

SPS Summer School 2022

« Plant cell walls in development, plant-microbe interactions and for the bioeconomy »

June 19-25, 2022 Versailles, France



Participant's guide

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Sponsors and partners





UNIVERSITE PARIS-SACLAY



Institut Jean-Pierre Bourgin INRAE Centre Île-de-France Versailles-Grignon Route de St-Cyr (RD 10) 78000 Versailles

Hôtel

Hôtel la Résidence du Berry 14, rue d'Anjou 78000 Versailles https://www.hotel-berry.com/fr/

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Summer School e-mail address

SPS-Summer-School@inrae.fr

Important information



Arrival of the participants - Sunday June 19

The meeting point is at the «Ferme Nature et Découvertes», Passage des Etangs Gobert, 78000 Versailles (see map on the page dedicated to the program on June 19).

Please be there a little before 3:30 PM. Don't be late!

Attention : During the Summer School, you will stay at the « Hôtel la Résidence du Berry », 14 rue d'Anjou, 78000 Versailles. The hotel check-in starts at 2 PM. Thus, we advise you to check-in before coming to the «Ferme Nature et Découvertes».

End of the Summer School - Saturday June 25

The Summer School will end around 1 PM, after the guided tour of the Versailles Castle. However, after the guided tour, you will be free to prolong your visit of the Castle.

Covid antigen test

Official website to find a place to get a Covid antigen test: https://www.sante.fr/covid19/external/depistage?region=iledefrance

According to this website, there is a pharmacy doing antigen tests without appointment close to the hotel: Pharmacie Martin-Gerbault, 33 Rue de Satory, 78000 Versailles https://www.sante.fr/pharmacie-dofficine/versailles/pharmacie-martin-gerbault

Visit of the SOLEIL Synchrotron - Monday June 20

Visit of the synchrotron hall is prohibited for people with electronic equipment that can have an impact on their health (pacemaker, insulin pump, neurostimulator for epileptic seizures, etc.), due to the presence of live magnets. Note that cochlear implants are authorized, even if the magnetic fields can cause crackling sounds without consequence.

Bus trip from the hotel to the INRAE Center

The bus tickets are not covered by SPS. However, here is some information.

The bus stops closest to the hotel are indicated on the map above. From there, you can take either of the buses 11, 40, 44 or 401, which will all take you to the INRAE stop.

To travel on these buses, you can buy a ticket directly to the driver (2 euros, prepare the exact change) **but as you are a large group, this solution is not recommended.**

You can also buy T+ tickets via the vending machines in the Paris or Versailles stations (https://www. ratp.fr/en/titres-et-tarifs/t-tickets). These tickets are valid for the bus network in the IIe-de-France region. These tickets can as well be bought and stored on you cell phone (if compatible) through the «Bonjour RATP» app (https://www.ratp.fr/en/achetez-vos-titres-de-transports-par-telephone).

Groups for the practical sessions

> Group 1:

Biomass multiscale characterization (coordinated by Matthieu Reymond, Guillaume Rivière and Stéphanie Baumberger)

> Group 2:

A cytological approach to cell wall polymer architecture and mechanics (coordinated by Kalina Haas, Herman Höfte and Alexis Peaucelle)

> Group 3:

Composition, architecture and specialized functions of cell wall polysaccharides in seeds (coordinated by Helen North and Aline Voxeur)

Group 1	Group 2	Group 3
KOLLULLY RADHAKRISHNAN Anjitha	DE CONINCK Tibo	BERENGUER Eduardo
MARTINEZ DIAZ Jimena	ECHEVARRÍA-POZA Alberto	CULLEN Erin
MOTA Thatiane	HARNVANICHVECH Yosapol	CURRY Thomas
RENSTRÖM Anna	HOU Xiaoyu	IVANOVA Anastasiia
	LESO Martina	MARTÍN-DACAL Marina
	PARKER Daniela	RIEGLER Stefan
	PFAFF Sarah	
	RAMSAY Nathan	

Map of the INRAE Center



Transports in Paris area

https://www.iledefrance-mobilites.fr/en

https://www.ratp.fr/en/

Travel instructions > Versailles

DEPARTURE



!! Warning !! Make sure the train or RER you take stops at the station you need to get off at (stops are indicated on screens or light panels).

* Attention RER B (https://www.transilien.com/en/page-lignes/ligne-b)

> Option 1 (recommended): Direct substitution buses will be set up between CDG 1/2 airports and Mitry-Claye (L1 and L1bis lines, ~25 min). After that, take the RER B from Mitry-Claye. On the weekend of June 18 and 19, modernization work will require the interruption of traffic in both directions, all day long between the stations CDG2 Airport and Aulnay-sous-Bois.

> Option 2: Substitution buses will be set up between CDG1 Airport and Aulnay-sous-Bois and will serve all stations (L2 line, ~45 min). After that, take the RER B from Aulnay-sous-Bois.

Planning at a glance

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	Sunday June 19	Monday June 20	Tuesday June 21	Wednesday June 22	Thursday June 23	Friday June 24	Saturday June 25
8 AM		0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0				• • • • • • • • • • • • • • • • • • •	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
9 AM		9 AM « Cell wall polysaccharide structures and interactions	9 AM « Cell wall and plant-pa- thogens interactions»	8:30 AM Practical session	9 AM « Studying cell wall struc- ture using spectroscopic techniques »	8:30 AM Practical session	« Hôtel du Berry » - Check out
		» « Cell wall modifying enzymes »	« Using genetics to study cell walls » « Studying cell wall nanos- tructure using super	10:30 AM Coffee break	« Studying cell wall polymer structures using X-ray diffraction »	10:30 AM Coffee break	
12 PM		11 AM Coffee break 11:30 AM « Hemicellulose-cellulose interactions through in vitro physico-chemical approaches »	resolution microscopy » 11:30 AM Coffee break 12 PM « Studying cell expansion using of sensors and live cell imaging »	11 AM Practical session	11 AM Coffee break 11:30 AM • «Studying cell wall syn- thesis and assembly using structural biology»	Practical session	To AM - 1 FM Tour of the Versailles Castle (Guided tour 11:15 AM > 1PM)
1 PM		12:30 PM Lunch	12:40 PM Lunch	1 PM Lunch	12:30 PM Lunch	1 PM Lunch	1 PM End of the Summer School
2 PM 3 PM	« Hôtel du Berry » - Check in	2 PM Bus trip to Saclay	2 PM Practical session	2 PM Practical session	2 PM Tour of the IJPB (Greenhouses) 3 PM « Cell wall micromechanics	2:30 PM Restitutions preparation	
4 PM	3:30 PM	2 :30 PM			and plant development »	3:30 PM Restitution of the	
	Welcome introduction	Tour of the	4 PM Coffee break	4 PM Coffee break	4 PM Coffee break	Practical sessions	
5 PM -	SPS presentation	« Soleil » synchrotron			4:30 AM «Ticnin structupe encinee-	1 and 2 5 PM Coffee break	
6 PM	Presentation of the 3 practical sessions' topics	UPSaclay presentation at the IPS2	4:30 PM Practical session	4:30 PM Practical session	 Compared utilizations Coll biology of cell wall synthesis and growth » 	5:30 PM Restitution of the Practical sessions 3	
7 PM	Flash-talks			6:30 PM		- Discussion	
8 PM	7:30 PM	7:30 PM	6:30 PM Social activity / Game	Poster session	6:30 PM Pétanque tournament		
Md 6	Dinner Restaurant in Versailles	Dinner Restaurant on the Plateau of Saclay	Buffet Dinner		Buffet Dinner		
		9 PM Bus trip back to Versailles					

Program

Sunday June 19

Please be at 3:30 PM at the «Ferme Nature et Découvertes», Passage des Etangs Gobert, 78000 Versailles (see map below). Don't be late!

Attention :

During the Summer School, you will stay at the « Hôtel la Résidence du Berry », 14 rue d'Anjou, 78000 Versailles. The hotel check-in starts at 2 PM. Thus, we advise you to check-in before coming to the «Ferme Nature et Découvertes».

3:30 PM – 7:30 PM: Welcome introduction SPS presentation Presentation of the the 3 practical sessions' topics Flash-talks of the participants' research (2 to 3 Powerpoint slides, 5 min max.)

7:30 PM: Diner at the «Ferme Nature et Découvertes»



Monday June 20

9 AM - 10 AM: Focus « Glycobiology and cell wall structure » (Building 7- 1st floor)

Marie-Christine Ralet (Biopolymères, Interactions, Assemblages - Nantes, France)

"Pectin: from jam to seed coat mucilage – a matter of interaction" - Interactions between cellulose, hemicelluloses, and pectin are key determinants of plant cell wall properties and function. Much remains to be understood concerning these interactions but pectin, a highly complex polyelectrolyte exhibiting a high degree of inter and intra-molecular heterogeneity, is known to play a central role. How pectin structural features drive its interaction properties will be illustrated through various examples.

10 AM - 11 AM: Focus « Cell wall modifying enzymes » (Building 7- 1st floor)

Estelle Bonnin (Biopolymères, Interactions, Assemblages - Nantes, France)

"Cell wall modifying enzymes" - Cell wall is made of many different families of polysaccharides. Due to their structural complexity, these polysaccharides are modifiable by many modifying- and degrading-enzymes, belonging to three families of Carbohydrate Active enZymes (CAZy): glycoside hydrolases, polysaccharide lyases and carbohydrate esterases. They are distinguished by their different mechanisms, action patterns and specificities. This talk will focus on some of these enzymes to illustrate their diversities, their physico-chemical and biological properties, as well as their use in various applications.

11 AM – 11:30 AM: Coffee break (Building 7)

11:30 AM – **12:30** PM: Focus « Hemicellulose-cellulose interactions through in vitro physico-chemical approaches » (Building 7– 1st floor)

Bernard Cathala (Biopolymères, Interactions, Assemblages - Nantes, France)

"Hemicellulose-cellulose interactions through in vitro physico-chemical approaches " - In the cell wall, cellulose microfibrils are closely associated with hemicelluloses to form complex networks. The relationships between the fine chemical structure of hemicelluloses and the final cellulose/hemicellulose network architecture are still poorly understood. Nevertheless, adsorption of hemicelluloses onto cellulose can be described as an entropic process modulated by kinetic effects and/or by the limited solubility of hemicelluloses in water and accordingly, in vitro physicochemical approaches can be used to address cell wall polymer organization issues. In this course, the adsorption of xyloglucan and xylan on cellulose model surface will be followed by quartz crystal microbalance with dissipation (QCM-D). The influence of the hemicellulose structure (i.e. molar mass and substitution patterns) will be discussed by modelling the adsorption process using a kinetic model.

12:30 PM - 1 PM: Lunch at the INRAE cafeteria

2 PM: Bus trip to the Plateau de Saclay

2:30 PM - 6:30 PM: Visits

Tour of the «SOLEIL» synchrotron - SEE WARNING ON PAGE 5

SOLEIL is a particle (electron) accelerator that produces the synchrotron radiation, an extremely powerful light that permits exploration of inert or living matter. It provides new perspectives in the study of matter with a resolution down to millionths of meters and a sensitivity to all types of materials. It is a cutting edge pluridisciplinary research laboratory, a service platform open to all scientific and industrial communities, and a centre for exchanges in order to spread scientific and technical knowledge.

UPSaclay presentation at the Institute of Plant Sciences Paris-Saclay (IPS2, member of the SPS network)

The IPS2 aims at better understanding the molecular and genetic mechanisms controlling plant growth and their regulation by endogenous and exogenous signals of biotic and abiotic origins. Analysis of these mechanisms is conducted in an integrated manner at cellular, organ and whole plant levels. IPS2 applies multidisciplinary approaches (combining genomics, molecular and cellular biology, bioinformatics, biochemistry, genetics, physiology) and develops tools (including bioinformatics and modelling) required to provide more predictive knowledge and facilitate «translational» research between model species and crops.

7:30 PM – 9 PM: Diner at the restaurant « L'invitation », 1 place de la Mairie 91190 Saint-Aubin

9 PM: Bus trip back to the "Hôtel Résidence du Berry"

Tuesday June 21

9 AM – 10 AM: Focus « Cell wall and plant-pathogens interactions » (Building 1 - Library)

Antonio Molina (Centro de Biotecnología y Genómica de Plantas – Madrid, Spain)

"Plant immunity triggered by cell wall derived signals: lessons and applications" - Despite the important role of plant cell wall derived signal in plant-pathogen interactions, a very limited number of carbohydrate-base ligands (glycoligands) are well characterized. We have identified novel glycoligands that trigger immune responses and disease resistance in Arabidopsis and crops, which further support their potential application in sustainable agriculture to replace chemical pesticides. The most recent advances in the characterization of the mechanisms of perception by plants of these glycoligands and their function in the regulation of plant immunity will be presented.

10 AM - 10:40 AM: Focus « Using genetics to study cell walls » (Building 1 - Library)

Herman Höfte (IJPB)

"Genetic tools for studying cell walls" - The use of genetics has revolutionized our understanding of cell wall metabolism and its role in biological processes in plants. I will present some striking examples but also discuss the limitations of many genetic approaches to dissect complex processes. Finally I will discuss some new strategies to perturb cell wall-related processes with high temporal and spatial resolution and how they can be harnessed to establish causality between genetic perturbation and phenotypic changes.

10:40 AM – 11:30 AM: Focus « Studying cell wall nanostructure using super resolution microscopy » (Building 1 - Library)

Alexis Peaucelle (IJPB)

"Quantitative cytological analysis of plant cell walls: multi-color immunohistochemistry and Atomic Force Microscopy" - We will present the immunohistochemistry technique using a case study and how it can be used for semiquantitative analysis of cell wall chemistry. We will finally discuss the limitations of this approach and potential artifacts. In the second part, we will discuss Atomic Force Microscopy as a tool to study the cell wall mechanical properties. We will again discuss its potential limits and numerous artifacts.

Kalina Haas (IJPB)

"Bring to light the cell wall nanosctructure by multicolor 3D dSTORM nanoscopy" - In my presentation, I will show how 3D stoichiometric multicolor nanoimaging empowers our understanding of plant growth by enabling high-density mapping of the cell wall polymers with molecular specificity. During the practical sessions we will explore the data analysis pipelines of the 3D multicolor dSTORM images. For this we will use a Matlab-based program Grafeo (Protocol for multicolor three-dimensional dS-TORM data analysis using MATLAB-based script package Grafeo. K.T Haas, A. Peaucelle, STAR protocols 2021, 2 (3) 100808).

11:30 AM – 12 PM: Coffee break (Building 1)

12 PM – 12:40 PM: Focus « Studying cell expansion using of sensors and live cell imaging » (Building 1 - Library)

Sébastjen Schoenaers (University of Antwerp, Belgium)

"Studying cell expansion using live cell imaging" - The cell wall is a remarkably dynamic structure. Understanding cell growth requires insight into when and how rapid changes in the cell wall are controlled during expansion. By combining advanced live cell microscopy, microfluidics and a range of sensors and dyes we can study the localization and timing of signaling events which control cell wall homeostasis during growth. In this module we will learn about state-of-the-art live cell imaging techniques and how they can be used to study cell wall signaling at unprecedented resolution.

12:40 PM - 2 PM: Lunch at the INRAE cafeteria

2 PM - 6:30 PM: Practical sessions - Part 1

6:30 PM - 9 PM: Buffet dinner and Social activity - Game (veranda of the INRAE cafeteria)

Wednesday June 22

8:30 AM - 1 PM: Practical sessions - Part 2

1 PM – 2 PM: Lunch at the INRAE cafeteria

2 PM - 6:30 PM: Practical sessions - Part 3

6:30 PM - 8 PM: Poster session (Greenhouse 71)

8 PM: End of the day

FOR THE PARTICIPANTS > Diner is not included in the Summer School FOR THE INVITED SPEAKERS > 8:30 PM: Diner at the restaurant « Le Bœuf à la mode «, 4, rue au Pain 78000 Versailles

Thursday June 23

9 AM – 10 AM: Focus « Studying cell wall structure using spectroscopic techniques » (Building 14 – Salle TP)

Laurent Heux (Centre de recherches sur les macromolécules végétales - Grenoble, France)

"Studying cell wall structure using spectroscopic techniques" - Spectroscopic techniques such as FTIR or solid state NMR (SS-NMR) provide quantitative molecular information on heterogeneous samples, including plant cell walls. We will discuss here the interest of using these techniques in combination with classical biochemical characterizations. We will see what kind of structural and dynamic information the 1D 13C CP-MAS techniques can provide with improved techniques such as 2D or DNP (Dynamic Nuclear Polarization), illustrated by some examples.

10 AM – **11** AM: Focus « Studying cell wall polymer structures using X-ray diffraction » (Building 14 – Salle TP)

Yoshiharu Nishiyama (Centre de recherches sur les macromolécules végétales - Grenoble, France)

"Cell wall polymer structures from X-ray diffraction" - X-ray/neutron/electron diffraction and scattering studies allow determination of spatial arrangement of molecules at different resolution depending on the structural regularity. Some highly crystalline samples give fine details of the stable structures, that helps understanding the molecular interactions at play. Complementarity with model building based on wider range of apriori knowledge will be discussed.

11 AM - 11:30 AM: Coffee break (Building 14)

11:30 AM – 12:30 PM: Focus « Studying cell wall synthesis and assembly using structural biology » (Build. 14 – Salle TP)

Julia Santiago (University of Lausanne, Switzerland)

"Studying cell wall synthesis and assembly and remodeling using structural biology" - Plants require of an array of proteins to build and remodel their complex cell wall structure. I this session we will revise the state-of-the art knowledge of the molecular mechanism of the enzymes involved in cell wall formation and remodeling.

12:30 PM - 2 PM: Lunch at the INRAE cafeteria

2 PM - 3 PM: Visit of the IJPB greenhouses

3 PM – 4 PM: Focus « Cell wall micromechanics and plant development » (Building 14 – Salle TP)

Arezki Boudaoud (LadHyX, Ecole polytechnique - Palaiseau, France)

"Cell wall micromechanics and growth" - This lecture will introduce basic principles in mechanics and discuss how to measure mechanical properties of the cell wall in relation with cell expansion and plant morphogenesis.

4 PM - 4:30 PM: Coffee break (Building 14)

4:30 PM – 5:30 PM: Focus « Lignin structure, engineering and utilization » (Building 14 – Salle TP)

Wout Boerjan (VIB - Gent, Belgium)

"Genetic engineering lignin to improve biomass processing" - The presence of lignin in the cell wall is a major factor that negatively affects the processing efficiency of biomass into fermentable sugars. By engineering lignin content or structure, plants with large increases in biomass processing efficiency can be obtained. It is as well possible to engineer completely novel lignin structures by expressing exotic genes that vouch for the biosynthesis of lignin monomer-like compounds that incorporate into the lignin polymer. Field trials are established to investigate whether the improvements are maintained under relevant agronomic cultivation practices.

5:30 PM - 6:30 PM: Focus « Cell biology of cell wall synthesis and growth » (Building 14 - Salle TP)

Staffan Persson (University of Copenhagen, Denmark)

"Cell biology of cellulose synthesis during plant growth" - Cellulose is a prominent component of plant cell walls and is produced at the cell surface by Cellulose Synthase (CESA) protein complexes. The last decades have seen a boost in our understanding of how plants produce cellulose with advances in the CESA structure, regulation and trafficking. The CESA complexes associate with the cytoskeleton, which regulates the secretion of CESAs and the direction of cellulose synthesis. In this talk, I will give a broad overview of the cell biology of cellulose synthesis and present new data from my lab that further our understanding of this fascinating process.

6:30 PM - 9 PM: Buffet dinner and Pétanque tournament (veranda of the INRAE cafeteria)

Friday June 24

8:30 AM - 1 PM: Practical sessions - Part 4

- 1 PM 2 PM: Lunch at the INRAE cafeteria
- 2 PM 3:30 PM: Preparation of the practical sessions restitutions

3:30 PM – 5 PM: Restitution of the practical sessions 1 and 2

- 5 PM 5 :30 PM: Coffee break (Building 10)
- 5:30 PM 7 PM: Restitution of the practical session 3 Discussion
- 7 PM: End of the day Diner not included in the Summer School

Saturday June 25

Check-out at the "Hôtel Résidence du Berry". Leave your luggage at the hotel.

10 AM – 1 PM: Tour of the Versailles Castle's gardens (free - self-guided tour - facultative)

11:15 AM – 1 PM: Guided tour of the Versailles Castle - BE ON TIME

«Versailles, private side»: Discover the "other Versailles", the Versailles of King Louis XV and his family, private apartments, and intimate life. Far from the pomp and splendour, luxury remains...and personalities are revealed.

1 PM: Tour of the rest of the Versailles Castle (self-guided tour - facultative)

End of the Summer School







© Château de Versailles - Christian Milet











Abstracts

Cell elimination in seeds

E. Berenguer and G.C. Ingram

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In angiosperms, seed development requires coordination action between the two zygotic tissues generated by double fertilization, the embryo, which will give rise the future plant, and the endosperm, which will nourish the embryo during its development. As the embryo expands, the endosperm cells are rapidly eliminated, leaving a single and highly specialized peripheral cell layer that surrounds the embryo at seed maturity. The aim of my project is to characterize some of the important characteristics of this cell elimination event in the model species *Arabidopsis thaliana*. In the endosperm a specific transcription factor-complex comprising ZHOUPI and INDUCER OF CBF EXPRE-SION (ZOU/ICE1) controls this lytic cell death. Both *zou* and *ice1* mutants, present a persistent endosperm and act upstream cell elimination. In *zou* mutants, cell wall thinning and cell separation do not occur and many modifying cell walls enzymes are not expressed. Based on these results, ZOU may regulate endosperm elimination by predisposing endosperm to crushing-mediated cell elimination upon invasive embryo growth. Our results will provide important transcriptomic, genetic and cellular data of the mechanisms controlling cell elimination in seeds.

Explosive seed dispersal in Cardamine hirsuta

Erin Cullen, Angela Hay

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Adaptations for dispersal are ubiquitous in nature. Plants have evolved many and varied ways to disperse their seeds, from the parachute of plumed dandelion seeds, to the exploding fruit of Cardamine hirsuta. Explosive seed dispersal in C. hirsuta is a rapid movement where elastic potential energy is stored in the fruit, released as kinetic coiling energy, and transferred to launch the seeds. This movement is so fast that high-speed cameras are needed to see the explosion - seeds are accelerated from zero to ten metres per second in approximately half a millisecond! Cellular innovations have been identified that are responsible for generating and rapidly releasing tension in the exploding fruit. Yet it is unknown how the kinetic energy in the coiling valve is transferred to the seeds. Based on computational modelling, it was hypothesised that a viscoelastic 'glue' provides transient adhesion between the valve and seeds and determines seed ejection. In this project I aim to elucidate the identity and nature of this 'glue', and link my findings with this viscoelastic model of seed launch. To address this guestion I will: (1) investigate how seed launch and dispersal is perturbed in a flightless (fli) mutant, where seed ejection fails despite explosive valve coiling, and (2) investigate whether the endocarp *a* cell layer (the valve surface which contacts the seed) acts as a 'glue' during seed launch. By following these two approaches I aim to provide new insights into the mechanism of seed ejection in C. hirsuta.

Development of a Versatile *In Vitro* One-Pot Multienzyme (OPME) Xylan Synthesis Platform

Thomas M. Curry, Hsin-Tzu Wang, Peter Smith, Jeong-Yeh Yang, Kelley Moremen, Breeanna Urbanowicz

Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA

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Xylan is a member of a group of complex, branching polysaccharides known as hemicelluloses, which constitute a major portion of plant cell walls. The structure of cell wall polysaccharides is determined by the synergistic action of various enzymes; however, the individual and cooperative impact of most cell wall biosynthetic enzymes on polysaccharide structure are poorly understood. In vitro biochemical characterization of these enzymes demystifies the relationship between enzyme activity and cell wall polysaccharide structure. Unfortunately, the availability and *in vitro* properties of substrates often complicates biochemical characterization of these enzymes. To address these issues, we are developing a modular one-pot multienzyme (OPME) system that allows synthesis of complex xylan from simple sugars and nucleotides in a cost-effective manner. Subsequently, this model system will be used to study xylan biosynthetic enzymes in vitro. We have expressed and purified recombinant proteins from a variety of organisms and have demonstrated the feasibility of our system by synthesizing nucleotide sugars and xylan in one pot from readily available substrates. Separately, we have concurrently synthesized and acetylated large xylan products to study enzyme variants involved in polysaccharide O-acetylation. Future development of the platform will focus on expanding the scope of the OPME to include other xylan modifying enzymes and substrate generation pathways. The creation of a tunable system to study xylan synthesis is a significant step toward building a comprehensive view of plant cell wall biosynthesis, influencing the future development of designer biomass based on manipulation of pathway enzymes.

Multi-domain proteins composed of a glycoside hydrolase and a ricin B lectin domain: a synergistic relationship?

Tibo De Coninck, Els JM Van Damme

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With an annual production over 730 million tons, accounting for 20% of the global energy intake, rice is considered a staple food. However, rice production is under pressure due to climate change, diseases and the increasing world population. Hence, there is strong need for applied and fundamental research in rice. To date, a wealth of completely sequenced plant genomes is available, creating an opportunity for scientific research. Through a comprehensive analysis of the genomes of Arabidopsis, rice, soybean and cucumber, the occurrence of a novel group of chimerolectins composed of a Ricin B domain linked to a glycoside hydrolase domain with α -galactosidase activity was discovered. These newly identified proteins comprise a unique protein domain architecture combining carbohydrate-binding and -cleaving activities. This raises the guestion whether or not a synergistic relationship exists between the domain domains, facilitating the cell wall-active α-galactosidase activity on e.g. galactomannan substrates. To date, the chimerolectin was expressed in multiple Escherichia coli and Pichia pastoris strains as well as Arabidopsis PSB-D cells, and we are optimizing the conditions to produce the protein recombinantly and to characterize it. Ongoing and future experiments encompass purification and recombinant expression of the whole protein and its individual domains, investigate biochemical characteristics, enzymatic activities and synergistic relationship of the proteins, subcellular localization studies in tobacco leaves and functional analysis of the biological role in rice by means of transgenic overexpression lines, knock-out and knock-down lines.

Polysaccharide analysis by SEC coupled to PACE reveals great heterogeneity in xylan chain length

Alberto Echevarría-Poza, Marta Busse-Wicher, Paul Dupree

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Xylan is the main hemicellulose in the secondary cell wall of eudicot plants. These xylan polymers are comprised of a β -1,4 xylose backbone decorated with acetate and glucuronic acid that can also be methylated. Although the length of the xylan chains is estimated to be around 100 xylose residues in *Arabidopsis thaliana*, the mechanisms that regulate this length during its biosynthesis are unknown, and the biological importance of the length is not fully understood either. In order to gain a better insight into the relevance of the length of xylan chains, we developed a novel method to easily visualise undigested xylan polymers. This approach comprises size-exclusion chromatography (SEC) coupled to pre-labelled polysaccharide analysis using carbohydrate gel electrophoresis (PACE). We report a great heterogeneity in the length of different xylan chains both in wild-type *A. thaliana* plants and in several xylan synthesis deficient mutants with shorter xylan, such as *irx9*, *irx10*, and *irx14*. In addition, this method allowed a simple comparison of xylan chain length between the aforementioned xylan synthesis deficient mutants. We also tested sequentially digesting SEC-fractionated xylan length subpopulations with specific hydrolases. This could eventually lead towards xylan domain and whole chain sequencing, which will prove exceptionally valuable to better understand the role of xylan and engineer cell walls for a better future.

The Arabidopsis embryo as a mechanobiology model

Yosapol Harnvanichvech^{1,2}, Cecilia Borassi², Vera Gorelova², Joris Sprakel¹ and Dolf Weijers²

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Plants perceive mechanical stimuli through their cell wall. While the stimulation has been shown to affect plants' intracellular structure, we lack an understanding of the mechanisms underlying how the mechanical stimulation influences genetic reprogramming. One of the main reasons is the absence of a simple plant model system for mapping between genetic reprogramming and mechanical changes. In this research, we developed the early Arabidopsis embryo, a comprehensive miniature plant, as a model system to study how plant response under mechanical stimulus. We first designed a microfluidic device as a tool to precisely apply mechanical force on isolated embryos. Next, we developed a method to quantify the mechanic changes of cell shape and cell wall mechanics in embryos. As a part of our investigations, we found that two-thirds of the cell volume is deformable, showing that embryo cell wall mechanics is highly adaptable. To further understand how mechanical stimulus affect to genetic reprogramming, we isolated RNA from mechanical stimulus embryos and control embryos to generate whole gene expression data. As a results, we found a group of cell wall modifying enzymes genes are differentially expressed. We are now validating the function of these genes in corresponding to mechanical stimulus in plants.

Functional analysis of type 2C protein phosphatase family during LRX1-mediated cell wall integrity sensing

Xiaoyu Hou, Amandine Guérin, Garbor Kadler, Shibu Gupta and Christoph Ringli

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The control of plant cell growth requires an elaborate system to monitor cell wall homeostasis and convey the signals to intracellular signaling cascades. The transmembrane protein kinase FERONIA (FER) functions in cell wall integrity sensing and interacts with RALF ((RAPID ALKALINIZATION FAC-TOR) peptides and the extracellular leucine-rich repeat extensins (LRXs). LRXs are high-affinity binding sites for RALF (Rapid Alkalinization Factor) peptide hormones. Therefore, our group proposed an LRX-RALF-FER module that regulates cell wall development. In Arabidopsis, *LRX1* and *LRX2* are predominantly expressed in root hairs and mutations in *LRX1* cause defects in root hair development. Our group uses *Arabidopsis* root hair as a model system to study the function of LRX1 in cell wall integrity sensing.

In order to decipher the negative regulation mechanisms of LRX1-mediated signaling pathway, we screened for *repressor of lrx1* (*rol*) mutants that reconstitute the root hair development in the root hair defective mutant background *lrx1*. One of the characterized mutants *rol23* has a mutation in the gene encoding for a type 2C protein phosphatase. Protein phosphatases have been shown to regulate signaling pathways by controlling kinases turnover. To this end, we aim to study how the induction and amplitude of LRX1-mediated cell wall integrity sensing signaling are regulated by type 2C protein phosphatase. Of a great interest, we will analyze the influence of *ROL23* on cell development and cell wall structure.

Characterizing the mode of action of the plant cell wall integrity maintenance mechanism in *Arabidopsis thaliana*

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The proposed PhD project is part of the ongoing Wall Integrity project (funded by the Norwegian Research Council). The Wall Integrity project is focused on understanding how plant cell wall damage responses are controlled by the cell wall integrity (CWI) maintenance mechanism in *Arabidopsis thaliana*. Cell wall damage can be caused by different stress conditions, both of biotic (i.e., pathogens and pests) and abiotic (e.g., drought, flooding, heat) origin. The proposed PhD project is aiming to investigate the part of the CWI mechanism responsible for coordinating pattern triggered immunity (PTI) active during biotic stress response, with hyper-osmotic stress response and adaptive alteration of cell wall and cellular metabolism. The aim of the project will be achieved by characterizing the functions of several candidate genes implicated in CWI maintenance through transcriptomics experiments performed by the host group. This will involve generation of transgenic lines (knockout / over-expression), analysis of cell wall composition and structure as well as biotic/abiotic stress assays of the transgenic plants, gene expression studies, cloning of reporter constructs for cell-biological studies. The results will provide the foundation for more targeted candidate-specific studies and identify leads for the future improvement of food and/or bioenergy crop performance.

Towards Understanding the Control of Coniferous Wood Formation

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Conifers dominating the northern hemisphere serve as a major terrestrial carbon sink by fixing carbon as secondary xylem (wood) through cambial activity. The coniferous wood consists mainly of tracheids of which structure, lifespan and chemical characters differ significantly from the xylem cells of hardwoods. However, the molecular mechanism causing these differences are not known. Hence this project, "Towards understanding the control of coniferous wood formation" aims at developing high-resolution transcriptomic data of Norway spruce cambium at single-cell type level to dissect the unique features in expression patterns during wood formation. In addition, the project aims to validate the conclusions recently made through network inference on genes that putatively regulate cell wall formation in conifers. Laser Capture Micro dissection and single-cell type RNA sequencing(scRNA-Seq) of the cambium followed by bioinformatic analysis will be used to study the global expression changes during the cell differentiation. This high-resolution transcriptome data sheds light into the molecular mechanism of cambium cell differentiation in conifers. Putative transcriptional regulators will be functionally validated by the generation of genetically modified lines of Norway spruce. The identification of gene targets of the studied transcription factors (TF) in co-expression network will be carried out. The putative conserved role of TFs will be studied by phenotyping Arabidopsis mutants. The functional validation of the putative TFs related to wood formation in Norway spruce will be novel. Conjointly, the findings from this work have a great potential in expanding the existing knowledge on conifer biology along with deep insights into evo-devo studies that further helps in the comparative genome study of angiosperms and gymnosperms.

Pectin modifications are important for efficient infection by the parasitic plant *Phtheirospermum japonicum*

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Parasitic plants are major pathogens that can severely affect agricultural yields by withdrawing nutrients from their host plants. To successfully infect their hosts, parasitic plants develop an invasive structure known as *haustorium*, which allows vascular connections and the exchange of nutrients and signalling molecules. A key step in haustorium development is the invasion of the host's tissues. This process relies on the modification of the host's cell-adhesion through the action of cell-wall modifying enzymes such as pectin methylesterases (PMEs), which are upregulated in many parasitic species during parasitism. Despite the importance of this process, the role of these enzymes has not been closely investigated in parasitic plants. My project aims to better understand the role of cell wall modifications and PME activity during the establishment of the haustorium. To explore this angle I am working with *Phtheirospermum japonicum*, a model for root facultative parasites that can also infect *Arabidopsis thaliana*. The approaches used include the analysis of transcriptomics data, pectin-specific antibody staining, chemical treatments and the generation of overexpression and reporter constructs for selected PMEs and PME inhibitors (PMEIs) for hairy root ransformation in *P. japonicum*. The results obtained so far suggest the involvement of PME activity in different stages of haustorium formation, from the early host invasion to the later development of the vascular connection.

Deciphering the signaling mechanisms triggered by mixed-linked-glucans (MLGs) in *Arabidopsis thaliana*.

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Plant cell walls (CW) are complex structures that play an essential function in disease resistance and stress adaptative responses. CWs are also a source of oligosaccharides (glycans) that are released during pathogen infection or wounding and are perceived by plants as Damage-Associated Molecular Patterns (DAMPs). DAMPs activate Pattern Triggered Immunity (PTI) upon their recognition by plant Pattern Recognition Receptors (PRRs), that can bind different types of ligands. Although plant peptidic DAMPs have been broadly studied, our knowledge of the perception of DAMPs of carbohydrate nature is scarce. To better understand plant mechanisms involved in the perception of glycan and to identify PRRs and/or downstream signaling components regulating glycan-mediated PTI, we have carried out a forward genetic screening in Arabidopsis thaliana. We implemented a high-throughput luminescence-based screening using ethyl methanesulfonate mutagenized seed population of Arabidopsis thaliana that was treated with different glycans, like the mixed-linked-glucans (MLGs). We have identified dozens of mutants which are either impaired in the perception of MLGs. Interestingly, img1-img3 fully lost responsiveness to MLGs. Characterization of the impaired genes is currently underway by genomic sequencing and the specificity of the corresponding genes on glycan perception in plants is under study by following a genetic approach. Additionally, we are also testing the contribution of these IMG components in the Arabidopsis disease resistance responses. Our strategy will help us to unravel the mode of action of MLGs in the activation of plant immunity. Moreover, these results will provide us with the knowledge to develop more sustainable crop protection strategies.

Identifying biomass parameters for processing with OrganoCat

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Fossil fuels are widely used for the supply of energy and manufacturing of several chemical products over decades. However, attention has been driven to alternatives derived from renewable sources. The valorisation of lignocellulosic biomass is in the spotlight for bio-based products and energy, as it does not interfere with food supply and is available in great abundance ^[1].

A crucial step in the valorisation of lignocellulose is the fractionation of biomass into its major components – cellulose, hemicelluloses and lignin– which can be converted into value-added products in biorefineries. Due to the recalcitrant nature of the cell wall, its fractionation is challenging ^[2]. The OrganoCat process holistically valorises the main components of lignocellulose. In a biphasic reaction system, hemicellulose is selectively depolymerised by a diluted acid catalyst at mild conditions. Lignin is *in situ* extracted by 2-methyltetrahydrofuran, while the cellulose-enriched pulp remains as a solid ^[3,4].

The aim of this project is to identify key parameters of the biomass for a better performance when subjected to OrganoCat. To do so, a collection of genetically different rapeseed samples will be analytically characterized and their performance in OrganoCat will be evaluated. Wet chemical characterization of the different accessions will be correlated with data of a high throughput Fourier-transform near-Infrared (FT-NIR) spectroscopy and statistically evaluated. Based on the results, a representative population will be selected to undergo the OrganoCat process. Subsequently, the downstream products will be assessed to identify potential correlation between chemical composition of the biomass and OrganoCat process performance.

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A field trial with transgenic poplar downregulated for CAFFEOYL SHIKIMATE ES-TERASE, a gene involved in lignification

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Lignocellulosic biomass is mainly composed by cellulose, hemicellulose and lignin, and it is an environment-friendly alternative feedstock for the biorefinery. The structural complexity of the cell wall and the presence of the lignin polymer hinder the polysaccharide conversion to fermentable sugars. Genetic modification of plants for genes involved in lignin biosynthesis has been used to generate plants with altered lignin levels and composition, and improved sugar release upon biomass saccharification. CAFFEOYL SHIKIMATE ESTERASE (CSE) plays an essential role in monolignol biosynthesis. It catalyzes the conversion of caffeoyl shikimate into caffeate. Our group demonstrated that the RNAibased silencing of CSE in hybrid poplar (Populus tremula x P. alba) resulted in plants with reduced lignification, increased cellulose levels and improved biomass saccharification, whereas these plants did not display any yield penalty when grown in a greenhouse. These poplar transgenic hpCSE lines were planted in June 2021 in a field trial in Belgium to evaluate how they cope with environmental conditions and whether these field-grown plants present favorable biomass traits for biorefining. During the first cycle of growth, the hpCSE lines grew similarly to wild type trees. The transgenic lines had similar bud closure and similar capacity to cope with rust infection and insect damage when compared to the wild type trees. The transgenic poplars are growing for another growth cycle and after harvesting in winter 2023 (2-year-old poplars), a multiple-level characterization of the wood will provide additional information about their cell wall composition and their potential for biorefining.

Biohybrid plants with electronic roots via in-vivo polymerization of conjugated oligomers

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In recent years there has been a growing interest in developing plant-based biohybrid systems¹ by integrating smart materials² and devices into plant structures³. The interest relies on the advantage that plants are well suited to sense and adapt to environmental stimuli, and they can self-repair via tissue regeneration. If we combine plants with the versatile characteristics of organic conductive polymers, we look forward to developing plant-biohybrid systems.

In our recent work⁴ we integrate mixed ionic-electronic conductors formed by conjugated polymers in intact plants root system. Cell wall peroxidases⁵ catalysed ETE-S polymerization on the root epidermis, self-organizing the polymer. The conductivity of the resulting p(ETE-S) roots reached the order of 10 S/cm, and it remains stable over the course of 4 weeks while the roots continue to grow. Plants adapted by developing a more complex root system after undergoing electronic functionalization. Biohybrid plants with electronic roots pave the way for autonomous systems with potential applications in energy, sensing and robotics.

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Secondary Cell Wall Formation in Protoplasts Points to Xylan's Role in Patterned Wall Formation

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Xylem vessel secondary cell walls (SCWs) are deposited in distinct banding patterns. Ectopic expression of the transcriptional regulator, VASCULAR NAC DOMAIN 7, initiates transdifferentiation into xylem vessels. Arabidopsis protoplasts, induced to regenerate xylem vessel-type SCWs, were used to investigate the role of xylan in patterned wall formation. SCW cellulose patterns were characterized in wild-type Arabidopsis protoplasts and then compared to those produced from xylan mutants (irx9 and *irx14*) and in protoplasts treated with an *endo*-1,4- β -xylanase during wall regeneration. The SCW cellulose patterning in the xylan mutant protoplasts showed a distinct blurring that was even more distorted in the SCW-regenerating protoplasts treated with an *endo*-1,4-β-xylanase (family GH11) during active wall synthesis. Immunofluorescence localization of xylan indicated that the disrupted cellulose banding patterns coincided with the locations of xylan deposition. Previous research showed that the deposition of xylan and lignin in SCW domains is independent of cellulose1. Our work shows that the deposition of cellulose into distinct SCW domains was severely disrupted by altered xylan structure. As it has been shown that xylan is deposited before cellulose appears in the regenerating SCW¹, the dynamics of microtubules (MTs) during SCW deposition are being investigated to determine if xylan disruption affects MT patterns. This work points toward a potential feedback mechanism between the cell wall and MTs that may influence the subsequent deposition of cellulose in SCW domains and also presents SCW-regenerating protoplasts as a novel platform for research into the deposition of xylem vessel-type SCWs.

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Properties of the cell wall affect freezing tolerance in Arabidopsis thaliana

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Freezing is a significant environmental stress which poses a threat to the future of global food security through frost-induced crop losses. Reducing such losses requires an understanding of the molecular mechanisms underpinning freezing tolerance so that frost-tolerant crop species may be engineered. There is growing evidence to suggest that the cell wall plays a major role in both cold acclimation and freezing tolerance in plants. Here, we show that various cell-wall mutants of Arabidopsis thaliana exhibit both increased cellular damage and decreased whole-plant survival when subjected to freezing stress. These mutants have defects in their cell walls such as decreased cross-linking of the pectic polysaccharide rhamnogalacturonan-II or partial inhibition of cellulose synthesis. We have developed a confocal-based method to quantify cell-wall porosity (the size of the spaces between cell-wall components), which involves measuring the porosity-dependent decrease in fluorescence of a plasmamembrane localised dye after application of an extracellular quencher. In some cases, we have shown that these cell-wall mutations result in a more porous cell wall and this is likely one of the factors contributing to the increase in freezing sensitivity. We have shown that chemical treatments, such as with the cellulose-synthesis inhibitor isoxaben, can also result in a significant increase in cellwall porosity. As some of these mutants are suspected of having altered cell-wall tensile strength, we next aim to use a confocal micro-extensometer to quantify the mechanical properties of their cell walls and elucidate another characteristic which likely contributes to freezing tolerance.

Effects of nitrogen source and availability on wood formation 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. Conversely, nitrogen source seems to have less importance in low N-conditions and amplifies as more N is added. 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%. How this is reflected in cell wall chemistry it yet to be assessed.

Unravelling auxin-cytokinin synergism-triggered cell wall modulation in plant nutrient starvation and plant-fungal mutualism

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Plants are capable of developing new organs and regulating their growth throughout their lifespan. Phytohormones, particularly auxin and cytokinin, play crucial roles in these processes. The Benkova lab recently discovered the SYNERGISTIC ON AUXIN AND CYTOKININ 1 (SYAC1) gene in Arabidopsis thaliana whose expression is strictly dependent on simultaneous activity of auxin and cytokinin signalling pathways. SYAC1 is the first gene whose expression in root tissue is known to be induced by synergistic action of both hormones. By inhibiting pectin secretion, SYAC1 renders cell walls softer and reduces elongation growth. While this functionality is important in several developmental contexts in shoot tissue, SYAC1's function in the root remained unknown at first. In setting out to unravel its function in root tissue we discovered that limited nutrient availability triggers SYAC1 expression. Plants adapt their root system architecture in response to nutrient starvation, a process requiring auxin, cytokinin and cell wall modification. SYAC1 expression increases further if a mutualistic fungal endophyte is present under nutrient starvation. The interaction with soil microbes such as this fungus involves auxin, cytokinin as well as cell wall regulation. We hypothesise that SYAC1, driven by auxin-cytokinin synergism, is involved in both, regulation of root system architecture upon nutrient starvation and in regulating interaction with a mutualistic fungus via softening the cell walls, thereby facilitating colonisation. Employing advanced imaging techniques we aim to unravel the cell wall mechanics at the intersection of low nutrient responsive root system architecture, fungal mutualism and auxin-cytokinin synergism.

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