Plant Molecular Genetics

The aim of the Plant Molecular Genetics Department is the study of the regulatory mechanism and pathways controlling plant development, adaptation to the environment, and defense responses to biotic and abiotic stresses.

Research lines focused on developmental processes include the study of root architecture and shoot branching. Plant adaptive responses to nutrient starvation, toxic concentrations of metals or defensive responses to pests and pathogens are also subject to intense research efforts. In addition to the basic interest of the key biological questions that underlie these processes, our work aims at generating new tools and knowledge for improving crop production. For this ultimate goal, we exploit natural diversity resources as well as genetic engineering, including CRISPR/Cas9 technology for precise genome editing, as promising tools and methods. Direct biotechnological applications of plants are also addressed, such as their use as biopharmaceutical factories or as tools for alleviating metal pollution and related environmental conditions. We use model species, such as the angiosperms *Arabidopsis thaliana, Nicotiana benthamiana* and the duckweed Lemna spp., and the liverwort *Marchantia polymorpha*. Crops such as tomato, potato and Prunus are also major subjects of our studies, to which knowledge generated in the model species is applied.

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Figure legend: Arabidopsis thaliana *mutants in SNOWY COTYLEDON 2 (Pablo Pulido).*

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Natural variation of plant development

The main goal of our laboratory is to understand the genetic, molecular and evolutionary mechanisms involved in plant adaptation. In particular, we are interested in understanding how developmental traits enable plant adaptation. To address this question we are exploiting the genetic variation that exists in nature in the wild, annual, and model plant *Arabidopsis thaliana*. In the past few years we have analysed the natural variation for the amount and distribution (pattern) of trichomes, showing that Arabidopsis has evolved trichomes in fruits exclusively in the Iberian Peninsula (Figure 1 and 2). Genetic analyses have demonstrated that three loci named as *MALAMBRUNO* (*MAU*) 2, 3 and 5, showing strong epistatic interactions, are necessary and sufficient to display this trait. Molecular analyses show that synergistic mutations in three genes encoding MYB transcription factors, TCL1, TRY and GL1, have driven evolutionary innovations in fruit trichome patterning in Arabidopsis (Arteaga *et al*., 2021).

Genome-wide association analyses for trichome pattern in other organs, such as leaves, stems and pedicels, have shown that partly independent genomic architectures underlie vegetative and reproductive phases. Furthermore, climatic associations suggest a precise fit between trichome patterning and climate throughout the Arabidopsis life cycle. In addition, in collaboration with other laboratories, we have also analysed Arabidopsis natural variation for flowering time and virus tolerance (Shukla *et al*., 2022), as well as for oxygen dependent traits (Abbas *et al*., 2022), thus identifying new adaptive mechanisms. Finally, we have also developed a new collection of natural strains of the Arabidopsis relative *Cardamine hirsuta*, which will enable future comparative genomic analyses of developmental traits (Fuster, 2022).

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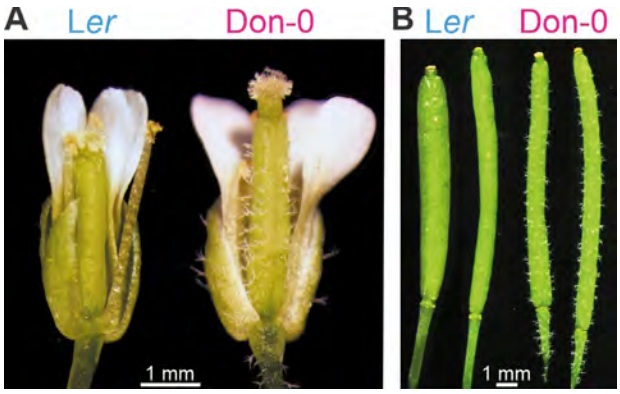

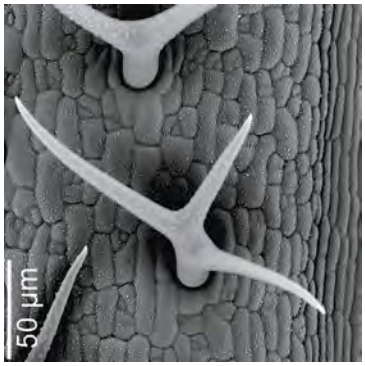

2

1 *Natural variation for trichome development in carpels (A) and fruits (B) of Arabidopsis. Ler and Don-0 are two natural accessions of Arabidopsis from Poland and Spain, respectively.*

2 *Branched trichome developed in a fruit of Don-0 accession from Spain.*

SELECTED PUBLICATIONS

Arteaga N, Savic M, Méndez-Vigo B, Fuster-Pons A, Torres-Pérez R, *et al*. MYB transcription factors drive evolutionary innovations in Arabidopsis fruit trichome patterning. Plant Cell 2021, 33, 548-565.

Arteaga N, Méndez-Vigo B, Savic M, Fuster-Pons A, Murillo-Sánchez A, *et al*. Differential environmental and genomic architectures shape the natural diversity for trichome patterning and morphology in different Arabidopsis organs. Plant Cell Environ 2022, 45, 3018-3035.

Abbas M, Sharma G, Dambire C, Marquez J, Alonso-Blanco C, *et al*. An oxygen-sensing mechanism for angiosperm adaptation to altitude. Nature 2022, 606, 565-569.

Méndez-Vigo B, Castilla AR, Gómez R, Marcer A, Alonso-Blanco C, Picó FX. Spatiotemporal dynamics of genetic variation at the quantitative and molecular levels within a natural *Arabidopsis thaliana* population. J Ecol 2022, 110, 2701-2716.

Shukla A, Pagán I, Crevillén, P, Alonso-Blanco C, García- Arenal F. A role of flowering genes in the tolerance of *Arabidopsis thaliana* to cucumber mosaic virus. Mol Plant Pathol 2022, 23, 175-187.

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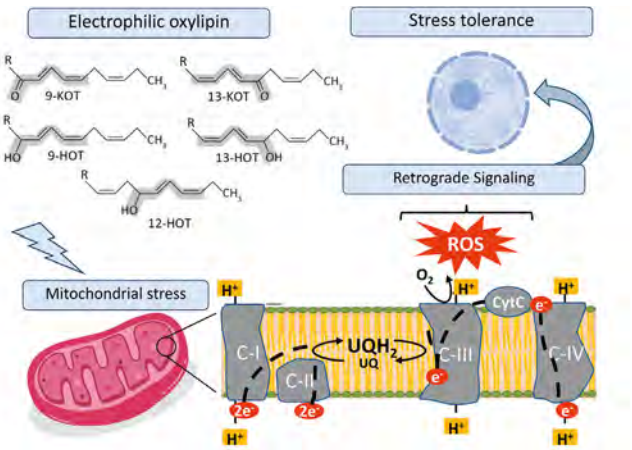
Plant immunity strategies against microbial pathogen infection

Plant pathogens cause diseases in many economically important crop plants leading to severe losses in food production that are also of fundamental importance for forestry, other plant-derived products and for the sustainability of natural environments. This circumstance, pose a severe threat to agriculture and plant sustainability. An important requirement for the development of successful plant disease control strategies is the understanding of host-pathogen interactions; a knowledge critical for the devise of effective approaches to minimise plant losses due to infection. We focus our research in exploring the activities of oxylipins, a family of lipid derivatives activating immune responses in plants. Our research has revealed that oxylipins produced by the biosynthetic pathways initiated by fatty acid alpha-dioxygenases (alpha-DOXs) and 9-lipoxigenases (9-LOXs) contribute to the activation of local and systemic defense. Analyses with 9-HOT and 9-KOT (two members of the 9-LOX pathway) and signaling mutants (*noxy* for *non-responding to oxylipins)* showed the role of mitochondria in 9-LOX signaling and the action of 9-HOT and 9-KOT by enhancing ROS production at complex III of the respiratory mitochondrial chain; a response that protects plants against subsequent mitochondrial damage and activates plant defense. Protection against mitochondrial damage was found in *noxy* mutants affecting mitochondria, cytoplasmic or chloroplast proteins. This indicated that mitochondrial protection is a common response to distinct stresses and likely a critical process to maintain energy supply and facilitate plant survival. Furthermore, our results support the action of oxylipins as inducers of retrograde signaling pathways mediating communication and functional coordination of organelles during the activation of plant immunity. Evidence suggests that these responses are mediated by covalently binding of oxylipins to their targets, presumably mitochondrial proteins. The characterisation of the processes mentioned will contribute to define new defense mechanisms, as well as the signals, pathways, and genes involved in controlling plant immunity.



In environmental stress conditions, electrophilic oxylipins from both enzymatic and non-enzymatic sources cause a mild, ROS-associated mitochondrial stress. This process triggers a retrograde signalling pathway, leading to induction of nucleus-encoded genes which protect mitochondria against subsequent stress.

SELECTED PUBLICATIONS

Izquierdo Y, Muñiz L, Vicente J, Kulasekaran S, Aguilera V, *et al*. Oxylipins from different pathways trigger mitochondrial stress signaling through respiratory complex III. Front Plant Sci 2021, 2:705373.

López Sánchez A, Hernández Luelmo S, Izquierdo Y, López B, Cascón T, Castresana C. Mitochondrial stress induces plant resistance through chromatine changes. Front Plant Sci 2021, 12:704964.

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Genetic control of shoot branching patterns in plants

We are studying the genetic basis of the control of axillary bud development in the model system Arabidopsis, and in the crop species tomato and potato in which control of lateral shoot branching is of great agronomical interest. We have characterised the Arabidopsis *BRANCHED1* (*BRC1*) gene, which acts as a central switch of axillary bud development and outgrowth. We have recently combined ChIP-seq, transcriptomic and systems biology approaches to characterise the Arabidopsis BRC1-regulated gene network. We have identified a group of *BRC1* direct target genes encoding transcription factors (BTFs) that orchestrate, together with *BRC1*, an intricate transcriptional network enriched in abscisic acid signalling components. The *BRC1* network is enriched in feed-forward and feed-back loops, robust against noise and mutation and reversible in response to stimuli. This knowledge is fundamental to adapt plant architecture and crop production to ever-changing environmental conditions.

In Solanaceae we are exploring new roles of BRC1-like genes in relation to the control of carbon allocation storage and usage, a process critical for plant growth and development. A good model system to study this is potato tuberisation, a developmental program important for both crop food production and CO2 capture. Potato tubers are natural carbon reserves in the form of starch that have evolved to allow propagation and survival over winter. We have shown that *BRANCHED1b* (*BRC1b*) acts as a tuberisation repressor in aerial axillary buds, which prevents buds from competing in sink strength with stolons. *BRC1b* loss of function leads to ectopic production of aerial tubers and reduced underground tuberization. In aerial axillary buds, *BRC1b* promotes dormancy, abscisic acid responses and a reduced number of plasmodesmata. This limits sucrose accumulation and access of the tuberigen protein SP6A. *BRC1b* also directly interacts with SP6A and blocks its tuber-inducing activity in aerial nodes. Altogether, these actions help promote tuberisation underground.

In potato plants a single gene, BRANCHED1b, blocks the accumulation of sugars in above-ground plant organs. Its loss results in tubers growing on aerial shoots.

SELECTED PUBLICATIONS

Muñoz A, Pillot JP, Cubas P, Rameau C. Methods for phenotyping shoot branching and testing strigolactone bioactivity for shoot branching in Arabidopsis and pea. Methods in Molecular Biology. Strigolactones: Methods and Protocols, 2021 2309:115-127.

Sánchez E, Cubas P, Cardinale F, Visentin I. Evaluation of the bioactivity of strigolactone-related molecules by a quantitative luminometer bioassay. Methods in Molecular Biology. Strigolactones: Methods and Protocols 2021, 2309:191-200.

Nicolas M, Torres-Pérez R, Wahl, V Cruz-Oró E, Rodríguez-Buey ML, *et al*. Spatial control of potato tuberisation by the TCP transcription factor BRANCHED1b. Nat Plants 2022, 8, 281–294.

Li Q, Sánchez Martín-Fontecha E, Khosla A, White A, *et al*. Degradation of SUPPRESSOR OF MAX2 1 (SMAX1) by the strigolactone receptor D14 in Arabidopsis thaliana Plant Comms 2022, 3, (2)10030.

Fichtner F, Barbier F, Kerr S, Dudley C , Cubas P, *et al*. Plasticity of bud outgrowth varies at cauline and rosette nodes in Arabidopsis thaliana. Plant Physiol 2022, 188:1586-1603.

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Regulation of gene expression in plants

Plant adaptation to environmental stimuli involves the activation of specific transcriptional cascades and networks that allow plants to reprogram their growth and development in a changing environment. Regulation of these networks relies on sequence-specific transcription factors (TFs), regulatory proteins responsible for the transcriptional activation or repression of target genes.

Research in our group is focused in the study of the components that determine specific recognition of TF target genes and which may influence in the levels of gene expression. During the last few years we have contributed to the characterisation of one of these components, the short DNA sequences bound by TFs, known as TF-binding sites (TFBS) and we have explored the role of some other components involved in this process. With this regard, we have demonstrated that binding of some TFs extends beyond the TFBS core sequence, as some distant nucleotides, likely determining DNA-shape, are necessary for protein binding. We are also studying the role of the cytosine methylation epigenetic mark in the TFBS region during TF-target recognition, as well as its genetic control, what will allow adding a new layer of regulation of gene expression.

In addition we are developing some bioinformatic and computational approaches for a biological interpretation of genomic data and for the prediction of TFBS involved in the regulation of biological processes. These tools would contribute to a better and faster interpretation of biological data for the plant biology community, particularly in the case of non-expert researchers in bioinformatics or in the study of non-model species.

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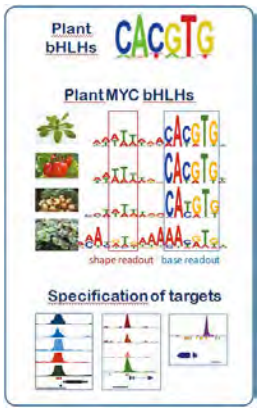
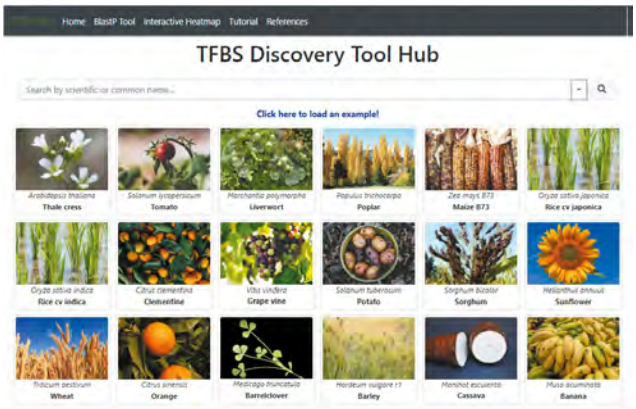


1 *Different components contribute to specification of bHLH targets.*



2

2 *TDTHub helps identifying relevant TFBS in model plants and crops*

SELECTED PUBLICATIONS

Grau J, Franco-Zorrilla JM. TDTHub, a web server tool for the analysis of transcription factor binding sites in plants. Plant J, 2022, 111(4), 1203.

López-Vidriero I, Godoy M, Grau J, Peñuelas M, Solano R, Franco-Zorrilla JM. DNA features beyond the transcription factor binding site specify target recognition by plant MYC2-related bHLH proteins. Plant Commun 2021, 2(6), 100232.

Franco-Zorrilla JM, Prat S. DAP-Seq Identification of Transcription Factor-Binding Sites in Potato. Methods Mol Biol 2021, 2354, 123.

Ramírez Gonzales L, Shi L, Bergonzi SB, Oortwijn M, Franco-Zorrilla JM, *et al*. Potato CYCLING DOF FACTOR 1 and its lncRNA counterpart StFLORE link tuber development and drought response. Plant J. 2021, 105, 855.

Kneeshaw S, Soriano G, Monte I, Hamberg M, Zamarreño AM, *et al*. Ligand diversity contributes to the full activation of the jasmonate pathway in *Marchantia polymorpha*. Proc Natl Acad Sci USA 2022, 119(36), e2202930119.

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Plant-pathogen-interaction in viral infections

Although metagenomic studies show that most viral infections do not produce noticeable damage in wild plant hosts, viruses also cause severe diseases in all type of cultured plants. Understanding the infection process is essential to uncover factors involved in viral pathogenicity and find weak points on which to act in order to prevent plant diseases of agronomical relevance. This is the general objective of our research line.

We are mainly interested in the family *Potyviridae*, the largest group of plant RNA viruses, especially in plum pox virus (PPV), which causes sharka, an important disease of trees of the genus *Prunus*. We have shown the peculiar nature of the P1 protein of the potyvirus sweet potato feathery mottle virus (SPFMV), which is able to functionally replace both P1 and HCPro of PPV. The wide variety of viral species selected in plants infected with the PPV-SPFMV chimera highlights the strong adaptation capacity of P1 and provides hints about the evolution of the family *Potyviridae*. We have also obtained evidence that the fusion of pyrophosphatase of non-canonical nucleotides to the viral polymerase facilitates adaptation of viruses to hosts of the family *Euphorbiaceae*. Also related with host adaptation, our results have shown that mutations emerged in a PPV isolate of the cherry strain during adaptation to *Arabidopsis thaliana* facilitates its infection in *Chenopodium foetidum*, a non-host species for this PPV strain. These results reveal how virus host jumping can be promoted by pre-adaptation into an intermediate host. Regarding engineering resistance, we have demonstrated that targeting the viral minus strand RNA contributes to the antiviral resistance against PPV conferred by artificial miRNAs (amiRNAs), and that in partially resistant plants, the selection pressure posed by the dual activity of both amiRNA strands on the genomic and minus viral RNA strands causes an evolutionary explosion resulting in the emergence of a wide range of virus variants. *(Research supported by grants of the Spanish government BIO2016-80572-R PID2019-109380RB-100 to J.A. García and C. Simón, and PID2019-110979RB-100 to A. Valli).*

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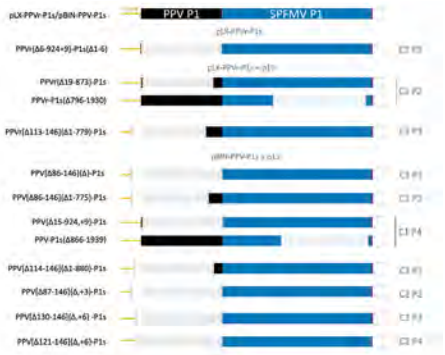

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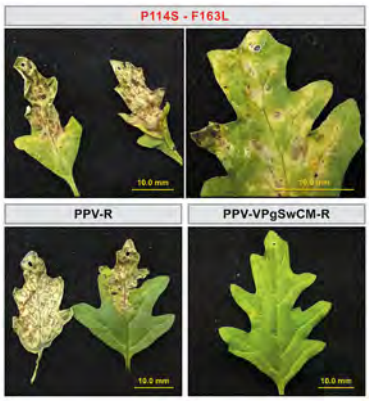

1 *Schematic representation of the different viral species found in plants infected with a chimeric plum pox virus carrying P1 of sweet potato feathery mottle virus.*

2 *Effect of VPg mutations emerged in* Arabidopsis thaliana *on* Chenopodium foetidum *infection by a plum pox virus chimera.*

SELECTED PUBLICATIONS

Rodamilans B, Casillas A, García JA. P1 of Sweet potato feathery mottle virus shows strong adaptation capacity, replacing P1-HCPro in a chimeric Plum pox virus. J Virol 2021, 95, e0015021.

Martínez-Turiño S, Calvo M, Bedoya LC, Zhao M, García JA. Virus host jumping can be boosted by adaptation to a bridge plant species. Microorganisms 2021, 9, 805.

Valli A, García López R, Ribaya M, Martínez FJ, García Gómez D, *et al.* Maf/ham1-like pyrophosphatases of non-canonical nucleotides are host-specific partners of viral RNA-dependent RNA polymerases. PLoS Pathogens 2022, 18, e1010332.

Rodamilans B, Oliveros JC, San León D, Martínez-García PJ, Martínez-Gómez P, *et al.* sRNA analysis evidenced the Involvement of different plant viruses in the activation of RNA silencing-related genes and the defensive response against Plum pox virus of 'GF305' peach grafted with 'Garrigues' almond. Phytopathology 2022, 112, 2012-2021.

Mesel F, Zhao M, García B, Simón-Mateo C, García JA. Targeting of genomic and negative-sense strands of viral RNA contributes to antiviral resistance mediated by artificial miRNAs and promotes the emergence of complex viral populations. Mol Plant Pathol 2022, 23, 1640-1657.

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Mechanisms underlying nutrient uptake and phytoremediation

Understanding the mechanisms underlying stress perception and growth adaptation to stress severity is a major goal of biology. This is particularly relevant at present, since climatic models predict the sudden availability of toxic compounds in the biosphere. Among all toxic compounds, the presence of arsenic in soils and waters is particularly serious in rice, being the most important entry of arsenic in the human food chain.

In our laboratory we are involved in the characterisation of the molecular mechanisms underlying arsenic perception in plants.

In the last two years we uncover a complex regulatory network that sense arsenite tightly coordinating the amount of arsenic perceived by plants with its detoxification capacity and accumulation (Navarro *et al*., 2021, Molecular Plant). Indeed, in collaboration with our colleague, Javier Paz-Ares, our results contributed to provide a perspective review of the cross talk between arsenate and phosphate uptake regulation (Paz-Ares *et al*., 2021, Molecular Plant). Finally, in collaboration with our colleague Carlos Alonso-Blanco, we performed a study of the natural variation of nitrogen, phosphorous and arsenic accumulation in a collection of duckweed natural isolates from the Iberian Peninsula. Currently, we are identifying regulators of the arsenic response critical for arsenic perception (Figure 1 and 2).

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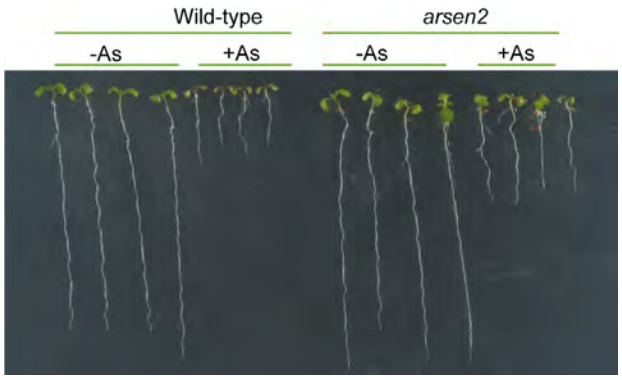
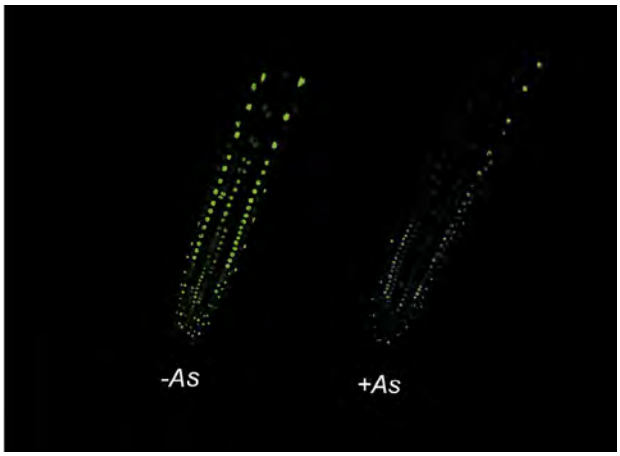


1 *ArSen2 mutant (arsen2) exhibits an arsenic tolerance phenotype. Wild-type and Arsen2 KO mutant plants (arsen2) were grown on MS vertical plates with (+As) or without (-As) arsenite.*

2



2 *ArSen2 is down regulated in response to arsenic. Confocal microscopic analysis of Arabidopsis plants expressing the ArSen2 tagged with GFP with or without arsenite (As).*

SELECTED PUBLICATIONS

Navarro C, Mateo-Elizalde C, Mohan TC, Sánchez-Bermejo E, Urrutia O, *et al*. Arsenite provides a selective signal that coordinates arsenate uptake and detoxification through the regulation of PHR1 stability in Arabidopsis. Mol Plant 2021, 6;14(9):1489-1507.

Paz-Ares J, Puga MI, Rojas-Triana M, Martinez-Hevia I, Diaz S, *et al*. Plant adaptation to low phosphorus availability: Core signaling, crosstalks, and applied implications. Mol Plant 2021,15(1):104-124.

Navarro C, Navarro MA, Leyva A. Arsenic perception and signaling: The yet unexplored world. Front Plant Sci 2022, 13:993484.

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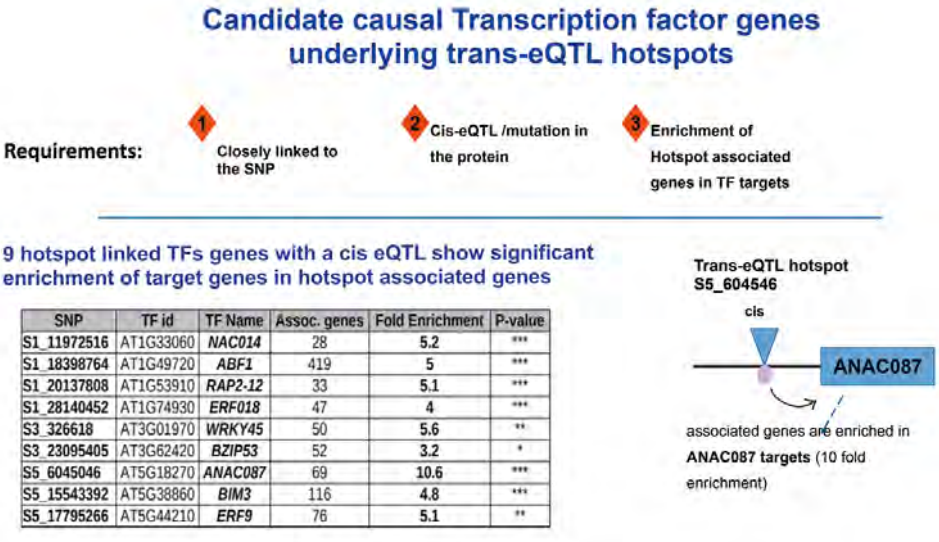
Regulation of gene activity in plants. The phosphate starvation rescue system

The phosphate starvation rescue system has been a model for studies on the regulation of gene activity since the beginning of molecular genetics. In plants this system has recently received great attention due to its potential to provide tools and strategies towards improving phosphate use efficiency, a key objective towards the effective implementation of sustainable agriculture practices. In the past, we identified several key components of the phosphate starvation signalling pathway following forwards genetics approaches (see Paz-Ares *et al* 2022)

Recently we embarked in the analysis of natural variation of the Pi starvation rescue system. Specifically, we performed a Genome wide Association Study (GWAs) of the Pi starvation transcriptome and metabolome in the Iberian collection of Arabidopsis accessions (kindly provided by Carlos Alonso-Blanco). We identified 15891 QTls associated to changes in 5999 metabolites (out of 8869 detected) and 18892 genes (out of 20808 detected), indication that a large proportion of the metabolome and transcriptome displays variation among different accessions. Of note is that we identified 1296 hotspots QTls each affecting the expression/accumulation more than 20 genes(eQTL)/metabolites (mQTL). Following the strategy depicted in Figure 1, we have identified 9 transcription factor (TF) genes as candidate causal genes underlying eQTL hotspots.



SNP	TF id	TF Name	Assoc. genes	Fold Enrichment	P-value
S1_11972516	AT1G33060	*NAC014*	28	5.2	***
S1_18398764	AT1G49720	*ABF1*	419	5	***
S1_20137808	AT1G53910	*RAP2-12*	33	5.1	***
S1_28140452	AT1G74930	*ERF018*	47	4	***
S3_326618	AT3G01970	*WRKY45*	50	5.6	**
S3_23095405	AT3G62420	*BZIP53*	52	3.2	*
S5_6045046	AT5G18270	*ANAC087*	69	10.6	***
S5_15543392	AT5G38860	*BIM3*	116	4.8	***
S5_17795266	AT5G44210	*ERF9*	76	5.1	**

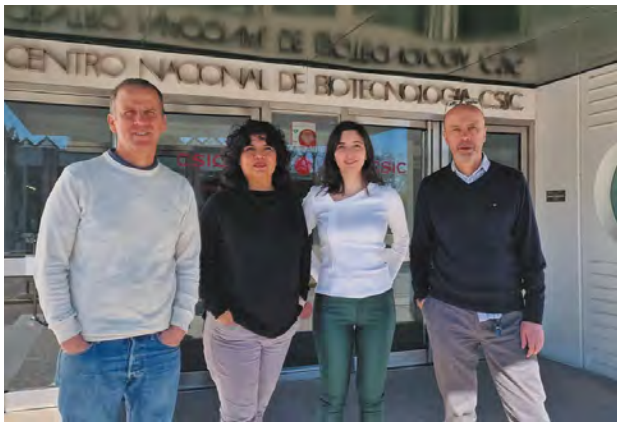
Strategy towards the identification of candidate causal TF genes underlying trans eQTL hotspots. *The causal TF gene should be 1) closely linked to the SNP; 2) the SNP should be associated to an alteration of the expression of the TF gene (cis-eQTL) or to a plausible strong mutation in the protein and 3) hotspot- associated genes should be enriched in targets of the candidate TF*

SELECTED PUBLICATIONS

Navarro C, Mateo-Elizalde C, Mohan TC, Sánchez-Bermejo E, Urrutia O, *et al*. Arsenite provides a selective signal that coordinates arsenate uptake and detoxification through the regulation of PHR1 stability in Arabidopsis. Mol Plant 2021 14:1489-1507.

Guo M, Ruan W, Zhang Y, Zhang Y, Wang X, *et al*. A reciprocal inhibitory module for Pi and iron signaling. Mol Plant 2022, 15:138-150.

Paz-Ares J, Puga MI, Rojas-Triana M, Martinez-Hevia I, Diaz S. Plant adaptation to low phosphorus availability: Core signaling, crosstalks, and applied implications Mol Plant 2022 15:104-124.



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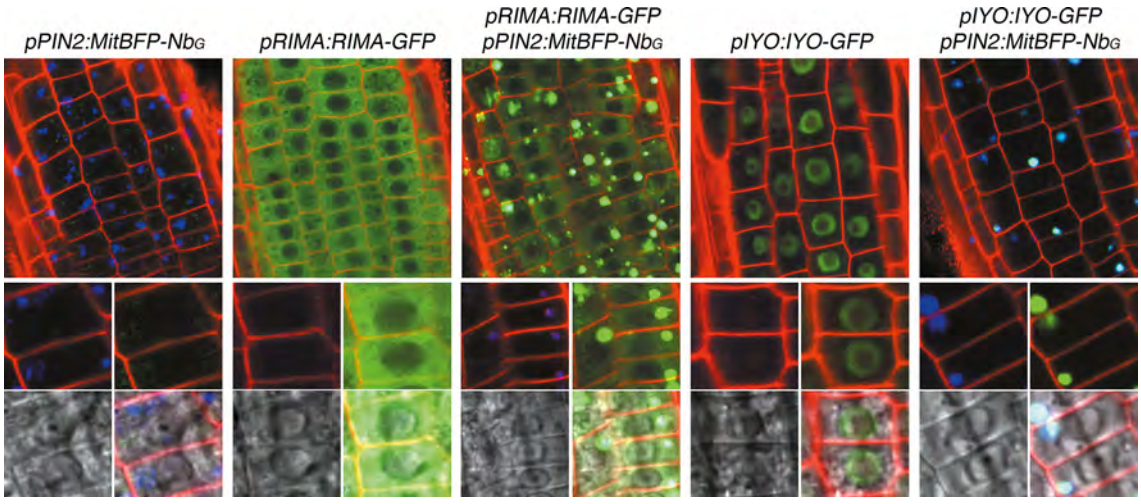
Signalling networks in plant development and defense responses

Plants are powerful model systems for genetically dissecting the mechanisms controlling stem cell fate. In our group we have identified two Arabidopsis genes, IYO and *RIMA*, which are required for initiating all events of stem cell differentiation in the plant. Our working model is that RIMA-dependent nuclear IYO accumulation functions as a switch that activates stem cell differentiation by directly regulating RNA Polymerase II activity and mediating transcriptional reprogramming in stem cell progeny. In the last two years, we have focused in determining the molecular mechanisms that regulate IYO nuclear migration and in identifying direct transcriptional targets of the IYO/RIMA module that drive cell differentiation. We are investigating whether the phosphorylation status of IYO determines its subcellular distribution and how it is controlled by endogenous and external stimuli. In addition, we have identified gene promoter regions bound by RIMA and are now developing genetic tools (Figure 1) for conditional activation and disruption of IYO and RIMA with the aim of determining early transcriptional responses to alterations in their activity. Ultimately, we want to use the knowledge gained on the function of this cell differentiation switch to alter organogenesis and/or regeneration capacity at will, and are currently pursuing some proof of concept experiments to demonstrate the usefulness of this technology for crop breeding.



Anti-GFP nanobody-mediated lockdown of IYO-GFP and RIMA-GFP in mitochondria of null mutants complemented with the GFP fusions (in collaboration with Dr. D. Van Damme, VIB).

SELECTED PUBLICATIONS

Chen L, Zhao M, Wu Z, Chen S, Rojo E, *et al*. RNA polymerase II associated proteins regulate stomatal development through direct interaction with stomatal transcription factors in Arabidopsis thaliana. New Phytol 2021, 230, 171-189.

Aniento F, Isono E, Rojo E, Russinova E. Highlights of ENPER 2019-European Network for Plant Endomembrane Research Meeting. Front Plant Sci 2021, 12, 12:719367.

Hu S, Li B, Wu F, Zhu, D, Zouhar J, *et al*. Plant ESCRT protein ALIX coordinates with retromer complex in regulating receptor-mediated sorting of soluble vacuolar proteins. Proc Natl Acad Sci USA 1 2022, 119(20):e2200492119.

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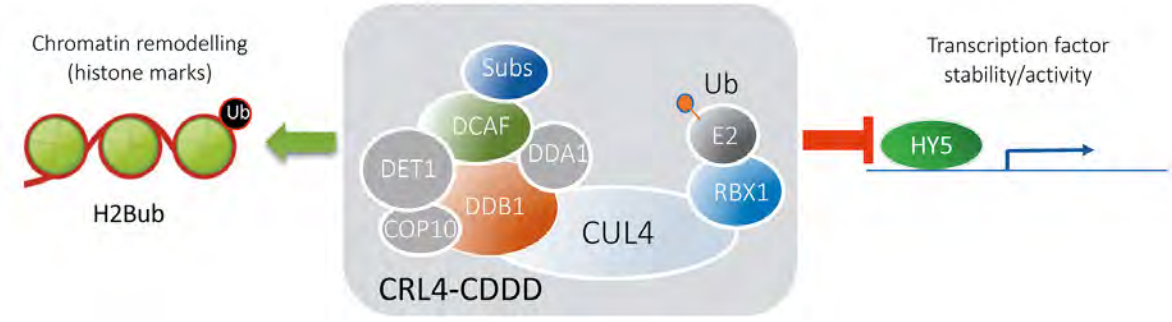
Role of ubiquitin in the control of plant growth and stress tolerance

Protein ubiquitination is an integral regulatory mechanism of many signaling pathways in plants. Ubiquitin (Ub) conjugation to proteins may trigger degradation of protein targets at the 26S proteasome or changes in their properties (e.g., protein activity, localization, assembly and interaction ability), depending on the extent or specific Ub chain configurations. Protein ubiquitination is mediated by an enzymatic cascade in which different types of E3 Ub ligases provide the substrate specificity. Among them, Cullin4 RING E3 ubiquitin ligases (CRL4) have been involved in biological processes spanning the plant´s whole life by promoting degradation of specific targets controlling those processes. Research at my group has been focused in the characterisation of CRL4 E3s that regulate developmental and stress responses in plants, including light and abscisic acid (ABA)-mediated stress signaling. Thus, we have reported novel mechanisms to modulate ABA responses in plants based on targeted destabilization of the ABA receptors (Irigoyen *et al*., 2014 Plant Cell; García-León *et al*., 2019 Plant Cell). Interestingly, CRL4 function is performed in close proximity to chromatin, which should enable rapid translation of environmental and stress signals into changes in gene expression. Indeed, we found that a CRL4-DET1 complex mediates a molecular pathway controlling epigenetic homeostasis (including Histone2B ubiquitination) in response to external stimuli (i.e. light conditions; Nassrallah *et al*., 2018 eLife). Our current objectives aim to identify and characterise new mechanisms by which CRL4 controls accumulation of specific epigenetic marks over the plant genome in response to environmental changes, to regulate expression of specific gene sets that lead to plant adaptation to changing climate conditions, as it is the case of COP1-DET1-complex-mediated destabilization of HY5 (Figure 1. Cañibano *et al*., 2021 Molecular Plant). As a highlight of our contributions to this field, we organised an International Conference on Plant Proteostasis in 2022 at the CNB-CSIC.



DET1 complexes play multiple roles at the plant chromatin by controlling accumulation of specific epigenetic marks and the stability of transcription factors.

SELECTED PUBLICATIONS

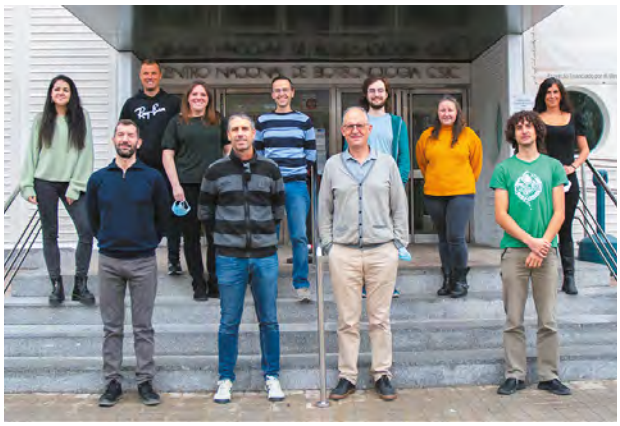
Grasser KD, Rubio V, Barneche F. Multifaceted activities of the plant SAGA complex. Biochim Biophys Acta Gene Regul Mech 2021, 2, 194613.

Cañibano E, Bourbousse C, García-León M, Garnelo Gómez B, Wolff L, *et al*. DET1-mediated COP1 regulation avoids HY5 activity over second-site gene targets to tune plant photomorphogenesis. Mol Plant 2021, 14, 1–20.

Lee B-D, Yim Y, Cañibano E, Kim S-H, García-León M, *et al*. CONSTITUTIVELY PHOTOMORPHOGENIC1 promotes seed germination by destabilizing RGA-LIKE2 in Arabidopsis. Plant Physiology 2021, kiac060

Theodoulou FL, Orosa-Puente B, Trujillo M, Rubio V. Plant proteostasis: a proven and promising target for crop improvement. Essays in Biochemistry 2022, 66, 75-85

Llamas E, Pulido P. A proteostasis network safeguards the chloroplast proteome. Essays in Biochemistry 2022, 66, 219-228.



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Jasmonate signalling in plants

Jasmonates (JAs) are fatty acid-derived signalling molecules essential for the survival of plants in nature since they are important activators of stress responses and developmental programs. The main focus of our lab is to understand mechanistically the JA signalling pathway in plants; knowledge that is basic to design biotech and agronomical applications that improve plant resistance to stresses and plant yield. We have traditionally worked in the model plant *Arabidopsis thaliana,* but have recently focused in the Liverwort *Marchantia polymorpha* due to its remarkable genetic advantages, such as very low gene redundancy.

Our major achievements in the last two years are:

- Identification of the binding determinants of the dn-OPDA co-receptor MpJAZ (Monte *et al*., PNAS, 2022)
- Discovery of a new ligand of the MpCOI1/MpJAZ co-receptor that co-operate with dn-OPDA in the activation of the JA pathway in *M. polymorpha* (Kneeshaw *et al*., PNAS, 2022)
- Elucidation of the biosynthesis pathway for dn-OPDA in M. polymorpha (Soriano *et al*., New Phytologist. 2022)
- Identification of a general antagonist of the JA receptor that functions in vascular and non-vascular plants (Chini *et al*., Plant Phys. 2021)
- Identification of a potent anti-SARS-Cov2 antiviral using Marchantia extracts (Jimenez-Aleman *et al*., *Pharmaceuticals*. 2021).
- Collaborated in the characterisation of DNA-binding determinants of MYC TFs (Lopez-Vidriero *et al*., Plant Communications, 2021)
- Collaborated in the discovery of the role of ANAC089 in seed germination and stress response (Albertos *et al*., Cell Reports, 2021).
- Collaborated in the characterisation of responses to Fusarium pathogens in *M. polymorpha* (Redkar *et al*., New Phytologist, 2021; Redkar *et al*., The Plant Cell, 2022)
- Collaborated in the characterisation of CDF1 and StFlore in tomato tuber development and drought (Ramirez *et al*., TPJ, 2021)

Like other bryophytes, Marchantia is dioecious, so the fertilisation occurs only by mating of male and female plants. The receptacle bearing female sex organs, called archegoniophore, is shown here.

SELECTED PUBLICATIONS

Chini A, Monte I, Fernandez-Barbero G, … Raikhel N, Solano R. A small molecule antagonizes jasmonic acid perception and auxin responses in vascular and nonvascular plants. Plant Physiology. 2021, 187(3), pp. 1399–1413.

Jimenez-Aleman GH, Castro V, Londaitsbehere A, Gutierrez-Rodríguez M, Garaigorta U, *et al*. SARS-CoV-2 Fears Green: The Chlorophyll Catabolite Pheophorbide A Is a Potent Antiviral. Pharmaceuticals 2021 14(10):1048.

Monte I, Caballero J, Zamarreño AM, …García-Mina JM, Solano R. JAZ is essential for ligand specificity of the COI1/JAZ co-receptor. Proc Natl Acad Sci USA 2022, 119(49): e2212155119.

Kneeshaw S, Soriano G, Monte I, …Jimenez-Aleman GH, Solano R. Ligand diversity contributes to the full activation of the jasmonate pathway in Marchantia polymorpha. Proc Natl Acad Sci USA 2022, 119(36), e2202930119.

Soriano G, Kneeshaw S, Jimenez-Aleman G, …García-Mina JM, Solano R. An evolutionarily ancient fatty acid desaturase is required for the synthesis of hexadecatrienoic acid, which is the main source of the bioactive jasmonate in *Marchantia polymorpha*. New Phytologist 2022, 233(3), pp. 1401–1413.