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# MORPHO-ANATOMICAL CONSIDERATIONS ON THE SPECIES *LIMONIUM TOMENTELLUM* (BOISS.) O. KUNTZE FROM ROMANIA

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***Abstract***

*Morphological and anatomical studies are of great importance to species of the genus Limonium, because they are essential for their correct differentiation. Limonium tomentellum (Boiss.) O. Kuntze. represents a plant of great sozological importance for Romania and it is mentioned in humid, halophilous meadows. A few specialized works include floristic and vegetation studies that mention this species, but data on the morphology and especially on the anatomy of the vegetative organs of this taxon are rare or absent.*

*The analysed vegetal material originated in the classic sampling location given for this species in Romania (Bratovoieşti - Dolj County). The specimens were collected in full bloom.*

*The roots of the specimens under study are taproots, vary in length, being comprised between 70 and 120 cm, and they have a secondary structure. At the level of the central cylinder, the stem has sclerenchyma, where the vascular bundles are located, being placed on three concentric circles. This tissue replaces the fundamental parenchyma. The leaves display an isolateral structure, their petiole having a semi-cylindrical shape, with a strongly convex lower part and a slightly concave upper part that extends on the two edges with a triangular formation with pointed end. It has six large steles towards the abaxial face and ten smaller steles towards the adaxial face, while each of the two extensions of the petiole contains two small steles that are placed one below the other; therefore, it is a polystelic petiole.*

***Key words****: characters, Limonium tomentellum, morpho-anatomy, Romania.*

## INTRODUCTION

The first data regarding the halophilic species within Romania are published by Prodan (1922). Subsequently, with the appearance of specialized field guides (Prodan, 1939; Ciocârlan, 2000, 2009; Sârbu et al., 2013), important information related to the taxonomy, chorology, morphology, anatomy and ecology of these species also appears (Dihoru, 1990; Popescu et al., 2000; Oprea, 2005; Simeanu, 2005).

An inventory of the species characteristic for the saline environments in northern Romania is published by Ţopa (1939) and a material that includes all species that vegetate on lands with different salt content within Romania is published by Grigore (2008).

Due to their adaptive heterogeneity in saline soils, halophytes are grouped into two categories, i.e. obligate and facultative halophytes (Ţopa, 1954; Braun-Blanquet, 1964; Ciocârlan, 2000, 2009). Those in the category of facultative halophytes are further divided into: preferential, supportive and accidental (Popescu et al., 2000).

The plants characteristic of Romanian saline environments belong to five botanical families: Plumbaginaceae, Frankeniaceae, several genera in the Poaceae, Chenopodiaceae and Tamaricaceae families.

The Plumbaginaceae is a cosmopolitan family that has many representatives in the temperate areas of the Northern Hemisphere, preferring dry or salty surfaces (Kubitzki, 1993).

At the European level, the Plumbaginaceae family (order Plumbaginales) includes eight genera: Plumbago, Ceratostigma, Acantholimon, Armeria, Limonium, Goniolimon, Limoniastrum and Psylliostachys (Moore in Tutin et al., 1972). Only four genera (Plumbago, Armeria, Limonium and Goniolimon) with fourteen species are present in the spontaneous flora of Romania. Out of these, the *Limonium* genus has the best representation (six taxa) (Ciocârlan, 2009).

Studies on Limonium species are present in several specialized papers (Fraine, 1916; Arisz et al., 1955; Pignatti, 1971, 1972; Erben, 1993; Czerepanov, 1995; Dolores et al., 2005).
Based on a vegetal material collected from the Poltava region (Ukraine), Orlova and Kotelevsky (2016) conducted certain studies concerning the intensity of physiological processes that take place in the vegetative organs of the *Limonium tomentellum* species (intensity of respiration, perspiration and photosynthesis). Following these analyses, it was found that the intensity of the above-mentioned processes varies depending on temperature, humidity and light.
*Limonium tomentellum* (Boiss.) O. Kuntze (syn. *L. alutaceum* (Stev.) O. Kuntze, *L. czurjukiense* (Klok.) Lavr. ex Klok., *L. tschurjukiense* (Klok.) Lavr. ex Klok., *Statice tschurjukiensis* Klok., (Czerepanov, 1995), *Statice tomentella* Boiss.) is a very rare taxon in the spontaneous flora of Romania and it is known from several places in Oltenia (Dihoru et Dihoru, 1994; Dihoru et Negrean, 2009; Păun et al., 1970; Răduțoiu 2013, 2014; Răduțoiu et al., 2016, 2018). It belongs to the halophyte plant category.
The specialized literature (Dihoru et Negrean, 2009) supplies a morphological description of this taxon, but it is incomplete. Data on the anatomy of the vegetative organs of this plant are absent at the national level. Few papers in the field literature deal with the anatomy of the vegetative organs of Plumbaginagee species (Fraine, 1916; Rao & Das, 1968, 1981).
The aim of this research is to examine the anatomical features of the root, flowering stem and leaves of this species, in order to identify new characters that would contribute to a better differentiation from other species and to explain its adaptation to the substrate on which it grows.

## MATERIALS AND METHODS

The analysed plant material originated in the classic sampling location given for this species in Romania (Bratovoieşti - Dolj County). The specimens were collected in full bloom.
The observations were conducted in a differentiated manner, i.e. the root system was analysed on a number of 20 plants, while the other elements were analysed on 100 specimens; both fresh and preserved material was used for these observations. Comparable segments were cut from the roots, stems and leaves of different specimens.
The root system was analysed through the total exhumation method. The length and the width of the vegetative organs were measured with a ruler. The anatomy of these organs was studied in cross sections, under a microscope. The epidermis was studied in tangential sections.
The microscopic measurements were performed with the ocular micrometer and a Nikon microscope was used for photography.
The following terms were used in the text: $V_M$ = the maximum individual value, $V_m$ = the minimum individual value, X = the arithmetic mean of the individual values.

## RESULTS AND DISCUSSIONS

The analysis of the morphological and anatomical characters in the specimens collected from this species revealed the elements presented below.
**The root** is a taproot; first order lateral roots have an irregular appearance, grow parallel to the soil surface and display branches (Figure 1). The length of the root is characterised by the following values: $V_M$ = 120 cm; $V_m$ = 70 cm; X = 91.8 cm.

Figure 1. Shows the aerial parts of plant

In cross section, the root has a secondary structure (Figure 2). The phellem is multilayered, it is 90 µm thick and the outer layers are dead and partially exfoliated. The phellogen is single-layered, consists of slightly elongated cells in a tangential direction and it has an average thickness of 4.5 µm. The phelloderm is multilayered, its cells are regularly arranged in radial rows, just like those

of the phellem, and the tangential walls of the first four - five layers of cells are collenchimatized. No strict delimitation can be made between the outer layers of the phelloderm and the outer part of the primary cortex, just as no delimitation can be made between the inner primary cortex and the phloem.
The primary cortex is made up of ovoid and spheroid shaped cells, with spaces of variable dimensions in between them, which make up the root aerenchyma. In the median area of the primary cortex there are four nodules made up of sclereids (or mineral salt deposits).
The primary phloem is not perceived as being distinct from the secondary one; together they are 78.75 µm thick. The size of the phloem vessels is characterised by: $V_M$= 13.5 µm; $V_m$ = 6.75 µm; X = 10.57 µm. The cambium consists of one - two layers of cells that are elongated in a tangential direction. The well-developed secondary wood consists of xylem vessels, xylem parenchyma and xylem fibres, grouped in bundles of varied dimensions, generally located on the outer part of the secondary wood. The xylem vessels are not regularly arranged and have a diameter of: $V_M$ = 38.25 µm; $V_m$ = 15.75 µm; X = 27 µm. The medullary parenchyma is missing and the medullary area of the root is occupied by small xylem vessels that belong to the primary wood (Figure 2).

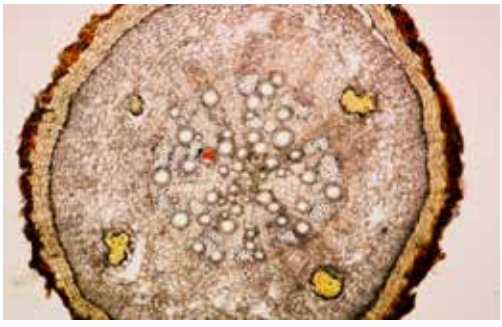

Figure 2. Root structure

The above-ground **stem** develops from a short rhizome, it is strongly branched starting with the lower third part and it has a length of: $V_M$ = 73 cm; $V_m$ = 34 cm; X = 56.1 cm.
On the cross section, the stem displays a primary structure (Figure 3) and has an average thickness of 1485 µm. The outward part of the stem displays a single-layered epidermis, which is 22.5 µm thick and has a 2.25 µm thick cuticle on its exterior side. The epidermal cells are tangentially elongated and they have cutinized walls. Many epidermal cells are transformed into elongated unicellular hairs.

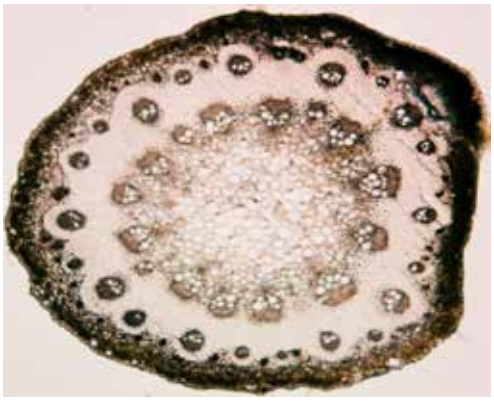

Figure 3. Stem structure

The cortex is 101.25 µm thick. The first three-four layers of cells located under the epidermis are radially elongated, arranged in palisades, with no spaces between them, with numerous chloroplasts inside. As we approach the central cylinder, the cortex cells become ovoid and then spheroidal in shape, containing few chloroplasts and small spaces between them. The starch sheath is not obvious.
The central cylinder is well developed and it does not display an obvious pericycle. The vascular bundles are arranged on three concentric circles, in the sclerenchyma, which occupies the place of the fundamental parenchyma; the circle located in the outer area of the central cylinder is made up of small bundles, the middle circle consists of larger bundles and the inner circle consists of much larger beams of the open collateral type.
The diameter of the xylem vessels in the outer circle is: $V_M$ = 6.75 µm; $V_m$ = 2.25 µm; X = 4.05 µm; that of those in the middle circle is: $V_M$ = 18 µm; $V_m$ = 6.75 µm; X = 11.7 µm, while that of the vessels in the inner circle is: $V_M$ = 27 µm; $V_m$ = 9 µm; X = 20.25 µm.
The diameter of the phloem vessels of the bundles located in the outer circle is: $V_M$= 4.5 µm; $V_m$ = 2.25 µm; X = 3.15 µm; that of those located in the middle circle is: $V_M$= 4.5 µm; $V_m$ = 2.25 µm; X = 3.6 µm, while the diameter of those in the inner circle is: $V_M$ = 6.75 µm; $V_m$ = 2.25 µm; X = 5.4 µm.

The outer circle displays vascular bundles that are completely surrounded by sclerenchyma, while others are only partially surrounded, the outer side remaining at the border with the parenchymal part that connects with the cortex. The middle circle is completely placed in the sclerenchyma and the bundles in the inner circle are incompletely surrounded by the sclerenchyma. The sclerenchyma cells located under the protoxylem have slightly thickened walls.
The central area of the stem is occupied by the medullary parenchyma, which consists of spheroidal cells with spaces of different sizes between them.

**The leaves** are arranged in a rosette at the upper part of the short rhizome and they are oblong-lanceolate, with the lamina gradually narrowing towards the petiole.
The basal ones are characterised by length values of: $V_M$ = 32.1 cm; $V_m$ = 11 cm; X = 17.13 cm and the width values of: $V_M$ = 5.3 cm; $V_m$ = 1.7 cm; X = 3.54 cm.
The adaxial epidermis has cells with straight and thick lateral walls, while the cuticle has numerous streaks on the outer walls, which represent its wrinkles. The values characteristic for the length of the epidermal cells are: $V_M$ = 47.25 µm; $V_m$ = 33.75 µm; X = 37.35 µm and those characteristic for the width are $V_M$ = 27 µm; $V_m$ = 13.5 µm; X = 22.05 µm.
The stomatal apparatus is of diacytic type, the stomata having length values of: $V_M$ = 31.5 µm; $V_m$ = 27 µm; X = 29.7 µm and density values of: $V_M$= 191.1 stomata/mm$^2$; $V_m$ = 127.4 stomata/mm$^2$; X = 161.35 stomata/mm$^2$. The trichomes are unicellular, elongated and they start from a common cell, as a group of 4-6 elements arranged in a rosette shape (Figure 4).
The values corresponding to the length of the trichomes are $V_M$ = 292.5 µm; $V_m$ = 117 µm; X = 184.5 µm.
The abaxial epidermis has epidermal cells with straight and thick lateral walls, and the cuticular streaks on the outer walls are less obvious than in the case of the adaxial epidermis. They are not regularly arranged and have length values of: $V_M$ = 60.75 µm; $V_m$ = 45 µm; X = 54 µm, while the width values are of: $V_M$ = 36 µm; $V_m$ = 22.5 µm; X = 28.57 µm. The stomata are of diacytic type, having length values of: $V_M$ = 31.5 µm; $V_m$ = 27 µm; X = 29.25 µm and density values of: $V_M$ = 127.4 stomata/mm$^2$; $V_m$ = 84.92 stomata/mm$^2$; X = 112.52 stomata/mm$^2$. Trichomes are unicellular, elongated and they start from the same cell in groups of 4-7, rarely fewer; they are arranged in a rosette and have length values of: $V_M$ = 234 µm; $V_m$ = 108 µm; X = 170.55 µm.

Figure 4. Trichomes unicellular

The leaf lamina is of isolateral structure type (Figure 5) and it is 301.5 µm thick. The single-layered adaxial epidermis consists of tangentially elongated cells, it is 22.5 µm thick and has a 2.7 µm thick cuticle.

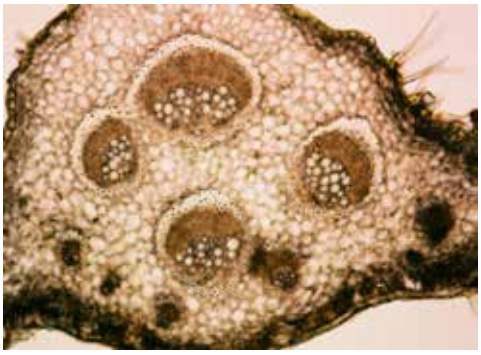

Figure 5. Leaf structure

The adaxial palisade parenchyma consists of three layers of cells that are radially elongated and do not display spaces between them; the substomatal chamber is located under the stomata. The lacunar parenchyma consists of cells that are slightly elongated in tangential direction and have small spaces between them.
The abaxial palisade parenchyma consists of two, rarely three layers of radially elongated cells. The abaxial epidermis is single-layered, it

is 20.25 µm thick and has a 2.25 µm thick cuticle on the outside. Beneath the stomata there is the substomatal chamber.
At the level of the midrib there are eight vascular bundles of open collateral type, of which four large ones are placed towards the abaxial face of the leaf and four small ones towards the adaxial face. All vascular bundles are surrounded by a sclerenchyma ring, which is much more developed around the large vascular bundles. The diameter of the phloem vessels within the four large vascular bundles has the following values: $V_M$ = 6.75 µm; $V_m$ = 2.25 µm; X = 3.83 µm, while that of the xylem vessels from the same vascular bundles is characterised by: $V_M$ = 24.75 µm; $V_m$ = 9 µm; X = 16.42 µm.
On the cross section, the **petiole** has a semi-cylindrical shape (Figure 6), with a strongly convex lower part and a slightly concave upper part that extends on the two edges with a triangular formation with pointed end.

Figure 6. Petiole structure

On the outside, the petiole has a single-layered epidermis that consists of cells with cutinized walls; it has an average thickness of 18 µm, the cuticle being 0.9 µm thick. Among the cells of the epidermis there are elongated unicellular hairs that become sharp at the tip.
The cortex is multi-layered and consists of spheroidal and ovoid cells with large spaces between them. The petiole is polystelic, having six large steles towards the abaxial face and ten smaller steles towards the adaxial face, while the two extensions of the petiole contain two small steles that are placed one below the other. The large steles and partially the small steles are surrounded by a multilayered sclerenchyma ring. On the outside of the phloem and xylem there is located the single-layered endoderm, which consists of cells with thickened walls, combined with cells with thin walls. The cambium is to be noticed between wood and phloem. Each large stele is characterised by the presence of numerous xylem vessels that are not orderly arranged (Figure 7).

Figure 7. The structure of the conducting beam

The diameter of the xylem vessels has the following values: $V_M$ = 18 µm; $V_m$ = 6.75 µm; X = 13.05 µm. The xylem vessels are accompanied by xylem parenchyma.
The phloem is made up of vessels with a diameter characterised by the following values: $V_M$ = 6.75 µm; $V_m$ = 2.25 µm; X = 4.5 µm and of phloem parenchyma.

## CONCLUSIONS

The branching of the root near the soil surface is due to the high humidity of the areas where this plant grows. The secondary structure of the root derives from the activity of the phellogen and of the one - two layers of tangentially elongated cells that belong to the cambium.
The flowering stem is strongly branched starting with the lower third part. It has a primary structure and numerous elongated unicellular hairs on the outside.
The leaves have an elliptical elongated shape and they are arranged in a rosette, at the basal part of the flowering stem. Their structure is isolateral and they have numerous elongated unicellular hairs that start from the same cell in groups of four to seven, rarely fewer.
The petiole has a semi-cylindrical shape, with a strongly convex lower part and a slightly concave upper part, which extends on the two edges with a triangular formation that sharpens towards the tip. The petiole is polystelic; it displays six large steles towards the abaxial face and ten smaller steles towards the adaxial face, while the two

extensions of the petiole contain two small steles that are placed one below the other.

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