

COMPARATIVE ANATOMY OF THE VEGETATIVE ORGANS OF SOME *EUPHORBIA* SPECIES (*EUPHORBIACEAE* JUSS.) FROM THE ROMANIAN FLORA

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This article compares the (subterranean and aerial) vegetative organs anatomy of 19 *Euphorbia* species from the Romanian flora, focusing on identifying the constant and variable histo-anatomical characters. The root structure differed by the type of primary stele structure and the secondary xylem organization. The rhizome characters which best distinguished among perennial taxa were: the absence or the presence of a peridermis, the secondary xylem organization and the pith conformation. In the stem the best diagnostic characters were: the pattern of epidermis cells, the presence or the absence of trichomes, the cortex organization and the repartition mode of secondary conducting tissues. The foliar limb structure differed by the pattern of epidermis cells, the mesophyll organization and the localization of stomata. The constant histo-anatomical characters of the taxa taken into study were: laticifers in all the vegetative organs, cordons of periphloemic sclerenchymatous fibers in the stem, stomata of anisocytic and anomocytic-type in the foliar limb, libriform gelatinous fibers, tangentially collenchyma at the periphery of the stem cortex. Anomalous structures occurred in a few taxa.

Key words: *Euphorbia*, vegetative organs, anatomy, laticifers.

INTRODUCTION

Following up our histo-anatomical researches on the vegetative organs of some *Euphorbia* taxa (Rotari, 2004; Galeș & Toma 2006), this paper presents the comparative anatomy of the root, rhizome, aerial stem and foliar limb of 19 *Euphorbia* taxa from the Romanian flora.

The existing literature on the anatomy of *Euphorbia* species is quite rich, if considering the researches on the origin, development and structure of non-articulated laticifers as well as on the cyathia structure and it might be considered poor, if one takes into account the papers exclusively devoted to the anatomy of the vegetative organs of the species from this genus.

The most ample paper which analyzes the structure of the stem and leaf of species from the *Euphorbiaceae* family is that of Gaucher (1902). Succinct references on the structure of the vegetative organs of some *Euphorbia* species are found in some general treatises which analyze the angiosperms anatomy (G. Bonnier & Leclerc du Sablon, 1905; Metcalfe and Chalk, 1950; Esau, 1965; Napp-Zinn, 1973, 1974).

Most of the studies published approach the structural peculiarities of some *Euphorbia* species, mentioned here being those of Dubard & Viguier (1905), in which the structure of the radicular system of *Euphorbia intisy* is investigated, as well as of Denis (1922), in which some data on the structure of the stem and leaf of the African species belonging to this family are found. Other authors make comparative studies of the phloem-xylem system of the foliar limb of species belonging to three orders (*Euphorbiales*, *Morales* și *Malvales*) (Dehay, 1938) or of the xylem structure of three groups belonging to the *Euphorbia* genus (Carlquist, 1970).

The Romanian literature in the field does not include any study exclusively on the structure of the *Euphorbia* species; there are only a few data on the structure of some vegetative organs (Ivănescu & Toma, 2003, Tudose, 2001) or general mentions in some lectures and manuals of Anatomy and Morphology of Plants (Grințescu, 1985; Șerbănescu-Jitariu & Toma, 1980; Toma & Gostin, 2000).

MATERIAL AND METHODS

The material utilized in the study belongs to 19 *Euphorbia* taxa (*E. helioscopia* L., *E. plathyphyllos* L., *E. falcata* ssp. *acuminata* (Lam.) Simonkai, *E. taurinensis* All., *E. maculata* L.; *E. pannonica* Host, *E. bazargica* Prod., *E. dobrogensis* Prod., *E. myrsinites* ssp. *myrsinites*, *E. myrsinites* ssp. *litardierei* Font Quer & Garcias Font, *E. nicaeensis* All., *E. amygdaloides* L., *E. cyparissias* L., *E. carniolica* Jacq., *E. agraria* Bieb., *E. agraria* var. *euboea* (Halácsy) Hay, *E. esula* L., *E. glareosa* Pallas ex Bieb., *E. virgata* W. et K.), collected from different parts of Romania (Bucovina, Moldova, Dobrogea, Transylvania).

The material subjected to analysis has been fixed and preserved in 70% ethyl alcohol, cross-sectioned with a microtome, coloured with iodine green and ruthenium red. The permanent slides obtained have been analyzed with a Novex (Holland) microscope and have been photographed on the same microscope with a Sanyo digital camera.

RESULTS AND DISCUSSIONS

The root structure

In all investigated taxa the root evidences a secondary structure resulted from the activity of both lateral meristems, *i.e.* the cambium and the phellogen.

The phellogen is generally differentiated from the pericycle cells or from an inner cortical layer with the exception of *E. plathyphyllos* root, in which the phellogen arises in the outer cortex, producing a few cork layers, the external ones being exfoliated. The cork has a typical organization and the phellodermis is mildly collenchymatous.

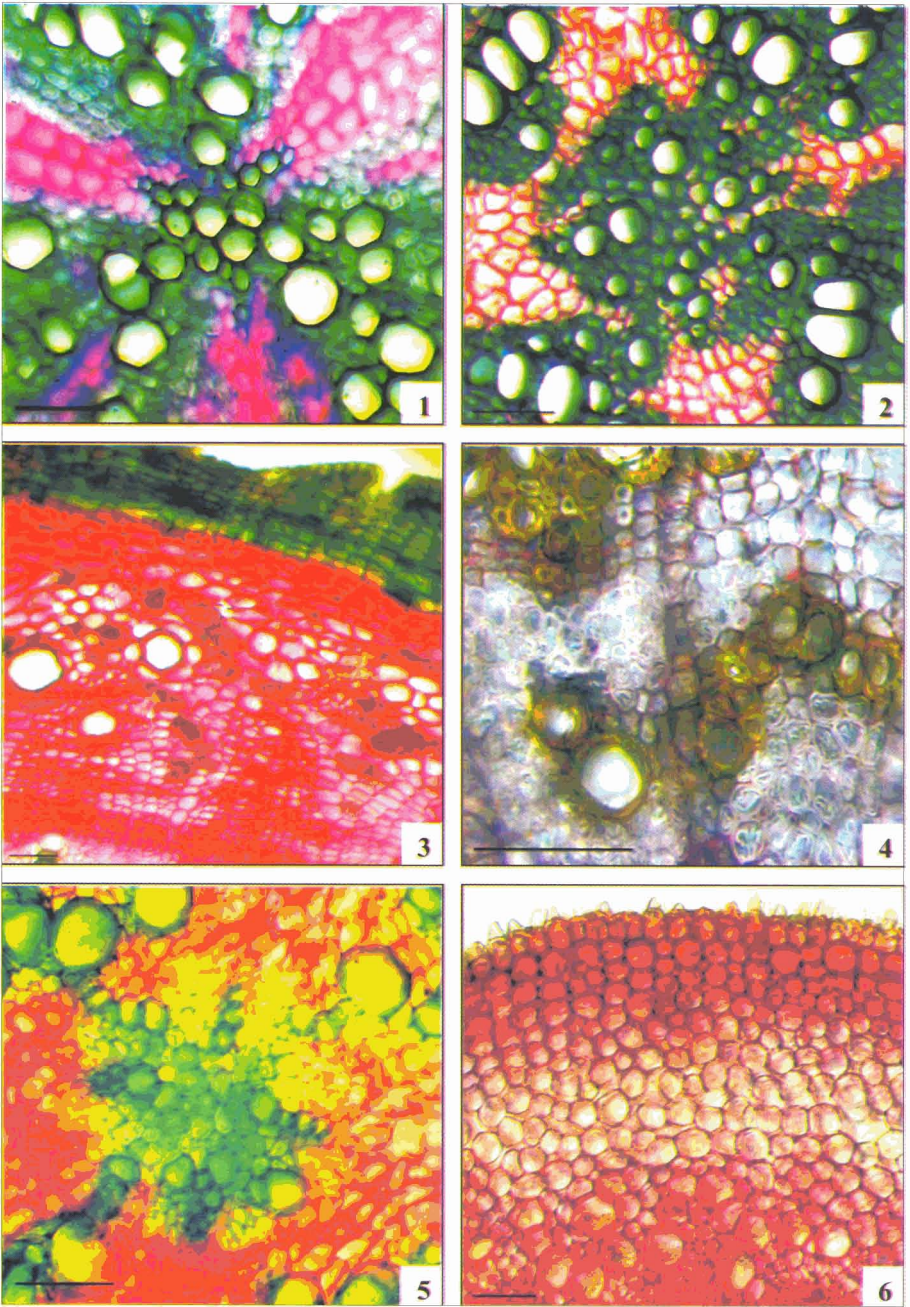


Plate 1

1. Transverse section of root of *E. helioscopia*. Scale bars=50µm; 2. Transverse section of root of *E. virgata*. Scale bars=50µm; 3. Transverse section of rhizome of *E. dobrogensis*. Scale bars=50µm; 4. Transverse section of rhizome of *E. agraria*. Scale bars=50µm; 5. Transverse section of rhizome of *E. dobrogensis*. Scale bars=50µm; 6. Transverse section of aerial stem of *E. bazargica* (upper level). Scale bars=50µm.

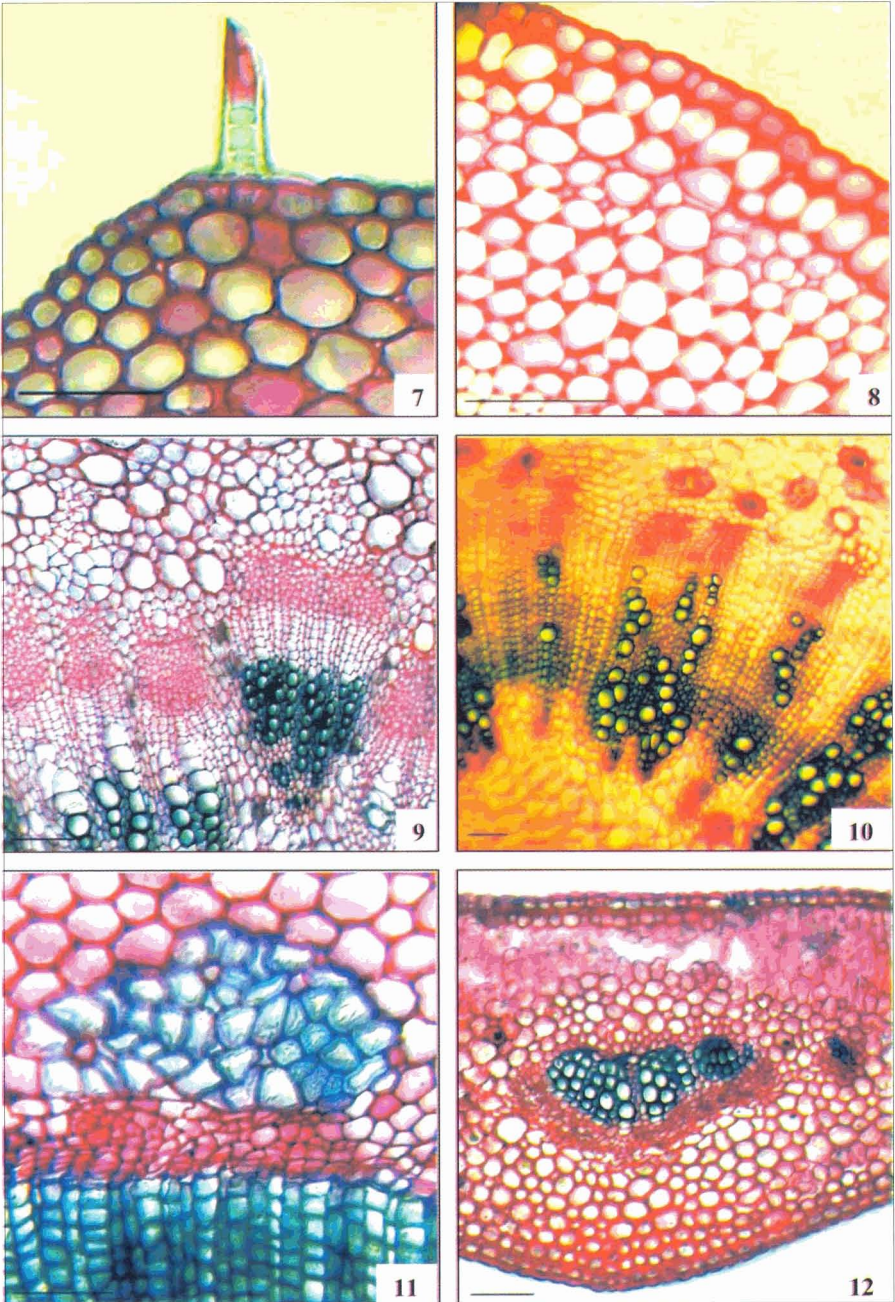


Plate 2

1. Transverse section of aerial stem of *E. maculata* (upper level). Scale bars=50μm; 2. Transverse section of aerial stem of *E. plathyphyllos* (upper level). Scale bars=50μm; 3. Transverse section of aerial stem of *E. nicaeensis* (upper level). Scale bars=50μm; 4. Transverse section of aerial stem of *E. myrsinites* ssp. *litardierei* (lower level). Scale bars=50μm; 5. Transverse section of aerial stem of *E. carniolica* (middle level). Scale bars=50μm; Transverse section of foliar limb of *E. agraria* var. *euboea*. Scale bars=50μm.

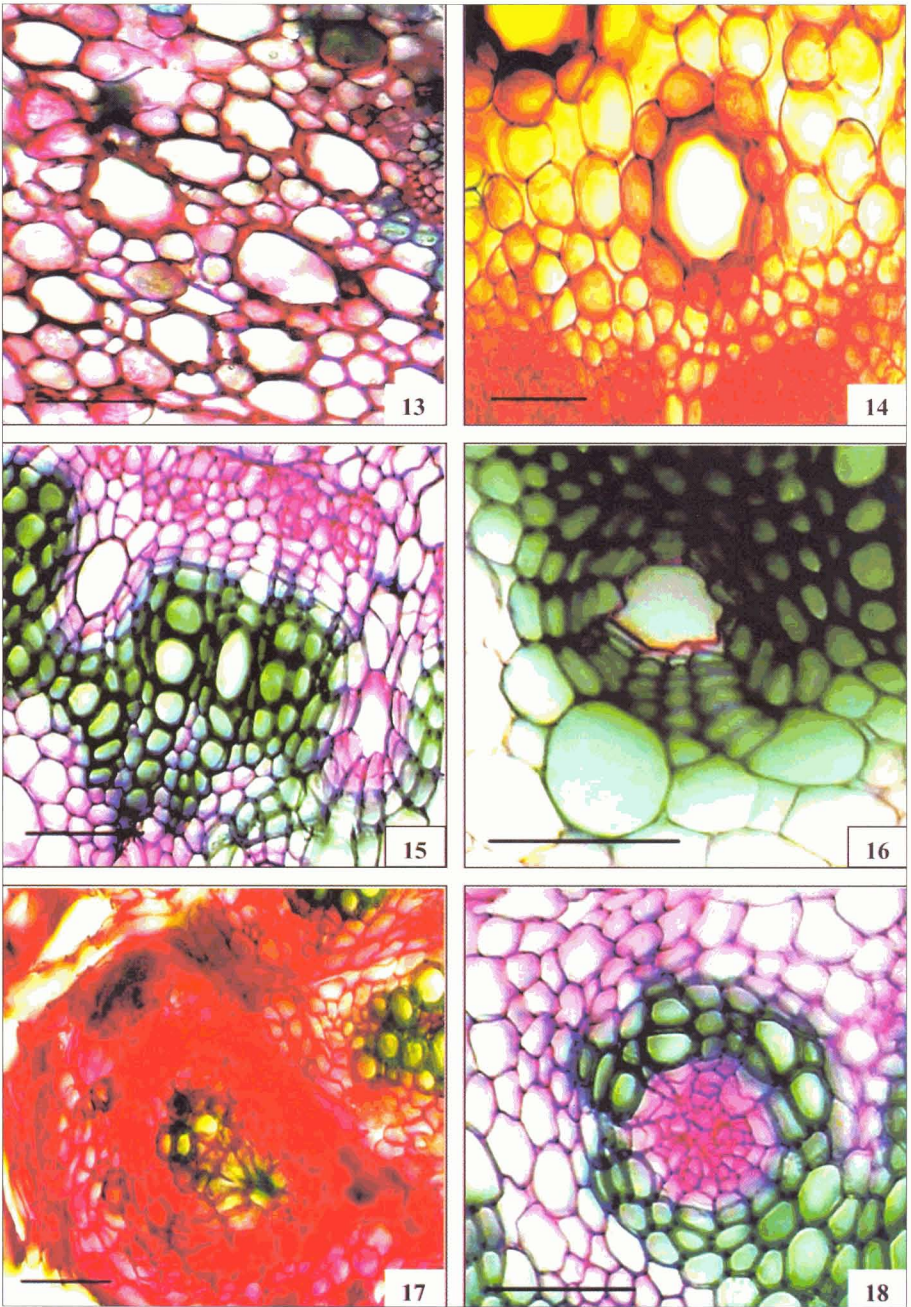


Plate 3

13. Transverse section of aerial stem of *E. myrsinites* ssp. *litardierei* (middle level). Scale bars=50 μ m; 14. Transverse section of aerial stem of *E. nicaeensis* (middle level). Scale bars=50 μ m; 15. Transverse section of aerial stem of *E. virgata* (upper level). Scale bars=50 μ m; 16. Transverse section of aerial stem of *E. virgata* (middle level). Scale bars=50 μ m; 17. Transverse section of rhizome of *E. dobrogensis*. Scale bars=50 μ m; 18. Transverse section of aerial stem of *E. virgata* (middle level). Scale bars=50 μ m.

The xylem is the most voluminous tissue of the root. The axle of the root comprises a variable number of primary vascular bundles, according to which the stele is of two types: 1—triarch in the root of *E. helioscopia*, *E. plathyphyllos*, *E. taurinensis* and *E. falcata* ssp. *acuminata* (Fig. 1); 2 — tetrarch in the root of *E. virgata* and *E. agraria* var. *euboea* (Fig. 2).

In most of the analyzed taxa, the secondary vascular tissues are traversed by parenchymatous cellulosic medullary rays, larger in the the secondary phloem, which follow up the axial primary vascular bundles. In the central region of the root of *E. taurinensis* and *E. falcata* ssp. *acuminata*, three small groups of elements with thick, but cellulosic, walls are present.

In the secondary xylem, the vessels have a different diameter and are irregularly dispersed. The libriform fibers have a very thick, weakly lignified secondary wall, most of it being gelified. The secondary xylem parenchyma is generally cellulosic. In the root of *E. taurinensis* and *E. plathyphyllos*, the cells of secondary xylem parenchyma are small, with moderately thick and lignified walls, organized in radiary continuous or discontinuous columns, rarely arranged around the large vessels.

The rhizome structure

In most of the investigated taxa, the rhizome has a secondary structure, resulting from the activity of the cambium and of the phellogen, except for the rhizome of *E. maculata*, *E. helioscopia* and *E. plathyphyllos*, in which only the vascular tissues are of secondary origin.

The phellogen is differentiated from an inner cortical layer, the cork and the phellodermis having a typical organization and a variable thickness (Fig. 3). The epidermis and the first cork layers are exfoliated with the exception of the rhizome of *E. panonica*, in which the epidermis cells are persistent and are cube shaped. In the *E. plathyphyllos* rhizome, the phellodermis is not collenchymatous. In the epidermis of *E. maculata* rhizome, stomata were identified.

The primary cortex does not end with a special type endodermis, except for the rhizome of *E. helioscopia* and *E. plathyphyllos*, in which an endodermis of Casparyan type was identified.

The stele is thick and comprises two concentric rings, the phloem one being relatively thin. The vessels of secondary xylem, of variable diameter, are irregularly dispersed in the libriform mass, constituted of fibers with very thick, moderately or not lignified, for the most part gelified walls (Fig. 4).

The cells of secondary xylem parenchyma with cellulosic walls form tangentially discontinuous bands, which penetrate in patches the libriform mass; in the rhizome of *E. plathyphyllos*, *E. maculata* and *E. amygdaloides* the secondary xylem parenchyma is lignified, its elements being arranged in radiary uniseriate discontinuous columns.

The pith is parenchymatous cellulosic of meatic type, except for the rhizome of *E. agraria* and *E. cyparissias*, in which the walls of the medullary cells are

partially or totally sclerified and lignified (Fig. 5). In the axle of *E. myrsinites* ssp. *litardierei* rhizome, two opposite large parenchymatous cellulosic rays, in which the elements of primary xylem penetrate, are connecting. In *E. amygdaloides* the central of the rhizome is occupied by a big aeriferous cavity.

The aerial stem structure

The epidermis has cells of cubic or tabular shape, with thick periclinal walls; the external wall is covered by a cuticle of variable thickness. In *E. myrsinites* ssp. *litardierei*, *E. myrsinites* ssp. *myrsinites*, *E. pannonica*, *E. dobrogensis*, *E. glareosa* and *E. bazargica*, most of the epidermal cells evidence a papilla-shaped prominence in the middle of the external wall, some of them forming very short prickle-shaped hairs (Fig. 6).

In most of the analyzed taxa, the stomata are located on the same level with the epidermis cells, with the exception of *E. cyparissias*, *E. myrsinites* ssp. *litardierei*, *E. myrsinites* ssp. *myrsinites* and *E. glareosa*, in which the stomata reveal a shallow suprastomatal chamber.

The stems of some taxa are pubescent, the trichomes being unicellular (*E. helioscopia*, *E. amygdaloides*, *E. agraria*, *E. glareosa*) or multicellular uniseriate (*E. maculata*) (Fig. 7).

In the most investigated taxa, except *E. maculata*, a variable number of cell layers just below the epidermis are tangentially collenchymatous. In *E. plathyphyllos* and *E. helioscopia*, the mass of the cortex is of angular collenchyma type (Fig. 8). The cortical parenchyma is of meatic type with the exception of *E. myrsinites* ssp. *litardierei*, *E. myrsinites* ssp. *myrsinites* and *E. glareosa*, in which, in the middle area of the cortex, numerous aeriferous cavities are present.

The stele has a primary structure in the upper third of the aerial stem, the genesis of the vessels being in progress. The stele is of eustelic type, comprising a variable number (30-55) of vascular bundles of collateral open type, disposed on a circle.

In most of the analyzed taxa, except for *E. myrsinites* ssp. *litardierei*, *E. myrsinites* ssp. *myrsinites* and *E. dobrogensis*, cordons of incipient sclerenchymatous fibers of polygonal contour in transverse section, with more or less thick and not yet lignified walls are present at the periphery of the phloem of primary vascular bundles (Fig. 9).

The pith is parenchymatous cellulosic with numerous aeriferous cavities, separated by uniseriate lamellae in *E. glareosa*, *E. myrsinites* ssp. *litardierei*, *E. myrsinites* ssp. *myrsinites*, and *E. cyparissias*. In *E. taurinensis* and *E. falcata* ssp. *acuminata* the pith is relatively compact.

The passing from the primary structure to the secondary structure, resulting from the activity of the cambium, may be observed along the aerial stem.

The secondary vascular tissues are generally of annular type, being traversed by large (in the secondary phloem), sclerified and lignified, uni- or biseriate (in the

secondary xylem) parenchymatous-cellulosic medullary rays. In some taxa (*E. glareosa*, *E. myrsinites* ssp. *litardierei*, *E. bazargica*) the secondary vascular tissues conserve the fascicular feature of those from primary structure; the medullary rays of variable width, from the secondary xylem, being parenchymatous cellulosic or lignified (Fig. 10).

In the thickness of the secondary xylem, the libriform fibers with a thick, moderately lignified and partially gelified secondary wall are predominant.

At the basis of the aerial stem, the periphloemic sclerenchymatous fibers are completely formed, having very thick walls, which are weakly or not lignified, most of them being gelified (Fig. 11).

The leaf structure

All investigated taxa present sessile bifacial leaves of two types: 1 – isofacial, centric-heterogeneous (*E. myrsinites* ssp. *myrsinites*, *E. myrsinites* ssp. *litardierei*, *E. dobrogensis*, *E. nicaeensis*, *E. glareosa*, *E. pannonica*, *E. agraria*, *E. agraria* var. *euboea*, *E. cyparissias*, *E. falcata* ssp. *acuminata*); 2 – heterofacial (*E. amygdaloides*, *E. esula*, *E. helioscopia*, *E. plathyphyllos*, *E. taurinensis*, *E. maculata*, *E. virgata*, *E. carniolica*).

Among the analyzed taxa, the number of palisade parenchyma layers below the both epidermis (in the bifacial isofacial, centric-heterogeneous foliar limb) or on the adaxial side (in the bifacial heterofacial foliar limb) is variable. In *E. agraria*, *E. esula*, *E. dobrogensis* and *E. glareosa* the lacunary tissue is relatively compact.

The median nervure comprises, in the fundamental collenchymatous parenchyma, a single vascular bundle, in which the xylem vessels disposed in radiary columns are separated by a few parenchymatous-cellulosic cells. In the foliar limb of *E. plathyphyllos* and *E. agraria* var. *euboea*, the median vascular tissues form an arc which is open at the adaxial side, in cross-section (Fig. 12).

In *E. maculata*, the vascular bundles of the lateral nervures are surrounded by a parenchymatous theca.

The characteristics of the foliar epidermis. In all investigated taxa, the surface of the foliar limb presents polygonal cells with generally straight or sinuous lateral walls. In the foliar limb of *E. plathyphyllos* and *E. taurinensis* the lateral walls of epidermis cells are zig-zag curved. In *E. pannonica*, *E. glareosa*, *E. dobrogensis*, *E. myrsinites* ssp. *litardierei*, *E. myrsinites* ssp. *myrsinites*, *E. nicaeensis* and *E. bazargica*, most of the epidermis cells evidence a papilla-shaped prominence in the middle of the external wall, some of them forming very short prickle-shaped hairs.

The stomata are of anisocytic and anomocytic-type, being localized in both epidermis, with the exception of *E. amygdaloides*, *E. cyparissias*, *E. taurinensis*, *E. falcata* ssp. *acuminata*, *E. bazargica* and *E. carniolica*, in which the foliar limb is hypostomatic. In the lower epidermis of the foliar limb of *E. virgata*, beside the common types, stomata of paracytic type are localized.

Most of the investigated taxa have glabrous leaves. In some taxa, unicellular (*E. agraria*) or multicellular uniseriate trichomes (*E. maculata*, *E. amygdaloides*, *E. esula*) may be observed on the surface of the foliar limb.

The distribution and morphology of laticifers

The laticifers are present in all the (subterranean and aerial) vegetative organs, being localized in certain tissues (cortical parenchyma, phloem, xylem, pith).

The laticifer walls are always cellulosic, uniformly thick or present centripetal thickenings in *E. pannonica*, *E. bazargica*, *E. myrsinites* ssp. *myrsinites* and *E. nicaeensis* (Fig. 13); only in *E. virgata*, the wall of the laticifers is thin.

In *E. myrsinites* ssp. *myrsinites* and *E. myrsinites* ssp. *litardierei*, the laticifers are surrounded by one or more cell layers of a shape that slightly differs from that of the adjacent cells, as a whole, taking the aspect of a secretory channel (Fig. 14).

In *E. virgata* aerial stem, some laticifers from the secondary xylem are surrounded by meristematic cells (Fig. 15) or by a parenchymatous cellulosic layer and more concentric layers of elements with sclerified and lignified walls (Fig. 16).

Anomalous structures

Among the anomalous structures, there are worth mentioning here: parenchymatous cellulosic structures, formed exogenously in the root of *E. agraria* var. *euboea*; medullary bundles, comprising some groups of xylem vessels, surrounded by parenchymatous cellulosic cells disposed on more circles, those from the periphery having thick walls (in the rhizome of *E. dobrogensis*) (Fig. 17); cortical bundles, centrally comprising some mechanic cells, surrounded by phloem elements and by a continuous or discontinuous theca of sclerenchymatous fibers (in the aerial stem of *E. virgata*); cortical bundles comprising only xylem vessels surrounded by cells with moderately sclerified and lignified walls (in the aerial stem of *E. pannonica*); phloem bundles surrounded by elements with sclerified and lignified walls, localized in the secondary xylem of *E. virgata* aerial stem (Fig. 18); vascular bundles, localized between the secondary phloem and the periphloemic cordon of sclerenchymatous fibers, comprising xylem vessels and a few parenchymatous cellulosic cells surrounded by sclerenchymatous elements, meristematic cells and elements of phloem, only on the external side (in the aerial stem of *E. nicaeensis* and *E. virgata*).

According to the speciality literature, the investigated *Euphorbia* taxa may be divided into two groups: 1 – annual taxa (*E. helioscopia*, *E. plathyphyllos*, *E. falcata* ssp. *acuminata*, *E. taurinensis*, *E. maculata*); 2 – perennial taxa (*E. pannonica*, *E. bazargica*, *E. dobrogensis*, *E. myrsinites* ssp. *myrsinites*, *E. myrsinites* ssp. *litardierei*, *E. nicaeensis*, *E. amygdaloides*, *E. cyparissias*, *E. carniolica*, *E. agraria*, *E. agraria* var. *euboea*, *E. esula*, *E. glareosa*, *E. virgata*).

Our histo-anatomical researches evidence the presence of a subterranean stem with typical secondary structure that follows up the main root of *E. helioscopia*,

E. plathyphyllos and *E. maculata* and a root with secondary structure in *E. virgata*, in which a rhizome was not identified.

E. bazargica, *E. dobrogensis* and *E. pannonica* are described as different species by Prodan (1953) in the third volume of the Romanian Flora; consequently, these species are considered synonymous with *E. nicaeensis* (Beldie, 1977; Ciocârlan, 2000). *E. glareosa* is mentioned in the Romanian flora by Ciocârlan (2000) and considered synonymous with *E. pannonica*. Until now, our investigations confirm these synonymies, for the most part, the structure of the vegetative organs of these species being similar. Further researches on the anatomy of cyathia and latex biochemistry are necessary in order to prove the validity of these synonymies.

The investigated taxa of this paper present a number of common characters, already mentioned in the literature on the anatomy of the *Euphorbia* genus (Gaucher, 1902; Metcalfe & Chalk, 1950; Esau, 1965).

In the stem of most of the analyzed taxa, periphloemic cordons of sclerenchymatous fibers with very thick, weakly or not lignified walls, most of which being gelified, are present. This is a constant characteristic in the *Euphorbiaceae* family (Gaucher, 1902).

The stem endodermis is usually not well defined (Metcalfe & Chalk, 1950). In all of the analyzed taxa, the primary cortex of the stem does not end with a typical endodermis. In the rhizome of *E. plathyphyllos* and *E. helioscopia* an endodermis of primary type with Casparyan spots in the lateral walls is present.

Gaucher (1902) mentions that the secondary xylem always forms a continuous ring, traversed by sclerified and lignified medullary secondary rays; but in some taxa (*E. glareosa*, *E. myrsinites* ssp. *litardierei*, *E. bazargica*), it was observed that the secondary tissues are of fascicular type, the medullary secondary rays of variable width which being parenchymatous-cellulosic or lignified.

The libriform fibers are often gelatinous, the thickness of their walls varying widely within *Euphorbia* (Carlquist, 1970). In all the investigated taxa, there was observed that the most libriform fibers of the subterranean organs have very thick, weakly lignified and mostly gelified secondary wall, comparatively with the stem libriform, in which the fibers with less thick, moderately lignified and partially gelified walls are predominant.

The pith is persistent even at the basis of the stem and in many cases it is partially or totally sclerified and lignified (Gaucher, 1902). In most of the analyzed taxa, in the secondary structure of the aerial stem, the pith conserves the parenchymatous cellulosic characteristic, some of its cells being disorganized, resulting in aeriferous cavities of variable size, only in the rhizome of *E. agraria* and *E. cyparissias*, the medullary cells are sclerified and lignified.

According to the structure of the foliar limb, the investigated taxa are divided into two groups: with bifacial, isofacial (centric-heterogeneous) leaves and

respectively with bifacial heterofacial leaves, the characteristics of the mesophyll being relatively constant in either group.

The mesophyll has a variable structure, in correlation with the plants' growth conditions, varying in the specimens of the same species, in the leaves of the same plant and in the same leaf, along the limb (Gaucher, 1902). In foliar limb of *E. pannonica* and *E. glareosa*, the mesophyll has a variable structure, being for mostly differentiated into palisade tissue below both the epidermis and a central region of lacunary tissue; in patches the mesophyll is totally of palisade type.

The stomata in European species of *Euphorbia* are predominantly ranunculaceous (Metcalf & Chalk, 1950), without guard cells of special type and localized generally at the level of the epidermis: at the cuticle level or at the half of the epidermis cell height (Gaucher, 1902). In the most analyzed taxa, the stomata are of anisocytic and anomocytic-type, being localized below the epidermis external level, resulting generally a deep suprastomatal chamber.

In the median nervure of the foliar limb, the grouping mode of the vascular tissues is variable, initially forming a complete ring and an open arc at the adaxial side of the limb, in the ends (Gaucher, 1902). In all investigated taxa, the median nervure comprises a single vascular bundle of variable size, being shaped as an arc which is open at the adaxial side, in the cross-section of the limb of *E. plathyphyllos* and *E. agraria* var. *euboea*.

The epidermis cells are cube shaped or tabular in transverse section, sometimes they are papillose (Gaucher, 1902). Most of the epidermis cells of the stem and leaf of *E. pannonica*, *E. glareosa*, *E. dobrogensis*, *E. myrsinites* ssp. *litardierei*, *E. myrsinites* ssp. *myrsinites*, *E. nicaeensis* and *E. bazargica* evidence a papilla-shaped prominence in the middle of the external wall, some of them forming very short prickle-shaped hairs.

The stems and leaves from most of the analyzed taxa are glabrous. In the pubescent vegetative organs, the trichomes are simple, unicellular or multicellular, uniseriate.

In all the vegetative organs of all the taxa taken into study, laticifers were observed, their number being less in the root. The species of the *Euphorbia* genus present non-articulated, branching and non-anastomosing laticifers (Esau, 1965). The laticifers have generally thin smooth or thick walls, in a few species, sometimes with distinct striations on the inner surface (Rudall, 1987). In most of the analyzed taxa, the walls of the laticifers are cellulosic, uniformly thick or present centripetal thickenings, with the exception of *E. virgata*, in which the laticifers have thin walls.

CONCLUSIONS

In conclusion, the taxa taken into study present the following constant characters: non-articulated laticifers with cellulosic generally thick walls in all the

vegetative organs; periphloemic cordons of sclerenchymatous fibers in the stem structure; stomata of anisocytic and anomocytic-type in the foliar limb; libriform gelatinous fibers; tangentially collenchyma at the periphery of the stem cortex.

The structure of the vegetative organs of the investigated taxa differs in several characters: the stem cortex organization; the pattern of epidermis cells; the presence or the absence of the trichomes; the structure of the rhizome pith; the primary structure of the root stele; the presence or the absence of the parenchymatous cellulosic medullary rays in the secondary xylem of the root; the (annular or fascicular) organization type of the secondary vascular tissues of the stem.

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