Programme des 14e journées du Réseau Français des Parois – Saint Malo 26-29 août 2024

Mardi	27 août
10h-12h	Visite du Minerallium
13h-14h	Accueil café et enregistrement
14h00-14h40	Cérémonie d'ouverture
Session 1	*Biosynthèse, architecture, composition des parois* *Mathilde Francin-Allami- Anne Laure Chateigner-Boutin*
14h40-15h05	Omics in the Garonne river: the aquatic plant cell wall *Thomas Berthelier*
15h05-15h30	Biochemical characterization of the first fucan synthase from brown algae *Ahlem Bourgerba*
15h30-15h55	Investigating the biochemical assembly of an extracellular protein-carbohydrate molecular network behind plant cell growth *Aurélie Dupriez*
15h55-16h35	Pause café
16h35-17h00	Control of secondary cell wall formation by Musashi-type translational regulators in trees *Hélène Fouassier*
17h00-17h25	Rôle de l'eau dans la structuration et le comportement hygroscopique d'assemblages lignocellulosiques *Anouck Habrant*
17h25-18h00	Présentations Flash *Elodie Rivet, Sophie Bouton, Muhammad-Moazzam Hussain, Valérie Lefebvre, Godfrey Neutelings, Fabien Sénéchal, Corinne Pau-Roblot, Yassin Refahi*
18h00-20h00	Session poster – Cocktail de bienvenue

Mercredi	28 août
Session 1	*Biosynthèse, architecture, composition des parois* *Mathilde Francin-Allami- Anne Laure Chateigner-Boutin*
9h00-9h25	Phenylboronic acid interacts with pectic rhamnogalacturonan-II and displays anti-auxinic effects during *Arabidopsis thaliana* root growth and development *Arnaud Lehner*
9h25-9h50	L'acylation des lignines : importante pour la physiologie de l'arbre, intéressante pour de nouvelles utilisations du bois ? *Laura Vimenet*
9h50-10h15	Functional analysis of PLATZ18 in the regulation of vascular tissues development in the poplar *Hugues de Gernier*
10h15-10h20	Présentation Cargill
10h20-11h00	Pause café
11h00-11h40	**Keynote : Bruno Clair**
Session 2	*Remodelage et dynamique des parois, notamment en situation de stress* *Helen North*
11h40-11h55	Caractérisation biochimique et fonctionnelle de MPPG2, une exopolygalacturonase de *Marchantia polymorpha* *Clémence Allard*
11h55-12h20	*Arabidopsis thaliana* leaf peptidomics: search for native peptides *Elisabeth Jamet*
12h20-14h00	Pause déjeuner

Session 2	*Remodelage et dynamique des parois, notamment en situation de stress* *Helen North*
14h00-14h25	Tomato resistance to *Botrytis cinerea* from the perspective of the cell wall *Luka Lelas*
14h25-14h50	Functional analysis of extensin motifs in the LRX protein family *Christoph Ringli*
Session 4	*Outils et méthodes de caractérisation, acquisition et traitement des données, modélisation* *Fabienne Guillon*
14h50-15h15	Solid-state NMR spectroscopy to characterize polysaccharide assemblies organization at molecular scale *Xavier Falourd*
15h15-15h40	A 4D quantification pipeline to characterize plant cell wall enzymatic hydrolysis in highly deconstructed samples *Solmaz Hossein*
15h40-16h10	Pause café
16h10-16h35	How to evaluate the mechanical properties of cell walls in cambium by atomic force microscopy *Liudmila Kozlova*
16h35-17h00	Spatio-temporal analysis of the spruce tree cell wall enzymatic hydrolysis *Khadidja Ould Amer*
17h00-17h25	Dynamique de la formation cellulaire du xylème secondaire chez le peuplier : approche couplée de transcriptomique et d'imagerie chimique des parois à l'échelle cellulaire *Yiyi Tan*
17h45-19h45	Visite de Saint-Malo
20h00	Repas de gala

Jeudi	29 août
Session 3	*Valorisation bioproduits, bioénergie, matériaux, alimentation, santé* *Gabriel Paës*
9h00-9h25	Wallderive : ingénierie de nouveaux oligosaccharides dérivés de la paroi cellulaire pour la protection des plantes *Romain Briatte*
9h25-9h50	Improvement of pectin gelling properties from sugar beet pulp using pectin acetylesterases *Juliette Chislard*
9h50-10h15	Pause café
11h10-11h35	Etude de la durabilité des fibres de lin grâce à l'analyse de textiles égyptiens anciens *Camille Goudenhooft*
11h35-12h00	3,6-anhydro-D-galactosidase caractérisation, enzymes involved in the carrageenans degradation of red algae cell walls *Marine Gourhand*
12h00-12h45	AG réseau français des parois
12h45-14h30	Pause déjeuner
Session 3	*Valorisation bioproduits, bioénergie, matériaux, alimentation, santé* *Gabriel Paës*
14h30-14h55	Metaproteomics identifies key cell wall degrading enzymes during flax dew retting *Suvajit Mukherjee*
14h55-15h20	Impact de la laccase fongique et des médiateurs sur les propriétés et la structure de la biomasse lignocellulosique pendant la saccharification *Fanny Vinter*
15h20-15h40	Remise du prix poster Clôture des 14e journées du Réseau Français des Parois
15h40-16h10	Pause café
16h10 -18h10	Visite Minerallium

Avec le soutien de

Présentations orales

Session 1 : Biosynthèse, architecture, composition des parois

OMICS IN THE GARONNE RIVER: THE AQUATIC PLANT CELL WALL

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450 MY ago, terrestrial plants emerged from aquatic environments[1,2]. This transition was made possible by evolutionary innovations that led to the development of specific traits such as cuticle, stomata, tracheids, and seeds, enabling them to adapt to life on land. More recently, some of these angiosperms have returned to an aquatic lifestyle by implementing new or already existing strategies[3]. This return was made possible, among other adaptations, by a remodeling of the extracellular matrix including the cell wall and the cuticle. The primary plant cell wall (CW) is composed of a polysaccharide portion (cellulose, hemicelluloses, and pectins) and a protein portion that gives it its dynamics.
To investigate the mechanisms allowing this transition back to aquatic life, we have selected *Potamogeton nodosus* as a model species. This native plant of French rivers was collected in the Garonne River. It exhibits heterophylly, *i.e.* a morphological distinction between its floating and submerged leaves (Figure 1). After sequencing and assembling its genome, we conducted transcriptomic and cell wall proteomic studies. Additionally, microscopy was utilized to characterize the differences between the two types of leaves. The images acquired allow us to suspect the presence of lignin beneath the surface of floating leaves. We found a differential accumulation of RNAs encoding enzymes involved in the lignin biosynthesis pathway as well as some CIII peroxidases. We will also discuss distinct patterns of polysaccharide distribution, which may be related to the adaptive differences between submerged and floating leaves.

Figure 1: Floating (FL) and submerged (SL) leaves of *P. nodosus*

References

[1] Nishiyama, T. *et al*., *Cell,* 174, 448-464.e24, 2018.
[2] Delaux, P.M., Nanda A.K., Mathé C., Séjalon-Delmas N. & Dunand C., *Perspectives in Plant Ecology, Evolution and Systematics,* 14, 49-59, 2012.
[3] Rascio, N., *Critical Reviews in Plant Sciences,* 21, 401-427, 2002.

BIOCHEMICAL CHARACTERIZATION OF THE FIRST FUCAN SYNTHASE FROM BROWN ALGAE

BOUGUERBA-COLLIN A.[a*], GROSSE A.[c], PRABHAKAR K.P.[b], URBANOWICZ R.B.[b], LEGENTIL L.[c] FERRIÈRES V.[b], HERVÉ C.[a]

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FUNCTIONAL ANALYSIS OF *PLATZ18* IN THE REGULATION OF VASCULAR TISSUES DEVELOPMENT IN THE POPLAR

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Poplar is a major tree for bioenergy and biomass production. We focus on elucidating the role of the transcription factor Plant A/T-rich protein and zinc-binding protein 18 (PLATZ18) in vascular system development and wood formation in *Populus tremula x P. alba*. The expression of *PLATZ18* in the xylem suggests a crucial role of the transcription factor in those processes. Previous work from Guérin et al. (2023)[1] highlights that disrupting the signalling cascade downstream of PLATZ18 positively impacts the secondary xylem thickness, thus denoting an increased cambial activity. The dominant repressor lines overexpressing the *PLATZ18-SRDX* fusion moreover display higher amount of lignin within wood tissues, hence indicating an alteration in cell wall composition within xylem cell types.
To gain better insights about the function of *PLATZ18*, we further overexpressed the wild-type allele. Strikingly, the overexpression lines show similar phenotypes to that of the *PLATZ18-SRDX* dominant repressor. Therefore, our results pinpoint towards a role of PLATZ18 as a potential repressor of key regulators of the cambium activity, and lignin biosynthesis and polymerization. Next, a RNAseq transcriptomic approach was carried on to identify the genes affected by the overexpression of *PLATZ18.* The pool of differentially expressed genes serves as a promising material for deciphering the pathways downstream of PLATZ18. Finally, CRISPR poplar mutants targeting *PLATZ18* were generated to be used as a toolbox to validate the central role of the transcription factor in the identified signalling cascades. We present an overview of our recent discoveries and the ongoing work aiming to unravel the core molecular mechanisms of wood formation.

References

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INVESTIGATING THE BIOCHEMICAL ASSEMBLY OF AN EXTRACELLULAR PROTEIN-CARBOHYDRATE MOLECULAR NETWORK BEHIND PLANT CELL GROWTH

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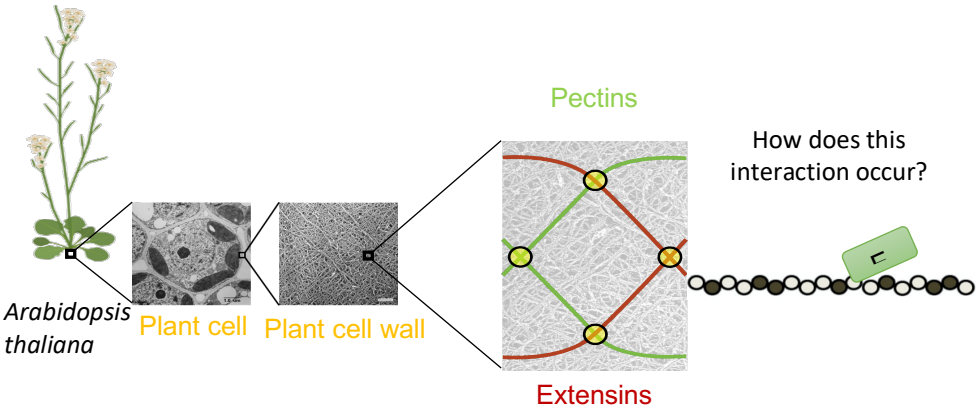
Cell growth is a tightly regulated phenomenon in living organisms. In plants, the cell-surrounding cell wall is a critical growth regulator thanks to its ability to change its composition and structure. Although the cell wall needs to be rigid to resist to turgor pressure, it must be plastic to allow for cell growth. These ambivalent properties are crucial to precisely control cell growth. To better understand cell wall properties, we must investigate its composition and organization. The cell wall is mainly composed of polysaccharides such as pectins, along with structural proteins like extensins. These two families of molecules can self-assemble to form independent molecular networks critical for cell wall integrity. Recently, some evidence showed that they can also interact with each other thanks to ionic interactions, forming the so-called pectins-extensins network [1].

This project aims to further characterize pectins and extensins association. In this context, we focus on *Arabidopsis thaliana* root-hair, as they are a model of choice to study cell wall expansion. To date, five root hair specific extensins have been identified as necessary for root-hair growth [2]. By combining molecular biology, biochemistry, microscopy and genetic tools, we want to (i) produce extensin-recombinant proteins then characterize this interaction *in vitro* and (2) *in vivo*, but also search for news partners that could interact with extensins and/or pectins and identify them by mass spectrometry. Different strategies to verify the production of these peculiar proteins will be discussed. Altogether, this project will extend our understanding of the cell wall networks dynamics behind plant cell growth.



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CONTROL OF SECONDARY CELL WALL FORMATION BY MUSASHI-TYPE TRANSLATIONAL REGULATORS IN TREES

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Deciphering the mechanism of secondary cell wall (SCW) formation in plants is crucial to understand their development, their plasticity in response to environment and the molecular basis of biomass recalcitrance. Although transcriptional regulation is essential for SCW formation, little is known about the implication of post-transcriptional mechanisms in this process. In a recent publication, Kairouani *et al*. characterized the function of two *bonafide* RNA-binding proteins homologous to the animal translational regulator Musashi (MSIL)[1]. They demonstrated that MSIL proteins belong to a multigenic family of 4 members and control SCW formation in Arabidopsis. MSILs mutation alter SCW formation in the fibers and decrease stem rigidity. This phenotype was associated to a reduction in lignin deposition, and an increase of 4-O-glucuronoxylan methylation. In accordance, quantitative proteomics of stems reveal an overaccumulation of glucuronoxylan biosynthetic machinery, including GXM3, in the msil2/4 mutant stem. We showed that MSIL4 immunoprecipitates GXM mRNAs, suggesting a novel aspect of SCW regulation, linking post-transcriptional control to the regulation of SCW biosynthesis genes. We identified several close orthologs of AtMSIL genes in trees genome which are preferentially expressed in stem tissues. Within the framework of the ANR project MusaWALL, we started to investigate the function of MSIL-like proteins in Eucalyptus using CRISPR-Cas9 mutants and overexpressors.

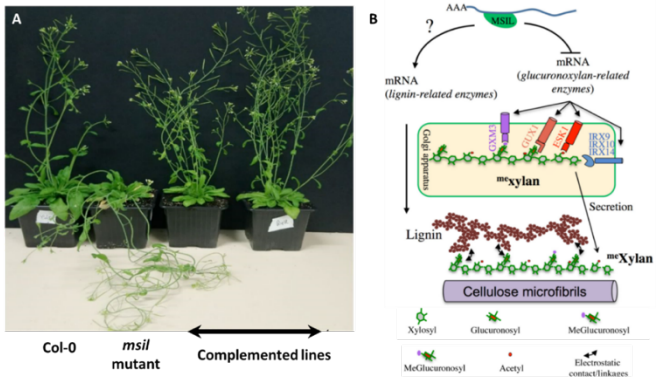

Figure 1 : MSIL RNA-binding proteins are involved in the control of SCW formation and stem rigidity. **A,** Phenotypes of MSIL KO mutants exhibiting pendant stems. **B,** Model of MSIL-dependent control of glucuronoxylan methylation in Arabidopsis.

Références

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ROLE DE L'EAU DANS LA STRUCTURATION ET LE COMPORTEMENT HYGROSCOPIQUE D'ASSEMBLAGES LIGNOCELLULOSIQUES

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Les fibres végétales et les polymères lignocellulosiques sont des candidats prometteurs pour le développement de nouveaux matériaux biosourcés. Cependant, la plupart de ces matériaux sont sensibles à l'humidité de l'air du milieu environnant, ce qui peut induire une instabilité de leurs propriétés structurales et mécaniques et ainsi réduire leur utilisation. Afin de mieux comprendre le rôle de l'eau dans la structuration et le comportement hygroscopique de ces polymères, notre stratégie a été de produire des assemblages bioinspirés de la paroi végétale, de complexité croissante et suivant différentes voies de synthèse, à base de polymères lignocellulosiques [1] et de les caractériser à l'aide de méthodes physico-chimiques (sorption dynamique de vapeur d'eau, DVS), mécanique (mesure du module d'Young) et spectroscopiques (IR, RMN du solide à haut champ et de relaxométrie T_2 par RMN à bas-champ). L'objectif est de caractériser ces systèmes macromoléculaires organisés en conditions d'humidité variable, à différentes échelles, de comprendre les interactions mises en jeu entre les chaines de polymères et les molécules d'eau en relation avec leurs propriétés mécaniques. Des corrélations entre les paramètres physico-chimiques et spectroscopiques mesurés dans le cas de mélanges binaires cellulose-glucomannane ont été établies : plus l'affinité du film pour l'eau est élevée (teneur en eau), plus les protons des polysaccharides sont mobiles (augmentation du T_2 par RMN) et plus la structuration de l'eau dans l'assemblage (diminution du temps de diffusion de spin des protons par RMN du solide) et sa capacité de rétention (hystérésis, dH) sont faibles [2]. Ces relations sont complétées par l'évaluation de l'accessibilité à l'eau des assemblages par IR couplée à une technique de deutération et par la détermination de leurs propriétés mécaniques en traction.

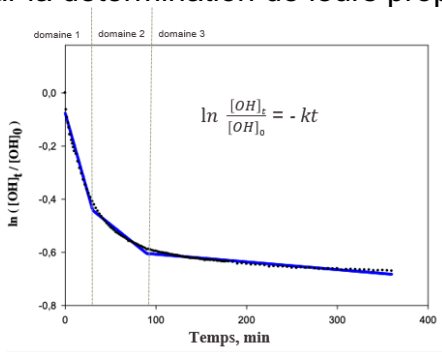

Figure 1 : Cinétique d'échange OD-OH d'un assemblage lignocellulosique mesurée en infrarouge par deuteration en condition saturante. Domaines 1 à 3: protons facilement à non échangeables.

Réferences

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PHENYLBORONIC ACID INTERACTS WITH PECTIC RHAMNOGALACTURONAN-II AND DISPLAYS ANTI-AUXINIC EFFECTS DURING *ARABIDOPSIS THALIANA* ROOT GROWTH AND DEVELOPMENT

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Boron dimerizes RG-II in the plant cell wall and is crucial for plant cell elongation. However, studying RG-II dimerization in plants is challenging because of the severe phenotypes or lethality of RG-II mutants. Boron deprivation abrogates both RG-II dimerization and plant growth, but whether or how these phenotypes are functionally linked has remained unclear. Boric acid analogues can serve as experimental tools to interfere with RG-II cross-linking. Here, we investigated RG-II dimerization and developmental phenotypes in *Arabidopsis thaliana* seedlings treated with a boric acid analogue, phenylboronic acid (PBA), to test whether the observed developmental phenotypes are attributable to alteration of RG-II dimerization or to other putative functions of boron in plants. We found that PBA treatment altered root development in seedlings while RG-II dimerization and distribution were not affected. Surprisingly, under low boron conditions, PBA treatment i) had no effect on root size but still prevented lateral root development and ii) restored RG-II dimerization. PBA treatment also disrupted auxin levels, potentially explaining the absence of lateral roots in seedlings treated with this analogue. We conclude that PBA interacts both with RG-II and other cellular targets such as auxin signaling components, and that the phenotypes caused by PBA arise from interference with multiple functions of boron.

L'ACYLATION DES LIGNINES : IMPORTANTE POUR LA PHYSIOLOGIE DE L'ARBRE, INTERESSANTE POUR DE NOUVELLES UTILISATIONS DU BOIS ?

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Dans un contexte de changement climatique et d'épuisement des énergies fossiles, la recherche de sources alternatives en carburants renouvelables est devenue une priorité. L'utilisation de biomasse lignocellulosique, telle que le bois pour produire du bioéthanol à partir de monosaccharides offre une solution prometteuse. En effet, le bois est composé à 75% d'oses organisés en polysaccharides pariétaux, qui peuvent être convertis en oses fermentescibles. Ces polysaccharides sont enrobés de lignines, qui contribuent à l'étanchéité des vaisseaux conducteurs de sève et influent sur les propriétés mécaniques des fibres de bois, indispensables à la croissance de l'arbre. Les lignines sont des polymères phénoliques complexes composées majoritairement de trois monomères appelés unités H, G et S[1], qui constituent un obstacle majeur à la production de bioéthanol, nécessitant de ce fait une étape de prétraitement coûteuse et polluante[2]. Chez certaines plantes, on peut observer la présence de groupements acyles au sein du polymère de lignines. Ces acylations se produisent principalement sur les unités S et leur présence de même que leur nature varient selon les espèces. Par exemple, les lignines de peuplier sont riches en *p*-hydroxybenzoate[3] tandis que celles des graminées sont décorées de *p*-coumarate[4]. Leur présence entraine des modifications aux niveaux de la structure et des propriétés des lignines : ainsi, la coumaroylation des lignines de peuplier par la *p*-CoumaroylCoA:monolignol transferase de *Brachypodium distachyon* (PMT) permet d'améliorer le rendement de saccharification[5]. Notre objectif est de mieux comprendre le rôle de l'acylation des lignines dans la physiologie de l'arbre et dans l'aptitude du bois à la saccharification. Nous présenterons les premiers résultats obtenus sur des peupliers transgéniques pauvres en *p*-hydroxybenzoate, mais également d'autres transgéniques dans lesquels nous avons modifié les proportions en monomères pour en observer l'impact sur l'acylation. Enfin, nous avons inséré le gène permettant l'expression de la PMT sur ces peupliers transgéniques pour en évaluer le rendement de saccharification.

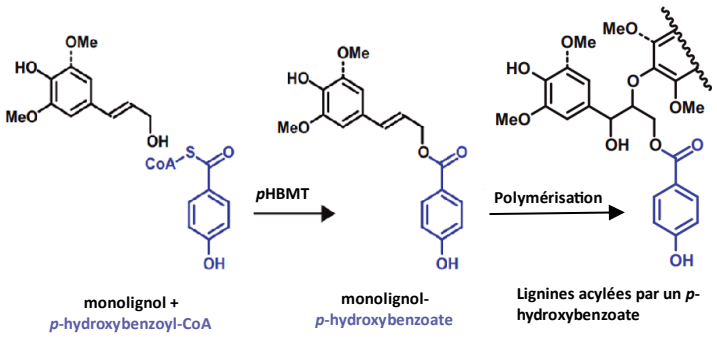

Figure 1 : représentation schématique des étapes de l'acylation d'un monomère de lignine avant polymérisation : exemple du *p*-hydroxybenzoate

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Session 2 : Remodelage et dynamique des parois, notamment en situation de stress

CARACTERISATION BIOCHIMIQUE ET FONCTIONNELLE DE MPPG2, UNE EXOPOLYGALACTRUONASE DE MARCHANTIA POLYMORPHA

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Les cellules végétales sont entourées d'une paroi complexe et dynamique intervenant dans l'acquisition de la structure, la communication intercellulaire, la régulation métabolique ainsi que la défense des végétaux face aux agents pathogènes. Parmi les composants de la paroi, les pectines sont des polymères clés, principalement constitués d'acides galacturoniques, comprenant les rhamnogalacturonanes I (RG-I), les rhamnogalacturonanes II (RG-II) et les homogalacturonanes (HG). Les HG subissent des modifications enzymatiques telles que l'hydrolyse des chaînes faiblement méthylées par les polygalacturonases (PG, EC 3.2.1.67 AN), permettant de moduler leur degré de polymérisation et de réguler la croissance cellulaire et le développement des plantes. Ce travail s'intéresse aux rôles des PG dans le développement de l'espèce modèle *Marchantia polymorpha* (présentant un nombre de PG moins important, qu'*Arabidopsis thaliana*) afin de mieux comprendre la contribution de leur spécificité. Dans un premier temps, nous avons pu montrer que MpPG2, produite et purifiée chez *Pichia pastoris,* présente une activité exo-polygalacturonase, en adéquation avec la caractérisation de sa structure tridimensionnelle. La surexpression de *MpPG2* chez *Arabidopsis thaliana* et *Marchantia polymorpha* induit des phénotypes de développement, tels qu'une réduction de la taille des plantes et des rhizoïdes. Ces résultats permettront à terme de relier la structure fine des pectines au contrôle du développement.

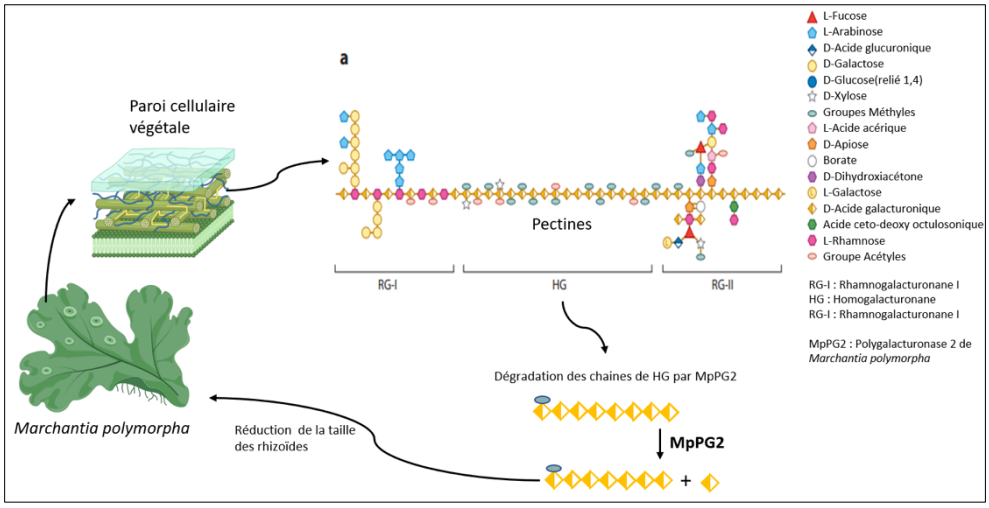

Figure 1 : Action de la MpPG2 chez *Marchantia polymorpha*.

Arabidopsis thaliana LEAF PEPTIDOMICS: SEARCH FOR NATIVE PEPTIDES

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Plant cell walls are extracellular matrices involved in cell-to-cell adhesion, plant support and signaling. They are constituted of complex networks of polysaccharides and structural proteins that can be rearranged during development and in response to environmental cues thanks to the activity of cell wall proteins (CWPs). These CWPs belong to diverse families among which proteases able to release signaling peptides from pro-proteins. This study aims at identifying such peptides in their native form. *Arabidopsis thaliana* was used as a model plant and extracts from healthy plants and of plants treated with a bacterial elicitor (flg22) were analyzed. Different strategies have been used to isolate fractions enriched in small proteins and peptides: (i) either extracellular fluids were extracted by low-speed centrifugation of leaves vacuum-infiltrated with a low molarity buffer; or (ii) total peptides were extracted with trichloroacetic acid (TCA) from ground frozen leaves. In the former case, small proteins were selected by centrifugation through a cut-off column with a size-exclusion of 10 kDa and there was no tryptic digestion. In the latter case, a partial tryptic digestion was applied. The proteins were identified using both the Araport11 database and a home-made database collecting known small proteins predicted to be secreted as well as newly-annotated ones based on prediction of short open reading frames and/or sequence homology. Among the identified proteins, we found Cys-rich proteins, SCOOP, RALF and CLE precursors. The length of the peptides was highly variable. In addition, we could localize the positions of hydroxyproline (Hyp) residues on some peptides and there were also found to be variable. These results will be discussed considering (i) proteolytic events occurring *in planta* or during the extraction procedure or (ii) regulatory mechanisms. Moreover, some of the identified peptides will be compared to those which have been shown to be active when synthesized and applied on plant organs.

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TOMATO RESISTANCE TO *BOTRYTIS CINEREA* FROM THE PERSPECTIVE OF THE CELL WALL

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The European Commission recently proposed an economical plan aiming to modify our agricultural systems, making them more sustainable and environmentally safer. In this context, the objectives of our research project are to explore novel cell wall derived oligosaccharides that are potentially correlated to resistance to phytopathogens and are able to stimulate plant immunity. Cell wall-originating oligosaccharides are especially interesting considering they can be sourced from plant matter coming from agronomic and food industries, allowing the development of a production process on a large scale. Furthermore, they not only have biodegradable properties but also present an underexplored topic that is becoming a prominent research subject in the field of plant cell signalling [1].

The foundation of our approach strategy is the comparison between the diversity of tomato cell wall-derived, *in planta* oligosaccharides generated upon infection in plants differing in resistance and susceptibility levels to *Botrytis cinerea*, the grey mould fungus. Using several tomato varieties, we were able to find a distinct pattern among them based on analysing their *in planta* generated oligosaccharide content, allowing us to explore the contribution of the cell wall to this division. Furthermore, for the purpose of exploring how commercially available cell wall-derived extracts might stimulate plant immunity and enhance resistance to *B. cinerea*, we have performed detached leaf infection assays on tomatoes. Results of these tests revealed activity of certain pectin-containing compounds at nanogram quantities, hinting at the possibility of effects on a molecular scale.

Our work aims to contribute to the development of bioactive molecules priming basal defense responses to diseases caused by necrotrophic phytopathogens, as well as contributing to development of biocontrol phytopharmaceutical products.

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FUNCTIONAL ANALYSIS OF EXTENSIN MOTIFS IN THE LRX PROTEIN FAMILY

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LRR-extensins (LRXs) are extracellular proteins that function as high-affinity binding sites for RALF peptide hormones and contribute to the compaction of pectin, hence, the modification of cell wall structures. While the function of the LRR domain as RALF binding site has been studied in great detail, the exact structure and function of the extensin domain remains largely elusive. Extensins are hydroxyproline-rich glycoproteins, highly repetitive structural cell wall proteins that can form self-organized networks and insolubilize in the cell wall via Tyr-mediated oxidative crosslinking. In the context of LRXs, the extensin domain appears to have an anchoring function (1). In the absence of a functional extensin domain (referred to as ΔE), LRXsΔE induce a dominant-negative phenotype, phenocopying the *lrx* knock-out mutants (2,3).
LRX1 of Arabidopsis is expressed in root hairs and the *lrx1* mutant develops a distinct root hair developmental phenotype. Complementation of the *lrx1* mutant allowed to define a minimal extensin domain that provides a functional LRX1. Based on this, we could demonstrate that Tyr residues potentially involved in Tyr-Tyr crosslinks, are important for protein function. At the same time, insolubilization of the LRX1 is not only dependent on Tyr residues (2). Here, we will present recent data we have obtained in the aim to compare activity and exchangeability of extensins of different tissues. *LRX* genes are expressed in a tissue-specific manner; this is reflected also by the extensin repetitive motifs that are tissue-specific. We have produced a number of extensin domain swap experiments to investigate whether extensin domains are functional in different types of tissues, or, whether their particular repetitive motifs reflect differences in the cell wall structures present in the different cell types.

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Session 3 : Valorisation bioproduits, bioénergie, matériaux, alimentation, santé

WALLDERIVE : INGENIERIE DE NOUVEAUX OLIGOSACCHARIDES DERIVES DE LA PAROI CELLULAIRE POUR LA PROTECTION DES PLANTES

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La paroi cellulaire végétale est une structure complexe en perpétuel remodelage. Elle permet aux cellules de supporter une forte pression intracellulaire, de participer à l'interaction cellulaire et sert de barrière pour les plantes face à l'attaque de potentiels agresseurs tels que les champignons. Ainsi, ces pathogènes ont au cours de l'évolution mis en place des moyens de contourner cette défense grâce à un arsenal enzymatique varié dirigé directement contre celle-ci, favorisant alors l'infection. Ce projet prend appui sur deux pathosystèmes, les modèles blé (monocotylédone)/*Zymoseptoria tritici* (causant la septoriose) et tomate (dicotylédone)/*Botrytis cinerea* (causant la moisissure grise), et vise à produire, *in vitro*, des oligosaccharides pouvant être utilisés comme éliciteur des défenses des plantes. L'analyse transcriptomique a permis d'identifier des gènes codant des glycosyl hydrolases surexprimés chez *B. cinerea* lors de l'infection, et notamment une exo-PolyGalacturonase, une endo-PolyGalacturonase, une endo-XyloGalacturonase ainsi qu'une RhamnoGalacturonane I Hydrolase. Ces enzymes ont été produites en système hétérologue, purifiées et caractérisées biochimiquement (substrats, température, pH). Les oligosaccharides libérés suite à l'action de ces enzymes ont été identifiés par LC-MS/MS. L'utilisation couplée de plusieurs de ces enzymes sur des co-produits de l'agriculture permettra, à terme, de mimer l'action des pathogènes sur les plantes et d'ainsi obtenir les oligosaccharides bioactifs. Une production à plus grande échelle des enzymes est prévue afin de pouvoir générer en quantité suffisante ces oligosaccharides et de confirmer leur bioactivité par des tests en champs.

IMPROVEMENT OF PECTINS GELLING PROPERTIES FROM SUGAR BEET PULP USING PECTIN ACETYLESTERASES

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France is the second biggest sugar beet (*Beta vulgaris spp. Vulgaris)* crop producer in the world and this industrial crop presents an interest in the Hauts-De-France region which produces over 50% of this culture.
Sugar beet pulp (SBP) is a by-product of the sugar industry, mainly composed of cell wall polysaccharides. SBP is low valorized and mostly used to feed animals. Its dry biomass comprises 20% of pectins. Pectins are a family of polysaccharides including homogalacturonan (HG), rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II), and xylogalacturonan (XGA)[1]. GalUA moieties from HG or RG-I can be acetylated at the O-2 and/ or O-3[1]. The degree of acetylation (DAc) can be modulated by pectin acetylesterase (EC 3.1.1.6; PAE) which removes acetyl groups on pectins[2].
Pectins are an economical interest because of their gelling properties. However, these properties depend on the acetylation degree (DAc): a low DAc increases pectin gelling skills. Pectins from SBP are high acetylated (19%) in comparison with the DAc of pectins from citrus (1-2%), which are effective gelling agents.[3,4]
The objective of this study is to improve the gelling properties of pectins from SBP using PAE. This work will provide a natural alternative to chemical gelling agent and avoid the pectins imported from citrus arising from distant countries (Asia, South America). That will thus decrease the carbon footprint.
In *Arabidopsis thaliana*, 12 PAEs have been annotated. Two of them (AtPAE7 and AtPAE11) were overexpressed in heterologous system and purified by affinity chromatography. The activity of recombinant AtPAE7 and AtPAE11 not only showed an acetylesterase activity but also a pectin acetylesterase activity, attesting their annotation. Their specific mode of action will be presented.

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ÉTUDE DE LA DURABILITE DES FIBRES DE LIN GRÂCE À L'ANALYSE DE TEXTILES ÉGYPTIENS ANCIENS

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Le lin est l'une des premières plantes domestiquées, et sa culture remonte à l'Égypte ancienne. À cette époque, le lin était utilisé pour l'habillement, les usages funéraires tels que les bandelettes des momies, ou encore les cordages filets de pêche.[1,2].
Certaines pièces de textiles de lin égyptien, datant des périodes pharaonique et romaine, ont été préservées pendant des millénaires jusqu'à nos jours. Les échantillons étudiés dans ce travail proviennent du Musée du Louvre (Paris, France) et du Museo Egizio, (Turin, Italie) ; ils ont été soigneusement sélectionnés pour leurs contextes archéologiques et pour les conditions de stockage auxquelles ils ont été soumis. Un textile de lin contemporain a également été examiné et utilisé comme référence pour l'étude.
Ce travail s'intéresse ainsi aux effets du vieillissement sur la morphologie et les performances mécaniques de fibres de lin provenant de ces échantillons anciens. Pour ce faire, une étude en microscopie électronique à balayage (MEB) est d'abord réalisée. Ensuite, les propriétés mécaniques des fibres sont évaluées par microscopie à force atomique (AFM) en mode « cartographie quantitative des propriétés nanomécaniques par peak-force » (PF-QNM). Ces techniques complémentaires permettent de mettre en évidence des hétérogénéités morphologiques et mécaniques pour les fibres de certains échantillons anciens (Figure 1). Pour d'autres, bien que le module d'indentation moyen de la paroi cellulaire de la fibre reste comparable au module moyen des fibres contemporaines, la topographie montre des défauts supplémentaires, tels que des fissures dans la paroi cellulaire qui peuvent être observées sur les fibres anciennes.

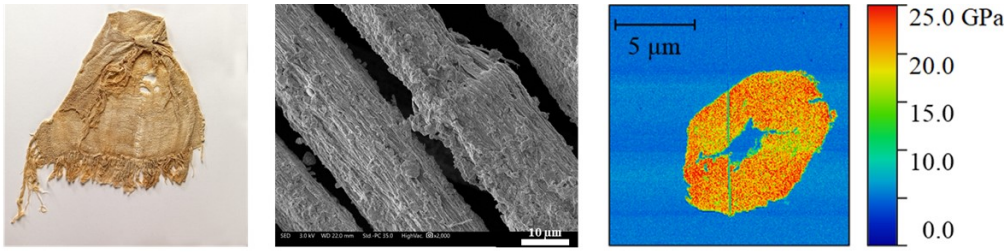

Figure 1 : Exemple de résultats sur fibres de lin issues d'un textile d'Egypte ancienne

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3,6-ANHYDRO-D-GALACTOSIDASE CARACTERISATION, ENZYMES INVOLVED IN THE CARRAGEENANS DEGRADATION OF RED ALGAE CELL WALLS

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Red algae are photosynthetic eukaryotic mainly organisms. Carrageenophyte red algae have carrageenan polysaccharides as major components of their cell walls. Carrageenans are complex, linear, sulfated galactans. They are organized into disaccharide units of D-galactopyranose and 3,6-anhydro-D-galactotpyranose, linked alternately by β-1,4 and α-1,3 glycosidic linkages. The structure of these polymers is at the origin of the texturizing rheological properties much exploited in the food, pharmacological and cosmetic industries. Marine microorganisms have evolved genes encoding enzyme functions for the catabolism of these macromolecules. Bacteria use these polysaccharides as a carbon source through multi-protein degradation systems dependent on several enzymatic activities, transporters and carrageenan-specific regulation. This project focuses on glycoside hydrolases : the endolytic-carrageenases, which releases neo series oligocarrageenans, and exolytic (α-1,3)-3,6-anhydro-D-galactosidases which remove of 3,6-anhydro-D-galactose from the non-reducing end of neo-oligocarrageenans. The activity of the 3,6-anhydro-D-galactosidases was only recently discovered and the specificities are not yet fully understood. Thus, in order to examine the different 3,6-anhydro-D-galactosidase specificities, we have developed a strategy for substrate production using natural hybrid carrageenans from different species of carrageenophyte red algae to have a diversity of carrageenans. Two carrageenases from *Zobellia galactanivorans* ι-carrageenase (GH82) and κ-carrageenase (GH16) were recombinantly produced in *E. coli* and purified. These enzymes were used to digest carrageenans extracted from *Chondrus crispus*, *Solieria chordalis* and *Furcellaria lumbricalis* to produce an oligosaccharide library. These oligosaccharides were then digested by four 3,6-anhydro-D-galactosidases (ZgGH129, ZgGH127-1, ZgGH127-2, ZgGH127-3) to study their fine enzymatic specificities and to produce modified oligosaccharides as molecules for bioactivity assays.

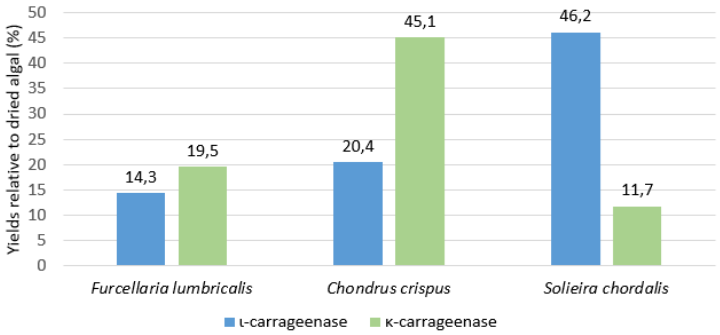

Figure 1. Carrageenan digestion yields, relative to the dry weight of red algae, treated with *Z. galactanivorans* endohydrolases.

METAPROTEOMICS IDENTIFIES KEY CELL WALL DEGRADING ENZYMES DURING FLAX DEW RETTING

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Ecosystems rely on highly complex populations of microorganisms to perform the essential functions of recycling carbon and nitrogen. And such microorganisms (decomposers) as fungi and bacteria, predominantly saprophytic and osmotrophic possess a variety of enzymes designed to efficiently break down complex plant cell wall polymers. Certain agricultural practices leverage these biological processes to exploit plant resources efficiently. One such example is flax dew retting, a customary technique used in northern France. During this process, microbial activity through enzymes strategically breaks down cell wall polymers that are crucial for separating bast fibers from the rest of the stem [1], [2]. In this study, we utilized meta-proteomics to highlight for the first time, specific proteins (CAZymes) associated with biochemically determined global enzyme activities (glucosidase, cellobiohydrolase; xylosidase, galactosidase; endo-polygalacturonase; peroxidase, phenoloxidase) involved in cell wall polymer degradation during the dew retting process in two different fields (at 250m distance) with contrasting industrial fiber qualities. Data-filtering, data-normalization, multiple annotations (including GO, CAZy, taxonomy) using R software and packages, and manual data curation led to the identification of 5702 nonredundant proteins present in 4 key retting stages (days-1, 6, 13, and 25). Of these proteins, 60 contained CAZy motifs belonging to 26 different families from all 5 CAZy classes. 16 families were putatively related to the degradation of different plant cell wall polymers including lignin (AA1), pectins (CE13, CE8, PL1, PL3, GH35), hemicellulose (GH2, GH26, GH35, GH55, GH3, GH5, GH17) and cellulose (GH7, GH3, GH35, GH94). Some of these enzymes (GH5, GH17, CE8, CE13) were present from the beginning (day-1), while others appeared sequentially during the different phases of the decomposition process. The comparison of retting processes in the two different fields showed that slight changes in enzyme presence and/or dynamics might result in different industrially evaluated "*in fine*" fiber characteristics.

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IMPACT DE LA LACCASE FONGIQUE ET DES MÉDIATEURS SUR LES PROPRIÉTÉS ET LA STRUCTURE DE LA BIOMASSE LIGNOCELLULOSIQUE PENDANT LA SACCHARIFICATION

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L'humanité est confrontée à un défi sans précédent alors que la terre est soumise à des changements climatiques croissants, principalement causés par les activités humaines. Cette situation s'aggrave alors que nos besoins en énergie ne cessent de croître. Dans ce contexte, les ressources énergétiques renouvelables et les produits biosourcés sont apparus comme des solutions indispensables pour remplacer le carbone fossile. Ces ressources renouvelables comprennent la biomasse végétale et notamment la biomasse lignocellulosique (LCB) située dans les parois cellulaires des plantes terrestres. La LCB se compose principalement de trois types de polymères : la cellulose et les hémicelluloses, toutes deux composées d'unités de sucre, et la lignine, un polymère phénolique complexe. La cellulose peut être convertie en éthanol après saccharification et fermentation. Néanmoins, la nature complexe des polymères structurels constituant les LCB pose un défi à leur conversion efficace en biocarburants et encourage les industriels à utiliser des techniques chimiquement polluantes et coûteuses en énergie. Le problème de la récalcitrance des LCB à la saccharification est principalement dû à la lignine, qui limite la conversion des monomères de sucre à partir de polysaccharides complexes et rend les biocarburants lignocellulosiques économiquement désavantageux par rapport aux alternatives dérivées du pétrole[1]. Malgré la disponibilité de cocktails enzymatiques commerciaux très efficaces pour dégrader les polysaccharides, ces derniers ne parviennent pas à décomposer correctement la fraction de lignine lors de la saccharification enzymatique. Face à ce problème, des prétraitements enzymatiques utilisant de la laccase ciblant la lignine ont déjà été employés pour réduire la récalcitrance des biomasses lignocellulosiques et ainsi améliorer les rendements de saccharification[2,3].

L'objectif de ce travail est de développer de nouveaux protocoles de traitement de la biomasse qui intègrent et améliorent ces systèmes laccase/médiateur, et de les utiliser pour optimiser la saccharification avec des cocktails d'enzymes commerciaux. Des essais ont été réalisés sur de la poudre de bois de peuplier, une espèce prometteuse pour la production de biocarburants. Nous avons testé différentes concentrations et conditions expérimentales afin d'obtenir un rendement de saccharification optimisé. Le meilleur rendement de saccharification a été principalement évalué en analysant la concentration de glucose dans les surnageants de dégradation, et en observant les modifications chimiques dans les résidus de biomasse par thioacidolyse, FTIR et RMN. Les résultats ont montré une augmentation de 35% de l'accessibilité aux sucres

lorsque la LCB était prétraité avec nos systèmes améliorés de laccase/médiateur avant la dégradation des polysaccharides avec un cocktail d'enzymes commerciales.

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Session 4 : Outils et méthodes de caractérisation, acquisition et traitement des données, modélisation

SOLID-STATE NMR SPECTROSCOPY TO CHARACTERIZE POLYSACCHARIDE ASSEMBLIES ORGANIZATION AT MOLECULAR SCALE

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Structural and dynamic information on polysaccharide assemblies at the nanometric scale can be assessed by solid-state ^{13}C NMR spectroscopy. Amongst the various NMR sequences, the VCT-CPMAS (Variable Contact Time-Cross Polarization Magic Angle Spinning) provides parameters ($T_{1\rho}^{H}$ spin lattice relaxation time in the rotating frame, T_{HH} spin diffusion time) that characterize macromolecular assemblies and can provide a better understanding of how water is structured through hydrogen bonds.
The VCT-CPMAS is generally underused due to time constraints. Moreover, extraction of the dynamical parameters from the experimental data relies on several complex mathematical models. An optimized VCT-CPMAS experimental and processing pipeline was developed [1], which includes the combination of different spin diffusion times to offer novel insights into water dynamics within the polysaccharide matrices, while $T_{1\rho}^{H}$ reflects the overall level of organization resulting from interactions (Figure 1). This processing approach was implemented on samples of increasing complexity including different celluloses [2] and potato starch assemblies whose water content has been modulated. Applied to binary films of cellulose and hemicellulose, this approach demonstrated the ability of T_{HH} to predict water retention capacity of the complex assemblies [3]. The initial results from real matrices (e.g., wood, vegetables, and fleshy fruit cell walls) demonstrate that spectral resolution can distinguish between the dynamics of the various macromolecular components (cellulose, lignins, cutins). This makes it possible to study the dynamic and structural characteristics of each component, which allows to better understand the origin of their functional properties.

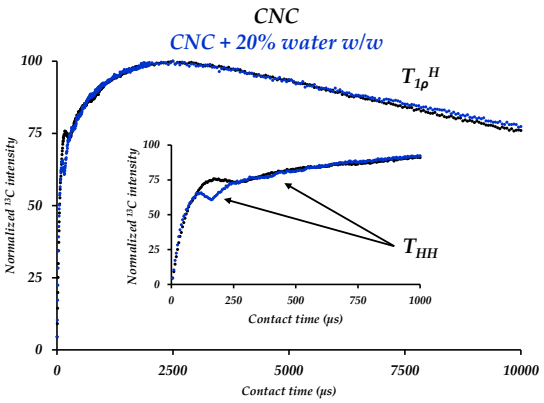

Figure 1: Evolution of the ^{13}C signal intensity as a function of contact time (Magnetization transfer from ^{1}H to ^{13}C). The increasing and decreasing parts allow to define spin diffusion times T_{HH} and relaxation time in the rotating frame $T_{1\rho}^{H}$, respectively.

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A 4D QUANTIFICATION PIPELINE TO CHARACTERIZE PLANT CELL WALL ENZYMATIC HYDROLYSIS IN HIGHLY DECONSTRUCTED SAMPLES

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Converting plant cell walls, as a renewable source of energy and materials, into bioproducts is an important step toward reducing dependence on fossil fuels. The primary obstacle in this conversion is overcoming the cell wall's inherent resistance to enzymatic breakdown called recalcitrance. Much of the research during past decades has concentrated on pinpointing markers of recalcitrance at the nanoscale. As a result, the enzymatic deconstruction of plant cell walls at the cell and tissue scales has been insufficiently studied. Our previous results have paved the way to fill this gap by connecting key parameters of poplar wood enzymatic deconstruction across nano and micro scales[1]. Building upon this work and to further investigate the recalcitrance across scales, we developed a protocol to acquire time-lapse images of spruce tree wood during enzymatic hydrolysis using fluorescence confocal imaging which generated images with highly deconstructed and deformed cell walls. Thus, it was necessary to develop a computational pipeline specifically designed to process this kind of images. The pipeline first segments the pre-hydrolysis image which can exhibit a tilt by applying spatial constraints on the watershed algorithm. The pipeline then employs an adapted spatial information propagation strategy to segment images of highly deconstructed samples by dividing the time-lapse images into sequential clusters where the final image of a cluster is also the first image of the subsequent cluster. Transformations are then computed within each cluster by registering the initial image of the cluster with the successive images within that cluster. Starting from the first cluster with the pre-hydrolysis image, these temporarily constrained transformations are then applied to the segmentation of the initial image of the cluster generating segmentations of the subsequent images. Overall, by limiting the registration to individual clusters, this approach successfully processes images of highly deconstructed samples (Figure 1). The quantification of cell and tissue scale morphological features using these segmentations sheds light on the underlying parameters of the enzymatic deconstruction. The pipeline also provides dynamics of cell wall autofluorescence intensity values to develop mathematical models of cell wall deconstruction.

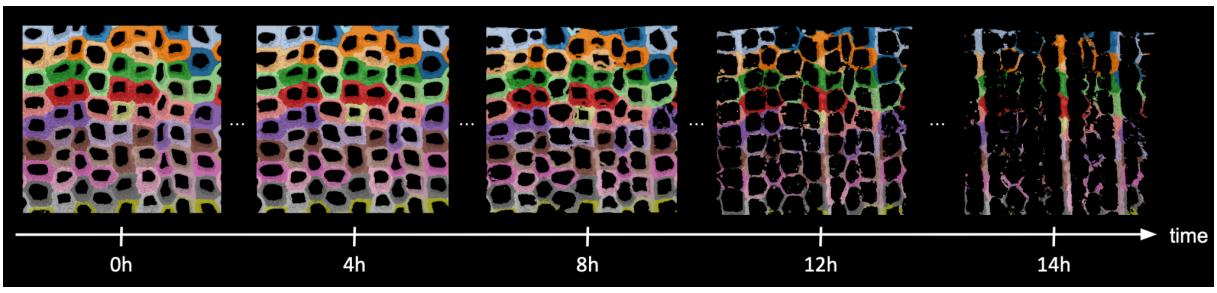

Figure 1 : 4D segmentation of spruce tree cell wall during enzymatic hydrolysis

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HOW TO EVALUATE THE MECHANICAL PROPERTIES OF CELL WALLS IN CAMBIUM BY ATOMIC FORCE MICROSCOPY

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Knowledge of the mechanical properties of plant cell walls is essential for studying plant development and morphogenesis, understanding the maintenance of plant structural integrity, plant hydraulics, signal generation and transduction, cell-cell adhesion, etc. It is also essential for the modelling of these phenomena.
A large number of elegant approaches have been developed to assess the mechanical properties of plant cell walls. However, the vast majority of those that focus on primary cell walls use primary meristems, or superficial cells of plant organs undergoing primary growth, as objects. Secondary meristems, and in particular the cambium, have been marginalized from these studies, and at present we have little or no information on cell wall properties in them. Although the cambium is largely responsible for the formation of conductive and mechanical plant tissues and determines plant architecture, it is also the meristem whose activity makes the greatest contribution to CO_2 sequestration by plants on a global scale. Therefore, it requires careful study.
The flagship technique for studying primary cell wall mechanics is atomic force microscopy. The complexity of cambium studies is dictated by the fragility of its cell walls combined with its location between the developed xylem and phloem tissues. In the case of woody species, the differences in stiffness between cambium, bark and wood can reach several orders of magnitude. Therefore, a key challenge is to make the cambium accessible for study without damaging or altering its properties. In my talk, I would like to describe the use of atomic force microscopy on the cambium of poplar, lime and flax using different sample preparation protocols and share dos and don'ts in the study of secondary meristems.

SPATIO-TEMPORAL ANALYSIS OF SPRUCE TREE CELL WALL ENZYMATIC HYDROLYSIS

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Plant cell wall recalcitrance poses a major challenge for the efficient transformation of lignocellulosic biomass into bio-products. Overcoming the recalcitrance is crucial for achieving plant based cost-effective sustainable alternatives to fossil fuels. Despite significant progress in identifying nanoscale markers of recalcitrance, the cell wall deconstruction at cell and tissue scales remains largely under-explored. Building upon our previous work combining 4D (3D + time) fluorescence imaging and image processing to characterize changes in cell wall properties of poplar samples during enzymatic hydrolysis, this study extends our approach to spruce tree wood, a valuable forest resource threatened by dieback. We collected time-lapse datasets of spruce tree wood samples using a fluorescence confocal microscope during hydrolysis using an enzyme cocktail of known composition with different activity levels (control, 7.5, 15, and 30 FPU/g sample). We then adapted our computational pipeline to effectively handle highly deconstructed datasets undergoing significant structural changes and deformations. This involved first dividing the time-lapse confocal images into sequential clusters with variable sizes, with smaller clusters corresponding to higher levels of deconstruction. We then used an adaptation of spatial information propagation strategy to segment the images during hydrolysis into individual cell walls [1] by temporally constraining the propagation to the clusters. To investigate the spatio-temporal structural changes at the tissue scale during enzymatic deconstruction, an adjacency graph representing the tissue's structure and intercellular connections was computed from segmented images. This graph helped to identify for each cell the number of neighboring cells. Subsequently, we computed dynamics of different cell wall structural parameters, namely cell wall volume, thickness and accessible surface area during enzymatic hydrolysis. We observed a significant reduction in the computed dynamics after 24 hours of hydrolysis in the presence of enzymes, whereas a slight decrease was observed in the initial hours for the control datasets due to photo-bleaching (Figure 1). We also computed autofluorescence intensity dynamics as a proxy for cell wall deconstruction at cell and tissue scales which revealed heterogeneous deconstruction. Altogether, our study identifies key cell and tissue scales parameters underlying spruce tree cell wall enzymatic deconstruction. This study also provides relevant parameters that can be used to develop computational mathematical models of plant cell wall deconstruction.

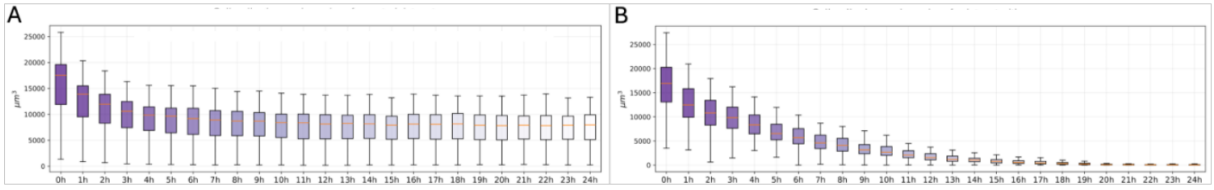

Figure 1. Cell wall volume dynamics during 24 hours in the absence (A) and presence of enzymes (B).

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DYNAMIQUE DE LA FORMATION CELLULAIRE DU XYLEME SECONDAIRE CHEZ LE PEUPLIER : APPROCHE COUPLEE DE TRANSCRIPTOMIQUE ET D'IMAGERIE CHIMIQUE DES PAROIS A L'ECHELLE CELLULAIRE

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Le bois, également appelé xylème secondaire, est un tissu complexe. Chez les arbres feuillus, le bois est composé de trois types de cellules, fibres, vaisseaux et rayons, assurant différentes fonctions pour l'arbre[1], se caractérisant par des trajectoires de différenciation et des parois cellulaires de compositions distinctes. Les cellules du xylème secondaire se différencient à partir des cellules produites par l'activité du cambium, permettant ainsi la croissance en diamètre de l'arbre[1].
Notre étude vise à mieux comprendre la dynamique de la formation des cellules du bois et particulièrement les mécanismes moléculaires impliqués dans leur différenciation ainsi que dans la formation de leurs parois cellulaires. En utilisant des approches à l'échelle de la cellule, telles que le Single-Nucleus RNA Sequencing[2] et l'imagerie ATR-FTIR[3], nous sommes capables de nous affranchir à la fois de la complexité cellulaire du bois et de suivre le gradient de différenciation de chaque type cellulaire. Plus précisément, nous souhaitons identifier : 1) les mécanismes moléculaires impliqués dans la différentiation cellulaire, 2) la composition chimique de la paroi cellulaire des différents types de cellules. Les approches mises en œuvre ont permis de générer des données d'expression génique à l'échelle cellulaire, ainsi que des spectres chimiques à l'échelle de la paroi cellulaire.
Grâce aux analyses bio-informatiques, en utilisant des packages R adéquats (Seurat, Monocle3, ClusterProfiler) nous avons identifié les sets de gènes exprimés au cours des différentes étapes des trajectoires de différentiation des cellules du xylème, depuis les cellules cambiales jusqu'aux fibres, vaisseaux et rayons. De même, nous avons acquis des informations précises sur la composition des parois des différents types cellulaires au cours de leur différenciation.
Nous travaillons maintenant à l'analyse de ces différents types de données pour mieux comprendre les mécanismes moléculaires impliqués dans les différentes trajectoires cellulaires des fibres, vaisseaux et rayons du xylème secondaire chez le peuplier. Nous présenterons ici l'avancement de nos analyses sur ce sujet.

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Posters

SUBCELLULAR CHARACTERIZATION OF GOLGI GLYCOSYLTRANSFERASE TARGETING MECHANISMS IN THE MICROALGAE *CHLAMYDOMONAS REINHARDTII*

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N-glycosylation is crucial for the biological functions and stability of proteins including their efficiency in biotherapy. As a co- and post-translational modification, *N*-glycosylation is taking place within both the Endoplasmic Reticulum (ER) and the Golgi apparatus. Recent studies have shown that our biological model, *Chlamydomonas reinhardtii*, a green unicellular microalga used as a biofactory, displayed specific Golgi steps of glycosylation (1) leading to potential immunogenic motifs in mammals. Engineering *N*-glycoprotein biosynthesis in *C. reinhardtii* was therefore mandatory to produce appropriate recombinant proteins for human applications.
Indeed, deciphering and understanding step-by-step the metabolic pathway of key players such as GlycosylTransferase (GT) is required to move forward. In eukaryotes, *N*-terminal portion of GTs are equipped with a Golgi-targeting domain so called Cytosolic tail Transmembrane domain Stem (CTS) responsible for enzyme addressing within different Golgi cisternae (2). The aim of the present work was to examine the addressing features of heterologous CTS in the *C. reinhardtii* cell factory.
Various mammalian CTS sequences were cloned and fused with reporter Fluorescent Proteins (FP). Resulting constructs were expressed in our microalgal strains, screened and selected through spectrophotometry. FP-CTS of interest were localized in the endomembrane system through confocal laser scanning microscopy and double-labeling experiments with ER and Golgi specific markers. In addition, combination of correlative light electron microscopy (FLIM-TEM) and 3D EM (FIB-SEM) will be performed to refine subcellular addressing of CTS domains in *C. reinhardtii* by allowing the characterization of the 3D organisation of organelles in several mutants.

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EFFETS DE NANOPLASTIQUES SUR LE DEVELOPPEMENT ET LA PAROI D'*ARABIDOPSIS THALIANA*

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Parmi les dernières avancées scientifiques, les nanomatériaux s'illustrent par leur capacité à multiplier les rendements de production, à protéger les cultures et à améliorer la qualité des aliments. Parmi-eux les polymères à empreintes moléculaires (MIP) représentent des nanoporteurs de molécules phytosanitaires innovants. La production de MIP plastiques à base de monomères pétrosourcés est majoritaire. Le devenir de ces nanoparticules plastiques sur les cultures en termes de biodégradabilité, stabilité ou décomposition est questionné. L'objectif du projet est de déterminer l'impact de nanoplastiques sur le développement et la paroi d'*Arabidopsis thaliana*. Pour ce faire, 4 nanoplastiques de charge et d'hydrophobie différentes sont synthétisés sous la forme de particules de composition similaire aux MIP et sont mises, *in vitro,* au contact de graines d'*A. thaliana*. L'analyse phénotypique des plantes cultivées au contact des nanoparticules concentrées à 10 et 100 µg.mL^{-1} a permis de conclure en l'effet stimulant ou inhibiteur de ces 4 nanoplastiques sur le développement végétal. Ainsi, le MAA, chargé négativement, semble présenter un profil négatif sur la croissance alors que le DEAEMA, chargé positivement et l'HEMA, molécule neutre hydrophile, affichent un profil totalement inattendu de « stimulateur de croissance » pour les plus fortes concentrations.

ROLE OF OLIGOSACCHARIDE OXIDATION BY FUNGAL ENZYMES IN THE ELICITATION OF PLANT DEFENSES

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Global food security as well as exploitable biomass stocks are threatened, among other factors, by various abiotic and biotic stresses (including fungal phytopathogens), causing significant production losses (up to 30% for some). Traditional crop protection methods mainly rely on the use of environmentally unfriendly chemical agents with significant health consequences. In recent years, approaches aimed at inducing plant resistance have emerged. In this context, new natural compounds capable of triggering a defense response in plants remain to be discovered. Among these potential bioactive compounds, oligosaccharides (often tested in mixtures) are considered as promising candidates. However, to better assess their elicitor power in plants, and consequently their biotechnological potential, the impact of the nature of the oligosaccharide, its degree of polymerization, and/or its degree of oxidation remains to be elucidated.

In this endeavour, we are currently creating a custom library of oligosaccharides using a library of fungal hydrolytic and oxidative enzymes (at BBF), and testing the effect of these oligosaccharides on plant immune response (at IJPB). We anticipate that the results of this project will contribute to the development of elicitor oligosaccharide cocktails to boost plant immunity, thereby enabling a more secure use of plant biomass, which is fundamental to human and animal nutrition and is a pillar of the emerging bioeconomy.

EXOGENOUS APPLICATION OF PGLR, A POLYGALACTURONASE FROM ARABIDOPSIS THALIANA, REVEALS THE CROSS-TALK BETWEEN CELL SIGNALLING, CELL WALL CHEMISTRY AND CELL-TO-CELL ADHESION

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Plant cell wall remodeling plays a key role in the control of cell elongation and differentiation. In particular, if homogalacturonans (HG) were previously reported to control a number of developmental processes, the high number of polygalacturonases (PG, 68 genes-coding in *Arabidopsis thaliana*), which hydrolyze HG, remains elusive. As a means to overcome potential compensation mechanisms among the gene family, we used exogenous application of a fully characterized plant PG, PGLR, applied over a short-time-course. Complementary technological approaches, including immunocytochemistry, analytical chemistry, RT-qPCR analyses, Atomic Force Microscopy (AFM) were used to relate the changes in cell wall chemistry and mechanics to their consequences on etiolated hypocotyl development, including cell elongation and cell adhesion. We showed that PGLR application had strong effects on the length of cells localized at the apical part of the hypocotyl but induced changes in cell wall composition at the basal part. In contrast, cell adhesion defects mainly localized at the basal part of the hypocotyl. Altogether, PGLR-mediated changes in cell elongation and cell wall integrity induced changes in the regulation of the expression of genes involved in cell wall remodeling and cell signaling.

ECTOPIC LIGNIFICATION IN FLAX FIBERS INDUCED BY SCARIFICATION

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Plants react to environmental stressors by exhibiting phenotypic plasticity, which can encompass both anatomical and metabolic alterations. The responses of plants to mechanical wounding and pathogen attacks are often very similar and include the rapid remodeling of the Plant Cell Wall (PCW). It has been shown that phenolic derivatives, and more particularly lignins, are deposited at the site of infection and wounding (Hawkins and Boudet, 1996) and so are considered as important contributors to the plant's defence mechanisms.
Tissue damage, particularly through a simple mechanical action, can also be considered as a means to evaluate the lignin production potential of tissues, even in cells that usually contain only small quantities. Among these cells, bast fibers (both primary and secondary extra-xylary fiber layers) are present in the cortex of some dicotyledonous plants. They are delimited by a thick secondary PCW and contain very low amounts of lignins, ranging between 1,5 % (Ramie) and 12 % (Jute) although the content may depend on the developmental stage or environmental conditions (Day et al., 2005). The amount of lignins can also increase tremendously when they are subjected to gene mutagenesis (Chantreau et al., 2014).
In this work, we characterized the PCW modifications occurring in the stem cortex following scarification (Fig. 1), with a special focus on the deposition of lignins in the bast fibers. The cell wall composition was determined by Raman spectroscopy and the dynamics of lignification assessed by a chemical biology approach and transcript accumulation.

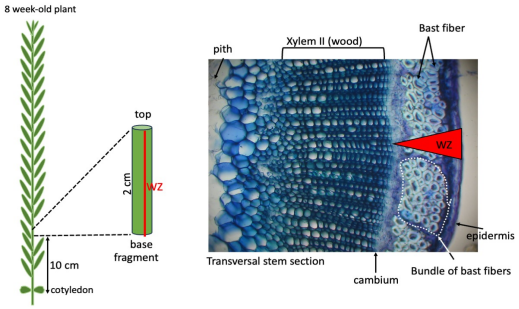

Figure 1: Stem cortex scarification protocol

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CHANGES IN CELL WALL PECTINS METABOLISM IN RELATION TO THE TEMPERATURE-INDUCED HYPOCOTYL GROWTH IN *ARABIDOPSIS THALIANA*

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Understanding mechanisms governing growth in the context of global warming is crucial since under high temperature plants establish fast growth of selective organs at the expense of crop yields. In dicotyledon plants such as *Arabidopsis thaliana*, seedlings developing under elevated temperature induce fast hypocotyl growth that notably results of phytochrome B (PhyB) inactivation and derepression of phytochrome interacting factors (PIFs), which modulate expression of growth-related genes [1]. In particular, PIF4 shares target genes with ARF6 and BZR1 transcription factors, respectively depending on auxin and brassinosteroids. These three transcription factors thus form a molecular signaling hub, and synergistically orchestrate hypocotyl growth [2-3]. Numerous cell wall-related genes were identified as putatively regulated through this molecular signaling hub and upon elevating temperature [4-5], and the importance of cell wall modifications for enabling growth was extensively shown in developmental models such as etiolated hypocotyl [6].

Unfortunately, the role of cell wall in the growth regulated by temperature remains poorly established, and thus led us investigating through morphological, biochemical and transcriptomic approaches changes in cell wall metabolism, especially those for homogalacturonan (HG)-type pectins, during the temperature-induced hypocotyl growth.

Our first analyses revealed that temperature induces fast growth, occurring heterogeneously in hypocotyl. Moreover, this growth response coincides with modifications in pectin metabolism that notably include the neutral and acidic sugar composition, the degree and patterns of HG methylesterification and the expression of HG-related genes. Collectively, our results suggest that elevating temperature triggers changes in cell wall pectins, which are likely needed in the establishment of the temperature-induced hypocotyl growth.

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OLIGOGALACTURONIDES FROM BIO-SOURCES SUBSTRATE INDUCED PROTECTION AGAINST WHEAT POWDERY MILDEW

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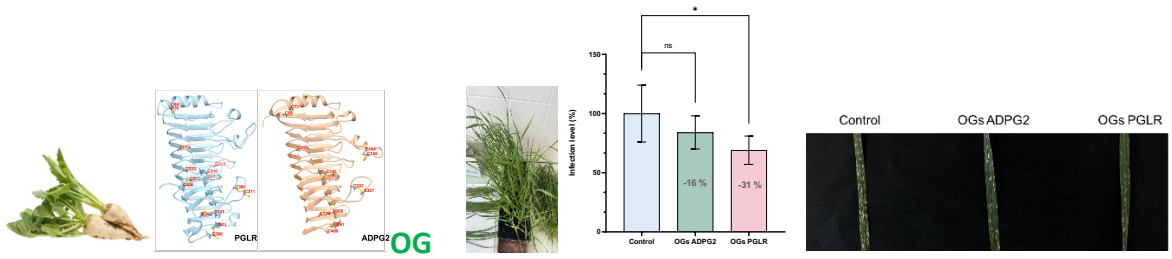
The objective of this work is to contribute to the ECOPHYTO II+ plan, by exploring safer alternatives to harmful phytosanitary products. Our focus is on developing bio-based molecules that can activate plant defenses. One promising approach is the use of oligogalacturonides (OGs) derived from plant cell wall pectins through polygalacturonases (PGs) activity. Although OGs have demonstrated their ability to elicit plant defences, the diversity of OGs tested so far has been limited [1,2].
In this study, we investigated the structure, dynamics, enzymatic kinetics, hydrolysis products of various plant PGs. We specifically focused on the digestion of pectins derived from agricultural waste by various plant PGs to produce diverse pools of OGs. LC-MS analysis of these OGs showed that the average degree of polymerization (DP) is depended on the substrate's origin and the enzyme used.
When applied concentrated OGs (5g/L) under controlled conditions to wheat plants two days before infection with *Blumeria graminis f.* sp. *Tritici (Bgt)*, the fungal pathogen responsible for powdery mildew disease (PMD). The results showed that the protection of wheat against PMD ranged from 16% to 31% and the activation of defense genes varied depending on the specific OGs utilized.
Our findings demonstrate the importance of characterizing OGs and highlight new ways of inducing defense mechanisms in wheat. By creating bio-based molecules with original structures, we aim to progressively substitute harmful phytosanitary products, fostering a healthier and more sustainable environment.



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BIOMODLAB: TOWARDS A DIGITAL TWIN OF PLANT CELL WALL DECONSTRUCTION

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The conversion of plant cell walls into bioproducts offers a sustainable alternative to petroleum-based products by recycling atmospheric CO2 and reducing our reliance on fossil carbon. However, the resistance of plant cell walls to deconstruction, known as recalcitrance, significantly increases the cost and complexity of this conversion process. Despite extensive research efforts, the markers of recalcitrance at the cell and tissue scales remain largely under-investigated and the prediction of plant cell wall deconstruction continues to be challenging. We have recently overcome the experimental and computational challenges of tracking the deconstruction of plant cell wall at cell and tissue scales by developing a 4D (space + time) imaging pipeline, which incorporates time-lapse fluorescence confocal imaging and 4D image processing enabling tracking and quantification of cell wall evolution during hydrolysis and generating detailed 4D virtual representation of cell wall deconstruction.

In parallel, we have developed image-based mathematical models of cell wall deconstruction which faithfully reproduces the 4D deconstruction datasets. The 4D datasets and the computational models of LB deconstruction are integrated into a digital twin of LB deconstruction called BIOMODLAB with a graphical user interface (Figure 1). BIOMODLAB aims to evolve into a comprehensive tool that bridges the gap between experimental observations and predictive modeling, thereby enhancing our understanding of plant cell wall deconstruction.

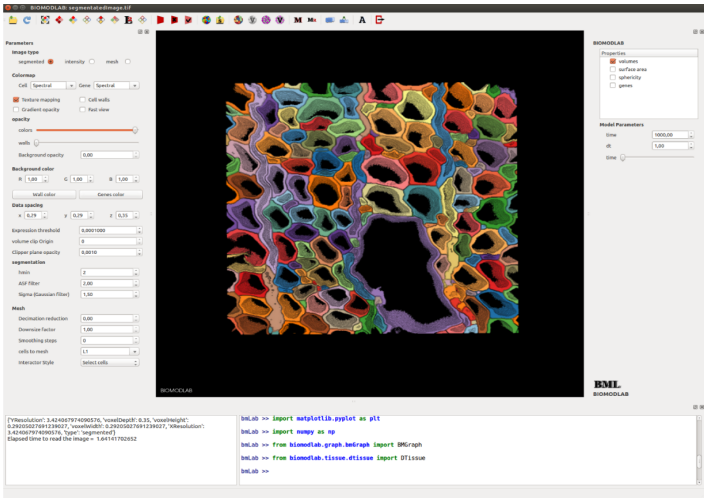

Figure 1 : Graphical user interface of BIOMODLAB

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