Inter- and intrapopulation variation in the response of tree seedlings to drought: physiological adjustments based on geographical origin, water supply and species

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Received: 5 November 2016; Editorial decision: 24 July 2017; Accepted: 27 July 2017; Published: 29 July 2017

Associate Editor: Rafael Oliveira

Citation: Carevic FS, Delatorre-Herrera J, Delatorre-Castillo J. 2017. Inter- and intrapopulation variation in the response of tree seedlings to drought: physiological adjustments based on geographical origin, water supply and species. AoB PLANTS X: plx037; doi: 10.1093/aobpla/plx037

Abstract. Initiatives to restore natural ecosystems have had little success in arid and hyperarid ecosystems. In this context, the natural seedling establishment is particularly affected by drought patterns and climatic variability. Likewise, the effect of plant provenance on forest restoration success remains unclear, although previous studies have concluded that some seed locations might be better able to tolerate water stress. In this study, we examined the physiological mechanisms involved in the drought stress resistance of Prosopis tamarugo and Prosopis alba seedlings from different arid and hyperarid locations of the Atacama Desert in northern Chile. We measured the xylem water potential ($\Psi$), cuticular transpiration ($E_c$), specific leaf area (SLA) and pressure–volume curves at the intrapopulation and interpopulation levels of seedlings of both species subjected to three drought-induced treatments. In addition, plant characteristics such as seedling height (Sh), stem diameter (Sd), leaf biomass (Lb), root biomass (Rb) and seedling survival (Ss) were measured during the treatments. Seedlings of most hyperarid habitats had the highest values of $\Psi$ and water content relative to the turgor loss point, as well as decreased SLA, especially during the strongest drought treatment. $\Psi$ was strongly correlated with Sh in both species, and soil humidity was correlated with Sd. This study highlights the high variability of physiological responses to water stress in both species at the interpopulation and intrapopulation levels, which provides us with a powerful seed selection tool for future reforestation programmes aimed at the early selection and genetic improvement of species of the Prosopis genus.

Keywords: Atacama Desert; drought stress; Prosopis; reforestation; seedling provenances; water relations.

Introduction
In general, drought stress in plants has been identified as a major limiting factor of growth and yield in arid and semiarid ecosystems. Water stress caused by summer drought is the main factor limiting the establishment of this species (Villagra et al. 2010). Physiologically, these limitations involve not only stomatal closure but also a decrease in stem water potentials ($\Psi$) and lower rates of carbon assimilation (Delatorre et al. 2008). Moreover, some species prone to water stress during periods of greater water availability tend not to control water loss to the same degree and hence are termed ‘water-spender species’ because they preserve water

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during dry periods but not during wet ones (Levitt 1980). Conversely, other species tolerate drought stress based on a ‘water conservative strategy’ by avoiding water loss during dry and wet periods adjusting cuticular transpiration (E.) and stomatal closure (Villar-Salvador et al. 2004). However, several authors agree that the main physiological drought adaptation strategy of arid climate plants seems to be the need to maintain a high water content and cell turgor, which might have a direct effect on their subsequent growth and seed production processes (López et al. 2013; Villalobos and Peláez 2015). Nevertheless, not all plants have the same strategies to respond to periods of water stress. For example, greater reductions in the growth of some species facing water limitations, such as Prosopis alpataco and Prosopis argentina, are affected in a negative way; however, their specific leaf areas (SLAs) are not significantly affected (Villagra and Cavagnaro 2006). On the other hand, under these same water limitations, the SLA of Erythrina velutina individuals—a member of the Fabaceae that can tolerate water stress—is significantly affected after long periods of drought stress (Da Silva et al. 2010).

Prosopis species are widely distributed in natural ecosystems in Africa, America and Asia and have important economic value due to their use as a source of food, seeds and agroforestry (Giordano et al. 2011). In the Atacama Desert in northern Chile, individuals of the endangered phreatophyte tamarugo tree (Prosopis tamarugo)— endemic to the Tarapacá region—are widely distributed in these arid lands, and, along with white mesquite (Prosopis alba) and Chilean mesquite (Prosopis chilensis), they are the most common phreatophyte species in the natural ecosystem known as ‘Pampa del Tamarugal’, which is considered an agroforestry system (Ormazábal 1991; Chiappa et al. 1997). Unlike P. tamarugo, P. alba forests are distributed in ecosystems of Argentina, Uruguay, Chile and Peru, and this species is classified as a vulnerable tree in Chile (Benoit 1989).

One of the main obstacles to the success of reforestation programmes in the Pampa del Tamarugal aquifer relates to the water table, which has shown a decline in the past few decades. This trend is associated with both the mining industry and the use of water by the urban population. The water flux into the aquifer is calculated to be between 880 and 1000 L s⁻¹, while the water outflow is estimated to be as high as 4000 L s⁻¹ (Calderon et al. 2015). Another global challenge of the Prosopis agroecosystem is its poor performance in natural regeneration compared with other desert species, due mainly to the endozoochory mechanism, since the rainfall is close to 0.6 mm year⁻¹ (Zelada 1986). In this context, there is no general consensus that explains the low natural regeneration rates of individuals of the Prosopis genus. Previous studies have concluded that the regeneration rates are extremely low mainly due to periods in which the El Niño phenomenon occurs, which induces erratic behaviour in the natural germination of seeds (López et al. 2006). However, other factors that could affect the regeneration rates relate to the ecological system, such as the pressure of predation from herbivores, the competition with grasses associated with agroforestry ecosystems of Prosopis or the water stress that constantly affects the ecosystem (Bush and Van Auken 1991; Weltzin et al. 1998; Shackleton et al. 2014).

Initiatives to restore natural ecosystems using mesquite (Prosopis) species have had little success in northern Chile ecosystems (Altamirano 2006). The effect of plant provenance on forest restoration success remains unclear, although previous studies have concluded that some seed locations—from the natural dispersal centre of origin—might be better able to tolerate water stress (Cony 1995; Cariaga et al. 2005). Thus, there is a need to increase the knowledge of the physiological strategy that seedlings from different locations of species from this genus utilize during periods of drought because an intrinsic feature of Prosopis is the high genetic variability of its populations, which might produce different responses to the same type of stress (Cony and Trione 1998; Carevic et al. 2015). These parameters are especially interesting because they can be used in reforestation plans when selecting seeds. Additionally, under natural conditions, Prosopis individuals are often exposed to periods of low temperature and frost during winter. Studies of water relations in Prosopis species of the arid zones of Chile are still emerging, and the physiological mechanism of the adaptation of seedlings to drought remains unclear (Altamirano 2006). A better understanding of the strategies used by different Prosopis plants for drought tolerance would help to develop comprehensive models of plant water requirements in arid zones and other drought-prone regions. These responses allow the species to survive and even to maintain some growth under adverse conditions, considering that the plant response depends on the nature of the water shortage that induces physiological responses to short-term changes. The goal of this study was to understand the mechanisms involved in drought stress resistance of P. tamarugo and P. alba seedlings from different locations. We hypothesized that both species will show different adaptive strategies in relation to their drought resistance and provenance. Furthermore, we hypothesized that the provenances of P. tamarugo and P. alba in the most driest region (Tarapacá) would be the most tolerant to drought because this zone is the area of the natural dispersion of this species. These findings will provide a theoretical basis for the ecological protection and restoration of the Pampa del Tamarugal basin and the surrounding arid region.
Methods

Study site

This research was carried out by collecting seeds of *P. tamarugo* and *P. alba* from nine random natural distribution zones of the Atacama Desert (Table 1). The Tarapacá region was the first area from which seeds were collected, with a total of five locations; in the second area, the Antofagasta region, four locations were selected for this purpose. In total, we were able to collect seeds of *P. tamarugo* from eight locations (five in Tarapacá and three in Antofagasta) and of *P. alba* from six locations (two in Tarapacá and four in Antofagasta). Regarding the different collection locations, we were able to identify a hyperarid area of distribution of *Prosopis* forests in which the precipitation does not exceed 0.6 mm year⁻¹ (Tarapacá) and an arid zone (Antofagasta) with a precipitation regime that exceeds 35 mm year⁻¹.

Safe collection zones between January and March of 2011 were selected to carry out the collection of seeds. This collection was achieved by installing fences and setting plastic traps under the crowns of the selected trees in the areas where seeds were collected before the seed dropping period.

Seedling breeding

Plant breeding was carried out in the Estación Experimental Canchones (EEC) of the Universidad Arturo Prat in northern Chile. After removing the cuticle with H₂SO₄, we germinated and transplanted the individual plants from both locations to plastic pots that were 15 cm in diameter and 90 cm deep during July 2011. During the entire period of the test, the plants were placed in a greenhouse with a roof of Raschel mesh so that the plants were exposed to the natural abiotic conditions (Table 2). The light level was 70–80 % of the outside light intensity. The soil used in the experiment was
a saline–sandy soil obtained from the La Tirana locality, where *P. tamarugo* and *P. alba* are the dominant species.

**Water supply treatments**

Each seedling was irrigated for a 1-year period (during the first year of growth) with 2.0 L of well water twice weekly based on full soil water capacity (FSWC). We estimated the FSWC based on moisture retention curves and wilting points following Orfánus (2005) and Delatorre (2008) for *Prosopis* species. The water supply treatments consisted of performing an experiment on plants from both regions (Tarapacá and Antofagasta) that were ~1 year old by decreasing the frequency of weekly irrigation of the individual plants. Thus, we used three watering supply periods: once a week until the FSWC was reached (T1) between 18 July and 20 August, 2012; once every 2 weeks until the FSWC was reached (T2) between 21 August and 23 September, 2012; and discontinuation of watering after this date (T3). Measurements for the last treatment (T3) were performed on 30 October 2012. Thus, a total of 240 *P. tamarugo* seedlings (30 for each of the eight locations) and 210 *P. alba* seedlings (35 for each of the six locations) were used for the test. In addition, we used a control group of seedlings for each species and location that was watered twice a week during the entire study period. Two physiological and morphological measurements were performed during each period (T1, T2 and T3).

Two soil humidity sensors (ECH2O®) were installed at a depth of 0.3 and 0.6 m for the plants selected for measurement. All of the sensors were positioned randomly in each treatment. The meteorological data from weather stations near the seed collection areas were available and were used to identify climate variables (absolute minimum and maximum temperatures as well as solar radiation) from the origin locations of the seeds.

**Seedling characteristics**

The following morphological measurements were carried out in the plants from each provenance during the entire study: seedling height (Sh), stem diameter (Sd), leaf biomass (Lb), root biomass (Rb) and seedling survival (Ss). Sh, Sd and Ss were measured in 28 individuals per treatment on a weekly basis. Seedling height was measured (in mm) with a ruler from the root collar to the terminal bud or to the highest point with green foliage for seedlings that had experienced dieback. Stem diameter (in mm) was measured with callipers at 0.5 cm above the root collar. Seedling survival (%) was quantified during each treatment by noting if the seedling was alive or dead. Resprouted individuals classified as dead were reclassified as alive. Dry biomass of leaves and roots (in g) was measured in 15 individuals per treatment per species in an oven at 65 °C during a period of 48 h. Root and leaf biomass was recorded after oven-drying at 65 °C for 48 h.

**Measures of plant water variables**

During the entire study, starting in July 2011, the water potential (Ψ) and cuticular transpiration (E<sub>c</sub>) measurements were carried out. The E<sub>c</sub> rates provided an estimate of the water that transpired when the stomata were closed, therefore allowing a leaf epidermis permeability assessment (Fernández et al. 2015). For these measurements, a homogenous quantity of 11 plants was randomly selected per location/species. Measurements of Ψ were performed using a pressure chamber (Model 1000, PMS Instruments, Corvallis, OR, USA) in mature leaves collected at dawn. The leaves were then placed into plastic bags, taken to the laboratory inside portable coolers, placed in the dark and refrigerated, and then measured immediately.

To measure E<sub>c</sub>, two healthy and fully grown leaves were selected from the same individuals and on the same sampling dates as the water potential measurements. After the leaves were selected, they were taken to the laboratory in a cooler inside glass tubes with the base of the short petiole submerged in water and were left in complete darkness for 24 h at 4 °C to be completely hydrated. Before performing the measurements, the leaves remaining inside the tubes were placed in an illuminated area of the laboratory to facilitate stomatal opening and temperature equilibration (Cape and Percy 1996). Cuticular transpiration was estimated using the free transpiration method (Quisenberry et al. 1982; Carevic et al. 2010). For this purpose, the leaves were placed on a plastic grille at 5 cm above the laboratory table. Next, their fresh weight was measured on a scale (±0.1 mg) at 5-, 10-, and 30-min intervals for ~7 h. These data were used to create transpiration decline curves for each leaf. The relative water content at the point of stomatal closure (RWC,) and cuticular transpiration (E<sub>c</sub>, µmol H<sub>2</sub>O kg<sup>-1</sup> s<sup>-1</sup>) were deduced from these curves. Then, after being placed in a heater at 65 °C until constant weight was reached, the surface area and dry weight were determined. The SLA (m<sup>2</sup> kg<sup>-1</sup>) was also calculated for each leaf.

**Pressure–volume curves**

To create pressure–volume curves, a total of two twigs were taken from the same individuals as those used for Ψ and E<sub>c</sub>. These twigs were selected from the seedlings and kept in a refrigerated chamber (3 °C) in darkness. The base of the twig was submerged in distilled water, and the twigs were covered with a polyethylene bag for at least 24 h to facilitate hydration until saturation occurred (Larcher 2003). Data collection was carried out...
using the free transpiration method (Robichaux 1984), which consists of measuring the $\Psi$ and fresh weight (FW) of the twig over short periods of time and at a constant temperature of 25 °C (±2 °C) until dehydration occurs. Afterwards, the twigs were placed in an oven at 70 °C to obtain their dry weight (DW) and relative water content (RWC = 100\[(FW - DW)/(FW_{sat} - DW)\]), where FW_{sat} is the fresh weight after water saturation. Once the pressure-volume curves were represented in a graph and it was established that there were no oversaturation points, the components of $\Psi$ (osmotic component and turgor or pressure component) could be determined for any hydration status. In addition, the following parameters were determined according to the methodology suggested by Peary et al. (1989): osmotic potential at full turgor ($\Psi_{100}$), osmotic potential at zero turgor ($\Psi_{0}$), water content relative to turgor loss point (RWC$_{0}$) and relative apoplastic water content (RWC$_{a}$).

**Data analysis**

We used a completely randomized experimental design with two fixed factors: seed origin and level of water supply (T1, T2 and T3). To evaluate the differences among origin locations and treatment effects regarding the morpho-physiological features measured (SLA and water parameters), repeated-measure ANOVA was used. To test the differences on the effect of water supply treatments on root and leaf biomass between species, an ANOVA test was performed. Normality and equality of variances were appropriately verified through the Kolmogorov–Smirnov test, and the data did not need to be transformed. When differences between subgroups were significant ($P \leq 0.05$), individual measurements were analysed using Tukey’s HSD test. Likewise, we evaluated possible relationships between physiological parameters by performing regression analyses. All statistical analyses were performed with SPSS software (Version 17.0, SPSS®, Chicago, IL, USA).

**Results**

Differences between pressure–volume curves during the study period were significant only for RWC$_{a}$ parameter estimated for P. Tamarugo at interpopulation level (Fig. 1). Therefore, we mainly focused on analysing the differences found between the other physiological and morphological parameters as well as the possible effect of treatments on water status.

**At interpopulation level for P. tamarugo**

Figure 1 shows the differences found between locations of P. tamarugo. In general, the significantly different parameters between the two locations (the Tarapacá and Antofagasta regions) for P. tamarugo included $\Psi$, SLA and RWC$_{a}$. Regarding the water potentials, significant differences occurred during the last measurement date, when the most aggressive drought treatment had already been applied. In this context, the minimal water potentials reached on that date were 2.41 ± 0.19 MPa for locations from the Tarapacá region and 2.73 ± 0.07 MPa for locations from the Antofagasta region. Regarding SLA, the only differences between the two locations were detected during T2 at the end of the most severe drought treatment (T3). The extreme values ranged from 59.3 ± 0.02 to 31.02 ± 0.02 m$^2$ kg$^{-1}$.

The other parameter that registered significant differences was the relative water content at the point of stomatal closure (RWC$_{c}$). The case regarding the values of the relative water content was the most evident. Differences started in T1, T2 and T3 (Fig. 1). Locations from Tarapacá always presented higher values than locations from Antofagasta, whose values never exceeded 80 %. During T1, significant differences were registered for the Sh ($F_{3,108} = 11.876; P < 0.01$) and Lb ($F_{3,108} = 39.801; P < 0.001$) parameters between populations from La Huayca and Toconao. The mortality rate at the end of the most aggressive drought treatment did not show significant differences, with rates being 68.3 % for Tarapacá and 66.2 % for Antofagasta ($F_{3,108} = 36.78; P = 0.124$).

**At intrapopulation level in P. tamarugo**

The intrapopulation analysis of P. tamarugo only showed significant differences in populations from Zapiga and La Tirana in Tarapacá and in Toconao in Antofagasta. The physiological parameters that showed differences were registered only at T3 in both regions (Table 3) and included the SLA, RWC$_{a}$ and $\Psi$ parameters. At the morphological level, during T3, the Ss parameter showed statistically significant differences in the populations of Salar de Llamara and La Tirana ($F_{3,108} = 231.876; P < 0.001; F_{3,108} = 189.760; P < 0.001$, respectively).

**At interpopulation level for P. alba**

In general, the significantly different parameters between the two locations (Tarapacá and Antofagasta regions) for P. alba were the xylem water potential and SLA. The xylem water potential showed significant differences when the T3 treatment was applied (Fig. 2). Extreme values from the two locations ranged from $-1.8 \pm 0.11$ to $-2.9 \pm 0.03$. Regarding the SLA parameters, the trends showed higher values in locations from the Antofagasta region than locations from the Tarapacá.
At the morphological level, Sh showed differences between the Toconao and La Tirana locations during T1 ($F_{3,108} = 122.765; P < 0.023$). The mortality rate at the end of the most aggressive drought treatment did not show significant differences, with values of 76.3 % for Tarapacá and 58.2 % for Antofagasta ($F_{3,108} = 87.78; P = 0.012$).

At intrapopulation level in *P. alba*

At the intrapopulation level, there were noteworthy differences in the estimated physiological parameters relative to $E_c$, SLA, RWC$_c$, and $\Psi$. Statistically significant differences were registered only during T3 in two populations from Antofagasta and one from Tarapacá (Table 4). Regarding the morphological parameters, seedling height was the only one that showed a significant difference during T3.

**Relationships among parameters**

The parameters that showed relationships in both species ($P < 0.01$) were the height of individuals and $\Psi$ measured during T1 (Fig. 3). Likewise, both species
registered a positive relationship between soil humidity and plant stem diameter during T1 \(R^2 = 0.57, F_{3,108} = 124.54; P < 0.01; P. tamarugo\) and \(R^2 = 0.59, F_{3,108} = 214.65; P < 0.001; P. alba\) and between \(\Psi\) and \(E_c\) \(R^2 = 0.74, F_{3,95} = 309.24; P < 0.001; P. tamarugo\) and \(R^2 = 0.79, F_{3,95} = 314.01; P < 0.001; P. alba\). The soil humidity ranged during the experiment (physiological measures) between 43 and 45%. No statistically significant differences were found between the dry biomass of roots in drought treatments and control groups in both species. However, statistically significant differences were found between the dry biomass of leaves in treatments between both species (Fig. 4).

**Discussion**

In the present study, we found strong evidence of higher xeromorphism in tamarugo (\(P. tamarugo\)) than in white mesquite (\(P. alba\)) at the species level, denoted by a lower ‘\(\Psi\)’ and the lowest values of dry leaf biomass obtained during drought treatment experiments. These physiological characteristics could explain the prevalence of \(P. alba\) in more mesic habitats found in the arid to semi-arid ecosystems of South America. These results are in accordance with those of other studies which reported stronger effects of decreased water supply on the morphological and physiological parameters of mesic \(P. alba\) than those of other xeric species (Vilela et al. 1996; Vilela et al. 2003). In terms of provenance, the clear differences obtained for parameters \(\Psi\) and SLA showed that the seeds which had originated north of the collection areas included in the present study were well adapted to drought. Thus, the tendency of \(P. tamarugo\) (originating

| Parameter   | La Huayca  | Zapiga  | Toconao  |
|-------------|------------|---------|----------|
| SLA (m² kg⁻¹) | 9.046 0.006 | 3.310 0.014 | 3.099 0.010 |
| RWCc (%)     | 19.555 <0.001 | 1.035 0.411 | 2.222 0.0435 |
| \(\Psi\) (MPa)| 0.444 0.511 | 14.680 <0.001 | 10.098 <0.001 |

*Figure 2. Comparison among provenances of \(P. alba\) in terms of xylem water potentials (A) and SLA (B). Provenances of Tarapacá and Antofagasta are denoted by white squares and white triangles, respectively. Control seedlings are represented by black figures. Watering supply treatments are denoted in x axis, each with two measurements (see text for information). For each watering supply treatment, statistically significant differences are represented by an asterisk.*

*Table 3. Intrapopulation parameters of \(P. tamarugo\). Statistically significant differences \((P < 0.05)\) were registered when T3 was applied.*
in Tarapacá) to maintain high $\Psi$ and RWC$_0$ values during T3 (~2.4 MPa and 90%, respectively) clearly indicates the presence of an adjustment strategy to withstand stress periods. These results from Tarapacá may be associated with the fact that this distribution area is the natural dispersal centre for this species (Calderon et al. 2015), which may have conferred critical ontogenic features to this species in order to adapt to the stress encountered in the Pampa del Tamarugal plain. Other studies which focussed on drought-tolerant species support the idea that seeds originating in natural dispersal areas for the species have genetic and morphological features that positively affect adaptation to the most severe environmental stresses typical of their place of origin (Cony 1996; Sánchez-Vila and Retuerto 2007; Andivia et al. 2012).

Table 4. Intrapopulation parameters of P. alba. Statistically significant differences ($P < 0.05$) were registered when T3 was applied.

| Parameter | Quillagua | Chiu-Chiu | La Huayca |
|-----------|-----------|-----------|-----------|
| $E$ ($\mu$mol H$_2$O kg$^{-1}$ s$^{-1}$) | 0.03 | 1.345 | 11.086 | <0.001 | 10.345 | <0.001 |
| SLA (m$^2$ kg$^{-1}$) | 9.111 | <0.001 | 2.123 | 0.023 | 1.000 | 0.657 |
| RWC$_c$ (%) | 16.432 | <0.001 | 11.111 | <0.001 | 9.456 | <0.001 |
| $\Psi$ (MPa) | 0.564 | 0.531 | 10.598 | <0.001 | 0.009 | 0.987 |
| Sh (mm) | 22.765 | <0.01 | 3.456 | 0.963 | 19.875 | <0.01 |

SLA is a parameter that varies depending on the water stress conditions that affect leaf growth, location of the crown (in terms of light) or nutrient availability (Mousseau 1999; Larcher 2003). In the present study, the origin location values of P. tamarugo and P. alba showed significant differences; these differences mainly appeared during the most severe drought treatments, with evidently higher values in the most humid locations (Antofagasta). This strategy may have been linked to a higher accumulation of non-structural leaf biomass in the driest locations (Tarapacá) as a response

Figure 3. Relationship between xylem water potentials ($\Psi$) and seedling height for P. tamarugo (black figures) and P. alba (white figures).
to reduce excessive water loss through the epidermis, which would allow the plants at this location to maintain higher RWC₀ values (Larcher 2003). This is a typical strategy of drought-tolerant species and can be used as an effective indicator of the degree of tolerance among species or their offspring. Similarly, in the same study area, Carevic et al. (2015) found that increase in leaf biomass is associated with SLA in mesquite (Prosopis burkartii) during stress periods caused by frost, which indicates the sensitivity of this parameter to the presence of environmental stress.

Conversely, RWC₀ has been identified as an important indicator of the water status of leaves under drought conditions (Koide et al. 1989). RWC₀ is strictly related to cell volume, and thus, it reflects the balance among the water content, water supply to the leaf and transpiration rate more accurately (Andivia et al. 2012). In the present study, the trends clearly indicated a water conservation strategy during the highest drought period, with maximum values close to 90 % in P. tamarugo. This strategy better outlines the way in which this species resists drought, which involves retaining a high percentage of water within its leaf tissues before stomatal closure. In P. alba, drought strategies were mostly reflected by higher accumulation of biomass at the moment of T3 application (indicated by decreased SLA values) and by maintenance of higher water potentials compared with others in more humid locations (Toconao and Chiu Chiu). In contrast, in the semiarid ecosystems of Argentina and under saline stress, P. alba utilizes strategies that are mainly associated with the suppression of its photosynthetic apparatus rather than those associated with water variables. This is because when faced with such a stress, P. alba possesses the ability to decrease its net photosynthesis and stomatal conductance at the expense of its Ψ, which decreases as stress becomes more severe (Meloni 2014). In contrast, when we compared Ψ of both the species during the most severe drought treatment, we observed that the extreme minimum values obtained in P. alba reached values close to −3.0 MPa, whereas P. tamarugo maintained extreme values of −2.4 MPa during T3. This fact is directly linked to the most efficient stomatal closure and lower E rate under minimal Ψ values, which was indicated by the positive relationship between Ψ and E in both species.

It is important to note that the relationships observed between plant height of both species and Ψ afford this parameter a significant influence that inhibits growth during the first months of seedling growth. Thus, maintaining values close to −1.0 MPa benefitted growth even more when seedlings of both species with values close to −3.0 MPa had a lower height. Similar trends were described by Vilela et al. (1996) in Prosopis spp. with regard to different watering rates, which directly affected the height and stem growth diameters of seedlings. The high variability of physiological response to water stress reported in the present study provides us with a powerful seed selection tool for species that are currently classified as endangered (P. tamarugo) and vulnerable (P. alba) by Chilean environmental agencies (Carevic et al. 2015). All of these responses, detectable at an age as early as 1 or 2 years, have an advantage that allows their use in reforestation and preservation programmes and represent an important source of information for future programmes aimed at early selection and genetic improvement of Prosopis spp.

Conclusions

In this research, we analysed the effects of different drought treatments on seedlings of two phreatophyte Prosopis spp. which grow in hyperarid climate. In general, Ψ, SLA and RWC₀ underwent rapid change under drought stress. These results indicate that in Prosopis spp. individuals, these physiological variables can be used as sensitive indicators for measuring drought stress and are good tools for investigating differences in physiological behaviour under different water supply periods. In addition, these results are useful for increasing the knowledge of the endogenous ecophysiological behaviour of Prosopis spp., which will be instrumental to elaborate upon well-planned forestation plans directed to regenerate and preserve these endangered woodlands. Nevertheless, further studies are required to elucidate the genetic mechanism of drought tolerance differences at interpopulation and intrapopulation levels in Prosopis spp. Thus, this intriguing and controversial topic warrants focus in future studies.
Sources of Funding
The authors acknowledge the financial support of FONDECYT project number 11130242 and CONAF project number 047/2011.

Contributions by the Authors
F.S.C. conceived the idea, performed the physiological measures and wrote the article; J.D.H. performed the statistical analyses; J.D.C. analysed the data and revised the article.

Conflict of Interest
None declared.

Acknowledgements
We thank the staff of National Forest Corporation (CONAF) at Pampa del Tamarugal National Reserve.

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