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RESEARCH PAPER

Silicification of the adaxial epidermis of leaves of a panicoid grass in relation to leaf position and section and environmental conditions

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ABSTRACT

Many studies relate silica content in plants with internal or external factors; however, few works analyse the effect of these factors on the silicification of different cell types. In this study, we examined the effect of leaf section and leaf position, and environmental conditions on the percentages of silicified epidermal cells of a native Pampean panicoid grass, *Bothriochloa laguroides* D. C. Pilger. Two different environmental situations were selected for the collection of plants: a natural wetland and a quartzite quarry, located in the southeast Buenos Aires province, Argentina. Clarification and staining methodologies were applied so as to study the distribution of silicified cells in different sections of leaves of the plants collected. Two and three-factor anovas were applied to the data. Between 13% and 19% of total cells of the adaxial epidermis of leaf blades were silicified. Typical silica short cells were the largest contributor to total silicified cells (53–98%), while the second largest contributor was bulliform cells (0–30%). Percentages of total silicified cells were higher in superior than in inferior leaves, while values from leaf sections varied. When collection sites were compared, plants growing in Los Padres pond, where the silica content in soils is higher, had the higher percentage of silicified cells. Among all types of cell, bulliform cells showed differences in the proportion of silicified cells between leaf position and section and collection site. These results show that silica availability in soils is an important factor that conditions silica accumulation and overlaps with the transpiration effect.

INTRODUCTION

Silica accumulation is a process commonly observed and well documented in grasses (*e.g.* Metcalfe 1960). In general, silica biomineralizations, also known as silicophytoliths, opal phytoliths or silica bodies, are more abundant in epidermal tissues; however, the parenchyma, sclerenchyma, xylem and phloem tissues have been found to be silicified in diverse species (*e.g.* Metcalfe 1960; Jones *et al.* 1963; Hayward & Parry 1973; Fernández Honaine *et al.* 2006; Mercader *et al.* 2010). Many studies have dealt with the different processes and factors involved in the ability of a species to take up silica from soils, and the relation between uptake and silica deposition (Ma & Takahashi 2002; Mitani & Ma 2005; Ma & Yamaji 2006; Mitani *et al.* 2009). However, scarce work has been done in relation to the factors involved in the silicification of different cells (Motomura *et al.* 2000, 2004; de Melo *et al.* 2010). Some of such studies have shown that the deposition of silica in a specific type of cell in leaf tissue depends on leaf side (adaxial or abaxial), plant species and leaf age; but no research involving environmental conditions has been found.

The silicification of certain cellular types, such as a bulliform cells or stomatal complex cells, implies loss of their functionality. In the case of bulliform cells, some authors have suggested that they take part in hygroscopic movement in the mature leaf (Jane & Tsai Chiang 1991), they are responsible for leaf rolling or folding during excessive water loss (Esau 1982) or they are water storage cells (Prat 1948). The accumulation of silica in this type of cell would imply a physiological or metabolic problem for the plant for any of the above possible functions; nevertheless, the silicification of this type of cell is common in various grass species (*e.g.* Metcalfe 1960; Ellis 1976; Zucol 1998; Ma & Takahashi 2002; Fernández Honaine *et al.* 2006; de Melo *et al.* 2010). Some studies have related the silicification of bulliform cells with water stress, senescence, transpiration processes or an increment in water availability in species that normally grow under dry conditions (Parry & Smithson 1958; Sangster & Parry 1969; Ma & Takahashi 2002; Bremond *et al.* 2005). However, until now there is no general agreement on the factors involved. In the case of stomata, no specific study has been carried out; however, some authors suggest that the silicification could be associated with transpiration (Ma &

Takahashi 2002). The other epidermal cells of grass leaves have not been analysed, except for Motomura *et al.* (2000, 2004), who studied Japanese bamboo species.

In order to advance knowledge of silicification in epidermal tissues of grass leaves and the factors affecting it, we study the native and abundant C_4 panicoid species, *Bothriochloa laguroides* D. C. Pilger. This species can be found on grasslands in North and South America and constitutes an important native grass with a wide distribution within the Pampean region (Soriano *et al.* 1991; Vega 2000). In this paper, we analysed the distribution of silicified cells in the adaxial epidermis of the first and second leaf blades in plants growing under two different field conditions. We studied the effect of leaf section or leaf position and environmental characteristics on the relative frequencies of silicified epidermal cells. Finally, we analysed different hypotheses proposed by some authors in relation to factors affecting the silicification of cells, which fall into two main groups: external (*e.g.* transpiration rate, water stress condition) or internal (genetic, metabolic) factors (*e.g.* Jones & Handreck 1967; Ma & Takahashi 2002; Motomura *et al.* 2004; Massey *et al.* 2007). The results obtained represent the first quantitative data in a Panicoideae species in relation to leaf ageing and environmental factors. Moreover, the present study constitutes one of the first antecedents where the effect of environmental conditions on silicification processes is evaluated in field conditions, since the majority of previous studies are controlled experimental works.

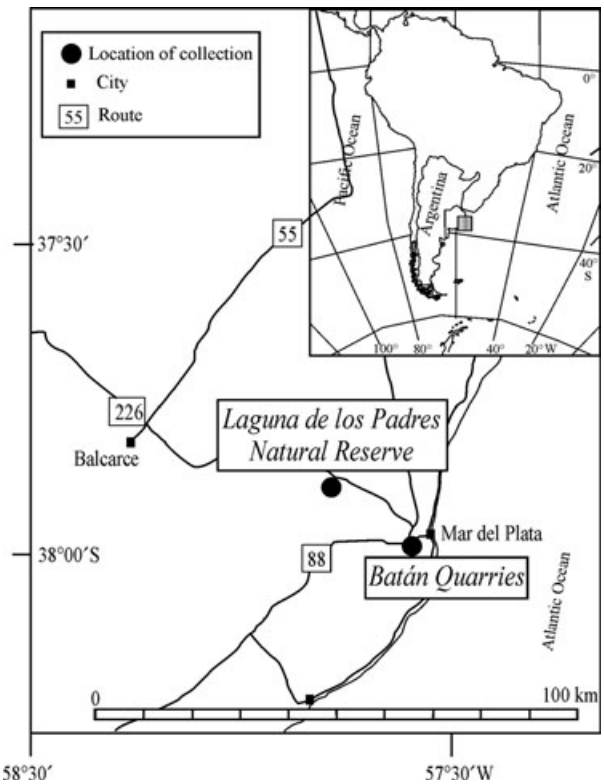

Fig. 1. Location of the sites of plant collection.

MATERIAL AND METHODS

Site selection and environmental characterisation

Two different environmental situations were selected for the collection of plants: a natural wetland (Los Padres Pond Natural Reserve) and a quartzite quarry (Batán quarries) (Fig. 1). Both sites are located in the southeast of Buenos Aires province, Argentina, an area characterised by hills belonging to the Tandilian Range and Perirange Eolian Fringe. The vegetation of the area belongs to the Rio de la Plata grassland, a temperate subhumid grassland that covers the vast plains of central-eastern Argentina, Uruguay and southern Brazil (Soriano *et al.* 1991). The climate is temperate, with mean annual precipitation of 940.6 mm, mean annual temperature of 13.8 °C, with a mean of 20 °C during the hottest month (January) and a mean of 7.3 °C during the coolest month (July) (Servicio Meteorológico Nacional 2010). Between January 2008 and March 2009, when most of the specimens where collected, unusual climate conditions occurred. These conditions were dominated by a monthly water deficit of 40–80% with respect to the historic mean, and an increment of 1 °C in mean monthly temperature, especially between October 2008 and March 2009 (Servicio Meteorológico Nacional, SMN 2009) (Fig. 2).

Los Padres Pond Natural Reserve (37°56′S, 57°50′W) is situated in Buenos Aires province, Argentina (Fig. 1). It is a natural area of 687 ha, where 319 ha correspond to an aeolic pond, surrounded by low aeolic hills. The soils where the plants were collected are Hapludolls and typical Argiudolls, developed from quaternary loess. Hapludolls are characterised by depths of 30 cm on average and a silt loam texture; Argiudolls are 60-cm deep on average, with a clay-silt loam texture (Fernández Honaine 2001). The silica (SiO_2) concentration of these soils was analysed by Borrelli *et al.* (2010) and determined with UV-VIS spectrophotometry according to the APHA–AWWA–WPFC (1998). The concentration was measured in leaching and capillary soil solutions. Capillary solutions are almost immobile, whereas leaching solutions flow through the soil and thus have a much shorter residence time. Therefore, capillary solutions may better reflect interactions with soil silicates and the chemistry of leaching and capillary solutions may be different (Gérard *et al.* 2002). Values of SiO_2 in the capillary soil solution range between 485–1106 μM for the 0–7 cm and 421–810 μM for the 7–30 cm of A horizons; while values for leaching soil solution range between 59–247 μM for the 0–7 cm and 59–369 μM for the 7–30 cm of A horizons (Borrelli *et al.* 2010). The vegetation is characterised by a grassy carpet, surrounding by thistles, shrubs such as *Baccharis* spp., *Rubus ulmifolius* and *Colletia paradoxa*, and small forests of *Eucalyptus* spp. The proximity to the pond and the presence of shrubs and arboreal species, make this site the wetter of the two environments selected (Fernández Honaine 2001).

Quartzite quarries, Batán city (38°30′S, 57°45′W), Buenos Aires province, Argentina, constitute anthropogenic diggings 20 m in depth on average, made for quartzite extraction. Since this activity finished, incipient soil horizons have developed (Osterrieth *et al.* 2005). These soils are Anthropic Regosols, characterised by a depth that ranges between some millimeters to more than 10 cm. The profiles that developed AC and CA horizons have sandy and sandy-silt textures, respectively (Osterrieth *et al.* 2005). The shallow depth, coarse texture and lax structure cause these soils to have low

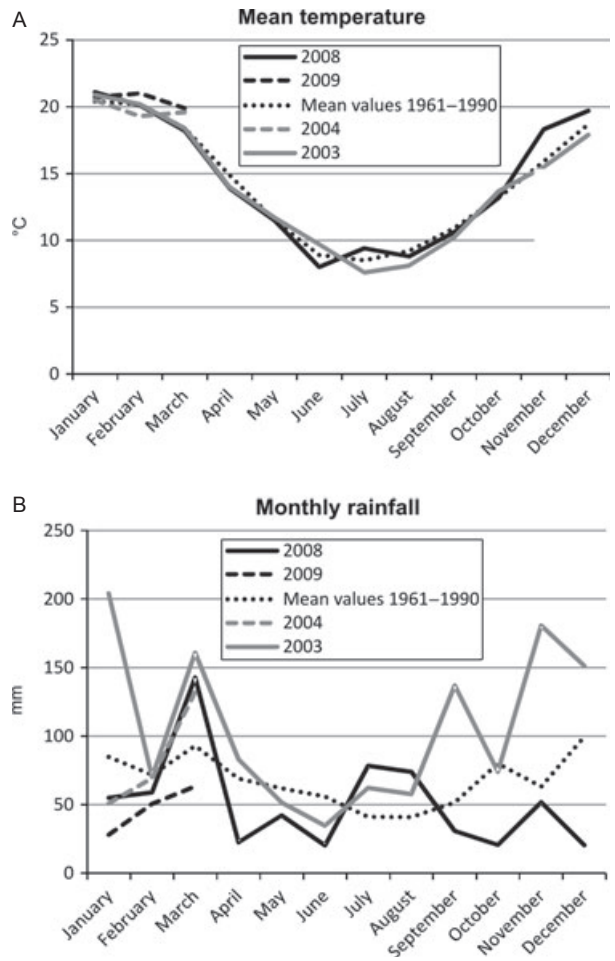

Fig. 2. A: Mean temperature for the years when plants were collected and the mean of the period 1961–1990. B: Total precipitation for the years when plants were collected and the mean of the period 1961–1990 (data obtained from Departamento Agrometeorología, Servicio Meteorológico Nacional, Argentina, Boletines Agroclimatológicos mensuales 2008–2009 and http://www.tutiempo.net/clima/Mar_del_Plata_Aerodrome/876920.htm).

hydric retention and rainwater is the main source of water for plants. Since water retention is very low and the soils are shallow, quantification of silica in soil solutions is unlikely. However, the availability of silica for plants in this site can be estimated from analysis of this element in the small and temporary water bodies present in the quarries. Values of SiO_2 concentration in these sites ranged between 135 and 276.6 μM (N. Borrelli, M. Osterrieth, M. F. Alvarez, M. Fernández Honaine, unpublished data), which are on average lower than the values obtained for Los Padres pond (see above). Besides these pedological differences from Los Padres pond, the quarries have special microclimate conditions. Since the floor and the vertical walls (10–20-m high) are made of quartzite, light reflection and temperature inside the quarries is higher than in the surrounding fields. For that reason, the quarries can be considered as a hotter and drier site than Los Padres pond.

Plant sampling and tissue silicification analyses

Plants from Los Padres Pond were collected between October 2003 and March 2004 and October 2008 and March 2009, whereas the quarries plants were collected between October 2008 and March 2009. From each site and each date, two to three plants at flowering stage and 30–50-cm tall were collected (Fig. 3A and B). From each plant, the two leaf blades below the leaf flag of the floral culm were sampled (Fig. 3B). Counting the leaves from the shoot apex downwards, the superior leaf is the first one (younger leaf) and the inferior is the second leaf (older leaf). The blades were divided in three equidistant sections: basal, median and apical. Fragments were cleared with acetic acid and hydrogen peroxide (1:1) for 48 h at 60 °C, according to Motomura *et al.* (2000), dehydrated in an ethanol series and then stained with phenol crystals. Phenol crystals were dissolved with a minimum quantity of ethyl alcohol before staining the fragments. The stained fragments were mounted in immersion oil and observed under a Leitz Wetzlar D35780 microscope (Ernest Leitz GMBH, Wetzlar, Germany) at ×400 magnification. Phenol crystals stain silica cells to a rose colour (Johansen 1940). From each slide (which included two to three fragments of a section of a blade), silicified and non-silicified cells in 20 fields of 0.11 mm^2 were counted. Relative frequencies of silicified and non-silicified cells were calculated.

Data analysis

Percentages of silicified cells from superior and inferior leaves from plants collected in three sites were subjected to two-factor ANOVA analyses. Since there was disproportional replication of the data, due to the quarries samples, three ANOVA

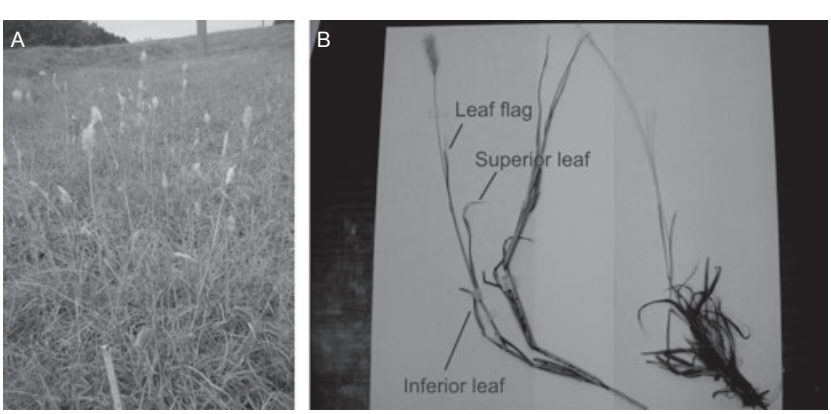

Fig. 3. A: Photographs of *Bothriochloa laguroides* in the field. B: Leaves of *B. laguroides* selected for the study.

analyses were carried out (Zar 1984). In each analysis, only two of the three samples from the quarries site were included, so as to obtained equal replications of each situation (n = 2). Differences in percentages of silicified bulliform cells between collection site, leaf position and leaf section were analysed through a three-factor ANOVA.

RESULTS

Description of the adaxial epidermis of the leaf blade

The adaxial epidermis of the leaf blade of *B. laguroides* can be divided in costal and intercostal zones. The costal zone is composed of silica cells *s.l.*, short cells (cork cells), long cells, hooks, prickles and bicellular hairs. The intercostal zone is composed of bulliform cells, long cells, short cells, stomata and bicellular hairs. In transverse section, the distribution of bulliform cells can be observed, which are three to six, grouped between costal cells (Fig. 4A and B).

Total percentage of silicified cells

Between 13% and 19% of total cells of the adaxial epidermis of leaf blades was silicified. ANOVA analyses showed that there were significant differences between percentages of superior and inferior leaves, while collection site significance varied according to the sample that was discarded (Table 1A). Moreover, there was no clear significant difference at P = 0.05, Fig. 5 shows that higher values were found in plants collected from Los Padres Pond in 2008–2009. Percentages of silicified cells in the different sections analysed varied; however, values in median and apical sections were slightly higher than those in basal sections (Table 2).

Distribution of silicified cells

In all leaves and sections, typical silica short cells, with bilobate or polylobate shapes, are the largest contributor to the total percentage of silicified cells (53–98%) (Table 3; Fig. 4C,

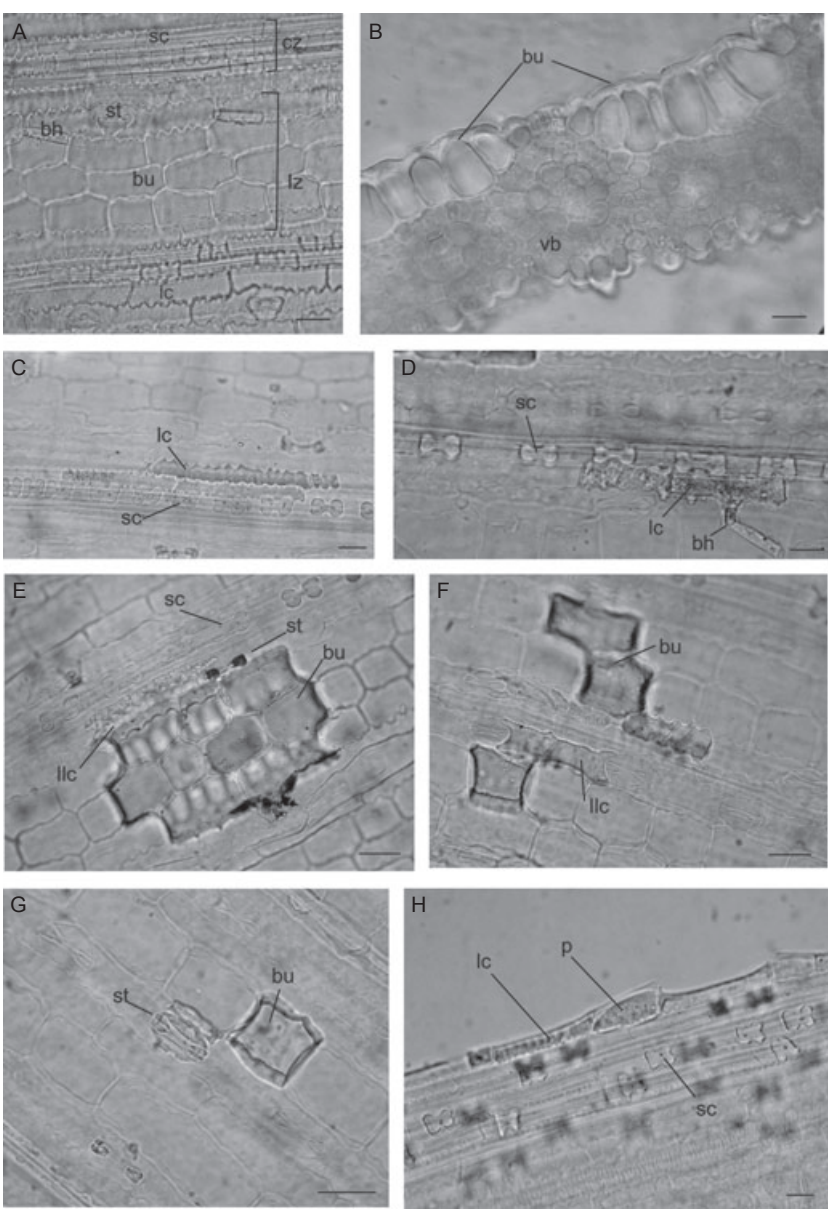

Fig. 4. A: Surfice view of adaxial epidermis showing costal (cz) and intercostal (iz) zones and the main types of cell. B: Transverse section of leaf showing bulliform cells (bu) and vascular bundle (vb). C–H: Silicified cells in costal (C, D, H) and intercostal zones (E, F, G). C: Long cells (lc) and typical silica short cells (sc) with silica deposits. D: Long cells (lc), typical silica short cells (sc) and bicellular hairs (bh) silicified in a costal zone. E and F: Interstomatic long cell (ilc), stomata (st) and bulliform cells (bu) silicified. G: Bulliform cell (bu) and stomata (st) silicified. (H) Prickle hair (p), long cells (lc) and typical silica short cells (sc) silicified at a leaf margin. Scale bar = 25 μm.

Table 1. ANOVA tables. (A) Two-factor ANOVA of total silicified cells. In each analysis only two of the three data points collected in quarry site were included SO as obtained proportionate replication (n = 2). (B) Three-factor ANOVA of percentages of bulliform cells silicified.

(A)

source of variation	sum of squares	df	mean square	F	P (F)	F (P = 0.05)
site collection	16.12	2	8.06	15.58	0.004**	5.14
leaf position	3.98	1	3.98	7.69	0.032*	5.99
site × leaf (interaction)	3.51	2	1.75	3.39	0.103	5.14
error	3.10	6	0.52			
total	26.72	11				
site collection	12.69	2	6.34	5.28	0.047*	5.14
leaf position	11.12	1	11.11	9.25	0.022*	5.98
site × leaf (interaction)	13.82	2	6.91	5.75	0.040*	5.14
error	7.21	6	1.20			
total	44.83	11				
site collection	12.47	2	6.24	4.11	0.075	5.14
leaf position	9.31	1	9.31	6.13	0.048*	5.98
site × leaf (interaction)	11.04	2	5.53	3.64	0.092	5.14
error	9.10	6	1.52			
total	41.94	11				

(B)

	sum of squares	df	mean square	F	P (F)
intercept	1418.51	1	1418.52	170.10	0
site collection	55.64	2	27.82	3.33	0.058
L. position	72.74	1	72.75	8.72	0.008**
L. section	118.31	2	59.16	7.09	0.005**
site × L. position	1.60	2	0.80	0.09	0.908
site × L. section	46.32	4	11.58	1.38	0.277
L. position × L. section	53.83	2	26.92	3.22	0.063
site × L. position × L. section	26.39	4	6.6	0.79	0.545
error	150.10	18	8.34		

*Significant differences at P < 0.05; **Significant differences at P < 0.01.

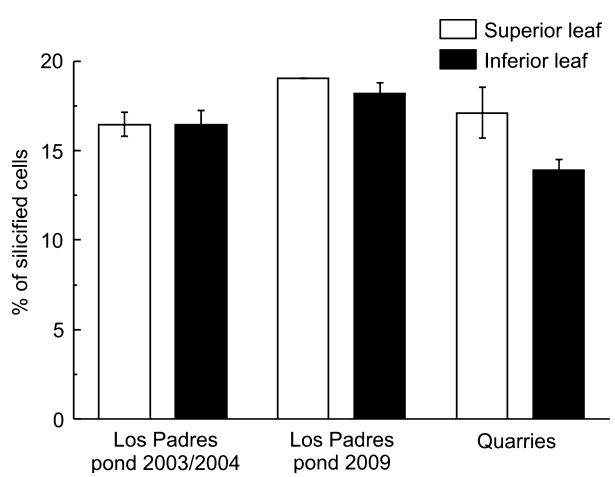

Fig. 5. Mean percentage ± standard error of silicified cells in relation to leaf position and site of collection.

D, E and H). Between 95% and 100% of typical silica cells had silica deposits, and these percentages remain constant along leaf position or section and collection site (Table 3). The second largest contributor to the pool of silicified cells (0–30%) was the equidimensional intercostal long cells, which correspond to the bulliform cells (Fig. 4E–G). The percentage of this cell type that was silicified (0–18%) generally increased from basal to apical section, and was higher in superior leaves to inferior leaves (Table 3). When the sites were compared, the proportion of bulliform cells that are silicified in Los Padres Pond plants was higher than that in the quarries plants (Table 3). Three-factor ANOVA analysis showed that silicified cell percentages between leaf positions and leaf sections were significantly different (Table 1B). Although the 'collection site' factor did not show a significant difference, the P-value obtained for the F statistic (P = 0.058) was very close to the level chosen for significance differences (P < 0.05). More data would need to be analysed to define the influence of this factor on the silicification of bulliform cells.

In the costal zone, the long cell contribution to total silicified cells was low (0–8.8%; Fig. 4C). As in the case of bulliform cells, the proportion of long cells that was silicified was greater in superior leaves and in Los Padres Pond plants than in inferior leaves and in the quarries plants (Table 3). The rest of the cell types in the costal zone made a lower contribution to the total silicified cells and did not show any difference between leaf sections, leaf positions or collection site.

Table 2. Mean ± standard error of the percentages of silicified cells in superior and inferior leaves in relation to leaf section and site of collection.

leaf position	leaf section	site collection: Los Padres pond 2003/2004	Los Padres pond 2009	quarries
superior	basal	15.38 ± 0.15	18.55 ± 0.55	17.01 ± 1.71
	median	16.36 ± 0.17	17.69 ± 0.41	19.34 ± 1.94
	apical	17.66 ± 2.33	20.83 ± 0.09	18.32 ± 0.67
inferior	basal	13.9 ± 0.35	18.36 ± 0.78	16.24 ± 1.39
	median	18.48 ± 0.45	19.39 ± 0.23	13.05 ± 1.24
	apical	16.96 ± 2.44	16.75 ± 1.29	12.69 ± 1.52

Most bicellular hairs and hooks were silicified, especially those on the margin of the leaf, but since they were not the most abundant cell type in the epidermis, their contribution to total silicified cells was poor (Fig. 4D and H).

Of the total silicified cells, cells from the intercostal zone (except for bulliform cells) had low values: non bulliform long cells (0–12%), short cells (0–4%), stomata (0–10%) and bicellular hairs (0–6%) (Fig. 4F and G). Some inconclusive differences between leaf position and site were observed; however, more studies are necessary to confirm these results. The percentage of long cells that were silicified was higher in superior than in inferior leaves, while stomatal silicification percentages were higher in most of the Los Padres Pond than in the quarries plants.

DISCUSSION

All of the cell types of the adaxial epidermis of leaf blades of *B. laguroides* were silicified in at least one plant in the present study. In a broad sense, the distribution of the silicified cells coincides with previous research, with some exceptions. Values of total silicified cells were higher (11%) than those found by Motomura *et al.* (2000), who studied the adaxial epidermis of the leaf blade of *Pleioblastus chino*. While in *P. chino* there were no differences between the three parts analysed (Motomura *et al.* 2000), we found slight differences between basal, median and apical sections. Also, silicified cell percentages in superior leaves were higher than in inferior leaves for *B. laguroides*. However, this finding is limited to the two leaf positions analysed in this work.

Silica accumulation in leaves has been explained with several hypotheses. The main studies on this issue relate cell silicification to transpiration, water stress, senescence and herbivory (Parry & Smithson 1958; Sangster & Parry 1969, 1971; Motomura *et al.* 2002; Ma & Yamaji 2006; Massey *et al.* 2007). The results obtained for *B. laguroides* leaves support some of these hypotheses. The higher accumulation in superior leaves contradicts the hypothesis that relates higher deposition of silica to the oldest organ, since the superior leaf is younger than the inferior leaf (Motomura *et al.* 2000; de Melo *et al.* 2010). These results could be explained by greater exposure to solar radiation, which in turn would increase evapotranspiration and allow the deposition of silica. In this case, the results would support the hypothesis of transpiration and silica deposition (Sangster & Parry 1971; Ma & Yamaji 2006). Instead, the higher accumulation in median and apical sections could be a consequence of senescence of the leaf, a higher transpiration rate (as in the case of superior leaves) or could be explained as a metabolic mechanism. Since the basal section is the zone of cellular growth and differentiation (Esau 1982), silica deposition could interrupt normal leaf development.

When plants from different environmental conditions were compared, the data showed that, even though quarries having soils developed from quartz rocks and higher temperatures than at Los Padres pond, the percentages of silicified cells were higher in the latter site. The reason of these results may lie in the lower availability of silica for plant uptake in soils of the quarries, where the soils are developed from quartz rocks, which is an ineffective silica source for plants (Jones & Handreck 1967). Moreover, as detailed in previous sections, the soils of this site are shallow and water retention is low, rain being the main source of this element for plants. Considering the low silica content in rainwater (57.3–126 μM; Borrelli 2008) and the youth of these soils (because they were developed after the quarries were abandoned), the silica content in this system is also low (Osterrieth *et al.* 2005). This can be verified by the low content found in the temporary water bodies formed at this site (see information in environmental characterisation section).

The main cells that were silicified in *B. laguroides* were typical silica short cells, as described by other authors (Motomura *et al.* 2000). According to previous studies (Motomura *et al.* 2000, 2004), silica short cells are silicified very early in leaf development, and this may explain their higher abundances in basal sections of leaves in the present study.

The second most abundant silicified cells were bulliform cells, which showed an increase from basal to apical sections and from inferior to superior leaves. Takeoka *et al.* (1984) also found an increase of silicification of bulliform cells from basal to apical sections of rice leaves. These authors related the accumulation of silica to sites of higher transpiration within the leaf surface. The results obtained in the present study show that within a single plant, bulliform cells increase their silica deposition in a senescence stage (median and apical sections) and/or as a consequence of a greater solar exposure – higher transpiration rate (superior leaf, median and apical sections), as suggested previously (Takeoka *et al.* 1984; Ma & Yamaji 2006). When different plants grown under different environmental conditions were compared, silica accumulation in bulliform cells seems to be favoured in the wetter site and with a higher silica availability in the soil, such as Los Padres Pond. These results contradict some findings of other researchers who related the silicification of

Table 3. Absolute frequencies (mean ± SE) of the different costal (A) and intercostal (B) cells of the adaxial epidermis of *Bothriochloa laguroides* in the three situations studied and according to leaf position and leaf section.

(A)

site of collection	leaf position	leaf section	costal cells									
			long cells		silica cells		cork cells		bicellular hairs		hooks	
			S	T	S	T	S	T	S	T	S	T
Los Padres pond 2003/2004	superior	basal	3 ± 3	304 ± 13	198 ± 20	198.5 ± 19.5	2 ± 2	183.5 ± 49.5	3 ± 3	5.5 ± 3.5	4.5 ± 1.5	10 ± 5
		median	11.5 ± 2.5	275 ± 20	197 ± 13	199.5 ± 13.5	2	173.5 ± 28.5	1.5 ± 0.5	3 ± 2	8 ± 3	20.5 ± 5.5
		apical	14 ± 13	228 ± 27	141 ± 27	142 ± 26	3 ± 1	134.5 ± 27.5	2.5 ± 0.5	4 ± 2	17.5 ± 8.5	40 ± 7
	inferior	basal	1 ± 1	258.5 ± 29.5	199.5 ± 12.5	208 ± 5	18.5 ± 1.5	269 ± 34	1.5 ± 0.5	6.5 ± 4.5	0	1.5 ± 1.5
		median	7	256 ± 32	250 ± 4	251 ± 4	7.5 ± 4.5	239.5 ± 9.5	0	0	3.5 ± 1.5	6 ± 3
		apical	3.5 ± 0.5	234.5 ± 4.5	199 ± 28	200 ± 27	4 ± 1	165.5 ± 7.5	0	0	4.5 ± 2.5	11.5 ± 2.5
Los Padres pond 2009	superior	basal	11 ± 2	284.5 ± 59.5	268.5 ± 5.5	268.5 ± 5.5	4.5 ± 1.5	316 ± 37	1 ± 1	1 ± 1	4 ± 3	4.5 ± 2.5
		median	10.5 ± 9.5	302.5 ± 36.5	292.5 ± 2.5	292.5 ± 2.5	5.5 ± 4.5	304.5 ± 35.5	0	0	2.5 ± 2.5	3 ± 3
		apical	22 ± 4	196 ± 20	187 ± 29	187 ± 29	16 ± 9	212.5 ± 45.5	0.5 ± 0.5	0.5 ± 0.5	4.5 ± 4.5	6 ± 3
	inferior	basal	7.5 ± 2.5	259.5 ± 17.5	292.5 ± 71.5	292.5 ± 71.5	2.5 ± 2.5	313 ± 34	0.5 ± 0.5	0.5 ± 0.5	0	0
		median	17 ± 7	216.5 ± 14.5	234.5 ± 29.5	234.5 ± 29.5	12 ± 9	208 ± 62	0	0.5 ± 0.5	2	2
		apical	13.5 ± 5.5	191.5 ± 17.5	225 ± 17	227 ± 15	6	239.5 ± 23.5	0	0	4.5 ± 2.5	12 ± 9
quarries	superior	basal	7 ± 9.2	347.67 ± 53.11	325.67 ± 34.88	325.67 ± 34.88	5.33 ± 6.85	328.67 ± 29.17	1 ± 0.82	1.67 ± 0.94	2.67 ± 3.09	3.33 ± 4.03
		median	13.33 ± 9.03	369.67 ± 85.32	320 ± 53.56	320 ± 53.56	24 ± 26.98	334.33 ± 50.86	0.33 ± 0.47	1 ± 0.82	5 ± 0.82	9.33 ± 3.4
		apical	2.67 ± 1.7	241 ± 83.76	207.33 ± 51.05	208.34 ± 50.74	7.33 ± 10.37	227 ± 65.26	0.67 ± 0.47	2.67 ± 3.09	5.33 ± 1.89	23.33 ± 13.02
	inferior	basal	2.33 ± 1.7	379.67 ± 93.1	359.33 ± 56.86	361.34 ± 55.36	6.33 ± 5.31	352.33 ± 73.51	0.33 ± 0.47	1.33 ± 1.88	0	1 ± 0.82
		median	6	341.33 ± 69.41	277 ± 65.91	282.33 ± 60.8	3.33 ± 4.03	283.67 ± 69.28	0.33 ± 0.47	2.67 ± 2.05	5.67 ± 3.86	11 ± 7.26
		apical	0	251 ± 88	198.5 ± 58.5	202 ± 55	1	206 ± 61	0	0	5.5 ± 5.5	10 ± 8.5

(B)

site of collection	leaf position	leaf section	intercostal cells									
			bulliform		short cells		stomata		long cells		bicellular hairs	
			S	T	S	T	S	T	S	T	S	T
Los Padres pond 2003/2004	superior	basal	36.5 ± 2.5	649.5 ± 72.5	7 ± 6	19 ± 16	9.5 ± 0.5	167.5 ± 23.5	9 ± 1	287 ± 61	12.5 ± 2.5	28.5 ± 3.5
		media	42 ± 3	637.5 ± 12.5	2	12.5 ± 5.5	8.5 ± 5.5	152 ± 5	6 ± 1	273.5 ± 68.5	12.5 ± 3.5	31.5 ± 2.5
		apical	93.5 ± 32.5	688 ± 15	0	6.5 ± 5.5	10.5 ± 3.5	147.5 ± 22.5	6 ± 5	288 ± 32	16 ± 5	43.5 ± 19.5
	inferior	basal	19 ± 12	655 ± 20	3 ± 1	11 ± 6	0.5 ± 0.5	121.5 ± 26.5	2 ± 1	218.5 ± 15.5	2 ± 2	26.5 ± 4.5
		media	29 ± 16	556.5 ± 139.5	1.5 ± 0.5	9 ± 7	17 ± 5	178 ± 9	5.5 ± 0.5	287 ± 57	13.5 ± 2.5	30.5 ± 2.5
		apical	59 ± 7	608 ± 52	1.5 ± 1.5	3 ± 2	13 ± 3	195 ± 22	4.5 ± 1.5	316.5 ± 41.5	10 ± 3	27 ± 1
Los Padres pond 2009	superior	basal	35 ± 10	596 ± 42	1.5 ± 1.5	8.5 ± 1.5	18.5 ± 18.5	177.5 ± 9.5	7.5 ± 7.5	256.5 ± 35.5	7 ± 3	19 ± 3
		media	56 ± 12	805.5 ± 80.5	0	1	5 ± 5	176.5 ± 15.5	3.5 ± 1.5	265.5 ± 68.5	9.5 ± 1.5	23 ± 1
		apical	89.5 ± 1.5	665.5 ± 94.5	1 ± 1	2.5 ± 2.5	2	89 ± 5	4 ± 4	206 ± 17	5 ± 3	27 ± 5
	inferior	basal	47 ± 7	763.5 ± 33.5	0	1.5 ± 1.5	19 ± 13	169 ± 42	14.5 ± 2.5	288.5 ± 102.5	4.5 ± 1.5	14.5 ± 0.5
		media	32 ± 18	591.5 ± 149.5	0	0	7.5 ± 3.5	140 ± 71	16 ± 15	275.5 ± 163.5	4 ± 3	13 ± 2
		apical	41 ± 14	782 ± 76	1 ± 0	10 ± 7	9.5 ± 4.5	135 ± 11	17 ± 12	279.5 ± 83.5	3 ± 2	23 ± 8

Table 3. Continued.

site of collection	leaf position	leaf section	intercostal cells									
			bulliform		short cells		stomata		long cells		bicellular hairs	
			S	T	S	T	S	T	S	T	S	T
quarries	superior	basal	28.67 ± 15.43	789.67 ± 138.86	0	2.33 ± 2.62	15.67 ± 16.58	189.66 ± 66.14	13.33 ± 4.11	394 ± 77.39	10.33 ± 4.78	25.67 ± 2.62
		media	51 ± 13.93	764.33 ± 159.47	0	0	19 ± 20.51	164.33 ± 69.18	19 ± 6.53	399.67 ± 70.49	2.67 ± 0.94	9 ± 2.45
		apical	80 ± 39.73	734.67 ± 310.93	0	0	16.33 ± 21.68	118.33 ± 42.68	21.33 ± 18.62	332.67 ± 97.31	3.33 ± 2.49	11.67 ± 8.22
	inferior	basal	39.67 ± 32.89	885 ± 109.99	1.33 ± 0.94	2.67 ± 2.49	4 ± 2.16	202.67 ± 42.97	6 ± 3.74	441.67 ± 34.12	6.67 ± 3.3	19 ± 5.89
		media	19.67 ± 7.84	838 ± 68.98	0.33 ± 0.47	2.33 ± 1.7	3 ± 1.63	205.67 ± 31.63	9.67 ± 3.85	491 ± 74.14	2.67 ± 1.25	16.67 ± 5.44
		apical	24 ± 24	639 ± 316	0	0	11 ± 11	149.5 ± 4.5	6.5 ± 6.5	413 ± 83	0.5 ± 0.5	3.5 ± 3.5

S = cells with silica deposits; T = total cells of each type.

bulliform cells with water stress conditions (Bremond *et al.* 2005). As described in the previous paragraphs, the differences in content of silica in soils may condition the silicification in bulliform cells. The soils of the quarries, besides having developed from quartz rocks, have a low silica content, where the silica material available for dissolution (quartz) is practical inert. Instead, soils from Los Padres pond have been developed from loessic sediments, which include important quantities of biogenic silica and volcanic ash, a more soluble silica source (Osterrieth *et al.* 2009; Borrelli *et al.* 2010). Also, these soils have an important input of biogenic silica from grasses growing in the area and from diatoms in the pond (Borrelli *et al.* 2010). So, besides transpiration rate determining the distribution of silica in cells, as documented in various research papers and confirmed in the present study, the availability of silica in soils represents a conditional factor for silicification processes. This aspect is very relevant, especially when the information is used for paleobotanical interpretation. A higher content of bulliform phytoliths in the fossil phytolith record could represent not only drier conditions (Bremond *et al.* 2005), but also a site with soils having higher silica content.

A last, but not insignificant, factor affecting bulliform silicification could be related to functionality. Considering the possible functions assigned to bulliform cells, especially those related to leaf movement and water storage, and the fact that silicification represents a loss of this functionality, it would be reasonable that in the drier and hotter site (*e.g.* the quarries), the silicification of bulliform cells occurs to a lesser degree. In this case, it may be possible that a metabolic control on the silicification process is involved.

In summary, the present results constitute the first quantitative data on the distribution of silicified cells in the adaxial epidermis of panicoid grass leaves. Silicification seems to be affected by external and metabolic factors and differs between cell types. Within a single plant, transpiration is probably mainly responsible for the proportion and distribution of total silicified cells in leaves, in relation to leaf section and leaf position, and in particular for bulliform cells. However, when environmental factors are included, silica availability in soils represents an important factor that contributes to silica accumulation and overlaps the transpiration effect. These results are especially relevant when silica cells (phytoliths) are used as indicators of plant communities and environmental conditions in the past (Alexandre *et al.* 1997; Bremond *et al.* 2005). Typical silica short cell silicification did not differ between sites or leaf positions and sections, probably because these cells are predetermined to be silicified and the silica deposition occurred very early in leaf development. The remaining cell types did not showed a clear pattern of variation or response to the site conditions evaluated.

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