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Leaf optical properties are affected by the location and type of deposited biominerals

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ABSTRACT

This study aimed to relate the properties of incrusted plant tissues and structures as well as biomineral concentrations and localization with leaf reflectance and transmittance spectra from 280 nm to 880 nm in the grasses *Phragmites australis*, *Phalaris arundinacea*, *Molinia caerulea* and *Deschampsia cespitosa*, and the sedge *Carex elata*. Redundancy analysis revealed that prickle-hair length on adaxial surface and thickness of lower epidermis exerted significant effects in *P. australis*; prickle-hair density at abaxial leaf surface and thickness of epidermis on adaxial leaf surface in *P. arundinacea*; thickness of epidermis on adaxial leaf in *D. cespitosa*; prickle-hair density on adaxial leaf surface and thickness of cuticle in *M. caerulea*; and prickle-hair density on adaxial leaf surface and cuticle thickness of the lower side in *C. elata*. Micro-PIXE and LEXRF elemental localization analysis show that all of these structures and tissues are encrusted by Si and/or by Ca. Reflectance spectra were significantly affected by the Ca concentrations, while Si and Mg concentrations and the Ca concentrations significantly affected transmittance spectra. High concentrations of Mg were detected in epidermal vacuoles of *P. arundinacea*, *M. caerulea* and *D. cespitosa*. Al co-localises with Si in the cuticle, epidermis and/or prickle hairs.

1. Introduction

In plant leaves, biominerals have multiple functions that depend on their structure, abundance, location and chemistry [1]. Biominerals can affect the water and energy balance of a plant [2,3], as well as increase the plant resistance to pathogens and herbivores [4–7]. Plant species of the families Poaceae and Cyperaceae are known to accumulate large amounts of different biominerals in their organs [8–10].

Amorphous silicon (Si) dioxide (silica), amorphous calcium (Ca) carbonate and Ca oxalate are the most common biominerals in plants [11,12]. Silica and Ca carbonate are important structural elements that increase the rigidity of the plant tissue, thus enhancing the strength [13,14]. Deposition of silica in plants occurs in intercellular and intracellular spaces and in the cell walls. Silica is not accumulated uniformly among various cell types and tissues, and it is found in high concentrations especially in the cell walls of epidermal cells, guard cells, bulliform cells and prickle hairs [8,10,15–17].

The depositions of biominerals in the upper layers of plant organs are known also as encrustations, the levels of which depend on leaf age and the side of the leaf (upper or lower leaf surface) [16,18,19]. Silica can also be found in the mesophyll tissue, although usually at lower concentrations than in the epidermal layers [18]. Silicon is deemed to be non-essential for plants, but it is well known for its beneficial effects [20–23]. It can substitute carbon (C) as a structural element [24,25] because the energy costs for Si structures are 10–20 times lower than those of C. It has been suggested that Si accumulation increased in the plants that developed in the Miocene (e.g., Poales), when the concentration of CO_2 in atmosphere were rather low [26]. Silicon also has an important role in stress mitigation, as it promotes an increase in the production of antioxidants and binds and co-precipitates metal ions [27–29]. The amelioration of aluminium (Al) toxicity by Si is well established [23,30–32], and many authors have observed co-deposition of Al and Si in epidermal cells [28,33,34].

Calcium is an essential nutrient that has a crucial role in several processes in plant cells, especially signalling and signal transduction [35]. Therefore intracellular Ca^{2+} concentrations need to be carefully regulated in order to maintain cellular homeostasis. For this reason, excess Ca^{2+} is first compartmented in the vacuole

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and/or endoplasmic reticulum, and can be also deposited in an inactive form as amorphous Ca carbonate and/or as crystal Ca oxalate [12]. Calcium carbonate bodies are known as cystoliths, and these are regularly distributed in the epidermis, and their deposition can also extend into the mesophyll [17]. Cystolith bodies are attached to the peripheral cell wall by a silicate stalk that has an important role in C and Ca storage and pH regulation [36]. Ca oxalate can be abundant in plants from most taxonomic groups [37]; however, Ca oxalate is rarely present in grasses and sedges [38]. In the mature leaves of *Morus australis* (Chinese mulberry), both Ca oxalate and Ca carbonate served as forms of Ca storage that can be reused during the growth of new leaves [39]. As well as Ca oxalate, magnesium (Mg) oxalate has also been reported in plants [1].

There is a lot of literature on different biominerals, although the studies on their ecological functions are rare, and there are almost no data about the role of biomineral encrustations in UV and photosynthetically active radiation reflection and transmission in plants [17,19]. Biominerals in the leaves can distribute the light flux more evenly inside the leaf tissue, which increases the energy availability for the lower tissues and prevents photoinhibition in the upper mesophyll [36]. Biominerals that appear at or close to the leaf surface can significantly affect the leaf optical properties [19]. Among such biominerals, Si and Ca appear to have the most important roles, as they usually reach high concentrations in the epidermal tissue, particularly in different grasses and sedges [3,15,17,18]. Our previous study showed that biomineralised structures at or close to the leaf surface affects the reflection and transmittance of light in the leaves of four grasses and a sedge [19].

In the present study, we assessed the spatial resolution of Si, Mg and Ca at the tissue level using micro-proton induced X-ray emission (micro-PIXE) spectroscopy. In addition, a method with high sensitivity for Al and higher spatial resolution, known as synchrotron low energy X-ray fluorescence (LEXRF) micro-spectroscopy [40–42] was used to probe the localisation of Si, Mg and Al in the upper leaf epidermis and in prickle hairs.

The aims of the present study were to show the differences in importance of the encrusted near-surface leaf tissues and structures that explain the leaf reflectance and transmittance spectra in *Phragmites australis* (common reed), *Phalaris arundinacea* (reed canarygrass), *Molinia caerulea* (purple moor grass) and *Deschampsia cespitosa* (tufted hairgrass), and in *Carex elata* (tufted sedge); and to localise and estimate the concentrations of the biominerals Si, Ca and Mg in the different layers of the leaf tissue, as well as to investigate the possible co-localisation of other elements with Si and Ca in near-surface leaf structures. We hypothesised (1) that the importance of near-surface encrustations for leaf optical properties differ among these species; (2) that the presence and amount of biominerals in different leaf tissues and structures is species specific; (3) that the presence of biominerals affect reflectance and transmittance spectra and (4) that possible co-localisation of biominerals with other elements might occur in selected species.

2. Materials and methods

2.1. Plant material

The plants were sampled in the area of an intermittent lake, Lake Cerknica (45° 46′ 15″ N, 14° 21′ 20″ E). The majority of the shallower parts of the lake are colonised by the cosmopolite common reed species *P. australis*, and other wetland species, while areas where the flooding occurs at the beginning of the growth season only are overgrown with mire and wet grassland species [43].

The leaves of the grasses *P. australis* (Cav.), *P. arundinacea* L., *M. caerulea* L., *D. cespitosa* L. and the sedge *C. elata* L. were collected in the summer of 2012 and 2013. Fully developed leaves were sampled. The measurements of morphological parameters were carried out on 10 to 40 replicates of leaves for each plant species. The micro-PIXE analysis was performed on three sub-samples for each plant species, and the mapping of the elemental distributions using LEXRF spectroscopy was performed on three or four samples of each plant species.

2.2. Measurements of spectral reflectance and transmittance

The optical properties of the leaves were measured on the day of sampling. The leaf reflectance and transmittance spectra were measured in a range from 280 nm to 880 nm, at a resolution of approximately 0.3 nm, using a portable spectrometer (Jaz Modular Optical Sensing Suite; Ocean Optics, Inc., Dunedin, USA) fitted with an integrating sphere (ISP-30-6-R; Ocean Optics, Inc.) and an optical fibre (QP600-1-SR-BX; Ocean Optics, Inc.). The reflectance and transmittance spectra were measured for the same part of the leaf.

The reflectance measurements followed the procedures in Klančnik et al. [44]. Adaxial leaf surfaces were illuminated with a UV–VIS-near infrared light source (DH-2000, Ocean Optics, Inc.). The spectrometer was calibrated to 100% reflectance using a white reference panel (Spectralon; Labsphere, North Sutton, USA).

For the transmittance spectra measurements, the integrating sphere was positioned at the abaxial leaf surface, while the adaxial surface was illuminated by the above-mentioned light source. Prior to the sample measurement, the spectrometer was calibrated to 100% transmittance with a light beam that passed directly into the interior of the integrating sphere.

2.3. Anatomical analysis

The thickness of the total mesophyll, cuticles and epidermis for the upper and lower leaf surfaces were measured on leaf transects at 100×magnification, using a CX41 microscope equipped with a XC30 digital camera and CellSens software (Olympus, Hamburg, Germany). The density and length of the prickle hairs on the upper and lower leaf surfaces were also determined using this microscopy system.

2.4. Sample preparation for mapping the element distributions

The leaves of each plant species were cut into small pieces (2 mm × 5 mm) with a razor blade, and these were immediately put inside 2-mm-wide stainless steel needles, embedded with a droplet of tissue freezing medium (Jung) to provide support during the cutting, and rapidly frozen in liquid propane cooled with liquid nitrogen [42,45,46]. The leaf pieces were then sectioned with a CM3050 cryo-microtome (Leica, Bensheim, Germany) with head and chamber temperatures in the range of −30 °C to −25 °C, and a section thickness of 50 μm for micro-PIXE analysis, and 20 μm for LEXRF analysis. The sections were placed in pre-cooled Al holders, and freeze-dried at −30 °C and 0.4 mbar pressure, for 3 days in an Alpha 2–4 Christ freeze dryer, using a cryo-transfer assembly that was cooled by liquid nitrogen [42,45,46]. Dry leaf cross-sections were then mounted between two layers of pioloform foil [45] for the micro-PIXE and LEXRF analyses. The images of the cross-sections were obtained with an Axioskop 2 MOT microscope (Carl Zeiss, Goettingen, Germany), using a visible and blue-light excitation source, an Axiocam MRc colour digital camera, and the AxioVision 3.1 software.

2.5. Micro-PIXE spectroscopy analysis

The spatial distributions of the mineral elements at the tissue level were determined in leaf cross-sections using micro-PIXE