

Adaptive variation of Patagonian *Senecio patagonicus* Hook. & Arn., *Adesmia boronioides* Hook.F. and *Lepidophyllum cupressiforme* (Lam.) Cass. as a plant response to environmental stress

[Variación adaptativa en respuesta al estrés ambiental de *Senecio patagonicus*, *Adesmia boronioides* y *Lepidophyllum cupressiforme* que habitan en dos localidades de la Patagonia chilena]

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Abstract

Different analyses were carried out on the following plant species: *Adesmia boronioides* Hook.F., *Senecio patagonicus* Hook. & Arn. and *Lepidophyllum cupressiforme* (Lam.) Cass. in order to compare the photosynthetic pigment, carotenoid and phenylpropanoid contents. Total phenolic content and antioxidant capacity were measured by using common established reagents: Folin-Ciocalteu and ABTS respectively. Plant samples were collected from specimens growing inside the National Park Pali Aike and the Botanic Garden Carl Skottsberg in Punta Arenas city. The results showed no marked tendencies between photosynthetic pigments. Nevertheless, a good correlation was found among the antioxidant capacity and phenolic compounds. Furthermore, species growing in the Pali Aike National Park seem to be more adapted to the environmental conditions.

Keywords: Environmental stress, antioxidants, phenolic compounds, Patagonia.

Resumen

Se realizaron diferentes análisis al contenido de pigmentos fotosintéticos, carotenoides y fenilpropanoides. El contenido de fenoles totales y la capacidad antioxidante se midieron usando reactivos ampliamente conocidos: Folin-Ciocalteu y ABTS respectivamente en las siguientes especies: *Adesmia boronioides*, *Senecio patagonicus* y *Lepidophyllum cupressiforme*. Estas plantas se encuentran en el Parque Nacional Pali Aike y en el Jardín Botánico Carl Skottsberg en la ciudad de Punta Arenas. Los resultados no muestran una tendencia clara entre las muestras de pigmentos fotosintéticos. Sin embargo, se pudo observar una buena correlación entre la capacidad antioxidante y el contenido de compuestos fenólicos. Además, aparentemente las especies que crecen en el Parque Nacional Pali Aike están más adaptadas a esas condiciones ambientales.

Palabras Clave: Estrés ambiental, antioxidantes, compuestos fenólicos, Patagonia.

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INTRODUCTION

In the few last decades, the search for antioxidants in nature has received much attention from researchers in order to find natural sources, substances that can be formulated to give nutraceuticals to prevent oxidative damage in biological organisms. It is well-known that plants must respond to stressful conditions in order to survive. Generally, these adaptations involve changes in their metabolic processes. In many cases, these stress factors produce reactive oxygen species (ROS) that can be used by plants in their own metabolism. However, when they increase to elevated concentrations, significant damage to the plants may occur (Buchanan *et al.*, 2002). Excess ROS in plants can lead to cumulative damage in proteins, lipids and DNA (Halliwell, 2006; Finkel and Holbrook, 2000). Hence the balance between antioxidation and oxidation is believed to be critical in order to maintain a healthy biological system. Plants expend metabolic energy and produce antioxidants that react with these ROS species generated in different metabolic pathways to reduce oxidative stress (Nicićforovic *et al.*, 2010).

The sites selected for this study are both part of Chilean Patagonia: the Pali Aike National Park (PANP), situated approximately 250 km to the west of the city of Punta Arenas, is a very unique place surrounded by volcanic structures and with highly degraded soil over an extension of 5000 ha. The last episodes of volcanic activity occurred almost 15000 years ago. In Pali Aike, strong winds blow persistently and rain precipitation is very low (200-250 mm per year). Its flora typically represents the Patagonian steppe in a semi-arid ecosystem (Dominguez *et al.*, 2004; Skewes, 1978). The Botanic Garden Carl Skottsberg (CSBG) is located at The University of Magallanes. This site has significant precipitation with more than 400 mm rain each year and is associated with the transition to highland weather and a more stable near-ocean temperature (Santana and Butorovic, 2010). The concept of this garden as a representation of the plant diversity of the Magellan Region was conceived by botanist Edmundo Pisano in 1970. The three species considered in this study are representative shrubs of the flora of the Magellan Region. *Senecio patagonicus* and *Lepidophyllum cupressiforme* are quite resinous. An infusion of *Adesmia boronioides* is used by local natives for its medicinal properties in treating some illnesses.

In this research, we examined intra-specific variations in the antioxidant capacity of *Senecio*

patagonicus, *Adesmia boronioides* and *Lepidophyllum cupressiforme* at these two climatically diverse sites.

MATERIALS AND METHODS

Plant material

Plant samples were collected in January and May 2011. Fresh leaves were kept in liquid nitrogen and transported to the laboratory for chemical analysis. Only leaves of the first floral stage that were exposed to full sunlight were used in this study.

Chlorophyll a, b and carotenoids

Leaves were chopped (1 cm²) and total chlorophylls and carotenoids were extracted with 5 ml of DMSO for 12 h at 65 °C in the dark as described in Hiscox and Israeltam (1979). The absorbance of the resulting samples (1 ml) was determined at 664, 648 and 470 nm. Photosynthetic pigment concentrations were calculated according to equations given in Chapelle *et al.*, (1992).

Phenylpropanoids

Flavonoids and phenylpropanoids (e.g., vacuolar compounds) were extracted by grinding leaves with a pestle and mortar, using 2 mL of the mixture MeOH:H₂O:HCl=79:20:1 (v/v) as in Mirecki and Teramura (1984). Fresh MeOH was added to the homogenates and their absorbance read at 300 nm.

Total phenolic content (TPC)

In this analysis, the Alvarez *et al.*, (2008) method was used. 50 µL of methanolic extract was added to 250 µL of Folin-Ciocalteu (undiluted). After 1 min, 750 µL of 20% (w/v) Na₂CO₃ solution was added and then made up to 5 mL with deionized water. After 2 h of incubation at room temperature, the absorbance was read at 760 nm. Methanolic solutions of gallic acid were used as standards. The phenolic concentrations were calculated using the regression equation indicated in Alvarez *et al.*, (2008):

$$Y = 0.0019X - 0.0271$$

Where:

Y = absorbance

X = phenolic concentration (GAE: mg of gallic acid/g of extract).

Antioxidant capacity

The free radical scavenging capacity was studied by using the ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation discoloration assay (Dudonné *et al.*, 2009), which is based on the reduction of ABTS+• radicals by antioxidants of plant extracts. ABTS was dissolved in deionized water to a 7 mM concentration. The radical cation (ABTS+•) was produced by reacting the ABTS solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. For the study the ABTS+• solution was diluted in deionized water to an absorbance of 0.7 (± 0.02) at 734 nm. A solvent blank reading was taken (A_B). After the addition of 100 μ L of methanolic plant extract solutions to 3 mL of ABTS+• solution, the absorbance reading was taken at

room temperature 10 min after initial mixing (A_E). The percentage of inhibition of ABTS+• was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_B - A_E)/A_B] \times 100$$

Where:

A_B = Blank absorbance

A_E = Extract absorbance.

Statistical analysis

All results are expressed as means \pm standard deviation and represent the mean of 3 replicates. T-Pearson correlation and t-test (2 variables) were used. The P value for all analysis was < 0.05 (Nícorovic *et al.*, 2010, Siatka and Kasparová, 2010). The software used in statistical analysis and graphics were Statistica 7 and MiniTab 16.

Table N° 1
Variation of photosynthetic pigment content^a.

		chlorophyll <i>a</i>	chlorophyll <i>b</i>	carotenoids
<i>A. boronioides</i>	1	8.99 \pm 0.84 ^c	7.35 \pm 0.92 ^c	1.30 \pm 0.08 ^c
	2	10.90 \pm 0.85	9.28 \pm 0.21	1.11 \pm 0.13
<i>S. patagonicus</i>	1	10.51 \pm 0.50 ^c	11.11 \pm 1.04 ^b	0.99 \pm 0.09 ^b
	2	8.03 \pm 1.18	5.17 \pm 0.35	1.67 \pm 0.07
<i>L. cupressiforme</i>	1	7.88 \pm 0.81 ^b	12.53 \pm 0.22 ^c	0.54 \pm 0.09 ^c
	2	10.56 \pm 0.16	11.30 \pm 0.59	1.16 \pm 0.26

^aMean concentration (μ g pigment/ml extract; N = 3) and standard deviation of plants from different localities: (1): PANP, (2): CSBG.

^bMeans are statistically different by T-test; P < 0.05.

^cMeans are not statistically different by T-test; P > 0.05.

RESULTS**Chlorophyll *a*, *b* and carotenoids**

The results of photosynthetic pigment analysis showed statistically significant differences between site samples in the chlorophyll *b* content of *S. patagonicus* and *L. cupressiforme*. In *S. patagonicus* the concentration of chlorophyll *b* ($11.11 \pm 1.04 \mu\text{g/ml}$) from PANP is almost double that of samples from the CSBG ($5.17 \pm 0.35 \mu\text{g/ml}$; Table 1). By contrast, the carotenoid content of both species were higher in samples from the CSBG than those from PANP. Likewise, samples of *A. boronioides* from the CSBG

showed higher content of Chl *a* and Chl *b* than samples from the PANP.

Phenylpropanoids

The absorbance at 300 nm of *A. boronioides* was higher for plants from PANP (2.3 ± 0.12 AU) than samples from CSBG (1.36 ± 0.12 AU) (Table 2). Likewise, samples of *S. patagonicus* from the PANP showed a higher absorbance (2.46 ± 0.04 AU) than those from the CSBG (1.71 ± 0.16 AU). All differences were statistically significant. The third species (*L. cupressiforme*) did not show any difference in this analysis.

Table N° 2
Variation of phenolics, antioxidant capacity and phenylpropanoids.

		Phenylpropanoids ^a		ABTS ^a		TPC ^a	
<i>A. boronioides</i>	1	2.30	± 0.12 ^b	22.72	± 0.95 ^c	54.53	± 0.26 ^c
	2	1.36	± 0.12	20.20	± 5.51	53.74	± 0.91
<i>S. patagonicus</i> ^b	1	2.46	± 0.04	34.08	± 4.56	75.18	± 4.61
	2	1.71	± 0.16	4.01	± 3.88	64.53	± 2.89
<i>L. cupressiforme</i>	1	1.55	± 0.04 ^c	30.48	± 0.54 ^b	67.26	± 0.64 ^b
	2	1.63	± 0.04	19.86	± 1.74	59.53	± 0.53

^aMean concentration (phenylpropanoids = absorbance at 300 nm; ABTS = % of inhibition; TPC = mg GAE/g extract; N = 3) and standard deviation of plants from different localities: (1): PANP, (2): CSBG.

^bMeans are statistically different by T-test; P < 0.05.

^cMeans are not statistically different by T-test; P > 0.05.

Total phenolic content (TPC)

The Folin-Ciocalteu method indicates the total phenol content and gives a general picture of the molecular content of phenolics in samples. In this assay *S. patagonicus* and *L. cupressiforme* from PANP showed higher values than samples from the CSBG. Conversely, *A. boronioides*' samples did not show any statistical differences.

Antioxidant capacity

According to the results of the ABTS assay (Table 2), the major antioxidant capacities were found in *S. patagonicus* (34.08 ± 4.56%) and in *L. cupressiforme* (30.48 ± 0.54%), both species from the PANP. The antioxidant capacity of *S. patagonicus* samples from PANP was almost 8 times higher than that of samples from the CSBG (4.01 ± 3.88%). *A. boronioides*' samples did not show any differences between locations.

DISCUSSION

In this study, we analyzed intra-specific variations in the antioxidant capacity of different species from two climatically diverse sites. These results are shown in table 1 and 2. Table 1 shows the pigment content of plants from both locations. It can be seen that *S. patagonicus* samples from PANP have higher content of Chl *b* than plants from CSBG. Similar variations are observed in the Chl *a* content of *A. boronioides* and *S. patagonicus*. These pigments are located in the light harvesting complex and their function is to collect light and direct it to the photosystems. It seems that

plants increase the amount of light harvesting pigments to dissipate the sun radiation from inner tissues thus avoiding irreparable damage to photosynthetically important membrane systems (Melis *et al.*, 1992). These findings are suggesting that environmental conditions such as the amount and quality of sun radiation may alter the photosynthetic pigments content. However, other environmental conditions such as wind, rain, and air temperature may also be inducing physiological problems (Salisbury and Ross, 1992).

Because of the absorption of flavonoids and phenylpropanoids ($\lambda = 270-310$ nm) these compounds are likely to participate in the protection of the plant against UV-B radiation (Cuadra and Harborne, 1996). Previous analyses of flavonoids and associated phenolics in plants subjected to UV-B stress have either been carried out on total leaf content or else on the internal leaf constituents (Cuadra *et al.*, 1997). In the present study on three Patagonian plants attention was focused on internal phenolics collected by grinding leaves in acidic aqueous methanol. Our results (Table 2) show that statistically significant differences occur in these internal components of *A. boronioides* and *S. patagonicus*. Apparently plants from much exposed environments (e.g. PANP) activate other mechanisms -different to those involved in the light harvesting complexes- to deal with enhanced levels of UV-B radiation. These findings tally well with those reported in the literature showing that plants accumulate unidentified UV-B absorbing compounds after UV-B exposure (Alenius *et al.*, 1995;

Cuadra *et al.*, 1997; Xiong and Day, 2001). These results also agreed with Cooper and Bhattacharya (1998) who have suggested that hydroxycinnamic acids and their derivatives play an important role in plant defense against UV-B radiation.

On the other hand, ABTS differences may be explained as a consequence of the above environmental variables that produce ROS species in these much exposed plants. The ABTS assay is particularly interesting in plant extracts because the wavelength absorption at 734 nm eliminates color interferences (Li *et al.*, 2008). The results of ABTS assay (Table 2) may suggest a relationship among phenolic compounds and free radical scavenging activity. This assumption is supported by statistical analysis of data. There is a good correlation among ABTS and TPC assays for all species: $r = 0.87$ for *A. boronioides*, 0.95 for *S. patagonicus* and 0.99 for *L. cupressiforme*. Similarly, a good correlation is observed in *S. patagonicus* among the phenylpropanoid content (A_{300}) and TPC ($r = 0.92$). Previous phytochemical studies on *S. patagonicus* (Villarroel *et al.*, 1991) and *A. boronioides* (Galleguillos *et al.*, 2008) have reported the presence of flavonoids which may sustain the above premise considering that phenolic compounds in plants may contribute significantly to their antioxidant potential. These findings are in agreement with previous reports on several medicinal plants (Wong *et al.*, 2006). In this work, the antioxidant properties are directly linked to their structure. Because of the presence of an aromatic ring bearing one or two hydroxyl groups they are potentially able to quench free radicals by forming resonance-stabilized phenoxyl radicals (Rice-Evans *et al.*, 1996; Bors and Michel, 2002). Similar results were found by Moura dos Santos *et al.*, (2012) in *Jathropa curcas* L. They compared the physiological adaptations in response to different climates. After measuring gas exchange, photochemical and biochemical efficiency, photoinhibition was detected in plants subjected to severe water deficit. A reduction in quantum yield was also observed. As a response, *J. curcas* increased catalase activity, accumulated proline, sugars and aminoacids which may represent a protection mechanism for plants under drought conditions. Likewise, Oh *et al.*, (2009) studied the responses of lettuce (*Lactuca sativa* L.) under water stress in order to examine the activation of genes involved in secondary metabolism and biosynthesis of antioxidants. They found a large increase in transcript level for PAL. After induced inhibition of PAL with 2-aminoindan-2-phosphoric acid, plants become more

sensitive to chilling and heat shock treatments. They concluded that activation of the secondary metabolism as well as the antioxidant metabolism is an integral adaptation to these conditions. In another study, Muñoz *et al.*, (2007), working on several aromatic plants, associated the free radical scavenging capacity with the chemical composition of plants with high levels of essential oils. All these findings are supporting our premise that correlates the antioxidant activity with secondary metabolites as a defense mechanism of plants to avoid damage to their structures.

In another context, Gutiérrez *et al.*, (2012) analyzed the antioxidant activity of several Mexican plants used in traditional medicine. Their purpose was to correlate the anti-inflammatory activity with the antioxidant capacity of methanolic and alkaloidal extracts. The results of Folin-Ciocalteu, DPPH and ABTS assays showed that the methanolic extract has higher antioxidant activity than the alkaloidal extract. These results were attributed to a larger amount of phenolics (111 g GAE/g) contained in the extract. As in our study, phenolic compounds are also involved in the antioxidant properties of plants.

CONCLUSIONS

Adverse environmental conditions may generate diverse plant responses in order to avoid potential damage. One of these responses may be the production of antioxidant substances which can capture reactive and dangerous molecules. They may also increase the concentration of constitutive metabolites. Nevertheless, it seems that plants use a combination of different strategies in order to survive in hostile environments.

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