Abscisic acid and its metabolites are involved in drought tolerance in four native species of Patagonian semiarid shrublands (Argentina)

Ana M. Cenzano[A,C]*, Mariana Reginato*[B]*, M. Celeste Varela*[B] *and M. Virginia Luna*[B]

[A]Laboratorio de Ecofisiología y Bioquímica Vegetal, Instituto Patagónico para el Estudio de los Ecosistemas Continentales (IPEEC), Centro Científico Tecnológico Consejo Nacional de Investigaciones Científicas y Técnicas-Centro Nacional Patagónico (CCT CONICET-CENPAT), Boulevard Brown 2915. 9120, Puerto Madryn, Chubut, Argentina.
[B]Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto. Ruta 36, Km 601. 5800, Río Cuarto, Córdoba, Argentina.
[C]Corresponding author. Email: cenzano@cenpat-conicet.gob.ar

Abstract. Drought is the main stress that affects growth of native species of Patagonian Monte. Physiological and biochemical traits with functional roles in adaptation to drought are still scarce in native species. The aim of this study was to evaluate changes in dry matter content and abscisic acid (ABA) and its metabolites (phaseic acid-PA, dihydrophaseic acid-DPA, ABA conjugated with glucose-ABA-GE) level in green leaves and fine roots of four native species during contrasting seasons regarding water availability. Results show that grasses had higher leaf dry matter content (LDMC) and root dry matter content (RDMC) than *Lycium chilense* shrub and LDMC decreased during autumn in *Larrea divaricata*. In green leaves, there was an interaction between species and seasons for ABA and PA contents. *L. divaricata* had high ABA content during spring and low ABA content during autumn; while *L. chilense* showed the opposite pattern. During spring, drought tolerant species had high PA content while drought avoidant species had low PA content, and during autumn these species showed the opposite pattern. ABA-GE content was highest in *L. chilense* green leaves. In fine roots, ABA content was highest in *L. divaricata* and during spring PA was higher in *L. divaricata* than *P. ligularis* and *L. chilense*. PA was the only metabolite that decreased in *L. divaricata* during autumn. Results showed that ABA homeostasis maintains the functioning of different life forms with different drought resistance strategies in Patagonia semiarid shrublands. In green leaves of the drought avoidant *L. chilense* shrub, the ABA conjugation pathway was active in both seasons. During spring, the most drought tolerant *L. divaricata* showed highest activation of ABA synthesis and ABA oxidation pathways. During autumn *L. chilense* showed highest activation of ABA synthesis and together with *P. ligularis* showed active the ABA oxidation pathway.

Additional keywords: abscisic acid homeostasis, *Larrea divaricata*, liquid chromatography-mass spectrometry, *Lycium chilense*, *Pappostipa speciosa*, phaseic acid, *Poa ligularis*.

Received 9 March 2018, accepted 16 October 2018, published online 3 December 2018

Introduction

Drought is the main stress that limits plant growth and determines the structure and functioning of the Patagonian Monte shrubland ecosystem (Paruelo *et al.* 1998). This shrubland is characterised by a semiarid to arid climate, with strong water deficits in spring and summer, unpredictable small rainfalls events and high evaporation enhanced by westerly winds (Fernández and Busso 1999), which influences plant physiological performance (Loik 2007).

Different plant strategies, considered as sets of traits, lead to successful growth and reproduction in a particular environment (Craine 2009). Drought stress tolerance is associated with

evergreen phenology, low specific leaf area (SLA), high water-use efficiency, low photosynthetic rates, low transpiration rates, osmotic adjustment, cell wall hardening, high production of secondary metabolites, high root allocation and slow relative growth rate. However, drought stress avoidance is associated with deciduous phenology, high SLA, low water-use efficiency, high photosynthetic rates, high leaf nitrogen concentration, low carbon investment in secondary metabolites, and fast relative growth rate (Carrera *et al.* 2000; Givnish 2002; Westoby *et al.* 2002; Campanella and Bertiller 2008; Pérez-Harguindeguy *et al.* 2013; Moreno and Bertiller 2015; Cenzano *et al.* 2016).

In the Patagonian Monte, the conservation of vegetation patches is crucial for preventing soil degradation processes and reducing desertification advancement (Chartier and Rostagno 2006). In this region, one of the typical vegetation patches comprises *Larrea divaricata* (chaparral, jarilla hembra – tall shrub) and *Lycium chilense* (coralillo, yaoyín – medium shrub) shrubs and *Pappostipa speciosa* (desert needlegrass, coirón duro) and *Poa ligularis* (bluegrass, coirón poa) grasses, which inhabit inside or around the patches (Bertiller *et al.* 1991; Campanella and Bertiller 2008; Cenzano *et al.* 2016).

It is well known that a suitable abscisic acid (ABA) level is necessary for successful plant growth under stress conditions (Spollen *et al.* 2000). During drought, ABA is important in root-to-shoot signalling through stomatal closure, reducing transpiration (Srivastava 2002) and maintaining shoot and root growth (Sharp *et al.* 1994). ABA is active in this free form but their metabolites produced by 8′-hydroxylation (phaseic acid, PA; dihydrophaseic acid, DPA) and sugar conjugation (ABA-glucose ester, ABA-GE) are inactive in stomatal closure signalling (Sauter *et al.* 2002; Srivastava 2002; Xu *et al.* 2002; Seiler *et al.* 2011).

Previously, it has been shown that ABA catabolic enzymes are upregulated under prolonged stress, leading to sequester high ABA level (Sreenivasulu *et al.* 2012). However, the conjugated form ABA-GE is a storage form of ABA, which after its hydrolysis produces ABA free, and/or a long distance hormonal signal (Sauter *et al.* 2002).

Detailed studies on ABA metabolite profiling of differential life forms under natural conditions of Patagonian Monte are still scarce and may contribute to the ability to classify plants ecophysiologically. Recently, Cenzano *et al.* (2014) have shown that a different ABA metabolite profile between two grass species of Patagonian Monte is correlated with leaf life span and drought-resistance strategy of each functional type. In this work, the highest ABA level in the green leaves of deciduous and drought avoidant grass *P. ligularis* and the highest DPA level in the evergreen and drought tolerant grass *P. speciosa* grown under controlled water availability were reported (Cenzano *et al.* 2014). However, in shrubs grown under natural field conditions, a high ABA level was reported in the green leaves of drought tolerant *L. divaricata* and a high ABA-GE was found in drought avoidant *L. chilense* during summer (Varela *et al.* 2018). Thus, the accumulation of ABA and their derivative metabolites (PA, DPA and ABA-GE) could be used as physiological or biochemical indicator traits to differ between life forms or between drought resistance strategies of native species inhabiting Patagonia rangelands.

The aim of this work was to study the changes in the vegetative biomass and ABA metabolic profiling (ABA synthesis and catabolism) of four native species from Patagonian Monte between two seasons. We hypothesised that ABA homeostasis is one of the main contributors to maintaining the functioning of different life forms belonging to different functional types that inhabit in natural drought conditions. Also, we theorised that an interaction effect between species and seasons determines a differential pattern of ABA accumulation or catabolism among the two groups of plants classifying according their drought resistance strategy. We predict a higher ABA level in shrubs (*Larrea divaricata*

and *Lycium chilense*) than grasses (*Pappostipa speciosa* and *Poa ligularis*), and a higher ABA level in drought tolerant and evergreen species (*L. divaricata* and *P. speciosa*) than drought avoidant and deciduous species (*L. chilense* and *P. ligularis*). Additionally, the ABA level would be higher in spring than autumn.

Materials and methods

Study site

The study was conducted in the Estancia San Luis (42°40′49.3″S, 65°21′33.6″W), located in north-east of Chubut Province, southern portion of the Monte Phytogeographic Province, Argentina (Fig. 1). This site is characterised by the dominate shrub *Larrea divaricata* Cav. (León *et al.* 1998). The climate is semiarid to arid, with water deficits in spring and summer, westerly winds, annual rainfalls between 200 and 260 mm, mean annual temperature of 13.9°C, and unpredictable rainfalls events (Fernández and Busso 1999; Campanella and Bertiller 2008, 2009).

Climatic conditions

Daily precipitation events and maximum and minimum air temperature were recorded using an automatic weather station with a programmable datalogger (21X Micrologger, Campbell Scientific, Logan, UT, USA). The weather station was programmed to record hourly temperature data and averaging daily data output. The data was downloaded onto a computer every 3 months over the 2 study years (2008 and 2009).

Study species and plant collection

The species selected for the study were *Larrea divaricata* Cav. and *Lycium chilense* Miers ex Bertero shrubs, present in a random patchy structure together with *Poa ligularis* Nees ex Steud. and *Pappostipa speciosa* (Trin. & Rupr.) Romasch. grasses.

Grasses

Larrea divaricata is an evergreen shrub, with vegetative growth during the whole year with a maximum in autumn (April) and reproductive growth from November to February and dimorphic root systems (shallow fine roots and deep roots reaching 3 m or more depth, Table 1), which allows access to shallow and deep-water pools (Bertiller *et al.* 1991). *L. chilense* is a deciduous shrub with vegetative growth depending on water soil availability (Bertiller *et al.* 1991) but frequently with maximum vegetative growth during autumn and roots reach 150 cm depth (Table 1), but none of them constitute a prominent tap root (Bucci *et al.* 2009). *P. speciosa* is an evergreen grass that maintains green leaves during summer but some plants (20–50%) senesce from summer (January). This species has vegetative growth from autumn to spring (Table 1). *P. ligularis* is a deciduous grass, with vegetative growth from autumn (May) to spring (November) and flowering in November, followed by senescence and summer dormancy (Campanella and Bertiller 2008; Table 1). Both grasses are shallow root species that reach 0.9–1.2 m of depth and they only can access shallow soil water (Bertiller *et al.* 1991). According to phenological phases of development, spring

Fig. 1.   View of Patagonian Monte shrublands dominated by *Larrea divaricata* shrub and grasses in a patchy structure alternating with bare soil.

corresponds to the season with the maximum vegetative growth. During summer dry season grasses are found in leaf senescence stage to minimise water loss and *L. chilense* lose their leaves.

Bunches of tillers from grasses – small branches with green leaves and fine roots from shrubs (corresponding to <2 mm diameter and located at 60 cm of depth) – were collected from five independent individuals of each species randomly arranged. The samples harvest was made during two seasons: spring (18 November 2008) and autumn (21 April 2009) and they were immersed in liquid nitrogen to reach the laboratory. After 72 h of lyophilisation, each part (green leaf and fine root) was weighed and the dry weight (DW) was obtained.

Determination of dry matter content in vegetative organs

Samples of green leaves and fine roots taken from four shrubs and five grasses of each species from each season were used for determination of leaf dry matter content (LDMC) and root dry matter content (RDMC). Fine roots were separated from soil and washed with tap water on a 2000 µm sieved mesh and dried with adsorbent paper. Fresh weight (FW) of green leaves and fine roots was obtained and each plant fraction was immediately lyophilised for 72 h and weighed to obtain DW.

Determination of endogenous levels of abscisic acid and metabolites

Between three and five lyophilised samples of green leaf and fine root of independent individuals (non-pooled samples) of each species grown in the field from each season were used for quantification of ABA and its metabolites. ABA and its metabolites were extracted and purified as described by Cenzano *et al.* (2014). The lyophilised samples (200 mg DW)

were ground in a mortar with liquid nitrogen, and 3 mL acetone : water : acetic acid (80 : 19 : 1, v/v/v) was added, then 50 ng of internal standards of d6-ABA, d3-PA, d3-DPA and d5-ABA-GE (NRC-Plant Biotechnology Institute, Saskatoon, Canada) were added. Extracts were centrifuged at 8000*g* for 15 min, and supernatants were collected and evaporated at 35°C under vacuum in a SpeedVac ISS110 (Thermo Savant; Thermo Fisher Scientific, Suwanee, GA, USA). Dried extracts were dissolved in 0.1 mL methanol : acetic acid (99 : 1, v/v) and then mixed with 0.9 mL 1% acetic acid. Samples were filtered through a syringe filter tip and purified with 3 mL Q3 BondElut-C18 cartridges (Varian, Palo Alto, CA, USA) on a vacuum manifold (Phenomenex, Torrance, CA, USA) at a flow rate <1 mL min^{-1}. Cartridges were conditioned with 1.5 mL methanol and equilibrated with 1.5 mL methanol : water : acetic acid (10 : 89 : 1, v/v/v). Samples (1.5 mL) were loaded onto cartridges and washed with 1.5 mL of the same mixture. ABA metabolites were eluted with 1.5 mL methanol : water : acetic acid (80 : 19 : 1, v/v/v), and collected in a 2-mL flat-bottom Eppendorf tube. The eluate was dried under vacuum by centrifugation (1000*g*, 30 min) at 35°C. Extracts were resuspended in 0.1 mL methanol (100%) and placed in vials. Samples (0.001 mL) were injected, and PA, DPA and ABA-GE were determined by liquid chromatography with electron spray ionisation (LC, Waters Corp., New York, USA) coupled to a tandem mass spectrometer (MS-MS) (Micromass, Manchester, UK) monitored with Masslink ver. 4.1 software (Manchester, UK). Measurements were performed in quadruplicate.

Liquid chromatography

Analyses were performed using a quaternary pump equipped with an auto-sampler; 10 µL of each sample were injected onto

Table 1.   Characterisation of species studied

	Larrea divaricata	*Lycium chilense*	*Pappostipa speciosa*	*Poa ligularis*
		Native species from Patagonian Monte		
Scientific name	*Larrea divaricata*	*Lycium chilense*	*Pappostipa speciosa*	*Poa ligularis*
Common name	Jarilla hembra	Yaoyin	Coirón duro	Coirón poa
Family	Zygophyllaceae	Solanaceae	Poaceae	Poaceae
Life form	Tall shrub[A,B,F,G]	Medium shrub[A,B,C,F,G]	Grass[A,B,D,F]	Grass[A,B,D,E,F]
		Phenological development		
Vegetative growth	Autumn (Apr)–late spring (Dec)[A,B]	Autumn (Jun)–late spring (Dec)[A,B]	Autumn (May)–spring (Oct)[A,B]	Autumn (May)–spring (Nov)[A,B]
Reproductive growth	Spring (Nov)–summer (Feb)[A,B]	Spring (Sep)–late spring (Dec)[A,B]	Spring (Nov)–summer (Jan)[A,B]	Spring (Nov)–summer (Jan)[A,B]
Senescence	Late Spring (Dec)–summer (Mar)[A,B]	Spring (Nov)–summer (Feb)[A,B]	Summer (Jan)–summer (Mar)[A,B]	Late spring (Dec)–summer (Feb)[A,B]
		Leaf traits		
Habit	Evergreen (11 month longevity)[A,B,F]	Deciduous (dry period)[A,B,D]	Evergreen[A,B,F]	Deciduous (summer)[A,B,E,F]
LMA	High (181.25 g m^{-2})[B]	Low (98.36 g m^{-2})[B]	High (237.94 g m^{-2})[B]	Low (143.99 g m^{-2})[B]
Nitrogen concentration	Low (1.77%)[B]	High (5.20%)[B]	Low (1.44%)[B]	High (2.24%)[B,E]
Lignin concentration	Intermediate (6.19%)[B]	Low (4.19%)[B]	High (9.57%)[B]	Low (3.70%)[B]
Phenolics concentration	High (14.45%, 50–80 mg g^{-1} DW)[B,G]	Low (1.50%, 15–25 mg g^{-1} DW)[B,G]	Low (0.68%)[B]	Low (0.66%)[B]
Relative water content	High (53–75%)[G]	Intermediate (40–63%)[G]	Intermediate (0.45 g g^{-1} FW)[D]	High (0.49 g g^{-1} FW)[D]
Root system	Shallow and deep (3 m depth)[A,G]	Shallow (1.1–1.5 m depth)[A,C,G]	Shallow (1.0–1.2 m depth)[A]	Shallow (0.9–1.0 m depth)[A]

[A]Bertiller *et al.* (1991).   [B]Campanella and Bertiller (2008).   [C]Bucci *et al.* (2009).   [D]Cenzano *et al.* (2014).   [E]Moreno and Bertiller (2015).   [F]Cenzano *et al.* (2016).   [G]Varela *et al.* (2016).

a Restek C18 (Restek, Bellefonte, Pennsylvania, USA) column at 28°C. The binary solvent system for the elution gradient was MeOH (solvent A) and 0.2% acetic acid in H_2O (solvent B) at a constant flow-rate of 200 µL min^{-1}. A linear gradient profile with the following proportions (v/v) of solvent A was applied with 7 min for re-equilibration (t (min), % A): (0, 40), (25, 80).

Mass spectrometry

A Micromass Quatro Ultima PT double quadrupole mass spectrometer (Micromass, Manchester City, UK) was used. Analyses were performed using a turbo ion electro spray source in negative ion mode with the following settings: capillary voltage: 3250 V, energy cone: 35 V, RF Lens1: 20, RF Lens2: 0.3, source temperature: 100°C, desolvation temperature: 350°C, gas cone: 100 l h^{-1}, gas desolvation: 701 l h^{-1}, collision: 50, and multiplier: 650.

MS/MS product ions were produced by a collision-activated dissociation of selected precursor ions in the collision cell of a double quadrupole mass spectrometer, and mass was analysed with a second analyser of the instrument. In the negative mode, the spectrum for ABA produced a deprotonated molecule [M–H]$^-$. Identification of ABA and its metabolites was performed by comparing the retention time of peaks in samples with those of a pure standard (ABA-GE Rt 2.30 min, DPA Rt 3.8 min, PA Rt 4.35 min and ABA Rt 9.55 min). Quantification was performed using the multiple reaction monitoring (MRM) mode following the 263 >153/ 269 >159 molecular masses, which corresponded to endogenous/standard ABA, 279 >139/ 282 >142 PA, 281 >171/284 >174 DPA and 425 >263/430 >268 ABA-GE. The MRM mode was required because several compounds might present the same nominal molecular mass. Thus, the combination of parent mass and unique fragment ions was used to selectively monitor each of the standards in crude plant extracts. Data were acquired and analysed using MassLynx 4.1 and QuanLynx 4.1 (Micromass, Manchester, UK) software. For quantification, values were obtained from a calibration curve previously constructed using pure compounds (Sigma, St Louis, MO, USA).

Statistical analyses

The significance of the differences in means of non-normally distributed response variables (LDMC and RDMC) between species and seasons were analysed by Kruskal–Wallis test. The interaction effect between species and seasons on the endogenous level of ABA and metabolites was analysed by 2-way ANOVA. For posteriori multiple comparisons we used Tukey's HSD test. Data were tested for normality by Shapiro-Wilk test and for homoscedasticity by Levene's test. Data were transformed into natural logarithm, cosine and square root (ABA, PA, DPA and ABA-GE) to meet the assumptions of ANOVA when was necessary. When there was no an interaction effect between factors, the significance of the differences between seasons (spring and autumn) for each species was performed by Student's *t*-test due to there were fewer than three groups for *post-hoc* tests. The significance level was set at $P < 0.05$ throughout the study.

Results

Climatic conditions

Climatic conditions corresponding to the years of sampling collection are presented in Table 2. The total precipitation 2008 was less than 2009 and the mean annual of maximum and minimum temperature were similar between both years. The total days with very small rainfall events (0.2–1.5 and 1.6–5.5 mm) were similar between both years. Only four and six rainfall events between 5.6–10.5 mm were registered in 2008 and 2009 respectively. An only rainy day for 20.6–30.5 category during 2008 and three rainy days for categories from 10.6 mm to 40 mm were observed during 2009 (Table 2).

The mean air temperature and cumulative monthly precipitation are presented in Fig. 2*a*. The maximum mean

Table 2. Climatic conditions of the study site corresponding to 2008 and 2009
Mean and total annual values

Climatic conditions	2008	2009
Total precipitation (mm)	133.86	190.54
Precipitation range (mm)	Total rainy days	
0.2–1.5	24	27
1.6–5.5	19	16
5.6–10.5	4	6
10.6–20.5	0	1
20.6–30.5	1	1
30.6–40.0	0	1
Maximum temperature (°C)	21.27	21.18
Minimum temperature (°C)	7.8	7.94
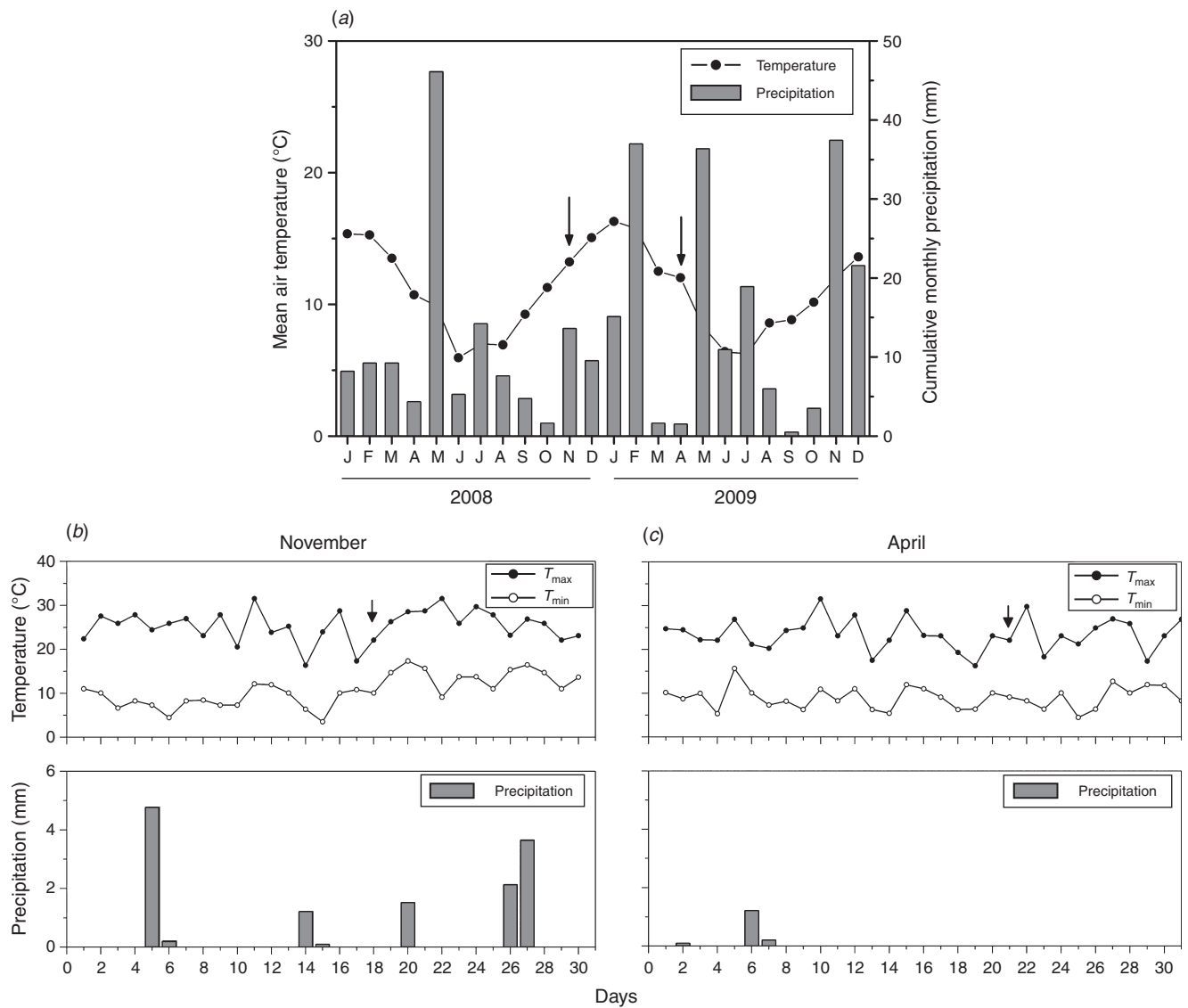
Fig. 2. (*a*) Monthly cumulative precipitation events (mm) and mean air temperature (°C) in 2008 and 2009 years. (*b, c*) Diary precipitation events (mm) and maximum and minimum air temperature (°C) for November 2008 (Spring) and April 2009 (Autumn). Arrow indicates sampling month (*a*) and sampling days of each season (*b, c*).

temperature occurred in summer (January 2008 and 2009) and the minimum mean temperature occurred in winter (June 2008 and July 2009). Small and fluctuating events of precipitation occurred along both years. During 2008, the cumulative monthly largest precipitation occurred in autumn (May, 46.12 mm) and the smallest precipitation was in October (1.62 mm). During 2009, there were three cumulative monthly precipitations (February, 36.98 mm; May, 36.37 mm and November, 37.44 mm) and the smallest precipitation was in September (0.51 mm).

During the first samples collection corresponding to spring, two days of precipitation occurred before the sampling day (day 5, 4.77 mm and day 14, 1.22 mm) were registered (Fig. 2*b*). During the second sample collection corresponding to autumn, only one day of precipitation (day 6, 1.22 mm) was registered (Fig. 2*c*).

Changes in dry matter content in vegetative organs

Significant differences ($P < 0.05$) were observed for dry matter content of green leaves and fine roots. Grasses had higher LDMC than *L. chilense* shrub (Fig. 3*a*). The LDMC of *L. divaricata* decreased during autumn, however no significant differences in the rest of the species were found between both seasons (Fig. 3*a*). *L. chilense* had the lowest RDMC and the grasses had the highest, but there were no significant differences between the grasses and *L. divaricata* and neither between both seasons (Fig. 3*b*).

Changes in abscisic acid and metabolites profiling

Despite the variability in the plant population, the levels of ABA and PA showed a statistically significant interaction effect (species × seasons) in green leaves (Fig. 4*a, b*). This interaction revealed a seasonal effect on ABA and PA content between species (Fig. 5*a, b*). The differences in ABA content of green leaves between seasons were evident for *L. divaricata* and *L. chilense* shrubs. *L. divaricata* showed a high ABA content during spring and a low ABA content during autumn; whereas *L. chilense* showed the opposite pattern (Fig. 5*a*). The differences in PA content were also evident between seasons but they were between drought tolerant species (*L. divaricata* and *P. speciosa*) and drought avoidant species (*L. chilense* and *P. ligularis*). During spring, drought tolerant species had high PA content while drought avoidant species had low PA content in green leaves, and during autumn these species showed the opposite pattern (Fig. 5*b*). In contrast, there was no significant interaction between species and seasons for DPA and ABAGE levels (Fig. 4*c, d*). DPA levels were not significantly different between species and seasons (Fig. 4*c*). ABA-GE levels were higher in *L. chilense* than *L. divaricata* and both grasses (Fig. 4*d*).

In fine roots, there was no an interaction effect between species and seasons (Fig. 4*e–h*). ABA endogenous level was higher in *L. divaricata* roots than the other species in both seasons (Fig. 4*e*). During spring, PA was higher in *L. divaricata* roots than *P. ligularis* and *L. chilense* but during autumn PA was the only metabolites that decreased in *L. divaricata* (Fig. 4*f*). However, DPA and ABA-GE levels did not significantly change between species and seasons (Fig. 4*g, h*).
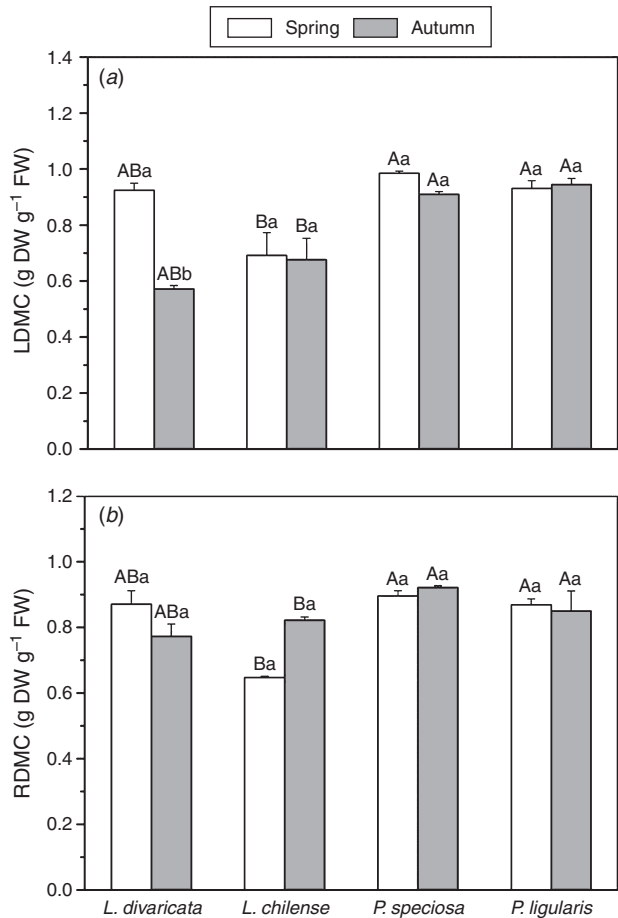

Fig. 3. Leaf dry matter content (LDMC) (*a*) and root dry matter content (RDMC) (*b*) of *Larrea divaricata*, *Lycium chilense*, *Pappostipa speciosa* and *Poa ligularis* in spring and autumn seasons. Means ± s.e. Different uppercase letters indicate significant differences between species and different lowercase letters indicate significant differences between seasons ($P \leq 0.05$).

The ABA metabolic pathway of each species was calculated considering as 100% the sum of the metabolites production in green leaves and fine roots of the individual. The ABA metabolic pathways expressed as percentage of ABA synthesis, ABA oxidation (PA production plus DPA production) and ABA conjugation (ABA-GE accumulation) with respect to total plant metabolite is shown in Fig. 6 for each season. During spring, the green leaves of evergreen species (*L. divaricata* and *P. speciosa*) showed the highest ABA percentage (15 and 7%, respectively, Fig. 6*a*). *L. divaricata* showed the highest ABA content (15%), activation of the ABA oxidation pathway (62%, PA + DPA), and the lowest ABA-GE percentage (4%); whereas the deciduous grass, *P. ligularis* showed the highest activation of the ABA conjugation pathway (55% ABA-GE, Fig. 6*a*). During autumn, ABA percentage was low for all species (6–8% for shrubs and 2% for grasses, Fig. 6*b*); *P. speciosa* showed lowest activation of the ABA oxidation pathway (23%), and other species showed similar percentage of ABA metabolites from oxidation pathway (PA + DPA) in values from 47 to 56%.
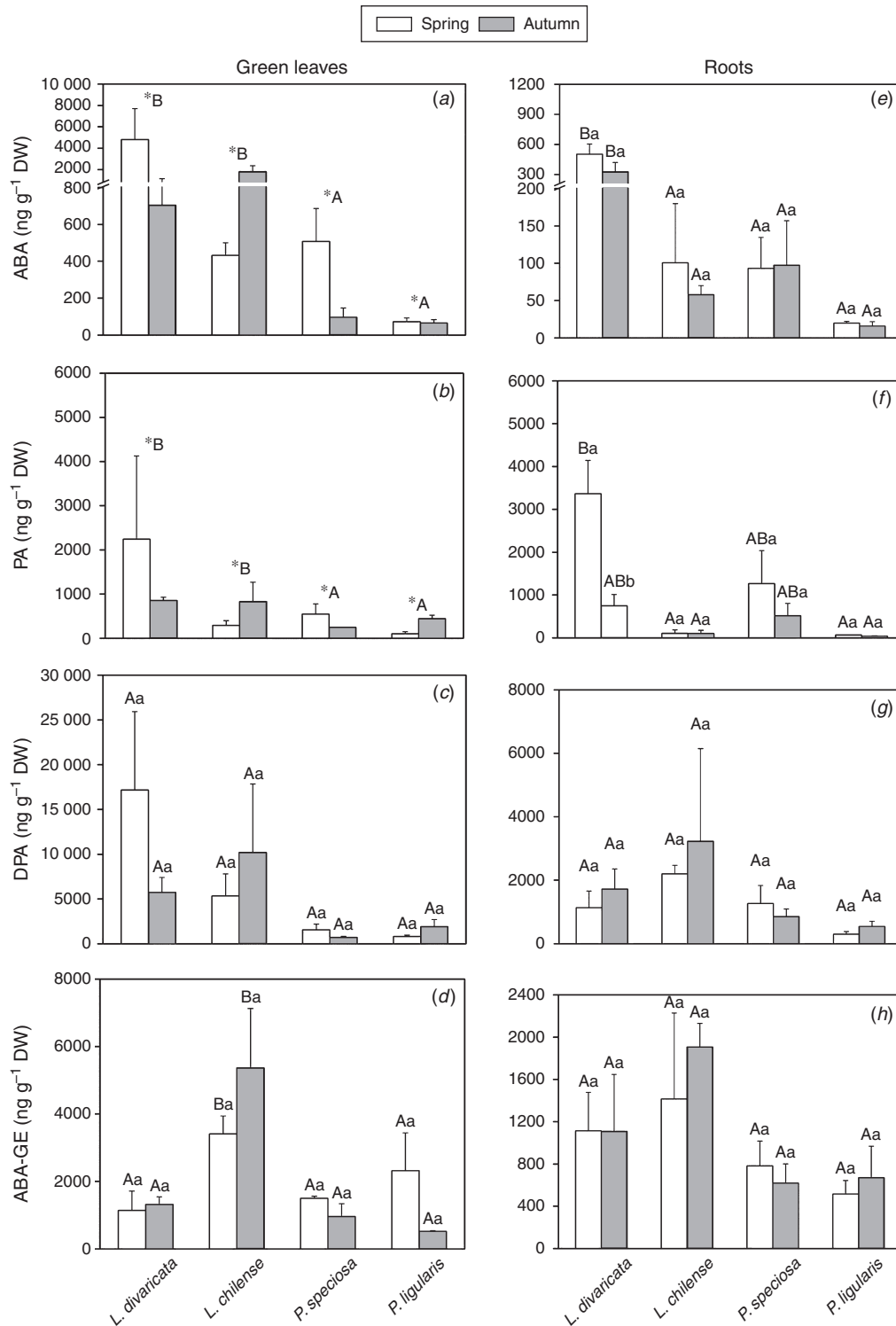
Fig. 4. ABA and metabolites levels in vegetative organs of *Larrea divaricata*, *Lycium chilense*, *Pappostipa speciosa* and *Poa ligularis* during spring and autumn seasons. Abscisic acid (ABA), Phaseic acid (PA), Dihydrophaseic acid (DPA) and abscisic acid conjugated with glucose (ABA-GE) in green leaves (*a–d*) and in fine roots (*e–h*) respectively. Means ± s.e. Asterisk indicates significant interaction effect in the 2-way ANOVA analysis performed for the graphed response variable. Different uppercase letters indicate significant differences between species and different lowercase letters indicate significant differences between seasons ($P \le 0.05$).
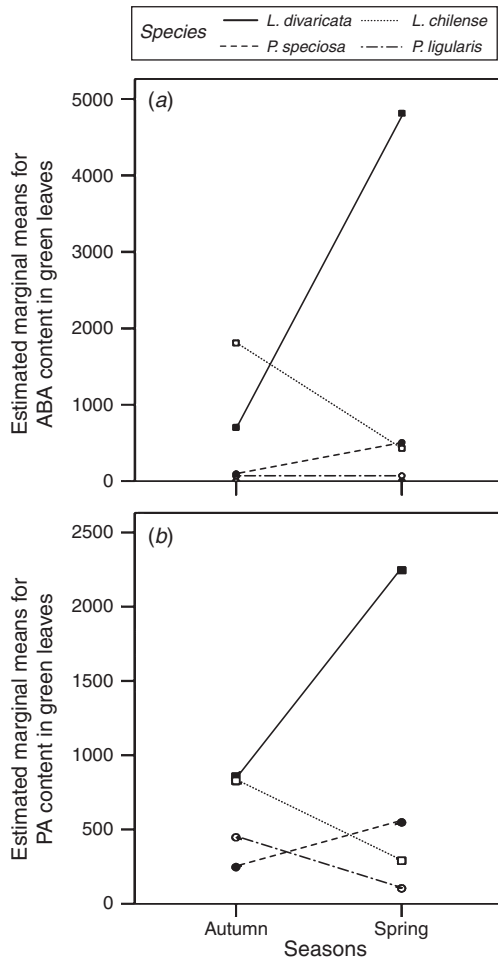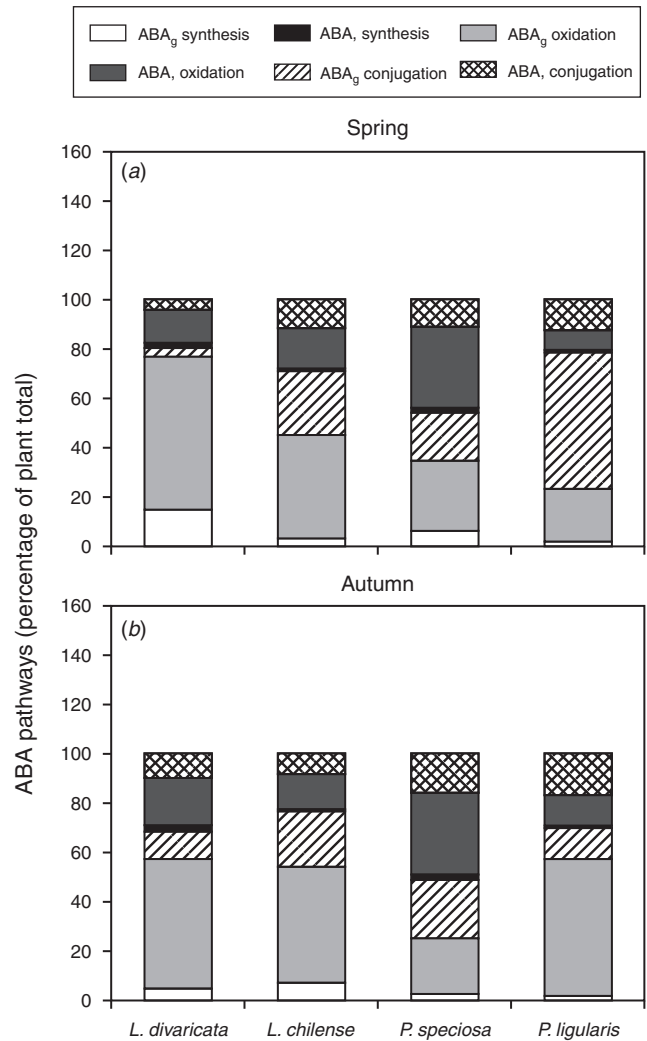
Fig. 5. Interaction effect (species × seasons) on ABA and PA content in green leaves. Non-parallel lines show an interaction effect between both factors.

Fig. 6. Percentage of ABA and its metabolites in the green leaves (gl) and fine roots (r) in relation to total metabolite per plant of *Larrea divaricata*, *Lycium chilense*, *Pappostipa speciosa* and *Poa ligularis* during spring (*a*) and autumn (*b*) seasons.

Finally, the ABA-GE percentage was similar for all species (11–23%, Fig. 6*b*).

In fine roots, evergreen species (*L. divaricata* and *P. speciosa*) had a double ABA percentage than deciduous species (*L. chilense* and *P. ligularis*) for both seasons (Fig. 6*a, b*). The ABA oxidation pathway was more active in *P. speciosa* during both seasons (33–34%) than other species (Fig. 6*a, b*). However, in these organs the ABA conjugation pathway was similar for all species except for *L. divaricata* which showed the lowest ABA-GE percentage during spring (Fig. 6*a*).

Discussion

In arid regions, an effective rain event activates biological processes. Light rains of 10–20 mm, at intervals of a few weeks, maintains moisture in the top 30 cm and will enhance shrub growth, but shrub survival depends on the replenishment of the deeper reserve at least once every 1–2 years by 40–100 mm falling in a short period (Noy-Meir 1973). Particularly, in the Patagonian steppe rainfall events larger than 10 mm are enough to generate a wet year (Golluscio *et al.* 1998). Throughout the present study there

were no rain events of that magnitude; thus, both seasons may be representative of dry seasons. Even the total precipitation during both years was less than 8-year average for this same study site (Campanella and Bertiller 2009). In agreement with our findings, the lack of precipitation events during autumn previously reported for this region (Coronato and Bertiller 1997; Varela *et al.* 2018) could explain the differential seasonal effect on ABA and PA endogenous levels between shrubs and between species with different drought resistance strategies (tolerant or avoidant) respectively. In addition, we found seasonal variation only in green leaf biomass of *L. divaricata*.

An indicator trait of plant resource use related to leaf lifespan is the leaf dry matter content (LDMC) and is considered a good predictor of specific leaf area (Grime *et al.* 1997; Poorter and Garnier 1999; Shipley and Vu 2002). In this sense, evergreen species such as *L. divaricata* have a long leaf lifespan, high LDMC and high potential for resource conservation. In contrast,

deciduous species such as *L. chilense* have short leaf lifespan, low LDMC and high potential for resource acquisition (Bertiller *et al*. 1991; Campanella and Bertiller 2008; Cenzano *et al*. 2016; Varela *et al*. 2016).

Changes in LDMC and RDMC that are complementary to water content in tissues are predicted to vary seasonally. The general pattern is the lowest water content in summer, whereas it tends to increase between autumn and winter (Saura-Mas and Lloret 2007) in accordance with highest rainfalls events registered in Patagonia during both seasons. During spring, the evapotranspiration primarily uses shallow water; while during winter a hydrological recharge occurs leading to non-water limited conditions for plants (Thomas *et al*. 2009). Our results showed that only the shrub *L. divaricata* modified dry matter content of green leaves with the seasons. The increased LDMC of *L. divaricata* in spring coincides with the vegetative growth stage after the winter rainfalls events (Campanella and Bertiller 2008); whereas in autumn the low LDMC correlates with the higher retention of water in the green leaves and reflects a water economic use for growth processes and the maintenance of metabolic activity. In addition, the high LDMC and RDMC in grasses compared with the shrub *L. chilense* could be attributed to the need for carbohydrate translocation to the sink organs to complete reproductive development and to use the carbon fixed in photosynthesis for growth, maintenance and dry matter root production (Farooq *et al*. 2009).

An increased investment in root growth and root dry matter has been observed to increase the water uptake under water stress (Reid and Renquist 1997; Poorter and Nagel 2000). In Patagonian steppe, most of the grasses and dwarf shrubs are shallow-rooted plants that use the water from the upper soil profile coming from recent rain events as a more efficient water transport system; whereas medium and tall shrubs are deeper-rooted plants that use deeper and stable in time water sources (Bertiller *et al*. 1991; Bucci *et al*. 2009).

In the present study, grasses had higher RDMC than *L. chilense*, the woody species, in agreement with high specific root lengths (Jackson *et al*. 1997) and faster use of superficial soil moisture (McLaren *et al*. 2004). A high RDMC could imply high shallow roots production that use deeper and stable in time water sources, as is the case of *L. divaricata* (Bertiller *et al*. 1991).

Drought-tolerant species maintain water-use efficiency reducing the water loss (Farooq *et al*. 2009). The role of ABA during short-drought stress in the green leaves is the induction of stomatal closure that prevent the transpiration water loss (Cutler *et al*. 2010; Tardieu *et al*. 2010); but under prolonged drought this hormone impairs photosynthesis and triggers senescence (Sreenivasulu *et al*. 2012). ABA, in coordination with other hormones, contributes to growth and maintenance of the primary roots (Sharp and LeNoble 2002), allowing adequate water supply (Sharp *et al*. 2004). Under prolonged drought stress an ABA homeostasis is required to attain photosynthetic sustainability and to maintain the stay-green phenotype (Sreenivasulu *et al*. 2012). This homeostasis consists in maintaining ABA near basal levels and regulates metabolites PA, DPA and ABA-GE production. However, plants unable to maintain ABA homeostasis shown high levels of ABA, which may deprive photosynthetic pigments,

mainly the carotenoids which are the ABA precursors, and triggers the growth retardation (Seiler *et al*. 2011; Sreenivasulu *et al*. 2012).

In the present study, *L. divaricata* could be considered as a model of a drought tolerant plant of the Patagonian Monte-natural desert ecosystems, because together with other described traits (high LDMC), this species showed the highest accumulation of ABA in green leaves and in fine roots during spring. In contrast, the drought avoidant *L. chilense* showed the highest accumulation of ABA-GE in green leaves during both seasons, suggesting a role of this metabolite as source of ABA active for physiological responses against natural drought conditions. A biological role for ABA-GE as an ABA source in response to environmental conditions has been proposed after the knowledge that β- glucosidases (BG1 and BG2) mediate the hydrolysis of ABA-GE to ABA (Yamashita *et al*. 2016). These authors found that ABA derived from ABA-GE was enough to produce decreasing transpiration rates and conferring drought tolerance.

Recently, it has been suggested that there is a role for the Patagonian shrubs leaves as safety valves to protect the integrity of the upstream hydraulic pathway (Bucci *et al*. 2013). Moreover, a role for lateral roots has been suggested preventing the reverse water flow from the plant to the soil, thereby decoupling portions of the plants from the drier shallow soil layers (Bucci *et al*. 2013). In particular, the stems of deep-rooted species from Patagonia are less protected from hydraulic dysfunction by leaves than shallow-rooted species that need to conserve stem hydraulic integrity to allow the production of new leaves and fine roots during drought periods (Bucci *et al*. 2013). The anisohydric behaviour of shallow-rooted species is associated with relatively unrestricted stomatal opening during summer drought. This behaviour could benefit gas-exchange rates during the growth season (Scholz *et al*. 2012). In contrast, isohydric species maintain higher leaf water potential under soil water deficit by stomatal closure and exhibit higher ABA accumulation in xylem sap (Coupel-Ledru *et al*. 2017).

In deeper rooted species (>2 m depth) from Patagonia such as *Schinus johnstoni*, a low leaf-specific hydraulic conductivity and high leaf water potential has been reported, whereas shallow species exhibited a high leaf hydraulic conductivity and low leaf water potential (Bucci *et al*. 2009). Thus, biochemical and hydraulic mechanisms are involved in the stomatal closure, through accumulation of ABA by a direct pathway and reducing leaf hydraulic conductance by an indirect pathway (Coupel-Ledru *et al*. 2017). Thus, we could classify the deepest root-evergreen species *L. divaricata* as an isohydric species according to high ABA content in green leaves, but the shallowest root-deciduous species *P. ligularis* as an anisohydric species with low ABA content. In addition, taking into account the seasonal effect on PA content, during spring the drought tolerant species showed activation of ABA oxidation pathway and the drought avoidant species showed the pattern opposite, demonstrating during autumn growth season the need of stomatal opening (through activation of ABA catabolism and decreasing ABA level) of drought avoidant- deciduous species.

During spring, the percentage of ABA in relation to total metabolites per plant was highest in the green leaves and fine roots of evergreen species (*L. divaricata* and *P. speciosa*). *L. divaricata* also showed a high induction of ABA oxidation pathway in the green leaves, suggesting that a balance between ABA synthesis and catabolism occur in this species and it is necessary to maintain others physiological processes such as leaf gas exchange through stomatal opening for carbon gain through photosynthesis (Hetherington and Woodward 2003; Xu and Zhou 2008; Okamoto *et al.* 2009; Pantin *et al.* 2012).

An active ABA conjugation pathway (high ABA-GE percentage in relation to total metabolites) was observed in the green leaves of *P. ligularis* during spring. This pathway activation could shuttle the ABA signal between organs (Sauter *et al.* 2002; Boursiac *et al.* 2013) and acts as a signal transport or storage form providing ABA active when is necessary the stomatal opening (Lee *et al.* 2006).

During autumn, the percentage of ABA synthesis in green leaves in relation to total metabolites per plant was low for both life forms, but in roots, the evergreen species (*L. divaricata* and *P. speciosa*) had the highest. *P. speciosa* had the lowest activation of ABA oxidation pathway in the green leaves and the maximum in the fine roots. The ABA conjugation pathway no changed between species in both tissues during autumn.

In conclusion, our findings support the idea that ABA homeostasis (a balance between synthesis and catabolism) contributes to maintain the functioning of different life forms (shrubs and grasses) that belong to different functional types (e.g. evergreen vs deciduous and drought tolerant vs drought avoidant) that inhabit in natural drought conditions of Patagonian Desert shrubland in Argentina. ABA derived from *de novo* synthesis as well as ABA derived from ABA-GE could have a role conferring drought tolerance under natural drought conditions of Patagonian Monte. The present study may help tin developing an understanding of the spatial distributions of species and the coexistence of different life forms based on physiologic and metabolic mechanisms mediated by ABA to tolerate drought stress. Thus, ABA homeostasis of native species inhabiting arid and semiarid ecosystems may be an important trait to be considered in ecophysiological studies and could prove useful in planning the conservation of this ecosystem.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

References

Bertiller MB, Beeskow AM, Coronato FR (1991) Seasonal environmental variation and plant phenology in arid Patagonia (Argentina). *Journal of Arid Environments* **21**, 1–11. doi:10.1016/S0140-1963(18)30722-5

Boursiac Y, Léran S, Corratgé-Faillie C, Gojon A, Krouk G, Lacombe B (2013) ABA transport and transporters. *Trends in Plant Science* **18**, 325–333. doi:10.1016/j.tplants.2013.01.007

Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Arce ME (2009) Soil water availability and rooting depth as determinants of hydraulic architecture of Patagonian woody species. *Oecologia* **160**, 631–641. doi:10.1007/s00442-009-1331-z

Bucci S, Scholz FG, Peschiutta ML, Arias NS, Meinzer FC, Goldstein G (2013) The stem xylem of Patagonian shrubs operates far from the point of catastrophic dysfunction and is additionally protected from drought-induced embolism by leaves and roots. *Plant, Cell & Environment* **36**, 2163–2174. doi:10.1111/pce.12126

Campanella MV, Bertiller MB (2008) Plant phenology, leaf traits and leaf litterfall of contrasting life forms in the arid Patagonian Monte, Argentina. *Journal of Vegetation Science* **19**, 75–85. doi:10.3170/2007-8-18333

Campanella MV, Bertiller MB (2009) Leafing patterns and leaf traits of four evergreen shrubs in the Patagonian Monte, Argentina. *Acta Oecologica* **35**, 831–837. doi:10.1016/j.actao.2009.08.007

Carrera AL, Sain CL, Bertiller MB (2000) Patterns of nitrogen conservation in shrubs and grasses in the Patagonian Monte, Argentina. *Plant and Soil* **224**, 185–193. doi:10.1023/A:1004841917272

Cenzano AM, Masciarelli O, Luna MV (2014) Abscisic acid metabolite profiling as indicators of plastic responses to drought in grasses from arid Patagonian Monte (Argentina). *Plant Physiology and Biochemistry* **83**, 200–206. doi:10.1016/j.plaphy.2014.07.024

Cenzano AM, Varela MC, Luna MV (2016) Ecophysiology of native species from Patagonian Monte, Argentina. In 'Sabkha ecosystems. Vol. V, The Americas (tasks for vegetation science)'. (Eds MA Khan, B Böer, M Clüsener-Godt, M Öztürk, B Gul) pp. 369–387. (Springer International Publishing AG: Cham, Switzerland)

Chartier MP, Rostagno CM (2006) Soil erosion thresholds and alternative states in northeastern Patagonian rangelands. *Rangeland Ecology and Management* **59**, 616–624. doi:10.2111/06-009R.1

Coronato FR, Bertiller MB (1997) Climatic controls of soil moisture dynamics in an arid steppe of Northern Patagonia, Argentina. *Arid Soil Research and Rehabilitation* **11**, 277–288. doi:10.1080/15324989709381479

Coupel-Ledru A, Tyerman SD, Masclef D, Lebon E, Christophe A, Edwards EJ, Simonneau T (2017) Abscisic acid down-regulates hydraulic conductance of grapevine leaves in isohydric genotypes only. *Plant Physiology* **175**, 1121–1134. doi:10.1104/pp.17.00698

Craine JM (2009) Resource strategies of wild plants. In 'The basis for plant strategies'. (Ed. JM Craine) pp. 1–14. (Princeton University Press: Princeton, NJ, USA)

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. *Annual Review of Plant Biology* **61**, 651–679. doi:10.1146/annurev-arplant-042809-112122

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development* **29**, 185–212. doi:10.1051/agro:2008021

Fernández OA, Busso CA (1999) Arid and semi-arid rangelands: two thirds of Argentina. *Rala Report* **200**, 41–60.

Givnish TJ (2002) Adaptive significance of evergreen vs deciduous leaves: solving the triple paradox. *Silva Fennica* **36**, 703–743. doi:10.14214/sf.535

Golluscio RA, Sala OE, Lauenroth WK (1998) Differential use of large summer rainfall events by shrubs and grasses: a manipulative experiment in the Patagonian steppe. *Oecologia* **115**, 17–25. doi:10.1007/s004420050486

Grime JP, Thompson K, Hunt R, Hodgson JG, Cornelissen JH, Rorison IH, Hendry GAF, Ashenden TW, Askew AP, Band SR, Booth RE, Bossard CC, Campbell BD, Cooper JEL, Davison AW, Gupta PL, Hall W, Hand DW, Hannah MA, Hillier SH, Hodkinson DJ, Jalili A, Liu Z, Mackey JML, Matthews N, Mowforth MA, Neal AM, Reader RJ, Reiling K, Ross-Fraser W, Spencer RE, Sutton F, Tasker DE, Thorpe PC, Whitehouse J (1997) Integrated screening validates primary axes of specialization in plants. *Oikos* **79**, 259–281. doi:10.2307/3546011

Hetherington AM, Woodward FI (2003) The role of stomata in sensing and driving environmental change. *Nature* **424**, 901–908. doi:10.1038/nature01843

Jackson RB, Mooney HA, Schulze ED (1997) A global budget for fine root biomass, surface area, and nutrient contents. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 7362–7366. doi:10.1073/pnas.94.14.7362

Lee KH, Piao HL, Kim HY, Choi SM, Jiang F, Hartung W, Hwang I, Kwak JM, Lee IJ, Hwang I (2006) Activation of glucosidase via stress-induced polymerization rapidly increases active pools of abscisic acid. *Cell* **126**, 1109–1120. doi:10.1016/j.cell.2006.07.034

León RJC, Bran D, Collantes M, Paruelo JM, Soriano A (1998) Grandes unidades de vegetación de la Patagonia extra andina. *Ecología Austral* **8**, 125–144.

Loik ME (2007) Sensitivity of water relations and photosynthesis to summer precipitation pulses for *Artemisia tridentata* and *Purshia tridentate*. *Plant Ecology* **191**, 95–108. doi:10.1007/s11258-006-9217-1

McLaren JR, Wilson SD, Peltzer DA (2004) Plant feedbacks increase the temporal heterogeneity of soil moisture. *Oikos* **107**, 199–205. doi:10.1111/j.0030-1299.2004.13155.x

Moreno L, Bertiller MB (2015) Phenotypic plasticity of morpho-chemical traits of perennial grasses from contrasting environments of arid Patagonia. *Journal of Arid Environments* **116**, 96–102. doi:10.1016/j.jaridenv.2015.02.007

Noy-Meir I (1973) Desert ecosystems: environment and producers. *Annual Review of Ecology and Systematics* **4**, 25–52. doi:10.1146/annurev.es.04.110173.000325

Okamoto M, Tanaka Y, Abrams SR, Kamiya Y, Seki M, Nambara E (2009) High humidity induces abscisic acid 8′-hydroxylase in stomata and vasculature to regulate local and systemic abscisic acid responses in *Arabidopsis*. *Plant Physiology* **149**, 825–834. doi:10.1104/pp.108.130823

Pantin F, Simonneau T, Muller B (2012) Coming of leaf age: control of growth by hydraulics and metabolics during leaf ontogeny. *New Phytologist* **196**, 349–366. doi:10.1111/j.1469-8137.2012.04273.x

Paruelo JM, Beltrán A, Jobbágy E, Sala OE, Golluscio RA (1998) The climate of Patagonia: general patterns and control son biotic processes. *Ecología Austral* **8**, 85–101.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, Wright IJ, Ray P, Enrico L, Pausas JG, de Vos AC, Buchmann N, Funes G, Quétier F, Hodgson JG, Thompson K, Morgan HD, ter Steege H, van der Heijden MGA, Sack L, Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino S, Cornelissen JHC (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* **61**, 167–234. doi:10.1071/BT12225

Poorter H, Garnier E (1999) Ecological significance of inherent variation in relative growth rate and its components. In 'Handbook of functional plant ecology'. (Eds FI Pugnaire, F Valladares) pp. 81–120. (Marcel Dekker: New York)

Poorter H, Nagel O (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO$_2$, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology* **27**, 595–607. doi:10.1071/PP99173_CO

Reid JB, Renquist AR (1997) Enhanced root production as a feed-forward response to soil water deficit in field-grown tomatoes. *Australian Journal of Plant Physiology* **24**, 685–692. doi:10.1071/PP96079

Saura-Mas S, Lloret F (2007) Leaf and shoot water content and leaf dry matter content of Mediterranean woody species with different post-fire regenerative strategies. *Annals of Botany* **99**, 545–554. doi:10.1093/aob/mcl284

Sauter A, Dietz KJ, Hartung W (2002) A possible stress physiological role of abscisic acid conjugates in root to shoot signaling. *Plant, Cell & Environment* **25**, 223–228. doi:10.1046/j.1365-3040.2002.00747.x

Scholz FG, Bucci SJ, Arias N, Meinzer FC, Goldstein G (2012) Osmotic and elastic adjustments in cold desert shrubs differing in rooting depth: coping with drought and subzero temperatures. *Oecologia* **170**, 885–897. doi:10.1007/s00442-012-2368-y

Seiler C, Harshavardhan VT, Rajesh K, Reddy PS, Strickert M, Rolletschek H, Scholz U, Wobus U, Sreenivasulu N (2011) ABA biosynthesis and degradation contributing to ABA homeostasis during barley seed development under control and terminal drought-stress conditions. *Journal of Experimental Botany* **62**, 2615–2632. doi:10.1093/jxb/erq446

Sharp RE, LeNoble ME (2002) ABA, ethylene and the control of shoot and root growth under water stress. *Journal of Experimental Botany* **53**, 33–37. doi:10.1093/jexbot/53.366.33

Sharp RE, Wu Y, Voetberg GS, Saab IN, LeNoble ME (1994) Confirmation that abscisic acid accumulation is required for maize primary root elongation at low water potentials. *Journal of Experimental Botany* **45**, 1743–1751. doi:10.1093/jxb/45.Special_Issue.1743

Sharp RE, Poroyko V, Hejlek LG, Spollen WG, Springer GK, Bohnert HJ, Nguyen HT (2004) Root growth maintenance during water deficits: physiology to functional genomics. *Journal of Experimental Botany* **55**, 2343–2351. doi:10.1093/jxb/erh276

Shipley B, Vu TT (2002) Dry matter content as a measure of dry matter concentration in plants and their parts. *New Phytologist* **153**, 359–364. doi:10.1046/j.0028-646X.2001.00320.x

Spollen W, LeNoble M, Samuels T, Bernstein N, Sharp R (2000) Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. *Plant Physiology* **122**, 967–976. doi:10.1104/pp.122.3.967

Sreenivasulu N, Harshavardhan VT, Govind G, Seiler C, Kohli A (2012) Contrapuntal role of ABA: does it mediate stress tolerance or plant growth retardation under long-term drought stress? *Gene* **506**, 265–273. doi:10.1016/j.gene.2012.06.076

Srivastava LM (2002) Abscisic acid and stress tolerance in plants. In 'Plant growth and development, Hormones and environment'. (Ed. LM Srivastava) pp. 381–412. (Academic Press: New York)

Tardieu F, Parent B, Simonneau T (2010) Control of leaf growth by abscisic acid: hydraulic or non-hydraulic processes? *Plant, Cell & Environment* **33**, 636–647. doi:10.1111/j.1365-3040.2009.02091.x

Thomas CK, Law BE, Irvine J, Martin JG, Pettijohn JC, Davis KJ (2009) Seasonal hydrology explains interannual and seasonal variation in carbon and water exchange in a semiarid mature ponderosa pine forest in central Oregon. *Journal of Geophysical Research* **114**, G04006. doi:10.1029/2009JG001010

Varela MC, Arslan I, Reginato M, Cenzano AM, Luna MV (2016) Phenolic compounds as indicators of drought resistance in shrubs from Patagonian shrublands (Argentina). *Plant Physiology and Biochemistry* **104**, 81–91. doi:10.1016/j.plaphy.2016.03.014

Varela MC, Reinoso H, Luna MV, Cenzano AM (2018) Seasonal changes in morphological and hormonal traits of two native Patagonian shrubs from Argentina with different drought resistance strategies.

Plant Physiology and Biochemistry **127**, 506–515. doi:10.1016/j.plaphy.2018.03.018

Westoby M, Falster D, Moles A, Vesk P, Wright I (2002) Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* **33**, 125–159. doi:10.1146/annurev.ecolsys.33.010802.150452

Xu Z, Zhou G (2008) Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of Experimental Botany* **59**, 3317–3325. doi:10.1093/jxb/ern185

Xu ZJ, Nakajima M, Suzuki Y, Yamaguchi I (2002) Cloning and characterization of the abscisic acid-specific glucosyltransferase gene from adzuki bean seedlings. *Plant Physiology* **129**, 1285–1295. doi:10.1104/pp.001784

Yamashita Y, Ota M, Inoue Y, Hasebe Y, Okamoto M, Inukai T, Masuta C, Sakihama Y, Hashidoko Y, Kojima M, Sakakibara H, Inage Y, Takahashi K, Yoshihara T, Matsuura H (2016) Chemical promotion of endogenous amounts of ABA in *Arabidopsis thaliana* by a natural product, theobroxide. *Plant & Cell Physiology* **57**, 986–999. doi:10.1093/pcp/pcw037

Handling Editor: Tim Brodribb