Journal of Applied Physics

Roles of silica and lignin in horsetail (Equisetum hyemale), with special reference to mechanical properties

Shigeru Yamanaka, Kanna Sato, Fuyu Ito, Satoshi Komatsubara, Hiroshi Ohata et al.

Citation: J. Appl. Phys. **111**, 044703 (2012); doi: 10.1063/1.3688253
View online: http://dx.doi.org/10.1063/1.3688253
View Table of Contents: http://jap.aip.org/resource/1/JAPIAU/v111/i4
Published by the American Institute of Physics.

Roles of silica and lignin in horsetail (*Equisetum hyemale*), with special reference to mechanical properties

Shigeru Yamanaka,[1,a)] Kanna Sato,[2] Fuyu Ito,[3] Satoshi Komatsubara,[4] Hiroshi Ohata,[4] and Katsumi Yoshino[4,5]

[1]*Faculty of Textile Science and Technology, Shinshu University, 3-15-1 Tokida, Ueda, Nagano 386-8567, Japan*
[2]*Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei, Tokyo 184-8588, Japan*
[3]*Satellite Venture Business Laboratory, Shinshu University, 3-15-1 Tokida, Ueda, Nagano 386-8567, Japan*
[4]*Shimane Institute for Industrial Technology, 1 Hokuryo-cho, Matsue, Shimane 690-0816, Japan*
[5]*Center for Advanced Science and Innovation, Osaka University, 2-1 Suita, Osaka 565-0871, Japan*

(Received 2 December 2011; accepted 26 January 2012; published online 29 February 2012)

This research deals with detailed analyses of silica and lignin distribution in horsetail with special reference to mechanical strength. Scanning electron images of a cross-section of an internode showed silica deposited densely only around the outer epidermis. Detailed histochemical analyses of lignin showed no lignin deposition in the silica-rich outer internodes of horsetail, while a characteristic lignin deposition was noticed in the vascular bundle in inner side of internodes. To analyze the structure of horsetail from a mechanical viewpoint, we calculated the response of a model structure of horsetail to a mechanical force applied perpendicularly to the long axis by a finite element method. We found that silica distributed in the outer epidermis may play the major structural role, with lignin's role being limited ensuring that the vascular bundle keep waterproof. These results were in contrast to more modern tall trees like gymnosperms, for which lignin provides mechanical strength. Lignin has the advantage of sticking to cellulose, hemicellulose, and other materials. Such properties make it possible for plants containing lignin to branch. Branching of tree stems aids in competing for light and other atmospheric resources. This type of branching was impossible for ancient horsetails, which relied on the physical properties of silica. From the evolutional view points, over millennia in trees with high lignin content, true branching, and many chlorophyll-containing leaves developed.
[http://dx.doi.org/10.1063/1.3688253]

I. INTRODUCTION

Extant horsetail (order *Equisetales*), which originated from an ancient large horsetail tree of the Carboniferous Period, has attracted the attention of botanists, physiologists, and physicists because of its high silica content,[1–6] and unique ultrastructures were the subjects of structural,[1–4] morphological, functional, and physiological studies.[5,6] The ancient horsetails, or class *Sphenosida*, were most widespread in the Carboniferous Period, when the surface of the earth was dominated by highly diverse forests.[7] The *Calamites*, a type of ancient horsetail, are believed to have grown to great heights of 30–40 ms, much taller than extant horsetails.[8]

Horsetail is a strong accumulator of silica, which has been found in terrestrial plants,[9–11] diatoms,[12–14] sponges, and mollusks.[15] One role of silica is considered to give mechanical strength to tissue. Other possible functions of silica in plants include prevention of excessive water loss through the epidermis,[4] reflection of excessive light,[11] and protection against predators. The silica content in horsetail amounts to 25% of the dry weight and is derived from silicic acid from the soil,[8] and a part of deposition mechanisms of silica has recently been reported.[16]

Lignin functions in binding other cell wall components such as cellulose microfibrils, and hemicelluloses and gives rigidity and/or mechanical strength to plants.[17,18] Mutants with less lignin content showed decreased mechanical strength.[19–21] Another function is reportedly to make xylem cell walls more hydrophobic. The lignin content of horsetail is reportedly low (about 11–13%),[8] compared with pine trees (26–30%),[18] for example. Therefore, it has been speculated that one function of lignin for providing strength has been taken over by silica in horsetail.[8]

Two species of horsetail, *Equisetum hyemale* and *Equisetum prealtum*, were cultivated in soil from April to December in Yokohama, Japan. *E. hyemale* grew upwards with scarce branching. *E. prealtum* likewise grew upwards, with branching at nodes only occurring perpendicularly. In this study, we have analyzed silica and lignin distribution using a scanning electron microscope (SEM) equipped with an energy dispersive X-ray spectroscope (EDX) and histochemical analysis with light microscope in *E. hyemale*.

II. METHODS AND MATERIALS

A. Plant samples

Stems of horsetail were obtained from a garden in Yokohama, Japan. The species was identified as *Equisetum hyemale* L. based on morphological characteristics according to

[a)]Electronic mail: s.y@jcom.home.ne.jp.

the descriptions of *Makino's Illustrated Flora in Colour*.[22] Another horsetail, *Equisetum prealtum*, was purchased from a nursery.

B. Horsetail cultivation

The two species of horsetail were cultivated in soil and in aquaculture from June to September in Yokohama, Japan. For aquaculture, horsetail was harvested from the field and put in glass vessels containing Hyponex Cute medium (Hyponex Co. Ltd., Osaka, Japan) and kept under sunlight. This medium contained urea and KH_2PO_4, KNO_3, Cu, Zn, and Mo ions, but the exact composition is proprietary.

C. Microscopic observations

The horsetail specimens were observed by an optical microscope (VHX-500 F, KEYENCE Co., Osaka, Japan), and Au coated specimens were observed by an SEM S-3000 scanning electron microscope (Hitachi Co.) equipped with an Emax Energy 7000 energy-dispersive X-ray analyzer (Horiba).

D. Histochemical observation of lignin

The Wiesner reaction was performed according to a standard protocol.[22] Samples of horsetail and rice stems

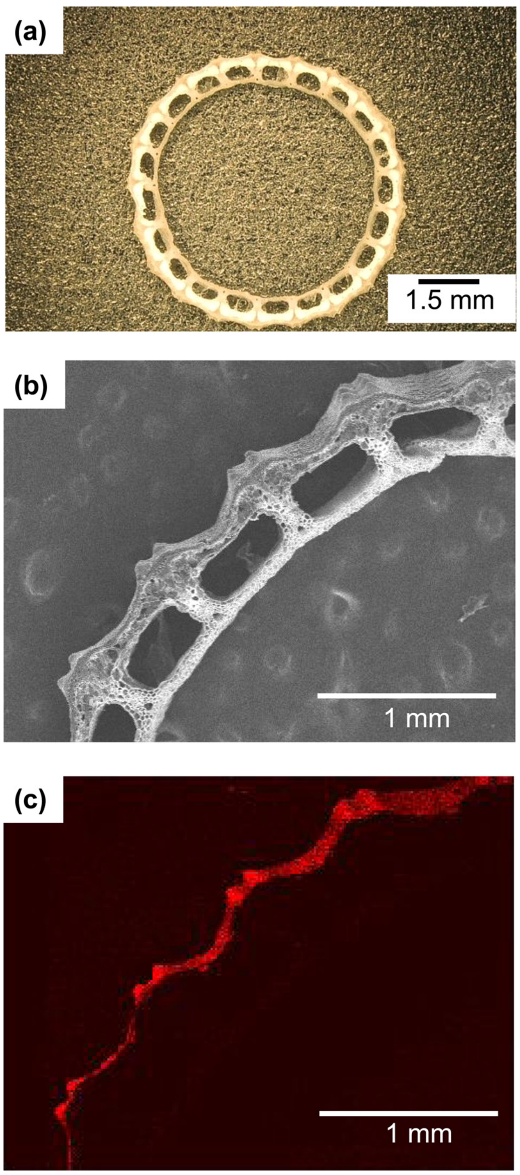

FIG. 1. (Color online) Microscopic analyses of horsetail, *Equisetum hyemale*. (a) Optical micrograph of a cross-section of a stem. (b) SEM image of a cross-section of epidermis. (c) Silicon (silica) distribution in (b), showing its concentration in the outermost part.

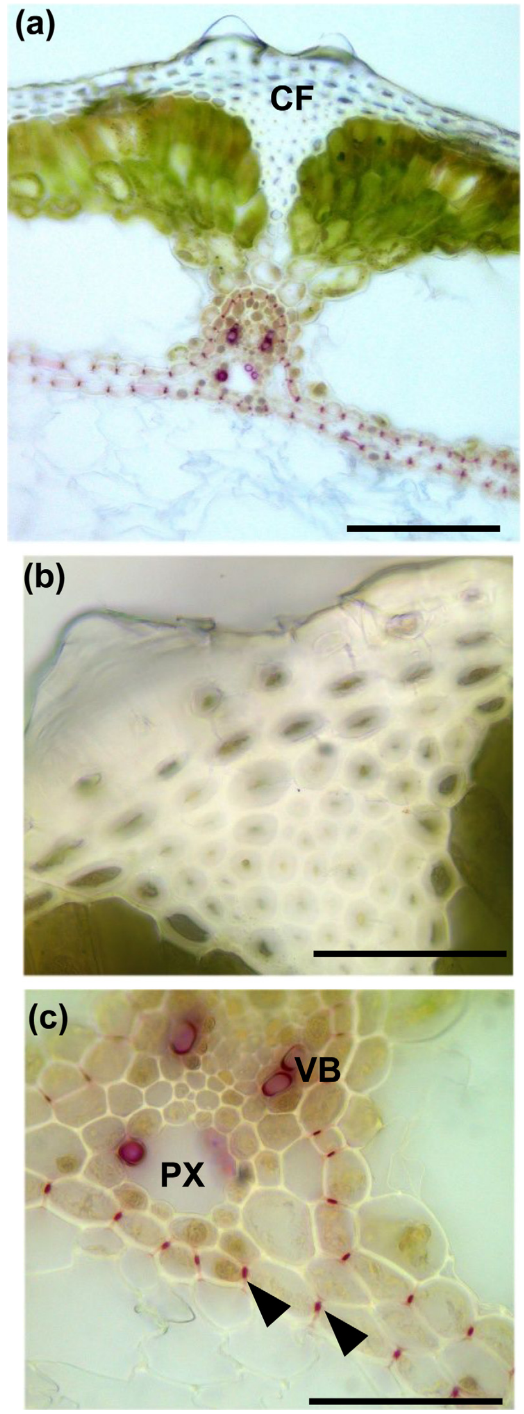

FIG. 2. (Color online) Histochemical analyses of lignin in internodes of horsetail using the Wiesner reaction. (a) Transverse section of horsetail showing limited localization of lignin in inner tissues (stained red). (b) Magnification of outer tissue showing no staining of cortical fibers (CF) in horsetail. (c) Magnification of inner tissue, showing a punctate lignin deposition (arrowheads) in horsetail. PX, protoxylem lacuna; VB, vascular bundle. Bars, 200 μm in (a), 100 μm in (b), and (c).

were fixed in formalin: acetic acid: 70% ethanol (1:1:18) and washed with water. Transverse sections were hand-cut with a razor blade. Longitudinal sections were cut on the freezing stage of a sliding microtome and sections with approximately 100 μm thickness were used for the Wiesner reaction,[23] which reacts with the cinnamaldehyde residues of lignin. Sections were incubated for 2 min. in phloroglucin solution (2% in ethanol), treated with a few drops of 12 N hydrochloric acid, and then observed with a DMLB light microscope (Leica, Wetzlar, Germany).

III. RESULTS AND DISCUSSION

A. Silica and lignin distribution in horsetail stem

In order to demonstrate silica distribution in the stem of horsetail, SEM observation and EDX mapping were performed. Optical micrograph (Fig. 1(a)) and SEM images (Fig. 1(b)) of a cross-section of an internode shows that a stem of horsetail consisted of outer thick tissue and inner thin tissues with large vallecular canals. EDX mapping of silicon indicates the presence of silica. Silica is deposited densely only around the outer epidermis (Fig. 1(c)).

Furthermore, lignin distribution in horsetail was analyzed by the Wiesner staining.[23] Though horsetail contains lignin (11–13%),[8] no detailed histochemical analyses have been reported. This reports the distribution of lignin in *Equisetum* in detail. As shown by histochemical analyses (Fig. 2), transverse sections of horsetail showed limited localization of lignin, stained in red (Fig. 2(a)). No lignin was detected in the silica-rich outer internodes of horsetail (Fig. 2(b)). In horsetail, a characteristic punctate lignin deposition (arrowheads) was visible (Fig. 2(c)).

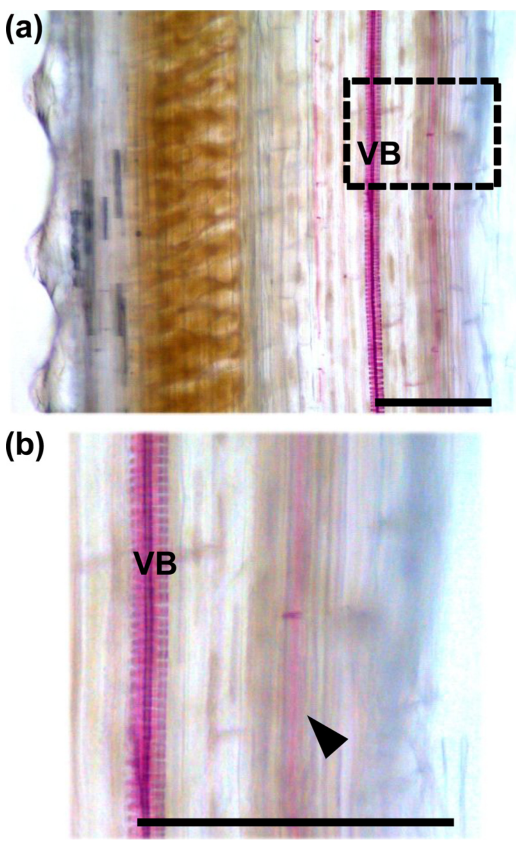

FIG. 3. (Color online) Histochemical analyses of lignin in longitudinal sections of horsetail with Wiesner reaction. (a) Longitudinal section of horsetail showing intense lignin deposition in the vascular bundle (VB). (b) Magnification of enclosed area in (a). Characteristic helical deposition of lignin was observed in the VB, and one layer stained by the Wiesner reaction was observed in the inner region of the VB (arrowhead). Bars, 200 μm.

To analyze this deposition of lignin, longitudinal sections were observed after staining by the Wiesner reaction. Intense lignin deposition was noticed in the vascular bundle (VB) (Fig. 3(a)). More detailed examination revealed characteristic helical deposition of the lignin observed in the VB, with one layer stained by the Wiesner reaction (arrowhead) in the inner region of the VB (Fig. 3(b)).

The young shoots emerging from the stems of plants cultivated on soil were mechanically strong due to the presence of silica (Fig. 4). However, shoots from plants cultivated hydroponically with no addition of silicic acid were fragile and withered within two or three days (Fig. 4(a), arrows). Figures 4(b) and 4(c) demonstrate SEM images of the surface of the young bud formed on the plant cultivated on soil and that cultivated hydroponically, respectively. The latter seems to be morphologically less differentiated than the former. Based on EDX analyses, their silica content was lower than for plants cultivated on soil (Figs. 4(d) and 4(e)), suggesting that silica is essential for normal shoot growth. No significant difference in lignin content was noticed between soil-grown and hydroponically cultured shoots (data not shown).

B. Structural analysis of mechanical strength in horsetail stem

To analyze the structure of horsetail from a mechanical viewpoint, we calculated the response of a model structure of horsetail to a mechanical force applied perpendicularly to the long axis by a finite element method.[24] We analyzed the anatomical distribution of silica and lignin in detail in order to infer their functions in tissues and calculated the mechanical strength of horsetail stems using two models of the silica and lignin components to identify their individual roles.

Using the cross-section of a horsetail indicated in Fig. 1 for reference, we used a coaxial two-pipe model connected with 18 ribs as the horsetail model. As indicated in Fig. 1, silica is mostly concentrated in the outer epidermis. Assuming that the outer pipe is composed of silica, as indicated in the inset of Fig. 5, and the inner pipe is composed of lignin (model 1), we calculated the mechanical responses. For comparison, we also calculated the mechanical response of the inverse structure of model 2 in which the inner tube is composed of silica.

In these cases, the mechanical constant of Young's modulus and Poisson's ratio described in the legend of Fig. 5 were adopted.[25] As shown in Fig. 5, upon application of a force perpendicular to the pipe axis, both the maximum principal stretching stress and deformation of model 1 with a structure similar to horsetail are much smaller than those of model 2. Therefore, from a mechanical viewpoint, the more likely structure of horsetail is with the silica content concentrated in the outer epidermis.

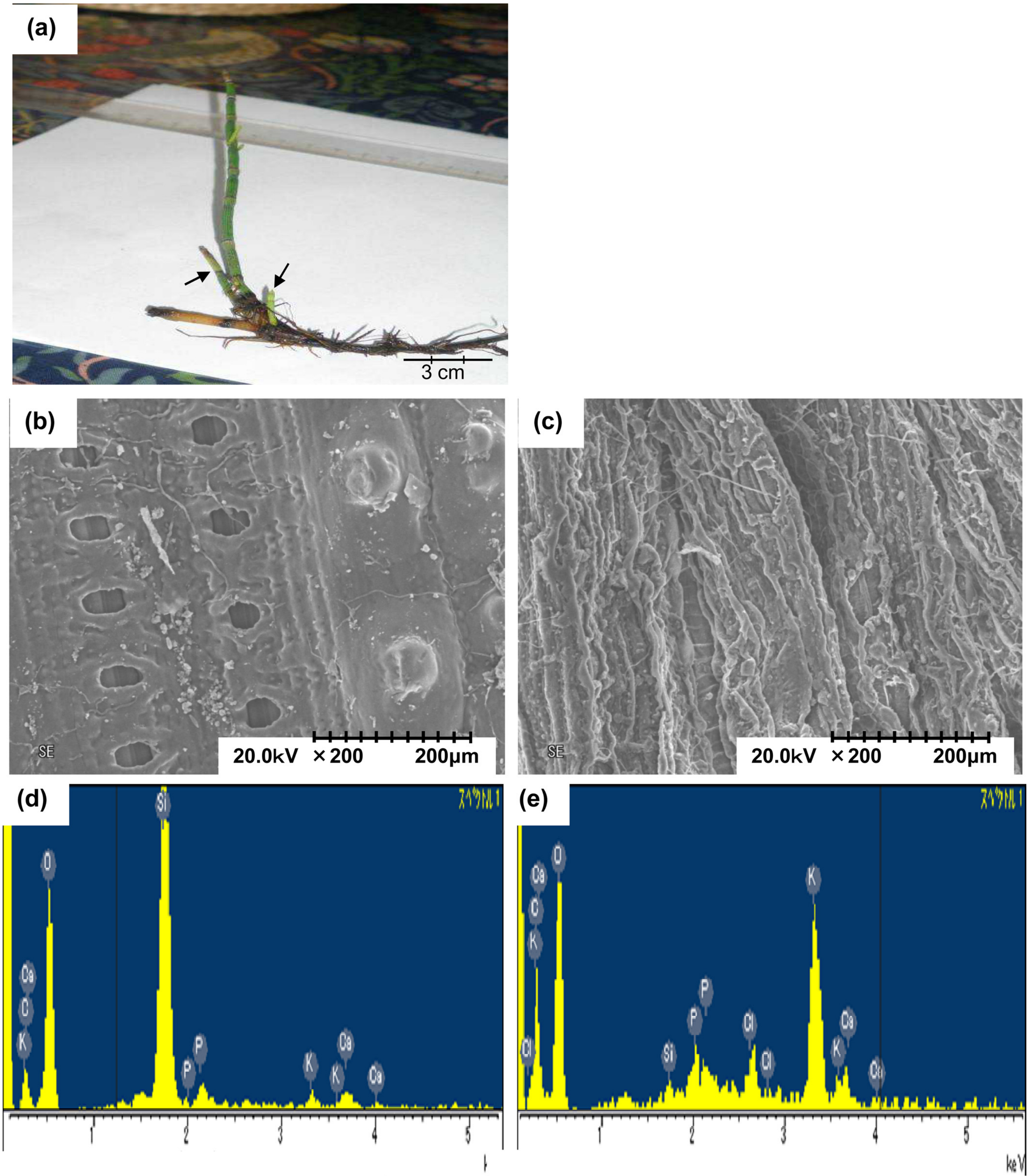

FIG. 4. (Color online) Analyses of young shoots of horsetail, *Equisetum hyemale*. (a) Horsetail cultivated hydroponically with no addition of silicic acid. Arrows show young shoots on a stem. (b), (c) SEM images of the surface of young buds formed on the internode of horsetail cultivated in soil and aquaculture, respectively. (d), (e) Silicon distribution on the surface of young shoots of horsetail cultivated in soil and aquaculture, respectively. Silicon was detected as the major constituent in (d), and at much lower levels in (e).

The horsetail *E. hyemale* had the morphology of model 1 in Fig. 5. The great height of the related Carboniferous horsetails may have been supported by a structural combination of silica and lignin; this zenith of horsetails may have been facilitated by an abundance of suitable silicon compounds.[26,27]

The distribution of lignin depends on the plant species.[28,29] Lignin has the advantage of sticking to cellulose, hemicellulose, and other materials. Such properties make it possible for plants containing lignin to branch, even in the horizontal direction. Branching of tree stems aids in competing

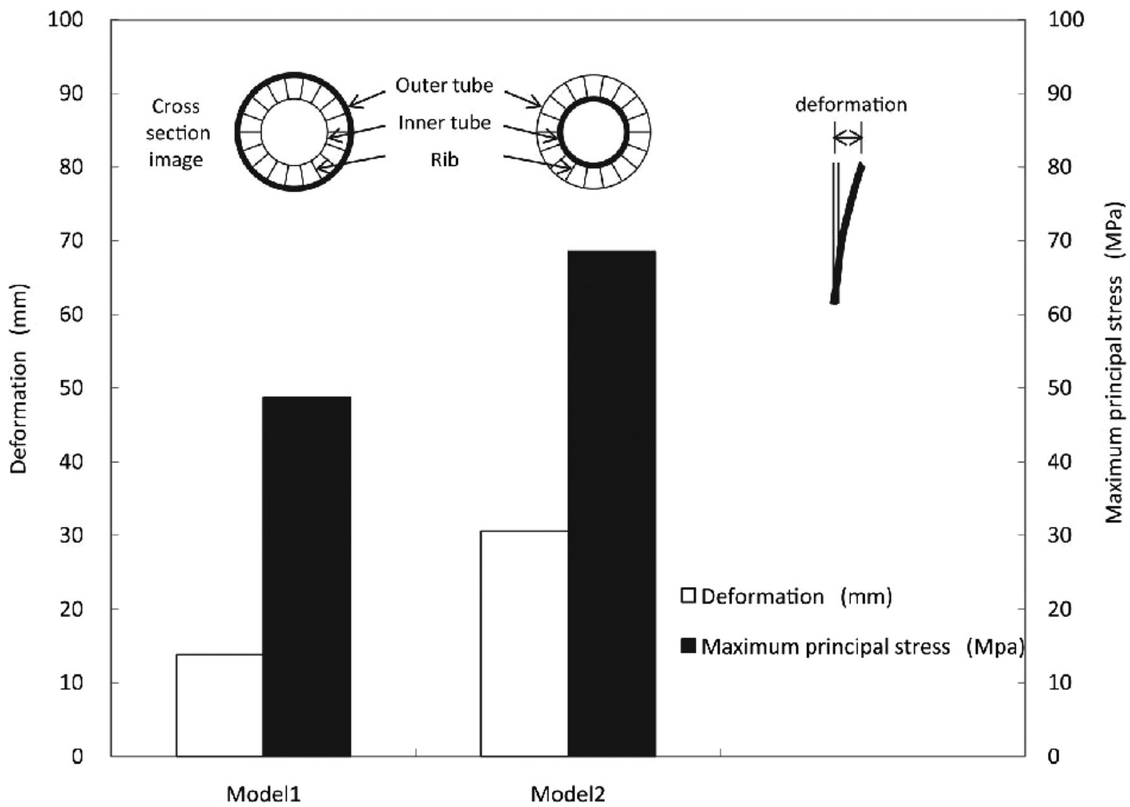

FIG. 5. Simulation of various combinations of silica and lignin demonstrating the deformation and maximum stretching stress. In cross-sections, broad lines indicate silica and normal lines, lignin. As a simple model of horsetail, two concentric tubes are shown in which the inner tube is connected to an outer tube with 18 ribs. Each structure is composed of silica or lignin. The diameter of the outer tube was assumed to be 4 mm and the inner tube 2.6 mm. The thickness of both the inner and outer tubes and the ribs was assumed to be 30 μm and the length of the model tree structure was 400 mm. For calculation, one edge of the model tree structure was fixed on the substrate (for theoretical calculation; any substrate is permissible as long as it is fixed completely) and a mechanical force of 0.1 N was loaded perpendicular to the tree; we adopted broadleaf tree values (Ref. 23) for silica of 68 000 MPa for the Young's modulus and 0.19 for Poisson's ratio, and for lignin, 9500 MPa for the Young's modulus and 0.37 for Poisson's ratio.

for light and other atmospheric resources.[30] The horizontal direction of branching was impossible for ancient horsetails, which relied on the physical properties of silica, considering our experiments with *E. hyemale* and *E. prealtum*. Over millennia, trees with high lignin content, true branching, and many chlorophyll-containing leaves developed.

In ancient times, giant Calamites trees grew in wetland forests. The largest anatomically preserved calamite specimen found has been attributed to the genus *Arthropitys*,[31] of the order Equisetales. *Arthropitys ezonata*, a plant of the extant horsetail relative, was reported to have trunk containing a silicified matrix that preserved the wood structure. Extant horsetail also contains silica as a main constituent, which may have originated from such ancient plants.

IV. CONCLUSIONS

Whereas lignin is usually the major contributor to mechanical support of land plants, experimental and empirical data showed that in horsetail, silica was primarily responsible for structural support with lignin providing waterproof properties. SEM images and EDX mapping demonstrated that silica is dense only around the outer epidermis. In detailed histochemical analyses of lignin, no lignin was detected in the silica-rich outer internodes of horsetail, while a characteristic lignin deposition was noticed in the vascular bundle. To analyze the structure of horsetail from a mechanical point, we calculated the response of a model structure of horsetail to a mechanical force applied perpendicularly to the long axis by a finite element method. The more likely structure of horsetail is with the silica content concentrated in the outer epidermis.

ACKNOWLEDGMENTS

We thank Y. Katayama (Nihon University, Japan) and H. Abe (Tokyo University of Agriculture and Technology, Japan) for helpful comments on the manuscript.

[1]L. Sapei, N. Gierlinger, J. Hartmann, R. Nöske, P. Strauch, and O. Paris, Anal. Bioanal. Chem. **389**, 1249 (2007).
[2]G. Holzhüter, K. Narayanan, and T. Gerber, Anal. Bioanal. Chem. **376**, 512 (2003).
[3]T. Speck, O. Speck, A. Emanns, and H.-Ch. Spatz, Bot. Acta. **111**, 366 (1998).
[4]P. B. Kaufman, W. C. Bigelow, R. Schmid, and N. S. Ghosheh, Amer. J. Bot. **58**, 309 (1971).
[5]C. C. Perry and M. A. Fraser, Philos. Trans. R. Soc. B **334**, 149 (1991).
[6]P. B. Kaufman, J. D. LaCroix, P. Dayanandan, L. F. Allard, J. J. Rosen, and W. C. Bigelow, Dev. Biol. **31**, 124 (1973).
[7]W. E. Friedman and M. E. Cook, Philos. Trans. R. Soc. B **355**, 857 (2000).
[8]T. E. Timell, Svensk. Pappers. **67**, 356 (1964).
[9]E. Epstein, Proc. Natl. Acad. Sci. USA **91**, 11 (1994).
[10]E. Epstein, Annu. Rev. Plant. Physiol. Plant. Mol. Biol. **50**, 641 (1999).
[11]S. Yamanaka, H. Takeda, S. Komatsubara, F. Ito, H. Usami, E. Togawa, and K. Yoshino, Appl. Phys. Lett. **95**, 123703 (2009).
[12]S. Yamanaka, R. Yano, H. Usami, N. Hayashida, M. Ohguchi, H. Takeda, and K. Yoshino, J. Appl. Phys. **103**, 074701 (2008).
[13]Q. Sun, E. G. Vrieling, R. A. van Santen, and N. A. J. M. Sommerdijk, Curr. Opin. Solid. State. Mater. Sci. **8**, 111 (2004).

[14]M. S. Hale and J. G. Mitchell, Aquat. Microb. Ecol. **24**, 287 (2001).
[15]K. Shimizu, J. Cha, G. D. Stucky, and D. E. Morse, Proc. Natl. Acad. Sci. USA **95**, 6234 (1998).
[16]C. Law and C. Exley, BMC Plant Biol. **11**, 112 (2011).
[17]H. S. Abreu, A. M. Nascimento, and M. A. Maria, Wood Fiber Sci. **31**, 426 (1999).
[18]M. M. Campbell and R. R. Sederoff, Plant Physiol. **110**, 3 (1996).
[19]B. Chabbert, B. Monties, N. Zieslin, and R. Ben-Zaken, Plant Physiol. Biochem. **31**, 241 (1993).
[20]L. Jones, R. Ennos, and S. R. Turner, Plant J. **26**, 205 (2001).
[21]I. Bjurhager, A.-M. Olsson, B. Zhang, L. Gerber, M. Kumar, L. A. Berglund, I. Burgert, B. Sundberg, and L. Salmén, Biomacromolecules. **11**, 2359 (2010).
[22]T. Makino, in *Makino's Illustrated Flora in Colour*, edited by H. M. Tokyo (Hokuryukan, Tokyo, 1982).
[23]L. M. Srivastava, TAPPI J. **49**, 173 (1966).
[24]K.-J. Bathe, in *Finite Element Procedures* (Prentice Hall, Upper Saddle River, NJ, 1996).
[25]*Wood Industrial Handbook* (in Japanese), Forestry and Forest Products Research Institute, Maruzen, Tokyo, 2004).
[26]M. Sommer, D. Kaczorek, Y. Kuzyakov, and J. Breuer, J. Plant Nutr. Soil Sci. **169**, 310 (2006).
[27]J. K. Schubert, D. L. Kidder, and D. H. Erwin, Geology **25**, 1031 (1997).
[28]O. Kawamura, N. Niijima, M. Niimi, and R. Akashi, Grassl. Sci. **47**, 578 (2002).
[29]H. P. S. A. Khalil, M. S. Alwani, and A. K. M. Omar, BioResouces **1**, 220 (2006).
[30]P. Sarkar, E. Bosneaga, and M. Auer, J. Exp. Bot. **60**, 3615 (2009).
[31]R. Rö*β*ler and R. Noll, Rev. Palaeobot. Palynol. **140**, 145 (2006).