Received: 18 July 2023 | Revised: 20 August 2023 | Accepted: 22 August 2023

DOI: 10.1111/ppl.14017

ORIGINAL RESEARCH

Physiologia Plantarum

Calcium sulphate biomineralisation: Artefact of sample preparation?

Andreu Cera[1,2] | Cristóbal Verdugo-Escamilla[3] | Juan A. Marín[4] | Sara Palacio[2]

[1]Centro de Ecologia Aplicada Prof. Baeta Neves (CEABN-InBIO), Instituto Superior de Agronomia, Universidade de Lisboa, Lisbon, Portugal

[2]Departamento Biodiversidad y Restauración, Instituto Pirenaico de Ecología, Consejo Superior de Investigaciones Científicas, Jaca, Spain

[3]Laboratorio de Estudios Cristalográficos, Instituto Andaluz de Ciencias de la Tierra, CSIC-Universidad de Granada, Armilla, Spain

[4]Departamento de Pomologia, Estación Experimental de Aula Dei CSIC, Zaragoza, Spain

Correspondence
Andreu Cera, Centro de Ecologia Aplicada Prof. Baeta Neves (CEABN-InBIO), Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal.
Email: andreucera@isa.ulisboa.pt

Funding information
Horizon 2020 Framework Programme, Grant/Award Numbers: H2020-MSCA-RISE-2017, 777803; Ministerio de Ciencia e Innovación, Grant/Award Number: PID2019-111159GB-C31

Edited by K.-J. Dietz

Abstract

Calcium biomineralisation is widely documented in plants. However, crystallisation of Ca-sulphate-containing minerals is closely related to water content, and sample processing, such as drying, alters the water balance of plant tissues. We hypothesised that common sample processing practices may favour the formation of crystals, leading to spurious crystallisation not observed in unaltered plant tissues. We selected three species (*Ononis tridentata*, *Helianthemum squamatum* and *Gypsophila struthium*) with reported gypsum biomineralisation. We used x-ray diffractometry on fresh intact or sliced leaves, and on the same leaves processed by subsequent drying, to address whether sample processing alters crystal formation. Ca-sulphate crystals were detected in dry samples of all species but not in fresh intact samples. Ca-sulphate crystallisation occurred in some cut fresh samples, although the accumulation greatly increased after drying. In addition, *G. struthium* exhibited Ca-oxalate crystals in both fresh and dry treatments, with a tendency for greater accumulation in dry treatments. Our results demonstrate that the Ca-sulphate crystals observed by x-ray diffractometry in these species are artefacts caused by common sample processing practices, such as excessive drying and slicing samples. We encourage future studies on the biomineral potential of plants to avoid the use of procedures that alter the water balance of tissues.

1 | INTRODUCTION

The interaction of plants with soils is an important field in plant sciences, with major consequences on ecosystem functioning and plant productivity. Plants mainly absorb mineral nutrients from the soil. Sometimes, these mineral nutrients are found in concentrations greater than the plant requirements (Kabata-Pendias, 2010), and plants must manage uptake and accumulation to avoid metabolic imbalances in cells (Lambers & Oliveira, 2019). Plants can prevent the acquisition of such excess nutrients by using roots as a nutritional barrier, and/or by tolerating them in aboveground parts (Lux et al., 2021; Tran et al., 2020), for example by forming biominerals (He et al., 2014).

Biomineralisation has been documented in plants for several centuries as the accumulation of calcium (Ca) and silica (Si) minerals in plant tissues (Bauer et al., 2011; Franceschi & Nakata, 2005; He et al., 2014). Ca precipitation in different mineral forms is widespread across plant lineages (He et al., 2012). Ca oxalate is the most documented Ca mineral biomineralised by plants. It is found in more than 215 plant families (Brown et al., 2013). However, other Ca minerals containing carbonate or sulphate have also been reported inside plant tissues (He et al., 2015). The evolutionary driving force for Ca mineralisation may be primarily the prevention of Ca ion accumulation inside the cell when Ca acquisition surpasses plant requirements (Bauer et al., 2011). Although other functions such as Ca storage, defence against herbivory or metal detoxification have been suggested for Ca biomineralisation in plants (Franceschi & Nakata, 2005; He et al., 2014), experimental evidence is still limited (Karabourniotis et al., 2020; Paiva, 2019, 2021). Elucidating the processes behind the

formation of mineral deposits in plants is crucial to understand plant–soil interactions, and has a huge impact on our understanding of plant functioning.

Ca-sulphate biomineralisation has been reported in plants growing worldwide. Depending on the conditions (e.g., saturation state, temperature, pressure and water activity), this group of evaporite minerals can precipitate to form anhydrite ($CaSO_4$), bassanite ($CaSO_4{\cdot}1/2H_2O$) or gypsum ($CaSO_4{\cdot}2H_2O$) (Van Driessche et al., 2017). Gypsum biomineralisation is the most commonly reported and is usually associated with plants growing on gypsum soils (Kayabaş & Yildirim, 2021; Moore et al., 2014; Muller et al., 2017; Palacio et al., 2014). However, gypsum biomineralisation has also been observed in *Acacia* spp. (He et al., 2015), *Sarcocornia carinata* (Rufo et al., 2021) and the lichen *Ramalina lacera* (Garty et al., 2002) growing off gypsum soils. Although no records have been found for anhydrite formation in plant tissues, bassanite has been observed in the wood of *Tamarix* sp. trees (Weiner et al., 2021).

Crystallisation is the organised precipitation of solids from a solution or gas, and is therefore tightly related to the liquid water content under normal conditions (101.3 KPa, 20°C in plants). For example, gypsum crystallises when the concentration of Ca-sulphate in solution surpasses 2.23 $g{\cdot}L^{-1}$ (Van Driessche et al., 2017). Therefore, osmotic conditions inside plant tissues may be key for biomineral precipitation. Most procedures commonly used to analyse plant biomineralisation involve drying or altering the osmotic conditions in plant tissues and, consequently, may affect crystal formation. For example, SEM microscopy involves sectioning plant tissues (which damages cells and consequently alters the osmotic conditions inside them) and drying or dehydrating (often by critical point drying with CO_2; He et al., 2012). FTIR spectroscopy, a technique widely used to study mineral components in plants (Palacio et al., 2014), requires drying and milling plant tissues, completely altering the osmotic conditions and water content of samples. Tissue sectioning, drying, dehydration and milling could significantly promote the formation of Ca-sulphate biocrystals, leading to artefactual crystal formation, with important consequences for subsequent physiological and ecological interpretations.

Conversely, x-ray diffraction (XRD) is a widely used procedure in crystallographic studies (Criado-Reyes et al., 2023). This technique can be applied directly to unaltered fresh tissues, hence providing information on the presence of mineral crystals in vivo. The application of this technique to plant samples could provide robust information on the presence of Ca-sulphate biominerals in plants. However, to our knowledge, no studies have evaluated the presence of Ca-sulphate biominerals in plants without altering the cell structure to date.

The aim of this study was to evaluate Ca-sulphate biomineralisation in three common species for which gypsum biomineralisation has been reported. We used XRD in fresh and dried samples and in entire or sliced samples, to address whether sample processing techniques widely used in biomineralisation studies alter the formation of crystals.

2 | MATERIALS AND METHODS

2.1 | Study species

We selected three species with previously reported gypsum mineralisation and high S concentration in leaves (Palacio et al., 2014): *Gypsophila struthium* L., *Helianthemum squamatum* Pers. and *Ononis tridentata* L (Figure 1). All are sub-shrubs and representative of the

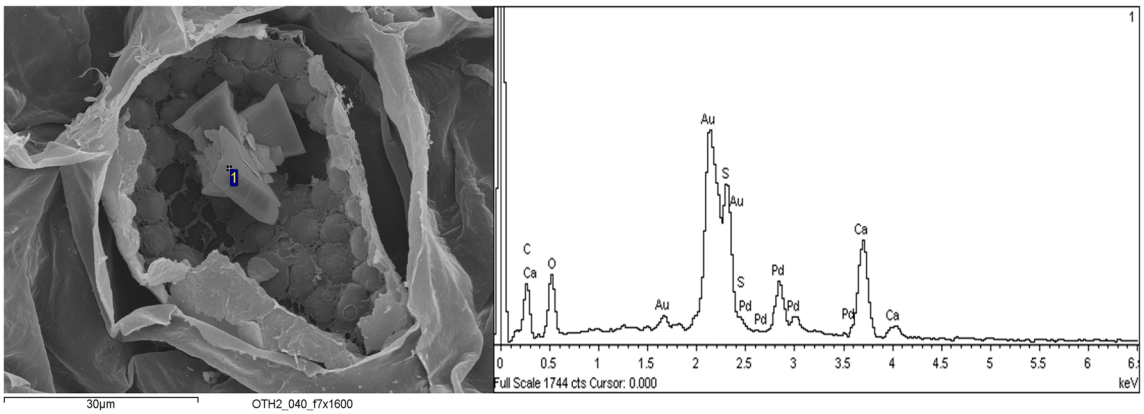


FIGURE 1 Gypsum crystals inside the leaf mesophyll cells of *Ononis tridentata* L., a gypsum endemic species in the Mediterranean region. On the right side, XRD measurements taken at point (1). The micrography was taken by a FE-SEM coupled to an x-ray diffractometer (Carl Zeiss MERLIN™) at the Analytical Services of the University of Zaragoza. Leaf samples had been collected in the field, fixed in 2.5% (v/v) glutaraldehyde in sodium phosphate buffer (0.03 M, pH 7.2) for 24 h, sliced with a razor blade under the stereomicroscope, sequentially dehydrated in increasing concentrations of acetone until 100%, CO_2 critical-point dried (Critical Point Dryer CPD7501 Polaron), mounted in SEM stubs and Paladium–Gold coated (Sputter Coater SP7610 Polaron) prior to observation.

dominant vegetation in gypsum ecosystems of Spain (Moore et al., 2014).

2.2 | Plant collection

We collected specimens in gypsum outcrops in south-eastern Spain. *H. squamatum* and *O. tridentata* were collected in Escúzar (Granada, Spain, 37°03′29.1"N 3°45′48.5"W; 930 m a.s.l.), whereas specimens of *G. struthium* were collected in Villanueva de las Torres (Granada, Spain, 37°30′35.0"N 3°06′03.3"W; 750 m a.s.l.). All collections were made on 3 July 2022.

Five specimens of each species were collected. We chose individuals located at least 2 m apart from each other. Selected individuals were healthy adult plants with their foliage exposed to full sunlight. We collected complete branches using secateurs and placed them individually in polyethylene bags and transported them to the laboratory, where plants were processed immediately.

2.3 | Sample processing

At the laboratory, branches were partly immersed in a bucket of tap water, and a 10-cm segment was cut off the most proximal part of the main stem of each branch under water to remove potential embolised xylem prior to branch re-hydration. After cutting off the basal segment of the stem, branches were immediately transferred to falcon tubes filled with tap water by submerging the stems. Branches were stored in the refrigerator at 4°C overnight, covered with a polyethylene plastic bag previously sprayed with tap water, to obtain plant samples at full hydration (Palacio et al., 2008).

Two sample processing treatments were evaluated: entire leaves or leaves cut into transverse sections. To observe entire leaves, a subsample of leaves (including petioles) was separated from each fragment and weighed on a precision scale (42 g/0.00001 g, MS105DU, Mettler Toledo). Senescent or damaged leaves were avoided. We separated five leaves of each individual for *G. struthium*, three for *H. squamatum* and two for *O. tridentata*. The amount of leaves was chosen according to the leaf size of each species and that of the slide for XRD observation. Leaves were mounted on the slide between two sheets of Mylar polyester film and sprayed slightly with tap water to keep them moist under XRD observation. Observations were made the next morning after plant collection in groups of three specimens, one of each species, changing the order each time. After XRD observation, samples were dismounted, dried to a constant weight at 50°C for 1 week, weighed and analysed again in the diffractometer. To study sectioned leaves, a subsample of leaves was separated and cut transversely with a razor blade into fragments approximately 2 mm wide. We separated the same number of leaves for each species, as described for entire leaves. Cut fragments were mounted on a slide between two sheets of Mylar polyester film and sprayed slightly with tap water. After XRD observation, they were dried and analysed as described for entire leaves.

2.4 | XRD analyses

Powder XRD data were collected using a Bruker D8 Advance Vαrio diffractometer (Bruker-AXS) equipped with a LYNXEYE detector, primary Ge (111) monochromator and Cu-Kα1 radiation (1.5406 Å). Diffractograms were acquired in transmission mode, at 40 kV acceleration voltage and 40 mA current, with 2θ scans spanning from 5 to 80° with a 2θ step of 0.02° s^{-1}. The observation time for each sample was 20 min. The detection limit of the diffractometer used depends on many variables and can reach a detection of 0.1% of the dry weight of the samples. DIFFRAC.EVA software (version 4.2) with the ICCD PDF-4+ (2021) database was used for phase identification.

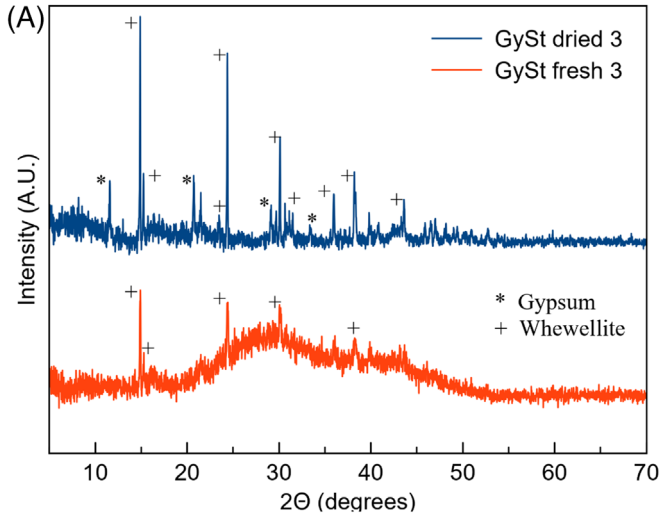
3 | RESULTS

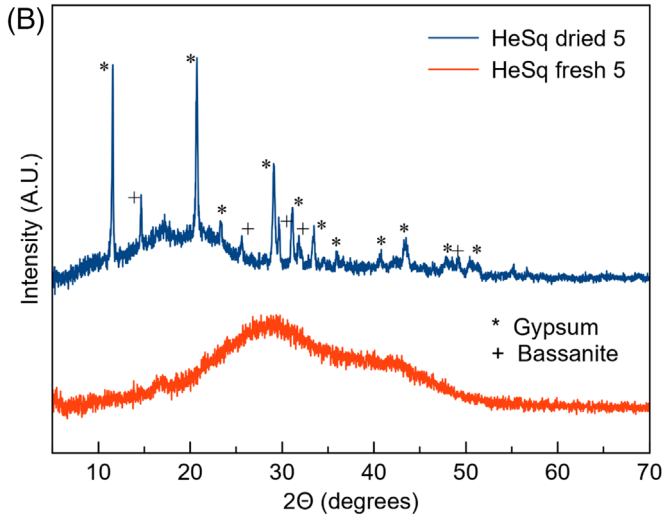
Ca-sulphate crystals were detected in dried samples of all species but not in fresh intact samples. Fresh samples had 72.02 ± 1.26% water content for *G. struthium*, 77.55 ± 0.53% for *O. tridentata* and 70.87 ± 2.08% for *H. squamatum*. None of the specimens analysed showed gypsum crystals in fresh entire leaves (Figure 2, see other replicates of diffractograms in Supporting Information). However, in all specimens of *H. squamatum* and *O. tridentata* and in one replicate of *Genista hispanica*, gypsum crystals were recorded on the same samples after drying. Some specimens of *H. squamatum* and *O. tridentata* also showed bassanite ($CaSO_4{\cdot}1/2H_2O$) crystals after drying (Figure 2B,C). All specimens of *G. struthium* had Ca-oxalate (Ca-OX) crystals (whewellite) either on fresh and dried treatments, with a trend of higher accumulation in dried treatments (Figure 2A).

Ca-sulphate crystallisation occurred in some cut samples, either in fresh or dried samples (Figure 3, see other replicates of diffractograms in Supporting Information). All specimens of *O. tridentata* had gypsum crystals in fresh cut samples, but the accumulation increased greatly after drying. Some replicates of *H. squamatum* showed weak peaks of gypsum crystals in fresh-cut samples, and all replicates showed strong peaks in dry-cut samples. Specimens of *G. struthium* had no gypsum crystals in fresh-cut samples, but these were formed in two specimens after drying. Ca-OX crystals were always present in fresh-cut leaves of *G. struthium* and after drying, but dried samples showed a greater accumulation than fresh ones.

4 | DISCUSSION

Drying samples to a constant weight at 50°C for 1 week (a common practice in plant science) was the main treatment altering the formation of Ca-sulphate crystals in our study, because this treatment removed all water in leaves. Target species have high concentrations of Ca and sulphate in leaves (Moore et al., 2014), and drying led to the precipitation of Ca-sulphate in the form of crystals. We mainly observed gypsum crystal formation because this is the stable phase of Ca-sulphate under 40°C, whereas anhydrite is the stable phase over 130°C, and bassanite is a transition phase (Van Driessche et al., 2017).





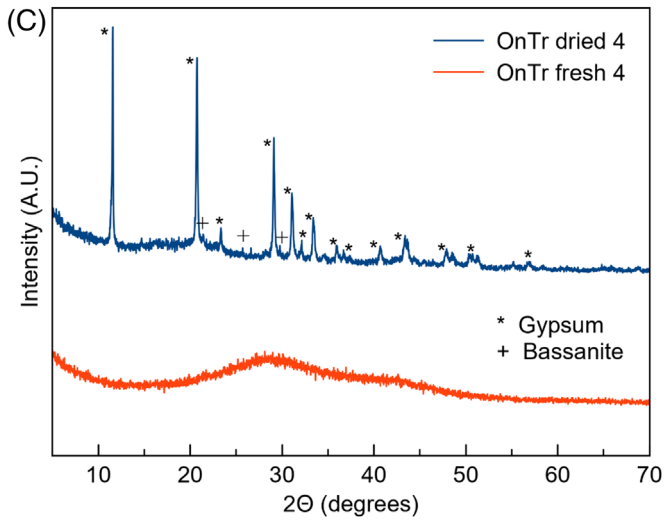
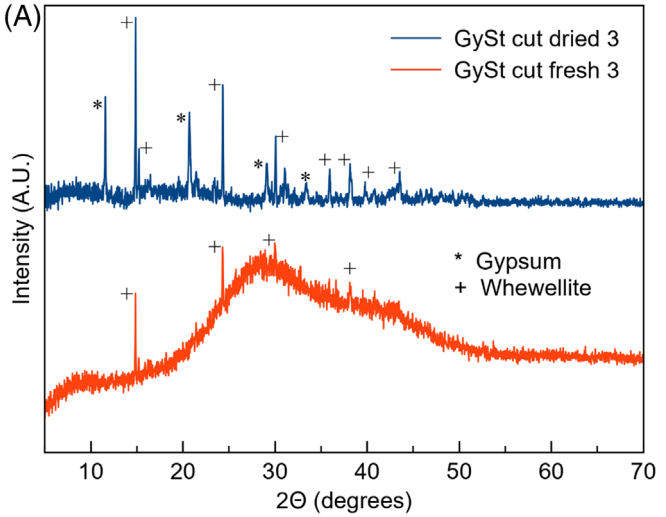
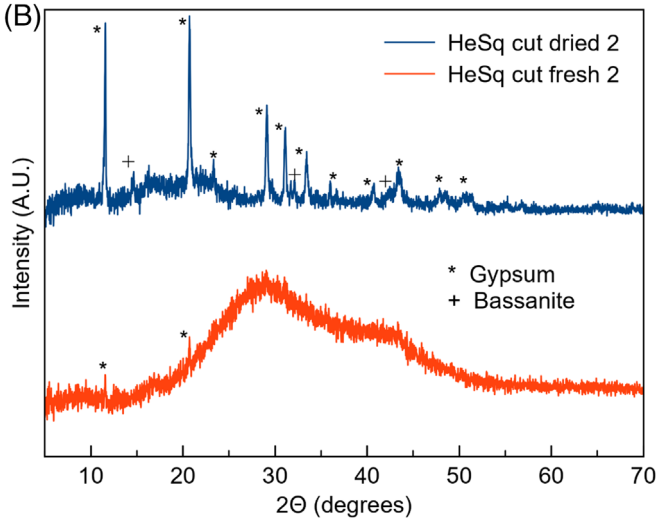


FIGURE 2 Powder x-ray diffractograms of *Gypsophila struthium* (A, GySt), *Helianthemum squamatum* (B, HeSq), *Ononis tridentata* (C, OnTr). Red lines are fresh entire leaves, and blue lines are the same leaves after being dried. Crystal peaks are marked and identified. Similar x-ray diffractograms were seen in four more replicates per species and treatment (see Supporting Information).





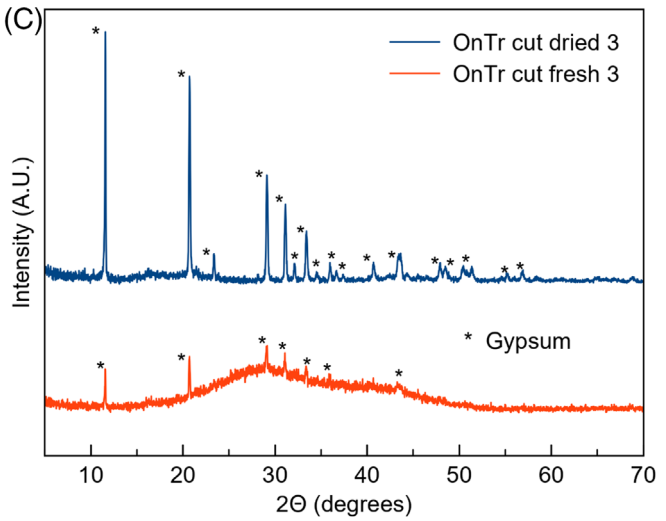


FIGURE 3 Powder x-ray diffractograms of *Gypsophila struthium* (A, GySt), *Helianthemum squamatum* (B, HeSq) and *Ononis tridentata* (C, OnTr). Red lines are fresh cut leaves, and blue lines are the same leaves after being dried. Crystal peaks are marked and identified. Similar x-ray diffractograms were seen in four more replicates per species and treatment (see Supporting Information).

Consequently, it is not surprising that we observed bassanite crystallisation in *H. squamatum* and *O. tridentata* samples dried at 50°C. Slicing leaves also slightly promoted the formation of crystals because cutting leaf tissues altered the leaf water balance. The effect of slicing was clearly observed in *O. tridentata* as this species has more succulent leaves and higher water content than *H. squamatum* and *G. struthium* (pers. observ.). In addition, in the sole species that formed Ca-OX crystals, crystals were also observed in fresh leaves, confirming biomineralisation, but the amount of Ca-OX crystals recorded increased remarkably after both processing treatments. This result is not surprising since Ca-OX is less soluble (0.00067 $g \cdot L^{-1}$ at 20°C) than Ca-sulphate, which explains its presence in intact leaves, but it also shows that it is a more sensitive mineral to water content.

These results have implications for the understanding of plant functioning. We have demonstrated that biomineralisation is tightly linked to leaf water content. This consideration is especially important when dealing with minerals with moderate or high solubility, as is the case with Ca-sulphate crystals. Therefore, any method altering foliar water balance may lead to spurious crystal formation, resulting in misleading or inaccurate conclusions about plant function. For example, the accumulation of Ca-sulphate in gypsum plants was previously interpreted as a way for plants to sequester excess Ca and sulphates in the form of biocrystals (He et al., 2014; Palacio et al., 2014), similar to the role of Ca-OX under conditions of excess Ca (He et al., 2014). Furthermore, the presence of Ca-sulphate biominerals has also been interpreted to play a role in preventing herbivory (Palacio et al., 2014). In contrast, our results indicate that the plants studied accumulated Ca-sulphates inside plant tissues at oversaturation. These plants probably use other elements, such as magnesium, which has also been found to accumulate profusely in gypsum plants (Muller et al., 2017) to reduce the nucleation and growth kinetics of Ca-sulphate crystals (Rabizadeh et al., 2017). This finding opens the window to new interpretations about the role of Ca-sulphate accumulation in plants, such as the case of plant specialisation to gypsum soils (Cera et al., 2023), where gypsum endemics would accumulate Ca-sulphate in oversaturation and not biomineralise.

5 | CONCLUSIONS

In conclusion, this communication highlights certain crucial aspects to be considered for future studies on plant biomineralisation. Future studies on the biomineral potential of plants should avoid the use of procedures that alter the water balance of plant tissues, such as drying, cutting, slicing, fixation in formalin–acetic acid, ethanol or acetone, or cell lysis. Future studies should also reduce the time elapsed between plant collection and analysis, as it may modify the water balance of plant tissues. The use of non-invasive techniques such as x-ray diffractometry or x-ray computed tomography is recommended over optic, transmission, or scanning microscopy (Matsushima et al., 2012). The cryo-preparation of samples and subsequent observation with low-temperature SEM or low vacuum environmental SEM in the presence of water vapour may be appropriate, although its potential interference with crystal formation should be further investigated. Histological techniques that do not involve cutting of tissues, such as diaphanisation and sodium hypochlorite clearing, should also be tested to observe crystals in plant organs. Further studies involving observation of the inside of fresh leaves with high vacuum SEM may also provide useful information on the origin of crystals. Small, disorganised crystals may be identified as artefacts of the effect of vacuum, while large euhedral single crystals present in specific cell types may be indicative of in vivo formation (e.g., Weiner et al., 2021).

AUTHOR CONTRIBUTIONS

All the authors developed the hypotheses, discussed the results, and wrote the manuscript. Andreu Cera, Sara Palacio and Cristóbal Verdugo-Escamilla designed the study. Andreu Cera and Cristóbal Verdugo-Escamilla analysed data. Andreu Cera led the manuscript writing.

ACKNOWLEDGMENTS

We are grateful to Carolina Ruiz for her help during field work; Duane Choquesillo and Francisco Javier Acebedo-Martínez for help with the analyses and Andrea Cotellucci and Fermín Otálora for contributions to the discussion. This study was funded by the Gobierno de España (PID2019-111159GB-C31) and by European Union's Horizon 2020 (H2020-MSCA-RISE-2017 GA No. 777803).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Bauer, P., Elbaum, R. & Weiss, I.M. (2011) Calcium and silicon mineralization in land plants: transport, structure and function. *Plant Science*, 180, 746–756.

Brown, S.L., Warwick, N.W.M. & Prychid, C.J. (2013) Does aridity influence the morphology, distribution and accumulation of calcium oxalate crystals in *Acacia* (Leguminosae: Mimosoideae)? *Plant Physiology and Biochemistry*, 73, 219–228.

Cera, A., Montserrat-Martí, G. & Palacio, S. (2023) Nutritional strategy underlying plant specialization to gypsum soils. *AoB Plants*, 15, plad041.

Criado-Reyes, J., Otálora, F., Canals, À., Verdugo-Escamilla, C. & García-Ruiz, J.-M. (2023) Mechanisms shaping the gypsum stromatolite-like structures in the Salar de Llamara (Atacama Desert, Chile). *Scientific Reports*, 13, 678.

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

Garty, J., Kunin, P., Delarea, J. & Weiner, S. (2002) Calcium oxalate and sulphate-containing structures on the thallial surface of the lichen *Ramalina lacera*: response to polluted air and simulated acid rain. *Plant, Cell & Environment*, 25, 1591–1604.

He, H., Bleby, T.M., Veneklaas, E.J., Lambers, H. & Kuo, J. (2012) Morphologies and elemental compositions of calcium crystals in phyllodes and branchlets of *Acacia robeorum* (Leguminosae: Mimosoideae). *Annals of Botany*, 109, 887–896.

He, H., Kirilak, Y., Kuo, J. & Lambers, H. (2015) Accumulation and precipitation of magnesium, calcium, and sulfur in two *Acacia* (Leguminosae; Mimosoideae) species grown in different substrates proposed for mine-site rehabilitation. *American Journal of Botany*, 102, 290–301.

He, H., Veneklaas, E.J., Kuo, J. & Lambers, H. (2014) Physiological and ecological significance of biomineralization in plants. *Trends in Plant Science*, 19, 166–174.

Kabata-Pendias, A. (2010) *Trace elements in soils and plants*. Boca Raton, FL: CRC Press.

Karabourniotis, G., Horner, H.T., Bresta, P., Nikolopoulos, D. & Liakopoulos, G. (2020) New insights into the functions of carbon–calcium inclusions in plants. *New Phytologist*, 228, 845–854.

Kayabaş, A. & Yildirim, E. (2021) Biochemical fingerprints of some endemic plants growing in gypsum soils: attenuated Total reflection-Fourier transform infrared (ATR-FTIR) spectroscopic study. *European Journal of Biology*, 80, 97–106.

Lambers, H. & Oliveira, R.S. (2019) Introduction: history, assumptions, and approaches. In: Lambers, H. & Oliveira, R.S. (Eds.) *Plant physiological ecology*. Cham: Springer International Publishing, pp. 1–10.

Lux, A., Kohanová, J. & White, P.J. (2021) The secrets of calcicole species revealed. *Journal of Experimental Botany*, 72, 968–970.

Matsushima, U., Hilger, A., Graf, W., Zabler, S., Manke, I., Dawson, M. et al. (2012) Calcium oxalate crystal distribution in rose peduncles: non-invasive analysis by synchrotron X-ray micro-tomography. *Postharvest Biology and Technology*, 72, 27–34.

Moore, M., Mota, J., Douglas, N., Flores-Olvera, H. & Ochoterena, H. (2014) The ecology, assembly, and evolution of gypsophile floras. In: Rajakaruna, N., Boyd, R. & Harris, T. (Eds.) *Plant ecology and evolution in harsh environments*. Hauppauge, NY: Nova Science Publishers, pp. 97–128.

Muller, C.T., Moore, M.J., Feder, Z., Tiley, H. & Drenovsky, R.E. (2017) Phylogenetic patterns of foliar mineral nutrient accumulation among gypsophiles and their relatives in the Chihuahuan Desert. *American Journal of Botany*, 104, 1442–1450.

Paiva, E.A.S. (2019) Are calcium oxalate crystals a dynamic calcium store in plants? *New Phytologist*, 223, 1707–1711.

Paiva, É.A.S. (2021) Do calcium oxalate crystals protect against herbivory? *Naturwissenschaften*, 108, 24.

Palacio, S., Aitkenhead, M., Escudero, A., Montserrat-Martí, G., Maestro, M. & Robertson, A.H.J. (2014) Gypsophile chemistry unveiled: Fourier transform infrared (FTIR) spectroscopy provides new insight into plant adaptations to gypsum soils. *PLoS One*, 9, e107285.

Palacio, S., Milla, R., Albuixech, J., Pérez-Rontomé, C., Camarero, J.J., Maestro, M. et al. (2008) Seasonal variability of dry matter content and its relationship with shoot growth and nonstructural carbohydrates. *New Phytologist*, 180, 133–142.

Rabizadeh, T., Stawski, T.M., Morgan, D.J., Peacock, C.L. & Benning, L.G. (2017) The effects of inorganic additives on the nucleation and growth kinetics of calcium sulfate dihydrate crystals. *Crystal Growth & Design*, 17, 582–589.

Rufo, L., Iglesias-López, M.T. & de la Fuente, V. (2021) The endemic halophyte *Sarcocornia carinata* Fuente, Rufo & Sánchez-Mata (Chenopodiaceae) in relation to environmental variables: elemental composition and biominerals. *Plant and Soil*, 460, 189–209.

Tran, T.K.A., Islam, R., Le Van, D., Rahman, M.M., Yu, R.M.K. & MacFarlane, G.R. (2020) Accumulation and partitioning of metals and metalloids in the halophytic saltmarsh grass, saltwater couch, *Sporobolus virginicus*. *Science of the Total Environment*, 713, 136576.

Van Driessche, A.E.S., Stawski, T.M., Benning, L.G. & Kellermeier, M. (2017) Calcium sulfate precipitation throughout its phase diagram. In: van Driessche, A.E.S., Kellermeier, M., Benning, L.G. & Gebauer, D. (Eds.) *New perspectives on mineral nucleation and growth: from solution precursors to solid Materials*. Cham: Springer International Publishing, pp. 227–256.

Weiner, S., Pinkas, I., Kossoy, A. & Feldman, Y. (2021) Calcium sulfate hemihydrate (Bassanite) crystals in the wood of the *Tamarix* tree. *Minerals*, 11, 289.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Cera, A., Verdugo-Escamilla, C., Marín, J.A. & Palacio, S. (2023) Calcium sulphate biomineralisation: Artefact of sample preparation? *Physiologia Plantarum*, 175(5), e14017. Available from: https://doi.org/10.1111/ppl.14017