J Wood Sci (2000) 46:376–380

ORIGINAL ARTICLE

Thi Bach Tuyet Lam · Kenji Iiyama

Characteristics of senescent straw cell walls of dwarf, semidwarf, and normal strains of rice (*Oryza sativa*) plants

Received: July 9, 1999 / Accepted: October 1, 1999

Abstract A normal variety of rice (*Oryza sativa* L. *cv. Taichung 65*, *T65c*), its isogenic dwarf line (*T65d$_1$*), and a semidwarf variety of a different line (*Oryza sativa* L. *cv. IR8*, *IR8*) were studied. The results were compared with those of an isogenic dwarf line (*Rh$_i$*) of wheat straw, which was previously reported. Expression of the dwarf gene, *d$_1$*, on the chemical composition and the structural features of lignin present in rice internodes differs from that in an isogenic dwarf line of wheat. The differences include the lignin content, total yield of alkaline nitrobenzene oxidation products, and distribution of wall-bound hydroxycinnamic acids. There was, however, no difference in the syringyl/guaiacyl nuclei (S/V) molar ratio and neutral sugar composition. The lignin composition of rice straw cell walls, particularly that of the dwarf variety, contained more of the condensed structure and fewer syringyl nuclei than lignin in wheat straw cell walls. It is suggested that crosslinking between lignin and polysaccharides by ester–ether bridges via ferulic acid contributes to the mechanical properties of the cell walls of rice straw. Thus the chemical and structural characteristics of lignin in rice straw differ to some extent from those of other temperate grasses, such as wheat (*Triticum aestivum*) and phalaris (*Phalaris aquatica*), as reported previously. This can probably be attributed to the water environment of rapidly growing rice seedlings, but it also depends on the genetic variety of the rice plant.

Key words Hydroxycinnamic acids · *Oryza sativa* L. · Isogenic dwarf line · Lignin · Partially methylated alditol acetate

T.B.T. Lam
School of Biochemistry, La Trobe University, Bundoora, Victoria 3083, Australia

K. Iiyama (✉)
Asian Natural Environmental Science Centre, the University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
Tel. +81-3-5841-2783; Fax +81-3-5841-5230
e-mail: aakenji@mail.ecc.u-tokyo.ac.jp

Introduction

There have been many trials of selective breeding of crop plants for the purpose of increasing productivity. The major aim of the selection is to introduce dwarfism to keep the plants from falling down. An *IR8* rice variety (*Oryza sativa* L. *cv. IR8*), which has been developed by the International Rice Research Institute (IRRI), is a typical semidwarf variety with significantly high rice productivity. This variety is widely cultivated in Southeast Asia, and there are large amounts of straw waiting to be utilized as biomass. However, there is no information on its cell wall composition. In addition, the chemical composition of rice straw walls is important for mechanical strength, but little information on the effect of dwarfism is available.

We have previously reported analysis of the chemical composition and structural characterization of lignin in the walls of normal wheat and an isogenic dwarf.[1] It has been found that ester–ether bridges, via ferulic acid, between polysaccharides and lignin provided the mechanical properties of cell walls during the developing stages of the plant.[1] The importance of covalent associations between cell wall polymers has been reviewed.[2–5] The dwarfism (*Rh* genes) of wheat reported in a previous paper[1] was caused reduced cell wall elongation of parenchymal cells.[6] In other systems dwarfism has been ascribed to decreased cell proliferation[7,8] or a reduction of both cell elongation and cell proliferation.[9–11] The causes of dwarfism in plants depend on both the species and the inheritance of various dwarf genes.[12]

In this study we investigated the chemical composition and structural characteristics of lignin present in the cell walls of rice expressing different dwarf genes and compared our previous results to the isogenic dwarf line of wheat, focusing on the formation of covalent associations between wall polymers.

Materials and methods

Plant materials

Senescent rice (*Oryza sativa* L.) stems, a normal variety *Taichung 65* (*T65c*) and its isogenic dwarf line (*T65d*$_1$), and a semidwarf variety of a different line *IR8* (International Rice Research Institute) were studied. The *IR8* has been hybridized with indica and japonica and is bred widely in southern and southeastern Asia. These varieties were kindly donated by Professor O. Kamijima, Department of Agriculture, Kobe University, Japan. The samples were dried in a vacuum oven at 40°C and separated into internode, leaf sheath, and leaf blade. Each fraction was milled in a Wiley mill to pass a 420-μm sieve. The ground samples were successively extracted with boiling 80% (v/v) aqueous ethanol (1 h × 3) and water overnight at 37°C then washed with absolute ethanol and dried in a vacuum oven at 40°C.

Analysis of chemical components

The lignin content was determined by an acetyl bromide procedure.[13] Neutral sugars were released by hydrolysis with 4% sulfuric acid for 1 h at 121°C after treatment with 72% sulfuric acid for 1 h at room temperature; the neutral sugars in the hydrolysate were analyzed as their alditol acetates[14] by a Hewlett-Packard HP5890 Gas chromatograph (BP225 capillary column 25 m × 0.25 mm i.d., column temperature 200°C). Uronic acids in the sulfuric acid hydrolysate were determined spectrophotometrically using the method of Scott.[15] The sample was finely ground in a Spex-mill (SPEX Industries, Metuchen, USA) under liquid nitrogen, then the partially methylated alditol acetate analysis of wall polysaccharides was performed using the procedure of Harris et al.[16] The products were identified by gas chromatography-mass spectrometry (GC-MS) using a Finnigan MAT INCOS-50 GC-MS system (BP225 capillary column 25 m × 0.25 mm i.d., with temperature programmed from 170°C to 230°C at 2°C/min), referring to Bjondal et al.,[17] Geyer et al.,[18] and Carpita and Shea.[19] It was then quantified using theoretical response factors calculated by Sweet et al.[20]

The chemical structure of lignin was examined by alkaline nitrobenzene oxidation.[21] The products were detected by a Hewlett-Packard HP5890 Gas chromatograph (BP1 capillary column 25 m × 0.25 mm i.d.) as trimethylsilyl (TMS) derivatives[21] using ethylvanillin as an internal standard. The yields of *p*-hydroxybenzaldehyde and vanillin were corrected for those produced from *p*-coumaric and ferulic acids ester- and ether-linked to wall polymers.[21] The content of ester-linked and total (ester-linked plus ether-linked) hydroxycinnamic acids released by alkaline hydrolysis at room temperature (1 M NaOH overnight)[22] and at 170°C (4 M NaOH for 2 h),[23,24] respectively, were determined by GC as TMS derivatives.[21] All of the chemical analyses, except the partially methylated alditol acetate analysis (which was duplicated), were triplicated.

Table 1. Lignin content and alkaline nitrobenzene oxidation products of cell walls of dwarf, semidwarf, and normal strains of rice internodes

Sample	Lignin content (% of ODM)	Alkaline nitrobenzene oxidation products	
		Total yield (% of lignin)	S/V
T65c			
Internode	12.2 (0.17)	10.8 (0.24)	1.10 (0.04)
Leaf sheath	15.6 (0.21)	8.4 (0.21)	0.98 (0.03)
Leaf blade	16.8 (0.32)	7.5 (0.34)	0.93 (0.02)
T65d$_1$			
Internode	10.9 (0.24)	8.4 (0.29)	1.45 (0.04)
Leaf sheath	12.9 (0.24)	7.9 (0.41)	0.93 (0.01)
Leaf blade	14.6 (0.29)	6.9 (0.21)	0.65 (0.02)
IR8			
Internode	15.6 (0.36)	7.5 (0.24)	1.37 (0.03)
Leaf sheath	15.4 (0.32)	6.7 (0.35)	1.29 (0.03)
Leaf blade	17.2 (0.29)	7.0 (0.16)	0.74 (0.02)

Numbers in parentheses are standard deviations (n = 3)
Lignin content was determined by an acetyl bromide procedure.[13] Alkaline nitrobenzene oxidation was carried out using the procedure of Iiyama and Lam.[21] Total yield of alkaline nitrobenzene oxidation products was corrected for products from hydroxycinnamic acids[21]. S/V, molar ratio of syringyl nuclei and guaiacyl nuclei; *T65c*, *Oryza sativa* cv. *Taichung 65* (normal); *T65d*$_1$, dwarf near-isogenic line of *T65c*; *IR8*, *Oryza sativa* cv. *IR-8*; ODM, original dry matter

Results and discussion

The length of the first internodes of rice plants was measured as an index of plant height. The distribution of length of the first internode (topmost) from 100 internodes showed nearly Gaussian distribution. The average lengths of the first internode of samples were *T65c*, 37.5 cm; *T65d*$_1$, 18.3 cm; and *IR8*, 27.1 cm. The lignin content of each plant part of *T65c* was higher than that of its isogenic dwarf variety, *T65d*$_1$ (Table 1). It was the same as for the isogenic dwarf lines of wheat reported in a previous paper[1] but different from the results reported by Kaneya et al.[25] for stems of a rice variety. They used a variety of *Fujiminori* (*O. sativa* L. *cv. Fujiminori*) and its dwarf mutant *F71* and determined the lignin content by the Klason procedure without adding the values for the acid-soluble lignin content. The difference between our results and theirs may be due to variation of expression of dwarf genes, to the lignin determination procedures as discussed in previous papers, or both.[26,27]

In dwarf wheat, the lignin content was found to be higher in the internodes than in leaf sheaths and leaf blades.[1] However, in the dwarf line of rice used in this study the lignin content was lower in the internodes than in the leaves. This may be due to the morphological characteristics of rice stems compared to those of wheat stems. The internodes of rice are more fragile than those of wheat. The internode may contribute less to the mechanical properties of rice than of wheat because rice plants are immersed in water during a significant period of their growth. Although the height of *IR8* is between that of *T65c* and *T65d*$_1$, the lignin

Table 2. Hydroxycinnamic acid contents of dwarf, semidwarf, and normal strains of rice plants determined by alkaline hydrolysis at room temperature[a] and at 170°C[b]

Sample	Ester-linked		Ether-linked		Total	
	PCA	FA	PCA	FA	PCA	FA
T65c						
Internode	0.46 (0.04)	0.20 (0.02)	0.84 (0.05)	0.93 (0.04)	1.30 (0.11)	1.13 (0.09)
Leaf sheath	0.30 (0.05)	0.24 (0.02)	0.59 (0.04)	1.05 (0.06)	0.89 (0.05)	1.29 (0.10)
Leaf blade	0.14 (0.05)	0.11 (0.03)	0.35 (0.02)	0.94 (0.03)	0.49 (0.06)	1.05 (0.05)
T65d$_1$						
Internode	0.40 (0.02)	0.18 (0.02)	0.69 (0.03)	0.75 (0.03)	1.09 (0.09)	0.93 (0.09)
Leaf sheath	0.51 (0.03)	0.55 (0.04)	0.18 (0.02)	0.56 (0.05)	0.69 (0.06)	1.11 (0.10)
Leaf blade	0.32 (0.02)	0.33 (0.04)	0.16 (0.01)	0.73 (0.02)	0.48 (0.04)	1.06 (0.11)
IR8						
Internode	0.44 (0.04)	0.23 (0.05)	0.79 (0.06)	0.97 (0.05)	1.23 (0.12)	1.20 (0.13)
Leaf sheath	0.63 (0.04)	0.68 (0.05)	0.16 (0.01)	0.80 (0.02)	0.79 (0.09)	1.48 (0.09)
Leaf blade	0.25 (0.03)	0.52 (0.04)	0.12 (0.03)	0.93 (0.05)	0.47 (0.05)	1.45 (0.10)

Results are percent of extract-free oven-dried sample. Numbers in parentheses are standard deviation (n = 3)
PCA, *p*-coumaric acid; FA, ferulic acid; PCA/FA. *T65c*: *Oryza sativa* cv. *Taichung 65* (normal); *T65d$_1$*, dwarf near-isogenic line of *T65c*; *IR8*, *Oryza sativa* cv. *IR-8*
[a] For ester-linked: 1 M overnight
[b] For total bound: 4 M NaOH for 2 hr

content of *IR8* was higher than that of *T65c*, probably owing to significant genetic differences.

The total yield of alkaline nitrobenzene oxidation products of the rice samples based on lignin content (Table 1) was significantly lower than that of the wheat samples in a previous study.[1,21] All of these values were about half that for the wheat samples, although the syringyl nuclei/guaiacyl nuclei (S/V) molar ratios were similar to those of wheat samples.[1,21] These results suggest that lignin in rice stems is rich in condensed guaiacyl nuclei and has small amounts of vanillin as an alkaline nitrobenzene oxidation product. Syringyl nuclei in rice lignins are fewer than in wheat lignins, which are characterized as lignin in immature plants.[22] The immature lignin may relate to the fragile nature of the internodes of rice stems.

The total yield of alkaline nitrobenzene oxidation products of *T65c* was higher than that of the isogenic dwarf line, *T65d$_1$*, indicating that the lignin in *T65c* is more mature than that in *T65d$_1$*. This result differs from that in wheat plants[1] in that the total yield of alkaline nitrobenzene oxidation products is independent of the plant height. There were no significant differences in the S/V ratio in internode walls for *T65c* and *T65d$_1$* for which the tendency is similar to that in isogenic dwarf lines of wheat plants.[1] These results indicate that lignin within the dwarf variety of rice is richer in the condensed guaiacyl nuclei than in the normal counterpart. The total yield and S/V ratio for leaf sheaths and leaf blades were lower than those for internodes, similar to what was found in wheat lines,[1] indicating that the lignin in leaf sheaths and leaf blades is richer in condensed structure than the lignin in internodes. The total yield of nitrobenzene oxidation products of *IR8* was lower than that for either *T65c* or *T65d$_1$*, and its S/V ratio was higher, indicating that the lignin in *IR8* stems is significantly richer in condensed guaiacyl nuclei.

The results of hydroxycinnamic acid analyses are shown in Table 2. The concentrations of the hydroxycinnamic acids, *p*-coumaric acid, and ferulic acid, in rice internodes were similar to those of the wheat samples.[28] The content of ester-linked *p*-coumaric acid, however, is significantly higher than that in the wheat and phalaris internodes[28] but considerably lower than in the internode cell walls of maize (*Zea mays*) and sorghum (*Sorghum vulgare*).[29] The level of *p*-coumaric acid in cell walls of grasses may reflect a classification of tropical (e.g., maize and sorghum), subtropical (rice), and temperate (wheat and phalaris). The contents of the etherified ferulic acid and etherified *p*-coumaric acid in the normal variety (*T65c*) were slightly higher than those of the dwarf variety (*T65d$_1$*). It is suggested that etherified ferulic acid may form covalent associations between lignin and polysaccharides, reinforcing the mechanical strength of rice stems. However, the difference between the ether-linked ferulic acid contents in *T65c* and *T65d$_1$* is smaller than that observed for wheat plants,[1] suggesting that the culm reinforcing mechanisms are not developed owing to the immersion of rice plants in water for significant periods of their growth.

Uronic acid contents in three types of rice straw were similar (Table 3), although the leaf blades gave the highest content among the plant parts. The glucose residue of the dwarf variety was lower than that of the normal variety of rice straws, similar to an isogenic dwarf line of wheat straw.[1] A significantly higher content of (1 → 4) linked glucose (i.e., mainly cellulose) and lower content of (1 → 4) linked xylose (i.e., arabinoxylan) were found in the normal variety (*T65c*) than in the dwarf (*T65d$_1$*) and semidwarf (*IR8*) varieties by partially methylated alditol acetate analysis (Table 4). The sugar composition of the semidwarf variety (*IR8*) of different genetic background from the *Taichung 65* was different from that in the *Taichng 65* line.

Table 3. Neutral sugar and uronic acids contents of straw cell walls of normal and dwarf varieties of rice plants

Sample	Neutral sugar							Uronic acid		
	Rha	Ara	Xyl	Man	Gal	Glc	Total	CalA	GlcA	Total
T65c										
Internode	0.6 (0.1)	3.1 (0.3)	17.5 (1.1)	– (0)	1.7 (0.3)	54.7 (1.4)	77.6 (1.8)	0.3 (0.1)	0.4 (0.1)	0.7 (0.2)
Leaf sheath	– (0)	3.3 (0.2)	14.9 (0.9)	– (0)	1.5 (0.4)	30.5 (1.1)	50.2 (1.4)	0.4 (0.1)	0.6 (0.2)	1.0 (0.2)
Leaf blade	– (0)	3.4 (0.2)	13.5 (0.6)	– (0)	1.3 (0.2)	23.1 (1.2)	41.3 (1.5)	0.5 (0.2)	0.7 (0.2)	1.2 (0.3)
T65d$_1$										
Internode	0.4 (0.1)	3.6 (0.3)	17.9 (1.0)	– (0)	1.7 (0.2)	49.7 (1.3)	73.3 (2.1)	0.5 (0.1)	0.3 (0)	0.8 (0.2)
Leaf sheath	– (0)	3.0 (0.3)	12.5 (1.1)	– (0)	1.4 (0.3)	31.2 (1.2)	48.1 (1.2)	0.8 (0.2)	0.4 (0.1)	1.2 (0.2)
Leaf blade	– (0)	3.4 (0.2)	13.5 (0.8)	– (0)	1.3 (0.2)	23.1 (1.0)	41.3 (1.6)	0.8 (0.3)	1.2 (0.3)	2.0 (0.3)
IR8										
Internode	0.6 (0.2)	3.3 (0.3)	22.9 (1.2)	0.3 (0.2)	1.3 (0.2)	43.5 (0.9)	71.9 (1.4)	0.7 (0.2)	0.4 (0.1)	1.1 (0.2)
Leaf sheath	– (0)	3.7 (0.3)	16.6 (0.9)	– (0)	1.0 (0.1)	28.1 (1.1)	49.3 (1.3)	0.7 (0.2)	0.7 (0.2)	1.4 (0.2)
Leaf blade	– (0)	3.2 (0.3)	15.7 (1.1)	– (0)	1.2 (0.3)	30.3 (0.8)	50.4 (1.1)	0.8 (0.3)	0.9 (0.2)	1.7 (0.3)

Numbers in parentheses are standard deviations (n = 3). Results are percents of extact-free oven-dried sample
Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; GalA, galacturonic acid; GlcA, glucuronic acid; *T65c*, *Oryza sativa* cv. *Taichung 65* (normal); *T65d$_1$*, dwarf near-isogenic line of *T65c*; *IR8*, *Oryza sativa* cv. *IR-8*

Table 4. Glycosidic linkages of polysaccharides of straw cell walls of normal and dwarf varieties of rice plants

Sugar residue and linkage position	*T65c* internode	*T65d$_1$* internode	*IR8* internode	Wheat internode
Arabinose				
Terminal	4.5 ± 0.3	5.6 ± 0.2	8.5 ± 0.2	3.8 ± 0.5
1,2-	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
1,3-	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.2 ± 0.1
1,5-	0.6 ± 0.0	0.8 ± 0.1	0.9 ± 0.2	0.6 ± 0.1
Total	6.0 ± 0.5	7.4 ± 0.4	10.2 ± 0.7	4.9 ± 0.5
Xylose				
Terminal	0.9 ± 0.1	1.2 ± 0.2	1.5 ± 0.2	2.2 ± 0.2
1,4-	10.4 ± 1.1	11.6 ± 0.9	18.2 ± 1.4	28.6 ± 1.8
1,2,4-/1,3,4-	1.2 ± 0.2	1.4 ± 0.2	2.3 ± 0.4	2.1 ± 0.3
Total	12.6 ± 0.4	14.2 ± 0.5	22.0 ± 1.2	33.0 ± 1.5
Galactose				
Terminal	–	–	–	–
1,3-	0.5 ± 0.1	0.8 ± 0.2	1.0 ± 0.1	0.2 ± 0.1
1,4-	–	–	–	–
Total	0.5 ± 0.1	0.8 ± 0.2	1.0 ± 0.1	0.2 ± 0.1
Glucose				
Terminal	2.8 ± 0.4	3.8 ± 0.4	1.6 ± 0.2	2.0 ± 0.1
1,4-	74.0 ± 1.4	68.7 ± 1.9	62.3 ± 1.4	57.2 ± 1.3
1,3-	2.0 ± 0.3	2.3 ± 0.1	0.8 ± 0.2	0.7 ± 0.2
1,2,4-/1,3,4-	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.2 ± 0.1
1,4,6-	1.3 ± 0.1	1.4 ± 0.2	0.9 ± 0.2	1.1 ± 0.3
1,2,6-	0.3 ± 0.0	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.3
1,3,6-	–	–	–	–
Total	80.6 ± 1.6	77.0 ± 2.1	66.3 ± 1.5	60.9 ± 1.7
Total	99.7 ± 1.3	99.4 ± 1.6	99.5 ± 1.3	98.9 ± 2.1

All data were duplicated
Results are percents of total neutral sugar determined by alditol acetate procedure

Thus the chemical composition and structural features of lignin and polysaccharides of rice straw are strongly dependent on the genetic variety in rice species and significantly different from those of other temperate grasses, such as wheat and phalaris straws. This observation may be influenced by the growth conditions of the plants, particularly the lengthy immersion of the plant in water during the period of plant growth.

Acknowledgment This study was partially supported by a Grant-in-Aid (10306011) from the Ministry of Education, Science, Sports and Culture, Japan.

References

1. Lam TBT, Iiyama K (1996) The role of ferulic acid bridge between lignin and polysaccharides in the growth of wheat internodes. Mokuzai Gakkaishi 42:157–162
2. Fry S (1986) Cross-linking of matrix polymers in the growing cell walls of angiosperms. Annu Rev Plant Physiol 37:165–186
3. Iiyama K, Lam TBT, Stone BA (1994) Covalent crosslinks in the cell wall. Plant Physiol 104:315–320
4. Lam TBT, Iiyama K, Stone BA (1990) Primary and secondary walls of grasses and other forage plants: Taxonomic and structural consideration. In: Microbial and Plant Opportunities to Improve Lignocellulose Utilizationby Ruminant. Elsevier, Amsterdam, pp 43–69
5. Taiz L (1984) Plant cell expansion: Regulation of cell wall mechanical properties. Annu Rev Plant Physiol 35:586–657
6. Chewdhry AR, Allan RE (1966) Culm length and differential development of the coleoptile, root, and subcrown internode of near-isogenic wheat lines. Crop Sci 6:49–51
7. Swenson SP (1940) Genetic and cytologic studies of a brachytic mutation in barley. J Agric Res 60:687–713
8. Nilson EB, Johnson VA, Gardner CO (1957) Parenchyma and epidermal cell length in relation to plant height and culm internode length in winter wheat. Bot Gaz 119:38–43
9. Hansen A (1957) The expression of the gene dwarf-1 during the development of the seedling shoot in maize. Am J Bot 44:381–390
10. Kamijima O, Takenaka Y (1982) Character expression in a dwarf isogenic line of rice. I. The effect of dwarf gene, d_2 on elongation of panicle, internodes, and leaves. Sci Rep Fac Agric Kobe Univ 15:35–42
11. Kamijima O, Takenaka Y, Nagasawa H (1985) Character expression in a dwarf isogenic line of rice. II. The action of dwarf gene d_2 on cell proliferation and cell elongation in parenchyma of the third internode. Sci Rep Fac Agric Kobe Univ 16:377–384
12. Pelton JS (1964) Genetic and morphogenetic studies of angiosperm single-gene dwarf. Bot Rev 30:479–512
13. Iiyama K, Wallis AFA (1990) Determination of lignin in herbaceous plants by an improved acetyl bromide procedure. J Sci Food Agric 51:145–161
14. Blakeney AB, Harris PJ, Henry RJ, Stone BA (1983) A simple and rapid preparation of alditol acetates for monosaccharide analysis. Carbohydr Res 113:291–299
15. Scott RW (1979) Colorimetric determination of hexuronic acids in plant materials. Anal Chem 51:936–941
16. Harris PJ, Henry RJ, Blakeney AB, Stone BA (1984) An improved procedure for the methylation analysis of oligosaccharide and polysaccharide. Carbohydr Res 127:59–73
17. Bjorndal H, Hellerqvist CG, Lindberg B, Svensson S (1970) Gas-liquid chromatography and mass spectrometry in methylation analysis of polysaccharides. Angew Chem Int Ed 9:610–619
18. Geyer R, Geyer H, Kunhardt S, Mink W, Stirm S (1982) Capillary gas chromatography of methyl hexitol acetates obtained upon methylation of *N*-glycosidically linked glycoprotein oligosaccharides. Anal Biochem 121:263–274
19. Carpita NC, Shea EM (1989) Analysis of Carbohydrates by GLC and MS. CRC Press, Boca Raton, FL, pp 157–216
20. Sweet DP, Shapiro RH, Albersheim P (1975) Quantitative analysis by GLC (gas-liquid chromatography) response-factor theories for partially methylated and partially ethylated alditol acetates. Carbohydr Res 40:217–225
21. Iiyama K, Lam TBT (1990) The reactivities of lignin units during alkaline nitrobenzene oxidation. J Sci Food Agric 51:481–491
22. Lam TBT, Iiyama K, Stone BA (1990) Distribution of free and combined phenolic acids in wheat internodes. Phytochemistry 29:429–433
23. Iiyama K, Lam TBT, Stone BA (1990) Phenolic acid bridges between polysaccharides and lignin in wheat internodes. Phytochemistry 29:733–737
24. Lam TBT, Iiyama K, Stone BA (1994) Determination of etherified hydroxycinnamic acids in cell walls of grasses. Phytochemistry 36:773–775
25. Kaneya K, Kitano H, Kawakami H, Futsuhara Y (1987) Developmental genetic studies on the dwarf rice. 3. Chemical component of the dwarf rice cell wall. Ikushugaku Zasshi (suppl) 71:212–213
26. Iiyama K, Stone BA, Macauley BJ (1994) Composition changes in composting and growth of *Agaricus bisporus*. Appl Environ Microbiol 60:1538–1546
27. Lam TBT, Iiyama K, Stone BA (1992) Cinnamic acid bridges between cell wall polymers in wheat and *Phalaris* internodes. Phytochemistry 31:1179–1183
28. Lam TBT, Iiyama K, Stone BA (1992) Changes in phenolic acid from internode walls of wheat and phalaris during maturation. Phytochemistry 31:2655–2658
29. Lam TBT, Iiyama K, Stone BA (1996) Lignin and hydroxycinnamic acids in walls of brown midrib mutants of sorghum, pearl millet and maize stems. J Sci Food Agric 71:174–178