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Effect of Submergence on the Cell Wall Composition of Deep-Water Rice Internodes[1]

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ABSTRACT

The cell wall composition of internodes of deep-water rice plants (*Oryza sativa* L. cv Habiganj Aman II) which were induced to grow rapidly by submergence in water was compared to that of nonsubmerged plants which grew slowly. No differences could be detected in cellulose, uronic acid, and lignin content expressed on a dry weight basis. Cell wall preparations of rapidly growing, submerged internodes contained more hydroxyproline and had a higher hydration capacity than those of control internodes. The silicon content of submerged rice internodes was considerably lower than that of air-grown plants. The role of silicon as a structural component of the cell wall of grasses is discussed in relation to lodging of deep-water rice plants after the flood waters have receded.

Internodal growth in deep-water rice is greatly enhanced by submergence. In Bangladesh, daily increases in plant height of 20 to 25 cm have been recorded (29). Internodal elongation in deep-water rice is based on stimulation of cell division and subsequent cell elongation (18). Ethylene is involved in the response of deep-water rice internodes to submergence. The ethylene concentration in the internodes rises from 0.02 to 1 μl/l after submergence, and ethylene applied to nonsubmerged rice plants causes an elongation response similar to that caused by submergence (17).

It is well established that *de novo* synthesis of cell wall-degrading enzymes is stimulated by ethylene during ripening of fruits and abscission of leaves (9). To find out whether ethylene causes cell wall changes in submerged rice internodes, we compared the cell wall composition of air-grown and submerged rice internodes. To obtain a representative picture of the changes occurring in the cell wall during elongation, typical components of each major plant cell wall constituent were analyzed. The major aim of this work was to investigate whether the cell wall composition of air-grown and submerged rice internodes provided an explanation for the extremely fast growth of submerged rice internodes and for the inability of deep-water rice to stay erect after the flood waters had receded.

MATERIALS AND METHODS

Plant Material. Seeds of a Bangladesh deep-water rice variety (*Oryza sativa* L. cv Habiganj Aman II) were obtained from the Bangladesh Rice Research Institute (Dacca, Bangladesh). Rice plants were germinated and grown as described by Métraux and Kende (17). All experiments were carried out with stem sections containing the expanding, youngest internode. Stem sections were cut as described by Raskin and Kende (20). Briefly, 20-cm-long stem sections were excised such that the second highest node of the culm was 2 cm above the basal cut, separated from the highest node by the youngest internode. The stem section above the highest node consisted of leaf sheaths which surrounded the youngest, developing leaves.

Growth of Stem Sections. Ten to 15 sections were placed upright in a 100-ml glass beaker containing 30 ml of distilled H_2O. For submergence treatment, the beakers with the sections were placed in 1-L volumetric cylinders, 42 cm deep. To keep stem sections submerged in water, the glass beakers were filled with glass beads which prevented the submerged sections from floating up. Control stem sections were incubated in open cylinders exposed to the air. Submerged and nonsubmerged sections were grown at 27°C under continuous light (70 $\mu E\ m^{-2}\ s^{-1}$) for 3 d.

Cell Wall Preparation. Ten to 15 stem sections were used for each cell wall preparation. In the case of air-grown stem sections, the whole internode consisting of more than 95% of tissue that did not elongate during the experiment (18) was used for the cell wall preparation. This fraction will be called control cell wall preparation. The internodes of the submerged stem sections were separated in two parts, the part present before submergence and the newly formed part. The cell wall preparations of these two fractions will be called preexisting and newly formed cell walls, respectively. The cell walls were prepared as described by Talmadge *et al.* (24) with minor modifications. The internodes were excised, chopped, frozen in liquid nitrogen, and subsequently ground in a mortar cooled with liquid nitrogen. The resulting fine powder was washed with water and centrifuged in a clinical centrifuge for 10 min. The supernatant was discarded, and the pellet successively washed with 10 volumes of water (3 times), ethanol (once), chloroform:methanol (1:2, v/v) (once), acetone (2 times), and water (3 times). The pellet was weighed, frozen, lyophilized, and weighed again. The cell wall preparations were stored over phosphorous pentoxide and KOH at 4°C in a desiccator under vacuum.

Analysis of Starch. Cell wall preparations were routinely tested for the presence of starch with the iodine reaction (28). If present, starch was removed by treatment with fungal α-amylase (Calbiochem-Behring) as described by Talmadge *et al.* (24).

Chemical Determinations. Cellulose was determined according to Updegraff (26). Uronic acids were measured as described by Ahmed and Labavitch (1). The lignin content was determined by phenol analysis and use of a conversion factor as suggested by Stafford (23). Hydroxyproline determinations were performed as described by Lamport (15). The hydration capacity of the cell wall was calculated from the weight of the cell wall preparation before and after lyophilization.

Neutral Sugar Analysis by Gas Chromatography of Alditol

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Table I. *Cellulose, Uronic Acid, Lignin, Hydroxyproline, and Dry Weight to Fresh Weight Ratio of Rice Internode Cell Walls*

Each Value is the average of three to four determinations carried out with different cell wall preparations (± SD). The values are expressed as μg/mg lyophilized cell wall preparation. The dry weight to wet weight ratio is given in per cent.

Tissue	Cellulose	Uronic Acids	Lignin	Hydroxyproline	$\frac{\text{Dry Wt}}{\text{Wet Wt}} \times 100$
			μg/mg		%
Air-grown internodes	479 ± 50	71 ± 6	146 ± 20	0.6 ± 0.2	5.2 ± 1.2
Submerged internodes, preexisting part	483 ± 50	71 ± 5	162 ± 46	0.6 ± 0.1	4.4 ± 1.0
Submerged internodes, newly elongated part	447 ± 50	82 ± 7	147 ± 24	1.4 ± 0.2	1.1 ± 0.4

Table II. *Neutral Sugar Composition of Air-Grown and Submerged Rice Internode Cell Walls*

Each Value is the average of three determinations carried out with different cell wall preparations (± SD). The values are expressed as μg/mg lyophilized cell wall preparation.

Tissue	Arabinose	Xylose	Galactose	Glucose	Total
			μg/mg		
Air-grown internodes	13.7 ± 1.6	62.4 ± 11.8	3.0 ± 0.7	52.6 ± 8.4	130.7 ± 19.8
Submerged internodes, preexisting part	13.9 ± 1.6	61.3 ± 7.6	3.2 ± 0.5	45.9 ± 6.6	126.2 ± 14.2
Submerged internodes, newly elongated part	22.8 ± 3.1	45.1 ± 0.9	6.1 ± 0.4	71.2 ± 13.2	145.2 ± 13.0

Table III. *Silicon Content of Air-Grown and Submerged Internodes as Measured by Neutron Activation Analysis*

The values are averages of two determinations carried out with preparations of different plants.

Tissue	Silicon
	μg/mg
Air-grown internodes	6.9
Submerged internodes, preexisting part	5.9
Submerged internodes, newly elongated part	2.1

Acetates. Alditol acetates were prepared following the method of Albersheim *et al.* (2). Analysis was carried out on a Perkin Elmer 910 gas chromatograph with He as carrier gas (flow rate 40 ml min^{-1}). The column (185 cm × 2 mm) contained polyethylene glycol succinate (0.2%), polyethylene glycol adipate (0.2%), and G.E. silcone XF 1150 (0.4%) on Gas Chrom Q.

Silicon Determination by Neutron Activation Analysis. Neutron activation analysis was carried out at the nuclear reactor of the Phoenix Memorial Laboratory (Ann Arbor, MI) as described by Jones *et al.* (12). Fifty to 100 mg of powdered plant material was used for each determination. A silicon standard was run with each experiment.

Silicon Distribution Analysis by X-Ray Microanalysis. Internodes were isolated from rice stem sections and cut into approximately 2-cm-long pieces with a razor blade. The tissue was fixed in 4% glutaraldehyde in H_2O, dehydrated with ethanol, and critical point dried with CO_2. The internode pieces were mounted on carbon stubs (2.54 cm in diameter) with tube coat and coated three times with carbon. The SEM[2] (JOEL 35 SEM) was operated at an acceleration voltage of 20 kev. For silicon microanalysis, the SEM was connected to an x-ray detector and amplifier system equipped with a multichannel analyzer. Selected areas of the internode were first photographed in the secondary emission mode and subsequently mapped for silicon (6).

[2] Abbreviation: SEM, scanning electron microscope.

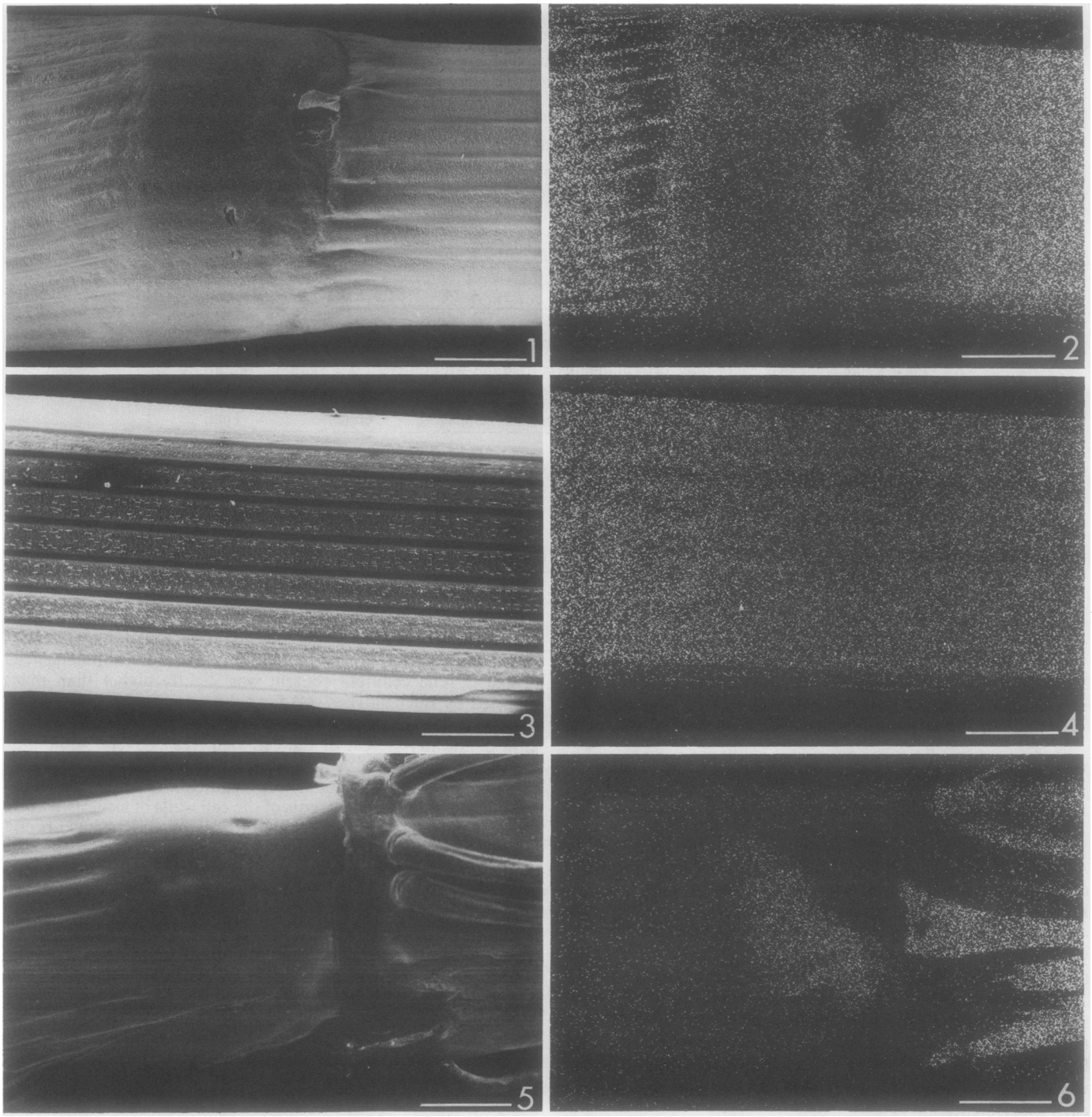
RESULTS AND DISCUSSION

The cellulose content of the internodal cell wall did not change significantly during elongation in response to 3 d of submergence (Table I). The cellulose content was slightly higher than that reported by Buchala *et al.* (4) for oat or by Franz (8) for bean cell walls. These authors reported cellulose reaching 40% and 23% of the dry weight of the cell wall, respectively, while we found that internodal cell walls of rice contained 45% to 48% cellulose. We also did not detect significant differences in the lignin and uronic acid contents of the cell walls of air-grown and submerged internodes. The amount of lignin might have been overestimated because of phenolic contamination of the cell wall preparation and because we measured the phenol content of the cell wall and calculated the lignin content using the conversion factor suggested by Stafford (23). Higuchi (10) pointed out that the number of phenolic hydroxyls per 100 C_9 units of lignin may vary between 14.5 and 25, the amount of phenolic hydroxyls per 100 C_9 units being specific for a given lignin. Even if our determinations were too high, there was no relative change between the internodal cell walls of submerged and control sections.

The hydroxyproline content of rice internodes was not significantly different in air-grown sections and preexisting parts of submerged sections but increased more than 2-fold in the newly elongated internodal region of submerged sections (Table I). Compared to the hydroxyproline content of dicotyledons, which is in the range of 20 μg/mg cell wall preparation (24), rice contains very low amounts of hydroxyproline. The same has been found for a number of other monocotyledons (5).

The hydration capacity of the cell wall preparation calculated as the ratio of dry weight to wet weight was not significantly different in the air-grown and preexisting parts of submerged rice internodes (Table I). However, the hydration capacity of the cell wall preparation from the newly elongated part of the internode was much higher than that of the already preexisting part.

In the neutral sugar fraction, only arabinose, xylose, galactose, and glucose were detected (Table II). This is in good agreement with data of Burke *et al.* (5) who could not detect rhamnose and fucose in any of the six monocotyledons that they analyzed. The

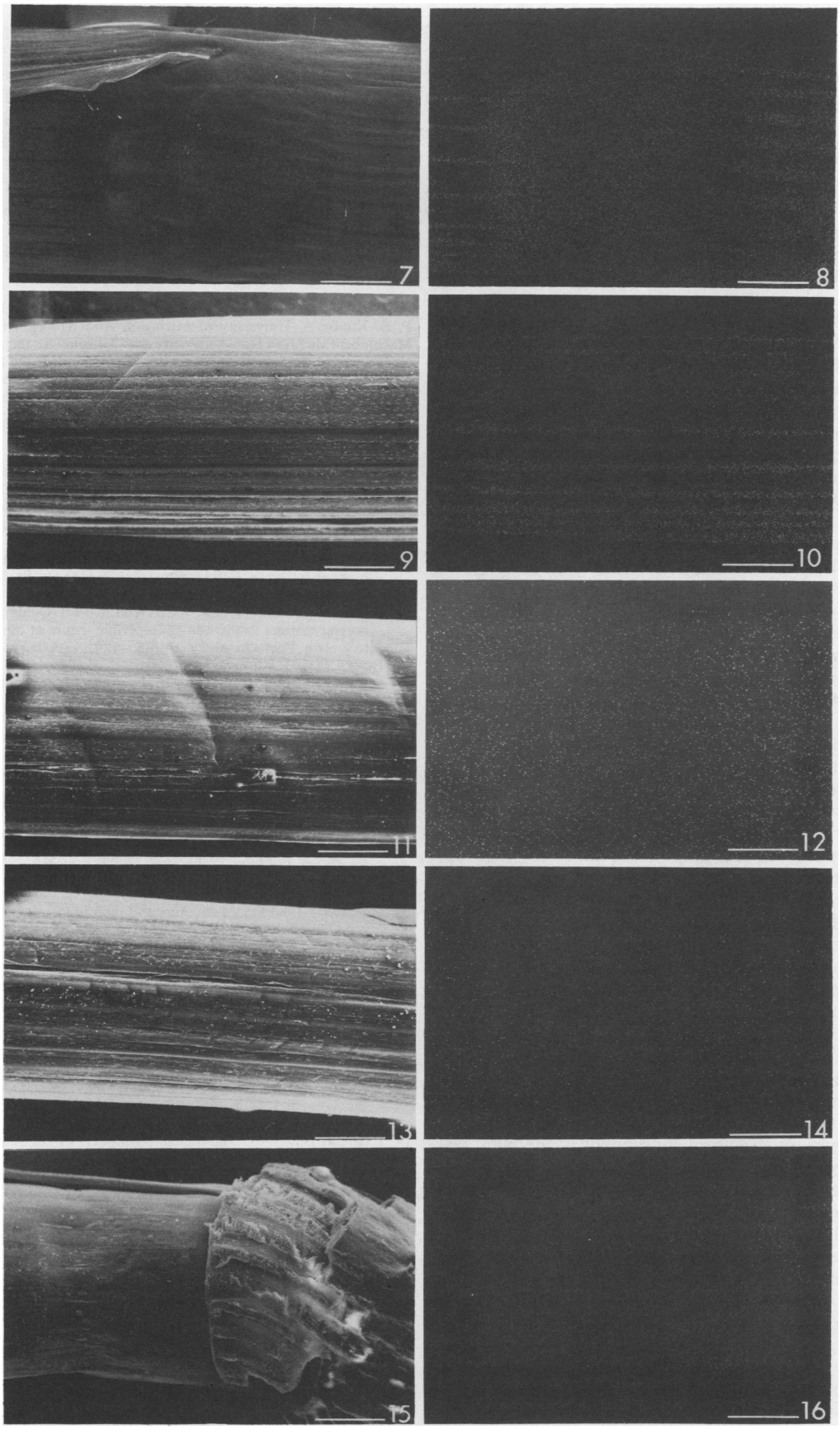


FIGS. 1–6. Scanning electron microscope views and x-ray mapping of silicon distribution in air-grown rice stem sections. 1,2: SEM view and Si x-ray map of the highest node. 3,4: Part of the same stem section 10 mm below the highest node. 5,6: Second-highest node of the same stem section. The internode including the two nodes was 39 mm long. The basal part of the internode is always on the right side of each picture. Bar: 1 mm.

total neutral sugar composition of the air-grown and the preexisting part of submerged internodal cell walls did not differ significantly. However, the cell walls of the newly elongated region of submerged internodes showed a rise in arabinose, galactose, and glucose and a decrease in xylose. The increase in total neutral sugars of the newly elongated part of submerged internodes was not significant.

More silicon was detected in air-grown internodes than in the preexisting part of submerged internodes, and very little silicon could be found in the newly elongated region of submerged internodes (Table III). The same could be seen in the x-ray photographs of rice internodes (Figs. 1–16). In air-grown stem sections, silicon was nearly uniformly distributed over the internode (Figs. 1–6). In submerged sections, the basal, newly elongated part of the internode contained almost no silicon (Figs. 11–16). Towards the top of the same internode (Figs. 7–10), the silicon density increased, reaching almost the same density as found in air-grown internodes.

The cell wall components that we determined account for 79% to 87% of the dry weight of the whole cell wall preparation. We did not determine the protein content of the internodal cell walls. The protein content of cell walls is in the range of 5% to 10%



FIGS. 7–16. SEM views and x-ray mapping of silicon distribution in a submerged rice stem section. 7,8: SEM view and Si x-ray map of the highest node. 9,10: Part of the same stem section 25 mm below the highest node. 11,12: Part of the same stem section 50 mm below the highest node. 13,14: Same stem section 75 mm below the highest node. 15,16: Second-highest node of the same stem section. Figures 11 to 16 show the part of the internode which has newly elongated during 3 d of submergence. The final length of the internode including the two nodes was 115 mm. The basal part of the internode is always on the right side of each picture. Bar: 1 mm.

(9). Cell walls of suspension-cultured rice cells have been reported to contain 17% protein (5); however, this value may not be representative for rice internodes because suspension-cultured cells contain more protein in their cell walls than do their parent plants (14). Assuming that the protein content of rice internodes is in the range of 5% to 10%, no major cell wall component has been omitted in our analysis.

The data presented in this paper indicate that submergence does not change the cell wall composition of the internodal parts present before submergence. No significant differences in cell wall composition could be detected between air-grown and preexisting part of submerged internodal cell walls. Cell division and cell elongation only take place at the base of the internodes (18), and only the newly formed parts of the internodes were altered in their cell wall composition.

From the altered neutral sugar composition of the newly formed parts of submerged rice internodes it is not possible to identify the hemicellulosic polysaccharides which have changed. The change in neutral sugar composition may not be due to a change in arabinoxylan content because arabinose increased and xylose decreased. Arabinoxylans are polymers found only in the hemicellulose of grasses (7). More detailed information about the hemicellulose fraction can only be obtained by digesting this cell wall fraction with purified hydrolytic enzymes (3). The doubling in the hydroxyproline content of the cell walls of the newly elongated parts of rice internodes is difficult to explain. In dicotyledons, hydroxyproline is found in cell wall proteins and arabinogalactan proteins which seem to be cross-linked extensively with each other (27). They have been suggested to form a network with the polysaccharide components of the cell wall (16). Therefore, an increase of hydroxyproline would be expected to cause a stiffening of the cell wall. However, in rapidly growing rice internodes, we found an increased hydroxyproline content in the cell walls which are expected to be softer than the cell walls of nongrowing internodes. It is known that hydroxyproline-rich glycoproteins accumulate in response to added ethylene (22, 25). Submergence causes ethylene accumulation in rice internodes (17). If ethylene promotes accumulation of hydroxyproline-rich protein, this accumulation would be expected in both preexisting and newly elongated parts of submerged internodes. An increase in hydroxyproline was only found in the cell walls of the newly elongated parts, however. Interestingly, rice coleoptiles, whose growth was stimulated under water, contain less hydroxyproline than the coleoptiles of air-grown seedlings, which grew less (11). The significance of hydroxyproline may not be the same in monocotyledons as in dicotyledons, since monocotyledons contain only about one-fifteenth of the hydroxyproline of dicotyledons (5).

Water is an important structural component of the plant cell wall. It forms a gel structure with the pectins of the cell wall matrix and reduces hydrogen bonding between cellulose fibrils and hemicellulose chains (9). Therefore, changes in the water content of the cell wall alter the texture of the cell wall matrix, changing it from a gel to a viscous solution (19). An increase in the hydration capacity of the cell wall, as found in the newly elongated parts of submerged rice internodes, probably leads to cell wall loosening.

The newly elongated part of submerged rice internodes contains considerably less silicon than the preexisting part of the same internode and air-grown internodes. Plants take up silicon by the root as $Si(OH)_4$ and transport it to the shoot in the transpiration stream. As water is transpired, the internal $Si(OH)_4$ concentration rises to the saturation value, and silicon precipitates as SiO_2 (21). This provides a good explanation for the fact that the newly formed parts of submerged rice internodes contain such small amounts of silicon. Water loss due to transpiration is reduced during submergence, and less silicon is deposited. Silicon is found in large quantities in the shoot of grasses (13) and seems to contribute to the stiffening of the cell wall. According to Raven (21) the energy cost of incorporating SiO_2 into the cell wall is 10- to 20-fold less than that of incorporating organic cell wall stiffeners (*e.g.* lignins). Lodging of rice after the flood waters recede may be caused by the absence of silicon in submerged rice internodes. Unless the rice plants show 'kneeing ability', *i.e.* the ability of straightening up at the uppermost node to keep the leaves above the water level, the plants decay, causing heavy crop damages (30).

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