INUPRAG Symposium on Integrative Plant Biology

21-23 February

Hotel Mimer in Umeå

Umeå Plant Science Centre

INRAE science for people, life & earth

CRAG CENTRE FOR RESEARCH IN AGRICULTURAL GENOMICS

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Day 1 - Tuesday, February 21, 2023

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Rozenn Le Hir (IJPB, INRAe, Versailles, France)

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Christian Dubos (IPSIM, INRAe, Montpellier, France)

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Chair person: Manvi Sharma (Postdoc in Petra Marhava's group)

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Elena Monte (CRAG, Barcelona, Spain)

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9:40 SUMO modulates senescence through the control of the ethylene signaling pathway in Arabidodpis
Maria Lois (CRAG, Barcelona, Spain)

10:00 The phosphoinositide signature guides the final step of plant cytokinesis
Marie-Cécile Caillaud (RDP, INRAe, Lyon, France)

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Chair person: Shahzad Anjam (Postdoc in Peter Marhavý's group)

11:10 Root growth against mechanical obstacle: the early growth response of a maize root facing an axial resistance agrees with the Lockhart model
Marie-Béatrice Bogeat-Triboulot

11:30 How wounded plants coordinate their healing and immune responses
Peter Marhavy (UPSC, SLU, Umeå, Sweden)

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Antoine Martin (UMR IPSIM, INRAe, Montpellier, France)*)*

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Alizée Malnoë (UPSC, UmU, Umeå, Sweden)

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Chair person: Justine Colou (Postdoc in Torgny Näsholm's group)

16:20 Do SSPs regulate ectomycorhizal symbiosis in response to biotic and abiotic nutrient signal in trees?
Clémence Bonnot (UMR IAM, INRAe, Université de Loraine, Nancy, France)

16:40 Root exudate simulation using microdialysis
Sandra Jämtgard (Dpt of Forest Ecology and Management, SLU, Umeå, Sweden)

17:00 From the study of fungal symbiotic effectors towards a central role of root terpenes for tree-microbes relationships
Claire Veneault-Fourrey (UMR IAM, INRAe, Université de Loraine, Nancy, France)

17:20 Conclusion and announcement of the poster award winner
18:00 Conference dinner

Session I

Plant cell wall properties and dynamics

4DWalls: understanding dynamism of plant cell walls

Laura Bacete[1,2]

[1]Umeå Plant Science Centre (UPSC), Department of Plant Physiology, Umeå University, Umeå, (Sweden); [2]Department of Biology, NTNU, Trondheim (Norway)

The cell wall is a complex and dynamic structure that plays an essential role in plant development and interaction with the environment. The discovery of the cell wall integrity (CWI) monitoring system is a good example of the change in the field of plant cell wall research that has occurred in the past two decades. The paradigm has shifted from considering this structure to be a passive structure to understanding it as a dynamic network in which both cell wall composition and structure are dynamic and vary between different cell types, developmental stages, and environmental conditions.

This dynamic behaviour of plant cell walls is known as cell wall plasticity. However, despite its importance, the mechanisms by which the cell wall responds to changes in composition and structure are not fully understood. For example, although it is intuitive that changes in cell wall composition impact cell wall structure and, consequently, cell wall mechanical characteristics, there is minimal experimental evidence supporting this idea. A reason for that is that studying dynamic processes is always challenging, with limited results obtained in the past years. Therefore, a holistic, multidisciplinary approach that examines changes in cell wall composition and structure over time is required.

In this talk, I will discuss recent advances in our understanding of cell wall dynamics, with a focus on how changes in cell wall composition impact cell wall structure and its mechanical properties. I will also highlight future directions for research in this field.

New model of plant growth and cell wall synthesis brought to light by optical nanoscopy

Kalina T. Haas[1]

[1]*Institut Jean-Pierre Bourgin, INRAE, AgroParisTech, Université Paris-Saclay, 78000 Versailles, France*

How plants grow and assume their shapes is one of the most pertinent questions in plant biology. The plant cell is surrounded by a dense pecto-cellulosic extracellular matrix, the cell wall (CW). The modifications of CW structure and chemistry are a prerequisite for plant cell expansion. To understand growth, it is mandatory to resolve the nanoarchitectures of the cell wall polymers. For a long time, the major limitation to solving this problem was the absence of appropriate microscopy that could resolve the chemical and the spatial organization of the polymer in tissue with nanometer resolution. In my presentation, I will show how 3D stoichiometric multicolor nanoimaging empowers our understanding of plant growth by enabling high-density mapping of cell wall polymers with molecular specificity[1,2].

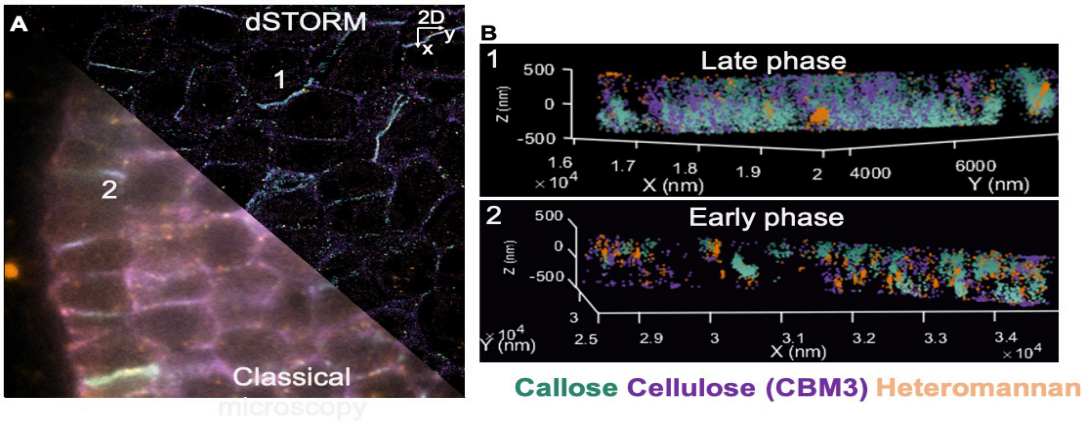

Figure 1: *Multicolor 3D dsTORM imaging of cell wall assembly in the apical meristem. A) A 2D (XY) epifluorescent image (bottom left corner) compared to 2D super-resolved dSTORM image (top right corner). Numbers show cell plates highlighted in B) in 3D view.*

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Mechanics and dynamics of cell-cell adhesion in plants

Stéphane Verger[1,2]

[1]Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Swedish University of Agricultural Sciences, Umeå, Sweden; [2]Department of Plant Physiology, Umeå Plant Science Centre, Umeå university, Umeå, Sweden.

How cell-cell adhesion is achieved is a fundamental question in the development of multicellular organisms. Surprisingly, this question remains largely under-explored in plants and much remains to be discovered. In plants, cell-cell adhesion is physically mediated by the cell wall. This is not only passive: in previous work we identified a signalling pathway for the active maintenance of cell-cell adhesion in plants.[1] Furthermore, we demonstrated how tensile stress in tissues tends to pull the cells apart in the epidermis during growth and development and may also act as an instructive cue for cell adhesion maintenance in plants.[2] In our current work we combine micromechanical approaches with molecular, cellular and developmental biology to investigates this question. Notably, we are developing and adapting a set of micromechanical tools to characterise the mechanics of cell-cell adhesion in plants.[3] We are also developing new transgenic lines in which we can impair cell adhesion in a controlled spatio-temporal manner (adhesion lines). In parallel, we study the processes of wood formation and in particular xylem fibers intrusive growth which requires a tight control of cell adhesion. We are generating new poplar reporter lines as well as mutants in which adhesion is impaired, to better understand this process. Altogether, our research aims to uncover fundamental molecular and biophysical mechanisms that regulate cell adhesion in plants from adhesion maintenance in Arabidopsis epidermis, to wood formation in poplar.

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The suberinteresting role of GELP proteins

Robertas Ursache[1], Valérie Dénervaud Tendon[2], Tonni Grube Andersen[3], Niko Geldner[2], Joop EM Vermeer[4]

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Plants roots take up the essential nutrients and block out the unwanted compounds from the soil by using a selective barrier in the roots known as the endodermis. Endodermis contains ring-shaped and lignin-based Casparian strips which act as a diffusion barrier and a checkpoint for water and solutes (Barbosa et al., 2019). Later in development, endodermal cells suberize to produce the so-called 'patchy' suberization that eventually leads to a zone of continuous suberin deposition (Serra and Geldner 2022). The two impermeable polymers, lignin and suberin, affect paracellular and transcellular transport, respectively. Suberin, differently from lignin, is a lipophilic polyester composed of fatty acids, glycerol and some aromatics. It's deposited as a hydrophobic layer between the primary cell wall and plasma membrane. Despite suberin being a major plant polymer, fundamental aspects of its biosynthesis and plasticity have remained unclear. We decided to investigate the suberin plasticity using lateral root formation as a tool. Plants shape their root system via lateral root formation, an auxin-induced process requiring local degradation and re-sealing of endodermal suberin. We demonstrated that differentiated endodermis has a specific, auxin-mediated transcriptional response, dominated by cell wall remodelling genes. We identified two sets of auxin-regulated GELP proteins (GDSL-lipases). Using an optimized CRISPR-Cas9 gene editing toolset (Ursache et al., 2021), we discovered that one set of GELPs is required for suberin polymerization, while the other set can drive suberin degradation (Ursache et al., 2021). These enzymes constitute novel core players of suberisation, driving root suberin plasticity during plant growth and in response to the environmental stress.

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Session II

Epigenetic regulation – transcription regulation

Concerted action of miRNA targets enables defense reprogramming upon pathogen-induced RNA silencing suppression

Luis Villar-Martin[1], Shubhada Kulkarni[2,3,4], Carlos Cáceres[1], Tamara Jiménez-Góngora[1], Detlef Weigel [5], Klaas Vandepoele[2,3,4] and Ignacio Rubio-Somoza[1]

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RNA silencing is an antiviral defense mechanism in plants and animals. In turn, viruses produce silencing suppressors interfering with different steps of that defense mechanism. It has been recently found that production of silencing suppressors is a general infection strategy also employed by pathogens as different as bacteria, oomycetes and fungi. Those distinct pathogens use different vectors and host colonizing strategies interacting with different host cell types throughout infection. The intracellular presence of silencing suppressors does not only disrupt a host´s defense mechanism but interferes with other endogenous processes orchestrated by this universal gene regulatory system. Of especial interest is their impact on micro RNA-mediated gene regulation (miRNA, a subclass of small RNAs). Plant miRNAs tend to regulate the expression of genes with pivotal roles in plant development and stress responses. Additionally, the repertoire of miRNAs and their targets is cell-type specific and therefore, host cell reprogramming might differ depending on the host cells targeted by different pathogens for RNA silencing intervention.

To ascertain the role of miRNA targets in cell-type specific defense upon silencing suppression, we first established the specific cell types targeted by two single stranded RNA (ssRNA) viral and a bacterial pathogen for silencing suppression in *Arabidopsis thaliana* leaves. Later on, we specifically induced the expression of silencing suppressors from those pathogens in those very same cell types and assayed their transcriptional reprogramming by isolating cells under direct reprogramming coupled to RNA-seq. Finally, we focused on determining the contribution of upregulated miRNA targeted transcription factors (TFs) to the overall reprogramming by leveraging ChIP- and RNA-seq approaches.

Our results show that upon pathogen-triggered RNA silencing suppression, the concerted action of miRNA targets enables a new layer of defense to cope with silencing suppressor producing pathogens. Thus, we propose that miRNAs constitute an intracellular surveillance system for the presence of pathogen threats through sensing foreign proteins that interfere with RNA silencing.

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The role of antisense transcription in plants

Peter Kindgren

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The development of novel sequencing technologies has enabled us to get an unprecedented insight into the blueprint of life - the DNA sequence. Surprisingly, although most of the eukaryotic DNA is non-protein coding, it is still transcribed into RNA. Thus, the role and purpose of non-coding transcription represents a key question in modern molecular biology. It has quickly become apparent that non-coding transcription events are triggered as an environmental response and can play diverse biological roles. Despite their emerging importance, the study of non-coding transcripts is challenging due to their rapid degradation. We have therefore recently established a Native Elongating Transcript sequencing (NET-seq) protocol that can robustly identify all transcription events in plants before any degradation occurs.

With NET-seq, we were able to detect thousands of novel transcription events on the antisense strand of coding genes. Most genes that have an associated antisense are highly expressed and responding to stress cues, indicating a general role for antisense transcription in the stress response in plants. We use available T-DNA lines and construct new CRISPR-Cas9 deletion mutants in the 3´-end of genes to disrupt the antisense transcription without affecting the sense transcription to study the role of antisense transcription. Our stress cue of choice is cold temperature, and we see that antisense transcription often have a positive role for the stress response of the host gene.

Our research points to a general role for antisense transcription in assisting stress acclimation in plants. Often, the RNA product synthesised from antisense transcription is degraded quickly by the cell, indicating that the act of transcription is the important step to remodel the chromatin environment.

ARGONAUTE shuttling processes in plants

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RNA silencing controls gene expression via 19-36 nucleotide (nt) small RNAs (sRNA) to regulate development, control stress responses, and preserve genomic integrity, among other essential processes. To fulfil their biological function, sRNAs are loaded into ARGONAUTE (AGO) proteins. The number of AGO genes varies among different organisms. The Arabidopsis thaliana genome encodes ten At-AGO, which can be grouped in three major phylogenetic clades. Whereas At-AGO1, -5, and -10 clade binds ~21nt molecules acting in cytoplasmic post-transcriptional silencing, the clade formed by At-AGO4, -6, -8, and -9 associates with 24nt sRNA mediating nuclear transcriptional silencing. A third clade composed of At-AGO2, -3, and -7 performs different functions including antiviral defense, tasiRNA biogenesis, and DNA repair.

At-AGO1 is the main effector of microRNA (miRNA)-mediated gene silencing. In plants, miRNAs are transcribed as miRNA precursors that are processed in the nucleus by DICER-LIKE1 releasing the mature miRNA. Instead, miRNA target regulation takes place in the cytoplasm, coincidental with At-AGO1 steady-state subcellular localization. Recently, we described a nucleo-cytoplasmic shuttling pathway for At-AGO1, where At-AGO1 loads the miRNA, and afterward At-AGO1:miRNA complexes are exported to the cytoplasm. This relies on a Nuclear Export Signal (NES) present in At-AGO1. Nonetheless, how At-AGO1 is imported to the nucleus has remained unclear. Here, we identify a Nuclear Localization Signal (NLS) in At-AGO1 recognized by an IMPORTIN protein, as the first step for nuclear import. At-AGO1 mutant version in this NLS fails to interact with the IMPORTIN and shows a cytoplasmic-only subcellular localization. In addition, immunoprecipitation experiments showed that At-AGO1 loading activities are affected when At-AGO1 shuttling is impaired. Altogether these results reveal the importance of At-AGO1 shuttling in the sRNA cellular partitioning in Arabidopsis. Currently, we are studying the mechanism beyond shuttling in At-AGO1 and other different eukaryotic AGO proteins.

Epigenetics, plasticity, and adaptation in forest trees

Stéphane Maury[1], R Fichot[1], MD Sow[1,8], J Vigneaud[1], A Delaunay[1], I Le Jan[1], A Duplan[1,2], V Segura[2], O Rogier[2], Leopoldo Sanchez-Rodriguez[2], Harold Durufle[2], C Ambroise[3], J Tost [4], I Allona[5], SH Strauss[6], JF Trontin[7], J Salse[8], C Plomion[9]

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Epigenetics refers to heritable changes associated with altered gene expression, silencing of transposable elements, and phenotypic variation that can arise independently of DNA sequence alterations. The inheritance of an epigenetic state can potentially range from high levels of stability, like a genetic variant, to complete instability from one generation to the next or even during the life of an organism. By modulating plant development and physiological responses to environmental conditions, (transient) epigenetic diversity can participate to plant plasticity while (stable) heritable epigenetic changes affecting fitness traits can be predicted, under natural or artificial selection, to participate in adaptation.

Forest trees represent a relevant and valuable model to study plant epigenetics[1,2] because of inherent characteristics such as sessile habit (driving high plasticity), wide ecological distribution (associated with high genetic diversity), long lifespan with continuous development or complex modes of reproduction (sexual *versus* vegetative). Current threats imposed by global changes on ecological and economic benefits provided by forests, such as drought-driven forest declines, reinforce the timely nature of studies on understanding all sources of flexibility available for trees.

Over the last decade, we developed pioneering work on the epigenetic variation (using DNA methylation as a reliable marker) occurring in trees [3,4,5] such as Poplar (for which genomic resources were readily available) in relation to phenotypic changes in response to environmental constraints such as temperature[6] or water availability[7,8]. More recently, we initiated population epigenetic studies to decipher how epigenetic variation can be related to qualitative and quantitative traits[2,9] (EPITREE ANR project, https://www6.inrae.fr/epitree-project_eng/). Our work opens perspectives on the use of epigenetics as a new source of phenotypic variation using integrative approach (Figure 1). Here, we will discuss epigenetic variation at two levels: intra-individual (plasticity) and inter-individual (adaptation), including recent data on mycorrhizal symbiosis[10] and somatic embryogenesis, before presenting potential applications for tree researchers and breeders[1,2,9].

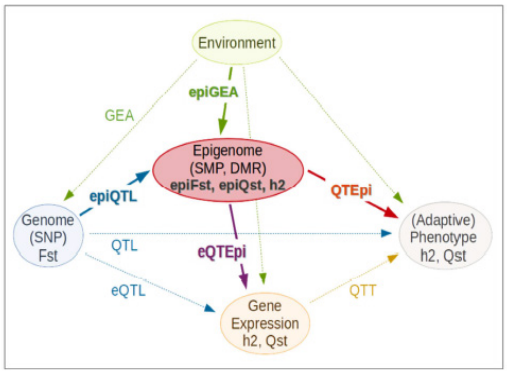

***Figure 1*: Quantitative and population genomics analyses for the integrative analysis of genetic, epigenetic and environmental effects1.** GEA: *gene-environment association*; *epi*GEA: *epigenetics-environment association*; QTL: *quantitative trait locus*; *epi*QTL: *epigenetic* QT; *e*QTL: *expression* QTL; QTEpi: *quantitative trait epilocus*; *eQTEpi: expression* QTEpi; QTT: *quantitative trait transcript*; h2: *heritability*; Fst: *genetic differentiation between populations*; *epi*Fst: *epigenetic differentiation between populations*; Qst: *quantitative differentiation between populations*; *epi*Qst: *epigenetic quantitative differentiation between populations*; SNP: *single-nucleotide polymorphism*; SMP: *single methylation polymorphism*; DMR: *differentially methylated region.*

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Unexpected NGATHA genes in gymnosperms

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RAV transcription factors are characterized by the presence of conserved AP2 and B3 DNA-binding domains [1]. The definition is often extended to include genes that encode the B3 domain only [2]. RAVs are present in all the major clades of land plants. Our current research involves expressing RAV genes from different species in Arabidopsis plants and studying the resulting phenotypes.

Arabidopsis plants expressing a RAV gene from *Ginkgo biloba* (*Ginkgo*RAV5) display a striking pleiotropic phenotype, including reduced size, narrow leaves, compact inflorescences and an aberrant silique morphology. These phenotypes are reminiscent of the overexpression of NGATHA, a small subgroup of genes belonging to the RAV superfamily and primarily involved in the development of style and stigma. NGATHA differ from other RAV genes in that they lack an AP2 domain and have been described only in angiosperms [3].

*Ginkgo*RAV5 belongs to a clade of gymnosperm RAV genes which seem to be phylogenetically closer to NGATHA genes than to other RAV genes. Preliminary results show that a similar phenotype is also observed in Arabidopsis plants expressing RAV genes from other gymnosperms, and these genes are able to complement Arabidopsis *ngatha* mutations. This may indicate that the function of NGATHA genes evolved in the common ancestor of seed plants, as has been previously suggested [4]. Therefore, the evolution to acquire new functions seems to predate the molecular changes. Gymnosperm RAV proteins (with both AP2 and B3 domains) are capable to act as angiosperm NGATHA (with only the B3 domain) in the development of style and stigma.

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Session III

Population Genetics

Landscape Breeding: Catching up with climate cline

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In the Nordic countries, up to 70% to 90% of productive Scots pine and Norway spruce are regenerated with improved forest reproductive material (FRM). In Sweden and Norway the aim is set to 100% by 2050. As for other efforts to build up the forest resource, also tree breeders must develop their tools for "breeding towards a moving target" as climate change will pose expected, unexpected and highly variable stress to the forest production. In this respect, the availability of flexible breeding strategies is becoming more important as we must make use of the large genetic variation present in our forests to obtain adapted and robust forests. In contrast to the long-term breeding strategies based on structured breeding populations, a Breeding without Breeding approach implemented at the Landscape level offer a more flexible breeding strategies to catch up with the consequences of climate change.

Demography and Genetic Diversity Analysis of Almond Germplasm

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Almond, Prunus dulcis (Miller) D.A. Webb (syn. *Prunus amygdalus Batsch*), is a self-incompatible tree species belonging to the Rosaceae family. Almond is the main nut-producing tree species worldwide, with an estimated cultivated area of 2.1 million hectares and a mean global production of 3.4 million tonnes (FAO, 2019). Almond is native to the Mediterranean region and its closest wild relatives are found in central and western Asia and way down into the eastern Mediterranean Basin. Centuries-long seed propagation of almond trees has led to early domestication of the species whereas its site of domestication is placed either in Levant or in central Asia. For our study we used a panel of 50 almond lines, comprised of modern/old cultivars sampled across the species geographical distribution, wild relatives and a peach (P. persica) variety as a species outgroup. Demographic studies suggest an ancient expansion of this species followed by a gradual population reduction during the last glacial period. A main pattern of variation across the axis West-East is observed according to PCA, although two main structured groups, defined as Asian and European/Mediterranean are detected using population structure algorithms. *P. webbi*, *P. bucharica* and *P. kuramica* become close related outgroups while and *P. fenziliana* join with European/Mediterranean varieties and are sharing most of variants with *P. dulcis*. Genome-wide variability analysis allowed us to detect candidate regions of the domestication process, infer the main demographic processes occurred among populations and study the evolutionary patterns that define the genetic architecture of the species.

Between and within species diversity of water use efficiency for *Q. petraea* and *Q. robur*: At the crossroad of ecology, ecophysiology and genetics

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Climate change predictions tend towards drier summers, challenging forest management to maintain biomass production and survival while reducing water use. Sustainable forest management practices need to take into account these future environmental conditions. In this respect, genetic variation for water use efficiency (WUE), the ratio between biomass production and water use, could be mobilized for adapting forests to these future climates. A strong within species adaptive diversity of WUE related to soil water conditions would support an assisted migration forest management strategy. Here, the case of the two predominant European species *Quercus robur* and Q. *petraea will be explored.*

A strong genetic control of WUE had already been shown for one Q. *robur* family. Here, the study of the genetic architecture of WUE was enlarged to Q. *petraea* and hybrid families, to establish the across species the genetic basis of WUE.

To further clarify the functional basis of the difference in WUE and underlying traits, within and between both species, detailed ecophysiological measurements were carried out. These ecophysiological studies were complemented by gene expression studies in leaves and in guard cells. These indicated a within species variation of the molecular response to drought stress[1].

Finally, an existing common garden of Q. *petraea* allowed to characterise within and among population diversity in WUE[2] Significant differences in WUE were found among populations but a much larger variability was observed within than among populations. The population plasticity of WUE to severe drought could be related to the soil type of the provenance sites, suggesting a local adaptation in terms of drought response strategies. The genotyping of these populations allowed to detect strong associations between WUE and its plasticity and sequenced genes.

An overview will be given on this research strategy, ranging from detailed ecophysiology and gene expression to functional ecology and genomics.

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Isolating adaptive variation from natural forest trees

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Conifers are ecologically dominant and economically important, but are succumbing to drought, disease, early-budding and other challenges globally because the climate has changed so that mature trees are no longer adapted to their environment. If we could predict how individual tree genotypes would respond to different environments, we could — given environmental predictions — plant the right tree in the right space. While agronomic approaches such as reciprocal transplant experiments and provenance trials can effectively estimate genotypic responses to environments, both the number of genotypes and the number of environments that can be evaluated are limited. My group takes advantage of tree increment core samples to estimate annual growth from tree-rings. For each genotype, we thus have a life-time's worth of experienced year-environments. This allows us to partition growth variation into generalizable environmental responses for years with historical weather or biotic information, using quantitative, genomic and ecological approaches to control for correlated responses. We focus on the economically and ecologically important conifer Norway spruce (*Picea abies*) to develop models and infrastructure to understand the fraction of annual growth that can be attributed to genotype, environment and genotype-by-environment interactions (GxE). These estimates can be used to map the genetic basis of adaptive response using estimates for GxE as a response in genome-wide association studies (GWAS) and predict genetic responses to novel environments. This approach will enable estimation of the genetic basis of adaptive responses in any population, providing the means to evaluate a tree's performance in any modeled environment. As environments shift under climate change, this will provide a powerful tool to select parents for healthy, resilient forests.

Session IV

Omics approaches to decipher plant development and adaptation

A systems genetics approach to identifying genes in the biosynthesis pathway of salicinoid phenolic glycosides in *Populus tremula*

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Most traits are genetically complex, being affected by multiple genes independently and by gene-gene and gene-environment interactions. Identifying the set of genes contributing to these complex traits is challenging since a range of genes and interactions can contribute to trait variation. Systems genetics aims to unravel the genetic architecture of complex traits by integrating genome-wide association studies from multiple quantitative phenotypes (including -omic levels). Systems genetics, therefore, allows us to study how information flows from one -omic level to the next, leading us from genotype to phenotype. We are using systems genetics to dissect salicinoid phenolic glycosides (SPG) biosynthesis in aspen (*Populus tremula*). SPGs function as defense compounds, providing protection from animals and insects. Elucidation of genes within the pathway would enable genetic engineering of the SPG pathway as a tool to help us better understand the ecological significance of these compounds.

Phenotypic prediction using multiomics in black poplar

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Multi-omics represent a promising link between phenotypes and genome variation. Few studies yet address their integration to understand genetic architecture and improve predictability. Our study used 241 poplar genotypes, phenotyped in two common gardens, with their xylem and cambium RNA sequenced at one site, yielding large phenotypic, genomic and transcriptomic datasets. For each trait, prediction models were built with genotypic or transcriptomic data and compared to concatenation integrating both omics. The advantage of integration varied across traits and, to understand such differences, we made an eQTL analysis to characterize the interplay between the genome and the transcriptome and classify the predicting features into CIS or TRANS relationships. A strong and significant negative correlation was found between the change in predictability and the change in predictor importance for eQTLs (both TRANS and CIS effects) and CIS regulated transcripts, and mostly for traits showing beneficial integration and evaluated in the site of transcriptomic sampling. Consequently, beneficial integration happens when the redundancy of predictors is decreased, leaving the stage to other less prominent but complementary predictors. An additional GO enrichment analysis appeared to corroborate such statistical output. To our knowledge, this is a novel finding delineating a promising way to explore data integration.

Using multi-omics to study of bud dormancy in apple tree

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Temperate trees are distributed over geographical regions that present wide seasonal environmental fluctuations all over the year. In order to optimize their reproductive success, temperate trees adjust their growth and flowering cycle patterns to these ever-changing conditions. This plasticity is conferred by mechanisms of environment perception (i.e. day-length and temperature) and signalling pathways that are integrated in molecular networks to modulate developmental responses. Dormancy is an essential developmental program that allows tree adaptation to low temperatures in winter. During dormancy, meristems are enclosed in protective buds and remain in a resting state until the end of the winter. After a prolonged cold period, buds reactivate their growth activity in response to the warm temperatures typical of the spring.

The mechanisms that regulate dormancy are highly heritable, suggesting a strong genetic control of this trait. However, the genetic networks controlling dormancy cycle in trees are still unknown. We have used diverse omics approaches to decipher how dormancy is controlled in fruit trees. We have made use of sequential DNA Affinity Purification sequencing (seq-DAP-seq) to gain knowledge on the function of a group of MADS transcription factors (TFs) in the control of winter bud dormancy of apple (*Malus domestica*). We found that these MADS TFs form transcriptional complexes that act in a gene regulatory network (GRN) to regulate dormancy cycle progression[1]. In parallel, we have investigated the potential role of small RNAs during the dormancy cycle. To this end, we have isolated small RNAs from apple buds during dormancy and described a miRNA/target regulatory module that could participate in the control of budbreak by acting on the sensitivity to abscisic acid[2]. Finally, we have performed a Genome Wide Association Study (GWAS) on a collection of 242 apple tree cultivars to identify genes involved in the control of dormancy cycle. Our GWAS resulted in the identification of candidate genes that are strongly associated to flowering time. The potential role of these genes in affecting dormancy cycle will be discussed.

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Characterization of the Arabidopsis peptidome and its role in flower development

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A substantial but uncharted fraction of plant proteomes is composed of small proteins (peptidome), of roles and functions -for the most part- yet to be discovered. Several putative peptides encoded in short Open Reading Frames (sORFs) have been found in intergenic regions and transcripts previously identified as non-coding by integrative analysis using omics-based approaches. In order to characterize the *Arabidopsis* peptidome and, in particular, its possible involvement in flower development, we performed tandem mass spectrometry of inflorescences of the floral homeotic mutants *apetala1*, *apetala2*, *apetala3*, *pistillata*, and *agamous*, and wild-type plants of *Arabidopsis thaliana*. For peptide identification we created an extensive database that includes putative sORF-encoded peptides (SEPs) from intergenic regions, UTRs, 'non-coding' RNAs and other transcripts. By combining the datasets from individual / wild type comparisons we were able to identify 132 novel peptide candidates expressed in flowers, from which 60 peptides are predicted to be specifically expressed, or at least enriched, in one type of floral organ. Around 25% of the novel SEPs belong to putative gene families in A. *thaliana* and almost 80% have putative homologs in other plant species such as A. *lyrata*, *Brassica oleracea* and *Camelina sativa*.

Recruitment of pre-existing translational networks during the evolution of C_4 photosynthesis

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Light is fundamental for almost every aspect in plant biology. Upon light induction, etiolated leaves rapidly respond to become photosynthetic and maximize their metabolic potential. Genes related to the highly efficient C_4 photosynthesis are heavily regulated by light responsive transcriptional networks that are also conserved in C_3 leaves. While most studies have focused in understanding how the light induced networks operate at the transcriptional level, less attention has been put into understanding the extent by which light induced translational networks modulate the expression of photosynthesis related genes in C_4 species compared to their ancestral C_3 relatives. In this regard, de-etiolation studies offer a good system to understand the establishment of the photosynthetic machinery in relatively short time periods.

Here, we used Ribosome profiling (Riboseq) in combination with RNAseq to capture translation dynamics and transcriptional activity during de-etiolation of a *Oryza sativa* (C_3) and a *Sorghum bicolor* (C_4). In general, transcription and protein synthesis are highly coupled in both species. However, our results revealed that genes related to light dependent reactions and C_3 photosynthesis in *Oryza sativa* and *Sorghum bicolor* are translationally enhanced in the dark, at a point where light induced transcriptional networks are not operational. This could be seen as a mechanism by which plants maximize translation efficiency of genes with low RNA pools. Interestingly, this effect was also observed in C_4 genes from sorghum but not in the corresponding orthologues from rice implying during the evolution of C_4 photosynthesis, C_4 genes were wired into pre-existing translational regulatory networks in addition to those transcriptional networks defined before.

Session V

Plant nutrition

Dissecting the components of root water transport

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Root water transport, which critically contributes to the plant water status and thereby plant productivity, has been the object of extensive experimental and theoretical studies. Following radial transport across peripheral cell layers (epidermis, cortex, and endodermis), soil water reaches the stele and xylem vessels where it is transported axially to the plant aerial parts. However, root systems represent an intricate assembly of cells in complex architectures, including many tissues at distinct developmental stages. Our comprehension of where and how molecular actors integrate their function in order to provide the root with its hydraulic properties is therefore still limited.

In our lab, over the recent years, we have been developing new techniques and new models, in addition to “standard” genetic approaches, to better understand root water transport. We implemented a model-assisted pressure chamber technique, called “cut-and-flow”, that allows to measure simultaneously both the axial and radial hydraulic conductivities of highly-branched root systems. A model of radial transport of a new type is also under development, which changes our conceptual point of view on this process. We aim at keeping a tight link between models and experimentation, in order to unravel the fine balances between permeabilities and between structures that have evolved and keep the root adapted to its environment while achieving its functions.

Deciphering the link between sugar transport and vascular system development: A SWEET story

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In plant cells, carbohydrates are the main building blocks for central metabolism, for soluble sugars storage and polysaccharides synthesis. Sugars also serve as signaling molecules and osmotica during abiotic stress responses. Intercellular and intracellular transport systems are therefore, not only critical for long-distance sugar allocation but also to modulate local sugar partitioning ranging from tissular to subcellular scales.

The vascular system including phloem, (pro)cambium and xylem, is an important tissue for sugar transport. A complex network of transcription factors controls its formation. At the cellular level, cytosolic sugar availability is regulated by sugar exchanges at the plasma membrane and the tonoplast, through the transport of sugars by plasmodesmata as well as active and/or facilitated transporters, suggesting a complexity in fine tuning of sugar transport and homeostasis in the vascular tissues. However, the balance between nutrient/sugars availability in the vascular cells and their use for the development of the vascular tissues remains to be addressed.

We are interested in the link between sugar transport and the plant vascular system development. Among the various families of sugar transporters, we are focusing our analysis on SWEET transporters, the latest sugar transporter family identified, that facilitate the movement of soluble sugars along the concentration gradient. By using an integrated approach, at the cell and the whole plant levels, we functionally characterized in *Arabidopsis thaliana* several members of the SWEET transporter family and show that they are important for the vascular system development and the secondary cell wall formation[1,2,3]. Additionally, our work shed new light on the central role of vascular parenchyma cells in these processes and further highlights the importance of finely regulated intercellular and/or intracellular sugar transport to assure appropriate plant growth and development.

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Iron nutrition in plants: towards a new paradigme?

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Iron (Fe) is an essential micronutrient for plant productivity and for the quality of their derived products. Although Fe is one of the most abundant elements in soil, plants often suffer from Fe deficiency, which most often occurs in alkaline soils, where Fe solubility is low. To meet their nutritional needs in Fe, plants have evolved elaborate strategies. According to the mechanism used to increase the solubility of Fe present in the surrounding medium, plants have been categorized into two phylogenetically distinct groups. Grass species secrete Fe chelating phytosiderophores and subsequently uptake Fe^{3+}-phytosiderophores complex via a transporter of the YSL family (Strategy II). In contrast, in Arabidopsis thaliana (and non-grass species), efficient Fe uptake from soil is ensured by a reduction-based mechanism in which Fe^{3+} is first reduced by FRO2 and the reduced Fe^{2+} is then transported across the plasma membrane by the IRT1 transporter (Strategy I).

In the last years, coumarins have captured the interest of researchers for their enigmatic functions in Strategy I plant Fe nutrition. Even though the importance of coumarins secretion in plant Fe nutrition is now well established, we lacked, until recently, information on their in planta localisation, dynamic and role in Fe acquisition.

By exploiting the natural fluorescence of coumarins we investigated in Arabidopsis the mechanisms by which these compounds are released from roots into the surrounding media. It highlighted the preponderant role of trichoblast in Arabidopsis Fe nutrition through the secretion of Fe mobilizing coumarins such as fraxetin. Strikingly, we discovered that once secreted, Fe mobilizing coumarins can be taken up by surrounding plants via an ATP dependent mechanism and that this mechanism was restricted to non-grass species. Importantly, plants defective in IRT1 activity, displaying Fe deficiency symptoms, can be complemented by exogenous supply of Fe mobilizing coumarins. The characterization of Fe chelating properties of Fe mobilizing coumarins highlighted that they can specifically form stable complexes with Fe^{3+} at neutral and alkaline pH.

Overall, our results demonstrate that at high pH, Fe mobilizing coumarins such as fraxetin can improve Fe nutrition by directly transporting Fe^{3+} into the root, circumventing the FRO2/IRT1 system, in a similar way as phytosiderophores do in grasses. Importantly, these data further confirm that the boundaries between Strategy I and Strategy II plants are much narrower than what is classically described since several decades.

Session VI

Development and Signaling

Convergence of Light and Chloroplast Signaling in the Regulation of Plant Development: a New Player in Town

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Light as an informational signal is of outmost importance for plant development. Light signal transduction pathways amplify and coordinate the response to ambient light to regulate plant physiology and development. In the first stage of a plant life after germination, light exposure activates several photoreceptors (among them the red-light absorbing phytochromes) that trigger a massive transcriptome reprogramming leading to autotrophic growth in a process termed deetiolation. Main regulatory factors of this developmental transition are the Phytochrome Interacting Factors (PIFs), which accumulate in the nucleus in the dark and are degraded by light-activated phytochromes. Deetiolation is characterized by the inhibition of hypocotyl growth and separation and expansion of the cotyledons, together with chloroplast development.

However, if light is in excess during this process it can damage the chloroplasts, triggering molecular communication between the altered chloroplast and the nucleus called chloroplast retrograde signaling (RS). In *Arabidopsis thaliana*, RS inhibits deetiolation. The chloroplast-localized protein GENOMES UNCOUPLED1 (GUN1) is a main regulator of this RS response. We have previously shown that convergence of phytochrome/PIF signaling and GUN1-mediated RS targets transcriptional regulators such as GLK1[1] and BBX16[2], which promote cotyledon separation during deetiolation under normal light and are repressed in photodamaging conditions.

More recently, we are focusing on understanding how other aspects of deetiolation might be regulated by light and RS. Interestingly, cotyledon expansion is promoted under normal light to maximize photosynthetic area, but is inhibited by RS. We have identified and are currently characterizing UCO, a novel PIF-regulated transcription factor that is necessary for cotyledon expansion. We have also found that GUN1-mediated RS targets UCO in potentially damaging high light to repress cotyledon expansion. Together, the picture that is emerging is that of a concerted action of transcriptional regulators at the convergence of photoreceptor and chloroplast signaling to optimize development in accordance to the prevailing light environment.

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Autophagy and chloroplast degradation

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Macroautophagy is induced during leaf senescence and in response to environmental stresses. Dedicated to cell clearance and organelle quality control, macroautophagy sequesters unwanted cytoplasmic material into vesicles named autophagosomes and drives them to lytic vacuoles. Facilitating cytoplasm clearance, it also facilitates macromolecule recycling and provides back small molecules as sugars, lipids and amino acids, that can be reused in the cell itself, or exported after phloem loading to the sink growing organs. As such, macroautophagy controls up to 60% of the nitrogen, sulfur and iron fluxes from senescing leaves to the seeds in Arabidopsis.

Chloroplasts that contain high amount of nitrogen and produce many deleterious reactive oxygen species under stress and during senescence, have been proposed to be targeted by autophagy during senescence. Specific autophagy of cytoplasmic material involves targetting of autophagic substrates (cargoes) by the ATG8 protein at the surface af autophagosome membrane, through protein-protein interaction. Organelles as peroxysome, mitochondria and chloroplast recognition should involve ATG8-interacting proteins that are associated to organelles membranes and could act as receptors. ATG8-receptor interaction could drive organelles or organelle portions inside the autophagosome.

Using comparative proteomic we identified potential autophagy cargo and recepor candidates that could play a role in chloroplast degradation. Recent advance in their characterization will be presented.

SUMO modulates senescence through the control of the ethylene signaling pathway in Arabidopsis

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In plants, senescence represents the final stage of leaf development. Ethylene is a volatile hormone that regulates many physiological processes including induction of leaf senescence and fruit ripening. The initial perception of ethylene signaling pathway as an initial linear transduction cascade has evolved to a more complex regulatory network including feedback regulations, multiple levels of protein stability control, and broad existence of signaling interplay and integration. In this complex regulatory network, the EIN3 transcription factor is a central component, which positively regulates senescence-associated genes (SAGs).

SUMO (small ubiquitin-related modifier) conjugation (i.e., SUMOylation) to protein substrates is a reversible posttranslational modification that regulates protein function. We will present our findings showing that SUMOylation negatively regulates ethylene signaling, which represents a novel regulatory mechanism to be added to the highly complex ethylene-signaling network. We observe that in SUMOylation of EIN3 in vivo represses its transcriptional activity. Moreover, SUMOylation of EIN3 results in the accumulation of this transcription factor in the cytosol, which represents a mechanism to control ethylene responses not previously described in plants. Importantly, we demonstrate that the crosstalk between SUMO and ethylene results essential for the development of an adequate senescence programme.

The phosphoinositide signature guides the final step of plant cytokinesis

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Plant cytokinesis, which fundamentally differs from that in animals, requires the outward expansion of a plasma membrane precursor named the cell plate. How the transition from a cell plate to a plasma membrane occurs at the end of the plant cytokinesis remains poorly understood. We investigated the acquisition of plasma membrane lipid identity after cell division through the lateral patterning of the phosphatidylinositol 4,5-bisphosphate PI(4,5)P2 at the newly formed cell plate membrane. We showed that during late cytokinesis, opposing polarity domains are formed along the cell plate. Exclusion of PI(4,5)P2 from the cell plate leading zone is controlled by SAC9, a putative phosphoinositide phosphatase. SAC9 colocalizes with and regulates the function of MAP65-3 at the cell plate leading zone, a key regulator of the cytokinesis. In the sac9-3 mutant, PI(4,5)P2 polar distribution at the cell plate is altered, leading to de-novo recruitment of the cytokinesis apparatus and resulting in the formation of an ectopic cell plate insertion site with a particular geometry. We proposed that PI(4,5)P2 acts as a polar cue to ensure a spatial separation between expansion and shift toward a functional plasma membrane during final step of cytokinesis.

Potato SP6A tuber induction correlates with enhanced cambial activity

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Plant storage organs supply a great part of the caloric intake for human and animal food needs. These specialized organs are formed by a relatively small number of plant species to which they serve as means of vegetative propagation, or as assimilates reservoir for later bolting. Depending on the species, they can differentiate from leaves (garlic and onion), specialized branches (potato tubers), the hypocotyl (ginger, horseradish), or the roots (cassava, radish, sweet potato). Swollen roots preserve most of the initial root morphology showing that sugar/starch-accumulating cells originate from the vascular cambium. However, which cells originate the anatomically more complex shoot-derived organs is still under debate.

Proteins of the FLOWERING LOCUS-T family act as inducing signals for potato and onion storage transition but lack a function into storage root initiation, which has led to believe that these developmental processes are unrelated. Bulbs and tubers include an inner shoot bud, or the stolon apical meristem /first axillary buds forming the tuber "eyes". Activity of these meristems is arrested during storage transition and go into a dormancy state, while are reactivated in the next spring and generate a clonal plant. This process largely reminds that of FT-regulated winter growth cessation in perennial trees, suggesting that function of FT paralogs in triggering shoot-derived storage organ formation has more to do with the seasonal alignment of this developmental transition than per se induction of a storage identity. Here, by analyzing the cell division patterns of early induced stolons we show that differentiation of starch accumulating cells is preceded by a division of the vascular cambium, consistently with a common developmental program involved at initiation of all storage organs. Single cell RNA-seq UMAP projections reveals storage parenchyma cells to cluster with the cambium and xylem precursor cells, in line with cambial derivative cells switch on tuberization transition their usual xylem specification program into a storage identity. Key vascular patterning and xylem differentiation regulators are indeed expressed in starch accumulating cells. Possible FT targets with a role at storage cell transition are discussed.

Session VII

Response to mechanical stress

Root growth against a mechanical obstacle: the early growth response of a maize root facing an axial resistance agrees with the Lockhart model

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At the root scale, a compact soil limits oxygen diffusion and opposes a homogeneous mechanical resistance, both impeding root growth. In field, the root elongation rate varies inversely with soil strength. The soil is also often physically heterogeneous and the growing root has to cope with obstacles of various size and rigidity such as grains, stones or hard aggregates, and the growth response of the root depends on the particle size (Kolb et al., 2017). After encountering a hard interface, the root tends to keep growing straight, pushing against it, then the growth axis may be reoriented. How the roots response to mechanical resistance is currently a hot topic, as evidenced by recent studies on mechanoperception or active growth axis reorientation (Jacobsen et al., 2021; Mousavi et al., 2021). Here we explore the biomechanical aspects of the root growth response to an obstacle.

Through a model experiment coupling force and kinematics measurements, we probed the force–growth relationship of a maize primary root contacting a stiff resisting obstacle, which mimics the strongest soil impedance variation encountered by a growing root. The growth of roots just emerging from a corseting agarose gel and contacting a force sensor (acting as an obstacle) was monitored by time-lapse imaging simultaneously to the force. The evolution of the velocity field along the root was obtained from kinematics analysis of the root texture with a particle image velocimetry derived technique. A triangular fit was introduced to retrieve the elemental elongation rate or strain rate. A parameter-free model based on the Lockhart law quantitatively predicts how the force at the obstacle modifies several features of the growth distribution (length of the growth zone, maximal elemental elongation rate and velocity) during the first 10 min. These results suggest a strong similarity of the early growth responses elicited either by a directional stress (contact) or by an isotropic perturbation (hyperosmotic bath).

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How wounded plants coordinate their healing and immune responses

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Roots are constantly surrounded by beneficial and necrotrophic bacteria as well as invading microorganisms like insects and nematodes, which may cause minimal tissue damage and injury. In the presence of thousands of plant-parasitic species, nematodes are omnipresent in soil. Many nematodes utilize their stylet (needle-like organ) to injure plant cells during initial attack, making them an attractive model for the study of primary root responses to early wounding. In the experimental procedure, laser ablation was used to mimic plant root cell injury similarly as caused by cyst nematodes. To explore stress responses on single cell level in the roots in spatial and temporal resolution, laser ablation offers an unique method for inducing mechanical injury. Here I will present a novel discoveries demonstrating how short-distance cell-to-cell signaling cascade acts as a crucial communication resource for alerting surrounding cells to invaders and preparing their self-defense and regeneration mechanism.

The primary eATP receptor P2K1 mediates responses to the impedance of the growth medium in Arabidopsis roots

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All known organisms use adenosine 5′-triphosphate (ATP) as an essential, cellular energy source to drive many biochemical reactions. ATP can also be released into the extracellular matrix, where it is referred to as extracellular ATP (eATP). Numerous studies have shown that eATP is involved in a variety of plant processes, including root hair growth, gravitropism, pathogen responses and thigmotropism. P2K1 -a lectin receptor-like kinase- has been identified as the primary eATP receptor in Arabidopsis and is known to trigger a calcium signaling pathway[1]. In this project, we are exploring the role that P2K1-mediated eATP signalling plays in mechano-responses. Our aim is to determine the effect of impaired P2K1 on the root response to changes in soil impedance.

To address this question, an original two-layer medium previously developed by the team has been used to mimic changes in growth medium strength, roots growing from the soft layer towards the hard one. Growth and trajectory of primary roots of *Arabidopsis thaliana* seedlings are investigated using in vivo image analysis. After contact with the harder layer, the root either penetrated it or underwent rapid curvature called buckling, enabling reorientation of the root growth. The penetration abilities of P2K1-1-3 and P2K1-1-4 primary roots were compared with those of wild-type roots in 0.2%/0.3% two-layer media. The spatiotemporal characterization of apex reorientation showed that the penetration ability is impaired in mutant primary roots, demonstrating that eATP- dependent signalling pathways are involved in the response to soil impedance in roots.

To determine the possible implication of P2K1 in the calcium signaling pathway triggered by the contact of the root with an obstacle, we are using the ratiometric $[Ca^{2+}]_{cyt}$ sensor R-GECO1-mTurquoise to monitor the $[Ca^{2+}]_{cyt}$ dynamics. Therefore we are currently developing a series of experiments using a vertical stage fluorescent microscope in order to correlate *in vivo* the spatiotemporal characterization of the root apex reorientation and the cytosolic calcium distribution, in P2K1 mutants and wild-type roots, when the root tip encounters an obstacle. In parallel, we are monitoring the variations of the cytosolic calcium distribution in response to a rapid mechanical stimulation applied on the tip of the root using micromanipulators.

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Session VIII

Adaptation to abiotic stress

Phenotypic plasticity and genetic variation underlying the response to the elevation of CO_2 in *A. thaliana*

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Atmospheric CO_2 concentration may rise up to 1000 ppm before the end of the century (IPCC, 2021). This will lead to profound changes in major physiological processes in plants (Gojon et al., 2022). On one hand, the stimulation of growth by the elevation of CO_2 increases the production of plant biomass. On the other hand, elevated CO_2 (eCO_2) negatively affects plant nutrition and mineral composition, and alters water use efficiency and heat tolerance. Here, we report the results from transcriptomic, genetic and genomic approaches done to understand the effects of eCO_2 on plant nutrition and responses to water deficit (WD) and elevated air temperature (HT), and to identify their genetic and molecular determinants. We notably demonstrated that eCO_2 targets regulatory modules specifically associated with the regulation of high-affinity root nitrate transport, resulting in a decrease in the efficiency of root nitrate uptake. We also used natural genetic diversity to explore the phenotypic plasticity of A. *thaliana* under eCO_2 and different combinations of WD and HT, and identified genotype-by-environment effects. Finally, we performed large-scale phenotyping of ionome content in A. *thaliana* natural populations under eCO_2 followed by GWAS to analyze the genetic architecture of the response of A. *thaliana* to eCO_2 and to identify genes involved in the adaptation to the current climate change.

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How plants deal with heat and cold: Molecular mechanisms of auxin transport in response to temperature stress

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Our planet is warming, and it is already evident that extreme weather events such as sudden heat and cold waves will become increasingly frequent. This has important implications for plants, which are sensitive to temperature. Temperature stress can severely affect plant distribution and productivity, thus threatening entire ecosystems and the services they provide. To date, most studies have concerned the big-picture elements of plant responses to climate change (e.g., biomass), but to improve our ability to support plant adaptation to heat and cold stress, future research need to focus on the molecular and cellular responses that help plants adapt to temperature stress. To adjust and adapt, plants rely on hormones such as auxin, which play essential roles in regulating plant growth and development. Auxin undergoes directional transport from one cell to another, which allows its asymmetric distribution in different cells and tissues. The different auxin concentrations are instrumental in both organ initiation and shape determination, but still the molecular mechanisms by which auxin transport is regulated at the cellular level and in different cell types to orchestrate the plant's response to temperature stress, remains poorly understood. My research will dissect the molecular mechanisms involved in auxin transport in different types of cells. To achieve this aim, my team will employ a novel combination of cutting-edge methods that can provide high-resolution information about proteins and cellular processes/responses that together orchestrate the regulation of auxin transport. We will perform experiments on the plant model organism *Arabidopsis thaliana*, particularly on its root system, which so far has been largely overlooked in the response to temperature stress. Taken together, the fundamental knowledge obtained through our research will contribute to the mechanistic understanding of plant responses to the temperature variability that will accompany climate change. Such understanding is key for anticipating the impacts of climate variability on agricultural and natural ecosystems.

Using the power of genetic screens to investigate plant photoprotection

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Light utilization is finely tuned in photosynthetic organisms to prevent cellular damage. The dissipation of excess absorbed light energy, a process termed NPQ, plays an important role in photoprotection. Our research focus is on the sustained or slowly reversible form(s) of NPQ which take hours or longer to relax. The *Arabidopsis thaliana* suppressor of quenching1 (*soq1*) mutant exhibits enhanced sustained NPQ[1], which we termed qH[2]. To identify molecular players involved in qH, we leveraged the power of forward genetic screens and isolated mutants affecting chlorophyllide a oxygenase, the plastid lipocalin (LCNP)[3], and a short chain dehydrogenase reductase (ROQH1)[4]. Analysis of the mutants showed that qH is localized to the peripheral antenna (LHCII) of photosystem II[5] and demonstrated that LCNP is required for qH, either directly (by forming NPQ sites) or indirectly (by modifying the LHCII membrane environment). qH operates under stress conditions such as cold and high light and is photoprotective, as it reduces lipid peroxidation levels. We are investigating the possible redox regulation of LCNP by SOQ1 through biochemical and structural studies[6] and further screened for other suppressors of qH. I will present a brief historical perspective on the evolution of methodologies used to determine causative mutations, including map-based cloning, mapping-by-sequencing and whole genome sequencing[7], as well as newly identified genes that alter qH. We are characterizing the impact of these new mutations on qH and screening for suppressors of *lcnp* or *npq1 lut2* to uncover new energy dissipation routes independent of qH or xanthophyll pigments zeaxanthin and lutein.

Keywords: *Photoprotection*, *NPQ*, *energy dissipation*, *Arabidopsis*, *genetic screens*

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Development of Chlamydomonas as a bioengineering platform

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Chlamydomonas reinhardtii is an established model organism for studying various cellular processes, including photosynthesis, metabolic engineering, and more recently as a promising synthetic-bio chassis. This green alga has a higher carbon dioxide fixation rate than conventional land plants, and a large amount of biomass can be easily obtained due to its rapid growth rate. As a result, it is commercially useful and even edible, and has great potential to be used to produce beneficial proteins and metabolites. Despite these advantages, *Chlamydomonas*'s industrial potential is limited by the underdevelopment of bioengineering tools such as lack of strong promoters compared to *E. coli*, yeast and other industrial hosts.

In this talk, I will present the current working projects to overcome these issues. Our lab has been investigating the synthetic promoters in microalgae using the high-throughput mutational screening. A massive number of synthetic promoters is generated to study the mutational effect on transcriptional activities. We are also modulating the expression of key genes involved in photoprotection and antioxidant response in order to generate high-light resistant strains with big potential for biotechnological purposes. Finally, I will introduce the update of UTRDesigner that can help to design UTR sequences for translation control in the prokaryotic system such as *E.coli* and cyanobacteria.

Session IX

Beneficial interactions

Do SSPs regulate ectomycorrhizal symbiosis in response to biotic and abiotic nutrient signals in trees?

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To improve their mineral nutrition, plants form symbiotic associations with microorganisms. Occurring between 6,000 tree species and 20,000 ascomycetes and basidiomycetes, ectomycorrhizal (ECM) symbioses dominate boreal and temperate forests[1]. Providing mineral nutrients in exchange for organic carbon, they have an energetic cost for the tree[2]. To use their resources efficiently, trees must integrate environmental and metabolic nutritional cues to regulate adequately their ECM interactions[3]. The signalling pathways leading to the regulation of ECM symbiosis in response to nutrient signals are unknown.

In herbaceous plants, Small Secreted Peptides (SSPs) transduce local and long-distance signals regulating their symbiotic associations in response to abiotic and biotic nutrient cues[4]. For example, in nitrate deficiency the secretion of C-TERMINALLY ENCODED PEPTIDEs (CEP) in the xylem sap drives a root-to-shoot starvation signal promoting nodulation in nitrate rich zones[5], while associations with arbuscular mycorrhizal (AM) fungi represses the expression of CEP genes[6]. Similarly, the secretion of CLAVATA3/ESR related (CLE) peptides transduces root-to-shoot signals repressing symbiotic associations with nitrogen (N)-fixing bacteria and AM fungi in response nitrate, inorganic phosphate or to prior associations with AM fungi and N-fixing bacteria [7,8,9,10].

SSPs are small peptides (<100 amino acids) produced from short open reading frames or by proteolytic cleavage of pre-proteins[11]. More than 4,000 SSP families exist in herbaceous, most remain to be functionally characterized[12]. Several of these families were found in trees[13,14]. To investigate their role in the regulation of ECM symbiosis in response to nutrient cues, we scanned the genomes of poplar and oak with an iterative method of HMM and blast to identify members of 21 SSP families known to regulate plant-microorganism interactions or induced during nutrient stresses in herbaceous. Using a transcriptomic approach, we selected a set of poplar SSPs transcriptionally regulated by ECM associations and/or nitrate stresses and tested their effects on ECM symbiosis through exogeneous application of synthetic peptides. We found that several CLEs and CEPs enhance the ECM association between poplar and the fungus *Laccaria bicolor*. This suggests that SSPs participate to the formation/regulation of ECM symbiosis in poplar. Further functional characterization of these peptides is required to understand their functions.

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Root exudate simulation using microdialysis

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A key aspect of plant root physiology is that plant roots exude carbon. This exudation stimulates microbial growth around roots, creating the rhizosphere. The microbial activity in the rhizosphere influences the amount and composition of nitrogen available for uptake. As atmospheric CO2 rises, future forest tree growth and capacity to bind carbon, will depend on the availability of nitrogen in the rhizosphere. The scale of these events occurs, in the root-soil interface, within millimetres around the roots. Previously available methods have lacked the precision to probe phenomena specifically at this interface, measurements of compounds in bulk soil provides little information of processes, substances and microbes at the root-soil interface. My research group works with a technique that provides new opportunities to study this interface, microdialysis[1]. The technique was originally developed in neuroscience and recently adapted for monitoring nitrogen fluxes in soil[2]. The method is still in its infancy with a lot of emphasis on optimization. So far our studies show that the technique can simulate root exudation through a semi-permeable membrane with a plant fine root-like size (diameter 0.5 mm) creating a 'probosphere' where we can release exudates and retrieve the effect on nitrogen availability[1]. Our goal is to connect root characteristics such as composition and amount of root exudates to the responses in microbial community composition and enzyme activity, to disentangle the underlying mechanisms of plant nitrogen availability.

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From the study of fungal symbiotic effector towards a central role of root terpenes for tree-microbes relationships

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As a consequence of limited availability of nutrients in forests soils, tree roots of boreal and temperate forests establish mutualistic interactions with hyphae of ectomycorrhizal (ECM) fungi to sustain their growth and health. ECM hyphae first aggregate on the surface of roots to form a sheath-like mantle, and then grow outwards into the soil to form extensive extramatrical hyphae that explore different soil niches for nutrients. A hyphal network, known as the Hartig net, is also formed inside the host root's apoplastic space and acts as the site of bidirectional nutrient exchanges between the two organisms (C from photosynthesis exchanged for N, P). The establishment of functional mycorrhiza required a strict coordination of development, immunity and physiology of both fungal and plant cells. Mycorrhiza-induced small secreted proteins (MiSSPs) were first identified 10 years ago through the combination of comparative genomics and transcriptomics. I will report on how the study of these MiSSPs have changed (or not) our view of mutualistic symbiosis and give the example on the study of *Laccaria bicolor* MiSSP7 effector points towards root terpene production as a regulatory mechanism of ECM symbiotic relationships.

Poster Presentations

1 Plants respond to single cell damage by depositing lignin in specific manner

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Cyst nematodes are biotrophic, sedentary endoparasites that invade the host root preferentially near the elongation zone, move through many cell layers to reach the nutrient-rich vascular cylinder, and then establish permanent, specialized feeding sites. During the migration phase, nematodes use their stylet and head force to move intracellularly, leaving behind a trail of mechanically injured cells. Upon detecting the disintegration of their own cell wall components and the discharge of cytoplasmic fluid, plants initiate a series of defensive responses. Here we identified a local defense response to the cells immediately next to the wounded cells by rapidly accumulating lignin in plant roots. Similarly to nematodes, the single cell laser ablation causes lignin induction, possibly enhancing mechanical strength of the cells against the infection. Furthermore, we discover that wound-induced localized lignin induction highly depends on *myb*15, and ethylene biosynthesis. Overall, we show that the local signaling triggered by single-cell wounding in terms of lignin deposition is not a default effect for general healing, but a specific mechanism to strengthen defense responses around vascular cylinder, thus appear to constitute a relevant root immune response against small invaders.

2 Root transcriptomics of boreal trees during cold stress

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Climate change in the boreal forest biome is expected to lead to warmer and more unpredictable weather conditions, resulting in reduced snow cover, lower soil temperatures and deeper and more prolonged soil frost. Even though native forest trees in the boreal regions are adapted to cold winters, the declined snow-soil-cover might have detrimental effects to the survival of these trees, when the decreased buffering effect of snow exposes the below-ground tissues to lowered soil temperatures during the winter.

What happens when roots are exposed to lower temperatures due to a reduced snow cover buffer? In our previous study with Norway spruce[1], its root transcriptional response to low temperature stress showed unique tissue specific responses compared to the above ground responses. We identified over 2000 root-specific up-regulated genes and a gene ontology analysis suggested that roots suffer from higher oxidative stress than needles during cold stress. These results led to our hypothesis that the cold stress transcriptional responses of roots could be conserved between boreal coniferous and deciduous tree species.

To gain insight into the low temperature, transcriptional responses of tree roots, we will perform genome-wide RNA-seq analysis from roots of four native boreal trees; Norway spruce (*Picea abies (L.) H. Karst*), Scots Pine (*Pinus sylvestris*), Silver birch (*Betula pendula*), Aspen (*Populus tremula*) and the model species *Arabidopsis thaliana* (Col-0 and an autumn germinating northern Swedish ecotype Ost-0). The aim of the study is to compare cold stress transcriptional responses between evolutionary distinct species and reveal both universal core cold mechanisms, as well as species-specific regulators in root. Our study will provide a comprehensive overview of the root transcriptome of the Boreal trees involved in cold stress and give insight to the root's adaptation to cold climate.

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3 Transcription start site selection, an important endogenous control system in trees

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The adaptation of perennials, such as trees, in temperate ecosystems is dependent on their remarkable plasticity which is controlled by various endogenous and environmental factors. Our knowledge of the endogenous factors, and their molecular mechanisms is particularly rudimentary until now. The gene expression profiles in plants are tightly controlled by various transcription-dependent RNA quality-control pathways. The regulation of transcriptional initiation is a crucial step in this process. Therefore, transcription initiation dynamics, the signals that specify them, and discovering the underlying molecular mechanisms associated with their selection is an important question. Here, by monitoring a genome-wide analysis of transcription start sites (TSSs) in spruce, *Picea abies*, using Transcription Start Site-sequencing (TSS_seq)[1], we show many novel TSSs on both sense and antisense strands with single nucleotid resolution. By clustering the TSSs into different TSS clusters (TCs), we also demonstrate relative level of transcripts initiated at different parts as alternative transcriptional initiation (ATI) in several specific genes. We also show that different tissues in spruce are exclusively using various TSSs with comparing TCs from needle, wood, and pool (PAM, embryo, xylem, and pollen) samples for the first time. These results provide an overview of the transcription start site usage as an important endogenous control factor. A better understanding of this important regulatory factor is crucial to design practicable approaches to address exigent topics in agriculture, horticulture, and forestry that can mediate solutions to issues caused by climate change.

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4 Quantifying cell-cell adhesion strength in plants

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Plant cell-cell adhesion is mediated by the cell wall which is constantly undergoing remodeling to allow cell expansion. Simultaneously, the mechanical tensions in plant tissues generated by turgor pressure and differential growth threaten cell-cell adhesion. We study the mechanical basis of cell-cell adhesion quantifying the force needed to separate adjacent cells, to understand how cells maintain their adhesion under chemical and mechanical stress-mediated stimuli. We use an interdisciplinary approach, integrating genetics, biophysics, and live imaging to better understand both the physics and the biology of the phenomena. We use a Micro Scale Extensometer (MSE) system that allows a very precise force application to the samples combined with high-resolution live imaging with confocal microscopes. Using MSE we pull the samples (wild-type and adhesion defective mutants like qua1-1, qua2-1, arp2) till the cells separate. We also exert a certain force and keep it for a long time and study how it affects the growth and adhesion of the adjacent cells. Our approach aims to get a deeper understanding of the mechanical implications of cell-cell adhesion in plants.

5 CDK8/CDKE1 modulates stress response through CAMTA3 regulation

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Plants are exposed to a wide range of environmental stresses, including extreme temperatures, drought, and pathogen attacks, which can significantly impact their growth and productivity. To cope with these stresses, plants have evolved complex molecular mechanisms that allow them to sense and respond to those environmental disruptions. The mediator complex, a large multi-subunit protein complex, plays a crucial role in this process by acting as a bridge between transcription factors and the basal transcription machinery. It has been previously shown that the kinase module of the mediator complex, which includes CDK8 and its associated subunits, plays a crucial role in regulating the stress response. However, the specific mechanisms by which CDK8 modulates gene expression in response to stress, specifically cold stress, are not well understood.

In this study, we aimed to investigate the role of CDK8, the only protein with kinase activity within the mediator complex, in the regulation of gene expression upon cold stress in plants. To address this task, we performed transcriptomic analysis of wild type (wt) and *cdk8* knock-out lines under cold stress conditions (4°C) at 0, 3, and 72h. The results of our studies revealed numerous misregulated genes related to both biotic and abiotic stress response in the *cdk8* mutant background relative to the wt plants, particularly in those genes linked to the CAMTA3 (Calmodulin-binding transcription activator 3) transcription factor. CAMTA3 has been shown to have a dual role in regulating both biotic (e.g., pathogen attacks) and abiotic stresses (e.g., low temperature). It is activated by calcium signaling and binds to the promoter regions of biotic stress-related genes and cold stress-related genes to repress or activate their transcription respectively. We further investigated the CDK8-CAMTA3 connection by proteomic analysis revealing that CDK8 binds to CAMTA3, phosphorylates it, and contributes to targeting it for proteasomal degradation in collaboration with other factors (e.g., MAPK3/6). Phosphorylation of CAMTA3 by CDK8 would result in the dissociation of CAMTA3 from the promoters of biotic stress-related genes releasing their repression, allowing it to bind to promoters of cold stress-related genes promoting their activation.

Our study provides novel insights into the regulatory mechanisms of plant stress responses and highlights the potential of this mechanism as a modulator of biotic and abiotic stresses.

6

Tissue specific co-factors promote FD binding to an unusual binding site

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The bZIP transcription factor FD plays a pivotal role in the correct timing of floral transition in *Arabidopsis thaliana* and many other species. We have recently shown that FD binds the canonical G-box motif (CACGTG), both when mis-expressed in phloem companion cells and in its normal expression domain at the shoot apical meristem (SAM)[1]. In addition, we found that FD binds another DNA-binding site specifically at the SAM. This atypical DNA-binding site of 16 nucleotides consists of two highly conserved palindromic sequences separated by a 6 nucleotide linker sequence. Screening public plant DNA-binding site databases did not reveal homology with any known motifs. For this reason, at the moment, we refer to this atypical binding site of FD as "new-box". Our goal is to find co-factors expressed at the SAM that promote the binding of FD to the new box. We found that the two highly conserved palindromic sequences of the new-box share partial similarity with the binding sites of some candidate genes. FD ChIP and ChIPseq in wild type and mutant plants for putative FD-interactors show that FD binds to the new box specifically at the SAM in a co-factor dependent manner. We further validated the interaction of FD with these candidate proteins by both *in vitro* and *in vivo* assays. In order to gain biological insights from the possible interaction of FD with these genes, we selected 22 FD-target genes containing the new-box in their promoter and phenotyped the corresponding mutant lines. Three of these mutants display interesting pleiotropic phenotypes and we are currently characterizing these lines in detail. If these interactions are confirmed, our research would add another layer of complexity to the regulation of floral transition and flower develompent as well as shed some light on the evolution of transcription factor binding sites.

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7 Functional characterization of MAX1 proteins in the Strigolactone biosynthetic pathway in pea

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Strigolactones (SL) are plant hormones that repress shoot branching. They are also known for their activity in the rhizosphere, in particular to stimulate symbiosis with endomycorrhizal fungi. More than thirty natural SLs with diverse structures have been characterised and plants can synthesize a cocktail of many SLs, sometimes specific to plant families or species. They can be classified into two groups according to their structures, the canonical SLs, with an "ABC" tricycle linked to the butenolide "D" and the non-canonical SLs without the "ABC" tricycle. The biosynthetic pathways of SLs begins with the successive conversion of trans-ß-carotenes via three enzymes. These steps, apparently common to all vascular plants, constitute what is known as the "CORE PATHWAY". The rest of the biosynthetic pathway, on the other hand, diversify and seems to vary greatly between species. Studies have highlighted the central role of the MAX1 protein (CYP711A) in the SL biosynthetic pathway. Its role is well described in Arabidopsis, tomato and rice, but the functions in other species are poorly described. There are two homologs in legumes, only one in other Dicotyledons whose genome has been sequenced, and at least five in Monocotyledons such as rice. Studying the MAX1 proteins in different species is important to understand the diversity of SLs. My project focuses on the two MAX1 homologs in Pea. We have obtained several mutants named *Psmax1s* by TILLING approaches. We are performing in vitro enzymatic tests and in planta SL quantifications to investigate the biochemical and biological functions of the pea MAX1. Unexpectedly, single and double mutants do not show the high branching phenotype characteristic of SLs mutants. However, these *Psmax1s* are deficient in the known canonical SLs present in Pea. Our results suggest that the branching inhibitory signal is not one of the strigolactones described so far in pea and that the real branching inhibitor signal remains to be discovered.

8 Structural and spectroscopic analysis of qH-dissipative antennae in Arabidopsis

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Photoprotection in plants is generally divided in an array of mechanisms affecting ("quenching") fluorescence emission. The most studied and described of these mechanisms generally represent short- or mid-term acclimation to changes in conditions. We still have a poor understanding, however, of how photosynthesis acclimate to longer stress, especially on a timescale of hours.

Recently, an increasing number of studies have brought to light various mechanisms of "sustained quenching", which affect photosynthesis with activation and relaxation timescales of tens of minutes to hours. One such mechanism, termed qH, has been shown to be independent of most well-known quenching pathways, as its activation requires neither lumen acidification, nor xanthophyll pigments, or, as far as is known, phosphorylation. Instead, several effectors have been described, whose exact role in qH remains to be characterized. These include LCNP, a lumenal lipocalin, SOQ1, a membrane multi-domain protein that operates upstream of LCNP and prevents quenching, and ROQH1, a possibly NADPH-dependent dehydrogenase-reductase, involved in qH relaxation.

In a recent study[1], quenched LHCII trimers (the main antenna of photosystem II) with active qH have been isolated directly from the model plant *Arabidopsis thaliana*. This very stable, native dissipative state does not depend on a single isoform of Lhcb1-2-3 proteins, composing the LHCII.

Here, we push further the investigation of the changes underlying the formation of this quenched state in plant antenna proteins. Using a combined approach of biochemistry, biophysics and structural biology, we explore the changes brought to LHCII trimers in qH conditions that could be at the origin of the dissipative state.

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9 Mechanical constraints regulate cell expansion and division in plant roots

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Cells evolved different mechanisms to sense the physical properties of the microenvironment and the mechanical forces that arise within it. In multicellular organisms, spatiotemporal sensing of mechanical cues plays a crucial role during development and regeneration. Plants developed extraordinary abilities to restore their tissues after injuries caused by abiotic or biotic environmental stimuli. Plant root can regenerate after excision of their tip, single-cell ablation, or nematode infection. Here, we use the primary root of arabidopsis as a simple experimental system to investigate the mechanical cues during the regeneration process. We used mutants related to cell wall biosynthesis and remodeling to study the involvement of the cell wall during the regeneration process. We show how mechanical stimuli activate cell division in pericycle cells after cell damage and how the mechanical cues are important in determining the orientation of cell division during regeneration.

10 New phytohormone derivatives as a modern tool for basic and applied plant research

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Cytokinins are an important group of plant hormones, regulating many growth and developmental processes in plants (Skoog et al., 1965). 6-benzylaminopurine (BAP) is one of the most exogenous applied CKs in plants for delaying senescence and reducing the stress impact. However, its endogenous natural and fast N9-glucosylation can also induce negative effects, which complicate micropropagation processes, especially in rare and susceptible medicinal plants (Bairu et al., 2009). To deal with that, the N9-glucosylation could be suppressed by appropriate N9 purine substitution of BAP or hydroxylation of its benzyl ring. Recently, a series of CK derivatives substituted at N9-position by various sugars and tetrahydropyranyl protective groups were prepared to improve their specific biological activity and are already routinely used in plant micropropagation (Plíhalová et at., 2016). Additionally, the replacement of the 2′ or 3′ hydroxyl groups of a nucleoside with a fluorine atom has also showed promising results in enhancing biological activity and increasing chemical or metabolic stability (Murvanidze et al., 2019). Moreover, by small change in cytokinin structure, a potent cytokinin antagonists and/or inhibitors of their inactivation have been obtained, including their isotopically and fluorescently labelled analogues (Plíhalová et at., 2016). Here, recent results of synthesis, characterization and biological activity testing of several new phytohormone derivatives will be presented and demonstrated that they can be used as an interesting new tool for plant biotechnology and agriculture.

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11 Low ambient temperature specific alternative splicing in *A. thaliana* regulates auxin homeostasis and is required for correct root patterning

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Organism biology strives to understand how complexity is achieved, whilst coordinating structure formation and organization. It has become evident that one key player in the establishment of organism complexity is a process known as alternative pre-mRNA splicing (AS). Besides providing the necessary variety of transcripts needed for correct development, an increasing amount of evidence suggests a prominent role of AS in abiotic and biotic stress signalling in plants. Although research in this area has grown, we have yet to integrate these observations into a coherent picture. Efforts in this direction are often hindered by the pleiotropic nature of mutations in central cellular mechanism such as RNA splicing.

In our research we focus on ambient temperature as an environmental stress signal. The core spliceosomal component SmE1/PORCUPINE (PCP) has been shown to be essential to ensure plant growth and development at low ambient temperatures (16 °C), while it appears to be largely dispensable at control temperatures (23 °C). The identified *pcp-1* mutant displays severe defects in plant architecture and meristem organization when grown at 16 °C. This observation raises the question whether, how and where the spliceosome is placed in the temperature signalling cascade and how the splicing output modulates plant development and temperature responses. To address this question we have implemented multiple experimental approaches, such as large-scale time-resolved RNA sequencing and phytohormone profiling. Interestingly, it seems that low ambient temperature specific AS regulates auxin homeostasis in the *A. thaliana* root, which could partially explain root patterning defects of *pcp-1* mutants. We could show that IAA and low ambient temperature have an additive negative effect on root development, and that *pcp-1* mutants are hypersensitive to exogenous IAA treatments. Taken together our results indicate that there is a refined cross-talk between AS and auxin signalling, which jointly regulate some aspects of plant development is response to low ambient temperature.

12 Concurrent time course of xylem hydraulic dysfunction and non-structural carbohydrates under contrasting water deficits and nitrogen supplies in poplar

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We compared the temporal dynamics of growth, leaf gas exchange, xylem hydraulic dysfunction and non-structural carbohydrates (NSC) of two poplar hybrid genotypes subjected to a differential nitrogen (N) supply under contrasting water deficits. Moderate water deficit generated intermediate embolism rates (45-70%) but had marginal effects on NSC. Severe water deficit generated progressive hydraulic failure and NSC reduction, leading to tree death within 90 days. At death, NSC in perennial tissues were not entirely depleted because of remaining soluble sugars. Higher N availability primarily affected growth and NSC dynamics (mainly starch), not embolism dynamics. The faster growing genotype benefiting most from N addition ceased growth and photosynthesis almost simultaneously, with no effect of N addition on process cessation, leading to progressive starch depletion during severe drought. In contrast, the slower growing genotype ceased growth before photosynthesis, leading to a transient increase in starch concentration during early drought stages, but this tended to be suppressed by higher N availability. Altogether, our findings indicate that carbon starvation alone is unlikely in hydraulically vulnerable species such as poplar even under prolonged moderate water deficit, but starch relative depletion covaries in time with progressive hydraulic failure under lethal water deficit. Nutritional status has the potential to shape drought responses, primarily through adjustments in carbon sink-source relations rather than in intrinsic xylem hydraulics, in a genotype-dependent manner. Exploring how hydraulic and carbon safety margins can be coordinated within species would be valuable in understanding how variation in drought response strategies can be exploited in breeding/selection.

13 Reciprocal transplantation of black poplar (*Populus nigra* L.) progenies reveals comparable growth but distinct underlying responses to environment between populations at early seedlings stage

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European black poplar (*Populus nigra* L.) is a key pioneer tree species colonizing alluvial sediments along rivers in temperate climate zones of Europe, northern Africa and western Asia. Changes in the patterns of river flow regimes and repeated droughts associated with ongoing climate change may however threaten seedlings establishment and population maintenance in the long-term. Adaptation potential within the species depends at least in part on the genetic variation and the phenotypic plasticity occurring for physiological functional traits. Previous work on established *P. nigra* populations grown in common gardens has revealed substantial genetic variation and phenotypic plasticity for growth and leaf traits (Chamaillard et al. 2011; Guet et al. 2015). However, there is no such data available data at early seedlings stage. In this study, we established a reciprocal transplant experiment in 1-m^3 containers to compare growth performance, biomass allocation, leaf traits and xylem functional anatomy in seedlings of two *P. nigra* populations originating from contrasting climates and river basins (Loire in Central-France under temperate influence *vs.* Drôme in Southern France under Mediterranean influence). For each population, seeds were collected from 10 individual open-pollinated female trees representing 10 progenies per population. Findings revealed substantial genetic variation within populations for all traits, at least comparable to previous findings observed on established populations, but also significant differences between progenies. Populations did not differ in final growth performance irrespective of the site, but differed in leaf traits and most importantly in the suites of traits that covaried in response to the environment. Altogether, our findings indicate no local adaptation for growth but distinct functional response strategies.

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14 Development of a HIgh Troughput Plant Phenotiping through Imaging Experiment (HIPPIE) for conifers

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Genetic degradation is a problem for the ecosystem, both ecologically and economically. The Swedish Forest is the outcome of a combination of natural regeneration and mainly human-driven planting with unenhanced and enhanced (breed) seeds from both Swedish and foreign origin. Little is known about the impact of breeding in genetic diversity, adaptability and resilience facing climate change. To answer this question, six Scots pine forests were selected. Three were naturally regenerated and three were planted with breed seeds originated from seed orchards. In those six sites buds were collected from a total of 600 trees. For assessing the resilience of those forests to climate change, seeds were collected and a drought essay using a novel method using a foam matrix. The phenotyping consisted in a new method using machine learning-based high throughput phenotyping to evaluate plant growth and development both in control and drought conditions. Thermal images were also captured to evaluate plant transpiration with thermal index for crop water stress (I_{CSWI}). The image dataset was analized using a machine learning approach and plant growth was modeled into sigmoidal growth curves to compare between different genotypes and conditions. Further analysis will include pigment interpolation during all the experiment using RGB images calibrated with a subset. This experiment will give information of how Scots pine plants from northern Sweden adapt to drought conditions and how well the genetic background of the population affects adaption to drought.

15

Ambient temperature and plant development: a view from the SM core assembly

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Splicing is a co-transcriptional process thanks to which plants react quickly to external changes by modulating the identity of the mature RNAs available in the cell. Thus, it is not surprising that many splicing related genes have been linked to the response to abiotic and biotic stresses, with mutants showing an unpaired ability to properly respond to stimuli compared to the wild type (Dikaya *et al.*, 2021). This is the case of SmEa, also named PORCUPINE and orthologue of human SNRPE and yeast SME, whose mutants show a wildtype phenotype when grown at 23°C but important developmental defects at 16°C (Capovilla *et al.*, 2018; Huertas *et al.*, 2019). SmEa is part of the heptameric Sm-ring, the core of the SnRNP spliceosome structure. In metazoan, the assembly of the Sm-ring is not spontaneous, but it proceeds in steps and it is assisted by multiple factors, members of the methylosome and the SMN-complex (Matera and Wang, 2014). Trying to shed light on the link between splicing and abiotic stress, we decided to specifically focus on the Sm-ring biogenesis to understand whether this process is involved in the response to temperature. So far, only two components of the methylosome and the SMN-complex have been described in plants, PRMT5 and GEMIN2, respectively. Interestingly, GEMIN2 mutants have been already reported to show a temperature-related phenotype (Schlaen *et al.*, 2015). We aim at understanding if other proteins exist in Arabidopsis that could be involved in the Sm-ring assembly and if this stepwise process is overall modulated by the temperature. Preliminary results from genetics, molecular and biochemical approaches suggest that high ambient temperatures help the plant to overcome defects in the Sm core assembly.

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Fine-tuning of plant amino acid uptake

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Fine-tuning of plant nutrient management can be executed on different levels such as on protein level. Significant progress has been made towards unraveling inorganic nitrogen (N) import, with respect to post-translational modifications of transport proteins. A common theory is that plants rely on inorganic N forms (NH_4^+ and NO_3^-) as the main contributors to plant N nutrition. However, it was shown that the contribution of amino acids (AAs) to soil N fluxes is much higher than previously thought, not only in poor soils but also in soils of higher fertility. Notably, N in the form of AAs has been shown to be absorbed by boreal forest plants, as well as by agriculturally important crops. Noteworthy is the low energetic cost for N assimilation, based on organic N forms. Lower assimilation costs harbor a range of important benefits for plants such as a larger root mass fraction.

It is noteworthy how little is known about the molecular underpinning of the AA import regulation, in spite of that transport proteins such as the central AA transporter AtLHT1 were described many years ago. We aim to unravel the protein-protein interactome of AtLHT1 in order to understand its regulation and, thus, AA uptake.

17 Genetic control of solid and chemical properties of the wood in two large progeny trials of Norway spruce in Sweden

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Genetic contol of wood quality properties and identification of their relationship with growth traits is of particular importance in breeding programs as it is a prerequisite for production of wood with higher quality. Norway spruce (*Picea abies*) is one of the most economically and ecologically important forestry species in Europe. Its increasing role for traditional forestry activities and the emerging bioenergy sector stimulated research into the investigation of genetic variation over its wood quality traits.

The main objective of this study is to gain a better understanding of the genetic control of chemical (cellulose, hemicellulose, and lignin content), solid (density, modulus of elacticity (MOE), microfibril angle (MFA)), and fiber properties of the wood as well as quantifying resin canals in Norway spruce.

Increment cores (12-mm) were sampled at breast height from 5600 trees in 524 open-pollinated families, using two 21-year-old Norway spruce progeny trials located in southern Sweden. High-resolution pith-to-bark radial variation of solid and fiber properties of the wood was measured using SilviScan instrument and the existing Silviscan measurements, coupled with machine learning methods, were applied to quantify resin canals of the samples. Similarly, the chemical composition of the samples was assessed using wet chemistry protocols.

Results of such study revealed that there is a moderate to high genetic variation for wood properties of Norway spruce, enabling its genetic improvement through selection and breeding. We further observed wood chemistry traits were inter-correlated (e.g., negatively between lignin and cellulose), while correlations with growth were not significant, indicating that growth and chemical properties could be improved independently. However, the unfavorable genetic correlation between solidwood (e.g., density and MOE) with growth traits remains as a constraint in simultaneous improvement of these traits in breeding programs.

18 Exploring the stomatal conductance and CO2 assimilation in ZTL and TOC aspen mutants with the IRGA and Thermal Imaging

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In plants, the circadian system regulates various processes, such as growth, organ movements, and gene expression. The circadian system also regulates the exchange of carbon dioxide and water vapour between leaves and the atmosphere by controlling stomata opening and photosynthesis. In this sense, stomatal movements are essential to balance gas exchange and water stress.

In this study, we analysed the circadian regulation of stomatal conductance (g_s), net photosynthetic rate (A_N) and growth of two transgenic 'mutant' aspen lines with reduced expression of *ZEITLUPE* (*ZTL*) and *TIMING OF CAB EXPRESSION 1* (*TOC1*) due to RNA interference. Strongly down-regulated and previously characterised representative lines *ztl*-5 and *toc*1-5 were used. We measured both parameters for 18 hours and filmed the plants with thermographic cameras in the same growth chamber. ZTL and TOC have previously been linked to stomatal movements, plant growth and fitness. Both peptides contribute to carbon fixation and biomass production by influencing the circadian clock's function. ZTL is the F-box component of an SCF complex shown in Arabidopsis to be involved in TOC1 and PSEUDO-RESPONSE REGULATOR 5 (PRR5) degradation. In general, our results showed that g_s and A_N tended to a cyclical variation of 24 hours, where the maximum of g_s in the mutants could occur in the morning and the minimum in the hours of darkness. For A_N, the maximum was maintained in wild-type (WT) and all the lines throughout the daytime and the minimum at night. We also calculated the water use efficiency (WUE), which was lower in the mutants than in the WT due to the high stomatal conductance. These results are still preliminary, and in future experiments, we plan to make measurements comprising 72 hours to test the g_s cycles and elucidate if thermal imaging could be a functional method to monitor stomatal conductance in aspen trees.

19 Exploring the role of *Arabidopsis* Mediator subunits in response to abiotic stresses

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As sessile organisms, plants are confronting various stresses from their environment and perception of these stresses is a crucial step for the survival of plants. Salinity and drought are stresses that prevails in arid and dessert areas and could hinder the development/productivity of crops. Mediator is a multi-subunit complex, which is essential for transcriptional regulation in eukaryotic cells. It serves as an interface between promoter-bound transcription factors and RNA polymerase II, to transduce signals for activation and repression of genes in response to changes in the environment, such as different types of stress. To understand the control and coordination of transcription by Mediator, we have selected five mediator subunit mutants (med9, med16, med18, cdk8/cdke1 and med25) to be studied in two relatable stresses: salt and drought. Plants were grown in short day conditions for 5 weeks and then exposed to salt/drought stress. To reveal differential gene expression pattern among wild type plants and mutants on a global level, RNA sequencing was performed at two time-points (as early and late) after stress. Differential genes expression pattern and salt/drought stress responses were evaluated for each genotype. Reprogramming of gene expression/pathways exhibiting similar and divergent stress responses will be presented.

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Tissue-specific expression of the COP9 signalosome CSN5A subunit restores the wild-type phenotype of *csn5a-1* knockout mutant

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Evolutionary conserved multiprotein complex, COP9 signalosome (CSN), plays an indispensable role in plant development. It controls the activity of CULLIN-RING E3 ubiquitin ligases (CRLs) which ubiquinate and target proteins for proteasomal degradation. CSN complex comprises eight subunits and the catalytic activity of the complex resides in the subunit 5 (CSN5). Certain plant species, including Arabidopsis, have duplicated the CSN5 (CSN5A; CSN5B) and CSN6 (CSN6A; CSN6B) subunits. CSN5A but not CSN5B has kept the catalytic activity and the knockout mutant csn5a-1 shows a dwarf phenotype and is unable to produce adventitious roots (AR). In Arabidopsis hypocotyl, ARs initiate from the pericycle cells adjacent to the xylem pole in a similar way as lateral roots. We transformed the csn5a-1 mutant with CSN5A:mCITRINE translation fusion expressed under its own promotor or the pericycle-specific GATA23 promoter. In both cases the AR phenotype was restored to wild type. Although it was expected to restore to WT the growth of the csn5a-1 mutant with the pCSN5A:CSN5A:mCITRINE construct, it was unexpected with the pGATA23:CSN5A:mCITRINE construct. We investigated further the expression pattern of pGATA23: GUS reporter gene and showed that it is not restricted to the hypocotyl or the root, but it is also expressed in the vascular tissue of the leaves. This raise the following question: how a tissue specific expression of the CSN5A subunit can fully restore the growth and development of the knock-out csn5a-1 mutant?

21 Maturation of iron-sulfur containing subunits of respiratory complex I in plants

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Complex I (CI) is a major protein complex in the oxidative phosphorylation system of most of the eukaryotic mitochondria, being essential for the electron transfer chain and for H^+ pumping into the intermembrane space of mitochondria that is required for the formation of ATP by the ATP synthase (complex V). Complex I has a conserved L-shape structure that is characterized by the presence of a membrane hydrophobic arm in the inner mitochondrial membrane, where the reduction of the coenzyme Q to ubiquinol and H^+ pumping takes place, and of a peripheral hydrophilic arm present in the mitochondrial matrix, where NADH oxidation and electron transfer occur. This electron transfer is possible due to the presence of 8 Fe-S clusters (two [2Fe-2S] and six [4Fe-4S] clusters) bound by 5 subunits (51 kDa, 75 kDa, 24 kDa, PSST and TYKY). It is thought that the Fe-S clusters and other cofactors like FMN, are first assembled in the subunits, and afterwards, the subunits are assembled stepwise in larger assembly intermediates which will form the whole holo-complex I.

The synthesis of Fe-S clusters in mitochondria is carried out by the Iron-Sulfur Cluster (ISC) assembly machinery. A [2Fe-2S] cluster is first *de novo* assembled on the scaffold protein (ISU). It may be directly transferred to [2Fe-2S] cluster-containing subunits. Additional transfer and conversion steps and players are likely needed for [4Fe-4S] cluster-containing subunits. However, the exact mechanisms by which these five CI subunits obtain their Fe-S clusters from the transfer proteins of the ISC machinery still need to be elucidated.

To clarify these mechanisms, we have expressed in *Escherichia coli* and purified recombinant proteins corresponding to both CI subunits and possible transfer proteins. Whenever proteins are soluble and stable, we intend to perform *in vitro* Fe-S cluster reconstitution and transfer assays using either specific protein couples or multiprotein complexes that are physiologically relevant.

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Differential Calcium and Hormonal Regulation by Localized Wound Stress

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Plants as sessile organisms are constantly exposed to cellular damage caused by mechanical stresses, herbivore feeding, or invading microbes. Plant defense response must be fast and restricted to the infected area to limit secondary negative effects. In plants, wounding triggers electrical and calcium (Ca^{2+}) signaling, which is mediated by glutamate-receptor-like channels (GLRs) and stretch-activated anion channel MSL10. Plant *GLRs* mediate the transport of Ca^{2+} and other cations as well as nutrient uptake, while MSL10 relates mechano-sensing, ion fluxes, membrane depolarization, and electrical signal propagation. Here, we report on the contribution of GLRs and MSL10 to localized early events after wounding performed by laser ablation. We discovered that calcium regulates both JA and ethylene signaling in response to local damage. Moreover, we establish a thorough interplay between JA and ethylene signaling in response to local injury, especially upon laser ablation. Therefore, we proposed an open and developing "calcium-hormone" model to explain in detail the early events in the local wounding region.

23 Ribosome footprint sequencing: translating *Plantae*

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Translation plays a prominent role in controlling the expression of protein coding genes. Several tools have been developed to study translational regulation, among which ribosome footprint sequencing (Ribo-Seq) is by far the most prominent method because of its ability to assess actively translating mRNAs in a quantitative and positional manner [1].

Ribo-Seq has been successfully used in plants, leading to a better understanding of molecular mechanisms underlying various biological processes and discovery of new open reading frames [2, 3, 4]. This has mainly been done in *Arabidopsis*, but releases of novel as well as improved genome annotations enables us to expand this to other plant species.

Here, we have for the first time developed the Ribo-seq protocol for several new plant species, including Norway spruce, aspen, tobacco. Several quality features of the Ribo-Seq data obtained by small scale sequencing were assessed, confirming that the data met the Ribo-Seq data quality requirement.

Improved understanding of translation in various plant species can help us tackle fundamental biological questions related to translational responses to biotic and abiotic stresses in plants in general.

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24 Molecular and protein characterization in different light conditions of the COL gene family in *Populus*

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Flowering time and other seasonal changes are finely regulated by molecular pathways able to perceive external environmental changes. Many studies have reported that flowering time in Arabidopsis is governed by the *CO/FT* module which is highly conserved among photoperiod-sensitive plants although its functions are distinct in different plants. In Arabidopsis, the *CONSTANS* (*CO*) gene is a key transcriptional regulator in the photoperiodic pathway and displays a diurnal regulation in which the mRNA accumulation peaks at the end of the day in long days leading to the translation of a stable protein. Under these long days conditions CO induces the expression of *FT*, a major component of the florigen signal.

In contrast to annual plants like Arabidopsis, *Populus* trees are perennials characterized by a multiple-year delay in flowering and in temperate zone they seasonally cycle between growth and winter dormancy. The purpose of our study is to investigate the function of the *CO/FT* module in *Populus* to determine its role in controlling flowering time and/or short-day induced growth cessation and bud set.

The *COL* gene family in Arabidopsis has many members: 17 genes have been characterised to belong to this family. As a results of phylogenetic analysis, COL proteins have been divided into three major groups. In our study we identify 19 genes belonging to the COL gene family in *Populus tremula*. We decided to focus on the genes belonging to group I, which in Arabidopsis are related to flowering induction, to study their diurnal expression pattern and found that their expression show distinct circadian regulation. Two genes, previously characterized from another group, show high identity with the *At*CO gene and therefore they have been named CO1 and CO2. For CO1 and CO2 CRISPR lines have generated respectively as well as a double mutant line for both genes and an overexpressing line of CO2 tagged with GFP. The initial phenotyping results don't show any significant variation between mutant lines compared to WT regarding vegetative growth or bud set in SD conditions. CO protein posttranslational regulation is trigged by specific light quality through multiple photoreceptors. To investigate this response, we measured CO2 protein level at different timepoints and light regimes. The results show that the exposure to white and blue light conditions leads to protein accumulation within 6 and 12 hours in contrast to red and far-red light conditions where protein levels are almost undetectable.

25 plaNET-seq reveals genes hosting antisense transcripts involved in the early cold stress response in *Arabidopsis thaliana*

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Antisense transcription occurs pervasively across eukaryotic genomes. Only a few antisense transcripts have been characterized and shown to control biological processes, albeit with idiosyncratic regulatory mechanisms. Thus, we largely lack knowledge about the general role of antisense transcription in eukaryotic organisms. Here, we characterized genes with antisense transcription initiating at the Poly(A) signal (PAS genes) in *Arabidopsis*. We compared plaNET-seq with RNA-seq during cold exposure and detected massive differences between the response in active transcription and steady-state levels of mRNAs of PAS genes. Among those, the induction by cold of transcription factors, *B-BOX DOMAIN PROTEIN 28* (*BBX28*) and *C2H2-TYPE ZINC FINGER FAMILY PROTEIN 5* (*ZAT5*), was detected by plaNET-seq, while their steady-state level being unaffected due to mRNA degradation dynamics. Knockdown of *BBX28* and *ZAT5* or of their respective antisense transcripts displayed compromised freezing tolerance. Decrease of antisense transcription resulted in a reduced cold response of *BBX28* and *ZAT5* revealing an unexpected positive role of both antisense transcripts. Our data expands the repertoire of noncoding transcription during cold stress and suggest that plaNET-seq can more precisely illustrate biologically important genome-wide transcriptional behaviours. Furthermore, our data indicate a positive regulatory role of antisense transcription to be important during abiotic stress response.

26 Modulation of *Arabidopsis thaliana* root system architecture by organic nitrogen

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Nitrogen (N) represents a life-essential macronutrient embedded in vital structures such as proteins, nucleic acids, vitamins or secondary metabolites and thus represents a growth-limiting factor for plants. As such, it has become the main component of synthetic fertilizers in its inorganic forms: nitrate and ammonium. However, the over-use of inorganic nitrogen (IN)-based fertilizers has resulted in severe environmentally harmful consequences. On the other hand, very little is known about how organic N (ON), a more environmentally friendly source of N, regulates plant development.

Our preliminary results show that ON in the form of the amino acid glutamine (GLN) has a promoting effect on plant growth, with a strong positive impact on total root biomass. ON modulates root system architecture (RSA) by inhibiting primary root growth while significantly promoting lateral root (LR) branching. Remarkably, the adaptation of root growth to the change of N source from IN to ON resulted in highly synchronized LR development 5 days after transfer. This adaptation effect was strikingly different compared to the basipetal gradient of LR development after continuous IN treatment. Microscopic analyses revealed that the N source differentially affected the spatial and temporal development of LR primordia. In presence of ON, the first LR initiation occurs closer to the root tip and LR primordia develop faster compared to IN. In addition, ON greatly impact LR primordia spacing, resulting in significantly higher LR primordia density when compared to IN.

In addition, our pilot studies indicate that the ON effect on root growth is mediated in concert with plant hormones. The hormonal analysis of plants grown under ON and IN conditions revealed that the levels of most auxin metabolites were significantly elevated in response to ON. However, in ON-treated roots the levels of bioactive auxin were higher. In addition, IN treatment resulted in higher levels of trans-zeatin (tZ) types of cytokinins (CKs), while ON induced the accumulation of cis-zeatin (cZ) types. These novel findings indicate that auxin and CK metabolism is selectively altered in response to different N sources. To conclude, our work has raised new questions on how plants modulate their RSA in adaptation to different N environments. The mechanisms of ON action will be further investigated in order to better understand how ON regulates LR initiation and development.

27 Plant chromatin regulation in response to chloroplast signals.

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Plants are photoautotroph organisms that harness light and turn it into chemical energy to sustain themselves. This essential process is called photosynthesis and occurs in chloroplasts, highly specialized organelles that, like mitochondria, contain their own genome. Due to the endosymbiotic origin, the photosynthetic machinery is built using proteins encoded both in the nucleus and plastids. In our laboratory we are interested in elucidating the regulatory networks that synchronize the different genomes in the plant cell. In particular, we use the process of chloroplast development and establishment of photosynthesis as a model for the genetic interplay between chloroplast and nucleus. Our work has demonstrated that besides an initial anterograde (nucleus-to-organelle) light-dependent response, a secondary retrograde (organelle-to-nucleus) signal originating in the maturing chloroplast is required for the transition into a fully activated transcriptional program for photosynthetic genes. Genome-wide analysis of the distribution of four histone marks uncovered a changing chromatin landscape through the process of establishment of photosynthesis. We observed highly dynamic epigenetic regulatory events in response to both anterograde and retrograde signals. In addition, by manipulating the process of chloroplast maturation we could confirm the influence of retrograde signals on the epigenetic status of the nucleus. Finally, by de novo motif prediction we have identified and characterized new transcription factors with the potential to accelerate or stall the greening process of plant seedlings. Overall, our work advances our understanding in how chromatin is regulated during plant development and how epigenetics can be influenced by organelle signaling systems.

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Why are conifers green in the dark?

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One of the most dramatic challenges in the life of a plant occurs when the seedling emerges from the soil and exposure to light triggers the establishment of photosynthesis. In angiosperms such as Arabidopsis, this process is tightly regulated as premature accumulation of light harvesting proteins and photoreactive chlorophyll precursors cause oxidative damage when the seedling is first exposed to light. Chloroplasts communicate their developmental and physiological status to the nucleus via retrograde signaling, ensuring proper nuclear gene expression. Some gymnosperms conifer species such as Norway spruce (*Picea abies*) adopted a different strategy where dark germinated seedlings possess the ability to accumulate chloropyll and, more surprisingly, they can produce rather differentiated chloroplasts with grana. GENOMES UNCOUPLED1 (GUN1)-dependent retrograde signal regulates the expression of several key transcription factors during the critical step of seedling emergence from darkness in Arabidopsis. We have preliminary data suggesting that GUN1 has a different function in conifers. Currently, we are looking for the answer to several questions associated with understanding the establishment of functional chloroplasts in Spruce: i) What characteristics of the establishment of photosynthetically active chloroplasts are light dependent?, ii) Could the greening strategy observed in Spruce be explained by a different function of GUN1 relative to Arabidopsis?, iii) Which components of the greening strategy in Spruce show a key role from an evolutionary point of view? Through this project we will contribute to the knowledge of the regulatory mechanisms details behind the strategies employed by Spruce and Arabidopsis.

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Adaptive clines in Norway spruce in response to light quality

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Under the vegetative shade, the pigments of the neighbouring plants absorb red light (R), while far-red light (FR) is reflected resulting in a low R:FR ratio. Shade-tolerant trees continue to become established, survive and thrive under the shade while trees that are shade intolerant require full sunlight for growth. During the growth season, northern forests in Sweden daily receive more hours of FR-enriched light/ twilight or shade-like conditions as compared to southern forests. Norway spruce is a shade-tolerant conifer species that has adapted to latitudinal variation in twilight characterized by a northward increase in FR requirement to maintain growth. We report the adaptations in Norway spruce under the local environmental conditions such as clinal variation in genes e.g. *PHYO* (perceives R/FR) and *MYB3* (suppressor of lignin pathway), defence strategies such as differential regulation of defence-related genes and lignin synthesis in response to shade like conditions that render protection to the tree.

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30 Nitrogen responses & cambial growth in aspen

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Nitrogen is often considered as the most important growth-limiting nutrient especially in boreal forests. It is well established that environmental stimuli, such as nutrient status, influence activity of the vascular cambium. Nitrogen fertilization stimulates the cambium and can cause alterations in wood structures formed during xylogenesis. The underlying molecular mechanisms driving cell expansion and the responses of xylem differentiation to nitrogen is unclear despite the economically and environmentally relevant implications.

Wood formation is orchestrated by onset of genes related to specific zones of the differentiating xylem. The corresponding influence on cell wall composition, chemistry and morphology will be analyzed in hybrid aspen trees cultivated in low, intermediate, and high nitrogen conditions and in response to inorganic versus organic nitrogen sources.

These experiments indicate how wood density decreases with increased N-availability. The decrease in density could partially be explained by thinner cell walls of xylem fibers. Image analysis estimated the difference in cell wall thickness between low and high N-treated trees to be as much as ~29%. But also vessel frequency and size is altered which could also contribute to the overall decrease. Furthermore, chemical assessments show a general decrease in lignin due to increased N-supply and more subtile differences in lignin compostition depending on the nitrogen source added. Next up will be further evaluation of the relationship between wood morphology and chemistry in respons to nitrogen along with the underlying molecular regulation.

31 What makes a tree a tree? Evolution of the gene regulatory network underlying wood formation

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What makes a tree a tree? Basic wood cells and processes are common to most trees, but there can be significant differences among the two main tree families. Surprisingly, most of the mutations affecting phenotypic variation are not located in protein coding regions and the wood transcriptional program is largely conserved between hardwood and softwood species. However, a pine is obviously very different from a poplar at many levels. Thus, modern studies imply that both speciation and intraspecific variation is mainly the result of differential gene expression regulation. If we could understand the regulatory network behind trees' growth, we would have a map of the trees development and evolution. A map of what makes a tree a tree, from a minuscule seed to a colossal sequoia

In my PhD research, I generate genetic network models from representative angiosperm and gymnosperm species and compare the gene regulation for wood development between those lineages.

Young wood samples from 3 angiosperm and 3 gymnosperm trees will be used in several genomic techniques, specifically, ATAC-sequencing, Hi-C, RNA-sequencing and DAPsequencing. The data obtained from the experiments will allow to generate high-resolution models of gene expression and wood development across many tissues. These models will be prepared by integrating the different genomic datasets and chromatin profiles with experimentally determined transcription factor binding sites using a novel computational framework to infer and compare regulatory networks and thus deduce the regulatory mechanisms explaining the evolution of trees. In parallel, we will identify important candidate genes for wood formation by conducting comparative phylogenomic analyses in a large set of woody and non-woody angiosperms, identifying previously undetected non-regulatory gene evolution of importance to wood-formation.

Outlining those interactions and their differences among species would provide useful information for targeted genome engineering to optimise the woody feedstock for custom uses, including biofuels, wood-based materials and reforestation programs.

Imagine if you knew trees as well as you know your best friend, someone that could lend a hand with all your problems. Fossil fuels pollute the air? With these networks, you know how oxygen production works at molecular level and can design machines and breeds of maximum efficiency. Why not let the forest do the hard work? Choose the best trees for reforestation and grow a jungle in a few years. This is but a little example. From this project, a thousand more can blossom.

32 Sm protein SmD3B is critical for *Arabidopsis* growth at low temperature and its expression is regulated by cis-intronic DNA.

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Modulation of pre-mRNA splicing has been widely associated with plant acclimation to temperature stress, yet we are just beginning to understand the underlying molecular mechanisms. Sm proteins are core components of the spliceosome small nuclear ribonucleoprotein complex (snRNPs). Sm proteins form a hetero heptameric ring that associates with U-rich snRNAs, around which other factors organise to form the spliceosome. We found that loss of *SmD3B* dramatically impairs growth and development of *Arabidopsis thaliana* at low temperature. This is in agreement with previous reports on the central role of Sm-mediated RNA splicing for survival under that environmental condition. To understand the role of SmD3B in response to low ambient temperature, we studied the *SmD3B* locus. Complementation analyses revealed that introns are required for proper expression of *SmD3B*. Ongoing experiments address the contribution of individual introns to *SmD3B* gene and protein expression, the underlying regulatory mechanism and whether this mode of regulation is specific to low temperature. We are also analysing which pre-mRNA splicing events are mediated by SmD3B at low temperature. Our analysis points towards unexplored mechanisms for plant response to low temperature that regulate core spliceosome components.

33 From single cell to root system shaping in response to nitrogen

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Plant roots show a high degree of plasticity, which allows plants to adapt to changing external conditions. Among other things, this root flexibility requires *de novo* organogenesis, the development of lateral roots (LRs). Generation of LRs is regulated by internal factors, such as plant hormones and external signal, such as nitrogen availability. Nowadays, inorganic nitrogen (IN) is extensively overused despite low nitrogen use efficiency and its harmful effects on ecosystems and environment. On the other hand, the role of organic nitrogen (ON), an abundant nitrogen source in the soil, in plant development has remained largely unexplored. According to our preliminary results, glutamine as a selected representative ON compound promotes lateral root development and increases root biomass in comparison to IN in Arabidopsis. Moreover, cytokinin signalling was altered by ON treatment compared to IN in the root zone where LR organogenesis occurs. GATA23 is a transcription factor which controls LR founder cells specification and that is expressed during the early stages of LR initiation. Interestingly, the *p*GATA23:GFP signal was observed not only at LR pre-branch sites, but also in xylem pole pericycle cells of the transition and elongation zone.

We propose the following hypothesis: Dormant LR founder cells are generated in the apical part of the root. These are stem cells that have the ability to start developing into LRs, depending on environmental stimuli. ON, being such an environmental signal, could promote re-programming of these dormant cells by blocking cytokinin-dependent inhibition, resulting in induction of LR organogenesis. The goal of our project is therefore to understand how ON controls LR organogenesis and thus the adaptive capacity of the root system. Single cell RNA sequencing (scRNAseq) will be used to study the early "invisible" stages of founder cells specification. *p*GATA23:*GFP* cells obtained from the apical part of the root will be processed by two main approaches a) single cell Fluorescence-Activated Cell Sorting (scFACS) into well plates following individual cDNA library construction with Smart-seq3 sequencing and b) bulk sorting and scRNAseq using the 10×Genomics technology. The aim of this project is to reveal re-programming of root system architecture under ON treatment at the single cell level and to understand the potential involvement of cytokinin in this process.

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Importance of organic nitrogen for shoot:root allocation

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Soil nutrients are critical elements for plant growth and productivity. Nitrogen (N), in particular, is a limiting factor for primary production in most terrestrial ecosystems. Furthermore, nitrogen nutrition is an important factor for the biomass allocation. For a long time, the importance of inorganic N was highlighted in the scientific community. However, in the last decades, it was possible to shift the focus away from inorganic N as sole important N source towards organic N such as amino acids (AAs). Here we analyzed differences in plant growth and shoot:root allocation of A. *thaliana* Col-0 plants when grown in a split root system. The plants were treated with the different N sources nitrate, glutamine (Gln) or both and were then analyzed regarding their 15N and 13C status.

Here we demonstrated that Gln, affects plant growth positively, when used as sole N source, and leads to a distinctive plant phenotype, characterized by increased root biomass, compared to inorganic N treatment. Besides the effect on the root biomass, we observed positive effects of Gln on the presence of root hairs and on the N uptake of plants compared to inorganic N. Interestingly, similar responses could be observed when the plant was treated with a combination of organic and inorganic N. The observed phenotypic characteristics of Gln grown plants resemble those of N deficient plants which raises the question of the existence of a specific amino acid phenotype.

Currently, we try to identify the role of carbon (C) provided by organic N and its effects on plant growth and development. We suggest that the observed phenotype was congruent with a significant contribution of C derived from organic N.

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The Role of Alternative Splicing During Photomorphogenesis

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Plants respond to light changes through a process known as photomorphogenesis by altering their growth and development. After germinating in darkness, etiolated seedlings are characterized by elongated stems and underdeveloped leaves where after a subsequent exposure to light significant transcriptional changes occur.

GUN1 has been characterized as a suppressor of photomorphogenesis and we previously showed that during early de-etiolation a negative GUN1-mediated retrograde signal restricts chloroplast development in darkness. Then, as the exposure to light progresses, it progressively stops inhibiting key TFs of photosynthesis establishment and chloroplast development and consequently downstream targets.

Alternative splicing (AS) plays a role in photomorphogenesis through the production of different protein isoforms in response to light that allows plants to respond during de-etiolation. Thus, AS of certain genes involved in light signalling can result in the production of different protein isoforms, allowing the plant to fine-tune its response.

Here, through the use of co-expression network and RNA-Seq analysis we identified new genes involved in photomorphogenesis and characterized at transcript level the changes involved in de-etiolation in wild type and *gun1* plants.

We herein show that splicing-related genes are down-regulated in *gun1* mutants, which led to changes in AS. We found in wild type plants 915 genes being affected by AS during de-etiolation and that AS landscape is affected in *gun1*. In addition, in darkness *gun1* plants showed AS alterations in 40 chloroplast related genes. We validated our results by qPCR and analyzed the functional consequences of AS changes using protein domains and structural analysis. Thus, our results characterize the transcriptome changes during de-etiolation and demonstrates that key genes for the establishment of photosynthesis are affected by AS during de-etiolation. Additionally, our findings show that GUN1 mutation affects AS during photomorphogenesis by an unknow mechanism and how this might influence the transcriptomics responses to light.

36 CHANGES IN SCOTS PINE GENETIC DIVERSITY ACROSS THE PAST CENTURIES – IN NORTHERN SWEDEN

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After the ice glaciers retreated in Sweden forest products have been imperative as resources for human needs in terms of heat, shelter, construction materials, and nutrient supply. Deforestation started in the mid-17th century and left large areas of heathland. Natural regeneration was the common way of renewing forests until the 19th century when extensive imports of seeds began. Then, reforestation was done predominantly with seedlings of Scots pine and Norway spruce imported from Russia, Finland, Norway, France, Poland and German origin. This project aims to study the original genetics of Northern Swedish forests of Scots pine before the imports of seeds initiated in the 19th century. Knowing the genotypes of the original forest can show if the imports made a drift in genetic diversity or the loss of locally adapted alleles. Core wood samples of old houses from the mid-centuries will be extracted for the remaining DNA using hexadecyltrimethylammonium bromide (CTAB) based DNA extraction followed by whole genome amplification. The samples will be then genotyped, either by traditional PCR or using new generation techniques such as SNP Arrays. Until now, we succeeded to extract the remaining DNA of Scots pine wood cores and amplifying SSR markers by PCR. The proposed protocol and method is a promising way to discover the missing link between the original Swedish forests and the modern forestry plantations, both for breeding and preserving the genetic diversity for future generations.

37 Gene regulation, a perspective from the UPSC Bioinformatics Facility

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The Umeå Plant Science Centre Bioinformatics Facility is offering support for bioinformatics, data-science and machine learning to research group at, and affiliated with, UPSC.

It provides data pre-processing as a service, data analysis support, as well as training of PhD and Post-Docs in learning the skills to perform their own data analysis. All these are subsidised by UPSC, meaning an access to competent and dedicated service at a fraction of the cost of commercial providers. The facility is highly familiar with plants genomics and in particular has developed strong skills for non-model organisms used in UPSC's research, such as spruce and (hybrid) aspen.

Besides being involved in genome sequencing projects, the facility has worked with all common data sequencing types, including long reads, and most sequencing protocols, including RNA-Seq, ChIP-Seq, NET-Seq, ATAC-Seq, ..., making it a useful bioinformatics resource hub at UPSC.

Furthermore, the facility has been involved in developing tools taking advantage of the large amount of data being generated by UPSC researchers to explore and visualise gene regulation, such as **seidr** [1] a tool to reconstruct gene networks from expression profiling data using an ensemble of 13 different network inference methods.

A major limitation of working with non-model organisms is their lack of annotation, which are in-silico lifted-over from public databases based on sequence similarities, making Arabidopsis thaliana, the almost only source of annotation for non-model plants, leading to less than half of Norway spruce genes to be annotated! The facility has been working on developing a method that combines semantic similarity (annotation information stored in knowledge bases such as Gene Ontology), with our gene network reconscruction to learn by artificial intelligence how to fill in this annotation gap.

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Biopolymer Analytical Platform - BAP

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The Biopolymer Analytical Platform (BAP) is dedicated to support research among UPSC groups on cell walls of terrestrial and aquatic plants, and biopolymer materials. Our competence lies in applying a large range of standard methods for the analysis of lignocellulose, as well as in fine detection of soluble sugars and starch. The methods include carbohydrate and lignin composition analysis using conventional wet chemistry and state-of-the-art analytical devices. The instrumental backbone for many of those methods is gas chromatography/mass spectrometry (GC/MS). Pyrolysis-GC/MS is one of the most important analytical tools that quickly yields highly reproducible and comprehensive chemical fingerprinting of carbohydrate and lignin types in samples in the lower microgram range.

Examples for applications are:

- Pyrolysis-GC/MS for carbohydrate and lignin (G, S and H types) content estimation and for identification of organic compounds in soil/sediment
- TMS/Alditol acetate sugar-GC/MS for monosaccharide composition analysis
- Updegraff cellulose/anthrone assay for crystalline cellulose determination
- Klason and acetyl bromide lignin assay for lignin determination
- Enzymatic assays for soluble sugars and starch detection
- Size exclusion chromatography (SEC) for determination of MW, DP etc. of lignocellulose polymers
- Sample preparation and extraction using accelerated solvent extractor (ASE) 350

We would be glad to have collaborations within the INUPRAG cooperation. Please contact the platform manager, Junko Takahashi-Schmidt (Junko.TS@slu.se).

Visit our homepage for more information: https://www.upsc.se/platforms/cell-wall-analysis/4845-biopolymer-%20analytical-platform.html

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