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Electron-Probe Microanalysis of Silicon in the Epidermis of Rice (*Oryza sativa* L.) Internodes

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Summary. Electron-probe X-ray microanalysis showed that significant amounts of silicon are accumulated in the entire epidermal system of the rice internode except in the stomatal apparatuses. Thus, there is a lack of specific sites for Si deposition from levels just above the base to the tip of the rice internode. In the intercalary meristem region, 1 cm above the base of the internode, point-count data indicate more Si accumulation in the dumb-bell shaped silica cells than in the long epidermal cells. Above this region, Si is accumulated essentially in a uniform pattern in all epidermal cells. Such a pattern for Si accumulation in rice internodes markedly contrasts with that for *Avena* internodes and may explain, in part, why rice plants have a higher percentage Si (dry weight basis) in their shoots. The adaptive significance of this silicification pattern in rice is discussed.

Introduction

The deposition of silicon (Si) in the various tissues of different organs of the rice plant have been studied by Yoshida *et al.* (1962a). They reported that the epidermis is the primary site for the deposition of Si in this plant. Using electron-probe microanalysis, Kaufman *et al.* (1969, 1971a), Soni *et al.* (1970, 1972a, b), and Laroche *et al.* (1968, 1969, 1970) studied the localization of Si in the epidermal cells of the developing and mature internode and leaf of *Avena*, *Oryza*, and *Equisetum*. These studies indicated that the various types of epidermal cells, *e.g.*, stomatal apparatuses (guard cells and subsidiary cells), trichomes, long epidermal cells, and cork-silica cell pairs or silica cells may accumulate significant amounts of Si in one part or on one surface of that part while these cells do not accumulate detectable amounts of Si in the other part or on the other surface of the part or organ of the same plant. Thus, the pattern of accumulation of Si in the epidermal system appears to be different in various plants.

Since rice accumulates sizeable amounts of Si as silica ($SiO_2 \cdot nH_2O$) (Lewin and Reimann, 1969), especially when compared with *Avena* (oats),

we were primarily interested as to whether Si was deposited in localized specific sites, as found in *Avena*, or deposited throughout the epidermal system, as in the scouring rush (*Equisetum hyemale* var. *affine*). Therefore, the present study was undertaken (a) to analyze the pattern of distribution of Si and (b) to determine the localization of specific deposition sites in the epidermal cells of the rice (*Oryza*) internode.

Materials and Methods

Seeds of *Oryza sativa* L. cv. 'Colusa' were obtained from the Rice Experiment Station, University of California, Biggs, Cal.

1. Culture of Rice Plants

The seeds were sterilized with 0.5% sodium hypochlorite ("Clorox", diluted 1:9) for 2 min, washed thoroughly with distilled water, and incubated at 30° C for 24 h. After this, the seeds were placed in plastic trays containing vermiculite, and incubated at 30° C in the dark. The seedlings were transferred to 1 gal. (ca. 4-l) porcelain crocks containing clay soil and grown in the greenhouse with a photoperiod of 18 h light (under incandescent light; intensity of 1600 ft.-c. at tops of plants) and 6 h dark. The average day temperature was 26° C and night temperature was 22° C.

2. Preparation of Epidermal Peels

For electron-probe microanalysis, we used the same methods for the preparation of tissue as cited in Kaufman *et al.* (1969) and Soni *et al.* (1970): (1) thin epidermal peels were obtained at 1-cm intervals from the base to the tip of a 25 cm long, nearly mature internode; (2) the tissue pieces were placed in aluminum foil envelopes 2 cm square, immediately frozen in liquid nitrogen, and then dried at 0° C in a Virtis automatic freeze dryer (No. 10–010) for 2 h; (3) the dried pieces were mounted on polished copper squares with electrically conductive silver paint, covered by evaporating a thin layer of carbon upon them, and stored in a desiccator until examination in the probe.

3. Electron-Probe Microanalysis

The electron microprobe (Applied Research Laboratories, Glendale, Cal. model EMX-SM) was operated at 15 keV, with a beam current of 0.5 μAmp, an emission current of 150 μAmp, and spot diameter = 0.5 μ, giving an electron-beam penetration into the tissue of about 15 μm. The average thickness of the epidermal layer is also 15 μm. The sample current was 0.02 μAmp. The X-ray spectrometer was peaked for the first-order silicon K α line using an ammonium dihydrogen phosphate (ADP) crystal. Secondary electron-scanning images were employed to examine the tissue and to select cells for analysis. Qualitative analysis of Si distribution was made by line scanning, and the data are expressed as counts per second (cps). Semi-quantitative analysis (point-count data) were obtained using an average of three locations per cell. At each locus, counts were made for 50 sec. At least three cells were counted at each locus.

Since there is clear evidence that Si is associated with oxygen as SiO_2 in biological material (Lewin and Reimann, 1969), and especially as non-crystalline isotropic silica gel ($SiO_2 \cdot nH_2O$) in grasses such as oats and rice (Jones and Milne, 1963; Lewin and Reimann, 1969), we assume this to be the case here. Because of the diffi-

Table 1. Semi-quantitative analysis (point-count data) for Si in different types of developing epidermal cells of the rice internode

Part of internode	Type of cell	Average counts (cps)[a]
0–1 cm above base	Long epidermal cells[b]	764
	Silica cells	
	Center region	1071
	Edges	1913
6–7 cm above base	Silica cells[b]	1205
	Center region	1435
	Edges	1329
4 cm from tip	Stomatal apparatus[b]	
	Edge of stomatal pore	1908
	In stomatal pore	258
	On epidermal papillae	937
	Between epidermal papillae	1353

[a] Mean count of Si for three point counts per cell (cps, counts per second) after subtracting background counts.
[b] At least three cells were counted at each locus.

culty of measuring oxygen directly with the electron microprobe, especially in biological material, we measured only Si. Therefore, in this paper, we refer to Si with reference to our direct measurements with the electron microprobe, and *silica* ($SiO_2 \cdot nH_2O$), as the probable form in which Si occurs in rice internodes.

Results

The epidermis of the rice internode is differentiated into long epidermal cells, stomata, trichomes, and dumb-bell-shaped silica cells, as seen in light microscopy (Juliano and Aldama, 1937) and scanning electron-microscopy (Kaufman *et al.*, 1971 b).

The present study with electron-probe microanalysis showed the presence of significant amounts of Si in the long epidermal cells (764 cps) and the silica cells (1071–1913 cps) within 1 cm from the base of the internode (Figs. 1, 2). At this level, the trichomes and stomata are not well-differentiated. Semi-quantitative analysis (point-count data) indicates that higher amounts of Si occur in the silica cells than in the long epidermal cells at this level. These data suggest at first sight that at this level of internode, within the intercalary meristem, there is some evidence for selective deposition sites for Si, namely, the silica cells. However, the fact that the long epidermal cells also accumulate Si, even if in lower amounts, tends to rule out this possibility.

Figs. 3 and 4 show the pattern of Si deposition at 6–7 cm from the base of the internode. At this level, the epidermis is differentiated into long epidermal cells, stomata, and silica cells. This microanalysis indicates the presence of Si in significant quantities in *all* the types of epidermal cells. Thus, the pattern of Si deposition is similar to that of higher portions of the internode (compare Figs. 3, 4 and Figs. 5, 6).

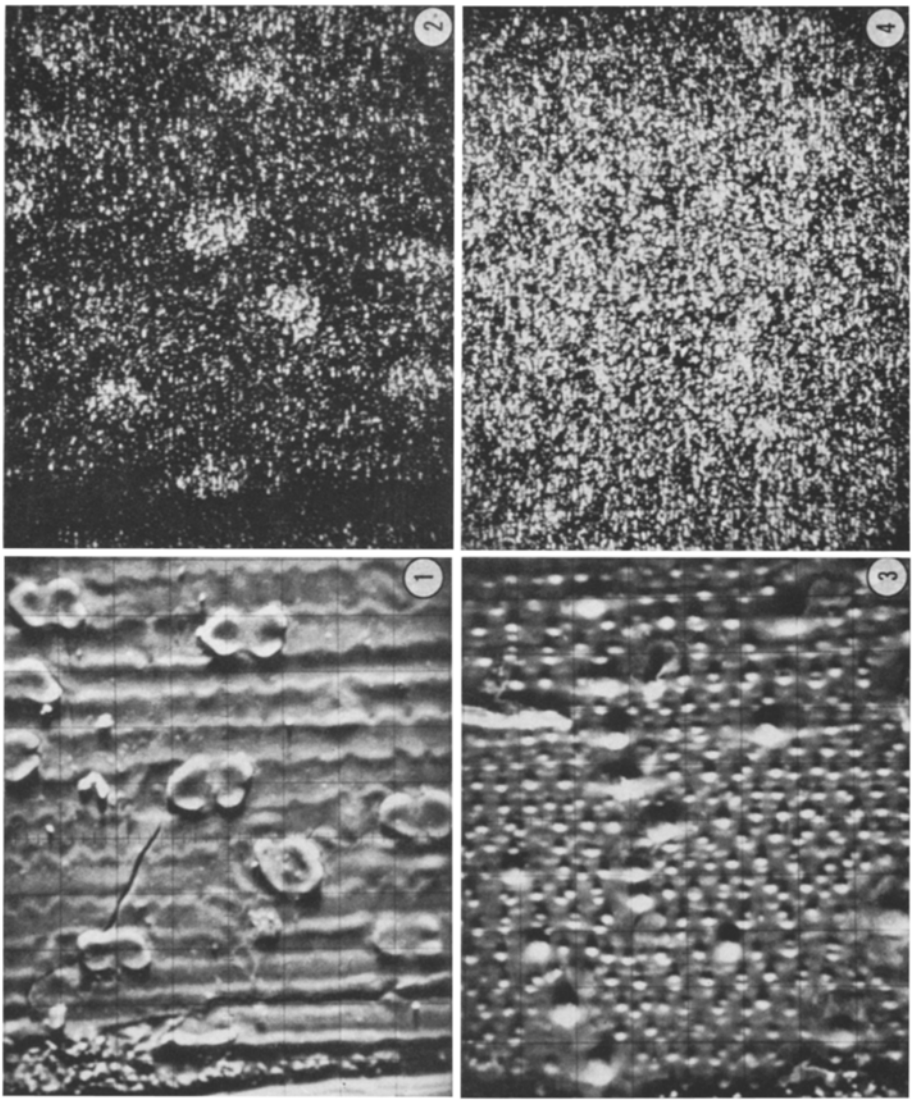
At a level of the internode 19–21 cm above the base (exposed portion near the top), it was observed that Si occurred in *all* types of epidermal cells. In the stomatal pores, significantly lower amounts of Si were measured (Table 1; Fig. 8). The only variation in amounts of Si occurred where slightly lower amounts of Si were found on the epidermal papillae rather than between them. This could be due to differences in density of the papillae and smooth portions of the outer wall of the epidermis. The main point here is that because Si is deposited on the entire epidermal system, there is no evidence for specific accumulation sites for Si as in the internodal epidermis of *Avena*.

Discussion

Soni *et al.* (1972b) observed significant amounts of Si in a few long epidermal cells, in the silica cells, and in the trichomes of both the surfaces of the leaf blade and sheath of the rice plant. In the ligule of this plant, they found significant amounts of Si in a few long epidermal cells. There are mostly specific sites of silicon deposition in the epidermis of the inflorescence parts (glume, lemma, palea) of the rice plant (Soni and Parry, unpublished). In other words, there are present specific sites for Si deposition in the epidermal system of the leaf blade and sheath, the ligule, and the inflorescence parts of this plant. In contrast, the present study indicates that significant amounts of Si occur in all the types of epidermal cells from the base to the tip of the developing internode. Thus, there is clearly a lack of specific sites for Si accumulation in the internode in contrast to the other parts of the shoot system in the rice plant.

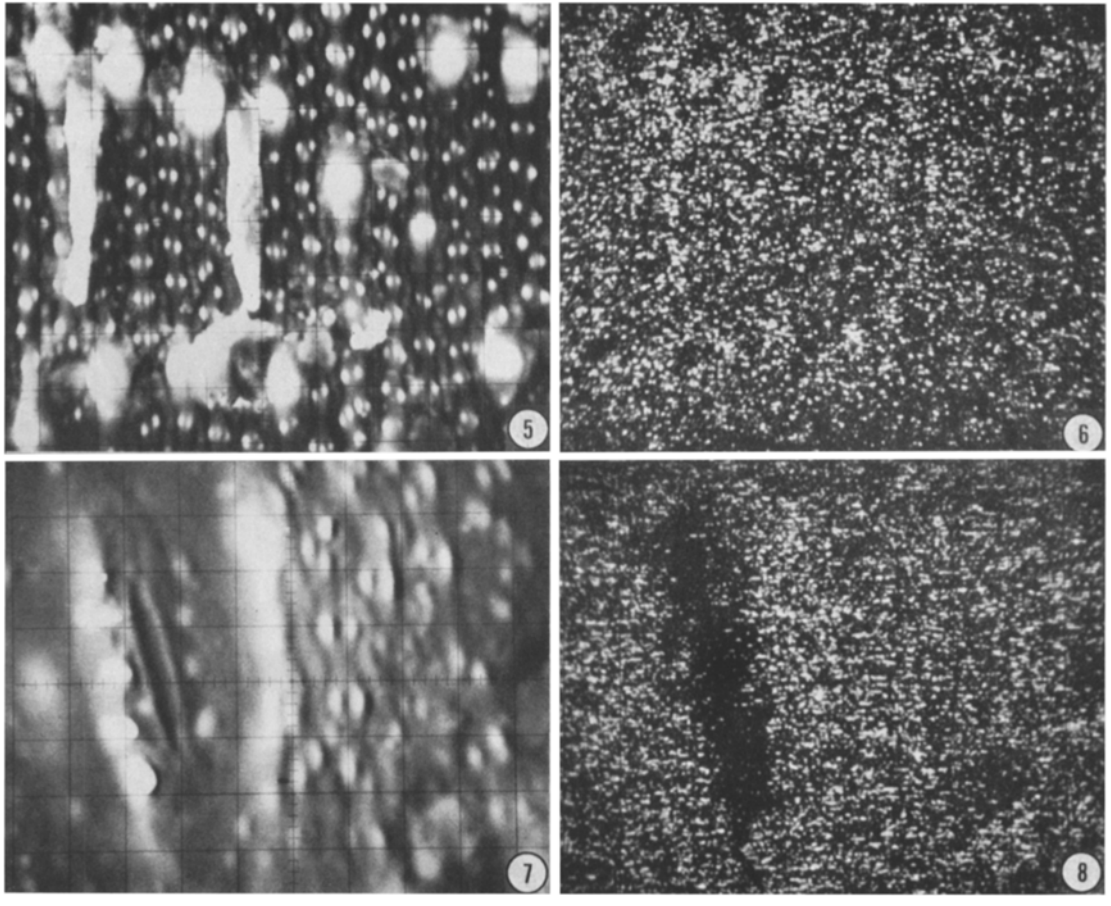
The silicification of the entire internodal epidermal system of rice indicates that the Si deposition must occur very rapidly. This deposition may be more rapid in the silica cells than in the long epidermal cells. This is supported by the occurrence of higher amounts of Si in silica cells than in the long epidermal cells at the base of the internode. The initial, gradual stage of the silicification process could not be studied here due to the deposition of Si from the very base of the internode. An extensive developmental study to analyze possible specific sites for Si accumulation in younger rice internodes is thus clearly needed.

The deposition of Si in the entire epidermal system of the developing internode is similar to that reported in *Equisetum hyemale* var. *affine* by



Figs. 1 and 2. Secondary electron and X-ray images for Si, respectively, in epidermis of the rice internode at least 1 cm above the base. Dumb-bell-shaped structures in Fig. 1 are silica cells. Greater amounts of Si are seen in these silica cells. ×825

Figs. 3 and 4. Same as Figs. 1 and 2, at least 6–7 cm above internode base. At this level, Si is deposited in all of the epidermal cells fairly uniformly. ×825



Figs. 5 and 6. Same as Figs. 1 and 2 at level 19–21 cm from the base of the internode. In Fig. 5, the round knobs are silica cells and the elongated structures are trichomes. At this level, the entire epidermal system is silicified. ×825

Figs. 7 and 8. Same level as in Figs. 5 and 6, illustrating a stomatal apparatus at left side (identified by slit-like aperture between the guard cells). The X-ray scan indicates absence of silicon both in the aperture as well as the guard cells (and probably the subsidiary cells) of the stomatal apparatus. ×1650

Kaufman *et al.* (1971 a). Kaufman *et al.* postulated that silicic acid might become distributed throughout the cell wall as well as on the surface via a diffusion process. Yoshida *et al.* (1962 b) reported that if Si were distributed through diffusion, it must have been located equally in all directions. Possible excretion pores on the surfaces of silica cells in rice internodes have already been described by Kaufman *et al.* (1971 b). Further work is clearly necessary to elucidate the process(es) by which silica deposition takes place in the epidermal system.

Because silica is deposited over the entire surface of the rice internode, rather than in specific types of epidermal cells, it is highly probable that this pattern of deposition has functional and adaptive significance. This is expressed in terms of providing further support for the internode in addition to that accounted for by the cellulosic framework and by lignin deposition. Siegel (1968) has pointed out that silica in the cell wall provides this support by becoming intercalated between the cellulose microfibrils (see Lewin and Reimann, 1969). The main point here is that both the leaf sheath (Soni *et al.*, 1972 b) and the internode are primary organs of support for the shoot system, and the generalized silicification pattern observed for the outer surfaces of these parts clearly provides this additional support that is required. In shoots such as *Avena*, this kind of support in the internode is provided by localized deposition of silica in the radial cell walls of the long epidermal cells (Kaufman *et al.*, 1969). The mechanism by which such a general deposition pattern for silica is achieved in the rice internode clearly needs to be elucidated and compared with those plants in which there are localized, more specific deposition sites.

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References

Jones, L. P. H., Milne, A. A.: Studies of silica in the oat plant. I. Chemical and physical properties of the silica. Plant and Soil **18**, 207–220 (1963).

Juliano, J. B., Aldama, M. J.: Morphology of *Oryza sativa* L. Philippine Agriculturist **26**, 1–134 (1937).

Kaufman, P. B., Bigelow, W. C., Petering, L. B., Drogosz, F. B.: Silica in developing epidermal cells of *Avena* internodes: Electron microprobe analysis. Science **166**, 1015–1017 (1969).

Kaufman, P. B., Bigelow, W. C., Schmid, R., Ghosheh, N. S.: Electron microprobe analysis of silica in epidermal cells of *Equisetum*. Amer. J. Bot. **58**, 309–316 (1971 a).

Kaufman, P. B., Soni, S. L., Bigelow, W. C., Ghosheh, N. S.: Scanning electron microscopy of the leaf and internodal epidermis of rice plant (*Oryza sativa* L.). Phytomorphology, in press (1971 b).

Laroche, J.: Contribution à l'étude de l'*Equisetum arvense* L. III. Recherches sur la nature la localisation de la silice chez le sporophyte. Rev. gén. Bot. **75**, 65–116 (1968).

Laroche, J.: État de la silice sur et dans la membrane épidermique des organes aériens stériles d'*Equisetum arvense* L. Rev. gén. Bot. **76**, 483–489 (1969).

Laroche, J., Guervin, C., Lecoq, C., Dao, Vo Thi.: Intérêt taxinomique de l'excrétion siliceuse chez les Equisétacées. C.R. Acad. Sci. (Paris) **270**, 2958–2960 (1970).

Lewin, J. C., Reimann, B. E. F.: Silicon and plant growth. Ann. Rev. Plant Physiol. **20**, 289–304 (1969).

Siegel, S. M.: Biochemistry of the plant cell wall. In: M. Florkin and E. H. Stotz (eds.), Comprehensive biochemistry vol. 26, p. 1–51. (1968). Amsterdam: Elsevier.

Soni, S. L., Kaufman, P. B., Bigelow, W. C.: Electron microprobe analysis of the distribution of silicon in leaf epidermal cells of the oat plant (*Avena sativa* L.). Phytomorphology **20**, 348–361 (1970).

Soni, S. L., Kaufman, P. B., Bigelow, W. C.: Electron probe analysis of silicon and other elements of leaf epidermal cells of the rice plant (*Oryza sativa* L.). Amer. J. Bot. **59**, 38–42 (1972a).

Soni, S. L., Kaufman, P. B., Jones, R. A.: Electron microprobe analysis of the distribution of silicon in rice leaf epidermis. Bot. Gaz., in press (1972b).

Yoshida, S., Ohnishi, Y., Kitagishi, K.: Chemical forms, mobility and deposition of silicon in rice plant. Soil Sci. and Plant Nutrition **8**, 15–21 (1962a).

Yoshida, S., Ohnishi, Y., Kitagishi, K.: Histochemistry of silicon in rice plant. II. Localization of silicon within rice tissues. Soil Sci. and Plant Nutrition **8**, 36–41 (1962b).

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