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Original Research

Calcium oxalate crystals in halo-xerophytic species and their macropatterns trends. The importance of a multivariate analysis considering soil characteristics

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ABSTRACT

The morphological characteristics of calciphytoliths and description of their macro and micropattern in leaves of halo-xerophytic species of the Salitral de la Vidriera, Argentina, were carried out. Additionally, a multivariate analysis was conducted to identify any relation between the form and distribution of calcium oxalate crystals and soil characteristics. Druses and polyhedral crystals were found. Among the species with druses five macropatterns were described while in the species with polyhedral crystals, only two. Regarding micropattern, crystals were distributed in different cells (chlorenchyma, idioblasts, bundle sheats, aqueous tissue). Species with druses were found in areas with different salinity and concentration of calcium while the polyhedral crystals were presented only in species inhabiting soils of lower salinity and ower concentration of calcium. The species inhabiting the area of lower salinity and concentration of calcium, had uniform distribution of crystals in their leaves; under other soil conditions the species presented variations in acropetal o centripetal direction.

1. Introduction

Bioliths are mineralized bodies found in different tissues of living organisms. Those occurring in plants are called phytoliths (Bertoldi de Pomar, 1975). The most abundant minerals found in plants are silica, calcium carbonate and calcium oxalate. Calcium comprises about 50% of known biominerals (Weiner and Dove, 2003) and these specific types of phytoliths are called calciphytoliths (Bertoldi de Pomar, 1975).

Calciphytoliths have been observed in different soils and rocks, and among multiple members of all of the five kingdoms (Monera, Protista, Fungi, Plantae, and Animalia). In plants, the common occurrence of inorganic calcium oxalate crystals in a wide variety of species has fascinated plant biologists for centuries (Bertoldi de Pomar, 1975; Franceschi and Horner, 1980; Genua and Hillson, 1985; Nakata, 2003; Zindler-Franck et al., 1988). More than 215 plant families posses these crystals, which have been observed in all organs, tissues and cell types, but their documentation has been described best for the Dicotiledoneae and, only for some Monocotiledoneae, like Araceae (Genua and Hillson, 1985; Sakai and Hanson, 1974). In all instances, the crystals are formed from environmentally derived calcium and from biologically synthesized oxalate, carbonate, sulfate, phosphate, silicate, citrate, tartrate and malate (Baran and Monje, 2008; He et al., 2012; Nakata, 2003; Prychid and Rudall, 1999). Calcium is an essential plant nutrient, which performs many fundamental functions in cellular metabolism: calcium can maintain cell wall strength and membrane integrity, regulates membrane permeability and ion transport and adjusts the metabolism as a cytoplasmic secondary messenger (Baran and Monje, 2008; Ci et al., 2010; Franceschi and Horner, 1980), and also forms part of crystals. It is evident that calcium fulfills different and varied functions in plants, so that the formation of calcium crystals can be the result of different cellular processes as well as a form of immobilization when the plant has an excess of this element (Baran and Monje, 2008; Brown et al., 2013; Choi et al., 2001; Franceschi and Horner, 1980; Lersten and Horner, 2005a; Mazen, 2004; Molano Flores, 2001; Nakata, 2003).

The commonly morphological types of calcium oxalate crystals encountered 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), polyhedral crystals and crystal sand (a mass of many tiny, individual crystals in a single cell). The first ones are cluster crystals while the rest are individual crystals which can appear solitary or grouped. One or more of these five types are found in most

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Angiosperm families (Franceschi and Horner, 1980; Franceschi and Nakata, 2005). Special combinations have been reported, like those called concretions in *Nauclea* L., which are druses embedded in crystal sand (Lersten and Horner, 2011) and druses found in some Chenopodiaceae formed by individual crystals with distinct characteristics (Pérez Cuadra and Hermann, 2013; Pérez Cuadra, 2012).

Calcium oxalate crystal distribution and accumulation within the plant are highly variable among species and throughout the growing season making them a significant anatomical component of an organ (He et al., 2012; Lersten and Horner, 2011). These reasons explain the importance of the macropattern descriptions, which are the gross location of crystals, either around vascular bundles, in the mesophyll, or in both regions, without considering specific cells or cell layers in which the crystals occur (Lersten and Horner, 2000, 2005a). Crystal localization in specific plant tissues requires more detailed anatomical analysis corresponds to the description of a micropattern (Lersten and Horner, 2000). Macro and micropatterns form part of important lines of research that allow for identifying some characteristics that result in a useful taxonomic tool for many families like Fagaceae, Nothofagaceae, Oleaceae, Punicaceae, Quillajaceae, Rosaceae (Prunoideae) and Ticodendraceae (Brown et al., 2013; Lersten and Horner, 2000, 2005a, 2005b, 2006, 2008a, 2008b, 2009).

Concentrations of calcium oxalate crystals vary among plants of different growth habit. For example, Ci et al. (2010) reported that shrubs and semi-shrubs had more calcium crystals in tissues than perennial herbs. Additional researches provided information about the concentrations of the crystals in different plant species living under the same ecological conditions or in specific stages in plant succession. In twelve different species from dissimilar successional stages, it was found that plants in their terminal stage had more calcium crystals in their tissues than those in early stages (Ci et al., 2010; Lerstern and Horner, 2004). Studies exploring the relationship between calcium oxalate crystal accumulation and rainfall are scarse; some are part of a series of wide-ranging dendrological studies, which have focused predominantly on the relationship between precipitation and wood anatomy. Other studies conducted on *Acacia* spp. determined that crystal morphology in these species was not influenced by rainfall. There were no differences found in crystal shape between species occupying different climate zones, but environmental and edaphic factors (influenced by water availability, and calcium concentration) played an important role on crystal formation (Brown et al., 2013).

The objective of this study is to describe morphological characteristics of calciphytoliths and their macro and micropatterns in xero-halophytic species to find out a posible relation between the form and distribution of calcium oxalate crystals and soil characteristics (specifically salinity and calcium concentration) in their natural habitats.

2. Material and methods

2.1. Study area

The study area, Salitral de la Vidriera and surrounding zone, is located between 38° 46′ and 38° 52′ of south latitude and 62° 34′ and 62° 20′ of west longitude. It is located approximately 30 km away from Bahia Blanca City, in the Partido of Villarino, Buenos Aires Province, and corresponds to the Espinal Phytogeographic Province within the Caldén District (Cabrera, 1971).

The climate in the area is generally mild and dry. The annual rainfall commonly varies between 400 and 500 mm, characteristic of a dry environment. The vegetation is distributed in patches that are represented by associations with xerophytes and halophytes of a definite floristic composition (Benedetti et al., 2010).

2.2. Sampling of plant species

Sampling of plant species was conducted in three floristic units. Within each unit, sampling of plant species was carried out in transects of 10 m, drawn from an access road used as a reference. Mature leaves, taken at random, of eight species belonging to seven botanical families were collected:

- Unit 1: *Sesuvium portulacastrum* (L.) L. (Aisoaceae), *Atriplex suberecta* I Verd. (Chenopodiaceae), *Cressa truxillensis* Kunth (Convolvulaceae), *Geoffroea decorticans* (Gillies ex Hook. & Arn.) Burkart (Fabaceae), *Prosopis strombulifera* (Lam.) Benth. (Fabaceae), *Spharealcea australis* Speg. (Malvaceae).
- Unit 2: *Schinus* L. sp. (Anacardiaceae), *Grahamia bracteata* Hook. & Arn. (Portulacaceae).
- Unit 3: *Atriplex suberecta* I Verd. (Chenopodiaceae).

The collection of the material was conducted over a period of four years in different seasons (fall, spring and summer). The reference specimens were deposited in the Herbarium BBB. Species scientific names and their classification followed Zuloaga et al. (2008).

Leaves of each species were fixed and preserved in FAA made of: 5%formalin; 5% glacial acetic acid and 90% ethanol (50%) (Jensen, 1962). To study the crystals and macropatterns, complete leaves were cleared (Dizeo de Strittmatter, 1973), mounted on a slide and observed in acropetal and centripetal directions. Safranin was used as contrast stain and the samples were mounted in glycerin gelatin.

To study the micropatterns, samples were dehydrated in an increasing concentration series of ethanol and tertiary butyl alcohol, infiltrated in paraffin and included in a compound of purified paraffin and plastic polymers (Johansen, 1940). Transverse sections of 10 μm thick were cut with a rotary microtome, stained with safranin-fast green and mounted in Canadian balsam.

In order to corroborate the calcium oxalate composition of the crystals, polarized light was used. The types of these crystals were named following the traditional nomenclature (Franceschi and Horner, 1980) except for those called prisms in it. In strict geometric terms the prisms are only a polyhedron with a polygonal base of "n" sides being outside of this nomenclature those that have irregular bases, so we prefere to use the nomenclature polyhedron instead of prism.

Five to ten samples per species were analyzed. The samples were observed with a Zeiss AxioLab (origin Mexico) compound microscope using bright-field and polarized optics, and observations were recorded with a Zeiss Axiocam.

2.3. Sampling of soils

Soil sampling was performed in the same three floristic units in which the plant species were sampled. For the sampling it was taken into account that at least seven days before had no precipitation in the zone, because this affects the distribution of salts in the soil with its consequent effect in the analysis. Samples were taken in spring, summer and autumn, obtaining between three and six replicates each time. These replicates were taken at random in the topsoil (0–40 cm) of each floristic unit, on the same transects plotted for the sampling of plant species. With each sample all remaining plant matter was remowed and packed in properly labeled plastic bags. These samples were sent to the INTA Hilario Ascasubi Experimental Station. The salinity was evaluated using an electrical conductivity meter and the concentration of calcium and magnesium was calculated by complexometry and flame photometry.

Table 1
Summary of the characteristics of the species included for the multivariate analysis (according to Pérez Cuadra and Hermann, 2013).

Species	Crystal type	Macropattern	Habitat
Atriplex undulata	Druses and polyhedral crystals	Areoles, the number increases toward de apex and toward the center of the leaf	Unit 2 and 3
Nitrophila australis	Druses	Areoles, the number decreases toward de apex and does not vary in any other direction of the leaf	Out of the units, spot samplimg
Suaeda divaricata	Druses	Areoles near the veins, the number decreases toward the apex and does not vary in any other direction of the leaf	Out of the units, spot samplimg

Table 2
Variables considered for the multivariate analysis with their binary statements.

Variables	0	1
Druses	absent	present
Polyhedral crystals	absent	present
Areoles	no	yes
On the veins	near	on
Number increases toward the apex	no	yes
Number decreases toward the apex	no	yes
Number increases toward the center	no	yes
Number decreases toward the apex	no	yes
Unit 1	absent	present
Unit 2	absent	present
Unit 3	absent	present

2.4. Multivariate analysis

For cluster and principal components analysis, in addition to the data for the eight species studied here, three species belonging to the Chenopodiaceae family (*Atriplex undulata* (Moq.) D. Dietr., *Nitrophila australis* Chodat & Wilczek and *Suaeda divaricata* Moq.) were included. They grow in the same area, and their characteristics of crystals, macropatterns and micropatterns were previously published (Pérez Cuadra and Hermann, 2013) (Table 1).

With the 11 species a matrix representing binary states of different variables (Table 2) was constructed. Under the variable "Unit" features of soil salinity (electrical conductivity) and concentration of the complex absorbing calcium/magnesium, were included. For the cluster analysis, the association index Simple Matching (by a default of the statistical program this index was transform in a distance one) and average linkages were used, and a covariance matrix for the principal components one. Both analyses were performed with the statistical program Infostat version 2011 (Di Rienzo et al., 2011).

3. Results

3.1. Characterization of crystals, description of macropatterns and micropatterns

In the eleven species only two types of calciphytholiths were found: druses and polyhedral crystals (Fig. 1A–H, L, O). In *Atriplex suberecta* (Fig. 1A), *Cressa truxilliensis* (Fig. 1B), *Grahamia bracteata* (Fig. 1C), *Schinus* sp., *Sesuvium portulacastrum* (Fig. 1D) and *Sphaeralcea australis*, druses were found, formed by polyhedral crystals, some with sharp ends (Fig. 1A, D, L, O). *Geoffroea decorticans* and *Prosopis strombulifera* displayed polyhedral crystals of various forms: rhombohedral (Fig. 1E), bipyramids (Fig. 1F), truncated bipyramids (Fig. 1G), rectangular and irregular polyhedra (Fig. 1H).

Among the species with druses five macropatterns were found, while in the species with polyhedral crystals, only two were found. Pattern I, characteristic of *G. bracteata*, corresponded to the presence of druses only in the areoles. Pattern II, described for *C. truxilliensis* and *S. portulacastrum*, showed the greatest number of druses in the areoles although some were found close to the minor veins (Fig. 1I). In Pattern III, characteristic of *A. suberecta,* druses were found at the base of the blade on both sides of the midvein, whereas in the rest of the leaf these were located in areoles (Fig. 1J). Pattern IV was characterized by the distribution of crystals mainly on or near the veins and in the areoles, showing two subtypes. In Pattern IVa, observed in *Schinus* sp., druses were arranged in rows (of two to eight crystals) primarily at both sides of the mainvein, secondary and tertiary veins, and in the areoles (Fig. 1K). Pattern IVb, described for *G. decorticans*, showed polyhedral crystals located on major veins (mainly at the margins) and in areoles (Fig. 1M). Pattern V was characterized by the distribution of the crystals only on or near the veins, showing two subtypes. In Pattern Va, observed in *S. australis,* druses were arranged on the minor veins (Fig. 1N) and only some on the midvein and major veins. In the Pattern Vb, characteristic of *P. strombulifera,* some crystals were distributed on the major veins and dense groups of crystals at the ends of the smaller veins (Fig. 1P). Some crystals were observed on the major veins in small groups (two to twelve crystals) arranged in irregular rows.

In *A. suberecta*, *Schinus* sp., *S. portulacastrum* and *S. australis*, druses were located in greater numbers in the leaf base, decreasing in acropetal direction, until the apex, with no crystals. Conversely, in *C. truxilliensis* the number of crystals increased from the base to the apex. In *A. suberecta*, *C. truxilliensis*, *Schinus* sp. and *S. australis*, the largest number of druses was observed in the area near the midvein and, crystals were absent at the margins of the leaf. In *S. portulacastrum* no variation was observed in centripetally direction. In *G. decorticans*, *G. bracteata* and *P. strombulifera* there were no changes in any direction.

Regarding micropattern, the distribution of crystals in *Schinus* sp. showed that druses were located in cells of the palisade and spongy chlorenchyma, whereas in *C. truxilliensis* they were present only in the spongy tissue. In *A. suberecta* the crystals were in idioblasts in the vicinity of the vascular bundles and scattered in the mesophyll. In *G. decorticans*, *P. strombulifera* and *S. australis* the crystals were found in the cells of the bundle sheaths and, in the first species they were also dispersed in mesophyll. In *G. bracteata* and *S. portulacastrum*, druses were in cells of the aqueous tissue (parenchyma that stores water), where the vascular bundles are immersed.

3.2. Soil characterization

The average result of the soil variables studied in each unit were:

- Unit 1: electrical conductivity of 11 dS m^{-1} and concentration of Ca + Mg, 33.3 mEq L^{-1}
- Unit 2: electrical conductivity of 47 dS m^{-1} and the concentration of Ca + Mg, 146.8 mEq L^{-1}
- Unit 3: electrical conductivity of 33 dS m^{-1} and the concentration of Ca + Mg, 107.7 mEq L^{-1}

The values of electrical conductivity found in all cases exceeded 2 dS m^{-1} (considered limit value for agricultural crop) but in any case the 50–55 dS m^{-1}, electrical conductivity of sea water (Ward, 2009). These values reflect the condition of strongly saline (Unit 1) and extremely saline soils (Units 2–3). This high electrical conduc-

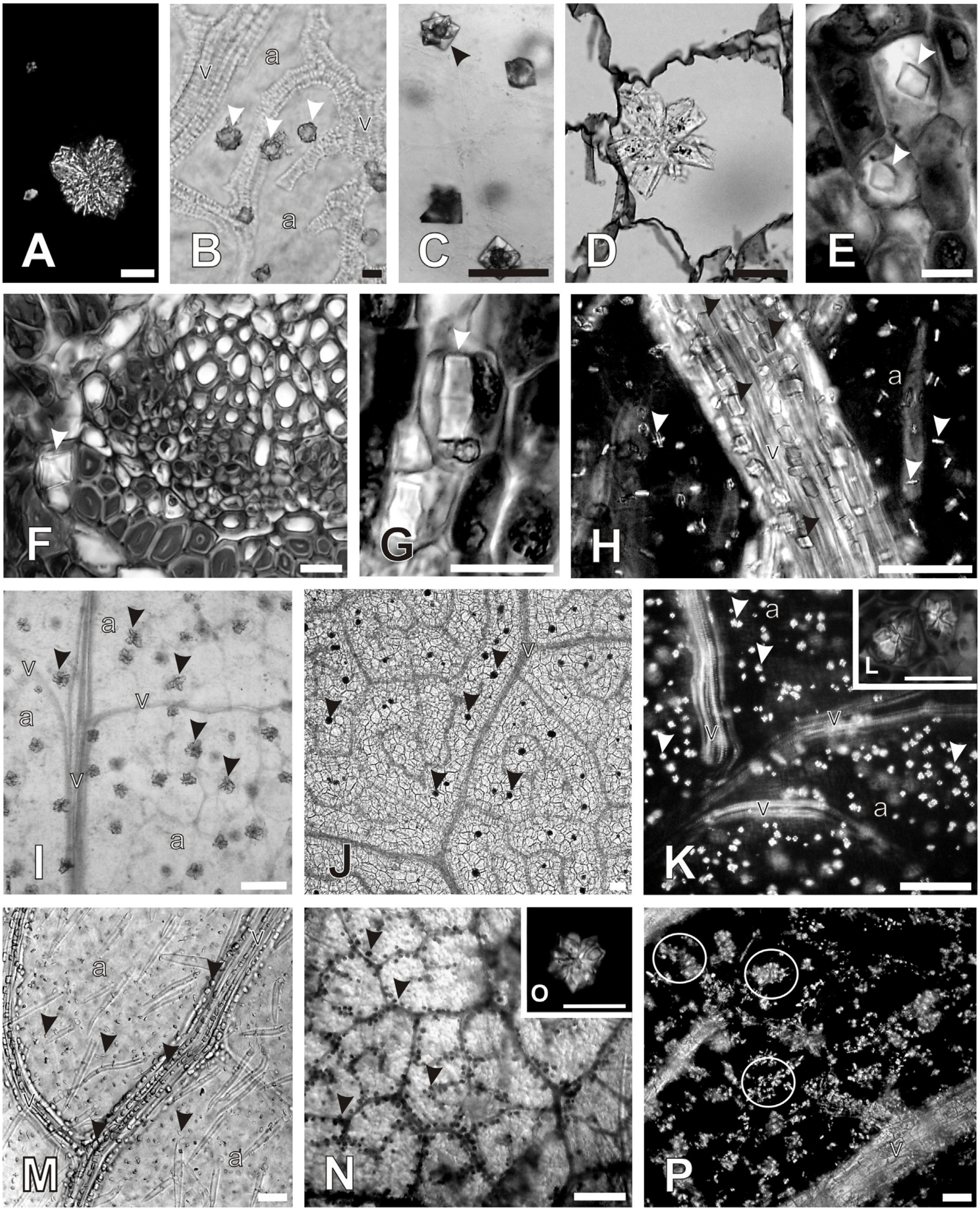

Fig. 1. Crystals types and macropatterns. A-D, L, O, Druses. E, Rhombohedral crystals in lateral view. F, Bipyramids in lateral view. G, Truncated bipyramids in lateral view. H, Rectangular and irregular polyhedral crystals. I, Pattern II. J, Pattern III. K, Pattern IVa. M, Pattern IVb. N, Pattern Va. P, Pattern Vb. A, J, *A. suberecta*. B, *C. truxilliensis*. C, *G. bracteata*. D, I, *S. portulacastrum*. E, G, P, *P. strombulifera*. F, H, M, *G. decorticans*. K-L, *Schinus* sp. N-O, *S. australis*. A, H, K-L, O-P, polarized light. B-G, I-J, M-N, normal light. Arrow heads indicate calcium oxalate crystals; Circles indicate dense groups of crystals at the ends of the smaller veins. Abbreviations: a, areoles; v, veins. Bars: A-G, L, O, 15 μm; H-K, M, N, P, 80 μm.

tivity in Units 2 and 3 is reflected in the presence of a salty layer on the soil surface.

3.3. Multivariate analysis in relation to crystals and soil characteristics

For the interpretation of multivariate analysis of the eleven variables used, three ("Areoles", "Number decreases toward the center" and "Unit 3") were not taken into account because they were poorly reconstructed by the analysis (Table 3).

Geoffroea decorticans and *Prosopis strombulifera*, in addition to *Nitrophila australis* and *Sesuvium portulacastrum*, were the species forming the two groups with major association index (Fig. 2). In the first one, species belonging to the same botanical family, had a similar and unique type of crystal (polyhedra) with no variation in density through the leave (mainly on the veins), plus both occurred

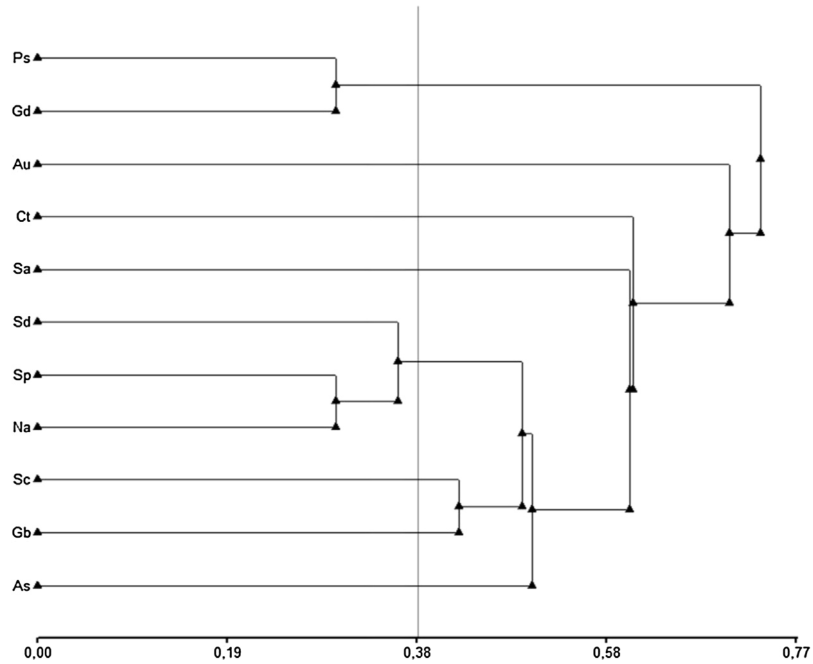

Fig. 2. Multivariate analysis of calciphytoliths, macropattern and soil characteristics. Cluster analysis (Cofenetic correlation 0.865). Gd and Ps had only polyhedra with no variation in density through the leaf (mainly on the nerves), plus both ocurred in Unit 1 (11 dS m^{-1} of electrical conductivity of and 33.3 mEq L^{-1} of concentration of Ca + Mg). Na and Sp had druses that decreased in number from the base toward the apex of the leaves as same as Sd. Gb and Sc had druses and ocurred in Unit 2 (electrical conductivity of 47 dS m^{-1} and the concentration of Ca + Mg, 146.8 mEq L^{-1}), being the character crystals linked to the previous group. The remaining species were grouped as outside the norm due to their greater variability in their characteristics. Abbreviations: As, *Atriplex suberecta*; Au, *Atriplex undulata*, Ct, *Cressa truxilliensis*, Gb, *Grahamia bracteata*; Gd, *Geoffroea decorticans*; Na, *Nitrophila australis*; Ps, *Prosopis strombulifera*; Sa, *Suaeda argentinensis*; Sc, *Schinus* sp.; Sd, *Suaeda divaricata*; Sp, *Sesuvium postulacastrum*.

Table 3
Percentage of variables reconstruction.

Variables	PC1-PC2	PC1-PC3	PC2-PC3
Druses	0.8273	0.8144	0.0929
Polyhedral crystals	0.797	0.365	0.4658
Areoles	0.4001	0.2977	0.2176
On the veins	0.774404	0.8228	0.048404
Number increases toward the apex	0.5713	0.1898	0.6553
Number decreases toward the apex	0.8242	0.2042	0.6922
Number increases toward the center	0.3298	0.8138	0.7018
Number decreases toward the apex	4.0009E – 06	0.0016	0.001604
Unit 1	0.5938	0.7693	0.1773
Unit 2	0.5305	0.3757	0.386
Unit 3	0.4426	0.2554	0.293

in Unit 1 (Fig. 3A and B). In the second, the species had druses distributed in numbers that decreased toward the apex (Fig. 3A); *Suaeda divaricata* sharing similar characteristics was included in this group. The group of *Grahamia bracteata* and *Schinus* sp. was defined because these species had druses and occurred in Unit 2 (Fig. 3A), being the character crystals linked to the previous group. The remaining species were grouped as outside the norm due to their greater variability in their characteristics.

Variables involving crystal form (druses and polyhedral crystals) determined the formation of the first groups (Figs. 2 and 3A and B) while the distribution of the crystals (under the veins) (Figs. 2 and 3A and B), only resulted a determining factor in the definition of the group of *G. decorticans* and *P. strombulifera*. Although *Sphaeralcea australis* had druses on their veins, like the members of the former group (Fig. 3A and B), it was grouped more closely with the other species because it shares most features in common with them (Fig. 2). *Atriplex undulata* presented druses and polyhedral crystals (Figs. 2 and 3A), and was grouped with the other species with druses (Fig. 2), but did not generate a greater relationship with the group of species with polyhedral crystals (Fig. 2) because it differed from them in other characteristics (for example: macropattern).

Species with druses were found in areas of high salinity and concentration of calcium (Unit 2: *G. bracteata* and *Schinus* sp.) as well as in others with lower salinity and calcium concentration (Unit 1: *S. portulacastrum* and *S. australis*). In contrast, the polyhedral crystals and the macropattern "On the veins" were presented only in species inhabiting the area of the lower values of salinity and calcium concentration (Unit 1: *G. decorticans* and *P. strombulifera*).

The species inhabiting the area of lower salinity and concentration of calcium had no variation in the amount of crystals in any direction; under other soil conditions the species presented variations in acropetal or centripetal direction.

4. Discussion

One of the more constant cellular contents of plants are the calcium oxalate crystals (Ancibor, 1992; Franceschi and Horner, 1980; Horner et al., 2009; Lersten and Horner, 2000, 2005a, 2006, 2008a, 2008b, 2009). Among the species studied in the present research, druses are the most common type of calcium oxalate crystals while polyhedral crystals (rhombohedral, bipyramids, truncated bipyramids, rectangular and irregular polyhedral) were found to a lesser extent in agreement with previous studies (García et al., 2008; Horner et al., 2009; Lersten and Horner, 2000, 2005a, 2006, 2008a, 2008b, 2009). Particularly in 12 *Acacia* species (Fabaceae) of an aridity region of Australia, the crystals found in greater amount were polyhedral ones (Brown et al., 2013); the only two species of the Salitral de la Vidriera that had polyhedral crystals were of the same family of the Australian *Acacia* species, one of the same subfamily too (*Prosopis strombulifera*, Mimosoideae) and other from a different one (*Geoffroea decorticans*, Papilionoideae). Furthermore, it should be noted that the diversity of crystal shapes found in the botanical families studied here is comparatively lower than reported for other families and subfamilies (Fagaceae, Nothofa-

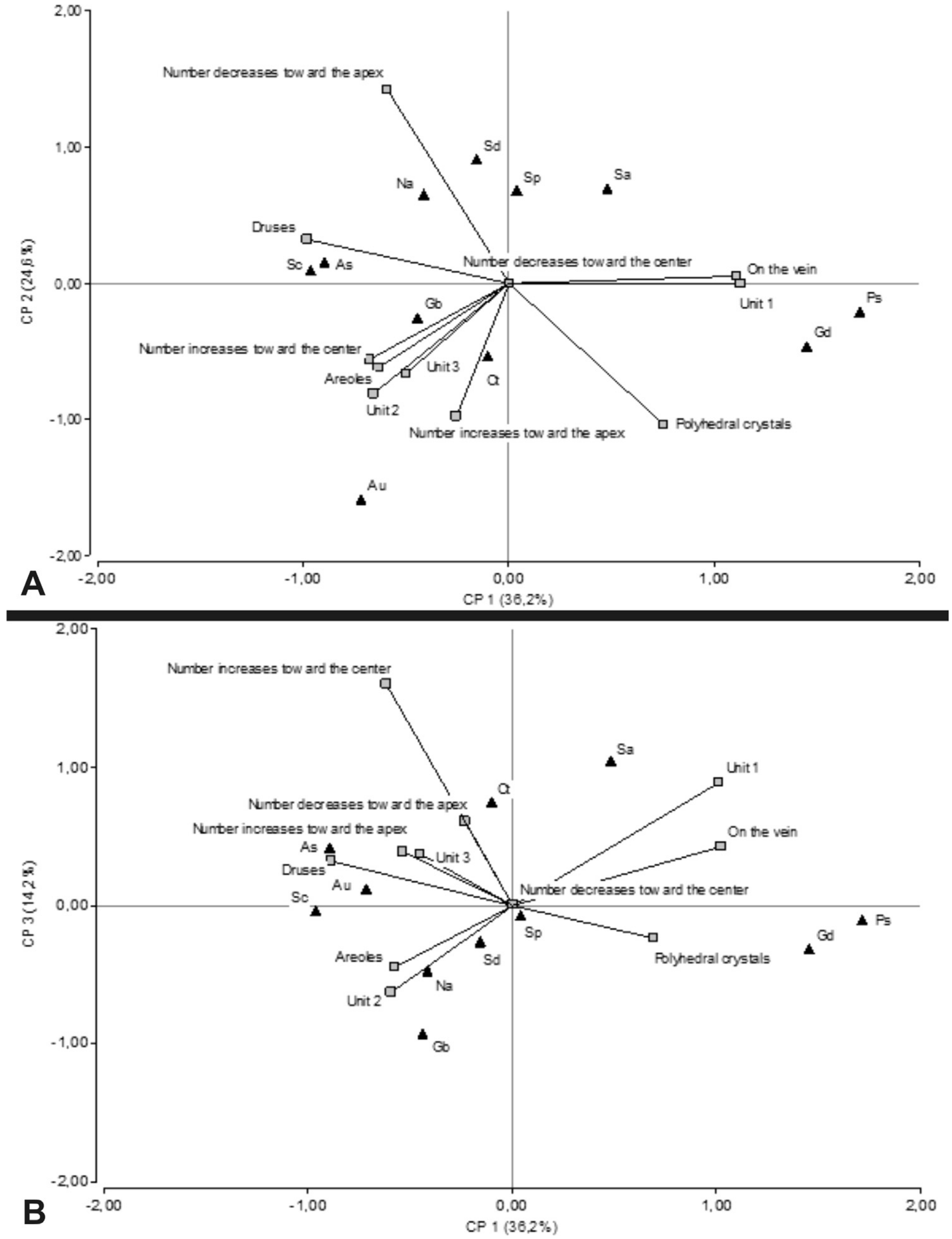

Fig. 3. Multivariate analysis of calciphytoliths, macropattern and soil characteristics. A-B, Principal component analysis (Cofenetic correlation 0.908). Variables involving crystal form (druses and polyhedral crystals) determined the formation of the first groups (Gd-Ps and Na-Sp); the distribution of the crystals "On the veins" only resulted a determining factor in the definition of the group Gd-Ps. Species with druses were found in areas of high salinity and concentration of calcium (Unit 2 electrical conductivity of 47 $dS\,m^{-1}$ and the concentration of Ca + Mg, 146.8 $mEq\,L^{-1}$: Gb and Sc) as well as in others with lower salinity and calcium concentration (Unit 1 11 $dS\,m^{-1}$ of electrical conductivity of and 33.3 $mEq\,L^{-1}$ of concentration of Ca + Mg: Sp and Sa). In contrast the polyhedral crystals and the macropattern "On the veins" were presented only in species inhabiting the area of the lower values of salinity and calcium concentration (Unit 1 11 $dS\,m^{-1}$ of electrical conductivity of and 33.3 $mEq\,L^{-1}$ of concentration of Ca + Mg: Gd and Ps). Abbreviations: As, *Atriplex suberecta*; Au, *Atriplex undulata*, Ct, *Cressa truxilliensis*, Gb, *Grahamia bracteata*; Gd, *Geoffroea decorticans*; Na, *Nitrophila australis*; Ps, *Prosopis strombulifera*; Sa, *Suaeda argentinensis*; Sc, *Schinus* sp.; Sd, *Suaeda divaricata*; Sp, *Sesuvium postulacastrum*.

gaceae, Oleaceae and Prunoideae) (Lersten and Horner, 2000, 2006, 2008a, 2009).

In leaf macropatterns of calcium oxalate crystals described in previous studies, generally these crystals were found both associated with major or minor veins or scattered in the areoles (Horner et al., 2012; Lersten and Horner, 2000, 2005a, 2006, 2008a, 2008b, 2009; Zindler-Frank, 1995). These general distribution patterns of crystals match those found in the species studied here. Only three of the species of the Salitral de la Vidriera showed the crystals associated with vascular bundles (in two cases were polyhedral crystals and in one, druses), whereas in studies of Australian species of *Acacia* of dry environments, the crystals were found mainly in close association with vascular bundles (Brown et al., 2013). The distribution of calcium oxalate crystals has a much closer relationship with phylogenetic aspects than with environmental variables, demonstrated in the variability of macropatterns detected in species of seven different botanical families growing in the same environment (Salitral de la Vidriera). That is why the adaptative significance of most aspects of the crystals macropatterns is difficult to interpret, as already mentioned for *Prunus* L. spp. (Lerstern and Horner, 2004).

In relating to the information extracted from macro and micropatterns, it is important to advertise that in the species in which the crystals are observed on the veins in macropatterns, these are always located in the cells of the bundles sheath. In the species in which the crystals were found in areoles or in the vicinity of the vascular bundles in macropatterns, the micropatterns become very important since they define in which tissue the crystals are found (aqueous tissue, chlorenchyma palide or spongy or both).

The extension of studies in different genera and species of diverse botanical families will help to use crystals macropatterns in leaves as a tool of phylogenetic importance (Horner et al., 2009). As studies of calciphytoliths advance in different families it will be possible to include this information in more phylogenetic analyses that contribute to a greater understanding of the relationships between organisms. The studies of characterization of calciphytoliths further suggest that crystals in plants are under genetic control due to the accuracy of their forms and specific localizations in a particular taxon (Lersten and Horner, 2011). Although the underlying factors controlling the type of crystal formed in any plant are still unknown, most certainly must be a combination of genetics, environmental conditions, and/or nutrient availability (Nakata and McConn, 2007).

To date, all studies performed involving analysis of macropatterns in specific taxa (Oleaceae, Fagaceae, Nothofagaceae, Prunoideae, etc.) had detected crystal homogeneity in morphology and distribution in each taxonomic group with slight variations. In this study, although the selected species taxonomically belonged to different families all were related by living in a common environment. The principal component analysis showed that the type of crystal (druse vs. polyhedral crystal) determined the separation of two groups, one including *Geoffroea decorticans* and *Prosopis strombulifera* (species that had one of the highest degrees of similarity) and the other consisting of the remaining nine species (a heterogeneous group). The case of *G. decorticans* and *P. strombulifera* is particularly important because these species belong taxonomically to the same botanical family, Fabaceae, and shared many characteristics that generated a well defined group. In another case four species belonging to the same family (*Atriplex undulata*, *A. suberecta*, *Nitrophila australis* and *Suaeda divaricata*, Chenopodiaceae) do not form a well defined group because of their differences in the crystals types. Moreover, two species (*A. undulata* and *A. suberecta*) even belonging to the same genus were not grouped closely. The taxonomic value of calciphytoliths is useful in families that have a limited variation in the diversity of crystal forms and such diversity is shared by all or most of the species of the taxonomic group. However, when members of a botanical family have varied morphologies not shared by all of its members, the variability affects crystal taxonomic utility, or at least more caution in the statements generated by these studies is required. Probably the taxonomic groups that present members with a great adaptive radiation (and thus possessing very diverse adaptive functional traits), are not the best examples for taxonomic use of calciphytoliths.

Investigations of Brown et al. (2013) in species of *Acacia* genus inhabiting an arid gradient in Australia determined that there were no consistent changes in the morphology of the crystals between species of different climatic zones. In the Salitral de la Vidriera, an area with no variations in climatic conditions but with strong differences in soil conditions (mainly related to the salinity), it was found that species with druses inhabited in regions of higher and lower values of salinity and calcium concentration respectively; whereas species with polyhedral crystals are only present in areas with low salinity and calcium concentration. Other studies on crystal formation under experimental conditions (Zindler-Frank et al., 1988; Zindler-Frank, 1991, 1995) concluded that the presence of calcium oxalate crystals depends on calcium supply, this means that at high or low concentrations of the ion in the nutrient solution, the formation of crystals differs in shape, abundance and location. In *Atriplex suberecta*, the only species studied in this work that grew under different calcium concentrations in a natural environment, showed no differences in shape or crystal location in different individuals grew in different units. Probably this means that plants respond differently under controlled experimental conditions than in natural conditions, which undergo environmental stress that are part of the conditions found and/or resources of their habitat.

The characterization of calcium oxalate crystals, the macropatterns and micropatterns described here constitute the first record for species included in this work, being also the first of its kind developed in a xero-halophytic community.

5. Conclusions

The descriptive studies on calcium oxalate crystals taking into account the natural distribution of the species according to soil characteristics, are scarce. In the leaves of the eight studied species, druses and polyhedral crystals, of various forms, were found and five distribution patterns were described. Species with similar macropatterns showed differences in their micropatterns. The species that presented druses were found to grow in soils with high or low salinity and concentration of calcium while those that presented polyhedral crystals were found in soils with low salinity and low concentration of calcium.

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