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RESEARCH PAPER

Early silicification of leaves and roots of seedlings of a panicoid grass grown under different conditions: anatomical relations and structural role

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ABSTRACT

- Grasses accumulate high amounts of silica deposits in tissues of all their organs, especially at mature stage. However, when and under which conditions do grass seedlings begin to produce these silica deposits and their relation with anatomy and development is little known. Here we investigated the silicification process in the first leaves and roots of seedlings of *Bothriochloa laguroides* grown in different substrate and Si treatments.
- The distribution and content of silica deposits in the organs of the seedlings grown under different conditions were analyzed through staining techniques and SEM-EDAX analyses.
- Leaf silica deposits were accumulated 3–4 days after the first leaf emergence, also under low silica solution (0.17–0.2 mM). Their location was mainly restricted to short costal cells from basal sectors, and scarcely in trichomes and xylem at tips. Silica content in leaves increased with the age of the seedlings. Roots presented dome-shaped silica aggregates, between 4–12 μm of diameter, located in the inner tangential wall of endodermal cells and similar to those produced at maturity.
- Silicification begins early in the first photosynthetic leaf, and silica distribution is opposite to that found in mature plants, mainly restricted to basal sectors, probably acting as a reinforcing element. The fast incorporation of solid amorphous silica in leaves and roots, may be useful for farm applications in species that are Si-fertilized.

INTRODUCTION

Plants incorporate amorphous silica in intra- or intercellular spaces, in cell walls, as well as on the cell surfaces to form the silica–cuticle double layer (Sangster *et al.* 1983; Lux *et al.* 2003; Zhang *et al.* 2013). Silicon is taken up by roots from the soil solution in the form of monosilicic acid and is transported through xylem as mono- and disilicic acid (Casey *et al.* 2003). The uptake and movement of silicic acid is mediated by proteinaceous transporters and also by passive diffusion, depending on the species involved (Mitani & Ma 2005; Ma *et al.* 2011). Regardless of the uptake system, silicic acid is translocated to the shoot *via* the xylem, where it is further concentrated by water loss through transpiration. Once the concentration exceeds 2 mM, it is polymerised, *i.e.* silicic acid is converted to colloidal silicic acid and finally to silica gel (Ma & Takahashi 2002).

Grasses, along with palms, sedges and horsetails, are the main producers of amorphous silica (Si) deposits (amorphous Si biomineralisations, Si phytoliths or silicophytoliths; Hodson *et al.* 2005; Gomes Coe *et al.* 2014; Katz 2015). In grasses the most frequently silicified tissue is the epidermis, however Si deposits can be found in the xylem, parenchyma, sclerenchyma and endodermis (Metcalfe 1960; Piperno 2006). Two groups of cells can be distinguished from the epidermis: those that are silicified early in plant development and in a regular way (silica short cells), and those that are silicified according to different factors such as phenology, transpiration rate and solar radiation (represented by bulliforms, long cells, stomatal complexes and trichomes; Blackman 1968; Takeoka *et al.* 1983; Motomura *et al.* 2000, 2002, 2006; Fernández Honaine & Osterrieth 2012; Fernández Honaine *et al.* 2013). Those silicophytoliths derived from short cells are considered as important palaeobotanical indicators in fossil records due to their taxonomic relevance, their early silicification and their good preservation in soils and sediments (*e.g.* Metcalfe 1960; Twiss 1992; Alexandre *et al.* 1997; Barboni *et al.* 2007).

Most studies related to the distribution of silicified cells in leaf tissues were carried on mature plants (*e.g.* Blackman 1968, 1969; Sangster 1970a,b, 1977; Motomura *et al.* 2002, 2004). The main results of these works were: (i) no silicification in plants grown in minimal Si solutions; (ii) Si deposit higher in sheaths than in blades; (iii) silicification acropetal in short cells and basipetal in prickles; and (iv) Si deposits occurred in relatively short time periods (between 8 and 20 days, depending on the species studied).

In order to advance knowledge of silicification processes and understand when and under which conditions grass seedlings

begin to produce Si deposits, we analysed silicification in the first leaves and roots of young seedlings of *Bothriochloa laguroides* D. C. Pilger grown under different conditions. *B. laguroides* is a native and abundant C_4 panicoid species with a wide distribution within the Pampean region (Argentina) and is also commonly present in grasslands of North and South America (Vega 2000). The leaves of *Bothriochloa* spp accumulate high amounts of silicophytoliths in different cell types, such as short cells, bulliforms, prickles and long cells (Gallego & Distel 2004; Fernández Honaine *et al.* 2006). Root tissues also deposit amorphous Si in endodermal sectors, as do all species in the Andropogoneae (Lux *et al.* 2003). Soil Si availability conditions Si accumulation in mature leaves of *B. laguroides*, and silicification of some cell types is related to ageing. The content of total silicified cells increases in older leaves and the proportion of silicified bulliform cells varies according to leaf position and section, and site collection (Fernández Honaine & Osterrieth 2012). Considering these previous findings, the present work aims to answer the following questions: (i) does the first leaf developed from *B. laguroides* caryopses produce silicified cells and how long does it take to do so; (ii) is there a relationship between the distribution of silicified cells and the growth, elongation and maturation sites in young leaves; and (iii) as mature plants produce Si deposits in bulliform cells of leaves and in endodermal cells of roots, does this also occur in young seedlings?

MATERIAL AND METHODS

Seed collection and germination conditions

Bothriochloa laguroides (Panicoideae) caryopses were obtained from plants collected in the Reserva Integral Laguna de los Padres, Buenos Aires province, Argentina, during summer 2011/2012. Caryopses were placed in paper bags and stored until the study was carried in November 2013. After surface sterilisation with 30% (v/v) hypochlorite for 15 min, caryopses were washed with de-ionised water and germinated on two different substrates (trial A: cotton and napkin, trial B: river sand) in an environment-controlled chamber at a 16-h light/8-h dark regime, at 25 °C and 70% relative humidity. Each substrate was placed in clear plastic trays of 16 cm × 10 cm, weighed and supplied with different Si solutions: 0 mM (distilled water), 0.5 mM and 1.8 mM Si. The solutions were prepared from sodium metasilicate (Na_2O_3Si). In trial A, 30 ml Si solution or distilled water was supplied per tray. In trial B, 300 g sand and 20 ml Si solution or distilled water was supplied per tray. In each trial 30 caryopses (including glumes) were sown, and each tray was wrapped in low- density polyethylene bags that were sealed to avoid water loss through evaporation.

Before the experiment, and in order to assess the Si content available for seedlings, Si content of the substrates was measured with UV-VIS spectrophotometry using the silicomolybdate method (American Public Health Association 1998). The values obtained were: 0.2 ± 0.1 mM Si for cotton/napkin substrate and 0.17 ± 0.1 mM Si for river sand substrate.

Seedling harvest and Si identification in tissues

Seedlings were collected at different stages (emergence of the first, second and, in cotton/napkin substrate, third leaf). Seedlings from cotton/napkin substrate were collected on days 8, 12 and 16 after sowing, while seedlings from river sand were collected on days 4, 7 and 11 after sowing. The organs collected were cleaned with distilled water, cleared with 50% (w/v) sodium hypochlorite solution, dehydrated in an ethanol series, stained with phenol crystals (slightly diluted in ethanol) and mounted in immersion oil. Phenol crystals stain Si in the cells a rose colour (Johansen 1940; Fernández Honaine & Osterrieth 2012). The stained organs (leaves and roots) were described using a Zeiss Axiostar Plus microscope at 400× magnification. Photographs were taken with a digital camera (Canon Powershot G10, Canon Inc., Tokyo, Japan). In each leaf a longitudinal transect along the entire leaf (blade and sheath) was traced. In some cases the transect did not cover the entire width, so two transects were traced (one in the middle and the other on the edge of the leaf). The number and type of silicified cells and the number of fields included in the transects were also counted. The number of silicified cells per mm^2 of leaf was calculated in two plants from each substrate, treatment and collection day (a total of 36 seedlings). Differences in the number of silicified cells per mm^2 in the first leaf of seedlings between substrates were analysed through a *t*-test. For each substrate treatment differences in the number of silicified cells per mm^2 in the first leaf of seedlings between silica treatment and day of harvest were analysed through a two-factor ANOVA.

Some leaf and root samples were observed under SEM (JEOL JSM-6460 LV; Tokyo, Japan) at Universidad Nacional de Mar del Plata, Argentina. Leaf samples were first fixed for 12 h with 3% glutaraldehyde, pH 7.2–7.4 phosphate buffered solution, followed by dehydration through an alcohol series (50, 70, 80, 90, 95 and 100%). Finally, samples were dried in HMDS (hexamethyldisilazane), mounted on aluminium discs and coated with gold. Other leaf samples and the roots were only dried at 65 °C for 24 h and observed under SEM, after gold coating. For root observation, the peripheral cortical tissues were mechanically removed with a razor blade, and the stele covered by endodermal walls was observed with SEM. The composition of the silicophytoliths was analysed using X-ray energy dispersive spectroscopy (EDS). The system used was an EDAX Genesis XM4-Sys 60, equipped with multichannel analyser EDAX mod EDAM IV, Sapphire Si (Li) detector and super ultra-thin window of Be, and EDAX Genesis version 5.11 software (Tokyo, Japan).

RESULTS

Leaf silicification

In both substrates, the emergence of the coleoptile and first leaf was observed 3–4 days after sowing. Seedling growth in cotton/napkin was slower than growth in river sand, however no statistical differences in the height of seedlings between substrate and/or Si treatment were detected (data not shown).

No Si deposition was found in coleoptiles. Silicified cells were observed in leaves of 26 out of 34 seedlings. No silicified cells were detected in leaves collected on day 4 in sand substrate (six plants) or in two seedlings collected on day 8 in cotton/napkin substrate. These results show that 7 days (in river sand treatment) and 8 days (in cotton/napkin treatment) after sowing (ca. 4 days after first leaf emergence) the leaves of seedlings of *B. laguroides* were capable of cell silicification, irrespective of

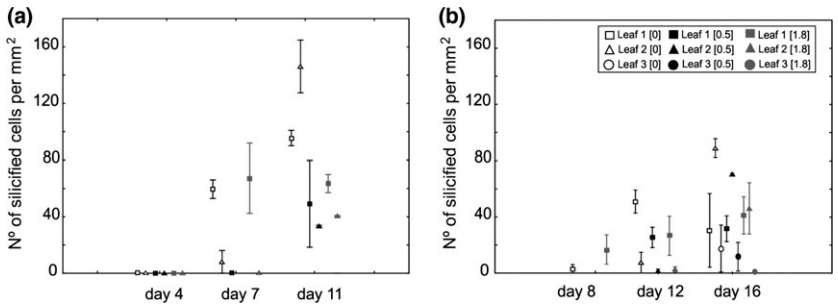

Fig. 1. Mean number of silicified cells per mm^2 in first, second and third leaves of *B. laguroides* seedlings grown in river sand (a) or cotton/napkin substrates (b), collected on different days and developed under different Si treatments: [0] = distilled water; [0.5] = 0.5 mM Si; [1.8] = 1.8 mM Si. Values are the mean of two individuals.

the Si solution treatment (Fig. 1). At the end of the experiment, the content of Si deposits in the first leaf of seedlings grown in river sand substrate was higher than in those grown in cotton/napkin substrate ($t = 2.3708$, $P < 0.05$).

Two-factor ANOVA showed that seedlings grown in river sand substrate have differences in the number of silicified cells in the first leaves between collection day and Si treatments ($P < 0.05$). The first leaf of seedlings grown in 0.5 mM Si produced no or a very low number of silicified cells per mm^2 than in the other Si treatments (Table 1, Fig. 1), probably due to some interaction between the substrate and the Si solution. In cotton/napkin treatment differences were observed between days ($P < 0.05$). The number of silicified cells per mm^2 increased with the age of the plants. A two-factor ANOVA was carried on second leaves of plants developed in cotton/napkin substrate, and only in the factor 'day of collection' were the differences significant ($F = 85.58$, $P < 0.001$ for day of collection factor; $F = 3.84$, $P = 0.08$ for Si treatment factor). In second leaves of plants developed in river sand substrate, only day 11 was evaluated. Kruskal–Wallis tests showed no differences between Si treatments ($X^2 = 4.57$, $P = 0.101$). The third leaf was only evaluated in plants from day 16, and no statistical differences were observed between Si treatments (Kruskal–Wallis, $X^2 = 3.42$, $P = 0.18$). In general, the fact that the additional applied Si concentration had no effect on total silicophytolith content may be simply interpreted as the response was already saturated by the background (substrate) concentration of 0.17–0.2 mM. However, considering that individuals evaluated in this study were young, it is possible that differences between Si treatments did not increase due to the age of the plants. Future studies including older plants will be necessary to corroborate these findings.

The cells that were silicified were the costal epidermal short cells, mostly located in the abaxial epidermis, and in middle as well as in edge sectors of leaves (Fig. 2). In the first leaf of 24 seedlings (from a total of 26 collected in different substrate and Si treatments), the silicified cells were mainly distributed at the base of the leaf, *i.e.* the sheath, ligule sector and part of the blade base (Figs 2, 3). In the second and third leaves, especially in those plants where the ligule was not defined, no clear silicification gradient was observed. In older leaves also the xylem and prickles were silicified at the tips and/or the edges of leaves (Fig. 2d, h).

Table 1. Two-factor ANOVA of the number of silicified cells per mm^2 in the first leaf of seedlings grown in river sand (a) or napkin/cotton (b) substrates.

Factor	Sum of square	df	Mean square	F	*P* (F)	F ($P = 0.05$)
(a)						
Collection day	14662.55	2	7331.27	19.78	0.0005**	4.26
Si treatment	4100.21	2	2050.11	5.53	0.0271*	4.26
Interaction	3559.75	4	889.94	2.40	0.1266	3.63
Error	3335.37	9	370.60			
Total	25657.87	17				
(b)						
Collection day	3208.73	2	1604.37	5.22	0.031*	4.26
Si treatment	335.18	2	167.59	0.54	0.598	4.26
Interaction	958.03	4	239.51	0.78	0.566	3.63
Error	2767.56	9	307.51			
Total	7269.50	17				

*Significant difference at $P < 0.05$.
**Significant difference at $P < 0.01$.

Root silicification

Silica deposits were identified in the basal section of roots of the oldest seedlings in both substrates (Fig. 2i, j). They were observed in seedlings collected from cotton/napkin substrate on day 12 in 1.8 mM Si treatment and on day 16 in all Si concentrations; also in seedlings from river sand substrate collected on day 11 in 1.8 mM Si treatment. These Si aggregates were dome-shaped, with a rugose/slightly granular surface. The diameter of these aggregates ranged between 3.98 and 12.4 μm, with a median of 7.34 (n = 40 aggregates measured in seven root fragments under SEM). They were regularly distributed in one, sometimes two, rows along the inner tangential wall of endodermal cells. Between three and seven silica aggregates were observed in each endodermal cell, depending on the length of the cell.

DISCUSSION

Silicification in the first leaves

Even though other research in grasses has shown early accumulation of amorphous Si in short cells of young leaves, those studies were on mature plants or seedlings developed from tillers, where probably other environmental/biological factors might affect the silicification process (Sangster 1970a, 1977; Motomura *et al.* 2006). The present study shows, in general terms, that the silicification process occurs in the first leaves of seedlings developed from caryopses collected in the field, between 7 and 8 days after sowing (*i.e.* as early as 3–4 days after first leaf emergence), regardless of substrate or Si treatment. It also proves that the ability for silicification is present in the first photosynthetic leaf, where numerous

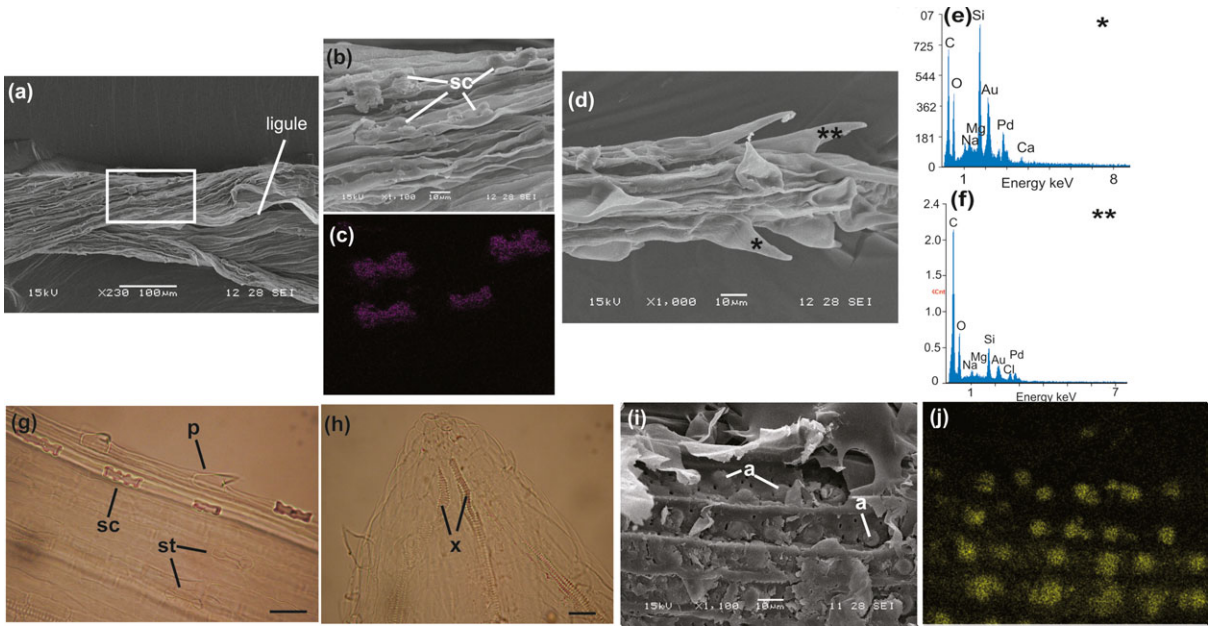

Fig. 2. Photographs of the epidermis of leaves and roots of *B. laguroides* seedlings taken under optical (g, h) and scanning electron (a–d, i–j) microscopes. a: Localization of Si cells (sc) in seedlings grown in river sand, [0] mM Si treatment and collected on day 11. b: Detail of the rectangle of (a). c: Mapping of Si of the sector shown in (b). D: Prickles located at the tip of the leaf in a seedling grown in river sand and collected on day 11. e, f: EDAX analysis of prickles marked with * in (d). g: Silica cells stained with phenol in leaves of seedlings grown in cotton/napkin substrate, [0.5] mM Si treatment and collected on day 12. h: Silicification of xylem at the tip of the leaf, in seedlings grown in river sand and collected on day 11. I: Silica aggregates (a) located in the inner tangential wall of endodermis in roots from seedlings grown in napkin/cotton substrate. j: Mapping of Si of the sector shown in (i). p: prickles; sc: silica cells, st: stomatal complexes; x: xylem. Scale bar in optical photographs: 20 μm.

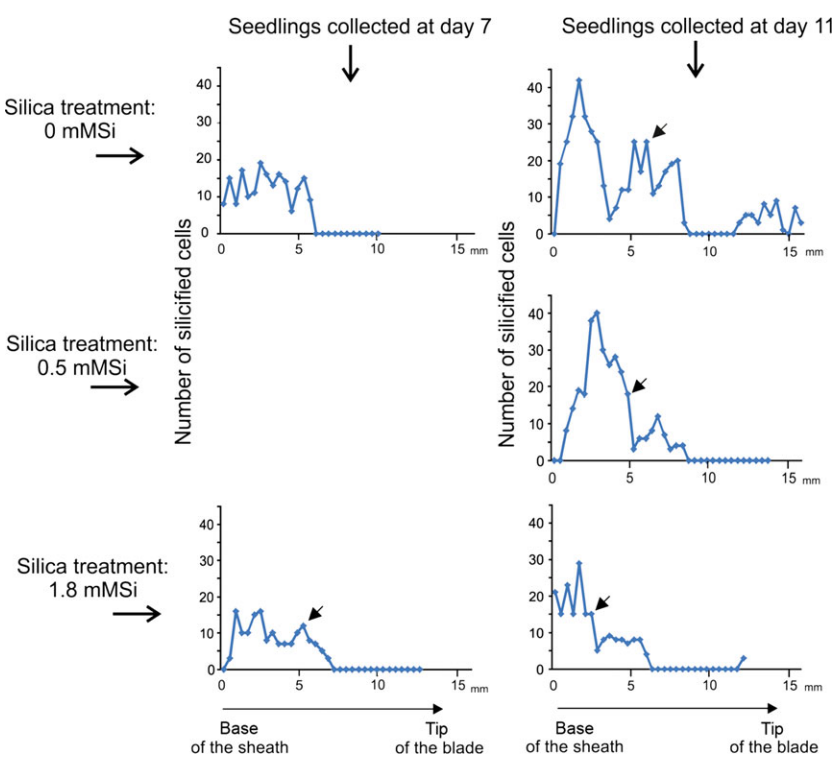

Fig. 3. Distribution of silicified cells along the first leaf of seedlings grown in river sand treatments and collected on days 7 and 11. Each value represents the number of silicified cells counted in a microscope field (see Methods section) in one plant. Arrow indicates the location of ligule (not present in leaf of day 7 and 0 mM Si solution).

metabolic processes are simultaneously generated (Taiz & Zeiger 2002). A process occurring so early in plant development is likely to have some importance, probably in relation to reinforcement of the structure in the first fragile leaves. Even though the first developmental stages of a plant may demand a significant investment of energy in order to ensure the success of photosynthesis, the silicification process also occurs. This means that this last process may be important

or necessary for plant development. The silicification process seems to be unaffected by the type of substrate or Si treatment, but rather by the time elapsed since leaf emergence. As time progresses, more short cells accumulate amorphous Si, until all of them are filled and, probably, other type of cell start to be silicified (trichomes).

The silicification pattern observed in the first leaves (dominance of silicified short cells at the base and some prickles at tips) is coincident with the description of Sangster (1977), who noted that Si deposits in short cells are acropetal while prickle deposits are basipetal. In mature plants, the distribution of silicified cells is opposite to that observed in young seedlings: the content of silicified cells increased towards the tip of the leaf (Blackman 1968; Motomura *et al.* 2006; Fernández Honaine & Osterrieth 2012). In mature leaves, practically all short Si cells have already been silicified and the gradient of silicification occurs because of the Si deposits in other cells, such as long cells, bulliforms, prickles and hooks, stomatal complexes, *etc.* (Motomura *et al.* 2006; Fernández Honaine & Osterrieth 2012). In this case, the higher Si content of silicified cells in the apical leaf sectors may be the consequence of a higher transpiration rate (due to higher solar radiation) and/or age. Instead, in young seedlings, the distribution of silicified cells may be due to the role that the first leaf plays in successful plant growth. Once the first leaf has emerged, it is the only organ capable for photosynthesis and is the responsible for developmental and growth initiation of subsequent leaves. The first leaf must elongate so as to reach the light and initiate the photosynthetic process. If Si is deposited in the lumen of cells from apical sectors, it is probable that elongation will be restricted and, hence, photosynthetic processes limited. Instead, it can be proposed that the first Si absorbed could account for elongation of cell walls of the first sectors (apical) of the leaf (Hossain *et al.* 2002, 2007). The presence of silicified cells in basal sectors and the sheath could also constitute a reinforcement structure, for the leaf as well as for the whole young plant. Raven (1983) suggested that Si deposition is a cheaper investment than lignin, so silicification in basal sectors would be a good strategy as a supporting structure in fragile and young plants.

Root silicification

Silica accumulation in the root endodermis of grasses has been described (*e.g.* Lux *et al.* 1999, 2003; Montti *et al.* 2009). Silica aggregates are a common feature of Andropogoneae and have also been associated with drought tolerance (Lux *et al.* 2002). Such deposits have been observed in *Sorghum* spp. and in mature plants of *B. laguroides* (*e.g.* Sangster & Parry 1976; Lux *et al.* 2002, 2003; M. Fernández Honaine, M. Paolicchi, M. Osterrieth, M.L. Benvenuto, unpublished). In the present study, Si aggregates were found in the oldest individuals (harvest on day 12 and 16 after sowing), in all Si treatments, indicating that Si accumulation in roots begins at early stages of plant development, and resembles mature plant morphologies.

Growing conditions and silicification

Our results show that Si accumulation in leaves and roots of seedlings of *B. laguroides* occurs even at low Si availability (0.17–0.2 mM Si), unlike previous studies (Sangster 1970a; Lux *et al.* 2003). These values represent the lower limit of the range of values commonly found in soils (0.1–0.6 mM; Epstein 2001). Caryopses were collected from plants grown on typic Argiudoll soils, which developed from quaternary loessic sediments, in the Pampean region (Borrelli *et al.* 2010). The average concentration of Si in the soil solutions is 0.7 mM, with a range between 0.4 and 1.25 mM Si (Borrelli *et al.* 2010). These data indicate that although normal development of this species occurs on soils with higher Si concentration, the production of silicophytoliths is also possible under conditions of low availability of this element.

In summary, the present study shows the ability of the first photosynthetic leaves of seedlings of *B. laguroides* to deposit amorphous Si in their tissues, even under lower Si availability conditions than those found in soils where it normally grows. It was shown that the accumulation of Si gradually increased over time and there were no effects of different silica/substrate treatments. The pattern of distribution of silicified cells in the first photosynthetic leaves is opposite to that found in leaves of mature plants, probably due to the role that early leaves play in development and growth. Instead, silicification of roots resembles the pattern observed in older roots. The rapid incorporation of solid amorphous Si in leaves and roots, even when Si availability is low, is useful knowledge for farm applications, especially for those species that are Si fertilised.

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REFERENCES

Alexandre A., Meunier J., Lezine A., Vincens A., Schwartz D. (1997) Phytoliths: indicators of grasslands dynamics during the late Holocene in intertropical Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **136**, 213–229.

APHA–AWWA–WPFC (American Public Health Association–American Water Works Association– Water Pollution Control Federation) (1998) *Keys to soil taxonomy*. 23rd edition. APHA, Washington, DC, USA.

Barboni D., Bremond L., Bonnefille R. (2007) Comparative study of modern phytolith assemblages from inter-tropical Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **246**, 454–470.

Blackman E. (1968) The pattern and sequence of opaline silica deposition in rye (*Secale cereale* L.). *Annals of Botany*, **32**, 207–218.

Blackman E. (1969) Observations on the development of the silica cells of the leaf sheath of wheat (*Triticum aestivum*). *Canadian Journal of Botany*, **47**, 827–838.

Borrelli N., Alvarez M.F., Osterrieth M.L., Marcovecchio J.E. (2010) Silica content in soil solution and its relation with phytolith weathering and silica biogeochemical cycle in Typical Argiudolls of the Pampean Plain, Argentina – a preliminary study. *Journal of Soils and Sediments*, **10**, 983–994.

Casey W.H., Kinrade S.D., Knight C.T.G., Rains D.W., Epstein E. (2003) Aqueous silicate complexes in wheat, *Triticum aestivum* L. *Plant, Cell and Environment*, **27**, 51–54.

Epstein E. (2001) Silicon in plants: facts vs. concepts. In: Datnoff L.E., Snyder G.H., Korndörfer G.H., (Eds.) *Silicon in agriculture*. Elsevier, Amsterdam, The Netherlands, pp 1–17.

Fernández Honaine M., Osterrieth M. (2012) Silicification of the adaxial epidermis of leaves of a panicoid grass in relation to leaf position and section and

environmental conditions. *Plant Biology*, **14**, 596–604.

Fernández Honaine M., Zucol A., Osterrieth M. (2006) Phytolith assemblage and systematic association in grassland species of the SE Pampean Plains, Argentina. *Annals of Botany*, **98**, 1155–1165.

Fernández Honaine M., Borrelli N., Osterrieth M., del Río L. (2013) Amorphous silica biomineralizations in sedges: their relation with senescence and silica availability. *Boletín de la Sociedad Argentina de Botánica*, **48**, 247–259.

Gallego L., Distel R.A. (2004) Phytolith assemblages in grasses native to Central Argentina. *Annals of Botany*, **94**, 865–874.

Gomes Coe H.H., Osterrieth M., Fernández Honaine M. (2014) Chapter 1: Phytoliths and their applications. In: Gomes Coe H., Osterrieth M., (Eds.) *Synthesis of some phytolith studies in South America (Brazil and Argentina)*. Series Botanical research and practices Nova Science Publishers, New York, USA, pp 1–26.

Hodson M.J., White P.J., Mead A., Broadley M.R. (2005) Phylogenetic variation in the silicon composition of plants. *Annals of Botany*, **96**, 1027–1046.

Hossain M.T., Mori R., Soga K., Wakabayashi K., Kamisaka S., Fujii S., Yamamoto R., Hoson T. (2002) Growth promotion and an increase in cell wall extensibility by silicon in rice and some other Poaceae seedlings. *Journal of Plant Research*, **115**, 23–27.

Hossain M.T., Soga K., Wakabayashi K., Kamisaka S., Fujii S., Yamamoto R., Hoson T. (2007) Modification of chemical properties of cell walls by silicon and its role in regulation of the cell wall extensibility in oat leaves. *Journal of Plant Physiology*, **164**, 385–393.

Johansen D.A. (1940) *Plant microtechnique*. McGraw-Hill, New York, USA.

Katz O. (2015) Silica phytoliths in angiosperms: phylogeny and early evolutionary history. *New Phytologist*, **208**, 642–646.

Lux A., Luxová M., Morita S., Abe J., Inanaga S. (1999) Endodermal silicification in developing seminal roots of lowland and upland cultivars of rice (*Oryza sativa* L.). *Canadian Journal of Botany*, **77**, 955–960.

Lux A., Luxova M., Hattori T., Inanaga S., Sugimoto Y. (2002) Silicification in sorghum (*Sorghum bicolor*) cultivars with different drought tolerance. *Physiologia Plantarum*, **115**, 87–92.

Lux A., Luxová M., Abe J., Tanimoto E., Hattori T., Inanaga S. (2003) The dynamics of silicon deposition in the sorghum root endodermis. *New Phytologist*, **158**, 437–441.

Ma J.F., Takahashi E. (2002) *Soil, fertilizer, and plant silicon research in Japan*. Elsevier, Amsterdam, The Netherlands.

Ma J.F., Yamaji N., Mitani-Ueno N. (2011) Transport of silicon from roots to panicles in plants. *Proceedings of the Japanese Academy, Series B*, **87**, 377–385.

Metcalfe C.R. (1960) *Anatomy of monocotyledons I. Gramineae*. Claredon Press, Oxford, UK.

Mitani N., Ma J.F. (2005) Uptake system of silicon in different plant species. *Journal of Experimental Botany*, **56**, 1255–1261.

Montti L., Fernández Honaine M., Osterrieth M., Graciano Ribeiro.D. (2009) Phytolith analysis of *Chusquea ramosissima* Lindm. (Poaceae: Bambusoideae) and associated soils. *Quaternary International*, **193**, 80–89.

Motomura H., Fujii T., Suzuki M. (2000) Distribution of silicified cells in the leaf blades of *Pleioblastus chino* (Franchet et Savatier) Makino (Bambusoideae). *Annals of Botany*, **85**, 751–757.

Motomura H., Mita N., Suzuki M. (2002) Silica accumulation in long-lived leaves of *Sasa veitchii* (Carriere) Rehder (Poaceae: Bambusoideae). *Annals of Botany*, **90**, 149–152.

Motomura H., Fujii T., Suzuki M. (2004) Silica deposition in relation to ageing of leaf tissues in *Sasa veitchii* (Carriere) Rehder (Poaceae: Bambusoideae). *Annals of Botany*, **93**, 235–248.

Motomura H., Fujii T., Suzuki M. (2006) Silica deposition in abaxial epidermis before the opening of leaf blades of *Pleioblastus chino* (Poaceae, Bambusoideae). *Annals of Botany*, **97**, 513–519.

Piperno D.R. (2006) *Phytoliths: a comprehensive guide for archaeologists and paleoecologists*. AltaMira Press, San Diego, CA, USA.

Raven J.A. (1983) The transport and function of silicon in plants. *Biological Reviews*, **58**, 179–207.

Sangster A.G. (1970a) Intracellular silica deposition in immature leaves in three species of the Gramineae. *Annals of Botany*, **34**, 245–257.

Sangster A.G. (1970b) Intracellular silica deposition in mature and senescent leaves of *Sieglingia decumbens* (L.) Bernh. *Annals of Botany*, **34**, 557–570.

Sangster A.G. (1977) Characteristics of silica deposition in *Digitaria sanguinalis* (L.) Scop. (crabgrass). *Annals of Botany*, **41**, 341–350.

Sangster A.G., Parry D.W. (1976) Endodermal silicification in mature, nodal roots of *Sorghum bicolor* (L.) Moench. *Annals of Botany*, **40**, 373–379.

Sangster A.G., Hodson M.J., Wynn Parry D. (1983) Silicon deposition and anatomical studies in the inflorescence bracts of four *Phalaris* species with their possible relevance to carcinogenesis. *New Phytologist*, **93**, 105–122.

Taiz L., Zeiger E. (2002) *Plant physiology* 3rd edn. Sinauer Associates, Sunderland, MA, USA.

Takeoka Y., Matsumura O., Kaufman P.B. (1983) Studies of silicification of epidermal tissues of grasses as investigated by soft X-ray image analysis. I. On the method to detect and calculate frequency of silica bodies on bulliform cells. *Japanese Journal of Crop Science*, **52**, 544–550.

Twiss P.C. (1992) Predicted world distribution of C_3 and C_4 grass phytoliths. In: Rapp G., Mulholland S.C. (Eds), *Phytolith systematics*. Plenum Press, New York, USA, pp 113–128.

Vega A.S. (2000) Revisión taxonómica de las especies americanas del género *Bothriochloa* (Poaceae: Panicoideae: Andropogoneae). *Darwiniana*, **38**, 127–186.

Zhang C., Wang L., Zhang W., Zhang F. (2013) Do lignification and silicification of the cell wall precede silicon deposition in the silica cell of the rice (*Oryza sativa* L.) leaf epidermis? *Plant and Soil*, **372**, 137–149.