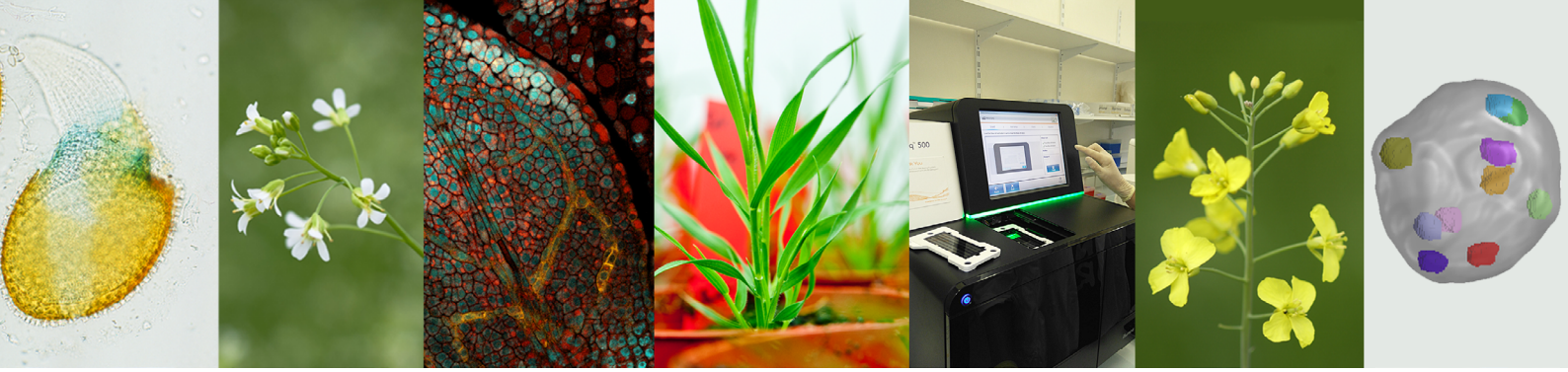

SACLAY PLANT SCIENCES

International SPS Conference 2018: Plant Sciences for the Future

ABSTRACT BOOK

July 4-6, 2018

Centrale-Supélec - Eiffel Building

https://symposium.inra.fr/sps-conference-2018/

SPS
SACLAY PLANT SCIENCES

Saclay Plant Sciences

French Graduate School of Research: research, teaching, training and innovation in plant sciences

Understanding the genetic, molecular and cellular mechanisms controlling the physiology and development of plants, as well as their interactions with fluctuating biotic and abiotic environments

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ACKNOWLEDGEMENTS

We would like to thank everyone involved in the organization of the International SPS Conference 2018: the members of the organizing and scientific committees, the session chairpersons, as well as the PhD students for their help. Our thanks also go to the conference participants, from speakers to poster presenters, for discussing their latest findings.

We are extremely grateful to the colleagues from the scientific committee. By proposing the invited speakers and selecting talks from the submitted abstracts, they greatly shaped the scientific program of this conference.

Finally, we gratefully acknowledge the contribution of our institutions and our other partners, including sponsors and exhibitors. Without their generous support, this event would not have been possible.

We hope that you will enjoy this conference and that it will fulfill its main objectives, providing the opportunity for plant scientists to gather and share science breakthroughs as well as new techniques or technologies, promoting interactions between young and more established scientists and highlighting the dynamism of the Paris-Saclay Plant Sciences community.

Loïc Lepiniec, chair of the conference and head of the SPS network
Marie-Jeanne Sellier, executive organizer of the conference and manager of the SPS network

CONTENT

PLANNING AT A GLANCE

	Wednesday July 4	Thursday July 5	Friday July 6
9 AM		Registration	Registration
10 AM	Poster setup and Registration	Session 1: NUTRITION, METABOLISM AND ENVIRONMENTAL INTERACTIONS	Session 2: DEVELOPMENTAL AND CELL BIOLOGY Session 3: BIOTECH AND PLANT BREEDING
11 AM		Coffee break	Coffee break
12 PM		Session 1: NUTRITION, METABOLISM AND ENVIRONMENTAL INTERACTIONS	Session 3: BIOTECH AND PLANT BREEDING
1 PM		Lunch	Lunch Poster removal
2 PM	Introduction Keynote lecture	Session 2: DEVELOPMENTAL AND CELL BIOLOGY	Session 3: BIOTECH AND PLANT BREEDING
3 PM	Session 1: NUTRITION, METABOLISM AND ENVIRONMENTAL INTERACTIONS		
4 PM	Coffee break	Coffee break	
5 PM	Session 1: NUTRITION, METABOLISM AND ENVIRONMENTAL INTERACTIONS	Session 2: DEVELOPMENTAL AND CELL BIOLOGY	
6 PM	Flash talks		
7 PM	Welcome cocktail Poster session	Poster session	6:45 PM - 11 PM Gala Dinner
8 PM			
9 PM			
10 PM			

For exact times, refer to the detailed schedule

PROGRAM

WEDNESDAY JULY 4

10:00 AM **Registration and Poster setup**

1:30 PM **Introduction talk**

KEYNOTE LECTURE

2:00 PM **David Baulcombe** (University of Cambridge)
Non coding RNA, epigenetics and non Mendelian inheritance in plants

SESSION 1: NUTRITION, METABOLISM AND ENVIRONMENTAL INTERACTIONS

Focus - Secondary metabolites

3:00 PM **Anne Osbourn** (John Innes Centre)
Finding drugs in the garden: Mining plant chemical diversity

3:35 PM **Adnane Boualem** (Institut de Sciences des Plantes Paris-Saclay)
Perfume plant innovation : how translational research helps to breed for more sent molecule

4:10 PM **Coffee break**

Talks selected on abstracts

4:40 PM **Dominique Arnaud** (University of Exeter)
The roGFP2-Orp1 biosensor reveals hydrogen peroxide signatures in plant cells during the immune response

5:00 PM **Florian Frugier** (Institut de Sciences des Plantes Paris-Saclay)
Local and systemic pathways regulating symbiotic nodulation in the Medicago truncatula legume

5:20 PM **Flash talks**

6:10 PM – **9:00 PM** **Welcome cocktail - Poster session**

THURSDAY JULY 5

8:30 AM **Registration and Poster setup**

SESSION 1: NUTRITION, METABOLISM AND ENVIRONMENTAL INTERACTIONS

Focus - Beneficial interactions

9:00 AM **Paola Bonfante** (Università di Torino)
At the root of the plant microbiota: the role of Arbuscular Mycorrhizal Fungi as biofertilizers and bioprotectors

9:35 AM **Bertrand Hirel** (Institut Jean-Pierre Bourgin)
Understanding and exploiting the beneficial impact of bacterial and fungal microorganisms for sustainable maize production

Focus - Photosynthesis and climate change

10:10 AM **Stephen L. Long** (University of Illinois)
Photosynthesis - The Final Frontier in Acheiving Increased Crop Yield Potential under Climatic Change

10:45 AM **Coffee break**

11:15 AM **Michael Hodges** (Institut de Sciences des Plantes Paris-Saclay)
Making photosynthesis great again

Talks selected on abstracts

11:50 AM **Sabine Zimmermann** (Biochimie & Physiologie Moléculaire des Plantes)
Unravelling nutrient exchange in ectomycorrhizal symbiosis contributing to plant potassium nutrition

12:10 PM **Mathilde Fagard** (Institut Jean-Pierre Bourgin)
Impact of nitrogen limitation on the Botrytis-Arabidopsis interaction

12:30 PM **Lunch**

SESSION 2: DEVELOPMENTAL AND CELL BIOLOGY

Focus - Biosensors

2:00 PM **Yvon Jaillais** (Ecole Normale Supérieure de Lyon)
A combinatorial lipid code shapes the electrostatic landscape of plant endomembranes

2:35 PM **Alexis de Angeli** (Institut de Biologie Intégrative de la Cellule)
Visualizing the in vivo activity of anion channels and transporters in guard cells with dynamic measurements of cytosolic pH and [$NO3^-$]

Talks selected on abstracts

3:10 PM **Ross Sozzani** (North Carolina State University)
Gene Regulatory Networks Controlling Root Stem Cells

3:30 PM **Faïçal Selka** (Institut Jean-Pierre Bourgin)
Towards a spatio-temporal atlas of 3D cellular parameters during leaf morphogenesis

3:50 PM **Coffee break**

4:20 PM **Guido Grossmann** (Heidelberg University)
Cellular growth regulation in roots - how to adapt in a complex environment

4:40 PM **Nicolas Arnaud** (Institut Jean-Pierre Bourgin)
A molecular framework for growth repression at boundary domains

Focus - Cytoplasm-nucleus interaction

5:00 PM **Dario Leister** (Ludwig-Maximilians-Universität München)
Chloroplast-nucleus communication: the special case of GUN signalling

5:00 PM **Françoise Budar** (Institut Jean-Pierre Bourgin)
Cytoplasmic natural variation and cytonuclear coadaptation: a hidden resource for plant breeding?

6:10 PM – **9:00 PM** **Poster session**

FRIDAY JULY 6

SESSION 2: DEVELOPMENTAL AND CELL BIOLOGY

Focus - Modeling

9:00 AM **George Bassel** (University of Birmingham)
Quantitative analysis of plant organ development

9:35 AM **Marie-Laure Martin-Magniette** (Institut de Sciences des Plantes Paris-Saclay)
A meta-analysis of transcriptomic data identifies a global response to stresses

SESSION 3: BIOTECH AND PLANT BREEDING

Focus - New breeding approaches

10:10 AM **Holger Puchta** (Karlsruhe Institut für Technologie)
Genome Engineering in Plants: Past, Present, Future

10:45 AM **Coffee break**

11:15 AM **Laurence Moreau** (Génétique Quantitative et Evolution - Le Moulon)
From Marker-Assisted Selection to Genomic Predictions: opportunities raised by new breeding approaches

11:50 AM **Lunch - Poster removal**

Talks selected on abstracts

1:30 PM **Pierre Hilson** (Institut Jean-Pierre Bourgin)
Mitochondrial respiratory chain dysfunction promotes in vitro morphogenesis in plant tissues

1:50 PM **Timothy Horn** (North Carolina State University)
Spatial control of plant cell growth and tissue differentiation with 3D-printing

Focus - Synthetic biology

2:10 PM **Jennifer Nemhauser** (University of Washington)
Plant Logic: Using Synthetic Biology to Understand and Redesign plant form

2:45 PM **Matthieu Jules** (Institut Micalis)
From Bacillus subtilis gene expression decomposition to synthetic biology

3:20 PM **End of the meeting**

6:45 PM **Gala dinner**
11:00 PM

ABSTRACTS : TALKS

Non coding RNA, epigenetics and non Mendelian inheritance in plants.

David BAULCOMBE

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Department of Plant Sciences, University of Cambridge

Eukaryotes contain small regulatory RNAs that have been referred to as the dark matter of genetics. They are typically 21-24 nucleotides long, associated with Argonaut or Piwi proteins. Some of these small RNAs guide the Argonaut/Piwi protein to a complementary RNA and they are negative regulators of gene expression acting at the level of messenger RNA turnover or translation. Others participate in more complex epigenetic systems affecting chromatin or they act as part of an RNA signal that moves between cells. In plants the posttranscriptional mechanism is involved in defense against RNA viruses. The chromatin effects play a role in defense against DNA viruses and transposable elements and it is associated with the establishment of heritable epigenetic marks.

However the importance of this defense system goes beyond suppression of transposons and viruses. There are secondary effects of the epigenetic marks that may influence the expression of adjacent genes in the sense of McClintock's "controlling elements". In most instances the effect is gene silencing and in some instances the effect may influence the biology of the affected plant. I will describe how RNA silencing may be particularly important following wide cross hybridisation and how it may influence hybrid vigour and transgressive segregation. I will describe our recent work that implicates paramutation like processes following wide cross hybridisation.

Finding drugs in the garden: Mining plant chemical diversity

Anne Osbourn

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John Innes Centre, Norwich Research Park, Norwich, UK

Plants produce a wealth of natural products that are valuable as industrial or pharmaceutical products. The growing reliance on chemicals from plants is driving demand for green, environmentally friendly and sustainable feedstocks across industrial sectors in order to enable us to reduce our dependence on products derived from chemical refineries. Importantly, many of the natural products that are produced by plants are structurally complex and beyond the reach of chemical synthesis. These compounds are commonly extracted from plant material either growing in the wild or in cultivation. Availability is limited by difficulties in accessing and cultivating source species, low yield and problems of purification. The scale of the economic opportunity for improving the supply of high value products from plants is therefore enormous.

The vast majority of the natural product diversity encoded by plant genomes remains as yet untapped. The explosion in plant genome sequence data, coupled with affordable DNA synthesis and new DNA assembly technologies, now offer unprecedented opportunities to harness the full breadth of plant natural product diversity and generate novel molecules in foreign hosts using synthetic biology approaches. The recent discovery that genes for the synthesis of different kinds of natural products are organised in biosynthetic gene clusters in plant genomes opens up opportunities for mining for new pathways and chemistries. This advance, in combination with powerful new transient plant expression technology, is enabling the development of rational strategies to produce known and new-to-nature chemicals tailored for particular applications. This presentation will focus on our work on developing a translational synthetic biology pipeline for rapid preparative access to plant natural products and novel analogs using synthetic biology approaches.

Perfume plant innovation: how translational research helps to breed for more sent molecule

Adnane Boualem

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The overall world market of cosmetics, perfume, scents and fragrances is projected to reach US$51.3 billion by 2022. In France, the perfumery and cosmetics is a strategic industry sector as it represents the second foreign trade surplus, twice that of the food industry. Nowadays, the perfume industry is facing a major problem, the secure and regular supply of all the necessary plant raw material to extract natural perfume ingredients. Natural perfume ingredients, essential oils or purified molecules, are defined as products that cannot be chemically synthesized. Among the perfume ingredient, ambergris, the metabolic product found in the gut of some blue sperm whales and the best-known amber odorant has been particularly used in perfume industry for its intense fragrance and unequalled fixative properties. Among the most synthetic equivalents of the scarce natural ambergris source, ambrox became the most important one. The natural pools of precursors for the synthesis of ambrox include mainly sclareol, a diterpene extracted from clary sage plant (*Salvia sclarea*), a herbaceaous plant of the Lamiaceae family (mint family). Despite the fame of the French perfume industry and the economic importance of the sclareol and essential oil, clary sage cultivation is declining due to the low yield of the cultivated varieties and to the lack of breeding programs and innovation. To rise to the challenge of improving sclareol production, we will establish and apply new breeding concepts to improve sclareol yield and production stability. We propose to improve our understanding on the molecular and genetic mechanisms of sclareol production and the temporal pic of its production. We aim also to deliver the tools to engineer high productive varieties by manipulating the sclareol pathway as well as agronomic traits such as clary sage vigor, flowering and plant habit.

At the root of the plant microbiota: the role of Arbuscular Mycorrhizal Fungi as biofertilizers and bioprotectors

Paola BONFANTE

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Plants, like humans, have their own microbiota, which may exert a powerful effect on their health.

Several studies on the plant microbiota focused on the identification of the biodiversity of microbiota present on both roots and epigeous organs, also detecting an influence of the plant genotype on the microbiota composition. Bacteria and fungi with beneficial functions, such as root symbionts and plant growth-promoting rhizobacteria, coexist with endophytes, saprotrophic microbes, as well as with a few pathogens. Arbuscular mycorrhizal (AM) fungi are common members of root microbiota in wild and agricultural ecosystems, where they improve the mineral plant nutrition, and in turn get back reduced carbon. They offer good tools to unravel how plants respond to beneficial microbes.

Using a combination of cellular, genetics and molecular approaches we demonstrate how model and crop (rice, wheat, tomato) plants respond to the presence of AM fungi thanks to a deep re-programming which involves cell organization, transcriptomics and proteomics profiles. The benefits of AM fungi as biofertilisers and bioprotectors are validated by the increased expression of genes involved in nutrient uptake and in defence responses. They often lead to relevant systemic effects. Lastly, some AM fungi contain endobacteria in their cytoplasm which may modulate not only the fungal responses, but also the host plant physiology.

In conclusion, by improving the nutritional status and by affecting the source-sink relationships of the whole plant, mycorrhizal fungi, as plant microbiota members, have a strong impact on plant nutrition and health.

Understanding and exploiting the beneficial impact of bacterial and fungal microorganisms for sustainable maize production

Bertrand Hirel[1], Liziane Cristina Brusamarello-Santos[1,2], Isabelle Quilleré[1], Lenaig Brulé[1], Julien Verzeaux[3], Thierry Tetu[3]

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Maize farming requires high amounts of nitrogen (N) fertilizer, with adverse environmental effects and insufficient agronomic sustainability. Certain maize genotypes can be colonized endophytically by atmospheric nitrogen (N_2)-fixing bacteria, but the agronomic potential of endophytic N_2-fixation is not fully exploited. Our hypothesis is that a scientific understanding of the mechanisms controlling these endophytic N_2-fixing associations could be useful to optimize endophytic N_2-fixation and exploit it in agriculture. The aim of our research is thus to better understand the interactions between bacterial endophytes fixing N_2 and maize combined with an assessment of maize genetic diversity and specificity with regards to this interaction, in order to identify and select maize genotypes that will be able to use the fixed N more efficiently and thus will be less dependent on mineral N fertilization. This is achieved by developing a multidisciplinary approach integrating molecular physiology, the assessment of whole-plant N responses to the endophytic interaction, molecular plant-microbe ecology and agronomy. We have started to characterize both at the physiological and molecular levels the atmospheric N_2-fixing endophytic interaction using a large-scale integrated transcriptomic, proteomic and metabolomic approach implemented with two established *Herbaspirillum* and *Azospirillum* models of N_2-fixing endophytic bacteria and 19 lines representatives of European and American maize genetic diversity. This will allow identifying the genetic and physiological determinants required for an efficient N_2-fixing endophytic association.
Within the frame of an ANR-funded project, a molecular screening is conducted in parallel to obtain effective endophytic N_2-fixing bacteria for agronomic improvement of maize cultivation at lower N input under temperate pedoclimatic conditions. The final goal of the project will be to produce innovative fertilizers based on bacterial inoculant technology. Such bacterial inoculants can be applied in the field to increase the capacity of commercial hybrids to use the N provided by the endophytic N_2-fixing bacteria.
Other beneficial microorganisms such as Arbuscular Mycorrhizal Fungi (AMF) are also known to play a major role in the uptake of nutrients, notably N, by crops. Some agricultural practices can interrupt fungal-plant interaction and thus impede the establishment of the mycorrhizal symbiosis. Both the absence of tillage and of N fertilization improve AMF colonization of roots. Moreover, under no-till conditions, N uptake and aboveground biomass production did not vary significantly between N-fertilized and N-unfertilized fields. This finding strongly suggests that, no-till farming is a sustainable agricultural system that allows a gradual reduction in N fertilizer use by promoting AMF functionality and at the same time increasing N uptake. As for the bacterial endophytes, we plan to identify maize genotypes that are able to establish a beneficial relationship with AMF in terms of N acquisition and then unravel the physiological and molecular mechanisms involved in this interaction.

Photosynthesis - The Final Frontier in Achieving Increased Crop Yield Potential under Climatic Change.

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Global demand for our major crops may rise 60% by 2050. This is at a time when the increases in yield seen over the past 60 years are stagnating while climatic and atmospheric change together with drought and pressures on irrigation water pose further threats to production. In reality we have little more than one crop breeding cycle in which to ensure against this projected global short-fall. The approaches of the Green Revolution are now reaching their biological limits. However, photosynthesis, which is among the best known of plant processes, falls far below its theoretical efficiency in current crop genotypes. Theoretical analysis and in silico engineering have suggested a number of points at different levels of organization from metabolism to crop canopy structure where efficiency of light, nitrogen and water use could be improved (Long et al. (2015) Cell, 161: 56-66), These have now been used to improve resilience under global change. Bioengineering both as a means and as a test of concept, has begun to validate some of these computationally predicted improvements with substantially greater crop productivity in the field under simulated conditions of global change (Kohler et al. (2017) J. Exp. Bot. 68: 715-726). Computationally guided bioengineered increases in productivity and water use efficiency in on-farm field trials will be illustrated from our recent work (Kromdijk et al. (2016) Science 354 : 857-860 ; Glowacka et al. (2018) Nature Comms. DOI: 10.1038/s41467-018-03231).

Making photosynthesis great again

Axel de JULIEN de ZELICOURT, Marine MESSANT, Yanpei LIU, Mathieu JOSSIER, Linda DE BONT, Bertrand GAKIERE, Michael HODGES

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Crop yields must increase by over 70% in the next 30 years to sustain human requirements and this should be attained without any detrimental effects on nutritional quality. To increase yield, a current major target is to improve photosynthetic CO_2 assimilation, however any beneficial effects could be limited by C3-plant acclimation to future elevated CO_2 (eCO_2) levels. Depending on different scenarios, atmospheric CO_2 is estimated to reach between 700 and 1000 ppm in 2100 if no action is taken. Although eCO_2 should enhance photosynthetic CO_2 assimilation, and lower photorespiratory carbon, nitrogen and energy losses and thus improve yield, many experiments have consistently shown that the increase in C3-plant yield in response to long term eCO_2 conditions is often 50% lower than predicted. This occurs because C3-plants adapt their metabolism, physiology and development to eCO_2 conditions. Acclimation mainly involves photosynthesis that can also negatively impact nitrogen assimilation. Photosynthetic acclimation has been associated with a lower stomatal conductance due to stomatal closure, lower amounts of ribulose-1,5-bisphosphate carboxylase/ oxygenase (RuBisCo) protein, and a general down-regulation of photosynthetic gene expression. Over the last few years, we have been investigating the phosphoregulation of the photorespiratory cycle (a target for improving plant photosynthesis and yield), the improvement of energy-metabolite production and more recently the impact of stomatal movement mutants (including *rhc1*, a key player in stomatal response to high CO_2 (Tian *et al*., (2015) Nat. Comm. 6: 1-10) on plant growth under normal and eCO_2 conditions. Our results will be discussed along with the available literature and in the context of climate change.

The roGFP2-Orp1 biosensor reveals hydrogen peroxide signatures in plant cells during the immune response.

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Reactive oxygen species (ROS) and particularly hydrogen peroxide (H_2O_2) are important signalling molecules for the plant immune response. In Arabidopsis, the NADPH oxidases RBOHD and RBOHF, as well as the apoplastic peroxidases PRX33 and PRX34 have been shown to play an important role in apoplastic ROS production after perception of Pathogen Associated Molecular Patterns (PAMPs) such as the peptide flagellin 22 (flg22). In this study, the ratiometric fluorescent H_2O_2 biosensor roGFP2-Orp1 was used to investigate cytoplasmic/nuclear H_2O_2 dynamics after flg22 treatment in leaves. Contrary to the fast and transient ROS burst generally observed in the apoplast, flg22 induced a delayed and long-lasting increase in intracellular H_2O_2. Introduction of the roGFP2-Orp1 biosensor into the *rbohD*, *rbohF*, *rbohD/F*, *prx33-3* and *prx34-2* mutant backgrounds revealed that RBOHD and PRX34 play only a minor role in flg22-triggered H_2O_2 accumulation in the cytoplasm/nucleus. The use of exogenous catalase also indicated that a large proportion of PAMP-induced H_2O_2 accumulated intracellularly. Moreover, to investigate specific H_2O_2 signatures in different subcellular compartments, we generated stable transgenic Arabidopsis lines expressing roGFP2-Orp1 targeted to the cytosol, nucleus, chloroplasts, mitochondria, peroxisomes and apoplast. The characterisation of these lines in response to different treatments such as H_2O_2, PAMPs and ABA is under investigation.

Local and systemic pathways regulating symbiotic nodulation in the *Medicago truncatula* legume

Pierre Gautrat, Carole Laffont, Sovanna Tan, Camille Fonouni-Farde, Anouck Diet, Mathias Brault and Florian Frugier

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Under nitrogen limiting conditions, legumes develop on their root system a symbiotic interaction with rhizobial bacteria leading to the formation of nitrogen-fixing nodules. Bacterial Nod Factors (NFs) are the initial trigger allowing infection in host root epidermal cells and nodule organogenesis in the root cortex, and plant regulatory pathways modulating NF responses are therefore critical to control nodulation efficiency.

The cytokinin phytohormone positively regulates, downstream of NFs, nodule organogenesis depending on different cytokinin receptors and notably MtCRE1 (1, 2), and negatively NF signaling in the root epidermis (3). Downstream of the cytokinin/MtCRE1 pathway, the MtDELLA-mediated gibberellin (GA) signaling pathway is critical for NF signaling and rhizobial infections in the root epidermis, and ectopic expression of a dominant active MtDELLA1 protein in the root cortex leads to the formation of nodule-like structures (4, 5). Overall, these results suggest a model where interactions between cytokinin/CRE1 and GA/DELLA1 signaling modules participate in the coordination between rhizobial infections and nodule organogenesis within the different root cell layers.

In addition to these local controls, nodule development is also regulated depending on environmental conditions by systemic pathways acting from shoots. This includes C-terminally Encoded Peptides (CEP) acting through the CRA2 (Compact Root Architecture 2) Leucine Rich Repeats – Receptor-Like Kinase (LRR-RLK; 6, 7). Integration between local and systemic pathways, as well as with feedback regulations, is essential for the dynamic regulation of root system architecture in a fluctuating environment.

Progress will be reported on some of these regulatory crosstalks.

(1) Plet et al. (2011) Plant J. 65: 622-33.
(2) Boivin et al. (2016) Plant Cell Environ. 39:2198-209
(3) Jardinaud et al. (2016) Plant Phys. 171:2256-76.
(4) Fonouni-Farde et al. (2016) Nat. Commun. 7:12636
(5) Fonouni-Farde et al. (2017) Plant Physiol. 175:1795-1806.
(6) Huault et al. (2014) Plos Genetics 10: e1004891.
(7) Mohd-Radzman et al. (2016) Plant Physiol. 171:2536-48

This work was funded by the French ANR “NodCCAAT” and “PSYCHE” projects.

Unravelling nutrient exchange in ectomycorrhizal symbiosis contributing to plant potassium nutrition

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A major role of mutualistic interactions between plant roots and soil fungi is the improvement of plant nutrition by an equitable exchange of nutrients leading also to tolerance of environmental stress. Ectomycorrhizal symbiosis established between woody plants and soil fungi, widespread in forest ecosystems, is crucial for the plant partner to efficiently take up poorly available nutrients. Physiological studies as well as recent genome sequencing projects (Kohler *et al*. (2015) Nature Genet 47: 410-415) and transcriptome analyses (Doré *et al*. (2015) New Phytol 208: 1169-1187; Doré *et al*. (2017) Environ Microbiol 19:1338-1354) have allowed progress towards the identification and characterization of the symbiotic transportome (Garcia *et al*. (2016) Trends in Plant Science 21: 937-950). One of the major nutrients is potassium (K^+), the most abundant cation in plant cells. We have shown improvement of potassium nutrition (Garcia *et al*. (2014) New Phytol 201: 951-960) under K^+ shortage conditions by ectomycorrhizal symbiosis using two model species from European forests, *Pinus pinaster* and *Hebeloma cylindrosporum*. Questions are raised to identify the transport systems involved in the uptake of nutrients from the soil and in their transfer towards the plant at the symbiotic fungus-plant interface, called Hartig net. In the case of potassium (Garcia and Zimmermann (2014) Front Plant Sci 5: 337), we have identified two types of K^+ transporters, Trk and HAK, as candidates to perform K^+ uptake from the soil by the fungal extraradical hyphae, and two types of K^+ channels, Shaker-like and TOK, that may release K^+ by the hyphae of the Hartig net into the plant apoplasm. We have studied the three TOK (Two-pore Outward K^+) channels identified in the genome of *H. cylindrosporum*, a channel family specific for fungi initially described in yeast (Ketchum *et al*. (1995) Nature 376: 690-695). These three TOK channels from the ectomycorrhizal fungus *H. cylindrosporum* belonging to two different subfamilies have been functionally characterized and localized (Carmen Guerrero-Galán *et al*. (2018) Env Microbiol , in press). Finally, we have analyzed whether these K^+ channels might play specific roles within the fungus and within the symbiosis.

Impact of nitrogen limitation on the Botrytis-Arabidopsis interaction

M.-C. Soulié[1], S. Miah Khoka[1], K. Floch[1], D. Barbe[1], S. Pateyron[2], M.-L. Martin-Magniette[2], and M. Fagard[1].

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Nitrogen (N) is essential for life and is a major limiting factor of plant growth. Because soils frequently lack sufficient N, large quantities of N fertilizers are added for crop production although they are a major source of global pollution. Furthermore, it has long been known that N fertilization has an impact on the incidence of crop diseases. However, the underlying mechanisms remain unclear and the variety of effects observed indicate that there is a complex relationship linking N availability to plant disease. Our goal is to understand the mechanisms that link the plant's N status to its response to necrotrophic pathogen infection and to identify key regulators that allow plants to adapt their biotic stress response to N availability. We previously showed that N limitation decreases the susceptibility to the fungus *B. cinerea* (Fagard et al, 2014, J. Exp Bot 65: 5643-5656). A transcriptomic approach of Botrytis-infected Arabidopsis plants grown in limiting or non-limiting N has enabled us to identify both fungal and plant genes for which expression is affected by the plant's N status. Characterization of corresponding fungal knock-out mutants led to the identification of novel Botrytis virulence genes, including a secreted protease and a secondary metabolite biosynthesis enzyme. On the plant side, we identified the jasmonate signaling pathway as a key player in the modulation of Arabidopsis susceptibility by N supply. Our current working model will be presented.

A combinatorial lipid code shapes the electrostatic landscape of plant endomembranes

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Many signaling proteins permanently or transiently localize to specific organelles for function. It is well established that certain lipids act as biochemical landmarks to specify compartment identity. However, they also influence membrane biophysical properties, which emerge as important features in specifying cellular territories. Such parameters include the membrane inner surface potential, which varies according to the lipid composition of each organelle. In particular, electrostatic interactions with negatively charged membranes contribute to the localization of many proteins containing polybasic clusters or cationic domains. Using a set of genetically encoded biosensors, we found that the plant plasma membrane (PM) and the cell plate of dividing cells are highly electronegative as compared to endomembranes. Phosphatidic acidic (PA), phosphatidylserine (PS) and phosphatidylinositol-4-phosphate (PI4P) are separately required to generate the electrostatic signature of the plant PM. In addition, we reveal the existence of an electrostatic territory that is organized as a gradient along the endocytic pathway. Within this territory, each compartment has a distinct electrostatic signature that is set-up by a combinatorial code of various anionic phospholipids. We propose that this “electrostatic code” may represent a fundamental patterning principle of the endomembrane system and acts as a key determinant of protein subcellular targeting and organelle identity.

Visualizing the *in vivo* activity of anion channels and transporters in guard cells with dynamic measurements of cytosolic pH and [NO_3^-].

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Anion transporters/channels of the plasma and intracellular membranes are key actors of guard-cells responses to environmental stimuli. In the last decade, it has been demonstrated that anion transport systems are fundamental in mediating stomata movements. During stomata opening and closure, guard-cells undergo massive fluxes of anions (like Cl^- and NO_3^-) in parallel to fluxes of K^+ and H^+. The ion fluxes during stomata opening and closure processes mainly involve three compartments: the apoplast, the cytosol and the vacuole. During opening, for example, the net ion flux is directed into the cell and induces an increase of the guard-cell osmotic potential leading to cell swelling and stomata opening. Notably, all the ionic fluxes between the apoplast and the vacuole has to pass through the cytosol. This opens the question of the regulation of the ionic gradients across the two major membranes delimiting the cytosol of the guard-cell: the plasma membrane and the tonoplast. To address this unexplored issue we developed the use of a fluorescent biosensor enabling to simultaneously measure the cytosolic pH and [NO_3^-] or [Cl^-] in Arabidopsis guard-cells. Unexpectedly, we observed fast responses of cytosolic pH and [NO_3^-] to extracellular conditions and stimuli. Further, we could visualize *in vivo* the impact of the activity of the vacuolar exchanger CLCa and of the plasma membrane channel SLAC1 on cytosolic pH and [NO_3^-]. Our results show that the activity of anion transport systems have a major impact on the cytosolic pH and [NO_3^-] homeostasis.

Chloroplast-nucleus communication: the special case of GUN signalling

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Activity changes in chloroplasts can have profound effects on the rest of the plant cell. Such intracellular responses are associated with signals originating in chloroplasts and conveying information to the nucleus, which leads to large-scale changes in nuclear gene expression (retrograde signalling). Genetic evidence obtained with *Arabidopsis thaliana* seedlings suggests that the chloroplast GUN proteins are required to integrate and transmit signals derived from perturbations in plastid redox state, plastid gene expression, and tetrapyrrole biosynthesis to the nucleus. This special type of retrograde signalling active only in early stages of plant development is still enigmatic and controversial although being the first type of retrograde signalling identified now more than 25 years ago. In this talk, recent findings will be presented and critically discussed.

Cytoplasmic natural variation and cytonuclear coadaptation: a hidden resource for plant breeding?

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Mitochondria and chloroplasts play crucial roles in energy metabolism and environment sensing and response. Although these roles heavily rely on nuclear genes, both organelles contain a tiny proportion of the genes necessary for their respective functions in energy metabolism, respiration and photosynthesis. Coadaptation between organelle genomes and the nuclear genome at the specific level has been observed for many years, in many eukaryotic lineages. This coadaptation is considered to result from responses, throughout evolution, to variation in either genetic compartment by compensating variation in the other (Rand et al, 2004, Trends in Ecology & Evolution, 19:645-653). More recently, evidence have accumulated for a role of natural cytoplasmic variation in environmental adaptation in plants (Bock et al, 2014, Molecular Ecology, 23:4899-4911) but also in several animal lineages. Although most of these reports relate to interspecific cytoplasmic variation, intraspecific mitochondrial variants with adaptive phenotypes have recently been reported in Drosophila (Camus et al, 2017, Molecular Biology Evolution, 34:2600-2612). Examples are still very scarce in plants. In addition, we know very little about adaptive polymorphisms in cytoplasmic genomes, how interaction with nuclear-encoded factors contribute to their phenotypic effect, and the traits under selection that contribute to shape these genetic polymorphisms.
As a first step to address these question in plants, we produced new genetic resources in *Arabidopsis thaliana*: cytolines combine cytoplasmic and nuclear genomes from different natural variants. We used them to assess the impact of natural cytoplasmic variation and of cytonuclear interactions on some traits in this species. I will present recently published and yet unpublished results with a focus on adaptive traits and some traits of interest in crop breeding.

Quantitative analysis of plant organ development

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Life originated as single celled organisms, and multicellularity arose multiple times across evolutionary history. Following this transition, a greater diversity of cellular arrangements were selected for, conferring organisms with adaptive advantages following structure-function relationships. By viewing organs as systems of interacting cells, the design principles and functional properties of these synergistic cellular configurations may be examined. Plants are well suited to investigate the complex organ design as their cells do not migrate during development. We have developed methods to capture all cellular associations within plant organs using a combination of high resolution 3D microscopy and computational image analysis. In this way, multicellular organs are abstracted into networks describing cellular connectivity, facilitating their quantitative analysis using network science. In this lecture I will discuss this quantitative analysis plant organs, and how this has been applied to bridge a structure-function relationship in epidermal patterning, and the principles of self-organization in the apical stem cell niche. This integration of network science and organ analysis provides a quantitative framework by which complicated developmental processes can be investigated.

A meta-analysis of transcriptomic data identifies a global response to stresses

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Plant response to stress is controlled by a variety of interacting molecular networks. These networks can be explored using guilt-by-association approaches on transcriptomic data to identify gene networks/modules controlling the plant physiology. The outstanding amount and diversity of transcriptomic datasets available in the public repositories is an invaluable source of information but this diversity is also the main limitation of transcriptome meta-analyses because it is associated with a large heterogeneity. To by-pass this limitation, we focused on almost 400 stress-related samples produced by the IPS2 transcriptomic platform over the last 15 years with standardized protocols (Gagnot *et al* (2008) NAR 36:D986-90). These samples were divided in 18 stress categories: 9 abiotic and 9 biotic. In each stress category, we identified clusters of co-expressed genes using mixture models. The 634 identified clusters have been annotated and show functional enrichments. They are available in the Gem2NET module (Zaag *et al* (2015) NAR 43:D1010–D1017). Extending the integration of the co-expression analyses of the 18 stress categories, a large network of more than 5600 genes co-regulated during stress responses was identified. In this network, 43 stable communities of co-regulated genes corresponding to various biological functions were identified, highlighting the global response of plants to stresses.

Gene Regulatory Networks Controlling Root Stem Cells

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Development in multicellular organisms requires not only the production of specialized cell types but also mechanisms of coordination among them. Stem cells are ultimately the source of all cell types, and the balance between self-renewal and differentiation of their progeny regulates organ growth. Transcription factors and cell-to-cell signaling have a key role in coordinating these processes; however, how these transcriptional networks control multicellular development is not completely understood. Moreover, while a number of transcription factors involved in root stem cell maintenance have been described, a comprehensive view of the transcriptional signature of the stem cells is lacking. Here, we used spatial and temporal transcriptomic data to predict interactions among the genes involved in stem cell regulation. To accomplish this, we transcriptionally profiled several stem cell populations, developed gene regulatory network inference algorithms, and leveraged the topology of our networks to infer potential major regulators. Specifically, through mathematical modeling and experimental validation, we identified *PERIANTHIA* (*PAN*) as an important molecular regulator of quiescent center function. Current work focuses on identifying additional players in the QC as well as if SHORTROOT and SCARECROW function to regulate QC maintenance. The results presented in this work show that our combination of molecular biology, computational biology, and mathematical modeling is an efficient approach to identify candidate factors that function in the stem cells.

Towards a spatio-temporal atlas of 3D cellular parameters during leaf morphogenesis

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In plants, cell division, growth and elongation are the major cellular mechanisms that determine organ size and shape. Cells also play a key role in organ morphogenesis by integrating genetic, mechanical, and environmental signals. Leaves, which are the main source of biomass in plants, are initiated from a few cells that subsequently undergo several rounds of cell division and expansion to reach the final organ shape and size. How cellular mechanisms are integrated at the organ scale to regulate leaf morphogenesis remains largely unknown. A fundamental bottleneck is the current lack of techniques to systematically quantify the spatio-temporal evolution of cell morphology during growth.

To answer this problem, we developed a comprehensive framework for automatic analysis and quantification of individual epidermal cell shape in 3D (Selka, et al. (2017) I*CCVW: 56-63)*. For each epidermal cell, multiple parameters are measured, including size (e.g., volume, directional extensions,..), shape (e.g., thickness, elongation, cell orientations...), and cell network topology. For each parameter, a map is generated that shows cell measures over the leaf surface. These surface maps can then be visualized in a 3D viewer (e.g., http://free-d.versailles.inra.fr) to reveal domains with specific characteristics.

Next, maps from individual leaves are integrated by averaging measurements on a prototypical leaf shape, which is the average surface of registered leaves. Comparing maps between different developmental stages will provide an integrative vision of the evolution of the cell morphology over time and space.
Our results show that epidermis cell is composed of a complex cellular organization with variations of cell shape parameters during the development. Furthermore, thanks to our framework we revealed a differential evolution of cell shape parameters that occur between the two sides of the leaf (Abaxial-Adaxial). This differential growth could participate to the evolution of the leaf shape and the apparition of blade curvature.

Cellular growth regulation in roots - how to adapt in a complex environment

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In nature, plant roots grow in highly heterogeneous conditions, which requires both systemic and local response mechanisms to modulate cellular growth and adapt organ morphology. How environmental cues are integrated into growth regulation and how systemic communication and cell-autonomous responses are interlinked in this process, remain challenging questions in plant biology.
To facilitate the investigation of root-environment interactions, we combine the use microfluidic lab-on-a-chip platforms with live-cell imaging of roots. Over the past years, we developed a series of microfluidic devices for on-chip root growth under precisely controlled conditions. Here, I present our dual-flow-RootChip, a microfluidic organ-on-a-chip platform for asymmetric perfusion of Arabidopsis roots to investigate root growth, development, and signaling under simulated environmental heterogeneity.
Using the dfRootChip, we traced molecular uptake into the root by selectively supplying one side of the root substrates and monitored physiological, signaling, and genetic responses under asymmetric biotic and abiotic stress conditions. Upon differential availability of inorganic phosphate (Pi), we revealed cell-autonomous regulation of root hair growth. Instead of root hair growth locally stimulated by Pi deficiency, we made the rather unexpected observation that, independent of the overall Pi status of the root, hair cells can triple their growth rate within minutes, when extracellular Pi levels rise. This finding points to a direct modulation of the tip growth machinery, resulting in hair growth that follows the nutrient gradient.

A molecular framework for growth repression at boundary domains

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The coordination of basal cellular processes such as cell growth and cell division drives growth of all living organisms. This is particularly true in plants, in which the production of distinct functional units within a tissue or an organ almost exclusively relies on differential growth. The CUP-SHAPED COTYLEDON transcription factors are expressed in boundary domains and have been involved in the control of differential growth but so far, little is known on their downstream targets and their precise cellular outputs. Here, we start to build the Gene Regulatory Network (GRN) controlling differential growth focusing on growth repression in boundary domains. We use of CUC2 inducible lines followed by RNAseq analyses to identify putative CUC2 targets. Our data show that CUC2 represses genes promoting cell expansion. Accordingly, CUC2 overexpression inhibits hypocotyl elongation in the dark and preliminary cellular live-imaging studies suggest that cell expansion could be the primary target of CUC TFs in leaves. Further results indicate a possible link between CUC2 and the BZR1/ARF6/PIF4 (BAP) module, a trio of transcription factors integrating hormonal pathways and environmental cues to regulate cell expansion. We show that CUC2 could antagonistically regulates targets of the BAP module by direct competitive binding to BZR1/ARF6/PIF4 binding site (Bai *et al.*, 2012; Oh *et al.*, 2012 ; Oh *et al.*, 2014). Interestingly, gibberellins signaling which regulate the BAP module, modify leaf shape indicating that this molecular module contributes to proper organ development. Altogether, our observations outline a molecular framework for the cell autonomous growth repression by CUC transcription factors, which was long-time hypothesized but never understood.

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Genome Engineering in Plants: Past, Present, Future

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More than 20 years ago, we were already able to show, that changes in the plant genome can be achieved by the induction of double strand breaks by sequence-specific endonucleases. Although the CRISPR/Cas system was first introduced only about five years ago, it almost instantly became the most efficient and widely used tool for genome engineering. For plants, efforts were centred on obtaining heritable changes in most transformable crop species by inducing mutations into open reading frames of interest, via non-homologous end joining. Now it is important to take the next steps and further develop the technology to reach its full potential. For plants, besides improving gene targeting by homologous recombination and avoiding off target effects, it will be desirable to apply the system for transcriptional control, imaging as well as for more complex genome rearrangements.

From Marker-Assisted Selection to Genomic Predictions: opportunities raised by new breeding approaches

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Since the early nineties, the development of molecular markers made it possible to position on the genome some of the genes involved in the architecture of traits of interest. Beyond the interest of such studies as a first step towards the identification of causal polymorphisms, once detected, marker-gene associations can be further used to select individuals carrying alleles of interest based on marker genotypes without the need of evaluating their performances, opening the way to marker-assisted selection (MAS) methods. First approaches of MAS, using low density genotyping, relied on the linkage disequilibrium between loci resulting from co-segregation within families. Identification of marker-trait associations therefore necessitated to develop large segregating families in order to be able to detect loci (QTL) with small individual effect involved in complex quantitative traits. In such approach, the poor accuracy of QTL positions and population specificity of marker-gene/QTL associations mostly limited the application of MAS to simple traits despite some reports of success for complex traits. Recently, the availability of cheap dense genotyping techniques has opened new prospects (i) for deciphering trait architecture by the use of association mapping and (ii) for breeding with the development of genomic predictions. Contrary to MAS that is focused on a few loci that have been detected in a previous step, genomic selection relies on the calibration of a prediction equation using marker effects estimated genome-wide with no explicit step of QTL detection. This approach that has been therefore often compared to a “black-box”, has been tested in different contexts and is now used in routine for breeding in a growing number of animal and plant species. In this talk, we will give an overview of the different marker-assisted selection approaches with a specific focus on genomic selection. We will present the different opportunities raised by this approach but also the issues that remain to be solved, more specifically for the calibration stage. We will present some recent developments, issues and opportunities for its use at the different levels of a breeding program. One major challenge for the future is to integrate the huge amount of functional data and the recent developments in the analysis of “omics” to open the “back-box” of genomic predictions. Reconciling predictive approaches with biological knowledge would help filling the gap from basic to applied research.

Plant Logic: Using Synthetic Biology to Understand and Redesign plant form

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The fundamental building blocks of plant development—cell-cell communication, cellular differentiation, cell growth and proliferation—are characterized by complex gene regulatory networks, many of which include plant hormones. By recapitulating the *Arabidopsis thaliana* forward auxin signal transduction pathway in *Saccharomyces cerevisiae*, we were able to identify and analyze the parameters of auxin response without interference from other networks. This work revealed that members of the large Aux/IAA family exhibit a range of degradation rates and that Aux/IAA degradation rates drive transcriptional dynamics. These synthetic experiments and subsequent experiments in transgenic plants demonstrate that Aux/IAA degradation rate can set the pace for critical developmental events. I will present work on the latest ways we are trying to capitalize on insights from the auxin system to semi-rationally engineer plant development.

From *Bacillus subtilis* gene expression decomposition to synthetic biology

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Synthetic circuits often fail to function as designed because of unwanted interactions between circuit components and the metabolic and regulatory environment of the host cell. A promising strategy to foster innovation in biotechnology involves (1) the construction of streamlined strains (i.e. chassis) in which the negatively interacting host functions have been minimized, and (2) rational engineering of the gene circuit expression (where "rational" means taking into account the physiological status of the host cell).

To address the first issue we develop novel genome engineering methods towards genome defragmentation and redesign. For instance, based on a repertoire of the *B. subtilis* chromosomal regions dispensable for growth, we rationally reduced the genome down to 2.73 Mb, which corresponds to an overall reduction of 35% in comparison with the reference genome (Tanaka K[$], Dervyn E[$], Planson AG[$] *et al.*, *unpublished*).

To address the second issue we decompose gene expression into distinct and well-characterized genetic elements (gene location, TATA box, transcription start, translation initiation region, etc.; Guiziou S *et al.*, Nucleic Acids Res, 2016). By doing so, we revisit the contribution of replication, transcription and translation to global mechanisms allowing bacteria to modulate abundance of single proteins with respect to the growth rate. For instance by combining model-based data analyses of transcript and protein abundances, we revealed a unique, hard-coded, growth rate-dependent mode of regulation by which translation in *B. subtilis* elicits a genome-wide, differential protein production (Borkowski O *et al.*, Mol Syst Biol, 2016). Such a mode of regulation opens novel avenues for bacterial synthetic biology, particularly in the potential it offers to bypass the need for multiple regulators to modulate expression of complex synthetic circuits (Sauveplane V *et al.*, *unpublished*).

Keywords: Gene expression; Genome engineering; Growth rate; Synthetic Biology; Systems Biology

Mitochondrial respiratory chain dysfunction promotes in vitro morphogenesis in plant tissues

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Mitochondrial respiration is vital because it provides the energy required to fuel cellular activities. But mitochondrial functions also affect developmental processes beyond the mere production of ATP. We have characterized the growth and development of Arabidopsis *ppr* mutants in which components of the mitochondrial electron transfer chain (mETC) are compromised. Each of these mutants lacks a different Pentatrico Peptide Repeat (PPR) protein encoded in the nuclear genome, but targeted to the mitochondria where it controls the expression of organellar gene(s) coding for mETC complex subunit(s). As expected, the *ppr* Arabidopsis mutant plants have growth retardation phenotypes, and so do calli derived from these mutants. However, *ppr* mutant calli also produced significantly more buds when transferred on shoot inducing medium. This enhanced caulogenesis phenotype can be phenocopied in wild-type explants treated with a chemical inhibitor targeting the same mETC complex that is compromised in the mutants, as demonstrated *in vitro* in Arabidopsis and tomato tissues. Furthermore, maize calli derived from immature *ppr* mutant embryos, in which the mETC is similarly dysfunctional, produced somatic embryos while the corresponding wild-type cultivar never produced embryogenic tissues in our hands. In the light of transcriptome signatures characterizing mutant, inhibitor-treated and control tissues at key stages of an *in vitro* regeneration protocol, we will discuss the mechanisms possibly linking mETC dysfunction and morphogenesis. Our findings may lead to novel approaches towards the production of transformed or edited plants in recalcitrant species or cultivars.

Spatial control of plant cell growth and tissue differentiation with 3D-printing

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Regulation of plant growth and development is dependent on the continuous generation of stem cells that differentiate to form all the cells, tissues, and organs of the plant. Understanding the spatial and temporal control of stem cell self-renewal and their differentiation is critical for generating predictable outcomes and redesigning plants for our future needs. To identify spatial and temporal mechanisms regulating stem cell proliferation and differentiation of their progeny into specific tissues, we are using 3D-bioprinting process to achieve precise placement of root plant stem cells, which we can now programmatically assembled in 3D space in nearly any arrangement. For this, we have developed 3D-bioprinting parameters for depositing plant stem cells on an agar substrate as well as protocols to quantitatively measure cell viability and cell wall formation in control and in 3D-bioprinted cells. A 2D mathematical model of the root stem cell spatio-temporal communications network has been developed. This mathematical model is a step forward to predict the necessary spatiotemporal distribution of the deposited stem cells to achieve engineered 3-D printed roots.

ABSTRACTS : POSTERS

The posters which will be the object of a flash-talk are indicated by “(FT)” after the title of the abstract.

Plant physiology and science in plant growth chambers (FT)

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Growth conditions for plants in nature are very different from those in indoor environments. Essential physiological conditions for good plant growth such as e.g. water evaporation, heat dissipation of irradiant energy, and gas exchange have to be considered and will be discussed. Generation of comparative quantitative data e.g. for different cultivars, genotypes, or different experimental treatments requires growth of plants in uniform environments. Data will be reviewed showing the impact of abiotic factors on plant growth performance and on gene expression at multiple levels in different model plants. Recommendations will be made for evaluation of plant growth equipment to achieve consistent and uniform plant growth for reliable science.

Functions of histone modification enzymes in metabolic control and plant stress

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Epigenetic modifications of chromatin usually involve consumption of key metabolites. Primary metabolic flux controls the activity of enzymes involved in epigenetic chromatin modifications, such as methylation and acetylation, which conversely have an effect on gene expression and/or enzymatic activity of specific metabolic pathways (Yuan S et al., 2016). Thus, coordination of metabolism and epigenetic regulation of gene expression is critical for controlling organism growth and development in the changing environment. Much has been learned from animal and yeast cells with regard to the interplay between metabolism and epigenetic regulation, and now the metabolic control of epigenetic pathways in plants is an increasing area of study.

Epigenetic mechanisms are quite similar between plant and mammalian cells, but plants being autotrophic, they display very important differences in both metabolism and metabolic signaling pathways. In our study, we will study the reciprocal regulation between plant epigenetics and metabolism in controlling plant growth and responses to environmental stimuli.

ChloroKB: an exploration tool of Arabidopsis metabolism (FT)

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Present plant metabolic pathway databases, though extremely useful, mainly focus on the set of chemical reactions and the enzymes that catalyze them. Important features such as subcellular compartmentation, transport reactions or short-term regulatory processes are left aside. However, these features are essential for modelling, understanding and manipulating plant metabolic network at both local and system levels.

ChloroKB is a manually reconstructed metabolic network freely available as a web-application (http://chlorokb.fr, Gloaguen et al. Plant Physiol (2017), 174:922-934) and represents a considerable improvement in the field of genome-scale metabolic modelling in plants (*Arabidopsis thaliana*). ChloroKB represents important features of a metabolic network not taken into account in other pathway databases (subcellular compartmentation, transports, regulatory processes, exhaustive interactions between processes, steps for which genes are still unknown, explicit reactions rather than classes of reactions…). The use of scalable vector graphic format allows a dynamic and intuitive exploration of the different levels of organization, from high-level representations, to detailed, interconnected zoomable maps with clickable icons. The use of standards of representation (based on CellDesigner graphical codes) allows fast understanding of complex processes, facilitating the acquisition of expert knowledge, interpretation of new data and the generation of hypotheses. Regulatory mechanisms involving protein-protein-metabolites interactions and post-translational modifications not available in other databases are displayed. In addition to representations as pathways, different points of view are provided with metabolic maps centered on key metabolites (hubs), allowing to show the multi-functionality of some metabolites and the different roles they play, depending on the compartment or cell type where they are. Shuttling of metabolites between compartments in integrated processes such as photorespiration, nitrogen fixation or redox exchanges are displayed. The web-like pathways of lipids synthesis and desaturation leading to a diversity of molecular species are represented explicitly. The integration, still under way, carried out during a period of 6 years and initially focused on chloroplast processes finally lead to the reconstruction of a metabolic network now encompassing nearly all biosynthetic and degradation processes and central metabolism, distributed among 10 subcellular compartments in Arabidopsis cell. Currently, the network contains 1274 proteins, 930 complexes, 272 unknown proteins, together involved in the synthesis/transport/degradation of 996 metabolites. However, despite this effort, grasping the global behavior of such a complex network is yet beyond human reach without the help of additional tools. New developments based on ChloroKB cartography now allow the automatic building of stoichiometry matrices from the maps displayed in chloroKB. A global stoichiometry matrix of Arabidopsis mesophyll cell metabolism can then be obtained for further structural analysis of the network. This matrix may be easily updated as new data are published and integrated in the reconstruction. Finally, ChloroKB can be used as a template to speed-up the reconstruction of other plant model metabolisms.

Conjugated sterols in flavonoid metabolism of Arabidopsis seeds : the endomembrane connection.

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Flavonoids play a central role in plant and seed adaptation to their environment, as antioxidants, signaling molecules, antimicrobial defense, light filters, pigments, allelochemicals, developmental regulators etc… They are synthesized during development and are also induced by various biotic and abiotic stresses (Treutter (2006) Environ. Chem. Lett. 4 : 147-57 ; Cheynier et al. (2013) Plant Physiol. Biochem. 72 : 1-20). In many plants including Arabidopsis, seed coat flavonoids are involved in reinforcement of seed dormancy and longevity (Sano et al. (2016) Plant Cell Physiol. 57 : 660-74). These polyphenols also have numerous nutritional and pharmacological properties with health benefits (Williamson (2017) Nutr. Bull. 42 :226-35). Consequently there is an obvious interest to breed crops with optimized flavonoid composition, especially in a context of climate global warming and with the urgent necessity to reduce chemical pesticide use for a sustainable agriculture. In contrast to the extensive knowledge available on biosynthesis and its transcriptional regulation, very little is known on how plants transport often toxic or highly reactive compounds from their site of synthesis to where they are ultimately stored. Thus proper trafficking and storage of phytochemicals is often a bottleneck in efforts aimed at the rational engineering of plant metabolism. It has been established, mainly with anthocyanins, that flavonoids synthesized at the endoplasmic reticulum traffick towards the central vacuole through at least two non-exclusive mechanisms, namely one involving a ligandin and a transporter and the other one involving vesicles from the endomembrane system. However, many questions remain to be answered (Zhao (2015) Trends Plant Sci. 20 : 576-85). In the present study, we take benefit from the Arabidopsis mutant *transparent testa 15* / *ugt80b1* having seed coats defective in proanthocyanidin (PAs) metabolism and previously shown to be affected in sterol glucosylation (DeBolt et al. (2009) Plant Physiol. 151 : 78-87 ; Stucky et al. (2014) J. Exp. Bot. 66 : 189-201) to explore further the role of the endomembrane network in flavonoid trafficking. Indeed sterols and their conjugates are important components of cell membranes (Ferrer et al. (2017) Prog. Lipid Res. 67:27-37). A live confocal imaging approach involving a thorough analysis of TT15 subcellular localization and FRAP experiments to assess membrane properties was realized. Moreover a genetic approach based on the subcellular detection of PAs in double mutants between *tt15* and other *tt* mutants was performed. A model integrating both sets of data will be presented and discussed.

Next step to water biomonitoring: are molecular biomarkers of the aquatic macrophyte *Myriophyllum alterniflorum* efficient to detect xenobiotic pollution? (FT)

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Surface water pollution by xenobiotics constitutes both problems for public and terrestrial/aquatic ecosystem health. European Water Framework Directive set objectives to achieve in 2027 for aquatic environment quality, management and protection. However, the upgrade of surface water quality involves a precocious and accurate detection of pollutions for which robustness, precision and lower cost are paramount parameters. To reach this goal, our laboratory works on the improvement of biomonitoring of xenobiotics contaminations in rivers.

Alternate watermilfoil (*Myriophyllum alterniflorum*), an immerged aquatic macrophyte of oligo-mesotrophic European rivers, constitutes our plant model for biomarker studies. Depending on trophic conditions and/or the xenobiotic availability, watermilfoil can exhibit morphological, histological, physiological and/or biochemical changes as it could be observed during cadmium, copper or arsenic exposures (Delmail *et al*. (2011) Envir. Exp. Bot. 72(2) : 174-181 ; Delmail *et al*. (2011) Hydrobiologia 664: 95–105; Krayem *et al*. (2016) Sci. Pollut. Res. 1–8; Krayem *et al*. (2018) Chemosphere 199: 427–434).

Watermilfoil biomarkers selection was performed in laboratory using *in vitro* culture. Alongside this system, the impact of hydro-dynamic conditions on biomarker response was evaluated through the development of a microcosm, an aquarium equipped with a water re-circulation, mimicing the environmental conditions. Finally, in keeping with previous field experiments, biomarker assays were performed during 28 days in rivers chosen for their land use in catchment area and their consecutive water composition. Natural arsenic contamination, urban impact such as domestic wastewater release, industrial activity, hydro-electrical dam and metal content were the selection criterions.

Whatever the scale of the studies system (culture box, aquarium or field experiment), physico-chemical parameters and watermilfoil biomarkers were analyzed. Alongside previously studied morphological, histological, physiological and/or biochemical biomarkers, we assessed xenobiotic effect on watermilfoil through molecular biomarkers like Rapid Amplification of Polymorphic DNA (RAPD). Gene expression of specific or unspecific xenobiotic-responsive genes are also under investigation. Indeed, phytochelatin synthase (*pcs*) gene appears as a good candidate to evaluate specifically the effect of metal or metalloid exposure on watermilfoil as it is involved in the synthesis of small peptides chelating metal ions. Another good candidate is the Glucose-6-Phosphate DeHydrogenase (*g6pdh*) gene involved in an essential metabolic pathway, the pentose phosphate ones. Both “classical” biomarker responses to xenobiotic and preliminary results of molecular biomarkers will be presented. These results indicate that alternate watermilfoil could constitute an efficient biomonitor of metal pollutions through biomarkers like malondialdehyde, proline and pigment content but also through RAPD analysis at least for cadmium exposure.

This research was supported by the European Union through the FEDER-Plan Loire project MACROPOD 2 2016EX-000856 for R. Decou post-doctoral fellowship.

Early phloem responses to *'Candidatus Phytoplasma solani'* infection in tomato are associated to major changes in the organic acid content of phloem exudates

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Phytoplasmas are severe plant pathogens responsible for causing important diseases in crops. These bacteria colonize only the phloem tissues of their host plants, and despite they can severely affect many crops, very little is known on their trophic relationship with plants. We exploited the pathosystem tomato/ *'Candidatus Phytoplasma solani'* stolbur phytoplasma to investigate the early plant responses occurring in the phloem of tomato plants in newly infected plants by performing metabolomic measurements. We observed that in infected plants the flow of sucrose translocated via the phloem was reduced, consistent with the callose deposits observed at subcellular level in the sieve plates of the sieve elements. To further determine the cause of the shortage of sucrose mass flow, we also analyzed the response to phytoplasma in antisense tomato lines downregulated for the two sucrose transporters, SUT1 and SUT2, which are involved in phloem sucrose loading. In infected antisense lines, we observed different susceptibilities to the infection but no reduction of the sucrose flow. However, an increase in organic acids, mostly glyoxylate, was correlated to the phytoplasma titer in the upper leaves. Further analysis confirmed that the infection by phytoplasma has a strong effect on the expression of genes involved in the metabolism of glyoxylate, in addition to several genes involved in the glycolysis. These data suggest that glyoxylate and another product of the glyoxylate cycle, glycolate, may act as forms of carbon for long distance transport as an adaptive process for plant response to stresses. Moreover, the differences of tolerance to the stolbur phytoplasma of the SUT1 antisense line suggest an interaction between phloem sugar loading and phytoplasma infection.

Regulation of plant bZIP transcription factors by the interconnected SnRK1 and TOR signaling pathways (FT)

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Plant growth and metabolism are modulated by two functionally antagonistic signaling pathways which are evolutionary conserved in eukaryotes: the Target of Rapamycin kinase (TOR) and the Sucrose non-fermenting 1-Related Kinase 1 (SnRK1) pathways. It is now clear that in plants the switch between activation/inactivation of TOR or SnRK1 pathways is triggered by energetic signals, which are linked to the daily oscillatory conditions of the photosynthetic activity, but also depends on responses to biotic and abiotic stresses. Both pathways lead to transcriptional reprogramming involving, among others, the transcription factors of the basic leucine zipper (bZIPs) family. While the bZIPs are conserved in Eukarya, its diversification relates to the evolution of complexity in multicellular organisms, regulating diverse developmental and physiological processes. These transcription factors are formed by a basic DNA-binding N-terminal region and the leucine zipper motif which is required for the bZIP homo- or hetero-dimerization. In this context, the bZIPs of C and S groups are known to form heterodimers for modulating the transduction of energetic signals. Intending to investigate the potential role of bZIPs as interacting nodes between TOR and SnRK1 pathways, Arabidopsis C and S group insertional mutants were used for evaluating the activation of TOR or SnRK1 pathways depending on the variations of the energetic status during day and night periods. Therefore, metabolite profiling experiments have been used to investigate the impact of C and S group bZIP mutations on plant adaptation to light/dark cycles.

Functional characterization of a *Brachypodium distachyon* cytochrome P450-encoding gene involved in strigolactone biosynthesis and in the interaction with *Fusarium graminearum* (FT)

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Fusarium Head Blight is one of the most economically problematic diseases of temperate cereals. It induces significant yield losses and constitutes a public health issue because of the ability of the main causal agent, *Fusarium graminearum*, to produce deoxynivalenol (DON), a mycotoxin harmful to human and animals. Based on transcriptomic studies investigating the response of *Brachypodium distachyon* both to fungal infection and to DON application, we identified a cytochrome P450-encoding gene which is highly and specifically induced. Through reverse genetics, we demonstrated that *BdCYP1* is involved in strigolactone (SL) biosynthesis since its overexpression in *B. distachyon* leads to significantly increased exsuded levels of orobanchol. In addition, transgenic expression of the *BdCYP1* gene in SL-deficient *Arabidopsis thaliana max1-1* allows significant complementation of mutant shoot phenotypes. Finally, qRT-PCR analysis on *B. distachyon* SL biosynthesis marker genes indicated a strong transcriptional activation at 12-24 h after DON treatment and at 96-168 h post inoculation by *F. graminearum*. Altogether, these results highlight the potential involvement of SLs in the *B. distachyon* - *F. graminearum* interaction. Experiments are underway to decipher more precisely their role in the interaction.

Functional and structural characterization of Starch Phosphorylases in potato leaves and tubers (FT)

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Starch is a major storage carbohydrate of higher plants. This polymer is composed by two macromolecules amylose and amylopectin. Amylopectin, the major component, is synthetized by the action of different enzymes: Starch Synthase (SS), Starch Branching Enzyme (BE), Starch Debranching enzyme (DBE) and Starch phosphorylase (PHS) (Ball and Morel, 2003). PHS catalyzes the reversible transfer of glucosyl units from glucose 1 phosphate to the non-reducing end of an α 1, 4 glucan chain. Two distinct forms of starch phosphorylases have been consistently observed in plants: cytosolic and plastidic phosphorylases. In potato two isoforms of plastidic phosphorylases are present. One (PHS1a) is expressed preferentially in tubers and the other (PHS1b) is expressed in leaves. Some studies have reported a role of starch phosphorylases in starch synthesis in potato (Albrecht *et al.,* 2001) and in rice (Satoh *et al.,* 2008). In this study, we investigated the function of PHS1b in potato. First, we determined enzyme activity *in vitro* and characterized the envelope (by SAXS analysis) of the recombinant protein. On the other hand, by the production of a varieties of potato mutants plants affected in the production of starch phosphorylases.

Deciphering the TOR signalling pathway controlling plant growth

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The conserved TOR (Target Of Rapamycin) kinase signalling pathway has emerged as a central hub controlling growth in response to nutrient availability. Dysfunction of the TOR kinase leads to cancer and metabolic diseases in humans. TOR is a member of the phosphatidylinositol 3-kinase (PI3K)-related kinase (PIKK) family. However, in plants the exogenous signals that act via TOR and the molecular mechanisms regulated by TOR remain poorly understood. The TOR kinase functions in a complex with two other proteins: RAPTOR (regulatory-associated protein of mTOR) and LST8 (lethal with SEC13 protein 8). The Arabidopsis genome encodes one *TOR* gene whereas *RAPTOR* and *LST8* are encoded by 2 genes. Arabidopsis *tor* mutants arrest growth early during embryo development. On the other hand, *lst8-1* and *raptor* mutants are viable and show an overlap between their phenotypes. *Lst8-1* mutants are hypersensitive to long days and have a reduced growth rate. This *lst8-1* phenotype provided the basis for an *lst8-1* suppressor screen. *Lst8-1* suppressors were isolated by screening *in vitro* a population of EMS (ethyl methanesulfonate) mutagenized M2 seeds under long-day conditions. The fastest growing M2 mutant plants were selected. Six putative *lst8* suppressors were so far isolated and sequenced by NGS (next generation sequencing) technologies. Additional analysis showed no modification in TOR activity but a reduced sensitivity to the specific TOR inhibitor AZD-8055 for two *lst8-1* suppressors compared to *lst8-1* mutants. These results suggest their accelerated growth was due to altered TOR signalling. These two *lst8-1* suppressor lines carry a mutation in the same kinase gene. Further characterization of these two *lst8* suppressors will be presented. This approach should lead to the identification of new molecular components of the plant TOR signalling pathway.

Fungal secreted peptides in the establishment of arbuscular mycorrhizal symbiosis (FT)

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Arbuscular mycorrhiza is a symbiosis between plants and members of an ancient phylum of soil fungi, the Glomeromycota. This symbiosis occurs in nearly all land plants including most crops. While colonizing plant roots, mycorrhizal fungi form extensive hyphal networks in the soil. The fungus provides water and minerals to the plant in exchange for photosynthetic sugars. These exchanges take place in highly branched structures within root cortical cells called arbuscules.
Secreted proteins are important regulators of plant - microbe interactions. We wonder whether fungal secreted proteins produced by the arbuscular mycorrhizal fungus *Rhizophagus irregularis* are potentially involved in the regulation of the symbiosis with the host plant *Medicago truncatula*. We previously identified *R. irregularis* transcripts encoding secreted proteins that preferentially accumulate in symbiotic tissues.
Two types of fungal proteins have caught our attention. The first class displays striking similarities with ascomycete sexual pheromones. These proteins are cleaved to produce small peptides in the extracellular space to initiate a molecular dialogue between compatible cells to promote cell fusion. Our fungus has no known sexual phase, thus these peptides may regulate yet unknown processes. The second class correspond to fungal proteins putatively coding for plant-like CLE peptides. So far, CLE peptide-encoding proteins were only identified in plants and plant-parasitic nematodes.
For the two classes, we are currently trying to elucidate the targets and the functions of the secreted peptides, mostly using biochemical analysis, genetic approaches, exogenous treatments and RNAseq studies. Our data sheds light on molecular regulations occurring during the most ancient plant-fungus symbiosis.

Neofunctionalization of acyltransferases from the BAHD family results in the accumulation of a novel phenolamide in the pollen coat of the Asteraceae (FT)

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Evolution within the plant kingdom is highly correlated with the apparition of new metabolic pathways, and notably those leading to synthesis of the so-called specialized metabolites. Indeed, their great diversity confers to plants the ability to adjust to their environment. Evolution of the reproductive structures was a key element during terrestrialization and emergence of pollen was a major event as well as the appearance of pollen coat among Angiosperms. Pollen coat assumes a wide range of functions such as protection against biotic or abiotic stresses, stigma-pollen recognition mechanisms or pollinator attraction but nonetheless, with a variable chemical composition depending on species. However, tri-substituted spermidines are specific phenolamides highly conserved in the Eudicots pollen coat. Still, to date, no clear function was assigned to these compounds. Nevertheless, their maintenance since appearance in pollen coat suggests their major significance in evolution of the green lineage. Otherwise, phenolamides constitute a class of specialized metabolites resulting from the conjugation of amines with phenolic acids that have been widely studied for their involvement in development, reproduction or biotic stress tolerance. Concerning synthesis of spermidine derivatives in pollen coat, it has been attributed to acyltransferases belonging to the BAHD family that catalyze acyl-CoA dependent acylation. Characterized enzymes from *Arabidopsis thaliana* or *Malus domestica* were up to now the only representatives of enzymes with ability to catalyze acylation of secondary amino-group of an aliphatic polyamine. Here, we identified new pollen coat phenolamides specific to the *Asteraceae* family that differ from the usual spermidine derivatives. In chicory (*Cichorium intybus* L.), we cloned and characterized two genes encoding BAHD acyltransferases involved in the metabolic diversification observed in this *Asteraceae* family. Further insights related to these new metabolites could give additional clues on the role of pollen coat phenolamides in regard to plant evolution.

Redox regulation of superoxide generation at photosystem I

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Photosynthetic electron flow is the main source of reactive oxygen species in the light. Leaves of *A. thaliana* plants grown under short day conditions (SD, 8 h light, 16 h dark) generate twice the amount of superoxide compared with plants grown under long day conditions (LD, 18 h light, 8 h dark). The site of superoxide generation was shown to be at photosystem I (PSI), indicating that superoxide was generated by the classical “Mehler reaction” or “pseudocyclic electron flow” (Michelet and Krieger-Liszkay, 2012). The Mehler reaction generated a higher proton gradient and more ATP as observedby comparing thylakoid membranes from SD and LD plants.

In plants lacking the chloroplast NADPH-dependent thioredoxin reductase (NTRC) the difference in superoxide production at the level of PSI between SD and LD was abolished (Lepistö et al., 2013). This observation points to a redox regulation of ROS generation at PSI. Thioredoxin m mutants (*trx m1m2*, *trx m4*) generated low amounts of superoxide independent of the growth conditions. In vitro assays using isolated thylakoids and recombinant proteins showed that superoxide production in thylakoids from long day plants was stimulated when they were reconstituted with Trx m4, NTRC and NADPH while these additives had no effect on thylakoids from short day grown plants. Immunoblots revealed that Trx m is attached to the thylakoid membrane in SD but not in LD plants. Mass spectrometry is performed to identify the difference in protein composition of PSI isolated from SD and LD plants. By this method the target of redox modification is identified. A model will be presented on the redox regulation of the Mehler reaction.

Michelet L, Krieger-Liszkay A (2012) Production of reactive oxygen intermediates produced by photosynthetic electron transport are enhanced in short-day grown plants. Biochim. Biophys. Acta 1817, 1306-1313

Lepistö A, Pakula E, Toivola J, Krieger-Liszkay A, Vignols F, Rintamäki E (2013) Deletion of chloroplast NADPH-dependent thioredoxin 1 reductase results in inability to regulate starch synthesis and causes stunted growth under short-day photoperiods. J. Exp. Bot 64, 3843-54.

The diversity of fungi associated with cabbage and their bio-control potential (FT)

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Endophytic fungi are increasingly used in biological control against various pests and diseases as agricultural producers attempt to reduce their environmental footprint.
This study aims to describe fungal communities associated with cabbage (*Brassica oleracea* var. *capitata*) and identify isolates with bio-control potential.
Putative endophytic fungi were isolated from cabbage grown in 3 regions in New Zealand. Fungal communities were described using metabarcoding data as well as direct isolation of fungi through culturing. Over 100 species were identified using culturing approaches, while metabarcoding approaches suggest the number of fungal species could be over 200. Selected fungal isolates were tested in bioassays to determine their potential for biological control of a fungal disease and insect pest of cabbage.

Systemic pathways regulating root architecture in the *Medicago truncatula* legume plant (FT)

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Legume plants adapt their root system architecture to environmental conditions by modifying root growth and lateral root number, as well as by developing new organs under nitrogen starvation and in response to Rhizobium symbiotic bacteria: the nitrogen-fixing nodules. Local and systemic regulatory pathways allow coordinating simultaneously the development of roots and nodules, notably depending on peptides perceived by Leucine-Rich Repeats Receptor Like Kinases (LRR-RLKs). A first systemic pathway regulating nodule number negatively rely on the action in shoots of the Super Numeric Nodules (SUNN) LRR-RLK and of CLAVATA3/embryo-surrounding region (CLE) peptide. We more recently identified in the *Medicago truncatula* model legume a second pathway systemically regulating nodule number, positively, depending on the Compact Root Architecture 2 (CRA2) LRR-RLK action in shoot and of C-terminally Encoded Peptides (CEP). Two antagonistic systemic pathways therefore control symbiotic nodulation. We are currently determining how the antagonistic SUNN and CRA2 systemic pathways regulating nodulation are coordinated, notably using a double mutant. In addition, we have identified targets acting systemically downstream of the CRA2 pathway in response to Rhizobia symbiotic bacteria using a transcriptomic approach. Ultimately, we aim to construct an integrated and dynamic model of nodule development regulation by systemic peptide/LRR-RLK pathways.

Phosphoregulation of photorespiratory enzyme SHMT1 in *Arabidopsis thaliana*

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Photorespiration is essential for the normal growth and development of plants in air (Bauwe *et al*., 2010). It can recycle the toxic metabolite 2-phosphoglycolate produced by Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RuBisCO) oxygenase activity. However, manipulate photorespiration has become a prime target for crop improvement because of losses of assimilated carbon, nitrogen and energy in this cycle. In the photorespiration cycle, there are eight core enzymes. At present, at least seven photorespiratory enzymes can be phosphorylated, and protein phosphorylation could be a key regulatory component in photorespiration (Hodges *et al,* 2013). But the knowledge about how it impacts plant metabolic pathways and functions and how they are coordinated is limited.
We have confirmed that the serine hydroxymethyltransfetase 1 (SHMT1) can be phosphorylated in response to different CO_2 content. We explored the effect of SHMT1 phosphorylation/non-phosphorylation events on plant physiology and metabolism using *shmt1* complemented mutants in respond to abiotic stresses.

Subvertion of organelle genes by bacterial PPR protein: study of *Erwinia amylovora*

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Mitochondria and chloroplasts are key players in cell-autonomous defense mechanisms of eukaryotic cells, which make them attractive targets for pathogens. To successfully infect their host, pathogens must suppress or by-pass the host defenses and subvert host functions. To do so, bacterial pathogens are using protein effectors. Among them are the pentatricopeptide repeat (PPR) proteins. PPR proteins are mainly found in land plants where they are involved in the regulation of organelle gene expression. They are also present in other eukaryotes but none of them are described in prokaryotes, mostly bacterial pathogens. So, as bacterial effectors, these PPRs are likely to suppress immune response mechanisms via the subversion of organelle gene expression. We focused on a bacterial PPR in *Erwinia amylovora*. Preliminary results show an enhanced susceptibility of *Malus communis* to the KO mutant ∆PPR of *Erwinia amylovora* compared to wild type bacteria. Plus, we confirm this result in an *Arabidopsis thaliana* pathosystem, which drove our decision to use *Arabidopsis* as a tool to study the role of PPR Ea *in planta*. To do so, overexpressing lines of *Arabidopsis* are used. PPR Ea was shown to interact in yeast with AtFES1c, a Nucleotide Exchange Factor (NEF) known to be targeted by various pathogen effectors from *Hyaloperonospora arabidopsidis*. The overexpressing lines as well as the ∆atfes1c KO mutant are more susceptible to *H. arabidopsidis*. Our data suggests that PPR Ea is an avirulence factor and AtFES1c is involved in a plant defense mechanism against various pathogens. A total and small RNA sequencing of the overexpressing lines coupled with segmentation analysis highlights a potential PPR footprint on psbA in chloroplast, without displaying any defect in photosynthesis under PAM fluorescence analysis.

Carotenoid and abscisic acid synthesis in *Arabidopsis thaliana* (FT)

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Abscisic acid (ABA) is a key element in seed development and germination as well as adaptive responses to environmental stresses. The tissue-specific modulation of its endogenous levels by fine-tuning of synthesis and catabolism determines its physiological action. In seeds, a major increase in ABA levels that occurs during the maturation phase regulates many developmental and maturation processes, including reserve storage, desiccation tolerance and dormancy. In vegetative tissues, ABA accumulation is triggered by water deficit, and induces stomatal closure to prevent water loss as well as longer-term responses. ABA is derived from the cleavage of C40 carotenoid precursors and to date genes coding enzymes responsible for most steps of the ABA biosynthesis pathway have been identified in Arabidopsis. ABA biosynthesis starts by the generation from β-carotene of oxygenated carotenoids termed xanthophylls. Xanthophyll conversions in plastids lead to the formation of *cis*-isomers of violaxanthin and neoxanthin and their cleavage into a C15 compound, xanthoxin, is the first committed step of ABA biosynthesis. Xanthoxin is then converted to ABA in the cytosol. Mutations in two different loci result in the absence of neoxanthin isomers (North et al. (2007) Plant J 50: 810-824; Neumann et al. (2014) Plant J 78: 80-93), however the function of the encoded proteins in the conversion of violaxanthin into neoxanthin remains obscure. Furthermore, despite similar defects of the two mutants *neoxanthin-deficient1* (*nxd1*) and the *ABA-deficient4* (*aba4*) in neoxanthin synthesis, only *aba4* was shown to be defective for ABA accumulation. Progress in the characterization of the respective roles of ABA4 and NXD1 will be presented.

Development of metabolomics techniques to study the Krebs and the Calvin cycles (FT)

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Measurements of metabolite levels and fluxes are useful for assessing the impact of perturbations in biological systems such as mutations and environmental stress conditions. Here we are interested in central metabolism, and especially organic acids and sugar phosphates as biomarkers of the Krebs cycle and Calvin cycle, respectively. Many of these metabolites are small polar molecules which are isobaric and therefore require a separation or a purification procedure prior to quantification. New advances leading to a higher resolution and a better sensitivity have been achieved in two techniques to improve the detection of such metabolites. First, nuclear magnetic resonance (NMR) spectroscopy is the favored technique to elucidate metabolite structure whereas quantification by NMR is often problematic. The measurement of organic acids in complex mixtures was possible by quantitative NMR ((q)-NMR) in 1-D experiments but to obtain a better trueness and sensitivity 2-D NMR spectra were analyzed using calibration curves and reference standards. Second, liquid chromatography coupled to electrospray ionization tandem mass spectrometry allows the detection of low concentrations of phosphorus compounds and an improved resolution of isobaric molecules. Since derivatization procedures are not required, both techniques allow a direct quantification without potential errors arising from differential or partial derivatization of molecules and therefore they are more suitable for fragile molecules. Specificity, linearity, precision, fidelity, quantification limits and accuracy will be discussed for each technique.

The Arabidopsis Target Of Rapamycin kinase: a coordinaTOR of stress responses (FT)

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Plants have to adapt their growth and metabolism to external stresses by regulating cellular processes such as mRNA translation and energy/nutrient homeostasis. The Target of Rapamycin (TOR) kinase is an evolutionarily conserved and central regulatory element that integrates nutrient, energy and hormone signals to regulate growth in yeast, animals and plants. Indeed, the TOR pathway controls essential biological outputs collectively contributing to growth and stress survival.
We will show that a decrease in TOR activity by genetic or pharmaceutical means has a strong effect on growth and affects the global transcriptome and proteome as well as primary metabolism. For example, mutations in the *Lst8* gene, coding for a component of the TOR complex 1, globally delays growth, flowering and impinges on the metabolic adaptations of Arabidopsis plants to long days. Moreover, TOR inhibition was found to decrease the expression and translation of a vast majority of nuclear genes coding for plastidic ribosomal proteins, which are regulated by many stresses in a coordinated way. TOR activity is also closely linked to hormone signaling and we have observed a decrease in ABA accumulation following TOR inhibition.
In conclusion, the TOR kinase is an important sensor and regulatory component in plants that is essential for growth and metabolic adaptations to the environment.

Phytotoxins produced by fungal pathogens of legumes.

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Legumes are annual and multifunctional crops with an extraordinary importance as food and feed sources worldwide, and also for their agricultural and environmental roles. Pathogenic fungi, e.g. those belonging to the genera *Ascochyta* or *Colletotrichum* are among the main biotic constraints of legume crops. Moreover, these genera are well-studied also because they produce a wide array of biologically active metabolites and phytotoxins belonging to different classes of natural compounds, differently involved in disease development and symptom appearance.
Our groups historically have been involved in studies on the production, purification and chemical and biological characterization of bioactive metabolites produced by plant pathogens, microorganisms and plants. We recently found interesting for our purposes two *Ascochyta* species, namely a strain of *A. lentis* var. *lathyri*, reported as a new pathogen of grass pea (*Lathyrus sativus*), and a strain of *A. lentis*, pathogen of lens (*Lens culinaris*). Furthermore, a strain of *Colletotrichum lupini*, an important and relatively unknown pathogen of lupin (*Lupinus albus*) proved to be particularly attractive for our scientific interests.
This communication will illustrate the isolation process, and the chemical and biological properties of the secondary metabolites produced by the two strains of *Ascochyta* and one of *Colletotrichum* mentioned above. The possible role of these metabolites in the development of disease symptoms will be also discussed.

The NTRC-2-Cys Prxs redox system controls the activity of functionally unrelated thioredoxins in Arabidopsis chloroplasts (FT)

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Thiol-dependent redox regulation of enzyme activity plays a central role in the rapid acclimation of chloroplast metabolism to ever-fluctuating light intensity. The chloroplast redox network is composed of a complex set of thioredoxins (Trxs), reduced by ferredoxin (Fdx) via a Fdx-dependent Trx reductase (FTR), and an NADPH-dependent Trx reductase with a joint Trx domain, NTRC, which efficiently reduces 2-Cys peroxiredoxins (Prxs). Thus, a relevant issue concerning chloroplast function is to establish the relationship between these redox systems and its impact on plant development. Based on the severe phenotype of the ntrc-trxx double mutant, lacking NTRC and x-type Trx and the ntrc-trxf1f2 triple mutant, lacking NTRC and f-type Trxs, we established that both systems act concertedly in plant development (Ojeda et al., (2017) Plant Physiol. 174(3) : 1436-1448). However, the dramatic growth inhibition phenotype of these mutants is suppressed by decreased contents of 2-Cys Prxs, as the ntrc-trxf1f2-Δ2cp (Pérez-Ruiz et al., (2017) PNAS 114 : 12069-12074) and ntrc-trxx-Δ2cp (Ojeda et al., *under revision*) mutants nearly recovered a wild type phenotype. Overall, our results show that the redox balance of 2-Cys Prxs, which is mainly controlled by NTRC, modulates the activity of functionally unrelated Trxs such as those of types f and x.

Mass spectrometry analysis of phytohormones produced by symbiotic fungi (FT)

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The arbuscular mycorrhizal symbiosis is a beneficial interaction between 80% of plant species and *Glomeromycota* fungi. The establishment of the symbiosis is engaged by a molecular dialog involving phytohormones (strigolactones, Gomez-Roldan *et al*., (2008) Nature 455:189-94. The phytohormones are secondary metabolites which modulate, at low concentration, important developmental and physiological processes in plants. They are produced by plants but also by some microorganisms (Chanclud *et al*., (2016) Molecular plant pathology 17:1289-97).

Our objective is to identify phytohormones produced by mycorrhizal fungi.
Ethylene production is measured by gas chromatography (GC). For auxins, cytokinins, abscisic acid, salicylate and jasmonate, we are using a global extraction and mass spectrometry analysis, using the Metatoul platform facilities (protocol adapted from Du et al., (2012) Anal. Bioanal. Chem. 403, 55-74). For strigolactones and brassinosteroids, the global analysis are under development.

Brassinosteroids regulate seed aging following priming in *Arabidopsis* natural accessions (FT)

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Seed germination and subsequent seedling growth are the initial steps in crop cultivation. To obtain vigorous seedlings as well as to synchronize their growth, a pre-sowing seed technique termed 'priming' is used commercially on post-harvest seeds. The treatment commences with imbibition of seeds in water under controlled conditions to trigger the metabolic processes that are normally activated during the early phase of germination (pre-germinative metabolism) and terminates by drying seeds prior to germination completion, or radicle emergence, so that seeds reenter a quiescent state. This technique often has an unwanted side effect of accelerating seed aging and thus seeds subjected to priming are less tolerant to long-term storage (Varier et al., 2010, Current Science 99:450–456). This loss of seed storability by priming is a disadvantage in terms of commercial seed storage and distribution.

In this study, the molecular mechanisms underlying seed ageing following priming were analyzed using *Arabidopsis* as a model to develop an innovative seed priming technique without undesirable accelerated aging. *Arabidopsis* is also a wild species and has many natural accessions with that exhibit marked genetic and phenotypic diversity. These accessions have been successfully employed to identify genes and causal mutations that are responsible for differences in phenotypes. A genome-wide association study (GWAS) was carried out using 169 accessions and detected 87 single nucleotide polymorphisms (SNPs) having significant correlation to seed aging after priming (FDR < 0.15, Benjamini-Hochberg method). Among these SNPs were located in 11 genic and/or intergenic regions at proximity to 20 genes that are annotated as sterol biosynthesis-related or signaling-related for the plant hormone brassinosteroids (BR) (unpublished data). In addition, an accession Est-1 that we have previously identified with better storability after priming than the reference accession Col-0 is being exploited (Sano et al., 2017, Sci. Rep 7:8095). Three major quantitative trait loci (QTL) have been identified, using recombinant inbred lines (RILs) derived from Est-1×Col-0, that correlate with the loss of seed viability on priming. RNA-Seq analysis of Est-1, Col-0 and RILs with the most extreme phenotypes revealed that genes related to BR biosynthesis/signaling and cell wall modification were highly expressed in primed seeds with shorter storage-life.

To examine the effect of BR on storage-life of the treated seeds, Col-0 seeds were primed with a bioactive BR (24-epibrassinolide; EBL) or a BR biosynthesis inhibitor (brassinazole; Brz). EBL accelerated aging following priming while Brz reduced it in a concentration dependent manner. Furthermore, primed seeds of BR biosynthesis-related gene mutants *cyp85a1/a2* and *det2* showed improved seed aging compared to wild type. These results suggest that BR have a negative effect on seed ageing after priming. Nevertheless, the germination speed of *cyp85a1/a2* and *det2* without priming was slower than that of wild type, whereas priming treatment efficiently enhanced the speed of both *cyp85a1/a2* and *det2*, to levels similar to wild type. The effect of BR on seed germination and aging following priming will be discussed.

Modification of sugar homeostasis and of organization of the vascular bundles in the floral stem of *Arabidopsis thaliana* under salt stress

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Sugar homeostasis is important for plant growth and abiotic stress responses and depends on adjusted carbon allocation between organs. The effects of the salt stress (150 mM NaCl) on levels of sugars and sugar transporters in leaves and floral stems of *Arabidopsis thaliana* L. plants of the Columbia-0 (Col-0) were studied. The beginning of salt stress was set up just before the floral transition to analyze the effect of the stress on the development of the floral stem, the main transport pathway for allocation of nutrients towards reproductive organs. Thirteen days after the beginning of salt stress, the content in sucrose and fructose was measured. We observed a higher sucrose and fructose content while starch content was lower in the floral stem in plants submitted to 150 mM of NaCl for 13 days. Expression analyses indicated that salt stress modulates the transcript levels of several target genes, including many genes involved in sugar homeostasis and sugar transport, some of which are already known to respond to salinity. The expression of the cell wall-invertase genes (*CwINV1* and *CwINV3*), cytosolic-invertase genes (*CINV1* and *CINV2*), fructokinase genes (*FRK1*, *FRK3*, *FRK4*, *FRK6*, *FRK7*) and a sucrose synthase gene (*SUSY3*) were significantly affected in the floral stem in response to stress. The expression of several sugar facilitator genes (*SWEET2*, *SWEET14*, *SWEET16*, *SWEET17*, *SWEET13*) were also affected, as well as a vacuolar monosaccharide transporter gene (*TMT2)*. Some of the responses differ significantly from those previously described in the rosette leaves at vegetative stage. We also observed that floral stem cross sections of plants subjected to salt stress exhibited lower xylem area than control plants, in addition to a significant number of collapsed xylem vessels and a modification of the composition of the secondary cell wall in xylem poles. In addition, we observed that an acclimation period of 4 days, with lower salt concentrations (50 mM and 75 mM) prior to beginning of salt treatment (150 mM) alleviated the effect of stress on the organization of the vascular bundles in the main floral stem and on the composition of the secondary cell wall of xylem cells. These results suggest that the plant response to a salt treatment on starch and sugar homeostasis is developmentally and spatially regulated, most probably through the regulation of sugar transporters and effects on carbon allocation in competing sink organs.

Key words: Salt stress, floral stem, sugar transport, sugar homeostasis, vascular bundle organization.

PII1: a protein involved in starch initiation that determines granule number and size in Arabidopsis chloroplast (FT)

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Most of photosynthetic organisms accumulate starch to store carbon and energy produced during photosynthesis. It is thus one of the most abundant storage polysaccharides on earth. Despite our knowledge of starch granule properties and metabolism, its initiation process remains poorly understood. Great progress in this field has been made through the discovery of the starch synthase 4 (SS4) as a major protein involved in starch priming in Arabidopsis chloroplasts. Indeed, this enzyme controls the number of the starch granules but also their shape and their size.

In this poster, we will focus on the analysis of a PROTEIN INVOLVED IN STARCH INITIATION (PII1 90KDa) that interact with SS4 within the chloroplast. This protein was identified as a partner of SS4 in a yeast-2-hybrid screen using SS4 as bait and BiFC experiments confirmed the interaction between the two proteins. As in *ss4* lines, *pii1*- mutants contain one large starch granule per chloroplasts while the wild-type contains 5 to 7 granules per plastid. The growth rate of the *pii1* mutants is equivalent to the wild-type plants and leaves display a dark green pigmentation, while *ss4* mutation induces a dwarf phenotype associated to pale-green leaves. This study reveals the involvement of the PII1 protein in the machinery determining starch granules number in *Arabidopsis thaliana* leaves.

Integration of high-throughput phenotyping and genomics data to explore Arabidopsis natural variation

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Most traits of interest to biologists and breeders are of quantitative nature, revealing the complex interplay of multiple genetic factors contributing to phenotypic variation, as well as their interaction with the environment.
Following a long history of quantitative genetics, it now becomes feasible to use naturally-occurring variation contained in Arabidopsis thaliana accessions as the source of quantitative genomics approaches, designed to map QTLs and resolve them at the gene level. The objectives we want to follow with this project consist in exploiting the wealth of data we have generated in the lab through quantitative genetics approaches on Arabidopsis natural variation, and make a strong contribution to dissecting the molecular mechanisms underlying plant responses to complex abiotic stress. The analysis of the molecular and functional variation leading to the targeted phenotype(s) in interaction with the environment will provide clues as to how and where in the pathways adaptation is shaping natural variation. Moreover, the new genes and physiological functions identified here will provide targets for stress-related breeding programs, as well as an integrative view of the biology of the species and its evolution.

This project thus starts from classical quantitative genetics/genomics approaches designed to map loci controlling any trait of interest. Skills and datasets in modern genetic approaches such as expression QTL analyses, metabolite GWAS analyses, bioinformatics identification of the most likely candidate genes or high-throughput phenotyping-based fine mapping and cloning, will be applied here to identify the genes and metabolites responsible for natural variation in multiple stress responses. Moreover, we can now start to integrate phenotypes at different levels of complexity to better dissect/reveal the underlying phenotypic variation.

The roles of different catalase isoforms in Arabidopsis

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Reactive oxygen species (ROS) are produced during plant development and environmental stress. Instead of a toxic byproduct, It has been well accepted as signaling players these years. Photoresipiration in peroxisome is one of the major pathways of ROS synthesis in plants. To keep ROS-related sinaling under good control, many antioxidative systems play important roles in cells. Among them H_2O_2 metabolism by catalases (CAT) in peroxisome has received particular attention. CAT possesses a high capacity of metabolizing H_2O_2. Its classical reaction is the dismutation of H_2O_2 to water and O_2. Among all three *CAT* genes identified in Arabidopsis to date, *CAT2* encodes the major leaf isoform and its function is closely linked to photorespiration.

Many work on CAT were focused on leaves before. While the roles of different CAT isoforms in roots remain to be elucidated. In our functional analysis of different *CAT* mutants, we found that CAT2 seems to be the most important isoform in roots as well as in leaves. CAT1 or CAT3 deficiency doesn't exacerbate the *cat2* phenotype significantly. The presence of sugar partly rescues the phenotype caused by *CAT2* mutation. Instead, hCO_2 rescues it almost completely. As is known before, hCO_2 condition could avoid the affect by photorespiration in *cat2* leaves, so *cat2* root phenotype is probably a secondary nutritional effect of insufficient catalase activity in leaves.

Histone deacetylase HDA9 and transcription factor WRKY53 are mutual antagonists in regulation of plant salt stress-response

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Epigenetic regulation of gene expression is important for plant adaptation to environment changes including stresses. How epigenetic regulators integrate environmental signals to targets to and to regulate chromatin modification at specific genomic loci remains largely unknown. Here, we show that Arabidopsis RPD3-like histone deacetylase HDA9 represses stress-tolerance by interacting and regulating activity of AtWRKY53 that functions a higher hierarchy positive regulator of salt stress resistance. We found that AtWRKY53 is post-translationally modified by lysine acetylation which is negatively regulated by AtHDA9. AtHDA9-mediated lysine deacetylation of AtWRKY53 is likely to result in inhibition of its transcription activity. In addition, we observed that AtWRKY53 also suppresses AtHDA9 histone deacetylase activity. The results indicate that AtHDA9 and AtWRK53 are reciprocal negative regulators of their activities and reveal a functional interplay between a chromatin regulator and a transcription factor to regulate stress tolerance in plants.

Impact on the xylem development of sugar transport modification in *Arabidopsis thaliana*

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During its development, the xylem tissue requires an important amount of sugars in order to sustain the formation of its secondary cell wall. However, the mechanisms by which sugars are transported to their site of use are far from being understood. Among the proteins that could achieve this task, two members of the SWEET (Sugar Will Eventually Be Exported Transporters) sugar transporters family were recently shown to be good candidates(1). Additionally two others *SWEET* genes has been shown to be expressed in the xylem cells of the floral stem(2,3) and could also be involved in the same processes. The purpose of this PhD project is to further characterize the role of these SWEET transporters in the vascular system development and during the xylem secondary cell wall formation. I will therefore characterize mutant lines of *SWEET11*, *SWEET12, SWEET16* and/or *SWEET17* (simple, double, triple and quadruple mutants). A phenotypical analysis in long-day growth conditions will be perform to analyze growth parameters kinetics, stem morphology and xylem anatomy. The composition of the xylem cell wall will also be considered by infrared spectroscopy (FTIR). Moreover potential modifications of the carbon metabolism will also be assessed in the different mutant lines by quantifying the amount of soluble sugars and by measuring the modification of key enzymes at the gene level (qPCR analysis) and/or at the protein level (enzymatic activities). The impact of mutations in these genes will also be addressed in abiotic stress conditions such as freezing tolerance and drought stress, which are known to impact the sugar metabolism. Finally, in order to better characterize the SWEET proteins function, the affinity of these transporters for precursors of the cell wall polysaccharides will be evaluated by a biochemical approach such as a yeast functional complementation and/or transport assays in Xenopus oocytes.

Keywords: SWEET, sugar allocation, vascular system development, secondary cell wall.

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Gene networks regulating the development of endothelium cells in Arabidopsis seeds

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In Arabidopsis, seeds are composed of three main compartments: an embryo, an endosperm and maternal tissues. The latter comprise in particular a seed coat, involved for instance in seed protection, nutrient transport and dormancy. The endothelium is the innermost cell layer of the seed coat, acting as the interface between seed coat and endosperm. Particularly, the endothelium is the production site of condensed tannins, a class of flavonoid compounds of physiological and agricultural interest that give their brown color to Arabidopsis seeds. To date, several genes involved in tannin accumulation in the endothelium have been discovered and named *TRANSPARENT TESTA* (*TT*), according to the yellow color of their respective mutant seeds. Whilst a handful of these genes encode key enzymes in the biosynthesis of tannins, several others code for transcription factors involved either in the fine-tuned transcriptional regulation of tannin accumulation, or in the proper development of endothelium cells. These developmental genes, which include in particular *TT16* (Nesi et al. (2002) Plant Cell 14:2463-79) and *TT1* (Sagasser et al. (2002) Genes Dev 16:138-149), are of special interest for they act upstream in tannin production. We previously demonstrated that *TT16* is also involved in the developmental patterning of the parenchymatic cell layer adjacent to the endothelium (Coen et al. (2017) Development 144:1490-1497). Furthermore, we recently characterized a cutin-like apoplastic barrier, situated in between the endothelium and the endosperm, whose deposition is part of endothelium developmental program and is controlled by both *TT16* and *TT1* (Coen et al. unpublished). We are now focusing our work on broader genetic networks regulating endothelium development, and involving TRANSPARENT TESTA GLABRA 2, the PHABULOSA HD-ZipIII, and the SEEDSTICK and SHATTERPROOFs 1/2 MADS-Box transcription factors.

PUX10 associates with CDC48A and regulates the dislocation of ubiquitinated oleosins from seed lipid droplets (FT)

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Storage lipids are compartmentalized in specialized organelles called lipid droplets (LDs, also referred to as oil bodies or oleosomes), which predominantly accumulate in many dicotyledonous seeds to provide carbon and energy for seedling growth. Originally considered as inert balls of fat, intracellular LDs are now recognized as dynamic organelles. Although LD dynamics has attracted attention for a few decades in mammals with regard to human pathologies like obesity, diabetes and steatosis, this topic has emerged only recently in plants. Very little is known with regard to the mechanistic details that control the turnover of LD proteins in plants.
Post-germinative mobilization of neutral lipids stored in seed LDs is preceded by the degradation of oleosins, the major structural LD proteins that stabilize LDs in dry seeds. We previously showed that Arabidopsis oleosins are marked for degradation by ubiquitination, and extracted from LDs before proteolysis (Deruyffelaere et al., 2015). However, the mechanisms underlying the dislocation of these LD-anchored proteins from the LD monolayer are yet unknown. Here, we report that PUX10, a UBX domain-containing protein, is an integral LD protein that associates with a subpopulation of LDs during seed germination. We phenotypically characterized and functionally complemented two independent T-DNA mutant lines of *PUX10* in Arabidopsis. Using quantitative immunoblotting and subcellular fractionation approaches, we showed that PUX10 deficiency delays the degradation of oleosins and ubiquitinated oleosins, and prevents the extraction of ubiquitinated oleosins from the LD monolayer. We also identified several functional domains of PUX10 by expressing truncated versions of the protein in tobacco leaves or in yeast. In addition to a membrane-anchoring domain, PUX10 possesses two functional UBA and UBX domains that mediate interactions with ubiquitin and the CDC48A AAA-ATPase, respectively. Collectively, these results strongly suggest that PUX10 is a LD-anchored scaffolding protein recruiting CDC48A to ubiquitinated oleosins, thus promoting the dislocation of oleosins from LDs by the segregase activity of CDC48A. We propose that PUX10 and CDC48A are core components of a novel LD-associated degradation machinery, tentatively named LD-associated degradation (LDAD) system. Importantly, our comprehensive analysis of PUX10 localization using Arabidopsis transgenic lines expressing PUX10-GFP under native promoter revealed that PUX10 is progressively expressed and sequentially targeted to a specific subpopulation of LDs in germinated seeds. PUX10 is therefore the first determinant of a LD subpopulation identified in plants, suggesting that the functional differentiation of LDs described for several years in mammals and yeast, occurs also in plants.

Deruyffelaere, C., Bouchez, I., Morin, H., Guillot, A., Miquel, M., Froissard, M., Chardot, T., and D'Andrea, S. (2015). Ubiquitin-Mediated Proteasomal Degradation of Oleosins is Involved in Oil Body Mobilization During Post-Germinative Seedling Growth in Arabidopsis. Plant Cell Physiol. 56, 1374-1387.

Dynamic, multiscale, *in vivo* imaging of *Arabidopsis* roots in microfluidic devices (FT)

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A recently developed microfluidic platform, the Rootchip, demonstrated a new technology for *in vivo* imaging of plant roots (Grossmann, *et al*. (2011) *Plant Cell* 23: 4234-4240, and Jones, *et al*. (2014) *Elife* 3:e01741). We have expanded on the use of PDMS microfabrication on coverslips to create a wide variety of microfluidic devices for *in vivo* dynamic imaging of model *Arabidopsis*, and potentially other, plant roots. These microponic chips offer access to detailed imaging, measurement and analysis of multiscale 4D cellular processes and events that may be unravelled thanks to the concomitant development of plant lines containing targeted, genetically encoded, fluorescent nanosensors. Current uses of these microponic chips combined with nanosensor imaging include, for example:

- the elucidation of key cell cycle and division events by imaging membrane, cytoskeleton, and cell-cycle markers, and finely mapping cellular parameters relevant to the temporal/spatial control of cell divisions in the root meristem (Schaefer, *et al*. (2017) *Science* 356:186-189),
- the study of cell elongation through the use of biosensors such as pH, calcium and ROS, and inhibition of cell elongation by drug application,
- the elucidation of early cellular events involved in autophagosome formation, and controlling the induction of autophagy through stress (Le Bars, *et al*. (2014) *Nat. comm*. 5:4121), and
- the study of ion and metal trafficking, concentration, localization and transport (Lanquar, et al. (2014) *New Phytol*. 202: 198-208).

Further microponic chips under development include mechanosensing chips, for studying the roles of mechanosensitive channels in response to stress (Peyronnet, *et al*. (2014) *Front. Plant Sci.* 5 : 558), through the use of integrated features and dynamic valves for inducing passive or dynamic mechanical constraints on the root, as well as microfluidic chips compatible with root study by deep-UV fluorescence microscopy.

Transcriptional regulation of flavonoid biosynthesis in Arabidopsis seed

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Flavonoids are secondary metabolites that are ubiquitously distributed in plants and known for the colours they confer to various tissues such as flowers, leaves, fruits, or seeds. They contribute to plant adaptation to their biotic and abiotic environments as antioxidants, antimicrobial, light filters, pigments, allelochemicals, or as developmental regulators. They also display nutritional and pharmacological properties with health benefits and impact on food quality. Consequently there is a strong interest to breed crops with optimized flavonoid composition, especially in the global context of climate changes and required limitation of synthetic chemicals for a more sustainable production. In addition, as convenient biological markers, flavonoids have been instrumental in major genetic and epigenetic discoveries. These positive biological, agronomic and societal attributes explain the increasing interest for the study of flavonoids in recent years.

Flavonoid biosynthesis pathway is well established and the elucidation of transcriptional regulations has greatly progressed during the recent years, including the characterization of various MYB transcription factors and more specifically of MBW (MYB–bHLH–WDR) regulatory complexes. These proteins are well conserved in higher plants. They participate in different types of controls ranging from fine-tuned transcriptional regulation by environmental factors to the initiation of the flavonoid biosynthesis pathway by positive regulatory feedback. Nevertheless, there is still little information on the environmental or developmental regulation of the *MBW* genes. On this line, the target genes and the mechanisms involving three Transparent Testa (TT) regulators, namely TT1 (WIP1 / Zn-finger), TT16 (ABS/AGL32, MADS box), and TTG2 (WRKY44) remains to be identified and characterized.

Thus, we started a new genetic screening based on the suppression of "*transparent testa*" (*tt*) phenotypes of these regulatory mutants. We expect to isolate a large number of suppressors since the mutated genes could be involved either directly in the regulation of flavonoid genes (e.g. induction of MYB or bHLH expression or activity, or repression of various inhibitors) or indirectly (cell differentiation or activation of biotic or abiotic signalling pathways). In parallel we wish to conduct a thorough analysis of the genetic and epigenetic regulation of *MBW* gene expression. The identification of new actors of the flavonoid regulatory network will pave the way for new routes of research ranging from the transcriptional control of secondary metabolites to seed development, and the production of flavonoids for improving the quality of plant products and the sustainability of plant production.

Seed tissue and nutrient partitioning: the role of the nucellus (FT)

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In angiosperms, seed architecture is shaped by the coordinated development of three genetically different components: embryo, endosperm, and maternal tissues. The relative contribution of these tissues to seed mass and nutrient storage varies considerably among species. The development of embryo, endosperm, or nucellus maternal tissue as primary storage compartment defines three main typologies of seed architecture: endospermic, non-endospermic and perispermic. The ancestral condition of angiosperm seeds is still debated between endospermic and perispermic as basal angiosperms display either a large nucellus or endosperm as primary seed storage compartment. This project aims at deciphering the genetic and molecular mechanisms underlying tissue and nutrient partitioning in A*maranthus* perispermic and *Arabidopsis* endospermic seeds, with an emphasis on the study of nucellus development. We rely on an integrated interdisciplinary approach that spans tissue three-dimensional reconstruction, cell-type-specific transcriptomic profiling, and gene network and metabolic analyses.

Genetic dissection of cell adhesion in Plants (FT)

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The mutants defective in QUASIMODOS present abnormal pectin biosynthesis resulting in a cell adhesion phenotype (Bouton *et al.* (2002) Plant Cell 14:2577-2590; Mouille *et al.* (2007) Plant J 50:605-614). We have previously performed a genetic screen to isolate mutants suppressing the *quasimodo* phenotype. We identified one suppressor mutated in *ESMERALDA1*. This gene encodes a putative O-fucosyltransferase similar to POFUT known to fucosylate the EGF containing protein in animals. POFUT is involved in the control of a range of processes such as cell differentiation, cell adhesion, cell proliferation. This work demonstrates that the cell adhesion is tightly tuned during plant development through a complex signal/transduction pathway (Verger *et al.* (2016) Development 143:2536-2540). The perception of a signal from the extracellular matrix probably mediates (through kinase(s) activity(ies)) transcriptional regulation of genes and a feedback control on the quality of the cell wall in order to modulate its properties and consequently, the cell-cell cohesion.

We recently showed that cell-cell cohesion is impacted or impacts the quality of the cell wall (pectin content), a specific transducing pathway, the cytokinins content, and the transcription of specific genes. This pleiotropic phenotype confirms the complexity and multilevel of the pathway involved in the control of cell adhesion. We then performed a new suppressor screen. The work presented here attempts to classified the mutants according to the mandatory features, listed above, implicated in the control of cell-cell cohesion.

An O-fucosyltransferase controls pectin methylesterification in the cell adhesion defective mutant *qua2* (FT)

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Cell adhesion between neighbouring plant cells is established by the pectin rich middle lamella. The most abundant pectic polymer in the middle lamella is homogalacturonan (HG). Severe decrease in HG content was linked with cell adhesion defects in *quasimodo1* (*qua1*) and *quasimodo2* (*qua2*) (Bouton *et al.* (2002) Plant Cell 14:2577-2590; Mouille *et al.* (2007) Plant J 50:605-614). But, the reduced HG content is unlikely to be the only actor causing the cell adhesion defects since: (i) mutants with reduced HG content have been described displaying proper adhesion (e.g. *irx8*) and (ii) mutants with proper HG content display cell adhesion defects (e.g. *frb1*). Further, a suppressor screen on *qua2* identified the *esmeralda1* (*esmd1*) allele, which restored cell adhesion without restoring HG content (Verger *et al.* (2016) Development 143:2536-2540). HG is delivered to the cell wall in a highly methylesterified form and is demethylesterified in the cell wall by the action of pectin-methylesterases (PME). The methylesterification pattern has been shown to play a mandatory role in plant development and impacts the mechanical properties of the cell wall (Pelletier *et al.* (2010) New Phytol 188:726-739); Peaucelle *et al.* (2015) Curr Biol 25:1746-1752).
In order to determine the potential role of the methylesterification pattern of the HG in cell to cell adhesion we used an oligo-profiling approach in various mutant backgrounds and investigated the expression of genes encoding PMEs (66) and PME inhibitors (PMEI, 64). The cell wall located PME determine the methylesterification pattern of HG. The altered expression of PME genes we observed correlates with altered pectin-methylesterification patterns, in the cell adhesion defective *qua2* strain and in its suppressor *esmd1*.
ESMD1 is a putative O-fucosyltransferase with homology to POFUT in animal cells (Verger *et al.* (2016) Development 143:2536-2540). POFUTs are fucosylating EGF-repeats which are required for cell-cell communication, protein trafficking, protein-protein interaction and cell adhesion (Haltom and Jafar-Nejad (2015) Glycobiology 25:1027-1042). A few plant proteins possess EGF-repeats in their extracellular domain (e.g. WAK). WAKs may recognize pectin and transduce feedback signals from the cell wall (Kohorn (2016) J Exp Bot 67:489-494). Our results suggest that ESMD1 might control cell adhesion by feedback signals originating from the cell wall in particular through the indirect control of esterification pattern of HG through the control of PME and PMEI expression level.

Functional characterization of the PPR336 protein, a plant-specific component of the mitoribosome (FT)

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Mitochondria are essential cellular organelles of eukaryotic cells that are mostly devoted to the production of ATP by the oxidative phosphorylation involving the electron transport chain (ETC). To express their genetic content and produce the subunits of the ETC, plant mitochondria use sophisticated gene expression processes involving many post-transcriptional processing steps. Many factors related to mitochondrial mRNA processing have been found in the last years. However, the process of mitochondrial translation, the last level of gene expression, is still largely unclear. The PPR336 protein was previously found to co-sediment with mitochondrial polysomes (Uyttewaal *et al.*, 2008). In this new analysis, it was found to be a plant-specific component of highly purified Arabidopsis mitoribosome, but its function in mitochondrial translation was not resolved. The function of PPR336 protein has been investigated and shown in this poster.

Modeling leaf morphodynamics

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In nature, there is a wide variety of leaf shapes. However, the leaf always appears at the margin of the meristem as a mass of cells with a simple finger-like shape called primordium. This shape changes during growth and can become extremely complex at the mature stage. Our goal is to study the mechanisms that are responsible for leaf diversity. Morphogenetic determinants and their interactions that control its shaping during growth remain largely unknown. There identification and characterization is a complex question that has captured the attention of many scientists (Kuchen et al., 2012 ; Rolland-Lagan et al., 2014 ; Rodriguez et al., 2014). Modeling is a strategy adapted to understand, and explain complex systems such as leaf growth and models were proposed for this purpose. In the literature, the models do explain not the precise shape of leaves observed in nature.

Moreover, to properly implement and challenge a model, it is necessary to precisely quantify the shape all over the development. MorphoLeaf (Biot et al., 2016) is an application that proposes tools for the quantification of individual leaf shape (area, length, width, perimeter), at global and at local levels. Further, MorphoLeaf proposes to compute average curves from contours of individual leaves to build a growth trajectory.

Our strategy is to propose a modeling framework for a better understanding of the mechanisms responsible for leaf shape changes, and that precisely explains the leaf morphology at all stages of the growth. A numerical model was implemented at the tissue scale. Moreover, this model allows us to simulate a growth trajectory. A method has been developed to optimize the model parameters, based on a measure of similarity between real and simulated trajectories.

We showed that the appearance and the teeth growth on the leaf margin are controlled by the same biological mechanisms, whatever the phenotypes of wild type leaves.

Impact of DCL3 in small RNA dynamics and epigenetic regulation of nitrogen fixing nodules in Medicago truncatula? (FT)

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Small RNAs are essential regulators of gene expression during plant development, responses to the environment and plant-microbe interactions. These non-coding RNAs, 21-24 nucleotides long, regulate the expression of target genes at the post-transcriptional (PTGS) or transcriptional (TGS) levels. Several microRNAs, mainly targeting transcription factors, have been functionally associated to the control of symbiotic nodule development in legumes. However, much less is known about the roles of siRNAs in this process. Small RNAs are produced after cleavage of long double-stranded or hairpin RNA precursors by enzymes of the ribonuclease III family, called DICER-LIKE proteins (DCL). DCL3 encodes the DCL involved in heterochromatic 24-nt sRNA biogenesis and RNA-directed DNA methylation (RdDM). In Medicago truncatula nodules, MtDCL3 expression is higher in the meristematic zone than in the differentiation region of the nodule, suggesting a specific role of DCL3 and putatively 24nt siRNAs in nodule meristems. Furthermore, we showed that mutations (Tnt1 insertional mutants) or RNAi lines of MtDCL3 allow the development of bigger, multi-lobed and more nitrogen-fixing nodules. The impact of DCL3 on nodule small RNAs is undergoing as well as the impact of DCL3 on epigenetic regulation of this organogenesis.

Functional GUS assay of GRAS transcription factor from *Medicago truncatula* (FT)

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GRAS gene family encodes transcriptional factors (TFs), which play vital role in different processes of plant growth and development of stems and roots, phytohrome A signal transduction (PAT), gibberellic acid signal transduction (GA), plant response to biotic and abiotic stress conditions and disease resistance. They could be induced by salt and drought treatment and in response to pathogen attack. Recently by using the data from *M. truncatula* genome and phylogenetic analyses, 68 *MtGRAS* genes were identified and classified in 16 groups, localized in 8 chromosomes. The expression profile of some of these genes in the response to different abiotic stress conditions was analysed. Comparing data from Phytozome (http//phytozome.jgi.doe.gov) and PLAZA3,0 data bases and blast analyses we have established that *MtGRAS* gene, previously identified on the basis of *Tnt1* insertion mutagenesis and later cloned (MT2g026250), corresponds to one of those 68 genes pointed as *MtGRAS7* (Medtr2g026250). During the GRAIN LEGUMES integrated project – GLIP, FP6 of EU *M. truncatula Tnt1* insertion mutant collection was created. Randomly selected mutant lines from *Tnt1* insertion mutant collection were screened by transposon display (TD) technique in order to confirm presence of new copies of *Tnt1* retrotransposon. Some of the randomly selected mutant lines obtained by research team of AgroBioInstitute (ABI), *Tnt1* insertions were sequenced. The plant genomic region that borders the *Tnt1* (Flanking Sequence Tag, FST) could be identified and some of them were located in the coding sequences of different genes. Investigated by us *MtGRAS* TF (MT2g026250, PLAZA3.0), corresponding to insert 6 of *Tnt1* mutant line So5945, corresponding to Medtr2g026250 (http//phytozome.jgi.doe.gov). In the frames of the project "Integrated functional and comparative genomics studies on the model legumes *Medicago truncatula* and *Lotus japonicus*" DO-02-268, funded by BNSF, transcriptional reporter plants carring the promoter region of the gene encoding *MtGRAS* TF (MT2g026250) fused to GUS and GFP reporter genes were obtained via *Agrobacterium*- transformation of suspension cultures. The activity of the both marker genes was detected in various parts and tissues of T1 generation of transformed plants. The expression of both marker genes was localized in root columnela and vascular system of the root and in the secondary root primordium, in the vasculature of leaves and stems and as a spot expression in the petiole. In mature plants in hydropon condition GUS signal was detected in meristematic zone of the induced nodules. Our preliminary obtained results, together with recently published information for the role of GRAS TFs in plant response to various abiotic and biotic stress, led us to continue with detailed analyses related to the expression pattern of investigated by us gene under stress conditions. In the frame of newly initiated project "Functional and bioinformatics analyses of *GRAS* transcription factors related to the response of abiotic and biotic stress in annual (*Medicago truncatula*) and perennial (*Medicago sativa*) alfalfa, funded by BNSF, transcriptional reporter plants (14-days-old) were grown in hydroponic system and divided into two groups - one were subjected to salinity stress (50 mM NaCl) and the other control group were grown in normal condition. Samples from control and treated plants were taken at the time points 0, 24, 48, 72 h after treatment. GUS signal was observed in both groups treated and not treated in root tips, secondary root branches and in leaves. Decrease in intensity of the GUS signal was observed with increasing the time for treatment.

The role of plant cell wall deposition and metabolism in the plant-pathogen arms race (FT)

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Plants produce specialized cell wall structures, the so-called papillae, that constitute the first line of defense against appressorium-forming fungal pathogens. Papillae are remarkably strong assemblies of cell wall polymers that can resist enzymatic degradation and the extreme pressures that are exerted by the penetration peg of the fungal appressorium (O'Connell (2004) Mol Plant Microbe Interact *17*, 272-282). The success of the initial immune response depends on the ability of the plant to lay down sufficiently strong papillae before the appressorium is formed. Very little is known on the structural determinants of these extreme mechanical properties of the papillae and the underlying cellular processes. On the other hand, fungal pathogens most likely evolve strategies to interfere with papillae formation by producing toxins or effector proteins that target these cellular processes (O'Connell (2012) Nat Genet *44*, 1060-1065). The identification of such fungal anti-defense compounds should provide interesting tools to study of the cell biological processes underlying papillae formation and may lead to new strategies for plant protection as well as sources of novel herbicides.

EPITRANS: Exploring, and creating genetic and epigenetics diversity (FT)

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Agricultural yields have been greatly enhanced in the past 50 years. However, climatic perturbations and the spread of pests and pathogens are major upcoming threats to agriculture worldwide. Crop selection led to a reduction in the allelic variability and thereby narrowed the possibilities for genetic improvement. In order to carry on the genetic improvement process, we need to identify new phenotypes (i), identify the genetic basis underlying those phenotypes (ii) and finally transfer those phenotypes in crops (iii). Thanks to last decades' efforts in plant sciences, many of the genetic and epigenetic regulators controlling key aspects of plants developments and plants interactions with their environments are known: stress resistance, photosynthesis, defense against pathogens etc. Despite this massive amount of data, the translation of the findings into traits in elite varieties is modest. This could be explained by the absence of a national infrastructure dedicated to translating plant research into leader alleles that breeders can use to create new plant prototypes.
To help to translate fundamental research into leader alleles we have established two platforms, the EPIGENOMICS platform and the TRANSLATIONAL RESEARCH platform.

The IPS2 EPIGENOMICS platform proposes state of the arts tools to investigate the contribution of the epigenome in the control of biological processes in crop species, with the final goal to identify epigenetic targets for crop improvement

The TRANSLATIONAL RESEARCH PLATFORM proposes state of the arts tools to genetically validate the concepts and to translate the finding into leader alleles.

Because the two platforms raise the challenge of helping the scientific community to investigate agronomic traits in model and crop species, the EPIGENEMOMICS and TRANSLATIONAL RESEARCH PLATFORMS merge in a single platform EPITRANS.

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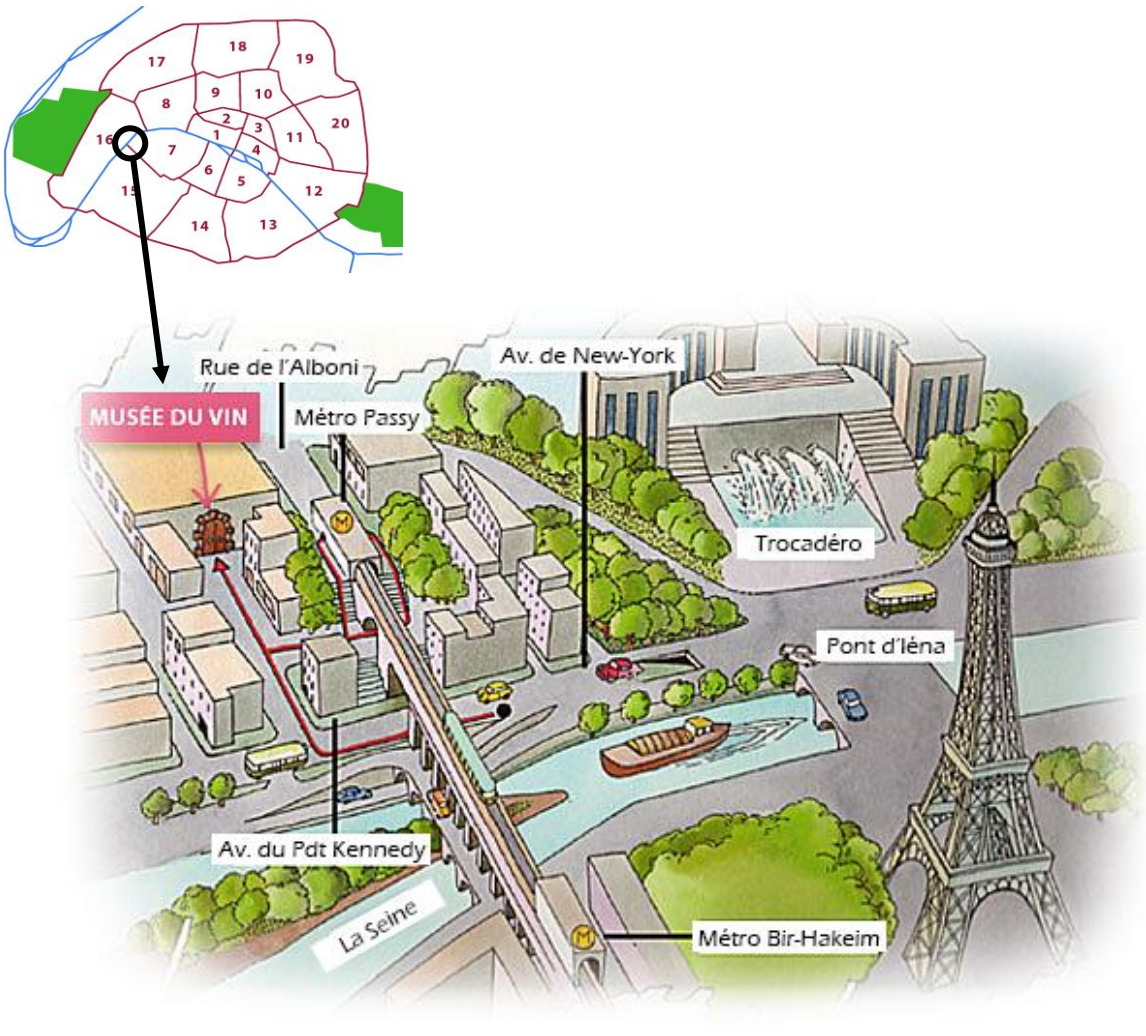

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