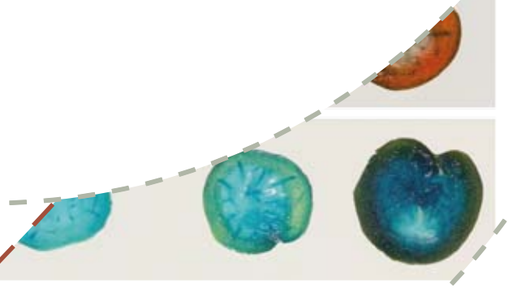
Plant Molecular Genetics

Research conducted by the Plant Molecular Genetics Department aims to uncover the signaling pathways underlying plant's ability to adapt to a variable environment and defensive responses against pathogenic diseases. Studies carried out by the different groups cover important aspects of Plant Biology such as stem cell function, root architecture, shoot branching, responses to day length duration and light quality, innate-immunity and defense responses to pathogens, as well as adaptive responses to nutrient shortage or heavy metals, since all these processes are known to be important determinants of biomass production and crop harvest yields. Biochemical, cell biology, genetic and genomic approaches are used to tackle the regulatory pathways underlying these developmental processes, in addition to the analysis of natural variation in these important responses or the role of targeted protein destabilization in their activation.



Plant Molecular Genetics

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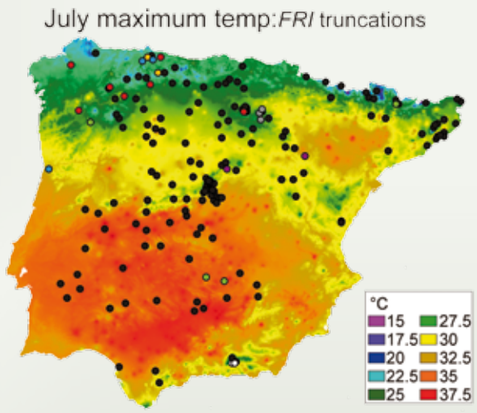
Genetic and Molecular Bases of the Naturally-Occurring Variation of Plant Development

The main objective of our research is to understand the genetic and molecular mechanisms involved in plant adaptation.

We are dissecting the 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 different 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 is currently focused on two specific objectives.

On the one hand, we are carrying out genetic analysis of the naturally-occurring variation for a key quantitative developmental trait such as the timing of flowering. We have developed several populations of recombinant inbred lines (RIL) and introgression lines (IL). We have analysed the relationship between the genetic basis of flowering initiation and the rate of vegetative growth in a new RIL population of 222 lines derived from the cross LerxFei-0 (Figure 1). This population has been used for QTL (quantitative trait locus) mapping analysis, which indicated 10 genomic regions that show two distinct patterns of pleiotropic effects on both traits (Méndez-Vigo et al., 2010).

On the other hand, we have developed a collection of wild genotypes of *A. thaliana* from the Iberian peninsula, which serve as a permanent experimental population on which to carry out genetic and environmental association analyses. As a first step in exploiting this population, we characterized 182 Iberian genotypes for their flowering response to vernalisation and have sequenced several flowering genes. We found new wild allelic series of some flowering genes by association mapping. In addition, analysis of the geographic and climatic distribution of gene polymorphisms is enabling us to identify alleles that might be involved in climatic adaptation.



Geographic and climatic distribution of FRI truncations in the Iberian Peninsula. FRI functional alleles are shown as black dots, while FRI loss-of-function (truncation) alleles are shown in other different colors. The climatic map of the Iberian Peninsula corresponds to maximum July temperature.

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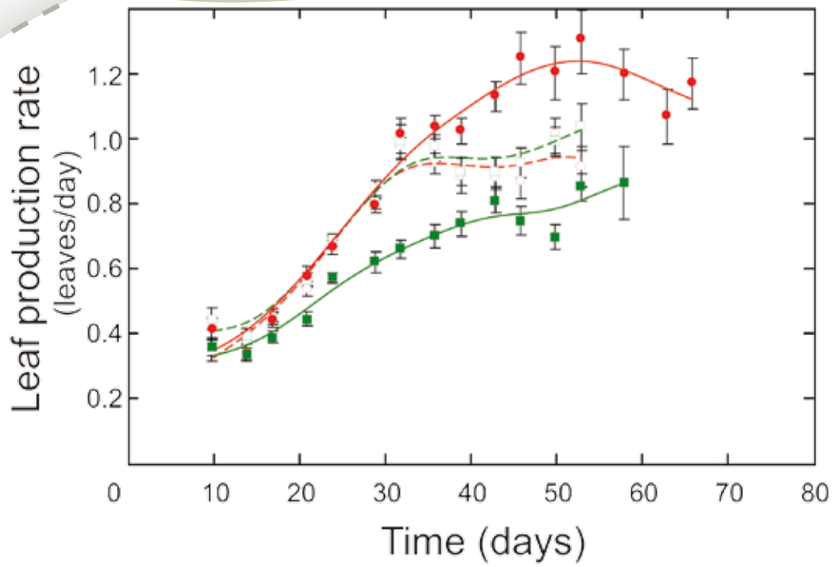

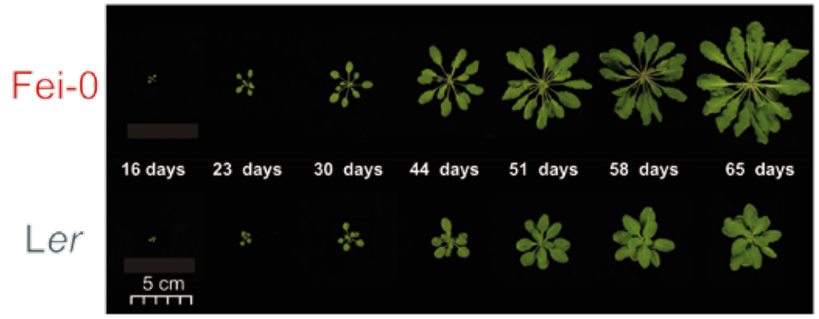

Rate of vegetative growth of Ler, Fei-0 and their reciprocal hybrids.

Plant Molecular Genetics

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Plant Immunity Strategies Against Microbial Pathogen Infection

An ubiquitous response of plants to pathogen attack is the generation of active lipid derivatives, collectively known as oxylipins, whose importance as regulators of plant defence is being established.

Such compounds can be formed by enzymatic peroxidation of fatty acids, catalysed by the activities of 9- and 13-lipoxygenases and α-dioxygenases, or non-enzymatically in the presence of singlet oxygen and free radicals. The importance of the oxylipin pathway initiated by 13-lipoxygenases (13-LOX) and its main product, jasmonic acid (JA), in plant fertility and in controlling resistance to necrotrophic pathogens has been demonstrated. In addition, increasing experimental evidence indicates the participation of oxylipins produced by the 9-LOX and α-DOX pathways, as well as of non-enzymatically generated oxylipins in plant defence. Nevertheless, with the exception of JA, the signalling mechanisms by which distinct oxylipins exert their function remain poorly understood.

We aim to define the role of oxylipins in protecting plants against pathogen infection and to dissect the signalling pathways mediating their actions. During this period we undertook a genetic approach to investigate the signalling processes regulated by 9-HOT, a 9-LOX-derivative that induces production of callose deposits, initiation of oxidative stress and transcriptional changes of defence-related genes. A *lox1 lox5* mutant, which is deficient in 9-LOX activity, and the mutant *noxy22* (*non-responding to oxylipins22*), which is insensitive to 9-HOT, were used for this purpose. Map-based cloning positioned the *noxy22* mutation at the *ETO1* (ETHYLENE-OVERPRODUCER1) locus with constitutive ethylene (ET) production. As predicted from these results, we found that ET acts as a negative regulator of the 9-HOT signalling and, reciprocally, that 9-HOT interferes with the activation of the ethylene (ET) pathway.

We found that the 9-HOT and ET pathways play a critical role in controlling oxidative stress and the response to the lipid peroxidation-inducer singlet oxygen. Thus, the massive transcriptional changes seen in wild type plants in response to singlet oxygen were greatly affected in the mutants examined. Accordingly, *lox1 lox5* and *noxy22* displayed enhanced susceptibility to singlet oxygen (Figure 1). Further studies revealed the participation the 9-LOX and ET in the defence of plants against *Pseudomonas* infection. Results showing enhanced susceptibility of *lox1 lox5* and *noxy22* to *Pseudomonas* attack and altered ROS homeostasis supported the role of the 9-LOX oxylipin pathway in controlling oxidative stress during plant defence against biotrophic bacteria, and the negative role of ET in the defence response against this type of pathogens.

Given the participation of the 9-LOX oxylipin pathway in plant defence and in controlling the response to singlet oxygen ($^1O_2$), we are investigating the role of this reactive molecule and of the $^1O_2$-formed oxylipins in the immune strategies of plants against microbial infection. Singlet oxygen is produced as part of an oxidative burst that takes place after pathogen attack, in which oxygen is converted in distinct reactive oxygen species (ROS), such as the superoxide anion radical (O_2-), the hydrogen peroxide (H_2O_2) and hydroxyl radical (OH*). Polyunsaturated fatty acids are a preferred target of $^1O_2$ attack, and several of its oxidation products (non-enzymatically generated oxylipins) could act as secondary messengers to trigger defence responses. In support of this idea, we found that production of $^1O_2$ (triggered by Rose Bengal) and application of $^1O_2$-formed hydroxy acids (12-HOT, 10-HOT, 10-HOD) induce a strong accumulation of callose, a marker of the plant response to pathogen attack, in leaves and roots of *Arabidopsis* (Figure 2). Moreover, we found that application of 12-HOT provoked a strong transcriptional response in which ~50% of genes are defence-related. Further studies are underway to define the role of singlet oxygen and of $^1O_2$-formed oxylipins in plant defence.

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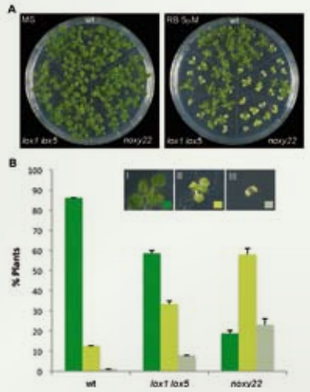
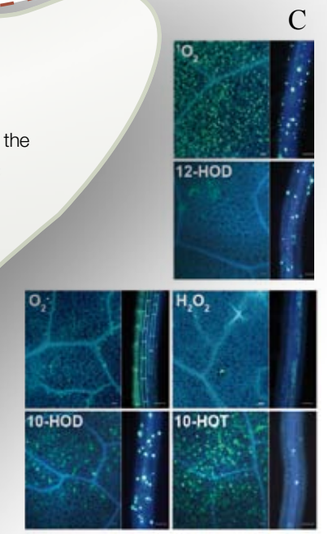


Analyses of phenotypic alterations in wild type, lox1 lox5, and noxy22 plants grown in the presence of Rose Bengal, used as generator of singlet oxygen. A. Phenotype of seedlings grown during 15 days in MS medium (left) or in MS RB-containing medium (5 µM) (right). B. Phenotypic alterations were scored in a three-point scale, designated as I, II and III, according to the severity of the symptoms. Bar graph indicates the percentage of seedlings showing the phenotypes observed after 15 days of growth in RB-containing medium. Sample means and standard errors from three independent experiments are shown.



Production of ROS in plant defense. A. Generation of distinct ROS during oxidative burst. B. Tight regulation of ROS production at different cellular compartments and cell types is critical for plant survival. C. Callose accumulation after production of singlet oxygen ($^1O_2$), superoxide anion radical (O_2-), hydrogen peroxide (H_2O_2) and specific $^1O_2$-derived hydroxy fatty acids: 12-HOD (12-hydroxy-octadecadienoic acid, 25 µM), 10-HOD (10-hydroxy-octadecadienoic acid, 25 µM) and 10- HOT (10-hydroxy-octadecatrienoic acid, 25 µM). Aniline blue staining in Arabidopsis thaliana leaves of 4-week-old plants (square-shaped panels) and in roots of in vitro grown seedlings (rectangular panels). Scale bars = 50 µm.

Plant Molecular Genetics

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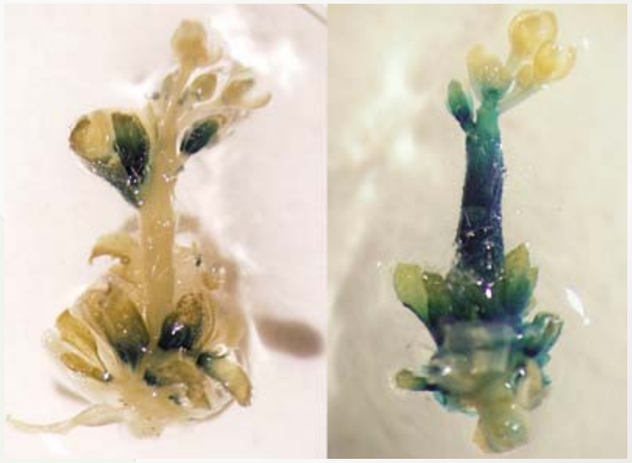
Genetic Analysis of Axillary Meristem Development

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 agronomical 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*. First, we identified and characterised two types of motifs conserved in the *BRC1*-like promoters, which act as transcriptional silencers of *BRC1*. One of these elements confers qualitative (spatial) regulatory information essential for driving *BRC1* expression in axillary buds; the others act as silencers, necessary to maintain low *BRC1* expression levels. We have identified two transcription factors that could bind to one of these elements and might be involved in the negative transcriptional regulation of *BRC1*. Second, we have begun to understand the protein-protein interactions involving *BRC1* and have isolated two factors that could modulate *BRC1* activity. In addition, we identified the *BRC1* protein domains mediating those interactions. Third, we compared the transcriptomic profiles of wild type and brc1 axillary buds and identified genes that could be controlled directly by *BRC1*. We are systematically analysing the genetic control of branch suppression in response to shade in *Arabidopsis* and testing the working hypothesis that during the SAS, *BRC1* is upregulated in response to reduction in the R:FR ratio and its activity is responsible for the branch suppression response.

Solanaceae is a family that includes a large number of species in which the control of branch outgrowth is of great agronomical interest, and for which understanding the function of some of the key players will help optimise plant architecture and yield. Our work shows that in tomato and potato, species with branching patterns divergent from those of *Arabidopsis*, two *BRC1*-like paralogues, *BRC1a* and *BRC1b*, are coexpressed in axillary buds. Reverse genetic analyses confirmed that tomato *SlBRC1b* plays a role in the promotion of axillary bud arrest. In contrast, *SlBRC1a*, 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* has evolved under a strong purifying selection in the clade comprising *S. Lycopersicum*, other closely related wild tomato species and potato, *BRC1a* has evolved at a faster rate under positive selection.



Left: Arabidopsis transgenic plants carrying the promoter of BRC1 fused to b-glucuronidase (GUS) display GUS activity in axillary buds. Right: similar plants carrying a mutated BRC1 promoter display ectopic GUS activity.

Scanning Electron Microscopy image of a young tomato axillary bud.

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PATENT

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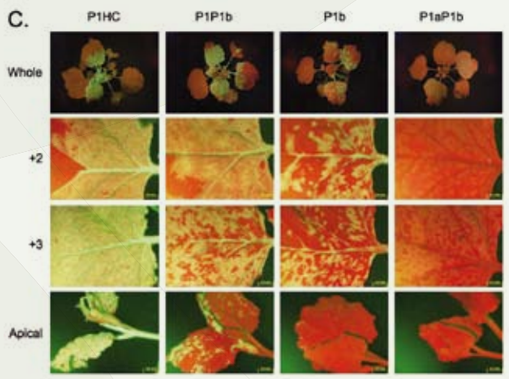
Plant-Pathogen Interaction in Viral Infections

Plant viruses depend largely on host factors to replicate in the cell and to propagate throughout the plant and between individual plants.



Infection of wild type PPV (P1HC) or chimeric PPV viruses in which P1HCpro has been replaced by CVYV P1b alone (P1b) or preceded by PPV P1 (P1P1b) or CVYV P1a (P1aP1b). All viruses are tagged with GFP. A western blot of the infected tissues is shown at the left.

Plants in turn have developed antiviral defence mechanisms that must be counteracted by viral factors. These 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 especially interested in defence responses related to RNA silencing and its viral suppressors.

HCPro is the typical silencing suppressor of potyviruses, but other silencing suppressors of *Potyviridae* family members have recently been discovered. We demonstrated that serine proteinase P1b of the ipomovirus *Cucumber vein yellowing virus* (CVYV) suppresses silencing by sequestering siRNA, and can replace HCPro functionally in PPV infection in a host-specific manner. Our results demonstrate that although potyviruses can exploit different sources of anti-silencing activity, their own silencing suppressors can contribute to defining 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 have an important role in viral long distance movement, and that an unknown resistance mechanism interferes with these interactions in Nicotiana species. We showed that the N-terminal region of PPV CP is *O*-GlcNAcylated and phosphorylated. An *O*-GlcNAcylation-deficient PPV mutant infects *Nicotiana clevelandii* and *P. persica* with no apparent differences compared to wild type PPV. In contrast, PPV accumulation is significantly lower in *Arabidopsis thaliana* Col-0 plants infected with the mutant virus, concurring with a fine-tuning effect of CP *O*-GlcNAcylation on PPV infection that facilitates its adaptation to different hosts and environmental conditions. We are interested in applying the information obtained in our research to design control strategies for sharka disease. We thus used RACE assays of viral RNA fragment accumulation in infected plants as well as deep sequencing of viral siRNA to design 20 PPV-specific amiRNA constructs. Cleavage activity on sensors and a high level of antiviral protection in agroinfiltration systems was shown for some of the amiRNA, which will be transferred to other PPV plant hosts. Finally, another target of interest is 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 infecting cherry trees in nature. Biological features of this cloned isolate are being analysed.

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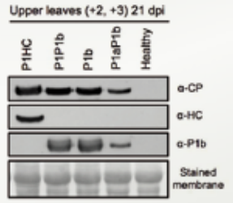

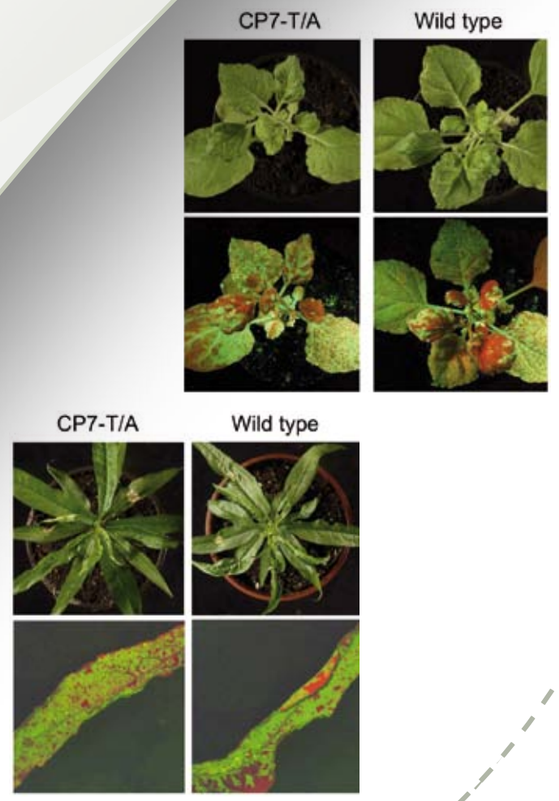

Infection of the O-GlcNacylation-deficient mutant CP7-T/A in Nicotiana benthamiana and Prunus persica. The mutant and the wild type PPV are tagged with GFP.

Plant Molecular Genetics

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Molecular Mechanisms Underlying Root Architecture and Arsenic Phytoremediation

We are developing two research lines in the laboratory.

Our first aim is to study the molecular mechanisms underlying arsenic perception and the second, to study the mechanisms involved in root architecture. For the latter, we performed a mutant screening in *Arabidopsis* to identify mutants altered in the spatial distribution of roots. From this screening, we identified several mutants altered in root architecture, including actin2-4, a new mutant allele of the ACTIN2 gene. This mutant shows an increase in actin dynamics, leading to the enhancement of tropic responses and auxin transcriptional responsiveness, mimicking the auxin/brassinosteroid synergistic response. In the first research line, mechanisms underlying arsenic perception, we made two key observations. Using a transgenic plant that expresses the Pi transporter promoter fused to the luciferase gene, we identified a set of genes inducible by Pi starvation that are repressed by As(V) with extraordinary speed (30 min), whereas repression by Pi takes 36 hours. The use of the transgenic line PHT1;1:LUC opens the possibility to identify mutants altered in the kinetics of repression by As(V), being a new approach for the characterization of the signalling pathway of As(V) and its possible cross-talk with that of Pi. Moreover, in the context of this project we have performed an analysis of the natural variability of tolerance to As(V) in a large collection of *Arabidopsis* ecotypes. In this analysis we identify a QTL that we are currently cloning.

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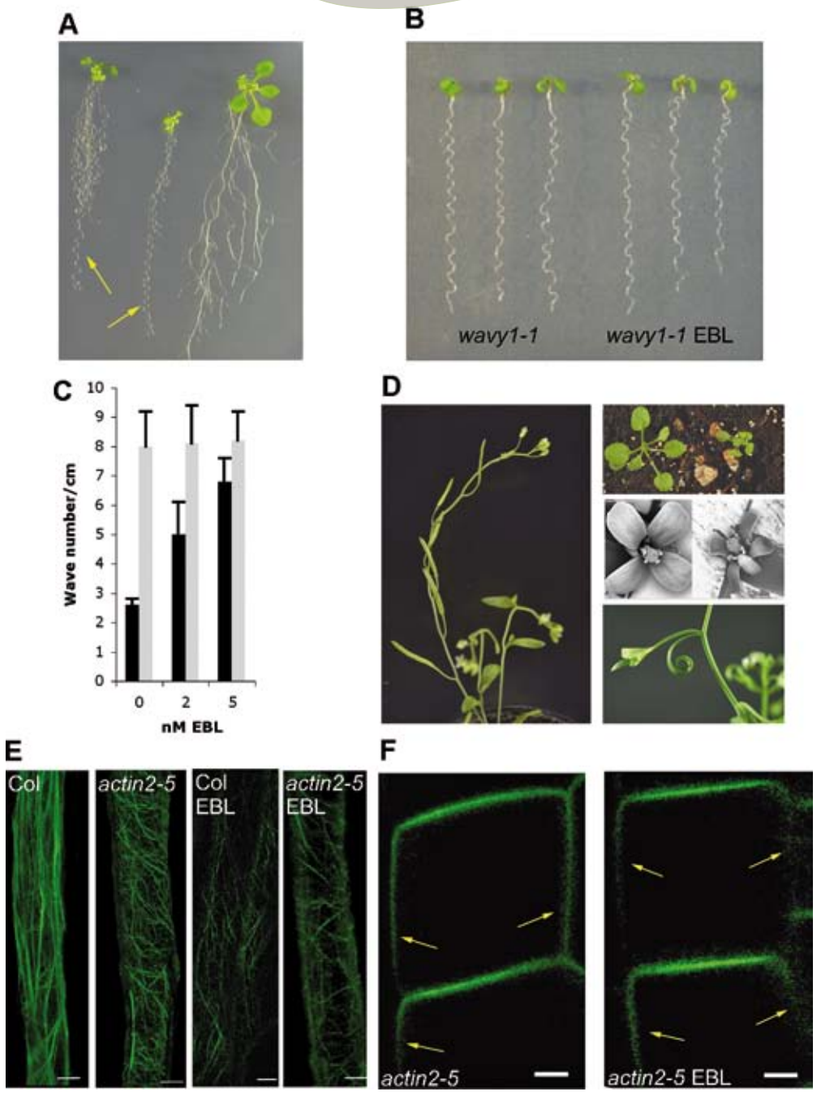

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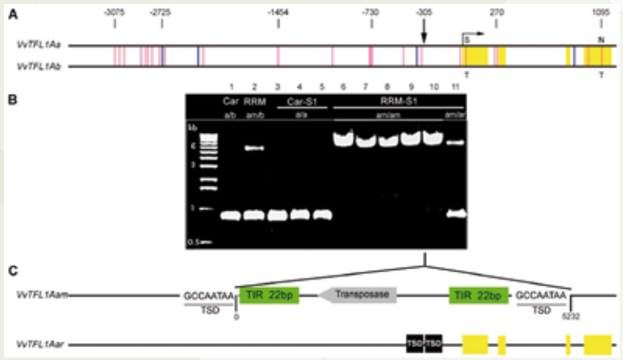
Grapevine Reproductive Biology

Starting in March 2009 most of our group moved to the recently created Instituto de Ciencias de la Vid y del Vino (Grapevine and Wine Research Institute) in Logroño.

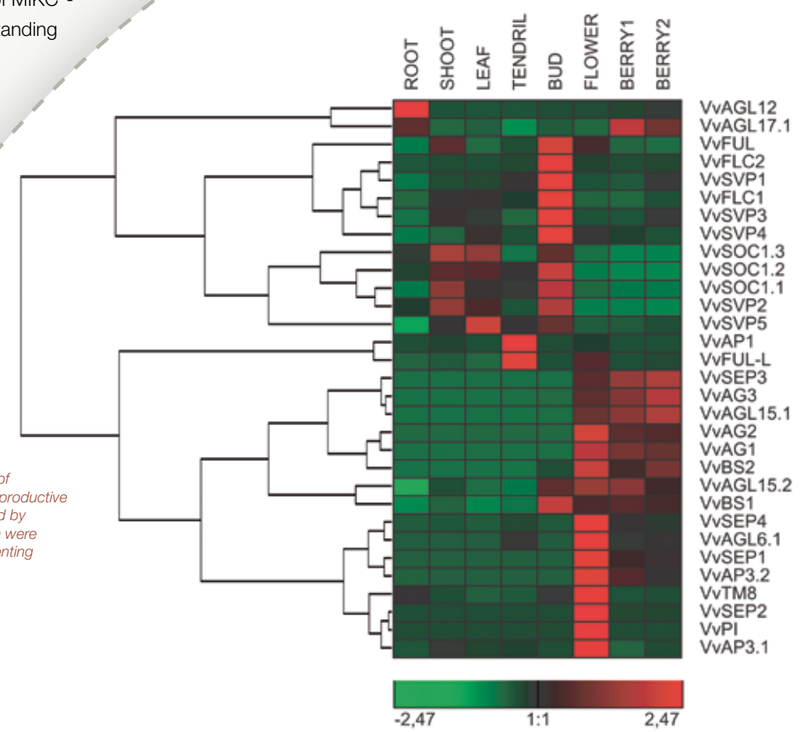


VvTFL1A alleles in cultivar Cariñena and RRM somatic variant. A. Wild type alleles. B. Mutant allele with transposon insertion. C. Revertant allele showing the target site duplication.

This Institute derives from a joint agreement between the Agencia Estatal CSIC, the University of La Rioja and the Government of La Rioja. However, part of the research group is still maintained at the CNB till the running projects reach to their end.

Our research interests focus on the understanding of the genetic and molecular mechanisms regulating reproductive development in grapevine and their contribution to final production and quality of grapes and wines. In the last two years our research activity has been centred on the characterization of grapevine natural genetic variation for reproductive traits and its use to identify some of the underlying loci and genes. With this purpose we are generating core collections of grapevine cultivars, collections of somatic variants and F1 hybrid progenies derived from crosses between different cultivars. The genetic analyses of these materials have allowed us the identification of QTLs for relevant reproductive traits such fertility, cluster structure, berry shape and size or seedlessness. In addition, the molecular analyses of somatic variants such as *Reiterated Reproductive Meristems* (RRM) in cultivar Cariñena has allowed the identification of a putatively active transposable element of the *Hatvine1* family as responsible for over-expression of the *VvTFL1A* in a natural activation tagging event. (Figure 1) Over-expression of *VvTFL1A* seems to be directly related to the RRM phenotype.

In addition we are involved in the genomic analyses of reproductive development in grapevine, performing both a manual annotation of gene families regulating the process as well as its transcriptional analyses. Annotation of the MIKC^{C}-type MADS box gene family in grapevine has shown the existence of at least 38 genes clustered in 13 subfamilies. Expression profiles of MIKC^{C}-type genes in vegetative and reproductive organs as well as during flower, tendril and fruit development show conserved expression domains for specific subfamilies but also reflect characteristic features of grapevine development. Expression analyses in latent buds and during flower development reveal common features previously described in other plant systems as well as possible new roles for members of some subfamilies during flowering transition (Figure 2). The analysis of MIKC^{C}-type genes in grapevine helps understanding the origin of gene diversification within each subfamily providing the basis for functional analyses of these MADS box genes in grapevine development.



Cluster analysis of gene expression profiles of grapevine MIKC^{C} genes in vegetative and reproductive organs. Expression analyses were performed by qRT-PCR, and relative gene expression data were gene-wise normalized. Color scales, representing signal values, are shown at bottom.

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Plant Molecular Genetics

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Control of Plant Responses to Phosphate Starvation

Plant responses to phosphate (Pi) starvation represent an emblematic system for studies on regulation of gene activity.

In plants, these responses involve both biochemical and developmental changes that improve Pi acquisition and recycling, and protect against the stress of Pi starvation. The induction of Pi starvation responses requires a sophisticated regulatory system that integrates information on external and internal plant Pi concentration, and on other nutrient content. Our aim is to contribute to the dissection of this regulatory system, which operates mainly at the transcriptional level but also involves post-transcriptional control.

Regarding transcriptional control, we have further demonstrated the key role of the transcription factor phosphate starvation response regulator1 (PHR1) and its binding site (P1BS) as a master trans-cis integrator module in the control of Pi stress responses. We also initiated large-scale phylogenomic footprinting to identify all cis-motifs involved in Pi starvation responsiveness. To identify regulatory mechanisms that act on PHR1, we carried out a screening for *phr1* suppressors, leading to the isolation of three mutants that partially suppress *phr1* mutant phenotypes. Cloning of one of the corresponding mutant genes revealed it corresponds to ALIX, a gene conserved from yeast to mammals. ALIX regulates ubiquitination and subsequent endocytosis of plasma membrane receptors, essential for signal propagation from these receptors (Fig. 1). Our hypothesis is that ALIX regulates Pi status signalling by direct interaction with a yet unknown Pi sensor.

Concerning post-transcriptional control, we started a program to study the biochemical and physiological functions of the ubiquitin (Ub) regulatory pathway in the control of plant responses to Pi starvation. We identified a small subfamily of E3 Ub ligases (PCE1-4) that control Pi starvation responses, as indicated by characterization of *Arabidopsis pce* null mutants. PCE proteins are nuclear-localized and its abundance is reduced by Pi addition, indicating a potential role as repressors of Pi signalling. Using a yeast two-hybrid approach, we identified a number of potential targets of PCE1 activity, including known transcription factors involved in control of plant growth and development. The biological relevance of these interactions is currently being addressed. As a complementary approach, we are analyzing variation in the abundance of nuclear proteins that depend on Pi supply and Ub-proteasome activity. We have focused on the nuclear proteome, since nuclear proteins probably have regulatory activities. Using 2-D fluorescence difference gel electrophoresis (DiGE) techniques (available at the CNB Proteomics Facility), we have identified four proteins with a potential regulatory role in Pi signalling whose characterization is ongoing (Fig. 2).

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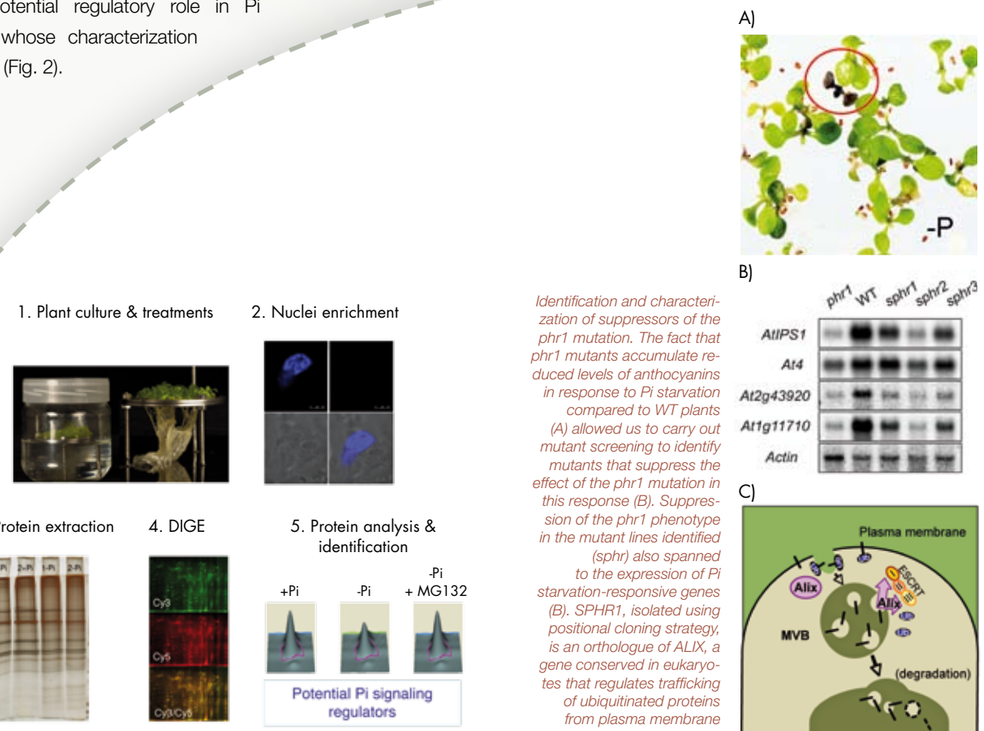

Analysis of changes in the nuclear proteome of Arabidopsis in response to variations in Pi supply. Study of the effect of ubiquitination and proteasomal degradation. To identify potential substrates of the Ub-proteasome pathway with a regulatory role in Pi signalling, we are analyzing variation in the abundance of nuclear proteins that depend on Pi supply. We have established both nucleus isolation and nuclear protein extraction protocols for Arabidopsis plants grown in hydroponic media under different Pi conditions (steps 1-3). Nuclear proteome separation, visualisation and protein abundance quantification are carried out by DiGE techniques (step 4). By adding proteasome inhibitors (e.g., MG132) in the culture media at specific time points?, we can determine the effect of ubiquitination and proteasomal degradation in the abundance of Pi-responsive proteins. The nature of each protein identified, using mass spectrometry techniques, provides hints about their potential regulatory function in Pi signalling (step 5). Functional characterization of the most interesting proteins will allow us to test their involvement in Pi homeostasis.

Identification and characterization of suppressors of the phr1 mutation. The fact that phr1 mutants accumulate reduced levels of anthocyanins in response to Pi starvation compared to WT plants (A) allowed us to carry out mutant screening to identify mutants that suppress the effect of the phr1 mutation in this response (B). Suppression of the phr1 phenotype in the mutant lines identified (sphr) also spanned to the expression of Pi starvation-responsive genes (B). SPHR1, isolated using positional cloning strategy, is an orthologue of ALIX, a gene conserved in eukaryotes that regulates trafficking of ubiquitinated proteins from plasma membrane to lysosomes (vacuoles in plants) through endosomes and multivesicular bodies (MVB). ALIX interacts with members of the ESCRT complexes, triggering degradation of specific plasma membrane proteins, which helps to control their abundance and function (C). In accordance, sphr1 mutants display defects in endocytic trafficking of high affinity Pi transporters fused to the green fluorescent protein (GFP) compared to wild-type (WT) plants (D).

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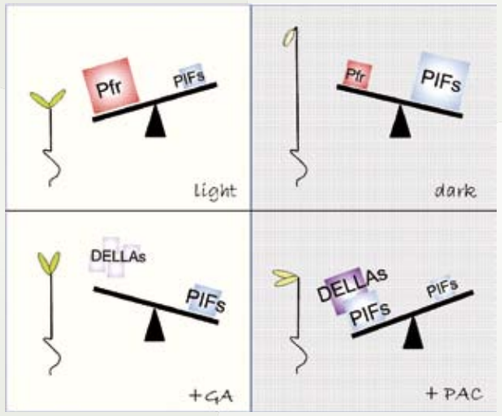
Hormonal Cross-Talk in Light Signalling and Day Length Control of Potato Tuber Formation

Light is a crucial environmental signal to plants since it provides energy for photosynthesis and in addition serves as an informational cue of the ambient in which the plant grows.

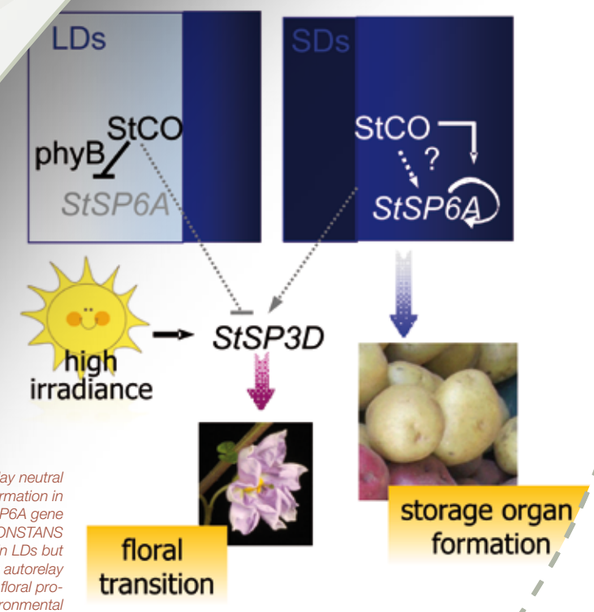
Important light-regulated responses are the de-etiolation of seedlings after seed germination and the response to shade in adult plants, aimed to cope with the competition imposed by other plants. These two developmental responses which are triggered by light/darkness or by changes in the R/FR light ratio are to a large extent regulated by the transcription factor PIF4 (PHYTOCHROME INTERACTING FACTOR 4), which is destabilized by the PHYB photoreceptor in the light. Work in our group aims to identify which signaling cascades govern etiolated seedling development in the dark and how these cascades are affected by light, with a particular focus in the mechanisms involved in integration of the light signal with the own running developmental programs of the plant. The plant hormones gibberellins (GA) and brassinosteroids (BRs) play a central role in transducing the light signal as judged from the dark de-etiolated phenotype of mutants with a block in the synthesis or response to these hormones. As for GA mutants, this phenotype is caused by the stabilization of the DELLAs, a family of nuclear proteins that repress GA-regulated gene expression and are rapidly destabilized in the presence of GAs. Evidence provided by our group showed that DELLAs bind the bHLH DNA recognition domain of the PIF factors and block DNA binding ability of these transcriptional regulators. PIF4 and its close homologue PIF5 regulate the expression of several genes driving cell elongation, by recognizing a G-box element in their promoters, the growth restraint imposed by DELLAs hence being mediated through a block in PIF4/PIF5-mediated activation of these genes.



PIF4/PIF5 integration of light and GA-signals. PIFs accumulate in darkness and promote hypocotyl elongation. In the light, the photoreceptor phyB marks these factors for degradation and represses hypocotyl growth. In the absence of GAs (+PAC), DELLAs bind to PIFs and block their DNA binding ability. GAs destabilize these repressors and lead to elongation of the hypocotyl.

Regarding BR-deficient/-response mutants an important finding was the observation that these seedlings are insensitive to GAs. This impaired response, however, does not correlate with an increased stability of DELLAs, suggesting that a regulatory step downstream of these repressors mediates GA-/BR- cross-talk. BRs were actually found to stabilize the PIF4/PIF5 factors in the light, these results pointing to a master regulatory role of these transcriptional regulators in the control of genes with a role in cell elongation, these TFs integrating not only light- (by PHYB-mediated destabilization) and GA- signals (by inactive complex formation with DELLAs) but playing a role also in BR signaling, thus serving as a molecular link between the signaling cascades controlling plant growth and elongation and the exterior.

A second important line of research in our group is day length control of storage organ formation in potato. It is well established that day length duration is perceived in the leaves, a tuberization signal known as tuberigen being synthesized in these organs and transported to the underground organs of the plant. Our work has demonstrated that this mobile signal is encoded by an FT-like gene (StSP6A), an additional member of the FT-like gene family (StSP3D/SFT) controlling floral transition independent of day length.



Two different FT paralogs control day neutral flowering and SD-dependent tuber formation in potato andigena species. The potato StSP6A gene encodes the mobile tuberigen signal. CONSTANS represses expression of this gene in LDs but promotes its activation in SDs, through an autorelay mechanism. The StSP3D plays a role in floral promotion and is induced in response to environmental cues other than day length.

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Plant Molecular Genetics

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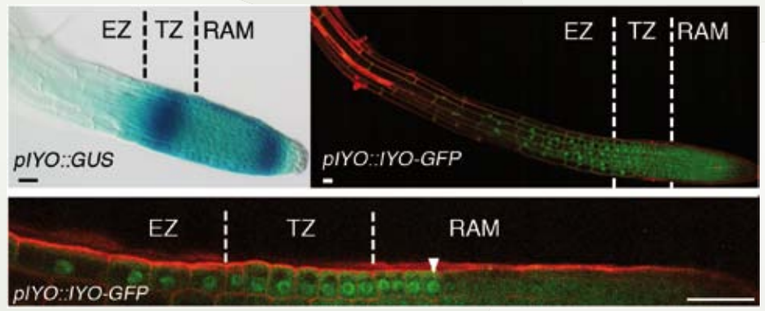
Intracellular Trafficking in Plants

Recent work in our group has been focused in two separate topics.

First, we have continued the genetic dissection of storage protein trafficking to the vacuole in plants, through isolation of the *mtv* mutants impaired in this transport process. The first *MTV* gene isolated was isolated was VTI12, a SNARE protein that forms a complex at the TGN required for transport of storage proteins, but not of other vacuolar cargo (Sanmartín et al., 2007). Other components of this pathway subsequently identified were an SM protein that positively regulates the VTI12 SNARE complex (Zouhar et al., 2009) and the sorting receptors for storage proteins (Zouhar et al., 20010). We are currently characterising other MTV proteins, such as MTV8, which contains a lipid-binding ENTH domain and is involved both in trafficking of storage proteins and in regulating plant senescence (Fig. 1). Several of the genes isolated are co-expressed and are activated during the massive deposition of storage proteins that occurs in maturing-seed embryos, suggesting that they are induced by a common mechanism to achieve full transport capacity in those cells. We have initiated a project to identify the cis regulatory elements and trans-acting factors that activate their transcription during seed maturation. The goal is to develop tools to activate in vegetative tissues the expression en *bloc* of this (limiting) transport machinery and determine whether storage capacity is altered.



Premature senescence in an mtv8 mutant.

A second topic in the lab was initiated through isolation of the *miniyo-1 (iyo-1)* mutant, which has delayed organogenesis in the shoot apical meristem. Functional characterisation of *MINIYO* revealed that it is a necessary and sufficient factor for initiating all events of differentiation in *Arabidopsis*. Moreover, our results suggest that the targeted nuclear accumulation of IYO in transition cells (Fig. 2) functions as a transcriptional switch for this fate transition. We are now studying how endogenous and environmental signals regulate IYO expression and subcellular localization to control the onset of cell differentiation and, consequently, regulate plant growth and development.

IYO is expressed in meristems and the protein is transferred to the nucleus in transition cells, initiating differentiation. RAM: root apical meristem; TZ: transition zone; EZ: elongation zone.

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Plant Molecular Genetics

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Phenotypes of wild type, single and enhanced mutant plants. (a) Genotypes are indicated for adult plants (top) and their corresponding rosette stage (bottom). (b) Wild type (left) and pp2ac-3 pp2ac-4 double mutant (right) plants. Plants were grown on MS plates with 1% sucrose for 15 days. Detailed of pp2ac-3 pp2ac-4 double mutant plantlets. Scale bar = 100 µm.

Wound Signalling in Plants

Our research focuses primarily on wound signalling in plants, with *Arabidopsis thaliana*, tomato and potato as model systems.

Upon wounding, defence-related gene expression is induced at both damaged and distal tissues well apart from the wound site. The plant hormone jasmonic acid (JA) plays a pivotal role in the complex pathway that triggers activation of wound-responsive genes.

Our laboratory studies the role of JA and related compounds (oxylipins) in plant defence responses. We determined the localisation of various biosynthetic enzymes and studied possible interactions among them that could regulate oxylipin synthesis (Farmaki et al., 2007). Using transgenic overexpression and co-suppression approaches, we modified JA and oxylipin biosynthesis in transgenic potato and tomato plants, and studied the consequences on defence gene activation. Allene oxide synthase, which catalyses the first committed step in JA synthesis, is an important target of our research to elucidate a role for the isoforms found in potato and tomato. Overexpression of w-3 fatty acid desaturases in tomato results in production of higher levels of 3-hexenal, a potent antimicrobial oxylipin and a major component of the aroma of tomato fruit (Dominguez et al., 2010).

Reversible protein phosphorylation is a common molecular mechanism in the regulation of signal transduction pathways. We established that a regulatory phosphorylation switch controls the spatial and temporal pattern of wound-induced gene expression. In this switch, dephosphorylation of target protein(s) is essential for JA-dependent gene activation. Pharmacological studies indicate that PP2A and/or PP4 are the most likely candidates for dephosphorylating target effector proteins.

We have undertaken a reverse genetic approach to identify loss-of-function mutants in PP2A catalytic subunits (PP2Ac). These mutants will help to elucidate the role of specific PP2A in the regulation of signal transduction networks in *Arabidopsis*. The *Arabidopsis* genome encodes five *PP2Ac* genes. Homozygous knockout mutant lines have been generated with T-DNA insertions in all *PP2Ac* genes. T-DNA-mediated disruption of the *PP2Ac-2* gene leads to ABA hypersensitivity. PP2Ac-2 thus appears to be a specific negative regulator of ABA signal transduction in Arabidopsis (Pernas et al., 2007). However, all other single *PP2Ac* mutants show no obvious phenotypes, suggesting that the encoded proteins might play largely redundant roles, consistent with their high degree of similarity.

Double mutants have also been generated for each gene pair. Thorough characterisation of these mutants is revealing PP2A involvement in the regulation of developmental pathways and in responses to environmental stress. Identification and characterisation of target proteins that are specifically dephosphorylated by PP2A in these signalling pathways is one main goal in our future work.

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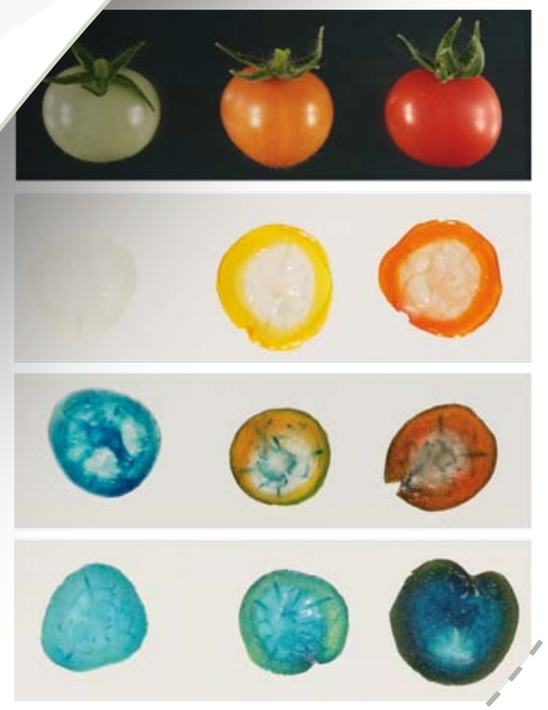

Histochemical localisation of GUS activity in 13-HPL promoter lines. *Tomato fruits at three developmental stages (mature green, orange and ripe red) were cut transversely and stained for GUS activity. WT: fruits from non-transformed MicroTom plants; Pro35S: fruits from the constitutive CaMV 35S promoter; PromHPL:GUS: fruit from a 2-kb HPL promoter transgenic line.*

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The Jasmonate Signalling Pathway in *Arabidopsis*

We are interested in understanding how 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 reprogramming of the cell. The main focus of my lab is to understand one of the pathways involved in this network, the jasmonate (JA) signalling pathway, in *Arabidopsis thaliana*. Jasmonates are fatty acid-derived signalling molecules essential for plant survival in nature, since they are important activators of stress responses and developmental programs. We aim to identify the components of this pathway and determine how they explain jasmonate pathway interactions with other pathways in the network. We are also particularly interested in the molecular mechanisms underlying JA-mediated activation of plant responses to necrotrophic pathogens. This type of defence requires the concerted cooperation of at least two phytohormonal signalling pathways: JA and ethylene (ET). Since the plant uses these two hormones to signal many other developmental and stress responses, ET/JA crosstalk constitutes a unique (simple) system to study the regulation of signalling networks that allow the plant to discriminate between different stresses (e.g., pathogens and wounding) and select the correct set of responses to each.

To understand these biological questions, we are using genomic, genetic, biochemical and molecular tools, following two approaches:

1. Dissection of the JA signalling pathway in *Arabidopsis*. We have discovered several components of this pathway:

- The transcription factors (TFs) ERF1 and AtMYC2/JIN1, which regulate expression of two subsets of JA/ET-related effector genes (Lorenzo et al., 2003; Lorenzo et al., 2004). The balance of activation of these two TF helps determine the type of response activated by the plant to a specific stress (pathogens or wounding; Lorenzo and Solano, 2005).

- SGT1b/JAI4, a regulator of SCF (Skip-Cullin-Fbox) E3 ubiquitin ligase complexes, including the JA-signalling component SCF^{COI1} (Lorenzo and Solano, 2005; Feys et al., 1994; Xie et al., 1998; Xu et al., 2002; Devoto et al., 2002; Feng et al., 2003).

- The JAZ family of nuclear repressors that regulate TF activity (i.e., AtMYC2) and are targeted by SCF^{COI1} for degradation by the 26S proteasome (Chini et al., 2007). Discovery of the JAZ family of repressors linked the previous steps in the pathway (SCF^{COI1} and the TFs) and facilitated an integrated view of the core JA signalling module composed by SCF^{COI1}-JAZs-MYC2. It also evidenced the similarity between the JA and auxin pathways. Based on the crystal structure of the auxin receptor (F-box TIR1; closely related to COI1), we hypothesised that COI1 might also be the JA receptor. A combination of genetic and biochemical analyses supported this hypothesis (Fonseca et al., 2009). Using a screen for bioactive jasmonates, we found that the majoritary form of the hormone [(-)-JA-L-Ile] was inactive as a ligand of COI1, and discovered the real endogenous bioactive form of the hormone, (+)-7-iso-JA-L-Ile.

- NINJA, novel interactor of JAZ, is an adaptor protein that connects JAZ repressors to the general corepressor TOPLESS (Pauwels et al., 2010).

2. Dissection of the interaction between the oomycete *Pythium irregulare* and the plant *Arabidopsis thaliana*. We characterised the infection process and the hormone pathways involved in plant defence. We identified the genes responsible for plant resistance to this oomycete, and analysed the contribution of each of the "classical" defence hormones (JA, ET and SA) to the activation of this defence gene set. Finally, we found that abscissic acid (ABA) is essential for the activation of defences against *P. irregulare* and found that it precedes JA biosynthesis in response to the oomycete (Adie et al., 2007).

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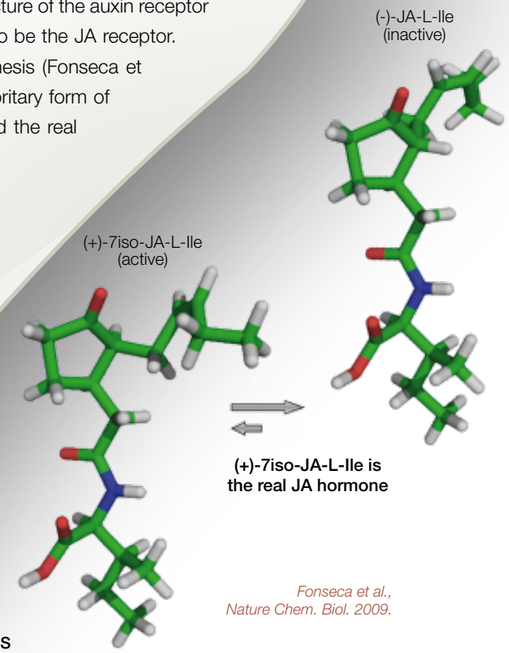

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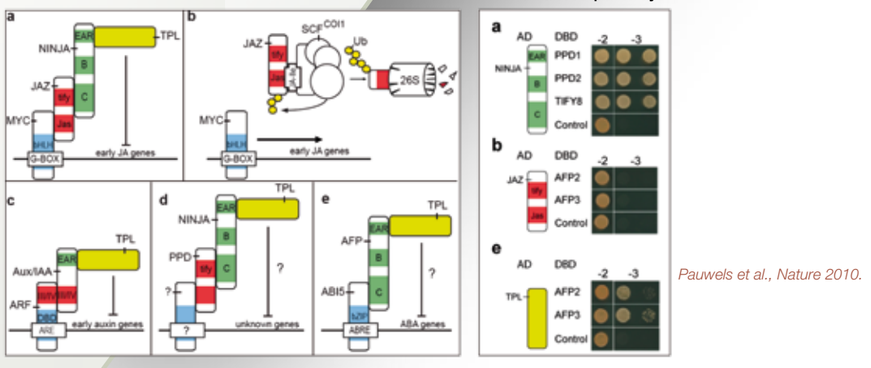
NINJA recruits TOPLESS to various pathways



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