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Plant Molecular Genetics

Research is conducted by the Plant Molecular Genetics Department to uncover the signalling pathways involved in the main growth and adaptive responses of plants to environmental changes and pathogenic diseases. The department includes groups whose research focusses on the control of root architecture, shoot branching, response to light quality or duration of day length, innate immune responses to pathogens and viruses, and adaptive responses to nutrient shortage or the presence of toxic metals. Besides the intrinsic fundamental interest in understanding key biological processes in plants, our research will allow the development of new tools and methods to improve crop production and quality, selection of new varieties more resistant to pathogens or their modification to reduce fertiliser needs. Biotechnological applications such as the use of plants as biopharmaceutical factories or as tools to fight environmental problems arising from spillages and/or accumulation of toxic substances are also being studied. Research is carried out mainly in the model species *Arabidopsis thaliana* and *Nicotiana benthamiana*, but crop species such as tomato, potato and Prunus are also studied.



GROUP LEADER:

Carlos Alonso Blanco

POSTDOCTORAL FELLOWS:

Belén Méndez de Vigo
Israel Ausin

PHD STUDENT:

Marija Savić

MASTER'S DEGREE STUDENT:

Ifigenia Kyrkou

TECHNICIANS:

Mercedes Ramiro
Jenifer Pozas

SELECTED PUBLICATIONS

Sánchez-Bermejo E, Méndez-Vigo B, Picó FX, Martínez-Zapater JM, Alonso-Blanco C. Novel natural alleles at FLC and LVR loci account for enhanced vernalization responses in *Arabidopsis thaliana*. *Plant Cell Environ.* 2012 Sep;35(9):1672-84

Gujas B, Alonso-Blanco C, Hardtke CS. Natural *Arabidopsis* brx loss-of-function alleles confer root adaptation to acidic soil. *Curr Biol.* 2012 Oct 23;22(20):1962-8

Méndez-Vigo B, Picó FX, Ramiro M, Martínez-Zapater JM, Alonso-Blanco C. Altitudinal and climatic adaptation is mediated by flowering traits and FRI, FLC and PhyC genes in *Arabidopsis*. *Plant Physiol.* 2011 Dec;157(4):1942-55

Gomaa NH, Montesinos-Navarro A, Alonso-Blanco C, Picó FX. Temporal variation in genetic diversity and effective population size of Mediterranean and subalpine *Arabidopsis thaliana* populations. *Mol Ecol.* 2011 Sep;20(17):3540-54

Cao J, Schneeberger K, Ossowski S, Günther T, Bender S, Fitz J, Koenig D, Lanz C, Stegle O, Lippert C, Wang X, Ott F, Müller J, Alonso-Blanco C, Borgwardt K, Schmid KJ, Weigel D. Whole-genome sequencing of multiple *Arabidopsis thaliana* populations. *Nat Genet.* 2011 Aug 28;43(10):956-63

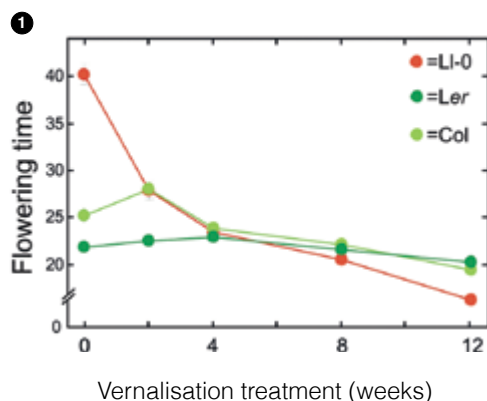
Genetic and molecular basis of naturally-occurring variation in plant development

The main objective of our research is to understand the genetic and molecular mechanisms involved in plant adaptation. We are dissecting existing genetic variation in the model annual plant *Arabidopsis thaliana* in nature. Similar to many other plant species, individuals and populations of *A. thaliana* living in distinct geographical regions differ in many developmental traits that are presumed to reflect adaptations to different environments. To exploit this genetic variation for understanding plant adaptation, our group currently focusses on two specific objectives.

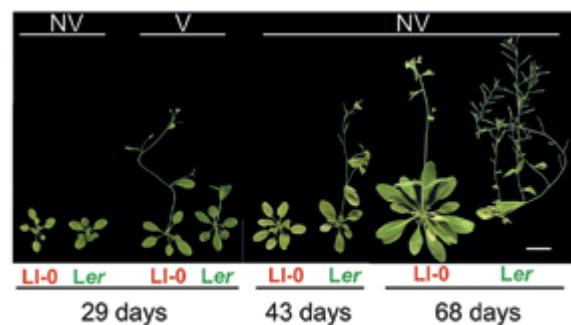
We are carrying out genetic analysis of naturally occurring variation for a key quantitative developmental trait, the timing of flowering. We analysed the genetic basis of the variation for flowering initiation relative to vernalisation, the induction of flowering by low winter temperatures. For these studies, we developed a new RIL population of 139 lines derived from the cross *LerxLi-0* and have grown them with different vernalisation treatments at 4°C. The study of this population by QTL (quantitative trait locus) mapping and by expression and association analyses led to

1. identification of small effect loci termed *Llagostera vernalisation response (LVR)*
2. identification of a cis-regulatory polymorphism in the *FLC* gene that might confer climatic adaptation by increasing vernalisation sensitivity (Sanchez-Bermejo *et al.*, 2012).

In addition, we developed a collection of wild *A. thaliana* genotypes from the Iberian Peninsula, which serves as a permanent experimental population for genetic and environmental association analyses. As a first step in exploiting this population, we characterised 182 Iberian genotypes for flowering behaviour and sequenced four flowering genes, *FRI*, *FLC*, *PhyC* and *CRY2*, involved in the vernalisation and photoperiod pathways. We found a new natural allelic series of *FRI* and *FLC* genes by association mapping. In addition, geographic and climatic association analyses showed that frequent Iberian alleles in *FLC* and *PhyC* are probably involved in climatic adaptation (Mendez-Vigo *et al.*, 2011).



1 Flowering behaviour of *Li-0*, *Ler* and *Col* accessions relative to vernalisation.





GROUP LEADER:
Carmen Castresana

POSTDOCTORAL FELLOWS:
Marta Martínez
Tamara Velloso
Jorge Vicente
Satish Kulasekaran
Verónica Aguilera

PHD STUDENTS:
Ruth Marcos
Yovanny Izquierdo

TECHNICIAN:
Tomás Cascón

SELECTED PUBLICATIONS

López MA, Vicente J, Kulasekaran S, Velloso T, Martínez M, Irigoyen ML, Cascón T, Bannenberg G, Hamberg M, Castresana C. Antagonistic role of 9-lipoxygenase-derived oxylipins and ethylene in the control of oxidative stress, lipid peroxidation and plant defence. *Plant J.* 2011 Aug;67(3):447-58

Vicente J, Cascón T, Vicedo B, García-Agustín P, Hamberg M, Castresana C. Role of 9-lipoxygenase and α -dioxygenase oxylipin pathways as modulators of local and systemic defense. *Mol Plant.* 2012 Jul;5(4):914-28

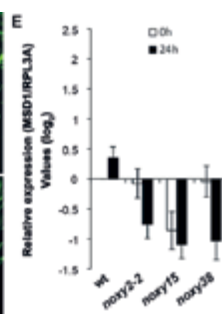
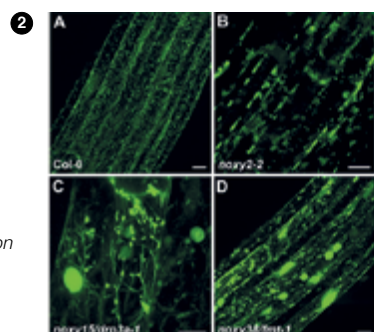
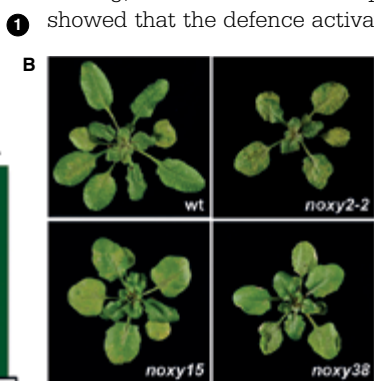
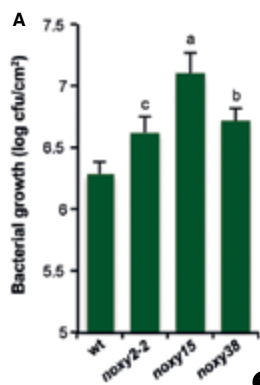
Velloso T, Aguilera V, Marcos R, Bartsch M, Vicente J, Cascón T, Hamberg M, Castresana C. Defense activated by 9-lipoxygenase-derived oxylipins requires specific mitochondrial proteins. *Plant Physiol.* 2013 Feb;161(2):617-27

Plant immunity strategies against microbial pathogen infection

Plant oxylipins are a class of lipid signalling molecules with a critical role in protecting plants against pathogen attack. Recent studies demonstrate the participation of the 9-LOX and alpha-DOX oxylipin pathways in the defence mechanisms activated by *Arabidopsis* following infection by hemibiotrophic bacteria, in which these enzymes collaborate to achieve full resistance against virulent strains. We showed that these oxylipin pathways participate in the three layers of defence –pre-invasion, apoplastic and systemic defence– triggered by plants to prevent *Pseudomonas syringae pv tomato* DC3000 infection. In these responses, oxylipins were found to act as regulators of oxidative stress, lipid peroxidation and hormone homeostasis. Our studies also showed high 9-LOX and alpha-DOX levels activity in roots of untreated *Arabidopsis* plants, suggesting that these oxylipin pathways participate in plant defence against root pathogens, a process that remains poorly understood.

Studies to characterise non-response to oxylipins (*noxy*), a series of *Arabidopsis* mutants insensitive to the 9-LOX product 9-hydroxy-10,12,15-octadecatrienoic acid (9-HOT), demonstrated the importance of cell wall modifications as a component of 9-LOX-induced defence. We found that a majority (71%) of 41 *noxy* mutants studied had added insensitivity to isoxaben, an herbicide that inhibits cellulose synthesis and alters the cell wall. The specific mutants *noxy2*, *noxy15*, and *noxy38*, insensitive to both 9-HOT and isoxaben, showed enhanced susceptibility to *Pseudomonas syringae* DC3000, as well as reduced activation of salicylic acid-responding genes. Moreover, map-based cloning, fluorescence microscopy and molecular analyses of the three *noxy* mutants showed that the defence activated by 9-lipoxygenase requires specific mitochondrial

proteins. Our results demonstrated that the defensive responses and cell wall modifications caused by 9-HOT are under mitochondrial retrograde control, and that mitochondria have a fundamental role in innate immunity signalling. These findings support our interest in examining 9-LOX and alpha-DOX oxylipin pathway functions in plant defence, as well as in identifying the molecular components that mediate their activity. Our studies could provide knowledge to help develop alternative strategies for disease control in crop plants, a major limiting factor that reduces agricultural productivity.



1 The mutants *noxy2*, *noxy15*, and *noxy38*, showed enhanced susceptibility to *Pseudomonas syringae* DC3000 infection. Bacterial growth (A) and lesions (B).

2 Images of mitochondria (green) in roots of Col-0 (A), *noxy2-2* (B), *noxy15/drp3a-1* (C) and *noxy38/fmt-1* (D). Expression of mitochondrial manganese superoxide dismutase (MSD1) in wild-type and *noxy* mutants (E).



GROUP LEADER:

Pilar Cubas

POSTDOCTORAL FELLOWS:

Michael Nicolas
Florian Chevalier

PHD STUDENT:

Eduardo González

MASTER'S DEGREE STUDENT:

Laura Gutiérrez
Sandra Martínez
Chidi Afamefule

TECHNICIAN:

Isabel Domínguez

▲ SELECTED PUBLICATIONS

Preston JC, Hileman LC, Cubas P. Reduce, reuse, and recycle: Developmental evolution of trait diversification. *Am J Bot.* 2011 Mar;98(3):397-403

Martín-Trillo M, Grandío EG, Serra F, Marcel F, Rodríguez-Buey ML, Schmitz G, Theres K, Bendahmane A, Dopazo H, Cubas P. Role of tomato *BRANCHED1*-like genes in the control of shoot branching. *Plant J.* 2011 Aug;67(4):701-714

★ PATENTS

EP11166057.7, PCT/2012/058892: *SpBRANCHED1a* of *Solanum pennellii* and tomato plants with reduced branching comprising this heterologous *SpBRANCHED1a* gene

P20090008, P201030915 DIV, PCT/ES09/070538. Genes regulating plant branching, promoters, genetic constructs containing same and uses thereof

Genetic analysis of axillary meristem development

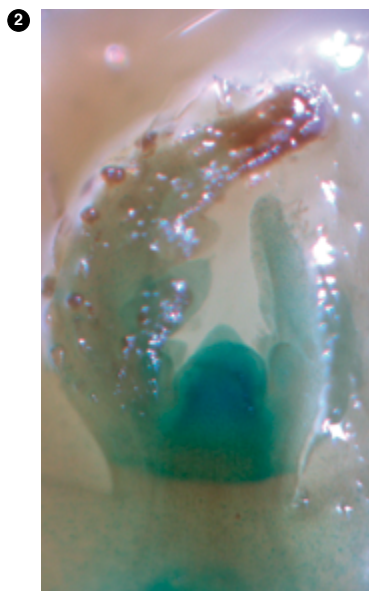
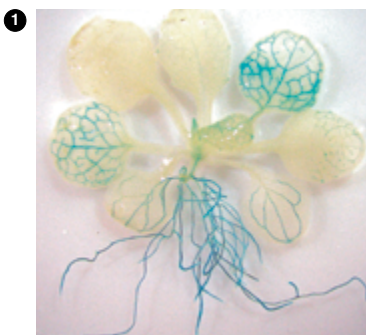
We are studying the genetic basis of the control of axillary bud development in the model system *Arabidopsis*, and in the crop species tomato and potato, in which control of lateral shoot branching is of great agronomic interest. We have characterised the *Arabidopsis* *BRANCHED1* (*BRC1*) gene, which acts as a central switch of axillary bud development and outgrowth. We are now expanding our knowledge of the genetic networks involving *BRC1* in *Arabidopsis*.

The *Arabidopsis thaliana* gene *BRANCHED1* (*BRC1*), expressed in axillary buds, is necessary for branch suppression in response to shade. *BRC1* is negatively controlled by phyB. Transcriptional profiling of wild-type and *brc1* buds of plants treated with simulated shade revealed a group of genes whose mRNA levels are dependent on *BRC1*. Among them there is a set of upregulated ABA-response genes, and a network of cell cycle- and ribosome-related downregulated genes. The downregulated genes have promoters enriched in TCP binding sites, suggesting that they are transcriptionally controlled by TCP factors. We are now testing whether they are *BRC1* direct targets.

We also used two mutageneses to identify new components of the pathways that control branching and identified a number of so-called *seto* mutants (bushy mutants in shade) and *sud* mutants (*SUPpressors of BRANCHED1*); we are now in the process of cloning and analysing them.

Solanaceae is a family including a large number of species in which the control of branch outgrowth is of great agronomic interest, and for which understanding the function of some of the key players will help optimise plant architecture and yield. Our work has shown that in tomato and potato, species with branching patterns different from those of *Arabidopsis*, two *BRC1*-like paralogues (*BRC1a* and *BRC1b*) are coexpressed in axillary buds. Reverse genetic analyses confirmed that tomato *SIBRC1b*

has a role in the promotion of axillary bud arrest. In contrast, *SIBRC1a*, which encodes a divergent protein with a novel C-t domain, has a still unclear role in this process. Evolution rate studies indicate that whereas *BRC1b* evolved under strong purifying selection in the clade comprising *S. lycopersicum*, *BRC1a* from other closely related wild tomato species and potato evolved at a faster rate under positive selection.



2 Axillary bud of a tomato transgenic plant carrying an *Arabidopsis* *BRANCHED1* promoter fused to beta-glucuronidase (*GUS*). *GUS* expression is detected by a histochemical staining that gives a blue product in the presence of *GUS*.

1 *SETO5* gene expression in *Arabidopsis* visualised by a promoter:beta-glucuronidase (*GUS*) fusion. *GUS* expression is detected by histochemical staining that gives a blue product in the presence of *GUS*.



GROUP LEADERS:
Juan Antonio García
Carmen Simón

POSTDOCTORAL FELLOWS:
Mingmin Zhao
Bernardo Rodamilans

PHD STUDENTS:
María Calvo
José de Jesús Pérez
Jon Ochoa
Fabio Pasin
Araiz Gallo
Frida Mesel
David San León

TECHNICIAN:
Beatriz García

SELECTED PUBLICATIONS

Valli A, Oliveros JC, Molnar A, Baulcombe D, García JA. The specific binding to 21-nt double-stranded RNAs is crucial for the anti-silencing activity of Cucumber vein yellowing virus P1b and perturbs endogenous small RNA populations. *RNA*. 2011 Jun;17(6):1148-58.

Simón-Mateo C, García JA. Antiviral strategies in plants based on RNA silencing. *Biochim Biophys Acta*. 2011 Nov-Dec;1809(11-12):722-31

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Maliogka VI, Salvador B, Carbonell A, Sáenz P, León DS, Oliveros JC, Delgadillo MO, García JA, Simón-Mateo C. Virus variants with differences in the P1 protein coexist in a Plum pox virus population and display particular host-dependent pathogenicity features. *Mol Plant Pathol*. 2012 Oct;13(8):877-86

Valli A, Busnadiego I, Maliogka V, Ferrero D, Castón JR, Rodríguez JF, García JA. The VP3 factor from viruses of Birnaviridae family suppresses RNA silencing by binding both long and small RNA duplexes. *PLoS One*. 2012;7(9):e45957

Plant-pathogen interaction in viral infections

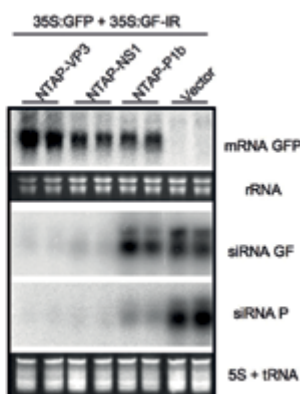
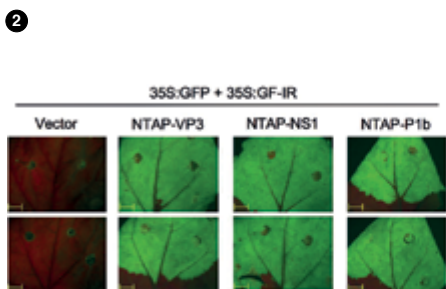
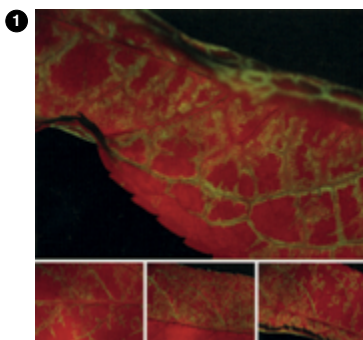
Plant viruses depend largely on host factors to replicate within the cell and to propagate throughout the plant and between individual plants. Plants have in turn developed antiviral defence mechanisms, which must be counteracted by viral factors. These viral factors appear to be preferred targets for alternative plant defences. In our laboratory, we try to understand this complex interplay, mainly in the infection of the potyvirus Plum pox virus (PPV), the causal agent of sharka, a damaging disease of *Prunus* trees.

We are interested in defence responses related to RNA silencing and its viral suppressors, and especially in distinguishing common and virus-specific features of these silencing suppression proteins in potyviral infection. Our results demonstrate that although potyviruses can exploit different sources of anti-silencing activity, their own silencing suppressors can help to define the specific host range of the virus. We also showed that single amino acid changes at the N-terminal region of the capsid protein (CP) control specific PPV adaptation to *Prunus persica* and *Nicotiana* species. Our findings suggest that species-specific interactions of the CP N-terminal region with host factors are important in viral long distance movement, and that an unknown resistance mechanism interferes with these interactions in *Nicotiana* species. We

are characterising the host counterparts and the molecular pathways involved.

Another main priority is to apply the information obtained in our research to designing control strategies for sharka and other viral diseases.

We are currently trying to establish principles for the design of effective, durable anti-viral resistance based on artificial miRNA, to obtain *N. benthamiana* and *P. persica* plants resistant to PPV infection. Finally, another target of interest is the development of PPV-based plant expression vectors. To broaden the range of plants susceptible to PPV-based vectors, we developed an infectious cDNA clone of a PPV isolate of strain C, the only one that infects cherry trees in nature. Biological features of this cloned isolate are at present being analysed.



1 Infection of GF-305 peach seedlings by a Plum pox virus (PPV)-derived virus expressing Cucumber vein yellowing virus (CVYV) P1b

2 Proteins from animal and plant viruses suppress RNA silencing. GFP fluorescence pictures and Northern blot analyses of GFP mRNA and GFP-derived siRNAs (right panels) in agroinfiltrated leaf patches.



GROUP LEADER:
Antonio Leyva

POSTDOCTORAL FELLOWS:
Gabriel Castrillo
Cristina Navarro

PHD STUDENTS:
Mohan TC
Bárbara del Llano

TECHNICIAN:
Yolanda Leo del Puerto

SELECTED PUBLICATIONS

Lanza M, Garcia-Ponce B, Castrillo G, Catarecha P, Sauer M, Rodríguez-Serrano M, Páez-García A, Sánchez-Bermejo E, T C M, Leo del Puerto Y, Sandalio LM, Paz-Ares J, Leyva A. Role of actin cytoskeleton in brassinosteroid signaling and in its integration with the auxin response in plants. *Dev Cell*. 2012 Jun 12;22(6):1275-85

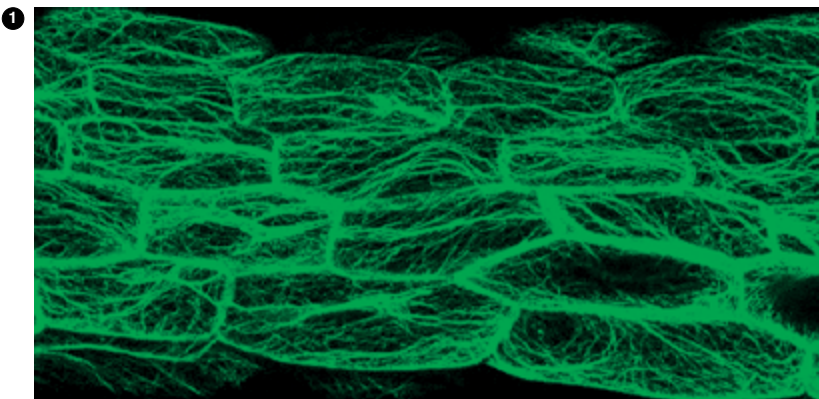
Molecular mechanisms underlying root architecture and arsenic phytoremediation

Our group has two research lines; in the first, we study the molecular mechanisms that underlie arsenic perception and in the second, we study mechanisms involved in root architecture.

For root architecture studies, we performed a mutant screening in *Arabidopsis* to identify mutants altered in the spatial distribution of roots. We identified several mutants altered in root architecture, including *actin2-4*, a mutant allele of the *ACTIN2* gene. This mutant has increased actin dynamics, leading to enhancement of tropic responses and auxin transcriptional responsiveness, and thus mimics the auxin/brassinosteroid synergistic response.

In the context of our research on the mechanisms underlying arsenic perception, using a transgenic plant that expresses the phosphate (Pi) transporter promoter fused to the luciferase gene, we identified a set of genes inducible by Pi starvation that are repressed by arsenate (As(V)) with extraordinary speed (30 min), while it takes 36 hours to be repressed by Pi.

The use of the transgenic line PHT1;1:LUC allows identification of mutants with kinetic altered by As(V). This is a new approach to the characterisation of the As(V) signalling pathway and its possible crosstalk with that of Pi. In the last year, we identified and characterised several transcription factors involved in arsenic perception. Moreover, we analysed the natural variability of As(V) tolerance in a large collection of *Arabidopsis* ecotypes; we identified a quantitative trait locus that we are currently cloning.



1 Confocal analysis of actin filament configuration in 5-day-old seedlings of *Col-0 Arabidopsis* hypocotyls grown on vertical MS plates.



GROUP LEADER:

Javier Paz-Ares

POSTDOCTORAL FELLOWS:

María Isabel Puga
Valerie Brigitte Chevalier

PHD STUDENTS:

Laura Cuyàs
Charukesi Rajulu
Miguel Miñambres

TECHNICIAN:

Erica Gil

LAB MANAGER:

Juani Monterrey

SELECTED PUBLICATIONS

Fernández P, Chini A, Fernández-Barbero G, Chico JM, Gimenez-Ibanez S, Geerinck J, Schweizer F, Godoy M, Franco-Zorrilla JM, Pawels L, Puga MI, Paz-Ares J, Goosens A, Reymond P, de Jaeger G, Solano R. (2011) The bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of JA responses. *Plant Cell*. 23, 701-715.

Bayle V, Arrighi JF, Creff A, Nespoulous C, Vialaret J, Rossignol M, Gonzalez E, Paz-Ares J, Nussaume L. (2011) *Arabidopsis thaliana* high-affinity phosphate transporters exhibit multiple levels of post-translational regulation. *Plant Cell*, 23, 701.

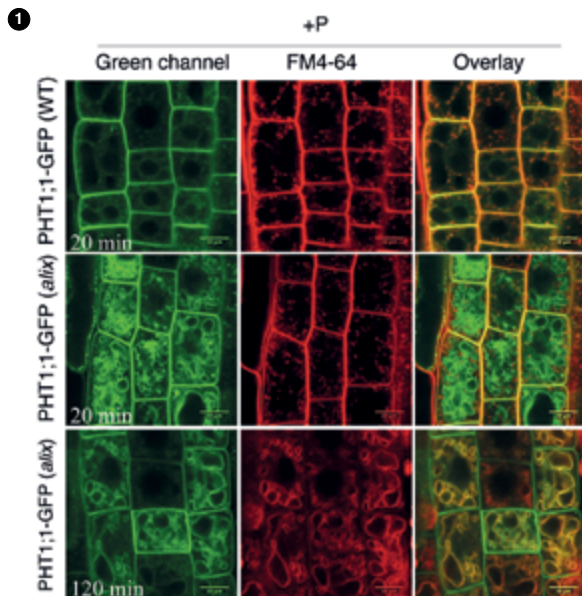
Castrillo G, Turck F, Leveugle M, Lecharny A, Carbonero P, Coupland G, Paz-Ares J, Oñate-Sánchez L. (2011) Speeding cis-trans regulation discovery by phylogenomic analyses coupled with screenings of an arrayed library of *Arabidopsis* transcription factors. *PLoS One*. 6:e21524.

Lanza M, Garcia-Ponce B, Castrillo G, Catarcha P, Sauer M, Rodriguez-Serrano M, Páez-García A, Sánchez-Bermejo E, Tc M, Leo Del Puerto Y, Sandalio LM, Paz-Ares J, Leyva A. (2012) Role of actin cytoskeleton in brassinosteroid signaling and in its integration with the auxin response in plants. *Dev Cell*. 22, 1275-1285.

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

Our research is focussed on the phosphate (Pi) starvation rescue system of plants, a model system for studies of gene activity, and with presumed biological potential in the context of low input agriculture. In previous years, we identified the transcription factor PHR1 as a master regulator of Pi starvation responses in plants. In the last two years, we advanced our knowledge of additional transcription factors and other regulators of this response and performed a genome-wide analysis of PHR1 targets *in vivo*:

1. Additional transcription factors that affect Pi starvation responses were sought in a library of transgenic plants conditionally expressing 1000 different transcription factors; these were generated in the context of the TRANPLANTA Project (CONSOLIDER INGENIO program), which we coordinated. We initiated the ionic analysis of these lines by ICP-OES and have now identified four transcription factors whose overexpression results in altered Pi content.
2. Additional regulators of Pi starvation responses were identified from a suppressor screen of the *phr1* mutant, leading to the isolation of AtALIX, a scaffold protein of the endomembrane system. Cell biological analysis of AtALIX showed that it alters the recycling of Pi transporters.
3. Genome-wide analysis of PHR1 targets. Using RNA-seq, we identified approximately 2400 targets of PHR1. Analysis of the bound region showed that PHR1 binds its targets via two sites (PHR1 binding site I and II; P1BSI and P1BSII), which are bound by PHR1 using two different modes, dimer and monomer. P1BSI- and P1BSII-containing targets show differential enrichment in ontology classes and in P1BS-unrelated motifs. This study allowed us to conclude that PHR1-based control uses a dual regulatory logic and that the underlying P1BSI and P1BSII motifs have different evolutionary constraints.



1 AtALIX regulates Pi transporter recycling. Root epidermal cells of 5-day-old seedlings of wild type (WT) and *Atalix* (*alix*) mutants overexpressing PHT1;1-GFP grown in Pi-rich conditions were observed by confocal laser scanning. Seedlings were treated with 2 μM FM4-64 for 5 minutes, and observations made after 20 and 120 minutes. PHT1;1-GFP in WT is located in plasma membrane and in sorting endosomes, as shown by PHT1;1-GFP and FM4-64 colocalisation in both compartments after 20 minutes. PHT1;1-GFP in the *Atalix* background is also found in tonoplasts, as shown by colocalisation of PHT1;1-GFP fluorescence with FM4-64 after 120 minutes, indicating that AtALIX function is needed for correct Pi transporter recycling.



GROUP LEADER:
Salomé Prat

POSTDOCTORAL FELLOWS:
Ana Espinosa
José A. Abelenda
Cristina Martínez
Cristina Nieto

PHD STUDENTS:
Vadir López
Stella Bernardo
Pilar Lasierra
Eduard Cruz

TECHNICIAN:
Carlos A. Cuéllar

▲ SELECTED PUBLICATIONS

Kloosterman B, Abelenda JA, Gomez M del M, Oortwijn M, de Boer JM, Kowitzanich K, Horvath BM, van Eck HJ, Smaczniak C, Prat S, Visser RG, Bachem CW. (2013) Naturally occurring allele diversity allows potato cultivation in northern latitudes. *Nature*. 495(7440):246-50

Soy J, Leivar P, González-Schain N, Sentandreu M, Prat S, Quail PH, Monte E. (2012) Phytochrome-imposed oscillations in PIF3 protein abundance regulate hypocotyl growth under diurnal light/dark conditions in *Arabidopsis*. *Plant J*. 71(3):390-401.

Bou-Torrent J, Martínez-García JF, García-Martínez JL, Prat S. (2011) Gibberellin A1 metabolism contributes to the control of photoperiod-mediated tuberization in potato. (2011) *PLoS One*. 6(9):e24458.

Navarro C, Abelenda JA, Cruz-Oró E, Cuéllar CA, Tamaki S, Silva J, Shimamoto K, Prat S. (2011) Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. *Nature*. 478(7367):119-22.

Abelenda JA, Navarro C, Prat S. (2011) From the model to the crop: genes controlling tuber formation in potato. *Curr Opin Biotechnol*. 22(2):287-92.

★ PATENT

PCT/ES2010/070265. Genes homologous to the FLOWERING LOCUS T (FT) gene and the use thereof for modulating tuberization. Extended to USA, EU, Brazil, India and Australia.

Hormonal control of light signalling

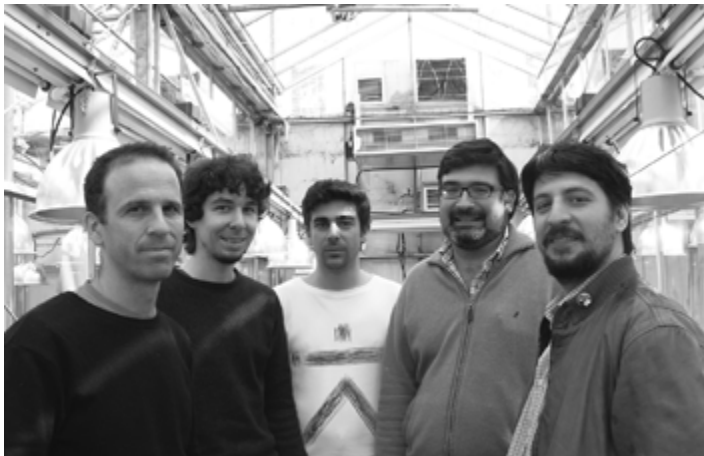
By perceiving changes in the quality of incident light or oscillations in diurnal light hours, plants are able to sense the surrounding environment and recognise year season progression. Signalling pathways implicated in response to shade proved to be closely related to those that control seedling de-etiolation; the phytochrome-interacting factor (PIF) family of bHLH factors has an important function in these responses. These factors accumulate in the nucleus in the dark or in FR spectrum-enriched light, but in red light they are rapidly destabilised by the light receptor phytochrome. PIF-regulated expression, on the other hand, is tightly regulated by the endogenous developmental programmes, which allows plants to adjust growth and architecture to variable light conditions and preclude excessive elongation. The gibberellin (GA) and brassinosteroid (BR) hormones are essential in regulating this crosstalk.

In previous studies, we showed that the DELLA repressors, which are central to GA signalling, repress cell elongation by sequestering PIF in an inactive complex unable to bind DNA. We also provided evidence that BR signalling is necessary for GA-induced growth, and that GA and BR pathways interact downstream of DELLAs. We uncovered a role of BRs in promoting PIF4 accumulation by inhibiting phosphorylation of this factor by the GSK3 kinase BIN2, with a negative role in BR signalling. Our results demonstrate that PIF4 and the BR signalling factors BES1/BZR1 are obligate partners for activation of cell elongation genes, the PIF/BES1/ BZR1 activation complex thus providing a robust mechanism for cell elongation control in response to light, GA, and BR.

In studies of the photoperiodic control of potato storage organ formation, we have shown that a conserved CONSTANS-FT module mediates SD-dependent tuberisation. Potato CONSTANS modulates expression of a FT homologue (SP5G), which acts as a tuberisation repressor by inhibiting activation of the *SP6A* gene or mobile tuberisation signal. Elevated temperatures, which inhibit tuber formation, block *SP6A* activation, and overexpression of this gene preserves high tuber yields in heat stress conditions. These transgenic lines are suitable for cultivation in tropical regions.

1 Increased yield in tubers of potato FT plants under heat-stress conditions





GROUP LEADER:
Enrique Rojo

POSTDOCTORAL FELLOWS:
Michael Sauer
Alfonso Muñoz
Silvina Mangano

PHD STUDENTS:
Guillermo Ruano
María Otilia Delgadillo

TECHNICIAN:
María López

VISITING PERSONNEL:
Ramón Contreras
Hiu Man Leung

▲ SELECTED PUBLICATIONS

Sanmartín, M. Sauer, M., Muñoz, A., Rojo E. (2012). MINIYO and transcriptional elongation: lifting the roadblock to differentiation. *Transcription* 3: 1-4.

Sanmartín, M. Sauer, M., Muñoz, A., Jan Zouhar, J., Ordóñez, A., van de Ven, W. Caro, E., Sánchez, M., Raikhel N.V., Gutiérrez, C., Sánchez-Serrano, J.J., Rojo E. (2011). A molecular switch for initiating cell differentiation in *Arabidopsis*. *Curr. Biol.* 21, 999-1008.

Rosado, A., Hicks, G.R., Norambuena, L., Rogachev, I., Meir, S., Pourcel, L., Zouhar, J., Brown, M.Q., Boirsadore, M.P., Puckrin, R.S., Cutler, S., Rojo, E., Aharoni, A., Raikhel, N.V. (2011). Sortin1-hypersensitive mutants link vacuolar-trafficking defects and flavonoid metabolism in *Arabidopsis* vegetative tissues. *Chemistry & Biology* 18, 187-197.

★ PATENT

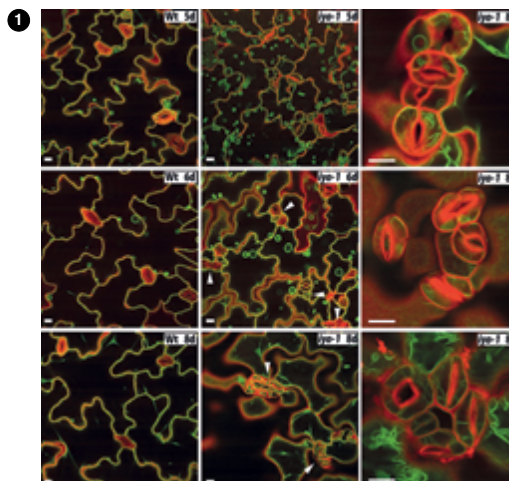
PCT/EP12/059312 Procedure to modify plant architecture and improve the crops yield through the control of entering into cell differentiation

Intracellular trafficking in plants

The subcellular localisation of proteins is a key parameter in defining their function. We are interested in studying how proteins are targeted and transported to their cellular destinations in plants, focusing on trafficking processes that are of particular relevance to these organisms.

A conspicuous peculiarity of the plant endomembrane system is the presence of very large vacuoles, which in most cells occupy the majority of the cell volume. The enlarged vacuoles of plants are necessary adaptations to autotrophy and immobility, as they provide an energy-cheap organ expansion mechanism for exploring the surroundings and a buffering organelle to maintain cell homeostasis. In addition, plants can have different types of vacuoles in a single cell. They develop specialised vacuoles in certain cells, such as the protein storage vacuoles in seed tissues, which constitute the main source of proteins for human and livestock nutrition. The molecular machinery responsible for the delivery of proteins and membranes to vacuoles is nonetheless largely uncharacterised. To define the mechanisms of vacuolar trafficking in plants, we designed a genetic screen in *Arabidopsis* for mutants with impaired vacuolar transport. In this way, we identified and characterised several factors involved in trafficking to the vacuole, including sorting receptors, SNARE and SM proteins, and phospholipid-modifying enzymes. We recently characterised an ENTH protein and an ARF GAP protein that act as key effectors for clathrin-coated vesicle-mediated trafficking of vacuolar proteins from the trans Golgi network to the late endosome in plants.

We also initiated a new line of research to study the mechanisms of nuclear-cytoplasmic partitioning of the IYO/ART protein complex. This dual localisation functions as a binary molecular switch that initiates cell differentiation when the IYO/ART complex is translocated to the nucleus. We are also studying the molecular activities of the IYO/ART complex and the structural basis of these activities. Our results suggest that, in the nucleus, the IYO/ART complex interacts with RNA polymerase II and activates productive transcriptional elongation of developmental regulators, triggering differentiation.



1 Epidermal development in wild type (Wt) and *iyo-1* cotyledons. Protoderm differentiation is delayed in *iyo-1* plants and as a result, stomatal patterning is disrupted. The plants express a tonoplast GFP marker (green) and are stained with propidium iodide (red).



GROUP LEADER:
Vicente Rubio

POSTDOCTORAL FELLOWS:
Marina Trigueros
Joaquín Iglesias
María Luisa Irigoyen

PHD STUDENTS:
Mónica Rojas
Elisa Iniesto
Amr A. Nassrallah
Diaa Abd El-Moneim

TECHNICIAN:
Jennifer Esteban

▲ SELECTED PUBLICATIONS

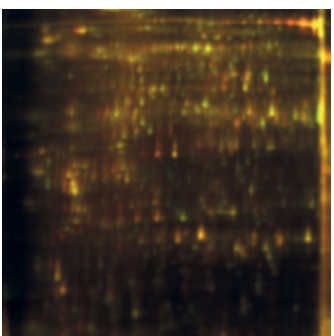
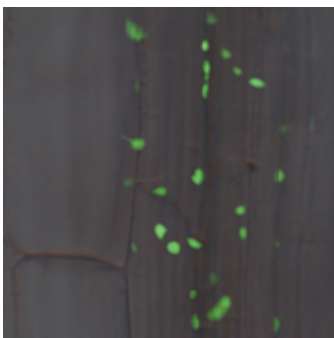
Rojas-Triana M, Bustos R, Espinosa-Ruiz A, Prat S, Paz-Ares J, Rubio V. Roles of ubiquitination in the control of phosphate starvation responses in plants. *J Integr Plant Biol.* 2013 Jan;55(1):40-53

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

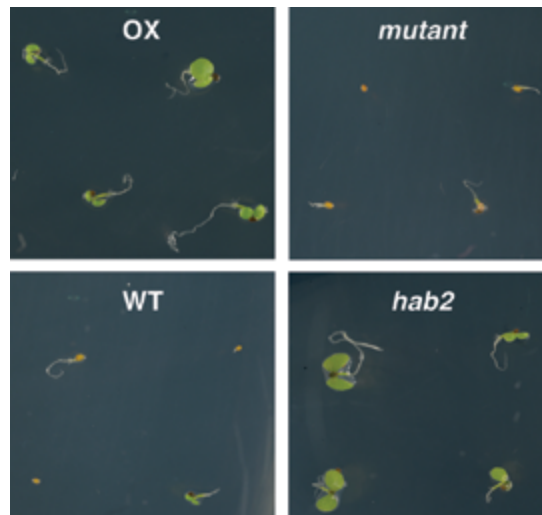
Ubiquitin conjugation to proteins is mediated by an enzymatic cascade in which E3 ubiquitin ligase enzymes provide substrate specificity. Proteins labelled with ubiquitin chains usually undergo degradation via the proteasome, although ubiquitination can also modify protein function by altering their subcellular localisation, assembly into complexes or enzyme activity. The potential regulatory relevance of the ubiquitin pathway in plants can be understood by the fact that proteins in the ubiquitination machinery comprise 5% of the *Arabidopsis* proteome. Protein ubiquitination affects many key aspects of plant biology, including phytohormone synthesis and signalling, floral organ formation and transition, defence against pathogens, and adaptive responses to numerous abiotic stresses. In accordance with this regulatory potential, the number of E3 ubiquitin ligases and targets reported in the control of specific plant biological processes is rapidly increasing. This trend parallels that observed in other eukaryotic organisms in which ubiquitination is conserved. Our knowledge of the molecular mechanisms that regulate ubiquitination machinery function and allow a coordinated response to environmental stimuli and stress conditions remains limited, especially in plant systems. Several mechanisms that control E3 activity have nonetheless been reported, many of them conserved in eukaryotes, such as control of the assembly of Cullin-RING E3 ligases by neddylation/deneylation cycles or regulation of the subcellular location of E3 components such as DDB1. The latter forms part of protein complexes involved in chromatin signalling and DNA damage repair, including several CUL4-RING ubiquitin ligases and ubiquitination-associated complexes.

The aim of our research is to characterise the molecular mechanisms that regulate the ubiquitination machinery in the control of plant development and stress responses associated with climate change events such as drought, high temperatures, salinity and UV radiation, using genetic, molecular and proteomic approaches. Regulatory proteins or their mutated versions identified by these approaches should help to develop modified crops with increased resistance to environmental stress.

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1 Genetic, molecular and proteomic approaches are used in our laboratory to characterise the function of specific components of the ubiquitin-proteasome system in the control of plant growth and stress tolerance.

2 *Arabidopsis* plants with gain (OX) or loss of function (mutant) for specific CUL4-RING ubiquitin ligases have opposite phenotypes for tolerance to salinity (100 mM NaCl in growth media) during seedling establishment. Wild type (WT) plants and tolerant mutants to salinity (*hab2*) are used as controls.



GROUP LEADER:
José J. Sánchez Serrano

POSTDOCTORAL FELLOWS:
Maite Sanmartín
Miguel Ángel López Carrasco

PHD STUDENT:
Marco Taurino

TECHNICIAN:
Pilar Paredes

VISITING SCIENTIST:
Fedra Francocci

SELECTED PUBLICATIONS

Sanmartín, M., Sauer, M., Muñoz, A., Zouhar, J., Ordóñez, A., van de Ven, WTG., Caro, E., Sánchez, MP., Raikhel, NV., Gutiérrez, C., Sánchez-Serrano, JJ., and Rojo, E. (2011) A molecular switch for initiating cell differentiation in *Arabidopsis*. *Current Biology* 21, 999-1008

Sanmartín M, Sauer M, Muñoz A, Rojo E. (2012) MINIYO and transcriptional elongation: lifting the roadblock to differentiation. *Transcription* 3, 25-28.

PATENT

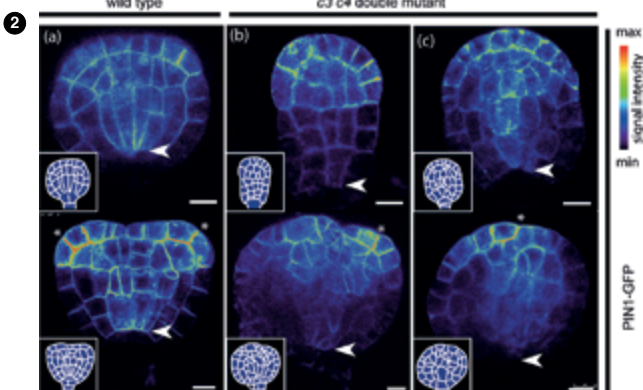
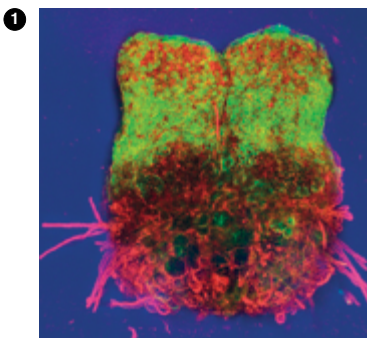
PCT/EP12/059312. Procedure to modify plant architecture and improve the crops yield through the control of entering into cell differentiation.
 M. Sanmartín, Jose J. Sanchez Serrano and E. Rojo

Signalling networks in plant development and defence responses

Plants adjust their development to accommodate cell differentiation and growth to fluctuating, often detrimental environmental conditions. Intricate signalling networks translate environmental cues into reprogramming of gene expression as a major adaptive response to biotic and abiotic stresses.

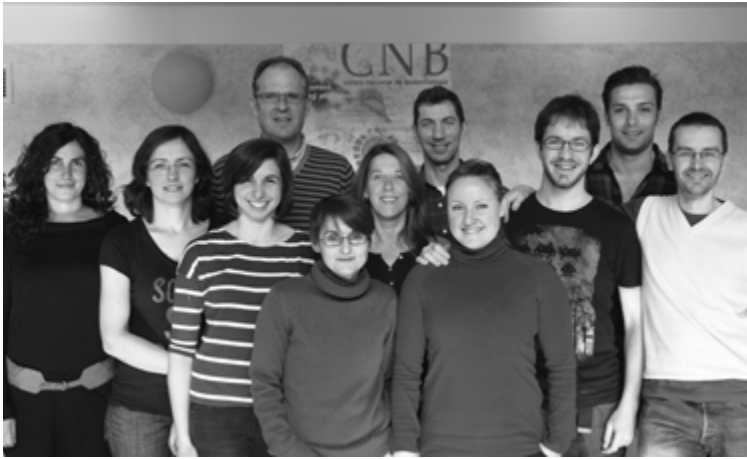
Reversible protein phosphorylation is a common mechanism in transduction pathways that connect external and/or internal signals to a given cell response. A phosphorylation switch consists of i) a target protein whose intrinsic characteristics are altered by its phosphorylation status, ii) a protein kinase, and iii) a protein phosphatase, which respectively phosphorylate/dephosphorylate their target in specific circumstances. Protein phosphatases of the subgroup 2A (PP2A) dephosphorylate proteins at serine/threonine residues. PP2A is a heterotrimer that consists of a catalytic subunit (PP2A-C) whose specific activity is regulated by the binding of A (PP2A-A) and B (PP2A-B) regulatory subunits. The *Arabidopsis* genome encodes five very similar PP2A-C, grouped in subfamily 1 (PP2A-C1, -C2, and -C5) and subfamily 2 (PP2A-C3 and -C4). The level of amino acid identity within the two subfamilies suggests redundancy in their roles. Single *pp2ac-3* and *pp2ac-4* mutant lines do not show any obvious phenotype, suggesting they indeed fulfil largely redundant functions. *pp2ac-3 pp2ac-4* plantlets are severely misshapen, however, with strong cotyledon and root primordia malformations. Our results indicate that PP2A-C3 and PP2A-C4 have redundant functions in controlling embryo patterning and root development, processes that depend on auxin fluxes. Moreover, polarity of the auxin efflux carrier PIN1 and auxin distribution are affected by mutations in PP2A-C3 and PP2A-C4. Our work indicates functional specialisation of subfamily 2 in the regulation of PIN protein polarity and hence, of auxin fluxes and plant patterning.

Following attack by pests and/or pathogens, plants turn on inducible defence responses. A major wound-signalling pathway involves the plant hormone jasmonic acid (JA). Large, transient increases in endogenous levels of JA after mechanical damage trigger the transcriptional activation of an array of defence genes. In potato, two genes (*StAOS1* and *StAOS2*) encode the putative 13-AOS, which catalyses the first committed step in JA biosynthesis. We showed that simultaneous cosuppression of *StAOS1* and *StAOS2* is necessary to lower the JA content of the plant significantly, resulting in altered responses to wounding and increased susceptibility to plant pathogens.



1 DR5pro:GFP fluorescence in *c3c4* seedlings. Confocal microscopy image of a *c3c4* seedling expressing the auxin reporter DR5pro:GFP. GFP fluorescence (green) essentially concentrates at the cotyledons (upper side) while propidium iodide staining and chlorophyll fluorescence (red) are mostly visualised in the hypocotyl and the defective root region (lower side).

2 PIN1-GFP in wild type and *c3c4* embryos. PIN1-GFP fluorescence in wild type (a) and *c3c4* double mutant embryos (b and c). Early embryonic stages on top, later stages at the bottom. Arrowheads indicate the border between embryo proper and the incipient root pole, asterisks mark PIN1-GFP accumulation in the protodermis at incipient cotyledon primordia. False colour coded with red for maximum, dark blue for minimum signal intensity. All bars are 10 µm.



GROUP LEADER:
Roberto Solano

SENIOR SCIENTIST:
José Manuel Chico

POSTDOCTORAL FELLOWS:
Andrea Chini
Selena Giménez Ibañez
Sandra Fonseca
Marta Boter
Patricia Fernández Calvo

PHD STUDENTS:
Isabel Monte
Guillermo Fernández Jiménez

TECHNICIAN:
Gemma Fernández Barbero

VISITING SCIENTIST:
Laurens Pauwels

SELECTED PUBLICATIONS

Godoy M, Franco-Zorrilla JM, Pérez-Pérez J, Oliveros JC, Lorenzo O, Solano R. Improved protein-binding microarrays for the identification of DNA-binding specificities of transcription factors. *Plant J.* 2011 May;66(4):700-11

Fernández-Calvo P, Chini A, Fernández-Barbero G, Chico JM, Gimenez-Ibanez S, Geerinck J, Eeckhout D, Schweizer F, Godoy M, Franco-Zorrilla JM, Pauwels L, Witters E, Puga MI, Paz-Ares J, Goossens A, Reymond P, De Jaeger G, Solano R. The *Arabidopsis* bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. *Plant Cell.* 2011 Feb;23(2):701-15

Hornitschek P, Kohlen MV, Lorrain S, Rougemont J, Ljung K, López-Vidriero I, Franco-Zorrilla JM, Solano R, Trevisan M, Pradervand S, Xenarios I, Fankhauser C. Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. *Plant J.* 2012 Sep;71(5):699-711

Llave C, Franco-Zorrilla JM, Solano R, Barajas D. Target validation of plant microRNAs. *Methods Mol Biol.* 2011;732:187-208

The jasmonate signalling pathway in *Arabidopsis*

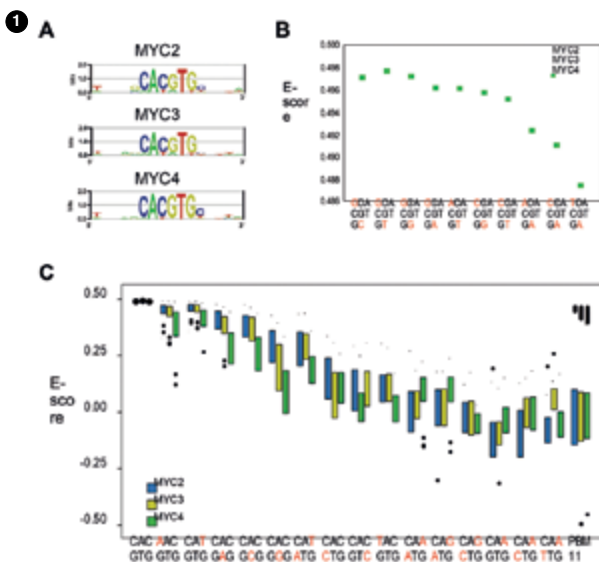
We are interested in understanding how plants perceive changes in their environment and integrate stress signals with their internal developmental programs to induce adaptive responses and survive in nature. This integration depends on complex signalling networks that regulate the genetic reprogramming of the cell. The main focus of my lab is the jasmonate (JA) signalling pathway in *Arabidopsis thaliana*. JA are fatty acid-derived signalling molecules essential for plant survival in nature, since they are important activators of stress responses and developmental programmes. We aim to identify the components of this pathway and understand how these components explain the molecular interactions of the jasmonate pathway with other pathways within the network. Understanding these molecular interactions is essential to decipher how a single hormone can activate so many physiological responses in the plant, and how the plant is able to discriminate between different stress types (e.g., pathogens and wounding) and select the correct set of responses to each.

We are using genomic, genetic, biochemical and molecular tools to dissect the JA signalling pathway and characterise its components. The major achievements of our group so far are:

- Identification of the first transcription factors (TF) that regulate JA responses (MYC2, MYC3, MYC4 and ERF1; Fernández Calvo *et al.*, 2011). They are essential for understanding the specificity of plant responses to the hormone.
- Discovery of the JAZ family of nuclear repressors of these TF. The discovery of the JAZ family of repressors linked the previous steps in the pathway (SCFCOI1 and the TF) and facilitated an integrated view of the core JA signalling module composed by SCFCOI1-JAZs-MYC2.
- Discovery of the active form of the hormone. Since its discovery about four decades ago, jasmonic acid was assumed to be the bioactive hormone. A combination of genetic and biochemical analyses allowed us to demonstrate that the real bioactive form of the hormone is (+)-7-iso-jasmonoyl-isoleucine.

- Discovery of SGT1b/JAI4, a regulator of SCF (Skip-Cullin-Fbox) E3 ubiquitin ligase complexes, including the JA-signalling component SCFCOI1.

- Identification of the mechanism of TF repression by JAZ proteins (recruitment of the general co-repressor TOPLESS by the adaptor protein NINJA; Pauwels *et al.*, 2010).



1 Identification of MYC2, MYC3 and MYC4 DNA-binding motifs in vitro. (A) Position weight matrix representation of the top scoring 8-mers corresponding to MYC2, MYC3 and MYC4. All three proteins showed highest binding affinity to a canonical G-box (CACGTG). (B) Enrichment scores (E-scores) of all the possible G-box-containing 8mers for the three MYC proteins tested, showing similar binding preferences of the three proteins for nucleotides at 5'. (C) Box-plot of E-scores of G-box variants including both single-site mutations and E-boxes (CANNTG). Boxes represent quartiles 25% to 75%, and black line within represents the median of the distribution (quartile 50%). Bars indicate quartiles 1 to 25% (above) and 75 to 100% (below), and dots denote outliers of the distribution. Boxes in blue correspond to MYC2, green boxes represent MYC3 and yellow ones correspond to data from MYC4.