Structural Analysis of Lignins and Hemicelluloses in Mature Bamboo *Phyllostachys Bambusoides* Culms for Comparison with Immature Bamboo and Other Grass Species

Yoko Okahisa-Kobayashi[1, 2*]

[1]JSPS Postdoctoral Fellow for Research Abroad: UMR 1318 INRA-AgroParisTech, Institut Jean-Pierre Bourgin, Versailles France

[2]Present affiliation; Institute for the Promotion of University Strategy Global Excellence, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

Abstract

An in-depth structural analysis of lignins, hemicelluloses and associated ferulic acid (FA) and *p*-coumaric acid (CA) in *Phyllostachys bambusoides* bamboo 2-year old culms and immature 150 cm-long culms were performed. This analysis was comparatively carried out with maize stems and wheat straw, selected as representatives of C_4 and C_3 grass species. Neutral sugar composition and CA/FA ratio were changed between the immature basal side and immature apical side as well as immature and mature bamboo. As compared to maize or wheat samples, this investigation revealed some original features of the native lignins from mature *Phyllostachys bambusoides* culms.

Keywords: Bamboo, Monosaccharide sugar composition, Lignins, S/G ratio, *p*-Coumaric acid (CA), Ferulic acid (FA), *p*-Coumaroylated Arabinose, Feruloylated Arabinose, Py-GC-MS, FTIR, GC-MS, HPLC

1 Introduction

In recent years, much attention has been paid to lignocellulosic feedstocks as bioenergy source or to develop effective biorefinery processes for high-value-added utilization of cell wall polymers. In this context, bamboo, a perennial woody grass, is a most promising lignocellulosic resource which grows in wet-seasoned zones and comprises more than 1200 species and 75 genera [1-3]. Some bamboo species may provide higher biomass yield than dedicated energy crops such as corn stover, switchgrass or miscanthus [4]. Among the 100 bamboo species growing in Japan, *Phyllostachys bambusoides* is second in distribution area and also economically important [5]. Its culms grow up to 10-15 m during the few months following shoot emergence. Bamboo culms progressively gain hardness and strength through the deposition of lignified and thick secondary cell walls in fibers, long parenchyma cells and vessels [6] - [8].

Similar to angiosperm lignins, bamboo lignins are composed of guaiacyl (G) and syringyl (S) units, together with the low amount of *p*-hydroxyphenyl (H) units [9,10]. These lignin units are linked by labile ether linkages, referred to as β-O-4 bonds, and by resistant carbon-carbon and biphenyl ether linkages [11]. In addition, bamboo lignins have two specificities of grass lignins. They are acylated by *p*-coumaric acid (CA) on the γ-OH side chain of lignin units [12,13]. They have covalently linked to the ferulic acid (FA) units that acylate the arabinose substituents of arabinoxylans [13] - [16].

In the present study, we performed an in-depth structural analysis of lignins, hemicelluloses and associated FA and CA in *Phyllostachys bambusoides* bamboo 2-year old culms. This analysis was comparatively carried out with immature 150 cm-long culms as well as with maize stems and wheat straw, selected as representatives of C_4 and C_3 grass species. The cell wall compositional changes of bamboo occurred drastically with maturation. As compared to maize or wheat samples, this investigation revealed some original features of the native lignins from mature *Phyllostachys bambusoides* culms.

2 Experimental

2.1 Materials

Three types of bamboo samples *(Phyllostachys bambusoides)* were selected from the Kyoto Prefecture, Japan, in May 2008. The mature bamboo sample was collected from 2-year old bamboo culms. Immature bamboo was collected from 150 cm high young shoots subdivided into two zones, the apical one and the basal one. The immature apical (Im-Ap) and the immature basal (Im-Ba) zones were analyzed as the youngest and oldest zones, respectively [17]. The brown skins of the immature bamboo shoots were removed. The 3 air-dried bamboo sample types were ground to 0.5 mm.

For comparison of the mature bamboo sample with other grass species, we used maize stem (*Zea mays*, F2; collected

*Corresponding author.
E-mail address : okahisa@kit.ac.jp

at the silage stage) and wheat straw (*Triticum aestivum*, cv Champlein) samples which were dried and ground similarly as for the bamboo samples. Before compositional cell wall analyses, every grass sample was exhaustively extracted with water, then ethanol to recover an extractive-free material. A dioxane lignin fraction (DL) was isolated from the extractive-free mature bamboo sample and by mild acidolysis, as previously described [18].

2.2 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the extractive-free cell wall samples as well as of the DL lignin fraction were recorded with a Thermo-Nicolet Nexus 470 spectrometer over the range 1800 to 800 cm^{-1}. Each spectrum was obtained from the co-addition of 5 to 10 acquisitions at a 4 cm^{-1}resolution.

2.3 Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS)

The extractive-free bamboo samples were subjected to Py-GC-MS analyses using a CDS model 5250 pyroprobe autosampler (CDS Analytical, Inc., Oxford, PA, USA) interfaced to an Agilent 6890/5973 GC-MS (Agilent Technologies Inc, Bellevue, WA, USA). The samples were pyrolyzed in a quartz tube and at 500°C for 10 s, using helium as the carrier gas with a flow rate of 1 mL/min. The volatile pyrolysis products were separated on a GC capillary column (5% phenyl methyl siloxane, 30 m, 250 µm i.d., 0.25 µm film thickness, Model Agilent 19091S-433). The pyrolysis and GC-MS interfaces were kept at 280°C and the GC-MS was temperature-programmed from 40°C (1 min) to 130°C at 6°C/min, then from 130 to 250°C at 12°C/min and finally from 250 to 300°C at +30°C/min (3 min at 300°C). The MS was operated in the electron impact mode (70 eV) for m/z 40 to 450. The various phenolic pyrolysis compounds were identified by comparison to the spectra of authentic compounds (when commercially available) or to published spectra of forage pyrolysis products [19].

2.4 Monosaccharide composition analyses of hemicelluloses

The neutral sugar composition of the amorphous polysaccharides from extractive-free samples or from the DL lignin fraction was measured after hydrolysis in 2.5 M trifluoroacetic acid (TFA) as previously described [20].

2.5 Lignin analyses

The lignin content of extractive-free grass samples was measured by the gravimetric Klason procedure as previously described [22] or by the spectrometric acetyl bromide method as previously described [23].

The evaluation of lignin structure was evaluated by thioacidolysis and by GC-MS analysis of the lignin-derived H, G and S thioacidolysis monomers according to a previously described procedure [22]. In addition, the frequency of free phenolic end groups in lignins was measured by thioacidolysis of permethylated samples as previously described [24].

2.6 Analyses of cell wall-linked p-coumaric and ferulic acids

The amount of ester-linked CA and FA was measured by mild alkaline hydrolysis followed by acidification, extraction on a solid phase extraction (SPE) cartridge (Waters Sep-pak tC18, Waters corporation) and high-performance liquid chromatography (HPLC), as previously described [25].

The sum of ester– and ether– linked FA was measured by severe alkaline hydrolysis of the extractive-free sample (about 20 mg) put in a Teflon vial together with 5 ml 4M NaOH. The vial was then heated in an autoclave reactor put at 170°C (oven) and for 2 hours. The cooled reaction mixture was subjected to acidification, SPE and HPLC as performed for FA releasable by mild alkaline hydrolysis. The ether-linked FA units were then evaluated as the difference between severe and mild alkaline hydrolysis.

The evaluation of CA and FA units ester-linked to arabinose was performed by GC-MS analyses of the *5-O-p*-coumaroyl –L-arabinofuranose (CA-Araf) and of *5-O*-feruloyl-L-arabinofuranose (FA-Araf) isomers released by a recently developed mild acidolysis procedure [26].

3 Results and discussion

3.1 Rapid evaluation of bamboo cell walls by FTIR and Py-GC-MS analyses

We first subjected the three bamboo extractive-free samples to FTIR and Py-GC-MS as these high-throughput methods can provide some valuable information. The FTIR spectra were examined in the fingerprint region (1800-800 cm^{-1}) (Figure 1). To unambiguously identify the FTIR signals from lignins and CA esters, we isolated a dioxane lignin fraction DL by mild

acidolysis of the bamboo mature extractive-free sample. When applied to grass cell walls, acidolysis carried out by refluxing the sample in dioxane/water mixture (9/1, v/v) containing 0.2 M HCl ensures the recovery of lignin fractions with a substantial yield (40 to 70% of the total lignin content) and a low degree of sugar contaminants (a few percents). In addition, this method essentially preserves the *p*-coumarate esters linked to grass lignins [18]. The bamboo DL fraction was recovered with a 42% isolation yield (when expressed on the basis of the Klason lignin content of the original sample). It contained only 2.5% of sugar contaminants, 9.74% of ester-linked CA and 0.46% of ester-linked FA.

In other words, we can ascertain that the FTIR signals quoted on the bamboo DL spectrum (Figure 1) can be essentially assigned to lignins and CA esters. The most diagnostic lignin peak certainly is the aromatic signal at about 1510 cm^{-1}, which has been used for lignin quantification in eucalyptus wood as this signal is not overlapped by polysaccharidic bands [27]. Signals from CA esters contribute to this aromatic signal and also to the 1700 and 1169 cm^{-1} bands [28]. While the FTIR spectrum of the extractive-free mature bamboo sample (Figure 1) clearly displays not only the 1510 cm^{-1} aromatic signal but also most lignin signals (such as bands at 1600, 1460 and 1425 cm^{-1}), lignin signals cannot be detected in the FTIR spectra of the Im-Ap and Im-Ba samples. This is due to the large predominance of polysaccharidic signals, the most prominent occurring in the 1200-900 cm^{-1} region. This result suggests that the lignin and CA levels of the immature samples are too low to allow the straightforward FTIR identification of these components. A closer examination of the immature FTIR spectra revealed the occurrence of more specific signals, such as a strong and wide band around 1640 cm^{-1} and a weaker one around 1550 cm^{-1}. These two 1640 and 1550 cm^{-1} FTIR signals are most likely assignable to the amide I and amide II vibrations of the protein backbone, respectively [29]. It is well established that immature bamboo shoots are rich in proteins, more particularly in the youngest apical area [17]. The occurrence of protein signals in the FTIR spectra of the immature extractive-free samples reveals that proteins survived the extraction step. This contamination has to be considered for the determination of lignin content as protein contaminants can lead to an overestimation of the Klason lignin content.

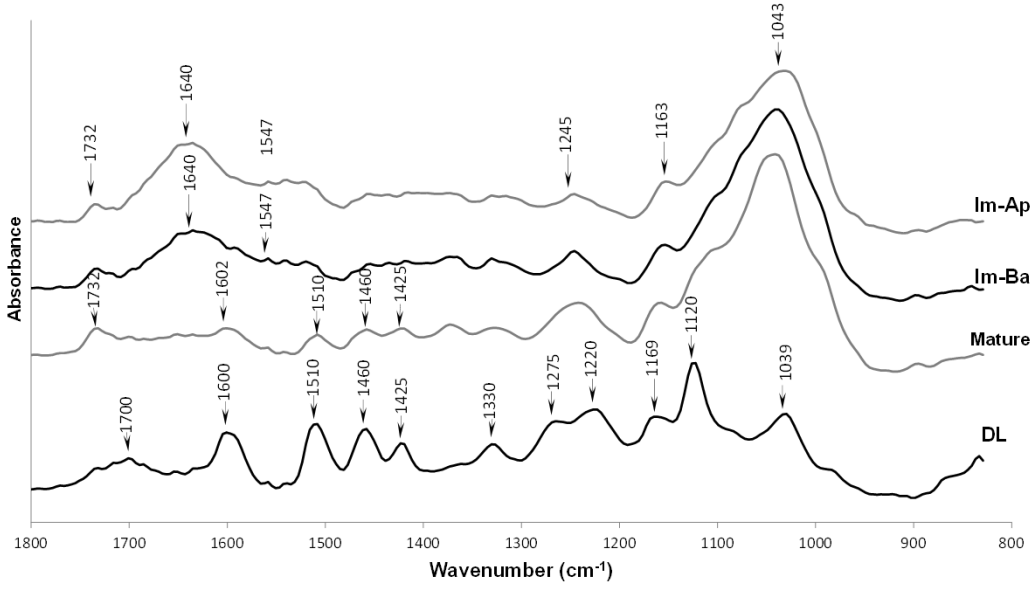

Figure 1 The FTIR spectra of the extractive-free cell wall samples and DL lignin fraction

Analytical Py-GC-MS has been used for many years as a high-throughput way to characterize complex lignocellulosic samples [19,30-32]. The two prominent peaks observed on the Py-GC-MS chromatograms from grass cell walls usually are vinylphenol and vinylguaiacol which mainly and respectively originate from the pyrolytic degradation of cell wall-linked CA and FA units [19]. Not unexpectedly, this is also the case for the mature and immature bamboo extractive-free samples (Figure 2). In addition, the GC-MS trace of the immature bamboo samples displayed 2 peaks identified as indole and 3-methylindole by comparison to published mass spectra (Figure 2) [19]. These indole derivatives issued from the pyrolysis of tryptophan are protein markers, which confirms the occurrence of protein contaminants in the extractive-free immature samples. When subjected to pyrolysis, G lignin units give rise to a series of G compounds, including vinylguaiacol and S lignin units give rise

to S compounds (including vinylsyringol). The pyrograms from the mature bamboo sample reconstructed over the m/z 115-220 range in order to eliminate most polysaccharide-derived pyrolysis compounds, displays many lignin-derived G and S peaks (Figure 2A). By contrast, only a few tiny lignin-derived peaks (mainly G ones) can be seen from the immature bamboo traces (Figure 2 B and C).

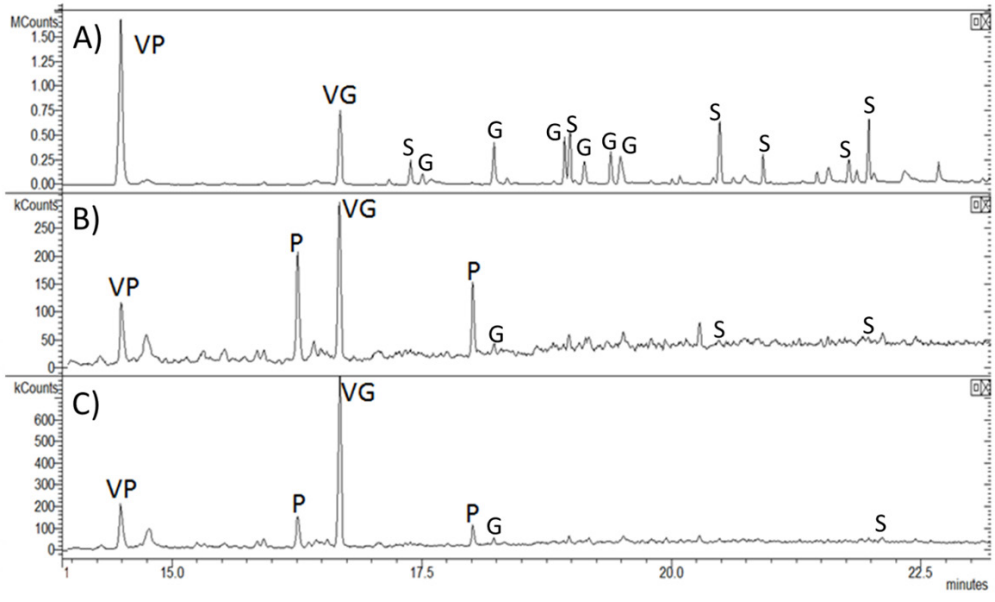

Figure 2 Py-GC-MS traces obtained from extractive-free samples of A) mature, B) immature apical and C) immature basal bamboo culms. The GC-MS traces correspond to the ions detected over the m/z 115-220 range, which eliminates most signals from sugars. VP: vinylphenol; VG: vinylguaiacol; P: protein-derived indole compound; G: guaiacyl compound; S: syringyl compound.

The relative quantitative determination of the main pyrolysis aromatic compounds (i.e. vinylphenol, vinylguaiacol, other G compounds and S compounds), (Table 1) confirmed the predominance of vinylphenol and vinylguaiacol for all samples together with the higher lignin level and S frequency of the mature sample. The low relative abundance of lignin-derived G and S compounds released from immature samples is diagnostic for a low lignin level. In addition, the recovery of the substantial relative amount of vinylphenol supports the hypothesis that the parent CA pool is ester-linked to polysaccharides in immature samples, a situation which contrasts from the predominant *p*-coumaroylation of lignins in the mature one. The S/G ratio of lignin-derived pyrolysis compounds was calculated without considering vinylguaiacol as this compound may originate either from FA units or from G lignin units. Not unexpectedly, this ratio was found to be lower in the immature samples relative to the mature one. It is indeed well established that G lignin units are deposited earlier than S lignin units (reviewed in [33]).

Table 1 Relative abundance (% of total area) of the main phenolics released by Py-GC-MS of extractive-free mature and immature bamboo culms.

Samples	Vinylphenol[a]	Vinylguaiacol[b]	Other guaiacyl G monomers	Syringyl S monomers	S/G
Immature culm					
Apical area	25.9 (3.2)	56.1 (1.2)	14.6 (1.3)	3.6 (0.6)	0.25 (0.02)
Basal area	27.8 (2.1)	62.3 (0.2)	9.1 (1.9)	1.2 (0.0)	0.14 (0.03)
Mature culm	33.3 (1.2)	9.5 (0.3)	25.6 (1.6)	31.6 (0.1)	1.24 (0.08)

Data are mean values (and standard errors SE) between duplicate analyses. [a] Vinylphenol originates from cell wall-linked *p*-coumaric acid; [b] Vinylguaiacol originates from cell wall-linked ferulic acid and from G lignin units. The S/G ratio is calculated without considering vinylguaiacol.

3.2 Study of bamboo hemicelluloses and their changes from immature to mature culms

Since a few years, bamboo hemicelluloses, their hydrolysis and potential applications have been extensively studied (reviewed in [34-36]). Mature bamboo contains up to 30% of hemicelluloses. The chief bamboo hemicelluloses are glucuronoarabinoxylans (GAX) which have a backbone of xylose residues bearing arabinose and glucuronic acid substituents as well as acetyl groups. In addition, other hemicelluloses occur in a lower amount, such as (1→3, 1→4)-β-D-glucan, xyloglucan and glucomannan. Bamboo GAX are both p-coumaroylated and feruloylated and bamboo xyloglucan may also be feruloylated [15, 37]. Ferulate esters mainly linked to the GAX arabinose residues may act as peroxidase-driven cross-links between hemicelluloses or between hemicelluloses and lignins [15]. We determined the neutral sugar composition of hemicelluloses from extractive-free mature and immature bamboo culms and by TFA hydrolysis, as compared to wheat and maize samples. Not unexpectedly and similarly to wheat or maize hemicelluloses, the major neutral sugar released from bamboo hemicelluloses was xylose (Table 2), a predominance diagnostic for GAX abundance. In mature grass stems, arabinose holds the second position and the xylose-to-arabinose ratio decreases when the substitution degree of GAX increases. According to the data of Table 3, GAX from the mature bamboo culm are less substituted than GAX from wheat straw or maize stem. By contrast, GAX of immature culms seems to be much more substituted than from mature culm. This result is consistent with literature results reporting the occurrence of highly substituted GAX in maize coleoptile [38] and in bamboo young culms [6]. Sugar analysis was also carried out for the DL lignin fraction isolated from mature culm. The results confirmed that this fraction had a low content of sugar contaminants (2.5 ± 0.1% by weight) and revealed that these sugars were predominantly represented by arabinose (30.7%) and xylose (63.4%) together with a small glucose amount (5.9%). This result emphasizes the close association between grass lignins and GAX, very likely mediated by FA cross-links.

Table 2 Neutral sugar composition of hemicelluloses from bamboo, maize and wheat extractive-free samples.

Sample	Total (% w/w)	Relative abundance (%)						
		Ara	Gal	Glc	Man	Rham	Xyl	Xyl/Ara
Bamboo culms								
Immature-apical	33.3 (1.0)	10.0	8.2	64.5	0.4	1.0	15.9	1.6
Immature-basal	32.2 (0.1)	7.4	6.3	61.3	0.3	1.3	23.4	3.2
Mature	20.4 (1.2)	5.5	1.9	20.8	0.6	0.3	71.0	12.9
Maize stem	25.0 (0.7)	11.9	2.9	17.9	1.9	N.D.	65.4	5.5
Wheat straw	22.0 (0.5)	12.1	3.9	10.6	2.3	N.D.	71.2	5.9

Data are mean values (and SE) from duplicate experiments. The relative amount of arabinose (Ara), galactose (Gal), glucose (Glc), mannose (Man), rhamnose (Rham) and xylose (Xyl) are calculated as anhydrous monosaccharides. N.D. means no detectable.

The amount of glucose released from the immature samples by TFA hydrolysis was found to be outstandingly high. This situation might reflect the abundance of mixed glucan and/or xyloglucan in bamboo shoots, a hypothesis supported by the fact that similarly high glucose levels have been obtained from hemicellulose hydrolysis [36]. It might also reflect that some starch has survived the extraction step as bamboo shoots contain substantial starch levels [17].

3.3 Cell wall-bound *p*-coumaric and ferulic acids and their variation with maturation

It is now well established that grass arabinoxylans are acylated on their arabinose substituents mainly by FA and, to a lower extent by CA [39]. We evaluated arabinose acylation and by a mild acidolysis procedure recently developed [26]. This analysis confirmed that FA is the main substituent of arabinose units and revealed that maize and wheat samples released substantially more feruloylated arabinose (FA-Araf) than the mature bamboo sample analyzed herein (Table 3). In addition, the relative importance of *p*-coumaroylated arabinose (CA-Araf) was found to be lower in the mature sample (Table 3). In the immature samples, about 10 to 17% of acylated arabinose units are *p*-coumaroylated, versus 6.5% in the mature one. This result is consistent with the Py-GC-MS data and suggests that arabinose *p*-coumaroylation is more pronounced in juvenile tissues than in mature ones.

Table 3 Determination of 5-O-*p*-coumaroyl-L-arabinofuranose (CA-Araf) and of 5-O-feruloyl-L-arabinofuranose (FA-Araf), released by mild acidolysis of extractive-free grass samples.

Sample	CA-Araf (mg/g)	FA-Araf (mg/g)	% CA-Araf/ (CA-Araf+FA-Araf)
Bamboo culms			
Immature-apical	0.10 (0.01)	0.92 (0.07)	9.8 (0.5)
Immature-basal	0.37 (0.00)	1.84 (0.13)	16.7 (0.8)
Mature	0.13 (0.03)	1.88 (0.20)	6.5 (1.8)
Maize stem	0.16 (0.01)	4.85 (0.07)	3.2 (0.2)
Wheat straw	0.47 (0.00)	2.50 (0.01)	15.8 (0.1)

Data are mean values (and SD) from triplicate experiments. The *Z* and *E* isomers of the *α* and β arabinofuranose acylated derivatives are considered.

The total amount of cell wall-bound CA and FA esters was evaluated by mild alkaline hydrolysis. In addition, a severe alkaline hydrolysis allowed the release of FA units ester-linked to GAX together with that of FA units both ether-linked to lignins and ester-linked to GAX. The difference between severe and mild alkaline hydrolyses gives thus an estimate of FA ethers acting as cross-links between lignins and GAX.

Not unexpectedly, ester-linked CA was found to be the higher in the mature bamboo culm (Table 4), in agreement with literature data reporting that most CA accretion occurs in parallel with grass cell wall lignification [40,41]. That CA of mature cell walls predominantly acylates bamboo lignin is confirmed by the high *p*-coumaroylation degree of the DL lignin fraction isolated from mature culm (mild-alkaline hydrolysis releasable CA: 97.1 ± 3.2 mg/g DL). Although bamboo and maize plants respectively have a C_3 and C_4 photosynthesis type, both species can generate high biomass yield. In addition, the bamboo mature culms analyzed herein display a level of *p*-coumarates on lignins similar as mature stems from most C_4 high productive grasses (maize in Table 4 and [42]) and much greater than that of wheat (Table 4) or other C_3 grasses [42].

Table 4 Determination of ester-linked CA, ester-linked FA and ether-linked FA by mild and severe alkaline hydrolyses of extractive-free grass samples.

Sample	CA esters[a] (mg/g)	FA esters[a] (mg/g)	FA ethers[b] (mg/g)
Bamboo culms			
Immature-apical	0.42 (0.01)	0.80 (0.00)	N.D.
Immature-basal	0.55 (0.04)	1.61 (0.02)	N.D.
Mature	15.93 (0.50)	1.21 (0.06)	4.34 (0.04)
Maize stem	17.85 (0.52)	5.73 (0.13)	4.20 (0.33)
Wheat straw	4.76 (0.10)	2.71 (0.01)	8.04 (0.19)

Data are mean values (and SE) between duplicate analyses. [a] Released by mild alkaline hydrolysis; [b] difference between severe and mild alkaline hydrolyses. N.D. means no detectable.

Contrary to CA units, the highest amount of ester-linked FA units was found in the basal immature sample (Table 4). Severe alkaline hydrolysis consistently revealed that most FA esters occurring in the mature bamboo sample were also ether-linked to lignins (Table 4). By contrast, FA ethers were not detectable from the immature and poorly lignified samples. This result confirms that GAX-linked FA esters act as initiation sites for lignins. In agreement with acidolysis data (FA-Araf in Table 3), total ferulates (FA esters + ethers) were released in lower amount from mature bamboo cell walls than from those of maize or wheat.

3.4 Lignification of bamboo cell walls and structural specificities of mature bamboo lignins

Lignin content was measured on extractive-free samples as extractives may interfere with lignins during the gravimetric or spectrometric lignin determination by the Klason or the acetyl bromide procedures [43,44]. Not unexpectedly, extractive-free samples were recovered with a higher yield from the mature bamboo culm (about 83%) than from the immature ones (about 53%). For mature bamboo, maize and wheat extractive-free samples, the ABL method gave about 20% higher lignin level than the Klason method (Table 5), a situation already reported for mature grass samples [44]. Whatever the method, the highest lignin level was consistently obtained for the mature bamboo sample. Not surprisingly, the two extractive-free immature bamboo samples displayed much lower lignin levels. However, their Klason lignin (KL) level was substantially higher than their acetyl bromide lignin (ABL) level. At this point, we cannot decide which method gives the more accurate results as both the KL and the ABL methods have their specific advantages and limitations. However and from the Py-GC-MS data revealing protein-markers from immature samples, we may hypothesize that the KL method leads to overestimated values as proteins can condense with lignins during the KL gravimetric determination [45].

Table 5 Determination of lignin content in extractive-free grass cell wall samples by the acetyl bromide lignin (ABL) method and by the Klason lignin (KL) method.

Sample	ABL %[a]	KL %
Bamboo culms		
Immature-apical	2.90 (0.15)	6.35 (0.25)
Immature-basal	4.38 (0.14)	6.89 (0.28)
Mature	31.77 (0.57)	26.74 (0.09)
Maize stem	19.58 (0.88)	16.39 (0.11)
Wheat straw	21.31 (0.52)	18.15 (0.09)

Data are mean values (and SD) between 3 to 6 analyses. [a] The ABL lignin level is calculated using an extinction coefficient of 20 $g^{-1}.L.cm^{-1}$ for grass lignin at 280 nm.

Lignin structure was evaluated by thioacidolysis, an analytical degradation method which provides H, G and S monomers from H, G and S units only involved in labile β-O-4 ether bonds [46-48]. When calculated on the basis of lignin content, the total yield of thioacidolysis monomers gives thus an estimate of the frequency of these parent structures. Conversely, when this yield is low, this may be diagnostic for a high frequency of resistant interunit linkages in lignins.

As compared to the mature bamboo sample, the yield of thioacidolysis monomers released from immature bamboo lignins was dramatically lower (Table 6). This difference holds when lignin level is estimated by the ABL method (Table 6) as well as by the Klason method (data not shown). This low yield may originate from a high frequency of resistant interunit bonds in the lignins from immature samples and from an overestimation of their lignin level.

Table 6 Lignin-derived H, G and S monomers released by thioacidolysis of extractive-free samples

Sample	(H+G+S) µmol/ g ABL[a]	Relative molar frequency		
		H%	G%	S%
Bamboo culms				
Immature-apical	80 (2)	1.0 (0.1)	59.6 (0.7)	39.4 (0.8)
Immature-basal	47 (2)	4.3 (0.5)	54.0 (0.0)	41.7 (0.6)
Mature	1271 (16)	4.7 (0.1)	48.3 (0.5)	47.0 (0.5)
Maize stem	725 (20)	1.5 (0.1)	49.5 (0.2)	49.0 (0.1)
Wheat straw	885 (17)	5.0 (0.2)	49.0 (0.5)	46.0 (0.4)

Data are mean values (and SE) between duplicate analyses. [a] The total thioacidolysis yield is calculated in µmoles monomers per gram of acetylbromide lignin

Consistently with many literature data and with Py-GC-MS data, the frequency of S monomers was higher in mature than in immature bamboo samples. This result confirms that S lignin units are deposited in higher relative amount during the late lignification stages [33]. The relative frequency of G and S thioacidolysis monomers released from bamboo, maize or wheat mature lignins was found to be similarly high (in the 46 to 49% range) while H monomers were systematically obtained in weak amounts (a few percents). Lignins from mature bamboo culms released about 50% more thioacidolysis monomers than lignins from mature maize or wheat stems. It is well established that G and H lignin units are prone to participate in resistant interunit bonds at their free C5 position. As compared to the lignins from other grass species, the 50% higher thioacidolysis yield of lignins from mature bamboo cannot, therefore be accounted for by a lower frequency of S units but seems to actually reflect their higher frequency in β-O-4 linked units.

We examined the proportion of H, G, and S β-O-4-linked lignin units that occurred as free phenolic terminal units or etherified internal units by thiacidolysis of permethylated samples. The principle of the overall procedure is outlined in Figure 3 [49]. As expected, the β-O-4-linked H units were essentially terminal units with free phenolic groups, S units were prominently internal units and G units displayed an intermediate behavior (Table 7). The proportion of β-O-4-linked G or S units with free phenolic groups was decreased in mature lignin compared to immature lignin, which indicates the frequency of internal units was increased with maturation.

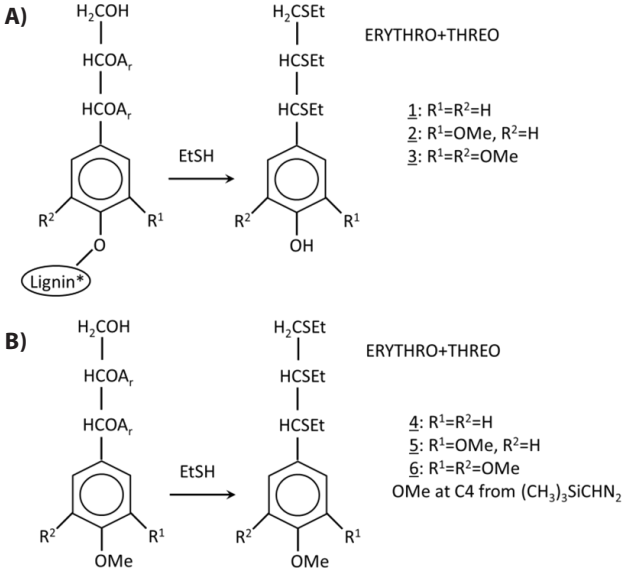

Figure 3 Thioacidolysis of $(CH_3)_3SiCHN_2$-methylated lignins: main recovered monomers from β-aryl ethers structures in A) non-terminal units and B) terminal units with originally free then methylated phenolic groups. Lignin*=aliphatic C in lignin.

Table 7 Percentage of free phenolic end-groups in β-O-4 linked H, G or S units, as revealed by thioacidolysis of permethylated extractive-free samples.

Sample	H % [4/(1+4)][a]	G % [5/(2+5)][a]	S %[3/(3+6)][a]
Bamboo culms			
Immature-apical	[b]-	33.6 (0.9)	6.9 (1.7)
Immature-basal	[b]-	25.2 (2.7)	7.7 (1.2)
Mature	85.0 (2.1)	27.5 (1.0)	3.7 (0.1)
Maize stem	86.5 (0.4)	47.6 (1.0)	8.6 (0.0)
Wheat straw	86.1 (1.6)	39.3 (3.0)	3.8 (0.6)

Data are mean values (and SE) between duplicate analyses. [a] Compound numbers refer to the numbers given in Figure 3; [b] compound 1 is obtained in too weak amount for an accurate calculation.

It was reported that bamboo culms gradually ceased elongation near the end of June, and lignin deposition was observed in the fibre walls and vascular bundles of bamboo collected in this month. While it was detected in both fibre and parenchyma cells in mature culms [50]. The samples, which were used in this study, were harvested in May. It can be considered that lignin deposition was progressed partially at that time. Moreover, according to the results of microspectrophotometric analysis, vessel walls mainly contained guaiacyl lignin, while both guaiacyl and syringyl lignin were present in the fibre and parenchyma cell walls of bamboo [7]. The structural changes of lignin, which were confirmed in this study, may be related to these morphological changes of cell walls with maturation.

The level of β-O-4-linked S units of DL lignin isolated from mature culm was increased significantly (12.2 ± 0.2 %) because β-O-4 linkages were mainly cleaved by acidic dioxane treatment. The frequency of G units with free phenolic groups in bamboo was lower than other grasses even in DL lignin fraction isolated from mature culm (37.4 ± 0.2 %). This shows that bamboo lignin contains higher ratio of internal units. Therefore, it was suggested that bamboo lignin has lower branching degree and/or higher polymerization degree, which mean larger lignified domains.

4 Conclusions

I investigated the lignins and hemicelluloses structure of immature and mature bamboo cell walls and compared to these of other grasses. The cell wall compositional changes of bamboo occurred drastically with maturation. Furthermore, some original features of the native lignins from mature bamboo culms as compared to maize or wheat samples were revealed.

A level of *p*-coumarates on lignins of the mature bamboo culms was similar as mature stem from C_4 grasses such as maize and much greater than that of wheat or other C_3 grasses. The levels of FA esters, arabinose, and FA-Ara were lower in bamboo culms, which suggest lower ferulate mediated cross-linking possibilities between arabinoxylans and lignins. Moreover, the frequency of β-O-4 linked G units with free phenolic groups in bamboo was lower than other grasses, which means higher ratio of internal units in bamboo lignin.

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