UNIVERSITY "ALEXANDRU IOAN CUZA" from IAŞI

FACULTY OF BIOLOGY

PhD Thesis

THE VERNAL FLORA FROM NATURAL RESERVATION GÂRBOAVELE FOREST – GALAȚI: MORPHO – BIOLOGICAL AND HISTO – ANATOMICAL INVESTIGATION

Scientific coordinator,

Academician Prof. Dr. Constantin TOMA

PhD,

Anca-Daniela Mereacre

(Married Clapa)

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Introduction

All protected areas from our country (scientific reserves, national parks, nature parks) were declared as protected areas after specialized studies published in different magazines by botanists, zoologists, foresters, paleontologists, geologists and specialist in geomorphology.

Among the natural reservations from Moldavia region we discover Gârboavele Forest, from Galati County, which is mentioned and described in different monographic papers (Pop and Sălăgeanu, 1965; Mohan and Ardelean, 2006). Flora and vegetation of this scientific reservation were researched, papers were published by several authors in recent 50 years (Mititelu et al. 1968, 1993; Sârbu et al. 1997), floristic inventory here counting over 470 species of angiosperms, from which 40 species are very rare from Moldavian flora, and 4 of them have a special regime being vulnerable (*Adonis vernalis*), or endangered (*Lathyrus pannonicus, Glanthus elwesii* and *Paeonia peregrina*), as resulting from the "Red Book" published by Dihoru and Negrean in 2009. Moreover, the species Paeonia peregrina was declared a monument of nature, as described and illustrated in the last illustrated terrain guide for determination by Sirbu et al. 2013.

Species from vernal, summer and autumnal season are included in the flora of Gârboavele Reservation.

In this PhD thesis it were studied, after suggestion of the scientific coordinator – academician Constantin Toma, the vernal flora from Forest - park Gârboavele, analyzing the anatomical structure of the vegetative organs from 42 species of angiosperm, 31 among the dicotyledonous species (belong to 14 families) and 14 among the monocotyledonous species (belong to 4 families).

The 42 vernal species belong to different biological types.

Ephemeral, Ephemeroids, Geoephemeroides

I. Ephemeral: are annual plants with a short vegetation period; they pass the vital cycle in a short and wet time; they have dwarf port, sparsely branched stems, small leafs, well-branched root system.

They are classified in annual spring or vernal (especially in steppe) which became alive in February, and annual autumn (autumnal).

II. **Efemeroids**: These are perennial plants, with capability to increase the vital processes in dryness time and restart their growth on much agreeable times. The only similarity with the ephemeral is the short vegetation period.

Some of the authors consider that the Ephemeroids and Geoephemeroides, which we will treat further, are equal.

III. **Geoephemeroides**: are perennial plants (most of them vernal), with underground organs (bulbs, bulbo - tuber, radicular and scorching tubers, rhizomes, tuber roots) rich in reserve substances.

These plants parts are deep in to the soil by using contractile roots (*Crocus, Allium species*), because of the positive geotropic growth – of stem (*Colchicum, Tulipa, Gagea, Muscari, Hyacinthus, Ornithogalum, Scilla, Convallaria, Polygonatum, Galanthus, Corydalis, Ficaria, Anemone species*).

Once with the completion of the thesis, I wish to thank all those who have guided and supported during the execution of it.

I extend thanks and gratitude, first to Academician Professor Doctor Constantin Toma, scientific leader of this work, for scientific guidance, patience, support and help in realization of this thesis.

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I. Physical-geographical characterization of GÂRBOAVELE RESERVATION (after Brezeanu I., 1980; Posea G. et al.,1982)

Gârboavele Forest was composed from the lands of the village people, lands which in 1948 were moved in the property of the state, and also from other terrains, wooded later.

Gârboavele Forest is located in Covurluiu Depression, at 17 km N-V from Galati city, with a surface of approximate 900 ha of natural forest, from which 250 are Forest-park, and the rest is acacia plantation and protection zone. At the first objective of Forest – park it was added also the objective of being botanical, zoological and sylvan reservation.

II. FLORA AND VEGETATION OF GÂRBOAVELE RESERVATION

The flora of Gârboavele Forest counts an approximate number of 470 angiosperm species, from which about 40 are very rare in the flora of the Moldavian Region.

In spring, in April we find many vernal plants bloomed: Crocus reticulatus L. (crocus) elwesii Galanthus L. (snowdrop), Scilla bifolia L. (violet), Corydalis solid (L.) Sw. (BREBENEL), Pulmonaria officinalis L. (honey bear), Viola odorata L. (violets), Anemone ranunculoides L. (pills), Stellaria holistic L. (rocoţeaua) cation Ranunculus L. (Figwort), Arum maculatum L. (arum) etc.

The vegetation of the forest is typical forest-steppe, consist of several associations (types) of forest, mostly degraded (cleared) with poor consistency brushes, abundant flora invaded the surrounding steppe, with low power regeneration (from offshoots) and being modified by the introduction of numerous species of trees cultivated and naturalized acacia (Sirbu et al., 1997).

III. HISTORY OF MORPHOLOGICAL AND HISTOANATOMICAL RESEARCHES REALIZED ON SPECIES OF VERNAL PLANT

At the beginning it were determined species under study, then it was realized the systematic classification framing, establishing whether annual, biennial or perennial (Ciocârlan, 2009; Sirbu et al., 2013). For the same guide for determination of the remembered authors, and the monumental opera "Flora RPR / RSR" it were taken the main morphological characteristics of under terrain and over terrain vegetative organs.

All the investigated plants under histo-anatomical report come from the natural reservation "Forest – Park Gârboavele" (Galați), so I consulted papers regarding flora and vegetation of this area (Mititelu et al., 1968, 1993; Sârbu et al., 1997). The data for the physical-geographical frame were token from Brezeanu, 1980 ; Posea et al., 1982.

The natural reservation from which the study material was prevailed is mentioned in several papers including the papers elaborated by Pop şi Sălăgeanu (1965); Posea et al. (1982); last one published, "Parcuri şi rezervații naturale din România", is elaborated by Mohan and Ardelean (2006). In "Cartea roșie a plantelor vasculare din România" (Dihor şi Negrean, 2009), the plants are considered endemic and sub-endemic, declared as rare, vulnerable or jeopardized Lathyrus pannonicus, *Adonis vernalis, Galanthus elwesii and Paeonia peregrina*, the last one being declared, monument of nature" (Sâbu et al., 2013); about this one it were already published anatomical dates by Filipescu (1972, 1993) and Mereacre et al. (2014).

Scientific papers which are referring to the anatomy of the plants from families in which the vernal species are framed: *Boraginaceae* (Jodin, 1903; Schmidt, 1888), *Caryophyllaceae* (Baillaud et al., 1964), *Fabaceae* (Cumbie, 1960; Streicher, 1902), *Lamiaceae* (Chaillot, 1912; Naidu şi Shan, 1981), *Liliaceae* (Chouard, 1931; Cutler şi Gregory, 1983; Fuchsig, 1911; Galap, 1933; Gatin, 1920; Oganezova, 1982; Răduțoiu, 2011), *Papaveraceae* (Bersillon, 1955; Harvey şi Gibson, 1917), *Primulaceae* (Decrock, 1901), *Ranunculaceae* (Brouland, 1935; Filipescu, 1972; Kuklina, 1961; Marié, 1885), *Valerianaceae* (Vidal, 1930).

Suggestive and useful were the papers which are referring straight to ephemeral and ephemerides plant species, dicotyledonous and monocotyledonous (Keller, 1934; Goryşina, 1965; Szynal, 1963; Ubaidulaev, 1959). Especially we focus on the papers published by Goryşina (1965), who had researches on the anatomical structure of the vernal ephemerides plants which grow in the oak forests.

We published four articles referring to the structure of vegetative organs from *Muscari* (Clapa, 2013), *Ornithogalum* (Mereacre și Galeș, 2010), *Paeonia peregrina* (Mereacre et al., 2014) and *Viola* (Mereacre et al., 2014).

IV. MATERIAL, METHODS AND WORK TECHNIQUES

The vegetal material for our researches is represented by hypogeal vegetative organs (roots, bulbs, rhizomes, tubers) and over terrain (stems, leafs) sampled from natural plants (in the anthesis state).

The preparation of the vegetal material protocol is different in relation with the provenience of the plants.

The vegetative apparatus of the live plants, collected in the field is fixed and conserved in ethyl alcohol 70%.

The protocol for realizing the permanent microscopically preparation is placed in the fallowing stages:

1. Cutting the plant material

2. Sections chlorination

3. Section coloring

4. Section build

5. Preparation analysis

The preparation with sections by hypogeal and aerial (over ground) was analyzed with the Dutch microscope NOVEX and the photography's were realized with the Canon A 560 camera on the same microscope.

V. Results of own researches

I. Class Magnoliatae (Dicotyledonatae)

7. Family Paeoniaceae

Paeonia peregrina Mill. (Romanian Peony)

A. Morphology

Perennial plant, with some radical tuber fiber, oblong ellipsoid. Erect body, 50 - 80 cm high, not ramificated, single flora, under cylindrical or under sulcatus, smooth. Inferior long petiolate leafs, two terns, with oblong oval leaflet, rare lanceolated, 2-3 cuts or more or less lobate penne, with oval lobes lanceolated, continuous or more or less indent, the successive superior one less cut, the supreme with involucres role. The finale leaflet more or less long petiolated, smooth, on front closed green, on side light green, glaucous. Blooms in May – June.

B. Anatomy

Root

The ridiculer system is represented by thin roots, some of the tuberous, all of them very rich in reserve substances (A. Nyárády, 1953).

a. **Thin root**. The structure is secondary on the sectioned level, as result of the activity of both lateral meristems: phellogen and cambium. Phellogen produces a relative thick blanket of Quercus suber (5-6 layers, the external ones in peeling process), and one on the phelloderm more thin (2 layers of collenchyma tangential cells).

The primary leather is an amyliphere relative thick parenchyma (5-6 layers).

The central cylinder (stellate) is very thick, largely the result of the bifacial work of cambium. This has produced a very thin freely ring (tubes ridden, annexes cells, cells of liberian parenchyma) and a central woody body very thick, with two under zones: an axial one thin (with less vessels, separated by amyliphere parenchyma cells) and one other very thick (with irregular vessels, separated by less cells of cellulose parenchyma amyliphere and liberiformes fiber with the wall extremely thick).

b. **Tuberous root.** Structure recalls, partially, by thy one of a rhizome. On periphery there is the same periderm, without the phelloderm cells be different by the one of the parenchyma cortical (thick) amyliphere.

The central cylinder (stellate) is very thick, mostly totally amyliphere. In his fundamental parenchyma, cellulosed, we observe plenty leading vein radial elongated, with primary structure, disposed on a circle and separate by pithy fascicle extremely long, parenchymatics – celulossed, by amyliphere type. All the fascicles have a little of primary liber (riddled tubes and cells annexes) and more primary wood (vessels and cells of parenchymatics – cellulosed). The organ axe has little vessels of wood, often solitary, dispersed in fundamental parenchyma amyliphere.

The Stem

The transversal section contour by grooved stem (80-85 high) is circular – down grades, with coasts less obtrusive.

Epidermis presents very small cells, with external wall very thick and covered by cuticle. Here and there is visible stoma, which is distinguishing easily over the epidermis cells between which he is.

The **leather** is relative thick (25-30 layers), collenchymatic on outside, parenchymatic and by meatus type otherwise, the size of the cells grows to the central cylinder; from point to point we distinguish some aeriphere cavities on irregular contour and different size.

The central cylinder (stellate) is very thick and includes: a very winding ring of leading beam very thick medulla, parenchyma – cellulosed.

The veins are numerous, very closed together, on different sizes, the biggest ones being localized on the steamy coasts, distinguish mostly in cortical parenchyma. All the fascicles are separated by medullar beam very contracted (sclerotizated and lignified) and they have on the freed periphery few cords by sclerenchimatic fiber with the wall extremely thick and lignified.

The leading fascicles, usually the big ones, have insofar primary structure (freed with tuber circuits and annexes cells, wood with vessels and cellulose parenchyma cells), as also the secondary structure (in freed we find also parenchyma cells, and in wood we discover also liberiformes fiber).

On the stem base (much thicker), the coasts are much attenuated, the big leading fascicle are distinguished very much on the primary cortical parenchyma. The freed from all fascicle realize a very thin ring, and the wood, a much gross ring, both of them very winding. More from the sclerenchimatic fiber periphloemic are very close, so then it results only 1-3 mechanical cords on the periphery of every leading fascicle.

The leaf

The leaf has leaflet obovate – oblong, 2-3 – cut more or less lobate, on superior front with short obtrusive coats alongside of the nerve.

Petiole. The contour of the transversal section is cyclical – elliptic, modified by two exterior – adaptable coasts; which bound a relative large and deep groove.

Epidermis has cells with the external wall and is covered with cuticle.

The fundamental parenchyma is collenchymatic under epidermis and meatus as for the rest. In this parenchyma there are inserted 7-8 leading vein disposed or arc, and those from the exterior – adaptable apices. In the median fascicle we observe parenchyma cells in free and liberiforme fibers on wood, as prove that it was passed to the secondary structure. On the periphery of the leading fascicle there are group of sclerenchimatic fibers.

The **limbo**. **Epidermis** viewed from front side has very big cells, irregular contour, with the side walls extremely sinuous, stoma, numerous on the surface unity, are localized only on inferior epidermis, therefore the limbo is hypo stoma. The epidemic cells from the front of the nerve are polytonally – oblong, with multiple interpunction on the side walls.

In transversal section the median nervure (sometimes also the laterally ones on I-st order) is obtrusive very much on inferior side of the limbo, and it contains a vein in the primary structure.

Epidermis has isodiametric cells on the median nervure, on different sizes and some of them oblong tangential between the lateral nervure, always bigger on superior side of the limbo.

Mesophyll contains a lower polisade layer cells on superior side and more cells of incomplete tissue on inferior side, therefore the limbo has a bifacial heterofacial structure (dorsoventral). Some of the polisade cells present relative deeper septum from the external wall on the lumen.

The superior order leading fascicle from the lateral nerve are smaller and very smaller, some of them having only liberian elements. On the studied material it wasn't observed oxalifère cells (mentioned by Filipescu in 1972).

II. Liliatae (Monocotyledonatae)

3. Family *Iridaceae*

Muscari neglectum Guss. ex Ten. (Muscari false botryde)

A. Morphology

Perennial plant, 10 - 25 (40) cm tall. Long bulb around (12) 15 - 20 mm, with brown tunic, more or less scarificated or persistent, with more or less numerous bulbs, leafless, forming in favorable conditions nests around the main bulb. Scapes 1 - 3, 3 - 14 cm long, on the base reddish like leafs. Leafs (3) 4 - 6 (10), usually appear in the autumn, (15) 20 - 25 (35) cm long, 2 - 4 (7) mm wide, canaliculus, on the base brown reddish, on front glaucescentes and smooth, on side with 10 - 12 coasts in relief. Blooms in March – April.

B. Anatomy

Adventitious root

Rhizoderma presents small cells, with bellied external wall and slightly thick than others.

The leather is thick, differenced by:

- Exoderma: big cells, with the walls moderate bellied and corky;

- Cortical parenchyma: thick (6 -7 layers), on meatus type;
- Endoderma: with small cells, tangential oblong, with Caspary thick on radial walls;

The laterall cilinder (stellate) is thin and from pericycle it brace 5 leading fascicle (tuber circuits and annexes cells) and so much wooden fascicle, with a central vase of metaxylem, which cover the centre of organs, therefore the bone is missing.

The bulb

A transversal section throw bulb it shows us the next structure:

- One discontinuous external leather, exfoliated on some places, most of his cells are moderate collenchymatic; some of the cells contains urchin of calcium oxalate.

- An intermittent zone cork uni or bi stratificated
- On liber zone, in which is obtrusive the leading fascicle and some adventitious root with endogen origin, but which had not yet broken the leather.

- A central cilinder (stellate) thick, with numerous cords of wooden vases, which have different orientation and are sectionated on longitude; more cells from fundamental parenchyma of the central cylinder contain raphid of calcium oxalate.

The nodes of the bulb surface have a simple structure:

- The epidermis with isodiametrics cells, having the external wall thicker beside the others;
- An fundamental parenchyma of meatus type, with a lot of amyliphere cells and less crystallized (raphid)

- In the thickness of the homogeny parenchyma, incomplete type, very amyliphre generous, there are encapsulated more leading fascicle very small, on collateral type closed.

Aerial stem

Epidermis presents isodiametric cells or rectangular oblong radial, with internal and external walls thicker than the others. From one place to one other stomata are observed

The **leather** is thin (5-6 layers), parenchymatic – cellulosed, on meatus type, without to be finished with a endoderm of a special type.

The central cylinder (stell) starts with a multi layer ring of polygonal cells, localized in pericyclic position and having thin walls on the stem level. On the aged plant, this ring will present cells with the walls thicker and lignified; therefore will become a real mechanical ring of sclerenchyme.

The leading tissue forms veins of a collateral closed type, of two categories:

- Some of them smaller, on an external circle, most of them having the liber in touch with the pericyclic ring; the most fascicles having only liberian tissue.

- Others bigger, internal, in number of 8-9, disposed on a circle, this fascicles have the a moderate liber, colenchymatic and the wood with less vase disposed in 1-2 radial strings covered by cells of cellulosed parenchyma.

The bone is thin, cellulosed parenchymatic, with the same form cells, but bigger than the one from cortical parenchyma.

Along the stem, throw his base, the structure remain the same, with the difference that the epidermis cells have the internal and external walls thicker, and the fascicles on the internal circle are bigger.

The leaf

In **transversal section**, the limbo has a semi moon form or the aspect of the "U" letter, with the arms slightly divergent, delimiting an adaxial groove large and deeper. The inferior side is waved, with a lot of obtrusive coasts, which delimit valecule very little deep.

The epidermis present isodiametric cells, with the external wall thicker than on the others, on the edge of the limbo the cells have the external wall very thick. From one place to one other we discover stoma in the both epidermis so the limbo is amphistomatic.

The mesophyll is relatively thick, differenced by polisadic tissue on the inferior side and lacunary tissue multi layer on the rest. The polisadic tissue is single or bi layered, with relative short and large cells, cells like that occupy also the edges of the limbo, being carried on partially also on the superior side; on the rest, on this side we have there has short assimilative cells or even isodiametric.

The central mesophyll is formed by very big cells, without the chloroplast.

Therefore the limbo has a bifacial structure, more or less heterofacial (dorsoventral), only with dorsoventrality reversed.

The leading tissue forms more (15) veins of collateral closed type, with the wood containing vase with the walls poor lignified.

Conclusions

► The vernal flora from the natural reservation Gârboavele – Galați is represented so much of annually species (some ephemeral), and especially the perennial (most of them ephemerides or geoephemeroides); from the all vernal plants, we investigated the anatomic structure of the vegetative organs of 31 dicotyledonous species (18 annually, 2 annually – biennial – perennial, 11 perennial: 8 with rhizomes, 3 with tuber roots) and 11 species of monocotyledonous (all perennial: 2 with rhizomes, 9 with tunicate bulb)

▶ On all 11 species of monocotyledonous and a little species of dicotyledonous (*Adonis vernalis*, *Ranunculus ficaria*, *Corydalis solida*), the **root** has and remains only with primary structure (with the stelle three arch or four arch on dicotyledonous, poly arch at monocotyledonous). On the most of the dicotyledonous species, the root structure (at least of the level sectioned by us) is secondary, as result of the activity of both lateral meristeme or only of the cambium, so only to the central cylinder level (*Lamium*, *Veronica*, *Vicia*, some species of *Viola*). In the tuberose roots, the leather presents auriferous cavities of irregular contour; same auriferous cavities we meet also in the leather of the normal roots (*Ranunculus, Hesperis, Convallaria*). The endodermis is of the casparien type on the all species of investigated vernal plants.

► The steam has only primary structure on the monocotyledonous species and at some dicotyledonous species from the genus *Androsace, Moehringia, Stellaria, Veronica, Corydalis, Ranunculus, Papaver, Fumaria, Adonis, Anemone, Lamium* and some *Viola* species. On the most of dicotyledonous, along the steam we observe a crossing to the primary structure (on the superior third of the organ) on secondary structure (on the base third of the organ) but only to the central cylinder level, therefore as result of the cambium activity; this secondary structure is evidentiated by the presence of the parenchyma cells in liber and libriforme fibers in wood.

► The foliated limbo is amphistomatic on most of the species studied, only at *Glaucium*, *Adonis*, *Anemone*, *Corydalis*, *Paeonia*, *Polygonatum*, *Viola hirta* is hypostomatic.

► The mesonphill is homogeneous, usually of lacunary type, having therefore a bifacial - isofacial structure (*Androsace, Arabidopsis, Myosotis, Corydalis, Convallaria, Crocus, Gagea, Galanthus, Muscari, Ornithogalum boucheanum, Polygonatum, Scilla*) so mostly on all species of liliacee; only *Ornithogalum orthophyllum* has leafs with bifacial dorsoventral structure, with reverse dorsoventrality, therefore with polisadic tissue on superior side of the limbo.

Our main target was to know as much deeply possible the structure of the vegetative organs, under terrain and over terrain, from most of the vernal species (ephemera and ephemerides) which grow in scientific reservation Gârboavele – Galați.

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