BIOLOGIA PLANTARUM 64: 234-243, 2020 DOI: 10.32615/bp.2019.155

Do soil and leaf silicon content affect leaf functional traits in *Deschampsia caespitosa* from different habitats?

M. GRAŠIČ*, T. SAKOVIČ, D. ABRAM, K. VOGEL-MIKUŠ, and A. GABERŠČIK

Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111,
SI-1000 Ljubljana, Slovenia

Abstract

The purpose of this study was to show the extent of phenotypic plasticity of the grass *Deschampsia caespitosa* from four habitats with different soil properties by comparing selected leaf traits and content of silicon and other elements. Morphological, biochemical, and optical properties were examined in leaves, but content of silicon and other elements also in soil samples. Plant-available silicon in the soil was determined following extraction in $CaCl_2$. Bulk element analysis was conducted using X-ray fluorescence spectrometry. The habitats of *D. caespitosa* differed significantly according to soil structure, which resulted in significantly different leaf traits, including leaf optical properties and content of minerals. There was no correlation between leaf silicon and plant-available or total soil silicon, while positive correlation was seen between leaf calcium and total soil calcium. In addition, plant-available silicon showed strong positive correlation with leaf calcium and phosphorus. The majority of *D. caespitosa* leaf and soil properties differed significantly among habitats.

Additional key words: leaf anatomy, leaf reflectance and transmittance, chlorophyll, plant and soil element composition, tufted hairgrass.

Introduction

Biomineralisation involves the deposition of amorphous silica, calcium carbonate, Ca phosphate, and Ca oxalate in plant tissues (Bauer *et al.* 2011). Biominerals have multiple functions in plants, as they can affect the plant water and energy balance, and increase plant resistance to pathogens and herbivores (He *et al.* 2014). The rate of mineral uptake is facilitated by leaf transpiration flow (Larcher 2003), and thus this might be more pronounced in plants that thrive in wet habitats.

Silicon (Si) is the most important plant biomineral. Plants can only absorb Si in the form of monosilicic acid (H_4SiO_4) (Haynes 2014), which is a result of chemical weathering of silicate-containing minerals (Basile-Doelsch *et al.* 2005). The concentration of plant-available Si in the soil solution is strongly influenced by different environmental factors, namely the soil pH and the amounts of clay, minerals, organic matter, and Fe/Al oxides/hydroxides, which are collectively related with the geological age of soil (Tubana *et al.* 2016). Si is a beneficial element for plants, as it alleviates negative effects of drought, extreme temperatures, toxic metals, high salinity and radiation, and pathogen attacks (Currie and Perry 2007, De Camargo *et al.* 2019), through improving the antioxidant capacity and phenolic metabolism of plants (Ribera-Fonseca *et al.* 2018). Silica bodies are deposits in plant tissues, where they function as structural support, as an alternative to lignin, and as defence against herbivory (Brizuela *et al.* 1986, Strömberg *et al.* 2016). Si also has an important role in gene regulation in plant development and defence (Frew *et al.* 2018).

Representatives of the families *Poaceae* and *Cyperaceae* can accumulate large amounts of silica in their tissues (Bauer *et al.* 2011). The extent of tissue silicification in grasses defines their importance in the global terrestrial silica cycle (Trembath-Reichert *et al.* 2015). In grasses, Si accumulates in or near the epidermis (Yoshida *et al.* 1962, Prychid *et al.* 2004), such as in the bulliform cells (Sangster and Parry 1969), in epidermal structures, in trichomes (Kaufman *et al.* 1981) and stomata (Lu *et al.* 2009), along the veins (Prychid *et al.* 2004), and in the mesophyll (Morikawa and Saigusa 2004). Once Si is deposited in the target cells, its translocation is no longer possible (Raven 1983, Epstein 1994). Si deposition in leaf tissues alters the sun radiation gradient within the tissues and thus affects the leaf energy balance. Dietrich *et al.* (2003) showed that the amount of accumulated Si in grasses varies greatly within the same species, and depends on Si availability in the soil.

Submitted 19 August 2019, *last revision* 7 November 2019, *accepted* 9 December 2019.
Acknowledgements: This study was funded by the Slovenian Research Agency (grant numbers P1-0212, 39096). The authors are grateful to Christopher Berrie for revision of the English writing.
* Corresponding author; e-mail: mateja.grasic@bf.uni-lj.si

Deschampsia caespitosa (L.) P.Beauv. is a perennial grass. It is widely important because of its global distribution in moist arctic and temperate regions of the world (St. John *et al*. 2011). Due to its high phenotypic plasticity and genecological variation, it is a very variable species (Davy 1980). Therefore, *D. caespitosa* can tolerate a wide range of environmental conditions (Davy and Taylor 1974, Grime *et al.* 1988) and can thus serve as a model system for studying the variability in Si/plant relations within a species. The level of biomineralisation, which depends on environmental conditions (Davy 1980, Gaberščik *et al.* 2018), might thus differ among *D. caespitosa* plants that thrive in different habitats.

In the present study, the aim was to examine selected biochemical and structural traits, and the content of Si and other elements in *D. caespitosa* leaves and the corresponding soils from different habitats. The first two habitats were river floodplain and intermittent lake on carbonate rock, while the other two habitats were alpine heath and alpine foothills forest edge with prevailing calc-alkaline volcanic rock. We expected that leaf Si content would differ according to habitat, as Si content in plants is closely related with soil Si content (Dietrich *et al.* 2003). This would also be the case for some other elements, namely calcium, phosphorus, and potassium. In addition, Si may impact element stoichiometry of plants (Brackhage *et al.* 2013). We also expected differences in the structural and functional traits of the leaves of plants from different habitats.

Materials and methods

Plant growth: Plants of *Deschampsia caespitosa* (L.) P.Beauv. were sampled from four different habitats: *1*) the floodplain of the river Rak, in Rakov Škocjan (45°47'44" N, 14°17'20" E; 515 m a.s.l.); *2*) the intermittent Lake Cerknica (45°45'43" N, 14°21'38" E; 548 m a.s.l.); *3*) an alpine heath below the summit of Mount Komen (46°24'58" N, 14°50'46" E; 1 625 m a.s.l.); and *4*) a forest edge at the foothills of Mount Komen (46°25'17" N, 14°49'46" E; 409 m a.s.l.). The plants from the first two habitats (*i.e.*, river floodplain, intermittent lake) grew on carbonate rock, while the other two habitats (*i.e.*, alpine heath, alpine foothills forest edge) were characterised by prevailing calc-alkaline volcanic rock. Ten plants were sampled randomly per each location during flowering, wherein the second youngest fully developed leaf from each of the sampled plants was used for analyses.

The data for the 24-h rainfall were from two meteorological stations close to the four chosen sampling locations (Fig. 1 Suppl.). For each plant sample, the rhizospheric soil was collected for further analysis from a depth of 0 to 10 cm. The soil samples were air dried in separate unsealed paper bags for ~60 d and later sifted through a 0.5 × 0.5 mm mesh sieve prior to the soil element analysis and the analysis of plant-available Si in the soil. For the soil structure analysis, these soil samples were combined to obtain a composite soil sample for each of the four habitats.

Leaf morphological properties: Leaf structure was analysed on the transverse sections of vital, fully developed, *D. caespitosa* leaves. The measurements included thickness of mesophyll, epidermis, and cuticle, and length and density of stomata. All the measurements were performed on the central parts of the leaves, using light microscope (*CX41, Olympus*, Tokyo, Japan), equipped with a digital camera (*XC30, Olympus*) and the *CellSens* software (*Olympus*). The specific leaf area was calculated as leaf area per unit of dry matter.

Leaf biochemical properties: The content of chlorophyll (Chl) *a*, Chl *b*, and carotenoids was determined in leaf extracts using 100 % (v/v) acetone and absorbance was measured at 470, 645, and 662 nm using a UV/VIS spectrometer (*Lambda 25, Perkin-Elmer*, Norwalk, CT, USA) according to Lichtenthaler and Buschmann (2001a,b). The anthocyanin content was determined in leaf extracts using 37 % HCl + 100 % methanol solution (1:99; v/v), as described by Drumm and Mohr (1978) and absorbance was measured at 530 nm. Total methanol-soluble UV-B-absorbing and UV-A-absorbing compounds were also extracted from the fresh plant material using 37 % HCl + distilled water + 100 % methanol solution (1:20:79; v/v), according to Caldwell (1968), and absorbance was measured from 280 to 319 nm, and from 320 to 400 nm, respectively. The coefficients of absorbance were integrated for each UV region. One arbitrary unit [a.u.] corresponds to one relative unit.

Leaf optical properties: The leaf optical properties were determined in the laboratory on vital, fresh, and fully developed *D. caespitosa* leaves. The reflectance spectra were measured from 290 to 880 nm, and the transmittance spectra from 290 to 800 nm, at a resolution of ~1.3 nm, using a portable spectrometer (*Jaz Modular Optical Sensing Suite, Ocean Optics*, Dunedin, FL, USA; grating, #2; slit size, 25 µm) with an optical fibre (*QP600-1-SR-BX, Ocean Optics*) and an integrating sphere (*ISP-30-6-R; Ocean Optics*). The leaf reflectance spectra were measured for the adaxial leaf surface by irradiation with a UV/VIS-near infrared (NIR) radiation source (*DH-2000, Ocean Optics*). The spectrometer was calibrated to 100 % reflectance using a white reference panel with > 99 % diffuse reflectance (*Spectralon, Labsphere*, North Sutton, NH, USA). The spectrometer was calibrated to 100 % transmittance with a light beam that passed directly into the interior of the integrating sphere.

Bulk element analysis: The content of Si, P, S, Cl, K, and Ca in the *D. caespitosa* leaves were determined using X-ray fluorescence spectrometry (*Peduzo T01, Jožef Stefan Institute*, Ljubljana, Slovenia). From 100 to 500 mg of dried and powdered leaves was pressed into pellets using a pellet die and a hydraulic press. ^{55}Fe (*25 mCi, Isotope Products Laboratories*, Valencia, PA, USA) was used as the primary excitation source for the analysis. The fluorescence radiation emitted was collected using a Si drift diode detector (*Amptek*, Bedford, MA, USA), with a 12-µm-thick beryllium window. The energy resolution of the spectrometer at count rates < 1 000 cps was 140 eV at 5.9 keV. The X-ray fluorescence spectrometry analysis was performed under vacuum, with the samples irradiated for 5 000 s to obtain spectra with sufficient statistics (Nečemer *et al.* 2008). The analysis of the X-ray spectra was performed using an iterative least-squares programme, as included in the quantitative X-ray analysis system software package (Vekemans *et al.* 1994). Element quantification from the measured spectra was performed using quantitative analysis of environmental samples based on fundamental parameters (Kump *et al.* 2011). Quality assurance for the element analysis was performed using standard reference materials: *NIST SRM 1573a* (tomato leaves as a homogenised powder) in the form of pressed pellets.

Total soil Si, S, Cl, K, and Ca content was measured from ~300 mg of dried and powdered soil samples following the above-described methodology and using an X-ray spectrometer (*Peduzo T02, Jožef Stefan Institute*, Ljubljana, Slovenia) equipped with an Rh tube and a Si drift diode detector with a 12-μm-thick Be window (*Amptek*).

Analysis of plant-available Si in the soil: Plant-available Si in the soil was extracted from ~300 mg of dried and powdered soil samples using 0.01 M $CaCl_2$, as described by Korndörfer *et al.* (1999). The samples were further processed using a commercially available kit (*Heteropoly Blue Method, Hach Lange*, Düsseldorf, Germany), with absorbance measured at 815 nm using a spectrophotometer (*DR 3900, Hach Lange*).

Soil structure analysis was conducted on composite samples from each of the four sampling habitats by the Infrastructural Centre for Pedology and Environmental Protection (Department of Agronomy, Biotechnical Faculty, University of Ljubljana). The soil parameters investigated were soil texture, pH in $CaCl_2$, available P and K (measured as P_2O_5 and K_2O, respectively), electrical conductivity, carbonates, carbon, nitrogen, C/N ratio, total C, organic matter, cation exchange capacity (measured as mmol charge kg^{-1}; *i.e.*, $mmol_c\ kg^{-1}$), Ca^{2+}, Mg^{2+}, K^+, Na^+, total exchangeable bases, and base saturation (Fig. 2 Suppl.).

Soil pH was measured in 0.01 M $CaCl_2$ solution. Organic matter and organic C were determined in a solution of excess $K_2Cr_2O_7$ in H_2SO_4, using the Walkley-Black method, with small modifications. The organic C was calculated by dividing the organic matter by 1.724. Total N was determined according to the Kjeldahl method, with small modifications. The C/N ratio was calculated as the proportion between organic C and total N in the soil. Available P and K were determined as described by Hoffmann (1991), following Ca-lactate-acetate extraction. P was measured colorimetrically using molybdenum blue staining, while K was measured using a flame spectrophotometer. The exchangeable basic cations (*i.e.*, Ca^{2+}, Mg^{2+}, K^+, Na^+) were extracted with ammonium acetate and determined by atomic absorption spectrophotometry. Exchangeable H^+ was extracted with $BaCl_2$ and determined by titration. Base saturation defines the ratio between basic and total measured exchangeable cations (basic ions and H^+), and was used to define the share of basic cations within all of the measured exchangeable cations. The proportions of sand, silt, and clay textural fractions were determined by the pipette sedimentation method. Electrical conductivity of the soil was measured in soil:water = 1:5.

Statistical analysis: Normal distributions of the data were evaluated using Shapiro-Wilk tests. Homogeneity of variance from the means was analysed using the Levene's tests. One-way analysis of variance (*ANOVA*) and the Duncan's *post-hoc* multiple range tests were used to assess differences between the four sampling habitats for each measured parameter. Pearson's correlation analysis was performed to investigate the relationships between selected measured parameters. *IBM SPSS Statistics 22.0* was used for these statistical calculations, with significance accepted at $P \leq 0.05$.

The figures were drawn using *Microsoft Excel 2016*, and the pictures were taken under a light microscope with a digital camera (*XC30, Olympus*) using the *CellSens software* (*Olympus*).

Detrended correspondence analysis was used for the exploratory data analysis, with the *CANOCO for Windows 4.5* programme package. Due to the gradient lengths obtained (< 3 SD) (Ter Braak and Šmilauer 2002), redundancy analysis was used to determine whether leaf reflectance and transmittance of *D. caespitosa* was related with variations in leaf morphological and biochemical properties. The significance of the effects of the variables was determined using Monte Carlo tests with 999 permutations. Forward selection of the explanatory variables was used to avoid co-linearity. All of the variables used in the analysis were standardised.

Results

The unique morphology of the silicified cuticle, phytoliths, and prickle hairs of the *D. caespitosa* leaves is shown on Fig. 2 Suppl. The four habitats of the sampling sites were classified into two distinct groups according to leaf thickness: leaves from the river floodplain (Rak) and the intermittent lake (Cerknica) were significantly thinner than those from both the alpine heath (Komen summit) and the alpine foothills forest edge (Komen foothills) (Table 1 Suppl.). The shortest long prickle hairs were seen in plants from the intermittent lake, and the longest from the alpine heath. Leaves from the intermittent lake had significantly greater specific leaf area compared to those from the alpine heath. However, the largest specific leaf area was seen in plants from the alpine foothill forest edges, which was significantly greater than from all other habitats.

The thinnest cuticles were seen in plants from the river floodplain and the alpine foothills forest edge (Table 1 Suppl.). For the upper leaf surface, the cuticle was thickest in plants from the alpine heath. Plants

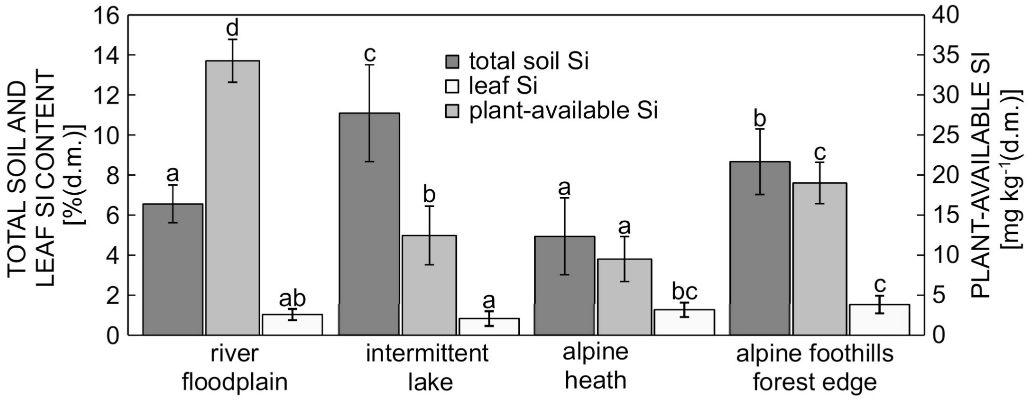

Fig. 1. Total silicon content in *Deschampsia caespitosa* leaves and in soil samples, and plant-available silicon content in the soil from four habitats. Means ± SDs, $n = 10$ for each habitat. Different letters above the columns indicate significant differences within each of the three parameters across the habitats ($P \leq 0.05$; the Duncan test).

from both of the alpine habitats showed significantly thicker leaf epidermis than from the river floodplain and especially intermittent lake. The stomata density only showed significant differences for the upper leaf surface, where it was lowest in plants from the alpine foothills forest edge. The plants from the intermittent lake showed the greatest stomata densities, although the differences were only significant when compared to the alpine heath. The upper leaf surface stomata length had a similar outcome to leaf thickness. For the lower leaf surface, the stomata length was significantly shorter in plants from the intermittent lake compared to the other three habitats. The upper leaf surface prickle hair density also grouped together the river floodplain and the intermittent lake, with significantly greater densities than on the leaves from the alpine heath and the alpine foothills forest edge. For the lower leaf surface, the prickle hair densities differed significantly between most of the habitats, except for the river floodplain and the alpine heath, where they were lowest. The highest prickle hair densities were seen in plants from the intermittent lake.

As for the leaf biochemical properties, the plants from the alpine foothills forest edge had significantly higher content of chlorophylls, carotenoids, and anthocyanins and significantly lower content of the protective UV-absorbing compounds compared to plants from other habitats (Table 1 Suppl.). Otherwise, the only significant differences were for the higher Chl *b* content in plants from the intermittent lake compared to the alpine heath, and for the higher content of the UV-A-absorbing compounds in plants from the alpine heath compared to the alpine foothills forest edge.

The leaf element composition showed significant differences between the four habitats for all of the elements studied, except for Cl (Table 1 Suppl.). As for the leaf Si content, the lower content was found in plants from river floodplain and the intermittent lake compared to both the alpine heath and the alpine foothills forest edge, with no significant difference between these latter two habitats. The leaf K and Ca content was highest in plants from the alpine foothills forest edge. The plants from alpine habitats had the lowest leaf Ca content and the second highest leaf K content. The plants from the river floodplain showed the highest leaf P content, followed by the alpine foothills forest edge, and finally the significantly lower leaf P content was found in plants from both the intermittent lake and the alpine heath. Leaf S content was highest in the plants from the alpine foothills forest edge, although significant difference was only *versus* the river floodplain and the alpine heath.

Plant-available Si ($CaCl_2$-extractable Si) content in the soil also differed significantly among the four habitats (Table 1 Suppl.). There were also significant differences among the four habitats in total soil element content. Total soil Si content was highest in plants from the intermittent lake. In plants from the river floodplain and the alpine heath, total soil Si content was similar, and it was significantly lower than in those from the alpine foothills forest edge. Total soil Ca content was significantly lower in plants from the alpine heath compared to the other three habitats. The total soil S content was significantly higher in soil from the alpine heath relative to the other three habitats. For total soil Cl, both of the alpine habitats had significantly higher content when compared to the intermittent lake. Total soil K content was lowest in intermittent lake and the alpine heath, followed by the river floodplain with intermediate total soil K content, and the highest in the alpine foothills forest edge. The comparisons between leaf and total soil Si content, and plant-available Si content are illustrated in Fig. 1. The comparisons between leaf and total soil Ca content are depicted in Fig. 2.

Soil structure analysis revealed marked differences between the four habitats (Table 2 Suppl.). However, the alpine heath stood out prominently from the other three sites with its heath character that defined its particular structural features: the low pH, high organic matter content, and low content of carbonates and Ca^{2+}. In contrast, the alpine foothills forest edge showed high content of carbonates and Ca^{2+}. The soils from the other three habitats primarily contained loam. Nevertheless, the soil samples from the river floodplain contained less sand and had a higher proportion of clay relative to the intermittent lake and alpine foothills forest edge. Soil organic matter content was lower in the intermittent lake compared to the river

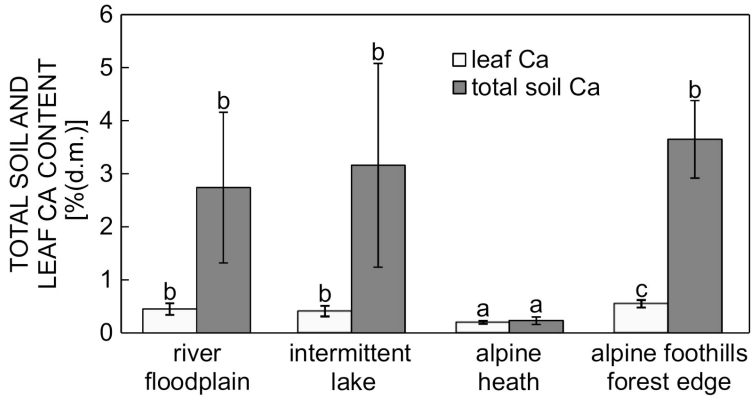

Fig. 2. Total calcium content in *Deschampsia caespitosa* leaves and in soil samples from four habitats. Means ± SDs, $n = 10$ for each habitat. Different letters above the columns indicate significant differences within each of the two parameters across the habitats ($P \leq 0.05$; the Duncan test).

floodplain; in contrast, electrical conductivity was highest for the soil of the river floodplain.

The reflectance spectra of the *D. caespitosa* leaves differed significantly across the four habitats, especially in the visible regions of the spectrum (Fig. 3, Table 3 Suppl.). In general, leaves from the alpine heath showed the highest reflectance from green to NIR. However, the differences in reflectance were not significant compared to the river floodplain for the red and NIR regions. The plants from alpine foothills forest edge showed the lowest leaf reflectance from green onwards, except for NIR. From UV-B to blue, leaf reflectance was highest in plants from the river floodplain, although the difference was significant only against those from the Mount Komen alpine habitats (except for the alpine foothills forest edge for the UV-A region).

The leaf transmittance spectra were generally similar among the plants from four sampling sites (Table 3 Suppl.). However, the alpine heath only differed significantly from the other three habitats from green to red. The intermittent lake generally showed the lowest leaf transmittance from violet onwards. Nonetheless, these differences were only significant compared to the alpine heath, and from blue onwards, and also when compared to the alpine foothills forest edge for NIR.

The redundancy analysis carried out for associations between leaf reflectance and biochemical leaf traits pointed out the following parameters as significant: Chl *a* content, which explained 28 % ($P = 0.001$), Chl *b*, which explained 15 % ($P = 0.002$), and carotenoids, explaining additional 10 % ($P = 0.005$) of the reflectance spectra variability. The second redundancy analysis was run for associations between leaf reflectance and morphological leaf traits (Fig. 4). It revealed that 19 % ($P = 0.001$) of

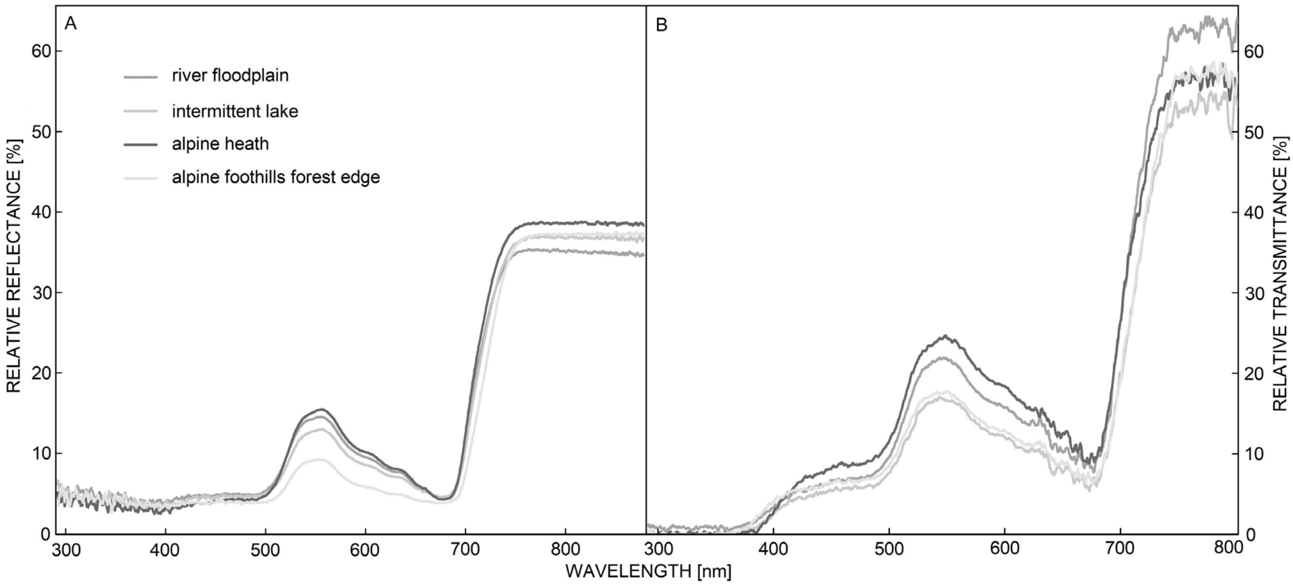

Fig. 3. Mean leaf reflectance spectra from 290 to 880 nm (*A*) and mean leaf transmittance spectra from 290 to 800 nm (*B*) for *Deschampsia caespitosa* plants from the four habitats. The data were smoothed using moving averages with a period of five consecutive measurements ($n = 10$).

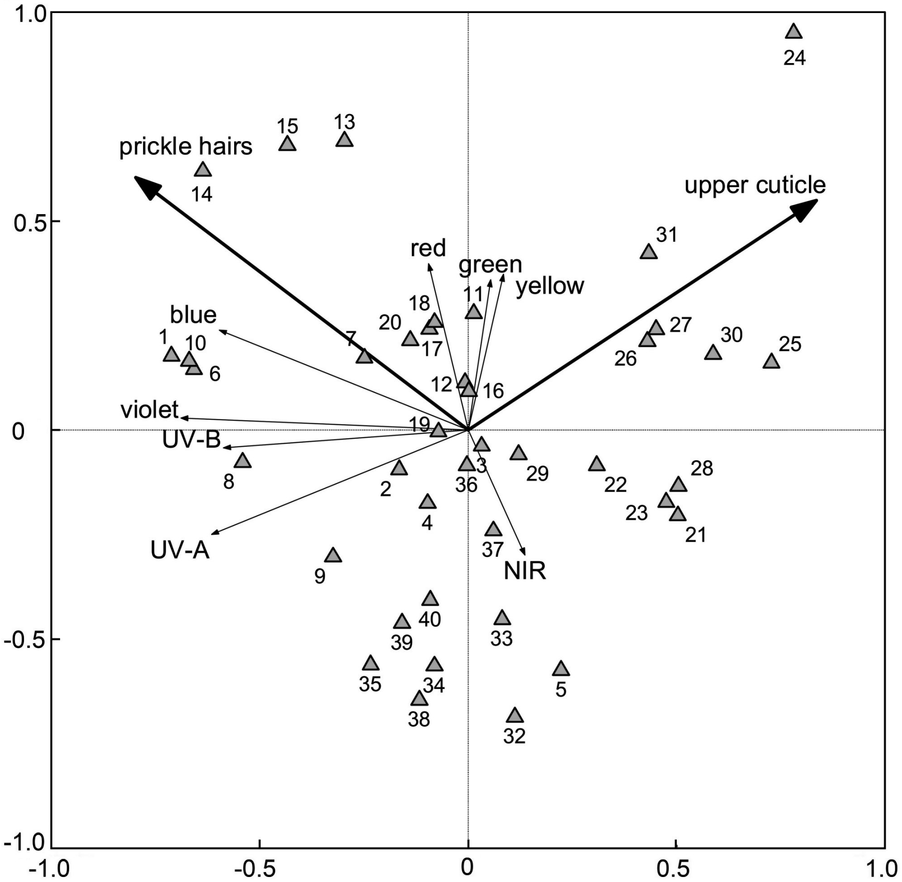

Fig. 4. A redundancy analysis plot showing the strength of the associations among leaf morphological parameters (*i.e.*, upper leaf surface prickle hair density and upper cuticle thickness), and the regions of reflectance spectra for *Deschampsia caespitosa* leaves. *Grey triangles* - samples from four different habitats: 1—10 - river floodplain (Rak); 11—20 - intermittent lake (Lake Cerknica); 21—30 - alpine heath (Mount Komen); 31—40 - alpine foothills forest edge (Mount Komen). Only the significant leaf parameters are shown.

the reflectance spectra variability was explained by upper leaf surface prickle hair density, and 12 % ($P = 0.002$) by upper cuticle thickness. The eigenvalues for the first two axes were 0.238 and 0.071, respectively. Here, the samples from the alpine heath (21 - 30), the alpine foothills forest edge (31 - 40), and the intermittent lake (11 - 20) formed three distinct groups, while the samples from the river floodplain (1 - 10) were more dispersed. Prickle hairs were positively related with leaf reflectance along the whole spectrum with the exception of the NIR region, while upper cuticle was negatively related with leaf reflectance at short wavelengths. This was also supported by Pearson's correlation analysis, where upper leaf surface prickle hair density turned out to be significantly positively related with leaf reflectance at short wavelengths, from UV-B to blue (UV-B, 0.514; UV-A, 0.429; violet, 0.604; blue, 0.560; $P \leq 0.01$). In the case of upper cuticle thickness, negative correlation with leaf reflectance was obtained from UV-B to violet (UV-B, -0.452; UV-A, -0.668; violet, -459; $P \leq 0.01$). Additional redundancy analysis was carried out for associations between leaf transmittance and other leaf traits. Only two parameters revealed to be significant there, namely long prickle hair density and short prickle hair length, which explained 12 % ($P = 0.016$) and 7 % ($P = 0.038$) of the transmittance spectra variability, respectively.

Considering the link between total soil and leaf element content for Si and Ca, Pearson's correlation analysis revealed strong positive correlation for Ca (0.559; $P \leq 0.01$) whereas no correlation was found for Si. Similarly, there was no correlation between plant-available Si content in the soil and leaf Si content. However, plant-available Si showed strong negative correlation with cuticle thickness (-0.651 for upper leaf surface and -0.569 for lower leaf surface; $P \leq 0.01$), and also turned out to be positively related with upper leaf surface prickle hair density (0.318; $P \leq 0.05$). Total soil Si displayed even stronger positive correlations with prickle hair density (0.439 for upper leaf surface and 0.583 for lower leaf surface; $P \leq 0.01$). In addition, total soil Si was negatively related with long prickle hair length (-0.627; $P \leq 0.01$). Strong negative correlation was seen between leaf Ca content and leaf reflectance from green to NIR (green, -0.627; yellow, -0.640; red, -0.576; NIR, -0.582; $P \leq 0.01$). Pearson's correlation analysis also revealed strong positive correlation between leaf Si content and the contents of some other elements in the leaves of *D. caespitosa*, namely for S (0.457; $P \leq 0.01$) and K (0.546; $P \leq 0.01$), but not for P, Cl, and Ca. In addition, plant-available Si showed significant positive correlation with leaf Ca (0.432; $P \leq 0.01$) and leaf P (0.757; $P \leq 0.01$).

Discussion

Deschampsia caespitosa colonises gleyed clay soils, brown soils, leached brown soils with mull or moder humus, calcimorphic brown soils, moderately acid to base-rich peats, chalk, and limestone soils with pH ranging from 3.7 to 8.3 (Davy 1980). The *D. caespitosa* plants examined in the present study showed wide variability in their habitat characteristics according to the soil structure analysis. The soil pH ranged from 4.1 for the alpine heath to 7.0 for the alpine foothills forest edge, while the soil organic matter content ranged from 6.6 % for the intermittent lake to 42.9 % for the alpine heath. The intermittent lake is affected by floodwater from an area with soluble Jurassic and Cretaceous limestone (Gaberščik *et al.* 2018). The soil from this habitat was the richest in total Si, which might have resulted from sedimentation of the Si-rich diatom remains in the intermittent lake. In spite of this, the plants growing there showed the lowest leaf Si content, which was partly a consequence of the low Si availability for these plants.

The importance of diatoms as a source of Si was shown previously by Desplanques *et al.* (2006), who measured 1 kg(Si) m^{-2} in a rice field in France, which originated from these freshwater algae in irrigation water. Even though the alpine heath lies on volcanic rock, it revealed significantly lower total soil Si content in comparison to the other three habitats. However, leaf Si content in the plants growing in both of the alpine sites (*i.e.*, alpine heath, alpine foothills forest edge) was higher than in those from both of the karst habitats (*i.e.*, river floodplain, intermittent lake). A high total soil Si content does not necessarily imply high availability of Si for plants, as most Si is insoluble and occurs in forms that are not available to plants (Balakhnina and Borkowska 2013). The content of plant-available Si in the soil differed significantly between the four habitats, with the highest value, 34.3 mg kg^{-1}, in the river floodplain, and the lowest, 9.5 mg kg^{-1}, in the alpine heath, which also displays the lowest soil pH. No correlation was seen between soil and leaf samples for Si, either for plant-available Si or total soil Si. Although the intermittent lake was the richest in total soil Si, the content of plant-available Si in this habitat was only 12.5 mg kg^{-1}.

Phytogenic cycling, namely the uptake of Si by plants, formation of phytogenic silica, and the subsequent return of this silica to the soil in the plant litter, are the main determinants of the Si concentration in soil solutions in natural forests and grasslands (Haynes 2014). In addition, geologically younger soils contain higher content of plant-available Si than highly weathered soils (Tubana *et al.* 2016). No correlation between soil and leaf samples for plant-available Si could be the consequence of the changes in its availability during the growing season, as the solubility of silica in the soil is affected by soil processes, soil pH, the presence of organic complexes and aluminium, iron, and phosphate ions, dissolution reactions, and soil moisture (Rao *et al.* 2017). In spite of the lower total soil Si content in the alpine heath soil from below the summit of Mount Komen, one would assume that the soil from this habitat was richer in plant-available Si, as it has been shown that the bioavailability of Si can increase with greater soil organic matter content (López-Pérez *et al.* 2018). However, this was not the case here. High soil organic matter content promotes high soil water capacity, which positively affects water availability for plants, increases transpiration flow, and thus the uptake of minerals, including Si (Grašič *et al.* 2019a,b). The solubility of plant-available Si increases markedly with increased pH (Milne *et al.* 2014, Klotzbücher *et al.* 2018). Miles *et al.* (2014) studied the relation between $CaCl_2$-extractable Si and soil pH for 112 soils, and showed that the content ranged from 10 to 100 mg kg^{-1} of soil dry matter as pH increased from 4 to 7.

The correlation between total soil and leaf Ca content was highly positive. Brackhage *et al.* (2013) showed that Si availability during plant growth affects Ca and micronutrient content in the leaves. Accordingly, strong positive correlation between plant-available Si in the soil and leaf Ca content was obtained in the present study, while no direct correlation was found between leaf Si and Ca content. Some other studies showeda negative correlation between these two elements (Klančnik *et al.* 2014b, Golob *et al.* 2017). Brackhage *et al.* (2013) reported about a decrease in Ca content at high Si availability by replacing Ca as a structural element with the more energy-efficient Si in order to avoid Ca stress. In this study, a positive correlation was obtained between leaf Si and S, and between leaf Si and K. This latter might be related with the important role of K in the transport of water and nutrients throughout the plant *via* the xylem (Prajapati and Modi 2012). Leaf K content showed the most pronounced differences, as it ranged from 1.0 to 2.9 % of leaf dry matter. Si decreases the permeability of cell membranes and improves the ability of plants to absorb K, thereby helping to maintain sufficient content of K in plants (Liang *et al.* 1996). There was no direct correlation between leaf Si and P in the present study. The results of Schaller *et al.* (2016), who studied different wetland plants, showed a positive correlation between Si and P, and a negative correlation between C and N. However, in our study leaf P was found to be strongly positively related with plant-available Si in the soil. It was shown previously in wheat that nutrient-use efficiency (including for P) was improved by Si availability, and the production of biomass was enhanced at a constant N supply in the substrate (Neu *et al.* 2017). In addition, Si fertilisation of soils with insufficient amount of P results in increased crop yield, as Si reduces the need of plants for P (Roy *et al.* 1971). Silicon may also be beneficial for plants at an excessive P content, which causes chlorosis. In the case of Si addition under sufficient or excessive supply of plants with P, Si negatively affects P uptake into plants by deposition in roots and reduction of transpiration rate (Ma and Takahashi 2002).

Differences in the habitat characteristics here resulted in differences in many leaf traits, among others in the leaf optical properties and the leaf content of other elements, as has also been shown in other studies (Téllez *et al.* 1998, Klančnik *et al.* 2014a,b). The leaves from the plants from the alpine heath stood out the most as they had the thickest cuticles, the highest content of UV-absorbing compounds, and the lowest reflectance at short wavelengths. Such high content of UV-absorbing compounds might be associated with increased UV-B radiation (*i.e.*, 290 to 320 nm) due to the altitude of the alpine heath (1 625 m a.s.l.), as compared to the river floodplain (515 m a.s.l.). It has been reported that UV-B radiation increases from 6 % (Caldwell *et al.* 1980, Roblek *et al.* 2008) to 20 % (Blumthaler *et al.* 1993) per 1 000 m of elevation.

The relationships between leaf reflectance and biochemical leaf traits were investigated using redundancy analysis. Most of the leaf reflectance spectra variability was explained by Chl *a*, Chl *b*, and carotenoid content. These relations are expected, since pigments play an important role in leaf reflectance (Klančnik *et al.* 2012). A second redundancy analysis, which was run to explain the relationship between the leaf reflectance spectra and morphological parameters, revealed the importance of upper leaf surface prickle hair density and upper cuticle thickness, as was also confirmed by Pearson's correlation analysis. The importance of prickle hairs in reflecting short wavelengths had already been proved by Klančnik *et al.* (2014a). The present analysis did not show direct relationships between leaf reflectance and leaf Si content. However, it is known for *D. caespitosa* that silicic acid accumulates in the outer epidermal cell walls of the leaves, in the cell vacuoles of the epidermal cells (as needle-shaped crystals), and in the intercellular spaces (Neumann *et al.* 1999). The highest amounts of Si in *D. caespitosa* leaves are accumulated in prickle hairs, as shown by Klančnik *et al.* (2014b). Our study demonstrated that plant-available Si was negatively related with both upper and lower leaf surface cuticle thickness, and positively with upper leaf surface prickle hair density. Garbuzov *et al.* (2011) demonstrated that the competitive balance between two grass species depends on the availability of Si in the soil, either directly through its species-specific effects on plant biomass, as well as indirectly through influencing herbivory patterns.

In conclusion, *D. caespitosa* revealed high phenotypic plasticity of leaf traits, including leaf content of biominerals. In contrast with expectations, leaf Si content did not correlate with plant-available Si content in the soil, while the correlation between total soil and leaf Ca content was highly positive. The absence of correlation between plant-available Si in the soil and leaf Si content may be a consequence of environmental effects on Si solubility, which may change during the season. To get a better insight into plant-available Si in the soil, we suggest monitoring of plant-available Si in the soil during the next experiment, especially at locations with changing water regime. Nevertheless, plant-available Si content in the soil was also related with specific silicified structures, namely cuticle and prickle hairs. Due to the substantial role of these structures in shaping leaf optical properties of plants, differences in Si availability in the soil might affect the fitness of plants in these specific environments. While no correlation was found between leaf Si and Ca, and between leaf Si and P, we obtained positive correlation between leaf Si and S, and between leaf Si and K. All of these findings reveal the importance of Si for leaf element stoichiometry. The outcomes of this research have wider importance, as they contribute to our knowledge about Si as a beneficial element from the aspects of plant ecology and biogeochemistry.

References

Balakhnina, T., Borkowska, A.: Effects of silicon on plant resistance to environmental stresses: review. - Int. Agrophys. **27**: 225-232, 2013.

Basile-Doelsch, I., Meunier, J.D., Parron, C.: Another continental pool in the terrestrial silicon cycle. - Nature **433**: 399-402, 2005.

Bauer, P., Elbaum, R., Weiss, I.M.: Calcium and silicon mineralization in land plants: transport structure and function. - Plant Sci. **180**: 746-756, 2011.

Blumthaler, M., Ambach, W., Huber, M.: Altitude effect of solar UV radiation dependent on albedo, turbidity, and solar elevation. - Meteorol. Z. **2**: 116-120, 1993.

Brackhage, C., Schaller, J., Bäucker, E., Dudel, E.G.: Silicon availability affects the stoichiometry and content of calcium and micronutrients in the leaves of common reed. - Silicon **5**: 199-204, 2013.

Brizuela, M.A., Detling, J.K., Cid, M.S.: Silicon concentration of grasses growing in sites with different grazing histories. - Ecology **67**: 1098-1101, 1986.

Caldwell, M.M.: Solar ultraviolet radiation as an ecological factor for alpine plants. - Ecol. Monogr. **38**: 243-268, 1968.

Caldwell, M.M., Robberecht, R., Billings, W.D.: A steep latitudinal gradient of solar ultraviolet-B radiation in the artic-alpine life zone. - Ecology **61**: 600.611, 1980.

Currie, H.A., Perry, C.C.: Silica in plants: biological, biochemical and chemical studies. - Ann. Bot. **100**: 1383-1389, 2007.

Davy, A.J.: Biological flora of the British Isles: *Deschampsia cespitosa* (L.) Beauv. - J. Ecol. **68**: 1075-1096, 1980.

Davy, A.J., Taylor, K.: Water characteristics of contrasting soils in the Chiltern Hills and their significance for *Deschampsia caespitosa* (L.) Beauv. - J. Ecol. **62**: 367-378, 1974.

De Camargo, M.S., Bezerra, B.K.L., Holanda, L.A., Oliveira, A.L., Vitti, A.C., Silva, M.A.: Silicon fertilization improves physiological responses in sugarcane cultivars grown under water deficit. - J. Soil Sci. Plant Nutr. **19**: 81-91, 2019.

Desplanques, V., Cary, L., Mouret, J.-C., Trolard, F., Bourrie, G., Grauby, O., Meunier, J.-D.: Silicon transfers in a rice field in Camargue (France). - J. Geochem. Explor. **88**: 190-193, 2006.

Dietrich, D., Hinke, S., Baumann, W., Fehlhaber, R., Bäucker, E., Rühle, G., Wienhaus, O., Marx, G.: Silica accumulation in *Triticum aestivum* L. and *Dactylis glomerata* L. - Anal. Bioanal. Chem. **376**: 399-404, 2003.

Drumm, H., Mohr, H.: The mode of interaction between blue (UV) light photoreceptor and phytochrome in anthocyanin formation of the *Sorghum* seedling. - Photochem. Photobiol. **27**: 241-248, 1978.

Epstein, E.: The anomaly of silicon in plant biology. - Proc. nat. Acad. Sci. USA **91**: 11-17, 1994.

Frew, A., Weston, L.A., Reynolds, O.L., Gurr, G.M.: The role of silicon in plant biology: a paradigm shift in research approach. - Ann. Bot. **121**: 1265-1273, 2018.

Gaberščik, A., Krek, J.L., Zelnik, I.: Habitat diversity along a hydrological gradient in a complex wetland results in high plant species diversity. - Ecol. Eng. **118**: 84-92, 2018.

Garbuzov, M., Reidinger, S., Hartley, S.E.: Interactive effects of plant-available soil silicon and herbivory on competition between two grass species. - Ann. Bot. **108**: 1355-1363, 2011.

Golob, A., Kavčič, J., Stibilj, V., Gaberščik, A., Vogel-Mikuš, K., Germ, M.: The effect of selenium and UV radiation on leaf traits and biomass production in *Triticum aestivum* L. - Ecotox. Environ. Safety **136**: 142-149, 2017.

Grašič, M., Malovrh, U., Golob, A., Vogel-Mikuš, K., Gaberščik, A.: Effects of water availability and UV radiation on silicon accumulation in the C_4 crop proso millet. - Photochem. Photobiol. Sci. **18**: 375-386, 2019a.

Grašič, M., Dobravc, M., Golob, A., Vogel-Mikuš, K., Gaberščik, A.: Water shortage reduces silicon uptake in barley leaves. - Agr. Water Manage. **217**: 47-56, 2019b.

Grime, J.P., Hodgson, J.G., Hunt, R.: The autecological accounts. - In: Grime, J.P., Hodgson, J.G., Hunt, R. (ed.): Comparative Plant Ecology: A Functional Approach to Common British Species. Pp. 220-222. Springer, Dordrecht 1988.

Haynes, R.J.: A contemporary overview of silicon availability in agricultural soils. - J. Plant Nutr. Soil Sci. **177**: 831-844, 2014.

He, H., Veneklaas, E.J., Kuop, J., Lambers, H.: Physiological and ecological significance of biomineralization in plants. - Trends Plant Sci. **19**: 166-174, 2014.

Hoffmann, G. (ed.): Methodenbuch Band I: Die Untersuchung von Böden. Ed. 4. - VDLUFA, Darmstadt 1991.

Kaufman, P.B., Dayanandan, P., Takeoka, Y., Bigelow, J.D., Jones, J.D., Iler, R.: Silica in shoots of higher plants. - In: Simpson, T.L., Volcani, B.E. (ed.): Silicon and Siliceous Structures in Biological Systems. Pp. 409-449. Springer, New York 1981.

Klančnik, K., Mlinar, M., Gaberščik, A.: Heterophylly results in a variety of "spectral signatures" in aquatic plant species. - Aquat. Bot. **98**: 20-26, 2012.

Klančnik, K., Vogel-Mikuš, K., Gaberščik, A.: Silicified structures affect leaf optical properties in grasses and sedge. - J. Photochem. Photobiol. B **130**: 1-10, 2014a.

Klančnik, K., Vogel-Mikuš K., Gaberščik, A.: Leaf optical properties are affected by the location and type of deposited biominerals. - J. Photochem. Photobiol. B **140**: 276-285, 2014b.

Klotzbücher, T., Klotzbücher, A., Kaiser, K., Merbach, I., Mikutta, R.: Impact of agricultural practices on plant-available silicon. - Geoderma **331**: 15-17, 2018.

Korndörfer, G.H., Coelho, M.N., Snyder, G.H., Mizutani, C.T.: Evaluation of soil extractants for silicon availability in upland rice. - Rev. bras. ciênc. Solo **23**: 101-106, 1999.

Kump, P., Nečemer, M., Rupnik, Z., Pelicon, P., Ponikvar, D., Vogel-Mikuš, K., Regvar, M., Pongrac, P. (ed.): Integration of Nuclear Spectrometry Methods as a New Approach to Material Research. IAEA, Vienna 2011.

Larcher, W. (ed.): Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups. Ed. 4. - Springer, Berlin 2003.

Liang, Y., Shen, Q., Shen, Z., Ma, T.: Effects of silicon on salinity tolerance of two barley cultivars. - J. Plant Nutr. **19**: 173-183, 1996.

Lichtenthaler ,H.K., Buschmann, C.: Extraction of photosynthetic tissues: chlorophylls and carotenoids. - Curr. Protocols Food anal. Chem. **1**: 165-170, 2001a.

Lichtenthaler, H.K., Buschmann, C.: Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. - Curr. Protocols Food anal. Chem. **1**: 171-178, 2001b.

López-Pérez, M.C., Pérez-Labrada, F., Ramírez-Pérez, L.J., Juárez-Maldonado, A., Morales-Díaz, A.B., González-Morales, S., García-Dávila, L.R., García-Mata, J., Benavides-Mendoza, A.: Dynamic modeling of silicon bioavailability, uptake, transport, and accumulation: applicability in improving the nutritional quality of tomato. - Front. Plant Sci. **9**: 647, 2018.

Lu, H., Zhang, J., Wu, N., Liu, K.B., Xu, D., Li, Q.: Phytoliths analysis for the discrimination of foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*). - PLoS ONE **4**: e4448, 2009.

Ma, J.F., Takahashi, E.: Soil, Fertilizer, and Plant Silicon Research in Japan. - Elsevier, Amsterdam 2002.

Miles, N., Manson, A.D., Rhodes, R., Van Antwerpen, R., Weigel, A.: Extractable silicon in soils of the South African sugar industry and relationships with crop uptake. - Commun. Soil Sci. Plant Anal. **45**: 2949-2958, 2014.

Milne, N.A., O'Reilly, T., Sanciolo, P., Ostarcevic, E., Beighton, M., Taylor, K., Mullett, M., Tarquin, A.J., Gray, S.R.: Chemistry of silica scale mitigation for RO desalination with particular reference to remote operations. - Water Res. **65**: 107-133, 2014.

Morikawa, C.K., Saigusa, M.: Mineral composition and accumulation of silicon in tissues of blueberry (*Vaccinum corymbosus* cv. Bluecrop) cuttings. - Plant Soil **258**: 1-8, 2004.

Nečemer, M., Kump, P., Ščančar, J., Jaćimović, R., Simčič, J., Pelicon, P., Budnar, M., Jeran, Z., Pongrac, P., Regvar, M., Vogel-Mikuš, K.: Application of X-ray fluorescence analytical techniques in phytoremediation and plant biology studies. - Spectrochim. Acta Part B At. Spectrosc. **63**: 1240-1247, 2008.

Neu, S., Schaller, J., Dudel, E.G.: Silicon availability modifies nutrient use efficiency and content, C:N:P stoichiometry, and productivity of winter wheat (*Triticum aestivum* L.). - Sci. Rep. **7**: 40829, 2017.

Neumann, D., Schwieger ,W., Lichtenberger, O.: Accumulation of silicon in the monocotyledons *Deschampsia caespitosa*, *Festuca lemanil* and *Schoenus nigricans*. - Plant Biol. **1**: 290-298, 1999.

Prajapati, K., Modi, H.A.: The importance of potassium in plant growth – a review. - Indian J. Plant Sci. **1**: 177-186, 2012.

Prychid, C.J., Rudall, P.J., Gregory, M.: Systematics and biology of silica bodies in monocotyledons. - Bot. Rev. **69**: 377-440, 2004.

Rao, G.B., Poornima, Yadav, P.I., Syriac, E.K.: Silicon nutrition in rice: a review. - J. Pharmacogn. Phytochem. **6**: 390-392, 2017.

Raven, J.A.: The transport and function of silicon in plants. - Biol. Rev. **58**: 179-207, 1983.

Ribera-Fonseca, A., Rumpel, C., Mora, M.L., Nikolic, M., Cartes, P.: Sodium silicate and calcium silicate differentially affect silicon and aluminium uptake, antioxidant performance and phenolics metabolism of ryegrass in an acid andisol. - Crop Pasture Sci. **69**: 205-215, 2018.

Roblek, M., Germ, M., Trošt Sedej, T., Gaberščik, A.: Morphological and biochemical variations in St. John's wort, *Hypericum perforatum* L., growing over altitudinal and UV-B radiation gradients. - Period. Biol. **110**: 257-262, 2008.

Roy, A.C., Ali, M.Y., Fox, R.L., Silva, J.A.: Influence of calcium silicate on phosphate solubility and availability in Hawaiian latosols. - In: Proceedings of the International Symposium on Soil Fertility Evaluation. Pp. 757-765. Indian Society of Soil Science, Indian Agricultural Research Institute, New Delhi 1971.

Sangster, A.G., Parry, D.W.: Some factors in relation to bulliform cell silicification in the grass leaf. - Ann. Bot. **33**: 315-323, 1969.

Schaller, J., Schoelynck, J., Struyf, E., Meire, P.: Silicon affects nutrient content and ratios of wetland plants. - Silicon **8**: 479-485, 2016.

St. John, L., Ogle, D.G., Darris, D., Parr, S.: Plant Guide for Tufted Hairgrass (*Deschampsia caespitosa*). - USDA-Natural Resources Conservation Service, Aberdeen 2011.

Strömberg, C.A.E., Di Stilio, V.S., Song, Z.: Functions of phytoliths in vascular plants: an evolutionary perspective. - Funct. Ecol. **30**: 1286-1297, 2016.

Téllez, T.R., Devesa, J.A., López, J.: Anatomical plasticity in species of *Deschampsia* P. Beauv. (*Poaceae*) in SW Europe (Iberian Peninsula). - Acta bot. gallica **145**: 281-305, 1998.

Ter Braak, C.J.F., Šmilauer, P. (eds.): CANOCO. Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (Version 4.5). - Microcomputer Power, Ithaca 2002.

Trembath-Reichert, E., Wilson, J.P., McGlynn, S.E., Fischer, W.W.: Four hundred million years of silica biomineralization in land plants. - Proc. nat. Acad. Sci. USA **112**: 5449-5454, 2015.

Tubana, B.S., Babu, T., Datnoff, L.E.: A review of silicon in soils and plants and its role in US agriculture: history and future perspectives. - Soil Sci. **181**: 393-411, 2016.

Vekemans, B., Janssens, K., Vincze, L., Adams, F., Vanespen, P.: Analysis of X-ray spectra by iterative least squares (AXIL): new developments. - X-ray Spectrom. **23**: 278-285, 1994.

Yoshida, S., Ohnishi ,Y., Kitagishi, K.: Chemical forms, mobility and deposition of silicon in rice plant. - Soil Sci. Plant Nutr. **8**: 15-21, 1962.