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ELECTRON MICROPROBE ANALYSIS OF SILICA CELLS IN LEAF EPIDERMAL CELLS OF *CYPERUS ALTERNIFOLIUS*

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SUMMARY

Electron microprobe analysis was used to examine relative amounts of selected accessory elements (Ca, Mg, K, Na, P, Mn, and Fe) associated with silicon in silica cells in leaf epidermal cells of *Cyperus alternifolius*. In the leaf epidermal cells, silicon is localised in significant quantities in silica cells and in a few long epidermal cells. Silicon could not be detected in the stomatal apparatuses. The accumulation of significant amounts of silicon in the silica cell appears to retard the accumulation of K and to enhance the accumulation of Ca, Mg, Mn, Fe, and P. Sodium was detected in very small traces in the silica cells. The possible functional significance of the altered deposition patterns for these elements in *Cyperus* is discussed.

INTRODUCTION

Silicon has been reported to occur in the form of silica (SiO_2) in most of the monocotyledonous families (chiefly, Gramineae and Cyperaceae); in Equisetum [9 10 11 12]; in diatoms; and in certain animals, *i.e.* radiolarians and sponges. It is deposited as opal, or, primarily as silica gel ($SiO_2 . nH_2O$) in the epidermis and other tissues of many plants [13]. By using electron microprobe x-ray analysis, the presence of silicon has been reported in a centriole in the section of the distal renal tubule of a healthy quinea pig [21] and in an early stage of bone calcification of the normal tibia from young mice and rats [4].

Silica has several salient functions in plants: (1) it controls the water loss from the plant, (2) keeps the leaves erect, (3) plays a role

in growth metabolism, and (4) protects the plant from fungal hyphae penetration [13]. In animals, silicon may be a possible factor in bone calcification [4]. Silica possesses greater diagonistic value in plant taxonomy when deposited as small bodies with distinctive shapes, *e.g.* silica bodies in Cyperaceae can at once be distinguished from those of grasses by the dome-shaped silica deposits of the former [15].

The distribution of silica in plants has been investigated by various techniques such as wet-way ashing [7], dry-way ashing [16], by different chemical reagents [18], scanning electron microscopy [20], and electron microprobe analysis [8 9 10 22]. Since the electron microprobe permits the simultaneous detection of several elements in a microstructure [1], we examined, in the present study, silica cells and adjacent cells of the leaf epidermis of *Cyperus alternifolius* for the relative amounts of accumulation of other accessory elements such as Ca, K, Mg, Na, Fe, Mn, and P associated with low and high amounts of silicon. The primary objective of this study was to determine whether cells which accumulate large amounts of silicon altered the accumulation patterns of these other elements.

MATERIAL AND METHODS

Plants of *Cyperus alternifolius* L. were obtained from greenhouse collections at the Matthaei Botanical Gardens, University of Michigan, Ann Arbor, Mich.

Tissue preparation for electron microprobe analysis essentially followed the same procedures described previously [8 9 22] except for the following minor modifications: (1) thin epidermal peels were made of the abaxial and adaxial epidermises of mature leaf blades; (2) tissue pieces were placed in aluminium foil envelopes 2 cm^2, immediately frozen in liquid nitrogen, dried at 0°C in a Virtis automatic freeze-dryer (No. 10-010) for 2 to 3 h; (3) the dried pieces were mounted on polished copper squares with electrically conductive silver paint, coated with carbon, and stored in a desiccator until ready for microprobe analysis.

The electron microprobe (model EMX-SM, Applied Research Laboratories) was operated at 15 kv, giving an electron beam penetration into the tissue of about 15 μm. The sample current was 0.02 μamps; the beam current = 0.36 μamps, the emission current = 150 μamps, and the spot diameter was 2 μ. The spectrometers were peaked for the first order Kα wavelengths using a lithium fluoride (LiF) crystal for K, Ca, Fe, and Mn, a potassium acid phthalate (KAP) crystal for Na and Mg, and an ammonium dihydrogen phosphate crystal (ADP) for Si and P. Secondary electron scanning images were employed to examine the tissue and to select cells for analysis. Point count data were obtained using an average of ten locations per cell.

RESULTS AND DISCUSSION

The leaf epidermis of *Cyperus alternifolius* is differentiated into long epidermal cells, stomatal apparatuses, and silica cells. In this study, all of these cell types in the epidermal system were examined. In the Cyperaceae, the stem and leaf are characterised by having subepidermal fiber strands, which are above and or between the veins [14]. The epidermis overlying a fiber strand is designated 'strand region', and that over the area between two strands, 'inter-strand strands' [14]. This terminology will be followed here.

Silicon

Electron microprobe analysis revealed a significant amount of deposition of silicon in silica cells (Figs. 1, 2) and in a few long epidermal cells (Figs. 3, 4). No significant amount of silicon was detected in the stomatal apparatuses * (Figs. 3, 4) in contrast to its presence in walls of cells of stomatal apparatuses in internodal epidermis of *Avena sativa* [8]. However, recent evidence shows no significant amount of silicon has been detected in the stomatal apparatuses of leaf epidermal cells of this plant.[22]

Point count data for silicon in the silica cells of *Cyperus* indicate that the concentration of silicon is much higher in the center (1364

TABLE 1

Point count data * for silicon in the different types of epidermal cells of leaf of *Cyperus alternifolius***.

Part of plant	Type of cell	Average counts per sec. (cps)	Background counts for silicon (cps)
Leaf blade	Epidermal cells	1032	63
Leaf blade	Silica cells		
	edges	486	7
	center region	1364	7

* Mean count of silicon per point count (cps, counts per second), average of 10 counts per locus. A silicon K alpha line was used for the counts.

** Three cells were examined to obtain an average for a particular cell.

* Following Esau [5], we use 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.

TABLE 2

Point count data * for silicon ** and other accessory elements *** in the adaxial (inner) surface of leaf blade of *Cyperus alternifolius*

Element	Type of cell	Mean counts (cps)	Background counts (cps)
Si	Silica cell	1331	4
Ca	Silica cells	244	4
	Other cells	80	4
K	Silica cell	127	4
	Other cells	354	4
P	Silica cell	47	2
	Other cells	4	2
Mg	Silica cell	37	8
	Other cells	33	8
Mn	Silica cell	21	6
	Other cells	7	6
Fe	Silica cell	13	6
	Other cells	8	6
Na	Silica cell	5	4

* Mean count of element per point count (cps, counts per second); average of 10 counts per locus.

** Three cells were examined to obtain an average for a particular cell.

*** Mean count of the element in the adjacent epidermal cells not accumulating significant quantities of silicon.

cps, Table 1) than at the edges (486 cps, Table 1) of these cells. Comparable findings were also reported for the silica cells of cork-silica cell pairs of internodal epidermis of Avena [8] and for the silica cells of cork-silica cell pairs in the abaxial surface of the leaf sheath base in Avena.[22]

No significant amounts of silicon were detected in the epidermal cells adjoining the silica cells over the strand region (Figs. 1, 2). These epidermal cells are considered to be potential silica cells [14].

Analysis of other associated elements, *i.e.* Ca, K, Mg, Mn, Fe, Na, and P, in silica cells which contained significant amounts of silicon indicated that the last five listed elements are present only in low quantities and Na in very minute traces (Table 2). In the special silica cells of the leaf sheath base, as well as in the sclerenchyma cells

in the leaf blade of *Avena sativa*, both of which contain large amounts of silica, Mn and Fe could not even be detected [22].

Calcium, potassium and magnesium

The point-count data in Table II for the different elements in the silica cells and in adjacent cells having no significant amount of silicon (Figs. 5, 6) suggest that Ca (Fig. 8) and K (Fig. 7) accumulation is significantly affected in cells which contain significant amounts of silicon. The amounts of calcium are about three times higher in the silica cells than in adjacent cells. This is in contrast to observations on amounts of calcium found in the special silica cells in the abaxial epidermis at the base of leaf sheath of Avena [22], where the accumulation of calcium is much more in the adjoining cells having no significant amounts of silicon as compared to special silica cells. Thus, the relative accumulation pattern for silicon and calcium seem to differ in different plants depending upon still unknown factors. The accumulation of potassium appears to be markedly repressed by the accumulation of silicon (Table 2). The amount of potassium in the adjoining cells (with no significant quantity of silicon) is about three times higher than in the silica cells (with significant amounts of silicon). In Avena, the amount of potassium in the adjoining epidermal cells (with no significant amount of silicon) is about ten times higher than in the special silica cells (with significant amount of silicon) in the abaxial epidermis at the base of leaf sheath [22]. The amount of magnesium in the silica cells is slightly greater than in the adjacent cells (with no significant amount of silicon). The accumulation of magnesium may be slightly promoted by the accumulation of silicon in a cell or tissue (Table 2).

In studies with rice plants [6], inclusion of silicon with other nutrients in the culture solution promoted the uptake of cations (Ca and Mg) but decreased potassium uptake. In the present study, the accumulation of calcium and magnesium is increased while that of potassium is decreased by the accumulation of silicon in a cell or tissue.

Iron and manganese

The accumulation of higher concentrations of certain mineral nutrients in aerial parts of silicon-deficient plants has been reported

by various investigators [13]. The accumulation of Mn and Fe in leaves of Si-deficient rice plants seems to be most striking. The analysis of silica cells in *Cyperus alternifolius* (Table 2) shows that the amount of manganese is about three times greater in the silica cells than in the adjoining cells (with no significant amount of silicon present). In the scherenchyma cells in the margin of leaf blade and in special silica cells (both types of cells with significant amount of silicon detectable) in the abaxial epidermis at the base of leaf sheath in Avena, Mn and Fe could not be detected [22]. It thus appears that the accumulation of silicon by a cell or tissue does not completely abolish the accumulation of Mn and Fe in the same cell or tussue. Silicon addition to the nutrient culture solution has been shown to decrease the iron and manganese contents of rice plants [6].

Sodium

Table 2 indicates very small traces of sodium present in silica cells, as also reported in the cells accumulating significant amount of silicon in Avena [22].

Phosphorus

Okuda and Takashi [17], using P^{32}, concluded that silicon seemed to retard the excessive uptake of P (as phosphate) by rice plants and to promote the translocation of P into the grain. Rothbuhr [19] used radioactive Si^{31} to study the uptake of silicic acid in the presence and absence of phosphoric acid. The uptake of silicic acid

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Plate 1 (opposite)

Figs. 1, 2, Secondary electron and x-ray images, respectively, of the adaxial epidermis of the leaf of *Cyperus alternifolius*, showing significant amounts of silicon deposited in the silica cells (× 167).

Figs. 3, 4, Secondary electron and x-ray images, respectively, of the adaxial epidermis of the leaf, indicating no significant amount of silicon in the stomatal apparatuses; there is a significant amount of silicon in a few long epidermal cells (× 250).

Figs. 5–8, Secondary electron image (Fig. 5) and x-ray images (Figs. 6–8) for Si (Fig. 6), K (Fig. 7), Ca (Fig. 8) through the silica cell in adaxial epidermis of the leaf blade of *Cyperus alternifolius*, showing deposition of significant amount of Si but less deposition of K and more accumulation of Ca in the vicinity of silica cells as compared to the adjacent epidermal cells with no significant amounts of silicon (× 334).

was found to be very slightly depressed in the presence of phosphate, whereas the uptake of P was slightly enhanced when silicic acid was present. Silicon addition to the nutrient culture solution increased the phosphorus concentration of rice plants [6]. The amount of phosphorus uptake by wheat plant was increased by the application of non-diffusable colloidal silica [3]. Silicon in the form of silicate promoted the efficiency of absorbed phosphorus in barley [2]. With microprobe analysis (Table 2), we find that the accumulation of P is about twelve times more in the silica cells than in the other epidermal cells (with no significant amount of silicon); it thus appears that silicon enhances the accumulation of phosphorus in a cell or tissue with significant amounts of silicon present.

Silicon may play regulatory role in ion uptake by particular cells. Its access in a cell or tissue may exclude certain elements, such as potassium, or enhance the accumulation of elements like calcium, phosphorus and magnesium. The exclusion of potassium needs further study, for it may occur in higher levels in cells adjacent to stomata, presumably where the silicon content is much lower. At higher levels, in these cells, potassium might function more effectively in regulating the opening and closing of stomata as in a light activated potassium pump [23].

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