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with II plates

Studies on silicon accumulation in developing internodal epidermal cells of *Cyperus alternifolius*

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Electron probe analysis was employed to study the accumulation and distribution of silicon in differentiating epidermal cells of the developing internode of Cyperus alternifolius. *The process of silicon accumulation was found to occur from the base to the tip of an elongating internode. The accumulation of silicon by long epidermal cells and silica cells in the elongating internode appears to occur in two stages: the first is gradual and takes place in the basal portion of the internode in regions within the intercalary meristem; the second is rapid and occurs at sites well above the intercalary meristem. The first stage is much more prolonged in the long epidermal cells than in the silica cells. This study indicates that in plants such as* Cyperus *the process of silicification does not proceed nearly as rapidly as in internodes of* Avena (*oat*) *and* Equisetum. *Possible explanations for these differences are discussed.*

La microsonde à rayons X fut utilisée pour l'étude de l'accumulation et de la répartition du silicium en vue de différencier les cellules de l'épiderme des entre-noeuds du Cyperus alternifolius *au cours de leur développement. Il fut établi que le silicium s'accumulait graduellement de la base aux extrêmités des entre-noeuds. L'accumulation du silicium par les cellules silicieuses dans la longueur des entre-noeuds semble s'effectuer en deux étapes. La première a lieu progressivement et se produit dans la partie de base de l'entre-noeud à l'intérieur du méristème intercalaire; la seconde est rapide et s'effectue dans des sites éloignés du méristème intercalaire. La première étape est beaucoup plus lente le long des cellules épidermiques que dans les cellules silicieuses. Cette étude montre que le processus de silicification dans des plantes telles que* Cyperus *n'est de loin pas aussi rapide que dans les entre-noeuds d'*Avena (*orge*) *et* Equisetum. *Différentes possibilités d'explication de ces différences sont avancées.*

Mit einer Elektronensonde wurden Anlagerung und Verteilung von Silizium in den differenzierenden Epidermiszellen des Zwischenknotens von Cyperus alternifolius *untersucht. Es wurde festgestellt, dass der Siliziumanreicherungsprozess vom Fusse bis zur Spitze eines sich verlängernden Knotens vor sich geht. In den langen Epidermiszellen und den Siliziumzellen des sich verlängernden Zwischenknotens geschieht der Anreicherungsprozess in zwei Phasen. Die erste ist langsam und findet im unteren Teil des Zwischenknotens in der Gegend des Intercalarmeristems statt. Die zweite ist rasch und geht weit oberhalb des Intercalarmeristems vor sich. Die erste Phase dauert viel länger in den langen Epidermiszellen als in den Siliziumzellen. Diese Arbeit deutet darauf hin, dass in Pflanzen wie* Cyperus *die Verkieselung viel länger dauert als in den Zwischenknoten von* Avena (*Hafer*) *und* Equisetum. *Die Bedeutung dieser Unterschiede werden erörtert.*

INTRODUCTION

The distribution of silica has been studied mostly in mature leaf and stem tissues of *Oryza* (Yoshida, Ohnishi and Kitagishi, 1962; Soni, Kaufman and Bigelow, 1972a);

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Secale (Blackman and Parry, 1968); *Sieglingia* (Sangster, 1970a); *Avena* (Jones, Milne and Wadham, 1963; Kaufman, Bigelow, Petering and Drogosz, 1969; Soni, Kaufman and Bigelow, 1970) and *Equisetum* (Kaufman, Bigelow, Schmid and Ghosheh, 1971). These studies indicate that the distribution of silica may not be uniform in different types of cells of the same organ or in different organs of the same plant (Lewin and Reimann, 1969; Soni *et al.*, 1970).

The studies on the distribution of silica by Blackman (1969), Kaufman *et al.* (1969) and Sangster (1970b) on developing or immature leaves and internodes indicate that silica is accumulated in specialized cells such as silica cells very rapidly. Such studies also indicate that the deposition of silica occurs in an ordered sequence of cell types related to the basipetal maturation gradient within the leaf or internode.

Using electron probe analysis, the present study on silica in the differentiating internodal epidermis of *Cyperus alternifolius* was undertaken to determine if in this plant, where intercalary growth persists for a long time in the developing internode (Fisher, 1970), the silicification process might be different in terms of rates and duration of silicification than in plants such as *Avena* and *Equisetum*. Thus, we examined the level or initial stage of silica deposition and the relative amounts of silicon present in immature and mature parts of elongating internodes.

MATERIALS AND METHODS

Young plants of *Cyperus alternifolius* were obtained for this study from greenhouse collections at the Matthaei Botanical Gardens, University of Michigan. A young elongating internode (ca.18cm long), and a mature, fully elongated internode (ca.40cm long) were excised and used for this study.

For microprobe analysis, we used the same methods for the preparation of tissue cited in Soni, Kaufman and Bigelow, (1972b). To study the level or stage of initial silica deposition in the young internode, peels were made at every 1cm, starting from the base to the tip of the internode. From the mature internode, peels were prepared within 3cm from the base and 3cm from the tip.

The electron microprobe (model EMX-SM, Applied Research Laboratories) was operated at 15kV, giving an electron beam penetration into the tissue of about 15μm. The sample current was 0.02μA. The X-ray spectrometer was peaked for first order silicon Kα line, using an ammonium dihydrogen phosphate (ADP) crystal for silicon. The tissue was examined using the back-scattered electron display and the cells selected for X-ray analysis.

Qualitative determinations were made by raster and line scanning for an average of ten counts per locus and three loci per cell. Statistical tests were run for elemental detection as reported by Lipps and Ribbe (1967).

Since there is clear evidence that silicon is associated with oxygen as SiO_2 in biological material (Lewin and Reimann, 1969) and especially as non-crystalline isotropic silica gel ($SiO_2.nH_2O$) in grasses such as oats and rice (see Jones and Milne, 1963; Lewin and Reimann, 1969), we assume this to be case here. Because of the difficulty of measuring oxygen directly with the electron microprobe, especially in biological material, we measured only silicon (Si). Therefore, in this paper, we refer to *silicon* (Si) in reference to our direct measurements with the electron microprobe, and *silica* ($SiO_2.nH_2O$), as the probable form in which silicon occurs in *Cyperus* internodes.

RESULTS

Epidermis of the mature internode. The mature internodal epidermis is composed of long epidermal cells, stomatal apparatuses*, and silica cells. This analysis reveals the presence of significant amounts of silicon in the long epidermal cells and silica cells (Figures 1 and 2). The relative amounts measured by point count analysis indicated that both long epidermal cells and silica cells contained sizeable amounts of silicon (Table 1). The silica cells had roughly twice the amount of silicon present as the long epidermal cells. No silicon could be detected in the stomatal apparatuses in the mature internode. Similarly, no significant amount of silicon has been detected in the stomatal apparatuses of the leaf epidermis of *Avena* (Soni *et al.*, 1970), and *Oryza* (Soni, Kaufman and Jones, 1972).

Epidermis of the immature internode. In the immature 18cm internode, silicon was detected in the long epidermal cells at about 3cm from the basal intercalary meristem region which is ensheathed and thus unexposed (Table I; Figures 1-4). At the next higher level in the same peel, silicon was revealed in higher amounts in the long epidermal cells (24 and 41cps) and in the silica cells (58cps). At this stage, the amounts of silicon measured in different silica cells were as follows: 24, 36, 44, 48cps. At the next higher level in the same peel, the amount of silicon increased more rapidly (Figures 3-6) in the silica cells (528cps) than in the long epidermal cells (77, 71cps). At about 14cm above the base of the same internode (exposed portion of internode), the amount of silicon was found to be almost two times that in the long epidermal cells and silica cells of the unexposed (sheath present) portion about 10cm below this level (Table I; cf. Figures 7-10). Point count data for silicon in the mature internode indicate about 12 times more silicon to be present in the long epidermal cells and about 3 times more in the silica cells than near the tip of the immature internode (Table I).

Table I indicates significant amounts of silicon (528cps) present in the silica cells of the unexposed and ensheathed portion of the internode. This has also been observed by Kaufman *et al.* (1969) in *Avena* internodes. Thus, the exposure of the internode together with the expected increase in amount of transpiration from the exposed portion of the internode may not be essential for the process of silicification in *Cyperus*, as in *Avena*.

DISCUSSION

Using counting technique, Blackman (1968) reported the pattern of silica deposition in the internode to be opposite to that in the leaves of the rye plant. In the internode, a high level is present at the apical end of the internode falling to a low level at the base near the intercalary meristem. In the leaves, a high level of silica occurs at the base of the sheath, but the level decreases towards the ligule. Sangster (1970b) found in immature leaves of Gramineae that deposition occurs in an ordered sequence of cell types related to the basipetal maturation gradient within the leaf. The present semi-quantitative analysis of the developing internodal epidermal cells of *Cyperus* reveals that the initial stages of silicification occur within 4cm of the basal intercalary meristem

* We follow Esau (1965) for the use of the term stomatal apparatus (complex) to include a stoma and the associated subsidiary cells. A stoma is comprised of guard cells and the pore or aperture between them.

region of the internode. The quantities of silicon increase from the base of the internode near the meristem towards the apex of the internode in both the long epidermal cells and the silica cells (Table I). Thus, the process of silicification extends from the base to the tip of the internode. This may be related to the rate of maturation of the

TABLE 1

Point count data for silicon in different types of developing internodal epidermal cells in Cyperus alternifolius.

Part of Plant	Type of Cell	Average Counts (cps)*
Internode **	Long epidermal cell ‡	24, 21
	Silica cell, centre region	n.d.
	Long epidermal cell ‡	24, 41
	Silica cell, centre region	58
	edge	12
	Long epidermal cell ‡	77, 71
	Silica cell, centre region	528
Internode †	Long epidermal cell ‡	172, 168
	Silica cell, centre region	1235
Internode ††	Long epidermal cell ‡	1620, 1656
	Silica cell, centre region	3648
	edge	2031

* Mean count of silicon per point count (cps, counts per second) after subtracting background counts.

** Immature internode at about 2-3cm from the base of the internode.

† Internode portion at 14cm of the same internode as in †† (mature internode).

†† Mature internode.

‡ Two counts per cell average given for these cells to indicate variation in amount of silicon at different levels.

n.d. =none detected.

tissues, as the apical region may mature more rapidly and thus contain a greater amount of silicon, while the ensheathed base matures more slowly (see paper by Fisher, 1970).

It appears from this semi-quantitative analysis of the differentiating internodal epidermal cells that during initial stages of silicification, silicon accumulates in a gradient series (such as 24, 36, 44, 48 and 58cps in silica cells). Later on (at higher levels of the internode), it accumulates much more rapidly (528cps in silica cells). Thus, there may be two stages for the process of silicification: first, a stage which involves a slow rate

of accumulation of silicon, followed by a second stage in which silicon is accumulated very rapidly. The former stage is much more prolonged in the long epidermal cells than that in the silica cells. This indicates that silicon is neither accumulated at the same time in both of these types of cells nor by the same mechanism. Comparable conclusions were also reached by Blackman and Parry (1968).

These observations in support of a two-phase accumulation process for internodes of *Cyperus* clearly are at variance with our recent observations for silicon accumulation in developing internodes of *Avena* (Kaufman *et al.*, **1969**) and of *Equisetum* (Kaufman *et al.*, 1970). In these two plants, we found no detectable silicon in the intercalary meristem. Then, as one progresses up the internode, just above the intercalary meristem, there are large amounts (up to 776cps in *Avena* and 982cps in *Equisetum*) of silicon present. In other words, it appears to be an "all-or none" type phenomenon. In fact, in *Avena*, one silica cell in a longitudinal series may have no detectable silicon and the next silica cell up in the series has large amounts detectable with the electron microprobe (Kaufman *et al.*, **1969**). In light of this, it is possible that in *Cyperus* internodes, the silicon is first accumulated, not as a result of breakdown of membranes and organelles as in *Avena* silica cells (Kaufman *et al.*, **1969**), but could involve a slow process in living cells, during the time that intercalary growth occurs (long time in *Cyperus*, relatively short duration in *Avena* and *Equisetum*); when nuclei and other organelles presumably do break down, it could then accumulate very rapidly. Studies by electron microscopy on developing silica cells in the elongating *Cyperus* internode are clearly needed to resolve this point.

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FIGURES 1-6

Mature and developing internodal epidermis of Cyperus alternifolius

Figure 1. Back-scattered electron image of the mature internodal epidermis. ×334.

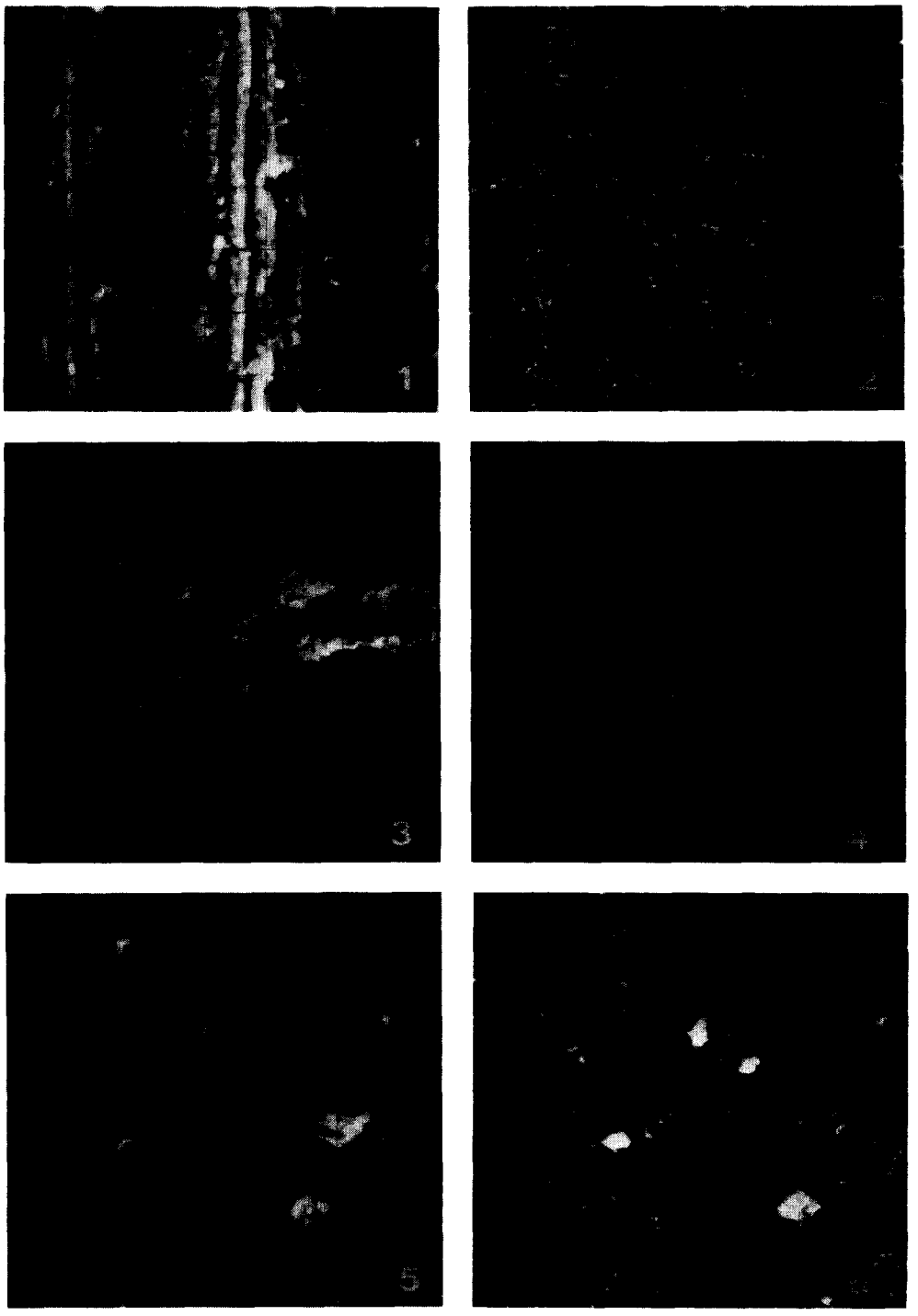
Figure 2. X-ray image for silicon of the same area as shown in Figure 1. ×334.

Figure 3. Back-scattered electron image of the developing internodal epidermis at about 3cm from the base of the immature internode. ×223.

Figure 4. X-ray image for silicon of the same area shown in Figure 3. There is no significant amount of silicon in the epidermal and silica cells. ×223.

Figure 5. Back-scattered electron image of the developing internodal epidermis at about 3cm from the base of the immature internode. ×200.

Figure 6. X-ray image for silicon of the same area shown in Figure 5. Significant amounts of silicon are present mainly in the silica cells. ×200.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FIGURES 7-10

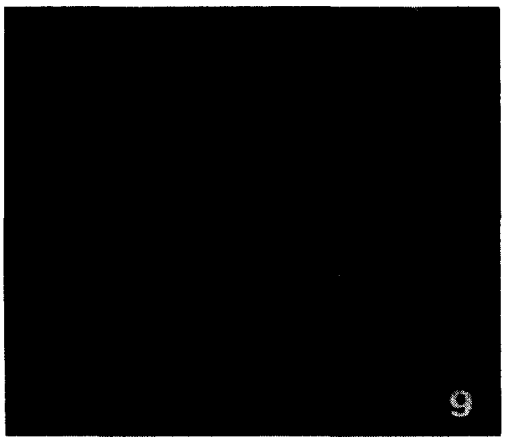
Developing internodal epidermis of Cyperus alternifolius

Figure 7. Back-scattered electron image of the developing internodal epidermis at about 10cm from the same immature internode shown in Figures 3-6. × 2,000.

Figure 8. X-ray image for silicon of the same area shown in Figure 7. × 2,000.

Figure 9. Back-scattered electron image of the developing internodal epidermis at about 14cm from the base of the same immature internode shown in Figures 3-6. × 1,000.

Figure 10. X-ray image for silicon of the same area shown in Figure 9. × 1,000.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