HISTO-ANATOMICAL OBSERVATIONS REFERRING TO SOME MELAMPYRUM SPECIES

ASPAZIA BĂEȘU*, C. TOMA **, IRINA STĂNESCU***

Abstract: The paper presents the results of some histo-anatomical research upon five hemiparasite species of Melampyrum genus belonging to the Romanian flora: M. arvense L. M. bihariense A. Kerner, M. cristatum L., M. saxosum Baumg and M. sylvaticum L. The authors investigate the structure of all vegetative organs and of the haustoria, focusing on the fact that they bear xylem vessels which facilitate the transport of the crude sap from the host plant to the root of the parasite. The root passes quite early to a secondary structure, due to the activity of both lateral meristems; the stem also presents a secondary structure, due only to cambium’s activity. The mechanic tissue is present only in a few species, represented by isolated or grouped periphloemic sclerenchymatic fibers and few weak collenchymatized elements in hypodermic position. The indumentums of leaves and bracts differs in all 5 species, representing very important taxonomic criteria. The mesophyll is different in all 5 species, strongly correlated with the environment of the species.

Key words: Melampyrum, anatomy, vegetative organs.

Introduction

In Europe, Melampyrum gender bears 24 epirhizoid hemiparasite facultative species, most of all polyphagous (ubiquist species which do not manifest any particular preferences regarding the host plant) [3, 7, 10]. In the Romanian flora there are 8 species [2, 9], from which we analyzed only 5: M. arvense L., M. bihariense A. Kerner, M. cristatum L., M. saxosum Baumg and M. sylvaticum L.

Epirizoid facultative hemiparasites are autotrophic plants which have mostly an independent life. Haustoria appear on some of their roots which penetrate the roots of the host plants in order to complete their requisite of crude sap.

The general anatomic architecture of the vegetative organs belonging to the species from Scrophulariaceae family is well known since the beginning of the 19th century; Koch [6] published in 1815 his PhD thesis regarding the anatomic characters of the scrophulariaceas. During two centuries there were numerous studies regarding this family, interested mostly by the hemiparasite species. Others [4] studied the morphology of the absorption organs of some hemiparasite from Rhinanthaceae species, including Melampyrum species. Weak references about the general structure of the haustoria are present in some botanic handbooks [1, 3, 11] or even of parasite species [7, 10]. The general structure characters of the vegetative organs of Scrophulariaceae family representants are explained in synthesis handbooks upon dicotyledons’ anatomy [5] or angiosperm’s [6]. In our country, there were made investigation of the structure of the vegetative organs [8], except the haustoria, of two Melampyrum species (M. sylvaticum, M. saxosum).

* “E. Hurmuzachi” National College, Rădăuți, Suceava, baeszaspazia@yahoo.com
**Faculty of Biology, “Alexandru Ioan Cuza” University of Iasi, ctoma@uaic.ro; irinagostin@yahoo.com
***“Anastasie Fătu” Botanic Garden, “Alexandru Ioan Cuza” University of Iasi, irinastanescu2005@yahoo.com
Material and methods

The studied species occupy various areas: *M. arvense* – cultures, crops, vineyards, bushes, from field to the mountain regions; the analyzed exemplars were collected from a lawn, Tulcea County; *M. bihariense* – lawns, bushes, borders of forests, from hills to spruce level; the analyzed exemplars were collected from the same lawn, Poeci, Neamț County; *M. cristatum* – lawns and forest borders, bushes, rockeries, from forest steppe to beech level; the analyzed exemplars were collected from the same lawn, Poeci, Neamț County as *M. bihariense*. *M. saxosum* – forests and subalpine lawns; the analyzed material comes from Ceahlău Massive; *M. sylvaticum* – beech and spruce level, forests, rockeries, lawns; the investigated material was collected from a lawn, Red Lake.

All material, collected in June 2004, has been fixed in ethylic alcohol 70%, sectioned (cross-sectioned and superficial) using a hand microtome and a cryotome, then it was coloured with ruthenium red and iodine green. The cuttings were mounted in gel and analyzed in a Novex (Holland) light microscope. The light micrographs were performed by a Minolta camera.

Results and discussions

The general structure characters are quite similar in all 5 analyzed species, the differences result from their various ecology, due to their different habitats.

**Lateral roots.** The transition to the secondary structure is quite early, due to both lateral meristems: cambium and phellogen; even the thinnest roots present secondary structure in the central cylinder (Fig. 1). The phellogen, differentiated due to an inner cortical layer, forms suber which exfoliates (Fig. 2). The pheloderm is represented by 1-2 layers of cells with moderately thickened walls (*M. arvense*) or by a thin region with cells similar to those belonging to the secondary phloemic parenchyma (*M. bihariense*); most of it is often exfoliated, being present only in a few regions (*M. cristatum, M. saxosum, M. sylvaticum*) (Fig.3), so, the primary cortex, in most of the species, is exfoliated, excepting the primary endodermis; its cells present Caspary thickenings in the radiary walls (*M. arvense*); the endodermis of primary type presents strongly tangentially elongated cells in *M. cristatum* and is absent in *M. bihariense*.

In the central cylinder the following sub-regions and secondary tissues resulted from cambium’s activity can be distinguished:

- an outer thin ring of phloem, formed by sieved tubes, guard cells and cells of phloemic parenchyma. In *M. bihariense* the proper conductive elements form small isles separated by cellulosed parenchyma (of phloem or of wide medullary rays). The phloem ring is swept by one-layered or bi-layered parenchymatic-cellulosed rays (*M. cristatum*); in *M. saxosum* the phloemic elements are strongly radiary flattened;

- a central xylem massive, with vessels irregularly dispersed in the libriform; this is more developed in the external part of the secondary xylem, formed by tangentially-elongated elements, disposed on radiary rays; their walls are moderately thickened and lignified; only a few vessels and cells of xylemic parenchyma with moderately thickened walls are present in the center of the root. In *M. saxosum* 5 rays of vessels are displayed in the center of the organ; the
elements present between the rays bear moderately thickened, but unlignified walls, representing libriform in progress.

**The main root.** The structure is similar to that of the lateral root, with the following peculiarities:

- at the periphery there could persist a thin region (often one-layered) of cells with weak thickened and suberified walls, which results from the activity of the phellogen differentiated from a profound cortical layer (Fig. 4);
- phelloderm is well represented, multi-layered, bearing big cells with moderately collenchymatised walls in *M. arvense* and *M. saxosum*;
- the ring of secondary phloem is thicker; there are groups of sclerenchymatic elements with moderately thickened and lignified walls in the center, periphery (*M. arvense*) and in the endodermis (*M. cristatum*);
- the secondary xylem massive is quite thick, bearing a big quantity of libriform with numerous vessels of various diameter, irregularly dispersed (Fig. 5). In *M. sylvaticum* the central xylem massive presents an axial region with vessels separated by lignified parenchyma and an outer thicker region where the vessels are separated by numerous libriform elements, with strongly thickened and moderately lignified walls (Fig. 6). In *M. bihariense*, in the central part of the xylem massive there are two compact groups of smaller vessels, with strongly lignified walls, which represent rests of the primary xylem and tell us that the stel from the primary structure is of diarchic type (Fig. 7).

**Haustoria** appear on the lateral roots and penetrate the root of other species (which belong to the same *Melampyrum* species or to other species). As a general form, the haustoria are spherical, with nodule-like profile.

The root which forms haustoria has an asymmetric shape (at least regarding the central cylinder) and the general structure is modified: the parenchymatic tissues (cortical, phloemic) prevail; the xylemic and phloemic tissues are weak developed (Fig. 8 and 9).

Some cross sections of the main root display a longitudinal section of the haustoria, so, a cordon of tracheidal elements surrounded by cellulosed parenchymatic cells could be distinguished (Fig. 10 and 11).

The longitudinal section through the haustoria of *M. cristatum* evidences the direct contact between the cordon of xylem vessels of the haustoria and the xylem tissue of the main root. Xylem vessels bear mostly screw thickenings, surrounded by long parenchyma cells, with weak thickened, but cellulosed walls. In *M. sylvaticum* the primary endodermis of the main root elongates in the haustoria. At the limit between the two partners (parasite and host), the cells of the haustoria present suberified and weak thickened walls; at the periphery of the haustoria almost all the cells are elongated, suchlike the root hairs, playing an important role in affixing on host’s root and penetrating it.

**The stem.** In the superior part, the cross section has quadrate-rectangular profile, with rounded angles (*M. arvense, M. bihariense, M. cristatum, M. sylvaticum*) or a hexagonal profile with rounded angles and numerous hairs on two opposite sides (*M. saxosum*) (Fig. 12).

In all 5 species, epidermis presents isodiametric cells of various dimensions, with the internal and external walls thicker than the others, the last one being covered by a thin cuticle; here and there, some multicellular unilayered protective hairs are present, bearing a narrowed terminal cell and very short multicellular secretory hairs, with unicellular pedicle
and bicellular gland (in cross section), excepting *M. arvese* which does not bear any secretory hairs.

The cortex is parenchymatic-cellulosed, of meatic type, formed by 3-7 layers of big cells with thin walls. Here and there, aeriferous lacunae are present in *M. arvense* and *M. bihariense*. The cortex does not present a special endodermis, while the central cylinder does not present pericycle.

The central cylinder displays two rings of conductive tissue with primary origin in *M. saxosum*, *M. sylvaticum* and *M. cristatum*, with phloem formed by small isles of sieved tubes and guard cells and the xylem formed by vessels irregularly dispersed and separated by cells of cellulosed xylemic parenchyma; the rings are separated by a continuous multi-layered thick region of procambial tissue (Fig. 13).

In *M. arvese* and *M. bihariense* the transition to the secondary structure happens quite early, due only to the cambium. The secondary phloem bears cells of phloemic parenchyma, too, while xylem has vessels disposed on radial layers separated by libriform (Fig. 14).

In the middle part of the stem, the structure differs as follows:

- the protective hairs are more numerous in *M. arvense* and *M. cristatum*, less numerous in *M. sylvaticum* and *M. bihariense*; sometimes their shape is different, being very short and unicellular in *M. bihariense*;
- numerous solitary or grouped sclerenchymatic fibers are present at the periphery of the phloem ring, bearing thick and lignified walls (*M. bihariense*);
- the rings of conductive tissue (of secondary origin, in all analyzed species) are thicker than in the anterior analyzed level;
- some species (*M. cristatum*, *M. saxosum* and *M. sylvaticum*) present aeriferous cavity, irregularly shaped, in the center of the pith, resulted by the disorganization of its cells; in *M. bihariense*, the medular region bear bigger cells, arranged in concentric layers, simulating a secretory canal (the cells will disorganize themselves in the inferior part of the stem in order to create the aeriferous cavity) (Fig. 15).

In the inferior part of the stem we underline the following differences:

- the protective hairs are less numerous, shorter and unicellular;
- in *M. arvense* the epidermis is exfoliated somewhere, and some lenticels appear with a lot of spongy tissue (Fig. 16);
- the cortical cells are tangentially elongated;
- the ring of secondary xylem is thicker; all its elements present strongly lignified walls; in *M. cristatum*, three sub-regions can be distinguished in the xylem ring: an internal region with libriform moderately sclerified and numerous vessels, a middle one, with more libriform intensively lignified and only a few vessels and an external one, moderately sclerified and weak lignified (Fig. 17);
- solitary or grouped sclerenchymatic fibers, with thickened and lignified walls are present at the outer part of the phloem ring;
- pith is thin, partially disorganized, forming a big aeriferous cavity of irregular shape.

**The leaf.** In *M. arvense* and *M. cristatum* the leaves are sessile, so our observations take into account the other 3 species.
The petiole. In cross section, the petiole has a semielliptic-crescent profile, with concave adaxial face. Epidermis displays isodiametric cells, with the external wall thicker than the others and covered by a fine stripped cuticle; here and there, short unicellular or bicellular protective hairs are present. In the fundamental mediate parenchyma, collenchymatised in hypodermic position, phloemic-xylemic bundles of collateral type are present, of various number and position: numerous and closed one to another, disposed on a curved sheath and 1-2 far away from the others (*M. bihariense*); 3, with the middle one bigger than the others (*M. saxosum*) (Fig. 18); a wide median arch with numerous bundles separated by uni- or multilayered parenchymatic rays and two lateral bundles (*M. sylvaticum*).

The foliar limb. In front side view, the epidermis displays cells of irregular shape, with strongly curved lateral walls; stomata of anomocytic type have various location: in both epidermis, so the foliar limb is amfistomatic (*M. arvense* and *M. sylvaticum*) or only in the lower epidermis, so the foliar limb is hypostomatic (*M. bihariense*, *M. cristatum* and *M. saxosum*). *M. bihariense* presents, in the lower epidermis, very big nectariferous extrafloral glands, located in deep excavations; those glands bear numerous, radiary elongated, secretory cells which form a continuous sheath beneath the basal part.

Both epidermis displays secretory and protective hairs, with different structure and position, as follows:

- *M. arvense*: numerous protective hairs, mostly unicellular, but bi- or multicellular, too, bearing a basal cell of circular profile; they are longer at the lower part of the foliar limb and the point is flexed; very short protective hairs with thick wall and obtuse point, at the edge of the foliar limb; the secretory hairs are shorter, sessile, with bicellular gland in cross section;

- *M. bihariense*: very short protective hairs, unicellular, rarely bicellular, aculeiform, oblique, narrow-pointed and with very thick walls; rare, short secretory hairs, with bicellular gland in cross section;

- *M. cristatum*: numerous uni- bi- or multicellular protective hairs in both epidermis; rare multicellular secretory hairs;

- *M. saxosum*: numerous short protective hairs, unicellular and mostly bicellular, located in the upper epidermis; numerous, short secretory hairs, with bicellular gland in cross section, more frequent in the lower epidermis;

- *M. sylvaticum*: short, unicellular protective hairs, narrow-pointed, more numerous in the upper epidermis; multicellular secretory hairs, more frequent in the lower epidermis.

In cross section of the foliar limb, the middle vein is very prominent at the abaxial face and consists of fundamental parenchyma and a vascular bundle or arch of conductive elements in *M. bihariense* (Fig. 19).

The mesophyll could be: 1) weak differentiated in one-layered palisade tissue with wide and short cells and multilayered lacunary tissue, so the foliar limb has a bifacial-heterofacial structure (*M. arvense*, *M. cristatum* and *M. saxosum*); the cells of the palisade tissue may have H form in *M. saxosum* and *M. arvense* (Fig. 20); 2) homogenous, of lacunary type, with the hypodermic adaxial layer bearing high and wide cells, fact that makes us consider that the foliar limb presents a transitory structure from bifacial-izofacial to bifacial-heterofacial (*M. sylvaticum*); 3) homogenous, typical and entire lacunary, so the foliar limb has a bifacial-izofacial structure (*M. bihariense*).
Conclusions

The root passes quite early to the secondary structure, due to the activity of both lateral meristems. Only a few species present mechanic tissue, represented by isolated or grouped periphloemic sclerenchymatic fibers and a few weak collenchymatized elements in hypodermic position. Haustoria present a structure adapted to their function, bearing xylem vessels which facilitate the transport of the crude sap from the host plant to the xylemic tissue of the parasite’s root.

The stem presents a secondary structure resulted only from the cambium’s activity; the secondary conductive tissues are of annular type.

The indumentums of leaves and bracts differs in all five species, being considered as very good taxonomic criteria. The mesophyll differs, too, in all five species, being correlated with the environment of the species.

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Explanation of plates

PLATE I
Fig. 1 – Melampyrum arvense: lateral root (cross section)
Fig. 2 – Melampyrum bihariense: lateral root (cross section)
Fig. 3 – Melampyrum cristatum: lateral root (cross section)
Fig. 4 – Melampyrum saxosum: main root (cross section)
Fig. 5 – Melampyrum sylvaticum: main root (cross section)
Fig. 6 – Melampyrum bihariense: main root (cross section)
Fig. 7 – Melampyrum bihariense: main root (cross section)
Fig. 8 – Melampyrum arvense: haustorul (cross section through the main root)
Fig. 9 – Melampyrum sylvaticum: haustorul (cross section through the main root)

PLATE II
Fig. 10 – Melampyrum saxosum: haustorul (cross section through the main root)
Fig. 11 – Melampyrum cristatum: haustorul (cross section through the main root)
Fig. 12 – Melampyrum bihariense: tulipina (cross section)
Fig. 13 – Melampyrum sylvaticum: stem (cross section)
Fig. 14 – Melampyrum arvense: stem (cross section)
Fig. 15 – Melampyrum bihariense: stem (cross section)
Fig. 16 – Melampyrum arvense: stem (cross section)
Fig. 17 – Melampyrum cristatum: pețiolul (cross section)
Fig. 18 – Melampyrum saxosum: pețiolul (cross section)
Fig. 19 – Melampyrum bihariense: limbul (cross section)
Fig. 20 – Melampyrum saxosum: limbul (cross section)
Fig. 1

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Fig. 7

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Fig. 9