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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.