

Morphology and anatomy of the seedlings of Malvaceae weed species

Morfología y anatomía de las plántulas de las malezas en la familia Malvaceae

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Abstract

The morphology and the anatomy of seedlings are described for three weed species, such as *Malvastrum coromandelianum* (L.) Garcke, *Sida regnellii* R. E. Fries and *Sida rhombifolia* L. (Malvaceae). Seedlings of the three species are phanerocotylar and epigeal in which cotyledons and eophylls present different shapes. Although the general anatomy of the seedlings among the species is uniform, there may be differences in the structure of the epidermis (epicuticular waxes and trichomes) as well as in the number of cell layers in the mesophyll (cotyledons and eophylls).

Key Words: root, hypocotyl, cotyledon, epicotyl, eophyll.

Resumen

La morfología y la anatomía de plántulas fueron descritas para tres especies de malezas, como *Malvastrum coromandelianum* (L.) Garcke, *Sida regnellii* R. E. Fries and *Sida rhombifolia* L. (Malvaceae). Las plántulas de las tres especies son fanerocotilares y epígeas en que los cotiledones y eofilas presentan formas diferentes. Aunque la anatomía general de las plántulas entre las especies es uniforme, puede haber diferencias en la estructura de la epidermis (cera epicuticular y tricomas) así como en el número de capas celulares en el mesofilo (cotiledones y eofilos).

Palabras clave: raíz, hipocótilo, cotiledone, epicótilo, eofila.

Introduction

The weed species can be considered as pioneer plants under the botanical-ecological point of view. In another way, they are plants evolutionary developed for colonization of areas where, for some reason, the original vegetation was deeply altered, occurring great richness of niches to the plant growth (Lorenzi, 2008). According to this author the weed function is to create an appropriate environment to the beginning of the population succession which will culminate in the re-establishment of the original vegetation.

Knowledge of life-cycle of Magnoliophyta (Angiospermae) is of vital importance, especially in the tropics, because of the high diversity of species. The germination period of the seed, the survivorship and the establishment of the plant in the initial stages

of development are critical in the preservation of a species. The first vegetative phase of a plant after the seed germination of the so-called seedling has enormous value in the study of the population dynamics, in the forestry, in the storage of seeds, in the arboretum works, and in the forest preservation and regeneration (Souza, 2009a). Failure in the adaptation process in the seedling stage may take the species to the extinction (Amorim-Rodrigues & Gomez-Pompa, 1976).

There is somewhat of literature dealing with the morphology of the Malvaceae seedling. Duke (1965) studied seedlings in Puerto Rico forests presenting descriptions of *Gossypium* and *Hibiscus* ones. Burger Hzn (1972) made methodical descriptions and illustrations of seedlings from a number of tropical trees and a few subherbaceous plants in south-east

Asia standing out seedlings of *Hibiscus* and *Thespesia*. Ng (1980) presented data on the germination of 335 species of Malaysian plants (trees, shrubs, lianas) presenting a *Hibiscus* species. Finally, Mourão *et al.* (2007) analyzed the seedling morphology and the juvenile stage of several species, among them *Sida rhombifolia* L. However, it was not found registration for the anatomical study of Malvaceae seedlings.

The morphological and anatomical investigation of weed leaves has been receiving the attention of researchers, being considered that the leaves are paths of herbicide penetration in the process of chemical control of plants. Cuticle (epicuticular waxes), trichomes and stomata should be studied to acquire a better understanding of the barriers each species imposes to herbicide penetration. Studies on the herbicide absorption in adult leaves have been made by several investigators (Audus, 1976; Devine, 1990; Hess & Falk, 1990; Ferreira *et al.*, 2003; Procópio *et al.*, 2003).

Here seedlings of *Malvastrum coromandelianum* (L.) Garcke, *Sida regnellii* R. E. Fries and *Sida rhombifolia* L. (Malvaceae) were presently investigated, not only to contribute to the understanding of the structure of root, hypocotyl, cotyledons, epicotyl and eophylls, but equally to supply leaf surface features for their use in weed control.

Material and methods

The fruits and seeds of *M. coromandelianum*, *S. regnellii* and *S. rhombifolia* were collected at the Universidade Estadual de Maringá and vacant lot, in Brazil (state of Parana). Voucher materials were deposited at the UEM Herbarium (BRASIL, PARANÁ: Maringá, 05-X-2008, fl. and fr., L. A. Souza 14191, 15703, 14192 (HUEM).

Seeds were washed in sodium hypochlorite solution and distilled water and finally air-dried. The seeds were laid out to germinate on moist filter paper in Petri dishes, which were placed in germinator TE 400 Tecnal with light- and temperature-controlled. The germinated seeds were sown in soil (mixture of soil and organic substratum in equal proportion) contained in plastic sacs in a greenhouse.

The term seedling was used for phase from the beginning of germination up to stage where it presents expanded eophylls (Souza, 2009a; Souza *et al.*, 2009). The terminology employed in the seedling morphology and classification was based on Rizzini (1977), Vogel (1980), Garwood (1996) and Souza *et al.* (2009). The venation pattern of the cotyledons and eophylls was based on the classification system of Hickey (1979).

For anatomical description of the seedling (root, hypocotyl, cotyledons, epicotyl and eophyll) was employed the terminology of Souza (2009b), and the trichomes were described according to Theobald *et al.* (1979). The seedlings were fixed in glutaraldehyde and FAA50 and latter transferred into alcohol 70%, following the protocol of Johansen (1940). Free-handed, semi-permanent slides with cross-sections (and occasionally longitudinal sections) were made and stained using safranin & astra blue. The fixed seedlings were embedded in historesin and sectioned (cross-sections) in a rotation microtome, carried out according to the usual techniques (Feder & O'Brian, 1968). Sections were stained in toluidine blue (O'Brien *et al.*, 1965). Specific microchemical tests were done for lipid substances (Sudan III) (Johansen, 1940), mucilage (methylene blue) (Costa, 2000), starch (iodine-potassium iodide test) and lignin (phloroglucin test) (Berlyn & Miksche, 1976).

Micromorphological analysis (Bozzola & Russel, 1992) was done with material fixed in glutaraldehyde (cotyledons and eophylls). After washing in 0.1M sodium cacodylate buffer, the samples were dehydrated in a graded acetone series, critical point dried with CO₂, and then mounted on aluminum stubbs, gold coated, and subsequently examined using scanning electron microscopy (Shimadzu SS-550 Superscan), obtaining digital images. The terminology of plant epicuticular waxes was made in agreement with Barthlott *et al.* (1998).

Figures were prepared using a light microscope (Willd M20) equipped with a reflex camera. Photographs were taken with the stereomicroscope Leica EZ4D and microscope Olympus BX50 with digital camera Canon Power Shot A95, and subsequently prepared using the

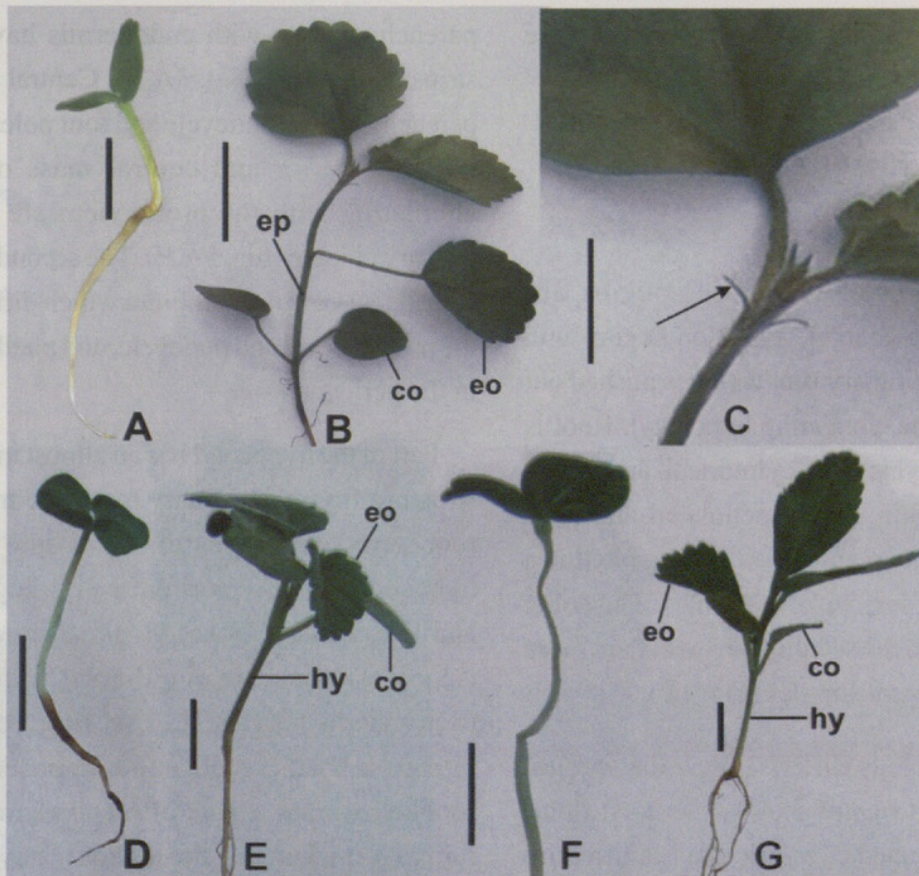


Fig. 1. A-G. Morphology of the seedling. A, B. *Malvastrum coromandelianum* (L.) Gaercke (seedlings 10 and 40 days old); C. detail of figure 1B showing stipule (arrow); D, E. *Sida regnellii* R. E. Fries (seedlings 4 and 20 days old); F, G. *Sida rhombifolia* L. (seedlings 4 and 24 days old);. (co=cotyledon; ep=epicotyl; eo= eophylls; hy=hypocotyl). Bars: Fig. 1.A = 0.15 cm; Fig. 1.B = 1.5 cm; Fig. 1.C = 0.2 cm; Fig. 1.D-G = 0.5 cm

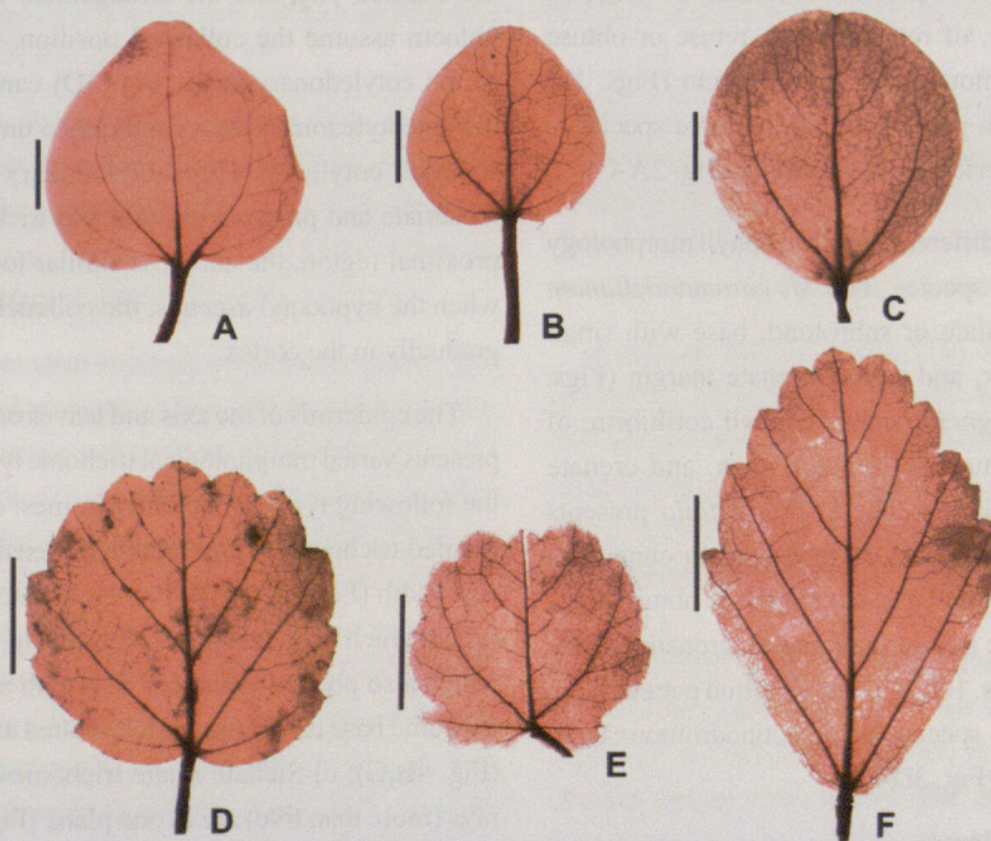


Fig. 2. A-F. Leaf morphology and venation patterns of the cotyledons (A-C) and eophylls (D-F). A, D. *Malvastrum coromandelianum* (L.) Gaercke; B, E. *Sida regnellii* R. E. Fries; C, F. *Sida rhombifolia* L. Bars: Fig. 2.A-C = 2 mm; Fig. 2.D-F = 5 mm

software Zoom Browser EX 4.6. All samples were prepared on the same micrometric scale.

Results

Seedling morphology

Seedlings (Fig. 1) of the three species are phanerocotylar and epigeal. Germination begins with the emergence of the primary root which is pushed out of the seed coat by the elongating hypocotyl. Root is axial and the hypocotyl is long, cylindrical, and green. The cotyledons are thin, green, petiolated and leaf-like. Epicotyl also is long and green. The eophyll is a simple and petiolated leaf without stipules. The collet is indistinguishable. In the seedling phase occurs more eophylls, but they present stipules (Fig. 1C).

Cotyledon morphology differs among the species. *Malvastrum coromandelianum* shows ovate cotyledons, of cordate base to truncate, retuse apex, and entire margin (Figs. 1A,B; 2A). The *S. regnellii* cotyledons are cordiform to orbiculate, of cordate base, obtuse apex, rounded or retuse, and entire margin (Figs. 1D; 2B). *Sida rhombifolia* presents orbiculate cotyledons, rounded or ovate, of rounded base, retuse or obtuse apex with small notch, and entire margin (Figs. 1F; 2C). The venation pattern among the three species is actinodromous perfect basal reticulate (Fig. 2A-C).

There are also differences in the eophyll morphology among the three species. The *M. coromandelianum* eophyll is orbiculate or subrotund, base with small notch, acute apex, and serrate crenate margin (Figs. 1B; 2D). *Sida regnellii* shows eophyll cordiform, of cordate base, obtuse or rounded apex, and crenate margin (Figs. 1E; 2E). The *S. rhombifolia* presents eophyll shape that varies of rhomboid to cuneiform oblong, of cuneiform base, cuneiform or obtuse apex, and margin entire inferior half and mucronate crenate superior half (Figs. 1G; 2F). The venation pattern is the same in the three species, being actinodromous basal perfect marginal (Fig. 2D-F).

Seedling anatomy

Primary root presents similar structure in the three species. Epidermis is uniseriate and the cortex is

parenchymatous with endodermis having Casparian strips on its walls (Fig. 3A,B). Central cylinder shows parenchymatous pericycle and four poles of protoxylem on the exterior and central mass of metaxylem; alternating with the protoxylem are the strands of primary phloem (Fig. 3A,B). The secondary root growth is made by vascular cambium which differentiates from the procambium and pericycle, and phellogen that arises in the pericycle.

Part of the hypocotyl has an almost indistinguishable structure from the primary root. The transition region root-stem commences in the middle portion of the hypocotyl. In this portion a pith begins to appear, and the primary xylem is spread into a continuous tangential ring surrounding the pith, with four projecting protoxylems (Fig. 3C). This ring becomes broken into four triads, each one composed of a median protoxylem and a pair of metaxylems. The phloem remains unchanged throughout, never undergoing division. This vascular tissue disposition stays even close to the cotyledonary node where it occurs the change of the exarch typical condition of the root to the endarch one, and the arrangement of xylem and phloem assume the collateral position. In the region of the cotyledonary node (Fig. 3D) can be observed the epicotyledonary traces and only a unilacunar trace for each cotyledon. Hypocotyledonary epidermis is uniseriate and presents stomata and trichomes. In the proximal region, the cortex is similar to the root, and when the hypocotyl ascends, the collenchyma appears gradually in the cortex.

The epidermis of the axis and leaves of the seedlings presents varied morphological trichome types. There are the following types of tector trichomes: a) Unicellular pointed trichomes with variable dimensions in length and width (Figs. 4A; 7B); b) Fasciculate trichomes or tufted which ends in a point with two to six trichomes which also present variations in length and width; the trichome base can present thick-walled and simple pits (Fig. 4B,G); c) Stellate rotate trichomes in which the rays (more than five) are in one plane (Fig. 7B); and d) Stellate multiangulate trichomes which arms radiate in two planes (Figs. 4E,F; 7A). The glandular trichomes are multicellular and capitate, being distinguished

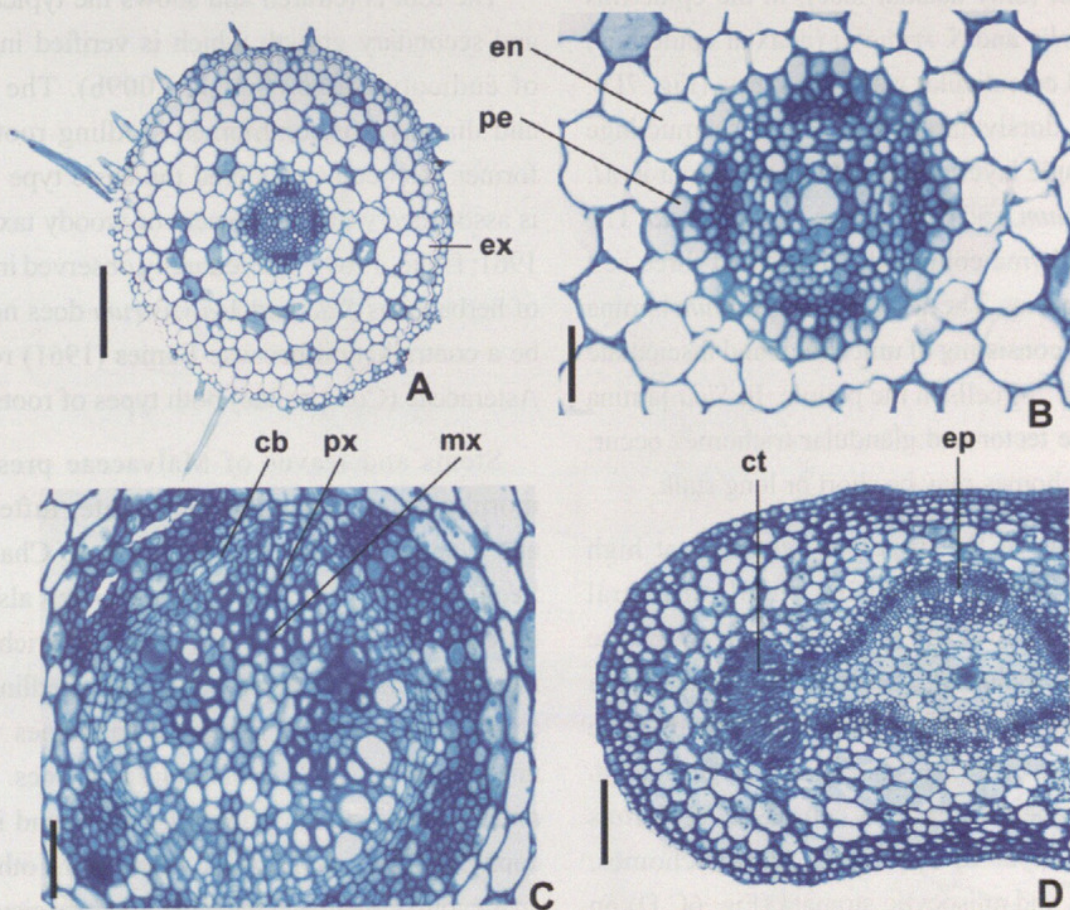


Fig. 3. A-D. LM micrographs of primary root and hypocotyl structure in cross-sections of *Sida regnellii* R. E. Fries (A, B) and *Sida rhombifolia* L. (C, D). A. root/hypocotyl; B. detail of A. C. middle region of hypocotyl; D. Hypocotyl in the cotyledon node region. (cb=cambium; ct=cotyledon trace; en=endodermis; ep=epicotyledonary trace; ex=exodermis; mx=metaxylem; pe=pericycle; px=protoxylem). Bars: Fig. 3.A, D = 150 μ m; Fig. 3.B, C = 40 μ m

two types: a) Short-stalked trichome (Fig. 4D) with rounded, ovate or ellipsoid multicellular apex; and b) Long-stalked trichome (Fig. 4C) with rounded unicellular apex. In the cotyledonar node it may be present uniseriate multicellular tector trichomes.

Epicotyl has stem structure in the three species. The uniseriate epidermis presents trichomes and stomata, the cortex possesses collenchyma and parenchyma, and the central cylinder shows collateral vascular bundles surrounding the parenchymatous pith (Fig. 5A,B). In the pith occur druses and mucilage cells. With reference to the trichome types, there is variability at the specific level. *Malvastrum coromandelianum* presents tector trichomes, the unicellular and fasciculate one with two to four trichomes, and glandular trichomes with short stalk and ovate or ellipsoid apex. In *S. regnellii* occur fasciculate with three trichomes and both types of capitate glandular trichomes. *Sida rhombifolia* presents all the trichome types described.

Epicotyl and petioles (cotyledons and eophylls) of the three seedlings present a subepidermal cortical layer composed of parenchyma with abundant intercellular spaces (Figs. 5B, 8A). This layer can be continuous or interrupted by collenchyma in the petioles.

Cotyledons present petioles (Fig. 5C) with uniseriate epidermis, consisting of stomata and trichomes. In *Sida* species the petiole presents only parenchyma or parenchyma and collenchyma. In *M. coromandelianum* occur collenchyma and parenchyma (Fig. 5C). Mucilage and druse cells occur in the petioles of the three species. In the petiole there is a single collateral vascular bundle (Fig. 5C) with cambium. The lamina has uniseriate epidermis in which the ordinary cells presented sinuous anticlinal walls being more sinuous in *Sida* species (Fig. 6A,B). The anisocytic stomata are present on both surfaces (Fig. 6A,B). Cotyledon epidermis presents epicuticular waxes as very thin covering (film) in *M. coromandelianum* (Fig. 7C)

and *S. regnellii* (only abaxial face); in the epidermis of *S. rhombifolia* and *S. regnellii* (adaxial epidermis) are developed epicuticular waxes as crusts (Fig. 7D). Mesophyll is dorsiventral (Fig. 5D) with mucilage cells and a single layer of palisade parenchyma in *M. coromandelianum*, and two or three layers in *Sida*. The spongy parenchyma commonly consists of three cell layers in the species. The *M. coromandelianum* lamina is glabrescent, consisting of unicellular and fasciculate trichomes with 2-4 cells in the petiole. In *Sida* lamina and petiole the tector and glandular trichomes occur; these latter trichomes may be short or long stalk.

The eophylls of the three species show at high structural similarity, exhibiting small structural variations. In the petioles (Fig. 8A) there are uniseriate epidermis with trichomes and stomata, collenchyma and parenchyma; the vascularization is made by three collateral bundles being the central one of larger calibre. The lamina of the three species consists of epidermis with a single layer of cells (Fig. 8D), trichomes, mucilage cells and anisocytic stomata (Fig. 6C,D) on both surfaces. Eophyll epidermis presents epicuticular waxes as thin covering (film) in *M. coromandelianum* (Fig. 7E) and formation of crusts in both species of *Sida* (Fig. 7F). Mesophyll is dorsiventral (Fig. 8D) with mucilage and druse cells. The leaf margin shows isodiametric cells. Midrib (Fig. 8B,C) exhibits collenchyma, parenchyma and a single collateral vascular bundle with fiber primordia in the phloem face.

Discussion

Seedlings of the three studied species are distinguished by lamina shape of the cotyledons and eophylls. Seedlings of these species may be framed at Macaranga type which was formulated by Vogel (1980). In agreement with this author, the Macaranga morphological characters are epigeal germination, phanerocotylar seedlings, thin and leaf-like cotyledons and the first leaves all spirally arranged. Each studied seedling may be classified as PEF (Phanerocotylar, Epigeal, Foliaceous) (Garwood, 1996), being taken into account the persistent foliaceous cotyledons and the pioneering behavior of weeds (Lorenzi, 2008).

The root is tetrarch and shows the typical primary and secondary growth which is verified in seedlings of eudicotyledons (Souza, 2009b). The tetrarchy and diarchy are common in seedling roots and the former has been considered the basic type because it is associated with arborescent or woody taxa (Eames, 1961; Duke, 1969). The tetrarchy observed in seedlings of herbaceous *Sida* and *Malvastrum* does not seem to be a contradiction because Eames (1961) registers in Asteraceae (Compositae) both types of roots.

Stems and leaves of Malvaceae present great morphological variation of stellate, tufted, peltate and glandular trichomes (Metcalfe & Chalk, 1957). Seedlings of three Malvaceae species also present different types of tector and glandular trichomes that may be specific diagnostic value. *Sida* seedlings possess capitate long-stalked glandular trichomes which are not present in *M. coromandelianum* ones. Rotate or multiangulate stellate trichomes are found in the first eophyll of *Sida rhombifolia*, whereas the other species are devoid of them. On the other hand, fasciculate tector trichomes in which the trichomes are more prolonged and wider, with thick-walled pitted cells that occur in *M. coromandelianum* were not verified in *Sida*.

The root-stem transition region of the three species begins approximately in the middle of the hypocotyl. In agreement with the classes of transition (Compton, 1912), the transition region of the *M. coromandelianum* and *Sida* seedlings may be included in the intermediate-high type. The low type of transition begins some distance the external collet and it seems to be common in eudicotyledons, being registered in investigated species of Bignoniaceae, Clusiaceae, Fabaceae and Annonaceae (Souza, 2009b).

The leaf-like cotyledons are similar to the first eophylls concerning *M. coromandelianum* and *Sida* as for the type of venation actinodromous, occurrence and types of tector and glandular trichomes, sinuous anticlinal walls of the epidermal cells, presence of anisocytic stomata, and dorsiventral mesophyll structure. According to Vogel (1980) the leaf-like photosynthetic cotyledons are possibly of different origin than other types, as either foodstoring or haustorial cotyledons.

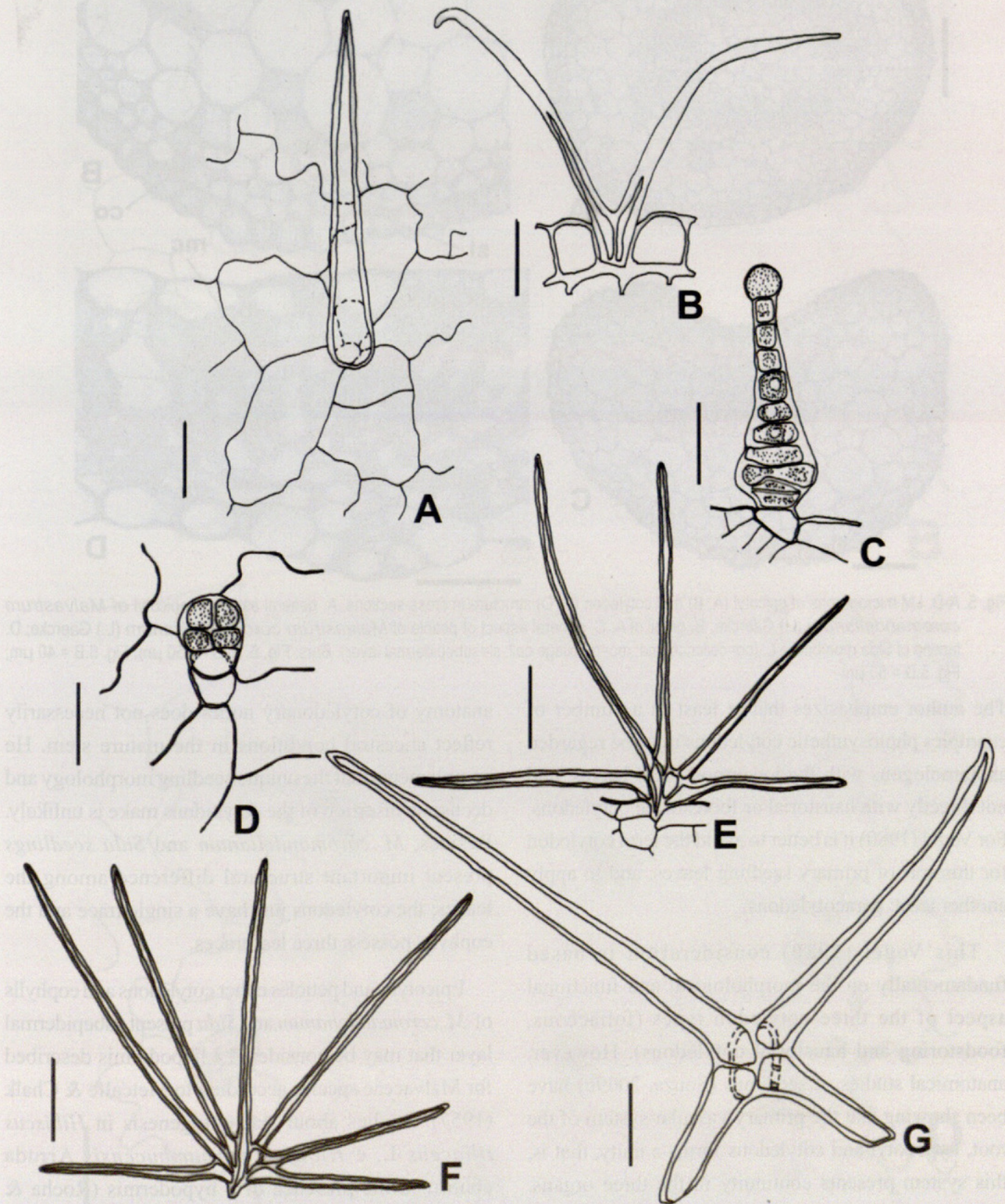


Fig. 4. A-F. Illustrations of trichomes types of *Malvastrum coromandelianum* (L.) Gaercke (D, G), *Sida regnellii* R. E. Fries (A) and *Sida rhombifolia* L. (B, C, E, F). A. unicellular trichome; B, G. fasciculate trichomes; C. long-stalked glandular trichome; D. short-stalked glandular trichome; E, F. stellate multiangular trichomes. Bars: Fig. 4.A-F = 30 μm

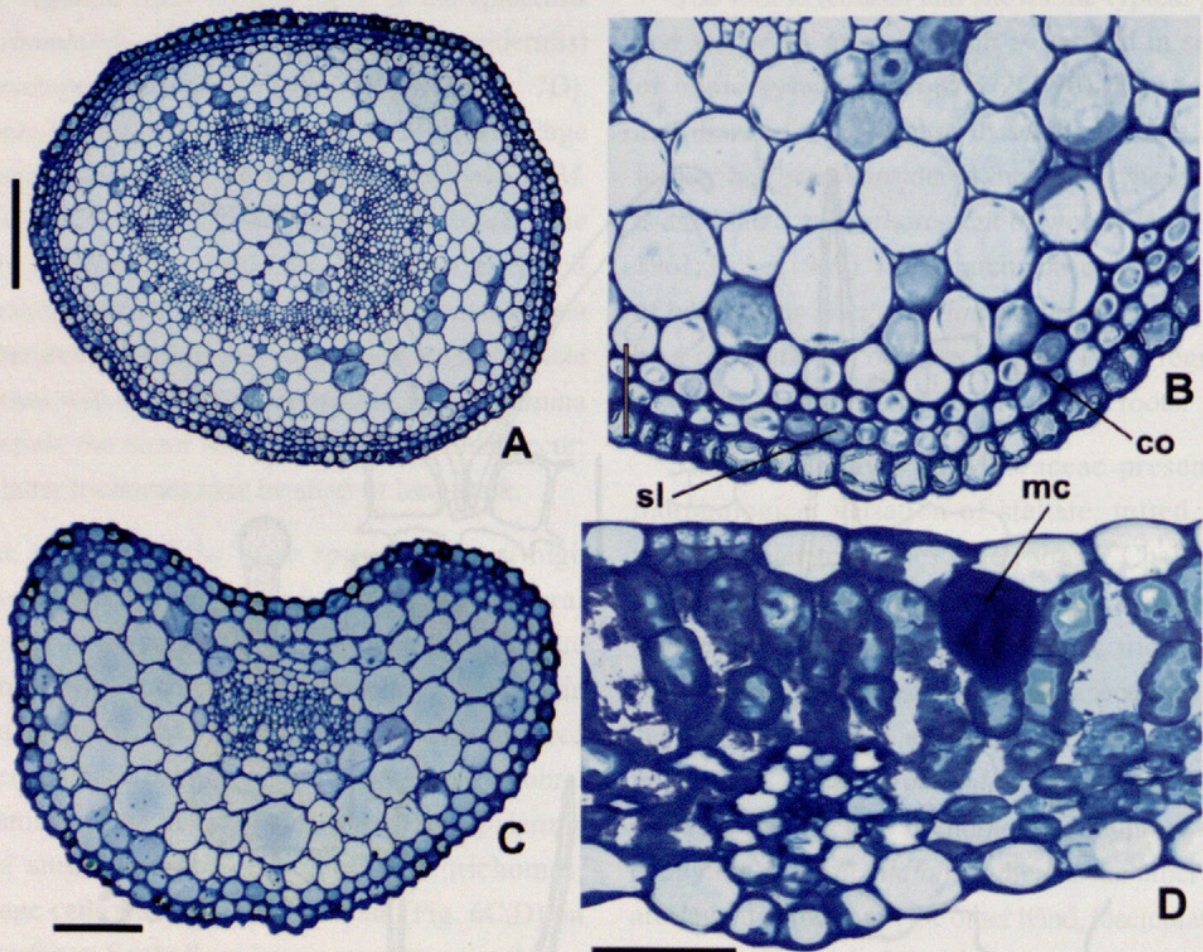


Fig. 5. A-D. LM micrographs of epicotyl (A, B) and cotyledon (C, D) structure in cross-sections. A. general aspect of epicotyl of *Malvastrum coromandelianum* (L.) Gaercke; B. detail of A; C. general aspect of petiole of *Malvastrum coromandelianum* (L.) Gaercke; D. lamina of *Sida rhombifolia* L. (co=collenchyma; mc=mucilage cell; sl=subepidermal layer). Bars: Fig. 5. A, C = 150 μ m; Fig. 5.B = 40 μ m; Fig. 5.D = 50 μ m

The author emphasizes that at least in a number of examples photosynthetic cotyledons must be regarded as homologous with the lowermost stem leaves, and not directly with haustorial or foodstoring cotyledons. For Vogel (1980) it is better to avoid the term cotyledon for this sort of primary seedling leaves, and to apply another term: paracotyledons.

This Vogel (1980) consideration is based fundamentally on the morphological and functional aspect of the three cotyledon types (foliaceous, foodstoring and haustorial cotyledons). However, anatomical studies of seedlings (Souza, 2009b) have been showing that the primary vascular system of the root, hypocotyl and cotyledons forms a unity, that is, this system presents continuity in the three organs. Another argument contrary to the interpretation of Vogel (1980) for paracotyledon is confirmed by the studies of Benzing (1967) and Takhtajan (1980) about nodal structure. Benzing (1967) correctly points out that

anatomy of cotyledonary nodes does not necessarily reflect ancestral conditions in the mature stem. He complements that the unique seedling morphology and decussate insertion of the cotyledons make is unlikely. Besides, *M. coromandelianum* and *Sida* seedlings present important structural difference among the leaves: the cotyledons just have a single trace and the eophylls possess three leaf traces.

Epicotyls, and petioles either cotyledons and eophylls of *M. coromandelianum* and *Sida* present subepidermal layer that may be considered a hypodermis described for Malvaceae species according to Metcalfe & Chalk (1957). Studies about leaf ontogenesis in *Hibiscus tiliaceus* L. e *Hibiscus pernambucensis* Arruda confirmed the presence of a hypodermis (Rocha & Neves, 2000).

General structural characters of leaves and stems of Malvaceae (Metcalfe & Chalk, 1957) may be verified in epicotyls (stems) and eophylls of *M. coromandelianum*

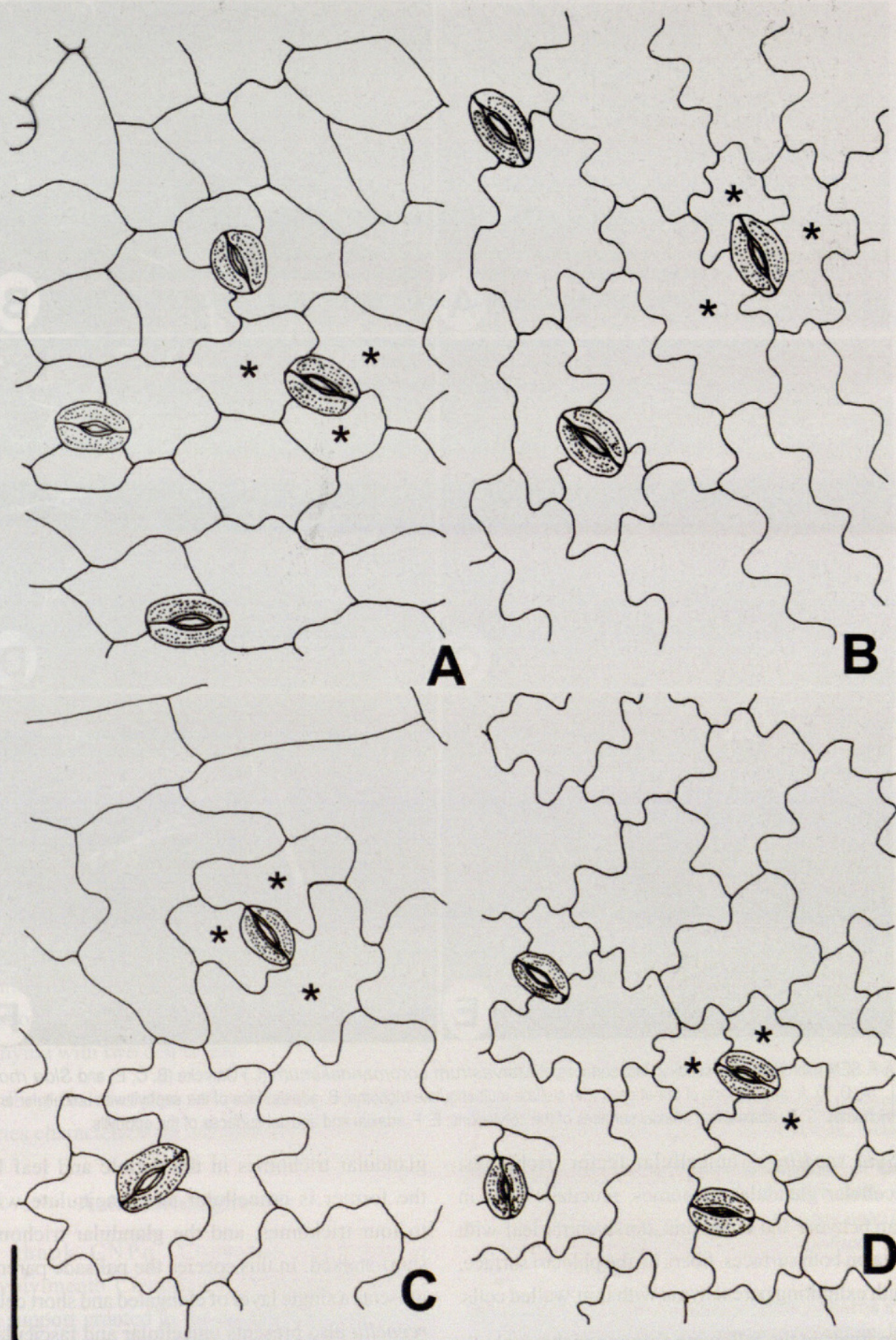


Fig. 6. A-D. LM illustrations of cotyledon and eophyll epidermis in frontal view (abaxial surface) of *Malvastrum coromandelianum* (L.) Gaerke (A, C), *Sida regnellii* R. E. Fries (D) and *Sida rhombifolia* L. (B). A, B. cotyledons; C, D. eophylls. (asterisk=subsidiary cells). Bars: Fig.6.A-D = 30 μ m

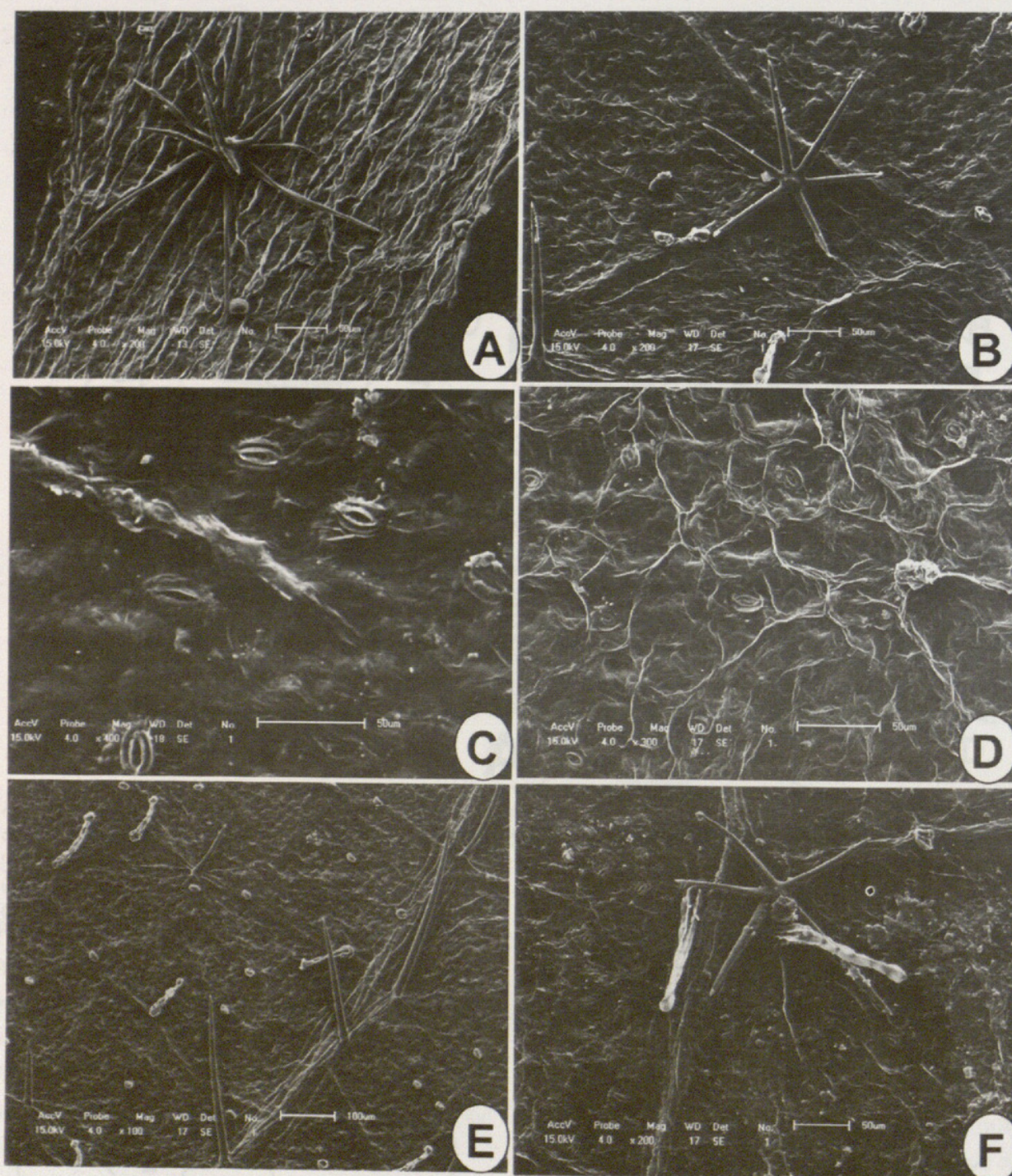


Fig. 7. A-F. SEM micrographs of seedling leaf surfaces of *Malvastrum coromandelianum* (L.) Gaercke (B, C, E) and *Sida rhombifolia* L. (A, D, F). A. abaxial face of the eophyll with stellate multiangular trichome; B. adaxial face of the eophyll with unicellular and stellate trichomes; C, D. abaxial and adaxial surfaces of the cotyledons; E, F. adaxial and abaxial surfaces of the eophylls.

and *Sida* seedlings: unicellular tector trichomes, multicellular glandular trichomes, mucilage cells in the parenchyma and epidermis, dorsiventral leaf with stomata on both surfaces, fibers on the phloem surface, and pith exhibiting parenchyma with thin-walled cells.

Variations in the following features of the eophylls are found to be of specific diagnostic value: trichome and layer number of palisade parenchyma. *Malvastrum coromandelianum* presents either nonglandular or

glandular trichomes in the petiole and leaf lamina; the former is unicellular and fasciculate with two to four trichomes, and the glandular trichomes are short-stalked. In this species the palisade parenchyma presents a single layer of elongated and short cells. *Sida regnellii* also presents unicellular and fasciculate (2-6 trichomes) nonglandular trichomes, and the glandular ones are short-stalked and long-stalked trichomes. In *S. regnellii* the palisade parenchyma three cell layers are registered. The same types of nonglandular trichomes

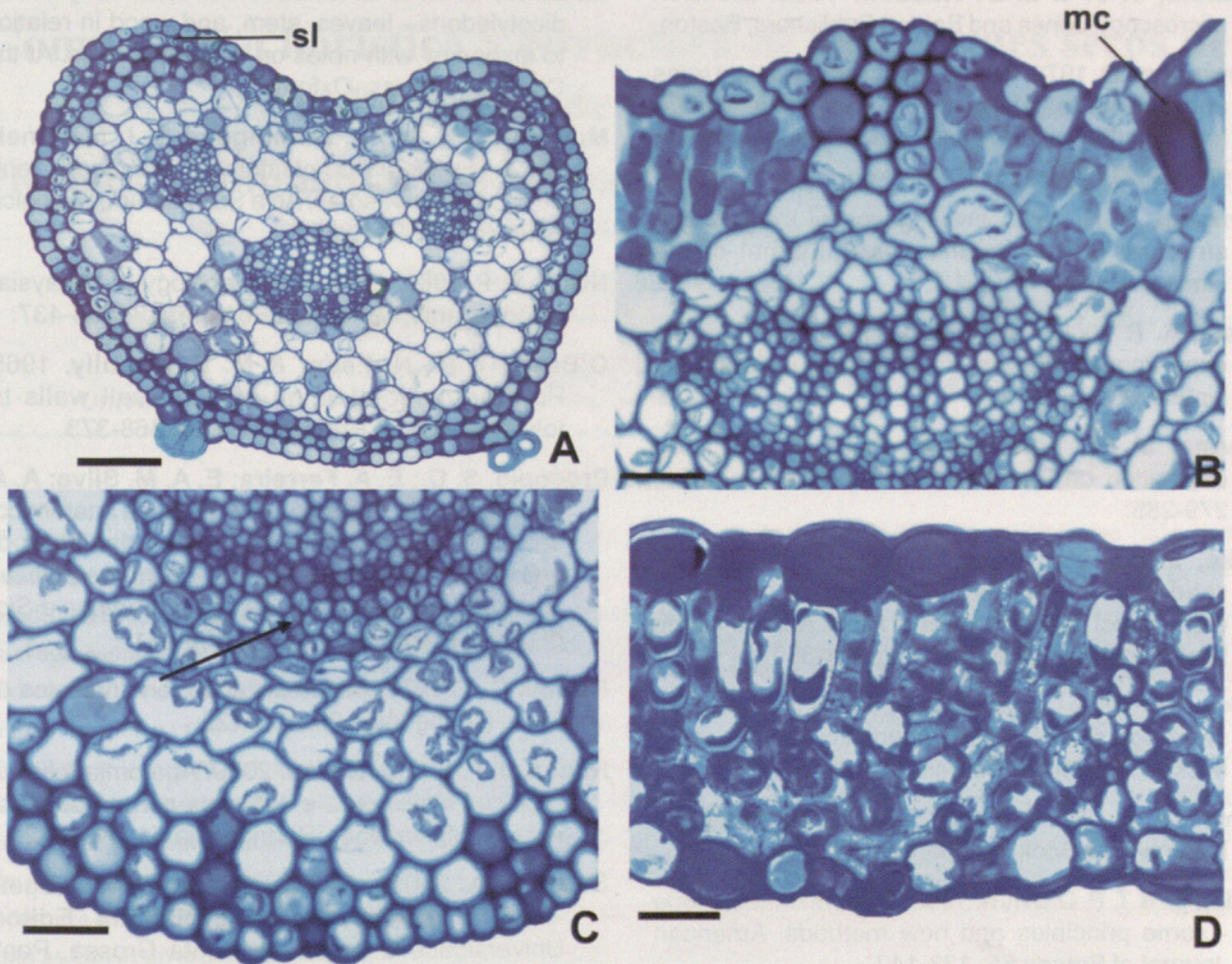


Fig. 8. A-D. – LM micrographs of eophyll structure in cross-sections. A, D. petiole and lamina of *Malvastrum coromandelianum* (L.) Gaercke; B, C. midrib of *Sida rhombifolia* L. (mc=mucilage cell; sl=subepidermal layer; the arrow indicates the fiber primordia). Bars: Fig. 8.A = 150 μ m; Fig. 8.B-D = 40 μ m

as in *S. regnellii* are observed in *S. rhombifolia*, but in the latter occurs other trichome types such as stellate rotate and stellate multiangulate. Still in *S. rhombifolia* occur short-stalked glandular trichomes and palisade parenchyma with two cell layers.

Seedling features that are potentially significant in species characterization are summarized in Tab. 1.

Acknowledgments

We thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil) for the support granted to the accomplishment of this work (PIBIC Grant to G. O. Santos and Research grant to L. A. Souza). We also thank Dr. O. A. Guimarães, UFPR, Brazil, and Dr. M. Bovini, Jardim Botânico do Rio de Janeiro, for the species identification.

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Santos, Gracielli de Oliveira et al. 2012. "Morphology and anatomy of the seedlings of Malvaceae weed species." *Arnaldoa : revista del Herbario HAO* 19(2), 155–166.

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