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Research article

Abscisic acid metabolite profiling as indicators of plastic responses to drought in grasses from arid Patagonian Monte (Argentina)

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ABSTRACT

The identification of hormonal and biochemical traits that play functional roles in the adaptation to drought is necessary for the conservation and planning of rangeland management. The aim of this study was to evaluate the effects of drought on i) the water content (WC) of different plant organs, ii) the endogenous level of abscisic acid (ABA) and metabolites (phaseic acid-PA, dihydrophaseic acid-DPA and abscisic acid conjugated with glucose ester-ABA-GE), iii) the total carotenoid concentration and iv) to compare the traits of two desert perennial grasses (*Pappostipa speciosa* and *Poa ligularis*) with contrasting morphological and functional drought resistance traits and life-history strategies. Both species were subjected to two levels of gravimetric soil moisture (the highest near field capacity during autumn-winter and the lowest corresponding to summer drought). Drought significantly increased the ABA and DPA levels in the green leaves of *P. speciosa* and *P. ligularis*. Drought decreased ABA in the roots of *P. speciosa* while it increased ABA in the roots of *P. ligularis*. *P. ligularis* had the highest ABA level and WC in green leaves. While *P. speciosa* had the highest DPA levels in leaves. In conclusion, we found the highest ABA level in the mesophytic species *P. ligularis* and the lowest ABA level in the xerophytic species *P. speciosa*, revealing that the ABA metabolite profile in each grass species is a plastic response to drought resistance.

1. Introduction

In arid ecosystems, drought is the major abiotic stress, and it affects physiological and biochemical processes in plants, leading to reduced growth and crop yield (Guo et al., 2010). Plant species have evolved adaptive mechanisms for drought resistance, including plant strategies related to drought avoidance or tolerance (Levitt, 1980). Among different physiological and biochemical traits, the accumulation of abscisic acid (ABA) has been described for several mesophytic species (Jiang and Zhang, 2001; Qin and Zeevaart, 2002; Seiler et al., 2011). In xerophytic species, although some genes encoding transcription factors involved in abscisic acid signalling pathway have been described (Zou et al., 2004), the role of ABA and its metabolites are essentially unexplored.

During drought, ABA is important in root-to-shoot signalling for stress-induced stomatal closure; thus, it reduces transpiration, maintains shoot growth and promotes root growth (Srivastava, 2002).

It is well known that a suitable ABA level is necessary for successful plant growth under stress conditions, and the endogenous pool of free ABA is dynamically regulated by the balance between synthesis, transport and degradation (Cutler and Krochko, 1999). ABA catabolic pathways mainly include 8′-hydroxylation and sugar conjugation (Nambara and Marion-Poll, 2005). In higher plants, ABA catabolism is initiated by ABA 8'-hydroxylase to form 8'-hydroxy-ABA. 8'-hydroxy-ABA is then spontaneously isomerised to phaseic acid (PA) that is reduced to dihydrophaseic acid (DPA; Cutler and Krochko, 1999), which is the end-product of the ABA degradation pathway (Seiler et al., 2011). The majority of ABA conjugation occurs with glucose by an ABA glucosyltransferase to produce ABA-glucose ester (ABA-GE) (Xu et al., 2002), which may function in transport and storage or as a long-distance hormonal signal (Sauter et al., 2002). ABA-GE must be hydrolised by β-glucosidases to produce free active ABA (Lee et al., 2006; Llanes et al., 2014).

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Few studies have reported changes in the ABA level in Patagonian grass species (Abernethy and McManus, 1998). The persistence of perennial grasses highly preferred by herbivores in arid ecosystems is essential for maintaining plant cover, preventing degradation processes such as water and wind soil erosion, and reducing desertification advancement (Chartier and Rostagno, 2006). In Patagonian Monte, *Poa ligularis* and *Pappostipa speciosa* are dominant perennial grasses (Pazos et al., 2007). Both grass species differ in morphological and functional traits; *P. ligularis* has the highest expression of mesophytic and acquisitive traits and is considered a drought avoiding species, while *P. speciosa* has xerophytic and conservative traits and is considered a drought tolerant species (Pazos et al., 2007; Cenzano et al., 2013). *P. ligularis* is highly preferred by native and domestic herbivores, while *P. speciosa* is less preferred by herbivores (Pelliza Sbriller et al., 1997).

Although many studies have identified plastic changes in phenology, leaf lifespan and seed longevity in response to elevated CO_2 (Nicotra et al., 2010), the identification of biochemical traits such as modifications in ABA metabolic pathways that act as plastic responses to drought has not been performed. Moreover, quantification of the level of endogenous ABA and its metabolites (PA, DPA and ABA-GE) in *P. ligularis* and *P. speciosa* grasses has not been performed, and its relation to different plant ecological strategies during drought remains unknown.

Therefore, focussing on aspects of the drought adaptation of two coexisting perennial grasses (*P. ligularis* and *P. speciosa*) of Patagonian Monte will improve our knowledge of plant plastic responses to drought mediated by hormones, which may be useful for the conservation and planning of rangeland management.

We hypothesised that the different ecological strategies used by some desert perennial grasses in response to drought involve different ABA metabolite profiles and total carotenoid concentration. We predicted that *P. ligularis*, which has higher expression of mesophytic traits, would have higher ABA metabolism and a higher total carotenoid concentration than *P. speciosa* in response to drought.

2. Materials and methods

2.1. Geographical localisation

This study was performed in the Northeastern region of Chubut Province (Southern portion of the Monte Phytogeographic Province, Argentina; Soriano, 1950). Experiments were performed within this area at the experimental site of the Centro Nacional Patagónico-CENPAT (42°47'11.68"S, 65°00'28.56") under a rainout shelter.

2.2. Study species and plant collection

The perennial grasses *P. ligularis* Nees. Ap. Steudel and *P. speciosa* (Trin. et Rupr.) Romaschenko were selected for this study. Plant harvesting was performed in Estancia San Luis (42°40'49.3"S, 65°21'33.6"W) in autumn 2009 by randomly collecting sixty bunches of each species for transplantation. The topsoil (0–20 cm) underneath each bunch was also extracted, pooled and sieved to 2 mm. Individual rooted tillers of each species were separated from each bunch (5–10 tillers per bunch). Tillers were pooled for each species, and 200 tillers of each species were transplanted in pots (one rooted tiller per pot) filled with 1400 g of topsoil and maintained in a greenhouse for one month up to the beginning of the experiment.

2.3. Experimental design

The experimental design was previously described by Cenzano et al. (2013). The experiment was performed during the period of August 2009–December 2010. Each species was submitted to two levels of gravimetric soil moisture (GSM): 16% (control) and 4% GSM (drought). The 16% GSM corresponded to the highest mean value near field capacity during autumn-winter and 4% GSM corresponded to the lowest value during summer drought, which were registered under natural field conditions in the Patagonian Monte (Coronato and Bertiller, 1997).

Pots were placed under a rainout shelter at the experimental site of CENPAT, and the soil moisture in each pot was controlled weekly during spring-summer and fortnightly during autumn-winter by weighing the pots and applying water to the target weight.

2.4. Determination of water content

Ten plants of each species from each watering level were randomly selected and harvested at the end of the treatment (December 2010). Roots were separated from soil and washed with tap water on a 1000 μm sieved mesh. After manually drying the plants, the roots were separated from the aboveground organs. The fresh weight (FW) of green leaves, senescent leaves, roots, panicles and the rest of the plant-tiller bases was obtained, and each plant fraction was immediately lyophilised for 72 h and weighed. Dry weight (DW) data were used to determine the water content (WC) of different plant organs using the equation $WC = (FW - DW)/FW$, which was described by Garnier and Laurent (1994).

2.5. Determination of the abscisic acid metabolite profile

Four plants of each species from each watering level were randomly selected and harvested at the end of the treatment (December 2010). Roots were washed with tap water on a 1000 μm sieved mesh. After manually drying, the green leaves, senescent leaves and roots were separated and frozen with liquid nitrogen and lyophilised for 72 h. Analyses of ABA and its metabolites were performed according to Zhou et al. (2003) with modifications. For ABA and extraction of its metabolites extraction, lyophilised material (200 mg DW) was ground in a mortar with liquid nitrogen, and 3 mL acetone/water/acetic acid (80/19/1, v/v/v) was added. Internal standards, 50 ng each of d6-ABA, d3-PA, d3-DPA and d5-ABA-GE (NRC-Plant Biotechnology Institute, Saskatoon, Canada) were added. Extracts were transferred to 50 mL tubes, they 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, USA). Dried extracts were dissolved in 100 mL methanol/acetic acid (99/1, v/v) and then mixed with 900 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, 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 v. 4.1* software. Measurements were performed in quadruplicate.

2.5.1. Liquid chromatography

Analyses were performed using a quaternary pump equipped with an auto-sampler; 10 μl of each sample was injected onto a Restek C_{18} (*Restek*, 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).

2.5.2. Mass spectrometry

An 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 temp.: 100 °C, desolvation temp.: 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).

2.6. Determination of total carotenoid concentration

Three plants of each species from each soil moisture level were harvested and lyophilised at the end of the experiment (December 2010). The concentration of total carotenoids was assessed in the above-ground organs (green and senescent leaves) and roots at the end of drought treatment according to the method proposed by Vernon (1960) and McKinney (1941).

2.7. Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine significant differences in water content, endogenous ABA and metabolite level, rates of ABA accumulation (ABA biosynthesis/catabolism), and total carotenoid concentration in two different species at different water levels. The factors considered were the following: species (*P. ligularis*, *P. speciosa*) and watering level (16% and 4% GSM) for each organ analysed (green leaf, senescent leaf, root, panicle and rest of plant-tiller base). For multiple comparisons, the Bonferroni test was used. Data were tested for normality by the Shapiro–Wilk test and for homogeneity of variance by Levene's test. Data were transformed into natural logarithm values (ABA, PA, DPA and ABA-GE) and cosine values (carotenoids) to meet the assumptions of ANOVA. The significance level was set to $P \leq 0.05$.

3. Results

3.1. Water content of different plant organs

The water content (WC) of green leaves and the rest of the tillers significantly differed between *P. speciosa* and *P. ligularis* (Fig. 1). Green leaves and the rest of the tillers of *P. ligularis* had a higher WC than *P. speciosa*. The panicles of the *P. speciosa* had a higher WC than *P. ligularis*. Changes in the WC of the senescent leaves and roots of both species were not observed, but a significant interaction between species and watering level was found for the WC. *P. speciosa* had a higher WC than *P. ligularis* at 16% GSM, while at 4% GSM, *P. ligularis* had a higher WC than *P. speciosa* (Fig. 1).

Drought treatment (4% GSM) decreased the WC in the roots of both species, but no significant changes were observed in other organs (Fig. 1).

3.2. Abscisic acid and metabolite profiling

3.2.1. Effect of drought on the abscisic acid metabolite profile

The endogenous level of ABA and its metabolites significantly changed in *P. speciosa* and *P. ligularis* during drought (4% GSM) compared with control treatment (16% GSM, Fig. 2). The ABA level in the green leaves of both grasses increased during drought (three-fold compared with control in *P. ligularis* and two-fold in *P. speciosa*) (Fig. 2a). Similarly, the DPA level increased during drought (three-fold compared with control in both species) (Fig. 2a). However, the level of the metabolic intermediary PA was unchanged during drought in both species. ABA-GE level was unchanged during drought in both species, but a significant effect of the interaction between species and water levels was found for this metabolite. At 4% GSM, the *P. speciosa* had a higher ABA-GE level than *P. ligularis*, while at 16% GSM, both species had similar ABA-GE levels (Fig. 2a).

Drought did not induce a change in ABA and metabolite level in senescent leaves of both species (Fig. 2b), but a significant interaction between species and water levels was found on the DPA level. *P. speciosa* had a higher DPA level than *P. ligularis* at 16% GSM, while at 4% GSM, both species had a similar DPA level (Fig. 2b).

In roots, drought decreased the ABA level in *P. speciosa* (two-fold compared with control), increased the ABA level in *P. ligularis* (two-

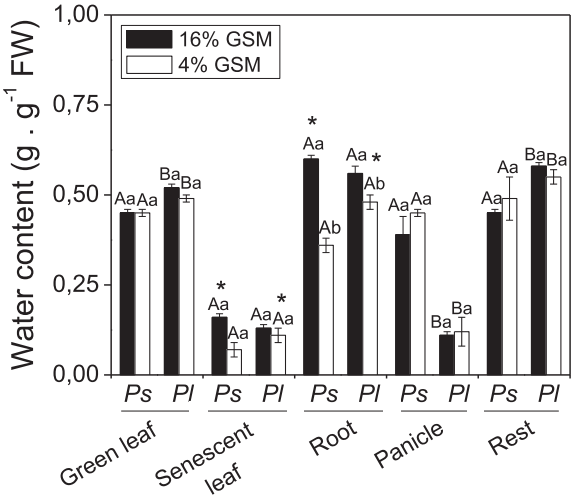

Fig. 1. Water content of different plant organs. Means ± SE ($n = 10$) of *Pappostipa speciosa* (*Ps*) and *Poa ligularis* (*Pl*) at the two water levels (16% GSM, solid symbols and 4% GSM, open symbols). The different uppercase letters indicate significant differences between species, and different lowercase letters indicate significant differences between water levels ($P \leq 0.05$). Asterisks indicate significant interaction between species and water level.

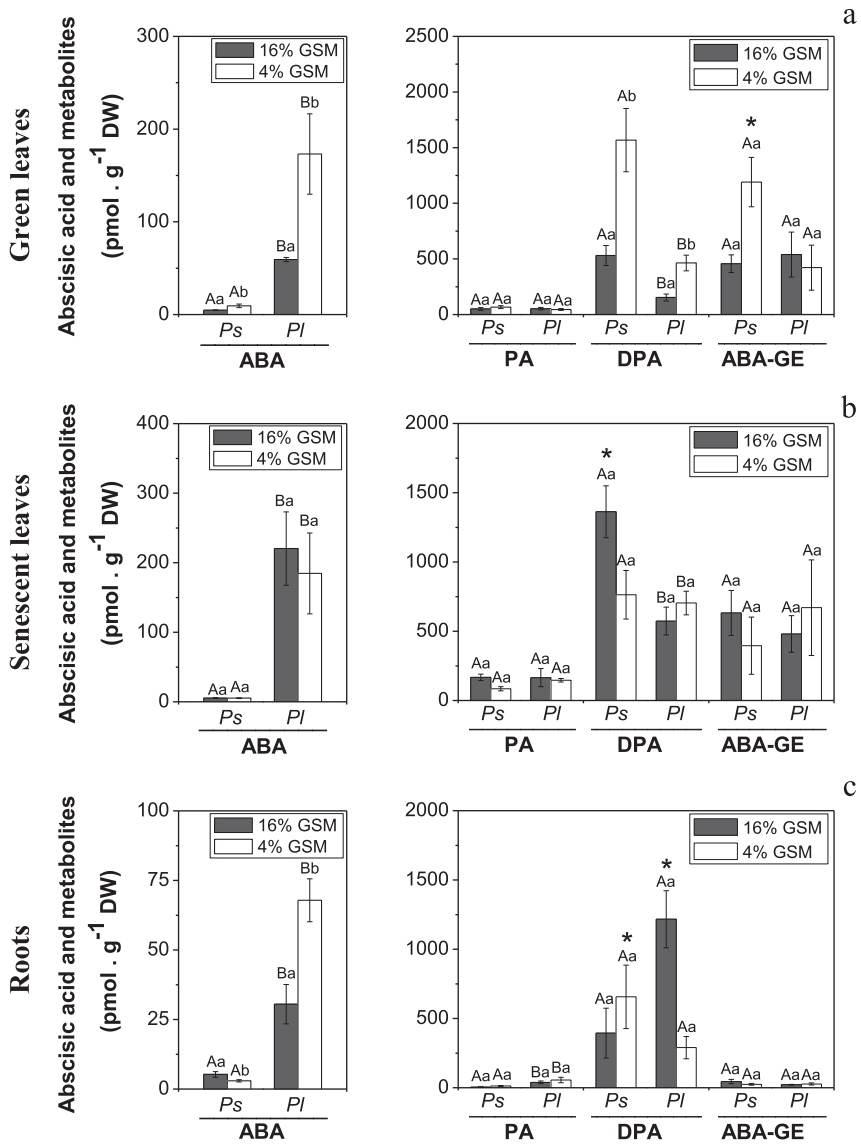

Fig. 2. Effect of drought on the level of ABA and its metabolites. Means ± SE ($n = 4$) of a. green leaves, b. senescent leaves, c. *Pappostipa speciosa* (*Ps*) and *Poa ligularis* (*Pl*) roots at the two water levels (16% GSM, solid symbols and 4% GSM, open symbols). The different uppercase letters indicate significant differences between species, and different lowercase letters indicate significant differences between water levels ($P \leq 0.05$). Asterisks indicate significant interaction between species and water level.

fold compared with control), and a significant interaction between species and water level was found for ABA (Fig. 2c). Although *P. ligularis* had a higher ABA level than *P. speciosa* under the GSM analysed, the differences were larger at 4% compared with 16% GSM (Fig. 2c). Drought did not induce changes in the PA level in both species, and no significant effects of the interaction between species and water level was found for this variable (Fig. 2c). The DPA level was unchanged during drought, but a significant interaction between species and water level was found for this variable. At 4% GSM, *P. speciosa* had a higher DPA level than *P. ligularis*, while at 16% GSM, *P. ligularis* had a higher DPA level than *P. speciosa* (Fig. 2c). The ABA-GE level was unchanged during drought, and the interaction between species and water level was not significant (Fig. 2c).

3.2.2. Differences in ABA metabolite profiles between species

The level of ABA and its metabolites significantly differed between *P. speciosa* and *P. ligularis* (Fig. 2). Green leaves from *P. ligularis* showed a higher ABA (12.8-fold) and DPA (3.5-fold) level than the *P. speciosa* control (16% GSM), while the PA and ABA-GE level was unchanged between the species (Fig. 2a). In senescent leaves, the ABA level was higher in *P. ligularis* (41-fold) than *P. speciosa*, but the level of DPA was higher in *P. speciosa* (2.4-fold) than *P. ligularis* (Fig. 2b). The PA and ABA-GE levels were similar in both species (Fig. 2b). In roots, *P. ligularis* had a higher ABA (5.8-fold) and PA (7.6-fold) level than *P. speciosa*. The DPA and ABA-GE levels were similar in both species (Fig. 2c).

3.2.3. Rates of ABA biosynthesis and catabolism

ABA accumulation was analysed as a function of synthesis and catabolism.

All *P. ligularis* organs had a higher rate of ABA biosynthesis than catabolism compared with *P. speciosa* (Table 1). The rate of ABA biosynthesis was higher in green and senescent leaves than roots. However, the rate of ABA biosynthesis and catabolism was unchanged during drought in all organs, and a significant effect of the interaction between species and water level was found for senescent leaves and roots. At 16% GSM, the senescent leaves of *P. ligularis* had higher ABA biosynthesis than catabolism, while at 4% GSM,

Table 1
Mean rates of ABA biosynthesis and catabolism of the 8'-hydroxylation (PA and DPA products) and conjugation (ABA-GE) pathways.

Organ	Species	ABA/PA + DPA		ABA/ABA-GE		ABA/PA + DPA + ABA-GE	
		16% GSM	4% GSM	16% GSM	4% GSM	16% GSM	4% GSM
Green leaves	*P. speciosa*	0.009 Ba	0.006 Ba	0.011 Ba	0.009 Ba	0.005 Ba	0.004 Ba
	P. ligularis	1.158 Aa	0.340 Aa	0.209 Aa	0.839 Aa	0.119 Aa	0.184 Aa
Senescent leaves	*P. speciosa*	0.004 Ba	0.007 Ba	0.009 Ba	0.022 Ba	0.003 Ba	0.004 Ba*
	P. ligularis	0.323 Aa	0.247 Aa	0.477 Aa	0.368 Aa	0.175 Aa*	0.118 Aa
Roots	*P. speciosa*	0.021 Ba*	0.005 Ba	0.306 Ba	0.129 Ba	0.019 Ba*	0.005 Ba
	P. ligularis	0.031 Aa	0.250 Aa*	1.445 Aa	4.450 Aa	0.030 Aa	0.229 Aa*

The different uppercase letters indicate significant differences between species, and different lowercase letters indicate significant differences between water levels ($P \leq 0.05$) for each organ. Asterisks indicate significant interaction between species and water level.

P. speciosa senescent leaves had higher ABA biosynthesis than catabolism. In roots, at 16% GSM, *P. speciosa* had higher ABA biosynthesis than 8'-hydroxylation catabolism and conjugation, while at 4% GSM, *P. ligularis* had higher ABA biosynthesis than 8'-hydroxylation catabolism and conjugation (Table 1).

3.3. Total carotenoid concentration

The concentration of total carotenoids in green leaves was unchanged during drought, and there was no difference between species and no significant effects of interaction between species and water level for this variable (Fig. 3). In senescent leaves, the concentration of carotenoids increased during drought in *P. ligularis* and *P. speciosa*; no significant differences were found between species, and no significant effects of the interaction between both factors was observed for this variable (Fig. 3). In roots, drought did not induce significant differences in the concentration of carotenoids, the carotenoid concentration was higher in *P. ligularis* than *P. speciosa*, and no significant effects of the interaction between both factors was observed for this variable (Fig. 3).

4. Discussion

Our findings suggest a relationship between the water status of plant organs, hormonal and biochemical traits (changes in ABA metabolism and total carotenoids concentration), and functional types (mesophytic and resource-acquisitive vs xerophytic and resource-conservative species) for drought resistance in two coexisting grasses in the arid Patagonian Monte.

In response to drought stress, plants develop adaptive strategies, including drought tolerance or drought avoidance strategies.

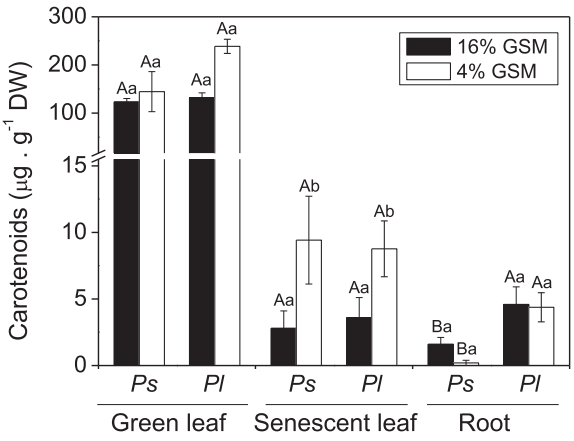

Fig. 3. Effect of drought on total carotenoid concentration. Means ± SE ($n = 3$) of green leaves, senescent leaves and root carotenoid concentration for *Poa ligularis* (*Pl*) and *Pappostipa speciosa* (*Ps*) at two water levels (16% GSM, solid symbols and 4% GSM, open symbols).

Plants may avoid drought stress by maintaining a favourable water status during drought either by increasing the capacity for water uptake in roots or reducing water loss from leaves (McCann and Huang, 2008).

Our results show that during drought, the green leaves and tiller base-maintained WC in both species reveal a mechanism for conserving water for the production of new tillers to survive, and this response was more evident in *P. ligularis* than *P. speciosa*. These findings are in agreement with fast-growing species that have higher leaf WC than slow-growing species, which favours leaf productivity in the first species and leaf persistence in the second (Garnier and Laurent, 1994).

Included in the root traits controlling water uptake are viability, length and number of roots (McCann and Huang, 2008). Our results suggest that the higher WC in *P. ligularis* compared with *P. speciosa* during drought may be due to a higher number of roots in the former species than the latter (Cenzano et al., 2013).

We report changes in the ABA metabolite profile produced by drought treatment (4% gravimetric soil moisture) equivalent to hydric conditions during periods of water shortage in habitats in which these species grow.

ABA plays a vital role in the plant stress response as evidenced by the fact that species adapting to arid environments have a high concentration of ABA (Turner and Hartung, 2012). Our results showed an increase in the ABA and DPA endogenous level in green leaves from *P. speciosa* and *P. ligularis*, a decrease in the ABA level in roots from *P. speciosa* and an increase in the ABA level in roots from *P. ligularis*. These results indicate that drought enhances ABA biosynthesis and catabolism, resulting in an increase in ABA and its metabolites. Modulation of the level of endogenous ABA in a particular tissue may result in ABA biosynthesis and catabolism (Nambara and Marion-Poll, 2005), but it may also be produced by the release of ABA from its inactive glucose conjugate (Lee et al., 2006). ABA catabolism is proportional to the tissue ABA level (Jia and Zhang, 1999). Thus, activation of hydroxylation and/or conjugation pathways may reduce the ABA endogenous level (Okamoto et al., 2009; Xu et al., 2002) and inactivate ABA (Seiler et al., 2011). In this study, the highest ABA level of the mesophytic and deciduous *P. ligularis* plant is due to a greater ratio of ABA biosynthesis/catabolism in all organs, while the xerophytic and evergreen *P. speciosa* plant has a lower ratio of ABA biosynthesis/catabolism due to activation of the 8'-hydroxylation pathway (highest DPA level) in green and senescent leaves. Drought-tolerant plants accumulate lower ABA levels, which correlate with lower ABA biosynthesis and higher ABA 8'-hydroxylase expression (Ji et al., 2011). In addition, the increase in catabolic rate leading to a basal level of ABA has been suggested as way to recover functional capability once the water deficit has been relieved (Ren et al., 2007).

At present, the role of ABA metabolites is poorly understood, and several authors have hypothesised that PA, DPA and ABA-GE are physiologically inactive and allow stomatal opening (Sauter

et al., 2002; Srivastava, 2002). During drought stress, PA and DPA are the major metabolites in barley leaves, and their levels are higher than that of ABA (Seiler et al., 2011). In addition, a maximum level of DPA has been reported under extreme desiccation in *Haberlea rhodopensis*, a resurrection plant (Djilianov et al., 2013). Taken together, these results would suggest that, if PA, DPA and ABA-GE are physiologically inactive, the 8'-hydroxylation and conjugation pathways could be involved in maintaining ABA levels below certain critical threshold. In our study, high ABA-GE levels in the green and senescent leaves of both grass species reveal an enhanced ABA conjugation pathway in these organs and reduced synthesis or accumulation of ABA-GE in roots, which is in agreement with other reports (Hansen and Dörffling, 1999; Turner and Hartung, 2012). These findings are consistent with the proposal by Jiang and Hartung (2008) that β-glucosidase activity, both in the apoplast and the cytosol of the mesophyll, play an important role in the regulation of ABA signal intensity thus contributing to fine tune stomatal aperture.

Moreover, high ABA levels in *P. ligularis* roots during drought may be correlated with its function in the maintenance of root growth (Sharp, 2002) and the enhancement of root-to-shoot ABA signalling (Srivastava, 2002). However, low ABA levels in *P. speciosa* roots during drought may be correlated with other functional traits of drought resistance such as a high root/leaf ratio and high root biomass (Cenzano et al., 2013), which could confer a fitness advantage in water limited habitats.

Our results suggest that the high ABA level together with high WC in the green leaves of the acquisitive species *P. ligularis* and high green leaf production (Cenzano et al., 2013) during drought may be correlated with its function in stomatal closure as way to retain resources during unproductive conditions (Grime et al., 1997). However, the low level of ABA in the green leaves of the conservative species *P. speciosa* could support the maintenance of high photosynthetic rate for continuing carbon assimilation (DaCosta and Huang, 2007).

In contrast, the fact that the ABA level in senescent leaves remained unchanged during drought may be because the leaf senescence observed at the beginning of summer is a natural process characteristic of the endogenous summer dormancy of deciduous species. This scenario may be the case for the summer deciduous species *P. ligularis*, and this process has been correlated with strategies for surviving drought for several grasses (Volaire and Norton, 2006). Nevertheless, high ABA metabolism was found in both grass species, indicating that ABA must play a role in leaf senescence as proposed by Lee et al. (2011).

Several lines of evidence suggest that stress-induced ABA is derived from epoxycarotenoids, particularly xanthophylls (Taylor et al., 2005). The epoxycarotenoids are present in relatively large quantities in most photosynthetic tissues, and their level is relative to the amount of ABA synthesised (Schwartz et al., 2003). The findings reported in this study show that carotenoids and the ABA level are higher in the green leaves and roots of *P. ligularis*, while in *P. speciosa*, carotenoids were higher in green leaves, and the ABA level was lower, revealing that the level of total carotenoids as an ABA precursor pool is not a suitable indicator for the amount of ABA synthesised.

During leaf senescence, most of the chlorophylls are lost, and carotenoids have to absorb light to maintain a basal level of photosynthesis (Biswal, 1995). Thus, the conversion of zeaxanthin and antheraxanthin to violaxanthin is a mechanism proposed to dissipate excess energy and provide photo-protection from oxidative damage (Ramel et al., 2013). In agreement with these studies, we found an increase in the carotenoid concentration in the senescent leaves of both grass species during drought conditions. We suggest that high carotenoid and endogenous ABA levels (the former to be considered as non-enzymatic antioxidants) may be involved in an anti-oxidative defence system in senescent leaves of Patagonian grasses.

Our results suggest that ABA and its metabolites may play a major role in the control of the adaptive responses that help plants tolerate water stress. ABA may be involved in the control of phenotypic plasticity via maintenance of the homeostatic ABA level in the plant tissues of these species, which is crucial for living in such an arid ecosystem. Thus, the highest ABA level found in the mesophytic species *P. ligularis* may be an adaptive plastic response to drought avoidance, and the highest ABA metabolite level in the xerophytic species *P. speciosa* could also be an adaptive response related to enhanced drought tolerance. In other words, lack of ABA peaks in *P. speciosa* could eventually be related to the rapid regulation of the water status during drought, and it could be associated with other constitutive traits such as the low specific leaf area (Campanella and Bertiller, 2008).

In conclusion, the highest ABA level was found in *P. ligularis*, a summer deciduous grass with acquisitive and mesophytic traits, and the lowest ABA level was found in *P. speciosa*, an evergreen grass with conservative and xerophytic traits. In addition, both ABA catabolic pathways, 8'-hydroxylation and ABA conjugation, were active in down regulating the ABA level. Thus, our findings reveal that the ABA metabolite profile of each grass species correlates with the strategy for drought-resistance for each functional plant type in Patagonian rangelands.

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Contributions

The identification of hormonal and biochemical traits with play functional roles in adaptation to drought would be useful to planning the conservation of this arid ecosystem. The species in study prevent the desertification and also they are pasture for local herbivorous. Our findings reveal that the abscisic acid metabolite profile of each grass species correlates with the strategy for drought-resistance for each functional plant type in Patagonian rangelands.

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