

## COMPARATIVE ANATOMICAL ASPECTS OF *EUPHORBIA MILLI* VAR. *SPLENDENS* (BOJER EX HOOK.) URSCH & LEANDRI AND *EUPHORBIA PULCHERRIMA* WILLD. EX KLOTZSCH SPECIES LEAVES

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Received 28 September 2016; accepted 5 December 2016

### ABSTRACT

The paper presents a comparative study concerning the leaf structure of two *Euphorbia* species belonging to Euphorbiaceae family: *Euphorbia milli* var. *splendens* (Bojer ex Hook.) Ursch & Leandri and *Euphorbia pulcherrima* Willd. ex Klotzsch. Anatomically, the leaves of the studied species are quite similar in the basic structure. The petiole has a single-layered epidermis, a collenchyma tissue – hypodermis - and three collateral bundles embedded in a basic tissue. Differences occur concerning the relationship between the collateral bundles. The *Euphorbia pulcherrima* bract petiole has almost the same structure as those of the leaf petiole. The blade is amphistomatic for *Euphorbia milli* var. *splendens* and hypostomatic for *E. pulcherrima*. The heterogeneous mesophyll is isobilateral in *Euphorbia milli* var. *splendens* blade and bifacial in *E. pulcherrima*. The vascular system of the mid rib is represented by one collateral bundle for both species, more developed in *Euphorbia milli* var. *splendens* blade. Differences appear concerning the epidermal cells cuticle thickness, the type of mesophyll, the abundance of the non-articulated laticifers and the development of the vascular system. The *Euphorbia pulcherrima* bract has the same organization plan structure as the leaf blade but some features differences occur.

**KEY WORDS:** anatomy, comparative, leaf, laticifers, *Euphorbia*

### INTRODUCTION

The studied species belong to Euphorbiaceae family, one of the largest and most complex genera in the world of flowering plants, sometimes known as spurges. It includes around 300 genera and 7.700 species, of which 870 are regarded as succulents herbs, shrubs and trees found in temperate, sub-tropical and tropical climates (Armstrong, 2003; Niculescu, 2009). The spurge family takes its name from the genus *Euphorbia* (Webster, 1987). Many species are more or less succulent and growing mostly in the tropical and subtropical regions of the world (Carter, 2002). Concerning *Euphorbia* genus, several botanists have made unsuccessful attempts to subdivide the genus into numerous smaller genera. According to the recent phylogenetic studies, *Euphorbia* can be divided into 4 subgenera, each containing

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several not yet sufficiently studied sections and groups (Bruyns *et al.*, 2006; Steinmann & Porter, 2002; Steinmann *et al.*, 2007).

Recent researches of Kenneth Wurdack, Benjamin van Ee and their contributors, in 2012, show clear support for the following relationships among the four main clades of *Euphorbia*: clade B (subgenus *Esula*) is the sister group to clade A (subgenus *Rhizanthium*) and that is in turn sister to both clades C and D (subgenus *Euphorbia* and subgenus *Chamaesyce*) (Horn *et al.* 2012). *Euphorbia* species (Euphorbiaceae family) contain poisonous milky-sap – latex - content in secretory structures named laticifers. Secretory structures are of great taxonomical interest and their restricted distribution has an important diagnosis value (Fahn, 1979; Metcalfe & Chalk, 1966; Batanouny, 1992).

*Euphorbia milli* var. *splendens* (Bojer ex Hook.) Ursch & Leandri is a variety of *E. milli*, commonly named crown of thorns, Christ plant or Christ thorn, native to Madagascar. It seems that the plant's scientific name was given in honor of Baron Pierre Bernard Millius, and then governor of Bourbon islands of French Guiana, which introduced this species in France in 1821 (Ombrello, 2009).

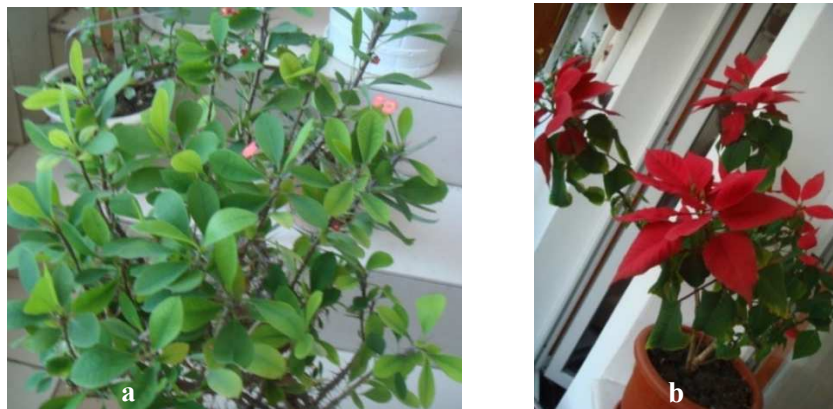


FIG. 1. Natural view of potted *Euphorbia milli* var. *splendens* (a) and *Euphorbia pulcherrima* (b).

It is a woody succulent perennial plant. The woody stems are greyish brown, branched, with prominent grey sharp spines, covering the stem and reaching up to 1.2 m. The leaves are obovate in shape and green, arranged in spiral (Fig. 1, a). The small female flower are surrounded by male flowers in a cup-shaped series of bracts known as a cyathium, subtended by a pair of conspicuous petal-like bracts, variably red, pink or white, up to 12 mm broad (Huxley *et al.*, 1999).

*Euphorbia pulcherrima*, known commonly as Christmas star, is small deciduous shrub up to 4 m tall in its native regions (Southern Mexico and Guadalupe), with few stout, hairless branches. The long petiolate, lobate, slightly denticulate leaves are relatively thin, usually 10–15 cm long with pointed tips. The male and female flowers grouped in cyathia which are surrounded by red bracts. Each cyathium bears a two-lipped, yellow gland. Flowers are borne at the stem tips in winter. It is known in our country and Europe as a pot plant but it can be grown directly in the soil in the greenhouse to produce floral stems (Fig. 1, b) (Minaune, 2004).

In literature information concerning the morphology and anatomy of a large number of *Euphorbia* species dated from the 19th century. The most ample study which analyzes the anatomical structure of the vegetal organs of species of Euphorbiaceae family belongs to Gaucher (1902). Succinct data on the structure of the vegetative organs of some *Euphorbia* species are found in some general studies concerning the angiosperms anatomy (Bonnier & Leclerc du Sablon, 1905; Dilcher, 1974; Esau, 1965; Metcalfe & Chalk, 1950; Napp-Zinn, 1973, 1974; Fahn, 1990; Jafari & Nasseh, 2009). Studies' concerning the non-articulated laticifers referring to their formation, development and distribution in some *Euphorbia* species belong to Mahlberg and Sabharwal (1968) in *Euphorbia marginata*, Metcalfe (1966) and Fahn (1990). A comparative study of the starch grains found in latex of different varieties of *Euphorbia pulcherrima* species was made by Mahlberg (1982). Some ultrastructure aspects of the primary sieve cells elements in *Euphorbia pulcherrima* belong to Thorsch and Esau (1988). Some studies of the leaf anatomy, especially the blade epidermises, of some *Euphorbia* species are those of Sehgal and Paliwal (1974), Stace (1984), Hassin *et al.* (2004), Essiett *et al.* (2012).

In Romania a most ample study concerning the morphology, anatomy and distribution of laticifers of 13 *Euphorbia* spontaneous species from our country belong to Galeș and Toma (2006, 2007). General mentions about *Euphorbia* laticifers occur in some lectures and manuals of Anatomy and Morphology of Plants (Serbanescu-Jitariu & Toma, 1980; Grintescu, 1985; Toma & Gostin, 2000; Bavaru & Bercu, 2002; Niculescu, 2004; Ianovici, 2010).

The purpose of this paper is to highlight the similarities and differences of the leaf anatomical features of two *Euphorbia* species with ornamental value namely *Euphorbia milli* var. *splendens* and *Euphorbia pulcherrima*.

## MATERIALS AND METHODS

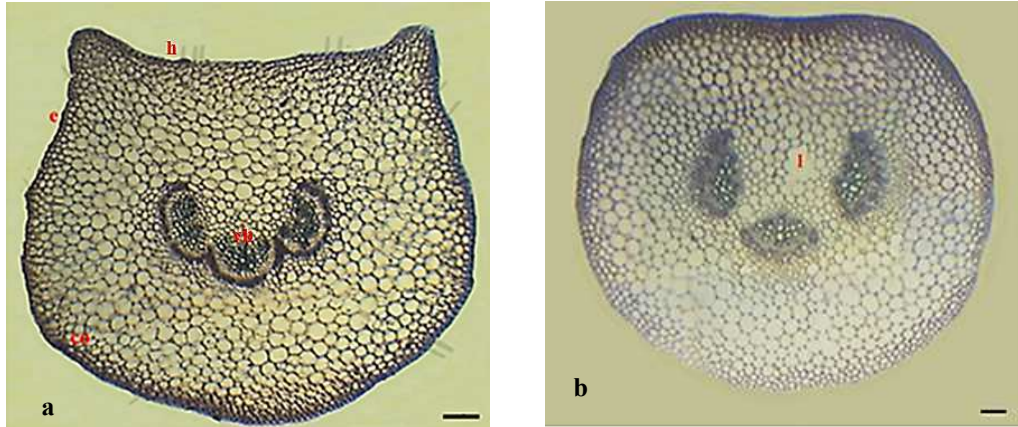
The mature leaves and inflorescence bracts, of *Euphorbia milli* var. *splendens* and *Euphorbia pulcherrima*, were collected from SC "Iris" International SRL greenhouse in December 2015. Small pieces of mature leaves were fixed in FAA (formalin: glacier acetic acid: alcohol 5:5:90). Cross sections of the leaf (petiole and blade) and bract were performed by hand made technique (Bercu & Jianu, 2003). The samples were stained with alum-carmin and iodine green and embedded in glycerinated gelatin. Anatomical observations and micrographs were performed with a BIOROM-T bright field microscope, equipped with a TOPICA 6001A video camera.

## RESULTS AND DISCUSSIONS

The petiole in transection has a semicircular shape determined by the presence of two lateral wings for *Euphorbia milli* var. *splendens* and circular for *E. pulcherrima* (Fig. 2, a, b). The one layered epidermis, in both species, presents slightly elongate cells for *Euphorbia milli* var. *splendens* and more or less isodiametric cells for *E. pulcherrima*, with external walls protected by a thick cuticle. It is interrupted by rare stomata for both species and rare simple short unicellular hairs for *Euphorbia milli* var. *splendens*.

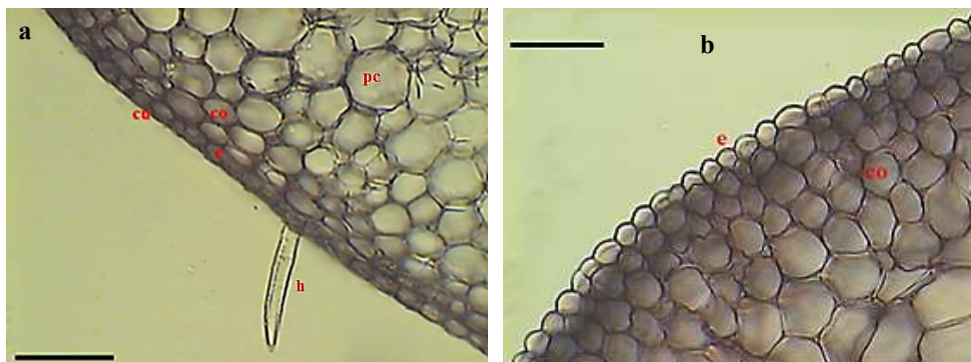
The epidermis is followed by a hypodermis represented by a collenchyma tissue with chloroplasts inside their cells. This mechanical tissue consists of 2-3 layers of cells in *Euphorbia milli* var. *splendens* and more developed (5-6 layers of cells) in *E. pulcherrima*.

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**FIG. 2.** Cross sections of *Euphorbia milli* var. *splendens* (a) and *E. pulcherrima* (b) petiole – ensemble: e- epidermis, co- collenchyma, h- hair, l- lacuna, vb- vascular bundle. Scale bar: 52  $\mu\text{m}$ ; 50  $\mu\text{m}$ .

The vascular system is represented by three collateral bundles embedded in a parenchyma tissue. The vascular bundles of *Euphorbia milli* var. *splendens* are close united by their phloem and sclerenchyma whereas those of *E. pulcherrima* are separated by the parenchyma cells of the basic tissue. The lateral vascular bundles are more developed than the abaxial one (Fig. 3, a, b).



**FIG. 3.** Cross sections of the petiole with epidermis and cortex of *Euphorbia milli* var. *splendens* (a) and *E. pulcherrima* (b) – details: e- epidermis, co- collenchyma, cu- cuticle, h- hair, pc- parenchyma cell. Scale bar: 45  $\mu\text{m}$ .

The vascular bundles are represented by xylem and phloem. Xylem consists of xylem vessels and xylem parenchyma to the epidermis and phloem elements (phloem vessels, companion cells and phloem parenchyma) to the center (Fig. 4, a, b).

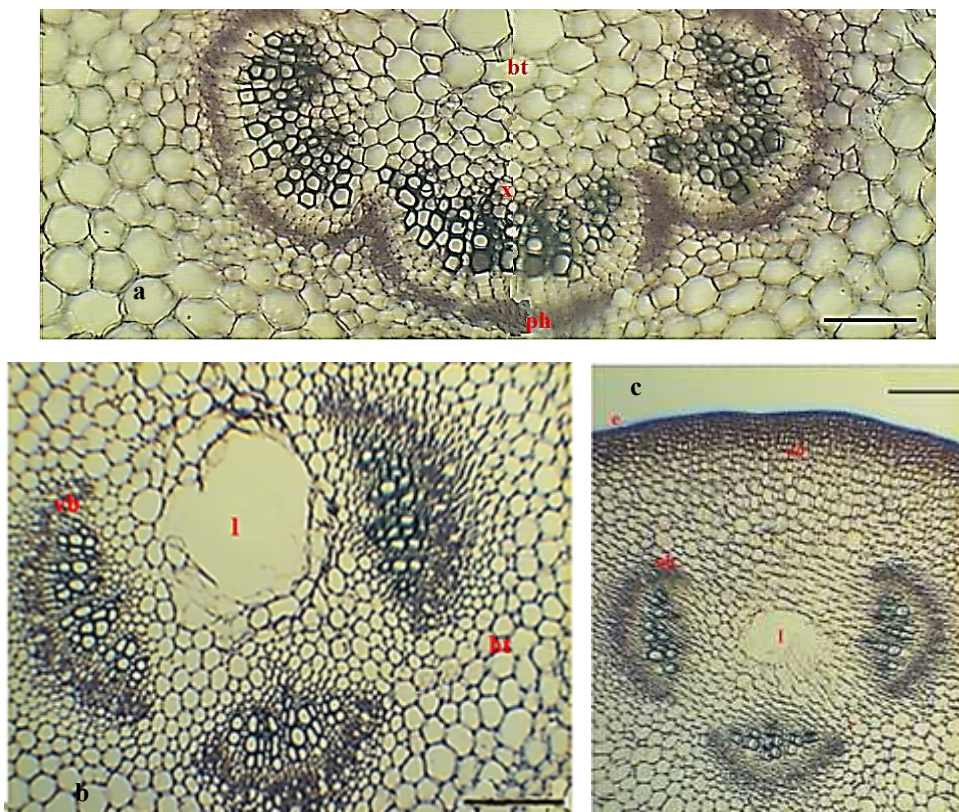


FIG. 4. Cross section of the petiole with vascular system of *Euphorbia milli* var. *splendens* (a) and *E. pulcherrima* (b). Cross section of the bract petiole of *E. pulcherrima* (c): bt- basic tissue, co – collenchyma, e- epidermis, l- lacuna, vb- vascular bundle. Scale bar: 45  $\mu$ m.

According to Esau (1965) and Hagel *et al.* (2008), the genus *Euphorbia* presents non-articulated, branching and non-anastomosing. The non-articulated laticifers begin their growth from the meristematic tissue of the embryo and are characterized by multi-nucleated cells and their intrusive apical growth (Mahlberg & Sabharwal 1968; Mahlberg, 1993). In our study, non-articulated and non-anastomosing laticifers are observed in the periphloemic region of *Euphorbia milli* var. *splendens* whereas those of *E. pulcherrima* are present both in the vicinity of phloem and few inside it (Fig 4, a, c). Laticifers are specialized cells with an exudate, a latex or milky sap. The latex consists of various chemical substances such as a complex solution of suspended particles, which are useful economically and bio-medicinally (Fahn, 1979). The non-articulated laticifers differentiation in *Euphorbia pulcherrima* has been studied by Fineran (1983) suggesting that, during the differentiation of laticifers, a large central vacuole develops and the latex particles (osmiophilic globules), which have been arising in small vacuoles (in the second-leaf growth seedling stage), are then released into this large central vacuole (formed by

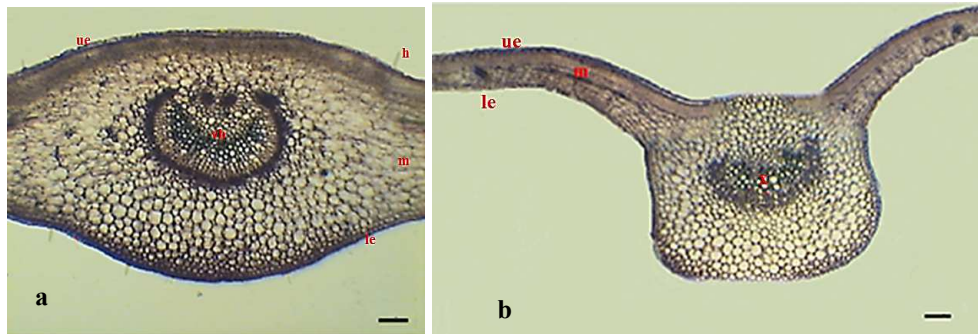
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the fusion of the small vacuoles). The matrix of the latex particles may be originated from the vacuolar sap (Fineran, 1983).

Even if the bract belongs to the reproductive parts of a plant, being a modified leaf, anatomically in *Euphorbia pulcherrima* it is more similar to a leaf than those of the other *Euphorbia* species. An anatomical comparison between these two vegetal organs (leaf and bract) is interesting. However, it must be noted that the petiole bracts of *Euphorbia pulcherrima*, structural organization is the same as those of the leaf petiole, but with smaller vascular bundles. Centrally, both in *Euphorbia pulcherrima* leaf and bract petiole an air lacuna is present (Fig. 4. c).

Cross sections of the blade of the two species, exhibits the usual succession of tissues (an upper and lower epidermis and the mesophyll with vascular bundles embedded). Such as in other *Euphorbia* species (Gales & Toma, 2006; Bercu 2015), both upper and lower epidermis, in are composed of more or less isodiametric cells (in *Euphorbia milli* var. *splendens*) and tangentially slightly elongated cells covered by cuticle (*Euphorbia pulcherrima*). The epidermis cells of *Euphorbia pulcherrima* are papillose as Kakkar and Paliwall (1974) confirmed for other *Euphorbia* species (e.g. *Euphorbia myrsinites*). The epidermal cells in *Euphorbia milli* var. *splendens* is not papillose, being covered by an epicuticle wax.

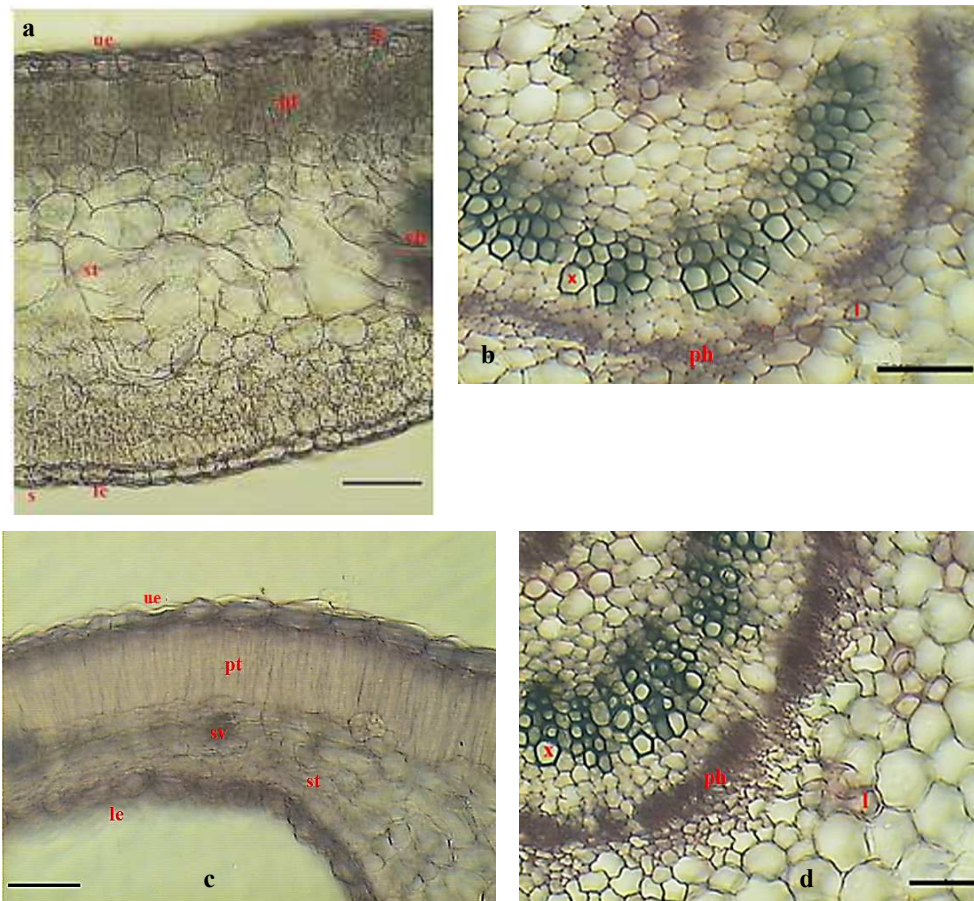
The one layered upper and lower epidermises of *Euphorbia milli* var. *splendens* is interrupted by the presence of stomata with evidently sub-stomatal cavities, whereas those of *E. pulcherrima* are present only in between the lower epidermis cells and have smaller sub-stomatal cavities. Both epidermises of *Euphorbia milli* var. *splendens* possess rare hairs with the same as those of the petiole. In the perixylemic and periphloemic zone, few non-articulate laticifers are present. The lower epidermis forms a slightly prominence to the adaxial and abaxial in the mid rib zone for *Euphorbia milli* var. *splendens* and a large prominence to the abaxial zone in *E. pulcherrima* blade (Fig. 5, a, b).



**FIG.. 5.** Cross section of the blade in the mid rib zone of *Euphorbia milli* var. *splendens* blade (a) and *E. pulcherrima* (b) – ensamble: h- hair, le- lower epidermis, m- mesophyll, ue- upper epidermis, vb- vascular bundle. Scale bar: 50  $\mu$ m.

The mesophyll is heterogeneous in *Euphorbia milli* var. *splendens*, the palisade tissue occurring adaxial and abaxial (two layers of cells) beneath the two epidermises, whereas in *E. pulcherrima* is one layer of palisade tissue with more or less slightly elongated cells below the

upper epidermis. The palisade tissue of *Euphorbia milli* var. *splendens* mesophyll is reduced to a single layer of cells in the mid rib zone while in *E. pulcherrima* is interrupted by the presence of collenchyma cells (Fig. 6, a, c).



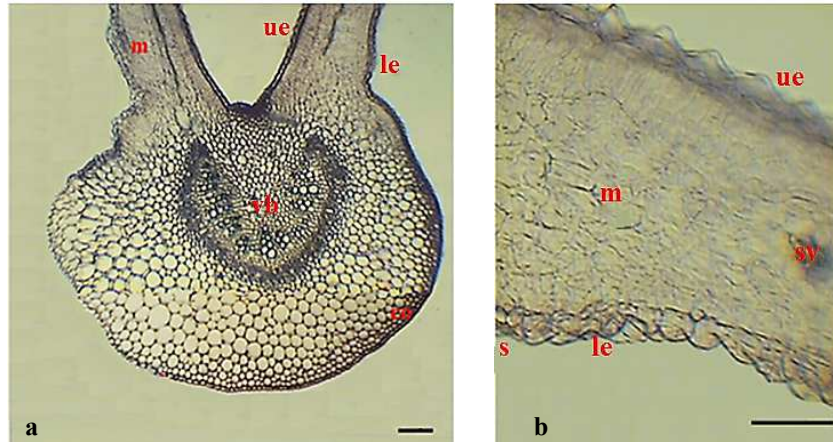
**FIG. 6.** Cross sections of the blade. Portion with mesophyll of *E. milli* var. *splendens* (a) and a vascular bundle (b). Portion with mesophyll of *E. pulcherrima* (c) and vascular bundle (d): l- laticifers, le- lower epidermis, ph- phloem, pt- palisade tissue, s- stoma, st- spongy tissue, sv- secondary vein, ue- upper epidermis, vb- vascular bundle, x- xylem. Scale bar a, b, d 45  $\mu$ m; c, 40  $\mu$ m.

The spongy tissue is composed by several numbers of round-oval cells in both species mesophyll. Differences appear concerning the number of spongy tissues layers, 5-6 for *Euphorbia milli* var. *splendens* and 4-5 for *E. pulcherrima*, with small intercellular spaces. The air spaces of *Euphorbia pulcherrima* are smaller than those of *E. milli* var. *splendens* (Fig. 6, a, c).

In the mesophyll are embedded the collateral bundles of the mid rib and the secondary veins. The mid rib collateral bundle is well developed in both species. They are represented by

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a single open arc vascular bundle more developed in *Euphorbia milli* var. *splendens*, with the same petiole bundles structure but with foliar arrangement of the conductive tissues. In periphloemic zone occur laticifers as well as in mesophyll with thicker wall than that of adjacent cells (Fig. 6, b, d).



**FIG. 7.** Cross sections of the bract mesophyll of *Euphorbia pulcherrima*. Ensemble (a). Portions with mesophyll (b): co – collenchyma, ph- phloem, l- laticifer, le- lower epidermis, m- mesophyll, s- stoma, ue- upper epidermis, vb- vascular bundle, x- xylem. Scale bar: a 50, b 45  $\mu$ m.



**FIG. 8.** Cross sections of the bract mesophyll of *Euphorbia pulcherrima* – a vascular bundle: ph- phloem, x- xylem. Scale bar: 45  $\mu$ m.

The bract mesophyll of *Euphorbia pulcherrima* has the same organization plan structure as the leaf blade but some differences occur. The upper and lower epidermis is papillose. The lower epidermis in the mid rib zone forms a larger prominence than those of the blade and a very small one in adaxial position (Figs. 7, a, b) The mechanical tissue of the mid rib zone is poor developed such as the vascular central bundle which is represented by few xylem and phloem elements (Fig. 8). Rare stomata with small sub-stomatal cavities are present to the lower epidermis. The mesophyll is almost homogenous with small air spaces (Fig. 7, b). The epidermis and mesophyll cells contain anthocyanins which gives red color to the bract.



## CONCLUSIONS

The studied *Euphorbia* species reveals both similarities and differences concerning their anatomical structure. The petiole has a single-layered epidermis covered by cuticle, a colenchymatous hypodermis and three collateral bundles embedded in a basic tissue. Differences occur concerning the relationship between the collateral bundles. The *Euphorbia pulcherrima* bract petiole has almost the same structure as the leaf petiole.

The blade of the two species has papillose epidermal cells in *Euphorbia pulcherrima* and not papillose in *E. milli* var. *splendens*. The blade is amphistomatic in *Euphorbia milli* var. *splendens* and hypostomatic in *E. pulcherrima*. The heterogeneous mesophyll is isobilateral in *Euphorbia milli* var. *splendens* blade and bifacial in *E. pulcherrima*. The vascular system of the mid rib is represented by one collateral bundle for both species, more developed in *Euphorbia milli* var. *splendens* blade. The *E. pulcherrima* bract has the same organization plan structure as the leaf blade but some features differences are present.

The secretory elements are represented by non-articulated laticifers present in petiole and blade of both species, the later mainly in the mid rib area. They are more abundant in *Euphorbia milli* var. *splendens*. The mechanical tissue is represented by collenchyma tissue which occurs in petiole and blade as well (in mid rib zone) for both studied species.

## ACKNOWLEDGEMENTS

We thank to Miss dr. Elena Bavaru, manager of SC Iris International SRL Greenhouse for kindly donating the plant material of *Euphorbia milli* var. *splendens* and *Euphorbia pulcherrima* for experimental use.

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