsNramp6) gene encoding an iron transporter from rice. Activation-tagged MIR7695 rice plants (MIR7695-Ac) exhibited enhanced resistance to M. oryzae infection. RNA-seq analysis revealed that blast resistance in MIR7695-Ac plants is associated with stronger induction of defense-related genes, including pathogenesis-related and diterpenoid biosynthetic genes. Furthermore, rice plants grown under high iron supply showed blast resistance, indicating that iron is a factor in controlling blast resistance. During pathogen infection, iron accumulates in the vicinity of fungal aspersoria, the sites of pathogen entry, and in cells surrounding infected regions of the rice leaf. This observation supports that rice plants use strategies to locally increase Fe content to prevent penetration and spread of the pathogen into the leaf tissue. A better understanding of the mechanisms that are regulated by miR7695 during rice immunity and crosstalk with iron homeostasis will help in designing novel strategies to control rice blast disease.

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Identification of three possible tomato susceptibility factors recruited into the pepino mosaic virus replication complexes

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Pepino mosaic virus (PepMV; genus Potexvirus) causes large economic losses in tomato crops worldwide. In order to determine target genes to engineer loss-of-susceptibility mutants, tomato proteins that interact with the PepMV coat protein (CP) and the triple gene block protein 1 (TGB1) were identified using a yeast two-hybrid (Y2H) screening. Here we present results for three of them: a glutathione S-transferase (IP24) interacting with CP, a putative transcription factor (IP9) interacting with TGB1, and an RNase H like protein (IP10) interacting with both CP and TGB1. Interactions were validated by directed Y2H and/or coimmunoprecipitation (CoIP). Confocal laser scanning microscopy (CLSM) of N. benthamianaliving cells showed that the 3 IPs relocalized to virus replication complexes (VRC) of infected cells. For IP9 and IP10 this phenomenon was especially dramatic as they localized to the nucleus when expressed alone. We hypothesized that the TGB1, which is also strongly localized to the nucleus when expressed alone, recruits IP10 and IP9 to the VRC suggesting a role for the IPs during virus replication. Moreover, IP10 and TGB1 relocalized in infected cells to plasmodesmata (PD) and within bodies associated with PDs which are similar to the cap structures described for PVX involved in coreplicational virus transport (Tilsner et al., 2013). IP24 may be recruited to the VRC to create an optimal redox environment for virus replication. PepMV susceptibility assays were carried out in IPs-transiently silenced tomato plants with encouraging results for IP24. IPs Micro-Tom knock-out mutants were then generated using the CRISPR/Cas9 technology; analysis of T2 homozygous mutants showed a reduction in the virus accumulation for IP24 and IP9 knocked-out plants. Further work focusing on editing IPs paralogs is in progress.

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Plant developmental pathways are modified by root-knot nematodes for their feeding sites organogénesis

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Root-knot nematodes (RKNs), a group of phyto-endoparasitic nematodes, cause serious losses in agriculture worldwide (Nicol et al., 2011). RKNs form a swelling on the root, a gall, where they stablish and induce their feeding cells, giant cells (GCs), from still not well-known precursor cells within the vascular cylinder. GCs expand and suffer repeated mitosis with aborted cytokinesis (Escobar at al., 2015).

The transcriptomes of early-developing Arabidopsis GCs/galls in Arabidopsis (Barcala et al., 2010) were enriched in genes characteristics of undifferentiated root cell types, i.e., the quiescent centre (QC), protoxylem and the lateral root (LR) initial cells (Cabrera et al., 2014). Accordingly, a characteristic marker of LR primordia founder cells, LATERAL ORGAN BOUNDARIES 16 (LDB16), was critical also for galls and GCs development. In this context, miR390, involved in LR development, mediated post-transcriptional gene silencing of ARF genes (ARF3) in the galls through TAS3 tasiRNAs (Cabrera et al., 2016). Yet, a global gene repression in early galls was also observed, therefore, we have explored the impact of epigenetic processes during gall formation, such as RNA directed Methylation pathways. Besides, we further analysed the expression and function of key genes determinant for LR development such as GATA23, several AUX/IAAs and ARFs during gall formation. Most of them were crucial also during gall development, but differences in some molecular transducers were also encountered. Other genes with essential roles in the root QC establishment and stem cell maintenance like SCHIZORIZA (SCZ), SCARECROW (SCR), SHORT ROOT (SHR) and WUSCHEL-RELATED HOMEBOX 5 (WOX5) were also induced and crucial in Arabidopsis galls, as was HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6), a protoxylem marker from the root apical meristem. Therefore, the cross-talk between the plant and the nematode effectors should alter epigenetic processes as well as central genes used during plant development for the new organogenesis of their feeding organs.

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Phloem pole identity in Arabidopsis thaliana is modulated through RECEPTOR LIKE PROTEIN KINASE 2 (RPK2) and its perception of CLE45

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As sessile organisms, plants have evolved the ability to adapt to positional information by modifying their cell fate and thus developmental trajectory. This developmental flexibility is important in the Arabidopsis phloem pole; comprised of proto- and meta-phloem sieve elements and adjacently positioned companion cells (CC). While molecular factors regulating Protophloem (PP) specification exist, less is known about the molecular mechanisms controlling PP surroudning cell identities. Two enzymes involved in the phosphoinositide biosynthetic pathway, COTYELDON VASCULAR PATTERN2 (CVP2) and CVP2 LIKE1 (CVL1) regulate PP differentiation. In cvp2 cvl1, protophloem differentiation is compromised, evident by the existence of undifferentiated cells (a.k.a gap cells) in its protophloem strand. Gap cells exhibit both PP and CC identity, resulting in a cell with a hybrid identity unable to become a functional protophloem element. A second site mutagenesis screen on cvp2 cvl1 enabled us to isolate a mutation in the kinase domain of RECEPTOR LIKE PRTOEIN KINASE 2 (RPK2) as a potential suppressor of cvp2 cvl1 vascular defects. Introgression of rpk2 mutation in a cvp2 cvl1 genetic background restored protophloem identity in cvp2 cvl1 gap cells. Moreover, we observed the ectopic expression of protophloem-specific genes in rpk2 phloem elements, suggesting that RPK2 is essential in creating a clear boundary between PP and surrounding cell identities. Additionally, we have found rpk2-2 to be resistant to the inhibitory effect of CLAVATA3/EMBRYO SURROUNDING REGION (CLE) 45 in suppressing PP formation. Interestingly, a short treatment with CLE45 switches the identity of PP cells to PP-surrounding elements. Recently, PP cells have been described as the main organizers of the phloem pole pattern by modulating the division rate of PP-surrounding cells1. Together, our results indicate that RPK2 acts in companion, pericycle and metaphloem cells to exclude protophloem identity through the perception of CLE45, thus supporting the correct patterning and function of the phloem pole.

Key words: Protophloem, cell identity, RPK2, phosphatidylinositol 4,5 bis-phosphate, CLE peptides

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Genome wide association mapping of apple fruit shape

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Apple shape is an important varietal trait. However, despite such importance, only few QTLs for fruit shape have been identified so far, while candidate genes and the genetic mechanisms underlying this trait are still unknown. Breeders, examination offices and post-registration organizations use standard shape descriptors, based on the visual estimation of the proportion between mid and distal fruit widths, to characterize the fruits. In this work we analyze measures of fruit dimensions obtained with Tomato Analyzer software in scanned fruit sections to define an objective fruit shape index (FSI) as the ration between mid height and mid widht. For this we cut and meassured 1507 apples of 94 accessions randomly sampled from an apple collection of 292 accessions with two replicates each, and genotyped with the Axiom® Apple 480k SNP array. FSI data obtained in the subsample was used in a GWAS analysis using General Linear Model and Mixed Linear Model. The limiting sample size did not allow to find significant SNPs after Bonferroni correction, however this approach has been highly useful to define a phenotyping and GWAS pipeline to study QTLs for apple fruit shape. Our data indicate that FSI is a good descriptor of fruit shape, however other measures and ratios provided by Tomato Analyzer will be also included in further GWAS analysis with the whole 292 apple accessions.

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Development of a Bioassay for the study of Hairy Root Disease of Tomato plants

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Hairy Root Disease (HRD) of hydroponically grown tomato plants is an important disease in the horticultural sector. This disease has been attributed to rhizogenic Agrobacterium harboring a root-inducing plasmid which encodes for several oncoproteins. Although, the root oncogenic loci oncoproteins (RolA, RolB and RolC) are thought to be essential in HRD formation their exact functioning and mode of action is relatively unknown. Moreover, also a suitable semi high-throughput bioassay for the study of HRD is currently lacking.
Therefore, the aim of the present study was to develop an optimized bioassay for the study of hairy root disease in tomato plants and commonly used rootstocks in greenhouse cultivation of tomato plants. Two important criteria for designing this bioassay were:
1) To confer a visual phenotype that points to HRD infection i.e., excessive root proliferation
2) Induce HRD on a shorter timescale in comparison to greenhouse-grown tomato plants
To achieve this, a sand-based bioassay was designed. Tomato cultivars and rootstocks grown in this system were infected with rhizogenic Agrobacterium strain ST15.13/012 isolated from a Belgian greenhouse infected with rhizogenic agrobacteria. Trial experiments carried out over 8 weeks with Maxifort rootstocks revealed a 2-2.5-fold increase in dry root biomass in comparison with uninoculated tomato roots, highlighting the efficacy of our bioassay to study HRD. Currently, we are testing this bioassay on wild-type rhizogenic agrobacteria strains as well as Rol gene knockouts of strain ST15.13/012 to elucidate the plant defense response towards HRD as well as to elucidate the contribution of Rol oncoproteins in the formation of HRD. Overall, data obtained from these studies will further our understanding into the HRD infection of tomato roots.

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Investigation of novel components of chloroplast-to-nucleus communication in the unicellular microalgae Chlamydomonas reinhardtii.

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Besides their well-described role in photosynthesis, chloroplasts act as environmental sensors that are able to regulate nuclear gene expression in response to developmental cues and different stresses. This chloroplast-to-nucleus communication is termed retrograde signaling (RS) and it is a key component in the control of plant growth and development. Despite its essential role, the RS pathway is still poorly understood and many molecular components remain uncharacterized. Most studies on RS have been done in the model plant Arabidopsis thaliana. Here, I present the green unicellular microalga Chlamydomonas reinhardtii as a simpler and advantageous model to address fundamental aspects of RS and find novel RS molecular components. To this end, I will describe the impact of RS in Chlamydomonas physiology and present specific genetic screening strategies and transcriptomic analysis to pinpoint gene regulators of putative positive and negative retrograde signals, and study the crosstalk and timing of light and retrograde control during chloroplast biogenesis, two outstanding questions in the RS field.

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Embryogenic competence acquisition in tamarillo – from tissue to single-cell based analysis

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Plant somatic embryogenesis (SE) is a developmental pathway in which a somatic cell acquires totipotency and evolves into an embryo. Somatic embryogenesis (SE) in tamarillo (Solanum betaceumCav.) is particularly relevant since it allows successful cloning but also developments in cryopreservation and genetic transformation protocols for this species. Also, it has several advantages for molecular analyses and experimental embryology approaches. SE induction in this solanaceous species is achieved by a two-step protocol, by first exposing leaf segments or mature zygotic embryos to MS media with an auxin and high concentrations of sucrose and then transferring the induced embryogenic masses (EM) to auxin-free medium to allow somatic embryos development. The EM formed can be isolated from surrounding non-embryogenic calli(NEC) and subcultured, and protocols for the proliferation of tamarillo EM cell suspension cultures were achieved. Based on this system a comparative proteomic profile of EM and NEC of tamarillo was obtained. Moreover, a protein with a putative inhibitory role in the acquisition of embryogenic competence was isolated and characterized. Besides the easy in vitro manipulation of this woody plant, the establishment of a protoplast isolation protocol is also an important tool for functional genomics studies in tamarillo, particularly for cell-type-specific transcript profiling. De novotranscriptome sequencing was used to generate sequences from embryogenic and non-embryogenic cells derived from SE induced tissues and obtained through FACS. The de novo assembly generated around 50 000 unigenes, of which 30% were annotated with a significant Blast against the databases. The differential expression of several transcription factors in sorted embryogenic cells revealed a strong epigenetic regulation of cell commitment to embryogenic competence. These results allow the formulation and test of novel fundamental hypotheses regarding the induction of SE.

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A tomato G-type lectin S-receptor-like serine/threonine-protein kinase is required for pollen and fruit development

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Cell-to-cell communication, through a signal transduction pathway, is an essential step for the development and survival of all multicellular organism. The large superfamily of Receptor-like protein kinases (RLKs) includes more than 15 families whose member play critical roles in perceiving external signals thus promoting different transduction pathways. RLKs are composed of an extracellular domain, a transmembrane domain and an intracellular kinase domain. The family of lectin receptor-like kinases (LecRLKs) have a lectin domain within the extracellular region and play important roles in plant development and stress responses. LecRLKs are subdivided as well into three types; depending on the class of lectin domain they contain (G-, L- and C-type). These proteins are also called S-domain RLKs owing to they have an S-locus glycoprotein domain and because they have a function in self-incompatibility in plants.

In a phenotypic screening of enhancer trap lines carried out in the Moneymaker background, we identified the small size fruit 1 (ssf1) mutant, displaying small and parthenocarpic fruits. Because the mutant phenotype did not segregate with the T-DNA insertion, we cloned the mutation through a mapping-by-sequencing approach, which allowed us to identify a G to A mutation in a gene encoding a G-type LecRLK.

We quantified the number of pollen grains produced by ssf1 plants that was lower than in the wild type. In vivo analysis of pollen development showed that wild-type pollen grains germinated and pollen tubes elongated normally on the stigma of ssf1 flowers; however, pollen of ssf1 plants were unable to form pollen tubes on the stigma of wild-type plants, indicating that SSF1 gene is needed for male sterility. To further characterize the defects in pollen development of ssf1, anther tissue sections at different stages of flower development were analysed . At the pre-meiotic and meiotic stages, ssf1 and Moneymaker anthers looked similar. However, from the tetrad stage to the dehiscence stage, aberrant developing anthers were evident in ssf1 but no in the wild type, which could explain its inability to produce viable pollen and hence, to yield fertile tomato fruits. In addition, SSF1 silencing phenocopies ssf1 plants proving that tomato SSF1 is required for pollen development and male fertility, and suggest that the parthenocarpy exhibited by the ssf1 mutant could be a consequence of the abnormal pollen development.

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Characterization of AGO N-terminal domain as regulators of subcellular shuttling mechanisms

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In eukaryotes, RNA silencing controls gene expression via 19-36 nucleotide (nt) small (s) RNAs. sRNAs are essential during developmental processes, adaptive responses to stress, to preserve genomic integrity by controlling transposon activity and general innate immune response to viruses. ARGONAUTE (AGO) proteins are the main component of RNA-induced silencing complexes (RISCs) recruiting sRNAs to interact with target mRNA or DNA in order to execute their final functions. Canonical eukaryotic AGOs contain four main domains: a variable N-terminal domain and three highly conserved PAZ, MID, and PIWI domains. AGOs fold into a bilobal structure displaying a central groove for sRNA binding. AGO proteins are the main RNA silencing effectors across kingdoms, and their gene numbers vary greatly, from 1 in Schizosaccharomyces pombe to 27 in Caenorhabditi. Elegans. Their functions range from cytoplasmic post-transcriptional gene silencing to nuclear processes such as transcriptional gene silencing, splicing modulation and DNA repair.

Although many functions of small RNA-guided gene silencing proteins in the nucleus and the cytoplasm have been recently reported, the mechanisms behind AGO subcellular transport routes remain largely unknown in all organisms. Recently, we have described a hitherto Arabidopsis AGO1 nucleo-cytosolic shuttling process required for mature miRNA translocation and miRNA-mediated silencing. Here, we characterized N-terminal regions from eukaryotic AGOs and propose a novel model; which could be used as a global mechanism for RNA transport among plants and metazoans, including humans.

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ABSTRACT BOOK

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