Genome-wide identification of poplar malectin/malectin-like domain-containing proteins and *in-silico* expression analyses find novel candidates for signaling and regulation of wood development

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Abstract

Background: Malectin domain (MD) is a ligand-binding protein motif of pro- and eukaryotes. It is particularly abundant in Viridiplantae**,** where it occurs as either a single (MD, PF1721) or tandemly duplicated domain (PF12819) called malectin-like domain (MLD). In herbaceous plants, MD- or MLD-containing proteins (MD proteins) are known to regulate development, reproduction, and resistance to various stresses. However, their functions in woody plants have not yet been studied. To unravel their potential role in wood development, we carried out genome-wide identification of MD proteins in the model tree species black cottonwood (*Populus trichocarpa*)*,* and analyzed their *in-silico* expression and co-expression networks.

Results: *P. trichocarpa* had 146 MD genes assigned to 14 different clades, two of which were specific to the genus *Populus*. 87% of these genes were located on chromosomes, the rest being associated with scaffolds. Based on their protein domain organization, and in agreement with the exon-intron structures, the MD genes identified could be classified into five superclades having the following domains: leucine-rich repeat (LRR)-MD-protein kinase (PK), MLD-LRR-PK, MLD-PK (*Cr*RLK1L), MLD-LRR, and MD-Kinesin. Whereas the majority of MD genes were highly expressed in leaves, particularly under stress conditions, eighteen showed a peak of expression during secondary wall formation and their co-expression networks suggested signaling functions in cell wall integrity, pathogen-associated molecular patterns, calcium, ROS, and hormone pathways.

Conclusion: *P. trichocarpa* MD genes exhibit a variety of domain organizations, and include genes apparently specific to *Populus*, as well as genes with potential involvement in signaling pathways regulating secondary wall formation.

Background

Plant cells are surrounded by cell walls made of cellulose, hemicelluloses, pectins and structural proteins, with lignin being present in cell types specialized for mechanical support (sclerenchyma) and water transport (xylem). Cell wall biosynthesis needs to be regulated so that its mechanical properties can be adapted to different circumstances according to the signals perceived. It is becoming generally accepted that there is constant feedback from the wall to the protoplast, mediated by different molecular pathways commonly termed cell wall integrity (CWI) signaling (Hématy et al., 2007; reviewed by Wolf and Höfte, 2014; Hamann, 2015; Voxeur and Höfte, 2016; Wolf, 2017; Rui and Dinneny, 2020). Perception of signals external to the protoplast is usually mediated by plasmalemma-localized proteins with various ectodomains. One large group of ectodomain-containing proteins is the receptor-like kinases (RLKs) that allow the plant cells to perceive external cues and transduce them**,** using a phosphorylation relay**,** into signals to initiate cellular responses (Gish and Clark, 2011; Engelsdorf and Hamann, 2014). Plant RLKs belong to the RLK/Pelle kinase family, one of the largest gene families in plants with more than 600 members in *Arabidopsis* (Shiu and Bleecker, 2001; 2003). It comprises both RLKs and receptor-like cytoplasmic kinases (RLCKs), and has been divided into 45 subfamilies, including wall-associated kinases, extensin-like RLKs, lectin RLKs, and leucine-rich repeat RLKs. RLCKs are cytoplasmic kinases without a transmembrane domain (TMD) and they recognize signaling molecules intracellularly. The RLKs usually function as heterodimers: one subunit with a large extracellular domain interacts with a ligand, and the other, which has a smaller extracellular domain, stabilizes this interaction and enhances signal transduction (Xi et al., 2019).

Among the different clades of plant RLKs, the *Catharanthus roseus* receptor-like kinase 1-like proteins (*Cr*RLK1Ls) have received significant attention as mediators of CWI (reviewed by Wolf and Höfte, 2014; Li et al., 2016; Franck et al., 2018). The family is conserved in all Streptophytes analyzed so far, including moss and liverwort, indicating its ancient origin (Galindo-Trigo et al., 2016). *Cr*RLK1Ls are characterized by two malectin ectodomains (MDs) forming a malectin-like domain (MLD), a transmembrane helix and a C-terminal intracellular Ser and Thr kinase domain. The *Arabidopsis* genome contains 17 *Cr*RLK1L genes and the majority of them have been functionally analyzed. THESEUS1 (THE1) was the first member to be identified as a mediator of dwarfism and ectopic lignification induced by defects in cellulose biosynthesis (Hématy et al., 2007; Merz et al., 2017). Other members of *Cr*RLK1L family including CURVY1 (CVY1), FERONIA (FER) and ANXUR1 (ANX1) are required for polar cell growth in different cell types. FER, ANX1/2 and BUDDHA'S PAPER SEAL1 and 2 (BUPS1 and 2) participate in sexual reproduction. FER mediates signaling by reactive oxygen species (ROS) and Ca^{2+} during pollen tube reception at the filiform apparatus (Escobar-Restrepo et al., 2007), whereas ANX1/2 together with BUPS1/2 form a receptor complex for RAPID ALKALINIZATION FACTOR (RALF) 4 or 19 in the growing tip of pollen tube and regulate ROS and Ca^{+2} gradients essential for its growth and CWI (Ge et al., 2017). In addition, *Cr*RLK1L proteins are involved in immune responses. FER positively regulates pathogen-associated molecular pattern (PAMP) - triggered immunity (PTI) by facilitating the formation of a receptor complex composed of BAK1-FLS2-FER or BAK1-EFR-FER (Stegmann et al., 2017), whereas ANX1 functions antagonistically in PTI and inhibits effector-triggered immunity (ETI) (Mang et al., 2017). The downstream responses of *Cr*RLK1Ls are

diverse and include Rho-GTPases activating NADPH oxidases involved in the production of apoplastic ROS (Foreman et al., 2003; Duan et al., 2010; Denness et al., 2011; Boisson-Dernier et al., 2013), RLCKs (Boisson-Dernier et al., 2015; Du et al., 2016), inhibition of the proton pump AHA1 (Haruta et al., 2014), Ca^{+2} signaling mediated by MLO proteins (Kessler et al., 2010; Meng et al., 2020), as yet unknown Ca^{+2} channels and a signaling cascade via intracellular kinases that eventually activate or repress gene transcription (Franck et al,. 2018).

The MLD, which is characteristic of *Cr*RLK1L proteins, and the MD, are also found in other types of plant RLKs (Zhang et al., 2016; Bellande et al., 2017). The MD was first identified in the protein called malectin residing in the endoplasmic reticulum of *Xenopus laevis* and other animals, where it monitors protein glycosylation by binding di-glucose motifs with a 1,4-, a 1,3-, and a 1,2-linkage in glycosylated proteins (Schallus et al., 2008; 2010). However, the crystal structure of MLD in ANX1, ANX2, and FER indicated an absence of the aromatic residues that interact with di-glucosides in animal MDs, and suggested different ligand specificities and/or functions of the MLDs in these proteins (Du et al., 2018; Moussu et al., 2018; Xiao et al., 2019). Several peptides from the RALF family have been demonstrated to bind to ectodomains of *Cr*RLK1L proteins in *Arabidopsis*: RALF34 to THE1 (Gonneau et al., 2018), RALF1, -17, -23, -32 and -33 to FER (Haruta et al., 2014; Stegmann et al., 2017), and RALF4 and -19 to the ANX1/2-BUPS1/2 receptor complex (Ge et al., 2017). Recently it has been shown that the binding of RALF23 to FER is stabilized by interaction with LORELEI-like GPI-ANCHORED PROTEINS (LLGs) and the formation of such a heterocomplex is required for PTI signaling (Xiao et al., 2019). Moreover, the ectodomain of FER has been shown to bind to the leucine-rich repeat (LRR) domain of LRR-extensin 1 (LRX1) (Dünser et al., 2019) and to pectin (Feng et al., 2018).

MD is classified as CBM57 in the CAZy database (http://cazy.org). Interestingly, the CBM57 family is greatly expanded in the model tree species *Populus trichocarpa* compared to the herbaceous model plant *Arabidopsis thaliana* (Kumar et al., 2019). Moreover, transcript of the CBM57 family members are highly upregulated in developing wood tissues of *Populus tremula* (Kumar et al., 2019) and *Eucalyptus grandis* (Pinard et al., 2015). These data suggest that MD/MLD-containing proteins (subsequently called MD proteins) have important functions in trees. We hypothesize that MD proteins are involved in the regulation of cell wall formation during secondary growth via pathways analogous to those reported for primary growth (Wolf and Höfte, 2014; Hamann, 2015; Li et al., 2016; Wolf, 2017), and that they participate in signaling cascades related to stress responses and developmental processes in trees. To find candidates for receptors active during secondary growth, we first carried out genome-wide identification of *P. trichocarpa* genes with predicted MD and MLD. Second, we used expression datasets from different organs (Sundell et al., 2015, Immanen et al., 2016) and high-resolution expression data for wood developmental zones in *P. tremula* (Sundell et al., 2017) to identify those MD proteins that are expressed during wood biosynthesis, and to classify them according to expression at specific stages of xylogenesis. Finally, we identified co-expression networks for the MD proteins expressed during secondary wall deposition, which include their putative interactors. Our analyses provide a framework within which to identify CWI monitoring, stress-response, and other signaling pathways operating during wood development.

Results And Discussion

Identification of MD proteins in *P. trichocarpa* and their classification

Searches of the *P. trichocarpa* and *A. thaliana* genomes for MD proteins resulted in the identification of 146 and 87 gene models respectively (Table S1 and S2). Previous analyses identified 62 MD genes in strawberry (Zhang et al., 2016), 74 in *A. thaliana* (Bellande et al., 2017; Sultana et al., 2019), and 84 in rice (Jing et al., 2020).

The *P. trichocarpa* proteins identified were analyzed for sequence similarity using protein sequence alignment and phylogenetic analysis, revealing the presence of 12 clades supported by at least 87 % of bootstrap replicates, and three ungrouped sequences, two of which had orthologous sequences in *A. thaliana*, and were therefore considered to be two single member clades III and XI (Fig. 1 and 2). The sequences were numbered *PtMD1* to *PtMD146* according to their sequential appearance in the intraspecific phylogenetic tree (Fig. 1). The predicted protein properties and probable subcellular localizations of *Pt*MD proteins are listed in Table S1. The deduced sequence lengths ranged from 274 to 1192 amino acids, and isoelectric points (pIs) ranged from 4.55 to 9.49. Seventy-six out of the 146 *Pt*MD proteins had a signal peptide (SP) cleavage site. The SP was not found in any members of clades I and XIV. Thirteen of the *Pt*MDs were predicted to be soluble proteins, with the predicted localization of six of them being extracellular, six - including all members of clade XIV - being cytoplasmic and one being peroxisomal. Out of 133 membrane proteins, one was predicted to localize in the endoplasmic reticulum.

Chromosomal distribution of *MD* genes in *P. trichocarpa*

127 out of the 146 poplar *MD* gene models were mapped to chromosomes, while 19 gene models were located on five different scaffolds (Fig. 4). The majority of chromosomal genes (79) were present in clusters comprising between two and eleven genes (Fig. 4; Table S3). Clusters were also present on the scaffolds. The clusters consisted of tandem repeats having the same or reverse orientations. This large number of tandem duplications strongly suggests that the main mechanism of MD family expansion in *P. trichocarpa* is via local gene duplication, rather than whole genome duplications. Gene multiplication at a given locus could occur via an unequal crossing over mechanism, which after multiple rounds would result in large numbers of tandemly repeated sequences. Such a mechanism was proposed as featuring particularly prominently in various *LRR* gene families (Schaper and Anisimova, 2015) including *LRR-RLK* (Shiu and Bleeker 2001; Zan et al., 2013; Zulawski et al., 2014; Zhang et al., 2016, Wang et al., 2019a) and *R* genes (Choi et al., 2016). Indeed, 11 out of our16 clusters of *PtMD* genes had members with LRR domain(s) (Table S3).

Tandem duplications allow rapid gene family expansion and the creation of novel alleles and are thought to be particularly important for the co-evolution of *R* and *Avr* genes in hosts and their parasites (Holub 2001; Choi et al., 2016). Partial duplications with omission of some domains form a key mechanism for neofunctionalization. Such a process apparently characterized the poplar MD family, since there were seven out of 16 clusters that included genes with LRR and kinase domains along with closely related members without LRR domains (Fig. 4; Table S3).

Analysis of exon-intron structures of *Pt*MD genes

Exon-intron structure reflects the evolutionary history of genes; hence we analyzed the exon-intron organization of *PtMDs*. Although the majority of clades displayed very diverse numbers of introns (Table S4; Fig. 1), the maximum number of introns for clades within a superclade was similar. The superclade LRR-MD-PK, comprising clades I-VIII, had genes with very large numbers of introns (maximum between 23 and 26); superclade MLD-LRR-PK (clades IX-XI) had at most 15 introns; superclade MLD-PK (clade XII or the *Cr*RLK1L group) contained genes with up to two, but typically without any, introns; and superclades MLD-LRR (clade XII) and MD-Kin (clade XIV) had at most 10 and 17 introns respectively (Table S4; Fig. 1). Lack, or low frequency, of introns in *Cr*RLK1L genes has also been observed in other species including strawberry, *Arabidopsis* and rice (Zhang et al., 2016; Bellande et al., 2017; Jing et al., 2020). Thus the exon-intron organization of poplar MD genes supported their grouping into superclades, which represent ancestral diversification of plant MD genes.

The phylogenetic tree of MD proteins was generally consistent between *P. trichocarpa* and *A. thaliana* with bootstrap values of greater than 76 % for the main clades (Fig. 2). Three exceptions were noted, however: one orphan protein *Pt*MD89, clade VI that included *Pt*MD41-*Pt*MD56, and clade VIII with *Pt*MD62-*Pt*MD71. These poplar genes apparently did not have orthologs in *A. thaliana*. Close homologs to *PtMD89* were found primarily among other trees, such as several *Populus* and *Prunus* species, *Quercus suber*, and *Juglans regia*, suggesting that *PtMD89* may have a specialized function in trees. Clade VI included clusters of tandemly duplicated genes located on chromosome 19 (Fig. 4; Table S3). BLAST searches using the PLAZA database (https://bioinformatics.psb.ugent.be/plaza/) revealed the presence of similarly replicated genes in some other species, such as *Hevea brasiliensis*, *Manihot esculenta, Ricinus communis* and *Prunus persica*, but not in Arabidopsis. Clade VIII is specific to some Rosids, where its genes are also tandemly and block duplicated. The largest representation of this clade outside poplar is found in *Hevea brasiliensis* and *Citrus clementina*, but there are no representative genes in *Arabidopsis* or other Brassicaceae. Taking into consideration the conclusion that the genes of clade VI and VIII had apparently undergone tandem duplication events in the *P. trichocarpa* lineage after its separation from that of *A. thaliana*, it is possible that they represent specialized genes, such as R genes important for immunity, that co-evolved with poplar symbionts and/or pathogens (Holub, 2001).

Besides identifying clades not represented in *Arabidopsis*, we found that the relative clade sizes (number of genes per clade relative to genome size) show some differences between the two species (Fig. 2). Clade IX was expanded in *A. thaliana*, whereas clade I was expanded in *P. trichocarpa* (c^2 test at $P \leq 0.05$).

Expression of *PtMDs* in different organs of *Populus*

RNA sequencing datasets available for aspen species were analyzed to reveal differential expression of *PtMD* genes among different organs and tissues. Out of the 146 genes, 145 were expressed at least in one of the organs and tissues tested, and the variance-stabilized transformation (VST) of expression values are shown in Table S5. Interestingly, the majority of *PtMD* genes (101) showed maximum expression in leaves. Moreover, many of them showed the highest expression in leaves exposed to abiotic/biotic stress, such as beetle (32), drought (8) or mechanical damage (11). Nine *PtMD* genes were most highly expressed in roots exposed to drought. Genes with maximal expression values detected in stressed organs were distributed among clades I, II, IV, V, VI, VII, VIII, X, XII, and XIII (Fig. 2), suggesting stress-response functions for these clades. Interestingly, no gene that was maximally expressed in stressed organs was

found in clades III, IX, XI or XIV, suggesting their involvement in other types of signaling. Several *PtMD* genes were most highly expressed in the vegetative growing organs: young roots (11) or leaves (4) (Fig. 5; Table S5: "root-control" and "expanding leaves", respectively). Eight genes, all from clades XII and XIII, were most highly expressed in female flowers at various developmental stages, and four in mature seeds. The genes highly expressed in expanding female flower buds or in mature seeds were in many cases also highly expressed in developing secondary tissues, vascular cambium or developing secondary xylem and phloem (Fig. 5 and Table S5).

PtMDs involved in wood biosynthesis

To investigate the expression of *PtMD*s during different stages of wood biosynthesis, we used the AspWood database (http://aspwood.popgenie.org/aspwood-v 3.0/), which provides data on high-spatial-resolution transcript abundance in developing secondary xylem and phloem tissues of aspen (Sundell et al., 2017). Only 89 *PtMD*s (61%) were found to be expressed in developing secondary vascular tissues (Table S6), with the majority exhibiting distinct patterns of expression, clustering in ten expression groups (Table S6; Fig. 6). This clustering indicates that certain sets of *PtMD*s have specific functions at certain stages of secondary vascular development. Some of the *PtMD* genes expressed in secondary vascular tissue also exhibited high expression under diverse stress conditions in leaves or roots (Table S6, Fig 2).

The largest group of *PtMD* genes (50) that were expressed in secondary vascular tissue showed a peak of expression in the phloem (Table S6; Fig. 4). These genes were mostly from superclades LRR-MD-PK, MLD-PK (*Cr*RLK1L), and MLD-LRR-PK. Cambium and radial expansion zones were the zones characterized by the greatest variety of *Pt*MD transcripts including members of superclades MD-Kin, LRR-MD-PK, MLD-LRR, and MLD-PK (*Cr*RLK1L). In contrast, *PtMD* genes having a peak of expression at the transition between primary and secondary wall deposition were mostly from the MLD-PK (*Cr*RLK1L) group. Intriguingly, the genes with maximum expression during secondary wall deposition were expressed at relatively low levels and many of them belonged to clade I of *Pt*MDs, which lacks LRR. *PtMD* genes with the highest expression in the maturation zone were mostly from clades V and XII.

Networks of xylogenesis-related *PtMD* genes

To find putative partners involved in signaling pathways together with the xylogenesis-related *PtMD* genes, we analyzed co-expression networks of *PtMD* genes identified as being expressed during xylogenesis. Ten *PtMD* genes having a peak of expression in the cambium-radial expansion zone and primary to secondary transition zone (CA-RE/PW-SW), and eight genes from clusters PW-SW/Secondary Wall and Secondary Wall (Fig. 6, Table S6), representing, respectively, the early and main stages of secondary wall deposition were used as baits for network analyses.

The baits for the CA-RE/PW-SW zones formed five separate networks (Fig. 7A; Table S7), the largest being that of *PtMD126* – one of the two poplar orthologs of *AtFER*. It included several candidates for functioning in signaling by phosphate relay and ROS, and for regulation of cell wall development. Apoplastic ROS in wood forming tissues could have a double role, in signaling and in regulation of lignin polymerization. Thus the *PtMD126* network included a homolog of *PBS1-LIKE 19* (*AtPBL19*), encoding a RLCK of subfamily VII-4, which signals a response to chitin perceived by CHITIN ELICITOR RECEPTOR KINASE 1 (*At*CERK1) through a phosphate relay (Bi et al., 2018), and ROS production (Rao et al., 2018). Homologs of *AtTGA1* and *AtTGA7*, which encode basic leucine zipper transcription factors involved in oxidative-stress mediated responses to biotrophic and necrotrophic pathogens (reviewed by Gatz, 2013), were, respectively, positively and negatively correlated with *PtMD126* (Fig. 7A; Table 1). The oxidation state of *At*TGA1 is regulated by a glutaredoxin, *At*ROXY19 (Li et al., 2019), the homolog of which has been found to respond to altered secondary wall xylan in aspen (Ratke et al., 2018), suggesting that the *PtMD126* network might include candidates for sensing secondary wall integrity. *At*TGA1 interacts with the BLADE-ON-PETIOLE 1 and 2 (*At*BOP1/2) transcription factors (Wang et al., 2019b), which are known to regulate xylem fiber differentiation (Liebsch et al., 2014). Moreover, the network includes a homolog of the *BEL1-LIKE HOMEODOMAIN 8* (*AtBLH8)* transcription factor, which controls expression of BOP1 (Khan et al., 2015) (Fig. 7A; Table 1). The network also includes a homolog of the gene encoding GROWTH-REGULATING FACTOR9 (*At*GRF9), a 14-3-3 protein that regulates developmental programs and stress signaling by binding phosphoproteins and regulating their activities (Mayfield et al., 2007; Liu et al., 2014; Omidbakhshfard et al., 2018). The presence of a homolog of *IMPORTIN b-4* (*AtIMB4*), which is required to transport GRF-INTERACTING FACTOR 1 (*At*GIF1) to the nucleus (Liu et al., 2019) (Fig. 7A; Table 1) further supports the involvement of *GRF/14-3-3* genes in the *Pt*MD126 network.

A separate large network was formed by neighbors of *PtMD98* – one of the two poplar orthologs of *AtTHE1* (Fig. 7A; Table 1; Table S7). This network comprised genes related to hormonal signaling by IAA and GA, and to the regulation of xylogenesis. One example is a homolog of *At*LAX3, which encodes an auxin influx carrier (Swarup et al., 2008). Another is a homolog of *AtAGC1-12*, encoding a kinase phosphorylating the auxin efflux carrier *At*PIN1 (Haga et al., 2018). We have also identified a homolog of *AtGASA4* involved in GA

responses and redox regulation (Rubinovich and Weiss, 2010). It is noteworthy that GA responses and *GASA* genes were also found to be upregulated in response to a secondary wall xylan defect in aspen (Ratke et al., 2018). Moreover, the co-expression network included an LRR-RLK homologous to *At*PXY-CORRELATED 1 (*At*PXC1), which is required for secondary wall deposition (Wang et al., 2013).

The network of *PtMD94* – the clade XII member related to *AtHERK1* and *AtCVY1* - included a homolog of *AtRALFL31* (Fig. 7A; Table 1; Table S7). *RALF* genes encode hormone peptides that signal developmental processes and stress responses by interacting with *Cr*RLK1Ls. *At*RALFL31 belongs to subfamily IIIA which includes as yet uncharacterized members, but both *At*RALFL31 and Potri.017G059500 have the conserved YISY motif essential for interaction with *At*FER (Campbell and Turner, 2017). Thus, *Potri.017G059500* could potentially encode a peptide hormone recognized by *Pt*MD94. The *PtMD94* network also included other candidates for signaling. For example, there was a homolog of *PLANT U-BOX 13* (*AtPUB13)*, which encodes an E3 ligase involved in signal-activated ubiquitination and subsequent degradation of different receptors including ABA INSENSITIVE 1 (*At*ABI1) (Kong et al., 2015), BRASSINOSTEROID INSENSITIVE 1 (*At*BRI1) (Zhou et al., 2018), LYSM-CONTAINING RECEPTOR-LIKE KINASE 5 (*At*LYK5) (Liao et al., 2017), and FLAGELLIN-SENSITIVE 2 (*At*FLS2) (Liu et al., 2012; Antignani et al., 2015). The ubiquitination of flg22-bound *At*FLS2 by *At*PUB13 depends on its interactor protein RAB GTPASE HOMOLOG A 4B (*At*RABA4B) (Antignani et al., 2015). Interestingly, a homolog to another *PtMD94* network member encodes ROOT HAIR DEFECTIVE 4 (*At*RHD4) which mediates polar localization of *At*RABA4B (Thole et al., 2008). Consequently, it seems likely that these *PtMD94* network members are indeed functionally linked within the same network.

Two other *PtMD* genes expressed during early secondary wall biosynthesis, *PtMD88* and *PtMD145*, formed small networks, which included important regulatory genes in xylem cell differentiation (Fig. 7A; Table 1; Table S7). One of them was the homolog of the master spatial regulator of vascular differentiation, PHLOEM INTERCALATED WITH XYLEM (*AtPXY*), encoding an LRR-RLK which signals tracheid fate upon binding the small CLE peptide *At*TDIF (Fisher and Turner, 2007). The other was a homolog of VASCULAR-RELATED RECEPTOR-LIKE KINASE 1 (*At*VRLK1) (Huang et al., 2018) which is probably responsible for the switch between xylem cell expansion and secondary wall deposition.

The late secondary wall-expressed baits formed five networks (Fig. 7B; Table 1; Table S8). The largest of these was associated with two *PtMD* genes, *PtMD129*, a clade XII member related to *AtANX1* and *AtANX2*, and *PtMD137*, which encodes an LRR-RLK, from clade XIII. Orthologs of key signaling-related genes were included within this network. One of them was *CALCIUM PERMEABLE STRESS-GATED CATION CHANNEL 1 (AtCSC1*). Stretch-activated Ca^{2+} channels have been predicted to be important players in CWI (Engelsdorf and Hamann, 2014). *At*CSC1 belongs to a newly characterized family of stretch-activated Ca^{2+} channels conserved in eukaryotes (Hou et al., 2014; Liu et al., 2018). In addition, we found *Potri.015G108700/AT5G61820*, encoding an uncharacterized NOD19-like protein, which has been implicated in responses to cold stress downstream of mechanosensitive Ca^{2+} channels (Mori et al., 2018). The aspen homolog of *At*CSC1 is thus a promising candidate for a secondary wall damage sensor. Another important signaling-related homolog is *TAPETUM DETERMINANT 1* (*AtTPD1*), which encodes a small peptide hormone that is recognized by an RLK complex consisting of *At*EMS1 and *At*SERK1/2 to activate transcription factors of the BES1 family (Chen et al., 2019). Moreover, a homolog of *Arabidopsis ROP (RHO OF PLANTS) GUANINE NUCLEOTIDE EXCHANGE FACTOR 7* (*AtROPGEF7*) was among the hits for *PtMD137*. *At*ROPGEF7 interacts with the kinase domain of *At*FER, mediating downstream NADPH oxidase-dependent ROS signaling which is needed for polarized cell growth (Duan et al., 2010). Finally, we identified a homolog of *Arabidopsis* GT-2 Like (*AtGT2L*), which encodes a Ca^{2+}-dependent calmodulin (CaM)-binding trihelix transcription factor involved in plant abiotic stress signaling (Xi et al. 2012). In addition to signaling-related genes, the *PtMD129-PtMD137* network included some key cell fate regulator proteins (Fig. 7B; Table 1; Table S8). One of these was the homolog of *WIP DOMAIN PROTEIN 5* (*AtWIP5*), which encodes a zinc-finger protein involved in root patterning downstream of auxin (Crawford et al., 2015) and ROS signaling (Miao et al., 2004). Another was a homolog of *Arabidopsis SQUINT* (*AtSQN*), which encodes a cyclophilin 40-like protein that promotes the accumulation of miRNAs miR156 and miR172, targeting master regulatory genes in organ development (Smith et al. 2009; Prunet et al., 2015). The network also included orthologs of two genes encoding master transcriptional regulators, *At*MYB46 and *At*MYB83, which activate the secondary wall program (Zhong and Ye, 2012). Both these genes showed positive correlation with *PtMD129*. In contrast, orthologs of three genes with roles in cell division were negatively correlated with *PtMD129* (Table 1). This supports the hypothesis that secondary wall integrity signaling results in coordination between cell division and secondary wall formation activities in developing wood (Ratke et al., 2018).

The network for *PtMD110*, which together with *PtMD111* forms a pair orthologous to *AtHERK2*, included a homolog of the *Arabidopsis* gene encoding the transcription factor BES1-INTERACTING MYC-LIKE1 (*At*BIM1) (Fig. 7B; Table 1; Table S8), which mediates brassinosteroid signaling (Chandler et al., 2009). Several other genes discussed above can be linked to BR-dependent or BES-related BR-independent signaling (Table 1). Intriguingly, a secondary wall xylan defect induced transcriptomic changes suggesting stimulation of BR signaling in aspen (Ratke et al., 2018), supporting the involvement of the *AtBIM1* homolog in sensing secondary wall integrity.

Conclusions

Malectin and malectin-like domains (MD/MLD) are lectin-like motifs found in proteins (MD proteins) of pro- and eukaryotes; they are particularly abundant in plants, where they carry out essential signaling functions in defense and development (Bellande et al., 2017; Franc et al., 2018). Recent studies have identified and classified *MD* genes in herbaceous plants (Zhang et al., 2016; Bellande et al., 2017; Sultana et al., 2019; Jing et al., 2020). Here we carried out a census of *MD* genes in the model woody species *P. trichocarpa* (Table S1) and expanded the set for *A. thaliana* (Table S2).

In total, 146 *MD* genes were found in *P. trichocarpa* and they were assigned to fourteen clades based on sequence similarity, and to five superclades based on predicted protein domain organization and intron-exon structures (Figs. 1 and 2). The variety of *MD* protein structures reflects their range of different functions in plants.

Certain *MD* genes appeared to be specific either to trees or to the *Populus* lineage and absent from *Arabidopsis*. The prevalence of tandem duplications within the *MD* gene family, which apparently led to family expansion, may have created conditions conducive to gene neofunctionalization and rapid evolution (Choi et al., 2016; Schaper and Anisimova, 2015).

The majority of the poplar *MD* genes were found to be highly expressed in leaves, particularly those subjected to biotic and abiotic stress conditions (Fig. 5), supporting their role in stress signaling. Detailed analysis of expression in wood forming tissues revealed subsets upregulated in xylem cells during secondary wall deposition (Fig. 6). These genes, not unexpectedly, include candidates for the sensing of cell wall integrity. We identified their co-expression networks revealing potential molecular pathways in which these *MD* genes might participate to ensure the coordination of secondary wall formation (Table 1). This study provides a framework for future investigations aiming at elucidating stress and developmental signaling pathways operating in trees.

Materials And Methods

Identification of *P. trichocarpa* proteins with malectin and malectin-like domains

The MD proteins of black cottonwood (*P. trichocarpa* Torr. & A. Gray) were identified by BLAST searches in the genome browser of the PopGenIE database (http://popgenie.org) containing *P. trichocarpa* genome assembly v3.0, using as baits the *P. trichocarpa* proteins containing Pfam domains 11721 and 12819, corresponding to MD and MLD respectively, retrieved from the Pfam database (https://pfam.xfam.org) (El-Gebali et al., 2019). The same approach was applied to *A. thaliana* using the TAIR database (v. 10.0) for BLAST searches (http://www.arabidopsis.org/). The presence of MDs/MLDs in the proteins selected for both *P. trichocarpa* and *A. thaliana* was confirmed using the CDvist web tool (http://cdvist.zhulinlab.org) (Adebali et al., 2015), which also served to identify other conserved domains in these proteins. The amino acid sequence lengths, molecular weights, isoelectric points and indices of protein stability of the putative proteins were calculated using the ProtParam tool provided on the ExPASy website (https://web.expasy.org/protparam/). The presence of signal peptides and subcellular localization were predicted with the SignalP 4.1 server (http:// www.cbs.dtu.dk/services/SignalP/) (Petersen et al. 2011) and DeepLoc-1.0 server (http://www.cbs.dtu.dk/services/DeepLoc/) (Armenteros et al. 2017) respectively. The exon-intron organization of the *PtMD* genes was determined using the PopGenIE GBrowse tool (http://popgenie.org/gbrowse) and their localization was mapped to *P. trichocarpa* chromosomes using the chromosome-diagram tool (http://popgenie.org/chromosome-diagram). Assignment of a gene to a gene cluster on each chromosome was based on the definition of Holub (2001).

Phylogenetic analysis and classification of the MD proteins of *P. trichocarpa*

All *Pt*MD proteins identified were classified into clades based on phylogenetic analysis with *A. thaliana*. The amino acid sequences were aligned by MUSCLE (http://phylogeny.lirmm.fr/phylo_cgi/index.cgi) and phylogenetic trees were constructed using the neighbor-joining (NJ) method in the MEGA7 software package with a bootstrap test with 1000 replicates (Kumar et al., 2016).

To identify the conserved residues in MD and MLD regions of poplar MD proteins, these regions were aligned with reference sequences using Jalview Version 2 (Waterhouse *et al*., 2009) with the MAFFT option (Katoh *et al*., 2005).

Expression of *PtMDs* in different organs of *Populus*

RNA-Seq datasets of expression values in different tissues/organs of outdoor and greenhouse grown aspen (*P. tremula* L.) and hybrid aspen (*P. tremula* L. x *tremuloides* Michx.) are available from the PlantGenIE website (Sundell et al., 2015), and those for secondary

tissues of greenhouse grown hybrid aspen are detailed by Immanen et al. (2016). The data retrieved were normalized and used for hierarchical clustering and determination of *tau* tissue/organ specificity scores as explained by Kumar et al., (2019). The customized R scripts used in analyzing these datasets are available at https://github.com/UPSCb/UPSCb/tree/master/manuscripts/Kumar2018 and the corresponding data are available from the FTP repository: ftp://ftp.plantgenie.org/Publications/Kumar2018. A heatmap was generated using ComplexHeatmap (Gu et al., 2016), an R (R Core Team, 2014) package designed for customizing heatmaps.

PtMDs involved in wood biosynthesis

The AspWood high-spatial-resolution RNA-Seq dataset (Sundell et al., 2017) was used for analysis of expression of *PtMDs* during wood biosynthesis. Identity of wood developmental zones was based on the expression of marker genes (Sundell et al., 2017). A heatmap of *PtMD* expression in wood developmental zones was constructed for one representative tree (tree 1) using the AspWood server (http://aspwood.popgenie.org/aspwood-v3.0/).

Co-expression analysis

Ten *PtMD* genes showing maximum expression in the CA-RE/PW-SW zone, and eight genes with maximum expression in either the PW-SW/secondary wall zone or the secondary wall zone (Figure 6; Table S6) were used as 'Guide Genes' to obtain co-expression networks for developing secondary tissues, using the AspWood website (http://aspwood.popgenie.org/aspwood-v3.0/). The corresponding GraphML files were generated using the ExNet tool (http://popgenie.org/exnet) with a Z-score threshold of 5.0, and visualized using Cytoscape 3.4.0 (Shannon et al., 2003).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

VK identified MD-genes and their chromosomal clustering, and wrote the first draft; VK and FB identified protein domains and the main clades of MD-genes; VK and SK analyzed exon-intron structures; VK and END analyzed gene expression; END, JU and VK analyzed conserved regions in MD and MLD of poplar; VK and FB analyzed co-expression networks; EJM conceived and coordinated the project, and finalized the manuscript with contributions from all authors.

References

1. Adebali O, Ortega DR, Zhulin IB. CDvist: a webserver for identification and visualization of conserved domains in protein sequences. Bioinformatics. 2015;31:1475-7.
2. Armenteros JJA, Sønderby CK, Sønderby SK, Nielsen H, Winther O. DeepLoc: prediction of protein subcellular localization using deep learning. Bioinformatics. 2017;33:3387-95.

3. Antignani V, Klocko AL, Bak G, Chandrasekaran SD, Dunivin T, Nielsen E. Recruitment of PLANT U-BOX13 and the PI4Kβ1/β2 phosphatidylinositol-4 kinases by the small GTPase RabA4B plays important roles during salicylic acid-mediated plant defense signaling in Arabidopsis. Plant Cell. 2015;27:243-61.
4. Asai T, Tena G, Plotnikova J, Willmann MR, Chiu W-L, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J. MAP kinase signalling cascade in *Arabidopsis* innate immunity. Nature 2002;415:977-83
5. Bella J, Hindle KL, McEwan PA, Lovell SC. The leucine-rich repeat structure. Cell Mol Life Sci. 2008;65:2307-33.
6. Bellande K, Bono JJ, Savelli B, Jamet E, Canut H. Plant lectins and lectin receptor-like kinases: How do they sense the outside? Int J Mol Sci. 2017;18:1164.
7. Bi G, Zhou Z, Wang W, Li L, Rao S, Wu Y, Zhang X, Menke FLH, Chen S, Zhou JM. Receptor-like cytoplasmic kinases directly link diverse pattern recognition receptors to the activation of mitogen-activated protein kinase cascades in *Arabidopsis*. Plant Cell. 2018;30:1543-61.
8. Boisson-Dernier A, Lituiev DS, Nestorova A, Franck CM, Thirugnanarajah S, Grossniklaus U. ANXUR receptor-like kinases coordinate cell wall integrity with growth at the pollen tube tip via NADPH oxidases. PLOS Biol. 2013;11:e1001719.
9. Boisson-Dernier A, Franck CM, Lituiev DS, Grossniklaus U. Receptor-like cytoplasmic kinase MARIS functions downstream of CrRLK1L-dependent signaling during tip growth. Proc Natl Acad Sci USA. 2015;112:12211–16
10. Campbell L, Turner SR. A comprehensive analysis of RALF proteins in green plants suggests there are two distinct functional groups. Front Plant Sci. 2017;8:37.
11. Chandler JW, Cole M, Flier A, Werr W. BIM1, a bHLH protein involved in brassinosteroid signalling, controls Arabidopsis embryonic patterning via interaction with DORNROSCHEN and DORNROSCHEN-LIKE. Plant Mol Biol. 2009;69:57-68.
12. Chen W, Lv M, Wang Y, Wang PA, Cui Y, Li M, Wang R, Gou X, Li J. BES1 is activated by EMS1-TPD1-SERK1/2-mediated signaling to control tapetum development in *Arabidopsis thaliana*. Nat Commun. 2019;10:4164.
13. Choi K, Reinhard C, Serra H, Ziolkowski PA, Underwood CJ, Zhao X, Hardcastle TJ, Yelina NE, Griffin C, Jackson M, Mézard C, McVean G, Copenhaver GP, Henderson IR. Recombination rate heterogeneity within *Arabidopsis* disease resistance genes. PloS Genet. 2016;12:e1006179.
14. Crawford BC, Sewell J, Golembeski G, Roshan C, Long JA, Yanofsky MF. Plant development. Genetic control of distal stem cell fate within root and embryonic meristems. Science. 2015;347:655-9.
15. Crooks GE, Hon G, Chandonia JM, Brenner SE. WebLogo: A sequence logo generator. Genome Res. 2004;14:1188-90.
16. Denness L, McKenna JF, Segonzac C, Wormit A, Madhou P, et al. Cell wall damage-induced lignin biosynthesis is regulated by a reactive oxygen species- and jasmonic acid-dependent process in Arabidopsis. Plant Physiol. 2011;156:1364-74
17. Draeger C, Ndinyanka Fabrice T, Gineau E, Mouille G, Kuhn BM, Moller I, Abdou MT, Frey B, Pauly M, Bacic A, Ringli C. *Arabidopsis* leucine-rich repeat extensin (LRX) proteins modify cell wall composition and influence plant growth. BMC Plant Biol. 2015;15:155.
18. Du C, Li X, Chen J, Chen W, Li B, et al. Receptor kinase complex transmits RALF peptide signal to inhibit root growth in *Arabidopsis*. Proc Natl Acad Sci USA. 2016;113:E8326-34
19. Du S, Qu LJ, Xiao J. Crystal structures of the extracellular domains of the CrRLK1L receptor-like kinases ANXUR1 and ANXUR2. Protein Sci. 2018;27:886-92.
20. Duan Q, Kita D, Li C, Cheung AY, Wu HM. FERONIA receptor-like kinase regulates RHO GTPase signaling of root hair development. Proc Natl Acad Sci USA. 2010;107:17821-6.
21. Dünser K, Gupta S, Herger A, Feraru MI, Ringli C, Kleine-Vehn J. Extracellular matrix sensing by FERONIA and Leucine-Rich Repeat Extensins controls vacuolar expansion during cellular elongation in *Arabidopsis thaliana*. EMBO J. 2019;38:e100353.
22. El-Gebali S, Mistry J, Bateman A, Eddy SR, Luciani A, Potter SC, Qureshi M, Richardson LJ, Salazar GA, Smart A, Sonnhammer ELL, Hirsh L, Paladin L, Piovesan D, Tosatto SCE, Finn RD. The Pfam protein families database in 2019. Nucleic Acids Res. 2019;47(D1):D427-D432.
23. Engelsdorf T, Hamann T. An update on receptor-like kinase involvement in the maintenance of plant cell wall integrity. Ann Bot. 2014;114:1339-47.
24. Escobar-Restrepo J-M, Huck N, Kessler S, Gagliardini V, Gheyselinck J, et al. The FERONIA receptor-like kinase mediates male-female interactions during pollen tube reception. Science 2007;317:656-60
25. Feng W, Kita D, Peaucelle A, Cartwright HN, Doan V, Duan Q, Liu M-C, Maman J, Steinhorst L, Schmitz-Thom I, Yvon R, Kudla J, Wu H-M, Cheung AY, Dinneny JR. The FERONIA Receptor Kinase Maintains Cell-Wall Integrity during Salt Stress through Ca^{+2} Signaling.

Curr Biol. 2018;28: 666-75

26. Fisher K, Turner S. PXY, a receptor-like kinase essential for maintaining polarity during plant vascular-tissue development. Curr Biol. 2007;17:1061-6.
27. Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, et al. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 2003;422:442-6
28. Franck CM, Westermann J, Boisson-Dernier A. Plant malectin-like receptor kinases: from cell wall integrity to immunity and beyond. Ann Rev of Plant Biol 2018;69:301-28.
29. Galindo-Trigo S, Gray JE, Smith LM. Conserved roles of CRRLK1L receptor-like kinases in cell expansion and reproduction from algae to angiosperms. Front Plant Sci. 2016;7:1269.
30. Galindo-Trigo S, Grand TM, Voigt CA, Smith LM. A malectin domain kinesin functions in pollen and seed development in Arabidopsis. J Exp Bot 2020, *advance access* doi:10.1093/jxb/eraa023
31. Gatz C. From pioneers to team players: TGA transcription factors provide a molecular link between different stress pathways. Mol Plant Microbe Interact. 2013;26:151-9.
32. Ge Z, Bergonci T, Zhao Y, Zou Y, Du S, et al. *Arabidopsis* pollen tube integrity and sperm release are regulated by RALF-mediated signaling. Science. 2017;358:1596-600
33. Gish LA, Clark SE. The RLK/Pelle family of kinases. Plant J. 2011;66:117-27.
34. Gonneau M, Desprez T, Martin M, Doblas VG, Bacete L, Miart F, Sormani R, Hématy K, Renou J, Landrein B, Murphy E, Van De Cotte B, Vernhettes S, De Smet I, Höfte H. Receptor kinase THESEUS1 is a rapid alkalinization factor 34 receptor in *Arabidopsis*. Curr Biol. 2018;28:2452-8.
35. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics. 2016;32: 2847-9.
36. Haga K, Frank L, Kimura T, Schwechheimer C, Sakai T. Roles of AGCVIII kinases in the hypocotyl phototropism of *Arabidopsis* seedlings. Plant Cell Physiol. 2018;59:1060-71.
37. Hamann T. The plant cell wall integrity maintenance mechanism–a case study of a cell wall plasma membrane signaling network. Phytochemistry. 2015;112:100-9.
38. Haruta M, Sabat G, Stecker K, Minkoff BB, Sussman MR. A peptide hormone and its receptor protein kinase regulate plant cell expansion. Science. 2014;343:408-11.
39. Hématy K, Sado PE, Van Tuinen A, Rochange S, Desnos T, Balzergue S, Pelletier S, Renou JP, Hofte H. A receptor-like kinase mediates the response of *Arabidopsis* cells to the inhibition of cellulose synthesis. Curr Biol. 2007;17: 922-31.
40. Holub EB. The arms race is ancient history in *Arabidopsis*, the wildflower. Nat Rev Genet. 2001;2:516-27.
41. Hou C, Tian W, Kleist T, He K, Garcia V, Bai F, Hao Y, Luan S, Li L. DUF221 proteins are a family of osmosensitive calcium-permeable cation channels conserved across eukaryotes. Cell Res. 2014;24:632-5.
42. Huang C, Zhang R, Gui J, Zhong Y, Li L. The receptor-like kinase AtVRLK1 regulates secondary cell wall thickening. Plant Physiol. 2018;177:671-83.
43. Immanen J, Nieminen K, Smolander OP, Kojima M, Alonso Serra J, Koskinen P, Zhang J, Elo A, Mähönen AP, Street N, Bhalerao RP, Paulin L, Auvinen P, Sakakibara H, Helariutta Y. Cytokinin and auxin display distinct but interconnected distribution and signaling profiles to stimulate cambial activity. Curr Biol. 2016;26:1990-7.
44. Jing XQ, Shalmani A, Zhou MR, Shi P-T, Muhammad I, Shi Y, Sharif R, Li W-Q, Liu W-T, Chen K-M. Genome-wide identification of malectin/malectin-like domain containing protein family genes in rice and their expression regulation under various hormones, abiotic stresses, and heavy metal treatments. J Plant Growth Regul. 2020;39:492–506.
45. Katoh K, Kuma K, Toh H, Miyata T. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 2005;33:511-8.
46. Kessler SA, Shimosato-Asano H, Keinath NF, Wuest SE, Ingram G, Panstruga R, Grossniklaus U. Conserved molecular components for pollen tube reception and fungal invasion. Science 2010;330: 968-71.
47. Khan M, Ragni L, Tabb P, Salasini BC, Chatfield S, Datla R, Lock J, Kuai X, Després C, Proveniers M, Yongguo C, Xiang D, Morin H, Rullière JP, Citerne S, Hepworth SR, Pautot V. Repression of lateral organ boundary genes by pennywise and pound-foolish is essential for meristem maintenance and flowering in *Arabidopsis*. Plant Physiol. 2015;169:2166-86.

48. Kong L, Cheng J, Zhu Y, Ding Y, Meng J, Chen Z, Xie Q, Guo Y, Li J, Yang S, Gong Z. Degradation of the ABA co-receptor ABI1 by PUB12/13 U-box E3 ligases. Nat Commun. 2015;6:8630.
49. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870-4.
50. Kumar V, Hainaut M, Delhomme N, Mannapperuma C, Immerzeel P, Street NR, Henrissat B, Mellerowicz EJ. Poplar carbohydrate-active enzymes: whole-genome annotation and functional analyses based on RNA expression data. Plant J. 2019;99:589-609.
51. Le MH., Cao, Y., Zhang, X. C., Stacey, G. LIK1, A CERK1-interacting kinase, regulates plant immune responses in *Arabidopsis*. PlosONE 2014;9: e102245.
52. Li C, Wu HM, Cheung AY. FERONIA and Her Pals: Functions and Mechanisms. Plant Physiol. 2016;171:2379-92.
53. Li N, Muthreich M, Huang LJ, Thurow C, Sun T, Zhang Y, Gatz C. TGACG-BINDING FACTORs (TGAs) and TGA-interacting CC-type modulate hyponastic growth in *Arabidopsis thaliana.* New Phytol. 2019;221:1906-18.
54. Liao D, Cao Y, Sun X, Espinoza C, Nguyen CT, Liang Y, Stacey G. Arabidopsis E3 ubiquitin ligase PLANT U-BOX13 (PUB13) regulates chitin receptor LYSIN MOTIF RECEPTOR KINASE5 (LYK5) protein abundance. New Phytol. 2017;214:1646-56.
55. Liebsch D, Sunaryo W, Holmlund M, Norberg M, Zhang J, Hall HC, Helizon H, Jin X, Helariutta Y, Nilsson O, Polle A, Fischer U. Class I KNOX transcription factors promote differentiation of cambial derivatives into xylem fibers in the Arabidopsis hypocotyl. Development. 2014;141:4311-9.
56. Liu J, Li W, Ning Y, Shirsekar G, Cai Y, Wang X, Dai L, Wang Z, Liu W, Wang GL. The U-Box E3 ligase SPL11/PUB13 is a convergence point of defense and flowering signaling in plants. Plant Physiol. 2012;160:28-37.
57. Liu J, Rice JH, Chen N, Baum TJ, Hewezi T. Synchronization of developmental processes and defense signaling by growth regulating transcription factors. PLoS ONE 2014;9: e98477.
58. Liu X, Wang J, Sun L. Structure of the hyperosmolality-gated calcium-permeable channel OSCA1.2. Nat Commun. 2018;9:5060.
59. Liu H, Xiong F, Duan C-Y, Wu Y-N, Zhang Y, Li Sha. Importin β4 mediates nuclear import of GRF-interacting factors to control ovule development in *Arabidopsis*. Plant Physiol 2019;179:1080-92.
60. Mang H, Feng B, Hu Z, Boisson-Dernier A, Franck C, et al. 2017. Differential regulation of two-tiered plant immunity and sexual reproduction by ANXUR receptor-like kinases. Plant Cell 29:3140-56
61. Mayfield JD, Folta KM, Paul AL, Ferl RJ. The 14-3-3 Proteins mu and upsilon influence transition to flowering and early phytochrome response. Plant Physiol. 2007;145:1692-702.
62. Meng J-G, Liang L, Jia P-F, Wang Y-C, Li H-J, Yang W-C. Integration of ovular signals and exocytosis of a Ca^{2+} channel by MLOs in pollen tube guidance. Nature Plants 2020;6:143-53.
63. Merz D, Richter J, Gonneau M, Sanchez-Rodriguez C, Eder T, et al. T-DNA alleles of the receptor kinase THESEUS1 with opposing effects on cell wall integrity signaling. J. Exp. Bot. 2017;68:4583–93
64. Miao Y, Laun T, Zimmermann P, Zentgraf U. Targets of the WRKY53 transcription factor and its role during leaf senescence in *Arabidopsis*. Plant Mol Biol. 2004;55:853-67.
65. Mineev KS, Bocharov EV, Pustovalova YE, Bocharova OV, Chupin VV, Arseniev AS. Spatial structure of the transmembrane domain heterodimer of ErbB1 and ErbB2 receptor tyrosine kinases. J Mol Biol. 2010;400:231-43.
66. Mori K, Renhu N, Naito M, Nakamura A, Shiba H, Yamamoto T, Suzaki T, Iida H, Miura K. Ca^{2+}-permeable mechanosensitive channels MCA1 and MCA2 mediate cold-induced cytosolic Ca^{2+} increase and cold tolerance in *Arabidopsis*. Sci Rep. 2018;8:550.
67. Moussu S, Augustin S, Roman A-O, Broyart C, Santiago J. Crystal structures of two tandem malectin-like receptor kinases involved in plant reproduction. Acta Cryst. 2018;D74, 671-80.
68. Omidbakhshfard MA, Fujikura U, Olas JJ, Xue GP, Balazadeh S, Mueller-Roeber B. GROWTH-REGULATING FACTOR 9 negatively regulates arabidopsis leaf growth by controlling ORG3 and restricting cell proliferation in leaf primordia. PLoS Genet. 2018;14:e1007484.
69. Pagnussat GC, Yu H-J, Ngo QA, Rajani S, Mayalagu S, Johnson CS, Capron A, Xie L-F, Ye D, Sundaresan V. Genetic and molecular identification of genes required for female gametophyte development and function in *Arabidopsis*. Development 2005;132: 603-14.
70. Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods. 2011;8:785-6.
71. Pinard D, Mizrachi E, Hefer CA, Kersting AR, Joubert F, Douglas CJ, Mansfield SD, Myburg AA. Comparative analysis of plant carbohydrate active enZymes and their role in xylogenesis. BMC Genomics. 2015;16:402.

72. Prunet N, Morel P, Champelovier P, Thierry AM, Negrutiu I, Jack T, Trehin C. SQUINT promotes stem cell homeostasis and floral meristem termination in *Arabidopsis* through APETALA2 and CLAVATA signalling. J Exp Bot. 2015;66:6905-16.
73. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2014;URL http://www.R-project.org/.
74. Rao S, Zhou Z, Miao P, Bi G, Hu M, Wu Y, Feng F, Zhang X, Zhou J-M. Roles of receptor-like cytoplasmic kinase VII members in pattern-triggered immune signaling. Plant Physiol. 2018;177:1679-90.
75. Ratke C, Terebieniec BK, Winestrand S, Derba-Maceluch M, Grahn T, Schiffthaler B, Ulvcrona T, Özparpucu M, Rüggeberg M, Lundqvist SO, Street NR, Jönsson LJ, Mellerowicz EJ. Downregulating aspen xylan biosynthetic GT43 genes in developing wood stimulates growth via reprograming of the transcriptome. New Phytol. 2018;219:230-45.
76. Rubinovich L, Weiss D. The *Arabidopsis* cysteine-rich protein GASA4 promotes GA responses and exhibits redox activity *in bacteria* and *in planta*. Plant J. 2010;64:1018-27.
77. Rui Y, Dinneny JR 2020. A wall with integrity: surveillance and maintenance of the plant cell wall under stress. New Phytol. 2020;225:1428-39
78. Schallus T, Jaeckh C, Fehér K, Palma AS, Liu Y, Simpson JC, Mackeen M, Stier G, Gibson TJ, Feizi T, Pieler T, Muhle-Goll C. Malectin: a novel carbohydrate-binding protein of the endoplasmic reticulum and a candidate player in the early steps of protein N-glycosylation. Mol Biol Cell. 2008;19:3404-14.
79. Schallus T, Fehér K, Sternberg U, Rybin V, Muhle-Goll C. Analysis of the specific interactions between the lectin domain of malectin and diglucosides. Glycobiology. 2010;20:1010-20.
80. Schaper E, Anisimova M. The evolution and function of protein tandem repeats in plants. New Phytol. 2015;206:397-410.
81. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498-504.
82. Shiu SH, Bleecker AB. Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. Proc Natl Acad Sci U S A. 2001;98:10763-8.
83. Shiu SH, Bleecker AB. Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in *Arabidopsis*. Plant Physiol. 2003;132:530-43.
84. Smith MR, Willmann MR, Wu G, Berardini TZ, Möller B, Weijers D, Poethig RS. Cyclophilin 40 is required for microRNA activity in Arabidopsis. Proc Natl Acad Sci USA. 2009;106:5424-9.
85. Song H, Guo Z, Chen T, Sun J, Yang G. Genome-wide identification of LRR-containing sequences and the response of these sequences to nematode infection in *Arachis duranensis*. BMC Plant Biol. 2018;18:279.
86. Stegmann M, Monaghan J, Smakowska-Luzan E, Rovenich H, Lehner A, Holton N, Belkhadir Y, Zipfel C. The receptor kinase FER is a RALF-regulated scaffold controlling plant immune signaling. Science. 2017;355:287-9.
87. Sultana MM, Hachiya T, Dutta AK, Nishimura K, Suzuki T, Tanaka A, Nakagawa T. Expression analysis of genes encoding malectin-like domain (MLD)- and leucine-rich repeat (LRR)- containing proteins in *Arabidopsis thaliana*. Bioscience, Biotechnology, and Biochemistry. 2019;doi:10.1080/09168451.2019.1661769.
88. Sundell D, Mannapperuma C, Netotea S, Delhomme N, Lin YC, Sjödin A, Van de Peer Y, Jansson S, Hvidsten TR, Street NR. The plant genome integrative explorer resource: PlantGenIE.org. New Phytol. 2015;208:1149-56.
89. Sundell D, Street NR, Kumar M, Mellerowicz EJ, Kucukoglu M, Johnsson C, Kumar V, Mannapperuma C, Delhomme N, Nilsson O, Tuominen H, Pesquet E, Fischer U, Niittylä T, Sundberg B, Hvidsten TR. AspWood: high-spatial-resolution transcriptome profiles reveal uncharacterized modularity of wood formation in *Populus tremula*. Plant Cell. 2017;29:1585-604.
90. Swarup K, Benková E, Swarup R, Casimiro I, Péret B, Yang Y, Parry G, Nielsen E, De Smet I, Vanneste S, et al. The auxin influx carrier LAX3 promotes lateral root emergence. Nature Cell Biol 2008;10: 946-54.
91. Thole JM, Vermeer JE, Zhang Y, Gadella TW Jr, Nielsen E. Root hair defective4 encodes a phosphatidylinositol-4-phosphate phosphatase required for proper root hair development in *Arabidopsis thaliana*. Plant Cell. 2008;20:381-95.
92. Voxeur A, Höfte H. Cell wall integrity signaling in plants: "To grow or not to grow that's the question". Glycobiology. 2016;26:950-60.
93. Wang G, Ellendorff U, Kemp B, Mansfield JW, Forsyth A, Mitchell K, Bastas K, Liu CM, Woods-Tör A, Zipfel C, de Wit PJ, Jones JD, Tör M, Thomma BP. A genome-wide functional investigation into the roles of receptor-like proteins in *Arabidopsis*. Plant Physiol. 2008;147:503-17.

94. Wang J, Kucukoglu M, Zhang L, Chen P, Decker D, Nilsson O, Jones B, Sandberg G, Zheng B. The Arabidopsis LRR-RLK, PXC1, is a regulator of secondary wall formation correlated with the TDIF-PXY/TDR-WOX4 signaling pathway. BMC Plant Biol. 2013;13:94.
95. Wang J, Hu T, Wang W, Hu H, Wei Q and Bao C. Investigation of evolutionary and expressional relationships in the function of the leucine-rich repeat receptor-like protein kinase gene family (LRR-RLK) in the radish. Sci Rep 2019a; 9:6937.
96. Wang Y, Salasini BC, Khan M, Devi B, Bush M, Subramaniam R, Hepworth SR. Clade I TGACG-motif binding basic leucine zipper transcription factors mediate BLADE-ON-PETIOLE-dependent regulation of development. Plant Physiol. 2019b;180:937-951.
97. Wolf S, Höfte H. Growth control: a saga of cell walls, ROS, and peptide receptors. Plant Cell. 2014;26:1848-56.
98. Wolf S. Plant cell wall signalling and receptor-like kinases. Biochem J. 2017;474:471-92.
99. Won S-K, Lee Y-J, Lee H-Y, Heo Y-K, Cho M, Cho H-T. Cis-element- and transcriptome-based screening of root hair-specific genes and their functional characterization in *Arabidopsis*. Plant Physiol. 2009;150:1459-73
100. Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ. Jalview Version 2 - a multiple sequence alignment editor and analysis workbench. Bioinformatics. 2009;25:1189-91.
101. Xi J, Qiu Y, Du L, Poovaiah BW. Plant-specific trihelix transcription factor AtGT2L interacts with calcium/calmodulin and responds to cold and salt stresses. Plant Sci. 2012;185-186:274-80.
102. Xi L, Wu XN, Gilbert M, Schulze WX. Classification and interactions of LRR receptors and co-receptors within the *Arabidopsis* plasma membrane - An overview. Front Plant Sci. 2019;10:472.
103. Xiao Y, Stegmann M, Han Z, DeFalco TA, Parys K, Xu L, Belkhadir Y, Zipfel C, Chai J. Mechanisms of RALF peptide perception by a heterotypic receptor complex. Nature. 2019;572:270-4.
104. Yang Y, Labbé J, Muchero W, Yang X, Jawdy SS, Kennedy M, Johnson J, Sreedasyam A, Schmutz J, Tuskan GA, Chen JG. Genome-wide analysis of lectin receptor-like kinases in Populus. BMC Genomics. 2016;17:699.
105. Yeh Y-H, Panzeri D, Kadota Y, Huang Y-C, Huang P-Y, Tao C-N, Roux M, Chien H-C, Chin T-C, Chu P-W, Zipfel C, Zimmerli L. The *Arabidopsis* Malectin-Like/LRR-RLK IOS1 is critical for BAK1-dependent and BAK1-independent pattern-triggered immunity. Plant Cell. 2016;28:1701-21.
106. Yuan N, Yuan S, Li Z, Zhou M, Wu P, Hu Q, Mendu V, Wang L, Luo H. STRESS INDUCED FACTOR 2, a leucine-rich repeat kinase regulates basal plant pathogen defense. Plant Physiol 2018;176: 3062-80.
107. Zan Y1, Ji Y, Zhang Y, Yang S, Song Y, Wang J. Genome-wide identification, characterization and expression analysis of *Populus* leucine-rich repeat receptor-like protein kinase genes. BMC Genomics. 2013;14:318.
108. Zhang Q, Jia MR, Xing Y, Qin L, Li BB, Jia WS. Genome-wide identification and expression analysis of MRLK family genes associated with strawberry (*Fragaria vesca*) fruit ripening and abiotic stress responses. PLoS ONE 2016;11:e0163647.
109. Zhong R, Ye ZH. MYB46 and MYB83 bind to the SMRE sites and directly activate a suite of transcription factors and secondary wall biosynthetic genes. Plant Cell Physiol. 2012;53:368-80.
110. Zhou J, Liu D, Wang P, Ma X, Lin W, Chen S, Mishev K, Lu D, Kumar R, Vanhoutte I, Meng X, He P, Russinova E, Shan L. Regulation of *Arabidopsis* brassinosteroid receptor BRI1 endocytosis and degradation by plant U-box PUB12/PUB13-mediated ubiquitination. Proc Natl Acad Sci USA. 2018;115:E1906-15.
111. Zulawski M, Schulze G, Braginets R, Hartmann S, Schulze WX. The Arabidopsis Kinome: phylogeny and evolutionary insights into functional diversification. BMC Genomics. 2014;15:548.

Tables

Table 1. Genes co-regulated with poplar *MD* genes expressed during secondary wall formation that were discussed in the text. All genes from network analyses are listed in Tables S7 and S8.

First neighbors	Poplar name	Best BLAST AGI codes	Ath-names	Baits							Ath short description	Pathway/process
				MD126 (Potri.006G110000)	MD94 (Potri.010G213200)	MD98 (Potri.001G405500)	MD88 (Potri.002G242700)	MD129 (Potri.008G105500)	MD137 (Potri.010G090800)	MD110 (Potri.001G467000)		
Potri.014G052700		AT5G47070	PBL19	+							PBS1-LIKE 19 - a RLCK phosphorylating MAPKKK5 and MEKK1 in response to chitin	PAMP, ROS, P, JA, and BR signaling, BOP1/2
Potri.002G090700		AT5G65210	TGA1	+							TGA-BINDING 1 - a bZIP TF, a redox-controlled regulator of SAR and development	PAMP, ROS, P, JA, and BR signaling, BOP1/2
Potri.005G170500		AT1G77920	TGA7	–							TGA-BINDING 7 - a bZIP TF, a redox-controlled regulator of SAR and development	PAMP, ROS, P, JA, and BR signaling, BOP1/2
Potri.004G213300		AT2G27990	BLH8	+							Bel1-like TF, regulating BOP1 and integrating stress signaling via JA	PAMP, ROS, P, JA, and BR signaling, BOP1/2
Potri.001G392200		AT2G42590	GRF9	+							GROWTH-REGULATING FACTOR 9, 14-3-3 gene. Binds Ca+2 and regulates development.	$Ca^{2+}$and P signaling and regulation
Potri.010G169800		AT4G27640	IMB4	+							IMPORTIN-b 4 transporting GRF-interacting factor 1 (GIF1) to nucleus	$Ca^{2+}$and P signaling and regulation
Potri.009G029600		AT3G46510	PUB13		+						PLANT U-BOX 13, an E3 ubiquitin ligase involved in ubiquitination of receptor FLS2.	PAMP signaling
Potri.005G100500		AT3G51460	RHD4		+						ROOT HAIR DEFECTIVE4, a phosphatidylinositol-4-P phosphatase required by root hairs	P signaling and regulation
Potri.015G108700		AT5G61820			+						Stress up-regulated Nod 19 protein;	$Ca^{2+}$and P signaling and regulation
Potri.017G059500		AT4G13950	RALFL31		+						RAPID ALKALINIZATION FACTOR LIKE 31 - peptide hormone	*Cr*RLK1L - mediated signaling
Potri.005G174000		AT1G77690	LAX3			+					Auxin influx carrier LAX3 (Like Aux1)	Auxin signaling
Potri.010G236200		AT3G44610	AGC1-12			+					Kinase involved in phototropism and gravitropism. Phosphorylates PIN1	Auxin signaling
Potri.017G083000		AT5G15230	GASA4			+					Encodes GA-regulated protein GASA4. Promotes GA responses and exhibits redox activity.	GA signaling
Potri.006G117200		AT2G36570	PXC1			+					Leucine-rich repeat protein kinase family protein	Xylogenesis and SW formation
Potri.001G057800		AT1G67310				–					Calmodulin-binding TF	Ca^{2+}-related signaling
Potri.001G126100		AT5G61480	PXY				+				PHLOEM INTERCALATED WITH XYLEM -a LRR-	Xylogenesis and SW formation

											RLK, receptor of TDIF regulating xylem cell fate	
Potri.006G114400		AT1G79620	VRLK1				+				VASCULAR-RELATED RLK1 - a LRR kinase regulating onset of secondary cell wall thickening.	Xylogenesis and SW formation
Potri.009G053900	MYB021	AT5G12870	MYB46					+			Master secondary wall TF MYB46	Xylogenesis and SW formation
Potri.001G018900		AT1G51220	WIP5					+	+		WIP domain 5. Target of WRKY53, involved cell fate determination in response to auxin via MP.	Auxin signaling
Potri.008G105600		AT4G24972	TPD1					+			TAPETUM DETERMINANT 1, peptide hormone perceived by EMS1-SERK1	P signaling and regulation
Potri.001G267300	MYB3	AT3G08500	MYB83					+			Master secondary wall TF MYB83	Xylogenesis and SW formation
Potri.016G104400		AT5G02010	ROPGEF7						+		ROP (RHO OF PLANTS) GUANINE NUCLEOTIDE EXCHANGE FACTOR 7	*Cr*RLK1L - mediated signaling
Potri.005G135500		AT2G15790	SQN						-		SQUINT - homolog of cyclophilin 40, involved in miRNA regulation	miRNA regulation
Potri.004G005900		AT4G22120	CSC1						+		CALCIUM PERMEABLE STRESS-GATED CATION CHANNEL 1- stretch activated cation channel.	Ca^{2+}-related signaling
Potri.005G051700		AT5G28300	GT2L					-			GT-2LIKE PROTEIN - a CaM-binding protein involved in cold stress signaling	Transcriptional regulation
Potri.001G295100		AT3G19590	BUB3.1					-			BUDDING UNINHIBITED BY BENZYMIDAZOL 3.1. - spindle assembly.	Cell division
Potri.001G394200		AT4G20010	PTAC9					-			PLASTID TRANSCRIPTIONALLY ACTIVE 9- a single-stranded DNA binding protein.	Cell division
Potri.013G079600		AT5G16750	TOZ					-			TORMOZ -rRNA processing required for cell division	Cell division
Potri.015G048000		AT5G08130	BIM1							+	BES1-INTERACTING MYC-LIKE 1-a BHLH TF involved in brassinosteroid signalig	BR signaling

Figures

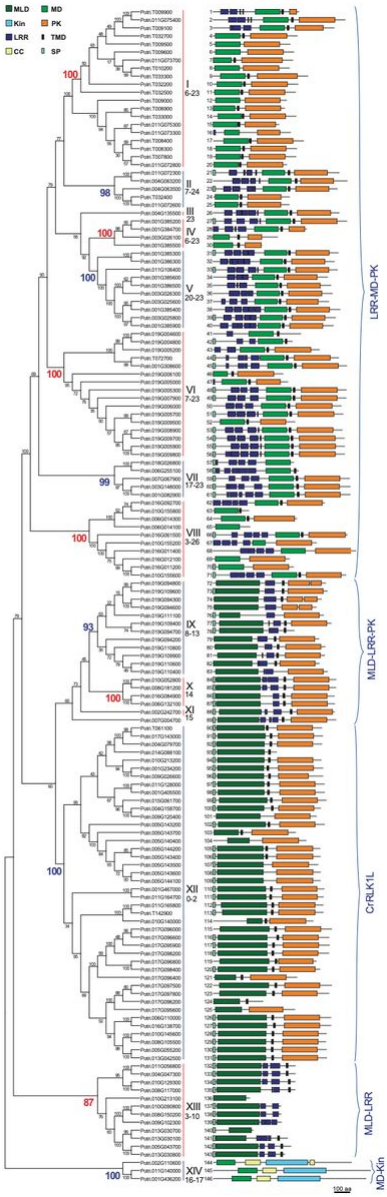

Figure 1

Phylogenetic tree of poplar malectin/malectin-like domain-containing proteins (PtMDs) showing their domain structures. The tree was constructed from MUSCLE-aligned amino acid sequences using the neighbor-joining method in MEGA 7.0 with 1000 bootstrap replicates and bootstrap support is displayed beside the nodes as percentages. PtMDs are identified by the number shown next to each protein structure. Domain abbreviations are: CC – coiled coil, Kin – kinesin, LRR – leucine-rich repeat, MD – malectin domain, MLD – malectin-like domain, PK – protein kinase, SP – signal peptide and TMD - transmembrane domain. Main clades are numbered with Roman numerals and their corresponding bootstrap values are colored in the phylogenetic tree. Numbers below the Roman numerals correspond to the number of introns observed within a clade. Five groups containing clades with similar protein domain structures are identified by the blue brackets: LRR-MD-PK (also known as poplar LRR-RLK XIII; Zan et al., 2013), MLD-LRR-PK (known as poplar LRR-RLK I; Zan et al., 2013), CrRLK1L, MLD-LRR, and MD-Kin.

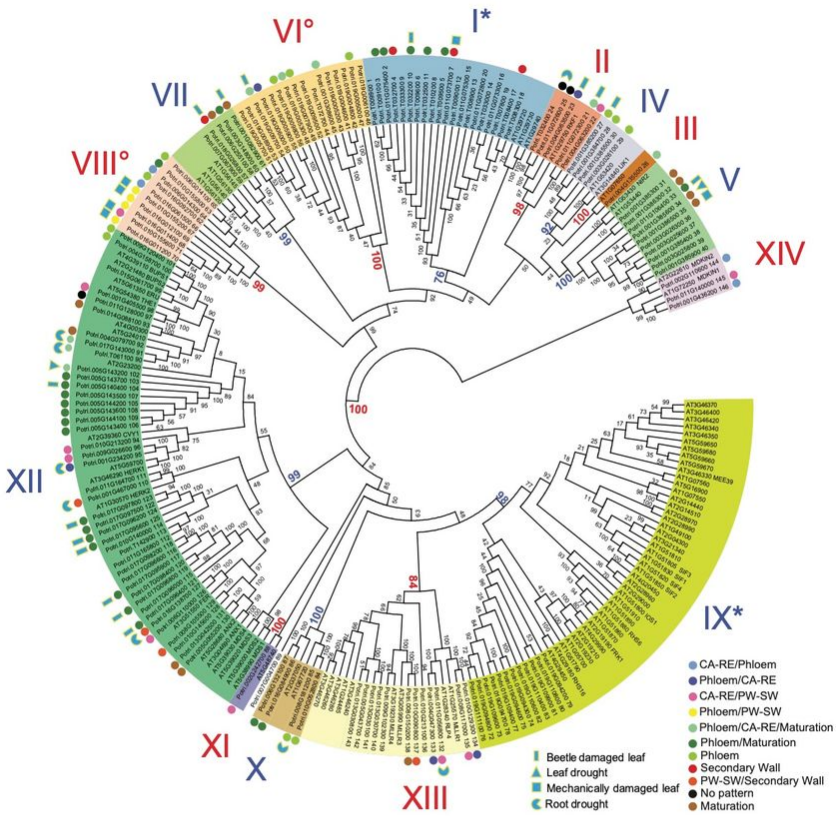

Figure 2

Phylogenetic tree of malectin/malectin-like domain-containing proteins in P. trichocarpa and A. thaliana. Each protein ID is followed by the name (A. thaliana) or PtMD protein number (P. trichocarpa). The phylogenetic tree was constructed based on MUSCLE-aligned amino acid sequences using the neighbor-joining method in MEGA 7.0 using 1000 bootstrap replicates, and the bootstrap support is displayed in percentages. Main clades are numbered with Roman numerals, and their supporting bootstrap values are shown in color. Colored dots beside PtMDs identify genes expressed in secondary vascular tissues based on the AspWood (http://aspwood.popgenie.org/aspwood-v3.0/) database and showing maximum expression in different developmental zones as indicated by colors. CA-RE - cambium-radial expansion zone, PW-SW - primary to secondary wall transition zone. Blue shapes with yellow outlines show stress-related expression based on the aspen expression atlas available at http://popgenie.org. Degree symbols and asterisks beside Roman numerals indicate clades that are represented by only one species or are significantly expanded in one species (χ^2 test, $P \leq 0.05$) respectively.

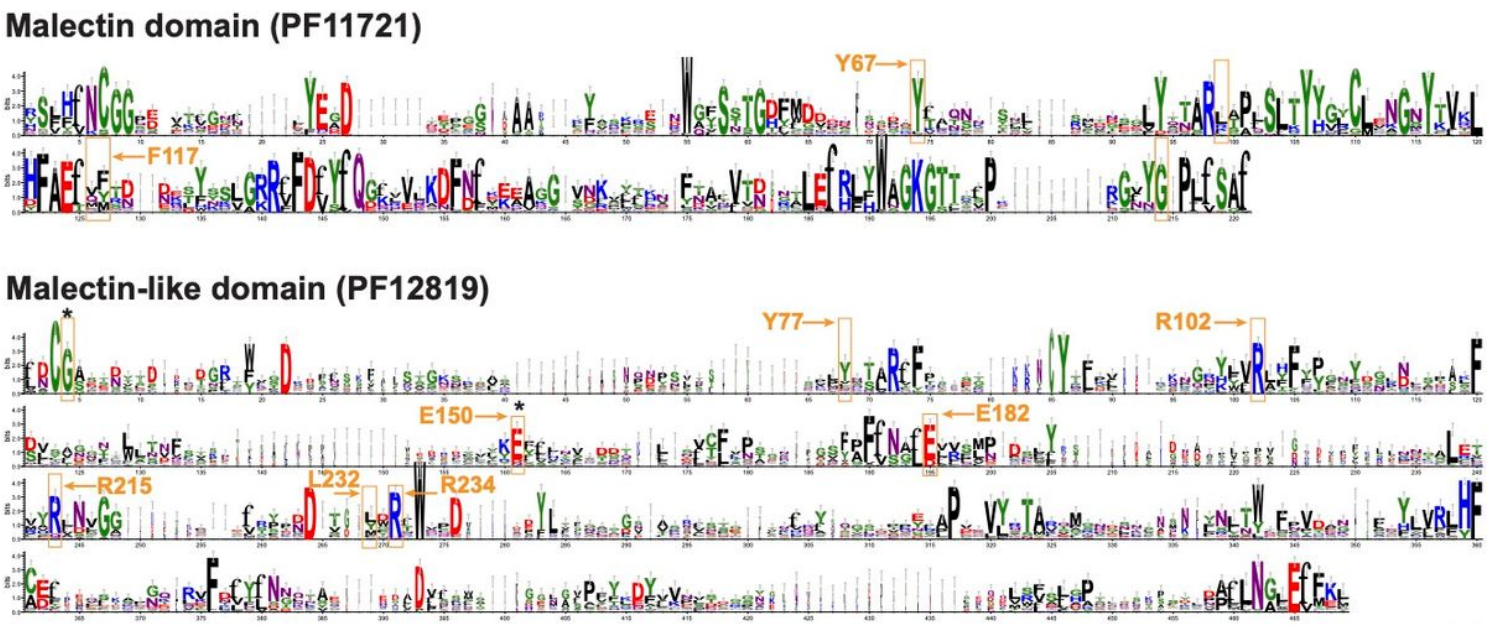

Figure 3

Conserved motifs in malectin (top) and malectin-like (bottom) domains of P. trichocarpa. The residues involved in interaction with ligands according to Schallus et al. (2008) and Moussu et al. (2018) are shown in orange boxes. The amino acids conserved in P. trichocarpa are marked by arrows. The mutated residues in the THE1 protein in the the1 mutants studied (Hématy et al., 2007) are indicated by black asterisks. The logos were created based on P. trichocarpa MD/MLD amino acid sequence alignments by using the WebLogo 3.7.4 online tool (http://weblogo.threeplusone.com, Crooks et al. 2004). The conservation of amino acid residues is represented in bits and residues are colored according to chemical properties. The associated multiple sequence alignments are shown in Supplementary Figures S1 and S2.

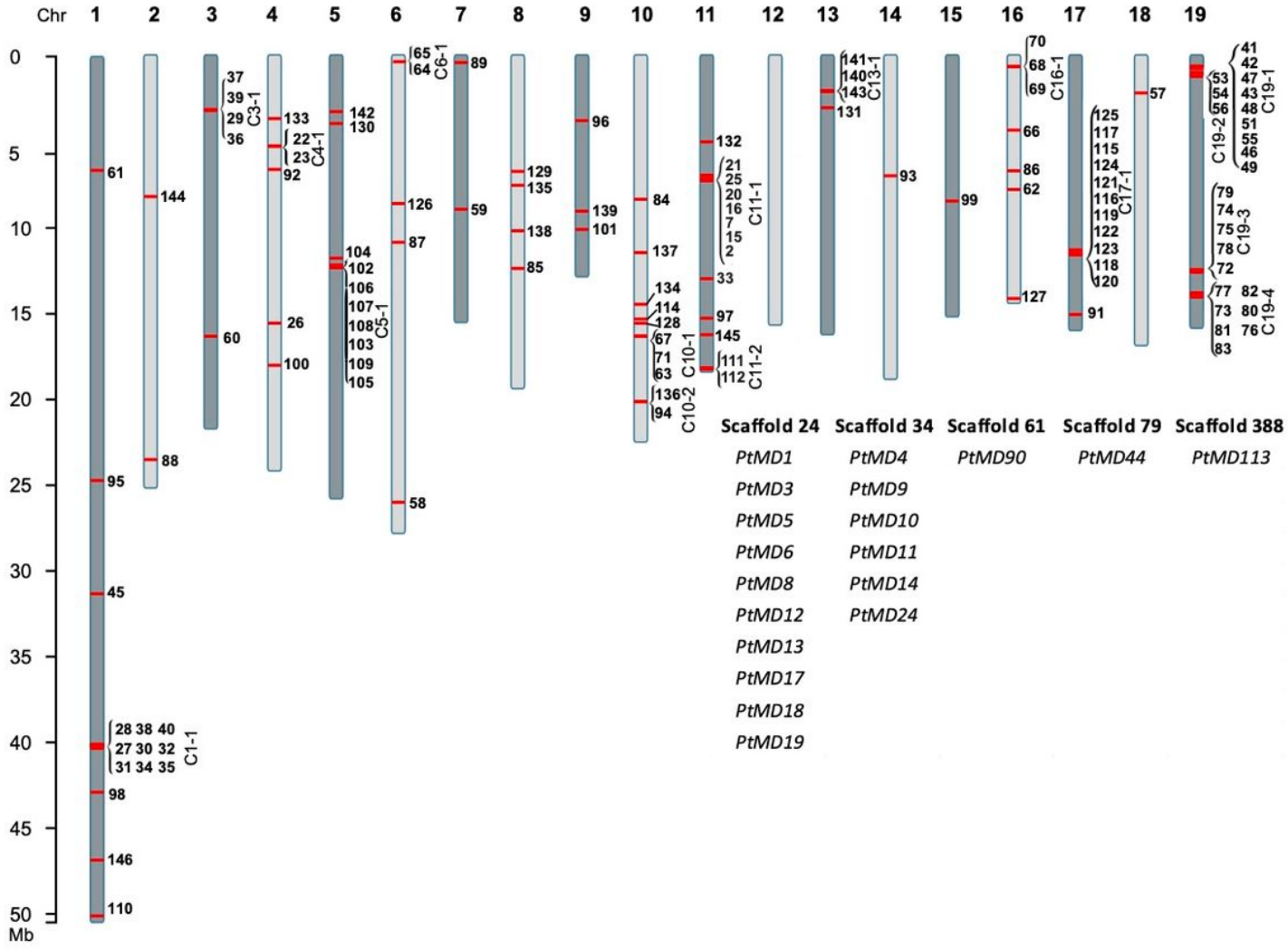

Figure 4

Chromosomal localization of the PtMD genes. The PtMD ID numbers are indicated beside chromosomes. Several PtMDs are found in clusters, shown by parentheses and labeled with a letter “C” followed by the chromosome number and the cluster number. Some PtMDs are on scaffolds, shown below the chromosomes.

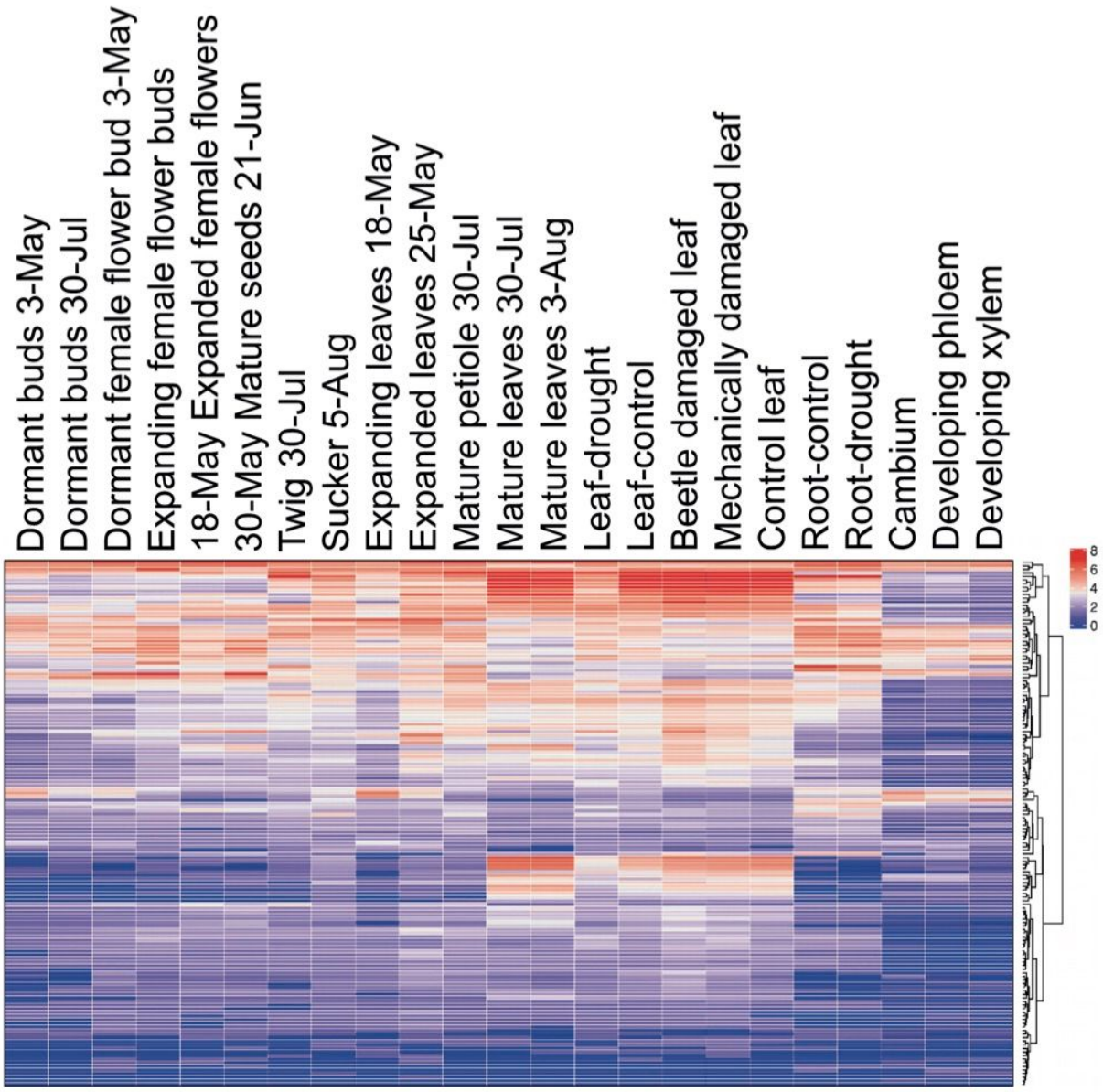

Figure 5

Heatmap of PtMD gene expression patterns in different organs of aspen. Data were retrieved from the aspen expression atlas available at http://popgenie.org and are listed in Table S5.

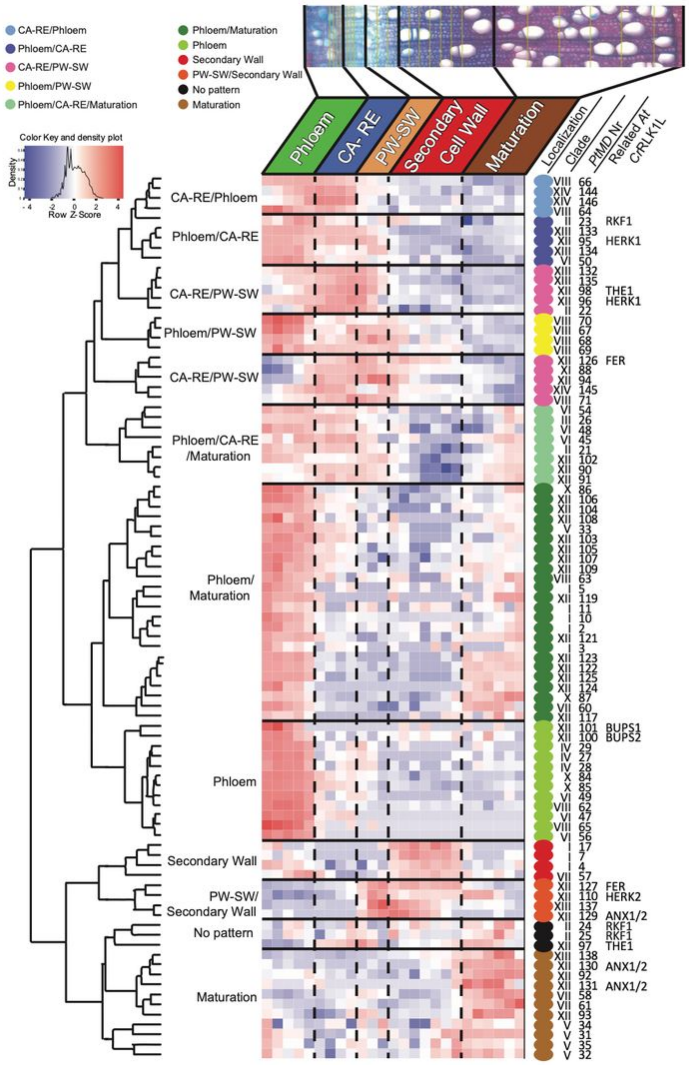

Figure 6

Heatmap of scaled PtMD expression patterns in developing secondary vascular tissues based on the AspWood database (http://aspwood.popgenie.org/aspwood-v3.0/). The majority of PtMD genes show maximum expression in the phloem and in the cambium-radial expansion zone (CA-RE). Smaller clusters of genes are expressed in the secondary wall formation zone, the transition between the primary and secondary wall zone (PW-SW), or the maturation zone. Specific wood developmental stages are defined based on the patterns of expression of marker genes (Sundell et al., 2017). Colored dots beside PtMDs identify groups with maximum expression in different developmental zones.

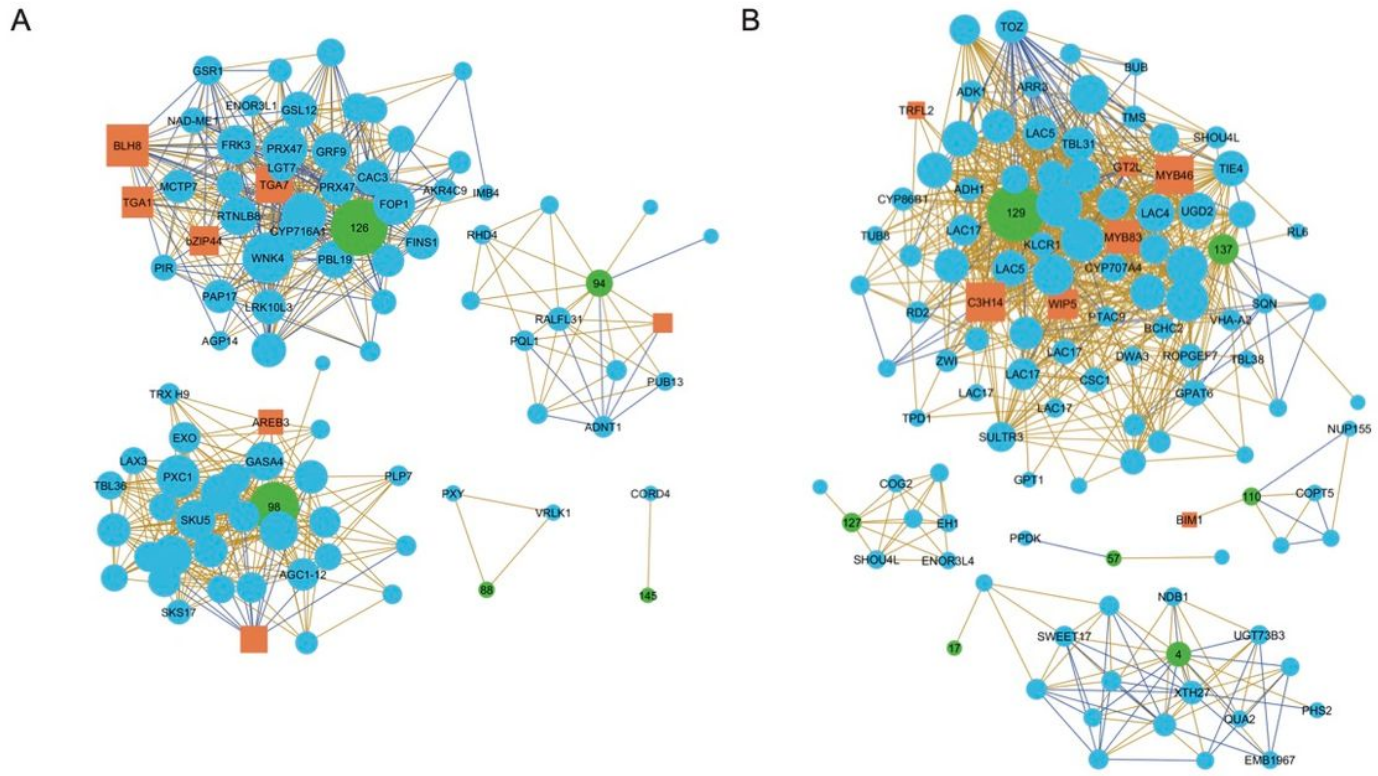

Figure 7

Co-expression networks of PtMDs in developing wood of aspen. The networks of genes expressed during the early (A) and main (B) stages of secondary wall deposition. The co-expressed genes were selected with threshold = 5 from the AspWood database (http://aspwood.popgenie.org/aspwood-v3.0/) and visualized in Cytoscape v 3.4.0. The transcription factors are marked by orange rectangles and the PtMD genes, used as baits, by green circles with PtMD ID numbers. PtMD22, 71, 96, 132 and 135 did not have any co-expressed genes (not shown). The corresponding Arabidopsis gene names are used in the figures. Positive and negative correlations are shown by beige and blue lines respectively. The size of each marker corresponds to the number of correlations associated with it.

Supplementary Files

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