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Histo-Anatomical Studies in *Allium saxatile*, a Specie with Ornamental Potential

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Abstract

The current paper presents some characteristics of the anatomy of vegetative organs (adventive root and leaf) of *Allium saxatile* M. Bieb., a species rare in the Romanian flora, which have not been described before in the literature. Histo-anatomical studies performed on *Allium saxatile* plants sampled from three local populations identified in the Dobrogea area, complete already existing data from other species of this genus, and represent the beginning of future studies on ecological anatomy, taxonomy and *ex situ* conservation strategies. Some of the obtained results confirm data from literature, but additional observations have been made that show features not reported previously for any specie of the *Allium* genus, such as the presence of colenchymatized cells in the internal mesophyll at the lamina level; or the presence, at sheath level, of epidermal cells with uniformly lignified and thick walls.

Keywords: *Allium saxatile*, vegetative organs, anatomy

Introduction

The taxonomic position of *Allium* and its related genera has been a controversial topic. In the past the genus was included in the family Alliaceae, but molecular systematics has led to some significant changes regarding its taxonomic position. In the first "Angiosperm Phylogeny Group" classification (APG, 1998) the genus was placed in a distinctive family, Alliaceae, which later in the third system of classification elaborated by the prestigious group (APG, 2009) was reconsidered as a subfamily, Alliodeae, within the family Amaryllidaceae. This latter system of classification has been broadly accepted in recent taxonomic and phylogenetic studies (Chase *et al*., 2009). Also the infrageneric classification of this large genus, including about 750 species (Stearn, 1992) is complex and controversial. The traditional system followed Regel (1875), but molecular data continued to bring new information. At present three main evolutionary lines within the *Allium* genus are considered: i. subgenera *Amerallium, Nectaroscordum, Microscordum*; ii. subgenera *Melanocrommyium, Caloscordum, Anguinum, Porphyroprason, Vvedenskia*; iii. subgenera *Rhizirideum, Butomissa, Cyathophora, Cepa, Polyprason, Allium s.str., Reticulatobulbosa s. str*. (von Berg *et al*., 1996; Kamenetscky and Fritsch, 2002; Fritsch and Friesen, 2002; Friesen *et al*., 2006). *Allium saxatile* M. Bieb. is placed in the third line of evolution, in subgenera *Polyprason*, sect. *Oreiprason* (Friesen *et al*., 2006).

Morphological and anatomical studies have a great importance in the *Allium* genus, since the shape, size, colour and texture of rhizomes, bulbs, adventives roots, leaves, scales, inflorescences, petals, stamina, ovaries, and seeds present a great variability, as well as their structure, which provide suitable data for diagnosis of different taxa (Stearn, 1980; Gregory *et al*., 1998; Fritsch and Friesen, 2002).

Data regarding the structure of organs of *Allium* taxa refer mostly to particularities of the root structure (Peterson and Perumalla, 1984; Zanoschi and Toma, 1985; Fritsch, 1992a; Uysal, 1999), the floral stem (Fritsch, 1993; Uysal, 1999; Namin *et al*., 2009), the leaf (Solereder and Meyer, 1928; Zanoschi and Toma, 1985; Fritsch, 1988; Uysal, 1999; Bogdanović *et al*., 2008), and the secreting structures (Uysal, 1999; Fritsch, 1992b). It is also worth mentioning the use of SEM and the importance of micro-morphological data of seminal tests carried out by Kruse (1984, 1986, 1988, 1994), and aspects related to the micro-morphology of pollen (Bogdanović *et al*., 2008).

Toma *et al*. (1994) published the results of their histo-anatomical work on the vegetative apparatus (root, aerial stem, leaf-sheath and foliar limb) of 13 *Allium* taxa, which are in the *ex situ* collection of "Anastasie Fătu" Botanic Garden in Iași: *A. angulosum* L., *A. cepa* L., *A. cyaneum* Regel., *A. cyathophorum* Bur. et Franch. var. *farreri* (Stearn.) Stearn., *A. montanum* Schmidt, *A. oleraceum* L., *A. porrum* L., *A. ramosum* L., *A. sativum* L., *A. schoenoprasum* L., *A. scorodoprasum* L., *A. sphaerocephalum* L. and *A.*

tauricum (Bess.) Pall.; this is the only study of this type in Romania.

Allium saxatile M. Bieb. (syn. *Allium globosum* M. Bieb. ex Redouté) ranges from Italy to the Pontic-Balkan region, extending to the north to Central Russia and Ukraine and to the East up to NW China (Tropicos database). It has been recently reported from Turkey (Özhatay *et al*., 2012) and in Romania has a statute of rare species (Oprea, 2005), present on rocky hills, rock cracks and especially on limestone (Zahariadi, 1966). Synonyms of the species are, among others, *A. caucasicum* M. Bieb.; *A. dshungaricum* Vved; *A. gmelinianum* Miscz. ex Grossh.; *A. stevenii* Willd, ex Ledeb (The Plant List, 2010). It is a small plant (15-35 cm high) and presents ornamental interest due to its globular umbelliform inflorescences, with pink coloured flowers and the hypsophile longer than the inflorescence (Ciocârlan, 2000). Since *A. saxatile* flowers from June till September it could be recommended both for garden decoration (stone designs, borders, groups, decorative pots), and also like cut flower.

In Romania, this species was identified in the counties: Botoşani (Stânca – Ştefăneşti), Constanța (Băltăgeşti at Alah – Bahir hill, Hagieni Forest, Fântânița – Murfatlar reservation, Gura Dobrogei reservation, Cheia Karst, Murfatlar at Serpla – Cula hill), and Tulcea (Cheia – Munții Măcinului, Culmea Pricopanului, Babadag, Popina island, Jurilovca at Capul Dolojman, Izvoarele at Consul hill, "Vârful Secaru" – Atmagea reservation, Greci at Piatra Îmbulzită, Țuțuiatu Mountain, between Babadag and Caugagia, Sarica, Beştepe, Tulcea, Greci) (Oprea, 2005).

Apart from classic taxonomical and morphological descriptions and considerations (Toma *et al*., 1994; Ciocârlan, 2000; Oprea, 2005; Friesen *et al*., 2006) as far as we know there are no other data published on this species. Therefore, the work described here will contribute to the knowledge, from a histo-anatomical perspective, on this rare taxon of the Romanian flora.

Materials and methods

Biological material used in the current study was represented by plants of *Allium saxatile* M. Bieb. sampled from natural habitats (Turcoaia and Babadag Forest - Tulcea County, Cheile Dobrogei - Constanța County) situated in Dobrogea area from SE Romania (Fig. 1) and planted in the experimental field of the University of Agricultural Sciences and Veterinary Medicine Iaşi (NE Romania), in the autumn of 2010 (September). To perform the morpho-anatomical studies, plants were harvested in May 2011.

The sampling areas are characterised by hot and dry climate specific to Dobrogea. Although there are no significant differences in the multi-annual temperature mean values (11.3 and 12 ˚C), the level of multiannual mean rainfall varies from 380 to 500 mm. The driest area is Babadag, characterised by an irregular rainfall regime: frequently the values of potential evaporation over-pass by 70% that of rainfalls, minimal values of annual rainfalls are lower than 300 mm, and the maximal ones over-pass 1000 mm (Bîndu, 1971).

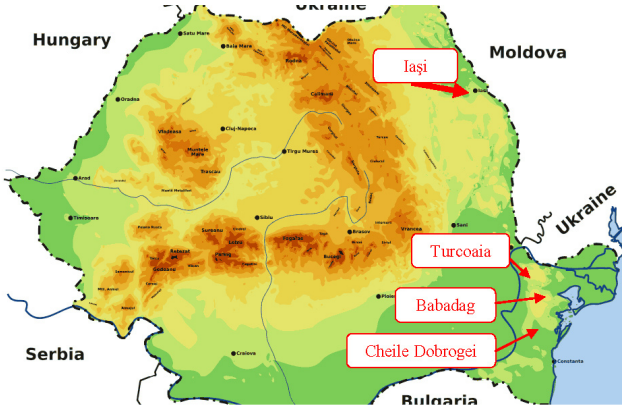

Fig. 1. Location of the studied population of *Allium saxatile* M. Bieb. (http://mapsof.net/uploads/static-maps/romanian_mountains.png)

Regarding the pedological conditions, at the sampling site in Turcoaia lithosols and skeletal soils predominate, alternating with pedological discontinuities represented by rocks (Florea and Munteanu, 2012; Geological Institute, 1966), frequently colonized by different species of lichens and bryophytes. The substrate of these soil are rocks quartz porphyry (Pîrvu, 1964), hard to be destroyed due to the high content in quartz. The rocky slopes are difficult to access, and this helps maintaining some endemic plant species in grazing areas. The presence of rocks helps the accumulation of fine material and the maintenance of the formed soil material, assuring a good protection against hydrological erosion processes.

In the area from Cheile Dobrogei narrow valleys with vertical walls are common; they are known as gorges and were formed after karstic erosion based on progressive dissolution processes (Tufescu, 1966). Some portions of the river bed frequently remain dry, because water is getting deep through various cracks and breaks which offer good water permeability to limes. Dominant soils in this area are rendsines (on plain fields or with a light slope), rendsinic and skeletal lithosols (on moderate or heavy slopes). The shape of rocks is not regular and their surface is colonized by different lithophytes, species installed on the rocks' surface, and chasmophytes, species installed inside the cracks (Anghel *et al*., 1972). On the irregular surface of rocks and in the micro-concavities formed by the progressive dissolution of calcium carbonate a diverse flora of lichens, bryophytes and high plants is installed.

The sampling area at Babadag Forest is situated on a plateau with low slopes. Here rendzines, lithosol rendzinics, kastanozioms, gleic chernozems, and brown chernozems are dominant (Florea and Munteanu, 2012; Geological

Institute, 1966). Soils with a high content in dust formed on loess and loessoid deposits have a greater susceptibility to erosion.

The plant samples were fixed and conserved in 70% ethanol for two days and then cross-sectioned by using a commercial razor blade and a hand microtone. The sections were stained with green iodine (Fluka) and red ruthenium (Merck) and studied using an Olympus VANOX AHBS3 optical microscope with attached photo camera. A number of seven plants from each natural habitat were analysed. Part of the seeds produced by the plants was included in the collection of Floriculture at the University of Agricultural Sciences and Veterinary Medicine Iaşi.

Results and discussion

Root (Figs. 2-4). The contour of the transversal section is circular. Rhizoderm is single-layered, with thin-walled small cells.

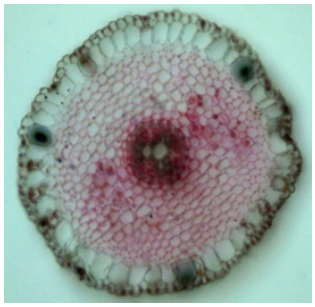

Fig. 2. *Allium saxatile* (Cheile Dobrogei), cross section through the root

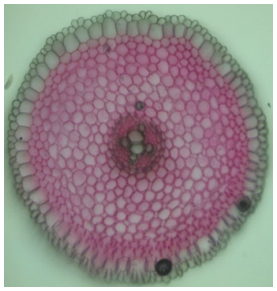

Fig. 3. *Allium saxatile* (Turcoaia), cross section through the root

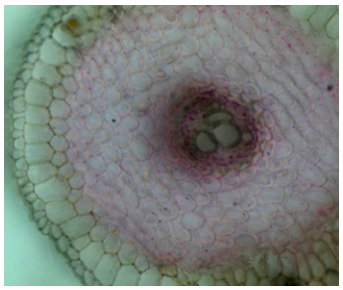

Fig. 4. *Allium saxatile* (Babadag Forest), cross section through the root

Bark, very thick (11-13 layers), is distinctly differentiated in exoderm, cortical parenchyma and endoderm. The exoderm presents one (in the material sampled from Cheile Dobrogei and from Turcoaia) or two layers (in the material collected from Babadag Forest) of polygonal cells, with thin and slightly suberized walls. Cells that form the exoderm and which are located under the rhizoderm, are very large. Cortical parenchyma is of the meatic type. In the plants sampled from Turcoaia, the endoderm is of the tertiary type, the cells have lignified internal and lateral walls thicker than the external ones. In the plants collected from Cheile Dobrogei and Babadag Forest, the endoderm is of the primary type, with Caspary points (the roots are very young with an endoderm in formation).

The central cylinder, much thinner than the bark, starts with a single-layered pericycle. The vascular system is represented by three wooden fascicles (Cheile Dobrogei and Babadag Forest) and four xylem fascicles (Turcoaia), which alternate with the same number of phloem fascicles; furthermore, a metaxylem vessel replaces the marrow, being located in the central position.

Leaf (Figs. 5-14). ***The sheath*** (Figs. 5-8). The contour of the transversal section is circular.

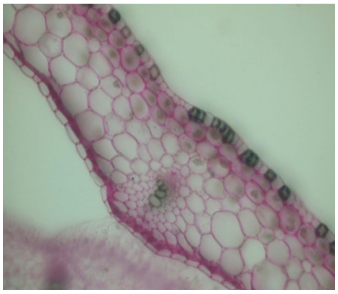

Fig. 5. *Allium saxatile* (Cheile Dobrogei), cross section through the sheath

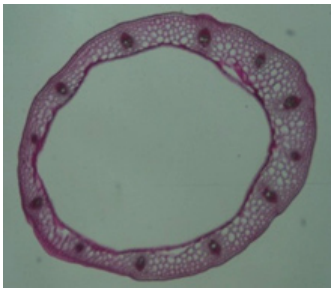

Fig. 6. *Allium saxatile* (Cheile Dobrogei), cross section through the sheath

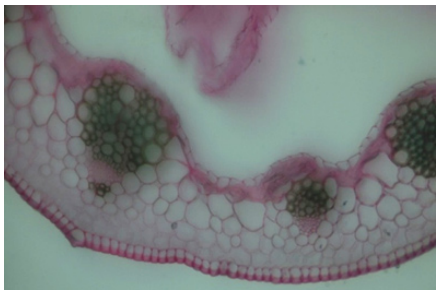

Fig. 7. *Allium saxatile* (Babadag Forest), cross section through the sheath

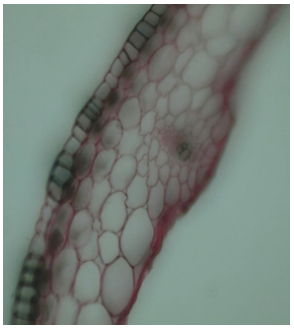

Fig. 8. *Allium saxatile* (Turcoaia), cross section through the sheath

The external epidermis has large cells, with thin and slightly cutinized external walls. Stomata are rarely present. Some epidermal cells have all the walls uniformly lignified and thickened (Cheile Dobrogei, Turcoaia). The internal epidermis has cells slightly elongated tangentially, with very thin walls, sometimes being under exfoliation.

The mesophyll is homogenous, lacuna type. Leading fascicles have a similar size (Cheile Dobrogei) or there are alternating large and small ones (Turcoaia, Babadag Forest). This fact demonstrates that this is the upper face.

The lamina (Figs. 9-14). The contour of the transversal section at the median level is semicircular unregulated, with 5-6 ridges on the inferior (underside) face and three dents on the superior (upper) face (Cheile Dobrogei and Turcoaia) or semicircular, with a flat upper face (sometimes two fine ridges could be observed in the biological material sampled from Babadag Forest).

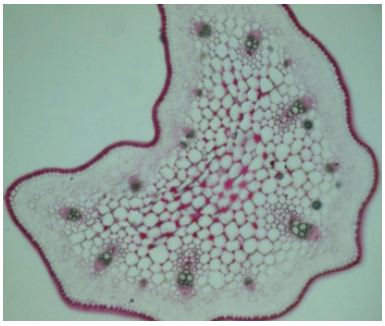

Fig. 9. *Allium saxatile* (Cheile Dobrogei), cross section through the lamina

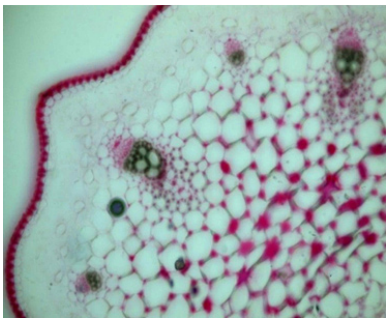

Fig. 10. *Allium saxatile* (Cheile Dobrogei), cross section through the lamina

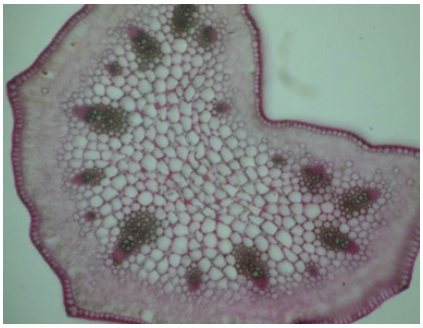

Fig. 11. *Allium saxatile* (Turcoaia), cross section through the lamina

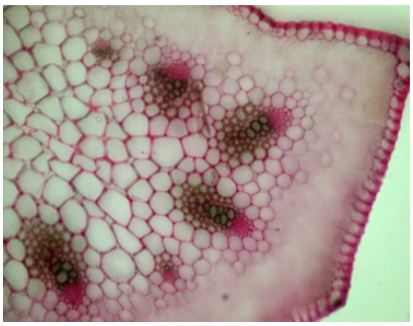

Fig. 12. *Allium saxatile* (Turcoaia), cross section through the lamina

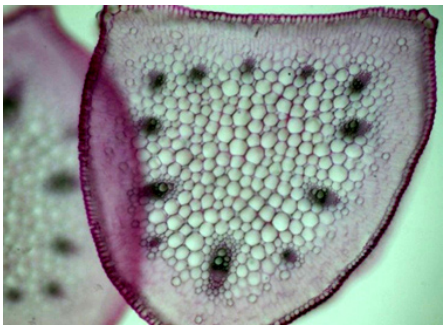

Fig. 13. *Allium saxatile* (Babadag Forest), cross section through the lamina

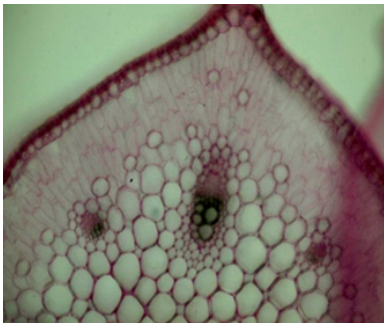

Fig. 14. *Allium saxatile* (Babadag Forest), cross section through the lamina

The epidermis presents cells more or less iso-diametric, with the external walls thickened and covered with a relatively thick cuticle with a ribbed aspect. Stomata are present, and very numerous in the material sampled from

Babadag Forest; they are of the xerophytic type, each having a relatively deep supra-somatic chamber.

The mesophyll is separated in two sub-zones: 2-4 layers of palisade-type cells, with high cells, in a hypodermic position, and a hard colenchimatized (Cheile Dobrogei), slightly colenchimatized (Turcoaia) or meatic type (Babadag Forest) parenchyma. The presence of some secreting elements (probably laticiferous) could be observed between these two types of tissues, but also in the palisade tissue.

Leading fascicles of the collateral type either vary in size, usually alternating some big ones with some small ones (Cheile Dobrogei, Turcoaia), or have almost the same dimensions (Babadag Forest). Mechanical elements of schlerenchimatic nature, with a weak lignification, are present only at the xylemic pole (Turcoaia) or are missing (Cheile Dobrogei, Babadag Forest). All the fascicles have the xylemic pole oriented to the median part of the mesophyll, a fact that attests the monofacial structure of the lamina from *Allium saxatile*.

Tab. 1 synthesizes histo-anatomical particularities of *Allium saxatile* plants from the three locations under study. A series of differences are evident, probably related to the different climatic conditions and different soil types in the three areas natural habitats under study. A general trait observed *Allium saxatile* is the predominance of xerophyte characters regardless of the natural habitat of plants (central cylinder with wooden vessels, stomata with xerophytes types with supra-stomata chamber, epidermis of lamina is made by cells with thick external walls and covered with a thick cuticle). Some of these characteristics could be partially correlated with the local specific environmental conditions, especially water stress. Temperature regime seems to be less responsible for those differences due to the fact the mean monthly temperatures recorded at Turcoaia, Cheile Dobrogei, Babadag are very close.

Occurrence of double-layered exoderma in plants from Babadag could be explained by the accentuated water deficit determined by the reduced rainfall and the soil type and also by the poorest vegetal coverage (Fig. 15). Monolayered exoderma observed at plants from Turcoaia and Cheile Dobrogei proves that plants were less exposed to soil water stress. In conditions from Cheile Dobrogei this could be explained by the presence of lime fragments in the soil, which increases the water retention capacity. Additionally, the presence of a bryophytes layer has a protec-

Tab. 1. Histo-anatomical characteristics of plants according to the source of plant material

Organ	Anatomical parts		Natural area of plants		
			Turcoaia	Cheile Dobrogei	Babadag Forest
Root	Rhizoderm		- single-layered with small cells, with thin walls	- single-layered with small cells, with thin walls	- single-layered with small cells, with thin walls
	Bark	Exoderm	- single-layered	- single-layered	- bi-layered
		Cortical parenchyma	- meatic type	- meatic type	- meatic type
	Central cylinder		- 4 xylem fascicules	- 3 wooden fascicules	- 3 wooden fascicules
Leaf	Sheath	External epidermis	- cells with all walls uniform thickened and lignified	- cells with all walls uniform thickened and lignified	- cells with all walls uniform thickened and lignified
		Mesophyll	- leading fascicules with different dimensions (alternates some large with small ones)	- leading fascicules with relatively equal dimensions	- leading fascicules with different dimensions (alternates some large with small ones)
	Lamina	Epidermis	- cells ± iso-diametric with external walls thickened and covered with a thick cuticle with a ribbed shape - stomata xerophytes type, with a deep supra-stomatic chamber	- cells ± iso-diametric with external walls thickened and covered with a thick cuticle with a ribbed shape - stomata xerophytes type, with a deep supra-stomatic chamber	- cells ± iso-diametric with external walls thickened and covered with a thick cuticle with a ribbed shape -stomata are very numerous, xerophytes type with a deep supra-stomatic chamber
		Mesophyll	- parenchyma lightly colenchimatized - leading fascicles varies as size, alternating some large with small ones - are missing mechanical elements of schlerenchimatic nature, which are present only in the xylem pole	- parenchyma strong colenchimatized - leading fascicles varies as size, alternating some large with small ones - are missing mechanical elements of schlerenchimatic nature	- parenchyma of meatic type - leading fascicles with almost same dimensions - are missing mechanical elements of schlerenchimatic nature

tive role upon vegetation by diminishing the transpiration process (Fig. 16). Although at Turcoaia the skeletal soil has a low capacity of water retention and the soil volume which could be explored by plants' roots is reduced, the negative effect of drought is improved by the plant coverage (Fig. 17).

In addition, some characters were found at the level of the sheath and the foliar limb, that have not been mentioned up to now in literature, regarding the structure of the external epidermis and the internal mesophyll.

Fig. 15. Babadag Forest - microhabitat with species of plants developed at the limits of forest

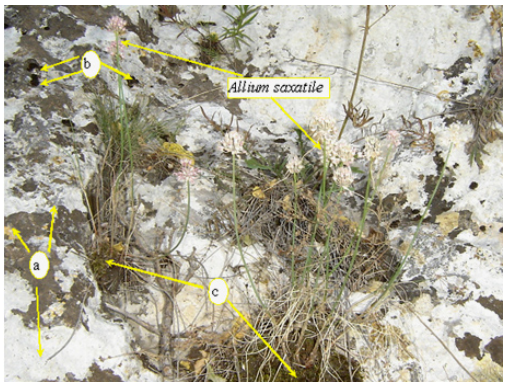

Fig. 16. Cheile Dobrogei - microhabitat with lithophytes (a-crusty lichens, b-bryophytes) and the chasmophyte *Allium saxatile* installed in soil material from rock cracks and protected by mulch layer, (c) installed at surface

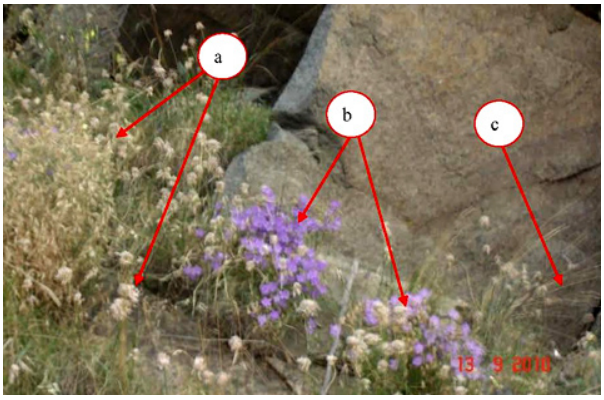

Fig. 17. Turcoaia - microhabitat on slope with hard alterable rocks due to high quartz content and plants installed in soil material accumulated in the spaces between the rocks: *Allium saxatile* (a), *Campanula romanica* (b) and xerophyles grasses (c)

Conclusions

This is the first report on anatomic characteristics in *Allium saxatile*. The analysis of anatomic characteristics at leaf level (sheath and foliar limb) revealed some specific histological characters: a) at the sheath level: the presence in the external epidermis of some epidermal cells with all the walls uniformly thickened and lignified (features not mentioned for any species of the *Allium* genus in the consulted scientific literature); b) at the lamina level: the existence of an internal mesophyll with colenchimatized cells. Other findings presented here, such as a xerophyte-type stomata, laticiferous, multi-layered exoderm and the structure of the central cylinder, confirm data already described in the literature.

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