Characterization and macropattern of calcium oxalate phytoliths in Argentinean endemic species of Chenopodioideae (Amaranthaceae)

V. Pérez Cuadra[*], P. Hermann

Lab. Plantas Vasculares. Dep. de Biología, Bioquímica y Farmacia. Universidad Nacional del Sur. Bahía Blanca, Prov. Buenos Aires, Argentina

ARTICLE INFO

ABSTRACT

The Chenopodioideae comprises species with a worldwide distribution, mainly confined to xeric or saline areas. Calcium oxalate crystals (calciphytoliths) are abundant in these species and relatively poorly studied. The aim of this work is to characterize the different types of calciphytoliths and describe their macropattern in *Atriplex undulata*, *Nitrophila australis* var. *australis* and *Suaeda divaricata* (Chenopodioideae). Mature leaves of the three species were collected in Salitral de la Vidriera (Argentina), killed and fixed in formalin-acetic acid-alcohol and processed using common techniques for anatomical studies and crystal macropattern identification. Cross sections of *A. undulata* show that crystals occur subepidermically in the chlorenchymatic tissue while in *N. australis* var. *australis* and *S. divaricata* they occur deeper in the aqueous tissue. Only *A. undulata* has two types of crystals: prisms and druses. The latter may be of two kinds: druses type A_1 and B according to the shape of the individual crystals involved. *N. australis* var. *australis* presents type A_1 druses while *S. divaricata* has a third type, A_2. In cleared leaves of *A. undulata* and *N. australis* var. *australis* crystals can be observed in intercostal areas; in *S. divaricata* they are associated with the vascular bundles. The characterization of crystals and their macropatterns are useful for the identification of these species, which indicates their taxonomic value.

© 2011 Elsevier Ltd and INQUA. All rights reserved.

1. Introduction

Bioliths are mineralized bodies found in animal and plant tissues. Those occurring in plants are called phytoliths (Bertoldi de Pomar, 1975) and they can be classified according to the nature of the mineralization agent: calcium carbonate or calcium oxalate in calciphytoliths, and silica in silicophytoliths (Bertoldi de Pomar, 1975).

The first phytoliths to be studied in greater detail were silicophytoliths due to their taxonomic value and duration associated to their chemical nature (Bertoldi de Pomar, 1975). Cyperaceae, Palmaceae and Poaceae were the first plant families to be known as silicophytolith producers. Later, however, new investigations demonstrated that many Pteridophyta and Dicotiledoneae produced them too (Bertoldi de Pomar, 1975). Typical silicophytoliths occur in epidermal cells of Cyperaceae and Poaceae, but particularly in cells adjacent to fibres or vascular bundles in Palmaceae and Scitaminaceae. They are generally constant in shape, and their location is predictable. Atypical silicophytoliths are the result of silicification of different cells such as bulliform, stomatal guard cells, trichomes,

vessels, etc., with a rather unpredictable location in the plant body (Bertoldi de Pomar, 1975; Ellis, 1979). For quite a long time, typical silicophytoliths were considered important diagnostic characters, particularly within Poaceae (Ellis, 1979). Presently, caution is recommended in this respect, as a special type of silicophytolith should be present in a taxon and absent in others to acquire systematic value (Madella et al., 2005). Silicophytoliths remain unalterable in the soil, mainly in upper soil horizons and in variable proportions depending on the plant population growing on it. They are frequently found in sediments of different ages, a fact that makes them valuable to reconstruct the sequence in plant succession through time, pointing out evolutionary or climatic changes, thus becoming of interest in paleoecology (Bertoldi de Pomar, 1975).

Calciphytoliths have been observed in rocks, soils, and among multiple members of all of the five kingdoms (Monera, Protista, Fungi, Plantae, and Animalia). In all instances, the crystals are formed from environmentally derived calcium and from biologically synthesized oxalate (Prychid and Rudall, 1999; Nakata, 2003). In particular, the fungal biomineralization of this type is of interest for their role in the calcium cycle in calcretes (Verrechia et al., 1993).

Calcium is abundant in the natural environment in which most plants grow, and plays important roles for growth and development. For example, it is a structural component of cell walls,

* Corresponding author. Fax: +54 291 4595101.
  E-mail address: vperezcuadra@uns.edu.ar (V. Pérez Cuadra).

a physiological messenger and a regulator of osmotic pressure. However, in most plant cells the levels of cytosolic free calcium must be restricted because higher concentrations interfere with a variety of cellular processes (Baran and Monje, 2008).

There are different explanations for the accumulation of calcium oxalate crystals. The first and widely accepted is related to intracellular calcium concentration equilibrium; an excess of it interferes with cellular metabolism and a viable way to reduce its concentration is to chelate calcium in the form of an oxalate, particularly in those species which, due to environmental conditions, present high transpiration rates (Franceschi and Horner, 1980; Molano Flores, 2001; Nakata, 2003; Lersten and Horner, 2005a; Baran and Monje, 2008). This occurs, for example, in some Fagaceae where minor order veins are found completely surrounded by crystals (Nakata, 2003; Lersten and Horner, 2008a) or in species of Cactaceae, which present a very high calcium oxalate crystal content, whereas the concentration may vary widely (Hartl et al., 2007). Another hypothesis considers that crystals are involved in the defence against herbivores, especially large ones (Franceschi and Horner, 1980; Molano Flores, 2001; Nakata, 2003; Lersten and Horner, 2005a; Baran and Monje, 2008); plants possessing crystals would have an adaptive advantage. Other functions have been attributed to crystals: detoxification of heavy metals, plant tissue support and, reflection and dispersion of light (Hartl et al., 2007). For most of these hypotheses, evidence is lacking (Baran and Monje, 2008; Franceschi and Nakata, 2005; Nakata, 2003). As mentioned by Lersten and Horner (2008a), given the functions ascribed to crystals and the different macropatterns that allow distinguishing genera and families, there should be some specific mechanism that determines their presence, not only being the result of simple, individual, cellular processes. Borrelli et al. (2011) compared the calcium and silica biomineralization production in some aquatic species of the Pampean Plain. They pointed out that those species with abundant calcium oxalate crystals have no silicophytoliths, at least in abundant proportions. Inversely, abundant silica producers do not have calcium oxalate crystals. These authors considered that both biomineralizations provide similar benefits to the plants that produce them and, because their production has an energetic cost, they hypothesized that each taxon has evolved in a different way and only one of these two biomineralization processes has been selected in each case.

The commonly encountered forms of calcium oxalate crystals are: druses (a spherical aggregate of individual crystals), raphides (needle shaped crystals occurring in bundles of many crystals per cell), styloids (elongated crystals with pointed or ridged ends), prisms and crystal sand (a mass of many tiny, individual crystals in a single cell). The first are cluster crystals while the rest are solitary crystals; one or more of these are found in most angiosperm families (both Monocotyledoneae and Dicotyledoneae) (Franceschi and Horner, 1980; Franceschi and Nakata, 2005). Concretions of calcium oxalate crystals in Naucleeae were recently described, with druses embedded in crystal sand. This is a new duplex combination (Lersten and Horner, 2011).

Knowledge of plant calcium oxalate crystals consists almost entirely of details of crystal structure, how a crystal forms within a cell, and distribution of crystals in mature organs; but it is important to describe the entire macropattern (crystal types and their specific distribution) (Lersten and Horner, 2005a). This type of studies have made it possible to characterize different genera of Fagaceae, Nothofagaceae, Oleaceae, Punicaceae, Quillajaceae, Rosaceae (Prunoideae) and Ticodendraceae (Lersten and Horner, 2000, 2005a, 2005b, 2006, 2008a, 2008b, 2009). They are the only macropattern descriptions of calciphytoliths existing to date. In Cactaceae, the authors did not find any evidence of distribution patterns of the crystals related to taxonomic levels. Species specific crystal macropatterns based on crystal type and their distribution and development found in other families were not detected in Cactaceae (Hartl et al., 2007). No references were found in the literature for Chenopodioideae in this respect, and thus the present study is the first report for this subfamily.

In contrast to silicophytoliths, calciphytoliths were considered as one more element within anatomical descriptions of different species without any additional value due to their cosmopolitan distribution among Monocotyledoneae and Dicotyledoneae and low duration of preservation (Lersten and Horner, 2008a). Presently, calciphytoliths are considered an integral part of the leaves and stems, emphasizing descriptions of their macropatterns for their taxonomic value (Lersten and Horner, 2008a).

The subfamily Chenopodioideae comprises species with a worldwide distribution, although most are confined to xeric or saline areas. Calcium oxalate crystals are abundant in these species in the form of large aggregates as well as crystal sand. Frequently, both forms coexist within a single leaf (Metcalfe and Chalk, 1957).

In the past, some species of Chenopodioideae, such as quinoa (*Chenopodium quinoa* Willd.) and caniihua (*Chenopodium pallidicaule* Aellen) were important highland food crops in the Lake Titicaca region. These species are common throughout the archaeological record (Cutler, 1968). Remains of Chenopodioideae have been found in a rock shelter site in Australia. That shows evidence of human occupation 40,000 BP. The use of these type of plants was more frequently found in arid and coastal regions, because these species occupy a wide range of saline soils and landforms where other useful plants cannot grow. In some specific regions, the chenopods were used as food during the last glacial maximum of the late Pleistocene (McConnell, 1998). The study of calciphytoliths could be helpful in this type of investigations, and others related to paleobotany, where the condition of the plant remains makes a correct identification difficult. The increase of detailed studies of calciphytoliths could be of interest too, for the identification of plant remains in archaeological ash layers. The identification of what was burnt and why could be a valuable tool to cultural information retrieved from artefacts (Canti, 2003). This application of calciphytoliths studies is possible because the crystal types present, combined with their distribution in foliar tissues (macropattern) have a definite taxonomic value, together characterizing some species observed (Lersten and Horner, 2000, 2005a, 2005b, 2006, 2008a, 2008b, 2009).

The aim of this work is to characterize the different types of calciphytoliths and describe macropattern in leaves of *Atriplex undulata* (Moq.) D. Dietr, *Nitrophila australis* Chodat and Wilczek var. *australis* and *Suaeda divaricata* Moq. (Chenopodioideae).

2. Materials and methods

The species studied grow in the Salitral de la Vidriera (Part. Villarino), approximately 30 km from Bahía Blanca City, Prov. of Buenos Aires, Rep. Argentina. The partido of Villarino is situated in the south of Buenos Aires province and occupies an area of 11400 km^2; the region of salt marshes is delimited by 38°35′ and 38°50′S, and 62°40′ and 63°15′W, extending approximately 1580 km^2 (Bonorino, 1970).

The climate is temperate and dry. January and July are the months in which extreme median temperatures occur. Seasonal climatic variation shows a cold season, winter, and a hot one, summer; spring and fall are seasons of transition. A year with a total annual rainfall higher than 600 mm is considered humid, whereas a dry year is that in which rainfall does not reach 500 mm. Annual rainfalls between 400 and 500 mm are most common. Maximum median monthly temperatures are 13 °C, in July, and 31 °C, in January. The minimum median monthly temperatures are 1 °C in June and 13.8 °C in Janaury (Bonorino, 1970).

The Salitral de la Vidriera shows a Quaternary stratigraphy of the Pospampean formation with silt-clay salty sediments and fossil remains of shells, related to marine ingression (Querandinense) (Bonorino, 1970). This region belongs to the phytogeographic province of the Espinal, within the Caldén district (Cabrera, 1971).

Eight species of Chenopodioideae occur here: *Allenrolfea patagonica* (Moq.) Kuntze, *Atriplex undulate* (Moq.) D. Dietr., *Heterostachys olivascens* (Speg.) Speg., *Heterostachys ritteriana* (Moq.) Ung.-Sternb., *N. australis* Chodat and Wilczek var. *australis*, *Sarcocornia ambigua* (Michx.) M. A. Alonso and M. B. Crespo, *Suaeda argentinensis* A. Soriano and *S. divaricata* Moq. Preliminary studies in all eight species showed the occurrence of crystals in only three: these are the ones studied in detail. To typify the crystals and their location in foliar tissues, the samples of leaves of *A. undulata*, *N. australis* var. *australis* and *S. divaricata* were obtained during October and November 2004 to 2009, fixed in formalin-acetic acid-alcohol, dehydrated in an ethyl alcohol-tertiary butyl alcohol series and embedded in Paramat. The sections (10 μm) were stained with safranin-fast green and mounted in Canada balsam. Other leaves, cleaned (Dizeo de Strittmatter, 1973) and stained with safranin, were mounted in gelatin-glycerin to determine their crystal macropattern. Five individuals of each species and five leaves of each individual were studied. Crystal size was measured on these samples obtaining a range between minimum and maximum possible value; median (for a total of 125 data for most abundant crystals and 50 for less frequent ones) and its standard deviation were calculated.
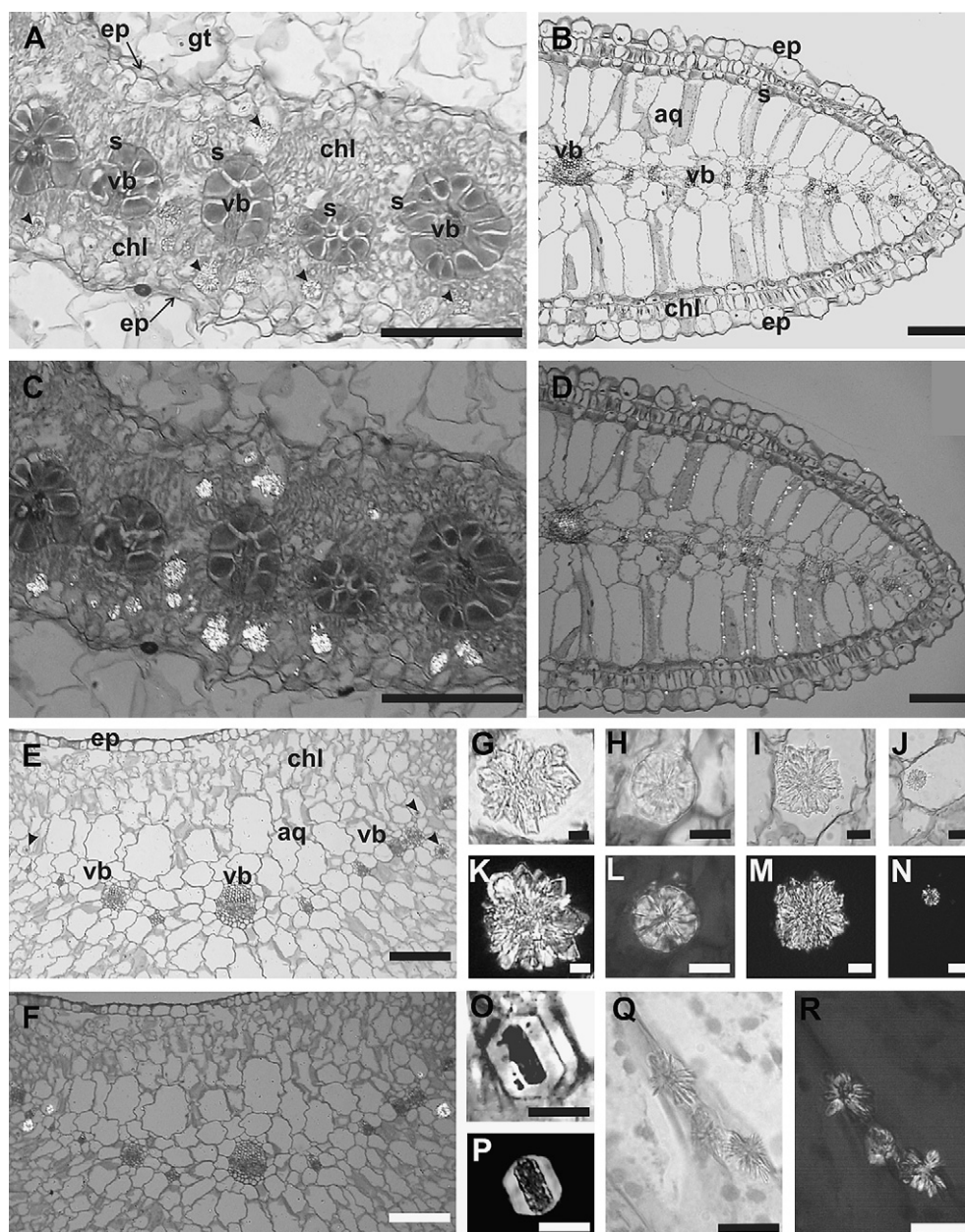

Fig. 1. Location and detail of calcium oxalate crystals in cross section of leaves. A, C, Druses in idioblasts (prisms not seen); B, D, Druses against the anticlinal walls of the aqueous tissue cells; E–F, Druses in the aqueous tissue cells. G, I–K, M–N, Type A_1 druses; H, L, Type B druses; O–P, Prisms; Q–R, Type A_2 druses. A, C, G–H, K–L, O–P, *A. undulata*; B, D, Q–R, *S. divaricata*; E–F, I–J, M–N, *N. australis* var. *australis*. C–D, F, K–N, P, R, Observation under polarized light. Abbreviations: aq: aqueous tissue; chl: chlorenchyma; ep: epidermis; gt: glandular trichomes (the terminal cells are broken); s: sheath; vb: vascular bundle. Arrowheads show calcium oxalate crystals. Barrs: A–F, 200 μm; G–R, 15 μm.

A compound microscope was used for the observations. Photographs were obtained with a conventional digital camera.

Selected leaves fixed in FAA, were sectioned transversely and dehydrated through an ethanol series (50%, 70%, 95%, 100%) at 4 h intervals. These were transferred to ultrapure 100% ethanol (two changes), CO_2 critical-point dried, sputter-coated with 30 nm gold and viewed in a scanning electron microscope LEO/EVO 40 VP SEM at 7 kV.

Herbarium material and permanent slides of the studied species studied are deposited in BBB (Herbario del Departamento de Biología, Bioquímica y Farmacia) and the Histoteca of the Laboratorio de Plantas Vasculares (Universidad Nacional del Sur), respectively.

3. Results

Cross sections of leaves of *A. undulata* show that crystals can be observed in the chlorenchyma beneath the epidermis (Fig. 1A, C). They occur in scattered idioblasts, frequently in their centre. In *S. divaricata* they appear deeper in the mesophyll, mostly against the anticlinal walls of the aquaeous tissue (parenchymatic tissue that accumulates water) cells within the Kranz sheath (Fig. 1B, D). In *N. australis* var. *australis*, crystals are seen in the aquaeous tissue that surrounds the vascular bundles, usually in the centre of the cells (Fig. 1E–F).

The crystals found in the species studied are prisms and druses. Prisms have quadrangular, prismatic bodies ending in quadrangular pyramids (Fig. 1O, P). Druses are of two types, A and B, the former showing two subtypes, A_1 and A_2. Under the light microscope, druses of type A (Fig. 1G, K, I–J, M–N, Q–R) are those having a contour with projections. In subtype A_1 (Fig. 1G, K, I–J, M–N) these projections resemble arrowheads whereas in subtype A_2 (Q-R) they resemble needles. Druses of type B (Fig. 1H, L) have a smooth contour without any projections. SEM observations show that subtype A_1 druses are formed by prismatic crystals with sharp pointed ends (Fig. 2A–B) concordant with the arrowhead projections observed under the compound microscope. Those of subtype A_2 are made up by rather elongated laminar crystals with angular endings (Fig. 2C), the thin edges of which look like needles under the light microscope (Fig. 2Q–R). Type B druses consist of prismatic crystals with blunt ends (Fig. 2D–E) in accordance with the smooth contour described previously.

A. undulata has two types of crystals: prisms $19–24 \times 12–19$ μm ($22,3 \pm 2,7 \times 18,48 \pm 1,4$) (Fig. 1O–P) and druses of types A_1 ((2) 17–55 μm ($20,7 \pm 4,18$) in diameter) (Fig. 1G, K) and B (10–24 μm ($14,9 \pm 3,4$) in diameter) (Fig. 1H, L). *N. australis* var. *australis* and *S. divaricata*, have only one type of crystals: druses. In *N. australis* var. *australis* type A_1 druses (20–42 μm ($31,1 \pm 9,6$) in diameter) (Fig. 1I–J, M–N) can be observed while *S. divaricata* presents type A_2 druses (9–17 μm ($13,5 \pm 5$) in diameter) (Fig. 1Q–R).

In cleaned leaves of *A. undulata* and *N. australis* var. *australis* crystals can be observed in intercostal areas (Fig. 3A–B). In *A. undulata* type A_1 druses are the most abundant, occur at random in the whole lamina (Fig. 3A), and their size increases both acropetally and centripetally, whereas prisms and type B druses are found only towards the margins of the leaf and they do not show any great variation in size. A few small type A_1 druses occur at the base of the lamina but only close to the midrib. Druses in *N. australis* var. *australis* (type A_1) are located in intercostal areas, in some cases almost reaching the midrib (Fig. 3B); few of them are found in the apical zone. In *S. divaricata* they occur in the median region of the lamina, and are seen associated with the veins (Fig. 3C). They are never observed in areas between veins.

4. Discussion

As mentioned in the Introduction, the study and characterization of crystal macropatterns is a relatively recent discipline within botany; therefore records in different families are scant and any new contribution becomes important for the advance of this type of research.

In all species studied belonging to different families, druses are the most frequent type of crystals; prisms, styloids, raphides and crystal sand are found in a lower proportion (Lersten and Horner, 2000, 2005a, 2005b, 2006, 2008a, 2008b, 2009; Horner et al., 2009). Crystals of an intermediate type are rather common in Fagaceae, but they have not been properly characterized (Lersten and Horner, 2008a). Some Oleaceae are distinctive in that they posses sphaerites, an uncommon type of crystal (Lersten and
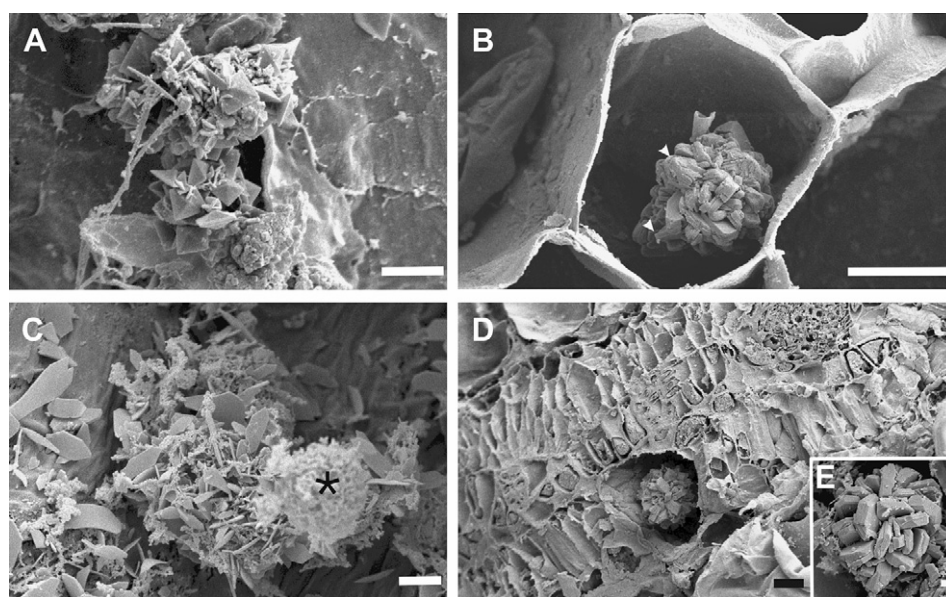

Fig. 2. Druses viewed with SEM. A, B, Type A_1 druses of *A. undulata* and *N. australis* var. *australis* (arrowheads show the crystals with sharp point ends); C, Type A_2 druses of *S. divaricata* (* debris); D–E, Type B druses of *A. undulata*. Barr: A, C, E, 2 μm; B, D, 20 μm.
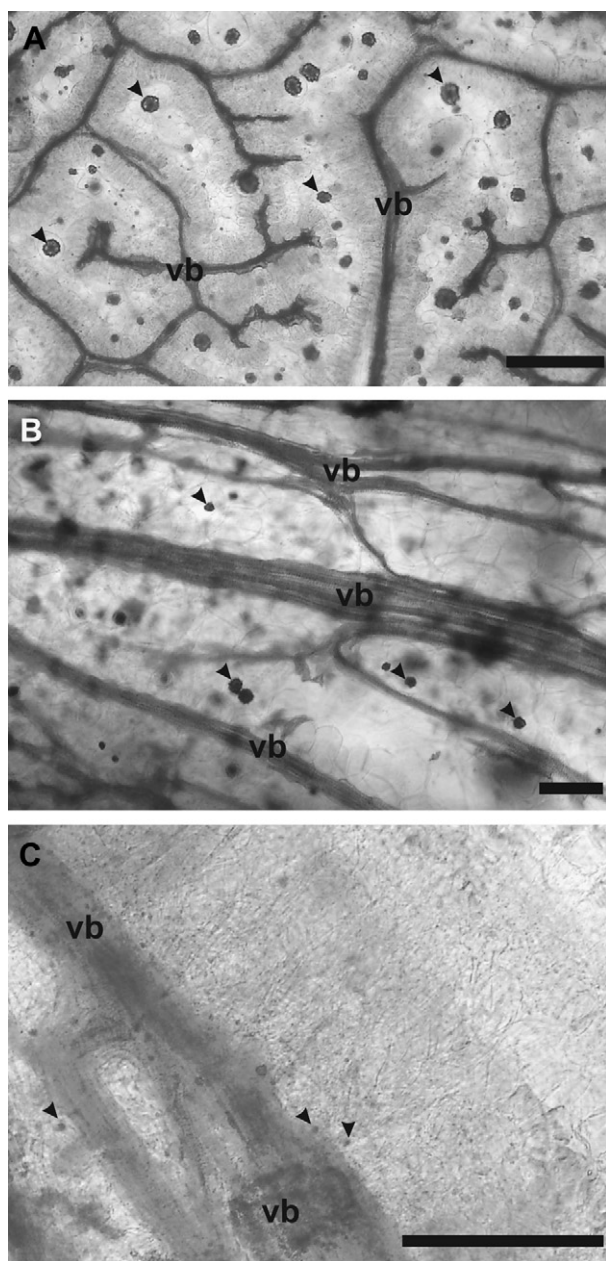
Fig. 3. Macropattern of calcium oxalate crystals in cleared leaves. A, *A. undulata*; B, *N. australis* var. *australis*; C. *S. divaricata*. Abbreviations: vb: vascular bundle. Arrowheads show calcium oxalate crystals. Barrs: 200 μm.

Horner, 2009). In the species of Chenopodioideae observed in this study only druses and prisms are found, showing a comparatively lower diversity respect to other families and subfamilies (Fagaceae, Nothofagaceae, Oleaceae and Prunoideae) (Lersten and Horner, 2000, 2006, 2008a, 2009). Metcalfe and Chalk (1957) pointed out that crystals in the shape of octahedrons or prisms are infrequent in the Chenopodioideae. Of the three species studied here, only *A. undulata* presents prisms, so this type of crystal could characterize either the genus or this species. More representatives should be analyzed to corroborate this.

None of the preceding studies refer to the specific shape of the individual crystals that form a druse. This aspect is relevant since in one species—*A. undulata*—two different druses (subtype A_1 and type B) occur while in *S. divaricata* there is another subtype (A_2). Up

to this time no mention of the existence of different types of druses according to the morphology of its contour or any other characteristic could be found in the literature, and this constitutes an important contribution to the characterization of plant crystals.

The classification of druses here offered is based on morphological characteristics only, and should help in anatomical descriptions. Hartl et al. (2007), from extensive studies in Cactaceae, corroborated that the definitions of different morphologies of crystals from the traditional classification (raphides, styloides, prisms, druses and crystal sand) based on microscopic findings are crystallographically not precise, due to the importance of the hydration state in relation with their morphology. However, a detailed morphological description based on studies at the light microscope and SEM levels should contribute new important data which, combined with the investigations with a different approach might lead to the complete characterization of these crystals.

Crystals are usually associated to main, secondary or minor veins or can be found in the mesophyll in spongy or palisade chlorenchyma. This can be observed in Fagaceae, Nothofagaceae and Punicaceae (Lersten and Horner, 2000, 2005a, 2005b, 2006, 2008a, 2008b, 2009). Less frequently, approximately in 18% of the angiosperms (Lersten and Horner, 2009), crystals occur in epidermal cells. In Oleaceae, however, this is quite frequent and it is characteristic of the family (Lersten and Horner, 2009). In the three species studied here the position of crystals varies considerably, therefore, there is no general pattern. In *A. undulata* crystals appear in idioblasts scattered in the chlorenchyma beneath the epidermis. In *N. australis* var. *australis* and *S. divaricata* druses occur in the aqueous tissue. This is why it is important to extend this type of research to different genera and species in the family to be able to compare and confirm or not, the existence of common macropatterns in different taxa of this group. The latter consideration is of significance since the Chenopodioideae present calcium oxalate crystals in practically all members of the subfamily (Metcalfe and Chalk, 1957) and they have not been studied to date.

Recent investigations use the leaf crystal macropattern as a phylogenetic important tool. Particularly, the genus *Peperomia* is characterized by an increasing complexity of the distribution of different crystal types, with few reversals. The occurrence of raphides and prisms and the presence of druses over the veins characterize only more advanced clades within this genus. This supports the hypothesis that calcium oxalate crystals are involved in strategies other than merely calcium regulation, including plant defence, protection, and photosynthetic efficiency (Horner et al., 2009). As studies on calciphytoliths increase in different families it might be possible to include these features in phylogenetic analyses contributing to a better understanding of relationships among related organism. The studies of characterization of calciphytoliths suggest that plant crystals appear to be under the control of gene expression because of their precise shapes and tissue locations within individual taxa (Lersten and Horner, 2011).

A possible and important field of application of research on calciphytoliths would be their utilization in the identification of archaeological plant remains. Canti (2003), however, said that the majority of plant species contain druses and prismatic crystals of very similar shape, their morphologies are generally too close to allow the identification at the species level; this is why silicophytoliths and cystoliths are used to identify plant remains in archeological, paleoenvironmental or paleobotanical investigations at the species level. Thus, detailed studies of these crystals and their macropatterns would help find features not considered up to now, such as those described for druses in this paper. These might allow a closer identification of plant remains at the level of genus or even species. Advances in this aspect would be important not only in the archaeological studies but also in quality control of food and

medicinal samples that include plant parts. Micrographic characters traditionally used are not always easy to see, or they do not lead to such identification.

The observations here described constitute the first report of macropatterns in the subfamily Chenopodioideae, and it was demonstrated that the characterization of crystals and their macropatterns are useful for the identification of these species, which implies their taxonomic value. The present study establishes a basis of comparison for future research on species belonging to other genera in the Amaranthaceae, a family which shows some taxonomic conflicts among its members. This type of investigation should contribute to their solution. The integrated studies of crystals, based on interdisciplinary research (chemical, geological, botanical, physiological) can promote a better crystal characterization for systematic, taxonomic, ecological and other applied approaches.

Acknowledgments

References

Baran, E., Monje, P.V., 2008. Oxalate biominerals. Metal Ions in Life Sciences 4, 219–254.

Bertoldi de Pomar, H., 1975. Los silicofitolitos: sinopsis de su conocimiento. Darwiniana 19, 173–206.

Bonorino, A.G., 1970. Contribución al conocimiento de las aguas subterráneas de la zona de Médanos, partido de Villarino (Provincia de Buenos Aires). In: Reunión sobre la Geología de las Sierras Australes Bonaerenses. Actas. Comisión de Investigaciones Científicas, La Plata, pp. 177–191.

Borrelli, N., Fernández Honaine, M., Altamirano, S.M., Osterrieth, M., 2011. Calcium and silica biomineralization in leaves of eleven aquatic species of the Pampean Plain, Argentina. Aquatic Botany 94, 29–36.

Cabrera, A.L., 1971. Fitogeografía de la República Argentina. Boletin de la Sociedad argentina de botanica 14, 1–48.

Canti, M.G., 2003. Aspects of the chemical and microscopic characteristics of plant ashes found in archeological soils. Catena 54, 339–361.

Cutler, H.C., 1968. Origins of agriculture in the Americas. Latin American Research Review 4, 3–21.

Dizeo de Strittmatter, C.G., 1973. Nueva técnica de diafanización. Boletin de la Sociedad argentina de botanica 15, 126–129.

Ellis, R.P., 1979. A procedure for standardizing comparative leaf anatomy in the Poaceae. II. The epidermis as seen in surface view. Bothalia 12, 641–671.

Franceschi, V.R., Horner, H.T., 1980. Calcium oxalate crystals in plants. Botanical Reviews 46, 361–427.

Franceschi, V.R., Nakata, P.A., 2005. Calcium oxalate in plants: formation and function. Annual Review of Plant Biology 56, 41–71.

Hartl, W.P., Klapper, H., Barbier, B., Ensikat, H.J., Dronskowski, R., Müller, P., Ostendorp, G., Tye, A., Bauer, R., Barthlott, W., 2007. Diversity of calcium oxalate crystals in Cactaceae. Canadian Journal of Botany 85, 501–517.

Horner, H.T., Wanke, S., Samain, M.S., 2009. Evolution and systematic value of leaf crystal macro patterns in the genus *Peperomia* (Piperaceae). International Journal of Plant Sciences 170, 343–354.

Lersten, N.R., Horner, H.T., 2000. Calcium oxalate crystal types and trends in their distribution patterns in leaves of *Prunus* (Rosaceae: Prunoideae). Plant Systematics and Evolution 224, 83–96.

Lersten, N.R., Horner, H.T., 2005a. Development of the calcium oxalate crystal macro pattern in pomegranate (*Punica granatum*, Punicaceae). American Journal of Botany 92, 1935–1941.

Lersten, N.R., Horner, H.T., 2005b. Macro pattern of styloid and druse crystals in Quillaja (Quillajaceae) bark and leaves. International Journal of Plant Sciences 166, 705–711.

Lersten, N.R., Horner, H.T., 2006. Crystal macro pattern development in *Prunus serotina* (Rosaceae, Prunoideae) leaves. Annales Botanici 97, 723–729.

Lersten, N.R., Horner, H.T., 2008a. Crystal macro patterns in leaves of Fagaceae and Nothofagaceae: a comparative study. Plant Systematics and Evolution 271, 239–253.

Lersten, N.R., Horner, H.T., 2008b. Subepidermal idioblasts and crystal macro pattern in leaves of *Ticodendron* (Ticodendraceae). Plant Systematics and Evolution 276, 255–260.

Lersten, N.R., Horner, H.T., 2009. Crystal diversity and macro patterns in leaves of Oleaceae. Plant Systematics and Evolution 282, 87–102.

Lersten, N.R., Horner, H.T., 2011. Unique calcium oxalate "duplex" and "concretion" idioblasts in leaves of tribe Naucleeae (Rubiaceae). American Journal of Botany 98, 1–11.

Madella, M., Alexandre, A., Ball, T., 2005. International code for phytolith nomenclature 1.0. Annales Botanici 96, 253–260.

Mc Connell, K., 1998. The prehistoric use of Chenopodiaceae in Australia: evidence from Carpenter's Gap shelter 1 in the Kimberley, Australia. Vegetation History and Archaeobotany 7, 179–188.

Metcalfe, C.R., Chalk, L., 1957. Anatomy of the Dicotyledons; Leaves, Stem and Wood in Relation to Taxonomy with Notes on Economic Uses. Clarendon Press, Oxford.

Molano Flores, B., 2001. Herbivory and calcium concentrations affect calcium oxalate crystal formation in leaves of *Sida* (Malvaceae). Annales Botanici 88, 387–391.

Nakata, P.A., 2003. Advances in our understanding of calcium oxalate crystal formation and function in plants. Plant Science 164, 901–909.

Prychid, C.J., Rudall, P.J., 1999. Calcium oxalate crystals in Monocotyledons: a review of their structure and systematics. Annales Botanici 84, 725–739.

Verrechia, E.P., Dumont, J.L., Verrechia, K.E., 1993. Role of calcium oxalate: biomineralization by fungi in the formation of calcretes: a case study from Nazareth, Israel. Journal of Sedimentary Petrology 63, 1000–1006.