Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 3, Jul.-Sep. 2011, pp. 563-571

VACUOLE FORMATIONS IDENTIFIED IN THE CELLS OF THE FOLIAR MESOPHYLL OF THE YOUNG LEAVES OF *SEDUM TELEPHIUM SSP. MAXIMUM* HARVESTED FROM NATURAL ENVIRONMENT

Mirela Ardelean[1], Dorina Cachiţă-Cosma[1], Constantin Crăciun[2]
1. "Vasile Goldiş" Western University from Arad, Romania
2. Electron Microscopy Center, „Babeş- Bolyai" University, Cluj-Napoca, Romania

SUMMARY

The examinations of the electronic microscopy made by us permitted the evidence in the vacuoles of some cells of the foliar mesophyll of the young leaves of *Sedum telephium* ssp. *maximum*, driven from the natural environment, of some measures hard to distinguish from the optical microscopy examinations, like the fine crystals of calcium oxalate, either as wise, either having attached corpuscles of phospholipidic nature, strong electron dense, making organic-molecular conglomerates, either filiform aggregates, scattered in the aggregation of the vacuole, either spheroid phlocular formations (we believe of mucilaginous nature), elaborated by the elements of the Golgi apparatus, which were moved towards the vacuole while being created, they crossed by the tonoplast and were spread in the vacuolar sap, by the golgi vesicles.

The transversal section practiced through the spheroid aggregations multifibriliar, present in the vacuolar sap of some mesophilic cells from *Sedum telephium* ssp. *maximum* leaves facilitated the discovery in the structure of the filiform aggregates in the spheres areas. So, the external area of the sphere remains multifibriliar, but made of fibers grouped in different light facsicles; towards, the interior of the sphere it comes out another optical clear area, without fibrils; but in the center of the sphere can be distinguished a third area occupied by multitude of fine floccular aggregates, apparently unorganized. Gradually, such spheroid formation constituted in three- phases proportion as they appear to invade the lumen of those cells and the cellular organites are disintegrated and the cells become „deposits", probably of mucilage's.

INTRODUCTION

The therapeutic qualities of the species of *Crasulaceae*, as *Kalanchoe pinnata, Sedum prealtum, Crassula argentea, Sedum acre, Sedum telephium* (which contain alkaloids, etheric oils, malic acid, resins, tannins, mucilage's, phormic acid, flavones, etc) is treating the acne, the corns, the carcinoma of the skin, the gangrenous plagues, the tumors, the rheumatism and spondilitis also some internal bleedings, endems, hemorrhoids, wounds, burns paresis of the peripheral nerves, etc. (Nordal şi Klewstrand, 1951; Paris şi Frigot, 1959; Soderstrom şi Thomas, 1962; Knoph and Kluge, 1979 etc.).

Because of the phytotherapeutic value of some Sedum species begins to grow, thanks to their richness from active principles in their great majority localized in the vacuolar sap that these plants contain, we came up with the idea of making a electronic microscopy studies, at the level of the foliar limbus cells, based on the examination of the aspects connected to the identification of some prominent ultra structural particularities in the vacuolar sap of the foliar mesophyll cells.

MATERIAL AND METHOD

For making optic and electronic microscopy examinations at the level of the foliar limbs of the young leaves of *Sedum telephium* ssp. *maximum* in the month of March we took leaves from the plants which were in the natural environment, 5 cm length and 3 cm wide from which we took fragments which were fixed and processed according to the transmission electron microscopy (TEM) specific techniques (*Hayat*, 2000). Fixation was made in 2.7% glutaraldehyde, for one hour, after which fragments were post-fixed in 2% osmic acid, and then dehydrated in increasing concentration of acetone baths; later, the vegetal samples were included in Epone 812. The sections in the Epone blocks were made with a Leica UC_6 microtome, and the coloration and the contrastation of the sections has been made with lead citrate and uranyl acetate solutions. The preparations were examined with transmission electron microscopy Tecnai 12, Biotwin and images were photographed with a brand digital camera Mega View 3. Later, photographs were processed through Corel Photo Paint 12.

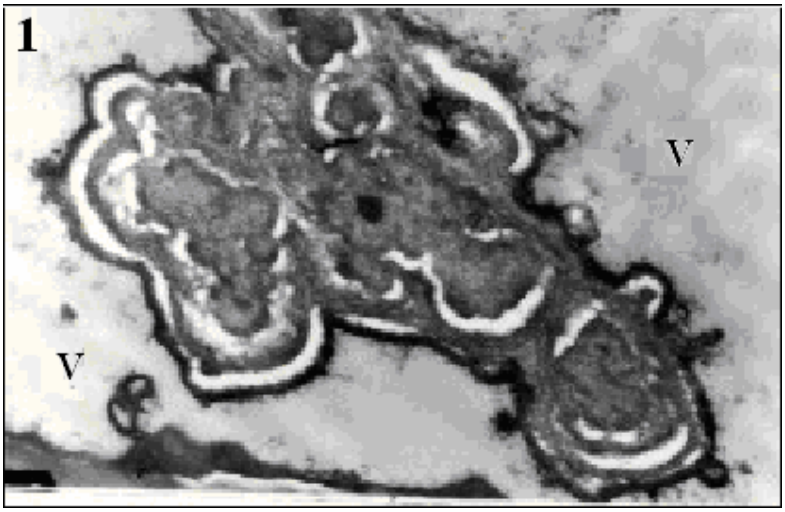
RESULTS AND DISCUSSIONS

We illustrated the most representative images of optic and electronic microscope identified by us, especially at the level of the vacuoles of the foliar mesophyll cells witch we exposed in the plate 2-7.

Mostly, the vacuolar sap of the vegetal cells has an homogeneous optical aspect and only some of the cells here and there (plate 1, fig. 1 and 2), can present crystals, phospholipids corpuscles, osmophilees, or follicular aggregations (diffusely scattered), or conglomerate. Most often, such aspects are met in the old cells or in senescent ones (*Cachiţă* şi *Crăciun*, 1990).

The examinations of electronic microscope, with transmission permitted the identification in the vacuolar sap of some cells of the foliar mesophyll of the young leaves of *Sedum telephium* ssp. *maximum*, taken from plants grown in a natural environment, of some diverse measures (see plate 3-7), some of these are not mentioned in the professional literature.

Optical microscopy examinations (plate 2, fig. 1 and 2), over the structure of a transversal section made through the foliar limb of *Sedum telephium* ssp. *maximum* young leaves taken from plants wich were grown in the natural environment and proceded the observations of electronic microscopy, refers to the fact that between the two epidermis of a leaf there is a homogeneous mesophyll.



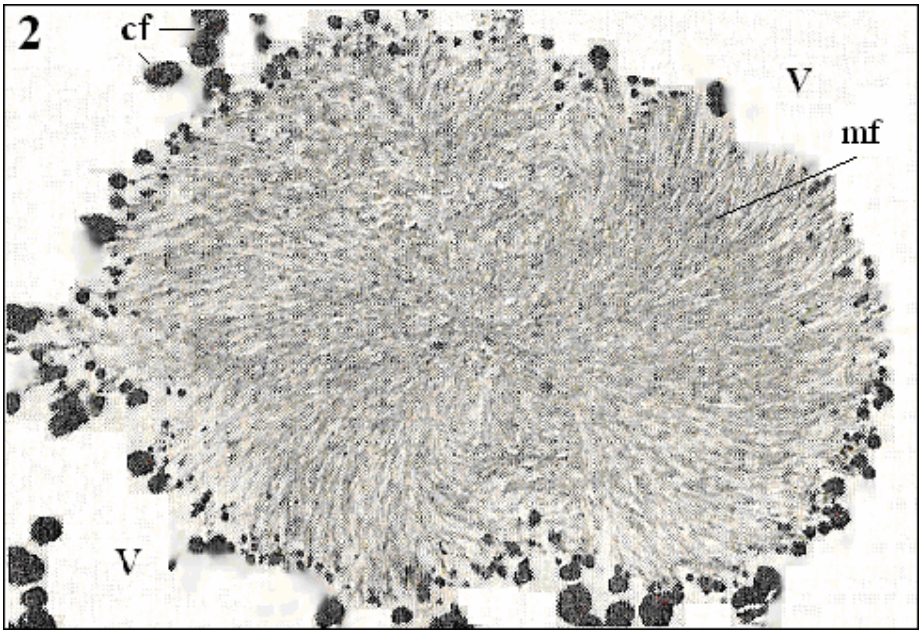


Plate 1, Fig. 1 and 2. The aspects seen in the vacuolar sap (V) of the foliar mesophyllic cells and of the hyperhidric vitro leaves of Forsythia, observed at the electronic microscope with transmission.

Fig. 1 – longitudinal section through an amorphous conglomerate of mineral salts, probably of Ca carbonate or Ca sulphate (21.000x); Fig. 2 – measure sphero-globular of microfibriles (mf) – seen from the exterior; at the periphery of which there are phospholipids corpuscles (cf) osmophiles (3.360x) (*Cachiță and Crăciun*, 1990).

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 3, Jul.-Sep. 2011, pp. 563-571

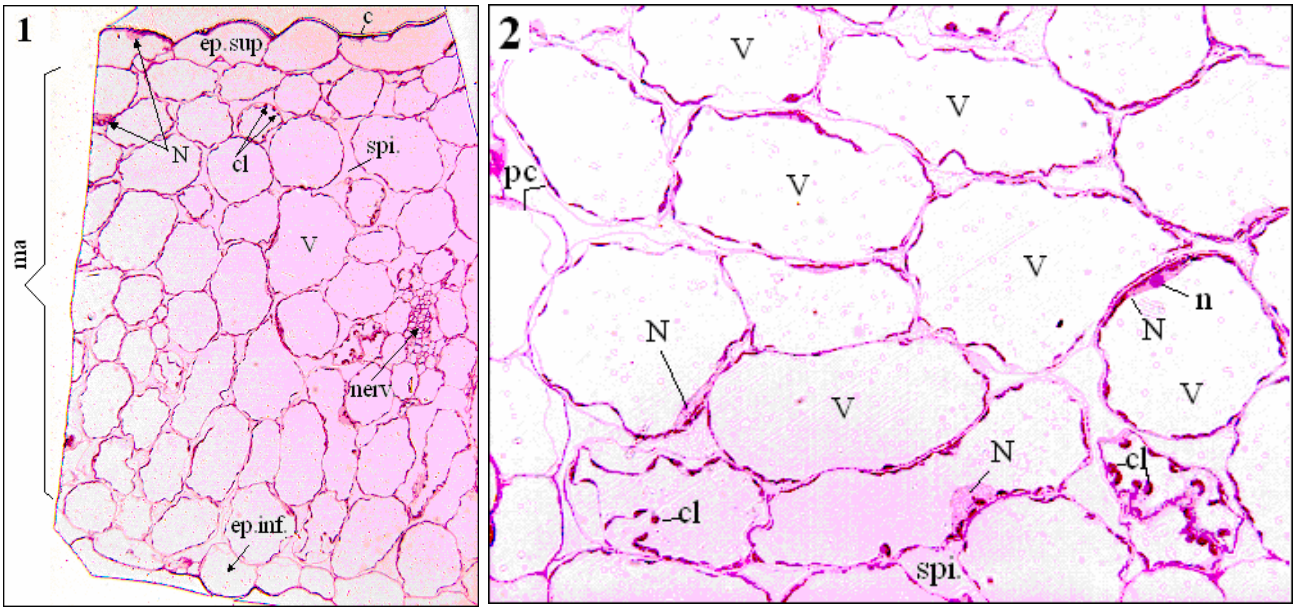


Plate 2 . Fig. 1 and 2. The aspects of optical microscopy observed in the transversal sections made in the limb of *Sedum telephium* ssp. *maximum* young leaves taken from plants which were grown in a septic environment (Fig. 1 – the superior epidermis (ep. sup.) and the inferior (ep. inf.) one of the foliar limb and of the assimilator mesophyll in which they are evident a nervure (20x); Fig. 2 – details of the foliar mesophyllic cells (240x), where: c- layer of wax; cl – chloroplasts ; pc – cellular wall; N – nucleus; nucleolus- n; spi – intercellular space; V – vacuole.

From an assimilator parenchyma, with oval shaped cells, amongst them there are seen intercellular spaces relatively big, but which don't have air gaps. The assimilator parenchyma has cells which are delimitated by pectocellular walls unmodified secondary, but which accede a pellicular cytoplasm rich in chloroplasts; the cells contain a unique vacuole which occupies most part of the cellular lumen. The chloroplasts lack from the epidermal cells. The external cellular wall of the superior epidermis is covered in wax (waxen).

The most interesting aspects that we observed is that some of the cells of the foliar mesophyll of the assimilator parenchyma of the young leaves of *Sedum telephium* ssp. *maximum*, after the examination with the electronic microscope, we identified in the vacuolar sap the presence of some crystals of Ca oxalate of a "sandy" nature (plate 3 and 4, fig. 1 and 2) many of them were not scattered in the vacuolar sap, but were conglomerated in some formations glued to each other and united through some compounds – we believe of phospholipidic nature – (plate 1, fig. 1) often these corpuscles of phospholipidic nature of spheroid shape float free in the vacuolar sap of the cells. Such osmophilic corpuscles of phospholipidic nature were described also by *Cachiță and Crăciun* (1990) as being present in the vacuolar sap of the cells of the foliar mesophyll of *Forsythia*, taken from plants wich were grown *in vitro*.

We took into consideration the particular aspects that we observed in the vacuolar sap of some of the mesophyllian cells of the leaves of *Sedum telephium* ssp. *maximum* which were made of small spheroid multifibrillar formations (plate 6, fig. 2 and plate 7, fig.1 and 2) that were described in the professional literature by *Cachiță and Crăciun* (1990), in the vacuoles of the cells of the foliar mesophyll of *Forsythia*, in tissular patterns taken from the leaves of some plantlets created *in vitro* that were affected by hyperhydration (plate 1, fig. 2). They form gradually, by the association of fibrilar aggregates, lonely, of small dimensions (plate 5, fig. 2), in spheroid fibriliar formations (plate 6, fig. 2).

As it can be seen from the plate 6, fig. 1, the follicular aggregates seem to be elaborated in the receiver of the Golgi apparatus (very numerous organites in such cells) from where they are vehicle (moved) and released in the vacuoles, by the Golgi vesicles. Gradually, the Golgi vesicles dismantled the tonoplast (plate 6, fig. 2) and their fibrillar content is released in the vacuolar sap, giving birth to flocculent aggregations, lonely or spheroid. They have a nature and effects that we still don't know, but we believe that these are made from mucilage. So as these fibrils are released in the vacuole they form spheres (to compare plate 2 from the plate 6 with fig. 1 and 2 from plate 7), uniformly structured, the fibrils being glued to each other mainly in the central area of the globular sphere. But, gradually the spheres - made of fibrils change their aspects and the central area of the sphere becomes less dense (plate 1, fig. 1 and 2), after which it fallows a circular region, optical clear, and only towards the exterior of the sphere we can still find fibrillar structures

but a lot shorter, which group themselves in tussocks of many dimensions.

On the way of these transformations the dimension of the sphere doesn't change too much their metamorphosis taking place in their interior. The existence of such formations was not mentioned in the professional literature.

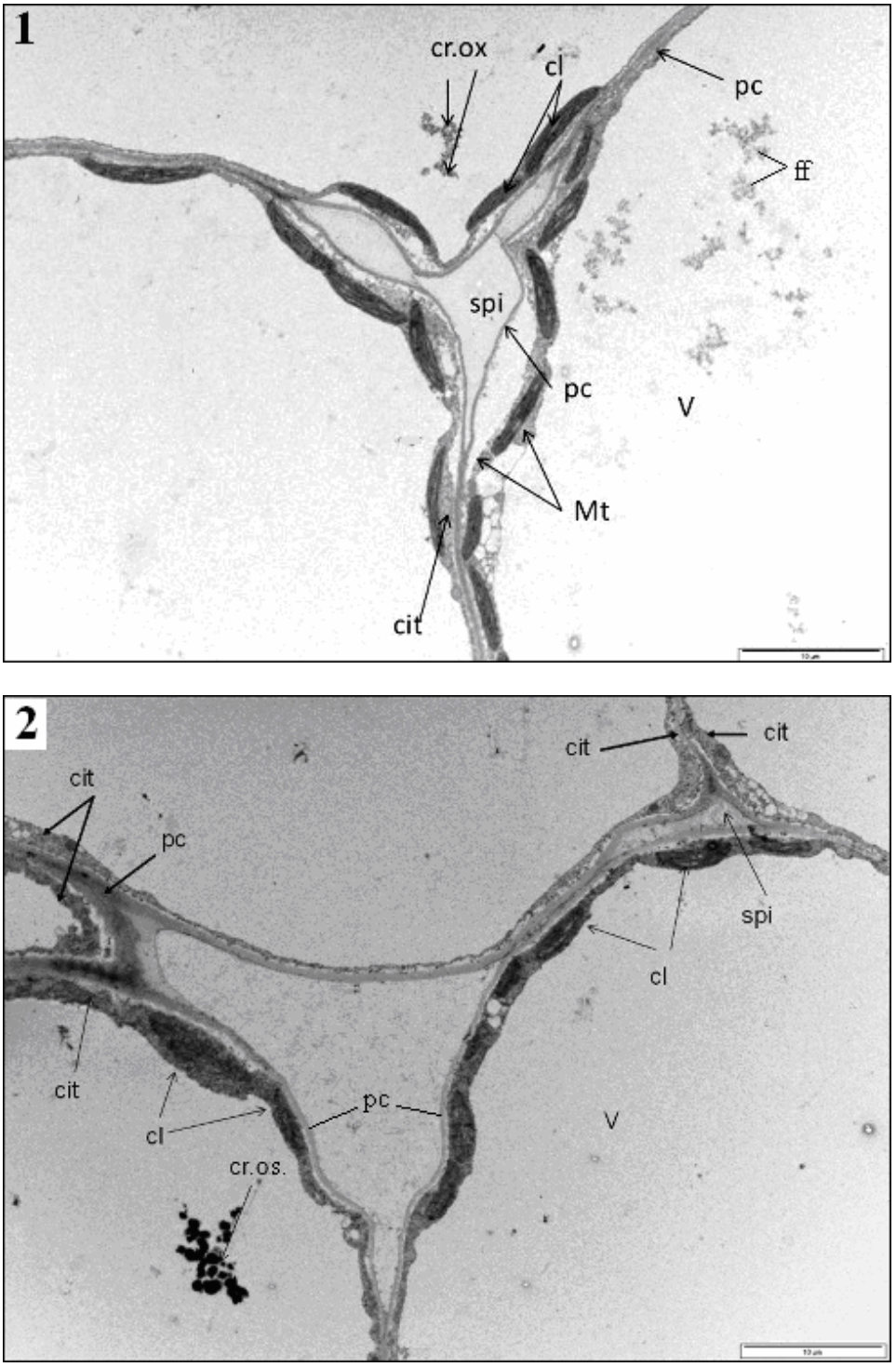


Plate 3. Fig.1 and 2. The electronic microscopy aspects of the cells of the foliar mesophy discovered in *Sedum telephium* ssp. *maximum* young leaves taken from nature, where: ci cytoplasm; cl- chloroplasts; cr.ox- crystals of Ca oxalate; cr.os – osmophilic corpuscles; ff – floculi formations; pc – cellular wall; Mt – mitochondria; spi– intercellular space; V – vacuole.

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 3, Jul.-Sep. 2011, pp. 563-571

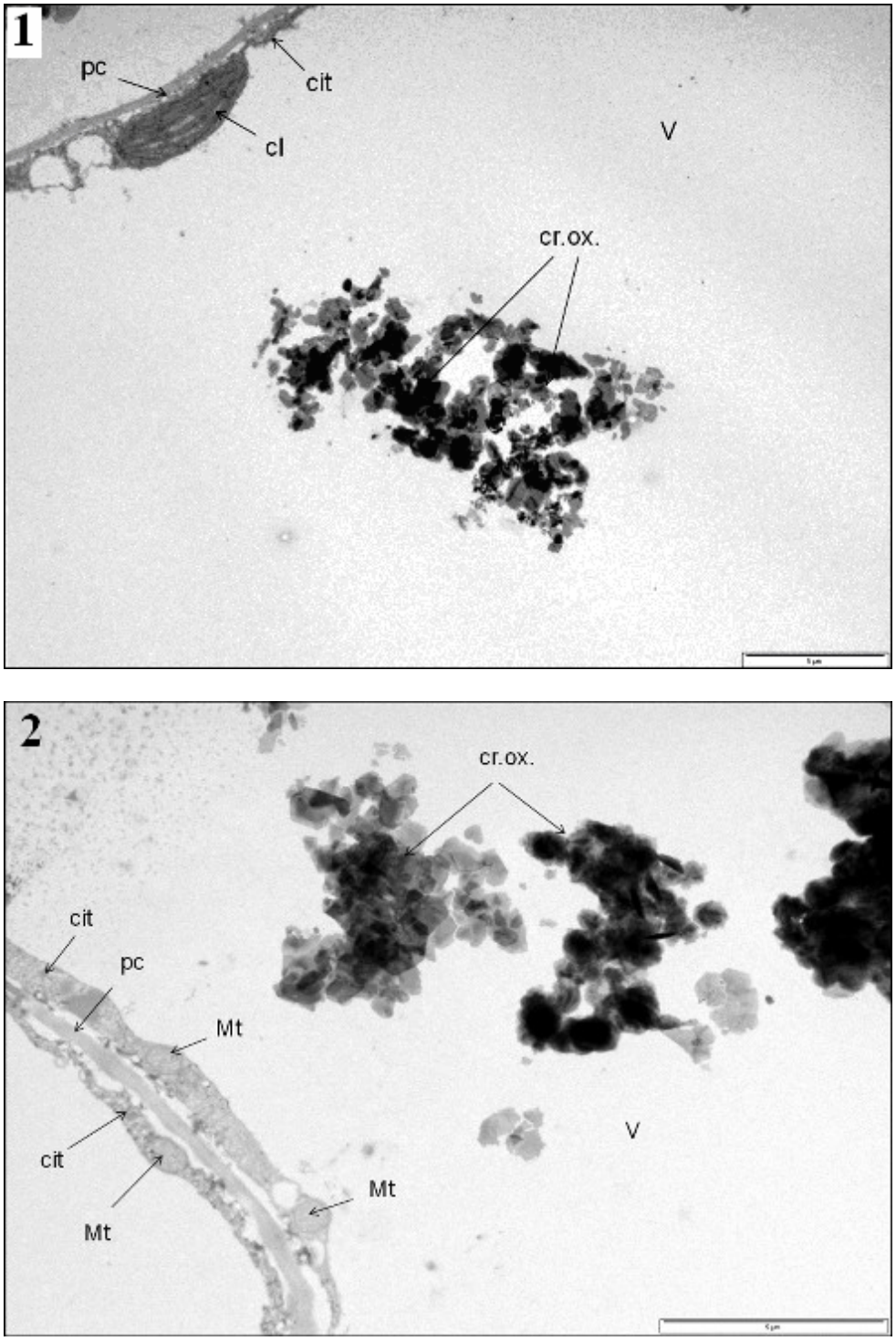


Plate 4. Fig. 1 and 2. The electronic microscopy aspects discovered in the vacuoles of the cells of the foliar mesophyll of *Sedum telephium* ssp. *maximum* young leaves taken from the natural environment, in which it can be seen many detailed images with the crystals of Ca oxalate in a sandy shape, where: cit – cytoplasm; cl – chloroplast; cr.ox.- crystals of Ca oxalate; Mt – mitochondria; pc- cellular wall; V- vacuole.

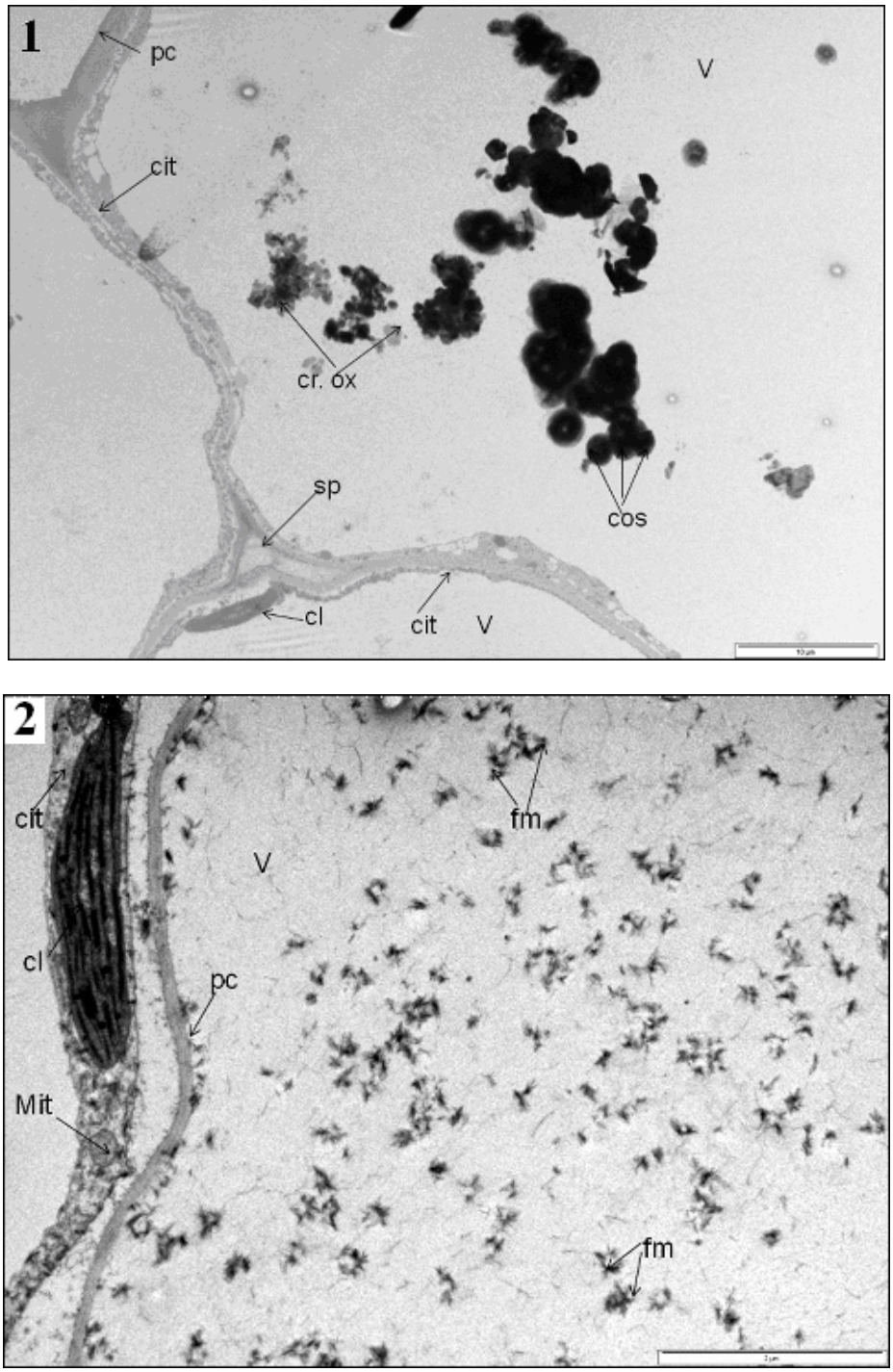


Plate 5. Fig.1 and 2. The cells of the foliar mesophyll seen at the electronic microscope in the *Sedum telephium* ssp. *maximum* young leaves taken from natural environment. In their vacuolar sap it can be distinguished osmophilic corpuscles of phospholipidic nature, some being adherent of crystals of Ca oxalate (cr.ox); Fig.2- flocular aggregates that we considered to be mucilage, where: cit – cytoplasm; cl – chloroplast; cr.ox.- crystals of Ca oxalate; pc- cellular wall; Mt – mitochondria; fm – mucilage formations; V- vacuole; cr.os – osmophilic corpuscles.

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 3, Jul.-Sep. 2011, pp. 563-571

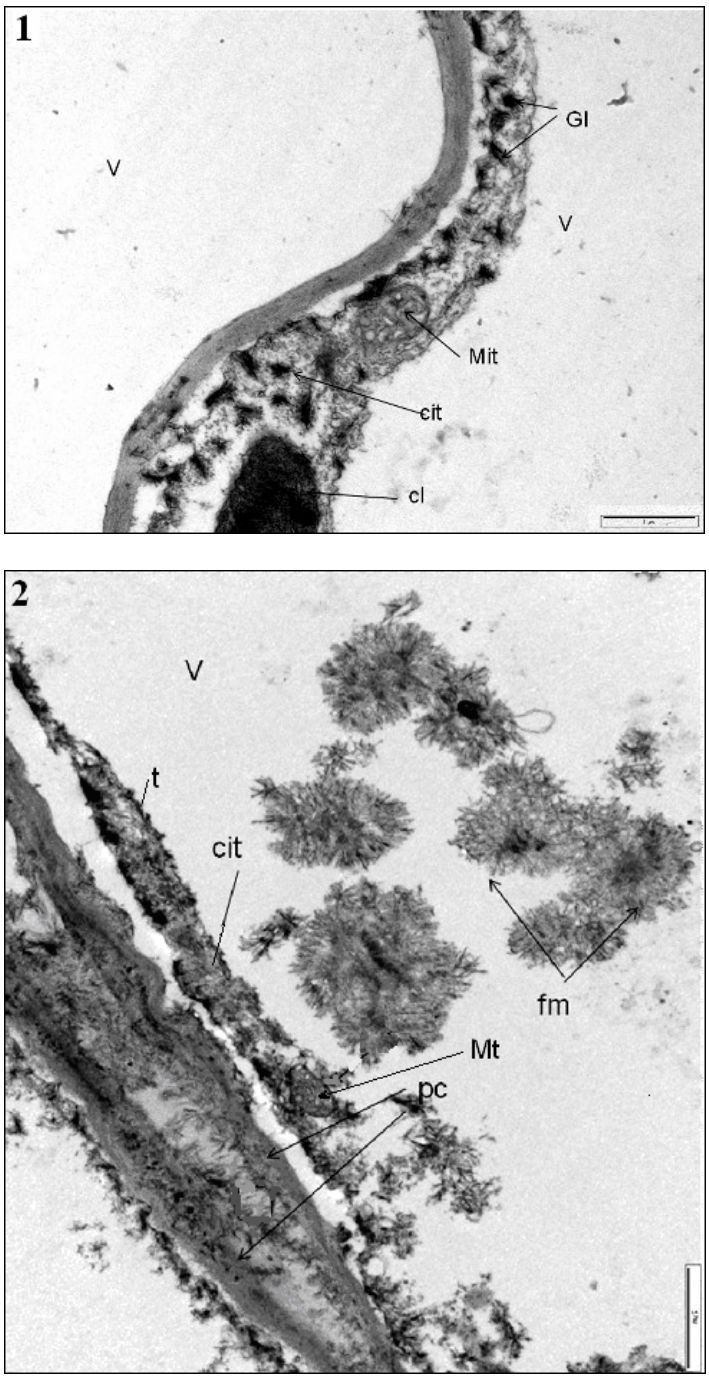


Plate 6. Fig. 1 and 2. Images of electronic microscopy of some cells of the foliar mesophyll of *Sedum telephium* ssp. *maximum* young leaves, taken from an natural environment, where we can distinguish Golgian formations, probably which secrete mucilage, present in the cytoplasm (Fig.1); Fig. 2 – flocular aggregates probably of mucilage which disorganizes the structure of the tonoplast and migrate in the vacuole, generating spheroid formations, where: cit – cytoplasm; cl – chloroplast; fm – mucilage formations; pc- cellular wall; Mt – mitochondria; V- vacuole; Gl- Golgi apparatus; t- tonoplast.

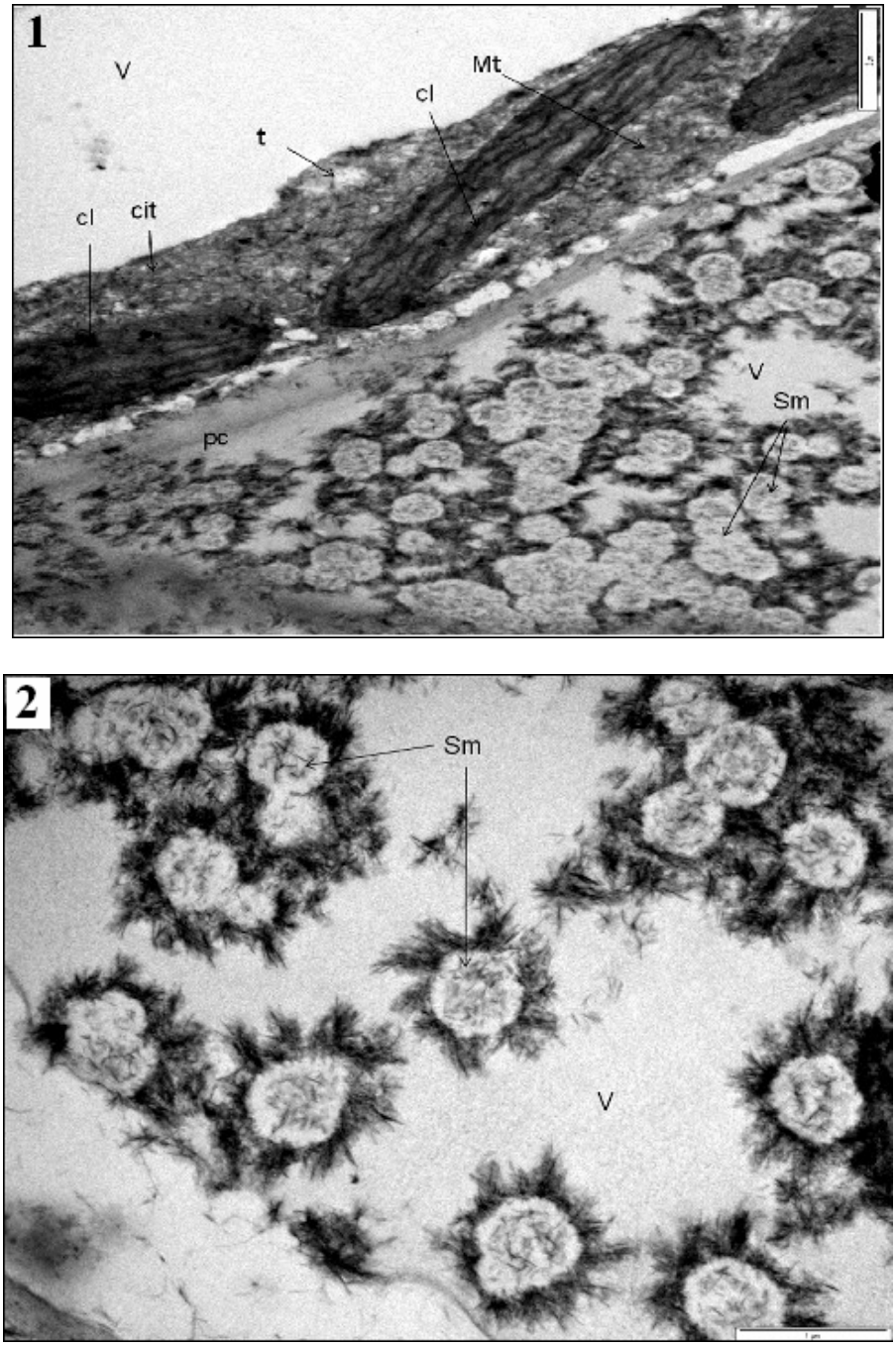


Plate 7. Fig. 1 and 2. Details of electronic microscopy from some cells of the foliar mesophyll of *Sedum telephium* ssp. *maximum* young leaves, taken from the nature, conglomerates made of spheroid formations of fibrillar mucilage which invaded the territory of the cells which contain them; the images show sections that were practiced through the spheroid aggregation of these, thing which permits us to observe three areas a central one, with a less dense content, where: cit – cytoplasm; cl – chloroplast; pc- cellular wall; Mt – mitochondria; V - vacuole; Sm - fibrils spheres of mucilage; t- tonoplast.

An interesting thing is the fact that as this kind of fibrils spheres, endovacuolizated, scatters in the whole cellular lumen – being very numerous- the rest of the cellular organites gradually disappear due to a disintegration process (plate 7, fig. 2).

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 3, Jul.-Sep. 2011, pp. 563-571

CONCLUSIONS

The electronic microscopy examinations made by us permitted us to see the evidence of the vacuoles of some cells of the foliar mesophyll of the young leaves of *Sedum telephium* ssp. *maximum*, taken from the nature, of formations difficult to distinguish from the optical microscopy examinations, such as the fine "sand" of calcium oxalate, either as wise, either having attached some spheroid corpuscles of phospholipidic nature, strong electrons dense and osmophillis in this way making the organo-mineral conglomerates. On the other hand, in other cells of the foliar mesophylle it was observed the presence of some spheroid, multifolicular formations that we believe to be of mucilage nature, elaborated of the elements of the Golgi apparatus, the fibriliar formations, which step by step were given birth were transported in the vacuole by the Golgian vesicles and in the vacuolar sap they appeared as flocculent aggregations.

Gradually, the flocular aggregates associate and make multifibriliar spheres with fibrils that are glued in the central area of these. In time, the internal structure of the spheres reorganizes it self, and inside it, three areas develop: and external area, multifibriliar, another area that is intermediary – optically clear –without fibriliar formations, and a third area which contains numerous fine formations, flocculent , associated in a small measure. It seems that once they reach such a structural stage, the spheres become stabile. These formations appear only in some of the cells of the foliar mesophyll and are in a great number, invading their lumen. In such cells the organites disintegrate and that cell becomes a "store" of spheroid formations, which in the external covering of the spheres presents numerous fibrils , of different lengths, random grouped. Their chemical composition and the physiologic role are still unknown.

REFERENCES

1. Cachiță C. D., Ardelean A. (2009) – *Tratat de biotehnologie vegetală,* vol II; Ed. Dacia Cluj-Napoca.
2. Kean Ch. I., (1924) - *The morphology and physiology of the leaves of some Crassulaceae* Trans. Bot. soc. Edinb., **29**: pp. 96-104.
3. Knopf O, Kluge M. (1979) - Proprieties of phosphoenolpyruvate carboxylase in Sedum species in relation to crassulacean acid metabolism (CAM), *Plant Cell and Environment 2*, pp.73-78.
4. Metcalfe C. R., Chalk L. (1972) - Crassulaceae, in *Anatomy of the Dicotyledons*, Clarendon Press, Oxford, pp. 578-581.
5. Nordal, A. and Klevstrand, R. (1951) -Studies of the constituents of crassulacean plants. I. Paperchromatographic investigation of the free sugars of some Sedum, Sempervivum, Echeveria, and Crassula species. *Acta Chem. Scand. 5*, pp. 85-88.
6. Paris, R. R.; Frigot, P. (1959) -Sur l'isolement d'une flavanone à partir d'une Crassulacée indigène, le Sedum altissimum Poir. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*, v. 249, pp. 560-561.
7. Soderstrom P.; Thomas M. (1962) - Smithsonian Institution, Washington, DC, The isocitric acid con- tent of crassulacean plants and a few succulent species from other families. *Amer. Jour. Bot*. 49 (8), pp. 850-855.
8. Stahl E. (1967) - *Dünnschichtchromatographie,* Springer Verlag Berlin Heidelberg New York, Ed. II, pp. 361-374.