INTRODUCTION

The majority of plant biomass consists of different cell wall polymers produced by living plant cells. In most cases, these polymers are energy-rich linked sugars that form the major structural components in plant cell walls, particularly in the thick secondary cell walls that characterize certain tissues. In addition to polysaccharides, another major cell wall polymer – lignin – limits access to cell wall sugars and negatively affects human utilization of biomass (livestock feed, paper manufacturing, and lignocellulosic biofuel production; Chapple et al., 2007). Because of its significant economic impact and central role in higher plant development, lignification is an important topic in plant biochemistry.

Despite a few exceptions (Martone et al., 2009), lignin is a phenolic biopolymer that is only detected in vascular plants. It is generated by radical coupling of hydroxycinnamyl alcohols named monolignols: coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol. When introduced in the lignin polymer the corresponding monolignols are named guaiacyl (G), sinapyl (S), and hydrogen-coumaryl (H) units, respectively. The biosynthesis of monolignols is initiated from the general phenylpropanoid pathway (Dixon et al., 2001). Although tyrosine was proposed to be the starting point of phenylpropanoid metabolism in some plants such as grasses (Nishi, 1961; Higuchi, 1990), it is generally recognized that monolignols are derived from phenylalanine via a series of enzymatic reactions, catalyzed by the following enzymes: phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate coenzyme A ligase (4CL), ferulate 5-hydroxylase (F5H), and producing substantial amounts of H units (Vanholme et al., 2009). The composition of lignin varies between plant species and tissues. In general, lignins from gymnosperms and related species are rich in G units and contains low amounts of H units, whereas dicot lignins are mainly composed of G and S units (Weng and Chapple, 2010). It is important to note that differences in monolignol content are controlled by key enzymes that are often limiting (e.g., FSH is limiting for S unit production; Weng and Chapple, 2010). Consequently, certain natural genotypes/varieties may show unusual proportions of monolignols as illustrated by the recent discovery of a Populus nigra variety containing a truncated HCT enzyme and producing substantial amounts of H units (Vanholme et al., 2013) that are usually almost completely absent in poplar lignin. Similarly, a loblolly pine naturally affected in cinnamyl alcohol dehydrogenase activity and showing modified lignin structure was also detected 20 years ago in a natural population (MacKay et al., 1995; Ralph et al., 1997).

From a developmental point of view, lignification is generally initiated during the formation of the secondary cell wall although cell/tissue-specific differences in the developmental pattern can occur. For example, lignification is initiated in the compound primary cell wall of xylem elements (middle lamella/cell corners) at the start of secondary cell wall formation (Donaldson, 2001; Fromm et al., 2003). In contrast, lignin may be deposited at later stages in the cell wall of middle lamella areas and in the corners of cells.
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Transport and compartmentation

Monolignols are synthesized in the cytoplasm and translocated to the cell wall for subsequent polymerization (Alejandro et al., 2012). For many years, the mechanism of transport remained unknown and different hypotheses were suggested (passive diffusion, exocytosis, active transport, etc.; Liu et al., 2011; Liu, 2012). An elegant biochemical study has recently demonstrated that the glycosylation status determines monolignol transport and subcellular compartmentation (Xiao and Liu, 2010). Plasma membrane-derived vesicles prepared from Arabidopsis and poplar cells transported coniferyl alcohol (the aglycone form), but not coniferin (the glycosylated form of coniferyl alcohol), in an ATP-dependent manner, whereas tonoplast-derived vesicles transported the glycosylated form but not the aglycone. Taken together these results would suggest that coniferyl alcohol is transported into the cell wall across the plasma membrane in an ATP-dependent process by an ABC-transporter and is subsequently polymerized by laccases (LACs) and/or peroxidases (PRXs). In contrast, the glycosylated form (e.g., coniferin) is transported into the vacuole for storage. Genetic confirmation of the involvement of ABC transporters in monolignol transport into the cell wall was recently reported (Alejandro et al., 2012). Co-expression studies in Arabidopsis identified an ABCG transporter gene (AtABCG29) co-regulated with phenylpropanoid gene expression and that was expressed in lignin-containing organs and tissues. Protein-fusion studies demonstrated that AtABCG29 was localized in the plasma membrane and transport studies in yeast suggested that the protein was involved in transporting p-coumaryl alcohol, but not coniferyl alcohol. A minor activity was also observed toward sinapyl alcohol. Abcg29 mutant lines showed reduced root growth when grown on medium containing p-coumaryl alcohol, but not coniferyl alcohol or sinapyl alcohol supporting the idea that AtABCG29 is involved in p-coumaryl alcohol transport. Lignin analyses of roots from two mutant lines revealed decreases in the amounts of H, G, and S subunits as compared to WT roots, an unexpected result since AtABCG29 is highly specific for p-coumaryl alcohol and it would be expected that only H units would be reduced. Interestingly, the mutant lines also showed metabolic modifications in soluble phenolics, flavonoids and glucosinolates together with reductions in associated gene expression. These modifications are reminiscent of changes observed in different lignin mutants (Vanholme et al., 2012b) underlining the fact that phenylpropanoid metabolism can be perturbed not only at the biosynthesis level, but also by modifications of monolignol transport. The results also raise the possibility that the observed cell-specific variation in lignin composition (e.g., vessel element walls are rich in G lignin whereas fiber walls contain S-G lignin) may also be regulated via specific ABC transporters. Plant genomes contain large ABC transporter gene families (e.g., 130 genes in Arabidopsis) but ABC transporters for coniferyl alcohol and sinapyl alcohol remain to be identified and characterized. In addition, it is probable that monolignol glucocones from the vacuole participate to tracheary element lignification following cell death and membrane disruption (Pesquet et al., 2013). Additional information on the potential role(s) of ABC transporters in the lignification process is discussed by Sibout and Hofte (2012).
FIGURE 1 | Schematic view of the monolignol biosynthetic pathway. The synthesis of monolignols from phenylalanine and shikimate involves cytosolic (PAL, HCT, 4CL, C4H, CAD, CCoAOMT, and COMT) and ER membrane-anchored (the cytochrome P450 enzymes F5H, C3H, and C4H) enzymes. Monolignols and lignans may be conjugated by UGTs and then transported to the vacuole or directly transported to the cell wall for oxidative cross-linking by apoplastic peroxidases and laccases into lignins. In grasses, some hydroxycinnamic acids are conjugated to arabinose by BAHD enzyme for export via the Golgi apparatus and future incorporation into polysaccharides (pectins or arabinoxylans) whereas some are conjugated to monolignols (mainly sinapyl alcohol) in the cytosol and incorporated into lignin. Other phenolic compounds (soluble phenolic esters and amides, flavonoids) are shown and may indirectly interact with lignification. Their production implies common steps (enzymes) with the monolignol pathway. Most of these transport routes and storage forms still remain to be discovered and the localization of some enzymes (GH, Perox, Lacc) confirmed. The dashed lines delineate putative pathways and full lines delineate known routes. Enzymes of the monolignol pathway are represented by circles. The brown color indicates involvement of the enzyme in the monolignol pathway; yellow indicates involvement in the flavonoid pathway; and pink indicates involvement in the sinapate ester pathway. PAL, phenylalanine ammonia lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-hydroxycinnamoyl-CoA ligase; HCT, hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase; C3H, p-coumaroyl shikimate 3′-hydroxylase; CCoAOMT, caffeic-CoA O-methyltransferase; CCR, caffeic acid/5-hydroxyferulic acid O-methyltransferase; COMT, catechol O-methyltransferase; F5H, ferulic acid 5-hydroxylase; COMT, caffeic acid/5-hydroxyferulic acid O-methyltransferase; CAD, cinnamyl alcohol dehydrogenase; UGT, UDP-glucosyltransferase; PH, beta-glucosidase; Perox, peroxidase; Lacc, laccase; DPs, dirigent proteins; BAHD, hydroxycinnamoyl acid transferase; ER, endoplasmic reticulum.
These enzymes play an important role in stabilization, enhancement of water solubility and deactivation/detoxification of a wide range of natural products including hormones and secondary metabolites (Jim and Bowles, 2004). A recent phylogenetic reconstruction (Caputi et al., 2012) of family 1 UGTs reveals a dramatic evolutionary increase in the number of family 1 GTs from one in the model green alga Chlamydomonas to 243 in poplar reflecting the increased complexity of plant life on Earth. Less than 20% plant UGTs have been functionally characterized (Sionskura-Sakulikura et al., 2012). A major problem is that plant GTs may be redundant. Functional analyses in Arabidopsis (Lanot et al., 2006) of the UGT72E2 clade suggested that the UGT72E2 gene product was responsible for monolignol glycosylation. Chemical analyses of soluble phenolics in light-grown roots from the triple mutant showed a significant decrease in the quantity of coniferol alcohol- and sinapyl alcohol-glucosides as compared to WT plants. Over-expression of the UGT72E2 gene resulted in a 10-fold increase in coniferin levels and a lower increase in sinapyl alcohol glucoside in roots suggesting that this is the principal gene involved in monolignol glycosylation in Arabidopsis. Interestingly, rosette leaves accumulated sinapyl glucoside to higher levels than coniferol alcohol glucoside hinting at organ-specific variations in glycosylation mechanisms. However, no impact on lignin content/composition was observed. Evidence suggesting a link between lignification and monolignol glycosylation was recently demonstrated (Wang et al., 2012) when the over-expression in tobacco plants of a poplar UGT (PtGT1) homolog to the Arabidopsis UGT72E1-3 genes was shown to be associated with an increased lignification as well as early flowering. Nevertheless, the observation that recombinant PtGT1 showed no activity toward monolignols and the fact that transformed plants did not contain higher amounts of monolignol glucosides led the authors to suggest that the observed impact of PtGT1 over-expression in tobacco was indirect. Further research is clearly needed to identify not only the UGTs specifically involved in monolignol glycosylation, but also to demonstrate a functional link between modifications in UGT gene expression and lignification. The potential importance of monolignol glycosylation in regulating lignin biosynthesis is also complicated by the fact that, in addition to glucose, plant UGTs can also use different sugars (glucose, rhamnose, galactose, galacturonic acid, etc.) to glycosylate monolignols (Caputi et al., 2012).

If monolignol glucosides give rise to monogalactans that are then incorporated into the lignin polymer, then enzymes (beta-glucosidases) capable of hydrolyzing monolignol glucosides were first identified in cell wall fractions of Picea abies hypocotyls and roots (Marciniowski and Gruszko, 1978). Subsequently, a beta-glucosidase showing high affinity for coniferin was co-localized with PXR activity to cell walls of young differentiating xylem in Pinus contorta (Burmeister and Hosel, 1981). The co-localization of beta-glucosidase activity and monolignol glucosides in differentiating xylem of conifers and some angiosperms led to the hypothesis that monolignol glucosides could represent lignin precursors. However, subsequent work in Ginkgo biloba (Tsuji et al., 2005) showing that a beta-glucosidase demonstrated a 10 times greater affinity toward coniferaldehyde as compared to coniferin threw doubt on this initial idea. Subsequent work with radioactive precursors indicated that coniferol alcohol derived from coniferin was only weakly incorporated into the lignin polymer and that greater amounts were converted into coniferaldehyde glucoside followed by subsequent deglycosylation and CAD-mediated conversion of the coniferaldehyde into coniferol alcohol. These results suggested that coniferin represented the storage form of coniferol alcohol and was not the direct precursor of coniferol alcohol for lignin. In Arabidopsis, three orthologs (BGLU45/At1g61810; BGLU46/At1g61820; BGLU47/At1g21760) of the Pinus contorta beta-glucosidase gene are present and belong to the group 10 of the Arabidopsis GH1 family (Xu et al., 2004). Heterologous expressions of BGLU45 and BGLU46 showed that BGLU45 is highly specific for the three monolignol glucosides, whereas BGLU46 preferentially uses pinocarvyl alcohol glucoside as well as showing broad activity toward other phenolic glucosides (Xu et al., 2004; Escamilla-Trevino et al., 2006). A recent functional study of Arabidopsis bglu45-2 and bglu46-2 T-DNA mutants (Chapelle et al., 2012) showed only slight reductions (8.5 and 6.5%, respectively) in global beta-glucosidase activity and protein amounts as estimated by Western blots following Concanavalin-A column chromatography, presumably because of functional redundancy—at least four other glucosidases are expressed in Arabidopsis stems—and antibody cross-reactivity. Lignin analyses showed a slight but significant increase in Klasson lignin for two BGLU45 mutants of the WS accession (bglu45-1 and bglu45-3) under long-day conditions only but not in Col bglu45 mutants. No significant modification in lignin content was observed for bglu46 mutants. Similarly, thioacidolysis revealed no changes in lignin subunit structure. Metabolomics (ultra performance liquid chromatography–mass spectrometry, UPLC–MS) identified around 100 different small glycosylated phenolic compounds, most of which were increased in the mutants. For the 10 most important differentials, five were identified/partially identified and all were derived from G units. Interestingly, an important ecotype effect was observed with, for example, an approximate 20-fold increase of coniferin in WS bglu45-1 mutants, but only a 3.4-fold increase in Col10 mutants. While coniferol alcohol, sinapyl alcohol, coniferaldehyde, sinapaldehyde, and syringin levels were not significantly modified, certain (neo)lignans increased [lariciresinol hexoside or isodiodehydrodiogenomethyl alcohol (IDDDC) hexoside, dehydrodiconiferyl alcohol (DDC) hexoside]. Based on these observations, the authors proposed that monolignol glucosides are the storage form of monogalactans and not the direct precursors. Nevertheless, it is interesting to note that BGLU45 and BGLU46 expression are deregulated under various biotic stresses suggesting that the monolignol storage pool might be used under pathogen attack in the fabrication of “defense lignin” and/or phytoalexins. Certainly, such a hypothesis allows a better understanding of the basic “spatial problem” associated with beta-glucosidase enzyme and substrate compartmentation (Figure 1). As indicated above, the Pinus contorta beta-glucosidase was localized to differentiating xylem walls and BGLU45/46 proteins are secreted to the cell wall (Chapelle et al., 2012), whereas glycosylated monogalactans are stored in the
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richest natural grain sources of certain lignans. The genus *Linum* (Onagraceae) seeds has been particularly studied since they are one of the major lignan producers. This species contains approximately 200 species worldwide that can be divided into two main groups depending upon the major lignan types produced: either *cinchona*- or *tubifera*-type lignans (Davin et al., 1997). (Note: two different nomenclatures are used to describe the same carbon atoms. A “β” indicates the carbon is involved in the β-lignin linkage). Lignans are optically active phenylpropanoid dimers that result from the stereo-selective dimerization of phenylpropanoid monomers (monolignols) in plants (Morant et al., 2008).

Taken together, these observations indicate that the glycosylation/deglycosylation of monolignols play key roles in determining their availability for subsequent lignin biosynthesis thereby representing potentially interesting engineering targets for plant biomass improvement. In addition, similar mechanisms are also likely to affect the availability of ferulic- and p-coumaric acids for lignin-herbicides. Hence, the balance between the monolignol glucosides and beta-glucosidases in the cell wall underlining the necessity for further research into the glycosylation/deglycosylation of monolignols and phenolic molecules.

**DIMERIZATION OF MONOLIGNOLS AND SYNTHESIS OF (NEO)LIGNANS**

Monolignols can also give rise to more than 3,000 different lignans and associated structures such as neo-, sesqui-, and flavanolignans (Schmidt et al., 2010). Lignans are optically active phenylpropanoid dimers that result from the stereo-selective dimerization of phenylpropanoids (monolignols) in plants (Davin et al., 1997). (Note: two different nomenclatures are used to describe the same carbon atoms. A “β” indicates the carbon is involved in the β-lignin linkage). Lignan formation has been widely studied since these molecules are natural anti-oxidants, possess anti-microbial activity and have numerous beneficial effects on human (Ford et al., 2001). Lignans are known to play a role in the durability, longevity, and resistance of the heartwood of many tree species against wood-rotting fungi and are believed to function as phytoalexins in other (non-woody) plant species (Pohjamo et al., 2003). The formation and accumulation of lignans in flax (*Linum usitatissimum*) L. seeds has been particularly studied since they are one of the richest natural grain sources of certain lignans. The genus *Linum* contains approximately 200 species worldwide that can be divided into two main groups depending upon the major lignan types accumulated – either *cinchona*- or *tubifera*-type lignans (Davin et al., 1997). Some plants in both groups can also accumulate dibenzyl-substituted *tropolone* (DBLP) lignans such as matairesinol and fururanol (FF) lignans such as pinosylvin. Both FF and DBBL lignans are precursors of the AT/ADN/AN lignans. In the seeds of flax, dimerization of two E-cinnamyl alcohol molecules leads to the formation of 85,8% (+)-pinoresinol, which is sequentially converted into 85,8S′-(-)-lariciresinol and 85,8S′-(+)-sclerosinol via the action of the enzyme pinoresinol lariciresinol reductase (PLR). (+)-Sclerosinol is then glycosylated to sclerosinol diglucoside (SDG) that accumulates to high levels in flax seeds (Hano et al., 2006, Hemmati et al., 2010; Venglat et al., 2011). In agreement with this organ-specific gene expression, 85,8S-SDG accumulates in flax seeds whereas aerial green parts accumulate 85,8R-SDG. Further analyses (Schmidt et al., 2012) of lignans in 16 different *Linum* species suggested that lignan stereochirality also depended upon the species analyzed and whereas 8R-SDG was mainly accumulated in the seeds from species containing relatively high amounts of ADN/AN cyclo-lignans, 8S-SDG was accumulated in seeds from species containing only trace amounts of cyclo-lignans.

A further insight into monolignol/lignan biology in flax was provided by a combined transcriptomics and metabolomics study (Huss et al., 2012) of flax inner- (xylem rich) and outer- (bark fiber rich) stem tissues (Figure 2). More than eighty different lignans and neolignans including pinosylvin, lariciresinol, secoisolariciresinol, DDC, and IDDC, as well as their glycosylated forms were detected in both tissues. In agreement with the presence of lignans and neolignans in the flax stem, transcripts corresponding to PLR, as well as the enzyme phenylcoumaran benzylic ether reductase (PCBER) catalyzing the reduction of DDC into IDDC and previously identified as the most abundant protein in poplar xylem (Gang et al., 1999; Vander Mijnsbrugge et al., 2008) were also highly abundant. Although PCBER transcripts were more highly abundant in inner stem tissues, IDDC was not detected in either inner- or outer-stem tissues, despite the presence of DCC in both tissues. In contrast IDCC hexosides were detected in both stem tissues suggesting that the neolignan is rapidly glycosylated. Overall, the glycosylated forms were more abundant in outer stem tissues in comparison with inner stem tissues. Further work in other species is necessary to know whether such differences in (neo)lignan glycochemistry are widespread and also the potential biological role. Interestingly, recent reports showing that lignans are detected in the phloem tissues (Ramos and Kato, 2012) and honey-dew of the stink-bug and that glycosylated flax lignans but not the aglycone forms significantly increased aphid *Myzus persicae* insect mortality (Saguez et al., 2012) raise the possibility that monolignol-derived compounds might be glycosylated and transported in phloem cells as a defense mechanism against insect predators.

The abundance of glycosylated (neo)lignans in flax and other species suggests the existence of different UGTs capable of glycosylating lignans. Lignan UGT activity was first identified in sesame (*Noguchi et al., 2008*) and more recently in Forsythia *Ono...*
FIGURE 2 | Monolignol metabolism in flax stems. Oligolignols were extracted from inner (IT) and outer (OT) tissues of flax stems (A) and analyzed by ultra-high performance liquid chromatography–Fourier transform–ion cyclotron resonance–mass spectrometry (UHPLC–FT–ICR–MS; Huis et al., 2012). Outer tissues are characterized by the presence of bast fibers with thick cellulose-rich secondary cell walls and extremely low lignin levels as indicated by limited red coloration with phloroglucinol-HCl staining (B). Inner tissues contain xylem tissue with heavily lignified cell walls (C). Aglycone and glycosylated forms of monolignols (1), lignans (2), and neolignans (3) were detected in both inner- and outer-stem samples. Glycosylated forms were generally more abundant in outer stems. DDC, dehydrodiconiferyl alcohol; IDDC, isodihydrodehydrodiconiferyl alcohol; PLR, pinoresinol lariciresinol reductase; PCBER, phenylcoumaran benzylic ether reductase. Red heat blocks indicate corresponding unigene expression in inner- versus outer-stem tissues. This figure illustrates the extremely rich metabolism post-monolignol biosynthesis.

(A) Courtesy of Anne-Sophie Blervacq, Université Lille 1.
Wang et al. Monolignol metabolism for engineering more efficient plant biomass. Of these mechanisms should lead to the elaboration of new targets for subsequent lignification. A better understanding of the mechanisms regulating the availability of synthesized monolignols in plant cells is currently lacking. It is clear that we have much to learn about the mechanisms involved in monolignol oxidation and participate in lignification (Figure 3). PRXs involved in lignification are exported to the apoplast in lignifying tissues where monolignol oxidation takes place (Koutaniemi et al., 2005; Vanholme et al., 2010). The oxidation reaction uses H$_2$O$_2$ as co-substrate which is probably provided by combined action of NADPH oxidase or germin-like proteins. Even though firm genetic evidence for the involvement of these enzymes is currently lacking (Davidson et al., 2009), PRX exist as large multigene families (73 and 138 in Arabidopsis and Oryza sativa, respectively; Welinder et al., 2002) making the clarification of the biological role of each member a considerable challenge. Clear characterization is also complicated by the fact that PRXs generally show low substrate specificities. Furthermore, recently studies suggesting that some PRX isoforms may be more specialized in the polymerization of guaiacyl or/and syringyl units could help to explain how plants are able to control lignin monomeric structure (Barcelo et al., 2007; Martinez-Cortes et al., 2012). Both the isoelectric point of the proteins and specific motifs in the PRX primary structure could support protein–substrate interaction with one monomer type over another and therefore influence lignin composition. Two examples of genetic manipulation illustrate this hypothesis. Antisense suppression of one PRX gene led to a global reduction of both G and S units in tobacco (Blee et al., 2003) whereas analyses of PRX down-regulated poplar (Li et al., 2003) revealed that lignin contained less G units but that S content was unchanged. More recently, Herrero et al. (2013a) used Zinnia elegans PRX protein sequence data to identify Arabidopsis orthologs for functional characterization. Histochemical analyses of the atprx72 mutant suggest that it has reduced overall lignin levels and a low syringyl unit content as compared to WT plants (Herrero et al., 2013b). However, the mutant shows a strong developmental phenotype and it would be necessary to confirm this result on a second mutant allele. In parallel, Shigeto et al. (2013) used similar strategies to functionally characterize other PRX gene candidates in

Beyond dimerization formation of lignins requires a polymerization step. The complex structure of the polymers built up of different subunits (H, G, and S), the presence of different types of linkages between subunits and the absence of repeated motifs suggest a highly unpredictable polymerization mechanism probably affected by its microenvironment (pH, polysaccharides, etc.; Wingender et al., 1999). In the developing cell wall, lignin polymerization occurs by radical coupling reactions (Freudenberg, 1959; Figures 3 and 4). Type III PRXs and LACs are probably both involved in monolignol oxidation and participate to lignification (Figure 3). PRXs involved in lignification are exported to the apoplast in lignifying tissues where monolignol oxidation takes place (Koutaniemi et al., 2005; Vanholme et al., 2010). The oxidation reaction uses H$_2$O$_2$ as co-substrate which is probably provided by combined action of NADPH oxidase or germin-like proteins, even though firm genetic evidence for the involvement of these enzymes is currently lacking (Davidson et al., 2009). PRX exist as large multigene families (73 and 138 in Arabidopsis and Oryza sativa, respectively; Welinder et al., 2002) making the clarification of the biological role of each member a considerable challenge. PRXs involved in lignification are exported to the apoplast in lignifying tissues where monolignol oxidation takes place (Koutaniemi et al., 2005; Vanholme et al., 2010). The oxidation reaction uses H$_2$O$_2$ as co-substrate which is probably provided by combined action of NADPH oxidase or germin-like proteins. However, the mutant shows a strong developmental phenotype and it would be necessary to confirm this result on a second mutant allele. In parallel, Shigeto et al. (2013) used similar strategies to functionally characterize other PRX gene candidates in
Arabidopsis. Chemical analyses indicated a significant decrease in the total lignin content and modified lignin structure in atprx2 and atprx25 mutants and altered lignin structure in atprx71 mutants.

The mechanism of oxidative radicalization of phenols followed by combinatorial radical coupling is well described (Vanholme et al., 2010; van Parijs et al., 2010). Nevertheless, the kinetics of monomer incorporation into the lignin polymer by PRX remains poorly understood. In a recent article, Demont-Caulet et al. (2010) propose a general scheme for S–G copolymerization coordinated by a PRX (PRX34) purified from Arabidopsis. The purified enzyme shows a high substrate specificity and seems unable to directly oxidize S monomers in the absence of G units. The authors studied kinetics of the monomer conversion and subsequent formation of di-, tri-, and tetramers by using monomer feeding experiments. Their results suggest that endwise and bulk polymerization mechanisms are likely to co-exist within the same system, the bulk mechanisms occurring after total consumption of the monomers. During endwise polymerization, monomers are added to the polymer one-by-one whereas during bulk polymerization different oligomers are linked together at the same time (Figure 4). Interestingly, Demont-Caulet et al. (2010) show that while monomer availability may influence the relative preponderance of the two mechanisms, it is the nature of the monomer that exerts the most influence. Indeed, under in vitro oxidation conditions, the presence of S monomer converted the PRX34-mediated polymerization of G monomers from a bulk to an endwise mechanism in agreement with data from Argyropoulos et al. (2002). These observations underline the central role of PRXs in controlling lignin biosynthesis. In a recent article, the group of Niko Geldner proposed a very nice model where PRXs are the last pieces of a puzzle that orchestrate lignification in Casparian strips in Arabidopsis roots (Lee et al., 2013). The authors show that the PRX enzymes colocalize with the very specific Casparian strip domain protein (CASP) and that their activity is indirectly under the control of an NADPH oxidase named respiratory burst oxidase homolog F protein (RBOHF) that provides \( \text{H}_2\text{O}_2 \) to PRXs via the action of an unknown superoxide dismutase (SOD). These fascinating results show for the first time that a trans-membrane protein...
Monolignol metabolism

Although there is not enough evidence to confirm that plant AtLAC2 levels in lignin are regulated during the transition from young to intermediate stage. Tomics (Ehlting et al., 2005) indicate that four LACs (AtLAC2, AtLAC4, AtLAC17, and AtLAC18) show moderate or high expression in Arabidopsis seeds. Transcriptomics (Ehlting et al., 2005) indicate that four LACs (AtLAC4, AtLAC12, AtLAC17, and AtLAC19) show moderate or high expression in Arabidopsis developing stems. These four genes are up-regulated during the transition from young to intermediate stage stems and were therefore considered as good targets for modifying lignin.

Direct evidence showing that specific LACs are involved in monolignol polymerization in plants was obtained by the characterization of Arabidopsis lac4lac17 double mutants (Berthet et al., 2011). The stems of double mutants possess collapsed vessels and show reduced G lignin deposition in fibers. Mutants also show a 20–40% decrease in total lignin content and a 130% increase in saccharification yields when compared to the wild-type control. Lignin composition (higher S/G ratio) is also modified and is mainly due to the decrease of G lignin in fibers. This is not surprising since LAC and lignin genes are tightly co-expressed (Berthet et al., 2011) and promoter-GUS analyses showed that LAC4 is expressed in vascular bundles and interfascicular fibers of stems while LAC17 is specifically expressed in fibers (Berthet et al., 2011). Phylogenetic analyses of Arabidopsis LACs showed that LAC4 and LAC17 are the best homologs (McCaig et al., 2005) suggesting redundant or at least similar activity for both proteins in lignification. Recently, Cesaro et al. (2013) identified a sugarcane LAC gene, SoLAC, putatively involved in lignification. Its transcripts are localized in the inner and outer-lignified zones of stem sclerenchymatous bundle sheaths. SoLAC was expressed preferentially in young internodes and the corresponding transcript level decreased along with stem maturity. The Arabidopsis lac17 mutant was partly complemented by expressing SoLAC under the control of the AtLAC17 promoter. SoLAC expression restored the lignin content but not the lignin composition in complemented lac17 mutant lines. These results suggest that specific LACs may also play a role in lignification in monocots.

Studies on mutants affected in different specific steps of monolignol biosynthesis indicate that plants are able to incorporate substantial amounts of components (e.g., aldehydes, 5-hydroxyconiferyl alcohol) that are poorly incorporated in “normal” conditions. Currently, it is not known whether incorporation is mediated by PRXs or LACs, or by both proteins. Some attempts have also been made to engineer plants so as to incorporate unusual compounds (e.g., rosmarinic acid or hydroxybenzaldehyde and hydroxybenzoate derivatives) into their lignins (Izudes et al., 2012; Tobiatsu et al., 2012). The incorporation of such specific compounds has been proposed as a strategy to reduce the degree of lignin polymerization. The incorporation of novel compounds together with the existence of large LAC and PRX gene families showing broad substrate specificities raises exciting new possibilities for the production of modified lignins and improved biomass.

ACYLATION

Plant lignins often contain various acids (acetates, p-hydroxybenzoates, and p-coumarates) in addition to conventional monolignols. It is usually accepted that some of them (ferulate, p-coumarate) are by-products or intermediates of the monolignol pathway since there is no evidence that an independent pathway exists. Several observations suggest that the degree of acylation could be reasonably expected to impact on biomass quality. First, amounts of acylated monomers show high natural variations between different species. Kiefel and Agarw possess up to 60% and 80% of acylated lignins, respectively when compared to maize and wheat that show 18 and 3% of acylated lignins, respectively.
Wang et al. Monolignol metabolism (reviewed by Withers et al., 2012). Second, it is possible that acylation may affect lignin structure during polymerization as well as subsequent degradation by chemical or biochemical processes such as saccharification and digestibility. In grasses, p-coumarates are linked to syringyl units and plants with low coumarate levels also generally show modifications in their S, or S and G content (Harrington et al., 2012). Importantly, coumaroylation of cell wall polysaccharides is most likely (or at least partly) catalyzed in the cytosol prior to export and polymerization in the cell wall strongly suggesting the existence of specific monolignol transferases and transporters (Withers et al., 2012; Molinari et al., 2013; Figure 1).

Until recently, the genetics underlying coumarylation of both lignin and arabinoxylans remained unknown, mainly because of the lack of suitable plants (specifically engineered to modify acylation) for functional characterization. It is generally accepted that the genes associated with such reactions would encode BAHD acyl-CoA transferase proteins. Recent work showing that transgenics have higher or lower feruloylation levels has suggested that some members of the BAHD acyl-CoA transferase protein family may be responsible for catalyzing the addition of ester-linked coumarate to cell wall arabinoxylans (Fernando et al., 2009; Withers et al., 2012; Bartley et al., 2013; Molinari et al., 2013). A member of the BAHD acyl transferase family was recently identified in the rice genome (Withers et al., 2012). Analyses of the recombinant protein produced in E. coli showed that it had a specific coumaroyl transferase activity. This enzyme OsPMT (O. sativa p-coumaric acid monolignol transferase) catalyzes the acylation of a monolignol with p-coumaric acid via p-coumaroyl CoA and the corresponding transcript is co-expressed with the monolignol biosynthetic 4CL gene. Based on catalytic efficiency
and reaction rate, the authors show that OsPMT would produce more \( \beta \)-coumaryl \( \beta \)-coumarate in saturating conditions but would favor the synthesis of sinapyl \( \beta \)-coumarate over coniferyl \( \beta \)-coumarate in plants. Interestingly this gene is grass specific and its enzymatic hallmark therefore perfectly correlates with the high level of coumaroylated sinapyl alcohols in these types of plant. In addition, the BAHD protein accepts coumarate, but not ferulate as a substrate. The production of rice plants with disrupted OsPMT expression may be difficult because of gene redundancy since there are at least two paralogs in this species. Nevertheless, it is interesting to note that there are less paralogs in *Brachypodium* (Withers et al., 2012) and the recent article published (Molinari et al., 2013) could help to identify suitable candidate genes for further expression analysis. The authors established a correlation between candidate genes and the recent article published (Molinari et al., 2013) could help to identify suitable candidate genes for further study. The authors also demonstrated that some of these key questions will be answered during the next decade.

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