



**HAL**  
open science

## **Breeding grasses for capacity to biofuel production or silage feeding value: an updated list of genes involved in maize secondary cell wall biosynthesis and assembly**

Audrey A. Courtial, Marçal Soler, Anne Laure Chateigner Boutin, Matthieu Reymond, Valérie Méchin, Hua Cassan-Wang, Jacqueline Grima Pettenati, Yves Y. Barrière

### ► **To cite this version:**

Audrey A. Courtial, Marçal Soler, Anne Laure Chateigner Boutin, Matthieu Reymond, Valérie Méchin, et al.. Breeding grasses for capacity to biofuel production or silage feeding value: an updated list of genes involved in maize secondary cell wall biosynthesis and assembly. *Maydica*, 2013, 58 (1-4), pp.67-102. hal-02651049

**HAL Id: hal-02651049**

**<https://hal.inrae.fr/hal-02651049>**

Submitted on 29 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Breeding grasses for capacity to biofuel production or silage feeding value: an updated list of genes involved in maize secondary cell wall biosynthesis and assembly

Audrey Courtial<sup>1,2,3</sup>, Marçal Soler<sup>2,3</sup>, Anne-Laure Chateigner-Boutin<sup>4</sup>, Matthieu Raymond<sup>5</sup>, Valérie Méchin<sup>5</sup>, Hua Wang<sup>2,3</sup>, Jacqueline Grima-Pettenati<sup>2,3</sup>, Yves Barrière<sup>1\*</sup>

<sup>1</sup>INRA, Unité de Génétique et d'Amélioration des Plantes Fourragères, 86600 Lusignan, France

<sup>2</sup>Université de Toulouse, UPS, UMR5546, LRSV, BP 42617, Auzeville, 31326 Castanet-Tolosan, France

<sup>3</sup>CNRS, UMR 5546, BP 42617, F-31326 Castanet-Tolosan, France

<sup>4</sup>INRA BIA, rue de la Géraudière, BP71627, F-44316 Nantes cedex 3, France

<sup>5</sup>INRA, Institut Jean-Pierre Bourgin, 78026 Versailles, France

\*Corresponding author: E-mail: yves.barriere@lusignan.inra.fr

### Abstract

In the near future, maize, sorghum, or switchgrass stovers and cereal straws will be a significant source of carbohydrates for sustainable biofuel production, in addition to the current use of grass silage in cattle feeding. However, cell wall properties, including the enzymatic degradability of structural polysaccharides in industrial fermenters or animal rumen, is greatly influenced by the embedding of cell wall carbohydrates in lignin matrix, and the linkages between lignins, *p*-hydroxycinnamic acids, and arabinoxylans. Breeding for higher and cheaper biofuel or silage production will thus be based on the discovery of genetic traits involved in each cell wall component biosynthesis and deposition in each lignified tissue. Due to its considerable genetic and genomic backgrounds, maize is the relevant model species for identifying traits underlying cell wall degradability variations in grasses. Maize genes involved or putatively involved in the biosynthesis of cell wall phenolic compounds, cell wall carbohydrates and regulation factors were therefore searched for using data available in grass, *Arabidopsis*, and woody species (mostly poplar and eucalyptus). All maize ortholog genes were searched for using protein sequences and a “blastp” strategy against data available in the [www.maizesequence.org](http://www.maizesequence.org) database. Genes were also mapped in silico considering their physical position in the same database. Finally, 409 candidate genes putatively involved in secondary cell wall biosynthesis and assembly were shown in the maize genome, out of which 130 were related to phenolic compound biosynthesis, 81 were related to cell wall carbohydrate biosynthesis, and 198 were involved in more or less known regulation mechanisms. Most probable candidate genes involved in regulation and assembly of secondary cell wall belonged to the MYB (45 genes) and NAC (38 genes) families, but also included zinc finger and HDZipIII encoding genes. While genes involved in ferulic acid cross-linkages with other cell wall components were little known, several families putatively involved in (arabino)-xylan chain biosynthesis and in feruloyl transfer were shown, including especially arabinosyl-CoA-acyltransferases, feruloyl-AX  $\beta$ -1,2-xylanyl transferases, and xylan-O-3-arabinosyl transferases. This candidate gene list, which focused on genes and orthologs known to be involved in cell wall component biosynthesis and regulation, cannot be considered as exhaustive. Other genes, whose role in cell wall lignification and deposition have not yet been defined, should very likely be added to the list of candidates required for secondary cell wall assembly. Genes encoding proteins of still unknown function should also be added to the list, as several of the latter are probably involved in lignified tissue biosynthesis and deposition.

**Keywords:** maize, cell wall, silage, biogas, bio-ethanol, lignin, degradability

### Introduction

Concerns over global climate changes, together with a growing worldwide demand for energy and the simultaneous “short term” depletion of fossil stocks, have demonstrated out the crucial need for alternative energy resources. In addition to solar, wind, ocean wave, and nuclear energies, second generation biofuels obtained from fermentation of lignocellulose materials have opened new avenues based either on dedicated crops or recovering a large part of agricultural and woody residues. First generation bio-

fuels based on cereal or oleaginous grains compete with food supplies and have been recently rejected by the European Union commissioner for “Climate Action”. Most species considered for biofuel production are grasses, with the use of plant straws of C3 and C4 cereals and sugarcane bagasse, and the whole plant use of maize, sorghum, or switchgrass. Grasses are also the basis of energy nutrition of dairy and meat cattle, with grazing during spring and early autumn and silage maize feeding during the long periods without meadow growth. In France, nearly

85% of milk is indeed produced by cows fed several months a year on silage maize. However, although green biomass contains almost the same amount of gross energy as do grains per unit of dry matter, the stover energy value is significantly lower, only reaching in wheat straws 33% of maize grain value both in the digestive tracts of animals and in industrial enzymatic fermenters. The biological conversion of cell wall carbohydrates, mainly located in the secondary lignified plant cell walls, into fermentable sugars is hindered by their association with lignins and *p*-hydroxycinnamic acids.

Lignification and the development of a vascular system have allowed plants to leave aquatic habitats and to acquire erect growth. Lignins also impart hydrophobicity to vascular elements allowing water and nutriment transportation. Lignins, with cellulose, contribute to the mechanical properties and structural integrity of tissues, and lignin-deficient tracheary elements may collapse as their cell wall cannot stand the negative pressure generated during long and/or intensive transpiration periods (Cochard, 2002; Cochard et al, 2008). Finally constitutive and neoformed lignins are involved in mechanisms related to disease and pest tolerances. The embedding and cross-linkages between phenolics and carbohydrates thus prevent physical access of enzymes to cell wall carbohydrates and strongly limit their enzymatic hydrolysis. Model studies, in which the degrees of polysaccharide-polysaccharide and lignin-polysaccharide cross-linkages are controlled, established that the former primarily impede the rate of carbohydrate degradation, while the latter impede both the rate and extent of carbohydrate degradation (Ralph, 2010). In addition, lignins adsorb hydrolytic enzymes on their surfaces, and consequently have a second negative effect on carbohydrate degradation. Moreover, lignin degradation products resulting from industrial pretreatment inhibit ethanologenic fermentations (Keating et al, 2006; Li et al, 2008).

Grain maize or “corn” is likely the plant species in which genetic improvements for agronomic traits were the most remarkable during the last century in the USA and over the last six decades in Europe. In forage maize (Barrière et al, 1987; Barrière et al, 2004a; Barrière et al, 2005), the genetic progress in yield was close to 0.17 t ha<sup>-1</sup> per year for hybrids registered in France between 1986 and 2000 and seemingly has continued up to now (1986 is the first year with registration tests including whole plant traits). Before 1986, forage yield improvement was correlative to the genetic progress in grain and was nearly equal to 0.10 t ha<sup>-1</sup> per year (Barrière et al, 1987). However, a significant drift of hybrid cell wall digestibility towards lower values was simultaneously observed during the same period. Based on data from the long term experiment on silage maize feeding value and cell wall digestibility (NDFD, Neutral Detergent Fiber Digestibility) measurements with sheep

at INRA Lusignan (Barrière et al, 2004a), hybrids with a lower NDFD than the well-known old early hybrid LG11 (NDFD = 50.2%) were 32, 63, 75, 84, and 93% in each of the registration periods before 1981, 1989, 1994, and 1999, and in or after 1999, respectively. This decline in average cell wall digestibility, which has indeed been mainly observed since 1980, was mostly a consequence of the introduction of lodent and BSSS genetic resources in early and medium-early line breeding, together with the phasing out of flint lines with high cell wall digestibility but with poor standability and yield. This decline, which was also related to the focus of breeders on (grain) yield and standability, has now ceased with the breeding of specialized silage maize and the use of a digestibility criterion in forage maize registration since 1986 in the Netherlands and since 1998 in France. While the cell wall degradability of the best modern hybrids does not yet equal that of the best older types such as INRA258, several currently registered French hybrids have cell wall digestibility close to that of DEA-type hybrids. However, unlike yield or stress tolerance for which steady improvements have been observed, the energy value of currently released silage maize varieties plateaus. This is due to the fact that cell wall degradability has not been sufficiently taken into account during this era of breeding. Maize cropping for silage use in Europe is therefore now mostly based on varieties bred for whole plant agronomic and quality traits, most of which have medium cell wall degradability. Significant improvements are nevertheless occurring with the registration in the Netherlands of early hybrids such as Aastar, Ayrro, LG30-225, etc, with a cell wall degradability equal or close to 110% of that of the control hybrids.

In any case, maize genetic improvement allowing increased animal or biofuel production based on the non-grain part of plants requires understanding cell wall building rules, as well as genetic and molecular mechanisms involved in secondary wall assembly and cell wall compound biosynthesis, and finally cross-linkage determinants. Gene discovery is thus a major pre-requisite in order to implement effective silage and biofuel breeding programs, based on marker assisted selection (MAS), genome-wide association studies and SNP-based (single nucleotide polymorphism) investigations, genetic resource management, and genetic engineering. Most cell wall (gene) research has been devoted to improving paper pulping conditions towards more environmental friendly processes in woody dicotyledonous and gymnosperms plants (poplar, eucalyptus, pine, spruce, etc). In addition, intensive investigations to decipher cell wall assembly have indeed been based on *Arabidopsis* model plant, and to a lesser extent alfalfa. A wide set of the genes involved in cell wall carbohydrate and phenolic compound biosynthesis, and their regulation, is thus currently available in *Arabidopsis*. However, a list mainly focused on

carbohydrate related genes has also been proposed for maize (Penning et al, 2009). Nevertheless, only limited information is indeed available for maize and grass cell wall gene regulation and lignified tissue patterning in leaves and stems. Firstly, this is due to the fact that the lignin pathway has not really been investigated in the rice model plant, or in the more recent *Brachypodium* or *Setaria* models, and secondly, because, in contrast to dicotyledonous plants, the vascular system of maize and non-woody monocotyledons is characterized by the absence of bifacial cambium and secondary growth. The major role of *p*-hydroxycinnamates in secondary wall structure is also specific to grass plants. However, the emergence and evolution of lignified and vascular tissues was indeed based on a preexisting poly-phenolic pathway (Boyce et al, 2003), followed by millions of years of divergent evolution in grasses and dicotyledons. Many results obtained in plant genetics and genomics of lignification have nevertheless illustrated a significant commonality in cell wall carbohydrate and phenolic compound biosynthesis in all plants. However, even if they have not yet been established, significant differences between species groups could be particularly expected for genomic traits involved in regulation and assembly of lignified tissues. The transcription factors families have disproportionally expanded, and the regulatory networks diverged, so that the eudicot model is not wholly generalizable to grasses (Handakumbara and Hazel, 2012).

### Objective and methodologies

The objective of this investigation was therefore to describe the composition and organization of the maize secondary cell wall, and then to list maize candidate genes possibly involved in the corresponding metabolic pathways. Maize orthologs of transcription and regulation factors described in other species, and mostly dicotyledonous species, were especially considered as candidates. An *e*-value at least lower than  $e^{-75}$  was retained during ortholog searches based on protein sequences. Maize candidate genes were finally put into eight groups including 1) genes involved in monolignol and *p*-hydroxycinnamic acid biosynthesis, and those involved in monolignol polymerization, 2) genes involved in cellulose biosynthesis and cellulose fiber assembly, 3) genes involved in arabinoxylan and related compound assembly, 4) genes involved in feruloylation and acylation of cell wall components, 5) genes of the shikimate pathway, 6) genes of the S-adenosyl-L-methionine cycles, 7) MYB, NAC, and transcription factors involved in cell wall regulation, and 8) miscellaneous genes involved in lignified tissue biosynthesis or assembly. These eight different sets of genes likely are not equally important in determining cell wall degradability variations. Gene GRMZM names and physical positions were based on the maize B73 sequence ([www.maize-esequence.org](http://www.maize-esequence.org), release v2 5b.60). The consensus

physical map used to illustrate gene repartition on the maize genome was drawn with markers mapped during the QTL investigations at INRA Lusignan (Méchin et al, 2001; Roussel et al, 2002; Génoplante unpublished data, 2007; Barrière et al, 2008; Riboulet et al, 2008a; Barrière et al, 2012; Courtial et al, 2013). Several markers were however added in areas surrounding centromeres based on their physical position given in the MaizeGDB database (all IDP markers), as these locations, in which recombinations are uncommon, were poorly marked.

### Phenolic constituents of maize and grass cell walls

The lignified secondary wall of grasses is a composite material with phenolic compounds, cellulose microfibrils, an amorphous matrix consisting predominantly of glucurono-arabinoxylans, and only very few pectins. Phenolic compounds are comprised of lignins and cross-linked *p*-coumaric (pCA) and ferulic (FA) acid derivatives, along with the array of FA dehydrodimer (diFA) derivatives.

Grass lignins result from the combinatorial radical coupling of *p*-coumaryl, coniferyl, and sinapyl alcohols, giving rise to *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) monomeric units. The average relative frequencies of each H, G, and S unit released by thioacidolysis of native lignins of mature maize internodes were shown to be nearly equal to 3, 37, and 60%, respectively (Lapierre, 1993). A large range of variation in monomer proportions was nevertheless shown between maize lines and the S/G ratio ranged between 1.0 and 1.8 (Méchin et al, 2005). In addition, significant variations in H unit proportions were also shown (Riboulet et al, 2008b). The H, G and S units of grass lignins are interconnected through labile  $\beta$ -O-4 ether bonds representing, depending on lines, from 15 to 25% of between-monomer linkages, and through a series of resistant carbon-carbon and biphenyl ether linkages. While lignins are often referred to as branched three-dimensional structures, lignins are in fact largely linear polymers. The two only known branching structures in lignins are the 5-5 and 4-O-5 bonding patterns, which cannot be formed without the participation of at least one G or H unit (Ralph et al, 2008a). Between these branching points, there are linear lignin fragments made of monomers essentially linked by  $\beta$ - $\beta$ ,  $\beta$ -5, and  $\beta$ -O-4 linkages. The low, but significant amount of H units, which is nearly five times higher than in dicotyledonous plants, impact the properties of the lignin polymer as these units increase the frequency of resistant inter-unit bonds. In addition, the degree to which lignin polymers incorporate various phenolics in place of the three regular constitutive monolignols is likely underappreciated (Ralph, 2010). The incorporation of free FA in lignins through bis-8-O-4 cross-coupling, which occurs at very low, but significant, levels in normal maize plants but may build up in CCR- and CAD-deficient plants,

provides a third branching point in lignin polymers (Ralph et al, 2008b; Barrière et al, 2013). In addition to FA and hydroxycinnamaldehydes, unusual monomers, including acylated hydroxycinnamyl alcohols, dihydro-hydroxycinnamyl alcohols, hydroxybenzaldehydes and other hydroxycinnamic acids, can be incorporated in lignins of wild-type plants (Vanholme et al, 2012). Moreover, currently obtained results seemingly show that plants could tolerate shifts in lignin composition with no or lower impact on growth than observed for reduced lignin contents (Eudes et al, 2012; Vanholme et al, 2012). The fact that plants can readily incorporate alternative unusual monomers with modified physicochemical and stereochemical properties could be a basis for original plant improvement for both animal feeding and biofuel production.

The participation of *p*-hydroxycinnamates in cell wall composition and organization is a specific trait of grass plant lignification. Among cell wall-linked *p*-hydroxycinnamates, *p*-coumarate is mainly esterified to the  $\gamma$ -position of the phenylpropane side-chain of S lignin units, even if 10% of *p*-coumarate can be found on maize G units (Grabber et al, 1996; Lu and Ralph, 1999). Most *p*-coumarate accretion occurs in tandem with lignification and *p*-coumarate accumulation is thus a relevant indicator of lignin deposition. In maize, S unit acylation occurs at the monolignol level, and from 25 to 50% of S lignin units may thus be acylated by pCA (Lu and Ralph, 1999; Morreel et al, 2004; Grabber and Lu, 2007; Martinez et al, 2008; Ralph et al, 2008a). Acylation has a marked influence on the bonding mode of S lignin units, on the spatial organization of lignins and consequently on their capacity to interact with polysaccharides. During monolignol polymerization, sinapyl alcohol is only slowly oxidized by maize peroxidases. Conversely, an oxidation shuttle operates in acylated conditions because the pCA component of the S-pCA conjugate is readily oxidized, with the subsequent transfer of its oxidation state to sinapyl alcohol (Boudet, 2000; Ralph et al, 2004; Hatfield et al, 2008).

Even if ferulate is at first the major *p*-hydroxycinnamic derivative in young grass cell walls, at least 50 to 70% of the alkali-labile ferulate deposition occurs during secondary wall lignification (Iiyama et al, 1990; Morrison et al, 1998; MacAdam and Grabber, 2002; Grabber et al, 2004). Ferulic units are primarily esterified to glucurono-arabinoxylans, and lignins and arabinoxylans are secondarily bridged through FA ether-linkages at the  $\beta$ -position of G units. Ferulates thus provide points of growth for the lignin polymer, acts as lignin nucleation sites, and direct cell wall cross-linking (Ralph et al, 1992; Jacquet et al, 1995; Ralph et al, 1995; Ralph, 2010). Moreover, the presence of ferulates linked to arabinosyl side-chains of arabinoxylans provides a convenient and reliable way of cross-linking these polysaccharide chains. Over 50% of wall ferulates can undergo dehydrodimerization and arabinoxylans are thus extensively cross-linked

by ferulate dimerization in mature cell walls (Grabber et al, 2004).

Large variations in phenolic component contents have been shown between maize inbred lines or between hybrids. The content in ADL/NDF nearly doubled between 4 and 8% in maize lines having extreme values [NDF and ADL are neutral detergent fiber and acid detergent lignin according to Goering and van Soest (1970), respectively]. A similar two-fold variation was also observed for pCA content, which could be partly related to the variation in lignin contents. Despite their (relatively) low amounts, significant variations have been shown for esterified and etherified FA (esterFA and etherFA) releases after alkaline hydrolysis, as was also highlighted for 8-O-4 and 5-5 diferulates. Finally, variation for the recovery yield of each H, G or S lignin-derived monomer after nitrobenzene oxidation was similarly of a two-fold range between maize lines, with significant consequences on the polymer arrangement and spatial organization.

As a consequence of variable phenolic component contents and organizations, large genetic variations in the *in vivo* or *in vitro* cell wall digestibility of maize plants have been shown, with small genotype x environment interaction effects compared to main effects. From a long term experiment based on 478 hybrids, the *in vivo* cell wall digestibility in maize (estimated as NDF digestibility, or NDFD) nearly doubled from 32.1 to 60.4% with an average value equal to 48.8 % (Barrière et al, 2004a). The *in vitro* cell wall digestibility (IVNDFD) also nearly doubled from 22.1 to 39.2% with an average value equal to 32.5% in a set of 26 lines (Barrière et al, 2009) representing probably the largest known variation for this trait [IVNDFD is estimated according to Struik (1983) as  $(100 \times (ES - (100 - NDF)) / NDF)$ , based on the enzymatic solubility (ES) of Aufrère and Michalet-Doreau (1983)].

Lignin content is the first trait that has been related to cell wall degradability, but breeding for a much reduced lignin content has too many negative consequences on other agronomic qualities. Moreover, variations in lignin content are not the only determinants explaining variations in cell wall degradability. This fact was especially highlighted after correlation and QTL analyses (Barrière et al, 2008; Riboulet et al, 2008b; Zhang et al, 2011a; Barrière et al, 2012), and from cell wall model studies (Grabber et al, 1998; Grabber et al, 2005). Variable colocalizations between cell wall degradability QTLs and phenolic compound QTLs were indeed shown, with several cell wall degradability QTLs that did not colocalize with lignin QTLs. Corroborating negative correlations between cell wall degradability and etherFA releases, and QTL colocalizations (Casler and Jung, 1999; Méchin et al, 2001; Lam et al, 2003; Riboulet et al, 2008b; Jung and Phillips, 2010; Taboada et al, 2010; Jung et al, 2011; Barros-Rios, 2012), the role of ferulate cross-linkages was tentatively estimated to account for nearly one half of the inhibitory effects of



lignin on cell wall fermentation" (Grabber et al, 2009). Breeding for a reduced level of ether-linked ferulate has thus improved cell wall degradability in perennial grasses (Casler and Jung, 1999; Casler et al, 2008). Similarly, breeding for reduced diferulate contents in maize pith increased cell wall polysaccharide degradability (Barros-Rios et al, 2012). Moreover, ferulate cross-linkages were shown to be involved in stalk stiffness (Grabber et al, 1995; Grabber et al, 2000; MacAdam and Grabber, 2002). While nearly one half of intake variations in cows were explained by cell wall degradability (Barrière et al, 2003), scattered but convergent results support the hypothesis that the rest of the genetic variations in intake are related to plant tissue friability and susceptibility to crushing. Consequently, ferulate cross-linkages likely impede silage maize intake as they decrease plant friability (Ciba-Semences, 1990, 1995; Barrière et al, 1995; Jung and Allen, 1995; Barrière et al, 2004b; Fernandez et al, 2004). Correlations and QTL colocalizations pointed out a negative effect of pCA content on cell wall degradability (Barrière et al, 2008; Riboulet et al, 2008b; Zhang et al, 2011a; Barrière et al, 2012). In addition to being a probable direct effect of S unit acylation on lignin polymer geometry, pCA content is likely also a relevant indicator of intensity and length of secondary tissue lignification. Finally, attempts to understand the impact of lignin structure, commonly described by ratios between H, G, and S units, on the susceptibility of the cell wall to enzymatic hydrolysis have led to conflicting results (Méchin et al, 2000; Grabber et al, 1997; Grabber et al, 2009). However, based on correlations and QTL colocalizations, increased proportions of H units and S units likely contribute, for different reasons, to lowering of cell wall degradability (Riboulet et al, 2008b). As was considered for pCA contents, a higher proportion of S units in lignins might indicate a higher proportion of mature secondary wall in tissues. Lignin structure can also be characterized by the yield of monomers released after thioacidolysis, and a greater proportion of  $\beta$ -O-4 linkage in the lignin polymer has been shown to be negatively correlated with cell wall degradability (Zhang et al, 2011a). This latter fact could be explained by the more extended shape of  $\beta$ -O-4 lignins, maximizing the masking effect on carbohydrate polymers, in comparison to the more globular shape of condensed 5-5 and  $\beta$ -5 lignins (Besombes and Mazeau, 2005).

### Genes involved in the upstream parts of cell wall carbohydrate biosynthesis

Cellulose and glucurono-arabinoxylans are the main constituents of lignified secondary walls. Putative genes encoding for enzymes catalyzing the early steps of cellulose and xylan synthesis have been identified in different plant species. The nucleotide sugar interconversion pathway comprises a set of enzymatic reactions by which plants synthesize ac-

tivated monosaccharides as precursor elements of cell wall polysaccharides from photosynthesis and D-fructose-6-P (Reiter and Vanzin, 2001; Reiter, 2008). UDP-D-glucose (UDP-D-Glc), which is at the basis of cellulose and arabinoxylan biosynthesis, is produced from D-fructose-6-P via D-glucose-6-P and D-glucose-1-P in three successive reactions catalyzed by phosphoglucose isomerases, phosphoglucomutases, and UDP-D-Glc pyrophosphorylases. UDP-D-Glc is also available from sucrose and uridine diphosphate (UDP) in the reversible reaction catalyzed by sucrose synthases. Nucleotide sugars are then the substrates which are used for the elongation of carbohydrate chains by UDP-glycosyltransferases (Kawakita et al, 1998; Gibeaut, 2000). All these genes, which are indeed key components in multiple plant metabolisms, were not *a priori* considered as putative candidates involved in variation of cell wall degradability. However, a member of one of these multigene families might be specifically involved in a metabolon devoted to cellulose or arabinoxylan biosynthesis, and therefore could be involved in cell wall variation with consequences on biofuel production capabilities. A nucleotide sugar transporter (OsNST1 or Os02g40030) was thus shown underlying the *brittle-culm-14* (*bc14*) mutation in rice (Song et al, 2011; Zhang et al, 2011b). Mutant *bc14* plants have reduced cellulose content, irregular orientation of cellulose microfibrils, and higher xylan extractability. This set of traits improves the extractability of all cell wall components. Only two close orthologs were shown in maize (Supplementary Table 1).

### Genes involved in cellulose biosynthesis and cellulose fiber organization

Cellulose is comprised of hydrogen-bonded  $\beta$ -1,4-linked glucan chains which are synthesized at the plasma membrane by large cellulose synthase (CesA) complexes, using UDP-d-glucose as a precursor. Twelve CesA genes have been described in maize (Appenzeller et al, 2004), and further shown in the maize sequence database. Deficiency in one or another CesA gene impedes cellulose biosynthesis and modifies the orientation or organization of cellulose microfibrils, with consequences on the mechanical quality of plant leaves or stems. In *Arabidopsis*, the two irregular xylem mutants *IRX1* and *IRX3* have a reduced stiffness of mature stems correlatively to a cellulose defect in secondary cell walls (Turner and Somerville, 1997). The *IRX1* and *IRX3* *Arabidopsis* genes encode the catalytic subunits of the cellulose synthase isoforms CesA8 and CesA7, respectively, with the latter being specifically expressed in xylem tissue (Taylor et al, 1999; Taylor et al, 2000). In addition to CesA genes, other genes also impact cellulose microfibril deposition and organization. The *fragile fiber FRA1* *Arabidopsis* mutant, which has a large reduction in fiber mechanical strength without appar-

ent alteration in cell wall composition, and the rice *brittle culm12* mutant are altered in kinesin proteins (Zhong et al, 2002a, Zhang et al, 2010). Kinesins are ATP-driven microtubule-based motor proteins with diverse functions in plant growth and developmental processes. These functions include the mediation of cortical microtubule activity and the orientation of cellulose microfibrils during differentiation of xylem cells (Zhong et al, 2002a). The fragile fiber *FRA2* mutant is altered in a gene encoding a katanin-like protein that regulates fiber cell length and wall thickness. The secondary walls of *FRA2* fiber cells lack distinct S1, S2, and S3 layers thus indicating that this katanin was considered to be essential for the formation of distinct layers of cellulose microfibrils during secondary wall thickening (Burk et al, 2001; Burk and Ye, 2002). *FRA1* and *FRA2* both have orthologous genes in maize, whose involvement in cellulose deposition is still unknown. Rice *brittle culm1* and maize *brittle stalk2* mutants, which have reduced mechanical strengths, are affected in orthologs of COBRA-like proteins encoding putative glycosylphosphatidylinositol-anchored proteins (Li et al, 2003a; Ching et al, 2006; Sindhu et al, 2007; Dai et al, 2011). These COBRA-like proteins were considered to be involved in a patterning of lignin-cellulose interactions that maintain organ flexibility rather than having a direct role in cellulose biosynthesis, even if the cellulose content was reduced in mutant plants (Sindhu et al, 2007). Supporting data is expected from studies of other rice *brittle* mutants which are similarly altered in cellulose deposition in the cell wall or in cellulose synthesis (Xu and Messing, 2008). In addition, KORRIGAN mutants have irregular xylem and the corresponding encoded protein is supposed to have a role in processing of the growing cellulose microfibrils or release of the cellulose synthase complex (Szyjanowicz et al, 2004). Moreover, it was considered that KORRIGAN activity facilitates cellulose biosynthesis in a way that increases the amount of non-crystalline cellulose (Takahashi et al, 2009), which is the preferentially hydrolysed part of cell wall cellulose. Finally, two chitinase-like proteins CTL1 (At1g05850) and CTL2 (At3g16920) have been shown to be involved in regulation and biosynthesis of cell wall carbohydrates (Zhong et al, 2002b; Hossain et al, 2011). Mutations in both *CTL1* and *CTL2* genes induced ectopic deposition of lignin (*CTL1* also named *ELP1* for *Ectopic Deposition of Lignin in Pith 1*, and *POM1*). The *CTL2* gene was shown predominantly expressed in stems. These two CTL genes encode proteins which have no chitinase activity, and have the same unique ortholog in the maize genome, located in bin 7.03. Associations of cellulose with hemicelluloses are important for microfibril spacing and for maintaining cell wall tensile strength. The two latter chitinase-like proteins were considered to play a key role in establishing interactions between cellulose microfibrils and hemicelluloses (Sánchez-Rodríguez et al, 2012). In

fact, many components are associated with the CesA complexes, some of which are specific to lignified secondary wall assembly (Endler and Persson, 2011). While several of these gene functions can induce differences in mechanical stem stiffness, it is still unclear whether such variations can induce differences in intrinsic cell wall degradability. However, variation in mechanical tissue quality intake have been considered to be involved in the duration of chewing and silage intake for dairy cows (Barrière et al, 2004b; Fernandez et al, 2004; Barrière et al, 2009). A lower mechanical resistance of tissue would also reduce the cost of biomass crushing in industrial processes. As a whole, 23 maize genes were shown with probable or putative function in cellulose biosynthesis, deposition, and fiber organization (Supplementary Table 1).

### Genes involved in arabinoxylan biosynthesis

Hemicellulose polysaccharides are formed from UDP-D-glucose in the Golgi apparatus and are exported to the external surface of the membrane in Golgi vesicles (Dennis and Blakeley, 2000). UDP-D-xylose is thus produced from UDP-D-glucose in a set of two reactions. UDP-D-glucose is converted into UDP-D-glucuronic acid (UDP-D-GlcA) in a reaction catalyzed by UDP-D-glucose dehydrogenases (G6DH). UDP-D-GlcA is next converted into xylose in a reaction catalyzed by UDP-D-GlcA decarboxylase. In addition, UDP-D-xylose can be converted into UDP-L-arabinose in a reversible reaction catalyzed by an UDP-D-xylose-4-epimerase. In *Arabidopsis*, a *UDP-D-xylose-4-epimerase* gene was shown to be affected in the *MUR4* mutant (Burget et al, 2003), which showed a 50% reduction in L-arabinose in leaf cell walls. The UDP-L-arabinose produced by the UDP-D-xylose-4-epimerase is in the pyran form and requires a UDP-arabinopyranose mutase (UAM) to be converted to UDP-L-arabinofuranose, which is the form transferred to the xylan backbone (Konishi et al, 2007; Konishi et al, 2010). UAM were shown to be encoded by genes of the reversibly glycosylated polypeptide/glycosyltransferase 75 family. RNA interference lines in rice targeting members of this family showed a decreased content in arabinofuranose in their wall (up to 44%) and a reduced level of xylan substitution (Konishi et al, 2011). In addition, the involvement of UDP-sugars in the biosynthesis pathway of hemicellulose polysaccharides could strengthen the possible and simultaneous involvement of UDP-arabinose in feruloylated arabinoxylan formation (Buanafina, 2009).

Biosynthesis of the  $\beta$ -1,4-xylan backbones is catalyzed by UDP-D-xylose:1,4- $\beta$ -D-xylan 4- $\beta$ -D-xylosyltransferase ("xylan synthases", GT43 glycosyltransferase family), using uridine 5'-diphosphoxylose (UDP-Xyl) as the donor substrate (Urahara et al, 2004). In *Arabidopsis*, the *IRX9* and *IRX14* mutations result in a deficiency in xylan xylosyltransferase (XylIT) activity, thus leading to a defect in the elongation

of the xylan backbone (Brown et al, 2007; Lee et al, 2007a). Furthermore, co-expression of *Arabidopsis* IRX9 and IRX14 in tobacco BY2 cells lacking xylan resulted in an increase in xylosyltransferase activity onto a Xyl4 acceptor (Lee et al, 2012). Several genes of the glycosyltransferase GT43 family were thus shown to be more specifically expressed in grasses (Mitchell et al, 2007), corresponding to plausible maize candidate genes encoding xylan xylosyltransferases. In addition, the *IRX10* mutants of *Arabidopsis* have similar characteristics to those of the IRX9 mutant, suggesting that IRX10-like glycosyltransferases (GT47 family) could also play a role in the elongation of the xylan backbone (Brown et al, 2009; Wu et al, 2009; Oikawa et al, 2010). Mutants in the rice IRX10 orthologous gene were recently reported not to be affected in the length of their xylan polymer, despite a reduction both in the xylose and the xylan contents (Chen et al, 2012a). A direct role of IRX10 in xylan elongation seems therefore unlikely. While no changes were simultaneously observed in lignin content, the rice *OsIRX10* mutant also displayed a higher cell wall saccharification efficiency (Chen et al, 2012a). IRX10-like genes may have indeed a different function in grasses than in *Arabidopsis*. Members of the GT47 family were proposed to carry out in grasses both xylan  $\alpha$ -1,2- and/or  $\alpha$ -1,3-arabinosyl transferase activities, allowing the transfer of an arabinosyl residue onto an X(X) chain (Mitchell et al, 2007). Nevertheless, the involvement of members of the GT47 family in the transfer of arabinose onto xylan is still only hypothetical. In addition, several members of the GT61 family (clade A) were shown to be xylan O-3 arabinosyltransferases in wheat and rice (Anders et al, 2012). Based on RNA interference, the deregulation of one GT61 gene induced a decrease in  $\alpha$ -1,3 linked arabinose, but also in total arabinoxylans. The latter fact suggested a substitution requirement for continued backbone synthesis. Other members of the GT61 family were found in rice to be  $\beta$ -(1,2) xylosyltransferase acting on arabinosyl residues linked to xylans on C3 (Chiniquy et al, 2012), with orthologs in maize. Members of the GT8 glycosyltransferase protein family are also involved in the biosynthesis of secondary cell wall xylans. The *Arabidopsis* *IRX8* mutant has thus a 60% reduction in xylan content (Persson et al, 2007). In poplar, two GT8 members were shown abundantly and specifically expressed in the differentiating xylem. RNAi down-regulated lines for both GT8 glycosyltransferase genes had a nearly 33% reduction in stem wood xylan content, no change in cellulose quantity, and an increase in lignin content ranging between 10 and 25%. These transgenic plants exhibit thinner fiber cell walls in stem xylem, a brittle wood phenotype, and reduced stem modulus of rupture (Li et al, 2011a). Xylan and carbohydrate content and organization in the secondary cell wall are thus likely, with ferulate cross-linkages, more important factors than lignin content affecting

the stiffness and fracture strength of tissue, with very probable consequences on forage intake by cattle.

Arabinoxylans in maize (and grasses) are in fact glucurono-arabinoxylans, with xylan substitution by arabinose on the C2 and/or C3 position, and by (4-O-methyl-) glucuronosyl on C2. Candidate genes for (4-O-methyl-) glucuronic transfer can be found in the GT8 family since xylans of *Arabidopsis* double mutants in the *GT8/GUX* genes were shown to be devoid of glucuronosyl substitution (Mortimer et al, 2010). Three orthologs of the *Arabidopsis* genes *GUX1* (At3g18660), *GUX2* (At4g33330), and *GUX3* (At1g77130), which encode proteins located in the Golgi apparatus, with a glucuronosyltransferase activity, were shown in the maize genome. The acetylation of xylan backbones at C-2 and/or C-3 positions could affect about 40% of xylosyl residues (Ebringerova et al, 2005; Lee et al, 2011a; Manabe et al, 2011; Gille and Pauly, 2012; Saulnier et al, 2012). The biological significance of polysaccharide O-acetylation is not fully known, but acetylation was shown to affect the physicochemical properties of cell wall xylans. Moreover, the presence of acetyl esters negatively impedes biomass enzymatic saccharification, and the release of acetate and conversion products of acetate also are inhibitory to the micro-organisms used during cell wall sugar fermentation into ethanol (Manabe et al, 2011; Gille and Pauly, 2012). Genes catalysing the O-acetylation of xylan have long been unknown in plants, while it has been shown that the *CAS1* gene from the yeast *Cryptococcus neoformans* was involved in the O-acetylation of its main capsular polysaccharide (Janbon et al, 2001). Four orthologs of the *Cap1s* encoded protein were recently shown in *Arabidopsis*, which are regulated by the NAC factor *SND1* (Lee et al, 2011a). These four *REDUCED WALL ACETYLATION* genes (*RWA1-4*) have close sequence similarities. *RWA1*, *RWA3* and *RWA4* genes were shown to be expressed in both xylem cells and interfascicular fibers, while *RWA2* was only expressed in xylem cells. *RWA2* mutant plants had an overall acetylation reduction of 20% in wall polymers including xylans (Manabe et al, 2011). A second family of protein involved in plant polysaccharide O-acetylation was identified, based on investigations with the *AXY4* mutants of *Arabidopsis* which lacked O-acetyl-substituents on xyloglucan chains (Gille et al, 2011). The *AXY4* (*TBL27*) and *AXY4*-like (*TBL22*) genes belong to the trichome birefringence-like (TBL) family (Bischoff et al, 2010a), which includes 46 members in *Arabidopsis*. Other members of the TBL family were proposed to encode additional wall polysaccharide specific O-acetyltransferases (Gille et al, 2011; Gille and Pauly, 2012). However, the latter fact has not yet been established, and similarly the possible role of maize orthologs in xylan O-acetylation is still hypothetical.

Taking into consideration the genes involved in xylose, arabinose, and arabinoxylan chain biosyn-



thesis and acetylation, 57 genes were shown in the maize genome, most of which had transferase activities ([Supplementary Table 2](#)).

### Genes involved in the shikimate pathway, upstream the monolignol pathway

In plants, the shikimate pathway links the carbohydrate metabolism to the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and, consequently, to the phenylpropanoid pathway. In the first step, phosphoenolpyruvate and erythrose-4-phosphate react to form 3-deoxy-D-arabinoheptulosonate-7-phosphate (DAHP), in a reaction catalyzed by the DAHP synthase. DAHP is transformed to 3-dehydroquinate (DHQ), in a reaction catalyzed by the DHQ synthase. DHQ is dehydrated to 3-dehydroshikimic acid by the dehydroquinase, which is finally reduced to shikimic acid by the shikimate dehydrogenase. The next enzyme involved is the shikimate kinase, which catalyzes the ATP-dependent phosphorylation of shikimate into shikimate 3-phosphate. The shikimate 3-phosphate is coupled with phosphoenolpyruvate to give the 5-enolpyruvylshikimate-3-phosphate in a reaction catalyzed by the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. Then, the 5-enolpyruvylshikimate-3-phosphate is transformed into chorismate by a chorismate synthase, and chorismate gives rise to prephenate and arogenate in two reactions, the order of which has not yet been established, catalyzed by the chorismate mutase and the prephenate aminotransferase, respectively, using glutamate as the nitrogen source. Phenylalanine and tyrosine are finally produced from arogenate in reactions catalyzed by the arogenate/prephenate dehydratase (ADT/PDT) and arogenate/prephenate dehydrogenase (ADH/PDH), respectively ([Maeda and Dudareva, 2012](#); [Vanholme et al, 2012](#)). Genes encoding enzymes of this pathway have been described in a more or less reduced number of plant species, allowing the search for orthologs in maize. The carbon flow in the shikimate pathway, the regulations of the shikimate pathway genes, and the phenylalanine (and tyrosine) supply to PAL (and TAL) enzymes, may thus directly affect biosynthesis of phenylpropanoid compounds, including monolignols. For the shikimate pathway, 28 genes were found in the maize genome, but all the members of each multigene family are probably not involved in aromatic amino acid supply to the monolignol pathway ([Supplementary Table 3](#)).

### Genes involved in the monolignol biosynthesis pathway

The first step of monolignol biosynthesis, which occurred downstream the shikimate pathway, is the deamination of L-phenylalanine into cinnamic acid. Successive steps including hydroxylation and methylation on the aromatic ring further lead to the production of the three monolignols which are then exported

to the cell wall and polymerized into lignins. Most genes involved in monolignol biosynthesis belong to small multigene families, with different members possibly involved in different metabolons corresponding to each monolignol biosynthesis and/or to their biosynthesis in each type of lignified tissue. However, not all family members are likely involved in constitutive lignin biosynthesis, and some members probably correspond to biotic or abiotic stress-response lignins. Some members of the upstream part of the pathway could also be specifically involved in the biosynthesis of other phenolic compounds such as suberins or flavonoids. Moreover, lignin pathway enzymes are likely organized as endoplasmic-reticulum-associated multi-enzyme complexes ([Winkel, 2004](#)). The probable different enzymatic complexes should function as different metabolons, each dedicated to the production of the different phenylpropanoid end-compounds ([Winkel, 2004](#)). Each member gene in a multigene family could thus be differentially involved in different metabolons, with differential regulation mechanisms.

The deamination of L-phenylalanine into cinnamic acid is catalyzed by a phenylalanine ammonia lyase (PAL) enzyme. Maize PALs also have a tyrosine ammonia lyase (TAL) activity ([Higuchi et al, 1967](#); [Roesler et al, 1997](#)), catalyzing tyrosine deamination into pCA. Ten PAL genes are present in the maize genome, corresponding to three groups of probably duplicated genes, plus an isolated gene which has a more distant sequence. A one-bp deletion in the second exon of the *ZmPAL* gene, introducing a premature stop codon, has been associated with higher plant digestibility ([Andersen et al, 2007](#)), possibly highlighting a major role of this member in the family. The hydroxylation of cinnamic acid is then catalyzed by a cinnamate 4-hydroxylase (C4H, two genes) and the resulting *p*-coumaric acid is then converted into coumaroyl-CoA by a 4-CoA ligase (4CL, two genes). In *Arabidopsis*, the down-regulation of the *4CL1* gene reduced the G unit content in lignins, but did not affect the S unit content ([Lee et al, 1997](#)). This fact strengthened the existence of metabolons with the preferential or specific involvement of different members of each multigene family in the biosynthesis of each monolignol and/or their biosynthesis in given tissues. The conversion of coumaroyl-CoA into caffeoyl-CoA through the formation of shikimate (or quinate) esters involves a hydroxycinnamoyl-CoA shikimate/quininate hydroxycinnamoyl transferase (HCT, two genes) also having a reverse action, and a *p*-coumaroyl-shikimate/quininate 3-hydroxylase (C3H, two genes) grafting the hydroxyl residue on the aromatic ring ([Schöch et al, 2001](#); [Hoffmann et al, 2003](#); [Hoffmann et al, 2004](#); [Mahesh et al, 2007](#); [Shadle et al, 2007](#)). An alternative route towards the 3-hydroxylation of the aromatic ring has been shown in poplar. The heterodimeric C4H/C3H protein complex catalyzes the conversion of *p*-coumaric acid into caf-

feic acid (Chen et al, 2011), allowing the production of caffeoyl-CoA in a reaction catalyzed by a 4CL. The conversion of caffeoyl-CoA into feruloyl-CoA is then catalyzed by caffeoyl-CoA O-methyltransferases (CCoAOMT, five genes), but CCoAOMT4, which is in duplicate position with CCoAOMT5, appeared to be of little importance in constitutive lignification (Guillaumie et al, 2007a; Guillaumie et al, 2007b; Riboulet et al, 2009). Caffeoyl-CoA and feruloyl-CoA are two hub-compounds, towards the synthesis of coniferyl and sinapyl alcohol, and also ferulate derivatives.

Conversions of activated *p*-coumaroyl-, caffeoyl-, and feruloyl-CoA compounds into aldehydes are mainly driven by the ZmCCR1 cinnamoyl-CoA reductase, even if a ZmCCR2 gene is present in the maize genome. Similarly, the reduction of *p*-hydroxy-cinnamaldehydes into alcohols is also mainly catalyzed by the ZmCAD2 cinnamyl alcohol dehydrogenase, while the role of ZmCAD1 is not really understood. These two types of CAD genes and proteins were described based on investigations in eucalyptus. EgCAD1-type proteins are short-chain alcohol dehydrogenases (Jornvall et al, 1995; Goffner et al, 1998), which are active as monomers (Hawkins and Boudet, 1994). EgCAD2-type proteins are zinc-containing long-chain alcohol dehydrogenases active as dimers (Jornvall et al, 1987; Hawkins and Boudet, 1994). An EgCAD1-type CAD activity has been described in maize by Kanazawa et al (1999), while an EgCAD1-type enzyme was proven to be involved in the synthesis of coniferyl alcohol in tobacco cell wall (Damiani et al, 2005). The major roles of both ZmCCR1 and ZmCAD2 in the two last steps of monolignol biosynthesis are highlighted by the effects of corresponding mutants. ZmCAD2 mutations have been associated with the maize brown-midrib *bm1* phenotype, inducing higher cell wall degradability, lower lignin content, an incorporation of aldehydes in the lignin polymer, and no change in the syringyl/guaiacyl (S/G) ratio (Halpin et al, 1998; Barrière et al, 2004c; Chen et al, 2012b; Barrière et al, 2013). Maize CAD down-regulated RNAi plants, which did not present the brown-midrib phenotype, nor changes in stem lignin content, were however shown to be more degradable, with an improved cellulosic bioethanol production (Fornalé et al, 2012). Even if similarly no brown-midrib phenotype was shown, a transposon-tagging mutation in the ZmCCR1 gene also induced reduced lignin content and higher cell wall degradability. In addition, H units were released in lower amounts from the ZmCCR1 mutant plants compared with the normal ones, with simultaneously an increase in the S/G ratio in mutants (Tamasloukht et al, 2011). Finally, other members of the CCR and CAD families could correspond to genes mainly involved in defense processes, which are likely able to partially compensate the impaired enzymatic activities in CCR1 and CAD2 mutant plants.

Ferulate 5-hydroxylase (F5H) catalyzes the 5-hy-

droxylation of coniferaldehyde (and to a lesser extent, coniferyl alcohol) into 5-hydroxyconiferaldehyde (5-hydroxyconiferyl alcohol, respectively). Two F5H genes are present in maize genome, F5H1 with a strong expression in maize stalks and F5H2 mostly expressed in roots (Guillaumie et al, 2007a; Riboulet et al, 2009). 5-Hydroxyconiferaldehyde is methylated into sinapaldehyde by the caffeic acid O-methyltransferase (COMT), which is the only gene of the maize monolignol pathway that does not belong in maize to a small multigene family. The COMT enzyme has a much greater affinity for the 5-hydroxyconiferyl aldehyde than for the alcohol (Li et al, 2000; Parvathi et al, 2001; Louie et al, 2010), and despite its denomination, the COMT enzyme has no *in vivo* activity on caffeic acid (Davin et al, 2008). The importance of the maize COMT gene in lignin biosynthesis was previously established from mutant or transformed plant investigations, and from association studies. The brown-midrib *bm3* mutation, which occurred in the COMT gene, first induced a reduced COMT activity and reduced lignin content (Grand et al, 1985; Vignols et al, 1995; Guillaumie et al, 2008). Plants with the *bm3* mutation also had greatly improved cell wall degradability, and reduced S/G ratio with a reduction to 40 % of S units released after thioacidolysis with significant incorporation of 5-hydroxy-coniferaldehyde (Kuc and Nelson, 1964; Barrière et al, 2004c). Similar results were shown in COMT down-regulated plants (Piquemal et al, 2002; He et al, 2003; Pichon et al, 2006).

Biosyntheses of coniferyl and sinapyl alcohols are possibly based on two different preferential routes starting from caffeoyl-CoA, at least in several plant species (Guo et al, 2001; Parvathi et al, 2001; Lee et al, 2011b). Coniferyl alcohol likely mostly originates from a synthesis of coniferaldehyde after a methoxylation of caffeoyl-CoA into feruloyl-CoA. Even if, depending on the species, a variable part of syringyl alcohol could also derive from the same pathway, syringyl alcohol could be produced from a first CCR-catalyzed reduction of caffeoyl-CoA into caffeoyl aldehyde, and then a methoxylation on this last compound. This methoxylation of caffeoyl aldehyde into coniferaldehyde has been considered to be catalyzed by the caffeic acid O-methyltransferase (COMT) in several studies on dicotyledonous plants (Li et al, 1997; Guo et al, 2001; Parvathi et al, 2001; Chen et al, 2006; Do et al, 2007; Lee et al, 2011b; Zhao and Dixon, 2011; Gray et al, 2012). The route duality and the involvement of COMT in a methoxylation step other than on 5-hydroxyconiferaldehyde have not been established in grasses. However, since the disruption of the COMT gene in maize *bm3* mutants did not completely prevent the synthesis of syringyl alcohol, an alternative methoxylation pathway should exist in maize. On the contrary, in the *Arabidopsis AtOMT1* mutant, the lignin content in S units is reduced to a value close to zero (Goujon et al, 2003). Because CCoAOMT en-

zymes have a strict affinity for CoA-esters (Martz et al, 1998; Meng and Campbell, 1998; Parvathi et al, 2001), they cannot be considered as candidates involved in 5-hydroxy-coniferaldehyde methoxylation. Conversely, several ZRP4-like OMT are expressed in lignifying tissue of maize stems (Guillaumie et al, 2007a,b). Consequently, their role is very likely not limited to methylation of suberin sub-unit precursors in plant roots as initially described (Held et al, 1993). At least one ZRP4-like OMT could thus contribute to methoxylate the C5 position of the phenolic ring during monolignol biosynthesis in maize. In agreement with this hypothesis, the expression of two ZRP4-like OMT was increased by nearly two fold in *bm3* young and silking plants (Guillaumie et al, 2007a; Guillaumie et al, 2008).

### Genes involved in monolignol transport and polymerization

After their biosynthesis, the three monolignols are sequestered into vacuoles as 4-O-glucosides, while monolignol aglycones are transported across membranes (Lim et al, 2005; Escamilla-Trevino et al, 2006; Miao and Liu, 2010; Alejandro et al, 2012; Liu, 2012; Vanholme et al, 2012). Maize genes were searched for as the orthologs of *Arabidopsis* uridine-diphosphate-glucosyltransferases (UGT), involved in glucosylation of coniferyl and sinapyl alcohols and strongly expressed in lignifying tissues (Lim et al, 2005; Lanot et al, 2006). Complementarily, orthologs of pine and *Arabidopsis*  $\beta$ -glucosidases ( $\beta$ -Glu45 and  $\beta$ -Glu46), which encode proteins involved in the release of monolignol aglycone from its glucosidic form at the cell wall with narrow specificity towards the three monolignol glucosides (Dharmawardhana et al, 1995; Escamilla-Trevino et al, 2006), were considered as involved in the corresponding maize monolignol metabolism. ABC transporters are involved in the transport of monolignols across membranes (Sanchez-Fernandez et al, 2001; Samuels et al, 2002; Ehling et al, 2005; Miao and Liu, 2010; Kaneda et al, 2011; Liu, 2012), a fact that was recently supported by the identification of *AtABCG29* as a gene encoding for an ABC *p*-coumaryl alcohol transporter in rice (Alejandro et al, 2012). The *AtABCG29* protein appeared with a great specificity to *p*-coumaryl alcohol, but its mutation nevertheless induced significant and mostly unexplained modifications in all lignin constituents. Other (ABC) monolignol transporters have thus to be evidenced, such as orthologs of eucalyptus ABC transporters expressed in xylem tissues (Rengel et al, 2009). In addition, out of the two maize mostly expressed ABC transporters in plantlets (Guillaumie et al, 2007a), one was significantly under-expressed in *bm2* plantlets, and could therefore be supposed to be preferentially involved in coniferyl alcohol transport (Guillaumie et al, 2007b).

Condensation of monolignols into the lignin polymer occurs via combinatorial radical-radical coupling

reactions (Freudenberg, 1959; Ralph et al, 2008a; Vanholme et al, 2012), despite the fact that it has also been considered to occur through an ordered radical coupling driven by dirigent proteins (Davin and Lewis, 2000; Davin et al, 2008). According to Vanholme et al (2012), and considering that radical coupling is a chemically driven process, independent of control by any protein, "any phenolic molecule entering the cell wall region and having the proper chemical kinetic, thermodynamic radical-generation, and cross-coupling propensities can couple into the lignin polymer". This fact helps explain the diversity of monomers that has been currently seen in the lignin macromolecules. In any case, the coupling modes leading to the structure and geometry of lignin polymers are primarily influenced by the polysaccharidic matrix in which tissue lignification occurs. This so called "template effect" is supported by experimental data obtained for gymnosperm and dicotyledonous angiosperm woods (Lapierre et al, 1991; Aimi et al, 2005; Lawoko et al, 2005). Such information is not available for grass cell walls, but a similar situation seems likely.

While class III peroxidases have long been considered as the unique class of oxidases involved in lignin polymerization, EST sequencing and expression studies based on lignifying tissues, and mutant investigations, have shown that both laccases and peroxidases are involved in cell wall lignification (Boudet, 2000; Nielsen et al, 2001; Boerjan et al, 2003; McCaig et al, 2005; Cai et al, 2006; Sasaki et al, 2006; Sato and Whetten, 2006; Tokunaga et al, 2009; Fagerstedt et al, 2010; Berthet et al, 2011). Class III peroxidases and laccases belong to multi-gene families, and consequently, redundancy in their activity has often been suspected. However, the importance of oxidase redundancy is greatly reduced by the fact that many peroxidases or laccases have specific spatio-temporal expression patterns. When considering genes expressed in maize vascular and lignifying tissues, and orthologs of *Arabidopsis* genes expressed in lignifying stems (de Obeso et al, 2003; Bakalovic et al, 2006; Caparros-Ruiz et al, 2006; Guillaumie et al, 2007a; Andersen et al, 2009; Barrière et al, 2009; Riboulet et al, 2009), only five peroxidase and fourteen laccase genes were currently considered in the maize genome. Whether all the latter genes are effectively involved in constitutive lignification, and whether some other members are still unidentified, especially for peroxidases, is not known. However, several investigations suggested that only a few members would be involved in secondary wall assembly. This fact is more likely a consequence of a regulated spatio-temporal expression of peroxidase and laccase genes, rather than a specificity of several family members towards monolignols. In addition, sinapyl alcohol is far more rapidly oxidized in the presence of *p*-coumarate, which is then oxidized by peroxidases and transfers the radical to sinapyl alcohol (Boudet, 2000; Hatfield et al, 2008).



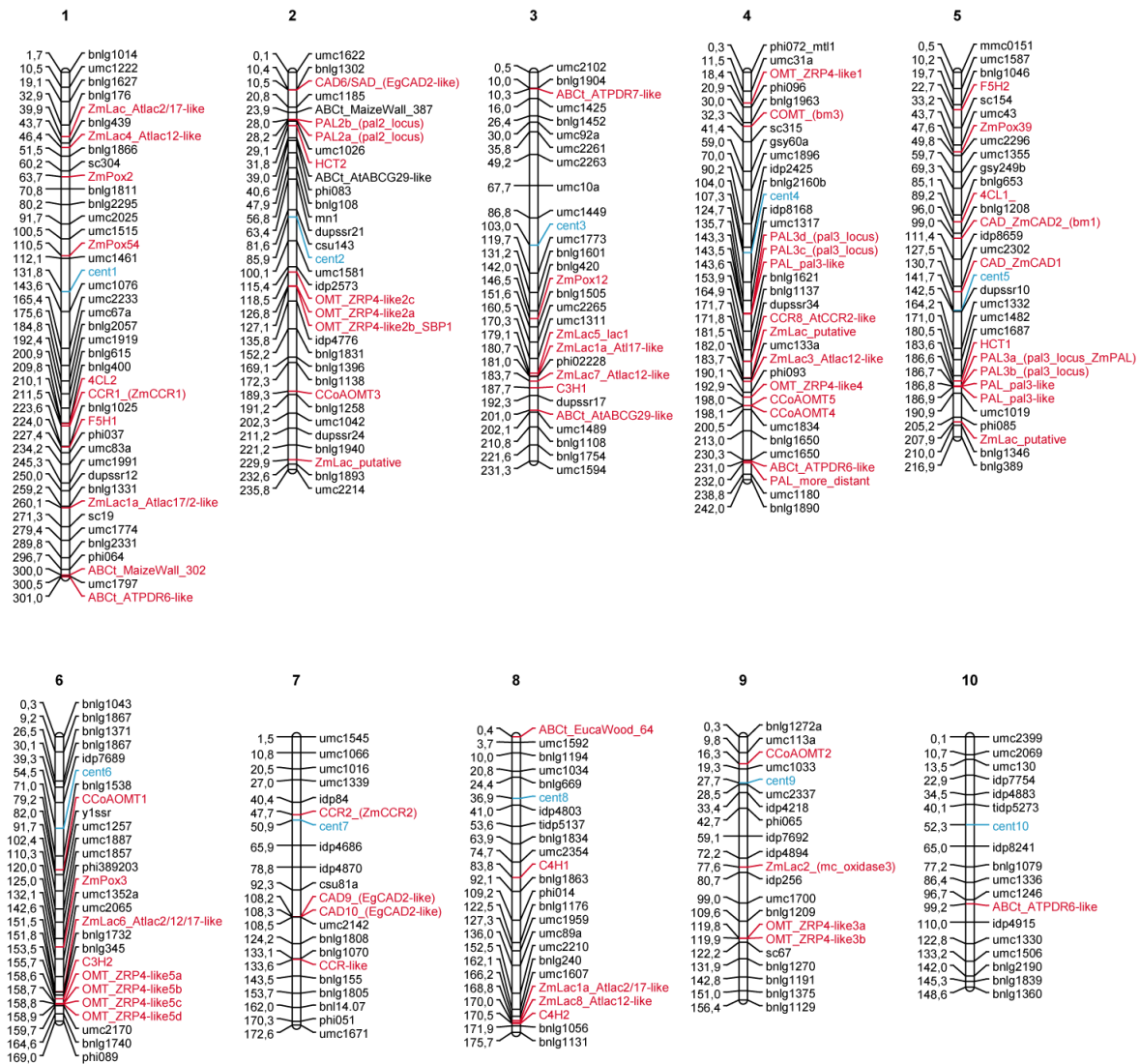


Figure 1 - Physical map of maize genes related to monolignol biosynthesis, transport, and polymerization.

Down-regulation of the tobacco peroxidase TP60 led to plants with lignin reductions of 20 and up to 40-50% of control plants. In the down-regulated line with the most robust changes in lignin content through several generations, plants have thin cell walls and limited secondary wall thickening with an abnormal S2 layer (Blee et al, 2003; Kavousi et al, 2010). Similar results were shown in aspen after deregulation of the PrxA3a peroxidase (Li et al, 2003b). In addition, a MITE insertion disrupting the *ZmPox3* gene was associated with higher cell wall degradability in a set of related maize European flint lines (Guillet-Claude et al, 2004), whereas *ZmPox2* was considered to be involved to a greater extent than *ZmPox3* in maize vascular vessels and epidermis lignification (de Obeso et al, 2003). While the lignin content was not changed, the down-regulation of the poplar *Lac3* laccase gene induced an important alteration of xylem

fiber cell walls, with an increase in soluble phenolic compounds (Ranocha et al, 2002). In *Arabidopsis*, laccase *AtLac4* and *AtLac17* double mutants had lignin content that were 35% lower than in control plants, with higher saccharification yields, while the reduction was nearly 13% in single mutants (Berthet et al, 2011). Over-expression of a cotton laccase in poplar plants induced an increase in lignin content in all tested transgenic lines in varying degrees, but as high as 21.5% (Wang et al, 2008). Observed effects on lignin content in (double) peroxidase or laccase mutants or transformants were thus of the same order of magnitude as those observed with monolignol genes, such as in the maize *bm3/COMT* mutant, and even higher than in the maize *bm1/CAD* mutant.

Considering the different steps involved in monolignol biosynthesis, transport, and polymerization (Figure 1 and Supplementary Table 4), 74 genes were



shown in the maize genome. The genes corresponding to the different enzymatic activities are scattered throughout the whole genome, while conversely clusters of paralogs, which likely correspond to gene duplications, were shown for PAL, ZRP4-like OMT, and laccase genes.

### Genes involved in the S-adenosyl-L-methionine cycles

The methylation of lignin precursors by SAM-dependent O-methyltransferases (S-adenosyl-L-methionine, SAM or AdoMet) consumes large amounts of methyl groups (Van der Mijnsbrugge et al, 2000). The formation of SAM from methionine and ATP is catalyzed by an S-adenosyl-methionine synthetase (SAMS). SAM-dependent transmethylation reactions release S-adenosyl-homocysteine (SAH or AdoHcy), which is a strong competitive inhibitor of COMT and CCoAOMT enzymes (Ravanel et al, 1998; Kocsis et al, 2003). SAH is thus promptly recycled into homocysteine and adenosine by an S-adenosyl-homocysteine hydrolase (SAHH) while an adenosine kinase (ADK) mediates the recycling of adenosine into adenosine monophosphate (Ranocha et al, 2000; Ranocha et al, 2001; Moffatt et al, 2002). The methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-methylTHF), a cosubstrate for homocysteine remethylation to methionine (Goyette et al, 1994). The methionine synthase (MS) then catalyzes the synthesis of methionine from homocysteine with the supply of the methyl radical from 5-methylTHF conversion into THF. The SAM pool is also regulated by the S-methylmethionine (SMM) "futile" cycle (Ranocha et al, 2001), with the synthesis of SMM by a methyl transfer from SAM to methionine catalyzed by a S-adenosylmethionine:methionine S-methyltransferase (MMT) and the release of SAH. SMM is reconverted to methionine by transferring a methyl group to homocysteine, in a reaction catalyzed by a homocysteine S-methyltransferase (HMT). The set of inter-dependent methionine-related cycles might therefore significantly impact the efficiency of methylation reactions in the lignin pathway and correlatively the quantity of lignins, the S/G ratio, and the ferulate contents. In lignifying tissues, PAL, CCoAOMT, and COMT expression profiles were thus highly correlated with SAMS and HMT profiles in maize or eucalyptus (Vincent et al, 2003; Kirst et al, 2004; Guillaumie et al, 2007a). Up until recently, no data had shown that methyl group availability could be a limiting factor in monolignol biosynthesis. However, the maize *bm2* mutation, giving plants with lower lignin content and lower G content in lignins (Chabbert et al, 1994; Barrière et al, 2004c), was shown to occur in a MTHFR gene (Tang, 2011). Because the methionine pathway is located upstream to coniferyl and syringyl alcohol biosynthesis, a similar effect on G and S lignin unit of the MTHFR mutation was nevertheless expected.

Considering the the S-adenosyl-L-methionine cycles, 16 genes were shown in the maize genome (Supplementary Table 5). Based on physical positions, there does not appear to be any tendency to clustering of methyltransferase genes and S-adenosyl-L-methionine cycle genes.

### Genes involved in p-hydroxycinnamate acylation and transfer onto cell wall components

The acylation of S units in grass lignin occurs at the monolignol level and before the transfer of S-pCA in the cell wall space (Ralph et al, 1994; Lu and Ralph, 1999; Grabber and Lu, 2007; Martinez et al, 2008). This process of *p*-coumaroylation is therefore dependent upon the production of the activated *p*-coumaroyl-CoA (pCA-CoA) which is a key-compound in the phenylpropanoid pathway, and upon specific transferase(s) acylating sinapyl alcohol with an activated *p*-coumaric acid. The corresponding acyltransferase(s) was shown to use an activated acid (*p*-coumaroyl-CoA) to form the corresponding sinapyl *p*-coumarate (Hatfield et al, 2009), and a candidate enzyme with higher affinity towards sinapyl alcohol than towards coniferyl alcohol was also described (Martinez et al, 2008; Hatfield et al, 2009). The rice gene *Os01g18744* (OsPMT, *Oryza sativa p*-coumarate monolignol transferase), which belongs to the BAHD acyltransferase family, was recently shown to encode an enzyme catalyzing the acylation of monolignols via the activated *p*-coumaroyl-CoA (Withers et al, 2012). Moreover, this gene was specific to grass species, and co-expressed in rice with genes of the monolignol pathway (Mitchell et al, 2007). A set of OsPMT orthologs were shown in maize, with two close orthologs and several other orthologs that were also previously found as orthologs of genes putatively involved in arabinoxylan feruloylation.

Results obtained with the maize *bm3* mutant strongly suggest that FA is not biosynthesized by a COMT-catalyzed methylation of a caffeic precursor. The disruption of the COMT gene does not affect the FA content of mutant plants (Barrière et al, 2004c), even if the lower lignin content in the *bm3* mutant may increase the yield of alkali-releasable FA (Grabber et al, 2000). As was shown for S unit acylation by the *p*-coumarate, the formation of feruloylated compounds does not occur at the free acid level, but involve conjugates of the ferulic acid which are likely CoA-esters (Fry et al, 2000). Corroborating this fact, a putative feruloyl-CoA-arabinoxylan-trisaccharide O-hydroxycinnamoyl transferase activity (Yoshida-Shimokawa et al, 2001) has been found in suspension-cultured rice cells fed feruloyl-CoA and arabinoxylan-trisaccharide (AXX), allowing the formation of feruloyl arabinoxylan-trisaccharide (FAXX). A reaction between feruloyl-CoA and UDP-arabinose was also considered, giving a FA-Ara-UDP intermediate which could be transferred to the arabinoxylan chain in a reaction catalyzed by a feruloyl-arabinose-

UDP transferase (Buanafina, 2009). Finally, another even less probable hypothesis for ferulate biosynthesis in grasses has been considered from investigations in the *Arabidopsis* REF1 mutant plants, which have a reduced content in soluble sinapate esters. This mutant is affected in a sinapaldehyde dehydrogenase gene and the REF1 protein exhibited *in vitro* both sinapaldehyde and coniferaldehyde dehydrogenase activities (Nair et al, 2004). The formation of free sinapic and probably ferulic acids in *Arabidopsis* is thus catalyzed via the oxidation of the corresponding cinnamaldehydes. In maize, three mitochondrial and two cytosolic ALDH (ALDH2C and 2D) orthologs of the REF1 gene have been described (Skibbe et al, 2002; Nair et al, 2004). However, their physiological role has not yet been determined. It is indeed not yet known if such an ALDH pathway is functional in maize and grasses for the cell wall linked ferulate metabolism.

Genes involved in arabinoxylan feruloylation were thus tentatively identified as acyltransferase (AcT) encoding genes specifically expressed in grasses in contrast to dicotyledons in which this particular function is supposed to be missing (Mitchell et al, 2007). One of the most differentially expressed groups of grass genes included members of the Pfam family PF02458 encoding CoA-dependent AcT including hydroxycinnamyl transferases. A study of gene deregulation in rice (Piston et al, 2010) supported the involvement of these putative feruloyl-transferases. Rice plants with an individually reduced expression of four members of this family had a reduced content of ester-linked ferulate in leaves and/or stems. As previously cited, maize orthologs of the rice PF02458 genes putatively involved in arabinoxylan feruloylation were for some of them the same as those found as orthologs of the rice gene involved in *p*-coumaroylation of S units. Acyltransferases enzymes share several conserved domains, a fact that could partly explain the close paralogs found in the maize genomes (D'Auria, 2006). Only 14 genes were shown in the maize genome (Supplementary Table 6) to be involved in *p*-hydroxycinnamate acylation and transfer onto cell wall components. This list is probably not exhaustive, and new genes or families are yet to be discovered.

Based on investigations in a transposon-tagging progeny of 12,000 plants, the *sfe* mutant (M04-21) was shown to have a low ferulate-ether phenotype in both leaves, sheathes, and stems, together with a lower lignin content (Jung and Philips, 2010). Moreover, dairy cows fed M04-21 *sfe* silage, which had similar lignin content but a 30% lower etherFA content than the control line, had a greater intake (+1.5 kg day<sup>-1</sup>) and a higher milk yield (+2.3 kg day<sup>-1</sup> FCM 3.5%) than cows fed the control W23 silage (Jung et al, 2011). The gene underlying the *sfe* mutation is not yet known, but its discovery would be one of the best ways to track specific mechanisms involved in lignin

and feruloylated arabinoxylan cross-linking. In addition, breeding for divergent ester-linked diferulate (diFA) concentration in maize stalk pith tissues induced, after two cycles of divergent selection, a 16% difference in diFA content between the two selected populations, with significant effect on rumen cell wall degradability (Barros-Rios et al, 2012). This significant diFA variation after breeding suggested that diFA deposition in maize pith parenchyma cell walls is a highly heritable trait. However, the corresponding involved genes are still unknown, all the more so given that divergent selection affected esterified and etherified FA contents differently, supporting the hypothesis that the metabolisms of these cell wall components are separately regulated (Barros-Rios et al, 2012).

Finally, reducing arabinosyl transferase activities would seem to be a relevant strategy for reducing ferulate cross-linkages in the walls. The ferulic acid is esterified to the  $\alpha$ -1,3 linked arabinofuranose. Rice *xax1* mutant plants are deficient in feruloylated arabinosyl residues on xylan chains. The *xax1* mutant plants exhibit an increased extractability of xylan and increased saccharification, probably as a consequence of a lower degree of diferulic cross-linkages (Chiniquy et al, 2012). Other genes compromise the grafting of arabinose on xylan chains, and consequently the ferulate cross-linkages, including UDP-arabinopyranose mutase (Konishi et al, 2011), may be considered as targets for increased saccharification in biofuel generation processes.

### Genes involved in regulation of phenylpropanoid biosynthesis and deposition

Genes involved in the regulation of monolignol biosynthesis have been described in different species, but little is currently known for maize or grasses. The transfer of data and knowledge related to tissue patterning and lignification from dicotyledons or gymnosperms to grasses is difficult due to the vascular specific traits in grasses. In contrast to dicotyledonous plants, the vascular system of non-woody monocotyledons is characterized by the absence of bifacial cambium and secondary growth. Monocotyledon lignification proceeds from an intercalary meristem in each internode, with vascular bundles scattered, penetrating radially and present in medulla and cortex (Terashima and Fukushima, 1993; Tomlinson, 1995). However, the emergence and evolution of lignified tracheids and vascular tissues was based for all vascular plants on the expression of a preexisting poly-phenolic pathway (Boyce et al, 2003), with lignin targeted deposition in different cell types. In addition, many results obtained in lignification genetics and genomics illustrated a large commonality in genes involved in cell wall carbohydrate and phenolic biosynthesis in all plant species. Orthologs of factors regulating lignin-related gene expression in woody species are therefore likely candidate genes for reg-

ulation of maize lignin biosynthesis and deposition, even if the targets have possibly changed during plant evolution. Moreover, with the possible selection of grass specific genes, as has been shown for genes involved in arabinoxylan biosynthesis, specific grass transcription factors might have emerged during plant speciation and evolution.

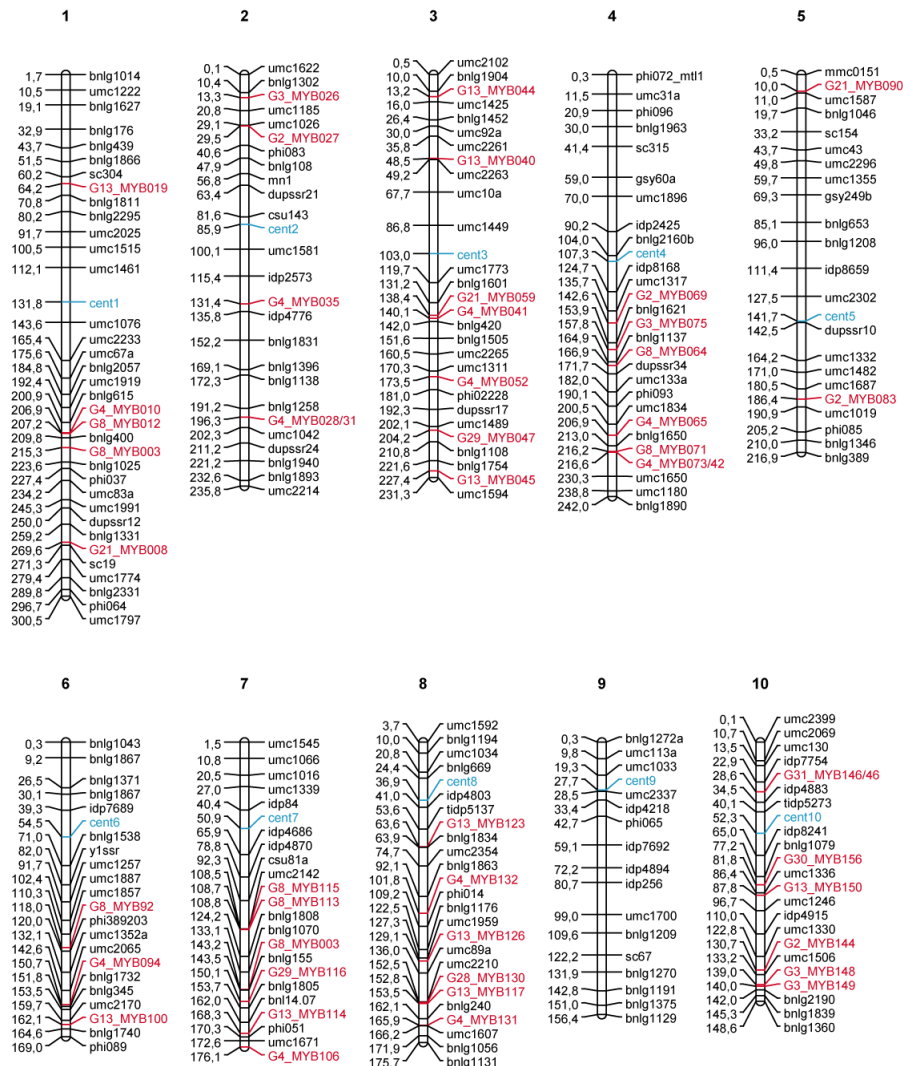
### MYB transcription factors

The regulation of phenylpropanoid gene biosynthesis was the first role identified for a plant R2R3-MYB transcription factor (Paz-Ares et al, 1987), which was first illustrated by the heavily reduced lignin content in mature parts of tobacco plants over-expressing an *Antirrhinum* MYB factor (Tamagone et al, 1998). R2R3-MYB genes recognize AC cis-regulating elements which are present in promoters of many phenylpropanoid genes (Sablowski et al, 1994; Peter and Neale, 2004), even if other interaction mechanisms also exist (Uzal et al, 2008). Other pathways are also regulated by R2R3-MYB, and based on aspen data, only 12% of the R2R3-MYB encoding genes showed the highest level of transcript abundance in differentiating xylem (Wilkins et al, 2009).

Only ZmMYB31, ZmMYB42, and ZmMYB46 have been proven to be related to the secondary wall formation in maize. ZmMYB31 and ZmMYB42 both have a repressive effect on the expression of several genes of the lignin pathway (Fornalé et al, 2006; Sonbol et al, 2009; Fornalé et al, 2010; Gray et al, 2012), while ZmMYB46 has an activator effect on secondary wall biosynthetic genes (Zhong et al, 2011). Similarly, PvMYB4, orthologous to ZmMYB42, was shown to have a repressive effect on lignin pathway genes, resulting in reduced lignin and pCA content in *Panicum virgatum* plants over-expressing this gene (Shen et al, 2010). Other MYB factors putatively involved in the regulation of maize lignification have been searched for as orthologs of lignin-related R2R3 MYB genes described in eucalyptus [EgMYB1, (Legay et al, 2007; Legay et al, 2010), EgMYB2 (Goicoechea et al, 2005)], poplar [PtMYB4, PttMYB21 or PtrMYB021 (Patzlaff et al, 2003; Karpinska et al, 2004; Wilkins et al, 2009)], pine [PtMYB1, PtMYB8 (Bomal et al, 2008)], barley [MYB hv5 and hv33, (Wissenbach et al, 1993)], and *Arabidopsis* [AtMYB46, AtMYB83, (Zhong et al, 2007; Zhong and Ye, 2012) AtMYB4, AtMYB7, AtMYB32 (Zhong and Ye, 2009; Zhou et al, 2009; Zhong and Ye, 2010; Zhong et al, 2010; Zhong and Ye, 2012), AtMYB20, AtMYB58, AtMYB63, AtMYB85 (Zhong et al, 2007; Zhou et al, 2009; Ohman et al, 2013), AtMYB52, AtMYB54, AtMYB69 (Zhong et al, 2008), AtMYB61 (Newman et al, 2004), AtMYB75 (Bhargava et al, 2010), and AtMYB103 (Ohman et al, 2013)]. In addition, a large overview of the R2R3-MYB gene family in maize has been recently proposed, with a comprehensive classification of all family members, including subgroups involved in regulation of lignified cell wall biosynthesis and deposition (Du et al, 2012). Maize

MYB orthologous to AtMYB4, which was shown to be a negative regulator of lignin gene expression, were gathered in the G4 subgroup "phenylpropanoid pathway" (Du et al, 2012). This group included the two ZmMYB31 (GRMZM2G050305) and ZmMYB42 (GRMZM2G419239) genes, and members of this group are also orthologs of EgMYB1 and hv5 MYB. ZmMYB31 was shown with a stronger repressing effect on COMT expression than ZmMYB42, but ZmMYB42 also negatively regulated the expression of several genes of the lignin pathway. Moreover, the over-expression of the ZmMYB42 gene in *Arabidopsis* plants generated a lignin polymer with a decreased S/G ratio due to a lower content in S units (Fornalé et al, 2006; Sonbol et al, 2009). Orthologs of AtMYB58 and AtMYB63, which are known for their activating role in lignin biosynthesis (Zhou et al, 2009), were gathered in the G3 subgroup "lignin biosynthesis", and in the G2 subgroup with genes considered to be involved in "defense" processes. Orthologs of AtMYB85, which is involved in cell wall thickening and lignin deposition (Zhong et al, 2008), were classified in the G8 subgroup "lignin deposition". Similarly, orthologs of AtMYB52, AtMYB54 and AtMYB69, which also have a role in cell wall thickening and lignin biosynthesis in *Arabidopsis* (Zhong et al, 2008), were gathered in the G21 subgroup. EgMYB2, AtMYB46, AtMYB83, which were shown to be transcriptional activators of lignification, and the only ZmMYB146 maize ortholog were classified by Du et al (2012) in the G31 "metabolism" subgroup, together with PtMYB4 [ZmMYB146 (or GRMZM2G052606, bin 10.03) is also named ZmMYB46 by Zhong et al (2011)]. The latter subgroup of AtMYB46/EgMYB2 genes was unexpectedly not related to any lignification process by Du et al (2012), despite their well known effect as an activator of secondary wall biosynthesis. In *Arabidopsis*, the two AtMYB46 and AtMYB83 genes, together with their NAC regulators and their direct targets, were indeed shown to be master genes of the secondary wall assembly. This set of genes regulates an array of downstream genes and thereby activates the secondary wall lignin and carbohydrate biosynthetic programs, in a multileveled feed-forward loop regulatory structure (Zhong and Ye, 2012). Other ZmMYB were considered as putative orthologs of AtMYB61, a gene that produced ectopic lignification when overexpressed in *Arabidopsis* plants (Newman et al, 2004). The latter were classified in the G13 "metabolism" subgroup (Du et al, 2012), which included the hv33 MYB gene expressed in lignifying tissue of barley (Wissenbach et al, 1993). In addition, the ZmMYB130 gene was the only maize MYB belonging to the G28 subgroup "phenylpropanoid pathway", and the closest *Arabidopsis* ortholog of ZmMYB130 is AtMYB5, a gene involved in anthocyanin metabolism. The AtMYB75 gene, also assigned to the G6 "anthocyanin biosynthesis" subgroup by Du et al (2012), was shown to have a role in stem lignifica-





**Figure 2** - Physical map of maize MYB genes putatively involved in maize secondary wall assembly [ZmMYB numbers and groups are given according to Du et al (2012)].

tion (Bhargava et al, 2010). It was thus considered to be "the first transcription factor reported so far that functions as a repressor of the entire secondary cell wall program" in *Arabidopsis* (Zhao and Dixon, 2011). In fact, while AtMYB75 was shown to be a positive regulator of anthocyanin accumulation, its physical interaction with KNAT7 was shown to increase the repressive effect of KNAT7 on secondary wall deposition (Bhargava et al, 2013). It is still unclear whether an ortholog of AtMYB75 exists or not in maize, but this group could be absent in grass genomes (Du et al, 2012). On the contrary, AtMYB103 was shown to positively and specifically regulate F5H expression in the lignin pathway, even if other roles in the cell wall assembly could not be ruled out (Ohman et al, 2013). The only maize ortholog of AtMYB103, located in bin 10.03, is also the only maize one belonging to the G30 group (Du et al, 2012), with genes involved in

"cell wall thickening". Unexpectedly, most *Arabidopsis* MYB genes described as involved in the regulation of secondary wall assembly are activator factors, while most of those currently described in maize are repressor factors. Finally, 45 MYB transcription factors putatively involved in the secondary wall assembly were thus found in the maize genome (Figure 2, Supplementary Table 7). This list is possibly incomplete because most of them were highlighted as orthologs of non-grass plant MYB.

### NAC transcription factors

Lignin and cell wall genes are regulated upstream the MYB transcription factor level. The first demonstrations that NAC transcription factors were involved in secondary wall assembly were likely the roles of NST1 and NST2 (NAC SECONDARY WALL THICK-



ENING PROMOTING FACTORS) in secondary wall thickening (Mitsuda et al, 2005) and the roles of VND6 and VND7 (VASCULAR-RELATED NAC-DOMAIN) in vessel xylem formation (Kubo et al, 2005). Over-expression of NST1, NST2 and SND1 thus induced ectopic lignified secondary cell wall thickenings in various *Arabidopsis* tissues (Mitsuda et al, 2005; Ko et al, 2007; Mitsuda et al, 2007; Zhong et al, 2006). Later, different *Arabidopsis* NAC proteins were shown to be master actors regulating the expression of several transcription factors (including MYB and other NAC genes) and/or genes involved in secondary cell wall biosynthesis (Zhong and Ye, 2007; Yamaguchi et al, 2008; Zhong et al, 2008; Zhong and Ye, 2009; Zhou et al, 2009; Zhong et al, 2010). NAC factors thus include NST 1-3 (with NST3 = SND1) which are specific to fibers, and the VND 1-7 which are specific to vascular vessels (Grant et al, 2010; Grima-Pettenati et al, 2012). Both NST and VND factors belong to the same NAC subfamily (Yamaguchi, 2010) and function as transcriptional activators. In addition, VNI2 (VND-INTERACTING2, and to a lesser extent VNI1) is a transcriptional repressor of vessel-specific genes regulated by VND7 (Yamaguchi et al, 2010). Interactions mostly occur between VNI2 and VND7, but also exist to a lesser extent with other VND proteins and possibly other NAC factors. In maize, four orthologs of *Arabidopsis* master NAC (ZmSWN2, 3, 6, and 7) were investigated and additional analysis has demonstrated that the latter ZmSWNs were functional orthologs of SND1 capable of activating the secondary wall biosynthetic program (Zhong et al, 2011). In addition, ZmSWN7 corresponds in fact to two closely duplicated genes in positions 28.04 and 28.14 Mbp on chromosome 9. Based on this set of data, 38 secondary wall NAC genes were shown in the maize, while only 11 were shown by Yao et al (2013), with the same limits as for MYB genes due to the search for non-grass plant orthologs (Supplementary Table 7).

### Partner and target genes of MYB or NAC transcription factors

The class II *KNAT7 Arabidopsis* gene, first described as *IRX11* (Brown et al, 2005), is one of the direct targets of SND1, VND6 and AtMYB46 (Zhong et al, 2008; Ko et al, 2009). The *KNAT7* gene was later shown to be a transcriptional repressor of secondary cell wall biosynthesis, in interaction with OFP1 and OFP4 (OVATE FAMILY PROTEIN 1 and 4, Li et al, 2011b), and AtMYB75 (Li et al, 2012; Barghava et al, 2013). *OFP4* loss-of-function mutants also have an irregular xylem phenotype and thinner interfascicular fiber cell walls (Li et al, 2011b). *Arabidopsis* *KNAT7* was considered to be involved in "a negative feedback loop within these regulatory networks governing secondary cell wall biosynthesis, working antagonistically to NAC and MYB positive regulators" (Li et al, 2012). In poplar, the class-I KNOX homeobox gene, ARBORKNOX2 (ARK2), which is ortholog

to BREVIPEDICELLUS (or KNAT1) in *Arabidopsis*, is involved in terminal cell differentiation during secondary growth and subsequent lignin and cellulose contents (Du et al, 2009). Protein-protein interactions in the phenylpropanoid metabolism also involve at least basic helix-loop-helix (bHLH), and WD40 proteins (Grima-Pettenati et al, 2012). bHLH transcription factors belong to a protein family for which many different functions have first been identified in animals, including the control of cell proliferation and development of specific cell lineages. In the *Arabidopsis* and rice genomes, 133 and 167 bHLH genes have been shown (Heim et al, 2003; Li et al, 2006), respectively, likely indicating that more than 200 are present in the maize genome. Based on bioanalysis investigations, the rice bHLH proteins can potentially participate in a variety of combinatorial interactions, endowing them with the capacity to regulate a multitude of transcriptional programs related to plant cell and tissue development as well as plant metabolism (Li et al, 2006), with likely only a few members involved in lignified tissue assembly. WD40 proteins are regulatory proteins which contain a domain of nearly 40 amino acids often terminating with tryptophan (W) and aspartic acid (D) These specific traits give them their "WD40" denomination (Ramsay and Glover, 2005). Protein complexes composed of MYB and bHLH transcription factors associated with WD40 proteins have been shown to initiate multiple cellular differentiation pathways in a range of plants (Ramsay and Glover, 2005). Coherent models of the network of interactions that lead to diverse cell fates through the activity of this protein complex were considered as one basis of flexibility in plant morphology, and consequently to have likely played a major role in angiosperm evolution and success. The complex appears to have arisen in the land plant lineage, although its component parts are considerably more ancient (Ramsay and Glover, 2005). It was thus hypothesized that such complexes are also involved in controlling of the regulation of lignin-related R2R3-MYB transcription factors, all the more so given that the role of such complexes has been shown for regulation of anthocyan biosynthesis (Zhao et al, 2008; Brueggemann et al, 2010), including in maize (Cone et al, 1993; Grotewold et al, 2000). As a whole, 28 partners and targets of MYB genes were shown in the maize genome (Supplementary Table 7).

### Zinc finger regulation factors putatively involved in lignified tissue assembly

Zinc finger proteins constitute one of the largest families of transcription factor regulatory proteins. They are involved in many regulations during plant development, including lignified tissue assembly. Zinc-finger C2H2 genes were the most frequently represented transcription factors in eucalyptus secondary xylem libraries (Rengel et al, 2009). The AtC3H14 zinc finger protein has been shown to activate all of the secondary wall phenolics and carbohydrate re-

lated genes tested. Both SND1 and AtMYB46 proteins were shown to bind to the AtC3H14 promoter, and AtC3H14 might function as master regulator of secondary wall biosynthesis, located downstream of AtMYB46 (Ko et al, 2009; Kim et al, 2012). Moreover, an AtC3H14 gene was shown to be a probable candidate gene underlying cell wall degradability QTLs in the Bur0 x Col0 progeny (Chavigneau et al, 2012). In addition, DOF type (DNA-binding with one finger) domain proteins, which are plant-specific zinc finger transcription factors involved as transcriptional activators or repressors in diverse plant growth and development processes (Yanagisawa, 2004; Kushwaha et al, 2011), are also involved in lignified tissue assembly. The high cambial activity of the HCA2 *Arabidopsis* mutant resulted from an elevated expression of a DOF transcription factor (AtDOF34) preferentially expressed in the cambium, phloem, and interfascicular parenchyma cells of stems (Guo et al, 2009). Ectopic lignification was also related to variation in DOF gene expression in *pom1*, *eli1* (ectopic lignification 1) and *det3* (de-etiolated 3) *Arabidopsis* mutants, in addition to expression variation of MYB genes (Rogers et al, 2005). As DOF type zinc finger, C3HC4 type RING zinc finger proteins also have important roles during plant growth and tissue assembly (Ma et al, 2009). Finally, LIM zinc finger proteins can also be involved in the regulation of plant lignification, as shown with the tobacco NtLIM1 acting as a positive regulator of the lignin pathway (Kawaoka and Ebinuma, 2001). LIM proteins are characterized by zinc-binding domains that ligate two zinc ions. Unlike the classical zinc fingers, these domains do not bind DNA, but mediate interactions with other proteins (Matthews et al, 2009). WRKY zinc finger proteins have highly conserved WRKYGQK amino acid sequences in their N-terminal part, followed by the C2H2 or C2HC zinc-finger motifs. WRKY proteins are involved in diverse physiological and developmental processes, especially including defense against biotic stresses (Wei et al, 2012). However, their role in cell wall constitutive lignification has not yet been established (Wu et al, 2005; Guillaumie et al, 2010; Rushton et al, 2010; Tripathi et al, 2012). Nonetheless, the AtWRKY12 (At2g44745) gene was highly expressed in lignifying stems (<http://genecat.mpg.de/database>), and its mutation induced secondary wall formation of pith cell (Wang et al, 2010). Similarly, the grapevine transcription factor WRKY2 was shown to be specifically expressed in cells undergoing lignification in young grapevine stems (Guillaumie et al, 2010). According to the Wei et al (2012) classification of maize WRKY genes, orthologs of VvWRKY2 and AtWRKY12 belonged to subgroups I and IIc, respectively. In addition, the use of artificial zinc finger chimeras, containing either an activation or a repression domain towards the *Arabidopsis* At4CL1 promoter region, resulted in a nearly 30% increase in lignin content with an ectopic lignin distribution, or a nearly

40% decrease in lignin content with a decrease in the S/G ratio, respectively (Sanchez et al, 2006).

### Other genes and regulation factors putatively involved in secondary wall assembly

The *Arabidopsis* COV1 (continuous vascular ring) gene encodes an integral membrane protein of unknown function which is supposed to be involved in a mechanism that negatively regulates the differentiation of stem vascular tissue by a mechanism independent of auxin (Parker et al, 2003). In addition to the firstly described COV1 gene (At2g20120), two COV1-like paralogs were later identified as the LCV2 and LCV3 genes [Like-COV-2 (At1g43130) and Like-COV-3 (At2g18460), TAIR database (<http://arabidopsis.org/>)]. Ten orthologous genes were found in the maize genome, including four, five, and one ortholog for COV1, LCV2, and LCV3, respectively. Colocalizations of cell wall degradability QTLs were shown with seven COV-like genes (out of nine), the only maize ortholog of COV LCV3 being one of the non-colocalizing genes (unpublished data). In addition, one of the LCV2 maize orthologs, located in bin 8.03, was 3.0 times over-expressed in bm3 ear lignifying internodes (Guillaumie et al, 2008), thus possibly illustrating an unknown form of inhibition in lignified tissue formation. The two latter facts likely corroborated the involvement of COV-like genes in maize and grass cell wall assembly.

Members of a small class III homeodomain-leucine zipper family, including AtHB8, AtHB9 (PHAVOLUTA), AtHB14 (PHABULOSA), AtHB15 (CORONA), and IFL1 (REVOLUTA), are expressed in vascular tissues and they have been considered to play regulatory roles in vascular differentiation (Talbert et al, 1995; Ratcliffe et al, 2000; Baima et al, 2001; McConnell et al, 2001; Green et al, 2005). The IFL1 *Arabidopsis* gene has two maize orthologs, the mutants of which have rolled leaf phenotypes (*RLD1* and *RLD2*). The maize *RLD1* gene is regulated by the Zm-miR166 miRNA (Juarez et al, 2004). The expression of the aspen *PtaHB1* gene, which is also orthologous to IFL1, is also inversely correlated with the level of miR166 miRNA (Ko et al, 2006). In addition, interactions between HDZIP III and KANADI gene family members were shown to be involved in the establishment of the spatial arrangement of phloem, cambium and xylem. It was considered that HDZIP III and KANADI transcription factors control cambium activity, with KANADI proteins acting on auxin transport, and HDZIP III proteins promoting axial cell elongation and xylem differentiation (Ilegems et al, 2010). Corroborating this assertion, the down-regulation of the poplar class III HD-ZIP gene *PtrHB7* led to plants displaying significant changes in vascular tissues with a reduction in xylem and an increase in phloem. On the contrary, *PtrHB7* over-expression enhanced differentiation of cambial cells toward xylem cells and inhibited phloem differentiation (Zhu et al, 2013). Transcrip-

tional analysis revealed that genes regulating xylem and phloem differentiations were correspondingly up- or down-regulated (Zhu et al, 2013). The *PtHb7* gene has six close orthologs in maize, including the two *RLD1* and *RLD2* genes. In addition, interactions between NAC and zinc finger homeodomain proteins have been reported (Tran et al, 2006).

The GRAS SCARECROW and SCARECROW-like proteins belong to a plant-specific transcription factor family which contains basic leucine zipper regions and are involved in complex regulatory pathways regulating tissue patterning and differentiation (Di-Laurenzio et al, 1996; Lee et al, 2008). SCARECROW proteins are thus involved in bidirectional cell signaling mediated by miRNA165/166. These proteins interfere with the transcription factor SHORT ROOT (SHR) equally expressed in stem and root, and HDZIP III proteins, towards the control of xylem patterning (Carlsbecker et al, 2010). These genes could be considered as candidates inducing cell wall degradability variation even if they are involved upstream in the pathway.

ROP family of Rho-like GTPases are important signalling proteins during plant growth and tissue differentiation, with very little data related to their role in xylogenesis. A member of the plant ROP family (EgROP1) was shown to be preferentially expressed in the cambial zone and differentiating xylem of eucalyptus (Rengel et al, 2009). Its over-expression in *Arabidopsis* altered vessel formation and fibre growth in secondary xylem, with changes in secondary cell wall thickness, lignin and xylan composition (Foucart et al, 2009). ROP/RAC/RAB genes encode geranylgeranylated GTP-binding proteins (GTPases) involved in the auxin proteolysis pathway. The latter are thought to provide a universal mechanism in the control of extracellular signal transmission to intracellular metabolic pathways related to growth, differentiation, development and defense responses (Gu et al, 2004; Nibau et al, 2006). Several of them are involved in autophagy and xylem development (Kwon et al, 2010). Consequently, the latter could be considered as putative candidates.

Candidate genes were also considered in the ERF/AP2 (ethylene responsive factor/APETALA2) SHINE family. After over-expression investigations in rice, an ERF/AP2 gene was considered as an upstream transcriptional regulator of both master and secondary target genes involved in the biosynthesis of cell wall phenolic and carbohydrate components. This ERF/AP2 transcription factor was supposed to directly bind promoter regions of NAC and MYB genes involved in regulation of cell wall assembly. Rice plants over-expressing *Arabidopsis* SHINE2 gene thus had lower lignin and higher cellulose and hemicellulose contents, without changes in plant strength or overall performances (Ambavaram et al, 2011).

The *Arabidopsis* SHP1 (SHATTERPROOF MADS-box) gene, which has been shown to specify with

SHP2 the lignified valve margin of mature siliques (Liljegren et al, 2000), likely has other roles in tissue lignification as it is also expressed in stems and down-regulated at the maturing stage (Ko and Han, 2004). While the roles of their maize orthologs are not known, the maize *ZmZAG5* gene was under-expressed to nearly the same level as the disrupted COMT gene in *bm2* plantlets, while it was 3.5 times over-expressed in the ear internode of *bm3* silking plants (Guillaumie et al, 2007b; Guillaumie et al, 2008).

The maize  $\alpha$ -expansin 5 (*EXPA5*) gene also probably has a function of interest in cell wall metabolism. Most expansins are involved in the disruption of hydrogen bonds between cellulose microfibrils and cross-linking hemicelluloses in the wall, restoring the long-term extension to cell walls (Li et al, 2003c). However, roles of expansins that do not involve wall expansion have already been shown. Expansins have been associated with the growth of protoxylem elements in *Zinnia* stems (Im et al, 2000). Similarly, several expansins appeared to be expressed during the differentiation of the tracheary elements, and the *ZmEXPA5* gene was expressed in leaf region where secondary cell wall deposition occurred (Miloni et al, 2001; Muller et al, 2007). The latter facts suggest their possible involvement in secondary wall formation. While ten genes are annotated  $\alpha$ -expansin in the maize sequence database and nine of them have numerous paralogs, the *EXPA5* gene (GRMZM2G361064) appears different from the others as it is the only one without any paralogs. Moreover, the *EXPA5* gene is located under a major cell wall QTL in bin 6.06 (Courtial et al, 2013) and it was at least 20 times more expressed in four RILs with high cell wall degradability than in the parental line with low cell wall degradability (Courtial et al, 2012).

The fasciclin-like arabinogalactan (FLA) proteins are characterized by a juxtaposition of glycosylated arabinogalactan-protein (AGP) domains and one or two fasciclin (FAS) putative cell-adhesion domains. Members of this gene family are implicated in many developmental roles, even if their functions remain largely undefined. However, among the 21 FLA *Arabidopsis* genes, the two *AtFLA11* (or *IRX13*) and *AtFLA12* genes were shown to be involved in secondary wall formation (Persson et al, 2005; MacMillan et al, 2010), with high transcript abundance in stem cells undergoing secondary-wall deposition. In addition, (double) mutant plants had altered stem biomechanics, altered cell wall architecture and composition, lower cellulose contents, and higher lignin contents. Other results obtained in their orthologs in poplar (Lafarguette et al, 2004), *Zinnia* (Dahiya et al, 2006), and eucalyptus (Qiu et al, 2008) strengthened the involvement of *AtFLA11/12*-like genes in secondary wall formation. In addition, another FLA gene, *AtFLA4*, was shown to be required for normal cell expansion, and the mutant *Salt Overly Sensitive5* (*SOS5*) have thinner



cell walls (Shi et al, 2003).

Finally, *Arabidopsis* MAP70 microtubule-associated proteins were shown to be essential for defining where secondary cell wall polymers were positioned and for determining the overall pattern of xylem vessel secondary cell walls (Pesquet et al, 2010; Pesquet et al, 2011).

This set of miscellaneous genes and regulation factors putatively involved in secondary wall assembly included 87 genes (Supplementary Table 8), which likely are of varying importance in cell wall assembly, and correlatively in cell wall degradability variation. SHATTERPROOF, HDZIP and COV genes can be considered of great importance in lignified tissue patterning, and thus could be relevant breeding targets to drive lignification in areas where this trait is essential for plant standability and disease or pest tolerance.

### Questioning the role of miRNA in cell wall and lignified tissue biosynthesis

The role of micro-RNA in regulation of plant lignified tissue assembly is little documented. Only a few miRNA have been shown to be involved or are thought to be involved in secondary cell wall formation. The miR166 and 165 families are well known for this developmental process. The two miR165 and miR166 have been shown to target and regulate the transcription HD-ZIP III genes, including those involved in vascular tissue differentiation (Juarez et al, 2004; Kim et al, 2005; Ko et al, 2006). The maize ZmmiR166 miRNA thus accumulates in phloem and regulates the maize *rolled-leaf1* gene (*RLD1*), which encodes an HD-ZIP III transcription factor. The *Arabidopsis* ortholog of *RLD1* is the IFL1/REVOLUTA gene, which is similarly involved in the differentiation of interfascicular fibers and secondary xylem (Ratcliffe et al, 2000; Juarez et al, 2004). Moreover, post-transcriptional regulation via miRNA-directed cleavage was also shown for several, but not all, NAC genes (Laufs et al, 2004; Guo et al, 2005; Yamaguchi et al, 2008; Zhang et al, 2009). In addition, miR164, as well as miR397, miR408, and miR528, were shown to target laccase genes (Zhang et al, 2009). Some of the latter are potentially involved in monolignol polymerization in the cell wall through the regulation of copper homeostasis (Abdel-Ghany et al, 2008). miR171, which targets SCARECROW genes (Zhang et al, 2009), could also be a possible candidate involved in variation of cell wall degradability. Finally, small interfering RNA derived from the 3'-coding region of *CesA6* cellulose synthase of barley were shown to be involved in the transition from primary to secondary cell wall programs (Held et al, 2008).

### The final candidate gene list

Based on the different considered functions required for the secondary cell wall assembly, 409 pu-

tative candidate genes were shown in the maize genome (Supplementary Tables 1-8), out of which 130 were involved in phenolic compound biosynthesis, 81 were involved in cell wall carbohydrate biosynthesis, and 198 were involved in regulation mechanisms. This candidate gene list, which focused on genes known to be involved in cell wall component biosynthesis and regulation, cannot be considered as complete. Other genes, whose roles in cell wall lignification and deposition have not yet been defined, should very likely be added to the list of candidates with a required activity in secondary cell wall assembly. Genes encoding proteins of still unknown function should be added to the list, as several of them are probably also involved in lignified tissue biosynthesis and deposition. As observed for all genes (Schnable et al, 2009), only a few genes related to cell wall biosynthesis and assembly were located around centromeres, and most of the latter were located in proximal and distal positions of chromosomes. When considering successive 30 Mbp long intervals all along chromosomes, a higher number of cell wall related genes were observed in the 0 - 30 Mbp areas of chromosomes 2 and 5. Similarly, a large number of cell wall genes were observed in the distal areas of chromosomes 5, 6, and 8 (Figure 3). Gene duplications in tandem positions were observed for several family members including especially PAL in bins 2.03, 4.06, and 5.05 (2, 3, and 4 genes, respectively), ZRP4-like OMT in bins 2.05, 6.06, and 9.04 (2, 4, and 2 genes respectively), or glycosyl transferase IRX10-like in 3.05, 6.07, and 8.06 (3, 2, and 2 genes, respectively).

### Discussion and conclusion

In the search for a biomass ideotype in maize, it is still open to debate whether breeding efforts should be focused on either biomass yield or rather on biomass degradability. A high biomass yield alone will certainly lead to disappointing results in dairy cow feeding, with reduced milk yields and/or the necessity of an extra cattle feeding with expensive concentrates due to the lower silage intake, digestibility and energy value. Similarly, for biofuel production, low degradable biomass will incur greater transport, processing and fermentation costs. However, fermentation costs could be expected to be lower if acid, alkaline, and/or heat pretreatments were used in biogas or bioethanol processes. But such treatments are both expensive and not environmentally friendly. Thus from an environmental and economic point of view, it is essential to breed more degradable plants, as well as plants with cell walls more susceptible to pretreatment processes. Using strategies allowing the introduction of alternative monomers (aldehydes, ester conjugates, ...) at reasonably low levels will not greatly alter the structural properties, as has been observed in maize *bm1* or sorghum *bmr6* plants. But these modifications will render the lignin polymer much easier to cleave into smaller fragments during pretreatments



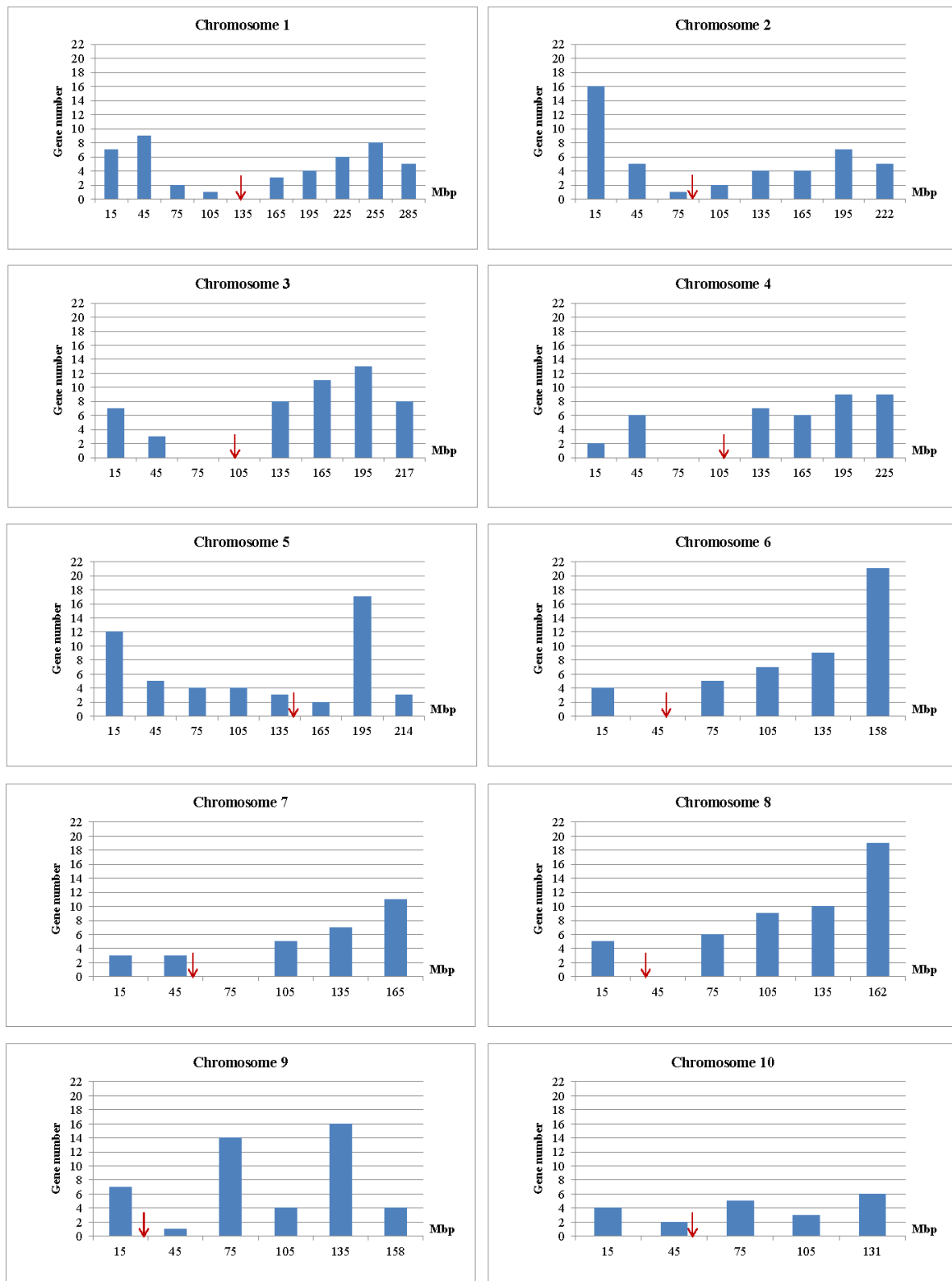


Figure 3 - Cell wall related genes distribution along maize chromosomes in 30 Mbp interval length (centromer positions are indicated by vertical arrows).

(Vanholme et al, 2012). Therefore, there is little doubt that cell wall qualities should be among the major targets for the improvement of silage and biofuel grass maize varieties. However, breeding grain maize varieties for the specific ability of their straw to be used as a bioproduct and converted into ethanol is likely not economically possible in the short term period. In grain maize hybrids, the elimination of very unfavorable lignin or cell wall structures should be progressively added as breeding traits for bi-functional grain and straw-ethanol maize plants.

Thanks to the huge advances in genetic and genomic tools and knowledge, maize is also an inescapable model plant for grass secondary wall lignification and degradability studies, and consequently for forage and biofuel production improvements. At present, similar research efforts are not being conducted on cell wall biosynthesis in other annual or perennial grass forage plants, nor in rice. The short-cycle model C3 grass *Brachypodium distachyon* (Garvin, 2007), and the recently sequenced and proposed as model plant for C4 grass *Setaria italica* (Bennetzen et al, 2012; Zhang et al, 2012) will both be complementary resources for gene mining and validation. The most important current limitation when it comes to using maize as a model system in plant genomics is the frequency of repeated elements, which represented between 75 and 80% of the genome, and the presence of several hundreds of gene copy number variations (CNV) together with several thousands of presence-absence sequence variations (PAV) in the maize genome (San Miguel et al, 1998; Meyers et al, 2001; Schnable et al, 2009; Springer et al, 2009; Belo et al, 2010; Lai et al, 2010; Swanson-Wagner et al, 2010). As a consequence, despite the fact that high throughput sequencing technologies are available, sequence comparison between lines is made difficult due to the difficulty of contiguating the obtained short-read sequence.

Among plant polymers, lignins have metabolic plasticity, with variable structures and non-conventional monomer incorporation, giving large variation in cell wall properties for which the underlying genetic determinants are mainly unknown. The lignin composition and structure in modern maize lines and hybrids are the result of long term grass evolution and short term maize breeding efforts towards high yield, high standability, and biotic or abiotic stress tolerances. To date, the challenge is to change cell wall and lignin polymer properties while keeping the high agronomic value of hybrids, that should be more hardy and drought tolerant than those of the previous decade, due to climatic changes.

Despite the fact that genes involved in cell wall carbohydrate and phenolic component biosynthesis have been listed, together with genes involved in their regulation, only a (very) few can be currently considered to be the relevant determinants of main variations in cell wall degradability. Discovery of the

relevant candidate genes involved in genetic variation of cell wall degradability, and to be used in marker assisted selection, will be based on a set of further investigations. One of the most promising investigative methods will probably be based on studies of colocalizations between cell wall trait QTLs and cell wall related genes, taking into consideration genes and QTL physical positions. Investigations in the F288 x F271 RIL progeny have highlighted the possible role of *ZmMYB42* and a *COV1*-like gene for two cell wall QTLs located in bin 4.09 (Courtial et al, 2012). Similarly, three MYB, the 4CL2, and the CCR1 genes are possible candidates for the cluster of cell wall QTLs shown in bin 1.07, in the F838 x F286 RIL progeny (Barrière et al, 2008). In the same progeny, the *ZmMYB46* is in close position to the cluster of QTLs located between bin 10.02 and 10.04. In contrast, no obvious candidate genes have yet been shown to explain numerous previously shown QTLs. The latter fact supports the possible role of genes of unknown function, of genes with known function but not yet related to cell wall assembly, as well as the probable role of non-coding sequence as the relevant determinants of variations in cell wall quality traits. Reverse genetics and transposon tagging, together with QTL fine mapping, are complementary essential strategies to understand the major traits involved in plant cell wall degradability variations.

In addition, the frequent clustering of QTLs for cell wall related traits raised the question of whether the underlying genetic determinant corresponds to a unique factor, or to a small set of highly linked and co-regulated genes. Colocalizations between cell wall degradability, core lignin content, and syringaldehyde QTLs could correspond to a shared mechanism involved in lignin biosynthesis and duration of lignin deposition. In addition, a greater proportion of S units in lignins could also correspond to a polymer richer in  $\beta$ -O-4 linkages and thus more linear, with greater masking effects on carbohydrates. Colocalizations between cell wall degradability, etherFA, and diFA QTLs could correspond to other genetic mechanisms involved in FA biosynthesis, in cross-linkages between arabinoxylan chains and between arabinoxylans and lignins. Occurrences of the simultaneous colocalizations between cell wall degradability, lignin content and structure, and ferulate related trait QTLs complicate the understanding and identification of the possible underlying genetic determinant(s). A cluster of linked genes involved in the different mechanisms of cell wall biosynthesis and assembly is likely the simplest situation to consider, but a single co-regulating "master" factor located upstream in the pathway of cell wall assembly can also be considered. Depending on colocalizing traits and QTLs, the two types of situation probably coexist in the maize genome. The fact that different genomic determinants are involved in cell wall degradability variation and are linked in close or identical positions strengthens the possibili-

ties of breeding for higher values of this trait without negative effects on agronomic value (yield, biotic and abiotic stress tolerance). Nevertheless, marker-assisted selection is essential in order to correctly identify the favorable recombinations, which require prior identification of the genes involved. Gene identification is all the more important given that there is often great gap in agronomic value between lines of interest for feeding value traits and elite modern lines. Finally, the germplasm currently used in maize breeding represents only a small share of the available genetic resources. Most of this germplasm corresponds to resources chosen for grain maize breeding, or to progenies of resources chosen for the latter, even if breeding companies also have programs devoted to silage and now biofuel purposes. Consequently, it is questionable whether it is of interest to carry out investigations on cell wall traits in unused accessions, old lines, and exotic resources, in order to discover new mechanisms or alleles allowing significant wall degradability improvement, without (too) negative effects on yield and agronomic value.

### Acknowledgements

This work was developed in the frame of the ZeaWall project, funded by INRA and by the breeding companies (Advanta, Caussade Semences, Limagrain Genetics, MaisAdour, Monsanto SAS, Pioneer Génétique, Pau Euralis, R2n RAGT Semences, SDME KWS France, Syngenta seeds) involved in the PROMAÏS - INRA network on maize cell wall lignification and degradability.

### References

- Abdel-Ghany SE, Pilon M, 2008. MicroRNA-mediated systemic down-regulation of copper protein expression in response to low copper availability in Arabidopsis. *J Biol Chem* 283: 15932-15945
- Alejandro S, Lee Y, Tohge T, Sudre D, Osorio S, Park J, Bovet L, Lee Y, Geldner N, Fernie AR, Martinoia E, 2012. AtABCG29 is a monolignol transporter involved in lignin biosynthesis. *Curr Biol* 10: 1207-1212
- Aimi H, Matsumoto Y, Meshitsuka G, 2005. Structure of small lignin fragments retained in water-soluble polysaccharides extracted from birch MWL isolation residue. *J Wood Sci* 51: 303-308
- Ambavaram MM, Krishnan A, Trijatmiko KR, Pereira A, 2011. Coordinated activation of cellulose and repression of lignin biosynthesis pathways in rice. *Plant Physiol* 155: 916-931
- Anders N, Wilkinson MD, Lovegrove A, Freeman J, Tryfona T, Pellny TK, Weimar T, Mortimer JC, Stott K, Baker JM, Defoin-Platel M, Shewry PR, Dupree P, Mitchell RA, 2012. Glycosyl transferases in family 61 mediate arabinofuranosyl transfer onto xylan in grasses. *Proc Natl Acad Sci USA* 109: 989-993
- Andersen JR, Zein I, Wenzel G, Krutzfeldt B, Eder J, Ouzunova M, Lübberstedt T, 2007. High levels of linkage disequilibrium and associations with forage quality at a Phenylalanine Ammonia-Lyase locus in European maize (*Zea mays* L) inbreds. *Theor Appl Genet* 114: 307-319
- Andersen JR, Asp T, Lu YC, Kloiber-Maitz M, Ouzunova M, Luebberstedt T, 2009. Development and mapping of gene-tagged SNP markers in lac-cases of maize (*Zea mays* L). *Plant Breeding* 128: 423-425
- Appenzeller L, Doblin MS, Barreiro R, Wang H, Niu X, Kollipara K, Carrigan L, Tomes D, Chapman M, Dhugga KS, 2004. Cellulose synthesis in maize: isolation and expression analysis of the cellulose synthase (CesA) gene family. *Cellulose* 11: 287-299
- Aufrère J, Michalet-Doreau B, 1983. In vivo digestibility and prediction of digestibility of some by-products, pp. 25-33. In: EEC seminar, Melle Gontrode. 26-29 September
- Bakalovic N, Passardi F, Ioannidis V, Cosio C, Penel C, Falquet L, Dunand C, 2006. PeroxiBase: a class III plant peroxidase database. *Phytochem* 67: 534-539
- Bhargava A, Mansfield SD, Hall HC, Douglas CJ, Ellis BE, 2010. MYB75 functions in regulation of secondary cell wall formation in the Arabidopsis inflorescence stem. *Plant Physiol* 154: 1428-1438
- Bhargava A, Ahad A, Wang S, Mansfield SD, Haughn GW, Douglas CJ, Ellis BE, 2013. The interacting MYB75 and KNAT7 transcription factors modulate secondary cell wall deposition both in stems and seed coat in Arabidopsis. *Planta* 237: 1199-1211
- Baima S, Possenti M, Matteucci A, Wisman E, Altamura MM, Ruberti I, Morelli G, 2001. The Arabidopsis ATHB-8 HD-zip protein acts as a differentiation-promoting transcription factor of the vascular meristems. *Plant Physiol* 126: 643-655
- Barrière Y, Gallais A, Derieux M, Panouillé A, 1987. Etude de la valeur agronomique en plante entière au stade de récolte ensilage de différentes variétés de maïs grain sélectionnées entre 1950 et 1980. *Agronomie* 7: 73-79
- Barrière Y, Emile JC, Traineau R, Hébert Y, 1995. Genetic variation in the feeding efficiency of maize genotypes evaluated from experiments with dairy cows. *Plant Breed* 114: 144-148
- Barrière Y, Guillet C, Goffner D, Pichon M, 2003. Genetic variation and breeding strategies for improved cell wall digestibility in annual forage crops. A review. *Animal Research* 52: 193-228
- Barrière Y, Emile JC, Traineau R, Surault F, Briand M, Gallais A, 2004a. Genetic variation for organic matter and cell wall digestibility in silage maize. Lessons from a 34-year long experiment with sheep in digestibility crates. *Maydica* 49: 115-126
- Barrière Y, Ralph J, Méchin V, Guillaumie S, Grab-

- ber JH, Argillier O, Chabbert B, Lapierre C, 2004b. Genetic and molecular basis of grass cell wall biosynthesis and degradability. II. Lessons from brown-midrib mutants. *CR Biol* 327: 847-860
- Barrière Y, Goncalves G, Emile J, Lefevre B, 2004c. Higher intake of DK265 corn silage by dairy cattle. *Journal of Dairy Science* 87: 1439-1445
- Barrière Y, Alber D, Dolstra O, Lapierre C, Motto M, Ordas A, Van Waes J, Vlaswinkel L, Welcker C, Monod JP, 2005. Past and prospects of forage maize breeding in Europe. I. The grass cell wall as a basis of genetic variation and future improvements in feeding value. *Maydica* 50: 259-274
- Barrière Y, Thomas J, Denoue D, 2008. QTL mapping for lignin content, lignin monomeric composition, *p*-hydroxycinnamate content, and cell wall digestibility in the maize recombinant inbred line progeny F838 x F286. *Plant Sci* 175: 585-595
- Barrière Y, Méchin V, Lafarguette F, Manicacci D, Guillon F, Wang H, Laressergues D, Pichon M, Bosio M, Tatout C, 2009. Toward the discovery of maize cell wall genes involved in silage maize quality and capacity to biofuel production. *Maydica* 54: 161-198
- Barrière Y, Méchin V, Lefevre B, Maltese S, 2012. QTLs for agronomic and cell wall traits in a maize RIL progeny derived from a cross between an old Minnesota13 line and a modern Iodent line. *Theor Appl Genet* 125: 531-549
- Barrière Y, Chavigneau H, Delaunay S, Courtial A, Bosio M, Derory J, Lapierre C, Méchin V, Tatout C, 2013. Different mutations in the *ZmCAD2* gene underlie the maize brown-midrib1 (bm1) phenotype with similar effects on lignin characteristics and have potential interest for bioenergy production. *Maydica* 58: 6-20
- Barros-Rios J, Malvar RA, Jung HJ, Bunzel M, Santiago R, 2012. Divergent selection for ester-linked diferulates in maize pith stalk tissues. Effects on cell wall composition and degradability. *Phytochemistry* 83: 43-50
- Beló A, Beatty MK, Hondred D, Fengler KA, Li B, Rafalski A, 2010. Allelic genome structural variations in maize detected by array comparative genome hybridization. *Theor Appl Genet* 120: 355-367
- Bennetzen JL, Schmutz J, Wang H, Percifield R, Hawkins J, Pontaroli AC, Estep M, Feng L, Vaughn JN, Grimwood J, Jenkins J, Barry K, Lindquist E, Hellsten U, Deshpande S, Wang X, Wu X, Mitros T, Triplett J, Yang X, Ye CY, Mauro-Herrera M, Wang L, Li P, Sharma M, Sharma R, Ronald PC, Panaud O, Kellogg EA, Brutnell TP, Doust AN, Tuskan GA, Rokhsar D, Devos KM, 2012. Reference genome sequence of the model plant *Setaria*. *Nat Biotechnol* 13: 555-561
- Berthet S, Demont-Caulet N, Pollet B, Bidzinski P, Cezard L, Le Bris P, Borrega N, Herve J, Blondet E, Balzergue S, Lapierre C, Jouanin L, 2011. Disruption of *LACCASE4* and 17 results in tissue-specific alterations to lignification of *Arabidopsis thaliana* stems. *Plant Cell* 23: 1124-1137
- Besombes S, Mazeau K, 2005. The cellulose/lignin assembly assessed by molecular modeling. Part 2: seeking for evidence of organization of lignin molecules at the interface with cellulose. *Plant Physiol Biochem* 43: 277-286
- Bischoff V, Nita S, Neumetzler L, Schindelasch D, Urbain A, Eshed R, Persson S, Delmer D, Scheible WR, 2010. Trichome birefringence and its homolog AT5G01360 encode plant-specific DUF231 proteins required for cellulose biosynthesis in *Arabidopsis*. *Plant Physiol* 153: 590-602
- Blee KA, Choi JW, O'Connell AP, Schung W, Lewis NG, Bolwell GP, 2003. A lignin specific peroxidase in tobacco whose antisense suppression leads to vascular tissue modification. *Phytochem* 64: 163-176
- Boerjan W, Ralph J, Baucher M, 2003. Lignin biosynthesis. *Ann Rev Plant Biol* 54: 519-546
- Bomal C, Bedon F, Caron S, Mansfield SD, Levasseur C, Cooke JEK, Blais S, Tremblay L, Morency MJ, Pavy N, Grima-Pettenati J, Seguin A, MacKay J, 2008. Involvement of Pinus taeda MYB1 and MYB8 in phenylpropanoid metabolism and secondary cell wall biogenesis: a comparative in planta analysis. *J Exp Bot* 59: 3925-3939
- Boudet AM, 2000. Lignins and lignification: selected issues. *Plant Physiol Biochem* 38: 81-96
- Boyce CK, Cody GD, Fogel ML, Hazen RM, Alexander CMO, Knoll AH, 2003. Chemical evidence for cell wall lignification and the evolution of tracheids in early Devonian plants. *Int J Plant Sci* 164: 691-702
- Brown DM, Goubet F, Vicky WWA, Goodacre R, Stephens E, Dupree P, Turner SR, 2007. Comparison of five xylan synthesis mutants reveals new insight into the mechanisms of xylan synthesis. *Plant J* 52: 1154-1168
- Brown DM, Zeef LA, Ellis J, Goodacre R, Turner SR, 2005. Identification of novel genes in *Arabidopsis* involved in secondary cell wall formation using expression profiling and reverse genetics. *Plant Cell* 17: 2281-2295
- Brown DM, Zhang ZN, Stephens E, Dupree P, Turner SR, 2009. Characterization of IRX10 and IRX10-like reveals an essential role in glucuronoxylan biosynthesis in *Arabidopsis*. *Plant J* 57: 732-746
- Brueggemann J, Weisshaar B, Sagasser M, 2010. A WD40-repeat gene from *Malus x domestica* is a functional homologue of *Arabidopsis thaliana* TRANSPARENT TESTA GLABRA1. *Plant Cell Rep* 29: 285-294
- Buanafina MM, 2009. Feruloylation in grasses: Current and future perspectives. *Mol Plant* 2: 861-872
- Burget EG, Verma R, Molhoj M, Reiter WD, 2003. The biosynthesis of L-arabinose in plants: Molecular cloning and characterization of a Golgi-localized UDP-D-xylose 4-epimerase encoded by the



- MUR4 gene of Arabidopsis. *Plant Cell* 15: 523-531
- Burk D, Liu B, Zhong R, Morrison W, Ye Z, 2001. A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. *Plant Cell* 13: 807-827
- Burk D, Ye Z, 2002. Alteration of oriented deposition of cellulose microfibrils by mutation of a katanin-like microtubule-severing protein. *Plant Cell* 14: 2145-2160
- Cai X, Davis EJ, Ballif J, Liang M, Bushman E, Haroldsen V, Torabinejad J, Wu Y, 2006. Mutant identification and characterization of the laccase gene family in Arabidopsis. *J Exp Bot* 57: 2563-2569
- Caparrós-Ruiz D, Fornalé S, Civardi L, Puigdomènech P, Rigau P, 2006. Isolation and characterization of a family of laccases in maize. *Plant Sci* 171: 217-225
- Carlsbecker A, Lee JY, Roberts CJ, Dettmer J, Lehesranta S, Zhou J, Lindgren O, Moreno-Risueno MA, Vaten A, Thitamadee S, Campilho A, Sebastian J, Bowman JL, Helariutta Y, Benfey PN, 2010. Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* 465: 316-321
- Casler MD, Jung HJG, 1999. Selection and evaluation of smooth bromegrass clones with divergent lignin or etherified ferulic acid concentration. *Crop Sci* 39: 1866-1873
- Casler M, Jung H, Coblenz W, 2008. Clonal selection for lignin and etherified ferulates in three perennial grasses. *Crop Sci* 48, 424-433
- Chabbert B, Tollier MT, Monties B, Barriere Y, Argillier O, 1994. Biological variability in lignification of maize: expression of the *brown midrib bm2* mutation. *J Sci Food Agric* 64: 455-460
- Chavigneau H, Goué N, Courtial A, Jouanin L, Reymond M, Méchin V, Barrière Y, 2012. QTL for floral stem lignin content and degradability in three recombinant inbred line (RIL) progenies of Arabidopsis thaliana and search for candidate genes involved in cell wall biosynthesis and degradability. *OJGen* 2: 7-30
- Chen F, Reddy MSS, Temple S, Jackson L, Shadle G, Dixon RA, 2006. Multi-site genetic modulation of monolignol biosynthesis suggests new routes for formation of syringyl lignin and wall-bound ferulic acid in alfalfa (*Medicago sativa* L). *Plant J* 48: 113-124
- Chen HC, Li Q, Shuford CM, Liu J, Muddiman DC, Sederoff RR, Chiang VL, 2011. Membrane protein complexes catalyses both 4-and-3-hydroxylation of cinnamic acid derivatives in monolignol biosynthesis. *Proc Natl Acad Sci USA* 108: 21253-21258
- Chen X, Vega-Sánchez ME, Verhertbruggen Y, Chiniquy D, Canlas PE, Fagerström A, Prak L, Christensen U, Oikawa A, Chern M, Zuo S, Fan L, Auer M, Willats WG, Bartley L, Harholt J, Scheller HV, Ronald PC, 2012a. Inactivation of OsIRX10 leads to decreased xylan content in rice stem cell walls and improved biomass saccharification. *Mol Plant* 6: 570-573
- Chen W, VanOpdorp N, Fitzl D, Tewari J, Friedemann P, Greene T, Thompson S, Kumpatla S, Zheng P, 2012b. Transposon insertion in a cinnamyl alcohol dehydrogenase gene is responsible for a *brown midrib1* mutation in maize. *Plant Mol Biol* 3: 289-297
- Ching A, Dhugga K, Appenzeller L, Meeley R, Bourett T, Howard R, Rafalski A, 2006. Brittle stalk 2 encodes a putative glycosylphosphatidylinositol-anchored protein that affects mechanical strength of maize tissues by altering the composition and structure of secondary cell walls. *Planta* 224: 1174-1184
- Chiniquy D, Sharma V, Schultink A, Baidoo EE, Rautengarten C, Cheng K, Carroll A, Ulvskov P, Harholt J, Keasling JD, Pauly M, Scheller HV, Ronald PC, 2012. XAX1 from glycosyltransferase family 61 mediates xylosyltransfer to rice xylan. *Proc Natl Acad Sci USA* 109: 17117-17122
- Ciba-Semences, 1990. Valorisation laitière d'une variété de Maïs ensilage. Synthèse d'une expérimentation conduite par l'EDE de Vendée en 1988-1989-1990
- Ciba-Semences, 1995. Comparaison de la valorisation par des vaches laitières de deux hybrides de maïs
- Cochard H, 2002. Xylem embolism and drought-induced stomatal closure in maize. *Planta* 215: 466-471
- Cochard H, Barigah ST, Kleinhentz M, Eshel A, 2008. Is xylem cavitation resistance a relevant criterion for screening drought resistance among Prunus species? *J Plant Physiol* 165: 976-982
- Cone KC, Cocciolone SM, Burr FA, Burr B, 1993. Maize anthocyanin regulatory gene *pl* is a duplicate of *c1* that functions in the plant. *Plant Cell* 5: 1795-1805
- Corea ORA, Ki C, Cardenas CL, Kim SJ, Brewer SE, Patten AM, Davin LB, Lewis NG, 2012. Arogenate dehydratase isoenzymes profoundly and differentially modulate carbon flux into lignins. *J Biol Chem* 287: 11446-11459
- Courtial A, Jourda C, Arribat S, Balzergue S, Huguet S, Reymond M, Grima-Pettenati J, Barrière Y, 2012. Comparative expression of cell wall related genes in four maize RILs and one parental line of variable lignin content and cell wall degradability. *Maydica* 57:56-74
- Courtial A, Thomas J, Reymond M, Méchin V, Grima-Pettenati J, Barrière Y, 2013. Targeted linkage map densification to improve cell wall related QTL detection and interpretation in maize. *Theor Appl Genet* 126: 1151-1165
- Dahiya P, Findlay K, Roberts K, McCann MC, 2006. A fasciclin domain containing gene, ZeFLA11, is expressed exclusively in xylem elements that have reticulate wall thickenings in the stem vascular

- system of *Zinnia elegans* cv. Envy. *Planta* 223: 1281-1291
- Dai X, You C, Chen G, Li X, Zhang Q, Wu C, 2011. OsBC1L4 encodes a COBRA-like protein that affects cellulose synthesis in rice. *Plant Mol Biol* 75: 333-345
- Damiani I, Morreel K, Danoun S, Goeminne G, Yahiaoui N, Marque C, Kopka J, Messens E, Goffner D, Boerjan W, Boudet AM, Rochange S, 2005. Metabolite profiling reveals a role for atypical cinnamyl alcohol dehydrogenase CAD1 in the synthesis of coniferyl alcohol in tobacco xylem. *Plant Mol Biol* 59: 753-769
- D'Auria JC, 2006. Acyltransferases in plants: a good time to be BAHD. *Curr Opin Plant Biol* 9: 331-340
- Davin LB, Lewis NG, 2000. Dirigent proteins and dirigent sites explain the mystery of specificity of radical precursor coupling in lignan and lignin biosynthesis. *Plant Physiol* 123: 453-461
- Davin LB, Jourdes M, Patten AM, Kim KW, Vassao DG, Lewis NG, 2008. Dissection of lignin macromolecular configuration and assembly: Comparison to related biochemical processes in allyl/propenyl phenol and lignan biosynthesis. *Nat Prod Rep* 25: 1015-1090
- Dennis DT, Blakeley SD, 2000. Carbohydrate metabolism, pp. 630-675. In: *Biochemistry and Molecular Biology of Plants*. Buchanan B, Gruissem W, Jones RL eds. American Society of Plant Biologist, Rockville, MD
- De Obeso M, Caparrós-Ruiz D, Vignols F, Puigdomenech P, Rigau J, 2003. Characterisation of maize peroxidases having differential patterns of mRNA accumulation in relation to lignifying tissues. *Gene* 309: 23-33
- Dharmawardhana DP, Ellis BE, Carlson JE, 1995. A beta-glucosidase from lodgepole pine xylem specific for the lignin precursor coniferin. *Plant Physiol* 107: 331-339
- DiLaurenzio L, WysockaDiller J, Malamy JE, Pysh L, Helariutta Y, Freshour G, Hahn MG, Feldmann KA, Benfey PN, 1996. The SCARECROW gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root. *Cell* 86: 423-433
- Do CT, Pollet B, Thevenin J, Sibout R, Denoue D, Barrière Y, Lapierre C, Jouanin L, 2007. Both caffeoyl Coenzyme A 3- O -methyltransferase 1 and caffeic acid O -methyltransferase 1 are involved in redundant functions for lignin, flavonoids and sinapoyl malate biosynthesis in *Arabidopsis*. *Planta* 226: 1117-1129
- Du J, Mansfield D, Groover AT, 2009. The *Populus* homeobox gene ARBORKNOX2 regulates cell differentiation during secondary growth. *Plant J* 60: 1000-1014
- Du H, Feng BR, Yang SS, Huang YB, Tang YX, 2012. The R2R3-MYB transcription factor gene family in maize. *PLoS one* 7: e37463
- Ebringerova A, Hromadkova Z, Heinze T, 2005. Hemicellulose. *Adv Polym Sci* 186: 1-67
- Ehltling J, Mattheus N, Aeschliman DS, Li EY, Hamburger B, Cullis IF, Zhuang J, Kaneda M, Mansfield SD, Samuels L, Ritland K, Ellis BE, Bohlmann J, Douglas CJ, 2005. Global transcript profiling of primary stems from *Arabidopsis thaliana* identifies candidate genes for missing links in lignin biosynthesis and transcriptional regulators of fiber differentiation. *Plant J* 42: 618-640
- Endler A, Persson S, 2011. Cellulose synthases and synthesis in *Arabidopsis*. *Mol Plant* 4: 199-211
- Escamilla-Trevino LL, Chen W, Card ML, Shih MC, Cheng CL, Poulton JE, 2006. *Arabidopsis thaliana* beta-glucosidases BGLU45 and BGLU46 hydrolyse monolignol glucosides. *Phytochem* 67: 1651-1660
- Eudes A, George A, Mukerjee P, Kim JS, Pollet B, Benke PI, Yang F, Mitra P, Sun L, Cetinkol OP, Chabout S, Mouille G, Soubigou-Taconnat L, Balzergue S, Singh S, Holmes BM, Mukhopadhyay A, Keasling JD, Simmons BA, Lapierre C, Ralph J, Loqué D, 2012. Biosynthesis and incorporation of side-chain-truncated lignin monomers to reduce lignin polymerization and enhance saccharification. *Plant Biotechnol J* 10: 609-620
- Fagerstedt KV, Kukkola EM, Koistinen VV, Takahashi J, Marjamaa K, 2010. Cell wall lignin is polymerised by class III secreted plant peroxidases in Norway spruce. *J Integr Plant Biol* 52: 186-194
- Fernandez I, Martin C, Champion M, Michalet-Doreau B, 2004. Effect of corn hybrid and chop length of whole-plant corn silage on digestion and intake by dairy cows. *J Dairy Sci* 87: 1298-1309
- Fornalé S, Sonbol FM, Maes T, Capellades M, Puigdomenech P, Rigau J, Caparrós-Ruiz D, 2006. Down-regulation of the maize and *Arabidopsis thaliana* caffeic acid O-methyl-transferase genes by two new maize R2R3-MYB transcription factors. *Plant Mol Biol* 62:809-823
- Fornalé S, Shi X, Chai C, Encina A, Irar S, Capellades M, Fuguet E, Torres JL, Rovira P, Puigdomenech P, Rigau J, Grotewold E, Gray J, Caparrós-Ruiz D, 2010. *ZmMYB31* directly represses maize lignin genes and redirects the phenylpropanoid metabolic flux. *Plant J* 64:633-644
- Fornalé S, Capellades M, Encina A, Wang K, Irar S, Lapierre C, Ruel K, Joseleau JP, Berenguer J, Puigdomenech P, Rigau J, Caparrós-Ruiz D, 2012. Altered lignin biosynthesis improves cellulosic bioethanol production in transgenic maize plants down-regulated for cinnamyl alcohol dehydrogenase. *Mol Plant* 5: 817-830
- Foucart C, Jauneau A, Gion JM, Amelot N, Martinez Y, Panegos P, Grima-Pettenati J, Sivadon P, 2009. Overexpression of EgROP1, a Eucalyptus vascular-expressed Rac-like small GTPase, affects secondary xylem formation in *Arabidopsis thaliana*. *New Phytologist* 183: 1014-1029

- Freudenberg K, 1959. Biosynthesis and constitution of lignin. *Nature* 183: 1152-1155
- Fry SC, Willis S, Paterson A, 2000. Intraprotoplasmic and wall-localised formation of arabinoxylan-bound diferulates and larger ferulate coupling-products in maize cell-suspension cultures. *Planta* 211: 679-692
- Garvin DF, 2007. Brachypodium: a new monocot model plant system emerges. *J Sci Food Agric* 87: 1177-1179
- Gibeaut DM, 2000. Nucleotide sugars and glycosyltransferases for synthesis of cell wall matrix polysaccharides. *Plant Physiol Biochem* 38: 69-80
- Gille S, deSouza A, Xiong G, Benz M, Cheng K, Schultink A, Reza IB, Pauly M, 2011. O-acetylation of Arabidopsis hemicellulose xyloglucan requires AX4 or AX4L proteins with a TBL and DUF231 domain. *Plant Cell* 23: 4041-4053
- Gille S, Pauly M, 2012. O-acetylation of plant cell wall polysaccharides. *Front Plant Sci* 3:12
- Goering HK, Van Soest PJ, 1970. Forage fiber analysis (Apparatus, reagents, procedures and some applications), pp. 1-20. US Dept Agri Sci Handbook n°379
- Goffner D, Van Doorselaere J, Yahiaoui N, Samaj J, Grima-Pettenati J, Boudet AM, 1998. A novel aromatic alcohol dehydrogenase in higher plants: molecular cloning and expression. *Plant Mol Biol* 36: 755-765
- Goicoechea M, Lacombe E, Legay S, Mihaljevic S, Rech P, Jauneau A, Lapierre C, Pollet B, Verhaegen D, Chaubet-Gigot N, Grima-Pettenati J, 2005. EgMYB2, a new transcriptional activator from Eucalyptus xylem, regulates secondary cell wall formation and lignin biosynthesis. *Plant J* 43:553-567
- Goujon T, Sibout R, Maba B, Nussaume L, Bechtold N, Lu F, Ralph J, Pollet B, Mila I, Charpentier JP, Barrière Y, Lapierre C, Jouanin L, 2003. A new Arabidopsis mutant deficient in the expression of O-methyltransferase 1: Impact on lignin and sinapic esters. *Plant Mol Biol* 51: 973-989
- Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, Rozen R, 1994. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. *Nat Genet* 7: 195-200
- Grabber JH, Hatfield RD, Ralph J, Zon J Amrhein N, 1995. Ferulate cross-linking in cell-walls isolated from maize cell-suspensions. *Phytochem* 40: 1077-1082
- Grabber JH, Quideau S, Ralph J, 1996. *p*-Coumaroylated syringyl units in maize lignin; implications for  $\beta$ -ether cleavage by thioacidolysis. *Phytochem* 43: 1189-1194
- Grabber JH, Ralph J, Hatfield RD, Quideau S, 1997. *p*-hydroxyphenyl, guaiacyl, and syringyl lignins have similar inhibitory effects on wall degradability. *J Agric Food Chem* 45: 2530-2532
- Grabber JH, Ralph J, Hatfield RD, 1998. Ferulate cross-links limit the enzymatic degradation of synthetically lignified primary walls of maize. *J Agric Food Chem* 46: 2609-2614
- Grabber JH, Ralph J, Hatfield RD, 2000. Cross-linking of maize walls by ferulate dimerization and incorporation into lignin. *J Agric Food Chem* 48: 6106-6113
- Grabber JH, Ralph J, Lapierre C, Barrière Y, 2004. Genetic and molecular basis of grass cell-wall degradability. I. Lignin-cell wall matrix interactions. *CR Biologie* 327:4 55-465
- Grabber JH, 2005. How do lignin composition, structure, and cross-linking affect degradability? A review of cell wall model studies. *Crop Sci* 45: 820-831
- Grabber JH, Lu FC, 2007. Formation of syringyl-rich lignins in maize as influenced by feruloylated xy-lans and *p*-coumaroylated monolignols. *Planta* 226: 741-751
- Grabber JH, Mertens DR, Kim H, Funk C, Lu F, Ralph J, 2009. Cell wall fermentation kinetics are impacted more by lignin content and ferulate cross-linking than by lignin composition. *J Sci Food Agric* 89: 122-129
- Grand C, Parmentier P, Boudet A, Boudet AM, 1985. Comparison of lignins and of enzymes involved in lignification in normal and *brown midrib (bm3)* mutant corn seedlings. *Physiol Veg* 23: 905-911
- Grant EH, Fujino T, Beers EP, Brunner AM, 2010. Characterization of NAC domain transcription factors implicated in control of vascular cell differentiation in Arabidopsis and Populus. *Planta* 232: 337-352
- Gray J, Caparrós-Ruiz D, Grotewold E, 2012. Grass phenylpropanoids: Regulate before using! *Plant Science* 184: 112-120
- Green KA, Prigge MJ, Katzman RB, Clark SE, 2005. CORONA, a member of the class III homeodomain leucine zipper gene family in Arabidopsis, regulates stem cell specification and organogenesis. *Plant Cell* 17: 691-704
- Grima-Pettenati J, Soler M, Camargo E, Wang H, 2012. Transcriptional Regulation of the Lignin Biosynthetic Pathway Revisited: New Players and Insights, pp. 173-218. In: *Advances in Botanical Research*. Jouanin L, Lapierre C eds. Academic Press, Volume 61, Chapter 6
- Grotewold E, Sainz MB, Tagliani L, Hernandez JM, Bowen B, Chandler VL, 2000. Identification of the residues in the Myb domain of maize C1 that specify the interaction with the bHLH cofactor R. *Proc Natl Acad Sci USA* 97: 13579-13584
- Gu Y, Wang Z, Yang Z, 2004. ROP/RAC GTPase: an old new master regulator for plant signaling. *Current Opin Plant Biol* 7: 527-536
- Guillaumie S, San-Clemente H, Deswarte C, Martinez Y, Lapierre C, Murigneux A, Barrière Y, Pichon M, Goffner D, 2007a. MAIZEWALL. Database and

- developmental gene expression profiling of cell wall biosynthesis and assembly in maize. *Plant Physiol* 143: 339-363
- Guillaumie S, Pichon M, Martinant JP, Bosio M, Goffner D, Barrière Y, 2007b. Differential expression of phenylpropanoid and related genes in *brown-midrib bm1*, *bm2*, *bm3*, and *bm4* young near-isogenic maize plants. *Planta* 226:235-250
- Guillaumie S, Goffner D, Barbier B, Martinant JP, Pichon M, Barrière Y, 2008. Expression of cell wall related genes in basal and ear internodes of silking *brown-midrib-3*, caffeic acid O-methyltransferase (COMT) down-regulated, and normal maize plants. *BMC Plant Biol* 8: 71
- Guillaumie S, Mzid R, Méchin V, Léon C, Hichri I, Destrac-Irvine A, Trossat-Magnin C, Delrot S, Lauvergeat V, 2010. The grapevine transcription factor WRKY2 influences the lignin pathway and xylem development in tobacco. *Plant Mol Biol* 72: 215-234
- Guillet-Claude C, Birolleau-Touchard C, Manicacci D, Rogowsky PM, Rigau J, Murigneux A, Martinant JP, Barrière Y, 2004. Nucleotide diversity of the *ZmPox3* maize peroxidase gene: Relationships between a MITE insertion in exon 2 and variation in forage maize digestibility. *BMC Genetics* 5: 19
- Guo D, Chen F, Inoue K, Blount JW, Dixon RA, 2001. Downregulation of caffeic acid 3-O-methyltransferase and caffeoyl CoA 3-O-methyltransferase in transgenic alfalfa: Impacts on lignin structure and implications for the biosynthesis of G and S lignin. *Plant Cell* 13: 73-88
- Guo HS, Xie Q, Fei JF, Chua NH, 2005. MicroRNA directs mRNA cleavage of the transcription factor NAC1 to downregulate auxin signals for Arabidopsis lateral root development. *Plant Cell* 17: 1376-1386
- Guo Y, Qin G, Gu H, Qu LJ, 2009. Dof5.6/HCA2, a Dof transcription factor gene, regulates interfascicular cambium formation and vascular tissue development in Arabidopsis. *Plant Cell* 21: 3518-3534
- Halpin C, Holt K, Chojecki J, Oliver D, Chabbert B, Monties B, Edwards K, Barakate A, Foxon GA, 1998. *Brown-midrib* maize (*bm1*) - a mutation affecting the *cinnamyl alcohol dehydrogenase* gene. *Plant J* 14: 545-553
- Handakumbura PP, Hazen SP, 2012. Transcriptional regulation of grass secondary cell wall biosynthesis: Playing catch-up with *Arabidopsis thaliana*. *Front Plant Sci* 3: 74
- Hatfield R, Ralph J, Grabber JH, 2008. A potential role for sinapyl *p*-coumarate as a radical transfer mechanism in grass lignin formation. *Planta* 228: 919-928
- Hatfield RD, Marita JM, Frost K, Grabber J, Ralph J, Lu F, Kim H, 2009. Grass lignin acylation: *p*-coumaroyl transferase activity and cell wall characteristics of C3 and C4 grasses. *Planta* 229: 1253-1267
- Hawkins SW, Boudet AM, 1994. Purification and characterization of cinnamyl alcohol-dehydrogenase isoforms from the periderm of *Eucalyptus gunnii* Hook. *Plant Physiol* 104: 75-84
- He X, Hall MB, Gallo-Meagher M, Smith RL, 2003. Improvement of forage quality by downregulation of maize O-methyltransferase. *Crop Sci* 43: 2240-2251
- Held BM, Wang HQ, John I, Wurtele ES, Colbert JT, 1993. An messenger-RNA putatively coding for an O-methyltransferase accumulates preferentially in maize roots and is located predominantly in the region of the endodermis. *Plant Physiol* 102: 1001-1008
- Held MA, Penning B, Brandt AS, Kessans SA, Yong W, Scofield SR, Carpita NC, 2008. Small-interfering RNAs from natural antisense transcripts derived from a cellulose synthase gene modulate cell wall biosynthesis in barley. *Proc Natl Acad Sci USA* 105: 20534-20539
- Heim MA, Jakoby M, Werber M, Martin C, Weisshaar B, Bailey PC, 2003. The basic helix-loop-helix transcription factor family in plants: a genome-wide study of protein structure and functional diversity. *Mol Biol Evol* 20: 735-747
- Higuchi T, Ito Y, Kawamura I, 1967. *p*-Hydroxyphenyl component of grass lignin and the role of tyrosine ammonia-lyase in its formation. *Phytochem* 6: 875-881
- Hoffmann L, Maury S, Martz F, Geoffroy P, Legrand M, 2003. Purification, cloning, and properties of an acyltransferase controlling shikimate and quinate ester intermediates in phenylpropanoid metabolism. *J Bio Chem* 278: 95-103
- Hoffmann L, Besseau S, Geoffroy P, Ritzenthaler C, Meyer D, Lapiere C, Pollet B, Legrand M, 2004. Silencing of hydroxycinnamoyl-coenzyme A shikimate/quinate hydroxycinnamoyltransferase affects phenylpropanoid biosynthesis. *Plant Cell* 16: 1446-1465
- Hossain MA, Noh HN, Kim KI, Koh EJ, Wi SG, Bae HJ, Lee H, Hong SW, 2011. Mutation of the chitinase-like protein-encoding *AtCTL2* gene enhances lignin accumulation in dark-grown Arabidopsis seedlings. *J Plant Physiol* 167: 650-658
- Iiyama K, Lam TBT, Stone BA, 1990. Phenolic acid bridges between polysaccharides and lignin in wheat internodes. *Phytochem* 29: 733-737
- Ilegems M, Douet V, Meylan-Bettex M, Uyttewaal M, Brand L, Bowman JL, Stieger PA, 2010. Interplay of auxin, KANADI and Class III HD-ZIP transcription factors in vascular tissue formation. *Development* 137: 975-984
- Im KH, Cosgrove DT, Jones AM, 2000. Subcellular localization of expansin mRNA in xylem cells. *Plant Physiol* 123: 463-470
- Jacquet G., Pollet B, Lapiere C, 1995. New ether-linked ferulic acid-coniferyl alcohol dimers identified in grass straws. *J Agric Food Chem* 43: 2746-



- 2751
- Janbon G, Himmelreich U, Moyrand F, Improvisi L, Dromer F, 2001. Cas1p is a membrane protein necessary for the O-acetylation of the *Cryptococcus neoformans* capsular polysaccharide. *Mol Microbiol* 42: 453-467
- Jornvall H, Persson B, Jeffery J, 1987. Characteristics of alcohol/polyol dehydrogenases. The zinc-containing long-chain alcohol dehydrogenases. *Eur J Biochem* 167: 195-201
- Jornvall H, Persson B, Krook M, Atrian S, Gonzalez-duarte R, Jeffery J, Ghosh D, 1995. Short-chain dehydrogenases reductases (Sdr). *Biochemistry* 34: 6003-6013
- Juarez MT, Kui JS, Thomas J, Heller BA, Timmermans MCP, 2004. MicroRNA-mediated repression of rolled leaf1 specifies maize leaf polarity. *Nature* 428: 84-88
- Jung HG, Allen MS, 1995. Characteristics of plant cell wall affecting intake and digestibility of forages by ruminants. *J Anim Sci* 73: 2774-2790
- Jung HG, Phillips RL, 2010. Putative seedling ferulate ester (sfe) maize mutant: morphology, biomass yield, and stover cell wall composition and rumen degradability. *Crop Sci* 50: 403-418
- Jung HG, Mertens D, Phillips RL, 2011. Effect of reduced ferulate-mediated lignin/arabinoxylan cross-linking in corn silage on feed intake, digestibility, and milk production. *J Dairy Sci* 94: 5124-5137
- Kanazawa K, Goodman MM, O'Malley DM, 1999. Genetic and biochemical analysis of maize CAD, pp. 17-21. Town & Country Hotel, San Diego, USA
- Kaneda M, Schuetz M, Lin B, Chanis C, Hamberger B, Western T, Ehrling J, Samuels A, 2011. ABC transporters coordinately expressed during lignification of *Arabidopsis* stems include a set of ABCBs associated with auxin transport. *J Exp Bot* 62: 2063-2077
- Karpinska B, Karlsson M, Srivastava M, Stenberg A, Schrader J, Sterky F, Bhalerao R, Wingsle G, 2004. MYB transcription factors are differentially expressed and regulated during secondary vascular tissue development in hybrid aspen. *Plant Mol Biol* 56: 255-270
- Kavousi B, Daudi A, Cook CM, Joseleau JP, Ruel K, Devoto A, Bolwell GP, Blee KA, 2010. Consequences of antisense down-regulation of a lignification-specific peroxidase on leaf and vascular tissue in tobacco lines demonstrating enhanced enzymic saccharification. *Phytochem* 71: 531-542
- Kawakita M, Ishida N, Miura N, Sun-Wada GH, Yoshioka S, 1998. Nucleotide sugar transporters: Elucidation of their molecular identity and its implication for future studies. *J Biochem* 123: 777-785
- Kawaoka A, Ebinuma H, 2001. Transcriptional control of lignin biosynthesis by tobacco LIM protein. *Phytochem* 57: 1149-1157
- Keating JD, Panganiban C, Mansfield SD, 2006. Tolerance and adaptation of ethanologenic yeasts to lignocellulosic inhibitory compounds. *Biotechnol Bioeng* 93: 1196-1206
- Kim J, Jung JH, Reyes JL, Kim YS, Kim SY, Chung KS, Kim JA, Lee M, Lee Y, Narry Kim V, Chua NH, Park CM, 2005. microRNA-directed cleavage of ATHB15 mRNA regulates vascular development in *Arabidopsis* inflorescence stems. *Plant J* 42: 84-94
- Kim WC, Ko JH, Han KH, 2012. Identification of a cis-acting regulatory motif recognized by MYB46, a master transcriptional regulator of secondary wall biosynthesis. *Plant Mol Biol* 78: 489-501
- Kirst M, Myburg AA, De Leon JPG, Kirst ME, Scott J, Sederoff R, 2004. Coordinated genetic regulation of growth and lignin revealed by quantitative trait locus analysis of cDNA microarray data in an interspecific backcross of eucalyptus. *Plant Physiol* 135: 2368-2378
- Ko JH, Han KH, 2004. *Arabidopsis* whole-transcriptome profiling defines the features of coordinated regulations that occur during secondary growth. *Plant Mol Biol* 55: 433-453
- Ko JH, Prassinis C, Han KH, 2006. Developmental and seasonal expression of *PtaHB1*, a *Populus* gene encoding a class III HD-Zip protein, is closely associated with secondary growth and inversely correlated with the level of microRNA (miR166). *New Phytol* 169: 469-478
- Ko JH, Yang SH, Park AH, Lerouxel O, Han KH, 2007. ANAC012, a member of the plant-specific NAC transcription factor family, negatively regulates xylary fiber development in *Arabidopsis thaliana*. *Plant J* 50: 1035-1048
- Ko JH, Kim WC, Han KH, 2009. Ectopic expression of MYB46 identifies transcriptional regulatory genes involved in secondary wall biosynthesis in *Arabidopsis*. *Plant J* 60: 649-665
- Kocsis MG, Ranocha P, Gage DA, Simon ES, Rhodes D, Peel GJ, Mellema S, Saito K, Awazuhara M, Li CJ, Meeley RB, Tarczynski MC, Wagner C, Hanson AD, 2003. Insertional inactivation of the methionine S-methyltransferase gene eliminates the S-methylmethionine cycle and increases the methylation ratio. *Plant Physiol* 131: 1808-1815
- Konishi T, Ohnishi-Kameyama M, Funane K, Miyazaki Y, Konishi T, Ishii T, 2010. An arginyl residue in rice UDP-arabinopyranose mutase is required for catalytic activity and autoglycosylation. *Carbohydr Res* 345: 787-791
- Konishi T, Takeda T, Miyazaki Y, Ohnishi-Kameyama M, Hayashi T, O'Neill MA, Ishii T, 2007. A plant mutase that interconverts UDP-arabinofuranose and UDP-arabinopyranose. A plant mutase that interconverts UDP-arabinofuranose and UDP-arabinopyranose. *Glycobiology* 17: 345-354
- Konishi T, Aohara T, Igasaki T, Hayashi N, Miyazaki Y, Takahashi A, Hirochika H, Iwai H, Satoh S, Ishii T, 2011. Down-regulation of UDP-arabinopyranose

- mutase reduces the proportion of arabinofuranose present in rice cell walls. *Phytochem* 72: 1962-1968
- Kubo M, Udagawa M, Nishikubo N, Horiguchi G, Yamaguchi M, Ito J, Mimura T, Fukuda H, Demura T, 2005. Transcription switches for protoxylem and metaxylem vessel formation. *Genes Dev* 19: 1855-1860
- Kuc J, Nelson OE, 1964. The abnormal lignins produced by the brown-midrib mutants of maize. *Arch Biochem Biophys* 105: 103-113
- Kushwaha H, Gupta S, Singh VK, Rastogi S, Yadav D, 2011. Genome wide identification of Dof transcription factor gene family in sorghum and its comparative phylogenetic analysis with rice and *Arabidopsis*. *Mol Biol Rep* 38: 5037-5053
- Kwon SI, Cho HJ, Jung JH, Yoshimoto K, Shirasu K, Park OK, 2010. The Rab GTPase RabG3b functions in autophagy and contributes to tracheary element differentiation in *Arabidopsis*. *Plant J* 64: 151-164
- Lafarguette F, Lepage JC, Dejardin A, Laurans F, Costa G, Lesage-Descauses MC, Pilate G, 2004. Poplar genes encoding fasciclin-like arabinogalactan proteins are highly expressed in tension wood. *New Phytol* 164: 107-121
- Lai J, Li R, Xu X, Jin W, Xu M, Zhao H, Xiang Z, Song W, Ying K, Zhang M, Jiao Y, Ni P, Zhang J, Li D, Guo X, Ye K, Jian M, Wang B, Zheng H, Liang H, Zhang X, Wang S, Chen S, Li J, Fu Y, Springer NM, Yang H, Wang J, Dai J, Schnable PS, Wang J, 2010. Genome-wide patterns of genetic variation among elite maize inbred lines. *Nat Genet* 42:1027-1030
- Lam TBT, Iiyama K, Stone BA, 2003. Hot alkali-labile linkages in the wall of the forage grass *Phalaris aquatica* and *Lolium perenne* and their relation to in vitro wall digestibility. *Phytochem* 64: 603-607.
- Lanot A, Hodge D, Jackson R, George G, Elias L, Lim E, Vaistij F, Bowles D, 2006. The glucosyltransferase UGT72E2 is responsible for monolignol 4-O-glucoside production in *Arabidopsis thaliana*. *Plant J* 48: 286-295
- Lapierre C, 1993. Applications of new methods for the investigation of lignin structure, pp 133-136. In: *Forage Cell Wall Structure and Digestibility*. Jung HG, Buxton DR, Hatfield RD eds. ASA-CS-SA-SSSA: Madison, Wisconsin, USA
- Lapierre C, Pollet B, Monties B, 1991. Heterogeneous distribution of diarylpropane structures in spruce lignins. *Phytochemistry* 30: 659-662
- Laufs P, Peaucele A, Morin H, Traas J, 2004. MicroRNA regulation of the CUC genes is required for boundary size control in *Arabidopsis* meristems. *Development* 131: 4311-4322
- Lawoko M, Henriksson G, Gellerstedt G, 2005. Structural differences between the lignin-carbohydrate complexes present in wood and in chemical pulps. *Biomacromolecules* 6: 3467-3473
- Lee D, Meyer K, Chapple C, Douglas CJ, 1997. Antisense suppression of 4-coumarate:coenzyme A ligase activity in *Arabidopsis* leads to altered lignin subunit composition. *Plant Cell* 9: 1985-1998
- Lee CH, O'Neill MA, Tsumuraya Y, Darvill AG, Ye ZH, 2007a. The irregular xylem9 mutant is deficient in xylan xylosyltransferase activity. *Plant Cell Physiol* 48: 1624-1634
- Lee H, Kim B, Song SK, Heo JO, Yu NI, Lee SA, Kim M, Kim DG, Sohn SO, Lim CE, Chang KS, Lee MM, Lim J, 2008. Large-scale analysis of the GRAS gene family in *Arabidopsis thaliana*. *Plant Mol Biol* 67: 659-670
- Lee C, Teng Q, Zhong R, Ye ZH, 2011a. The four *Arabidopsis* reduced wall acetylation genes are expressed in secondary wall-containing cells and required for the acetylation of xylan. *Plant Cell Physiol* 52:1289-1301
- Lee Y, Chen F, Gallego-Giraldo L, Dixon RA, Voit EO, 2011b. Integrative analysis of transgenic alfalfa (*Medicago sativa* L.) suggests new metabolic control mechanisms for monolignol biosynthesis. *Plos Comp Biol* 7: e1002047
- Legay S, Lacombe E, Goicoechea M, Briere C, Seguin A, Mackay J, Grima-Pettenati J, 2007. Molecular characterization of EgMYB1, a putative transcriptional repressor of the lignin biosynthetic pathway. *Plant Sci* 173: 542-549
- Legay S, Sivadon P, Blervacq AS, Pavy N, Baghdady A, Tremblay L, Levasseur C, Ladouce N, Lapierre C, Séguin A, Hawkins S, Mackay J, Grima-Pettenati J, 2010. EgMYB1, an R2R3 MYB transcription factor from *Eucalyptus* negatively regulates secondary cell wall formation in *Arabidopsis* and poplar. *The New Phytologist* 188:774-786
- Li LG, Popko JL, Zhang XH, Osakabe K, Tsai CJ, Joshi CP, Chiang VL, 1997. A novel multifunctional O-methyltransferase implicated in a dual methylation pathway associated with lignin biosynthesis in loblolly pine. *Proc Natl Acad Sci USA* 94: 5461-5466
- Li L, Popko JL, Umezawa T, Chiang VL, 2000. 5-hydroxyconiferyl aldehyde modulates enzymatic methylation for syringyl monolignol formation, a new view of monolignol biosynthesis in angiosperms. *J Biol Chem* 275: 6537-6545
- Li Y, Qian Q, Zhou Y, Yan M, Sun L, Zhang M, Fu Z, Wang Y, Han B, Pang X, Chen M, Li J, 2003a. Brittle culm1, which encodes a COBRA-like protein, affects the mechanical properties of rice plants. *Plant Cell* 15: 2020-2031
- Li Y, Kajita S, Kawai S, Katayama Y, Morohoshi N, 2003b. Down-regulation of an anionic peroxidase in transgenic aspen and its effect on lignin characteristics. *J Plant Res* 116: 175-182
- Li Y, Jones L, McQueen-Mason S, 2003c. Expansins and cell growth. *Curr Opin Plant Biol* 6: 603-610
- Li X, Duan X, Jiang H, Sun Y, Tang Y, Yuan Z, Guo J, Liang W, Chen L, Yin J, Ma H, Wang J, Zhang D,

2006. Genome-wide analysis of basic/helix-loop-helix transcription factor family in rice and *Arabidopsis*. *Plant Physiol* 141: 1167-1184
- Li X, Weng JK, Chapple C, 2008. Improvement of biomass through lignin modification. *Plant J* 54: 569-581
- Li Q, Min D, Wang JP, Peszlen I, Horvath L, Horvath B, Nishimura Y, Jameel H, Chang HM, Chiang VL, 2011a. Down-regulation of glycosyltransferase 8D genes in *Populus trichocarpa* caused reduced mechanical strength and xylan content in wood. *Tree Physiol* 31: 226-236
- Li E, Wang S, Liu Y, Chen JG, Douglas CJ, 2011b. OVATE FAMILY PROTEIN4 (OFP4) interaction with KNAT7 regulates secondary cell wall formation in *Arabidopsis thaliana*. *Plant J* 67: 328-341
- Li E, Bhargava A, Qiang W, Friedmann MC, Forneris N, Savidge RA, Johnson LA, Mansfield SD, Ellis BE, Douglas CJ, 2012. The Class II KNOX gene *KNAT7* negatively regulates secondary wall formation in *Arabidopsis* and is functionally conserved in *Populus*. *New Phytol* 194: 102-115
- Liljegren SJ, Ditta GS, Eshed HY, Savidge B, Bowman JL, Yanofsky MF, 2000. SHATTERPROOF MADS-box genes control seed dispersal in *Arabidopsis*. *Nature* 404: 766-770
- Lim EK, Jackson RG, Bowles DJ, 2005. Identification and characterization of *Arabidopsis* glycosyltransferases capable of glucosylating coniferyl aldehyde and sinapyl aldehyde. *FEBS Lett* 579: 2802-2806
- Liu CJ, 2012. Deciphering the enigma of lignification: Precursor transport, oxidation, and the top-chemistry of lignin assembly. *Mol Plant* 5:304-317
- Louie GV, Bowman ME, Tu Y, Mouradov A, Spangenberg G, Noel JP, 2010. Structure-function analyses of a caffeic acid O-methyltransferase from perennial ryegrass reveal the molecular basis for substrate preference. *Plant Cell* 22: 4114-4127
- Lu FC, Ralph J, 1999. Detection and determination of *p*-coumaroylated units in lignins. *J Agric Food Chem* 47: 1988-1992.
- Ma K, Xiao J, Li X, Zhang Q, Lian X, 2009. Sequence and expression analysis of the C3HC4-type RING finger gene family in rice. *Gene* 444: 33-45
- MacAdam JW, Grabber JH, 2002. Relationship of growth cessation with the formation of diferulate cross-links and *p*-coumaroylated lignins in tall fescue leaf blades. *Planta* 215:785-793
- MacMillan CP, Mansfield SD, Stachurski ZH, Evans R, Southerton SG, 2010. Fasciclin-like arabinogalactan proteins: specialization for stem biomechanics and cell wall architecture in *Arabidopsis* and *Eucalyptus*. *Plant J* 2: 689-703
- McConnell JR, Emery J, Eshed Y, Bao N, Bowman J, Barton MK, 2001. Role of PHABULOSA and PHAVOLUTA in determining radial patterning in shoots. *Nature* 411: 709-713
- McCaig BC, Meagher RB, Dean JF, 2005. Gene structure and molecular analysis of the laccase-like multicopper oxidase (LMCO) gene family in *Arabidopsis thaliana*. *Planta* 221: 619-636
- Maeda H, Dudareva N, 2012. The shikimate pathway and aromatic amino acid biosynthesis in plants. *Ann Rev Plant Biol* 63: 73-105
- Mahesh V, Million-Rousseau R, Ullmann P, Chabrilange N, Bustamante J, Mondolot L, Morant M, Noirot M, Hamon S, de Kochko A, Werck-Reichhart D, Campa C, 2007. Functional characterization of two *p*-coumaroyl ester 3'-hydroxylase genes from coffee tree: evidence of a candidate for chlorogenic acid biosynthesis. *Plant Mol Biol* 64: 145-159
- Manabe Y, Nafisi M, Verherbruggen Y, Orfila C, Gille S, Rautengarten C, Cherk C, Marcus SE, Somerville S, Pauly M, Knox JP, Sakuragi Y, Scheller HV, 2011. Loss-of-function mutation of REDUCED WALL ACETYLATION2 in *Arabidopsis* leads to reduced cell wall acetylation and increased resistance to *Botrytis cinerea*. *Plant Physiol* 155: 1068-1078
- Martinez AT, Rencoret J, Marques G, Gutierrez A, Ibarra D, Jimenez-Barbero J, del Rio JC, 2008. Monolignol acylation and lignin structure in some nonwoody plants: A 2D NMR study. *Phytochemistry* 69: 2831-2843
- Martz F, Maury S, Pincon G, Legrand M, 1998. cDNA cloning, substrate specificity and expression study of tobacco caffeoyl-CoA 3-O-methyltransferase, a lignin biosynthetic enzyme. *Plant Mol Biol* 36: 427-437
- Matthews J, Bhati M, Lehtomaki E, Mansfield R, Cubeddu L, MacKay J, 2009. It takes two to tango: the structure and function of LIM, RING, PHD and MYND domains. *Cur Pharmaceutical Design* 15: 3681-3696
- Méchin V, Argillier O, Menanteau V, Barrière Y, Mila I, Pollet B, Lapierre C, 2000. Relationship of cell wall composition to in vitro cell wall digestibility of maize inbred line stems. *J Sci Food Agric* 80: 574-580
- Méchin V, Argillier O, Hébert Y, Guingo E, Moreau L, Charcosset A, Barrière Y, 2001. Genetic analysis and QTL mapping of cell wall digestibility and lignification in silage maize. *Crop Sci* 41: 690-697
- Méchin V, Argillier O, Rocher F, Hébert Y, Mila I, Pollet B, Barrière Y, Lapierre C, 2005. In search of a maize ideotype for cell wall enzymatic degradability using histological and biochemical lignin characterization. *J Agric Food Chem* 53: 5872-5881
- Meng H, Campbell WH, 1998. Substrate profiles and expression of caffeoyl coenzyme A and caffeic acid O-methyltransferases in secondary xylem of aspen during seasonal development. *Plant Mol Biol* 38: 513-520
- Meyers BC, Tingey SV, Morgante M, 2001. Abundance, distribution, and transcriptional activity of repetitive elements in the maize genome. *Genome*

- Res 11: 1660-1676
- Miao YC, Liu CJ, 2010. ATP-binding cassette-like transporters are involved in the transport of lignin precursors across plasma and vacuolar membranes. *Proc Acad Sci USA* 107: 22728-22733
- Milioni D, Sado PE, Stacey NJ, Domingo C, Roberts K, McCann MC, 2001. Differential expression of cell-wall-related genes during the formation of tracheary elements in the *Zinnia mesophyll* cell system. *Plant Mol Biol* 47: 221-238
- Mitchell RAC, Dupree P, Shewry PR, 2007. A novel bioinformatics approach identifies candidate genes for the synthesis and feruloylation of arabinoxylan. *Plant Physiol* 144: 43-53
- Mitsuda N, Seki M, Shonozaki K, Ohme-Takagi M, 2005. The NAC transcription factors NST1 and NST2 of *Arabidopsis* regulate secondary wall thickenings and are required for anther dehiscence. *Plant Cell* 17: 2993-3006
- Mitsuda N, Iwase A, Yamamoto H, Yoshida M, Seki M, Shinozaki K, Ohme-Takagi M, 2007. NAC transcription factors, NST1 and NST3, are key regulators of the formation of secondary walls in woody tissues of *Arabidopsis*. *Plant Cell* 19: 270-280
- Moffatt BA, Stevens YY, Allen MS, Snider JD, Pereira LA, Todorova MI, Summers PS, Weretilnyk EA, Martin-McCaffrey L, Wagner C, 2002. Adenosine kinase deficiency is associated with developmental abnormalities and reduced transmethylation. *Plant Physiol* 128: 812-821
- Morreel K, Ralph J, Kim H, Lu FC, Goeminne G, Ralph S, Messens E, Boerjan W, 2004. Profiling of oligolignols reveals monolignol coupling conditions in lignifying poplar xylem. *Plant Physiol* 136: 3537-3549
- Morrison TA, Jung HG, Buxton DR, Hatfield RD, 1998. Cell-wall composition of maize internodes of varying maturity. *Crop Sci.* 38: 455-460
- Mortimer JC, Miles GP, Brown DM, Zhang Z, Segura MP, Weimar T, Yu X, Seffen KA, Stephens E, Turner SR, Dupree P, 2010. Absence of branches from xylan in *Arabidopsis gux* mutants reveals potential for simplification of lignocellulosic biomass. *Proc Natl Acad Sci USA* 107: 17409-17414
- Muller B, Bourdais G, Reidy B, Bencivenni C, Massonneau A, Condamine P, Rolland G, Conéjéro G, Rogowsky P, Tardieu F, 2007. Association of specific expansins with growth in maize leaves is maintained under environmental, genetic, and developmental sources of variation. *Plant Physiol* 143: 278-290
- Nair RB, Bastress KL, Ruegger MO, Denault JW, Chapple C, 2004. The *Arabidopsis thaliana* reduced epidermal fluorescence1 gene encodes an aldehyde dehydrogenase involved in ferulic acid and sinapic acid biosynthesis. *Plant Cell* 16: 544-554
- Newman LJ, Perazza DE, Juda L, Campbell MM, 2004. Involvement of the R2R3-MYB, *AtMYB61*, in the ectopic lignification and dark-photomorphogenic components of the *det3* mutant phenotype. *Plant J* 37: 239-250
- Nibau C, Wu HM, Cheung AY, 2006. RAC/ROP GTPases: 'hubs' for signal integration and diversification in plants. *Trends Plant Sci* 11: 309-315
- Nielsen KL, Indiani C, Henriksen A, Feis A, Becucci M, Gajhede M, Smulevich G, Welinder KG, 2001. Differential activity and structure of highly similar peroxidases. Spectroscopic, crystallographic and enzymatic analyses of lignifying *Arabidopsis thaliana* peroxidase A2 and horseradish peroxidase A2. *Biochemica* 40: 11013-11021
- Ohman D, Demedts B, Kumar M, Gerber L, Gorzsás A, Goeminne G, Hedenström M, Ellis B, Boerjan W, Sundberg B, 2013. MYB103 is required for FERULATE-5-HYDROXYLASE expression and syringyl lignin biosynthesis in *Arabidopsis* stems. *Plant J* 73: 63-76
- Oikawa A, Joshi HJ, Rennie EA, Ebert B, Manisseri C, Heazlewood JL, Scheller HV, 2010. An integrative approach to the identification of *Arabidopsis* and rice genes involved in xylan and secondary wall development. *PLoS One* 5: e15481
- Parker G, Schofield R, Sundberg B, Turner S, 2003. Isolation of *COV1*, a gene involved in the regulation of vascular patterning in the stem of *Arabidopsis*. *Development* 130: 2139-2148
- Parvathi K, Chen F, Guo DJ, Blount JW, Dixon RA, 2001. Substrate preferences of O-methyltransferases in alfalfa suggest new pathways for 3-O-methylation of monolignols. *Plant J* 25: 193-202
- Patzlaff A, McInnis S, Courtenay A, Surman C, Newman LJ, Smith C, Bevan MW, Mansfield S, Whetten RW, Sederoff RR, Campbell MM, 2003. Characterisation of a pine MYB that regulates lignification. *Plant J* 36: 743-754
- Paz-Ares J, Ghosal D, Wienand U, Peterson PA, Saedler H, 1987. The regulatory *c1* locus of *Zea mays* encodes a protein with homology to myb proto-oncogene products and with structural similarities to transcriptional activators. *EMBO J* 6:3553-3558
- Penning BW, Hunter CT 3rd, Tayengwa R, Eveland AL, Dugard CK, Olek AT, Vermerris W, Koch KE, McCarty DR, Davis MF, Thomas SR, McCann MC, Carpita NC, 2009. Genetic resources for maize cell wall biology. *Plant Physiol* 151: 1703-1728
- Persson S, Wei HR, Milne J, Page GP, Somerville CR, 2005. Identification of genes required for cellulose synthesis by regression analysis of public microarray data sets. *Proc Natl Acad Sci USA* 102: 8633-8638
- Persson S, Caffall KH, Freshour G, Hilley MT, Bauer S, Poindexter P, Hahn MG, Mohnen D, Somerville C, 2007. The *Arabidopsis irregular xylem8* mutant is deficient in glucuronoxylan and homogalacturonan, which are essential for secondary cell wall integrity. *Plant Cell* 19: 237-255



- Pesquet E, Korolev AV, Calder G, Lloyd CW, 2010. The microtubule-associated protein AtMAP70-5 regulates secondary wall patterning in Arabidopsis wood cells. *Cur Biol* 20: 744-749
- Pesquet E, Korolev AV, Calder G, Lloyd CW, 2011. Mechanisms for shaping, orienting, positioning and patterning plant secondary cell walls. *Plant Signal Behav* 6: 843-849
- Peter G, Neale D, 2004. Molecular basis for the evolution of xylem lignification. *Curr Opin Plant Biol* 7: 737-742
- Pichon M, Deswartes C, Gerentes D, Guillaumie S, Lapierre C, Toppan A, Barrière B, Goffner D, 2006. Variation in lignin and cell wall digestibility in caffeic acid O-methyltransferase down-regulated maize half-sib progenies in field experiments. *Mol Breeding* 18: 253-261
- Piquemal J, Chamayou S, Nadaud I, Beckert M, Barrière Y, Mila I, Lapierre C, Rigau J, Puigdomenech P, Jauneau A, Dignonnet C, Boudet AM, Goffner D, Pichon M, 2002. Down-regulation of caffeic acid O-methyltransferase in maize revisited using a transgenic approach. *Plant Physiol* 130: 1675-1685
- Piston F, Uauy C, Fu L, Langston J, Labavitch J, Dubcovsky J, 2010. Down-regulation of four putative arabinoxylan feruloyl-transferase genes from family PF02458 reduces ester-linked ferulate content in rice cell walls. *Planta* 231: 677-691
- Qiu D, Wilson IW, Gan S, Washusen R, Moran GF, Southerton SG, 2008. Gene expression in Eucalyptus branch wood with marked variation in cellulose microfibril orientation and lacking G-layers. *New Phytol* 179: 94-103
- Ralph J, Helm RF, Quideau S, Hatfield RD, 1992. Lignin feruloyl ester cross-links in grasses .1. Incorporation of feruloyl esters into coniferyl alcohol dehydrogenation polymers. *J Chem Soc [Perkin 1]* 1: 2961-2969
- Ralph J, Hatfield RD, Quideau S, Helm RF, Grabber JH, Jung HJG, 1994. Pathway of p-coumaric acid incorporation into maize lignin as revealed by NMR. *J Am Chem Soc* 116: 9448-9456
- Ralph J, Grabber JH, Hatfield RD, 1995. Lignin-ferulate cross-links in grasses - Active incorporation of ferulate polysaccharide esters into ryegrass lignins. *Carbohydr Res*. 275: 167-178
- Ralph J, Bunzel M, Marita JM, Hatfield RD, Lu F, Kim H, Schatz PF, Grabber JH, Steinhart H, 2004. Peroxidase-dependent cross-linking reactions of p-hydroxycinnamates in plant cell walls. *Phytochem Reviews* 3: 79-96
- Ralph J, Brunow G, Harris PJ, Dixon RA, Schatz PF, BoerjanW, 2008a. Lignification: are lignins biosynthesized via simple combinatorial chemistry or via proteinaceous control and template replication? pp. 36-66. In: Recent advances in polyphenol research. Daayf F, El Hadrami A, Adam L, Ballance GM eds. Wiley-Blackwell Publishing, Oxford, UK
- Ralph J, Kim H, Lu F, Grabber JH, Leple JC, Berri-Sierra J, Derikvand MM, Jouanin L, Boerjan W, Lapierre C, 2008b. Identification of the structure and origin of a thioacidolysis marker compound for ferulic acid incorporation into angiosperm lignins (and an indicator for cinnamoyl CoA reductase deficiency). *Plant J* 53: 368-379
- Ralph J, 2010. Hydroxycinnamates in lignification. *Phytochem Rev* 9: 65-83
- Ramsay NA, Glover BJ, 2005. MYB-bHLH-WD40 protein complex and the evolution of cellular diversity. *Trends Plant Sci* 10: 63-70
- Ranocha P, Bourgis F, Ziemak MJ, Rhodes D, Gage DA, Hanson AD, 2000. Characterization and functional expression of cDNAs encoding methionine-sensitive and -insensitive homocysteine S-methyltransferases from Arabidopsis. *J Bio Chem* 275: 15962-15968
- Ranocha P, Mcneil SD, Ziemak MJ, Li CJ, Tarczynski MC, Hanson AD, 2001. The S-methylmethionine cycle in angiosperms: ubiquity, antiquity and activity. *Plant J* 25: 575-584
- Ranocha P, Chabannes M, Chamayou S, Danoun S, Jauneau A, Boudet AM, Goffner D, 2002. Laccase down-regulation causes alterations in phenolic metabolism and cell wall structure in poplar. *Plant Physiol* 129: 145-155
- Ratcliffe OJ, Riechmann JL, Zhang JZ, 2000. Interfacicular fiberless1 is the same gene as REVOLUTA. *Plant Cell* 12: 315-317
- Ravanel S, Gakiere B, Job D, Douce R, 1998. The specific features of methionine biosynthesis and metabolism in plants. *Proc Natl Acad Sci USA* 95: 7805-7812
- Reiter WD, Vanzin GF, 2001. Molecular genetics of nucleotide sugar interconversion pathways in plants. *Plant Mol. Biol.* 47: 95-113
- Reiter WD, 2008. Biochemical genetics of nucleotide sugar interconversion reactions. *Curr Opin Plant Biol* 11: 236-243
- Rengel D, San Clemente H, Servant F, Ladouce N, Paux E, Wincker P, Couloux A, Sivadon P, Grima-Pettenati J, 2009. A new genomic resource dedicated to wood formation in Eucalyptus. *BMC Plant Biology* 9: 36
- Riboulet C, Fabre F, Dénoue D, Martinant JP, Lefevre B, Barrière Y, 2008a. QTL mapping and candidate gene research for lignin content and cell wall digestibility in a topcross of a flint recombinant inbred line progeny harvested at silage stage. *Maydica* 53: 1-9
- Riboulet C, Lefèvre B, Denoue D, Barrière Y, 2008b. Genetic variation in maize cell wall for lignin content, lignin structure, p-hydroxycinnamic acid content, and digestibility in a set of 19 lines at silage harvest maturity. *Maydica* 53: 11-19
- Riboulet C, Guillaumie S, Méchin V, Bosio M, Pichon M, Goffner D, Lapierre C, Pollet B, Lefèvre B, Martinant JP, Barrière Y, 2009. Kinetics of phen-

- ylpropanoid gene expression in maize growing internodes: Relationships with cell wall deposition. *Crop Sci* 49: 211-223
- Roesler J, Krekel F, Amrhein N, Schmid J, 1997. Maize phenylalanine ammonia-lyase has tyrosine ammonia-lyase activity. *Plant Physiol* 113: 175-179
- Rogers LA, Dubos C, Surman C, Willment J, Cullis IF, Mansfield SD, Campbell MM, 2005. Comparison of lignin deposition in three ectopic lignification mutants. *New Phytol* 168: 123-140
- Roussel V, Gibelin C, Fontaine AS, Barrière Y, 2002. Genetic analysis in recombinant inbred lines of early dent forage maize. II - QTL mapping for cell wall constituents and cell wall digestibility from per se value and top cross experiments. *Maydica* 47: 9-20
- Rushton PJ, Somssich IE, Ringler P, Shen QJ, 2010. WRKY transcription factors. *Trends Plant Sci* 15: 247-258
- Sablowski RWM, Moyano E, Culianezmacia FA, Schuch W, Martin C, Bevan M, 1994. A flower-specific Myb protein activates transcription of phenylpropanoid biosynthetic genes. *EMBO J* 13: 128-137
- Samuels AL, Rensing KH, Douglas CJ, Mansfield SD, Dharmawardhana DP, Ellis BE, 2002. Cellular machinery of wood production: differentiation of secondary xylem in *Pinus contorta* var. *latifolia*. *Planta* 216: 72-82
- Sanchez JP, Ullman C, Moore M, Choo Y, Chua NH, 2006. Regulation of *Arabidopsis thaliana* 4-coumarate : coenzyme-A ligase-1 expression by artificial zinc finger chimeras. *Plant Biotechnol J* 4: 103-114
- Sanchez-Fernandez R, Davies TGE, Coleman JOD, Rea PA, 2001. The *Arabidopsis thaliana* ABC protein superfamily, a complete inventory. *J Bio Chem* 276: 30231-30244
- Sánchez-Rodríguez C, Bauer S, Hématy K, Saxe F, Ibáñez AB, Vodermaier V, Konlechner C, Sampathkumar A, Rüggeberg M, Aichinger E, Neumetzler L, Burgert I, Somerville C, Hauser MT, Persson S, 2012. Chitinase-like1/pom-pom1 and its homolog CTL2 are glucan-interacting proteins important for cellulose biosynthesis in *Arabidopsis*. *Plant Cell* 24: 589-607
- SanMiguel P, Gaut BS, Tikhonov A, Nakajima Y, Bennetzen JL, 1998. The paleontology of intergene retrotransposons of maize. *Nat Genet* 20: 43-45
- Sato Y, Whetten RW, 2006. Characterization of two laccases of loblolly pine (*Pinus taeda*) expressed in tobacco BY-2 cells. *J Plant Res* 119: 581-588
- Sasaki S, Baba K, Nishida T, Tsutsumi Y, Kondo R, 2006. The cationic cell-wall-peroxidase having oxidation ability for polymeric substrate participates in the late stage of lignification of *Populus alba* L. *Plant Mol Biol* 62: 797-807
- Saulnier L, Guillon F, Chateigner-Boutin AL, 2012. Cell wall deposition and metabolism in wheat grain. *J Cereal Sci* 56: 91-108
- Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, Liang C, Zhang J, Fulton L, Graves TA, Minx P, Reily AD, Courtney L, Kruchowski SS, Tomlinson C, Strong C, Delehaunty K, Fronick C, Courtney B, Rock SM, Belter E, Du F, Kim K, Abbott RM, Cotton M, Levy A, Marchetto P, Ochoa K, Jackson SM, Gillam B, Chen W, Yan L, Higinbotham J, Cardenas M, Waligorski J, Applebaum E, Phelps L, Falcone J, Kanchi K, Thane T, Scimone A, Thane N, Henke J, Wang T, Ruppert J, Shah N, Rotter K, Hodges J, Ingenthron E, Cordes M, Kohlberg S, Sgro J, Delgado B, Mead K, Chinwalla A, Leonard S, Crouse K, Collura K, Kudrna D, Currie J, He R, Angelova A, Rajasekar S, Mueller T, Lomeli R, Scara G, Ko A, Delaney K, Wissotski M, Lopez G, Campos D, Braidotti M, Ashley E, Golser W, Kim H, Lee S, Lin J, Dujmic Z, Kim W, Talag J, Zuccolo A, Fan C, Sebastian A, Kramer M, Spiegel L, Nascimento L, Zutavern T, Miller B, Ambroise C, Muller S, Spooner W, Narechiana A, Ren L, Wei S, Kumari S, Faga B, Levy MJ, McMahan L, Van Buren P, Vaughn M, Ying K, Yeh C, Emrich S, Jia Y, Kalyanaraman A, Hsia A-P, Barbazuk WB, Baucom RS, Brutnell TP, Carpita NC, Chaparro C, Chia J-M, Deragon J-M, Estill JC, Fu Y, Jeddelloh JA, Han Y, Lee H, Li P, Lisch DR, Liu S, Liu Z, Nagel DH, McCann MC, SanMiguel P, Myers AM, Nettleton D, Nguyen J, Penning BW, Ponnala L, Schneider KL, Schwartz DC, Sharma A, Soderlund C, Springer NM, Sun Q, Wang H, Waterman M, Westerman R, Wolfgruber TK, Yang L, Yu Y, Zhang L, Zhou S, Zhu Q, Bennetzen JL, Dawe RK, Jiang J, Jiang N, Presting GG, Wessler SR, Aluru S, Martienssen RA, Clifton SW, McCombie WR, Wing RA, Wilson RK, 2009. The B73 maize genome: complexity, diversity, and dynamics. *Science* 326: 1112-1115
- Schöch G, Goepfert S, Morant M, Hehn A, Meyer D, Ullmann P, Werck-Reichhart D, 2001. CYP98A3 from *Arabidopsis thaliana* is a 3'-hydroxylase of phenolic esters, a missing link in the phenylpropanoid pathway. *J Bio Chem* 276: 36566-36574
- Shadle G, Chen F, Reddy MSS, Jackson L, Nakashima J, Dixon RA, 2007. Down-regulation of hydroxycinnamoyl CoA: Shikimate hydroxycinnamoyl transferase in transgenic alfalfa affects lignification, development and forage quality. *Phytochemistry* 68: 1521-1529
- Shen H, He X, Poovaiah CR, Wuddineh WA, Ma J, Mann DG, Wang H, Jackson L, Tang Y, Stewart CN Jr, Chen F, Dixon RA, 2012. Functional characterization of the switchgrass (*Panicum virgatum*) R2R3-MYB transcription factor PvMYB4 for improvement of lignocellulosic feedstocks. *New Phytol* 193: 121-136
- Shi H, Kim YS, Guo Y, Stevenson B, Zhu JK, 2003. The *Arabidopsis* SOS5 locus encodes a putative

- cell surface adhesion protein and is required for normal cell expansion. *Plant Cell* 15: 19-32
- Sindhu A, Langewisch T, Olek A, Multani DS, McCann MC, Vermerris W, Carpita NC, Johal G, 2007. Maize *Brittle stalk2* encodes a COBRA-like protein expressed in early organ development but required for tissue flexibility at maturity. *Plant Physiol* 145: 1444-1459
- Skibbe D, Liu F, Wen T, Yandeau M, Cui X, Cao J, Simmons C, Schnable P, 2002. Characterization of the aldehyde dehydrogenase gene families of *Zea mays* and *Arabidopsis*. *Plant Mol Biol* 48: 751-764
- Sonbol FM, Fornalé S, Cappellades M, Encina A, Tourino S, Torres JL, Rovira P, Ruel K, Puigdomenech P, Rigau J, Caparrós-Ruiz D, 2009. The maize ZmMYB42 represses the phenylpropanoid pathway and affects the cell wall structure, composition and degradability in *Arabidopsis thaliana*. *Plant Mol Biol* 70:283-296
- Song X, Zhang B, Zhou Y, 2011. Golgi-localized UDP-glucose transporter is required for cell wall integrity in rice. *Plant Signal Behav* 6: 1097-1100
- Springer NM, Ying K, Fu Y, Ji T, Yeh CT, Jia Y, Wu W, Richmond T, Kitzman J, Rosenbaum H, Iniguez AL, Barbazuk WB, Jeddloh JA, Nettleton D, Schnable PS, 2009. Maize inbreds exhibit high levels of copy number variation (CNV) and presence/absence variation (PAV) in genome content. *PLoS Genet* 5: e1000734
- Struik PC, 1983. Physiology of forage maize (*Zea mays* L) in relation to its production and quality, pp. 1-252. Ph Dissertation, Agricultural University, 6700 GW Wageningen, The Netherlands
- Swanson-Wagner RA, Eichten SR, Kumari S, Tiffin P, Stein JC, Ware D, Springer NM, 2010. Pervasive gene content variation and copy number variation in maize and its undomesticated progenitor. *Genome Res* 20: 1689-1699
- Szyjanowicz PMJ, McKinnon I, Taylor NG, Gardiner J, Jarvis MC, Turner SR, 2004. The *irregular xylem 2* mutant is an allele of KORRIGAN that affects the secondary cell wall of *Arabidopsis thaliana*. *Plant J* 37: 730-740
- Taboada A, Novo-Uzal E, Flores G, Loureda M, Ros Barceló A, Masa A, Pomar F, 2010. Digestibility of silages in relation to their hydroxycinnamic acid content and lignin composition. *J Sci Food Agric* 90: 1155-1162
- Takahashi J, Rudsander UJ, Hedenström M, Banasiak A, Harholt J, Amelot N, Immerzeel P, Ryden P, Endo S, Ibatullin FM, Brumer H, del Campillo E, Master ER, Scheller HV, Sundberg B, Teeri TT, Mellerowicz EJ. 2009. KORRIGAN1 and its aspen homolog PttCel9A1 decrease cellulose crystallinity in *Arabidopsis* stems. *Plant Cell Physiol* 50: 1099-1115
- Talbert PB, Adler HT, Parks DW, Comai L, 1995. The REVOLUTA gene is necessary for apical meristem development and for limiting cell divisions in the leaves and stems of *Arabidopsis thaliana*. *Development* 121: 2723-2735
- Tamagnone L, Merida A, Parr A, Mackay S, Culianez-Marcia FA, Roberts K, Martin C, 1998. The AmMYB308 and AmMYB330 transcription factors from *Antirrhinum* regulate phenylpropanoid and lignin biosynthesis in transgenic tobacco. *Plant Cell* 10: 135-154
- Tamasloukht B, Lam MS-JWQ, Martinez Y, Tozo K, Barbier O, Jourda C, Jauneau A, Borderie, G, Balzergue S, Renou JP, Huguet S, Martinant JP, Tatout C, Lapierre C, Barrière Y, Goffner D, Pichon M, 2011. Characterization of a *cinnamoyl-CoA reductase 1 (CCR1)* mutant in maize: effects on lignification, fibre development, and global gene expression. *J Exp Bot* 62: 3837-3848
- Taylor N, Scheible WR, Cutler S, Somerville CR, Turner SR, 1999. The *irregular xylem3* locus of *Arabidopsis* encodes a cellulose synthase required for secondary cell wall synthesis. *Plant Cell* 11: 769-779
- Taylor N, Laurie S, Turner S, 2000. Multiple cellulose synthase catalytic subunits are required for cellulose synthesis in *Arabidopsis*. *Plant Cell* 12: 2529-2539
- Tang HM, 2011. The *brown midrib 2 (bm2)* gene of maize encodes methylenetetrahydrofolate reductase. Iowa State University, Graduate Theses and Dissertations, Paper 12482, (<http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=3489&context=etd>)
- Terashima N, Fukushima K, 1993. Comprehensive model of the lignified plant cell wall, pp. 247-270. In: Forage Cell Wall Structure and Digestibility. Jung HG, Buxton D, Hatfield R, Ralph J eds. Madison, Wisconsin
- Tokunaga N, Kaneta T, Sato S, Sato Y, 2009. Analysis of expression profiles of three peroxidase genes associated with lignification in *Arabidopsis thaliana*. *Physiol Plantarum* 136: 237-249
- Tomlinson PB, 1995. Non homology of vascular organization in monocotyledons and dicotyledons, pp. 589-622. In: *Monocotyledons, Systematic and Evolution*. Rudall PJ, Cribbb PJ, Cutler DF, Humphries CJ eds. Royal Botanic Gardens, Kew
- Tran LSP, Nakashima K, Sakuma Y, Osakabe Y, Qin F, Simpson SD, Maruyama K, Fujita Y, Shinozaki K, Yamaguchi-Shinozaki K, 2006. Co-expression of the stress-inducible zinc finger homeodomain ZFHD1 and NAC transcription factors enhances expression of the ERD1 gene in *Arabidopsis*. *Plant J* 49: 46-63
- Tripathi P, Rabara RC, Langum TJ, Boken AK, Rush-ton DL, Boomsma DD, Rinerson CI, Rabara J, Reese RN, Chen X, Rohila JS, Rushton PJ, 2012. The WRKY transcription factor family in brachypodium distachyon. *BMC Genomics* 13: 270
- Turner S, Somerville C, 1997. Collapsed xylem phe-

- notype of *Arabidopsis* identifies mutants deficient in cellulose deposition in the secondary cell wall. *Plant Cell* 9: 689-701
- Urahara T, Tsuchiya K, Kotake T, Tohno-oka T, Komae K, Kawada N, Tsumuraya Y, 2004. A beta-(1-4)-xylosyltransferase involved in the synthesis of arabinoxylans in developing barley endosperms. *Physiol Plant* 122: 169-180
- Uzal EN, Gomez-Ros LV, Pomar F, Bernal MA, Paradela A, Albar JP, Ros-Barcelo A, 2008. The presence of sinapyl lignin in *Ginkgo biloba* cell cultures changes our views of the evolution of lignin biosynthesis. *Physiol Plant* 135: 196-213
- Van der Mijnsbrugge K, Meyermans H, Van Montagu M, Bauw G, Boerjan W, 2000. Wood formation in poplar: identification, characterization, and seasonal variation of xylem proteins. *Planta* 210: 589-598
- Vanholme R, Morreel K, Darrah C, Oyarce P, Grabber JH, Ralph J, Boerjan W. 2012. *New Phytol* 196: 978-1000
- Vignols F, Rigau J, Torres MA, Capellades M, Puigdomenech P, 1995. The *brown midrib 3 (bm3)* mutation in maize occurs in the gene encoding caffeic acid O-methyltransferase. *Plant Cell* 7: 407-416
- Vincent D, Lapierre C, Pollet B, Cornic G, Negroni L, Zivy M, 2005. Water deficits affect caffeate O-methyltransferase, lignification, and related enzymes in maize leaves. A proteomic investigation. *Plant Physiol* 137: 949-960
- Wang J, Zhu ML, Wei ZM, 2008. Cotton laccase gene overexpression in transgenic *Populus alba* var. *pyramidalis* and its effects on the lignin biosynthesis in transgenic plants. *Fen Zi Xi Bao Sheng Wu Xue Bao* 41: 11-18 (Article in Chinese)
- Wang H, Avci U, Nakashima J, Hahn MG, Chen F, Dixon RA, 2010. Mutation of WRKY transcription factors initiates pith secondary wall formation and increases stem biomass in dicotyledonous plants. *Proc Natl Acad Sci USA* 107: 22338-22343
- Wei KF, Chen J, Chen YF, Wu LJ, Xie DX, 2012. Molecular phylogenetic and expression analysis of the complete WRKY transcription factor family in maize. *DNA Res* 19: 153-164
- Wilkins O, Nahal H, Foong J, Provart NJ, Campbell MM, 2009. Expansion and diversification of the *Populus* R2R3-MYB family of transcription factors. *Plant Physiol* 149: 981-993
- Winkel BSJ, 2004. Metabolic channeling in plants. *Ann Rev Plant Biol* 55: 85-107
- Wissenbach M, Uberlacker B, Vogt F, Becker D, Salamini F, Rohde W, 1993. MYB genes from *Hordeum vulgare* - Tissue-specific expression of chimeric MYB Promoter/Gus genes in transgenic tobacco. *Plant J* 4: 411-422
- Withers S, Lu F, Kim H, Zhu Y, Ralph J, Wilkerson CG, 2012. Identification of grass-specific enzyme that acylates monolignols with *p*-coumarate. *J Biol Chem* 287: 8347-8355
- Wu KL, Guo ZJ, Wang HH, Li J, 2005. The WRKY family of transcription factors in rice and *Arabidopsis* and their origins. *DNA Research* 12: 9-26
- Wu AM, Rihouey C, Seveno M, Hornblad E, Singh SK, Matsunaga T, Ishii T, Lerouge P, Marchant A, 2009. The *Arabidopsis* IRX10 and IRX10-LIKE glycosyltransferases are critical for glucuronoxylan biosynthesis during secondary cell wall formation. *Plant J* 57: 718-731
- Xu JH, Messing J, 2008. Organization of the prolamin gene family provides insight into the evolution of the maize genome and gene duplications in grass species. *Proc Natl Acad Sci USA* 105: 14330-14335
- Yamaguchi M, 2010. Transcriptional regulation of secondary wall formation controlled by NAC domain proteins. *Plant Biotechnol* 242: 237-242
- Yamaguchi M, Kubo M, Fukuda H, Demura T, 2008. Vascular-related nac-domain7 is involved in the differentiation of all types of xylem vessels in *Arabidopsis* roots and shoots. *Plant J* 55: 652-664
- Yamaguchi M, Ohtani M, Mitsuda N, Kubo M, Ohme-Takagi M, Fukuda H, Demura T, 2010. VND-INTERACTING2, a NAC domain transcription factor, negatively regulates xylem vessel formation in *Arabidopsis*. *Plant Cell* 22: 1249-1263
- Yanagisawa S, 2004. Dof domain proteins: plant-specific transcription factors associated with diverse phenomena unique to plants. *Plant Cell Physiol* 45: 386-391
- Yao D, Wei Q, Xu W, Syrenne RD, Yuan JS, Su Z, 2012. Comparative genomic analysis of NAC transcriptional factors to dissect the regulatory mechanisms for cell wall biosynthesis. *BMC Bioinformatics* 13 Suppl 15:S10
- Yoshida-Shimokawa T, Yoshida S, Kakegawa K, Ishii T, 2001. Enzymic feruloylation of arabinoxylan-trisaccharide by feruloyl-CoA: arabinoxylan-trisaccharide O-hydroxycinnamoyl transferase from *Oryza sativa*. *Planta* 212: 470-474
- Zhang L, Chia JM, Kumari S, Stein JC, Liu Z, Narechania A, Maher CA, Guill K, McMullen MD, Ware D, 2009. A genome-wide characterization of microRNA genes in maize. *PLoS Genet* 5(11):e1000716
- Zhang M, Zhang B, Qian Q, Yu Y, Li R, Zhang J, Liu X, Zeng D, Li J, Zhou Y, 2010. Brittle Culm 12, a dual-targeting kinesin-4 protein, controls cell-cycle progression and wall properties in rice. *Plant J* 63: 312-328
- Zhang Y, Culhaoglu T, Pollet B, Melin C, Denoue D, Barrière Y, Baumberger S, Méchin V, 2011a. Impact of Lignin Structure and Cell Wall Reticulation on Maize Cell Wall Degradability. *J Agric Food Chem* 59: 10129-10135
- Zhang B, Liu X, Qian Q, Liu L, Dong G, Xiong G, Zeng D, Zhou Y, 2011b. Golgi nucleotide sugar transporter modulates cell wall biosynthesis and plant growth in rice. *Proc Natl Acad Sci USA* 108: 5110-



- 5115
- Zhang G, Liu X, Quan Z, Cheng S, Xu X, Pan S, Xie M, Zeng P, Yue Z, Wang W, Tao Y, Bian C, Han C, Xia Q, Peng X, Cao R, Yang X, Zhan D, Hu J, Zhang Y, Li H, Li H, Li N, Wang J, Wang C, Wang R, Guo T, Cai Y, Liu C, Xiang H, Shi Q, Huang P, Chen Q, Li Y, Wang J, Zhao Z, Wang J, 2012. Genome sequence of foxtail millet (*Setaria italica*) provides insights into grass evolution and biofuel potential. *Nat Biotechnol* 13: 549-554
- Zhao M, Morohashi K, Hatlestad G, Grotewold E, Lloyd A, 2008. The TTG1-bHLH-MYB complex controls trichome cell fate and patterning through direct targeting of regulatory loci. *Development* 135: 1991-1999
- Zhao Q, Dixon RA, 2011. Transcriptional networks for lignin biosynthesis: more complex than we thought? *Trends Plant Sci* 16: 227-233
- Zhong R, Burk DH, Morrison WH, Ye ZH, 2002a. A kinesin-like protein is essential for oriented deposition of cellulose microfibrils and cell wall strength. *Plant Cell* 14: 3101-3117
- Zhong R, Kays SJ, Schroeder BP, Ye ZH, 2002b. Mutation of a chitinase-like gene causes ectopic deposition of lignin, aberrant cell shapes, and overproduction of ethylene. *Plant Cell* 14: 165-179
- Zhong RQ, Ye ZH, 2007. Regulation of cell wall biosynthesis. *Curr Opin Plant Biol* 10: 564-572
- Zhong R, Richardson EA, Ye ZH, 2007. The MYB46 transcription factor is a direct target of SND1 and regulates secondary wall biosynthesis in *Arabidopsis*. *Plant Cell* 19: 2776-2792
- Zhong R, Lee C, Zhou J, McCarthy RL, Ye ZH, 2008. A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in *Arabidopsis*. *Plant Cell* 20: 2763-2782
- Zhong R, Ye ZH, 2009. Transcriptional regulation of lignin biosynthesis. *Plant Signaling Behavior* 4:1-7
- Zhong R, Ye ZH, 2010. The poplar PtrWNDs are transcriptional activators of secondary cell wall biosynthesis. *Plant Signaling Behavior* 5: 469-472
- Zhong R, Lee C, Ye ZH, 2010b. Global analysis of direct targets of secondary wall NAC master switches in *Arabidopsis*. *Molecular Plant* 3: 1087-1103
- Zhong R, Lee C, McCarthy RL, Reeves CK, Jones EG, Ye ZH, 2011. Transcriptional activation of secondary wall biosynthesis by rice and maize NAC and MYB transcription factors. *Plant Cell Physiol* 52: 1856-1871
- Zhong R, Ye ZH, 2012. MYB46 and MYB83 bind to the SMRE sites and directly activate a suite of transcription factors and secondary wall biosynthetic genes. *Plant Cell Physiol* 53: 368-380
- Zhou J, Lee C, Zhong RQ, Ye ZH, 2009. MYB58 and MYB63 are transcriptional activators of the lignin biosynthetic pathway during secondary cell wall formation in *Arabidopsis*. *Plant Cell* 21:248-266
- Zhu Y, Song D, Sun J, Wang X, Li L, 2013. *PtrHB7*, a class III HD-Zip gene, plays a critical role in regulation of vascular cambium differentiation in *Populus*. *Mol Plant* first published online January 2, 2013