ASPECTS REGARDING THE CALCIUM OXALATE CRYSTALS AT THE GRAPEVINES CULTIVATED IN IAŞI AND COTNARI VINEYARDS

Camelia IFRIM[2], Carmenica Doina JITĂREANU[1], Cristina SLABU[1], Alina Elena MARTA[1]

e-mail : camicris@yahoo.com

Abstract

The major function of calcium oxalate crystals in plants implies the adjustment of calcium (Ca) metabolism and the protection against herbivorous animals. The main types of crystals considered to play a protective role are the raphides, their shape being essential in this sense. Recent studies suggest that the crystals' aspect depends of the species, their shape and dimensions being characteristic. The types of *Vitis* studied in this paper make themselves noticed due to the morphological variety of the crystals, their structure in vegetative organs or in the specialized tissues of the same organ. From the five types of crystals mentioned in the specialized literature, three of them were noticed: raphides, druses and prismatic crystals. The differences noticed between the varieties can suggest they had been influenced by the characteristics of the soil, the environmental conditions and even by some genetic factors.

Key words: *Vitis,* calcium oxalate, raphides, druses, idioblasts

The living organisms for which calcium represents a key element in growing and developing have created intracellular control mechanisms for calcium concentration. This is determined by the toxicity of free calcium when present in high concentration levels.

Among the calcium salts the most frequent in plants is calcium oxalate which is present in most botanical families, with few exceptions. Calcium oxalate is present as mono and dihydric salts in more than one crystalline from. Monohydrate is a lot more stable and frequent in plants.

A large number of studies sustain the hypothesis that the formation of calcium oxalate crystals counteracts the excess of calcium and eliminates it from the metabolic process so to regain the normal calcium level of the cell. Studying the development of crystals and the rapid evolution of a large number of crystals results that the formation of calcium oxalate represents a cellular controlled process and not a simple precipitation phenomenon (Li, X., D. Zhang, V. J. Lynch-Holm, T. W. Okita and V. R. Franceschi, 2003).

Different forms of crystals perform different functions as providing protection against herbivorous, adjusting calcium metabolism, increasing tissue resistance, collecting and reflecting light and it is associated to heavy metal tolerance (Prychid Christina J., Jabaily Rachel Schmidt, Rudall Paula J., 2008).

The most frequent types of calcium oxalate crystals are: a) prismatic crystals, prisms of various shapes, usually one in a cell; b) raphides, acicular crystals present in fascicles; c) druses, spherical or prismatic aggregates of crystals; d) styloids, oblong crystals with sharp or dentated, one or two in a cell and e) crystalline sand, very small crystals, usually in high number.

The localization and the types of calcium crystals within the same taxon can be constant, situation in which they become useful for taxonomic classifications.

Calcium oxalate crystals develop according to specific anatomic patterns indicating that their formation is controlled by the plant. The environmental conditions as the changing of the seasons can influence the amount of oxalate produced as well as the number of crystals formed (Prychid, Christina J., Rudall Paula J., 1999).

MATERIAL AND METHOD

The botanical material included in this study consisted in the fertile and sterile young shoots of seven grapevine varieties (*Vitis vinifera* L*)*: *Băbească gri*, cultivated in Iaşi; *Fetească albă*, cultivated in Cotnari; *Fetească regală*, cultivated in Iaşi; *Frâncuşă*, cultivated in Iaşi; *Grasă de Cotnari*, cultivated in Iaşi and Cotnari; *Riesling italian*, cultivated in Iaşi; *Tămâioasă românească*, cultivated in Iaşi and Cotnari. The material was collected during the blooming phenophase.

The material subjected to the histological analysis (Şerbănescu-Jitariu & colab., 1983) has been fixed and preserved in 70% ethylic alcohol. The sections were cut by microtome, subsequently coloured with iodine green and rutheniu red, then analyzed in a Optika light microscope. The light micrographs were performed by means of the same light microscope, using Canon A540 camera.

RESULTS AND DISCUSSION

The representatives of the *Vitacae* family have in their vegetative and reproductive organs (especially in the fruits) a variety of crystals, the presence of raphides being characteristic to them (Metcalfe, C.R., L. Chalk, 1950, Zanoschi, V., C. Toma, 1985). From the five types of crystals recognized by the specialized literature, raphides, druses and prismatic crystals were noticed in the material included in our study. Styloids and sand crystals were not present, confirming the data from the specialized literature.

Crystals develop in idioblasts, specialized cells that isolatedly differentiate from one another. One idioblast presents only one type of crystal, raphide, druse or prismatic crystal.

Raphides are fascicles of needlelike crystals, representing the most common type of crystals in monocotyle plants, while druses (cluster-type crystals) are almost spherical aggregates, present especially in dicotyle plants. The development of raphides is bidirectional, while for the druses, the deposit of calcium is relatively simultaneous on all the crystal's surfaces.

The idioblasts with raphides are obviously larger than the surrounding cells, so that they are easily noticed; on the other hand, the dimensions of the dimensions of the idioblasts presenting druses or prisms is comparable to those of the surrounding cells. The idioblasts with raphides are axially-elongated cells, almost cylindrical, each of them presenting a crystalline chamber (known as Metcalfe and Chalk bag) with numerous acicular crystals oriented along the cell's axis.

The number of druses varies as well as their dimensions from few small crystals to one big crystal. Actually, each druse represents an aggregate of numerous crystals with different aspect.

The position of the druses in certain tissues with similar dimensions can be correlated with the environmental conditions in which the plant develops and which are related to the transpiration rate and the calcium level at a specific moment in the plant's development (Prychid Christina J., Jabaily Rachel Schmidt, Rudall Paula J., 2008).

The defense role against herbivorous is accomplished by the raphides, their acicular shape being the key factor of this defense mechanism.

Depending of the micromorphological characteristics of the raphides, more than one type of raphides can be presented (Saadi A. I., A. K. Mondal, 2012).

Type I raphides - are the most frequent one and they consist of four-sided single crystals with two symmetrical pointed ends.

Type II raphides – are also four-sided; they present one pointed end while the other one is bidentate or forked. The bidentate end resulted from crystal twinning.

Type III raphides – are crystals with six to eight sides and symmetrical pointed ends.

Type IV raphides – includes h shaped twinned crystals in transversal section and asymmetrical ends (one end is wedge-shaped while the other is sharply pointed).

In general, raphides are present in the idioblast in groups with a very uniform aspect (*fig. 1*), the acicular crystals having similar lengths and parallel distribution. There have also been noticed situations in which this uniformity is not present, the crystals having variable lengths and disordered distribution (*fig. 3*).

The distribution of idioblasts with raphines corresponds to the information present in the specialized literature (Metcalfe, C.R., L. Chalk, 1950). In the stem they are more frequent in the medullary parenchyma, especially in the outer layers near the wood parenchyma, and the shape of the cells is similar to the surrounding cells, situation present at the level of the petiole. In the leaf, elongated idioblasts, with an aspect different from that of the surrounding cells, were noticed in the main parenchyma from the median nervure as well as between the nervures, in the mesophile. In this second case, the idioblasts are positioned at the border between the palisade and the lacunose tissue, most frequent being parallel to the epidermis (*fig. 2*) and in a small number of cases, almost perpendicular.

This type of idioblasts in the mesophile is similar to the flexible tubular structures noticed in the leaves of some *Vitis* species (*V. labrusca, V. mustangensis* and *V. lupina*) (Webb M. A., Cavaletto J. M., Carpitan N., Lopez L., Arnott H.J., 1995).

The raphides present in the idioblasts presented above belong to the second type of raphides described in the literature dealing with this subject and observed in a small number of families, among which we can mention *Vitaceae.*

In the material included in this study, we also observed cells with a very low number (4-5) of unparallel acicular crystals (*fig. 4*) which resemble more type I raphides.

In the material taken into consideration by us were also noticed cells with a very low number (4-5) of unparallel acicular crystals (*fig. 4*) which resemble more to type I raphides.

Druses are present in the stem and the petiole and less in the leaf's limb. Their distribution is varied in the cellulosic parenchyma, especially in the proximity of the pericyclic fibers, in the medullary rays of the phloem or in the phloem (*fig. 5*). From the observations of the biological material resulted the presence of these crystals in collenchyma cells (*fig. 6*) and in the medullary rays of the xylem (*fig. 7*). Parenchyma cells with crystals may have lignified walls with secondary thickenings, but the wood most often presents prismatic or rhomboidal crystals.

The specialized literature (Everet R.F., 2006) mentions situations in which the parenchyma cells are divided, each section having one crystal, in our case we refer to druses situated in the petiole (*fig. 8*).

The prismatic crystals analyzed taken from the material included in the study were found in different positions (in the parenchyma and the phloem) and presented various shapes (rhomboidal or cubic – *fig. 9*) and dimensions.

The parenchyma cells of the petiole presented crystals with different aspect, intermediate between druses and prisms (*fig. 10*).

After analyzing the botanical material that was cultivated in different locations, the varieties of grapevines presented various differences, as well as their fertile and sterile young shoots. Thus, at the fertile shoot of *Grasă de Cotnari* cultivated in Iasi no crystals were noticed, while at the *Riesling* variety cultivated in Iasi only the fertile shoot presented druses next to the pericyclic fibers.

The *Fetească albă* variety cultivated in Cotnari, presented in its sterile shoot fewer crystals than the fertile shoot, while the morphological and location diversity are diminished.

The correlation between the dimension of the crystals and the taxon they belong to is difficult because size can vary along with type, partly due to some intrinsic factors (genetic), but partly due to some environmental factors that include the amount of calcium available during the formation of the crystal.

Dimension, localization and other characteristics of the crystal can be influenced by physical, chemical and biological conditions (Meric, Ciler, 2009) as temperature, light, pressure, pH level, ion concentration or the presence of herbivorous.

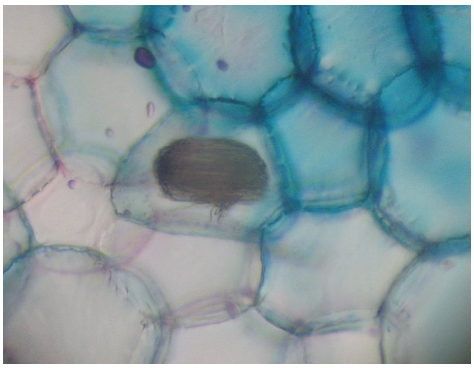

Figure 1. **The *Fetească regală* variety, cultivated in Iaşi, (fertile shoot) – Cross-section of the stem for underlining the raphides (x 1200)**

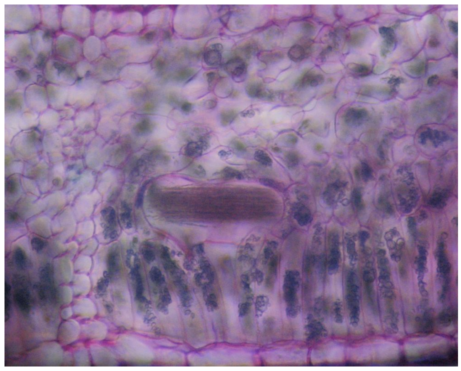

Figure 2. **The *Băbească gri* variety, cultivated in Iaşi, (fertile shoot) – Cross-section of the limb for underlining the raphides (x 700)**

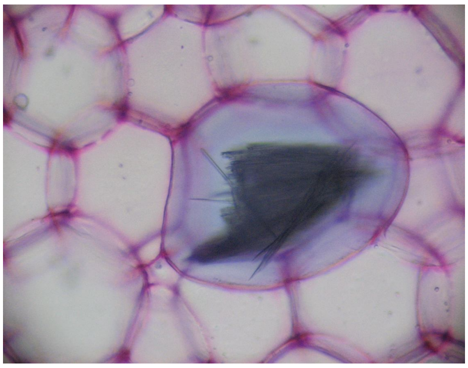

Figure 3. **The *Tămâioasă românească* variety, cultivated in Iaşi, (fertile shoot) – Cross-section of the petiole for underlining the raphides (x 1200)**

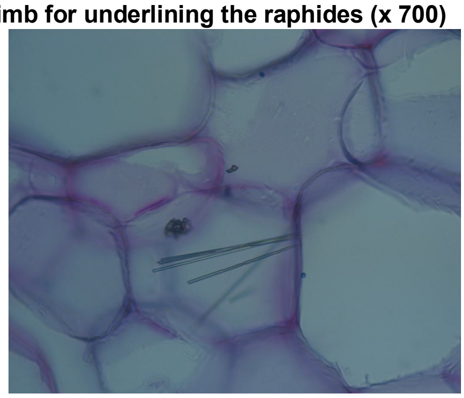

Figure 4. **The *Fetească albă* variety, cultivated in Cotnari, (fertile shoot) – Cross-section of the petiole for underlining the raphides (x 1200)**

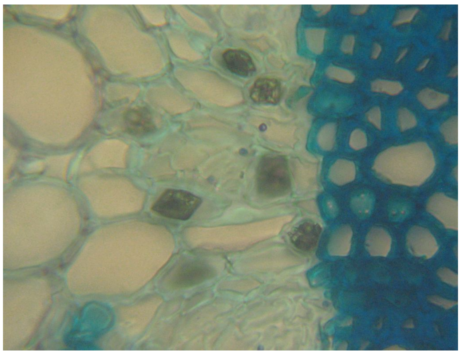

Figure 5. **The *Fetească albă* variety, cultivated in Cotnari, (fertile shoot) – Cross-section of the petiole for underlining the crystals (x 700)**

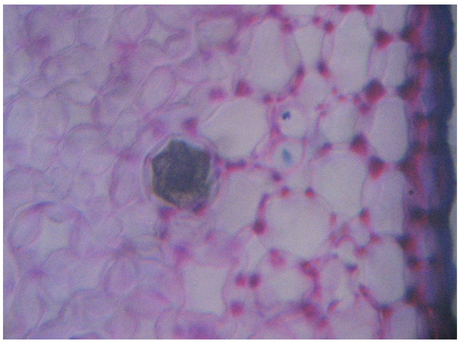

Figure 6. **The *Frâncuşă* variety, cultivated in Iaşi, (fertile shoot) – Cross-section of the petiole for underlining the druses (x 700)**

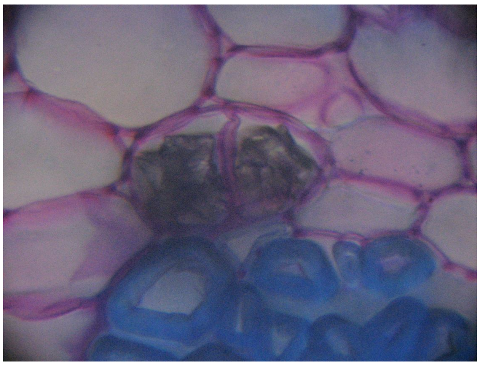

Figure 7. **The *Fetească albă* variety, cultivated in Cotnari – Cross-section of the fertile shoot for underlining the druses (x 1200)**

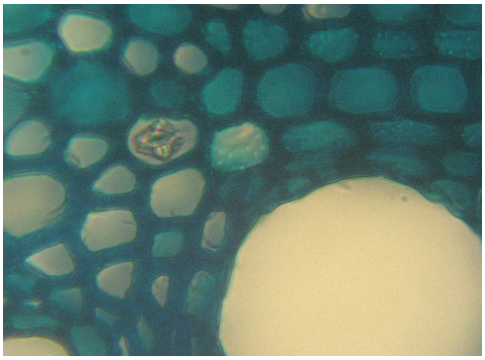

Figure 8. **The *Frâncuşă* variety, cultivated in Iaşi, (fertile shoot) – Cross-section of the strain for underlining the druses (x 1200)**

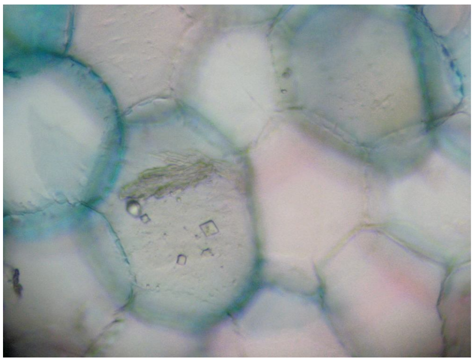

Figure 9. **The *Fetească regală* variety, cultivated in Iaşi, (fertile shoot) – Cross-section of the strain for underlining prismatic crystals (x 1200)**

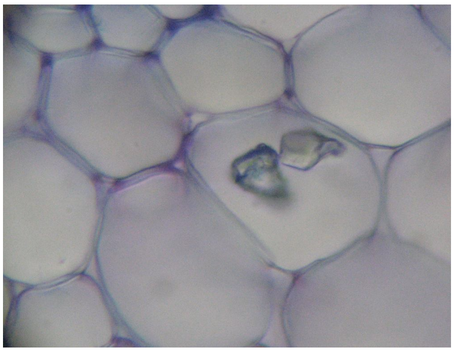

Figure 10. **The *Tămâioasă românească* variety, cultivated in Cotnari, (fertile shoot) – Cross-section of the petiole for underlining the crystals (x 1200)**

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CONCLUSIONS

1. The varieties of grapevines analyzed showed the presence of calcium oxalate crystals in an obvious morphological variety, being three

2. types of crystals: raphides, druses and prismatic crystals.

3. The localization of calcium oxalate crystals is also varied, them being present in parenchyma, phloem and even xylem tissue.

4. The morphological diversity and the localization of crystals is smaller in the leaf's limb (especially in the mesophyle) compared to the stem (annual shoot) and the leaf's petiole.

5. Various differences have been pointed out between the varieties studied as well as between the fertile and sterile shoots of the same variety as far as the type of crystals and their distribution is concerned, differences that are caused by genetic and environmental factors.

REFERENCES

Arnott, H. and M. Webb, 2000 - *Twinned raphides of calcium oxalate in grape (Vitis): Implications for cristal stability and function*. Int. J. Plant Sci., vol. 161, p. 133-142.

Evert R.F., 2006 - *Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function and Development*, 3rd ed. (Hoboken, NY: John Wiley & Sons).

Li, X., D. Zhang, V.J. Lynch-Holm, T.W. Okita and V.R. Franceschi, 2003 - *Isolation of a crystal matrix protein associated with calcium oxalate precipitation in vacuoles of specialized cells*. Plant Physiol., vol. 133, p. 549-559.

Meric, Ciler, 2009 - *Calcium oxalate crystals in some species of the tribe Inuleae (Asteraceae*). Acta Biologica Cracoviensia Series Botanica, vol.51(1), p. 105–110.

Metcalfe, C.R., L. Chalk, 1950 - *Anatomy of the Dicotyledons*. 1-2, Clarendon Press, Oxford.

Prychid, Christina J., Jabaily Rachel Schmidt, Rudall Paula J., 2008 - *Cellular ultrastructure and crystal development in Amorphophallus (Araceae)*. Ann. Bot., vol.101(7), p. 983-995.

Prychid, Christina J., Rudall Paula J., 1999 - *Calcium oxalate crystals in monocotyledons : a review of their structure and systematics*. Ann. Bot., vol. 84, p. 725-739.

Saadi A. I., A. K. Mondal, 2012 - *Studies on the calcium oxalate crystals (Raphides) and idioblast of some selected members of Araceae in Eastern India,* African. Journal of Plant Science, vol. 6(9), p. 256-269.

Şerbănescu-Jitariu & colab., 1983 - *Practicum de biologie vegetală*. Editura Ceres, Bucureşti.

Zanoschi, V., C. Toma, 1985 - *Morfologia şi anatomia plantelor cultivate*. Editura Ceres, Bucureşti.

Webb M.A., Cavaletto J.M., Carpitan N.C., Lopez L.E., Arnott H.J., 1995 - *The intravacuolar organic matrix associated with calcium oxalate crystals in leaves of Vitis*. Plant Journal, vol. 7, p. 633-648.