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Calcium Oxalate Crystal (CaOx) Composition at Different Growth Stages of Petiole in *Vitis Vinifera* (Vitaceae)

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Abstract

Most plants accumulate inorganic inclusions called calcium oxalate crystals (CaOx) in both vegetative and reproductive organs. In these studies, the similarities and differences for the type and distribution of calcium oxalate (CaOx) crystals were examined in both young and mature petiole *Vitis vinifera* L. (Vitaceae). Transverse, radial, and tangential sections were obtained from epidermis, cortex, xylem and phloem tissue of different specimens. Crystal measurements of young and mature petiole were statically analyzed. Significant differences were determined between young and mature petiole in terms of distribution in the tissues and varieties of crystal.

Keywords: Anatomy, Calcium oxalate, Crystal, *Vitis vinifera*

1. Introduction

Most plants accumulate inorganic inclusions called calcium oxalate crystals (CaOx) in both vegetative and reproductive organs. They represent storage forms of calcium that have environmentally supplied and oxalic acid that have produced by the plant's own metabolism [1]. This inorganic substance is produced in vacuole of specialized cells called idioblast although extracellular crystals have also been reported [2]. They are present in higher plants in addition to ferns and algea and are widespread in dicotyledons and monocotyledons, including both herbaceous and woody form [3, 4, 5].

Calcium oxalate crystals appear in a variety of shapes occurring as crystals of either in monohydrate (whewellite) form or di-(or tri-) hydrates (weddelite) form [6]. Most crystals can be classified as crystal sand, raphide, druse, styloid, and prismatic based on their morphology. Calcium oxalate crystals develop in specific anatomical and developmental patterns, indicating that their formation is controlled genetically by plants [7, 8, 9]. For this reason, crystal distribution and shape have been used as taxonomical characters for some of plant taxon although the amount of crystal may be influenced from environmental condition.

Calcium is essential to biological process in plant such as middle lamella formation and stomatal movement. İn addition calcium is used as a cofactor of proteins. But high concentration is toxic for plants. Some investigations indicated that calcium deficiency may cause organelle degradation, chromosomal anomaly and abnormally mitotic activity. It has been proposed that CaOx may serve as a regulatory of calcium concentration in plant. Several reports in support of this hypothesis have shown that the amount of CaOx in tissue decreased associated with environmental calcium reduction [7, 10].

Biological function of CaOx is not clearly understood. But based on crystal shapes and sizes, prevalence and spatial distribution, there have been a number of hypotheses regarding crystal function in plants. Previous research indicates that calcium oxalate crystals have variety of important functions, such as to calcium regulation [11], help prevent herbivory [12] and act metal detoxification [11]. In addition they may have more specific function that promote air space formation in some aquatic plants or protect the photosynthetic tissue from UV radiation [13].

Although there are many studies on various aspects of crystal chemical characterization and synthesis of calcium oxalate crystals in *Vitis*, reports to enlighten its possible function, temporal and spatial distribution are rare. Previous observations have shown in grape that raphide crystals of *V. vinifera* berries are composed of calcium oxalate monohydrate. This work also established ascorbic acid as the biosynthetic precursor of both oxalic and tartaric acids via two distinct pathways operating within the same organ [14]. According to [15], organic matrix associated with calcium oxalate crystals in grape are discussed in relation to crystal nucleation and growth. On the other hand it is suggested that the raphides are twinned along their length in *Vitis* may increase mechanical strength and they may have function in plant defense against herbivory [16].

In this study we carried out the similarities and differences between for the type and distribution of calcium oxalate (CaOx) crystals in both young and mature petiole *Vitis vinifera* L. (Vitaceae). This research is valuable to obtain a more comprehensive understanding of calcium oxalate function in plants tissue.

2. Materials and Methods

Grape leaves materials were obtained from different specimens were collected in Middle Black Sea Region of Turkey. Young and mature petioles were collected from native plants in May and September respectively. For light microscopy, the materials were fixed in 70 % ethyl alcohol and left in 95% ethanol for one night. The hand-sliced sections of fixed petiole were examined. Transverse, radial, and tangential sections were obtained from epidermis, cortex, xylem and phloem tissue of different specimens. Crystals were viewed using an Olympus microscope (Tokyo, Japan) and images were photographed with an Olympus digital camera (Camedia C5060). Selected images were processed in PhotoShop 7.0 (Adobe, San Jose, California). The diameters of the druses, the lengths and widths of the raphide and prismatic crystals measured for the analysis. Measurements of the crystals were taken with Image-Pro Plus, version 5.1 (Media Cybernet-ics, Silver Spring, MD). Fifteen crystals for each tissue and each crystal type were measured from randomly chosen 50 specimens. Druse, raphide and prismatic crystals measurements of young and mature petiole were analyzed using SPSS software (SPSS software version 18.0; SPSS Inc., Chicago, IL). The averages and standard deviations of data were calculated.

3. Results

Calcium oxalate crystals observed in grape typically form three main types of crystal morphologies: raphides or needle-shaped crystals that form in bundles; druses or multiple crystal forms of spherical conglomerates; and prismatic crystals consisting of regular prismatic shapes [17]. In further investigation, significant differences were determined between young and mature petiole in terms of distribution in the tissues and varieties of crystal in *Vitis vinifera* (Vitaceae) specie. CaOx morphology and distribution in tissues are shown in Table 1. Druse crystal density was higher than the other crystal types in both petiole (Figure 3 and 7). No prismatic crystals were found in young petiole, while they were present in some parts of mature petiole (approximately 9.5 crystals per mm^2). On the other hand Druse crystals and raphide in mature petiole is much more intense than those in young petiole.

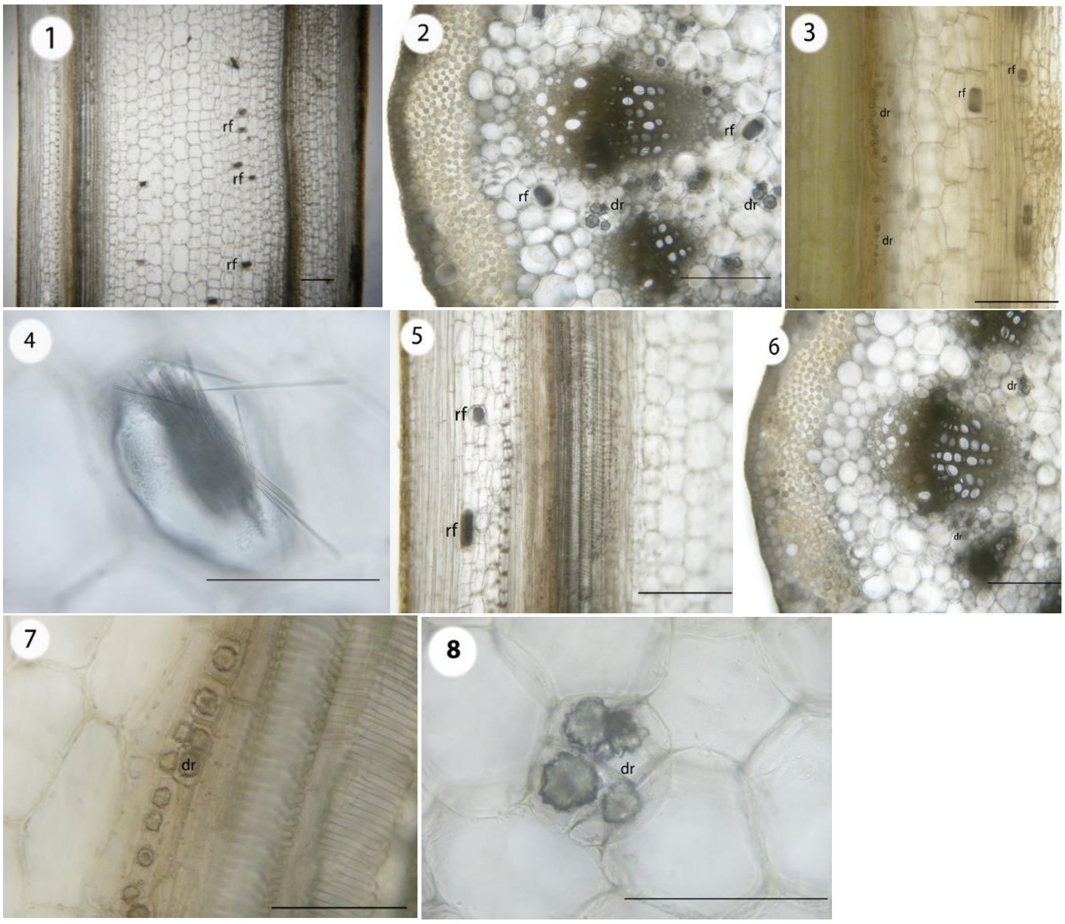

Figure 1-8. The transverse and tangential sections of young and mature petioles in *Vitis vinifera*. (Bar: 50µm) 1-4: mature petiole, 5-8: young petiole, (dr: druse crystal; rf: raphide crystal).

Using T-test, the diameters of the druses, the lengths and widths of the raphide crystals were compared between young and mature petiole (Table 2). Statistical analysis of the crystals indicated that there was no significant difference between both samples in terms of raphide heights and druse diameters, while the width of raphide clusters were quite different (Figure 9) (raphide width → t:2.132, df:27.726, $P<0.05$ raphide height→ t:0.404, df:23.655, P=690 druse diameter→ t:1.784, df:27.844, P=0.085).

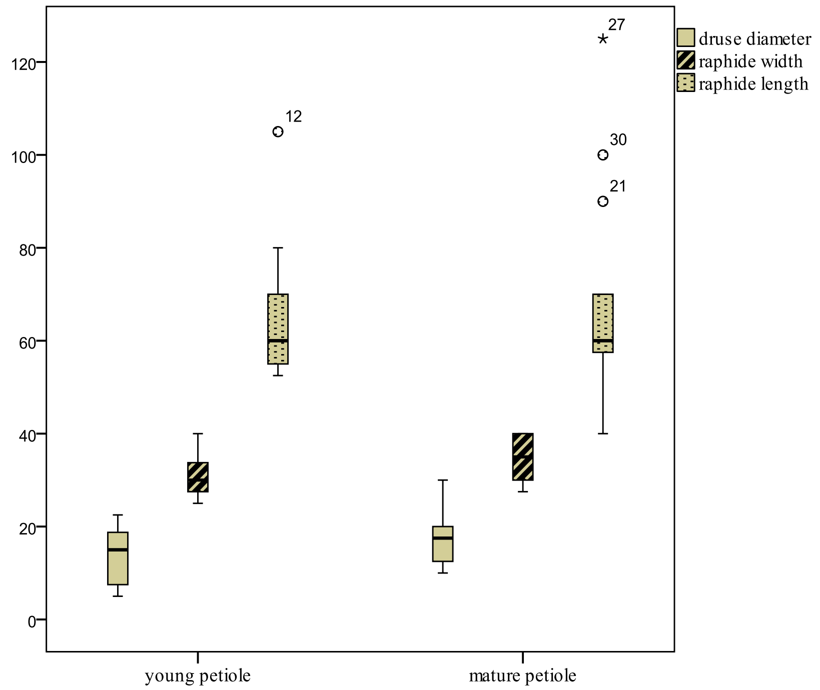

Figure 9. Calcium oxalate crystal measurement of druse diameter (shown in empty bar), raphide width (shown in striated bar) and raphide length (shown in pointy bar) of young and mature petiole.

Table 1. Crystals intensity and distribution at different tissue in both petiole

	Young petiole			Mature petiole		
	Druse	Raphide	Prismatic	Druse	Raphide	Prismatic
Epidermis	-	-	-	-	-	-
Cortex	+	+	-	++	++	+
Phloem	+	-	-	+++	+	+
Xylem	-	-	-	+	-	-
Pit	+	+	-	+	++	-

Table 2. Anatomical measurement of raphide, druse and prismatic crystals in both petiole

	Young petiole			Mature petiole		
	Druse	Raphide	Prismatic	Druse	Raphide	Prismatic
Amount of crystals per mm^2	53,75	25	-	140	41,25	9,5
Length/diameter width (mean±se)	13.33±6.09	65.33±13.65	-	17.167±5.65	68±21.59	-

Tissue distributions of these crystals were observed under light microscopy. Druse crystal was rich around vasculary bundles, especially phloem tissue and the diameters of these crystals were measured as 13.333 ± 6.0994 µm diameter (Figure 7) in young petiole. They were as clusters or single crystal in cell (Figure 8). Raphides were observed in cortex and pith cells were 31.333 ± 5.1640 width µm and 65.333 ± 13.6561µm length in young petiole. But they more abundant in mature petiole tissue (Figure 4). In addition, the lengths of these prismatic crystals were rarely determined in cortex and phloem of mature petiole (Figure 2). Compared with samples of mature petiole, the crystal densities of all types were less in young samples (Figure 6). Furthermore, a comparison between the tissues demonstrated that crystals were found more intense in phloem and cortex (Figure 3 and 5). No crystals were found in epidermis of the petiole. Druse crystals were densely present near the vasculary tissue, while raphide bundles dispersed in parenchymatic tissue (Figure 1). However, prismatic crystal was not observed in any tissue of young petiole samples (Table 2).

4. Discussion

Previous studies have reported that CaOx crystals in plant tissues show various functions, In this species, the fact that druse crystal have amorphous shape and the observation that druse crystals are the most common were compatible with the hypothesis that druse crystal had a main function protecting plant tissue against attacks by herbivores, which there are many articles that support this function.
Distribution of calcium oxalate crystal types between tissues at young and mature leaves petiole of *Vitis vinifera* show significant differences. In our study, it was determined that the crystal density at mature leaves was considerably higher than the young leaves. Furthermore, mature petiole contains the prismatic crystals, while no crystals are found at young petiole. Thus, they may act as a storage form of excess Ca, which is compatible with many studies.
Druse crystals are more common in both petioles than the other crystal types. They are mainly common at cells near to the vasculary component. Thus the excess calcium may carry with vasculary system and storage in parenchymatic cells so that they act as a protecting factor against collapse. Our results confirm the findings of Franceschi and Horner [3] with recpect to giving strength to the tissues. According to Okoli and Green [18], the crystals polymerize in relation to the some organic matter such as starch particles. Druse crystals especially localize in the tissues around the phloem are support the hypothesis of Okoli and Green [18]. The fact that significant difference is observed in terms of raphide bundles width between young and mature petiole, while crystals length are not different at statistical analysis suggest that raphide accumulation increases in crystal idioblast. Probably, as the development progresses, the new raphides join to the bundle.
In summary, we report the similarities and differences between the type and distribution of calcium oxalate (CaOx) crystals in both young and mature petiole *Vitis vinifera* L. (Vitaceae). These results are important in studying possible function of calcium oxalate crystals in plant to design experiments.

References

[1] H. Ilarslan, R.G. Palmer, J. Imsande and H.T. Horner, Quantitative determination of calcium oxalate and oxalate in developing seeds of soybean (Leguminosae), *American Journal of Botany*, **84** (1997), 1042 - 1046. http://dx.doi.org/10.2307/2446147

[2] H. Kinzel, Calcium in the vacuoles and cell walls of plant tissues, *Flora*, **182** (1989), 99 - 125.

[3] V.R. Franceschi and H.T. Horner, Calcium oxalate crystals in plants, *Botanical Review*, **46** (1980), 361- 427. http://dx.doi.org/10.1007/bf02860532

[4] A.C. Morrow and R. R. Dute, Crystals associated with the intertracheid pit membrane of the woody fern *Botrychium multifidum*, *American Fern Journal*, **92** (2002), 10 - 19. http://dx.doi.org/10.1640/0002-8444(2002)092[0010:cawtip]2.0.co;2

[5] D. Ward, M. Spiegel and D. Saltz, Gazelle herbivory and interpopulation differences in calcium oxalate content of leaves of a desert lily, *Journal of Chemical Ecology*, **23** (1997), 333 - 346. http://dx.doi.org/10.1023/b:joec.0000006363.34360.9d

[6] H.J. Arnott, An SEM study of twinning in calcium oxalate crystals of plants, *Scanning Electron Microscopy*, **3** (1981), 225 - 234.

[7] H. J. Arnott and F. Pautard, *Biological Calcification: Cellular and Molecular Aspects*, Meredith, New York, 1970.

[8] A.P. Kausch and H.T. Horner, Development of syncytial raphide crystal idioblasts in the cortex of adventitious roots of *Vanilla planifolia* L. (Orchidaceae), *Scanning Electron Microscopy*, **2** (1983), 893 - 903.

[9] A.P. Kausch and H.T. Horner, The development of mucilaginous raphide crystal idioblasts in young leaves of *Typha angustifolia* L. (Typhaceae), *American Journal of Botany*, **70** (1983), 691 - 705. http://dx.doi.org/10.2307/2443123

[10] L. A. Sunell and P. L. Healey, Distribution of calcium oxalate crystal idioblasts in leaves of taro (*Colocasia esculenta*), *American Journal of Botany*, **72** (1985), 1854 - 1860. http://dx.doi.org/10.2307/2443602

[11] V. R. Franceschi and P. A. Nakata, Calcium oxalate in plants: Formation and

function, *Annual Review of Plant Biology*, **56** (2005), 41 - 71. http://dx.doi.org/10.1146/annurev.arplant.56.032604.144106

[12] B. Molano-Flores, Herbivory and calcium concentrations affect calcium oxalate crystal formation in leaves of *Sida* (Malvaceae), *Annals of Botany*, **88** (2001), 387 - 391. http://dx.doi.org/10.1006/anbo.2001.1492

[13] H. T. Horner and V. R. Franceschi, Calcium oxalate crystal formation in air spaces of the stem of *Myriophyllum*, *Scanning Electron Microscopy*, **2** (1978), 69 - 76.

[14] S. DeBolt, J. Hardie, S. Tyerman and C.M. Ford, Composition and synthesis of raphide crystals and druse crystals in berries of *Vitis vinifera* L. cv. Cabernet Sauvignon: Ascorbic acid as precursor for both oxalic and tartaric acids as revealed by radiolabelling studies, *Australian Journal of Grape and Wine Research*, **10** (2004), 134 - 142. http://dx.doi.org/10.1111/j.1755-0238.2004.tb00016.x

[15] M. A. Webb, Cell-mediated crystallization of calcium oxalate in plants, *The Plant Cell*, **11** (1999), 751 - 761. http://dx.doi.org/10.1105/tpc.11.4.751

[16] H. J. Arnott and M. A. Webb, Twinned raphides of calcium oxalate in grape (*Vitis*): Implications for crystal stability and function, *International Journal of Plant Science*, **161** (2000), 133 - 142. http://dx.doi.org/10.1086/314230

[17] D. Jáuregui-Zúñiga, J. P. Reyes-Grajeda, J. D. Sepúlveda-Sánchez, J. R. Whitaker and A. Moreno, Crystallochemical characterization of calcium oxalate crystals isolated from seed coats of *Phaseolus vulgaris* and leaves of *Vitis vinifera*, *Journal of Plant Physiology*, **160** (2003), 239 - 245. http://dx.doi.org/10.1078/0176-1617-00947

[18] E. B. Okoli and O. G. Blessing, Histochemical localization of calcium oxalate crystals in starch grains of yams (*Dioscorea*), *Annals of Botany*, **60** (1987), no. 4, 391 - 394.

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