Determination of Stomatic Density, Index, and Area as Exposition Biomarkers of Pollution in *Deschampsia Antárctica* Desv. (*Poaceae*)

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Abstract

Deschampsia antarctica Desv. (Poaceae) is the only native grass described so far for Antarctica, with a distribution mainly centered on the Antarctic Peninsula.

The plants were collected at Argentinian Scientific Station Carlini, 25 de Mayo (King George) Island, to determine and evaluate in their leaves: the stomatic index (SI), density (SD), and area (SA) as pollution biomarkers. Samples were taken within the Station's influence area: (A) Supply Area (loading and unloading of fuel and supplies), (B) the area adjacent to the Electric Power Station, and (C) area of Fuel Tanks. Besides, other samples were taken from a pristine area called Peñon 7 (D). The results of SD showed significant differences only on the leaf abaxial face from the plants of the 4 studied sites: Peñón 7 (57.36 ± 2.03), Supply Area (61.30 ± 2.32), Electric Power Station adjacent area (69.56 ± 2.23) and Fuel Tanks area (80.11 ± 2.42). The SI as well as the SA did not have significant differences ($p > 0.05$) for all the analyzed sites. However, correlation analyses between SD and SI showed a positive and significant association only for the leaf adaxial face from all sites.

From the obtained results, we could suggest that the correlation (SD-SI) on the adaxial side of the leaves was a good biomarker to estimate the degree of anthropogenic impact in each studied area.

Introduction

The development of Antarctic vegetation is limited by climatic factors such as extremely low temperatures (ranging from − 35 oC to -65 oC on the Central Plateau and − 10 oC to -20 oC on the Antarctic Peninsula), winds that can exceed 70 km/h, high sun incidence (mainly due to ice reflection) and low water availability (SMN, 2019). These conditions determine that the flora consists mainly of mosses, lichens, and only two vascular plants: *Deschampsia antarctica* and *Colobanthus quitensis*. All these types of plants are distributed and can be found on rocky slopes, moraines, and simple soils, ice-free zones, even on cliff ledges and in cracks. Besides, slight declines in the Earth's landscape can generate a mosaic of microclimates that favors the distribution of plant life (Parnikoza et al. 2011).

If only the two vascular plant species are considered, *Deschampsia antarctica* Desv. (Poaceae) is the only native grass described so far for Antarctica, with a distribution mainly centered on the Antarctic Peninsula (Komárková et al. 1990; Convey 1996; Montiel et al. 1999; Barcikowski et al. 2001, 2003; Bravo et al. 2001; Chwedorzewska et al. 2008; Vera 2011; Casanova-Katny and Cavieres 2012). This species is abundant in ice-free zones, areas that also coincide with the scientific stations´ settlements, both permanent and temporary, where specific foci of pollution (chemical, physical, etc.) is generated by anthropogenic activities. *D. antarctica* has been the subject of several studies, including eco-physiological and genetic analyses, association with endophytes, medical applications and bioindicator of climate change (Bennett et al. 1982; Lewis Smith 1994; Alberdi et al. 2004; Rosa et al. 2009; Parnikoza et al. 2011; Navrotska et al. 2014; Domaciuk et al. 2016; Gonzalez et al. 2016; Köhler et al. 2017; Malvicini et al. 2018; Zamarrón et al. 2019).

The effects or early signs of exposure to the contaminants can be estimated via biomarkers, which can be used for risk assessment from the molecular level down to the level of populations and communities (Ernst and Peterson, 1994; Sandermann, 2000; Pastor et al. 2003; Ferrat et al. 2003; Ratola et al. 2014; Mena Torres et al. 2017). Moreover, exposure to xenobiotics was reported to produce direct, measurable, and quantifiable effects on plants (Ellis 1979; Meister and Bolhàr Nordenkampf 2003). The responses of plants to stress are reflected in morphological variations and show insufficient adaptation to changing environmental conditions. Generally, these responses can be caused by natural processes: volcanic eruptions, floods, salt dispersion (Oosting 1945; Collins 1969; Grimoldi et al. 1998; Hotes et al. 2004; Pardos 2004; Dale et al. 2005; Jiménez et al. 2013; Sakagami et al. 2020; Shao et al. 2020) or by anthropogenic activities (Bacci and Gaggi 1987; Sandermann 1992; García et al. 2006; Collins et al, 2011; Burden et al. 2020).

Leaves are the plant organs that best reflect the changes of environmental conditions (Mooney et al. 1991; Pedrol et al. 2000; Abbruzzese et al. 2009; Huang et al. 2011; Lázaro Nogal et al. 2015; Jumrani et al. 2017; Idris et al. 2018). In the presence of adverse conditions, most plants commonly respond with premature loss of leaves or modification of their color, which usually varies from pale green to yellow (chlorosis) or from yellowish to brown (necrosis) (Ernst 2003). Authors like Pastor et al. (2003), Dimitrova and Yurukova (2005), Kardel et al. (2010), Komolafe et al. (2015), Idaszkin et al. (2019), have determined and reported that both alterations in leaf morphology and leaf structures were reliable as stress' biomarkers. Among the leaf structures studied, stomas were the most chosen to detect the effects of environmental variations (Salas et al. 2001; Bruno et al. 2007; Rivera et al. 2013; Ferriol et al. 2014; Ganem et al. 2014; Naizaque et al. 2014).

Stomas are essential for the homeostasis of plants, and their number can vary concerning each plant species (Evert 2006; Ernst 2003). Some factors controlling the opening and closing of stomas include the concentration of CO2 inside the leaf, wind, relative humidity, ambient temperature, and water availability. Besides, many mechanisms, including stomatic development and stomatic density (SD), are genetically regulated and differ from species to species (Willmer and Friecker 1996; Bergmann et al. 2004; Casson and Gray 2008; Casson and Hetherington 2010; Araújo et al. 2011; Zoulias et al. 2018; Wu et al. 2019). Moreover, it had been seen that biotic parameters such as plant age, and abiotic parameters such as drought, UV-B radiation, presence of metals or excess potassium, can modify SD (Ernst 2003). Besides, several studies have described and characterized the stomas of *D. antarctica* (Romero et al. 1999; Barcikowski et al. 2003; Alberdi et al. 2004; Giełwanowska et al. 2005; Parnikoza et al. 2007).

Taken into account all the information stated in the previous paragraphs, the present work aimed to determine and evaluate the stomatic´s index (SI), density (SD), and area (SA) in leaves of *D. antarctica*, as biomarkers of exposition to pollution.

Material And Methods

Plants of Deschampsia antarctica were collected during the southern summer of 2017 in and around the Argentine Carlini Research Station, Potter Peninsula, 25 de Mayo Island (King George), South Shetlands, Antarctica (Fig. 1A,B). Within the area of the Station, samples were taken from “tussocks” located in zones of high anthropogenic impact: Supply Area (loading and unloading of fuel and supplies) (Fig. 1C), the lateral area adjacent to the Electric Power Station (Fig. 1D) and area of Fuel Tanks (Fig. 1E). Other plants were also taken at Peñón 7 (62°15'9.82 "S, 58°40'23.05 "W) which is within ASPA 132 (Antarctic Specially Protected Area) an area considered free of anthropogenic activity (Fig. 1F). From each sampling site, 6 to 7 plants along with a small amount of substrate were randomly selected.

At the Argentinean laboratory of the Carlini Station, 2 leaves were taken from each plant and stained with Feulgen (Dopchiz and Poggio 1999). The leaves were also photographed, with a blue filter, on both sides, using an Olympus® BX53 photomicroscope, for analyzing the potential effect of the Scientific Station activities on the stomas´ structure. Besides, the coloration of the leaves was observed and classified.

The other collected plants were transported to the Antarctic Institute laboratories (Buenos Aires, Argentina), in small containers to preserve the natural conditions of humidity and environmental temperature. The laboratories also have a cold chamber to maintain the temperature conditions, with irrigation and photoperiod suitable for each time of the year. Two leaves were selected from each plant and, to estimate stomatic density (SD) and stomatic index (SI), impressions were taken from both leaf sides with transparent adhesive tape. Then, from each leaf, 10 fields per side were randomly and systematically analyzed. Stoma counting was performed on a Motic® BA310R optical microscope, using the 40X objective lens corresponding to a leaf area of 0.188 mm2. Stomatic aperture (OS) was measured from photomicrographs taken with a Leica® DM 2500 photomicroscope. Fifty stoma pores were measured per individual and, in each of them, the major axis (MA) and minor axis (ma) were determined. The ImageJ 1.51 k software (Rasband 2017) was used to perform all the measurements. The SD was calculated as the number of stomas per leaf area. The stoma density ratio (SDR) between the abaxial and adaxial sides of the plant leaves was also calculated for each of the 4 studied sites.

The stomatic index (SI) was estimated as SI = (NS/ NS + EC) x 100, where NE = number of stomas and EC = number of epidermal cells. The stomatic opening (SO) was estimated as the ratio of the major axis divided by the minor axis, SO = MA / ma.

Statistics

The comparison between means was made with *Scheffé's test*. Pearson correlation and linear regression analyses were performed to determine the possible association of the variables SO, SD, and SI for each sampling site using the Infostat software (Di Rienzo et al. 2020).

Results

The leaves of *Deschampsia antarctica* were found to be amphistomatic. The stomatal complex was paracytic (Prabhakar 2004; Evert 2006). The stoma was formed by a pair of guard cells with bulbous ends and filiform nuclei. The subsidiaries cells were shaped like a dome (Fig. 2). No differences in the structure or size of the stomatal pores on either side of the leaves were found, nor were any particles obstructing the stomatal pore observed, for any of the sampled sites. The means of SD and SI were significantly different ($p < 0.05$) comparing the different studied sites (Table 1). The Peñón 7 area is the one that presented the lowest SD on both sides of the analyzed leaves. These values did not differ significantly ($p > 0.05$) from the SD values recorded for both leaf sides of the Supply Area. On the other hand, the SD values determined in the vegetation of the Electric Power Station and the Fuel Tanks varied between both leaf sides and to the other sampled sites. Significant differences ($p < 0.05$) were recorded on the abaxial side compared to those analyzed in Peñón 7 and the Supply Zone. In contrast, the SD values on the adaxial side showed overlapping differences between the studied sites. The stoma density ratio (SDR) showed that Electric Power Station had the lowest ratio (SDR = 3.46), meaning that for each stoma on the adaxial side there were 3.46 stomas on the abaxial side; meanwhile, the Fuel Tanks area had the highest ratio (SDR = 5.95) (Table 1).

If all sampling sites were compared, the abaxial face SI values varied between 16.22 ± 0.40 (Peñón 7) and 19.33 ± 0.41 (Fuel Tanks area), without any significant differences ($p > 0.05$). Similarly, the adaxial face did not present significant differences ($p > 0.05$) among the 4 studied areas.

Correlation analyses applied to the obtained results revealed a positive and significant association between SI and SD at each of the studied sites (Fig. 3, Table 2). The SD in Peñón 7 correlated positively with the SI of the abaxial leaf face, while the correlation of the adaxial face was high although it was not adjusted to a straight line but a polynomial curve. In the Fuel Tanks area, there was a high positive and significant ($p < 0.05$) ratio between SD and SI on the adaxial and abaxial leaf faces. On the other hand, when both leaf sides were considered at the same time the ratio was very low. For the other two sampled sites, the correlations determined for each leaf side were positive and significant ($p < 0.05$), although very low.

None of the studied sites showed differences ($p > 0.05$) in the SA values determined for each leaf side.

When the coloration of the plants was considered, green cushions were observed at Peñón 7, while the cushions at all other sites studied were light green to yellowish.

Discussion

Deschampsia antarctica has paracytic stomas, a structure that is common in Poaceae (Abid et al. 2007; López and Devesa 1991; Sanchez Anta et al. 1988; Dahlgren et al. 1985; Finot et al. 2006; Zarinkamar 2006). Previous works on this species have shown that *D. antarctica* is susceptible to factors such as snow cover, soil type, and temperature rise (Lewis Smith 1994; Parnikoza et al. 2007, 2011; Romero et al. 1999). In all the studied sites, our observations showed that the stomas were distributed on both sides of the leaves (amphystomatic), with high numbers on the adaxial side, as were observed by Romero et al. (1999) and Giełwanowska et al. (2005) in plants from other sites of the Antarctic Peninsula. However, in some sampled areas, plants showed leaves with stomas only present on the adaxial side (Barcikowski et al. 2003).

The results obtained in the Peñón 7 plants (Table 2) differ from those observed by other authors in other areas of the Antarctic Peninsula, for example, in Robert Island the SD of the adaxial side epidermis was: 170.10 (number of stomas *per* mm^2) and in the abaxial side epidermis was: 382.50 (number of stomas *per* mm^2) (Alberdi et al. 2004); while in the vicinity of the Polish Station Arctowski (Admiralty Bay, 25 de Mayo Island), the SD in the adaxial epidermis was: 5.56 (number of stomas *per* mm^2) (Barcikowski et al. 2003). These differences could be due to morphological variations between populations and subpopulations. Therefore, we consider that SD values should only be used as a reference for the specific sites studied. Morales Rodríguez et al. (2016) observed that the decrease in SD was related to the expansion of the leaflet as the leaf ages. Peñón 7 is an area free of anthropogenic impact where leaves can age and expand and therefore a low SD is observed. The plants in the areas near and at Carlini Station were exposed to different types of stress (fuel, vapors, trampling) and, as a form of protection, they expanded their leaves less.

Our results showed a clear trend towards an increase in SD, mainly on the abaxial side of the leaves (Table 1). The increase of stomas could cause a failure in the protection of the leaves as it would make them more susceptible favoring, for example, the pathogens entry (Morales Rodríguez et al. 2016), a fact that was also reported for other species (Husen and Iqbal 1999; Bruno et al. 2007; Fusaro et al. 2015). The morphological differences, observed among the plant leaves from the different sites, would not be of genetic origin, since the populations and subpopulations had a low rate of genetic differentiation and were distinguished using specific molecular markers (Holderegger et al. 2003; Chwedorzewska et al. 2004, 2008; Rabokon et al. 2019). In summary, the variation in SD among the impacted sites would then form a plastic response to the present stress conditions (Alberdi et al. 2004; Giełwanowska et al. 2005).

The increase in SD recorded in the plant leaves of the Supply Zone was due to the effect of continuous trampling through the periods of unloading food and fuel during the summer months, a fact that continuously occurred since the installation year of the Jubany (Carlini) Scientific Station (1953). This effect was similar to that observed by Lewis Smith (1988) on Signy Island (South Orkney) in response to trampling by a natural biological agent, the Sea Lion *Arctocephalus gazella*. As a result of the trampling in the supply area, the vegetation cover has disappeared and, besides, the effect of the melting snow further erodes this transit area. Jägerbrand and Alatalo (2015) reported that trampling, even with low frequency, produces alterations in the ecosystem.

Exposure to anthropogenic activity produces effects on plants that are reflected in altered SD and SI values, which, also, depending on leaf age, plant type, and species, among other parameters (Pourkhabbaz et al. 2010; Kardel et al. 2010). Occasional fuel spills, which may occur mainly when the Scientific Station is restocked, would be directly responsible for the damage caused on the leaves. Therefore, an increase in SD, chlorosis, and the formation of small mats was observed in the leaves, but without epidermal rupture or reduction in the size of the stomas, as was observed in other species such as *Sorghum bicolor* L. (Komolafe et al. 2015).

We did not find significant differences in SI among all the sampled sites (Table 1). This parameter would be influenced by the incidence of sunlight during leaf development (Schoch et al. 1980) and, therefore, was not affected by anthropogenic impact. Although leaf size was not measured, the high positive correlation between SD and SI ($r = 0.92$, $p < 0.00$) recorded in Peñón 7 indicated that leaves were large in that area. The decrease in correlation at the impacted sites would be associated with a decrease in leaf size which would provide an adaptive advantage in *D. antarctica* by protecting its leaves from non-specific cell damage (Ferriol et al. 2004).

The somatic aperture is associated with physiological factors such as K^+ and Ca^{2+} ion regulation, xylem pH variations, CO_2 concentration and osmotic potential regulation (Ψω) among others (Dayanandan and Kaufman 1975; Suarez Moya and Fernández González 1984; Cadena Iñiguez et al. 2001; Eisenach and De Angeli 2017). We did not find differences in the opening of the leaves´ stomas from plants collected at the four studied sites. This would be explained by the phenotypic plasticity of the leaves of *D. antarctica* leaves that allowed it to acclimatize to a micro-environment by varying the number and distribution of stomas.

The present work showed that the correlation between SD and SI on the adaxial side of the leaves could be a good biomarker for the anthropogenic impact estimation since they did not experience morphological changes. Therefore, the data obtained could be used as reference values to adopt, in time, the appropriate strategies to avoid vegetation loss and changes in the surveyed areas.

Declarations

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Compliance with ethical standards

The authors declare to have no conflicts of interest. All applicable international, national, and institutional guidelines for sampling, care and experimental use of plants for the study were followed as established by the Article III, Annex II of the Madrid Protocol, Law 24.216 (Taking, Harmful Intrusion and Introduction of Species) within the framework of the projects evaluated by the Environment Office of the IAA and Dirección Nacional del Antártico (DNA).

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Tables

Table 1. *Deschampsia antarctica* stomatic density ratio (SDR) and stomatic index (SI) for each sampled site. AB: abaxial side. AD: adaxial side. Values are expressed as means± standard error. Different letters means significate differences ($p < 0.05$). N= 150 (number of observations by zone).

Sampled site	SD		SDR	SI	
	AB	AD		AB	AD
Peñón 7	57.36 ± 2.03 a	12.66 ± 1.58 a	4,53	16.22 ± 0.40 a	3.18 ± 0.5 a
Supply Area	61.30 ± 2.32 ab	16.23 ± 1.65 ab	3,78	16.70 ± 0.54 a	3.61 ± 0.31 a
Electric Power Station	69.56 ± 2.23 b	20.08 ± 2.16 b	3,46	16.98 ± 0.47 a	3.76 ± 0.4 a
Fuel Tanks	80.11 ± 2.42 c	13.47 ± 1.92 ab	5,95	19.33 ± 0.41 a	3.05 ± 0.38 a

Table 2. Estimated Pearson's correlation coefficients between the variables: Stomatic Density (SD) and Stomatic Index (SI) for each sampling site. ab: abaxial side. ad: adaxial side. Significant values at $p < 0.05$.

	Peñon 7	Fuel Tanks	Electric Power Station	Supply Area
DE ab/ IE ab	r=0,62 p=0,00	r=0,65 p<0,00	r= 0,67 p<0,00	r=0,52 p<000
DE ab/ IE ad		r=0,23 p=0,02		
DE ad/ IE ab		r=0,29 p<,000		r=-0,16 p=0,05
DE ad/ IE ad	r=0,92 p=0,00	r=0,85 p<0,00	r= 0,68 p<0,00	r=0,45 p<0,000
IE ad/ IE ab		r=0,34 P<0,000		

Figures

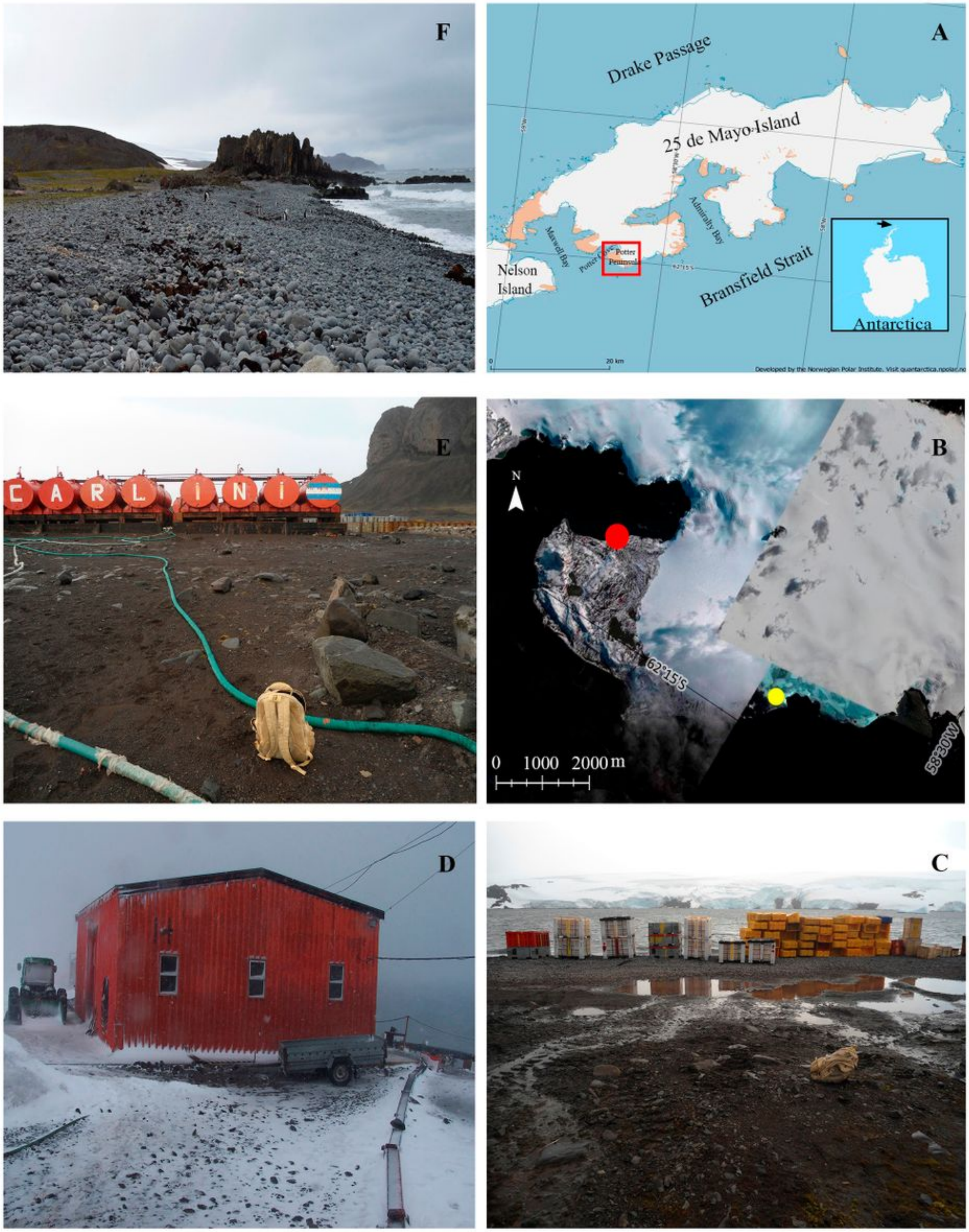

Figure 1

Sampling sites in Carlini Science Station, Antarctica. A: Map showing location of Potter Peninsula in May 25 Island (King George Island) (QGIS.org, 2020). B: Map showing Potter Peninsula. In yelow circle Science Station, in red circle Peñon VII. C: Supply area. D: Lateral area adjacent to the electric power station. E: Fuel tanks. F: Peñón VII. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

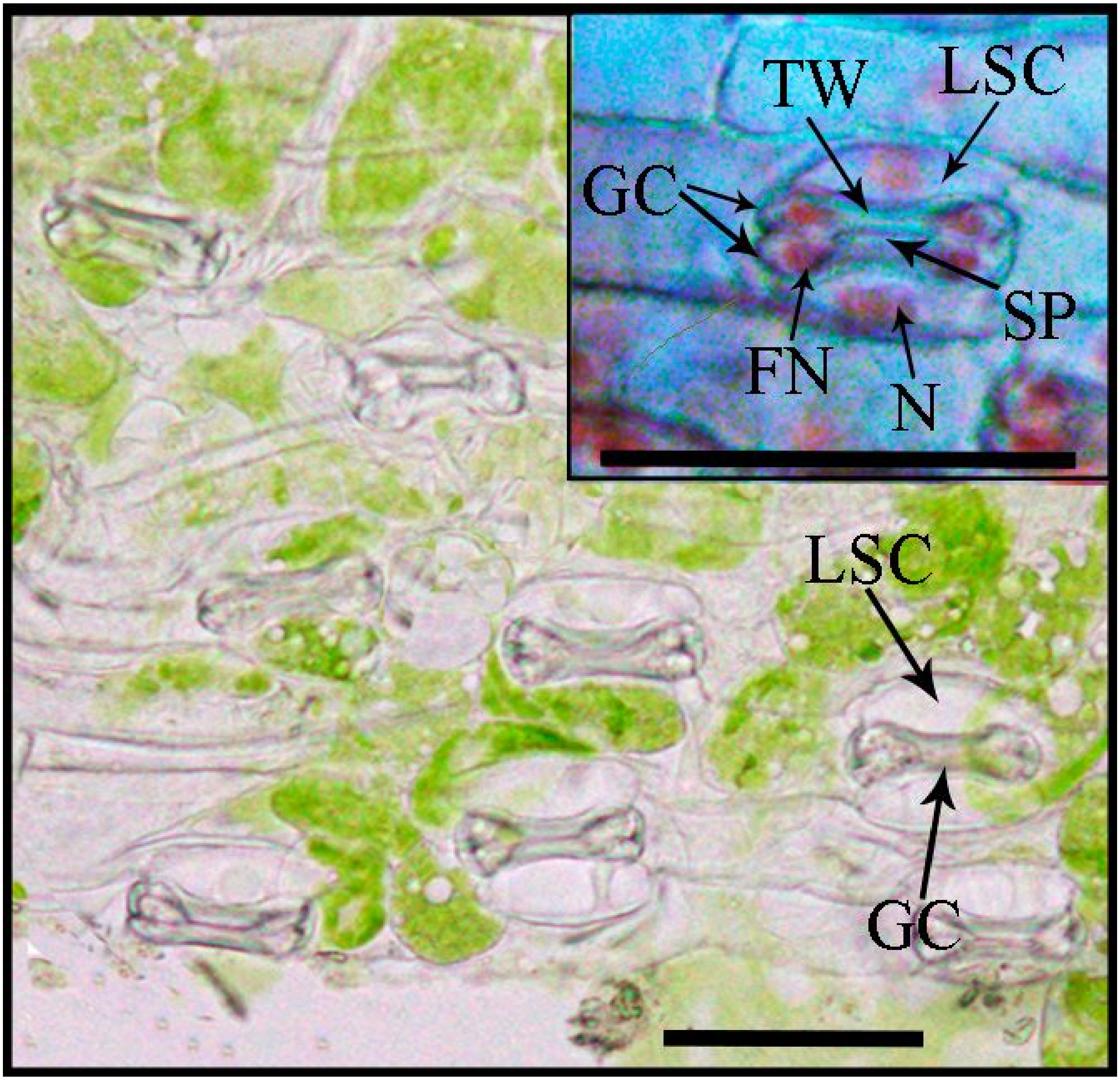

Figure 2

General view of the leaf abaxial side of Deschampsia antarctica. Many stomas are observed. The arrows show guard cells of a stoma and lateral subsidiary cell. Inset: Detail of stomata. GC: guard cell, LSC: lateral subsidiary cell, N: nucleus of the lateral subsidiary cell, NF: nucleus filiform, SP: stomatal pore, TW: Thickened wall. Scale bar (Fig and inset): 50µm.

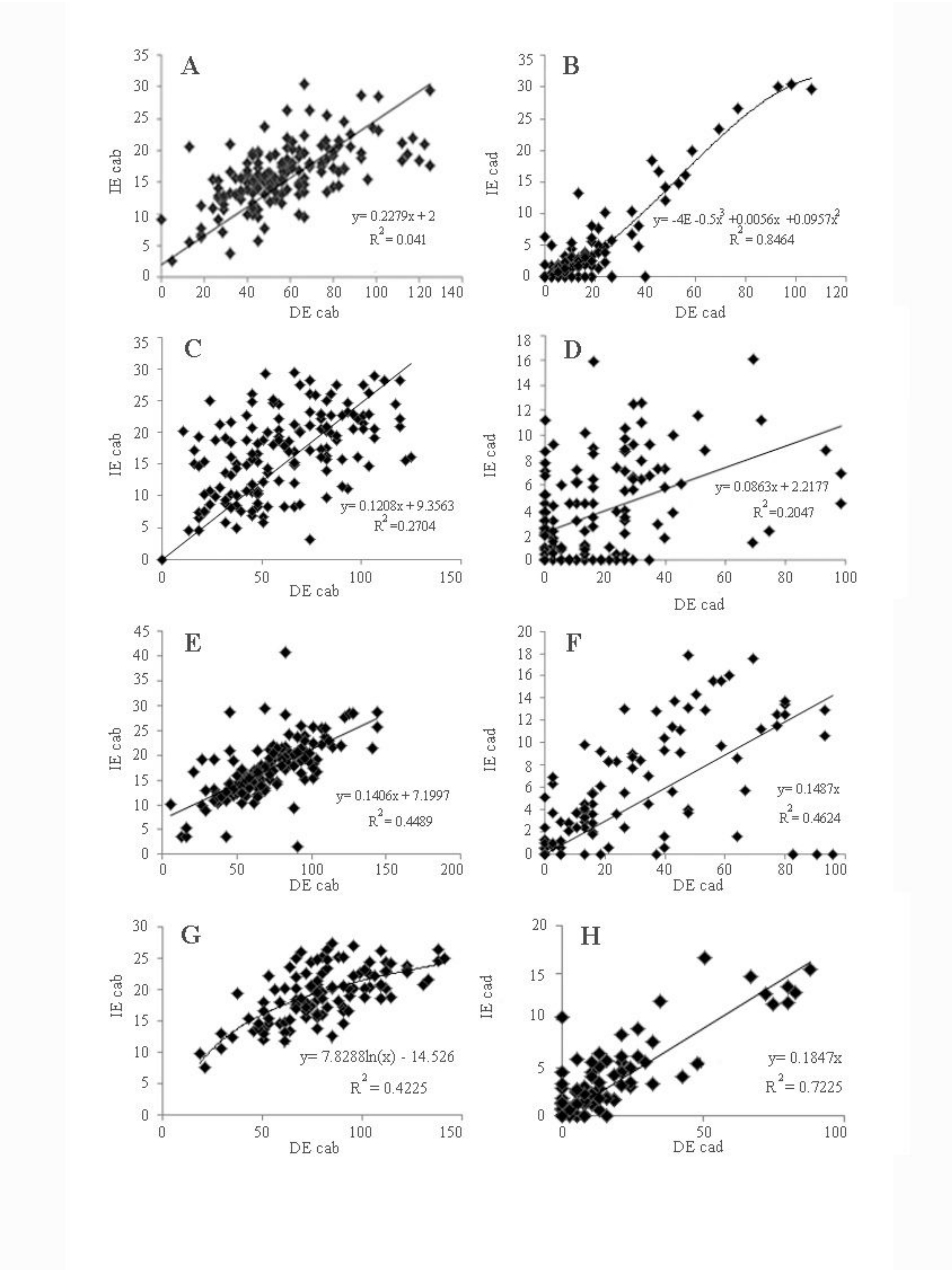

Figure 3

Correlations between stomatic density (SD) and stomatic index (SI) of the sites studied. A-B: Peñón 7. C-D: Supply area. E-F: Electr ic power station. G-H: Fuel tanks. ab: abaxial side; ad: adaxial side.