Silicon location through backscattered electron imaging and X-ray microanalysis in leaves of *Cyperus ligularis* L. and *Rhynchospora aberrans* C. B. Clarke (Cyperaceae)

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RESUMO
(Localização de sílica por elétrons retroespalhados em folhas de *Cyperus ligula*ris L. and *Rhynchospora aberrans* C. B. Clarke (Cyperaceae)). As Cyperaceae têm a capacidade de incorporação do ácido silícico mediante depósito de sílica coloidal, que é registrada pela ocorrência de projeções sob a forma de cones, nas paredes tangenciais internas de determinadas células epidérmicas ou "células de sílica". Folhas de *C. ligularis* e *R. aberrans* foram analisadas pela técnica de elétrons retroespalhados. *C. ligularis* além de acumular sílica nas "células de sílica", acumula também em alguns estômatos, nos tricomas e nas paredes das células que contornam as cavidades do aerênquima. Este último apresenta formas diversas, entretanto, as células que se localizam junto aos feixes vasculares mostram projeções cônicas, semelhantes àquelas da epiderme. *R. aberrans* contém "células de silica" cujas projeções cônicas têm "satélites". A sílica também ocorre em estômatos específicos e em células epidérmicas contíguas aos mesmos. Constata-se que a deposição de sílica ocorre tanto em combinação com a parede (sem modificações estruturais aparentes), como em estruturas de secreção da mesma, ou seja, projeções da parede. Essas variações de ocorrência estrutural e de localização nas espécies devem estar relacionadas com a funcionalidade, fatores ambientais, com destaque ao solo, além da relação com grupos taxonômicos.

Palavras-chave: estômatos, células epidérmicas, tricomas, parede celular

ABSTRACT
(Silicon location through backscattered electron imaging and X-ray microanalysis in leaves of *Cyperus ligula*ris L. and *Rhynchospora aberrans* C. B. Clarke (Cyperaceae)). The Cyperaceae show the ability to incorporate silicon by depositing colloidal silica, which is recorded by the occurrence of projections in the form of cones, in inner tangential walls of some epidermal cells or "silica cells". Leaves of *C. ligularis* and *R. aberrans* were analyzed through the technique of electron backscatter. *Cyperus ligularis* accumulates silica, in addition to "silica cells", in some stomata, trichomes and the cell walls that surround the cavities of the aerenchyma. The silica in the latter occurs in various forms; however, the cells located near the vascular bundles have conical projections, similar to those of the epidermis. *Rhynchospora aberrans* presents "silica cells" whose projections have tapered "satellites". In this species, silica also occurs in stomata and certain epidermal cells adjacent to them. It appears that the silicon deposition occurs in combination with the wall (with no apparent structural changes), and structures of secretion, or projections of the wall. These structural changes in the species, and location, are probably related to functional and environmental factors, especially the soil, in addition to relation with taxonomic groups.

Key words: stomata, epidermal cells, trichomes, cell walls

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Introduction

The deposition of minerals in plants is more common in the form of crystals (usually calcium oxalate) and occurs in various organs, including flowers (Macnish *et al.* 2003). The silica found in various plant groups is the result of the absorption of silicon that is deposited in the cells as colloidal silica (Jones & Milne 1963). According to Netolitzky (1929), the earliest records of silica in plants were reported by Struve (1835 apud Netolitzky 1929) from soil samples, and were called phytoliths because they considered the silica to be skeletons or organisms that lived in plants.

According to Sakai & Thorn (1979), silica is deposited in the middle lamina wall and primary regions of the primary wall, a region with a high concentration of pectin and hemicellulose, where it interacts with these substances, as well as lignin and phenolic substances, when present. Based on this fact, there have been numerous papers reporting biochemical studies that seek to clarify the polymerization of silica, since the silicon in plants is not deposited in crystalline form, and to analyze its activity after it is incorporated into the cell wall (Currie & Perry 2007).

The presence of silicic acid has beneficial effects on various plants in which it accumulates; these studies focus mainly on Poaceae (grasses) and date back many decades. In 1938, Lipman linked the presence of silica to the increased cultivation of barley. It is also known that the addition of steel slag to siliceous soil increases the production of sugarcane (Fox *et al.* 1969). Silica affects the absorption of mineral nutrients in various species, such as *Cyperus alternifolius*, where it excludes some and favors the accumulation of others (Soni *et al.* 1972). In addition, it controls the toxic effects of certain elements, for example, in soy it prevents the appearance of necrotic spots on the leaves caused by manganese, and reestablishes the growth of the plants (Kluthcouski & Nelson 1980).

The impregnated silica cell wall is also important for retaining water, thereby reducing cuticular transpiration (Yoshida *et al.* 1959). It can also protect the plant against pathogenic fungi (Lewin & Reimann 1969), particularly rusts (Kim *et al.* 2002), insects (Yoshida *et. al.* 1959) and herbivores (Hansen *et al.* 1976; Hunt *et al.* 2008). Also, depending on the type and location of deposition, it is an important taxonomic characteristic (Metcalfe 1969; Dahlgreen & Clifford 1982).

In Brazil, studies have concentrated on the morphology of phytoliths, such as the works of Labouriau and colleagues in the nineteen sixties, which focused predominantly on grasses (Sendulsky & Labouriau 1966; Teixeira-da-Silva & Labouriau 1970). More recently, Ribeiro *et al.* (2001) analyzed *Otachyrium* species through backscattered electron imaging and X-ray microanalysis.

In Cyperaceae, the presence of silica has been studied in the stems, leaves (Metcalfe 1971), floral bracts (Browning & Gray 1995) and achenes (Ernst *et al.* 1995). In the latter case, it has been studied more in relation to its application in the taxonomy of various genera (Shah 1968; Menapace 1991). Almost all of the records of silica in Cyperaceae refer to conical projections on the inner tangential walls of the epidermal cells or specific "silica cells". The occurrence and variations of these projections is important for the ecology, taxonomy and phylogeny of the plants. An example of the latter two is the comparative analyses of silica deposition in the leaves of species of *Cyperus* and *Lipocarpha* (Govindarajalu 1974) and in achenes of species of *Eleocharis* (Menapace 1991) and *Carex* (Starr & Ford 2001).

The study assessed the occurrence of silica in Brazilian species of Cyperaceae and determined whether this occurrence included other sites beyond the known "silica cells".

Material and methods

Cyperus ligularis L. comes from coastal sand dunes of the Bertioga region, in the state of São Paulo, and *Rhynchospora aberrans* C. B. Clarke comes from a region of Caatinga vegetation, on the Almas farm, in São José dos Cordeiros, in the state of Paraíba.

Analyses were performed on the third leaf of the apical bud of three specimens. Fragments were fixed in FAA 70 (Johansen 1940) and subsequently stored in 70% alcohol. Samples of each leaf, for the front view of the epidermis and cross sections of the lamina, were dehydrated in an ethanol series to 100% ethanol, and critical point dried with CO_2. The material was fixed on specific supports and sputter coated with carbon (Baltec, model SCD 005). Four samples from the middle of the leaf blade were analyzed. The location of the silica was determined with equipment used for backscattered electron analysis (Brandenberg *et al.* 1985), which was coupled to a scanning electron microscope (SEM) (model LEO 440i). For the qualitative analysis, an Energy Dispersive X-ray Spectometer (Motomura *et al.* 2000), model Oxford Microanalysis Group, with a solid-state silicon-lithium detector, was coupled to an SEM.

Results and discussion

Certain species of Cyperaceae have been widespread on the dunes of the northern coast of Brazil for nearly thirty years; nowadays, many of them are restricted to unspoilt areas, as is the case with *C. ligularis*. This species, however, is cited as a ruderal species in northeastern Brazil (Martins & Alves 2009). *Rhynchospora aberrans* is endemic to the *Caatinga* vegetation of northeastern Brazil and grows mainly in the states of Pernambuco, Paraíba and Rio Grande do Norte. It is small, growing to around 10 cm in height, and is found only during rainy periods. Therefore, it has a relatively short life cycle. These characteristics, associated with others, mean the species is considered rare by researchers of the family, like Dr. Wayt Thomas (personal communication).

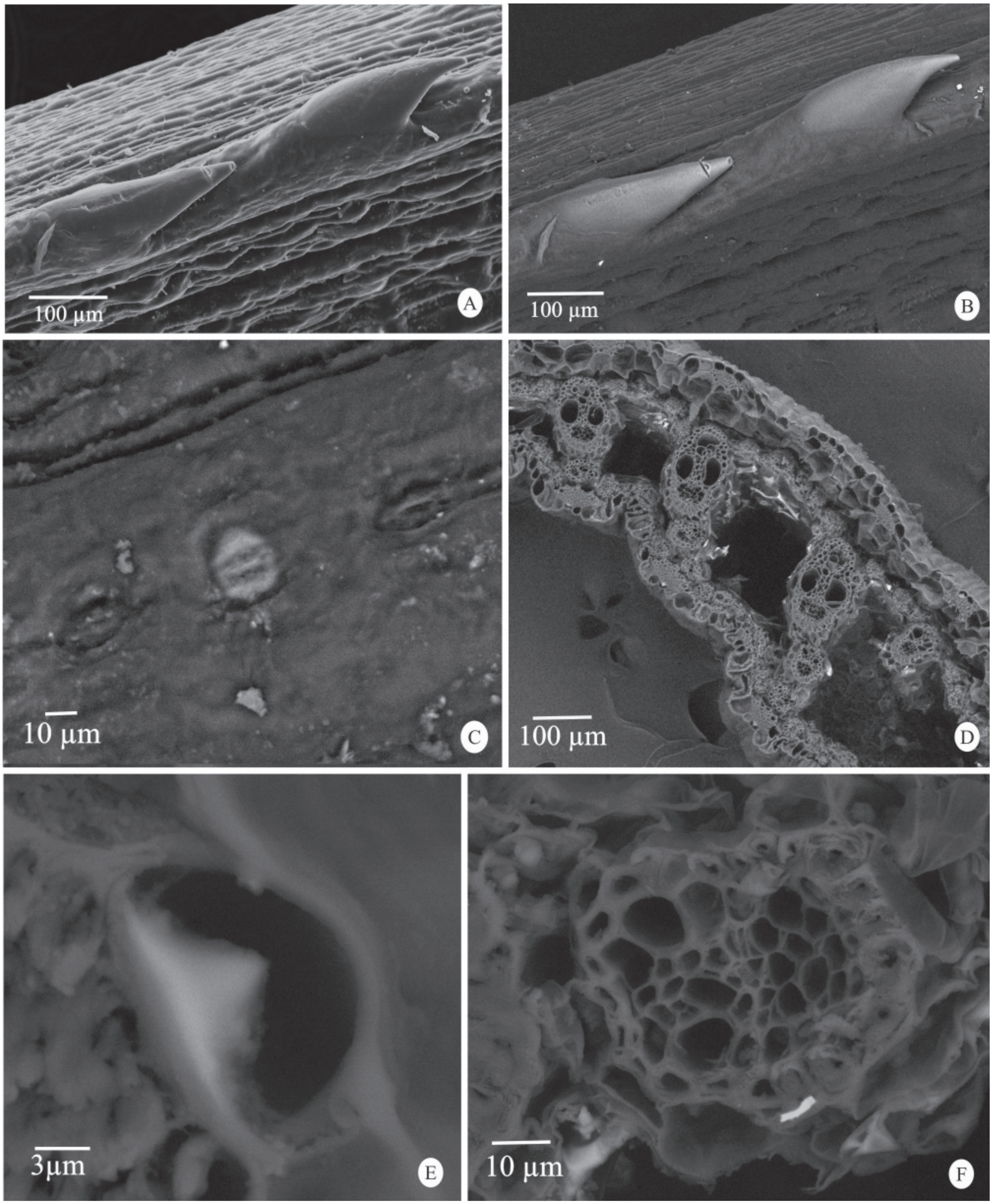

Figure 1. *Cyperus ligularis* L. A. Scanning electron microscopy of the frontal view showing leaf edge with aculeiform, unicellular trichomes; (B-F) location of silica using a backscattered electron detector; (B) silica in the trichomes; (C) frontal view of abaxial epidermis showing silicified and not silicified stomata; (D-F) cross section of blade; (D) silica in several cells that surround the aerenchyma channels; (E) conical projection with satellites of the inner tangential wall, in a "silica cell" of the epidermis of the abaxial surface. (F) silica cells surrounding the vascular bundles; some are conical projections similar to those of the epidermis.

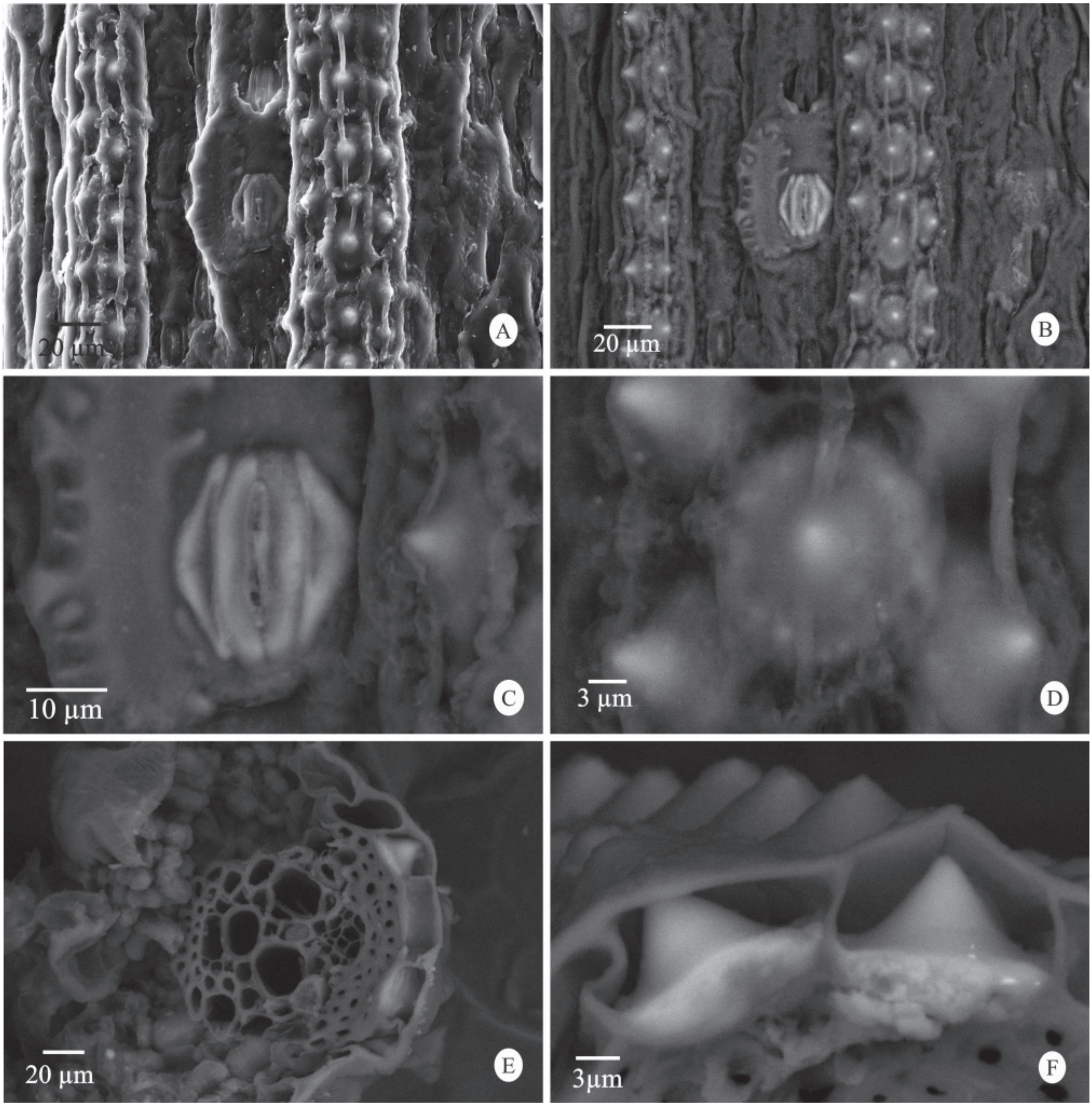

Figure 2. *Rhynchospora aberrans* C. B. Clarke. (a) Scanning electron microscopy of the frontal view of the abaxial epidermis showing stomata and other epidermal cell; (B-F) location of silica using a backscattered electron detector; (B-D) frontal views; (B) stomata (guard and subsidiary cells) and adjacent cells with silicified walls, and "silica cells"; (C) detail of the presence of silica in the stomatal apparatus and cell adjacent to it; (D) "silica cells" with conical projections of the inner tangential walls; in the central projection many satellites are seen; (E-F) cross sections; (E) "silica cell" with conical projections opposite to the fibers adjacent to the vascular bundles; (F) conical projections in two neighboring cells.

In the frontal view of the leaf blade, it was observed that the epidermis was formed by cells arranged in rows where the abaxial side and stomata are interspersed with "silica cells" (Figure 1A-C, Figure 2A-C and Figure 3). *Cyperus ligularis* also presents aculeiform, unicellular trichomes (prickles) among the other epidermal cells and also along the edge (Figure 1A, B). The analysis, using backscattered electrons showed silicified trichomes (Figure 1B). This information is uncommon in the literature on Cyperaceae, however, among the scant information, the paper of Bruhl (1995) is highlighted. Several stomata were silicified, including guard cells and subsidiary cells (Figure 1C, 2B, C), a fact that has not been previously published. Govindarajalu (1969) cited the presence of silica in the stomata of five species of *Rhynchospora* and emphasized that the stomata accumulated large amounts of silica, and became deformed and difficult to identify. In *C. ligularis* and *R. aberrans*, the stomata do not change; the silica is

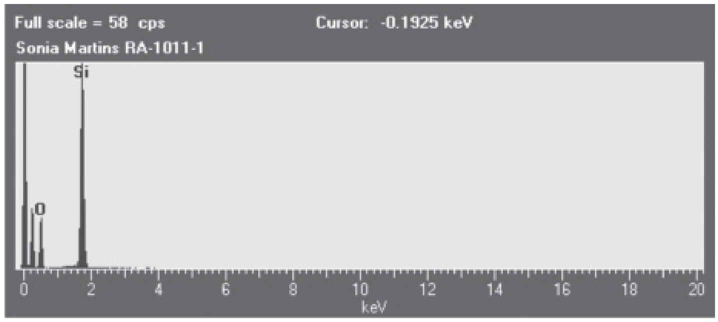

Figure 3. Spectrogram obtained by energy dispersion of X-ray corresponding to Fig. 2 (B) showing the presence of silica and oxygen.

combined with the cell wall and can be verified by more accurate methods, such as those used here. Also relevant in the two species is the presence of silicified stomata interspersed with non-silicified stomata (Figure 1C), which are distributed along the leaf blade. These stomata are similar in structure when observed in frontal view and in cross section, but probably perform different functions. In both species, there are frequently "silica cells", where the silica is deposited on conical projections of the inner tangential wall of epidermal cells of the abaxial surface (Figure 1e, 2B-F), and are always opposed to the fibers adjacent to the larger caliber vascular bundles (Figure 2E). The conical projections occur in clusters of cells (Figure 2B, D, F) and are usually surrounded by much smaller projections called "satellites" (Figure 1E, 2D).

The vast majority of works, including the comprehensive article by Metcalfe (1971), cite only "silica cells", often without conducting any specific test, such as that of Mehra & Sharma (1965) that analyzed "silica cells", in thirteen genera of Cyperaceae in India, and Kukkonen (1967). These authors used the number of cells with conical projections, and other characters, to separate *Uncinia* species. In addition, Rajbhandari & Ohba (1988) analyzed twenty-six species of *Kobresia* based on "silica cells," but lacked a method for identifying silicon. Other works, which include more specific methods also relate only to the "silica cells", such as Lanning & Eleuterius (1989), who carried out X-ray analyses of *Rhynchospora plumosa* and *Scirpus cyperius* Bruhl (1995) and studied numerous Cyperaceae leaves detailing the epidermis and making several records about the "silica cells". More recently, Prychid *et al*. (2003), in an article on silica in monocots, reported only "silica cells" in Cyperaceae.

In cross section, it was observed that *C. ligularis* also accumulates silica in its cell walls, which surround the aerenchyma channels (Figure 1D). Some of these cells are located near the vascular bundles of higher caliber, and can also present impregnation of the wall and conical projections similar to those of the epidermis (Figure 1f). Only Metcalfe (1969), Govindarajalu (1969) and Bruhl (1995) cite the occurrence of silica in Cyperaceae in other places, besides "silica cells". Metcalfe (1969) states that "silica-bodies are associated with sinuosities in the anticlinal walls of cells of epidermis"; however, the locations cited by these authors were not the same in this study.

It is observed that silica deposition may occur in different locations within a species, and not only in the "silica cells" as the vast majority of works suggest. This occurrence is revealed both by impregnation of the silica in wall (no structural changes) and secretion of the structures (with morphological changes). These structural changes occur in the same species and between species. Their location is probably related to environmental factors, including the soil, since according to Jones and Handreck (1965), the same species may absorb silica in different quantities, according to soil type. Besides environmental factors, variations in location and silica deposition may also be related to taxonomic groups.

The above comments relate to Cyperaceae, however, they are also relevant to the wide occurrence of silica in other plants. Authors such as Metcalfe & Chalk (1983) and Carlquist (2001) refer to the presence of silica in several taxa of dicotyledons. The study by Dahlgren & Clifford (1982) shows a distribution map of silica in monocots, among other information.

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