THE CELLULAR STRUCTURE OF THE FOLIAR LIMBS OF SPONTANEOUS
SEDUM TELEPHIUM SPP. MAXIMUM (L.) KROCK PLANTS HARVESTED
FROM NATURE

Mirela ARDELEAN1,2*, Violeta TURCUŞ1,2, Dorina CACHIŢĂ1, Iulian-Octavian
STANA2, Ciprian-Valentin MIHALI1

Abstract: We investigated structural aspects of cells of the Sedum telephium L. ssp. maximum (L.) Krock
foliar limbs, plant that belongs to the Crassulaceae family. These plants were grown under natural
conditions. Our research allowed us to evidence at the level of leaf mesophyll cells spheroid fibrillary
bodies present in the vacuolar juice and calcium oxalate crystals by using the optical microscope and the
transmission electron microscopy (TEM). These fibrillary bodies seem to be mucilage, which is
characteristic of the plants belonging to the Crassulaceae family, the biological role of these organic
components being less known.

Key words: Sedum telephium ssp. maximum (L.) Krock, osmiophilic corpuscles, vacuolar juice, spheroid
fibrillary bodies.

Introduction

In this study we present our contribution regarding tissular and cellular structure of the Sedum telephium ssp. maximum (L.) Krock species, which was less investigated anatomically until now. Sedum plants are frequently found in the spontaneous flora of Romania (Ştefan and Oprea, 2007). Belonging to Crassulaceae family, Sedum species have special particularities as the content of their vegetative organs and secondary metabolism products (Metcalfe and Chalk, 1972). In the near future, this species could become of general interest for the biotechnical procedures to obtain the phyto-pharmaceutical products (Mulinacci et al., 1993). In a paper that we published in 2009 we presented the main aspects regarding the structure of the vegetative-subterranean and aerial-observed organs at the plants gathered from a natural environment (Ardelean et al., 2009a). In a second paper from 2009, we have made a comparative and histo-anatomical analysis of the plants collected from nature and of the seedlings gathered from a vitro culture (Ardelean et al., 2009b).

In general, the studies in optical microscopy helped us broaden our knowledge gathered from the above-mentioned studies (Cachiţă and Crâciun, 1990). In this study, benefiting from the advantages of different techniques of microscopic preparations, we used a cutting system of the preparations in semi-fine sections which we analysed both optical microscope and electron microscope.

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1* Vasile Goldiș Western University of Arad, Department of Plant Biotechnology, Institute of Life Sciences,
Liviu Rebreanu 86, Arad, Romania; mirela.ardelean1@yahoo.com (corresponding author)
2 Vasile Goldiș Western University of Arad, Faculty of Natural Sciences, Engineering and Computer Science,
Liviu Rebreanu 91-93, Arad, Romania
**Materials and methods**

The vegetal material that we analysed for obtaining the semi-thin sections, in order to research the structural and ultrastructural aspects at the optical and transmission electron microscopy was represented by the leaves taken from young plants, grown in Milova Forest, Arad County.

The semi-thin sections of *Sedum telephium* ssp. *maximum* plants were obtained through an ultramicrotome, using diamond knives (Şerbănescu-Jitariu et al., 1983; Andrei and Paraschivoiu, 2003). The reactive used to make the colouring agent for the semi-thin sections of *Sedum telephium* ssp. *maximum* was the Epoxy tissue stain. The preparations thus obtained have been analysed both at a MC1 Romanian photon microscopy, with mirror of sign of projection (Projektionszeichenspiegel), and at NOVEX photon microscopy (Holland), with a Canon A95 digital camera.

The main stages which were extremely relevant in terms of accuracy for the gathering of reality-based information, from the cells of *Sedum telephium* ssp. *maximum* were the following: harvesting, binding, infiltration and inclusion, encapsulation, modelling, sectioning, contrasting, examination and digital recording of the images and their processing in the computer. For the examination of the preparations at the transmission electron microscopy, the contrasting sections have been introduced in the microscope and the chosen images are captured successively and stocked in a database by using the Megaview III camera software- Soft Imaging Analysis.

**Results and discussions**

*Sedum telephium* ssp. *maximum* plants grown in a normal, natural environment conditions have the leaves strongly certified, especially in their superior epidermis (Toma and Rugină, 1998). In the epidermal cells, both superior (Plate I and Plate II, Fig. 4) and inferior (Plate III, Fig. 3 and 4), we observed the presence of stomata, whose ultrastructural aspect is more complex, the nucleus is big – as compared to the cell’s volume (Plate III, Fig. 4); there are chloroplasts in the cells, and the vacuoles are of small calibre (similar to the fact that the volume of the stomata cells is smaller than that of other cells, be it epidermal or mesophyllian; at the same time, there is a substomatal cavity in the stomata subcells).

In the plates IV and V, we can see the electron-microscopical images of the chloroplasts which are spread in the pellicular cytoplasm that covers the inside of the cellular wall. The nuclei (Plate III and Plate VI) have a normal structure, they are not amoeboidal, but fusiform.

In the figures 1 and 2 from the plate VI, in the transversal section of the nervure, we can see an area with very young ligneous vases, accompanied by an already constructed woody parenchyma, neighboured by the foliar mesophyll cells which contain chloroplast, but much more rarefied than in the case the foliar mesophyll cells that carry out the chlorophyll assimilation. In plate VI, figures 4-6, we can see that the xylem is made of scalariform tracheids (Fig. 3 and 5), lacking cellular constituents, while the annex parenchyma is made of lively cells, rich in cytoplasm, with a well-constructed nucleus, but lacking chloroplasts.
Most of the times, the vacuolar juice of the vegetal cells have a homogenous optical aspect and only sometimes, some cells can have crystals, phospholipid or osmiophil corpuscles, or floccular masses spread diffusely or conglomerated. More frequently, these aspects can be found in aged or senescent cells.

The most interesting aspects that we have observed in some cells of the foliar mesophyll of the parenchyma assimilating the leaves of *Sedum telephium ssp. maximum* harvested from nature, after their examination at an electron microscope consisted in the identification in the vacuolar juice the presence of some *calcium oxalate crystals* in form of sand (Plate VII, Fig. 1 and 2), many of whom were not being dispersed in the vacuolar juice, but were conglomerated in incorporated formations, being reunited one with the other by some phospholipid compounds - osmiophil; at times, these corpuscles of phospholipid nature and spherical from, flow freely in the vacuolar juice of the vegetal cells (Plate VII, Figs. 1-7). In the figure 7 of plate VIII we have some electron microscopy details like conglomerate composed of spheroids mucilage that are invaded inside cells that contain them and sections performed through spheroidal mass of these configurations.

**Conclusions**

The investigations about the transmission electron microscopy carried out in this research, allowed us to highlight the random presence in the depth of the foliar mesophyll of some cells whose vacuolar content had been filled with various fibrillatory bodies. These ultrastructural aspects that we have discovered in the vacuoles of the foliar mesophyll cells of the *Sedum telephium ssp. maximum* leaves are for the first time presented in the specialty literature and we think that they are due the mucilage which is specific to the *Crasulaceae* family, the biological role of these organic compounds being less known.

**REFERENCES**

Plate I, Figs. 1 - 3

Optical microscopy images surprised in semi-thin sections of *Sedum telephium* ssp. *maximum*, obtained by transversal sectioning of the adult foliar limbs, harvested from plants grown in a natural conditions; (abbreviations: cit- cytoplasm; cl- chloroplasts; ep. sup- upper epidermis; ep. inf- lower epidermis; mez. fol- foliar mesophyll; N- nucleus; nr- nervure; pc- cellular wall; sp- intercellular space; V- vacuole).

Figure 1. Image of a foliar limb structure that contains both epidermis and assimilating mesophyll that has at its centre a nervure (ob. 10x).

Figure 2 and Figure 3. Details of the foliar mesophyll structure (Fig. 2 - ob. 40x; Fig. 3 - ob. 100x).
Plate II, Figs. 1 - 4

Optical microscopy images surprised in the semi-thin cross-sections prepared from the foliar adult of the plants *Sedum telephium* ssp. *maximum*, harvested from the natural conditions; (abbreviations: cl- chloroplasts; fl- phloem; mez. fol- foliar mesophyll; st- stomata; cst.- substomatal cavity; V- vacuole; il- ligneous rings).

Figure 1. Transversal section applied through the level of the nervure (ob. 40x).
Figure 2. Longitudinal section carried out through the nervure of an adult leaf (ob. 40x).
Figure 3 and Figure 4. Aspects of the lower epidermis and the foliar mesophyll (Fig. 3 - ob. 40x; Fig. 4 - ob. 100x).
Plate III, Figs. 1 - 4

Electron microscopic aspects observed in the cells of the superior epidermis of the *Sedum telephium* ssp. *maximum* adult leaf taken plants which was grown in natural conditions; (abbreviations: w- wax; cl- chloroplasts; cit- cytoplasm; cst.- substomatal cavity; lp- leucoplasts; M- mitochondria; N-nucleus; ox- calcium oxalate; o- osteola; pc- cellular wall; V – vacuole).

Figure 1. External cellular wall of a upper epidermal cell in which we highlighted the presence of the wall- towards the exterior- of a thick layer of wax, on its internal side being covered with cytoplasm (scale 10 µm).

Figure 2. Fragment from an epidermal cell in which in the cytoplasm there is a nucleus incorporated in a cytoplasmatic mass where there are proplastids and mitochondria (scale 10 µm).

Figure 3. Two epidermal cells, in their contact area, on the external side of the cellular wall are wax deposits, in the vacuolar juice there is oxalate under the form of sand (scale 5 µm).

Figure 4. Stomata with two stomatal cells and substomatal cavity clearly highlighted (scale 5 µm).
Plate IV, Figs. 1 and 2

Transmission electron microscopy images of the chloroplasts which are spread in the pellicular cytoplasm that covers the inside of the cellular wall, (abbreviations: w- wax; cl- chloroplasts; cit- cytoplasm; pc- cellular wall; V- vacuole) (scale 10 µm).
Transmission electron microscopy images which illustrate in the foliar mesophyll cells at the *Sedum telephium* ssp. *maximum* harvested from the natural environment, respectively, assimilating parenchyma, cells rich in chloroplasts and mitochondria; the cytoplasm is pellicular and sometimes it disorganizes and gives birth to deposits of calcium oxalate crystals; (abbreviations: cit- cytoplasm; cl- chloroplasts; pc- cellular wall; sp- intercellular space; V- vacuole; M- mitochondria; ox- calcium oxalate) (scale 5 µm).
Plate VI, Figs. 1 - 6

Tissues highlighted in the leaves harvested in nature. The ultrastructural aspects surprised at the electron microscopy in a freely-woody fascicle of tissues that compose the nervures and that are highlighted in the leaves harvested in nature; (abbreviations: cit- cytoplasm; m.f- foliar mesophyll; cl- chloroplasts; pal- woody parenchyma; V- vacuole; x- xylem; xf- xylem in formation; N- nucleus; n- nucleolus; il- ligneous rings, whole or sectioned).

Figure 1 and Figure 2. Transversal section through the nervure (scale 10 µm).

Figures 3 - 6. Longitudinal section through the nervure (scale 10 µm).
Plate VII, Figs. 1 and 2

Transmission electron microscopy images which illustrate in the foliar mesophyll cells at the *Sedum telephium* ssp. *maximum* harvested from natural conditions, the presence of some fine calcium oxalate crystals, which are known as “sand”; (abbreviations: cit- cytoplasm; cl- chloroplasts; cr. ox.- calcium oxalate crystals; pc- cellular wall; V-vacuole) (scale 10 µm).
Plate VIII, Figs. 1 - 7

Transmission electron microscopy images which illustrate leaf mesophyll cells of the *Sedum telephium* Ssp. *maximum* plants harvested from the natural conditions; (abbreviations: cit- cytoplasm, cl - chloroplast, pc- cell wall, fm- mucilaginous fibrils; fsm- mucilaginous spheroids, V - vacuole, zc - central area,zp - peripheric area) (scale 5 µm).