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Seedling structure of *Euphorbia* L. and *Chamaesyce* Gray species

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ABSTRACT. *Chamaesyce prostrata* (Aiton) Small, *Euphorbia heterophylla* L. and *Euphorbia graminea* Jacq. are weedy species that occur with relative frequency in the region of Maringá, Paraná State, Brazil. Data about the morphology and anatomy of seedlings of these species were obtained, with emphasis on significant structural differences of seedlings between species of *Euphorbia* and *Chamaesyce*. Seedlings are phanerocotylar and epigeal. Root is axial and triarch. Cotyledons are leaf-like and dorsiventral. Eophylls and prophylls are simple and dorsiventral. Morphological variations occur in the cotyledon shape of *Euphorbia* seedlings. It is remarkable the Kranz structure of leaves that occurs only in *Chamaesyce*.

Keywords: weeds, root, hypocotyl, cotyledons, epicotyl, eophylls.

Estrutura da plântula de espécies de *Euphorbia* L. e *Chamaesyce* Gray

RESUMO. *Chamaesyce prostrata* (Aiton) Small, *Euphorbia heterophylla* L. e *E. graminea* Jacq. (Euphorbiaceae) são espécies invasoras frequentes na região de Maringá, Paraná. Foram obtidos dados sobre a morfologia e anatomia das plântulas dessas espécies, com ênfase se há caracteres estruturais significativos das plântulas entre as espécies de *Euphorbia* e *Chamaesyce*. As plântulas são fanerocotiledonares e epígeas. A raiz é axial e triarca. Os cotilédones são foliáceos e dorsiventrais. Os eofilos e profilos são folhas simples e dorsiventrais. As variações mofológicas ocorrem no formato dos cotilédones das plântulas de *Euphorbia*. Sob o aspecto anatômico é especialmente notável a estrutura Kranz das folhas que ocorrem somente em *Chamaesyce*.

Palavras-chave: plantas invasoras, raiz, hipocótilo, cotilédones, epicótilo, eofilos.

Introduction

The subtribe Euphorbiinae contains genera that are universally accepted, as *Euphorbia* L., *Endadenium* L.C. Leach, *Monadenium* Pax, *Synadenium* Boiss., *Pedilanthus* Neck. ex Poit., and *Cubanthus* (DC.) Millsp., and genera that are frequently less accepted, as *Chamaesyce* Gray, *Elaeophorbia* Stapf, and *Poinsettia* Graham. Euphorbiinae with approximately 2000 species is dominated by *Euphorbia*, which accounts for around 80% of species and occurs throughout the geographic range of the subtribe. With about 300 species, *Chamaesyce* is the largest segregate genus from *Euphorbia*, and has a wide distribution, but most species are confined to the New World (STEINMANN; PORTER, 2002).

Chamaesyce species are distinguished with basis on its vegetative morphology, with cyathia nearly identical to those of many species of *Euphorbia* subg. *Agaloma* (Raf.) House (STEINMANN; PORTER, 2002). *Chamaesyce* is characterized by many unusual synapomorphies: apical abortion of the main shoot and subsequent sympodial growth; interpetiolar stipules; opposite and frequently asymmetrical leaves; and C_4 photosynthesis (KOUTNIK, 1984, 1987).

A study on seedling structure of *Euphorbia* and *Chamaesyce* species would help in the identification of these species and is also likely to be useful in the separation or not of both genera. Therefore, this research deals with morphology and anatomy of seedlings of the weedy species *Chamaesyce prostrata* (Aiton) Small, *Euphorbia heterophylla* L. and *E. graminea* Jacq., which occur with relative frequency in the region of Maringá, Paraná State, Brazil. In this analysis it was emphasized significant structural differences of seedlings between species of *Euphorbia* and *Chamaesyce*.

Material and methods

Seeds of the three species were collected at the campus of the State University of Maringá, Paraná State, Brazil. Voucher materials were deposited at the UEM Herbarium, collection numbers: *C. prostrata* – 19464 HUEM, *E. graminea* – 19462HUEM and *E. heterophylla* – 14505HUEM.

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

The materials (root, hypocotyl, cotyledons, epicotyl, eophylls and prophylls) were fixed in glutaraldehyde (1% in 0.1 M phosphate buffer, pH 7.2) (KARNOVSKY, 1965) and later transferred to ethyl alcohol 70%, following the protocol of Johansen (1940). The material was embedded in hydroxymethacrylate (GERRITS, 1991), sectioned (cross- and longitudinal sections) in an American Optical rotation microtome, and stained in Toluidine Blue (O'BRIEN et al., 1964). Seedlings were also analyzed in freehand sections stained in Astra Blue and Safranin (SOUZA et al., 2005).

Photographs were taken with the stereomicroscope Leica EZ4D and microscope Olympus BX50 with digital camera Canon Power Shot A95, and subsequently prepared using the software Zoom Browser EX 4.6. All samples were prepared on the same micrometric scale.

Results and discussion

Seedling morphology

Seedlings of the weedy species (Figure 1) are phanerocotylar and epigeal. Root system is axial and the hypocotyl is long, cylindrical, and green/red. Cotyledons (Figure 1) are thin, green (purple in *C. prostrata*), petiolate and leaf-like. Cotyledons have different shape among species: oblong with obtuse apex and base, entire margin in *C. prostrate* (Figure 1A); orbicular with rotundate apex and base, entire margin in *E. graminea* (Figure 1B); and broadly lanceolate with acute apex and base, of entire margin in *E. heterophylla* (Figure 1C).

Seedlings of Euphorbiaceae tropical trees are usually phanerocotylar with long-petiolate cotyledons (DUKE, 1969); these characters may be extended to the studied weed species with herbaceous habit. Leaf-like and photosynthetic cotyledons at least in a number of exemplars are considered by Vogel (1980) as homologous with the lowermost stem leaves, and not directly with haustorial or foodstoring cotyledons. In still agreement with this author, it is better to avoid the term cotyledon for this sort of primary seedling leaves, and apply another term: paracotyledons.

Absence of epicotyl was recorded in seedlings of *C. prostrata*, in which two buds are present in the cotyledonary axils, from the two non-equivalent shoots developed (Figure 1A). The abortion of the main shoot and subsequent sympodial growth in the adult plant was registered by Koutnik (1984) as a unusual synapomorphy. In the other two studied species the epicotyl is green and cylindrical, with edges only in *E. graminea*.

Seedlings of *E. graminea* and *E. heterophylla* present eophylls, while *C. prostrata* do not. Eophylls (Figure 1B and C) of two species have stipules, petiole, and the leaf blade shows lanceolate-ovate shape, of acute apex, acute or obtuse base and entire margin. The *C. prostrata* prophylls (Figure 1A) of two shoots that develop in the cotyledonary axils are not stipulate; they have green or dark red color, with obovate shape, obtuse or retuse apex, acute base, and entire and crenate margin only in the apical region.

Seedlings of *C. prostrata*, *E. graminea* and *E. heterophylla* may be included into the 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 are all spirally arranged. Seedlings of *C. prostrata*, *E. graminea* and *E. heterophylla* may also be classified as PEF (Phanerocotylar, Epigeal, Foliaceous) (GARWOOD, 1996), considering the persistent foliaceous cotyledons and the pioneering behavior of weeds (LORENZI, 2008).

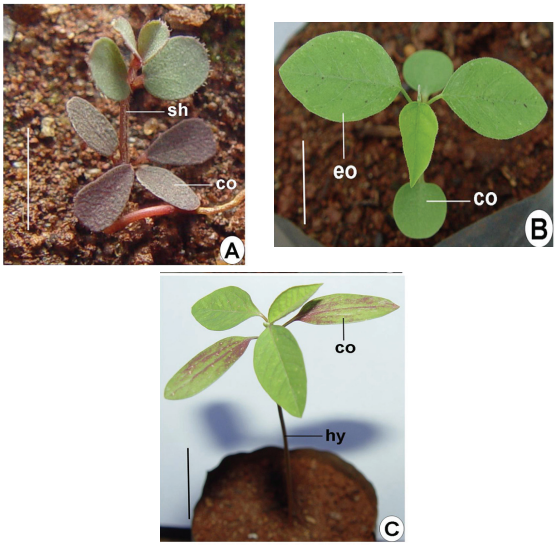

Figure 1. Seedling morphology of *C. prostrata* (A), *E. graminea* (B) and *E. heterophylla* (C). (co = cotyledon; eo = eophyll; hy = hypocotyl; sh = shoot). Bars = 0.5 cm (A), 2 cm (B), 3 cm (C).

Seedling anatomy

Primary root is triarch (Figure 2A) and the structure quite similar among the three species. The epidermis is uniseriate with thin-walled cells and

root hairs (Figure 2A and B). The cortex (Figure 2A and B) consists of exodermis that may be collapsed in *C. prostrata* and *E. graminea*, pluriseriate parenchyma and endodermis with Casparian strips. Central cylinder is composed of uniseriate parenchymatous pericycle and three strands of primary xylem and phloem (Figure 2A and B). A vascular cambium arises very early in the seedling.

The vascular cylinder of the primary root of seedlings is commonly diarch or tetrarch; other types seem to represent modifications of these basic types (EAMES, 1961). The triarchy observed in *Chamaesyce* and *Euphorbia* species seem to be slightly frequent in dicotyledon according to the author. The literature registers triarch roots in *Ormosia* Jacks. (Fabaceae) (GURSKI et al., 2012), *Arrabidaea mutabilis* Bureau and K. Schum. (Bignoniaceae) and *Vismia guianensis* (Aubl.) Pers. (Hypericaceae) (SOUZA, 2009).

The root/stem transition region of the three species (Figure 2C) is short and starts in the base of the root. In the base of the hypocotyl occurs the change of the exarch, typical condition of the root to the endarch one, and the arrangement of xylem and phloem assumes the collateral position (Figure 2D and E). In *E. graminea* and *E. heterophylla* three strands of primary xylem and phloem coming from the root separate into six collateral vascular bundles in the hypocotyl. Similar to the other two species, *Chamaesyce prostrata* has the same hypocotyledonary vascular structure, but may present three or four collateral bundles.

The level of the transition from the exarch to the endarch condition of the three Euphorbiaceae species is usually low according to the Compton (1912) classification. The low and intermediate types of transition seem to be common of the families Amaranthaceae, Annonaceae, Bignoniaceae, Cactaceae, Fabaceae and Hypericaceae (SOUZA, 2009).

The hypocotyl epidermis (Figure 2D and E) is uniseriate and contains stomata; the non-glandular trichomes occur in *C. prostrata* and *E. heterophylla*. The hypocotyl cortex (Figure 2D and E) shows parenchyma and eventually collenchyma; there is no endodermis with Casparian strips. The central cylinder consists of collateral vascular bundles (Figure 2D and E) which enclose the parenchymatous pith. The pith is partially destroyed in *E. graminea* (Figure 2D) and *E. heterophylla*, while in *C. prostrata* it is complete and reduced.

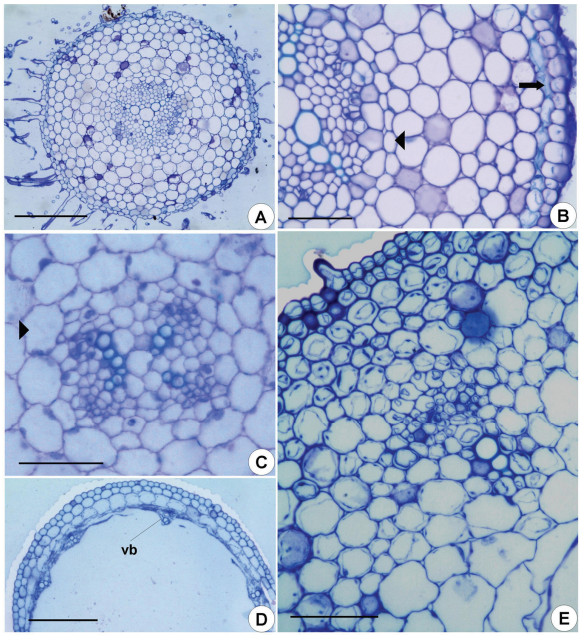

Figure 2. Root and hypocotyl structure of *C. prostrata* (C), *E. graminea* (D) and *E. heterophylla* (A, B, E), in cross-sections. A, B. Root; black arrow and arrow head indicate exodermis and endodermis, respectively. C. Root and stem transition region; black arrow head indicates endodermis. D, E. Hypocotyl (vb=vascular bundle). Bars = 50 μm (C), 70 μm (B, E), 200 μm (D), 300 μm (A).

Cotyledons of the three Euphorbiaceae species have three leaf traces (Figure 3A). The trilacunar condition seems to be uncommon among the studied seedlings (SOUZA, 2009). Bailey (1956) registered that of the 99 dicotyledon families studied, 77% of seedlings have an even number of traces at the cotyledonary node, and 60% of cotyledons have two independent traces related to a single gap.

The epicotyl of both species of *Euphorbia* has the stem structure (Figure 3B). The epidermis is uniseriate and the cortex presents parenchyma and collenchyma (Figure 3B). Numerous collateral vascular bundles occur in the central cylinder of *Euphorbia* (Figure 3B), approximately 12 in E. graminea and 16 in *E. heterophylla*. The parenchymatous pith of *E. heterophylla* may be partially destroyed. Seedling of *C. prostrata* are devoid of epicotyl, made up of stem (Figure 3C) with uniseriate epidermis presenting thick-wall cells and non-glandular trichomes, parenchymatous and collenchymatous cortex, central cylinder with six or seven collateral vascular bundles, and parenchymatous pith.

Cotyledons (Figure 4A and C) of the three species consist of uniseriate, glabrous and amphistomatic epidermis, and dorsiventral leaf with a layer of palisade parenchyma and bi- or multiseriate spongy parenchyma.

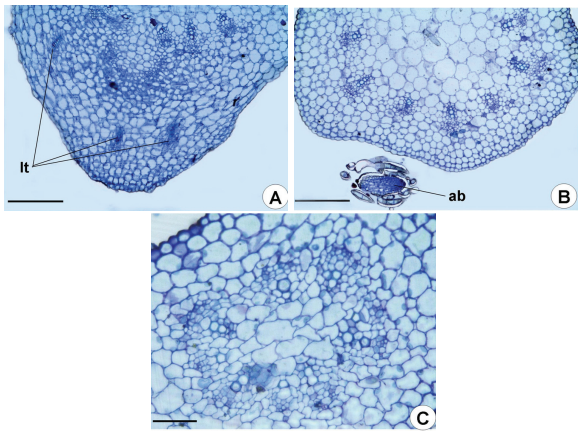

Figure 3. Structure of the aerial axis of *C. prostrata* (C) and *E. graminea* (A, B) seedlings, in cross-sections. A. Cotyledonary node. B. Epicotyl. C. Shoot. (ab=bud in the axil of the cotyledon; lt=leaf traces of the cotyledon). Bars=250 μm (A, B), 40 μm (C).

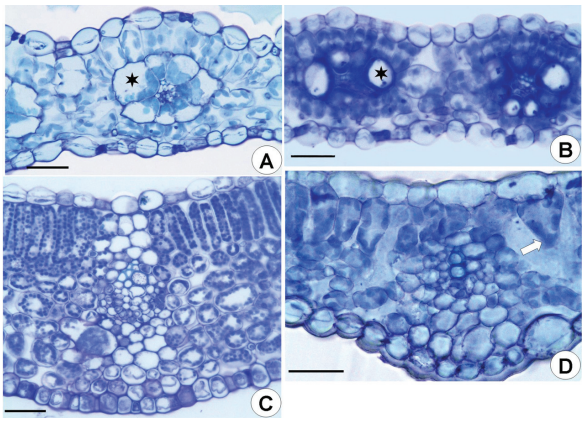

Figure 4. Leaf structure of *C. prostrata* (A, B) and *E. heterophylla* (C, D) seedlings, in cross-sections. A, C. Cotyledons. B, D. Prophyll and eophyll. Asterisk indicates bundle sheath with cells that radiate away from the sheath (Kranz structure); arrow indicates funneled cell. Bars = 40 μm (A), 70 μm (B, C, D).

Typical palisade parenchyma is wanting in *C. prostrata* in which cells are commonly elongated but much larger, except in the vein regions (Figure 4A). Small and medium-sized veins are immersed in the mesophyll and present endodermis (bundle sheath). Cotyledons of *C. prostrata* present Kranz-type leaf anatomy with prominent bundle sheath cells and a layer of radial mesophyll cells that surrounds the vascular bundles (Figure 4A).

Eophylls (Figure 4B) of both *Euphorbia* species consist of uniseriate and amphistomatic epidermis; multicellular non-glandular trichomes are present in *E. graminea*, never in *E. heterophylla*. Mesophyll is dorsiventral and made up of a palisade parenchyma layer in *E. graminea*, and parenchyma with funneled cells in *E. heterophylla* (Figure 4D). The structure of *C. prostrata* prophylls resembles the cotyledons, besides presenting Kranz structure (Figure 4B)

The Kranz type of leaf anatomy, observed in cotyledons and prophylls of *C. prostrata*, has been registered in Euphorbiaceae (FAHN, 1990; LAETSCH, 1974). For Koutnik (1984) *Chamaesyce* is characterized by unusual synapomorphies, among them the C_4 photosynthesis (Kranz structure).

The current taxonomic trend is going towards the split of the genus *Euphorbia* (STEINMANN; PORTER, 2002). This has already been observed with the removal of *Chamaesyce* by Webster (1967). The present study points out few differences among the seedlings of both genera. Morphological variations occur in the cotyledon shape in the seedlings of *Euphorbia*, and epicotyl and eophylls are entirely lacking in *Chamaesyce* seedlings. Under the anatomical aspect it is especially remarkable the Kranz structure of the leaves that occurs only in *Chamaesyce*. Other potentially significant seedling anatomical features for species characterization are listed in Table 1.

Table 1. Other seedling anatomical features potentially significant for the characterization of *Chamaesyce prostrata* (Aiton) Small, *Euphorbia graminea* Jacq. and *E. heterophylla* L.

Characters Species	*C. prostrata*	*E. graminea*	*E. heterophylla*
Root exodermis	Collapsed	Collapsed	Non collapsed
Hypocotyledonary epidermis	Non-glandular trichomes	Absent	Non-glandular trichomes
Number of bundles in the hypocotyl	Three or four	Six	Six
Hypocotyledonary pith	Complete	Partially destroyed	Partially destroyed
Number of bundles in the epicotyl/shoot	Six or seven	Twelve	Sixteen
Epicotyledonary pith	Complete	Complete	Partially destroyed
Typical palisade in cotyledons	Wanting	Present	Present

Conclusion

The study showed morphological variations in the cotyledon shape in *Euphorbia* seedlings. Epicotyl and eophylls are lacking in *Chamaesyce* Kranz structure just occurs in *Chamaesyce.*

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