

1 / Plant Molecular Genetics

Research in the Plant Molecular Genetics Department studies the molecular basis of the regulatory pathways that control plant development, environmental adaptation and protective responses to biotic and abiotic stresses. Interests of our research groups focus on the control of root architecture, shoot branching, photomorphogenesis and photoperiodism, adaptation to nutrient shortage or to toxic metals, and responses to pathogens and pests. Besides the intrinsic fundamental interest in understanding key biological processes in plants, our research seeks to derive new tools and methods to improve crop production, based on the use of natural biodiversity and on genetic engineering innovations. Biotechnological applications such as the use of plants as biopharmaceutical factories or as tools to fight environmental problems are also being studied. Our research is routinely carried out in the model species *Arabidopsis thaliana* and *Nicotiana benthamiana*, but crop species such as tomato, potato and Prunus are also major subjects of study.



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

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SELECTED PUBLICATIONS

Alonso-Blanco C, Méndez-Vigo B. Genetic architecture of naturally-occurring quantitative traits in plants: An updated synthesis. *Curr Opin Plant Biol* 2014; 18:37-43

Sánchez-Bermejo E, Castrillo G, Del Llano B, Navarro C, Zarco-Fernández S, Martínez-Herrera DJ, Leo-Del Puerto Y, Muñoz R, Cámara C, Paz-Ares J, Alonso-Blanco C, Leyva A. Natural variation in arsenate tolerance identifies an arsenate reductase in *Arabidopsis thaliana*. *Nat Commun* 2014; 5:4617

Brennan AC, Méndez-Vigo B, Haddioui A, Martínez-Zapater JM, Picó FX, Alonso-Blanco C. The genetic structure of *Arabidopsis thaliana* in the south-western Mediterranean range reveals a shared history between North Africa and southern Europe. *BMC Plant Biol* 2014; 14:17

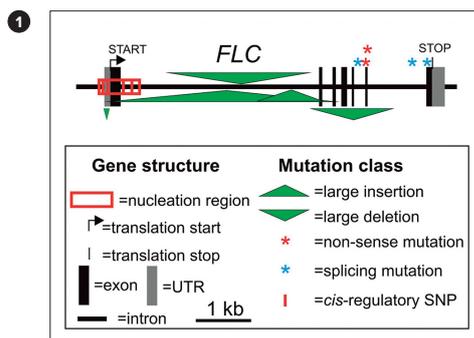
Barboza L, Effgen S, Alonso-Blanco C, Kooke R, Keurentjes J, Koornneef M, Alcazar R. Wild *Arabidopsis thaliana* semi-dwarfs evolved from independent mutations in GA20ox1, orthologue to semi-dwarf green revolution alleles in rice (*sd1*) and barley (*sdw1/denso*). *Proc Natl Acad Sci USA* 2013; 110:15818-15823

Méndez-Vigo B, Martínez-Zapater JM, Alonso-Blanco C. The flowering repressor SVP underlies a novel *Arabidopsis thaliana* QTL interacting with the genetic background. *PLoS Genet* 2013; 9:e1003289

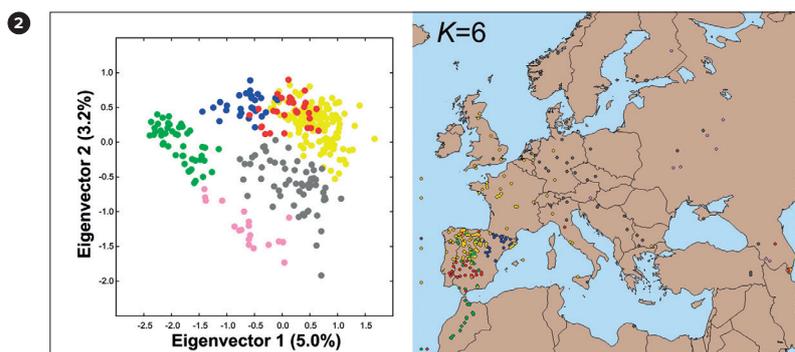
The main objective of our research is to understand the genetic and molecular mechanisms involved in plant adaptation. We are particularly interested in determining how developmental traits allow plant adaptation to different climates. To this end, we are dissecting genetic variation in the model annual plant *Arabidopsis thaliana* in nature. Our group currently focusses on two specific objectives.

On the one hand, we are performing genetic analysis of naturally-occurring variation for a key quantitative developmental trait, the timing of flowering. We have analysed the genetic basis of the variation for flowering initiation in relation to several environmental factors (Alonso-Blanco and Mendez-Vigo, 2014). Genetic mapping identified the locus *Flowering Arabidopsis* QTL 1 (FAQ1) as a major effect QTL that strongly affects the flowering response to photoperiod. Fine mapping and complementation demonstrated that FAQ1 is the gene *short vegetative phase* (SVP), a key flowering regulator that encodes a MADS transcription factor. We identified a new functional allele caused by a single amino acid substitution in the MADS domain, which produces SVP loss-of-function and early flowering. This natural allele appears to be distributed exclusively in Asia and its effect depends on genetic background (Mendez-Vigo *et al.*, 2013).

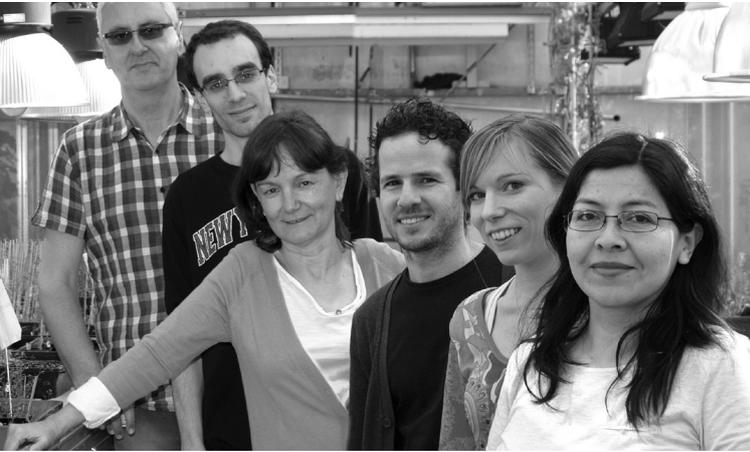
On the other hand, we developed a collection of wild *A. thaliana* genotypes from the Iberian Peninsula, which is a permanent experimental population for genetic and environmental association analyses (Fig. 1). Genetic analysis of this collection previously showed that Iberia is the world region with the largest known diversity of *A. thaliana*, which supports the hypothesis of multiple Iberian refuges during the last glaciations. To understand how *A. thaliana* has adapted to the climates of its southern limit, we have extended this collection to Northern Africa. Analyses identified a genetic lineage distributed exclusively in Iberia and Morocco, which did not contribute to the colonisation of the rest of Europe (Fig. 2). Further gene diversity analyses suggest that Morocco was a refuge for *A. thaliana* during the last glaciations and that the species colonised the Iberian Peninsula from Africa (Brennan *et al.*, 2014).



1 Functional nucleotide variation in the flowering repressor *FLC* of *Arabidopsis*



2 Genetic and geographic structure of *A. thaliana* at a global scale. The six groups detected by clustering analysis are shown in different colours in a scatter plot displaying the first two eigenvectors estimated by PCA (left) and in a worldwide collection of accessions. Accessions located outside the Eurasian map are shown at their corresponding latitude on the left (American accessions) and right (Asian accessions) edges.



Plant immunity strategies against microbial pathogen infection

PLANT MOLECULAR GENETICS / 15

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SELECTED PUBLICATIONS

Vellosillo 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; 161:617-627

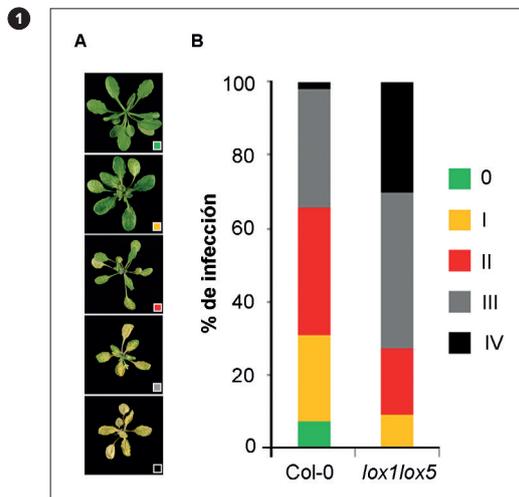
Montillet JL, Leonhardt N, Mondy S, Tranchimand S, Rumeau D, Boudsocq M, Garcia AM, Douki T, Bigeard J, Laurière C, Chevalier A, Castresana C, Hirt H. Identification of an ABA-independent oxylipin pathway that controls stomatal closure and immune defense in Arabidopsis. *PLoS Biol* 2013; 11:e1001513

Ozalvo R, Cabrera J, Escobar C, Christensen SA, Borrego EJ, Kolomiets MV, Castresana C, Iberkleid I, Horowitz SB. Two closely related members of Arabidopsis 13-LOXs, *LOX3* and *LOX4*, reveal distinct functions in response to plant-parasitic nematode infection. *Mol Plant Pathol* 2013; 15:319-332

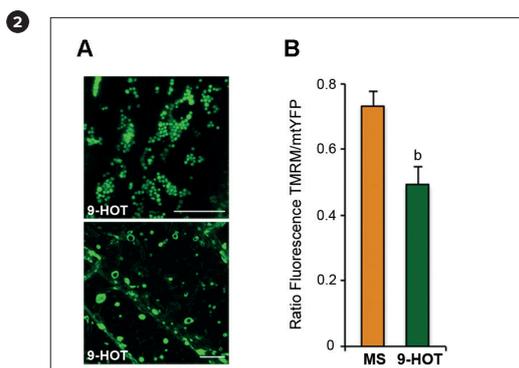
Coego A, Brizuela E, Castillejo P, Ruíz S, Koncz C, Del Pozo JC, Jarillo JA, Paz-Ares J, León J; The TRANSPLANTA Consortium. The TRANSPLANTA Collection of Arabidopsis Lines: A resource for Functional Analysis of Transcription Factors based on their conditional overexpression. *Plant J* 2014; 6:944-953

Plant oxylipins are a class of lipid signals involved in regulating plant development and immunity. Recent studies demonstrate the importance of the 9-LOX and alpha-DOX oxylipin pathways in the defence mechanisms activated by Arabidopsis following infection by hemibiotrophic bacteria; these oxylipin pathways participate in the three layers of defence (pre-invasion, apoplastic and systemic) triggered by plants to prevent *Pseudomonas syringae* pv *tomato* DC3000 infection. Our studies also showed high 9-LOX and alpha-DOX levels activity in roots of untreated Arabidopsis plants and participation of the 9-LOX pathway in the defence mechanisms against the root pathogen *Fusarium oxysporum* (Fig. 1). In these responses, oxylipins were found to act as regulators of oxidative stress, lipid peroxidation, hormone homeostasis and cell wall integrity. Characterisation of a series of *noxy* mutants (*non-responding to oxylipins*) insensitive to the 9-LOX products 9-hydroxyoctadecatrienoic acid (9-HOT) and 9-ketooctadecatrienoic acid (9-KOT) provided further support for the role of the 9-LOX pathway in plant defence and in signalling cell wall damage. We found that the defensive responses and cell wall modifications caused by 9-LOX products are under mitochondrial retrograde control and that mitochondria have a fundamental role in innate immunity signalling (Fig. 2). Additional experiments with mutants defective in brassinosteroids (BR), a class of plant hormones necessary for normal plant growth and cell wall integrity, showed that 9-LOX-derived oxylipins activate cell wall-based defence responses such as callose deposition by inducing BR synthesis and signalling. Studies with antioxidants showed that oxylipin-dependent activation of BR signalling is limited by the lipophilic antioxidant trolox, which indicated that lipid peroxidation helps trigger BR signalling. These results show interaction between the 9-LOX and BR pathways and point to participation of both oxylipins and brassinosteroids as part of the plant

response involved in controlling cell wall-based plant defence. The results of these studies support our interest in examining the action of 9-LOX and alpha-DOX oxylipins in plant defence, whose understanding will help to develop new strategies for disease control in crop plants, a major limiting factor in reducing agricultural productivity.



1 The *lox1lox5* mutant lacking 9-LOX activity displays enhanced susceptibility to the root pathogen *Fusarium oxysporum*. (A) Representative examples of infection symptoms scored on a five-point scale according to their intensity. (B) Percentage of leaves showing each of the symptoms evaluated.



2 Mitochondrial aggregation and decrease in membrane potential in response to 9-HOT. (A) Mitochondrial visualisation of 9-HOT (25 µM)-treated transgenic lines expressing 35S:Mt-YFP showed formation of aggregates (top) and annular structures (bottom). (B) TMRM (tetramethyl rhodamine methyl ester) and mt-YFP fluorescence intensities measured in untreated and 9-HOT-treated roots.



Genetic control of shoot branching patterns in plants

16 / PLANT MOLECULAR GENETICS

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SELECTED PUBLICATIONS

Chevalier F, Nieminen K, Sánchez-Ferrero JC, Rodríguez-Buey ML, Chagoyen M, Hardtke C, Cubas P. Strigolactone promotes degradation of AtD14, an α/β hydrolase essential for SL signalling in *Arabidopsis thaliana*. *Plant Cell* 2014; 26:1134-1150

González-Grandío E, Cubas P. Identification of gene functions associated to active and dormant buds in *Arabidopsis*. *Plant Signal Behav* 2014; 9:e27994

Coego A, Brizuela E, Castillejo P, Ruíz S, Ruíz C, del Pozo C, Pineiro M, Jarillo J, Paz-Ares J, Leon J. TRANSPLANTA Consortium. The TRANSPLANTA Collection of *Arabidopsis* Lines: A resource for Functional Analysis of Transcription Factors based on their conditional overexpression. *Plant J* 2014; 77:944-953

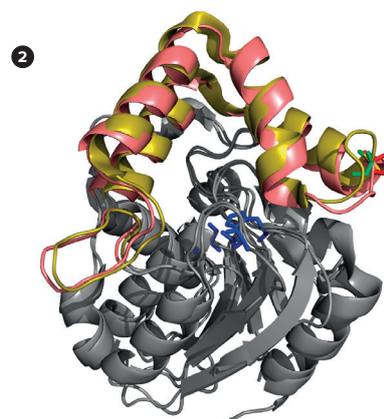
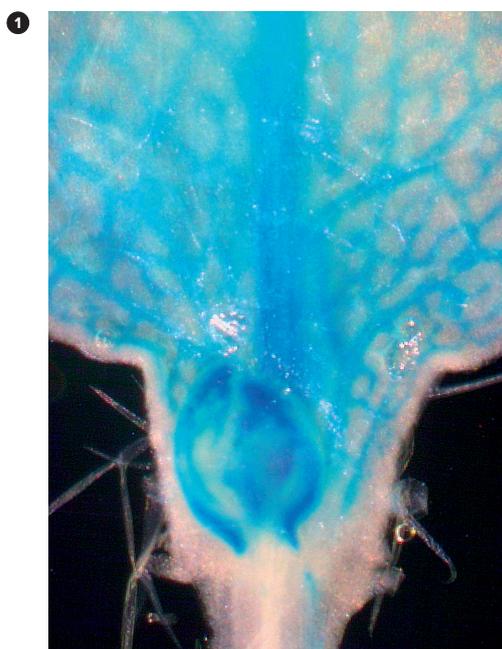
González-Grandío E, Poza-Carrión C, Sorzano COS, Cubas P. BRANCHED1 Promotes Axillary Bud Dormancy in Response to Shade in *Arabidopsis*. *Plant Cell* 2013; 25:834-850

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. A decrease in the red to far-red light ratio (R:FR) –a sign of shading by neighbouring vegetation– triggers a set of developmental plant responses termed shade avoidance syndrome. One of these responses is suppression of axillary bud outgrowth.

The *Arabidopsis* gene *BRANCHED1*, which encodes a TCP transcription factor, is a point at which signals that suppress shoot branching are integrated in axillary buds. We showed that *BRC1* is necessary for branch suppression in response to shade, and *BRC1* transcription is positively regulated after exposure to low R:FR.

To understand the growth-to-dormancy transition in axillary buds, we compared transcriptomic profiles of wild-type and *brc1* axillary buds, and identified sets of genes that are probably controlled by *BRC1*. We distinguished a network of upregulated abscisic acid response genes and two networks of cell cycle- and ribosome-related downregulated genes. The downregulated genes have promoters enriched in TCP binding sites, which suggests transcriptional regulation by TCP factors. We compared our transcriptomic data with two additional “active vs. dormant bud” transcriptomic data sets and found “core” coregulated gene networks closely associated to each condition.

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 characterise its function. Our data showed that D14 protein 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 plant. 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 GUS histochemical activity of *Arabidopsis* *D14* promoter fused to GUS (*D14pro:GUS*) in an *Arabidopsis* transgenic plant. A detail of an *Arabidopsis* young axillary bud is shown

2 3D models of the strigolactone receptor *AtD14* and karrikin receptor *KAI2*, based on crystallographic data (Hamiaux et al 2012; Kagiyama et al. 2013). Helical caps of *D14* and *KAI2* are highlighted in salmon pink and yellow, respectively. Active site residues are in blue. In red *D14* Pro-169, a residue whose mutation causes a complete loss of function of the *AtD14* protein.



Plant-pathogen interaction in viral infections

PLANT MOLECULAR GENETICS / 17

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SELECTED PUBLICATIONS

Carbonell C, Maliogka VI, Pérez JJ, Salvador B, San León D, García JA, Simón-Mateo C. Diverse amino acid changes at specific positions in the N-terminal region of the coat protein allow *Plum pox virus* to adapt to new hosts. *Mol Plant Microbe Interact* 2013; 26:1211-1224

Calvo M, Malinowski T, García JA. Single amino acid changes in the 6K1-CI region can promote the alternative adaptation of *Prunus*- and *Nicotiana*-propagated *Plum pox virus C* isolates to either host. *Mol Plant Microbe Interact* 2014; 27:136-149

Zhao M, San León D, Delgadillo MO, García JA, Simón-Mateo C. Virus-induced gene silencing in transgenic plants: transgene silencing and reactivation associate with two different patterns of transgene body methylation. *Plant J* 2014; 79:440-452

Valli A, Gallo A, Calvo M, Pérez JJ, García JA. A novel role of the potyviral helper component proteinase contributes to enhance the yield of viral particles. *J Virol* 2014; 88:9808-9818

Pasin F, Simón-Mateo C, García JA. The hypervariable amino-terminus of P1 protease modulates potyviral replication and host defense responses. *PLoS Pathog* 2014; 10: e1003985

Plant viruses depend on host factors to replicate and to propagate throughout the plant and between individual plants. Plants in turn have developed antiviral defences, 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 infection by the potyvirus *Plum pox virus* (PPV), the causal agent of sharka, a damaging disease of *Prunus* trees.

We are interested in virus-host interactions that can help to define virus host range. We have demonstrated that single alterations in the 6K1 and CI proteins (corresponding to the cleavage site recognized by the viral protease NIa in the PPV polyprotein) are involved in alternative host adaptation of atypical PPV isolates to *Nicotiana benthamiana* and *Prunus avium*. These results suggest that fine regulation of polyprotein processing might depend on specific host factors and contribute to adaptation to specific hosts. By studying resistance of *Arabidopsis thaliana* and *Chenopodium foetidum* to cherry strain PPV isolates, we showed that defects in interactions between translation initiation factors and potyviral proteins might not only prevent infection in resistant varieties of susceptible host species, but also contribute to non-host resistance. We also demonstrated that self-cleavage of PPV P1 is negatively regulated by its highly disordered N-terminal region and relies on a specific host factor for its activation. Based on these results, we speculate that host-dependent regulation of P1/HCPPro protease processing evolved to attenuate virus virulence and thus alleviate antiviral responses.

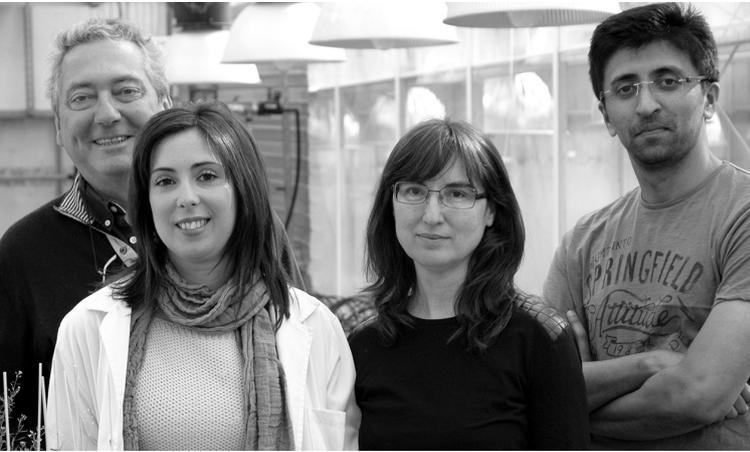
We apply the information obtained in our research to design control strategies for sharka and other viral diseases, and to develop PPV-based biotechnological tools. We have generated *N. benthamiana* transgenic lines that express different PPV-specific artificial miRNAs; they are being used to study the effectiveness and durability of antiviral resistance based on these small RNAs, and the side effects of the use of this technology on the virus evolutionary potential.



1 *Prunus avium* (left) and *Nicotiana benthamiana* (right) leaves infected with *Prunus*- or *Nicotiana*-adapted *Plum pox virus C* isolates, respectively. The background image shows a magnification of an infected *P. avium* leaf displaying vein chlorosis. (Cover, *Mol Plant Microbe Interact* 2014; 27:136)



2 Necrotic lesions caused by *Plum pox virus* (PPV) strain D (Rankovic isolate) in *Chenopodium foetidum*. (Cover, *Mol Plant Microbe Interact*. 2014; 27:1291)



Genes involved in root architecture and in arsenic phyto remediation

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SELECTED PUBLICATIONS

Castrillo G, Sánchez-Bermejo E, de Lorenzo L, Crevillén P, Fraile-Escanciano A, TC M, Mouriz A, Catarecha P, Sobrino-Plata J, Olsson S, Leo Del Puerto Y, Mateos I, Rojo E, Hernández LE, Jarillo JA, Piñero M, Paz-Ares J, Leyva A. WRKY6 Transcription Factor Restricts Arsenate Uptake and Transposon Activation in Arabidopsis. *Plant Cell* 2013; 25:2944-2957

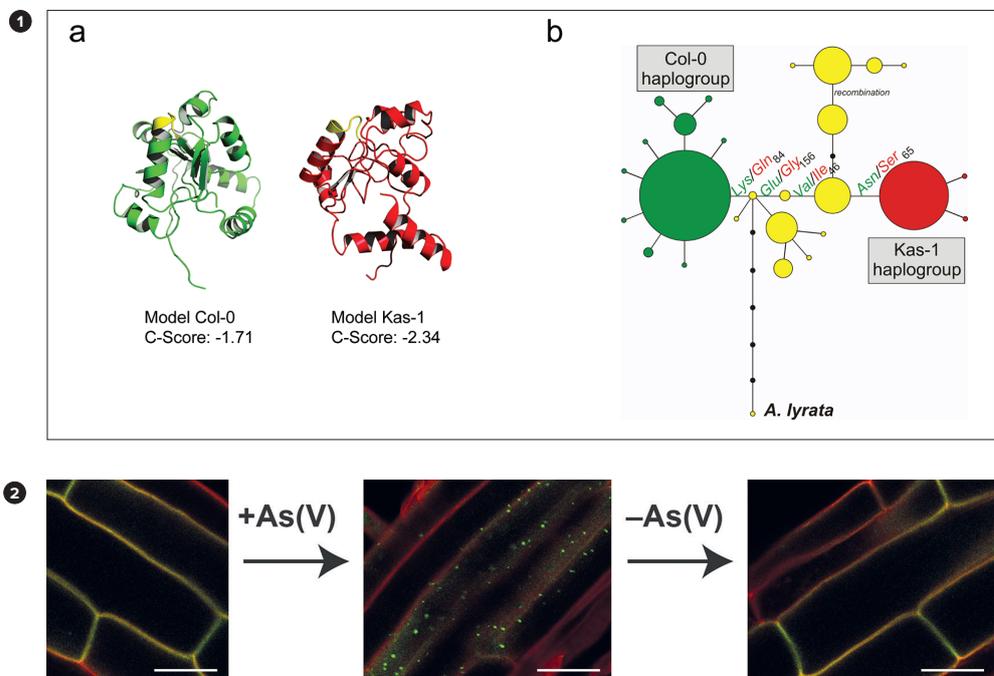
Coego A, Brizuela E, Castillejo P, Ruiz S, Koncz C, Del Pozo JC, Piñero M, Jarillo JA, Paz-Ares J, León J; The TRANSPLANTA Consortium. The TRANSPLANTA Collection of Arabidopsis Lines: A resource for Functional Analysis of Transcription Factors based on their conditional overexpression. *Plant J* 2014; 6:944-953

Sánchez-Bermejo E, Castrillo G, Navarro C, del Llano B, Zarco-Fernández S, Martínez-Herrera DJ, Leo-del Puerto Y, Muñoz R, Cámara C, Paz-Ares J, Alonso-Blanco C, Leyva A. Natural variation in arsenate tolerance identifies an arsenate reductase in *Arabidopsis thaliana*. *Nat Commun* 2014; 5:4617

Puga MI, Mateos I, Charukesi R, Wang Z, Franco-Zorrilla JM, de Lorenzo L, Irigoyen ML, Masiero S, Bustos R, Rodríguez J, Leyva A, Rubio V, Sommer H, Paz-Ares J. SPX1 is a phosphate-dependent inhibitor of PHOSPHATE STARVATION RESPONSE1 in Arabidopsis. *Proc Natl Acad Sci USA* 2014; 111:14947-14952

In our group, we are interested in the characterisation of the molecular mechanisms involved in arsenic perception in plants. Arsenic contamination is responsible for the worst mass poisoning ever suffered by man, and is considered a silent threat to public health. Clean-up of arsenic-contaminated soils or arsenic entry into the food chain from crops irrigated with arsenic-contaminated water, of particular importance in rice, is therefore a priority concern for the World Health Organisation (Mead. *Environ Health Perspect* 2005; 113:A378). This chemical threat was particularly critical for the evolution of sessile organisms such as plants, which were forced to develop rapid tolerance responses when As(V) was present. We recently identified a QTL (quantitative trait locus) that accounts for the genetic variability for As(V) tolerance among Arabidopsis accessions worldwide. Molecular isolation and characterisation of this locus showed it encodes a plant arsenate reductase with potential applications in arsenic phyto remediation (Sanchez-Bermejo *et al.* *Nat Commun* 2014; 5:4617). We also identified a transcriptional repressor that modulates expression of the arsenate transporter, thus identifying the molecular basis of an alternative strategy for plant adaptation to arsenic (Castrillo *et al.* *Plant Cell* 2013; 25:2944).

In collaboration with other groups, we contributed to the identification of a phosphate sensor (Puga *et al.* *Proc Natl Acad Sci USA* 2014; 111:14947) and participated in the production of a collection of transgenic plants that conditionally express more than 600 transcription factors (Coego *et al.* *Plant J* 2014; 77:944). The research lines currently in progress in our laboratory will allow us to understand the mechanisms that underlie arsenic perception, which will open up new possibilities for phyto remediation of arsenic-contaminated soils and waters.



1 Allelic variation at the arsenate reductase (*AtARQ1*) involves structural and regulatory polymorphisms. (a) 3D structural models of the arsenate reductase proteins from *Col-0* (Green) and *Kas-1* (red) predicted by I-TASSER. The catalytic sites are in yellow. (b) Haplotype network analysis using *AtARQ1* proteins from the 1001 Arabidopsis genome project and *Arabidopsis lyrata*. Each line segment corresponds to an *a.a.* substitution. Areas of circles are proportional to frequencies; red and green depict the two major haplogroups.

2 Arsenate [As(V)] delocalises and relocates the As(V)/Phosphate transporter *PHT1;1*. Analysis of *PHT1;1-GFP* localisation after two pulses of 30 μM As(V) (±As(V)) in *PHT1;1-GFP*-expressing Arabidopsis root cells. Duration of each pulse and gaps between them were 1.5 h.



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

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Iglesias J, Trigueros M, Rojas-Triana M, Fernández M, Albar JP, Bustos R, Paz-Ares J, Rubio V. Proteomics identifies ubiquitin-proteasome targets and new roles for chromatin-remodeling in the Arabidopsis response to phosphate starvation. *J Proteom* 2013; 94C:1-22

Castrillo G, Sánchez-Bermejo E, de Lorenzo L, Crevillén P, Fraile-Escanciano A, Te M, Mouriz A, Catarecha P, Sobrino-Plata J, Olsson S, Leo Del Puerto Y, Mateos I, Rojo E, Hernández LE, Jarillo JA, Piñeiro M, Paz-Ares J, Leyva A. WRKY6 transcription factor restricts arsenate uptake and transposon activation in Arabidopsis. *Plant Cell* 2013; 25:2944-2957

Coego A, Brizuela E, Castillejo P, Ruiz S, Koncz C, del Pozo JC, Piñeiro M, Jarillo JA, Paz-Ares J, León J & Transplanta consortium. The TRANSPLANTA Collection of Arabidopsis Lines: A resource for Functional Analysis of Transcription Factors based on their conditional overexpression. *Plant J* 2014; 77:944-953

Sánchez-Bermejo E, Castrillo G, del Llano B, Navarro C, Zarco-Fernández S, Martínez-Herrera DJ, Leo-del Puerto Y, Muñoz R, Cámara C, Paz-Ares J, Alonso-Blanco C, Leyva A. Natural variation in arsenate tolerance identifies an arsenate reductase in *Arabidopsis thaliana*. *Nat Commun* 2014; 7:4617

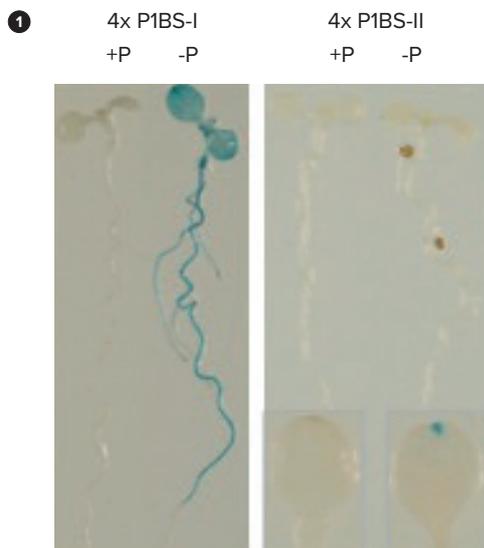
Puga MI, Mateos I, Charukesi R, Wang Z, Franco-Zorrilla JM, de Lorenzo L, Irigoyen ML, Masiero S, Bustos R, Rodríguez J, Leyva A, Rubio V, Sommer H, Paz-Ares J. SPX1 is a phosphate-dependent inhibitor of phosphate starvation response 1 in Arabidopsis. *Proc Natl Acad Sci USA* 2014; 111:14947-14952

Our research is focussed on the phosphate (Pi) starvation rescue system of plants, a model system for studies of gene activity, with presumed biological potential in the context of low-input agriculture. In previous years, we identified transcription factor PHR1 as a master regulator of phosphate starvation responses in plants. In the last two years, we carried out functional analysis of the two PHR1 binding sites, previously detected in a ChIP-seq assay with PHR1. We also identified a component of the Pi sensing system, SPX1, and revealed its mode of action. Finally, we began the analysis of natural variation of transcriptomic responses to Pi starvation.

To study the function of P1BSI and P1BSII, the two binding sites used by PHR1 to regulate transcription of Pi starvation-responsive genes, we prepared artificial promoters consisting of four tandem copies of P1BSI or P1BSII upstream of the -45 minimal promoter from the 35S gene of CaMV. These promoters were fused to the GUS coding region. We found that the two binding sites, which are recognised by PHR1 in dimeric and monomeric forms, respectively, are *bona fide* Pi starvation-responsive elements, with P1BSI promoting the strongest response, in line with higher affinity binding by PHR1 (Figure 1).

We also identified SPX1, whose gene is highly Pi starvation-responsive, interacting with PHR1 as a component of the Pi sensing system. Indeed, SPX1 interaction with PHR1 is Pi-dependent both *in vivo* and *in vitro*, and causes inhibition of PHR1 binding to DNA. The high SPX1 accumulation during Pi starvation is considered to provide a mechanism for rapid shutdown of Pi starvation responses once Pi is resupplied.

In a study of natural variation of molecular responses to Pi starvation, we examined the Pi starvation responsive transcriptome of four ecotypes in addition to the reference Col ecotype, and found great interecotypic differences in Pi starvation-responsive genes. The CT ecotype showed the largest differences with Col (~1000 genes differentially expressed in CT vs Col in Pi-grown plants). At present we are performing transcriptomic analysis of 100 recombinant inbred lines from a ColxCT cross to identify expression quantitative trait loci corresponding to Pi starvation-responsive genes.



① *P1BSI and P1BSII are bona fide Pi starvation response elements. Transgenic Arabidopsis plants were prepared harbouring constructs consisting of an artificial promoter with four tandem copies of P1BSI or P1BSII motifs upstream of the minimal 35S promoter and fused to the GUS coding region. Plants were grown in +P or -P conditions for 10 days before GUS staining.*



Light signalling and day length control of potato tuber formation

20 / PLANT MOLECULAR GENETICS

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Bernardo S, de Lucas M, Martínez C, Espinosa-Ruiz A, Davière JM, Prat S. Negative regulation of PHYTOCHROME-INTERACTING FACTOR 4 by the kinase BIN2 mediates seedlings cyclic elongation and brassinosteroid and gibberellins cross-talk. *Genes Dev* 2014; 28:1681-1694

de Lucas M, Prat S. PIFs get BRright: PHYTOCHROME INTERACTING FACTORS as integrators of light and hormonal signals. *New Phytol* 2014; 202:1126-1141

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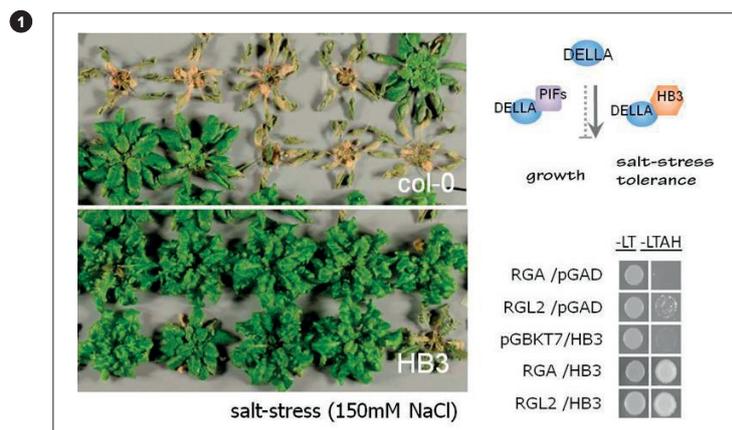
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Our group is interested in understanding the mechanisms by which hormone signalling controls plant growth and development in response to diurnal photocycles, changes in light spectra, or adverse environmental conditions. Our work focusses on gibberellins (GA), a group of hormones that control plant growth by triggering degradation of the DELLA repressors. We showed that DELLAs inhibit plant GA-regulated gene expression via interaction with PHYTOCHROME-INTERACTING FACTOR 4 and 5 (PIF4 and PIF5). DELLA interaction with the PIF bHLH domain prevents PIF binding to the promoters of their gene targets and suppresses growth. DELLAs also mediate increased tolerance to salt stress via a largely unknown pathway. In genetic screens, we identified several transcription factors whose overexpression confers increased tolerance to salt and drought stress, and which bind the DELLAs. We are currently studying

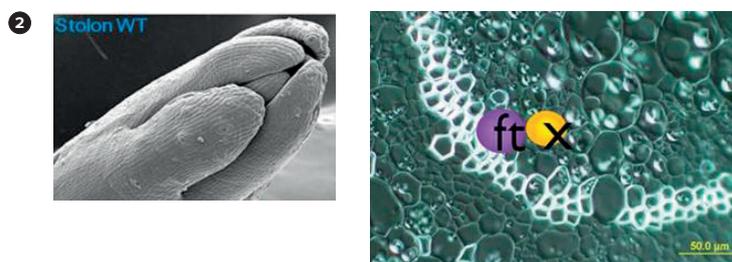
- The regulatory pathways controlled by these regulators
- The molecular mechanisms by which DELLAs modulate activity of these factors
- DELLA allelic mutations that interfere with PIF interaction but do not alter binding to these stress-related factors

Introduction of these allelic mutations into cultivated species will help to generate new cultivars more tolerant to drought and salt stress, and increase crop production in adverse climate conditions.

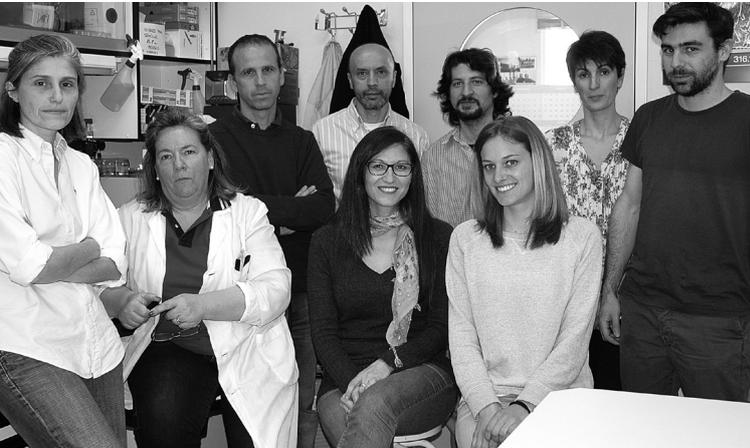
Our second line of research is the control of storage organ formation in the potato. We showed that potato tuberisation is triggered by a member of the potato FLOWERING LOCUS T (FT) gene family, *SP6A*. Temperatures >25°C inhibit storage organ formation by suppressing *SP6A* expression; this inhibitory effect is reversed by *SP6Aox*, which highlights the *SP6A* pathway as one of the primary targets for increased potato productivity. We aim to analyse the signalling events that lead to *SP6A* suppression at warm temperatures and to identify the *SP6A* downstream pathway that controls tuber formation and, by extension, storage organ formation in other bulb-, rhizome- or tuberous root-forming species.



1 The DELLA repressors confer increased tolerance to drought and salt stress. We identified several transcription factors that interact directly with DELLAs and whose overexpression increases plant survival in high salt conditions. DELLA allelic variants that interfere with PIF interaction but do not affect binding to these stress-related factors will enable us to uncouple growth inhibitory effects of these repressors from their stress tolerance function.



2 During stolon-to-tuber transition, cell division and starch accumulation is observed in cells around the vascular cambium. Identification of *SP6A* interactors in these cells will allow us to understand how formation of these important organs is triggered.



Signalling networks in plant development and defence responses

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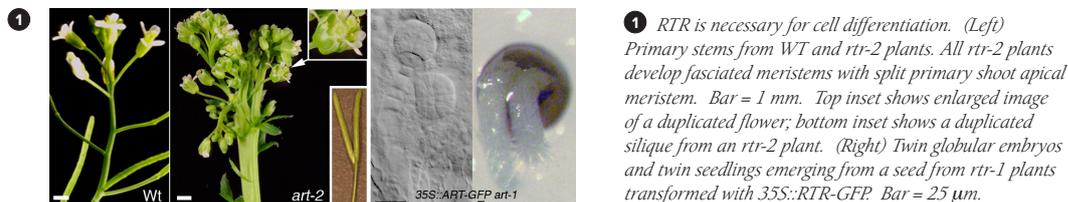
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Plants adjust their development to accommodate cell differentiation and growth to fluctuating environmental conditions. Intricate signalling networks translate environmental cues into reprogramming of gene expression as a major adaptive response to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

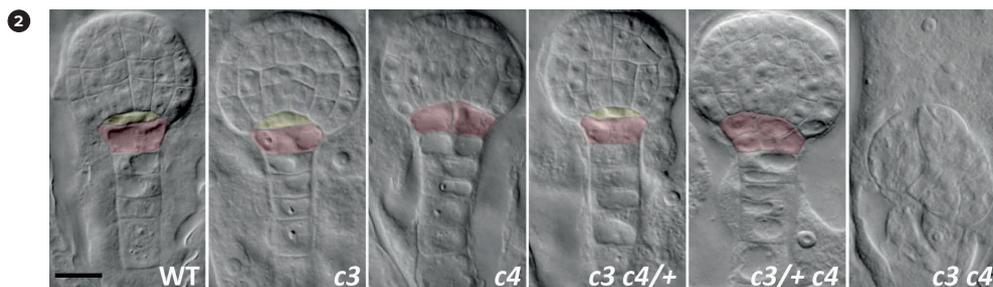
Stem cell differentiation implies large-scale transcriptional modifications. We have identified the RNA polymerase II (Pol II) phosphatase ART and its interacting partner MINIYO (IYO) as essential factors for initiating cell differentiation in *Arabidopsis*. Our results suggest that coupled uptake of ART and IYO into the nucleus switches on this cell fate transition through direct regulation of Pol II gene transcription.

Reversible protein phosphorylation is a common molecular switch in signalling pathways. Protein phosphatases 2A (PP2A) dephosphorylate proteins at serine/threonine residues. PP2A is a heterotrimer consisting of a catalytic (PP2A-C), a scaffold (PP2A-A), and a regulatory (PP2A-B) subunit. The *Arabidopsis* genome codes for five very similar PP2A-C proteins. These are grouped in subfamily 1 (PP2A-C1, -C2, and -C5) and subfamily 2 (PP2A-C3 and -C4). Whilst single *pp2ac-3* and *pp2ac-4* mutant lines do not display any obvious phenotype, *pp2ac-3 pp2ac-4* plantlets are severely misshapen, with severe malformations of cotyledon and root primordia. 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 both affected by mutations in *PP2A-C3* and *PP2A-C4*. Our work shows 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 that entail large changes in transcriptional profiles. A major wound-signalling pathway involves jasmonic acid (JA). Large, transient increases in endogenous JA levels occur after mechanical damage, and high JA levels trigger the transcriptional activation 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 *StAOS1* and *StAOS2* must be cosuppressed simultaneously to lower plant JA levels significantly, resulting in altered cell wall structure and increased susceptibility to pathogens.



1 RTR is necessary for cell differentiation. (Left) Primary stems from WT and *rtr-2* plants. All *rtr-2* plants develop fasciated meristems with split primary shoot apical meristem. Bar = 1 mm. Top inset shows enlarged image of a duplicated flower; bottom inset shows a duplicated silique from an *rtr-2* plant. (Right) Twin globular embryos and twin seedlings emerging from a seed from *rtr-1* plants transformed with 35S::RTR-GFP. Bar = 25 μ m.



2 PP2A-C3 and PP2A-C4 are essential for embryo patterning. Differential interference contrast microscopy of cleared embryos at late globular stage. Genotypes are indicated at the bottom. Bar = 12.5 μ m. Suspensor cells are coloured pink; lens-shaped cells produced after asymmetric division of the hypophysis are in yellow.



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

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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 Integ Plant Biol* 2013; 55:40-53

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Puga MI, Mateos I, Charukesi R, Wang Z, Franco-Zorrilla JM, de Lorenzo M, Irigoyen ML, Masiero S, Bustos R, Rodríguez JF, Leyva A, Rubio V, Sommer H, Paz-Ares J. SPX1 is a phosphate-dependent inhibitor of PHOSPHATE STARVATION RESPONSE1 in Arabidopsis. *Proc Natl Acad Sci USA* 2014; 111:4947-14952

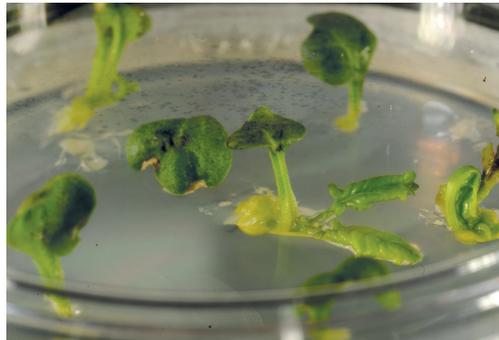
PATENT

PCT/EP2014/061214. DDA1 gene for mitigating negative ABA effects on growth during abiotic stress

Targeted destabilisation of proteins by the ubiquitin proteasome system regulates key developmental and stress responses in plants, including their adaptation to abscisic acid (ABA)-mediated abiotic stresses such as drought, high salinity and low temperatures. 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 climatic change events. Thus, we recently showed that CULLIN4-RING E3 ubiquitin ligases (CRL4) promote ubiquitination and degradation of PYR/PYL/RCAR ABA receptors to modulate ABA signalling in Arabidopsis. For this function, CRL4 require DDA1, a type of substrate adaptor conserved in higher eukaryotes. DDA1 provides substrate specificity for CRL4 by interacting with PYL8, as well as other PYR/PYL/RCAR family members, and facilitates its proteasomal degradation. We found that DDA1 negatively regulates ABA-mediated developmental responses, including inhibition of seed germination, seedling establishment and root growth. DDA1-triggered destabilisation of PYL8 is counteracted by ABA, which protects PYL8 by limiting its polyubiquitination. In sum, our data identify a mechanism for desensitisation of ABA signalling based on the control of ABA receptor stability (Fig. 1).

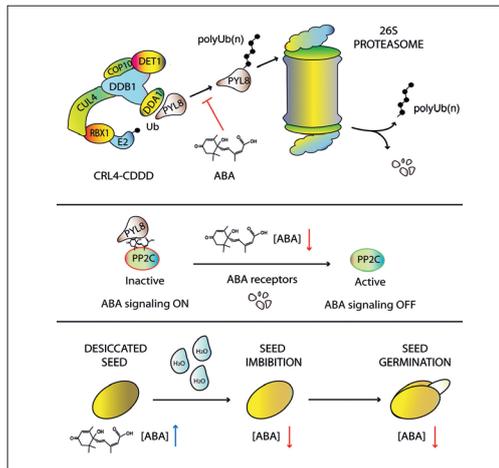
We are currently characterising the protective mechanism by which ABA impedes degradation of its receptors. In addition, we are exploring the role of CRL4-DDA1 complexes in the control of chromatin dynamics, including chromatin remodelling, gene expression and DNA damage repair. For this we use various genetic, molecular and proteomic approaches. Regulatory proteins or their mutated versions of them, identified and characterised through these approaches, should help to develop modified crops with increased resistance to environmental stresses (Fig. 2). This is the case of DDA1, for which a technology to generate plants more tolerant to water stresses has been patented and licensed.

1

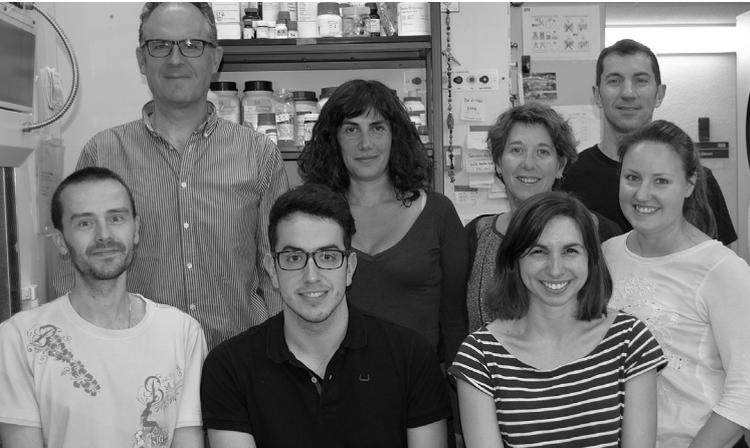


1 *Transgenic lines of colza (Brassica napus) that overexpress DDA1. Tolerance to water stresses (salinity, drought, osmotic stress) will be assayed in DDA1-overexpressing colza lines generated at the CNB's in vitro culture facility. Similar analyses will be performed in rice.*

2



2 *Model for DDA1 role in ABA desensitisation. DDA1 allows recognition and ubiquitination of ABA receptors (e.g., PYL8) by CRL4 Ub ligases. Polyubiquitinated ABA receptors are then degraded at the 26S proteasome. ABA, on the contrary, stabilises the receptors by limiting their polyubiquitination (top). Low ABA levels enable DDA1-mediated destabilisation of ABA receptors, promoting the release and activation of PP2C phosphatases, which act as negative regulators of ABA signalling (centre). In this way, DDA1 contributes to ABA desensitisation when levels of this hormone diminish; that is, when stress conditions disappear or during seed imbibition and germination (bottom).*



The jasmonate signalling pathway in Arabidopsis

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Plants are able to 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 re-programming of the cell. The main focus in my lab is to understand one of the pathways involved in this network, 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 JA pathway with other pathways within the network. Understanding these molecular interactions is essential to decipher how one single hormone can activate so many different physiological responses in the plant, and how the plant is able to discriminate between different stresses and select the correct set of responses to each. This knowledge is basic for the design of biotechnological and agronomic applications.

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

- Identification and characterisation of the bacterial effector HopX1, which enhances plant susceptibility to biotrophic pathogens by activating the JA signalling pathway. HopX1 has a protease activity that degrades the key JA-repressors JAZ (Gimenez-Ibanez *et al.* PLoS Biol 2014)
- Design and characterisation of a potent, specific antagonist of JA perception, COR-MO, with important biotechnological potential (Monte *et al.* Nat Chem Biol 2014)
- Determination of the DNA-binding sequence specificities of over 60 plant transcription factors, through the use of a protein-binding microarray (Franco-Zorrilla *et al.* Proc Natl Acad Sci USA 2014; Boer *et al.* Cell 2014)
- Discovery of the mechanism by which canopy shade reduces JA-dependent defences (Chico *et al.* Plant Cell 2014)
- Identification and characterisation of three bHLH transcription factors that repress JA responses (Fonseca *et al.* PLoS One 2014)
- Discovery of the mechanism by which MYC transcription factors (MYC2, 3 and 4) regulate glucosinolate biosynthesis (Schweizer *et al.* Plant Cell 2013)
- Analysis of protein disorder in the *Arabidopsis thaliana* proteome (Pietrosemoli *et al.* PLoS One, 2013)

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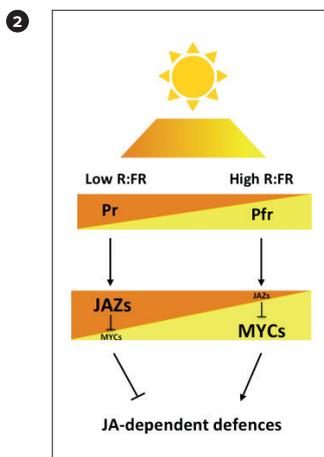
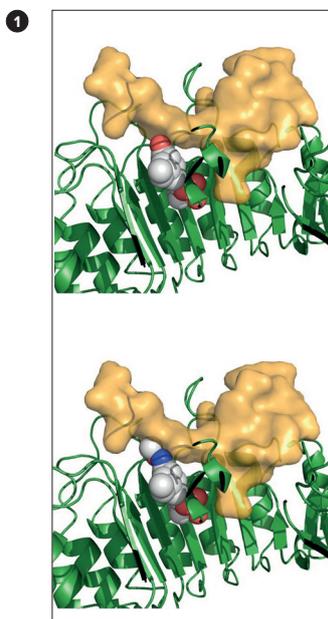
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PATENT

EP 13382362.5. Novel compounds antagonizing JA-Ile perception



2 Model of regulation of JA-mediated defences by light quality through modulation of MYC and JAZ stability. R:FR ratios determine the balance of activation/deactivation (Pr/Pfr) of phytochromes, which differentially regulate the stability of MYC TF and their JAZ repressors, and therefore, the defence output of the plant. Ambient light (high R:FR ratios) shifts the Pr/Pfr equilibrium to the active Pfr form, which enhances JA-dependent defences by mediating MYC stabilisation and allowing JA-mediated degradation of their JAZ repressors. Conversely, in shade conditions (low R:FR ratios), the phytochrome equilibrium is shifted towards the inactive Pr form, thus reducing JA-dependent defences by destabilising MYC and stabilising JAZ. The balance of active phytochromes, which depends on the R:FR ratio, thus regulates the relative amount of MYC and JAZ proteins and therefore define the JA-dependent defence output of the plant.

1 COR-MO is an antagonist of jasmonate perception. (Top) Molecular model of the interaction of COR with the co-receptor COII (green; ribbon representation) and JAZ degnon (yellow; surface representation) based on crystal structure described by Sheard *et al.*, 2010. (Bottom) The same complex with COR-MO instead of COR, showing the steric impediment of JAZ interaction.

