Unraveling the function of MYB090 and MYB156 in secondary cell wall formation in poplar using both ChIP-SEQ and in planta gene modification approach

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ROLE OF THE LIPID TRANSFER PROTEIN 2 (LTP2), A DUAL-LOCALIZED PROTEIN TO THE CELL WALL AND PLASTIDS, IN CUTICLE FORMATION IN ARABIDOPSIS THALIANA

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The cuticle is a hydrophobic layer that covers the outer periclinal wall of epidermal cells of aerial organs of land plants. It constitutes the first physical barrier for plant interactions with their environment and it plays multiples roles in plant physiology, including the prevention of non-stomatal water loss and the defense against biotic and abiotic stresses [1]. The major cuticle component is cutin either combined with or covered by waxes [1]. While the intracellular biosynthesis of cutin and waxes lipid components is well documented, the mechanisms underlying their extracellular assembly and deposition beyond the plasma membrane and across the hydrophilic polysaccharidic cell walls, remain to be solved. Cell wall-localized Lipid Transfer Proteins (LTPs) have been hypothesized to play a role in the transport of cuticular lipids across the cell wall given the presence of a hydrophobic pocket in their 3D structure [2] to lead to the cuticle formation. In this study, we took advantage of the Arabidopsis thaliana etiolated hypocotyls model (i) where LTP2 was identified as the unique LTP member in the cell wall proteome [3] and (ii) which is characterized by a thick cuticle [4], to go further in the understanding of the involvement of LTP2 in cuticle formation. To decipher the role(s) of LTP2, several complementary approaches have been carried on. By in situ hybridization, LTP2 has been shown to display a gene expression pattern restricted to epidermal cells of aerial organs, which is in agreement with the place of cuticle deposition. Using electron microscopy (TEM and SEM), we have shown that ltp2 mutants have an altered and disorganized cuticle. ltp2 mutants also display modifications in cuticle permeability / lipophilic staining. Finally, we have shown that LTP2 has a dual localization, both in cell walls as expected from proteomics data, but also and more surprisingly, in plastids. Using truncated versions of LTP2 fused to a fluorescent protein, we could identify the peptidic sequences enabling this original dual targeting through the secretory pathway. Our results demonstrate the involvement of LTP2 in cuticle formation in etiolated hypocotyls and highlight a new mechanism of dual targeting whose role remains to be elucidated.

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[2] Liu et al. (2015) JXB Vol. 66, No. 19 pp. 5663–5681 ;
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UNRAVELING THE FUNCTION OF MYB090 AND MYB156 IN SECONDARY CELL WALL FORMATION IN POPLAR USING BOTH CHIP-SEQ AND IN PLANTA GENE MODIFICATION APPROACH

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Plant R2R3-MYB transcription factors play an important role in plant secondary cell wall formation, by activating or repressing their target genes in a coordinated manner within a complex regulatory network. They belong to a large gene family - 180 genes in poplar -, with some members already identified as master switches in the regulation network (Zhang et al, 2014). Here, we combined genetic engineering and chromatin immuno-precipitation technique (ChIP-SEQ) in order to elucidate the function of 2 R2R3-MYB transcription factors in poplar, MYB090 and MYB156. MYB090 potentially regulates a high number of target genes in planta through a highly conserved motif, similar to GAMYB. These target genes are involved in the biosynthetic pathways of lignin, cellulose and xylans, which are the major components of secondary cell walls. Overexpression of MYB090 in poplar reduced stem growth and its total lignin content; lignification was severely impacted in wood parenchyma rays. By contrast, overexpression of MYB156 in poplar showed no growth defect, at least for some transgenic lines, while the total lignin content was decreased of 15% in the stem. These plants contained hypolignified fibers while the lignification of vessels seemed unaffected. These different results led to the conclusion that both MYB factors are transcriptional repressors of

lignification, but act at different levels of the regulatory network. This work opens new avenues on the building of transcriptional regulatory networks involved in secondary cell wall formation. It also generated new poplar transgenic lines with interesting traits for bioethanol production. This work was carried out in the frame of AMC2B Région Centre Project and TreeForJoules Plant KBBE Project. WL received a PhD fellowship from Région Centre and INRA-EFPA.

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VARIATIONS IN THE POLYSACCHARIDE COMPOSITIONS OF COLLENCHYMA CELL WALLS ALONG CELERY (APIUM GRAVEOLENS L.) PETIOLES

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Collenchyma is a common, simple tissue in plants, composed of elongated cells with thick, primary walls, and probably functions to support organs. Different types of collenchyma cells are recognized depending on the morphology of the cell walls and include angular, tangential, lacunar and annular. Although numerous studies have described the structures of collenchyma walls, much less is known about their polysaccharide compositions. In leaf petioles of celery (Apium graveolens L.), collenchyma is the angular type and occurs as strands made up of a number of cells just under the epidermis of the abaxial ridges and adjacent to the phloem in the vascular bundles. Only the strands in the ridges were examined in the present study. These strands run from the base to the tops of the petioles, with a significantly smaller cross-sectional area at the top than the bottom. Celery petioles were found to elongate throughout their lengths when young, but as they mature, elongation was found to be increasingly confined to the top, until elongation ceased. In the present study, fully elongated petioles were used and divided along their lengths into three regions of equal length: top, middle and bottom. Bright-field light microscopy and variable pressure scanning electron microscopy (VPSEM) were carried out on transverse sections from midway along these three regions and showed that the cross sectional areas and numbers of cells in the collenchyma strands decreased from bottom to top, whereas the wall thickness increased. Cell-wall preparations were isolated from collenchyma strands obtained from each of the three regions, and fractionated by sequential extraction with the following: 20 mM, pH6.7 potassium HEPES (4-(2-hydroxyenthyl)-1-piperazineethanesulfonic acid) buffer, 50 mM CDTA (trans-1,2-diaminocyclohexane-N, N, N, N-tetraacetic acid), 50 mM Na2CO3 (4°C, 16 h; room temperature, 4 h), 1 M KOH, and 4 M KOH. Monosaccharide compositions were determined for the polysaccharides present in the isolated wall preparations and the fractions, including the insoluble residue obtained at the end of the fractionation. These compositions indicated the cell walls contained ~30% cellulose, ~55% pectins, and ~15% hemicelluloses. In the top region, the pectins had a higher degree of methyl esterification and there were more hemicelluloses compared with the other two regions. Additionally, solid-state 13C nuclear magnetic resonance (NMR) spectrometry was done on the isolated cell-wall preparations using cross-polarization/magic-angle spinning (CP/MAS) and spin-pulse excitation (SPE) to examine the relative mobilities of the different polysaccharides. Linkage analysis will be used to facilitate the interpretation of the NMR spectra.

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XYLOGLUCAN DEFICIENCY DISRUPTS MICROTUBULE STABILITY AND CELLULOSE MICROFIBRIL ORGANIZATION IN ARABIDOPSIS THALIANA, ALTERING CELL GROWTH AND ORGAN MORPHOGENESIS

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Xyloglucan contains a glucan backbone that is decorated with side chains and comprises most of the hemicellulose in eudicot primary cell walls. It has been proposed to interact with cellulose to regulate cell wall mechanics. The Arabidopsis thaliana xxt1 xxt2 mutant displays an absence of detectable xyloglucan, moderate growth defects, and altered mechanical properties that are suggestive of alterations in wall integrity. To probe the mechanisms underlying these defects in xxt1 xxt2 plants, we used atomic force microscopy (AFM), field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), and confocal microscopy to analyze cellulose arrangement, cellulose biosynthesis, and microtubule patterning and dynamics. We used real-time quantitative PCR to detect the expression levels of microtubule- and wall-integrity-related genes. We found that cellulose microfibrils are highly aligned and bundled in the cell walls of xxt1 xxt2 etiolated hypocotyls, that GFP-CELLULOSE SYNTHASE3 motility is reduced in xxt1 xxt2 cells, corresponding with a reduction in cellulose content, and that microtubule patterning and stability are