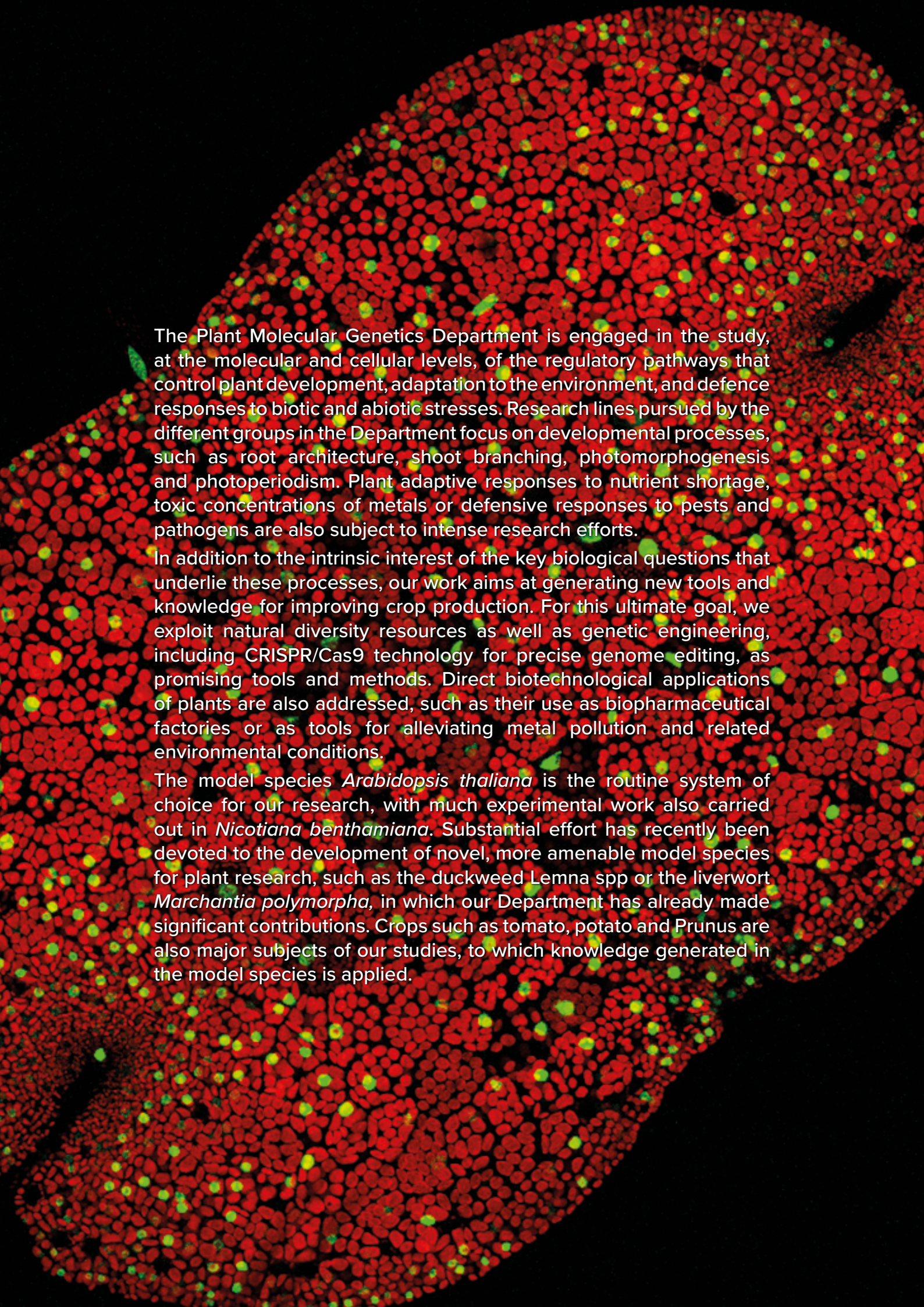

The Plant Molecular Genetics Department is engaged in the study, at the molecular and cellular levels, of the regulatory pathways that control plant development, adaptation to the environment, and defence responses to biotic and abiotic stresses. Research lines pursued by the different groups in the Department focus on developmental processes, such as root architecture, shoot branching, photomorphogenesis and photoperiodism. Plant adaptive responses to nutrient shortage, toxic concentrations of metals or defensive responses to pests and pathogens are also subject to intense research efforts.

In addition to the intrinsic 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.

The model species *Arabidopsis thaliana* is the routine system of choice for our research, with much experimental work also carried out in *Nicotiana benthamiana*. Substantial effort has recently been devoted to the development of novel, more amenable model species for plant research, such as the duckweed Lemna spp or the liverwort *Marchantia polymorpha,* in which our Department has already made significant contributions. Crops such as tomato, potato and Prunus are also major subjects of our studies, to which knowledge generated in the model species is applied.

# Plant Molecular Genetics

**HEAD OF DEPARTMENT**
José Juan Sánchez Serrano

**RESEARCH GROUPS**

1. **Natural variation in plant development**
   Carlos Alonso-Blanco
2. **Plant immunity strategies against microbial pathogen infection**
   Carmen Castresana
3. **Genetic control of bud dormancy**
   Pilar Cubas
4. **Plant-pathogen interaction in viral infections**
   Juan A. García & Carmen Simón
5. **Mechanisms underlying nutrient uptake and phytoremediation**
   Antonio Leyva
6. **Regulation of gene activity in plants: the phosphate starvation rescue system**
   Javier Paz-Ares
7. **Light signalling and day length control of potato tuber formation**
   Salomé Prat
8. **Role of ubiquitin in the control of plant growth and stress tolerance**
   Vicente Rubio
9. **Signalling networks in plant development and defence responses**
   José J. Sánchez-Serrano & Enrique Rojo
10. **Jasmonate signalling and plant defence**
    Roberto Solano

# Natural variation in plant development



**GROUP LEADER**
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## SELECTED PUBLICATIONS

Durvasula A, Fulgione A, Gutaker RM, Alacakaptan SI, Flood PJ, Neto C, Tsuchimatsu T, Burbano HA, Picó FX, Alonso-Blanco C, Hancock AM. African genomes illuminate the early history and transition to selfing in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 2017; 114: 5213-5218.

Lee CR, Svardal H, Farlow A, Exposito-Alonso M, Ding W, Novikova P, Alonso-Blanco C, Weigel D, Nordborg M. On the post-glacial spread of human commensal *Arabidopsis thaliana*. Nat Commun 2017; 8:14458.

Tabas-Madrid D, Méndez-Vigo B, Arteaga N, Marcer A, Pascual-Montano A, Weigel D, Xavier Picó F, Alonso-Blanco C. Genome-wide signatures of flowering adaptation to climate temperature: Regional analyses in a highly diverse native range of *Arabidopsis thaliana*. Plant Cell Environ 2018; 41: 1806-1820.

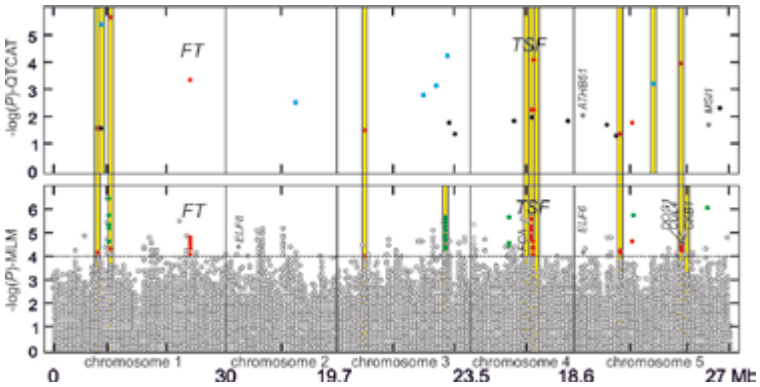
Expósito-Alonso M, Brennan AC, Alonso-Blanco C, Picó FX. Spatio-temporal variation in fitness responses to contrasting environments in *Arabidopsis thaliana*. Evolution 2018; 72: 1570-1586.

Marcer A, Vidigal DS, James PM, Fortin MJ, Méndez-Vigo B, Hilhorst HW, Bentsink L, Alonso-Blanco C, Picó FX. Temperature fine-tunes Mediterranean *Arabidopsis thaliana* life-cycle phenology geographically. Plant Biol 2018; 20: 148-156.

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, such as flowering time, seed dormancy or vegetative growth, allow plant adaptation. To address this question, we are exploiting the genetic variation that exists in nature within the wild, annual, and model plant *Arabidopsis thaliana*.

Given the relevance of climate change, our research is currently focused on identifying new genes and natural alleles that are involved in the adaptation to different climates. To this end, we are exploiting an *A. thaliana* regional collection of more than 400 wild accessions collected in the Iberian Peninsula (Tabas-Madrid *et al.*, 2018; Marcer *et al.*, 2018). We are carrying out multiple phenotypic and environmental genome-wide association analyses (GWAS), using the genome sequence of 174 Iberian accessions (Figure 1). We have identified known genes, such as *TWIN SISTER OF FT (TSF),* and new genes, like *VOLTAGE DEPENDENT ANION CHANNEL 5 (VDAC5)* as candidates for adaptation to climate temperature by altered flowering time (Figure 2). Furthermore, we are analysing the adaptive and demographic history of *A. thaliana* not only in this region (Exposito-Alonso *et al.*, 2018), but also in Eurasia (Lee *et al.*, 2017) and Africa (Durvasula *et al.*, 2017).

❶

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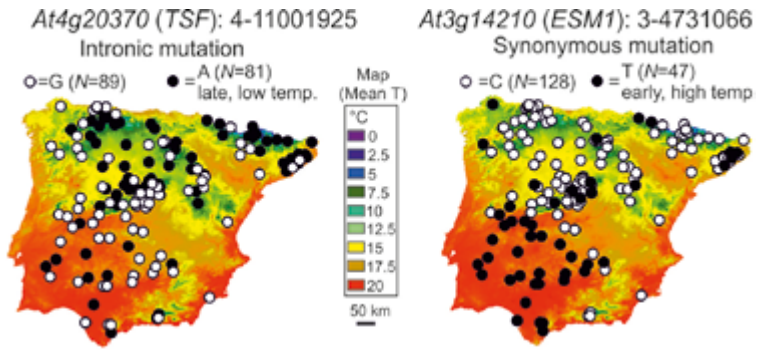


❶ *GWAS of flowering time in the Iberian Peninsula. Genomic regions associated with flowering time and climate temperature are shown in yellow colour.*

❷ *Geographic and climatic distribution of genes involved in flowering adaptation. Each panel shows the Iberian distribution of polymorphisms at TSF (left panel) and VDAC5 in relation to mean or maximum temperature, respectively.*

# Plant immunity strategies against microbial pathogen infection



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Pablo Benito

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**SELECTED PUBLICATIONS**

Sesma A, Castresana C, Castellano M. Regulation of Translation by TOR, eiF4E and eiF2α in Plants: Current knowledge, challenges and future perspectives. Front Plant Sci 2017; 8: 644.

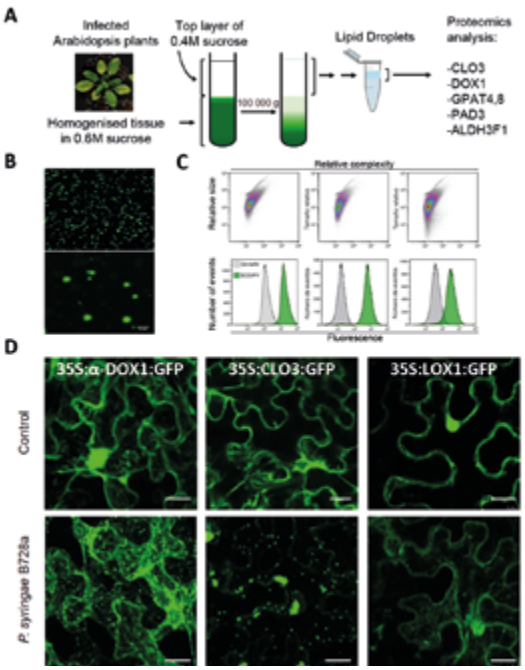
Izquierdo Y, Kulasekaran S, Benito P, López B, Marcos R, Cascón T, Hamberg M, Castresana C. Arabidopsis nonresponding to oxylipins locus NOXY7 encodes a yeast GCN1 homolog that mediates noncanonical translation regulation and stress adaptation. Plant Cell Environ 2018; 41: 1438-1452.

Vicente J, Mendiondo GM, Pauwels J, Pastor V, Izquierdo Y, Naumann C, Movahedi M, Roone D, Gibbs DJ, Smart K, Bachmair A, Gray JL, Dissmeyer N, Castresana C, Ray RV, Gevaert K and Holdsworth MJ. Distinct branches of the N-end rule pathway modulate the plant immune response. New Phytol 2019; 221: 988-1000.

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, together with an increasing augmentation of the world population, 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 and, in particular, of the molecular mechanisms evolved in plants to avoid pathogen infection. This knowledge will be critical to devise effective approaches to minimise plant losses due to infection by microbes.

To this end, we focus our research on exploring the activities of oxylipins, a family of lipid derivatives activating immune responses in plants. Over the last years, our research has revealed that oxylipins, produced by the biosynthetic pathways initiated by fatty acid alpha-dioxygenases (alpha-DOXs) and 9-lipoxygenases (9-LOXs), contribute to the activation of local and systemic defence. Moreover, we found that specific derivatives from these pathways act as inducers of defence responses and modulators of hormone homeostasis. In our recent studies, we showed that cellular organelles such as lipid droplets (Figure 1) and mitochondria are important players during the response to pathogen infection and that global translational reprogramming contribute to activation of plant immunity. Presently, we focus our research in examining the participation of oxylipins in these defence mechanisms and in defining the relevance of these processes as part of the plant defence responses to control pathogen infection. Our experiments are performed in Arabidopsis and tomato plants helping to examine the translation of results from a model plant to crops as well as to compare the responses and defence mechanisms of both types of plants. The characterisation of the processes mentioned will contribute to define new defence mechanisms, as well as the signals, pathways, and genes involved in controlling plant immunity.

**1**




**1** *Formation and characterisation of lipid droplets (LDs) in* Arabidopsis thaliana *leaves responding to* Pseudomonas syringae *pv. tomato DC3000 avrRpm1 inducing a hypersensitive defence reaction (HR).*
*(A) The protocol allows reproducible isolation of highly LD-enriched fractions from limited amounts of tissue. Mass spectrometry analyses showed the LD proteome of Pseudomonas-infected and senescent leaves.*
*(B) Confocal microscopy and flow cytometry of unstained (grey) and BODIPY stained (green) LD samples.*
*(C) LD-localisation of selected proteins was examined by transient expression in leaves of* N. benthamiana.
*According to our proteomic analyses, alpha-DOX and CLO3 localise in LDs whereas LOX1 was not detected in these cellular structures.*

# Genetic control of bud dormancy



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Coral Fernández

**SELECTED PUBLICATIONS**

Tarancón C, González-Grandío E, Oliveros JC, Nicolas M, Cubas P. A conserved carbon starvation response underlies bud dormancy in woody and herbaceous species. Front Plant Sci 2017; 8: 788.

González-Grandío E, Pajoro A, Franco-Zorrilla JM, Tarancón C, Immink RG, Cubas P. Abscisic acid signaling is controlled by a BRANCHED1/HD-ZIP I cascade in Arabidopsis axillary buds. Proc Natl Acad Sci USA 2017; 114: E245-E254

Yang Y, Nicolas M, Zhang J, Yu H, Guo D, Yuan R, Zhang T, Yang J, Cubas P, Qin G. The TIE1 transcriptional repressor controls shoot branching by directly repressing BRANCHED1 in Arabidopsis. PLoS Genet 2018; 14: e100729.

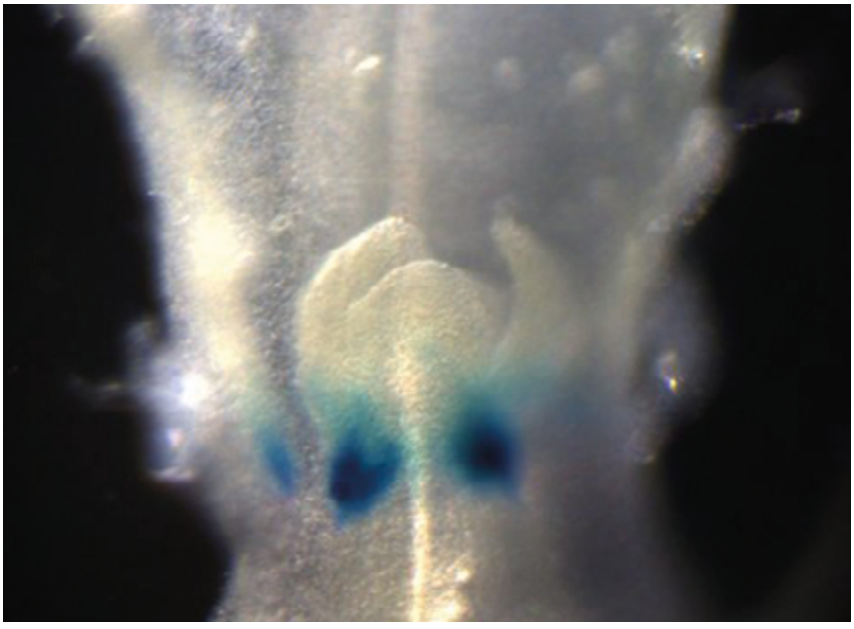
Sanchez E, Artuso E, Lombardi C, Visentin I, Lace B, Saeed W, Lolli ML, Kobauri P, Ali Z, Spyrakis F, Cubas P, Cardinale F, Prandi C. Structure-activity relationships of strigolactones via a novel, quantitative in planta bioassay. J Exp Bot 2018; 69: 2333-2343.

Martín-Fontecha ES, Tarancón C, Cubas P. To grow or not to grow, a power-saving program induced in dormant buds. Curr Opin Plant Biol 2018; 41: 102-109.

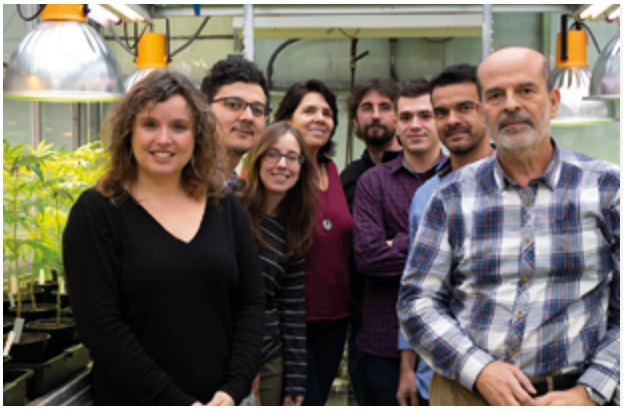
Shoot branching patterns depend on a key developmental decision: whether axillary buds grow out to give a branch or remain dormant in the leaf axils. This decision is controlled by hormone-mediated endogenous and environmental stimuli. The Arabidopsis gene BRANCHED1, which encodes a TCP transcription factor, negatively controls shoot branching. We are trying to understand the growth-to-dormancy transition in axillary buds and the role of BRC1 in this process by using transcriptomic and ChIP-seq studies to build transcriptional networks induced in dormant Arabidopsis axillary buds. We have identified several direct BRC1 targets that mediate BRC1 function and are essential for ABA synthesis and signalling in bud entering dormancy. We are also studying the evolution and divergence of the BRC1 function in *Solanaceae*, in particular in potato and tomato.

Strigolactones (SL) are phytohormones that regulate shoot branching. SL perception and signalling involve the F-box protein MAX2 and the hydrolase D14, proposed to act as a SL receptor. We are using strong loss-of-function alleles of the D14 gene to characterise its function. Our data showed that D14 distribution overlaps that of MAX2 at tissue and subcellular levels, allowing physical interactions between these proteins. Grafting studies indicated that neither D14 mRNA nor the protein move upwards over a long range in the host. Like MAX2, D14 is needed locally in the aerial part of the plant to suppress shoot branching. We also identified a mechanism of SL-induced MAX2-dependent proteasome-mediated D14 degradation. This negative feedback loop would cause a substantial drop in SL perception, which would effectively limit SL duration and signalling intensity.

**1**



**1** *Beta-glucuronidase expression driven by the HB53 promoter in* Arabidopsis thaliana *axillary buds. HB53 is a direct target of the BRANCHED1 gene and promotes bud growth arrest in short day photoperiods and under canopy shade.*



# Plant-pathogen interactions in viral infections



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**TECHNICIAN**
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Alfonso González de Prádena

**MASTER STUDENT**
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**UNDERGRADUATE STUDENTS**
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Magdalena de Arriba Sánchez de la Campa

**VISITING SCIENTISTS**
Zhenyu Liu
Andrés Mauricio Pinzón Núñez

A complex plant-virus interactive network modulates infectivity and symptom severity. Most plant viruses cannot infect all their potential hosts, and when they do, serious illness is not the norm, as revealed by recent metagenomic studies. Our laboratory studies this interaction network, which facilitates virus replication and propagation, but also induces plant defense responses and disease symptoms. *Plum pox virus*, our main subject of study, belongs to the family Potyviridae, the largest group of plant RNA viruses and causes sharka, a serious disease of stone fruit trees. We are especially interested in defense responses related to RNA silencing and its viral suppressors. The typical silencing suppressor of potyvirids is HCPro, but the existence of additional silencing suppressors in different potyvirids, prompted us to suggest that escaping RNA silencing-mediated antiviral defenses is a powerful driving force of virus evolution. Potyvirid genomic RNAs are expressed through the synthesis of large polyproteins, processed by viral-encoded endopeptidases. We are studying how host-specific modulation of this processing can contribute to potyviral pathogenicity and host range definition. Encapsidation of potyvirid genome is an active process. We have demonstrated a functional link between potyvirus RNA replication and virion assembly, and we are studying how posttranslational modifications of the capsid protein can contribute to sort the potyviral RNA into translation, replication or encapsidation. An important goal of our laboratory is applying our basic research results to control viral diseases through novel strategies. For instance, we are attenuating plant viruses by recoding their genomes in order to use them as cross-protection agents. We are also interested in developing other valuable biotools, such as a novel T-DNA delivery system, which can be used to efficiently inoculate plants with infectious viral cDNA clones, among other multiple applications.

**SELECTED PUBLICATIONS**

Pasin F, Bedoya L, Bernabé-Orts JM, Gallo A, Simón-Mateo C, Orzaez , García JA. Multiple T-DNA delivery to plants using novel mini binary vectors with compatible replication origins. ACS Synth Biol 2017; 6: 1962-1968.

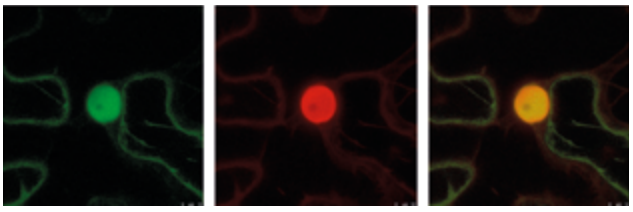
Martínez-Turiño S, Pérez JJ, Hervás M, Navajas R, Ciordia S, Udeshi ND, Shabanowitz J, Hunt DF, García JA. Phosphorylation coexists with O-GlcNAcylation in a plant virus protein and influences viral infection. Mol Plant Pathol 2018; 19: 1427-1443.

Shan H, Pasin F, Tzanetakis IE, Simón-Mateo C, García JA, Rodamilans B. Truncation of a P1 leader proteinase facilitates potyvirus replication in a non-permissive host. Mol Plant Pathol 2018; 19: 1504-1510.

Gallo A, Valli A, Calvo M, García JA. A functional link between RNA replication and virion assembly in the potyvirus *Plum pox virus*. J Virol 2018, 9: e02179-17.

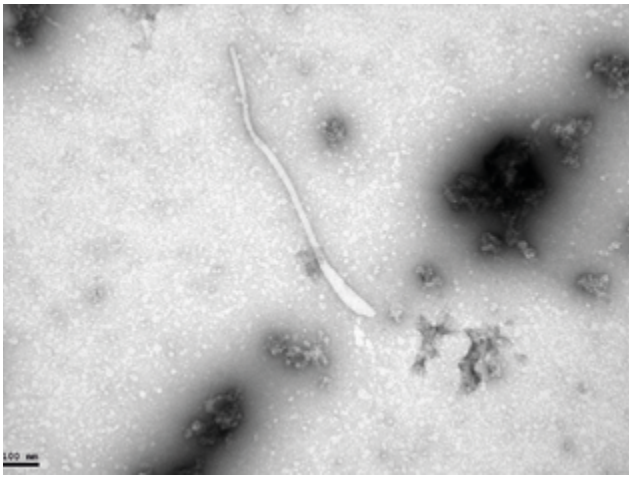
Rodamilans B, Valli A, Mingot A, San León D, López-Moya JJ, García JA. An atypical RNA silencing suppression strategy provides a snapshot of the evolution of sweet potato-infecting potyviruses. Sci Rep 2018; 8: 15937.

**1**



**1** *Confocal microscope image of the nuclear interaction between Cucumber vein yellowing virus, P1b and Importin 7 of* Nicotiana benthamiana, *observed by Bimolecular fluorescence complementation.*

**2**



**2** *Distorted virion of a* Plum pox *virus mutant emulating phosphorylation at the capsid protein N-terminal region*

# Mechanisms underlying nutrient uptake and phytoremediation



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Odette Den Rozalén
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**SELECTED PUBLICATIONS**

Puga MI, Rojas-Triana M, de Lorenzo L, Leyva A, Rubio V, Paz-Ares J. Novel signals in the regulation of Pi starvation responses in plants: facts and promises. Curr Opin Plant Biol 2017; 39: 40-49.

Navarro C, Arbaoui S, Mateo C, Bettaieb T, Leyva A. Arsenic, the Silent Threat: New Phytoremediation strategies for contaminated soils and waters. In Phytoremediation Methods, Management and Assessment, Environmental Research Advances 2017; ed. Vladimir Matichenkov, pp 27-59. Nova Science Publishers, New York.

In our group, we are interested in the characterisation of the molecular mechanisms involved in arsenic perception in plants. Our aim is to improve the efficiency of plants to extract arsenic from contaminated waters and soils. Plants have an extraordinary capacity to capture large quantities of nutrients and toxic compounds, including heavy metals and arsenic. Indeed, arsenic contamination is responsible for the worst mass poisoning ever suffered by man and is considered a silent threat to public health. Arsenic can enter into the food chain through water consumption or crops irrigated with arsenic contaminated water, particularly rice. This chemical threat was critical for the evolution of sessile organisms such as plants, which were forced to develop rapid tolerance responses when arsenic was present.

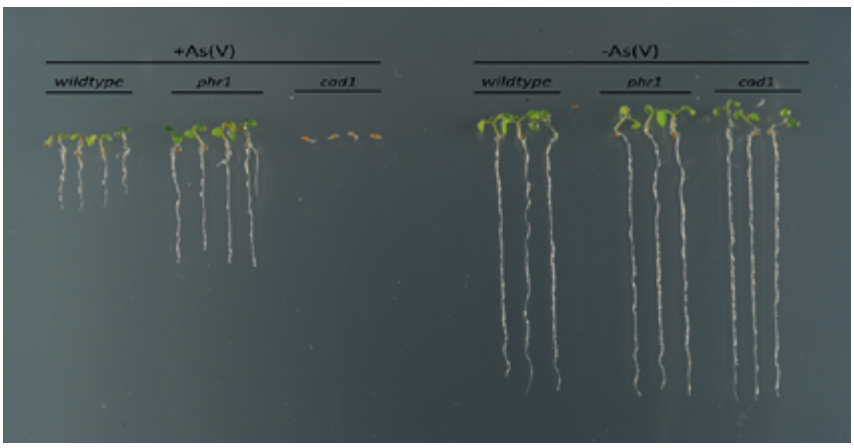
For the last two years we kept working on the characterisation of the molecular mechanisms involved in arsenic perception and detoxification using genetic and molecular approaches. We have identified several candidates of the signal transduction pathway that are currently being functionally characterised.

We have published two reviews in collaboration with other groups which outline the latest discoveries of the phosphate starvation response and the new strategies of arsenic phytoremediation.

In collaboration with Dr. Carlos Alonso Blanco at the CNB, we also study the application of natural isolates of duckweed aquatic plants for water phytoremediation. In particular, our laboratory is currently involved in the project "Duckweed technology for improving nutrient management and resource efficiency in pig" (www.life-lemna.eu), funded by the LIFE Programme of the European Commission. The collection is currently being used to identify highly efficient natural isolates for arsenic phytoextraction from contaminated waters.

The research lines in progress within our laboratory will allow us to understand the mechanisms that underlie arsenic perception, which will open up new possibilities for phytoremediation of arsenic-contaminated soils and waters.

**1**




**1** *Arsenic tolerance phenotype of Arabidopsis wildtype and mutant plants altered in arsenate uptake (phr1) or in arsenic detoxification (cad1) in the presence [+As(V)] or without [-As(V)] arsenate.*

# Regulation of gene activity in plants: the phosphate starvation rescue system



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César Poza

**TECHNICIAN**
Erica Gil

**PhD STUDENTS**
Miguel Miñambres
Sergio Díaz

**SELECTED PUBLICATIONS**

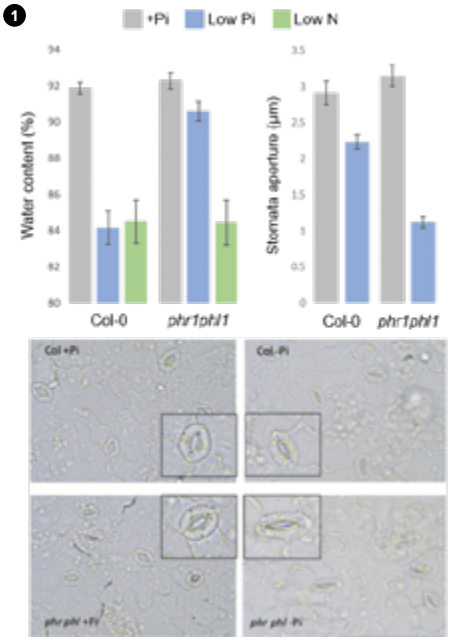
Castrillo G, Teixeira PJ, Paredes SH, Law TF, de Lorenzo L, Feltcher ME, Finkel OM, Breakfield NW, Mieczkowski P, Jones CD, Paz-Ares J, Dangl JL. Root microbiota drive direct integration of phosphate stress and immunity. Nature 2017; 543: 513-518.

Puga MI, Rojas-Triana M, de Lorenzo L, Leyva A, Rubio V, Paz-Ares J3 Novel signals in the regulation of Pi starvation responses in plants: facts and promises. Curr Opin Plant Biol 2017; 39: 40-49.

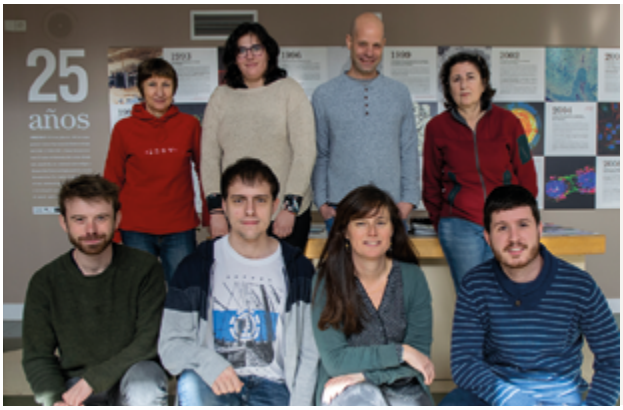
Our research is focussed on the phosphate (Pi) starvation rescue system (PSR) of plants, a model system for studies of gene activity. In addition, the study of this rescue system is important to generate tools and strategies towards the effective implementation of low-input, sustainable agricultural practices. Significant progress has been made in the last 20 years in the dissection of the signalling pathway governing the activation of this system, in which our laboratory has made key contributions, including among others the identification of PHR1 and related transcription factors as master regulators of PSR; the implication of SPX1 and related proteins in the Pi sensing system, and the identification of the targets and mechanisms of regulation of miRNAs by non-coding RNAs.

In the past two years, we have found that PHR1 targets induced by Pi starvation are also induced by drought. In line with this observation, we found that Pi starvation reduces water content (Figure 1). This effect on water content is mediated by PHR1, which strongly supports the notion that plant water content management contributes to the PSR. In fact, an obvious consequence of water content reduction is a concomitant increase in Pi concentration. Our studies on PHR1 targets have also served to identify a key role of PHR1 in the attenuation of plant defence responses to pathogens, indicating that plants prioritise Pi homeostasis over plant defence during Pi starvation.

Additionally, we embarked on a project to study the effect of Pi starvation on the formation of extra-chromosomal circular DNA (ecc DNA). So far, we have implemented the method for eccDNA isolation and characterisation (based on paired end sequencing) and found that there are more than 1,000 eccDNAs produced during Pi starvation. The Pi starvation specificity of these eccDNAs and their biological significance is currently under investigation.




**1** *Phosphate starvation promotes reduction in plant water content, a process dependent on PHR1/ PHL1 master transcription factors.*




# Light signalling and day length control of potato tuber formation



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## SELECTED PUBLICATIONS

Martínez C, Espinosa-Ruíz A, de Lucas M, Bernardo-García S, Franco-Zorrilla JM, Prat S. PIF4-induced BR synthesis is critical to diurnal and thermomorphogenic growth. EMBO J 2018; 37: e99552.

Martínez C, Nieto C, Prat S. Convergent regulation of PIFs and the E3 ligase COP1/SPA1 mediates thermosensory hypocotyl elongation by plant phytochromes. Curr Opin Plant Biol 2018; 45: 188-203.

Lasierra P, Prat S. Transient Transactivation Studies in Nicotiana benthamiana Leaves. Methods Mol Biol 2018; 1794: 311-322.

Pucciariello O, Legris M, Costigliolo Rojas C, Iglesias MJ, Hernando CE, Dezar C, Vazquez M, Yanovsky MJ, Finlayson SA, Prat S, Casal JJ. Rewiring of auxin signaling under persistent shade. Proc Natl Acad Sci USA 2018; 115: 5612-5617.

Ibañez C, Delker C, Martínez C, Bürstenbinder K, Janitza P, Lippman R, Ludwig W, Sun H, James GV, Klecker M, Grossjohann A, Schneeberger K, Prat S, Quint M. Brassinosteroids dominate hormonal regulation of plant thermomorphogenesis via BZR1. Curr Biol 2018; 28: 303-310.

Espinosa-Ruiz A, Martínez C, de Lucas M, Fàbregas N, Bosch N, Caño-Delgado AI, Prat S. TOPLESS mediates brassinosteroid control of shoot boundaries and root meristem development in *Arabidopsis thaliana*. Development 2017; 144: 1619-1628.

Day-length and temperature vary both daily and seasonally. Plants are not simply passive onlookers to these environmental fluctuations; in fact they constantly monitor their environment to pre-empt and prepare for the challenges that lie ahead. For example, a gradual decrease in temperatures and low light indicate the coming of night, and decreasing daylength and temperatures suggest that winter is just around the corner.

Plants sense the quality and quantity of daylight through a suite of photoreceptors: UVR8, cryptochromes, phototropins, zeitlupes and phytochromes. Information from these photoreceptors is integrated with the circadian clock and this enables plants to adjust their growth and development to match diurnal light-cycles and seasonal variation. Developmental processes tightly regulated by light and temperature cues and diel conditions include seed germination and early seedling development, the transition to flowering, and in potato, the differentiation of storage organs (tuberisation).

Research by our group aims to decipher the mechanisms by which these external cues interact with endogenous growth and hormonal programs. Our work spans across multiple developmental stages, from de-etiolation and seedling establishment, through to stress tolerance and storage-organ formation. We achieve this through work in the model species Arabidopsis and potato. Understanding how environmental factors are integrated into plant development is of utmost importance if we are to develop crops that are resilient to the negative impact of global warming.

In this context, specific biological questions addressed by our research are:

- How do warm temperatures and light affect plant morphology and how are these signals integrated with hormonal pathways?
- How are temperature oscillations sensed by the endogenous clock and what are the consequences of this for early plant development?
- How do elevated temperatures suppress the function of the clock “evening complex” whilst promoting the activity of the COP1 E3 ligase?
- What are the mechanisms underlying suppression of thermomorphogenic hypocotyl elongation by drought and salinity?
- Can we de-couple the effects of plant hormones on morphology and their effects on stress tolerance?
- What are the mechanisms responsible for temperature suppression of the FT mobile tuberisation signal?
- Are potato tubers derived from the vascular cambium?

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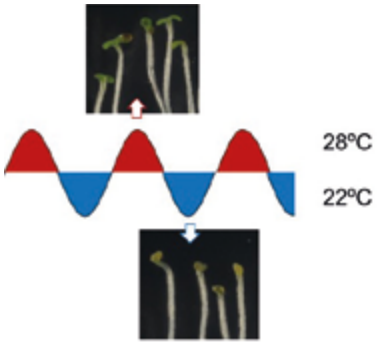


❶ *Oscillations in temperature entrain seedlings to de-etiolation on exposure to light.*

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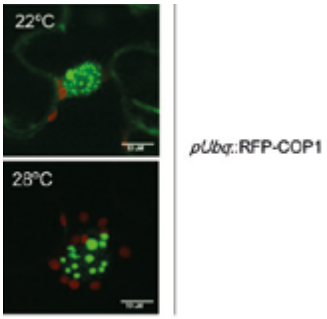
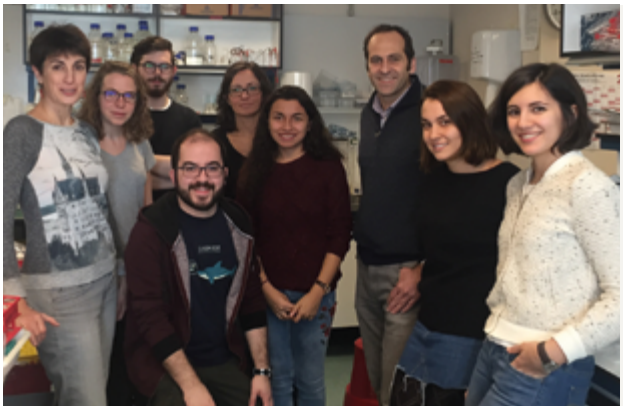



❷ *Effect of temperature on COP1 distribution.*



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



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**SELECTED PUBLICATIONS**

Perea-Resa C,... Iniesto E, Rubio V, Salinas J. Prefoldins Negatively Regulate Cold Acclimation in *Arabidopsis thaliana* by promoting nuclear proteasome-mediated HY5 degradation. Mol Plant 2017; 10: 791-804.

Puga MI,... Rubio V, Paz-Ares J. Novel signals in the regulation of Pi starvation responses in plants: facts and promises. Curr Opin Plant Biol 2017; 39: 40-49.

García-León M, Iniesto E, Rubio V. Tandem affinity purification of protein complexes from Arabidopsis cell cultures. Methods Mol Biol 2018; 1794: 297-309.

Park J, ... Iniesto E, Rubio V, ... Yun DJ. Epigenetic switch from repressive to permissive chromatin in response to cold stress. Proc Natl Acad Sci USA 2018; 115: E5400-E5409.

Nassrallah A, ... Fonseca S, Iniesto E, ... Bowler C, Rubio V, Barneche F. 2018 DET1-mediated degradation of a SAGA- like deubiquitination module controls H2Bub homeostasis. Elife 2018; 7: e37892.

The relevance of protein ubiquitination as an integral mechanism of many signalling pathways in plants has been demonstrated extensively. Ubiquitin (Ub) conjugation to proteins (i.e. ubiquitination) may trigger degradation of protein targets at the 26S proteasome or changes in their properties (e.g., protein activity, localisation, 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 plants' whole life, including embryogenesis, seedling photomorphogenesis, circadian clock function, flowering and tolerance to different stresses (i.e., drought, high salinity, cold, osmotic stress) by promoting degradation of specific targets controlling those processes.

As an example, we have recently shown that DDA1, a substrate adaptor of CRL4-CDDD complexes, recognises abscisic acid (ABA) receptors, triggering their ubiquitination and proteasomal degradation (Irigoyen *et al.*, The Plant Cell 2014). Therefore, CRL4-CDDD complexes act as repressors of ABA-mediated water stress responses under optimal growth conditions. Interestingly, CRL4-CDDD function is performed in close proximity to chromatin, which should enable rapid translation of environmental and stress signals into changes in gene expression. Indeed, recent results from our laboratory showed that CRL4-CDDD complexes are part of a molecular pathway controlling epigenetic homeostasis (including Histone2B ubiquitination) in response to external stimuli (i.e., light conditions; Nassrallah *et al.*, eLife 2018). Our current objective is to identify and characterise additional mechanisms by which CRL4-CDDD controls the accumulation of specific epigenetic marks across the plant genome in response to environmental changes, thereby regulating the expression of specific sets of genes that confer plants the ability to adapt to changing climate conditions.

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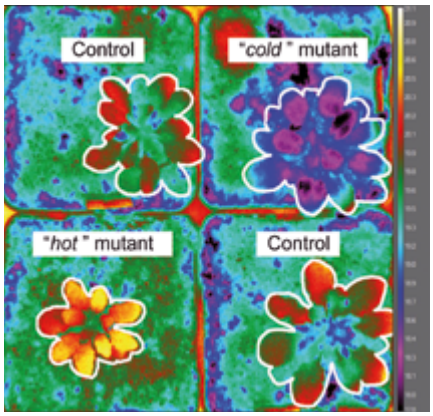


❶ *We investigate the molecular mechanisms by which light conditions influence growth and development through plants´ life.*

❷ *Infra-red imaging techniques allow quantitation of foliar temperature, which is indicative of water loss by transpiration, in mutant and control plants.*

# Signalling networks in plant development and defence responses



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## SELECTED PUBLICATIONS

Muñoz A, Mangano S, González-García MP, Contreras R, Sauer M, De Rybel B, Weijers D, Sánchez-Serrano JJ, Sanmartín M, Rojo E. RIMA-dependent nuclear accumulation of IYO triggers Auxin-irreversible cell differentiation in Arabidopsis. Plant Cell 2017; 29: 575-588.

Creighton MT, Sanmartín M, Kataya ARA, Averkina IO, Heidari B, Nemie-Feyissa D, Sánchez-Serrano JJ, Lillo C. Light regulation of nitrate reductase by catalytic subunits of protein phosphatase 2A. Planta 2017; 246: 701-710.

Sánchez Serrano JJ. Plant responses to wounding. In: eLS, John Wiley & Sons Ltd, Chichester UK, 2017.

Taurino M, Costantini S, De Domenico S, Stefanelli F, Ruano G, Delgadillo MO, Sánchez-Serrano JJ, Sanmartín M, Santino A, Rojo E. SEIPINs mediate lipid droplet biogenesis to promote pollen transmission and reduce seed dormancy. Plant Physiol 2018; 176: 1531-1546.

Lynch, CJ, Bernad R, Calvo I, Nobrega-Pereira S, Ruiz S, Ibarz N, Martinez-Val A, Grana-Castro O, Gomez-López G, Andres-Leon E, Espinosa Angarica V, Del Sol A, Ortega S, Fernandez-Capetillo O, Rojo E, Munoz J, and Serrano M. The RNA Polymerase II factor RPAP1 is critical for mediator-driven transcription and cell identity. Cell Rep 2018; 22, 396-410.

Our group studies how plants adjust their growth and development to changes in the environment and, in particular, to challenges from pests and pathogens.

One of our research lines is focused on understanding the molecular mechanisms that initiate cell differentiation and organogenesis in plants. Our previous work identified MINIYO (IYO) and RIMA as essential genes for cell differentiation in Arabidopsis. Analysis of these two genes has led to a working model suggesting that, upon displacement of stem cells from the meristem core, IYO and RIMA migrate from the cytosol to the nucleus where they modulate RNA polymerase II activity and reprogram the transcriptome to activate cell differentiation. We are now trying to elucidate how the IYO/RIMA nuclear switch is controlled by developmental and environmental cues and how it activates downstream targets to drive cell differentiation. IYO and RIMA homologues are found throughout eukaryotes, and the IYO transcriptional reprogramming module for stem cell differentiation appears to be conserved in mammals, suggesting that findings made on this molecular switch in Arabidopsis will be broadly applicable to crop species and potentially to animal systems.

A second line of research in our laboratory focuses on the signalling pathways that activate defence responses in plants. Our work has unravelled a crucial role of protein phosphatases 2A (PP2As) at the crossroads of hormonal and innate immunity signalling in plants. Previously, we have demonstrated the importance of PP2As in auxin and abscisic acid signalling, as well as in plant immunity against bacteria. We have now gained evidence that PP2As also play a key role in jasmonate and brassinosteroid signal transduction. By learning how these phosphatases integrate hormonal and defence signalling, we hope to develop technology to improve crop protection without negatively affecting plant growth and yield.

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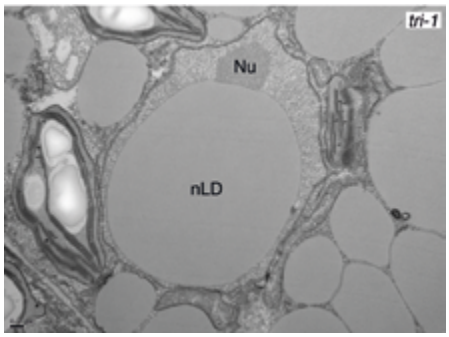


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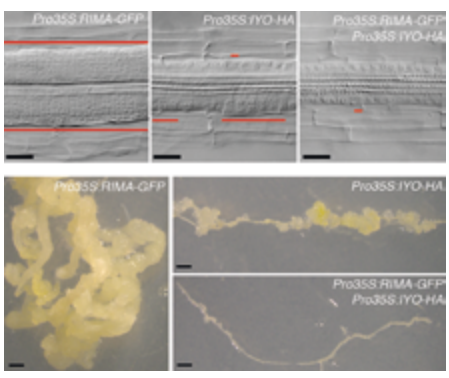


❶ *Nuclear lipid droplets accumulate in seipin triple mutants. Nu: nucleolus; nLD: nuclear lipid droplet.*

❷ *Concomitant overexpression of IYO and RIMA blocks auxin-induced callus growth. Root explants from plants overexpressing RIMA-GFP, IYO-HA or RIMA-GFP and IYO-HA under a constitutive 35S promoter were incubated for 5 days (upper panels or 51 days (lower panels) in media containing 300ng/ml 2,4D.*

# Jasmonate signalling and plant defence



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**SELECTED PUBLICATIONS**

Bowman JL, .... Monte I, Solano R,..., *et al.* Insights into Land Plant Evolution Garnered from the *Marchantia polymorpha* Genome. Cell 2017; 171: 287-304.

Gimenez-Ibanez S, Boter M, Ortigosa A, García-Casado G, Chini A, Lewsey MG, Ecker JR, Ntoukakis V, Solano R. JAZ2 controls stomata dynamics during bacterial invasion. New Phytol 213: 1378-1392.

Chini A, Monte I, Zamarreño AM, Hamberg M, Lassueur S, Reymond P, Weiss S, Stintzi A, Schaller A, Porzel A, García-Mina JM, Solano R. An ORP3-independent pathway uses 4,5-didehydro-jasmonate for jasmonate synthesis. Nat Chem Biol 2018; 14: 171-178.

Monte I, Ishida S, Zamarreño AM, Hamberg M, Franco-Zorrilla JM, García-Casado G, Gouhier-Darimont C, Reymond P, Takahashi K, García-Mina JM, Nishihama R, Kohchi T, Solano R. Ligand-receptor co-evolution shaped the jasmonate pathway in land plants. Nat Chem Biol 2018; 14: 480-488.

Ortigosa A, Gimenez-Ibanez S, Leonhardt N, Solano R. Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of SlJAZ2. Plant Biotechnol J 2019; 17: 665-673.

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:

- Discovery of a new pathway for JA biosynthesis (Chini *et al.*, Nature Chem Biol 2018)
- Discovery of the bioactive jasmonate in Bryophytes (Monte *et al.*, Nature Chem Biol 2018)
- Participation in the sequencing and analysis of the genome of *Marchantia polymorpha* (Bowman *et al.*, Cell 2017)
- Identification of the tomato orthologue of AtJAZ2 and design of tomato plant resistant to *Pseudomonas syringae* by CRISPR/Cas9-based mutation of SlJAZ2 (Gimenez-Ibanez *et al.*, New Phytol 2017; Ortigosa *et al.*, Plant Biotechnol J 2018).
- Filing of a patent for the biotech application of JAZ2 to improve resistance to plant pathogens: PCT/EP17/078493 owned by the CSIC and licensed to PBL (Plant Biotech Limited, UK).
- Characterisation of the TIFY family in wheat (Ebel *et al.*, PLoS One 2018).
- Identification and characterisation of a new fungal jasmonate (Chini *et al.*, J Exp Bot 2018).
- Collaboration in the characterisation of a JAZ subtype-selective agonist of jasmonate perception (Takaoka *et al.*, Nat Commun 2018).
- Identification of the DNA target sequence of many plant transcription factors using previously developed tools and in collaboration with several groups (Molina-Hidalgo *et al.*, J Exp Bot 2017; Gallemi *et al.*, New Phytol 2017; Hichri *et al.*, Front Plant Sci 2017; Yan *et al.*, PNAS 2017; Matthijs *et al.*, EMBO J 2017).

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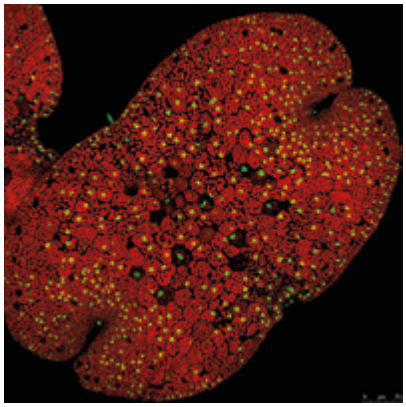

**1** *Air pore in the epidermis of WT* Marchantia polymorpha *plants*

**2** *Nuclear localisation of JAZ protein in young* Marchantia polymorpha *plants.*