J Wood Sci (2003) 49:255–261

ORIGINAL ARTICLE

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Structural characteristics of cell walls of kenaf (*Hibiscus cannabinus* L.) and fixation of carbon dioxide

Received: April 22, 2002 / Accepted: July 24, 2002

Abstract Kenaf (*Hibiscus cannabinus*) plants are widely known for their contribution to the global and regional environment because of their ability to fix CO_2. On the other hand, some scientists have doubts about CO_2 fixation by kenaf and have misgivings about the effect of kenaf on the ecosystem. We have characterized the structural characteristics of cell walls of bast fibers, cores, roots, and leaves of kenaf during the maturation of plants and investigated the rate of photosynthesis. During maturation of the kenaf plant the cellulose (bast fiber 52–59%, core 44–46%) and lignin (bast fiber 9.3–13.2%, core 18.3–23.2%) contents increased significantly. The aromatic composition of the lignin of bast fiber was significantly different from that of the core lignin and of other plants. The lignin of bast fiber appears similar to pure syringyl lignin. Fixation of CO_2 by kenaf plants and their contribution to the global environment are discussed. A significaly high rate of photosynthesis of kenaf plants was observed compared to that of woody plants in Japan, but the amount of CO_2 fixation depends on the characteristics of the plantation. If the kenaf was planted in high density, about twice as much CO_2 was fixed as was fixed by trees in a tropical rain forest.

Key words Kenaf · *Hibiscus cannabinus* · Lignin · Neutral sugar · Cell wall · Rate of photosynthesis · Fixation of carbon dioxide

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Introduction

Kenaf (*Hibiscus cannabinus* L.) has attracted increasing interest from the viewpoint of preserving the global environment because of the significantly high rate of CO_2 accumulation.[1,2] There have been reports on the management of cropping and cultivation[3–8] and on the morphological characterization of fiber.[9–11] It would be expected that as a raw material it might be an alternative to wood fiber in the pulp and paper industry to avoid destruction of forests.[12–16] Furthermore, the lignin in the cell walls has been of interest for its chemical structural features, as an unusually high ratio of syringyl/guaiacyl nuclei has been reported.[13,17–20]

Products such as paper,[21–26] charcoal, nonwoven matting in the automotive industry,[27] textiles,[28–31] carbon fiber,[32] graft polymer,[33,34] particleboard,[35] composites with minerals,[36] composites with synthetic polymers,[27,37,38] potting soil,[39] liquefaction,[40] and animal bedding can all be produced from kenaf. In addition, the kenaf plant is being watched with keen interest as a source of phytoremediation.[41–46]

In this paper the chemical composition of the cell walls of bast fiber, core, and roots of kenaf plants being grown in a greenhouse and matured kenaf plants were analyzed, and their structural features were characterized. In addition, the rate of photosynthesis and fixation of CO_2 by the kenaf plant were examined.

Materials and methods

Plants

Seeds of varieties Chinpi-3 and Everglades of kenaf (*Hibiscus cannabinus*) were kindly supplied by the Japan Kenaf Association. Both varieties were sown on 13 April 1999 in pots with commercial mixed fertilizer in a greenhouse at the Forestry and Forest Products Research Institute, Tsukuba, Japan. Then 5–10 plants were harvested at 30, 36, 43, 49, 52, 59, 68, 90, and 121 days. The plants were frozen immediately after harvesting and then freeze-dried. The dried plants were separated into bast fibers, stem cores, leaves, and roots. The weight of each tissue was determined after further drying in a vacuum oven for 48h at 40°C. Kenaf fibers for rope production from matured plants (variety VN-1, harvested in Vietnam) and fiber and core for paper-making (variety Chinpi-3, harvested in China) were gifts of Thai Binh Jute Co. Vietnam and Japanese Association of Non-Wood Paper Promotion, respectively. Dried tissue was milled with a Wiley mill to pass 420μm sieves, extracted with boiling 80% aqueous EtOH (1h, ×3), followed with hot water overnight at 65°C.

Chemical composition

The lignin content of the extract-free sample was analyzed by an acetyl bromide procedure.[47] The neutral sugar and uronic acids composition in the hydrolysate after H_2SO_4 hydrolysis of the sample was determined by alditol-acetate[48] and colorimetric[49] procedures, respectively. Cellulose content was estimated as the content of anhydroglucosyl residue. Hydroxycinnamic acids that were ester- and ether-linked to cell wall polymers were quantified by the procedure of Lam and coworkers.[50] The acetyl content was determined by an ^{1}H-nuclear magnetic resonance (NMR) procedure after hydrolysis with 25% (v/v) D_2SO_4/D_2O using EtOH as an internal standard.[51] The aromatic composition of lignin was investigated by an alkaline nitrobenzene oxidation procedure.[52] The sample was combusted for 3h at 700°C to quantify the total ash content. The ash was dissolved in 0.5M HCl with heating, and calcium and magnesium were quantified using atomic absorption spectroscopy (Hitachi Z-6100).

Isolation and characterization of native (Björkman) lignin

Extract-free samples of bast fibers, cores, and leaves of Chinpi-3 variety grown for 121 days were finely ground with a vibration ball mill for 72h with no solvent;[53] they were cooled by water flow. Lignin was isolated with the Björkman procedure[54] and then characterized by acetyl determination.[51] ^{13}C-NMR spectra of the lignin were measured in $(CD_3)_2SO$; and the ^{1}H-NMR spectra in $CDCl_3$ were investigated after acetylation with acetic anhydride-pyridine.

Rate of photosynthesis and biomass production

The rate of photosynthesis of kenaf leaves in the greenhouse and some leaves of other outdoor plants were determined at 31, 38, and 28 days, using the Shimadzu potable photosynthesis-evaporation analyzer SPB-H3. The rate was corrected for a photon density of 1000μmol/cm^2/s. Kenaf (*H. cannabinus* L. var. VN-1) was cultivated under various cultivation densities (222000–555000 plants/ha) at Thu Duc District, Ho Chi Minh City by Dr. Quang Hung Le, College of Agriculture and Forestry, Ho Chi Minh City, Vietnam.

Results and discussion

Chemical composition

The chemical compositions of matured kenaf samples supplied from Vietnam and China are shown in Table 1 and compared with those of typical temperate woody species: spruce [*Picea jezoensis* (Sieb. et Zucc.) Carr, *var. jezoensis*]

Table 1. Composition of kenaf samples

Parameter	VN kenaf bast fiber	VN kenaf fabric	CH kenaf bast fiber	CH kenaf core	Spruce	Beech
Ash	8.6	2.0	16.7	9.6	2.8	5.0
Calcium	1.5	1.3	1.4	2.7	1.3	1.0
Magnesium	0.4	0.5	0.4	1.0	0.1	0.5
Lignin	111	117	130	200	327	258
Neutral sugars						
Fucose	1	0	0	0	0	0
Rhamnose	4	4	3	0	0	4
Arabinose	3	3	4	0	12	5
Xylose	171	158	162	195	81	216
Mannose	10	8	19	6	146	29
Galactose	8	6	11	2	38	14
Glucose	344	348	345	308	283	261
Total	541	528	544	511	560	529
Uronic acid	45	56	52	63	12	47
Nitrobenzene oxidation						
Total yield	528	505	513	483	235	382
S/V ratio	6.97	7.02	7.48	4.20	0.0	2.12

Results are given as grams per kilogram of oven-dried matter
VN kenaf bast fiber, bast fiber of variety VN-1 cultivated at Thu Duc district, Ho Chi Minh City,Vietnam; VN kenaf fabric, purified VN kenaf bast fiber; CH kenaf bast fiber and CH kenaf core, bast fiber and core of variety Chinpi-3 cultivated in China, respectively; Spruce, *Picea jezoensis* (Sieb. Et Zucc.) Carr, var. *jezoensis*; Beech, *Fagus crenata* Blume; S/V, molar ratio of syringyl nuclei to guaiacyl nuclei

and beech (*Fagus crenata* Blume). Bast fiber was characterized by a significantly low lignin content and a high glucose content (i.e., cellulose content). These results were similar to those reported by Neto et al.[55] for neutral sugar composition and by Pappas et al.[18] for lignin content determined by near-infrared (NIR) spectroscopy. The structural features of bast fiber lignin were characterized by a high total yield of alkaline nitrobenzene oxidation products and a markedly high syringyl/guaiacyl nuclei molar ratio (S/V ratio). Abbott et al.[56] reported the S/V ratio of the kenaf bast fiber to be 2.0, whereas Ralph[17] reported a significantly high S/V ratio of kenaf bast fiber lignin (7 to 9), similar to the results of this study. The high total yield of alkaline nitrobenzene oxidation products of bast fiber lignin suggests less condensed intermonomer linkages. The calcium content in kenaf samples, which may be related to the presence of pectin, was not significantly different from those of spruce and beech.

Biomass accumulated in proportion to the exponential increase in days after sowing during maturation of the plants (Fig. 1), reaching 100 g/plant (oven-dried weight) and 200–230 cm height at 121 days after sowing. These results are similar to the results of Ahmad et al.[57]

The relative contents of lignin and cellulose increased, on the other hand, both 80% EtOH and H_2O extracts decreased during maturation of kenaf plants for all fractions of the tissues (Tables 2, 3). There were no significant differences in the major chemical composition between cores and roots for both Chinpi-3 and Everglades (data not shown for Everglades). The lignin content of bast fiber was about half that of the cores of roots, and the same level as in the matured samples described above for elder plants more than 50 days after sowing (Table 1). Morrison et al.[58] reported a low content of ester- and ether-linked hydroxycinnamic acids (*p*-coumaric acid and ferulic acid) at the bottom core of kenaf plants harvested 96 and 151 days after sowing. However, hydroxycinnamic acids comprised less than 0.1 % of the extract-free samples in this study (data not shown) probably because of significantly immature samples compared with those reported by Morrison et al.[58] The acetyl content was almost constant throughout the extract-free samples: 2.2 % for bast fiber and 1.5 % for stem core and root.

It has been reported that lignin of kenaf bast fiber is characterized by an unusually high SV molar ratio (7 to 9).[14,17–20] It would be interesting to make clear how lignin with a high S/V ratio is deposited during maturation of the plant. A significantly high S/V ratio was observed in bast fiber lignin even in young plants (36 days after sowing), but it leveled off at 50 days after sowing, whereas the levels of core and root lignins remained low. The total yield of alkaline nitrobenzene oxidation products increased with

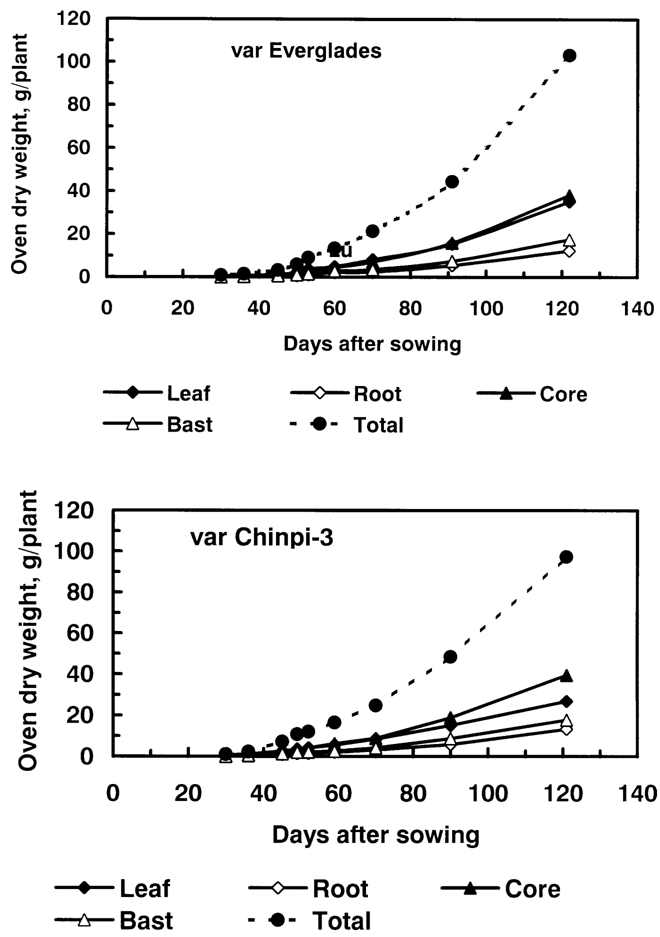

Fig. 1. Growth of kenaf varieties (Everglades and Chinpi-3), measured by dry weight

Table 2. Changes in extractives during maturing of kenaf plant (Chinpi-3)

Days	Bast fiber			Core			Root		
	80% EtOH	H_2O	Total	80% EtOH	H_2O	Total	80% EtOH	H_2O	Total
30	160	100	259	160	100	259	158	105	363
36	187	80	267	217	87	304	191	53	244
44	170	96	266	213	100	315	131	52	183
49	143	83	226	218	106	316	151	54	205
52	135	65	200	181	79	260	107	58	165
59	135	80	215	174	89	263	121	64	185
68	115	72	187	158	82	240	122	52	174
90	98	75	173	128	78	206	98	44	142
121	103	50	153	132	38	170	117	27	144

Results are given as grams per kilogram of oven-dried sample

Table 3. Changes in neutral sugar composition, uronic acid, and lignin content of kenaf (*Hibiscus cannabinus* L. var Chinpi-3) during maturation

Days after sowing	Change in neutral sugars							Change in uronic acid	Change in lignin
	Rha	Ara	Xyl	Man	Gal	Glc	Total		
Core									
36	10 (1.5)	26 (1.2)	122 (9.3)	22 (1.7)	36 (3.1)	521 (15.1)	738 (13.1)	83 (4.2)	93 (3.5)
49	9 (1.5)	24 (1.7)	141 (7.0)	19 (2.1)	28 (3.6)	580 (10.7)	801 (7.6)	60 (6.1)	110 (6.4)
59	10 (1.2)	25 (1.5)	139 (3.5)	19 (2.9)	23 (4.0)	598 (9.0)	813 (6.4)	62 (4.5)	121 (7.0)
90	8 (1.2)	24 (2.3)	131 (6.4)	16 (1.2)	28 (4.5)	597 (10.6)	804 (15.4)	71 (3.1)	124 (1.5)
121	10 (2.1)	22 (1.5)	135 (5.6)	22 (3.1)	21 (2.5)	591 (12.1)	801 (7.6)	69 (4.2)	132 (7.2)
Bast fiber									
36	10 (2.1)	14 (2.0)	205 (8.9)	20 (1.2)	32 (3.2)	442 (6.1)	723 (9.8)	52 (5.5)	183 (3.5)
49	10 (4.4)	13 (1.1)	208 (6.5)	18 (1.7)	28 (3.5)	449 (5.9)	726 (11.2)	57 (5.5)	203 (4.6)
59	9 (2.6)	13 (1.5)	211 (3.0)	17 (1.7)	22 (1.5)	448 (3.5)	721 (5.0)	44 (2.6)	232 (4.5)
90	9 (1.1)	11 (1.5)	208 (6.6)	19 (1.5)	17 (1.7)	452 (6.8)	716 (3.8)	46 (3.6)	230 (6.7)
121	10 (1.1)	9 (1.5)	210 (6.6)	17 (1.5)	16 (1.7)	461 (6.8)	723 (3.8)	42 (3.6)	232 (6.7)
Root									
36	8 (2.0)	18 (2.5)	199 (11.7)	13 (1.0)	23 (2.1)	411 (9.5)	672 (3.5)	62 (3.6)	192 (3.0)
49	8 (1.0)	21 (2.0)	198 (4.6)	10 (1.5)	22 (1.5)	422 (8.0)	681 (13.5)	50 (4.6)	204 (2.6)
59	8 (1.0)	17 (2.1)	196 (5.2)	11 (1.0)	21 (1.5)	443 (9.3)	696 (16.6)	44 (2.0)	212 (6.1)
90	10 (1.5)	16 (0.6)	200 (6.4)	9 (2.1)	18 (1.7)	449 (6.1)	702 (5.9)	42 (4.9)	209 (3.8)
121	7 (2.6)	14 (1.5)	195 (2.0)	10 (1.5)	21 (1.5)	456 (5.5)	703 (4.2)	40 (5.9)	218 (6.4)

Results are given grams per kilogram of extract-free sample: means of three samples and the SD (in parentheses)

Table 4. Changes in alkaline nitrobenzene oxidation products of kenaf (*Hibiscus cannabinus* var. Chinpi-3) during maturation

Days after sowing	Bast fiber		Core		Root	
	Yield	S/V	Yield	S/V	Yield	S/V
30	48 (4.6)	2.38 (0.070)	–	–	226 (4.2)	1.28 (0.082)
36	49 (2.5)	4.03 (0.056)	237 (8.5)	1.20 (0.091)	269 (7.0)	1.59 (0.069)
45	88 (5.0)	4.63 (0.053)	244 (3.5)	1.30 (0.067)	262 (4.0)	1.78 (0.054)
50	137 (3.1)	4.94 (0.074)	278 (7.5)	1.29 (0.048)	266 (4.6)	1.80 (0.088)
53	193 (3.8)	5.33 (0.092)	293 (6.6)	1.32 (0.040)	263 (4.2)	1.82 (0.074)
60	248 (3.5)	5.57 (0.087)	299 (5.5)	1.34 (0.055)	268 (3.0)	1.84 (0.061)
70	262 (1.5)	5.87 (0.053)	305 (4.0)	1.42 (0.060)	272 (7.0)	1.92 (0.048)
91	291 (7.1)	5.67 (0.075)	324 (5.0)	1.48 (0.040)	269 (8.4)	1.88 (0.060)
122	297 (6.1)	5.99 (0.096)	325 (9.3)	1.58 (0.079)	276 (5.8)	1.95 (0.082)

There were three samples for each measurement reported
Yield, total yield of alkaline nitrobenzene oxidation products (grams per kilogram of lignin determined by an acetyl bromide procedure); S/V, molar ratio of products with syringyl nuclei/products with guaiacyl nuclei

Table 5. Characterization of Björkman lignin prepared from maturing kenaf (*Hibiscus cannabinus* L. var. Chinpi-3) 121 days after sowing

Parameter	Fiber	Core	Leaves
Yield (g/kg of AcBr lignin)	422	389	154
Acetyl group (g/kg of sample)	121	33	30
Nitrobenzene oxidation			
Yield (g/kg)[a]	264	293	224
S/V ratio	5.78	1.44	1.78

[a]Total yield of alkaline nitrobenzene oxidation products (g/kg of sample)

Table 6. Rate of photosynthesis of leaves of maturing kenaf (*Hibiscus cannabinus* var. Chinpi-3 and var. Everglades) and other plants

Plant	Latin name	PN	SD
Kenaf			
Chinpi-3	*Hibiscus cannabinus* var. Chinpi-3	23.4	2.99
Everglades	*Hibiscus cannabinus* var. Everglades-3	22.6	1.97
Konara	*Quercus serrata* Thunb.	8.7	0.33
Ubamegashi	*Quercus phillyraeoides* A. Gray	7.3	1.24
Ichijiku	*Ficus carica* L.	7.0	1.00
Sangojyu	*Viburnum awabuki* K. Koch	6.2	0.92
Ajisai	*Hydrangea macrophylla* Seringe	5.2	0.42
Ichyou	*Ginkgo biloba* L.	4.3	0.30
Nanakamado	*Sorbus commixta* Hedlund	4.3	0.35
Sudajii	*Shiia Sieboldii* Makino	3.4	0.57
Kunugi	*Quercus acutissima* Carruth	2.5	–

PN, rate of photosynthesis (μmol $CO_2/m^2/s$) at a photon density of $1000\,\mu$mol $/cm^2/s$

maturation of the kenaf plant (Table 4), but there were no significant differences between the bast fiber, cores and roots.

Structural features of Björkman lignin of maturing kenaf

Björkman lignins were isolated from bast fiber, cores and leaves of maturing kenaf (121 days after sowing) with the Björkman's procedure.[54] The structure of the lignins was characterized by an alkaline nitrobenzene oxidation, acetyl group determination, and measurement of ^{1}H-NMR spectra after acetylation with acetic anhydride/pyridine. The ^{13}C-

Table 7. CO_2 fixation by plants

Plant	C (t/ha/y)	CO_2 (t/ha/y)	Biomass (t/ha/y)	Accumulation			
				m^3/ha	Specific gravity	Tons	% Increase
Japanese tree[a]							
Takayama	0.67	2.5	1.4	120	0.4	48	3.5
Kiyosumi	1.36	5.0	2.7	120	0.4	48	7.1
Tropical rain forest[b]	4.5	16.5	9.0	180	0.7	126	8.9
Melaleuca cajuputi[c]							
Dry acidic swamp	5.7	20.9	11.4	180	0.8	144	9.9
Wet acidic swamp	10	36.7	20.0	180	0.8	144	17.4
Philippines tree[d]							
Mahogany	3.8	13.8	7.5	180	0.6	108	6.9
Narra	5.0	18.3	10.0	180	0.6	108	9.3
Kenaf[e]	18–37	66–135	37–75	Cultivation density: 220,000 – 500,000 plants/ha			

t/ha/y, tons per hectare per year
[a] *Cryptomeria japonica* D. Don. Determined by Tange et al.[59] at Takayama and Kiyosumi
[b] *Dipterocarpaceae* spp. Determined by Tange et al.[59] in Malaysia
[c] Determined by Yamanoshita et al.[60] in dry and wet acidic swamps
[d] Determined by Iiyama et al. at Los Banos, Philippines for mahogany (*Swietenia macrophylla* King) and narra (*Pterocarpus indicus* Willd.)
[e] Determined by L.Q. Hung in Thu Duc District, Ho Chi Minh City, Vietnam

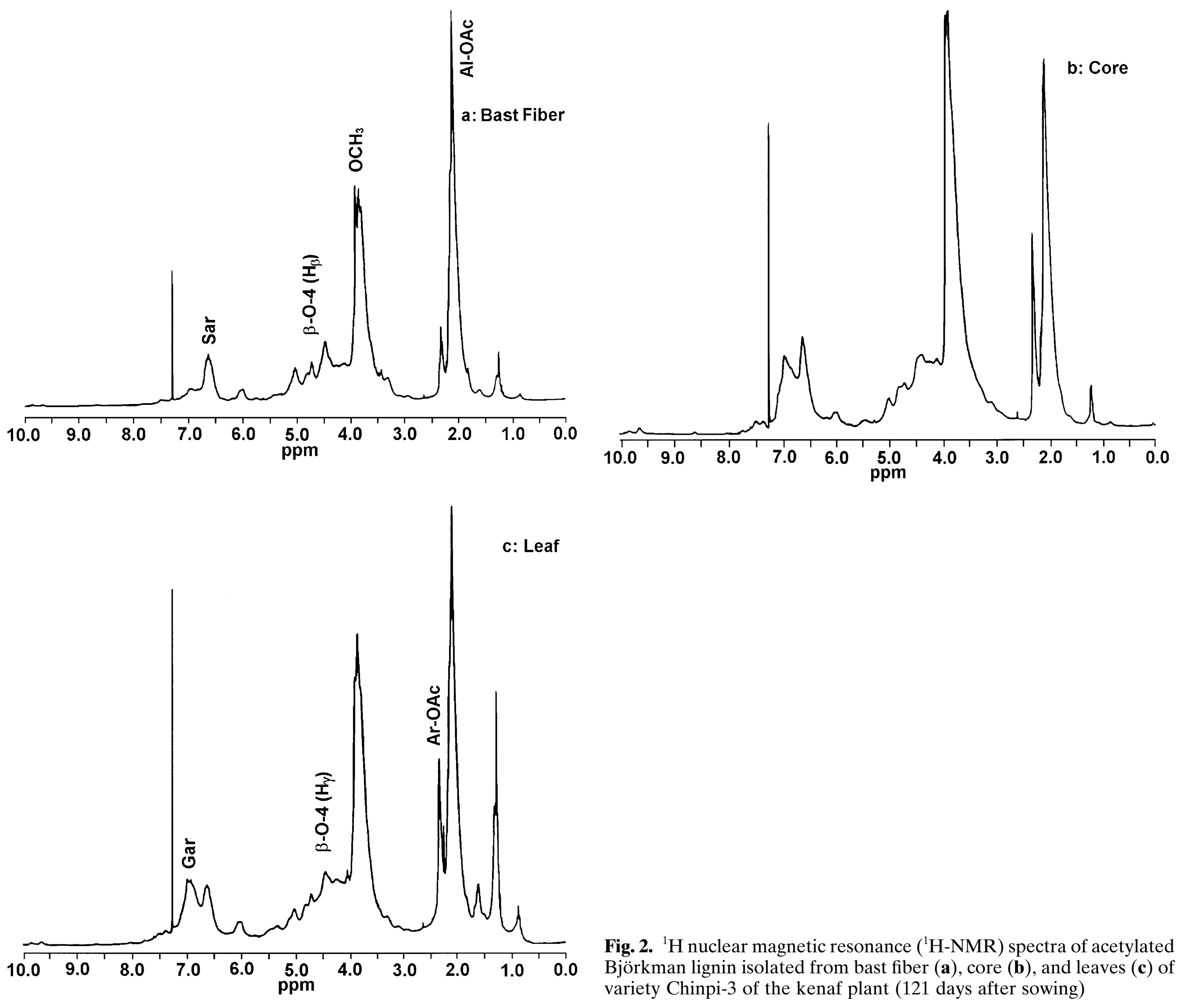

Fig. 2. ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectra of acetylated Björkman lignin isolated from bast fiber (**a**), core (**b**), and leaves (**c**) of variety Chinpi-3 of the kenaf plant (121 days after sowing)

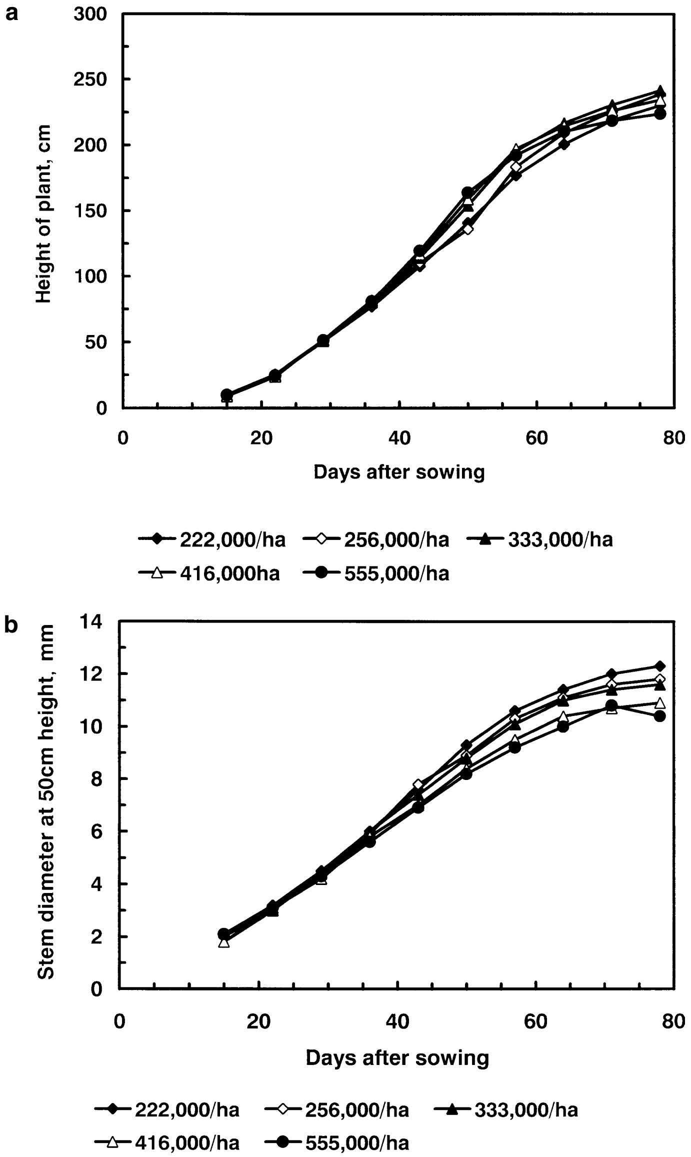

Fig. 3. Growth of kenaf, measured by the height (**a**) and diameter (**b**) of plants, in Vietnam under different cultivation densities of plants

NMR spectra of Björkman lignins were also measured before acetylation. The results are shown in Table 5 and Fig. 2. Lignin of kenaf bast fiber is characterized by a high S/V ratio, as described above, and it was confirmed by the ^{1}H-NMR spectrum that the aromatic unit of Björkman lignin of bast fiber is mostly composed of syringyl nuclei. Furthermore, the hydroxyl group of lignin is highly substituted by the acetyl group, as reported by Ralph and Lu.[19] The ^{1}H-NMR spectrum of bast fiber lignin suggested more β-ether linkages and fewer phenolic hydroxyl groups.

Photosynthesis rate and accumulation of biomass

The photosynthesis rate is a linear function of photon density. The photon density changes markedly with the intensity of the light. Hence the photosynthesis rate was corrected for photon density and calculated to be 1000μmol/cm^2/s. Kenaf plants showed an extremely high rate of photosynthesis, about 3–10 times higher than those of temperate plants (Table 6).

Fixation of CO_2 is a function not only of the photosynthesis rate of plants but also of their cultivation density. Kenaf (*Hibiscus cannabinus* L. var. VN-1) was cultivated under various densities (222 000–555 000 plants/ha) at Thu Duc District, Ho Chi Minh City, Vietnam. The growth of the plants was not affected by the cultivation density (Fig. 3), and the biomass accumulated was 100 g/plant (dry weight), with a standard deviation of 8.3 g, which corresponds to the net fixation of 183 g CO_2/plant. The CO_2 fixation by kenaf was compared with that of trees grown under different conditions (Table 7).

Acknowledgments This project was supported by the Science and Technology Agency (STA) fellowship of the Japan International Science and Technology Exchange Center (JISTEC), which has been successfully applied by Dr. Shuji Hosoya, Forestry and Forest Products Research Institute. We thank Dr. Toshio Sumizono and Mr. Masao Sakurai, Forestry and Forest Products Research Institute, for their kind help in determining the rate of photosynthesis and cultivating the kenaf plants, respectively. We also express our appreciation to Dr. Quang Hung Le, College of Agriculture and Forestry, Ho Chi Minh City, Vietnam for offering information about the cultivation of kenaf at Thu Duc District, Ho Chi Minh City.

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