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The Anatomy of Vegetative Organs of the Species *Trifolium repens* L.

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Abstract: The anatomy of vegetative organs of the species *Trifolium repens* L. The investigations covered a large number of individuals from nine populations of the species from different localities. The investigations were carried out on the materials preserved in the mixture of formalin, glacial acetic acid and alcohol. The study presents, the analyzed anatomical features and their relation with ecological factors.

Key words: Populations, leaf, stem, root, mesomorphic status, xeromorphic elements, amfistomata

INTRODUCTION

Trifolium repens L. is very rich with species. This species has been the object of research of many scientists: Hoglind (1997), Lindroth *et al.* (1999) and Yamada *et al.* (1989). *Trifolium repens* L. is widely spread starting from the seashore area up to the mountains area (it reaches the altitude of 1400 m above sea level). This species grows on wet and dry meadows, beaten places, cracks of concrete area and along the roadside.

There is a lot of information on morphologic, physiologic and ecologic features, but this is not the case for anatomic features; there is a lack of information on anatomic features of the species *Trifolium repens* L.

Taking that into the consideration, it was recognized that there is a need for detailed research of the anatomic features of white clover and so they could be presented in this study.

MATERIALS AND METHODS

In order to have a real picture of the features of a species especially, the anatomic features, it is very necessary to investigate a large number of individuals from a large number of the population. Therefore, a large number of individuals found in nine populations from the Prishtina are used to investigate the anatomy of vegetative organs of the species *Trifolium repens* L.

At least 10 individuals from each population were investigated. Plants were collected during the period Jun-August 1998/1999.

The collected materials were preserved in F.O.A, the mixture of (formalin, glacial acetic acid and alcohol).

The preserved materials were used for the preparation of a large number of microscopic sections. Thin sections were thoroughly rinsed in Javell lime and afterwards were treated with Congo Red (for indicating the cellulosed walls) and Methyl Green dyes (for indicating the lignin in the walls). For preparation of the thin and qualitative sections a manual microtome was used. In order to have comparable results the sections were made from parts from the same level of leaf and the same distance from the ground to the stem and root. Micro-photographs were made which present-certain details of the anatomy of the vegetative organs of the species *Trifolium repens* L.

RESULTS AND DISCUSSION

Leaf anatomy: The outer part of the leaf is covered by one-layer cell epidermis, the epidermis consists of cells whose external walls are more or less thickened. If we compare thickness of the upper epidermis to the lower epidermis we can come to the conclusion that the upper epidermis is thicker than the lower one. The highest average value of the epidermis thickness from the both sides of the leaf was noted at the population from the locality number 6. This indicates the connection with the dry condition of the locality where this population grows. Individuals of the populations that grow on dry meadows, beaten places and on the cracks of concrete areas have smaller epidermal cells compared to the populations growing on wet meadows. The cuticle is thin, from 2-4 μm. According to Bacic and Milicic (1985) the cuticle thickness of *Quercus ilex* as a xeromorphic plant is on average 5.7 μm. Thus the thickness of cuticle in the

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Trifolim repens L. leads to the conclusion that this species is a mesomorphic one. Anticlinal walls of epidermis cells are flat, slightly undulated up to significantly undulated. It is noted that the degree of undulation is not the same at the anticlinal walls of epidermis cells from different individuals belonging to the different populations and growing in locations with different degrees of moisture. Stomata are placed on the upper and lower epidermis (Fig. 1 and 2), therefore leaves are amfistomata.

If we see the structure of the guard cells it is obvious that such stomata belong to the Amarilis type. The number of stomata is not the same on the upper and lower epidermis (Table 1), normally, the number of stomata on the upper epidermis is bigger than on the lower epidermis. The same concluded by Soljan (1982) on several Genera of Edrainthus. Whereas, the stomata size on the upper epidermis is smaller. So, the stomata size stands in a negative correlation with the number of stomata.

The highest average number of stomata was found at the individuals of the population growing in locality number six, with a low degree of moisture (170.8 mm^{-2}) Table 1.

On the lower epidermis of the leaf there are simple, multi-cell trichoma with live content. The presence of trichoma is an indication of a xeromorphic feature of the species *Trifolium repens* L. (Fig. 3).

Mesophyll is very well differentiated on the *palisade layer* and sponge layer. The palisade layer consists of two layers of cells (Fig. 4). The three layer palisade tissue was found in the leaves of individuals inhabiting areas poor with moisture (beaten places, cracks of concrete, etc.).

The cells of the *palisade layers* are mostly prolonged, cylindrical, thus the height exceeds two ore three times the width of the cell. Beneath the palisade layer is the *spongy layer*. The cells of the spongy layer are more rounded and not so tightly packed. There are large intercellular air spaces. These cells contain fewer chloroplasts than those of the palisade layer.

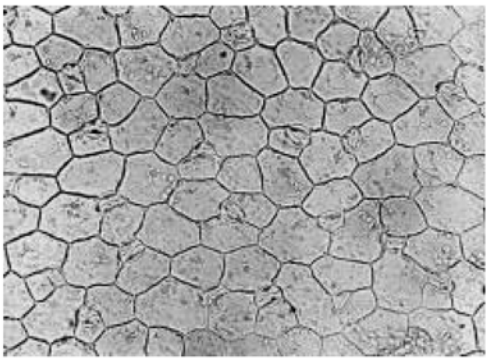

Fig. 1: Upper epidermis

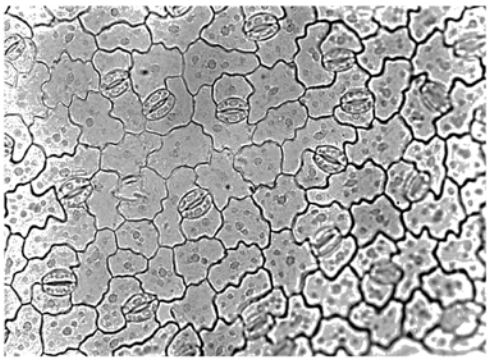

Fig. 2: Lower epidermis

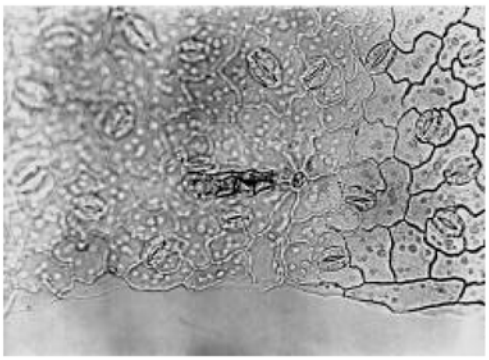

Fig. 3: Trichoma

Table 1: The average values of some quantitative anatomic features per population

The number of populations	The thickness of upper epidermis (μm) $\bar{X}$	The thickness of lower epidermis (μm) $\bar{X}$	The number of stomata on the upper epidermis (mm^2) $\bar{X}$	The number of stomata on the lower epidermis ($1mm^2$) $\bar{X}$	The length of stomata on the upper epidermis (μm) $\bar{X}$	The length of stomata on the lower epidermis (μm) $\bar{X}$	The width of stomata on the upper epidermis (μm) $\bar{X}$	The width of stomata on the lower epidermis (μm) $\bar{X}$
1	17.8	15.5	75.1	58.3	14.6	22.2	12.1	16.0
2	19.4	16.7	72.0	50.2	15.1	23.4	12.1	18.8
3	20.5	16.3	80.0	41.4	18.0	24.6	13.4	18.2
4	18.7	14.5	73.4	59.1	17.6	24.8	13.6	18.6
5	19.6	15.5	89.3	56.2	16.3	22.4	11.7	16.8
6	21.0	18.0	89.0	81.7	17.2	22.1	12.3	17.5
7	20.4	17.6	76.2	56.1	15.8	22.4	12.5	17.8
8	19.1	16.1	86.4	75.1	14.9	20.8	11.9	18.5
9	20.8	15.7	70.2	51.2	16.3	23.0	12.2	16.7

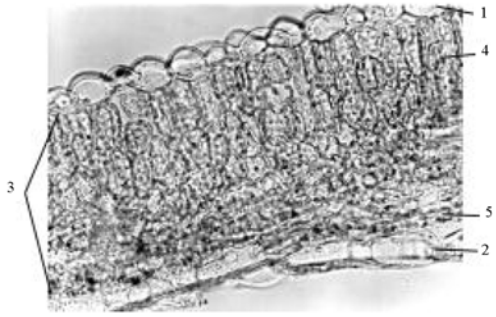

Fig. 4: Mesophyll the leaf cross section, 1): Upper epidermis, 2): Lower epidermis, 3): Mesophyll, 4): Palisade layer, 5): Spongy layer

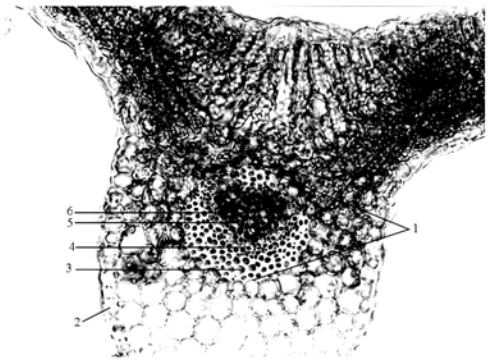

Fig. 5: The cross section of the leaf on the level of central vein, 1): Vascular bundle, 2): The Epidermal cells, 3): The mechanic tissue, 4): Phloem, 5): Xylem, 6): Trachea

Along the middle of the leaf elongates a well developed central vein (Fig. 5).

Xylem is very well presented in the vascular bundles; it is placed toward the upper side of the leaf. Hardness of the leaf is created by the presence of the mechanic tissue in the form of a cap situated on the top each vascular bundle (Fig. 5).

The stem anatomy

Primary structure: The young part of the stem reflects the primary structure (Fig. 6).

The stem from the outside is covered by epidermis-the primary protection tissue. Usually, epidermis consists of one layer of cell but there are certain cases when the epidermis is 2 layers. There is a thin cuticle existing on the outer walls of the epidermal cells. The number of stomata is relatively small. The primary cortex consists mostly of parenchyma. The last layer of the primary cortex-endodermis consists of tightly linked cells, without intercellular. The central stele occupies the medium part of the stem. The first outer part of the stele is called pericycle. Beneath the pericycle there are vascular

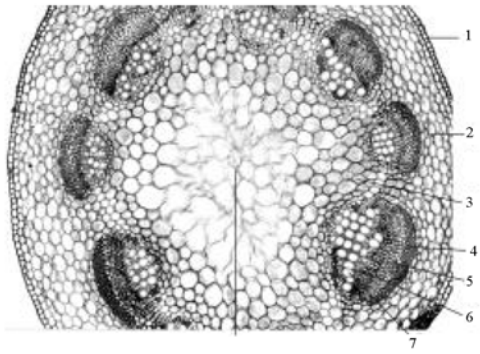

Fig. 6: Primary stem growth, 1): Epidermis, 2): Primary cortex, 3): Central stele, 4): Mechanic cap, 5): Phloem, 6): Cambium, 7): Xylem, 8): Pith

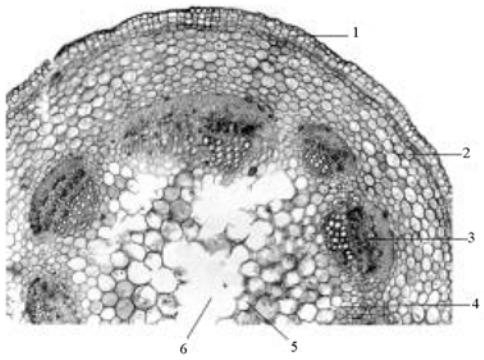

Fig. 7: The secondary stem growth, 1): The cork, 2): The primary cortex, 3): Vascular bundle, 4): The central stele, 5): Pith, 6): The central gape

bundles placed in a cylindrical order. The vascular bundles belong to the collateral opened type. Pith occupies the central part of the stem and it consists of the basic tissue (parenchyma) which very often serves as a reserve tissue. The mechanic tissue creates mechanic capes on the top of each vascular bundle.

Secondary structure: The older multiyear part of the stem shows a secondary structure (Fig. 7).

The secondary structure is characterized by: secondary protection tissue-peryderm which consists of a large number of cork cell layers and it goes deep into the cortex. In some places epidermis still remains, but it is fragmented. The primary cortex remains as well.

The secondary growth is linked with the activity of special lateral meristema called cambium. Here, cambium originates partially from the primary merystema and partially from the secondary meristema.

At the same time on part of the primary cortex felogen is created, which will give rise to the cork and felodermis. Pith very often bursts and creates the central gap.

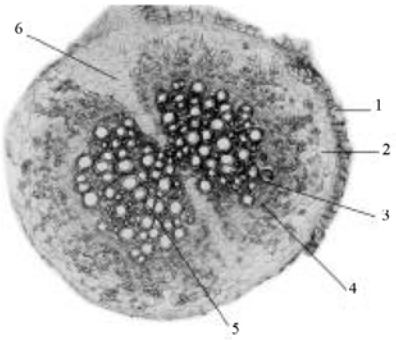

Fig. 8: The young root cross section, 1): Exodermise, 2): The parenchyma of primary cortex, 3): Central stele, 4): Phloem, 5): Xylem, 6): The beginning of creation of the lateral root

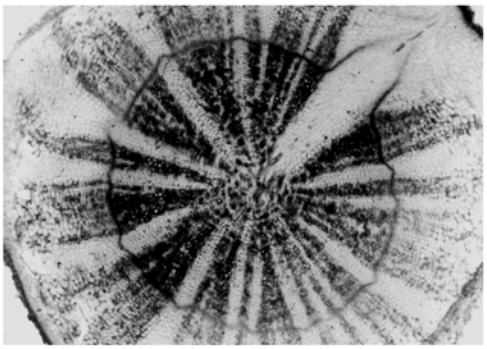

Fig. 9: The root cross section-secondary growth

The root anatomy: The root is the basic organ of cormophyta plants. The main role of the root is to support the plant and absorb water and mineral materials from the soil. Since, the white clover belongs to the dicots group of plants, it is understandable that the root shows primary and secondary growth. At the young root, the transition from the primary to the secondary growth can be noticed (Fig. 8).

On the outer part of the root with secondary growth the periderma is created. Usually periderma is created very early, thus the pericylcle cells intensively multiply, where their first superficial layer gives felogen which gives raise to the cork and to the small amount of feloderm (Fig. 9).

Phloem and xylem elements are structured in radial rows alternating with the pith rays.

CONCLUSION

Since, a large number of individuals from nine populations of the species *Trifolium repens* L. were used during the research of the anatomy of vegetative organs, it was possible to obtain information on the detailed anatomic structure and at the same time disclose the inter-population variability of the following anatomic features:

- The thickness of cuticle
- The thickness of upper and lower epidermal cells and the degree of the undulation of anticlinal walls
- The size of the epidermal cells
- The number and the size of the stomata on the upper and lower epidermis
- The degree of differentiation of mesophyll
- The thickness of palisade tissue and sponge tissue
- The number of the vascular bundles in the leaf
- The thickness of the stem, the surface dimensions of the stem's vascular bundles and the thickness of the phloem and xylem of the stem

Since, long time ago, for most of the presented features, it was find out that are closely linked with the complex of ecological factors where the given populations grows, through which the disclosed variability of some anatomic features can be explained.

From the ecological point of view, the anatomic features of vegetative organs of *Trifolium repens* L. mostly shows the mesomorphic status with certain elements of xeromorphosis, i.e., the amfistomata leaves, trichomes, etc.

Not the same degree of mesomorphosis and xeromorphosis of individuals of different populations was found out, what indicates the ecological-anatomic differentiation of the investigated populations.

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