

# Effects of CTL2 down-regulation on tension wood formation in GM poplars

Marie-Claude Lesage Descauses, Veronique Laine-Prade, Françoise F.
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# XIV <ell Wall Meeting

# Programme and Book of Abstracts

Chania, Greece 12 - 17 June, 2016

ORGANIZERS Dr. Panagiotis Kalaitzis, Dr. Georgia Drakakaki

Chania 2016

The organization of the meeting is supported by:
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## Committees

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#### **Mary Tierney**

University of Vermont, USA

#### Panagiotis Kalaitzis

Mediterranean Agronomic Institute of Chania, Greece

#### Georgia Drakakaki

University of California, Davis, USA

#### Scientific Sessions Committees

#### 1. Plant Cell wall structure and Evolution

Paul Dupree, Zoe Popper, Alison Roberts, Marie Christine Ralet

#### 2. Biosynthesis of cell wall components

Vincent Boulone, Monika Doblin, Henrick Sheller, Olga Zabotina

#### 3. Cell biology and dynamics of plant cell wall

Frederica Brandizzi, Erik Nielsen, Staffan Persson, Ariel Orelana

#### 4. Functions of plant cell walls: growth, morphogenesis & development

Alan Showalter, Paul Knox, Daku Demura, Charles Anderson

#### 5. Uses of plant cell walls

John Ralph, Maureen Mccann, Simon McQueen, Shawn Mansfield

#### 6. Biomechanics of plants

Anjia Geitman, Sioban Braybrook, Ingo Burget

- 7. Emerging approaches in cell wall biology and Computational modeling of plant cell wall
  Michael Hahn, Silvia Coimbra, Jerome Pelloux
- **8.** Cell wall interactions with microorganisms and the environment / Signaling and defense Azeddine Driouich, Georg Seifert, Antonio Molina, Bruce Kohorn

# Programme

## SUNDAY 12th June 2016

18:00 Registration

20:00-23:00 Welcome Reception - Minoa Palace Hotel

## MONDAY 13th June 2016

7:30		Attendee Registration
8:00-8:30		Welcome Addresses (Imperial Main Hall)
0.00 0.30		<b>Prof. Ch. Kasimis</b> , Secretary General of Agricultural Policy & Management of European Funds, Ministry of Rural Development and Food
		I. Malandrakis, Mayor of Platanias
8.30-10:10		ORAL SESSION – 2 Biosynthesis of cell wall components
		Moderator: <b>Dan Cosgrove</b> , Pennsylvania State University, USA
8:30	S2-01	The structure of Arabidopsis Xyloglucan Xylosyltransferase I and enzymatic activity of Xyloglucan Xylosyltransferase 5 Zabotina Olga, Iowa State University, USA
8:50	S2-02	Purification, crystallisation and structure of AtFUT1, a xyloglucan $\alpha$ -1,2-fucosyltransferase from Arabidopsis Lerouxel Olivier, Univ. Grenoble Alpes, CERMAV, France
9:10	S2-03	Secondary Cell Wall Cellulose Deposition - Simple Polymer, Complicated Biosynthesis Mansfield Shawn, University of British Columbia, Canada
9:30	S2-04	Extensive S-acylation of the cellulose synthase complex drives its plasma membrane integration Kumar Manoj, University of Manchester, UK
9:50	S2-05	Golgi-localized STELLO proteins regulate the assembly and trafficking of cellulose synthase complexes in Arabidopsis Dupree Paul, University of Cambridge, UK
10:10-10:40		Morning Tea/Coffee Break
10:40-	12:20	ORAL SESSION - 3 Cell biology and dynamics of plant cell wall
		Moderator: Anja Geitmann, McGill University, Canada
10:40	S3-01	Hetero-trans-ß-glucanase, a new wall remodelling enzyme that covalently attaches cellulose or mixed-linkage glucan to xyloglucan Frankova Lenka, The University of Edinburgh, UK
11:00	S3-02	A Principle for how Cortical Microtubules Withstand Forces Generated by Motile Cellulose Synthase Persson Staffan, University of Melbourne, Australia

## MONDAY 13th June 2016

11:20 S3-03 Regulation of secondary cell woof xylem vessel elements Demura Taku, Nara Institute of S	vall formation/patterning during differentiation
	science and recimology, Japan
11:40 S3-04 <b>Functional Analysis of the role deposition in Arabidopsis</b> Nielsen Erik, University of Michi	es of CSLD proteins during plant cell wall
sensing in growth contro	re Bourgin, Université Paris-Saclay, France
12:20-14:00 Lunch Break	
14.00-15:40 ORAL SESSION - 5 Uses of p	plant cell walls
Moderator: <b>Stephen Fry</b> , The	e University of Edinburgh, UK
14:00 S5-01 <b>Stacking of traits for improve</b> Scheller Henrik, Lawrence Berke	
14:20 S5-02 <b>A new generation of chromog</b> <b>carbohydrate active enzymes</b> Schückel Julia, University of Cop	<del>-</del>
14:40 S5-03 <b>Molecular, nanoscale and mes</b> McCann Maureen, Purdue Unive	soscale determinants of biomass recalcitrance ersity, USA
15:00 S5-04 <b>Light-weight load-bearing aer</b> Tenkanen Maija, University of H	ogels from plant-derived mannans elsinki, Finland
15:20 S5-05 <b>Engineering Crops for Whole- the Cell Wall Polymer Callose</b> Hanak Tobias, University of Han	Plant-Usage concepts through Modification of nburg, Germany
15:40-17:00 Poster Session and Coffee (	Imperial 1,2,3,4 Halls)
17:00-18:40 ORAL SESSION - 5 Uses of p	plant cell walls
Moderator: <b>Harholt Jesper</b> , (	Carlsberg Research Laboratory, Denmark
17:00 S5-06 <b>CESA9 Conserved-site Mutatio Cellulose Biosynthesis in Rice</b> Peng Liangcai, Huazhong Agricu	
multispectral autofluorescend	crofluidic approach using multimodal ce and infrared imaging to follow enzyme nges during biomass hydrolysis France
formulated food products: str	n cereal grains as functional ingredients in fucture – function relations of Food Chemistry and Biochemistry, Aristotle
18:00 S5-09 <b>The complexity of the Rumino expansion in glycan recognitio</b> Venditto Immacolata, Newcastle	
18:20 S5-10 <b>Genetics of plant cell walls: fo</b> Trindade Luisa, Wageningen Un	rage maize as a model and a crop iversity, Netherlands

## **TUESDAY** 14<sup>th</sup> June 2016

8.30-10.10		ORAL SESSION – 8 Cell wall interactions with microorganisms and the environment/Signaling and defense Moderator: Georg Seifert, University of Natural Resources and Life Science,
8:30	S8-01	Vienna, Austria  Xyloglucan is released by plant roots and has the capacity to increase soil particle aggregation  Galloway Andrew, University of Leeds, UK
8:50	S8-02	<b>Cell Wall Sensing By Wall Associated Kinases</b> Kohorn Bruce, Bowdoin College, USA
9:10	S8-03	PMEI10, PMEI11 and PMEI12 are novel regulators of pectin remodeling in plant immunity Lionetti Vincenzo, Sapienza University of Rome, Italy
9:30	S8-04	Modulation of cell wall synthetic machinery in barley papillae induced during infection with Blumeria graminis f. sp. hordei alters papillae cell wall composition and susceptibility Little Alan, University of Adelaide, Australia
9:50	S8-05	Root defense, role of the root extracellular matrix Driouich Azeddine, Normandie University at Rouen, France
10:10-10:40		
10:10-10	:40	Morning Tea/Coffee Break
10:10-10 10:40-12		Morning Tea/Coffee Break  ORAL SESSION - 2 Biosynthesis of cell wall components
		<i>G</i> ,
		ORAL SESSION - 2 Biosynthesis of cell wall components
10:40-12	:20	ORAL SESSION - 2 Biosynthesis of cell wall components  Moderator: Markus Pauly, University of Duesseldorf, Germany  Elucidation of the catalytic mechanisms of monolignol biosynthetic enzymes to enable protein engineering-based cell wall modification
<b>10:40-12</b> 10:40	: <b>20</b> \$2-06	ORAL SESSION - 2 Biosynthesis of cell wall components  Moderator: Markus Pauly, University of Duesseldorf, Germany  Elucidation of the catalytic mechanisms of monolignol biosynthetic enzymes to enable protein engineering-based cell wall modification  Vermerris Wilfred, University of Florida, USA  Progress Toward Structural Understanding of Cellulose Synthesis by Plants
<b>10:40-12</b> 10:40 11:00	:20 S2-06 S2-07	ORAL SESSION - 2 Biosynthesis of cell wall components  Moderator: Markus Pauly, University of Duesseldorf, Germany  Elucidation of the catalytic mechanisms of monolignol biosynthetic enzymes to enable protein engineering-based cell wall modification  Vermerris Wilfred, University of Florida, USA  Progress Toward Structural Understanding of Cellulose Synthesis by Plants  Nixon B. Tracy, Pennsylvania State University, USA  Pectin Biosynthetic Activity of Arabidopsis GAUTs Heterologously Expressed in HEK293 Cells
<b>10:40-12</b> 10:40 11:00 11:20	:20 S2-06 S2-07 S2-08	ORAL SESSION - 2 Biosynthesis of cell wall components  Moderator: Markus Pauly, University of Duesseldorf, Germany  Elucidation of the catalytic mechanisms of monolignol biosynthetic enzymes to enable protein engineering-based cell wall modification  Vermerris Wilfred, University of Florida, USA  Progress Toward Structural Understanding of Cellulose Synthesis by Plants  Nixon B. Tracy, Pennsylvania State University, USA  Pectin Biosynthetic Activity of Arabidopsis GAUTs Heterologously Expressed in HEK293 Cells  Engle Kristen, University of Georgia, USA  Hemicelluloses Produced by MUCILAGE-RELATED Proteins Determine the Organization of Polysaccharides in the Arabidopsis Seed Coat Epidermis

## **TUESDAY** 14<sup>th</sup> June 2016

14:00-15:40		ORAL SESSION - 3 Cell biology and dynamics of plant cell wall
		Moderator: Mary Tierney, University of Vermont, USA
14:00	S3-06	Mechano-chemical polarization of cell walls drives shape coordination Majda Mateusz, UMEÅ, Sweden
14:20	S3-07	XTH-mediated covalent-linking between cellulose and cellulose Nishitani Kazuhiko, Tohoku University, Japan
14:40	S3-08	Using Live-Cell Imaging to Compare Cellulose Biosynthesis in Primary Cell Walls and Induced Secondary Cell Walls Li Shundai, Pennsylvania State University, USA
15:00	S3-09	Identification of a sphingolipid mannosyltransferase that is required for normal cellulose deposition in Arabidopsis Mortimer Jenny, Joint BioEnergy Institute, USA
15:20	S3-10	RUBY PARTICLES IN MUCILAGE (RUBY) is a putative glyoxal oxidase required for cell wall strengthening Sola Kresimir, The University of British Columbia, Canada
15:40-16	:10	Afternoon Coffee Break
15:40-16: 16:10-17:		
		Afternoon Coffee Break  ORAL SESSION - 3 Cell biology and dynamics of plant cell wall  Moderator: Christoph Ringli, University of Zurich, Switzerland
		ORAL SESSION - 3 Cell biology and dynamics of plant cell wall
16:10-17	:10	ORAL SESSION – 3 Cell biology and dynamics of plant cell wall Moderator: Christoph Ringli, University of Zurich, Switzerland Actin and myosins XI are involved in trafficking of cellulose synthase complexes in Arabidopsis
<b>16:10-17</b> : 16:10	: <b>10</b> S3-11	ORAL SESSION - 3 Cell biology and dynamics of plant cell wall Moderator: Christoph Ringli, University of Zurich, Switzerland Actin and myosins XI are involved in trafficking of cellulose synthase complexes in Arabidopsis Staiger Christopher J., Purdue University, USA Secretion of pectin and cell wall proteins in seed coat epidermal cells of Arabidopsis

## **WEDNESDAY** 15<sup>th</sup> June 2016

8:30-10:30		ORAL SESSION - 2 Biosynthesis of cell wall components
		Moderator: Antony Bacic, University of Melbourne, Australia
8:30	S2-11	Recovery of the cse and ccr1 mutant phenotype via vessel-specific complementation Vanholme Ruben, University of Gent, Belgium
8:50	S2-12	Molecular Structure of a Cellulose Synthase Plant-Conserved Region by X-ray Crystallography and Small Angle X-ray Scattering and Possible Function in the Catalytic Domain Carpita Nicholas C., Purdue University, USA
9:10	S2-13	<b>Designer Lignins: Inspirations from Nature</b> Ralph John, DOE Great Lakes Bioenergy Research Center, USA
9:30	S2-14	<b>Cell wall polysaccharide biosynthesis in oomycete pathogens</b> Bulone Vincent, University of Adelaide, Australia
9:50	S2-15	Role of Arabidopsis thaliana KNOTTED-LIKE (KNOX) transcription factors in the regulation of cell wall biosynthesis Douglas Carl, University of British Columbia, Canada
10:10	S2-16	Understanding the mechanism of (1,3; 1,4)-β-D-Glycan Synthesis in cereals Doblin Monika, Australian Research Council Centre of Excellence in Plant Cell Walls, Australia
10:30-1	11:00	Morning Tea/Coffee Break
<b>11:00-</b> 1	12:20	ORAL SESSION - 4 Functions of plant cell walls: growth morphogenesis & development
		Moderator: <b>Paul Knox</b> , University of Leeds, UK
11:00	S4-01	Genetic and chemical genomic dissection of the cell adhesion mechnanisms in plants  Mouille Gregory, INRA, Versailles, Finland
11:20	s4-02	Pectin dynamics regulated by endogenous pectate lyases, leading to proper control of cell proliferation in Arabidopsis Ohtani Misato, Nara Institute of Science and Technology (NAIST), Japan
11:40	S4-03	Hydroxyproline O-arabinosyltransferase mutants oppositely alter tip growth in Arabidopsis thaliana and Physcomitrella patens MacAlister Cora, University of Michigan, USA
12:00	S4-04	How the walls are made in the stem cell niche of the plant shoot Wightman Raymond, University of Cambridge, UK
<b>12:20-</b> 1	12:50	Noon Coffee Break

## **WEDNESDAY** 15<sup>th</sup> June 2016

12:50-14:10		ORAL SESSION – 4 Functions of plant cell walls: growth morphogenesis & development
		Moderator: <b>Helen North</b> , INRA
12:50	S4-05	The effect of cell wall polymer interactions on seed mucilage architecture Harpaz-Saad Smadar, Hebrew University of Jerusalem, Israel
13:10	S4-06	Establishment of polarity in subsidiary cell mother cells of Zea mays stomatal complexes begins with the differentiation of the matrix cell wall materials Giannoutsou Eleni, University of Athens, Greece
13:30	S4-07	Two small multigene hydroxyproline-O-galactosyltransferase families function in arabinogalactan-protein glycosylation, growth and development in Arabidopsis Showalter Allan, Ohio University, United States
13:50	S4-08	The Role of xylem morhology on freezing tolerance as indicated by the Arabidopsis Xylan O-acetyltransferase mutant tbl29/ esk1 and its suppresor ess1 Pauly Markus, University of Duesseldorf, Germany
14:10-15:30		Lunch
16.00		Technical Tour

## **THURSDAY** 16<sup>th</sup> June 2016

8:30-9:50		ORAL SESSION - 1 Plant Cell wall structure and Evolution
		Moderator: Breeanna Urbanowicz, University of Georgia, USA
8:30	S1-01	Biosynthesis of a novel mixed-linkage arabinoglucan cell wall polysaccharide in Physcomitrella patens Alison W. Roberts, University of Rhode Island, USA
8:50	S1-02	Biochemical Evidence of Fucosylated Xyloglucan in Charophycean Green Algae Mikkelsen Maria Dalgaard, Technical University of Denmark, Denmark
9:10	S1-03	A new monoclonal antibody to pectic type I arabinogalactan Ralet Marie-Christine, INRA, Nantes, France
9:30		Best Poster Award and Presentation of candidatures for the organization of the next Cell Wall Meeting
9:50-10	0:20	Morning Tea/Coffee Break
10:20-11:40		ORAL SESSION - 1 Plant Cell wall structure and Evolution
		Moderator: <b>Zoë Popper</b> , National University of Ireland, Galway, Ireland
10:20	S1-04	Cell-wall assembly during growth and cell elongation in brown algae Siméon Amandine, Marine Glycobiology group, Roscoff, France
10:40	S1-05	Supramolecular 3D Nano-Architecture of Arabidopsis Plant Cell Walls by Cryo-Electron Tomography of Vitreous Sections Auer Manfred, Lawrence Berkeley Nat'l Lab, USA
11:00	S1-06	Variation in xylan structure: implications for the evolution and biosynthesis of monocot cell walls Peña Maria J., University of Georgia, USA
11:20	S1-07	Algal xylan synthase proves functional orthology between algal and plant cell walls Harholt Jesper, Carlsberg Research Laboratory, Denmark
11:40-13:00		Poster Session (Imperial 1,2,3,4 Halls)/ Open table discussion: Increase Participation and Diversity (Imperial Main Hall) Coffee Break
13:00-14:40		Lunch Break

## **THURSDAY** 16<sup>th</sup> June 2016

14:40-16:20		ORAL SESSION – 4 Functions of plant cell walls: growth morphogenesis & development Moderator: Alison Roberts, University of Rhode Island, USA
14:40	S4-09	Pollen tubes and pathogens: who's got the moves like JAGGER? Coimbra Sílvia, University of Porto, Portugal
15:00	S4-10	Mechanics of lobe formation in interlocking plant cells Anja Geitmann, University of Montreal, Canada
15:20	S4-11	Integration of cell wall feedback signalling with intracellular growth regulation Wolf Sebastian, COS Heidelberg, Germany
15:40	S4-12	A tension-microtubule feedback controls plant shapes through cellulose synthase guidance Verger Stéphane, INRA, CNRS, ENS, UCB Lyon 1, France
16:00	S4-13	While reaching for the sky, what controls hypocotyl growth? Bou Daher Firas, University of Cambridge, UK
16:20-16:50		Coffee Break
16:50-18	<b>8:10</b>	ORAL SESSION - 2 Biosynthesis of cell wall components
		Moderator: Rachel Burton, University of Adelaide, Australia
16:50	S2-17	The identification of Golgi UDP-Aarabinofuranose transporters in Aarabidopsis Ebert Berit, The University of Melbourne, Australia
17:10	S2-18	UDP-uronic acid transporters are important providing galacturonic acid and arabinose, but not xylose, inot the cell wall Ariel Orellanaa, Universidad Andrés Bello, Chile
17:30	S2-19	Identification of genes that regulate mixed-linkage glucan biosynthesis in Brachypodium distachyon Wilkerson Curtis, Michigan State University, USA
17:50	S2-20	Post-translational control of cellulose biosynthesis: comparing the function of CESA5 and CESA6 in seed coat mucilage biosynthesis Griffiths Jonathan, Institute Jean-Pierre Bourgin, France
18:10	S2-21	The BIN2 protein kinase directly phosphorylates Arabidopsis CesA1 and negatively regulates cellulose biosynthesis Wallace Ian, University of Nevada, USA

## FRIDAY 17th June 2016

8:30-9:50		ORAL SESSION - 6 Biomechanics of plants
		Moderator: Laigeng Li, ChineseAcademy of Sciences, China
8:30	S6-01	Distinctive Microfibril Motions Visualized by AFM during Mechanical Extension and Cell Wall Loosening Zhang Tian, The Pennsylvania State University, USA
8:50	S6-02	Pollen Tube: From Cell Wall Composition and Ultrastructure to Cell Mechanics and Growth Ndinyanka Fabrice Tohnyui, University of Zurich, Switzerland
9:10	S6-03	<b>Effects of CTL2 down-regulation on tension wood formation in GM poplars</b> Šećerović Amra, 1INRA, France
9:30	S6-04	Stomatal Cell Wall Crystallinity: Distinctive structural patterns in diverse phylogenetic groups Shtein Ilana, The Hebrew University of Jerusalem, Israel
9:50-10	0:20	Morning Tea/Coffee Break
10:20-11:40		ORAL SESSION - 7 Emerging approaches in cell wall biology and Computational modeling of plant cell wall
		Moderator: Hahn Michael, University of Georgia, USA
10:20	S7-01	An integrated computational and experimental approach to discover systems-level controls of morphogenesis Szymanski Dan, Purdue University, USA
10:40	S7-02	Combined experimental and computational approaches reveal distinct pH- controlled inhibiting capacities of Arabidopsis Pectin MethylesteraseInhibitors (PMEIs) Lefebvre Valérie, Université de Picardie, France
11:00	S7-03	From Octamer to Rosette: Structural rearrangements determine activity of the (1,3)-β-glucan synthase complex Voigt Christian, University of Hamburg, Germany
11:20	S7-04	An updated collection of Arabidopsis Glycosyltransferase (GT) clones and an enzymatic characterisation platform for plant cell wall research Zeng Wei, The University of Melbourne, Australia
11.40-1	12:10	Noon Coffee Break

## FRIDAY 17th June 2016

12:10-13:30		ORAL SESSION - 7 Emerging approaches in cell wall biology and Computational modeling of plant cell wall Moderator: Kim Johnson, University of Melbourne, Parkville, Australia
12:10	S7-05	Synthetic plant carbohydrates as a toolbox for cell wall biology Pfrengle Fabian, Max-Planck-Institute of Colloids and Interfaces, Germany
12:30	S7-06	In vivo tagging of non-cellulosic plant cell wall glycans using tagged CBM and scFv probes Hahn Michael, University of Georgia, USA
12:50	S7-07	Raman microscopy combined with multivariate data analysis to reveal plant cell wall structure and the deposition of hydrophobising substances on the micro- and nanoscale Gierlinger Notburga, University of Natural Resources and Life Sciences, Austria, Hochschule Zurich, Switzerland, EMPA-Swiss Federal Laboratories for Material Testing and Research, Switzerland
13:10	S7-08	Modelling Plant Cell Walls: linking specific wall structures with plant response to stress and fitness Eva Miedes, Universidad Politécnica Madrid., Spain
13:20-15:20		Light Lunch

## EFFECTS OF CTL2 DOWN-REGULATION ON TENSION WOOD FORMATION IN GM POPLARS

<u>Šećerović Amra</u><sup>1</sup>, Lesage-Descauses Marie-Claude<sup>1</sup>, Lainé-Prade Véronique<sup>1</sup>, Laurans Françoise<sup>1</sup>, Leplé Jean-Charles<sup>1</sup>, Segura Vincent<sup>1</sup>, Badel Eric <sup>2</sup>, Clair Bruno<sup>3</sup>, Pilate Gilles<sup>1</sup>, Déjardin Annabelle<sup>1</sup>

<sup>1</sup>INRA, UR0588, Unité Amélioration, Génétique et Physiologie Forestières, Orléans, France, <sup>2</sup>INRA, UMR Physique et Physiologie Intégratives de l'Arbre Fruitier et Forestier, Clermont-Ferrand, France, <sup>3</sup>CNRS, UMR Ecologie des Forêts de Guyane, Kourou, French Guiana,

Angiosperm trees are able to reorient their axes thanks to their capacities to differentiate tension wood on the upper side of stems and branches. In poplar, tension wood fibres develop an extra cell wall layer, named G-layer, responsible for the peculiar mechanical properties of tension wood. G-layer is composed of highly crystalline cellulose microfibrils embedded in a polysaccharide/glycoprotein matrix devoid of lignin. During the G-layer deposition, cellulose microfibrils get oriented parallel to the fibre axis and placed under the state of high tensile stress. Chitinase-like proteins (CTL) have been proposed to play different roles in plant growth and development. Genes encoding CTL have been associated to cellulose synthesis and their mutation causes ectopic deposition of lignin. Transcripts of CTL2 gene were shown to be highly abundant in differentiating tension wood (Déjardin et al., 2004). Its homologue in Arabidopsis is specifically expressed in stems and co-expressed with the CesA genes involved in cellulose synthesis in secondary cell walls (Persson et al., 2005). In our study, we investigated potential function of CTL2 in the formation and mechanical properties of tension wood. Expression of CTL2 was quantified in different tissues and the effects of CTL2 down-regulation have been characterized in GM poplar trees. CTL2 gene appeared highly expressed in xylem with no difference in expression between tension and opposite wood. Downregulation of CTL2 did not seem to impact growth and development of trees in greenhouse conditions. However, stems appeared to be highly breakable in the transverse direction. Anatomical observations of xylem showed altered formation of Glayers in vessel-surrounding fibres in transgenic poplars. Further analyses indicated a substantial increase in wood lignin content. Cellulose crystallinity, microfibril angle and stem mechanical properties are currently assessed. Results will be presented and discussed in the light of present hypothesis regarding CTL2 function in plant development.

**S6-04** 

## STOMATAL CELL WALL CRYSTALLINITY: DISTINCTIVE STRUCTURAL PATTERNS IN DIVERSE PHYLOGENETIC GROUPS

Shtein Ilana<sup>1</sup>, Shelef Yaniv<sup>2</sup>, Marom Ziv<sup>2</sup>, Bar-On Benny<sup>2</sup>, Popper Zoë A. <sup>3</sup>, Zelinger Einat<sup>4</sup>, Schwartz Amnon<sup>1</sup>, Harpaz Smadar<sup>1</sup>

<sup>1</sup>The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, The Robert H. Smith Faculty of Agriculture, Food & Environment, Rehovot 7610001, Israel, Rehovot, Israel, <sup>2</sup>Department of Mechanical Engineering, Ben-Gurion University of the Negev, Beer Sheva, Israel, <sup>3</sup>Botany and Plant Science, Ryan Institute for Environmental, Marine and Energy Research, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland, <sup>4</sup>The Interdepartmental Equipment Unit, The Robert H. Smith Faculty of Agriculture, Food & Environment, Rehovot, Israel,

Stomata evolved ~400 million years ago - and have remained a key feature of plant anatomy and physiology. Stomata offer a unique research system, where the function has remained largely the same, even though various cell wall features have evolved and changed. We attempted a renewed look at stomatal cell wall structure utilizing digitalized polar microscopy and confocal microscopy. We investigated the distribution patterns of cellulose, including microfibril orientation and crystalinity, lignin and phenolic compounds in the stomata of vascular plants from different phylogenetic groups. In addition, we applied a numerical mechanical Finite-Element simulation to understand the mechanical anisotropy of the stomatal cell wall. Stomata of the six species chosen for study cover a broad structural, ecophysiological and evolutionary spectrum: two ferns and two angiosperm species with kidney-shaped stomata, and two grass species with dumbbell-shaped stomata. Surprisingly, we observed three distinct patterns of cellulose crystallinity in stomatal cell walls: the ferns exhibited pattern Type I, angiosperm kidney-shaped stomata exhibited pattern Type II and the grasses presented Type III. Our data demonstrates for the first time the existence of distinct spatial patterns of varying cellulose crystallinity in guard cell walls. Guard cell walls undergo reversible deformations during opening/closing of the pore and thus must be both extremely strong and flexible. Different cellulose crystallinity patterns could influence those properties. Such spacial patterns could imply different biomechanical function, which in its turn could be a consequence of different environmental