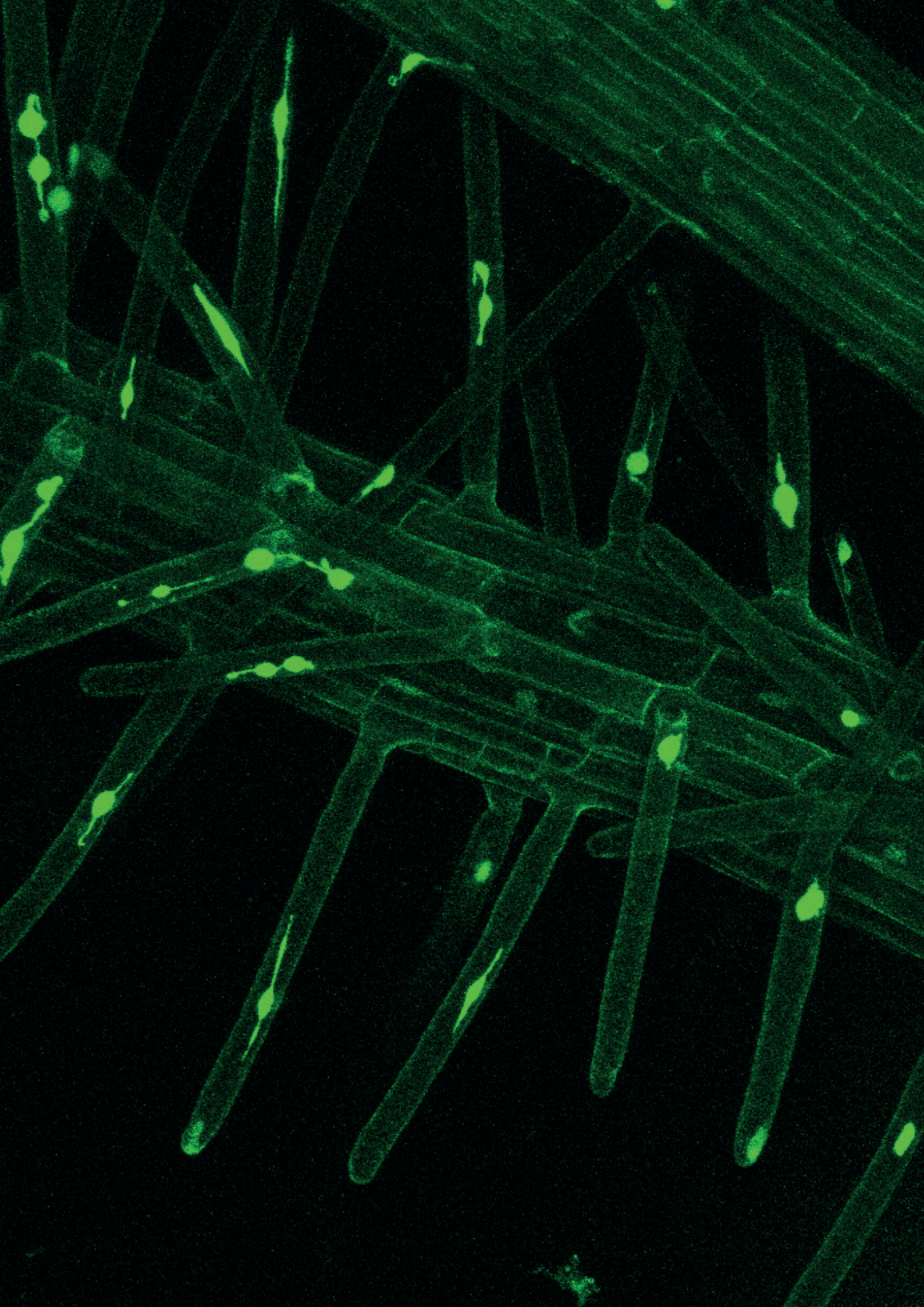


4

PLANT MOLECULAR GENETICS

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, to 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 liverwort *Marchantia polymorpha* or the duckweed *Lemna spp*. Crops such as tomato, potato and Prunus are also major subjects of our studies, to which knowledge generated in the model species is applied.

HEAD OF DEPARTMENT

Juan Antonio García

OUR 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 Antonio García and Carmen Simón
5. **Molecular mechanisms underlying arsenic phytoremediation**
   Antonio Leyva
6. **Regulation of gene activity in plants: the phosphate starvation rescue system**
   Javier Paz-Ares
7. **Hormonal and environmental control of plant development**
   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**
   Jose Juan Sánchez-Serrano and Enrique Rojo
10. **Jasmonate signalling in plants**
    Roberto Solano

Analysis of JAZ-GFP protein turnover in plant roots confirms the key role of JAZ repressors in the jasmonate signalling pathway (Chini et al., Nature 2007)

Natural variation in plant development

GROUP LEADER

Carlos Alonso-Blanco

POSTDOCTORAL SCIENTISTS

Belén Méndez de Vigo
Israel Ausín

PHD STUDENTS

Noelia Arteaga
Marija Savic

TECHNICIAN

Mercedes Ramiro

SELECTED PUBLICATIONS

Zhu W**, Ausin I**, Seleznev A, Mendez-Vigo B, Pico FX, Sureshkumar S, Sundaramoorthi V, Bulach D, Powell D, Seemann T, Alonso-Blanco C*, Balasubramanian S* (*corresponding authors, ** equal contribution). Natural variation identifies ICARUS1, a universal gene required for cell proliferation and growth at high temperatures in *Arabidopsis thaliana*. PLoS Genet 2015; 11: e1005085

Abe M, Kaya H, Watanabe-Taneda A, Shibuta M, Yamaguchi A, Sakamoto T, Kurata T, Ausín I, Araki T, Alonso-Blanco C. FE, a phloem-specific Myb-related protein, promotes flowering through transcriptional activation of *FLOWERING LOCUS T* and *FLOWERING LOCUS T INTERACTING PROTEIN 1*. Plant J 2015; 83: 1059-1068

Méndez-Vigo B, Savic M, Ausín I, Ramiro M, Martín B, Picó FX, Alonso-Blanco C. Environmental and genetic interactions reveal *FLOWERING LOCUS C* as a modulator of the natural variation for the plasticity of flowering in Arabidopsis. Plant Cell Environ 2016; 39: 282-294

Vidigal DS, Marques AC, Willems LA, Buijs G, Méndez-Vigo B, Hilhorst HW, Bentsink L, Picó FX, Alonso-Blanco C. Altitudinal and climatic associations of seed dormancy and flowering traits evidence adaptation of annual life cycle timing in *Arabidopsis thaliana*. Plant Cell Environ 2016; 39: 1737-1748

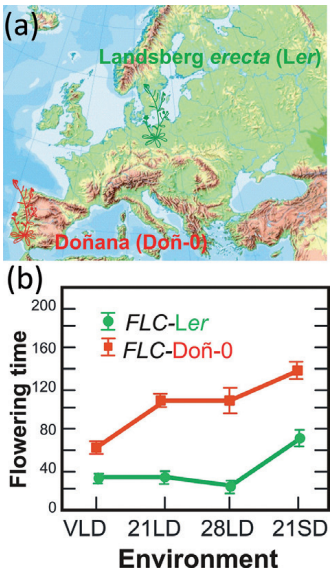
The 1001 Genomes Consortium. 1135 sequenced natural inbred lines reveal the global pattern of polymorphism in *Arabidopsis thaliana*. Cell 2016; 166: 481-491

The main goal of our laboratory is to understand the genetic, molecular and evolutionary mechanisms involved in plant adaptation. We are specifically interested in determining how developmental traits such as flowering time or vegetative growth allow plant adaptation to different climates. To address this question, we are mainly exploiting the natural genetic variation within the wild annual model plant *Arabidopsis thaliana*. Our research currently focuses on two specific objectives.

First, we aim to identify the genes and nucleotide polymorphisms that account for the natural variation of plant development. We recently identified the *ICARUS* gene, which encodes a t-RNA-His guanylyl transferase, as responsible for severe growth defects that are reversible depending on temperature (Zhu *et al.*, 2015). In addition, we showed that *FLC* is a modulator of the natural variation for plasticity of flowering relative to multiple environmental factors (Méndez-Vigo *et al.*, 2016).

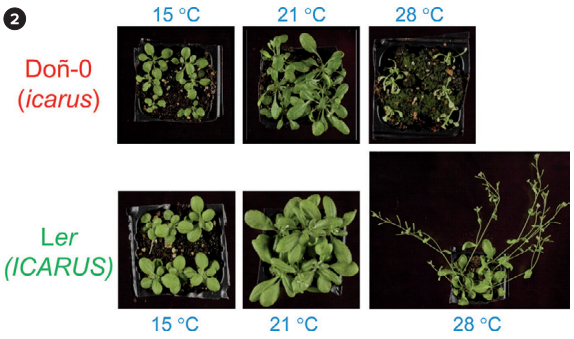
Second, given the current importance of climate change, we aim to identify new genes and natural alleles involved in adaptation to different climates. We are exploiting an *Arabidopsis thaliana* regional collection of more than 300 wild accessions collected in the Iberian peninsula. The genome sequence of 174 of these Iberian accessions was obtained as part of the 1001 Arabidopsis Genome Project (The 1001 Genomes Consortium, 2016). We have analysed this collection to determine the relevance of flowering time and seed dormancy in climatic adaptation and to identify some of the genes that might drive this adaptation (Vidigal *et al.*, 2016).

1

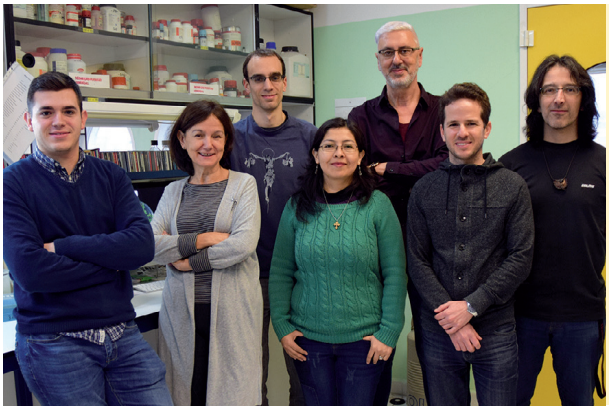


1 Natural variation in the *FLC* gene affects the plasticity of flowering. (a) Wild accessions Doñ-0 and L*er* (a) derive from distinct climatic regions in Spain and Poland, respectively. (b) An introgression line bearing the *FLC* allele from Doñ-0 (*FLC*-Doñ-0) on a L*er* genetic background flowers later and responds less to temperature than L*er* (FLC-L*er*). Flowering time in days.

2



2 Natural variation between wild accessions of *Arabidopsis thaliana* for vegetative growth and flowering time at different temperatures. The accession Doñana (Doñ-0) carries a natural loss-of-function allele of the *ICARUS1* gene (*icarus1*) that leads to near-arrest of growth compared to accession L*er*.



Plant immunity strategies against microbial pathogen infection

GROUP LEADER:

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Tomás Cascón

SELECTED PUBLICATIONS

Machado L, Castro A, Hamberg M, Bannenberg G, Gaggero C, Castresana C, Ponce de Leon I. The *Physcomitrella patens* unique alpha-dixoygenase participates in both developmental processes and defense responses. BMC Plant Biol 2015; 15: 45

Ponce de León I, Hamberg M, Castresana C. Oxylipins in moss development and defense. Front Plant Sci 2015; 6: 483

Marcos R, Izquierdo Y, Vellosillo T, Kulasekaran S, Cascón T, Hamberg M, Castresana C. 9-Lipoxygensases-derived oxylipins activate brassinoesteroid signaling to promote cell wall-based defense and limit pathogen infection. Plant Physiol 2015; 169(3): 2324-34

The development of alternative strategies to minimize crop losses due to infection by microbes requires understanding of host-pathogen interactions. We focus our research on the study of the defence mechanisms that allow plants to control pathogen attack. In particular, we explore the action of oxylipins in plant immunity. This family of lipid derivatives plays critical roles in activating the immune response in plants and in limiting pathogen infection. Recent studies demonstrate participation of the 9-LOX and alpha-DOX oxylipin pathways in the defence mechanism activated by Arabidopsis following infection by hemibiotrophic bacteria. We showed that these oxylipin pathways participate in the three layers of preinvasive, apoplastic and systemic defence triggered by plants to prevent *Pseudomonas syringae* pv *tomato DC3000* infection. We found high 9-LOX and alpha-DOX activity levels in roots of untreated Arabidopsis plants, and showed that these oxylipin pathways participate in plant defence against the root pathogen *Fusarium oxysporum*. Our findings indicate that, in these responses, oxylipins act as regulators of oxidative stress, lipid peroxidation, cell wall damage and hormone homeostasis. The availability of pure oxylipins and of mutants impaired in the synthesis and signalling of oxylipins (*noxy* mutants) are valuable tools that facilitate our studies. The *noxy* mutations we identified indicate the importance of mitochondria and of protein synthesis in 9-LOX signalling, which prompted us to analyse the role of these organelles and associated regulatory processes in plant defence (Figure 1).

In addition, we opened up a new line of experiments to examine the participation of alpha-dioxygenases and of additional oxylipin pathways as components of lipid bodies. We plan to study the function of these cell structures as an oxylipin reservoir and the function of oxylipins in plant defence (Figure 2). A major part of our experiments will be performed in Arabidopsis, and tomato plants will be used to determine the biotechnological value of the results.

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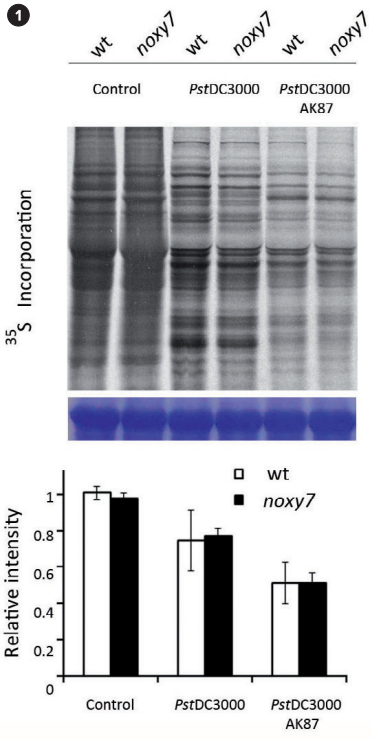
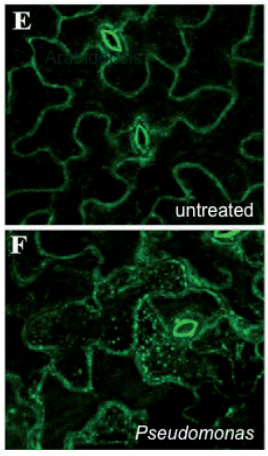


1

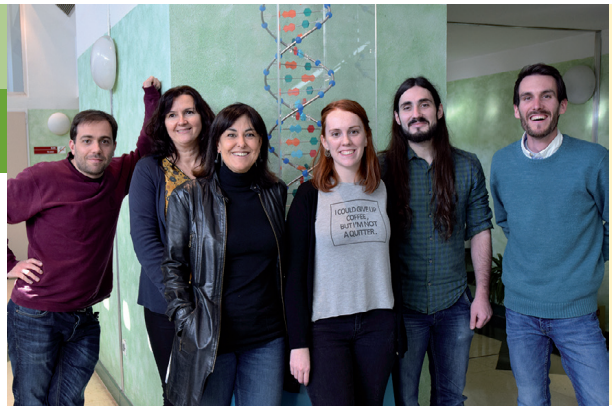
Analysis of de novo protein synthesis in Arabidopsis seedlings, responding to the virulent bacteria PstDC3000 and the attenuated strain PstDC3000 AK87. Representative examples of radiolabelled proteins are shown. Protein staining with Coomassie Blue was used as loading control.

2



2

Production of lipid bodies in Arabidopsis leaves responding to *Pseudomonas* infection.



Genetic control of bud dormancy

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Esther Canibano

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Isabel Domínguez

SELECTED PUBLICATIONS

Nicolas M, Rodríguez-Buey ML, Franco-Zorrilla JM, Cubas P. A Recently Evolved Alternative Splice Site in the BRANCHED1a Gene Controls Potato Plant Architecture. Curr Biol 2015; 25(14): 1799-809

Oliveros JC, Franch M, Tabas-Madrid D, San-León D, Montoliu L, Cubas P, Pazos F. Breaking-Cas – Interactive design of guide RNAs for CRISPR-Cas experiments for ENSMBL genomes. Nucleic Acids Res 2016; 44(W1): W267-71

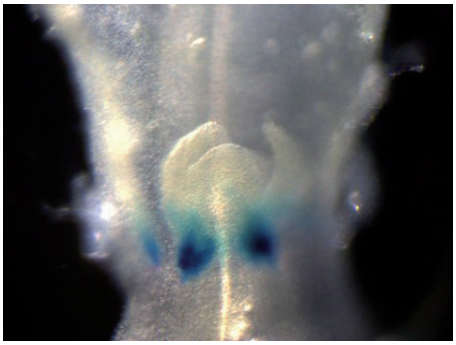
Nicolas M, Cubas P. TCP genes: new kids in the signaling block. Curr Opin Plant Biol 2016; 33: 33-41

In flowering plants, lateral shoots develop from axillary buds formed at the base of leaves. These buds can become quiescent or dormant at this stage, or continue development to form branches. The Arabidopsis TCP gene *BRANCHED1* acts inside axillary buds to promote bud growth arrest. We are studying the gene regulatory networks (GRN) controlled by *BRC1*. Among the GRN downregulated in response to *BRC1,* one is enriched in DNA synthesis-, cell cycle-, and cytokinesis-related genes and another in protein synthesis-related genes. *BRC1* represses these GRN directly or indirectly by competition with other TCP factors. We also characterized a *BRC1*-dependent GRN related to abscisic acid (ABA) signalling. To control this network, BRC1 binds to and activates directly three genes encoding homeodomain leucine zipper (HD-ZIP) transcription factors. With BRC1, these factors trigger a genetic cascade that promotes ABA accumulation and response, which is essential for bud dormancy in light-limiting conditions (González-Grandío *et al.*, Proc Natl Acad Sci USA 2017, 114:E245).

We also study the conservation and evolution of bud dormancy and the role of *BRC1* in the Solanaceae family. In potato, we discovered the recent evolution of an alternative splice site in one of the BRC1 paralogues, *BRC1a*, which generates two BRC1a protein isoforms with distinct C-terminal regions. This constitutes a multi-level mechanism of post-transcriptional and post-translational regulation of *BRC1a* that effectively modulates its activity in response to environmental and endogenous cues.

Strigolactones (SL) are phytohormones that regulate shoot branching. SL perception and signalling involves the F-box protein MAX2 and the hydrolase D14, proposed to act as a SL receptor. We used strong loss-of-function alleles of the *D14* gene to characterize its function. We also identified a mechanism of SL-induced MAX2-dependent proteasome-mediated D14 degradation. This negative feedback loop might cause a substantial drop in SL perception, which would effectively limit SL duration and signalling intensity.

1



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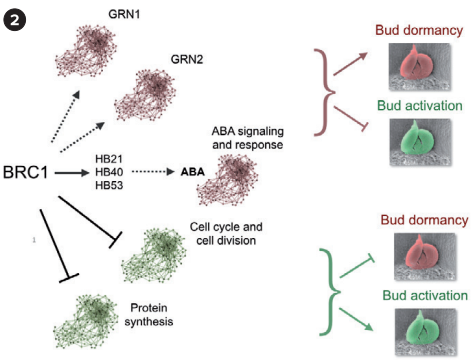

1

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.

2

Scheme of proposed BRANCHED1 function in the control of bud dormancy as activator and repressor of several bud dormancy gene regulatory networks.

Plant-pathogen interaction in viral infections

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Hongying Shan
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VISITING SCIENTIST

Leonor Bedoya Rojas

SELECTED PUBLICATIONS

Shan H, Pasin F, Valli A, Castillo C, Rajulu C, Carbonell A, Simón-Mateo C, García JA, Rodamilans B. The *Potyviridae* P1a leader protease contributes to host range specificity. Virology 2015; 476: 264-70

Revers F, García JA. Molecular biology of potyviruses. Adv Virus Res 2015; 92: 101-9

Rodamilans B, Valli A, Mingot A, San León D, Baulcombe D, López-Moya JJ, García JA. RNA polymerase slippage as a mechanism for the production of frameshift gene products in plant viruses of the *Potyviridae* family. J Virol 2015; 89: 6965-7

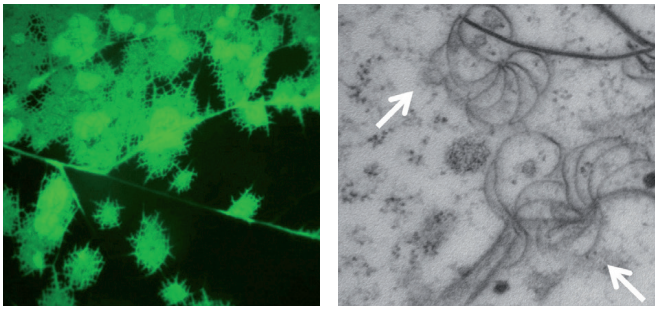
Zhao M, San León D, Mesel F, García JA, Simón-Mateo C. Assorted processing of synthetic trans-acting siRNAs and its activity in antiviral resistance. PLoS One 2015; 10: e0132281

Mingot A, Valli A, Rodamilans B, San León D, Baulcombe DC, García JA, López-Moya JJ. The P1N-PISPO trans-frame gene of sweet potato feathery mottle potyvirus is produced during virus infection and functions as RNA silencing suppressor. J Virol 2016; 90: 3543-3557

Plant viruses can infect a limited range of hosts, which is determined mainly by the formation of compatible interactions within the plant. These interactions are often neutral or even beneficial for the host, but the infection can also cause devastating diseases with great ecological and economic impact. A successful infection depends on the ability of the virus to recruit the host factors necessary for its replication and propagation, and on its capacity to escape from host defence responses.

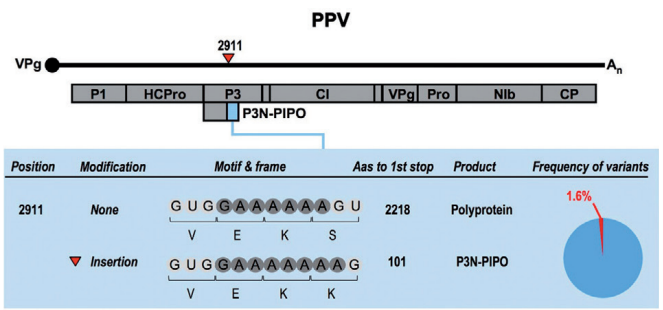
The general objective of our research is to unravel the complex network of virus-host interactions that determines whether the virus can establish a productive infection. Plum pox virus is our main subject of study, as it causes sharka, the most serious viral disease for the European stone fruit industry, and because this virus belongs to the genus Potyvirus, the largest group of plant RNA viruses. We are particularly interested in defence responses related to RNA silencing and its viral suppressors. The typical silencing suppressor of potyviruses is HCPro, but other silencing suppressors of Potyviridae family members have recently been discovered, and we are now clarifying common and virus-specific features of their contribution to infection. We also study factors that contribute to host range definition, especially the viral proteins P1 and capsid protein (CP), which are relevant for pathogenesis and adaptation to specific hosts. A specific target of interest, in the case of CP, is deciphering the meaning of the crosstalk between *O*-GlcNAcylation and phosphorylation at its N terminus, and how it affects virion assembly and viral RNA sorting to different fates during infection. We aim to use this knowledge to design new strategies for control of sharka and other viral diseases. In addition, we explore the potential of plant viruses as biotechnological tools.

1

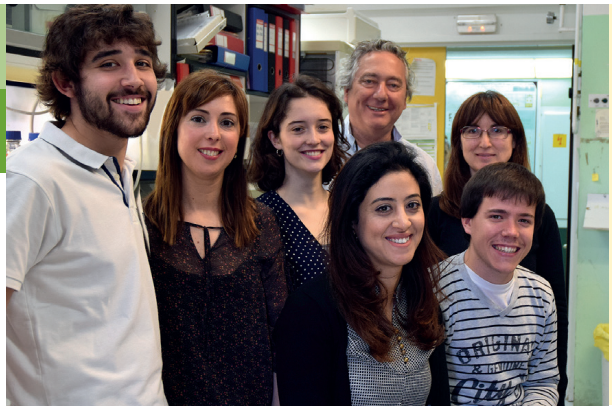


1 *Prunus domestica* leaf infected with a modified Plum pox virus (PPV) that expresses GFP (left); infected *Nicotiana benthamiana* cell, showing characteristic PPV pinwheels (right).

2



2 Alternative protein production in Plum pox virus through RNA polymerase slippage.



Molecular mechanisms underlying arsenic phytoremediation

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(Higher Institute of Agronomic Sciences of Chott-Mariem, Tunisia)

SELECTED PUBLICATIONS

Mohan TC, Castrillo G, Zarco-Fernandez S, Navarro C, Ramireddy E, Zamarreño AM, Paz-Ares J, Muñoz R, García-Mina JM, Hernandez LE, Schmülling T, Leyva A. Cytokinin determines thiol-mediated arsenic tolerance and accumulation. Plant Physiol 2016; 171(2): 1418-26

At the origin of life, arsenic was major a challenge to organism survival. Today, groundwater and soil contamination with this metalloid are still a global threat for all organisms, including humans. In plants, arsenic tolerance has been a major factor in plant adaptation and distribution. Some plant species show an extraordinary ability to accumulate arsenic, which highlights their potential for bioremediation. Some hyperaccumulator species are staple crops such as rice, which would compromise food safety and quality. Preventing undesirable arsenic levels in the food chain and boosting selection of efficient hyperaccumulator plants for phytoremediation strategies requires understanding of the molecular mechanisms that underlie arsenic perception and tolerance. In recent years, the molecular mechanism involved in plant arsenic tolerance is being studied extensively. Most proteins studied were nonetheless identified based on sequence homology with other proteins previously isolated from bacteria and yeast. Genetic approaches to uncover biologically relevant As(V)-tolerant mechanisms are scarce. Moreover, no regulatory protein involved in arsenic signalling has yet been isolated. In our laboratory, we are currently identifying transcriptional activators of the arsenic response using molecular, genetic and *in silico* approaches. We also aim to characterize the function and phytoremediation potential of a new arsenate reductase that contributes to the natural variation of arsenate tolerance. Finally, we study the application of natural isolates of aquatic plants for water phytoremediation.

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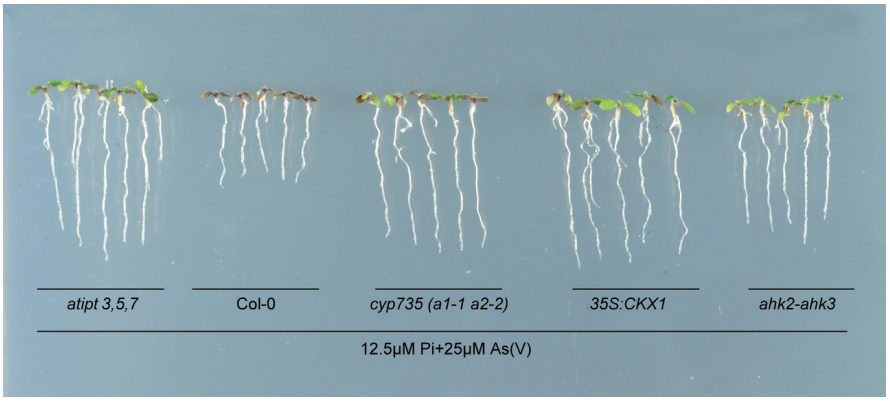

1 Reduction of endogenous cytokinin confers As(V) tolerance.

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

GROUP LEADER

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PHD STUDENTS

Miguel Miñambres
Sergio Diaz

TECHNICIAN

Erica Gil Velasco

SELECTED PUBLICATIONS

Thieme CJ, Rojas-Triana M, Stecyk E, Schudoma C, Zhang W, Yang L, Miñambres M, Walther D, Schulze WX, Paz-Ares J, Scheible WR, Kragler F. Endogenous Arabidopsis messenger RNAs transported to distant tissues. Nat Plants 2015; 1(4): 15025

Chen J, Wang Y, Wang F, Yang J, Gao M, Li C, Liu Y, Liu Y, Yamaji N, Ma JF, Paz-Ares J, Nussaume L, Zhang S, Yi K, Wu Z, Wu P. The Rice CK2 Kinase Regulates Trafficking of Phosphate Transporters in Response to Phosphate Levels. Plant Cell 2015; 27: 711-23

Cardona-López X, Cuyas L, Marín E, Rajulu C, Irigoyen ML, Gil E, Puga MI, Bligny R, Nussaume L, Geldner N, Paz-Ares J, Rubio V. ESCRT-III-Associated Protein ALIX mediates High Affinity Phosphate Transporter Trafficking to Maintain Phosphate Homeostasis in Arabidopsis. Plant Cell 2015; 27: 2560-81

Kalinowska K, Nagel MK, Goodman K, Cuyas L, Anzenberger F, Alkofer A, Paz-Ares J, Braun P, Rubio V, Otegui MS, Isono E. Arabidopsis ALIX is required for the endosomal localization of the deubiquitinating enzyme AMSH3. Proc Natl Acad Sci USA 2015; 112(40): E5543-51

Mohan TC, Castrillo G, Navarro C, Zarco-Fernández S, Ramireddy E, Mateo C, Zamarreño AM, Paz-Ares J, Muñoz R, García-Mina JM, Hernández LE, Schmülling T, Leyva A. Cytokinin Determines Thiol-Mediated Arsenic Tolerance and Accumulation. Plant Physiol 2016; 171(2): 1418-26

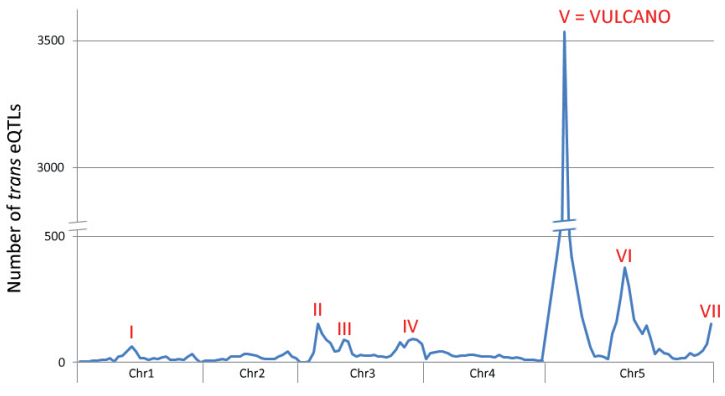
Our research focuses on the phosphate (Pi) starvation rescue system in plants, a model for gene activity studies with presumed biological potential in the context of low-input agriculture. We previously identified the transcription factor PHR1 as a master regulator of phosphate starvation responses in plants. In the last two years, we identified ALIX as another component of the plant Pi signalling pathway. We examined the inter-organ mobility of RNAs and the impact of Pi starvation on RNA movement. Finally, we analysed natural variation of molecular responses and identified several hotspot expression quantitative trait loci (eQTL) in a cross between two Arabidopsis ecotypes (Col and Ct).

ALIX involvement in Pi starvation signalling was detected in a study of suppressor mutants of *phr1*, with impaired function of the central transcription factor that regulates Pi starvation responses. We found that ALIX mediates Pi transporter trafficking to the vacuole in *Arabidopsis thaliana* and that, as a consequence, the partial loss-of-function mutant alix-1 displays reduced vacuolar degradation of Pi transporter PHT1;1. alix-1 also shows altered vacuole morphogenesis, which implies a new role for ALIX proteins in vacuolar biogenesis.

To study inter-organ RNA mobility, we devised a strategy to identify RNA distant from its synthesis site, based on the use of heterografts between phylogenetically distant ecotypes. Interecotype polymorphisms allowed identification of RNAs from one ecotype present in tissues generated from the other. As much as 20% of total RNA was mobile. We found that Pi starvation alters the mobile transcriptome, and not only of the Pi starvation-responsive transcript set, but also that not responsive to this stress.

To study the natural variation of molecular responses to Pi starvation, we performed transcriptomic analyses of 100 recombinant inbred lines from a ColxCT cross to identify eQTL of Pi starvation-responsive genes. We found 7 hotspot eQTLs, including VULCANO, which affects expression of >3,500 genes, a large proportion of which are Pi starvation-responsive. We are now in the process of identifying VULCANO and evaluating its effect on growth performance in low Pi growth regimes.

1



1

Distribution of *trans* eQTLs across the Arabidopsis genome and detection of hotspots in low Pi-grown plants. The number of trans eQTLs (y-axis) is plotted against the physical position of the 1Mb-window where they peak (x-axis; chromosome position indicated). Intervals with an excess of eQTLs relative to the threshold estimated by permutation were classified as hotspots.

Hormonal and environmental control of plant development

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Pilar Lasierra
Alex Navarro
Ana Beloso

SELECTED PUBLICATIONS

Navarro C, Cruz-Oró E, Prat S. Conserved function of FLOWERING LOCUS T (FT) homologues as signals for storage organ differentiation. Curr Opin Plant Biol 2015; 23: 45-53

Nieto C, López-Salmerón V, Davière JM, Prat S. ELF3-PIF4 interaction regulates plant growth independently of the Evening Complex. Curr Biol 2015; 25: 187-93

Abelenda JA, Cruz-Oró E, Franco-Zorrilla, JM, Prat S. Potato StCONSTANS-like1 suppresses storage organ formation by directly activating the FT-like StSP5G repressor. Curr Biol 2016; 26: 872-81

Martínez C, Espinosa-Ruiz A, Prat S. Gibberellins and plant vegetative growth. Annual Plant Reviews 2016, 49: 285-312 (eds P. Hedden and S. G. Thomas), John Wiley & Sons, Ltd, Chichester, UK.

A temperature increase from 22ºC to 28ºC causes etiolated growth like that observed in shade-grown plants. The light receptor phytochrome B (phyB) is a main temperature sensor through faster dark reversion of its nuclear bioactive Pfr form. In the cell nucleus, phyB Pfr binds PHYTOCHROME-INTERACTING FACTORS (PIF) to trigger their phosphorylation and degradation by the 26S proteasome. PIFs accumulate in darkness or shade, and directly activate expression of auxin biosynthesis and response genes that promote hypocotyl elongation. The circadian clock controls PIFs expression via the “evening complex” (EC) formed by the ELF3, ELF4 and LUX proteins, which suppresses PIF4 and PIF5 expression at night.

One of our main research lines is to determine how warmer temperatures inactivate the EC. Our results also support a critical EC function in ABA-regulated gene expression, and we are studying how increased temperatures affect the response of plants to drought and salt stress. We have shown DELLA repressors directly bind different salt-stress related factors to co-activate expression of their targets. Therefore, identification of DELLA mutations that impair interaction with PIFs, but do not affect binding to these "stress" factors, will provide novel strategies to improve tolerance of plants to salt, without affecting growth.

We also study the mechanisms by which warm temperatures lead to an acute drop in potato tuber yield. We showed that the FT SP6A gene, whose expression is activated in leaves in short days, triggers tuber formation. The SP6A protein is transported to underground stems or stolons to signal storage organ formation. Elevated temperatures impair SP6A expression by inhibiting an unknown activator of this gene. We intend to identify this transcriptional regulator and characterize the meristem cells that respond to the SP6A-inducing signal; our working hypothesis is that the vascular cambium drives tuber formation. Molecular characterization of this developmental process will help understand the function of this lateral meristem in plant secondary growth.

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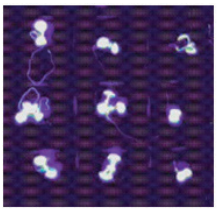

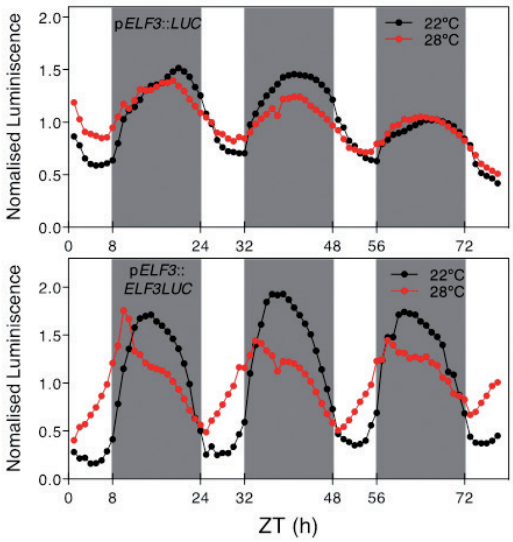

1

Elevated ambient temperatures increase ELF3 stability during the day and immediately after dusk

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

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SELECTED PUBLICATIONS

Cardona-López X, Cuyas L, Marín E, Rajulu C, Irigoyen ML, Gil E, Puga MI, Bligny R, Nussaume L, Geldner N, Paz-Ares J, Rubio V. ESCRT-III-Associated Protein ALIX Mediates High-Affinity Phosphate Transporter Trafficking to Maintain Phosphate Homeostasis in Arabidopsis. Plant Cell 2015; 27: 2560-81

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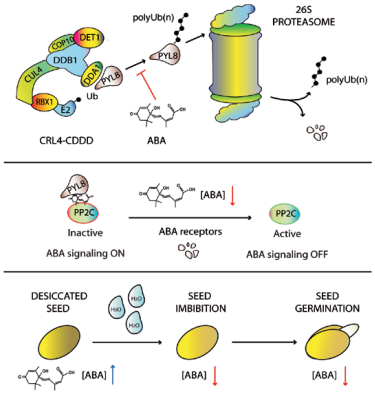
The main research interest of our laboratory is to characterize the molecular mechanisms by which the ubiquitin proteasome system (UPS) controls plant development and adaptation in response to different environmental stimuli and abiotic stresses.

In this regard, we aim to dissect the signal transduction routes that underlie plant growth and development in changing environmental (i.e., light, photoperiod) and stress conditions that limit water availability (drought, high salinity, extreme temperatures) and to characterize their components, with special emphasis on regulatory proteins. All these processes are modulated by the UPS, which controls the stability and thus the activity of hundreds if not thousands of proteins in plants through specific E3 ubiquitin ligases that recognize different protein targets. These findings lead to several intriguing questions we intend to answer:

- How is modulation of different signalling pathways coordinated by the same UPS components?
- Is there a hierarchy among signalling pathways regulated by the same UPS components?
- How is the UPS itself regulated in response to environmental and intracellular signals?

To respond to these questions, for our studies we chose the Cullin4-RING ligases (CRL4) as a representative E3 group, since they coordinately control different biological processes in plants, including photomorphogenesis, abscisic acid (ABA) signalling, DNA damage repair, circadian clock function, and flowering. To date, our studies have defined new CRL4 functions and identified an ABA-mediated regulatory mechanism to control their activity, as well as direct connections with chromatin remodelling events. More recently, we have become interested in the applied aspects of our research. We realized that manipulation of the genes we work with could generate interesting traits for molecular plant breeders and farmers, which led us to undertake a new research line devoted to the generation of stress-tolerant crops.

1



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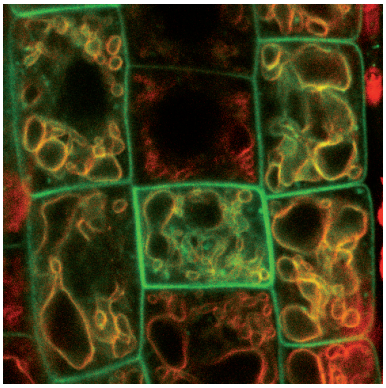
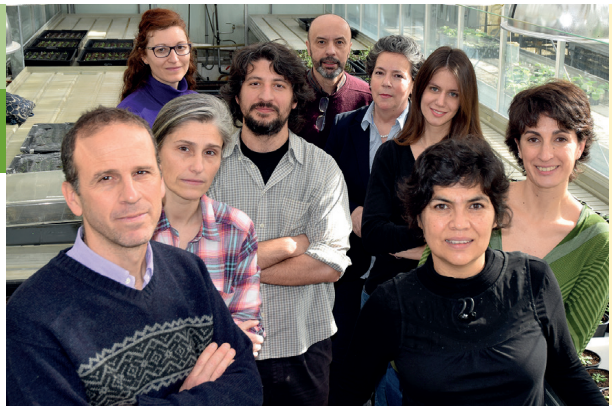


1

Model for a DDA1 role in ABA desensitization. DDA1, as part of CRL4 Ub ligases, allows the ubiquitination of ABA receptors (e.g., PYL8) and their degradation at the 26S proteasome. In contrast, ABA stabilizes the receptors by limiting their polyubiquitination (top). Low ABA levels enable DDA1-mediated destabilization of ABA receptors, promoting the release and activation of PP2C phosphatases, which act as negative regulators of ABA signalling (centre). DDA1 thus contributes to ABA desensitization when levels of this hormone decrease (when stress conditions disappear or during seed imbibition and germination) (bottom).

2

Defects in ALIX protein function alter protein cargo trafficking in plants. In normal conditions, high affinity phosphate transporter PHT1;1-GFP is internalized from the plasma membrane (green) and sorted into endosomes, to be degraded in the vacuole lumen. In the alix-1 mutant, however, PHT1;1-GFP does not enter the vacuole but is retained in the vacuole membrane (yellow), indicating a defect in protein cargo trafficking in this mutant.



Signalling networks in plant development and defence responses

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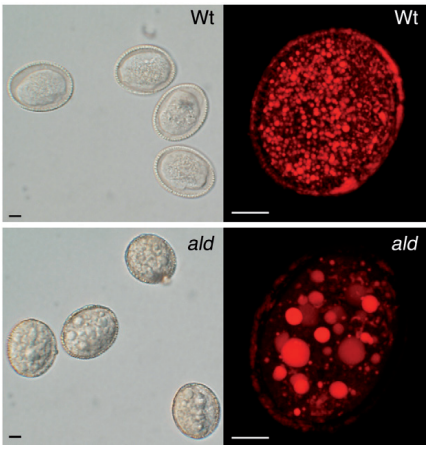
Scalschi L, Sanmartín M, Camañes G, Troncho P, Sánchez-Serrano JJ, García-Agustín P, Vicedo B. Silencing of OPR3 in tomato reveals the role of OPDA in callose deposition during the activation of defense responses against *Botrytis cinerea*. Plant J 2015; 81: 304-15

Our group is interested in studying how plants adjust their development to changing environmental conditions and, in particular, in response to biotic stresses.

One line of our research focuses on the regulation of stem cell proliferation and differentiation in plants, which is central to controlling development and growth. In a genetic screen for *Arabidopsis thaliana* mutants with delayed onset of differentiation, we identified *IYO*, which is necessary for initiating all cell differentiation events in the plant. We isolated an IYO-interacting factor, RIMA, which is homologous to RNA polymerase II phosphatases in yeast and mammals. Our evidence supports the idea that IYO and RIMA are co-transported into the nucleus, where they jointly regulate RNA polymerase II transcription to trigger cell differentiation (Muñoz *et al.*, Plant Cell 2017, in press). We are now studying the control of nuclear/cytosolic partitioning of IYO and RIMA, how the IYO/RIMA complex modulates RNA polymerase II activity, and which are the direct transcriptional targets of the IYO/RIMA/POL II complex. In addition, we are analysing how IYO and RIMA control the transition from the mitotic cycle to endoreduplication, a particularly prevalent phenomenon in plants that greatly influences cell expansion and organ growth.

Another long-standing research line in our laboratory centres on the signalling networks that activate defence responses in plants. We are pursuing the functional characterization of protein phosphatases type 2A (PP2A) in plants, in particular relative to hormone and defence signalling. We have reported PP2A functions in ABA and auxin signalling, as well as in innate immunity against bacteria. We are now studying the role of specific PP2A isoforms in brassinosteroid and jasmonate signalling. In addition, in collaboration with the group of Dr. Angelo Santino (ISPA-CNR, Lecce, Italy), we have initiated a project to define the role of oil bodies in lipid signalling and responses to stress in plants.

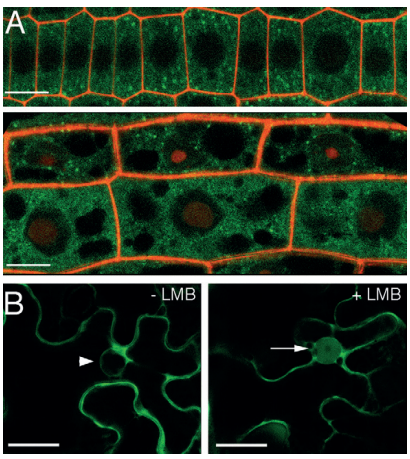
1



1

Nomarski and confocal images of pollen from wild type plants (Wt) and from a mutant isolated in the lab (*ald*) that displays enlarged oil bodies.

2



2

***RIMA* shuttles through cytosol and nucleus.** (A) Confocal images of Arabidopsis roots expressing *ProRIMA:RIMA-GFP*. (B) Confocal images of *Nicotiana benthamiana* leaves transformed with *Pro35S:RIMA-GFP* and mock treated (-LMB) or incubated (+LMB) with the nuclear export inhibitor leptomycin B.

Jasmonate signalling in plants

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Jasmonates (JA) are fatty acid-derived signalling molecules essential for the survival of plants in nature, since they are important activators of stress responses and developmental programmes. The main focus of my laboratory is to understand the JA signalling pathway in plants, knowledge that is essential for designing biotechnological and agronomical applications that improve plant resistance to stresses and plant yield. We have worked traditionally in the model plant *Arabidopsis thaliana*, but recently focused on the liverwort *Marchantia polymorpha*. We are using Marchantia for evolutionary studies as well as a model system in basic research due to its low gene redundancy.

The major achievements of our group in the last two years have been:

- Identification of a new family of transcription factors (YABBY) regulating responses to JA (Boter *et al.*, Plant Cell 2015)
- The demonstration that the COI1-JAZ2-MYC2,3,4-ANAC19,55,72 module regulates stomatal aperture in leaves. This module is hijacked by bacteria using the JA-mimic coronatine to open stomata and promote infection. This discovery provides novel strategies for crop protection against biotrophs without compromising resistance to necrotrophs (Giménez-Ibáñez *et al.*, New Phytol 2016).
- We participated in the analysis of the genome of *Marchantia polymorpha* (Bowman *et al.*, under review) and in defining the gene nomenclature in this species (Bowman *et al.*, Plant Cell Physiol 2016).
- We also established numerous collaborations with other groups that have elucidated the function of MYB & ZML genes in maize (Velez-Bermudez *et al.*, Plant Cell 2015), the role of jasmonates in carnivory feeding (Böhm *et al.*, Curr Biol 2016), the regulation of JAZ12 by KEG (Pauwels *et al.*, Plant Physiol 2015) and contributed to identifying the DNA sequence target of plant transcription factors using previously developed tools (Raines *et al.*, Plant J 2016; Medina-Puche *et al.*, Plant Physiol 2015; Baud *et al.*, Plant Physiol 2016).

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Vélez-Bermúdez IC, Salazar-Henao JE, Fornalé S, López-Vidriero I, Franco-Zorrilla JM, Grotewold E, Gray J, Solano R, Schmidt W, Pagés M, Riera M, Caparros-Ruiz D. A MYB/ZML complex regulates wound-induced lignin genes in maize. Plant Cell 2015; 27: 3245-59

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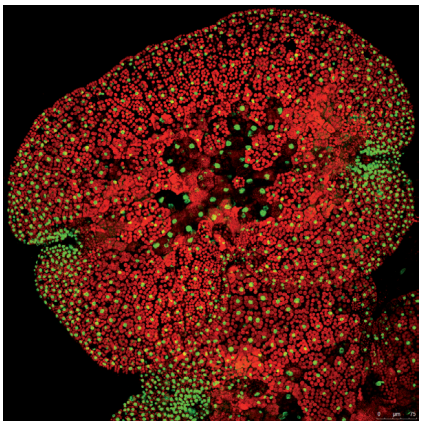

1 Nuclear localization of JAZ protein in young *Marchantia polymorpha* plants.

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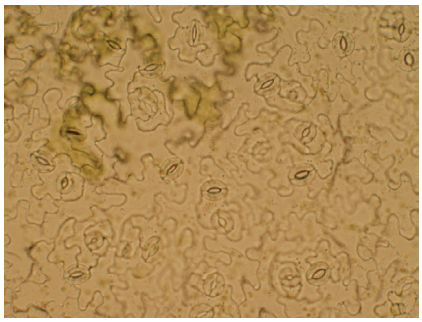

2 Stomata: entry ports of bacterial infection in plant leaves.