

Leaf surfaces of *Gomphrena* spp. (Amaranthaceae) from Cerrado biome

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ABSTRACT: The leaf structure and micromorphology characterize plant species and reflex its interactions with the environment. Leaf epidermis sculptures aid high transpiration plants on light reflection. The form and distribution of epicuticular wax crystalloids are important to characterize the surface. Aiming to know the micromorphology and the ultrastructure of *G. arborescens*, *G. pohlii* and *G. virgata*, leaves of these Cerrado native species were collected in Brasília, Distrito Federal, Brazil, at the Olympic Center of the Universidade de Brasília and at Reserva Ecológica do Roncador. Leaves of *G. globosa*, an Indian native species, were also studied for comparison. Leaves were fractionated, fixed and treated for observation under optical and scanning electron microscope. A description of the leaf epidermis is provided, along with some quantitative data to help the species taxonomy and support future studies on their physiology: all species are amphistomatic and have Stomatal Index between 7.27 and 18.99. The *Gomphrena* spp. studied have epicuticular wax platelets and wax sculptures over their larger trichome, which are relevant for their taxonomy. Over the Cerrado species cuticle, epicuticular wax is damaged by fungi hyphae development. The presence of epicuticular wax on *Gomphrena* spp. leaves corroborates the phylogenetical alliance between Amaranthaceae and Chenopodiaceae.

Introduction

The Amaranthaceae is a cosmopolite family and occurs at disturbed, arid or saline areas; one of the characteristics that ensure its survival in adverse environments is the operation of C₄ pathway of photosynthesis (Judd *et al.*, 1999; Borsch *et al.*, 2001), with the presence of the Kranz syndrome and the characteristic ultrastructure (Carolin *et al.*, 1978; Estelita-Teixeira and Handro, 1984). Other characters could collaborate on the wide distribution of the Amaranthaceae, like lifespan, plants habit and leaf structure, indumentum and venation types (Judd *et al.*, 1999).

A broad definition of the Amaranthaceae family

includes Chenopodiaceae, which is supported by morphology, *rbcL* and ORF2280 sequences, chloroplast structure and its DNA restriction sites, in a total of 2000 species and 170 genera, from which 100 spp. and 20 genera are native of Brazil (Judd *et al.*, 1999; Souza and Lorenzi, 2008). Leaves and roots of some species of the family are edible (*Chenopodium* spp., *Spinacia oleracea*, *Beta vulgaris* and *Amaranthus* spp.) (Judd *et al.*, 1999). Seeds of South American species of *Chenopodium* and *Amaranthus* (quinoa) are used to make flour and others are ornamental, such as *Celosia* spp., *Gomphrena* spp. and *Iresine* spp. (Judd *et al.*, 1999). From a total of 120 spp. of the *Gomphrena* gender, 46 species occur in Brazil and 41% of it is found in the Cerrado biome (Siqueira, 1992 and 2002; Marchioretto *et al.*, 2002). This biome raises species of the genera *Alternanthera*, *Amaranthus*, *Chamissoa*, *Froelichia*, *Froelichiella*, *Hebanthe*, *Pfaffia* and *Xerosiphon*

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(Siqueira, 1992; Mendonça *et al.*, 1998; Cavalcanti and Ramos, 2001; Marchioretto *et al.*, 2002; Marchioretto, 2008). Twenty species of the family are used in Brazil as food or as popular medicine plant, mostly species from *Alternanthera*, *Amaranthus* and *Gomphrena* genera (Siqueira, 1987). Amaranthaceae species present striated stem and entire alternate or opposite leaves and a great number of them are considered pioneer, growing in clay or sandy soil, frequently among rocks (Joly, 1998; Ribeiro *et al.*, 1999).

The Cerrado biome has a landscape that varies from tall savanna woodland to low open grassland with no woody plants and supports the richest flora among the world's savannas and has a high degree of endemism (Simon and Proença, 2000; Klink and Machado, 2005). This Brazilian biome is considered one of the hotspots for the conservation of global biodiversity, with 10,000 plant species (almost 50% of it are endemic), completely adapted to survive adverse conditions of soil and climate, and only 30% of its biodiversity is reasonably known (Myers *et al.*, 2000; Paiva, 2000; Klink and Machado, 2005). Coutinho (1980) believes that frequent fire is one of the most important factors to determine this biome vegetation, alternating communities and maintaining its biodiversity. The expansion of crops at Cerrado biome, which occupies around 40% of its area, has accelerated the natural habitat fragmentation, leading to local biodiversity extinction, introduction of exotic species, soil erosion, water pollution and alterations in vegetation and hydrologic conditions (Miranda and Miranda, 2000; Ratter *et al.*, 2000; Klink and Machado, 2005).

The leaves are the most plastic structures of the plant and their modifications help to understand how structure, function and metabolism are related, since leaves play a vital role in photosynthesis (Mauseth, 2008). The outermost layer of the leaf is the cuticle, which recovers the epidermis and acts as a barrier against mechanical damages, insects, excessive light and loss of water (Bird and Gray, 2003). The cuticle is a film of soluble and polymerized lipids (cutin and suberin) and the wax sculptures that rise from the cuticle plant are influenced by the environment, helping the species to reflect excessive light and to waterproof the epidermis, retaining water and improving the defenses against fungi (Salatino *et al.*, 1986; Rudall, 2007; Mauseth, 2008). Plants can reach the same adaptation pattern by different character combinations. The leaves epidermis is influenced by the plant needs for performing efficient photosynthesis, which includes the compact arrangement of cells and the absence or presence of some epi-

dermal characters, like cuticle and stomata (Esau, 1977; Rudall, 2007; Mauseth, 2008).

Scanning electron microscopy methods are not suitable to detect the thin wax films that are probably present in all terrestrial angiosperms but are good for determining local wax projections and detailed views of the surfaces (Wilkinson, 1979; Engel and Barthlott, 1988). When appears as crystalloids, the individual form, distribution and orientation of the epicuticular wax can be important to characterize the surface as a valuable systematic character (Engel and Barthlott, 1988; Barthlott *et al.*, 1998). The first survey on Amaranthaceae *stricto sensu* family did not find epicuticular wax on species of the genera *Aerva*, *Allmania*, *Alternanthera*, *Amaranthus*, *Celosia*, *Deeringia*, *Gomphrena*, *Iresine* and *Ptilotus*, while Chenopodiaceae members presented small epicuticular platelets (minor than 2 µm) with parallel orientation and not restricted to stomata neighbourhood (Engel and Barthlott, 1988).

In order to improve the knowledge of the biodiversity of the Cerrado biome and to understand the structural characteristics plants developed on evolutionary adaptations to this environment, the leaf epidermis of three Cerrado native species (*Gomphrena arborescens* L.f., *G. pohlli* Moq. and *G. virgata* Mart.) and of one cultivated plant - *G. globosa* L. - where structurally described under optical and scanning electron microscopy in search for taxonomically and environmentally relevant characteristics.

Material and Methods

Data research on native species and sample collection

Along with a survey on Herbaria data (Herbarium of the Universidade de Brasília/UB, Herbarium of Reserva Ecológica do Roncador/IBGE, Herbarium of the Universidade Vale dos Sinos/PACA and Herbarium of Empresa Brasileira de Pesquisa Agropecuária/CEN), numerous field searches were carried out in cerrado *sensu stricto* areas of Distrito Federal (Parque Ecológico Olhos D'água, Reserva Ecológica do Roncador, Centro Olímpico of Universidade de Brasília and other areas on road vicinities of Park Way and Jardim Botânico) in order to locate the native species in the Cerrado biome.

The Cerrado biome is a tropical savanna-like ecosystem that occupies about 2 millions of km² (from 3-24° S and from 41-43° W), presenting a hot, semi-humid, and notably seasonal climate, with a dry winter season and a rainy summer, raising typical vegetation

(Dias, 1992; Simon and Proença, 2000; Eiten, 2001).

Leaves of the 3rd to 5th nodes of the Cerrado native adult species were collected in the cerrado *sensu stricto* areas of the Olympic Center of the Universidade de Brasília (*G. arborescens*, *G. pohlii* and *G. virgata*) near the coordinates 15°45.936' S and 047°51.187' W and at the Reserva Ecológica do Roncador - RECOR/IBGE (*G. arborescens* and *G. virgata*), both in Brasília/DF, while the similar material of *G. globosa* was harvest from public gardens, in Brasília/DF, near 15°45.950' S and 047°53.006' W coordinates. Testimony material was deposited at the IBGE, PACA and UB Herbaria. Leaves of the 3rd to 5th nodes of *G. arborescens* juvenile species (6-9 months old), cultivated from seeds at vegetation house conditions were also collected and submitted to the same treatment as the adult ones.

Sample and methodology

The species leaves were fractionated and the middle part of them was fixed in Karnovsky solution (Karnovsky, 1965) and in FAA70 or FPA solutions (Kraus and Arduin, 1997) for 24 h. The Karnovsky fixed sample was post fixed in 1% osmium tetroxide in 0.05M sodium cacodylate buffer pH 7.2 for 1 h. Small pieces of fresh tissue were directly fixed over a stub for scanning electron microscopy and air dried without any preparation, as control pieces (Paiva, 2005). Part of the material of both fixatives were dehydrated in a series of ascending acetone and/or ethanol gradient (30%-100%) and critical point dried to the in a Critical Point Drier CPD 030 (Balzers) and, along with air dried samples, were gold sputtered in a SCD 050 (Balzers), followed by the observation under the scanning electron microscope JEOL JSM 840-A.

Part of the material of FAA70 fixative was hand free sliced and submitted to a Sudan III histochemical test to detect lipids, cutin and suberin on the epidermis of the leaves and to a solution of acid floroglucin to detect lignin (Kraus and Arduin, 1997). Other leaf rectangular pieces were washed in distilled water and submitted to 50% nitric acid solution and boiled until the mesophyll was dissolved, sparing the adaxial and abaxial epidermis apart. Then, the epidermis was washed again, stained with safranin and mounted between histological slides and cover slips with Kayser's glycerinated gelatin (Kraus and Arduin, 1997). As this technique was not good enough for all the studied species, some pieces of the leaves were cleared with a sequence of sodium hydroxide and sodium hypochlorite solutions, followed by safranin staining (Kraus and Arduin, 1997) and mounted between glass pieces with Verniz Vitral Incolor

of Acrilex™ (Paiva *et al.*, 2006). All samples were observed under a Zeiss Axiophot equipped with AxioCam and the AxioVision software.

Stomata and trichome density were determined by counting random areas of cleared leaves and expressed as the medium number by mm² plus the standard deviation on adaxial and abaxial epidermis surfaces of at least three leaves. The Stomatal Index were determined for all the four species on the middle adaxial and abaxial epidermis of the same leaf surfaces, calculating the percentage of stomata in relation of the other epidermal cells as $SI = [S/(E+S)] * 100$, where SI is the Stomatal Index, S is the number of Stomata per unit area and E is the number of Epidermal cells (including basal cells of trichomes) per same unit area (Metcalfe and Chalk, 1979).

The areas occupied by the ordinary epidermal cells, by the basal cell of the largest trichome and by the stomata complex (formed only by the 2 guard cells) were measured with the AxioVision software, on the adaxial epidermis surface at the middle part of the leaves, in 25 cells of each type. The results were expressed as the µm² medium area plus standard deviation. The larger trichome length of each species was measured at 25 of these epidermis appendixes, and length was expressed in µm plus standard deviation (Theobald *et al.*, 1979).

Results

Species origin, morphology and phenology

Three of the *Gomphrena* species used in this study are Brazilian Cerrado native species: *Gomphrena arborescens* L.f., *G. pohlii* Moq. and *G. virgata* Mart. The fourth species, *Gomphrena globosa* L., is an annual species, native from India and cultivated as ornamental. All the Cerrado *Gomphrena* species can be classified as hemicryptophytes as they are perennial, the renewal buds are located on top of storage roots, the aerial parts dry out in the dry season (Fahn and Cutler, 1992) and the morphology of the subterranean organs suggest they have xylopodia. Leaves are opposite and hairy, with mucronate apex and entire margins. Cerrado species have sessile leaves, while *G. globosa* leaves are petiolate. During the dry season the seeds of the Cerrado species are dispersed, what seems appropriate since all of them are dispersed by the wind.

Gomphrena arborescens L.f. (Fig. 1A) is known as *paratudo-do-campo* and is a subshrub of 50 cm high, with coriaceous leaves of 10 cm long and 7 cm wide, found in the mid-west and southeast regions of Brazil

and in Paraguay and used in folk medicine and in dried flowers arrangements (Barros, 1982; Pio Corrêa, 1984; Siqueira, 1987, 1992, 2002; Salles and Lima, 1990; Lorenzi and Matos, 2002). The species was found at bloom time at RECOR/IBGE and at the Olympic Cen-

ter of Universidade de Brasília, in Brasília/DF, usually in the rainy station (from November to April) and at vegetative stage from August to October. Some variations of the leaf position were found on *G. arborescens*, from alternated just below the apical inflorescence to

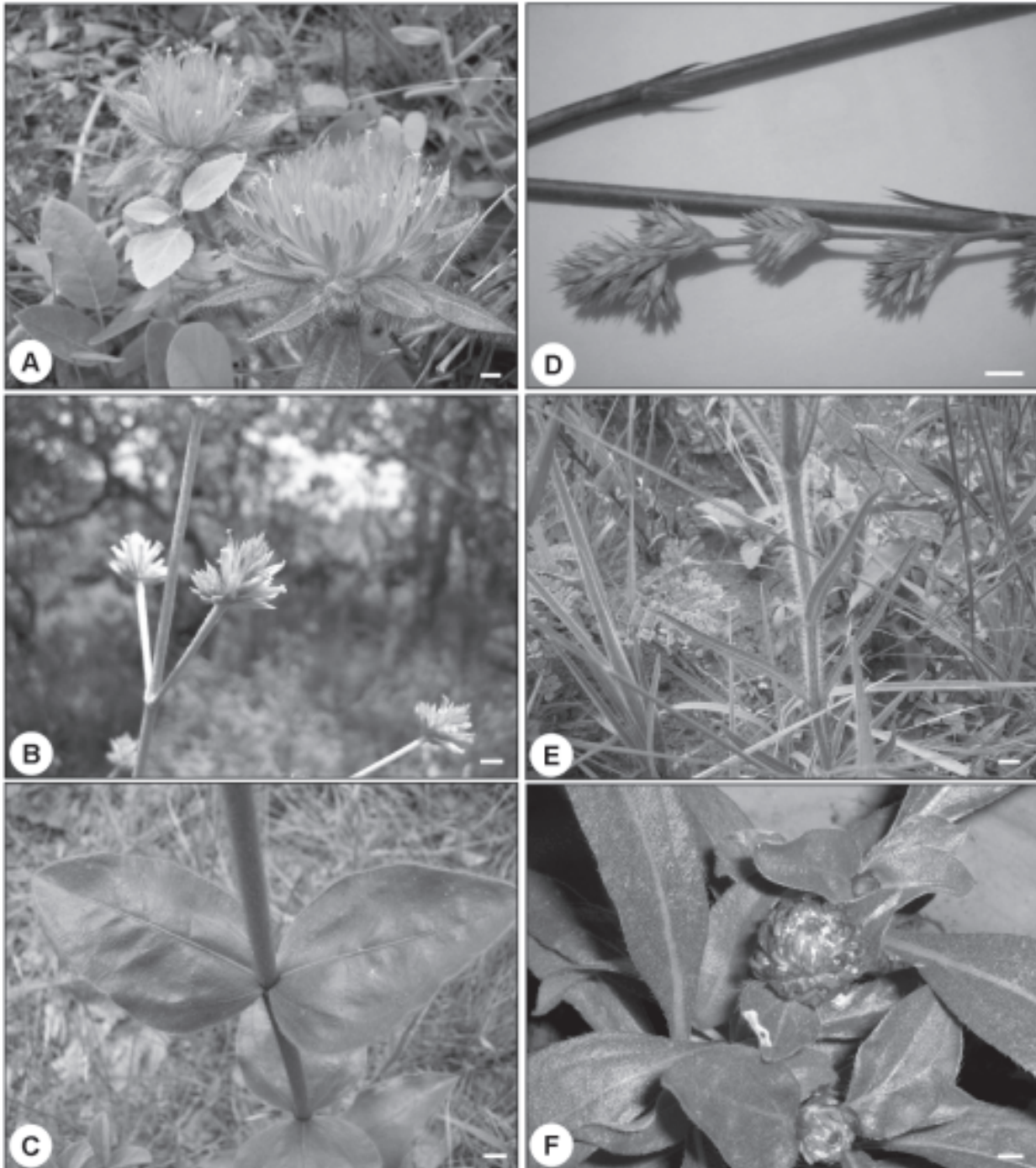


FIGURE 1. Studied species. Figures 1A to 1E: Brazilian Cerrado native species. Figure 1F: Indian native species, cultivated in Brazil. **1A:** *Gomphrena arborescens* L.f., known as *paratudo-do-campo*, is a subshrub of 50 cm high, with coriaceous leaves. **1B:** Inflorescence of *G. pohlii* Moq., known as *paratudinho*, a subshrub of 180 cm high. **1C:** *G. pohlii* Moq. coriaceous leaves. **1D:** Inflorescence of *G. virgata* Mart., known as *cangussu-branco*, a subshrub of 200 cm high. **1E:** *G. virgata* linear coriaceous leaves. **1F:** *G. globosa* L., known as *perpétua*, is a ornamental herb with membranaceous leaves. Bar = 1 cm.

opposite towards the base in adult Cerrado native plants, tending to be decussate. The leaves of a young plant growing at a vegetation house were always decussate. In this species, leaves are always attached to the stem at the same time as the flowers and fruits. Usually, during mostly of the dry season its aerial parts dry out.

G. pohlii Moq. (Fig. 1B – flowers and Fig. 1C – leaves) is a subshrub of 180 cm high with coriaceous leaves of 10 cm long and 7 cm wide, found in the mid-west and southeast regions of Brazil and in Paraguay and known as *paratudinho*, also used in folk medicine (Siqueira, 1988, 1992). The species was found only at the Olympic Center of Universidade de Brasília, in

Brasília/DF, at the same time as *G. arborescens*. The phenologies of both species are related and leaves are always attached to the stem, at the same time as the flowers and fruits. The bloom time occurs from December to April and the species was found at vegetative stage from August to November. During the dry season the aerial parts of the plant dry out.

G. virgata Mart. (Fig. 1D – flowers and Fig. 1E – leaves) is a subshrub of 200 cm high with linear coriaceous leaves of 15 cm long and 1 cm wide, broadly distributed in Brazil and known as *cangussu-branco*, also used in folk medicine (Pio Corrêa, 1984; Siqueira, 1992). The species was found at RECOR/IBGE and at

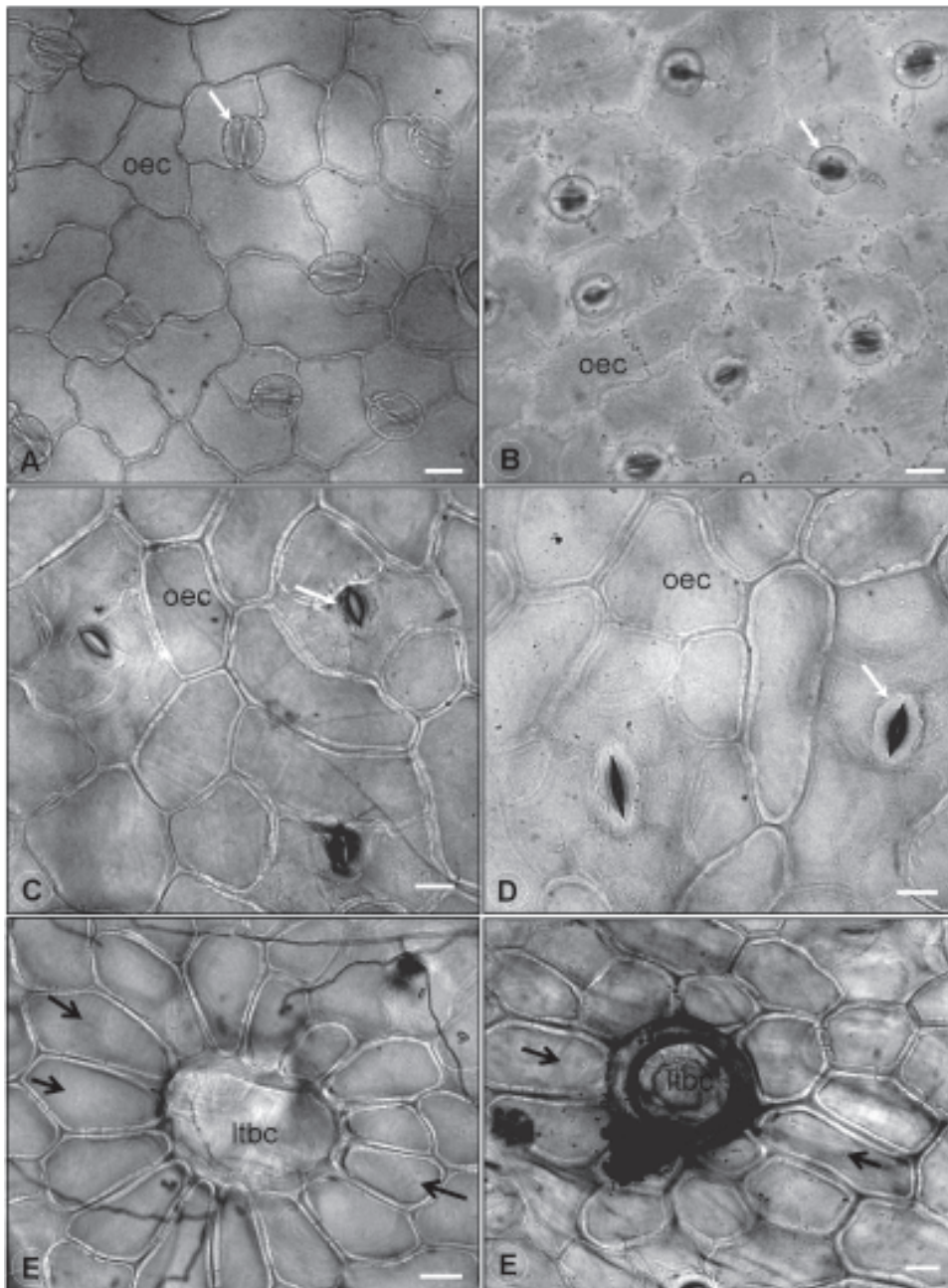


FIGURE 2. Leaf epidermal surfaces under optical microscope. **2A:** Abaxial surface of *G. globosa* leaf. **2B:** Abaxial surface of *G. arborescens* leaf. **2C:** Abaxial surface of *G. pohlii* leaf. **2D:** Abaxial surface of *G. virgata* leaf. **2E:** Long trichome basal cell and subsidiary ones on *G. pohlii* leaves. **2F:** Long trichome basal cell and subsidiary ones on *G. virgata* leaves. **Legend:** ltbc: long trichome basal cell; oec: ordinary epidermal cell; black arrow: trichome subsidiary cell; white arrow: stoma. Bar = 20 μm .

the Olympic Center of Universidade de Brasília, in Brasília/DF, from March (end of the rainy season) to July in vegetative stage. The bloom time of the species begins at July (middle of the dry season) and fructification goes until September (end of the dry season). During the bloom period, leaves enter in a senescence process and are disposed by the stem. After the blooming and dispersing the seeds, the aerial parts of the species also dry out, until almost the end of the rainy season (around March), when they re-sprout.

G. globosa L. (Fig. 1F) is a small herb with membranaceous leaves about 10 cm long and 4 cm wide, native of India and known in Brazil as *perpétua*, cultivated as ornamental (Siqueira, 1992; Lorenzi and Souza, 1995), including in Brasília squares and gardens, at the core region of the Cerrado biome. The species was collected in January, during the rainy season, when it is cultivated in public gardens of Brasília.

Anatomy

The studied species have uniseriate epidermis and tomentose amphistomatic leaves, with anomocytic stomata dispersed over the whole adaxial surface, except over the midrib and on the leaf ledges, where cells are elongated. Trichomes are always nonglandular and have at least two sizes and shapes. On the abaxial surface, stomata do not occur over the midrib and secondary veins, where cells are elongated, as well as over elongated cells that reinforce the leaves edges. All the studied species present stomata surrounded by three to five cells, but these cells are not different from other ordi-

nary epidermal cells, so they were not considered as stoma subsidiary cells.

On paradermal view, *G. globosa* (Fig. 2A) presents the highest stomatal index of all the studied species (Table 1), followed by *G. arborescens* (Fig. 2B), *G. pohlii* (Fig. 2C) and *G. virgata* (Fig. 2D). *G. arborescens* and *G. pohlii* presents the highest number of cell per area unit (Table 1), but *G. arborescens* presents the largest area occupied by just one epidermal cell (Table 2). *G. virgata* presents the minor number of cells by area (Table 1), and the major medium area occupied by each ordinary epidermal cell and stomata complexes (Table 2).

The ordinary epidermal cells are polygonal in all four species, but their anticlinal walls are thicker and have a straight path on both epidermis surfaces of *G. virgata* (Fig. 2D, F) and *G. pohlii* (Fig. 2C, E) leaves. *G. virgata* also presents elongated epidermal cells around the basal cells of its trichomes. The epidermis leaves of *G. arborescens* have more sinuous anticlinal walls on ordinary epidermal cells of abaxial surface (Fig. 2B), while the same cells on adaxial surface have straight anticlinal walls. Thicker anticlinal walls are restricted to the elongated cells that recover the edges and the major veins on *G. globosa* epidermis and this species also tends to have more sinuous anticlinal walls on ordinary epidermal cells towards the edges of both surfaces (Fig. 2A) and more straight anticlinal walls towards the mid vein.

Around the longer nonglandular trichomes of *G. arborescens*, *G. pohlii* (Fig. 2E) and *G. virgata* (Fig. 2F), the epidermal cells have rectangular gross shape, thicker

TABLE 1.

Quantitative data on trichome and stomata density and Stomatal Index (SI) of both surfaces of the studied leaves.

Species	Leaf Surface	Number of Trichomes/ mm ² ± SD	Number of Stomata/ mm ² ± SD	Number of Cells/ mm ² ± SD	SI
<i>Gomphrena arborescens</i> L.f.	Adaxial	5±0	138±17	905±49	15.23
<i>Gomphrena arborescens</i> L.f.	Abaxial	5±0	140±25	1020±92	13.71
<i>Gomphrena globosa</i> L.	Adaxial	16.50±1.42	136±30	771±70	18.99
<i>Gomphrena globosa</i> L.	Abaxial	28.05±2.85	126±24	675±162	18.7
<i>Gomphrena pohlii</i> Moq.	Adaxial	5.9 ± 1.5	137±38	1109±107	12.35
<i>Gomphrena pohlii</i> Moq.	Abaxial	6.22 ± 1.5	113±41	844±310	13.35
<i>Gomphrena virgata</i> Mart.	Adaxial	2.6 ± 0.4	32±11	442±14	7.27
<i>Gomphrena virgata</i> Mart.	Abaxial	1.4 ± 0.6	45±26	394±89	11.53

anticlinal cell walls and are lignified, what could characterize a kind of trichome subsidiary cells, specially in *G. arborescens* and *G. pohlii*, which are very similar in shape and disposition. This aspect was not found on *G. globosa* leaves.

Analyzing leaf transversal sections (data not showed), the Cerrado native species *G. arborescens*, *G. pohlii* and *G. virgata* have a thicker cuticle than the

cultivated species *G. globosa* and than a juvenile sample (6-9 months old) of *G. arborescens* cultivated under vegetation house conditions. The two stoma guard cells have prominent outer and small inner ledges. Cutin and suberin are restricted to the periclinal external wall of most of the epidermal cells, except at *G. virgata* epidermis, where the water proof cuticle extends itself towards to the inner sub-stomatal chamber, covering also the

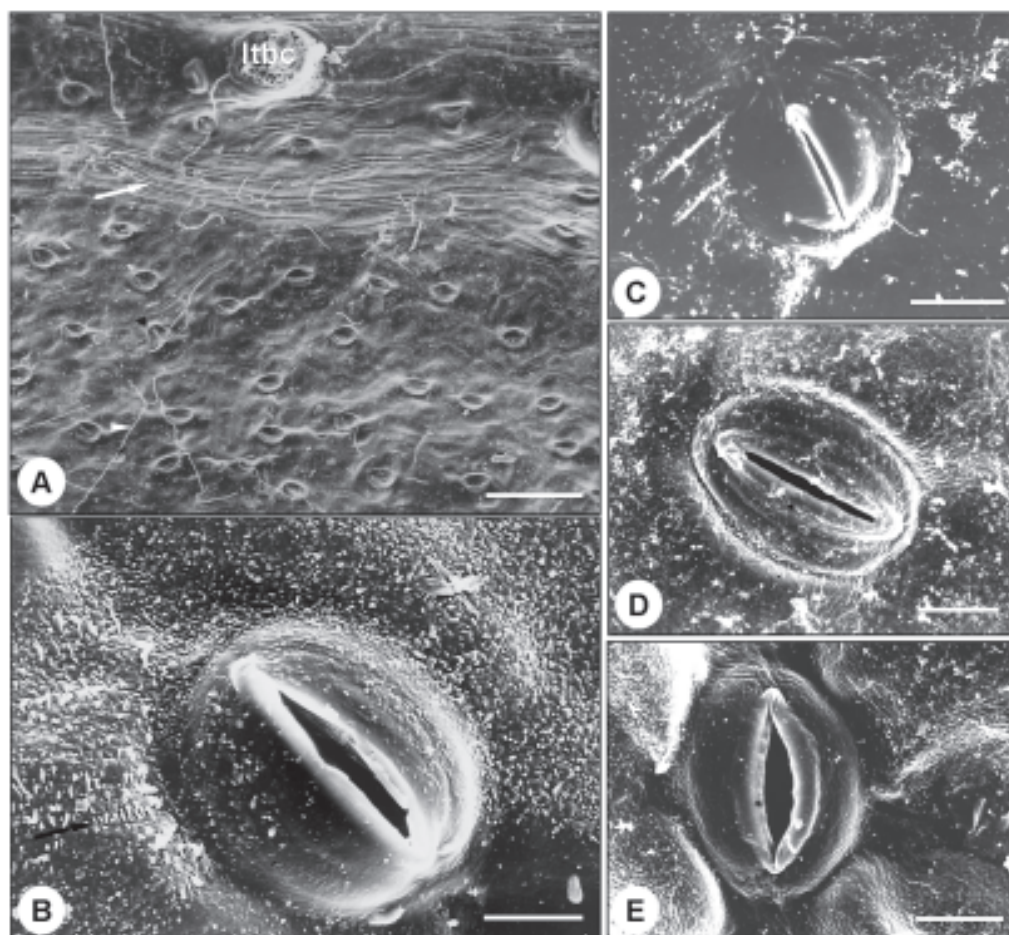


FIGURE 3. Leaf epidermis and stomata under scanning electron microscopy. **3A:** *G. virgata* adaxial epidermis with striae also over the secondary order vein (white arrow) and fungi hyphae (arrow head). **3B:** *G. arborescens* stoma and epicuticular wax (black arrow). **3C:** *G. pohlii* stoma. **3D:** *G. virgata* stoma. **3E:** *G. globosa* stoma. **Legend:** ltbc: long trichome basal cell. Bars: 3A = 100 μm ; 3B to 3E = 10 μm .

TABLE 2.

Quantitative data on area occupied by each kind of epidermis cell and size of stoma and large trichome of the studied leaves.

Species	OEC area: $\mu\text{m}^2 \pm \text{SD}$	Stoma cell area: $\mu\text{m}^2 \pm \text{SD}$	LTBC area: $\mu\text{m}^2 \pm \text{SD}$	Stoma length (μm) $\pm \text{SD}$	Large trichome length (μm) $\pm \text{SD}$
<i>Gomphrena arborescens</i> L.f.	1,681.93 \pm 328.66	541.46 \pm 46.56	17,654.04 \pm 8,400.06	26.56 \pm 2.46	3,060.00 \pm 880.00
<i>Gomphrena globosa</i> L.	2,416.53 \pm 492.70	490.08 \pm 48.19	3,836.13 \pm 1,213.15	27.03 \pm 2.83	676.78 \pm 274.32
<i>Gomphrena pohlii</i> Moq.	2,717.98 \pm 641.02	522.35 \pm 103.09	6,819.26 \pm 3,453.07	31.12 \pm 3.61	817.84 \pm 357.69
<i>Gomphrena virgata</i> Mart.	3,036.77 \pm 840.42	840.82 \pm 180.00	7,374.09 \pm 3,677.23	38.43 \pm 5.37	1,076.25 \pm 313.22

Note: LTBC: long trichome basal cell; OEC: ordinary epidermal cell; SD: standard deviation. Expressed as medium value of 25 elements of adaxial surface.

anticlinal wall of the guard cell and the proximal cells on both epidermis surfaces (adaxial and abaxial).

Micromorphology

All the studied species present a thin film of wax covering the cuticle of the outermost wall of the epider-

mal cells and appendixes, sometimes superimposed by crystalloids of epicuticular wax. The cuticle has striations over the first and second order veins and on the edge of the leaves (abaxial surface) and over the mid vein (adaxial surface) of all species, markedly on Cerrado species (Fig. 3A).

In the studied species the stomata guard cells are

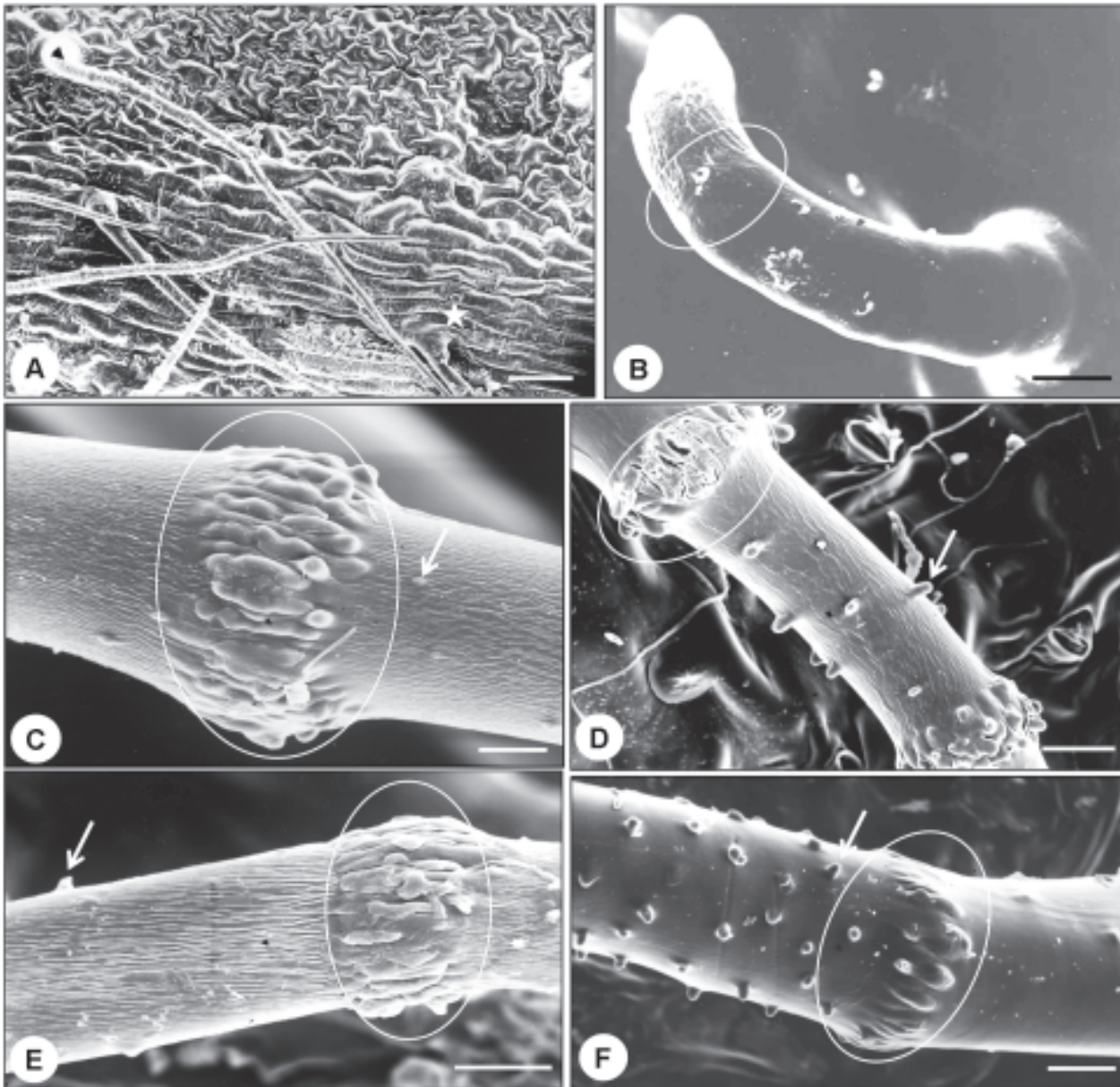


FIGURE 4. Leaf trichomes under scanning electron microscopy. **4A:** *G. globosa* abaxial surface showing a large trichome (arrow head) and elongated cells over the veins (star). Sample fixed in Karnovsky mix and dehydrated in ethanol. **4B:** Short trichome of *G. arborescens* with no wax ornamentation and non nodose articulation (ellipsoid). Sample fixed in FAA70 and dehydrated in ethanol. **4C:** *G. arborescens* long trichome epicuticular wax ornamentation near a nodose articulation (ellipsoid) and the smallest teichodes (arrow). Sample not fixed. **4D:** *G. pohlii* long trichome epicuticular wax scarce striation, the largest teichodes (arrow) and a nodose articulation (ellipsoid). Sample not fixed. **4E:** *G. virgata* long trichome densely ornamented by epicuticular wax striations, few intermediary sized teichodes (arrow) and a nodose articulation (ellipsoid). Sample not fixed. **4F:** *G. globosa* long trichome presents only few intermediary sized teichodes (arrow) and nodose articulation (ellipsoid). Sample not fixed. Bars: 4A = 100 μ m; 4B to 4F = 10 μ m.

leveled to the surrounding cells (Fig. 3A-D), tending to be more elevated on abaxial surface. The anomocytic stomata of the four species have guard-cells with prominent outer ledges (Fig. 3B-D). Only on *G. virgata* adaxial surface the stomata are slightly depressed between the ordinary epidermal cells. The guard cells of *G. arborescens* have ledges with thin and irregular borders (Fig. 3B) and there are some particles of epicuticular wax nearby, some recognizable as platelets. The ledge borders of *G. pohlii* guard cells are thin and regular and near its stomata some perpendicular ridges and crystals of epicuticular wax can be found (Fig. 3C). The stoma of *G. virgata* has ledges with regular and thick borders and processing artifacts do not allow epicuticular waxes to be observed (Fig. 3D). The ledges of the stoma guard cells of *G. globosa* have thick and regular borders and it is possible to see that the epidermal outer cell walls of neighbors' cells are convex (Fig. 3E). This last feature was not observed for any other of the studied species, which epidermal outer cell walls tend to be flat.

Over the elongated epidermal cells, that reinforces the leaf edges and veins, both types of trichomes are more frequent and there are no stomata (Fig. 4A). There are two types of nonglandular trichomes: a long uniseriate hair with osteolate thick cell walls and acuminate apex (Fig. 4A, Table 2) and a short uniseriate recurved hair with thin cell walls and a soft (not acuminate) apex (Fig. 4B).

The longest trichomes of the four species have similar morphology: round basal cells, pustulate osteolate intermediate cells with nodose articulation and acuminate apical cell (Fig. 4A). This trichome type usually has live basal cells (all the 2-3 round ones and at least one of the elongated intermediate cells) and these cells are translucent. The intermediate and apical cells give the trichome color: white in *G. globosa* and rusty (brown-orange) in the three Cerrado species.

The short trichomes present smaller and usually collapsed cell walls and there is no epicuticular wax ornamentation (Fig. 4B) over it or on either of the other

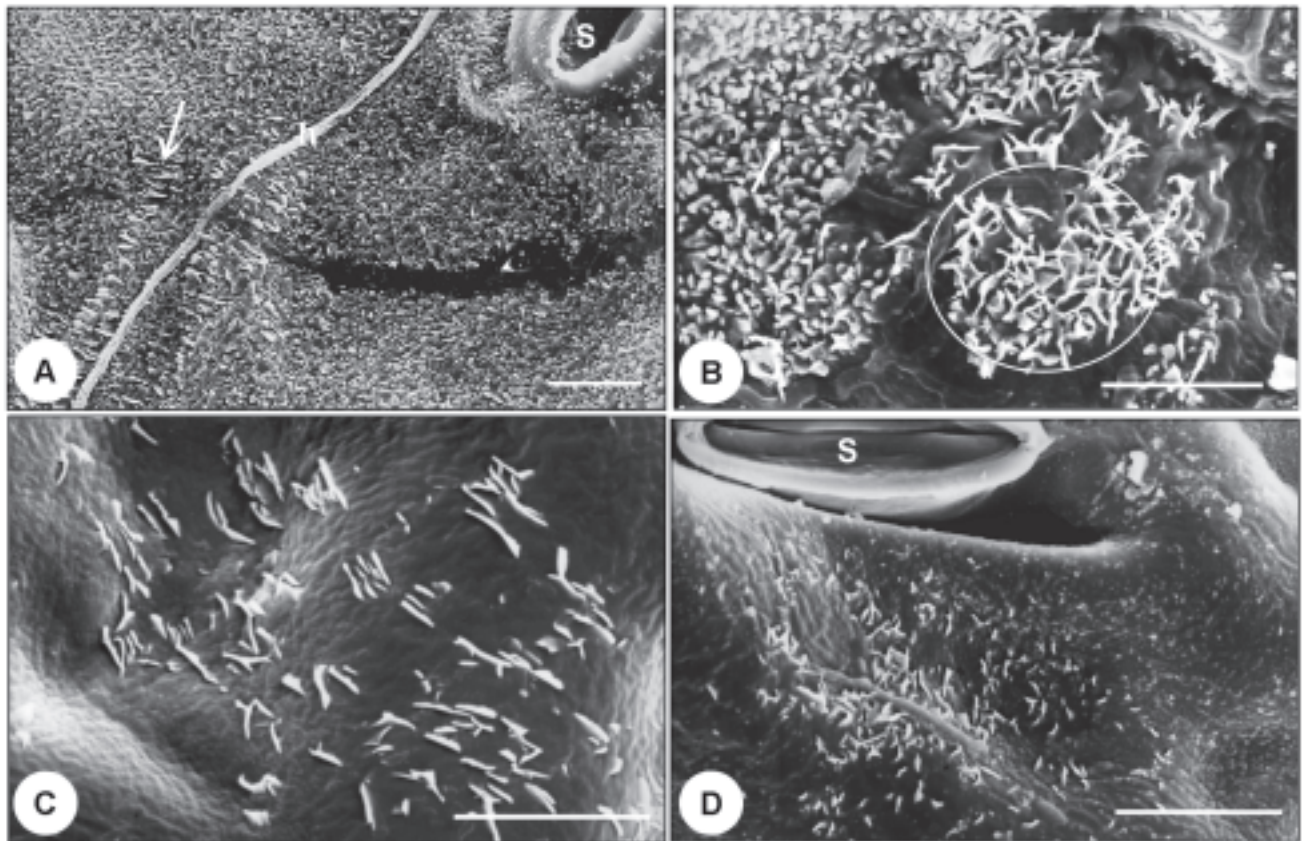


FIGURE 5. Epicuticular waxes over leaf cuticles under scanning electron microscopy. **5A:** *G. arborescens* abaxial surface with fungi hyphae (h) and epicuticular wax organized as a row of parallel platelets (arrow) near a stoma (s) and damaged by a detached hyphae (arrow head) on sample fixed with FPA and dehydrated with ethanol. **5B:** *G. arborescens* adaxial surface epicuticular wax granules (arrow) and rosettes (ellipses) on control sample (not fixed). **5C:** *G. pohlii* epicuticular waxes on adaxial surface a control sample (not fixed). **5D:** *G. virgata* epicuticular waxes near the stoma (s) on adaxial surface of a control sample (not fixed). Bar = 10 μ m.

species. This kind of trichome is formed only by living and thin walls translucent cells and is usually destroyed by fungi hyphae when the plant is living at the Cerrado biome. The articulation of the uniseriate cells is always simple, not nodose (Fig. 4B).

The large trichome presents no epicuticular wax ornamentation over the basal and round cells. However, over the anticlinal walls of the osteolate intermediate and apical cells a network of epicuticular wax streaks and teichodes can be present. This can be useful to discriminate species, because the morphology and ornamentation is slightly different in each one of them. The osteolate cells of the larger trichome of *G. arborescens* have dense epicuticular wax striations with small and rare teichodes (Fig. 4C). These cells in *G. pohlii* trichome have a few epicuticular wax striations and well developed but scarce teichodes of around 10 μm long (Fig. 4D). The same cells in *G. virgata* trichome have dense epicuticular wax striations and rare small teichodes (Fig. 4E). *G. globosa* cells have no epicuticular wax striations and have the greatest amount of teichodes of intermediate size (Fig. 4F). In all the four species, the articulation between the intermediate osteolate cells is nodose, characterized by the interlaced digitorum projections of the periclinal cell walls of neighboring cells (Fig. 4C-F).

G. arborescens leaves present the greatest amount of epicuticular wax particles over the ordinary epidermal cells, normally as entire parallel platelets (Fig. 5A), all over the leaves. Typically, over the subsidiary cells of the largest trichome and sometimes near or over stomata, the platelets can be disposed as single arrows radiating to the adjacent cells (Fig. 5A). This epicuticular wax disposition was not found in any of the other species of the study. Non entire platelets, granules and rosettes were also found (Fig. 5B). Epicuticular wax over guard cells of *G. arborescens* stoma is less concentrated and can be disposed in different arrangements. Platelets also can have irregular margins. *G. arborescens* epicuticular waxes had been damaged by fungi hyphae (Fig. 5A).

G. pohlii leaves present rare platelets of epicuticular wax (Fig. 5C), more frequently over the ordinary epidermal cells near the stomata and over the guard-cells. On *G. virgata* ordinary epidermal cells, the rare epicuticular wax crystals were restricted to the stomata neighbor cells of the adaxial surface of the leaf epidermis (Fig. 5D). Epicuticular wax particles are very rare over *G. globosa* leaf cuticles (data not shown).

The three species of the Brazilian Cerrado presented high fungal contamination (Fig. 4A and 5A) while *G. globosa* did not.

Discussion

Although all the *Gomphrena* Cerrado species have coriaceous leaves and share the same seasonally dry habitat, the anatomical data suggest no water restriction in these organs. All the *Gomphrena* Cerrado species vegetate under full sunlight, in open environment and tend to have from medium to large stomata, leveled to the other epidermal cells. According to Wilkinson (1979) larger stomata are coincidental with full sunlight and drier conditions while smaller stomata are coincidental with shade, humid atmosphere and moist soil conditions.

The Cerrado *Gomphrena* spp. present xeromorphic features as thick cuticle and a kind of hypodermis under the larger trichomes, as verified in *G. arborescens* L.f. leaves (Fank-de-Carvalho and Graciano-Ribeiro, 2005). Only in *G. virgata* the stomata are slightly sunken while on the other species it is leveled to the other epidermal cells on adaxial surface. *G. virgata* has narrower leaves and aerial parts that vegetate longer during the Cerrado dry season. Sunken stomata are considered important to minimize water loss while the thick cuticle and hypodermis diminish the intensity of light to the photosynthetic tissue (Rudall, 2007), although in *G. arborescens* it was suggested this tissue could help the light diffusion under the large trichome basal cells and to reinforce the epidermis to maintain this appendix (Fank-de-Carvalho and Graciano-Ribeiro, 2005).

The Amaranthaceae species of this study present similar Stomatal Index (SI) and number of cells per unit of area of the ones studied by Padmini and Rao (1995). Although they found SI of 19,5 on abaxial surface and 18,2 on adaxial surface and our data corresponds to 18,7 and 18,99 for the same surfaces (Table 1). This variation could be explained by the differences of the surrounding environment that plants cultivated in Brazil and in India are subjected to.

The difference between the *G. arborescens* and *G. pohlii* are smaller than the difference between both species and *G. virgata* on the SI and number of cells per area. When these data are related to the size and shape as well as the duration of the leaves of the Cerrado species, they could suggest that the briefer is the lifetime of the organ and smaller the width of the leaf, smaller the number of epidermal cells per square millimeter and the stomatal index (Table 1), leading to increase the area occupied by each cell. So, if larger cell area implies in bigger water loss (Fahn and Cutler, 1992), it is important to add to this relation the morphology of the roots of these species: all of them have well developed sub-

terranean systems (Siqueira, 1992) which guarantee water supply during the aerial re-sprout and flowering for *G. arborescens* and *G. pohlii* and re-sprout of *G. virgata* (since the latter species loses leaves during the bloom dry period). Even for *G. globosa*, that has a shorter lifetime and is not perennial, as the other species, this statement can be verified: it has the highest Stomatal Index, the second smaller leaf width, and the second medium number of epidermal cells per area.

All the *Gomphrena* species studied presented anomocytic stoma. There is a literature controversy about which stomata type is primitive and which is derivate (Wilkinson, 1979) so this classification was used only for diagnostic purposes. The stoma size is medium for most of the species (*G. arborescens*, *G. globosa* and *G. pohlii*), since the length of them is among 26-31 micrometers. Only *G. virgata* stoma can be considered large, with 38 micrometers of medium length. In this study, the three species with medium sized stoma have similar densities of them by square millimeter, while the species with larger stoma has the smaller density. Our data agrees with Wilkinson (1979), who verified that most authors agree that stoma size is stable enough to be used as a diagnostic character and that small stomata are correlated with high density while large stomata are correlated with low density.

All the species of this study have trichome over the foliar surfaces. The density and length of them is variable (Tables 1 and 2) and is interesting to note that the translucent and larger cells are near the leaf surface. *G. arborescens* leaves have the larger basal cells that bear the lengthiest trichomes and one of the smaller densities of this appendages among the studied species. The Brazilian *Gomphrena* spp. species will be studied in the future to verify if there are other anatomical shared features, as for the trichome types. According to Theobald *et al.* (1979), the trichome type is often valuable for taxonomic purposes, and the provided electron scanning microscopy figures and descriptions can be useful to identify the species.

The foliar surface studies under the scanning electron microscope revealed the existence of crystalloid wax projections on both foliar surfaces of *G. arborescens*, with greater concentration on the adaxial one. All the four species presented platelets of epicuticular wax over the periclinal outer wall of the leaf epidermal cells, although in different patterns and density. The streaked cuticle over the first degree venation and borders of Cerrado *Gomphrena* spp. leaves must represent a way to guarantee the integrity of its epidermis on great tension zones, the same way as the elongated shape of some

ordinary epidermal cells and the thicker anticlinal walls of the same cells.

When studying Cerrado species, sometimes it can be necessary to cultivate them in a controlled environment to get a better description of the epicuticular wax patterning, since fungi hyphae can difficult this objective. The Cerrado environment, moist and hot during the rainy season, favors the fungi development over leaves. The micromorphology of juvenile and adult *G. arborescens* leaves was very similar. As the individual form of the epicuticular wax crystalloids, their distribution and orientation can characterize the surface of the species (Engel and Barthlott, 1988; Barthlott *et al.*, 1998), the juvenile leaves were chosen to study the distribution pattern of epicuticular waxes. Engel and Barthlott (1988) studied Amaranthaceae, including some *Gomphrena* spp. and did not find epicuticular waxes. They considered the absence of these structures a feature to distinguish Amaranthaceae and Chenopodiaceae, as the later one always presented small and lobed platelets of epicuticular wax, normally with parallel orientation and not restricted to the stomata vicinity.

The methodology of sample preparation was very important in relation to epicuticular wax findings. Most of the studied species showed no epicuticular wax when dehydrated with ethanol or acetone, even if fixated with glutaraldehyde or formaldehyde plus ethanol. Only *G. arborescens* epicuticular waxes were more resistant to the dehydration process, using both acetone and ethanol, but the best preservation of it was also observed in the control sample. So, the use of the control sample, just dried out over a stub, was very relevant for this study. The use of control sample must be complimentary to the use of fixated sample, because there is no preservation of the shape of the ordinary epidermal cells and stomata. There was found no significant difference only due to the fixative used on our sample.

The presence of epicuticular wax did not prevent the development of fungi hyphae on leaves of none of the three Cerrado native species. There were hyphae all over the epidermis, sometimes penetrating into the mesophyll through some epidermal cells, walls of the smallest trichomes and stomata pores. Usually the epidermis is damaged near the fungi infected areas and, under the hyphae, the epicuticular wax can loose its shape or melt (Fig. 5A). The leaf surface sculptures should improve the protection of the surface against fungi infections (Salatino *et al.*, 1986), and maybe this is one of the strategies of the Cerrado species, which present more sculptures and epicuticular waxes in relation to *G. globosa*.

Cerrado *Gomphrena* spp. must have adapted to the Cerrado environment through the strategy of enhancement of the photosynthesis process since they do not present a significative reduction on the leaf area or on the epidermal cells sizes. Besides, the indumentum of these species is not so dense. They present amphistomatic leaves and Stomatal Index or stomatal density similar or higher than other species of higher photosynthetic rates, like the C4 *G. globosa* (Padmini and Rao, 1995) and *Zea mays* (Mauseth, 2008). Photosynthesizing organs can present two strategies of adaptation to arid conditions and the second strategy would be the reduction in the transpiration rate (Fahn and Cutler, 1992), what implicates in the study of the mesophyll structure, which is on development. On the other hand, these species have well developed cuticle and thick anticlinal walls and the structure of the epicuticular wax tends to be of thin plates, features considered xeromorphic and inducible by drought (Fahn and Cutler, 1992).

Conclusions

Two of the Cerrado species can be misidentified during the very early vegetative stage (*G. arborescens* and *G. pohlii*), due to the similarity of their stem and leaves. This study shows differences between the epidermal cells and other anatomic and micromorphology features of the epidermis that can be useful to better identify the species. The quantitative data for all four studied species complete the description and can represent good indicatives for the physiology of the species.

The finding of epicuticular wax in all the *Gomphrena* spp. leaves studied reinforces the phylogenetical relationship between Chenopodiaceae and Amaranthaceae families, which has been treated as Amaranthaceae *sensu lato*. The platelets over the cuticle did not represent an effective barrier against fungi hyphae penetration, although it must have improved it. These results indicate the need of increasing studies on other species of the Amaranthaceae *sensu lato* family, especially the Cerrado native ones, to improve the understanding of the taxonomy value of the sculptures arising from the leaf surfaces and its relationship with the plant adjustment to the environment.

This study shows that the Brazilian Cerrado biome raises unique vegetation with different strategies to adapt themselves to the environment, not only structural but also phenological. All the studied Cerrado species vegetate under full sunlight (open environments of the Cerrado biome) and present leaves only during

the rainy season, even though stem and flowers can be found still green during the dry season. So, all the leaves have a good supply of water, even though the atmosphere is not always humid during these days, because the rainy season coincides with the warmer tropical and sunny days. Plant life strategy must be correlated with the morphology and the structural data, as Stomatal Index, so these data can be correlated with the species physiology.

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