Trees
DOI 10.1007/s00468-012-0798-0

ORIGINAL PAPER

Drought stress tolerance of *Prosopis chilensis* and *Prosopis flexuosa* species and their hybrids

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Received: 14 May 2012 / Revised: 18 October 2012 / Accepted: 24 October 2012

Abstract Natural hybridization commonly produces individuals with intermediate morphological and genetic characteristics, but their response to environmental stress is still uncertain, with some studies showing that transgressive performance would be common. *Prosopis chilensis* and *Prosopis flexuosa* are the most important tree species from Arid Chaco, South-America. Both species occupy different ecological niches in terms of water availability. Genetic and morphological studies have demonstrated the existence of interspecific hybrids in contact areas between these species. Hybrids are characterized by clear intermediate morphological characteristics, which have taxonomical value, and genetic structure compared to both parental species. We studied mechanisms implicated in drought stress tolerance in seedlings of *P. chilensis*, *P. flexuosa* and their interspecific hybrids trying to elucidate if hybrids have a morpho-physiological, growth and survival intermediate response to drought compared to differential parental responses or if they out-perform both parental species when subjected to drought. Our results suggest that hybridization does not result in individuals with intermediate mechanisms related to drought resistance, but with a unique trait combination leading to high growth when water availability is high (similar to the most vulnerable parental species) and high survival under drought stress (similar to the more resistant parental species). Certain uncoupling between symplastic and apoplastic resistence to drought was observed in hybrids, as well as decreased physiological-wood anatomical plasticity compared to parental species. The long-term consequences in terms of adaptive response to drought of this particular trait combination of hybrids remain still unknown.

Keywords Hybridization · Drought stress · Phenotypic plasticity · *Prosopis*

Introduction

Natural hybridization is the spontaneous random mating among populations with a prior divergence history (Grant 1989). Hybridization may have evolutionary consequences, such as increased intraspecific genetic diversity, adaptive traits transfer from one species to another, or even the origin of a new species (Rieseberg 1997). There are several conditions that facilitate hybridization, such as no reproductive isolation between species and the existence of an appropriate niche where hybrids can thrive (Grant 1989). Antropogenic disturbed areas (e.g., cleared forest areas, overgrazed land, agriculture abandoned areas, etc.) seem to be an appropriate niche for hybrids, where they usually present higher fitness than parental species (Anderson 1948). In these cases, the hybrids would replace parental species. These hybrid zones are recognized as centers of

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Communicated by L. Gratani.

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diversity and evolution of plant species (Arnol 1992; Rieseberg et al. 2000).

The study of the physiological differences related to fitness between pure species and their hybrids is essential for understanding the genetic differentiation and niche preferences of different taxa, as well it may provide important information for the management and restoration of degraded environments. There are several recent papers comparing the adaptation of related woody species and their interspecific hybrids (Swenson et al. 2008; Gao et al. 2009). In these studies, hybrids produced transgressive or extreme phenotypes and their fitness under stress conditions was higher than that of the parental species. In fact, transgression seems the rule rather than the exception (Rieseberg et al. 1999). This is particularly important in arid regions, where the distribution of tree species is strongly influenced by the resistance of seedlings to drought stress (Li and Wang 2003). The behavior of seedlings under drought conditions, especially during the establishment stage, is subjected to strong selective pressure; therefore, populations that evolved in different environments would differ physiologically considering their water stress resistance (Villagra and Cavagnaro 2005).

The greatest resistance to a particular stress factor by a certain species or genotype can be attributed not only to the different drought-tolerant mechanism, but also to presenting different degrees of phenotypic plasticity (Valladares et al. 2000). In recent years, phenotypic plasticity has been of great interest to predict the responses of different species or populations to climate change (Valladares et al. 2006; Gimeno et al. 2009) and their adaptation to heterogeneous and changing environments (Ramírez-Valiente et al. 2010). In this regard, very scarce information is about potential differential phenotypic plasticity in hybrids compared to their parental species.

In Chaco region of Argentina, South-America, there are many areas where hybridization occurs among several species of the genus *Prosopis*. *Prosopis chilensis* (locally named "algarrobo blanco") and *P. flexuosa* ("algarrobo negro") are the most important *Prosopis* species in the Arid Chaco, the driest eastern portion of the region (Verga 1995). The former species thrives along dry watercourses and river beds in the foothills, where there is an extra water supply from ground or superficial water. On the other hand, *P. flexuosa* grows in a wider range, occupying flatland areas, where no extra water contribution exists (Cabido et al. 1992). This niche differentiation by water availability suggests differences in susceptibility to drought stress between species. Genetic studies have demonstrated the existence of interspecific hybrids in contact areas between these species, which can be identified by combining morphological studies and biochemical and molecular markers (Mottura et al. 2005; Verga and Gregorius 2007). Several leaf and fruit morphological traits present clear intermediate values between both parental species (Verga and Gregorius 2007), being useful for hybrid determination in the field. From these studies, *P. chilensis* and *P. flexuosa* are considered components of a biological entity or "homogametic complex" (according to Grant 1989).

The aim of this study was to understand the mechanisms involved in drought stress resistance of seedlings of *P. chilensis*, *P. flexuosa* and their interspecific hybrids. The hypothesis was that each species presents different adaptive strategies in relation to drought resistance. Furthermore, in relation to their specific niches, *P. flexuosa* would be the species most tolerant to water stress and *P. chilensis* the most susceptible. Considering the hybrids, two alternative hypotheses were stated: (a) based on their intermediate morphological position between parental species, hybrids present an intermediate performance (in terms of growth and/or survival) under drought stress, and (b) based on studies in other woody species hybrids, transgressive performance is expected, the hybrids out-performing both parental species when it is subjected to drought.

Materials and methods

Genetic characterization

Fourteen trees of *P. chilensis*, thirty-one of *P. flexuosa* and twenty-two of interspecific hybrids were sampled in the Southern area of Arid Chaco region of Argentina (Fig. 1). From these trees, pod and leaf samples were collected. Interspecific hybrids were sampled in sympatric populations, where both taxonomic species exist in close neighbourhood. In contrast, *P. chilensis* and *P. flexuosa* individuals were sampled in allopatric populations. A numerical taxonomic analysis was made with the herbarium sample. Fourteen quantitative and qualitative characters were scored for ten leaves and five pods from each sampled tree and morphological distance was calculated (Verga and Gregorius 2007).

To detect possible genetic differences between the morphological classes, allele frequencies were determined in the offspring (seeds) for the isozyme locus ADH-A. This locus was earlier shown by Verga (1995) to be diagnostic for the differentiation between both studied *Prosopis* species in the Argentinean arid Chaco. Thereby, ADH-A2 allele is almost fixed at *P. flexuosa* and ADH-A3 in *P. chilensis*, so hybrids have heterozygosity for both alleles at this locus. Therefore, *P. chilensis* and *P. flexuosa* mother trees were considered as "pure" species and their seeds used for producing pure species seedlings when their seeds did not show ADH-A heterocygous frequency greater than 0.05. Interspecific hybrid trees were selected for providing seeds to produce hybrid seedlings when their seeds present

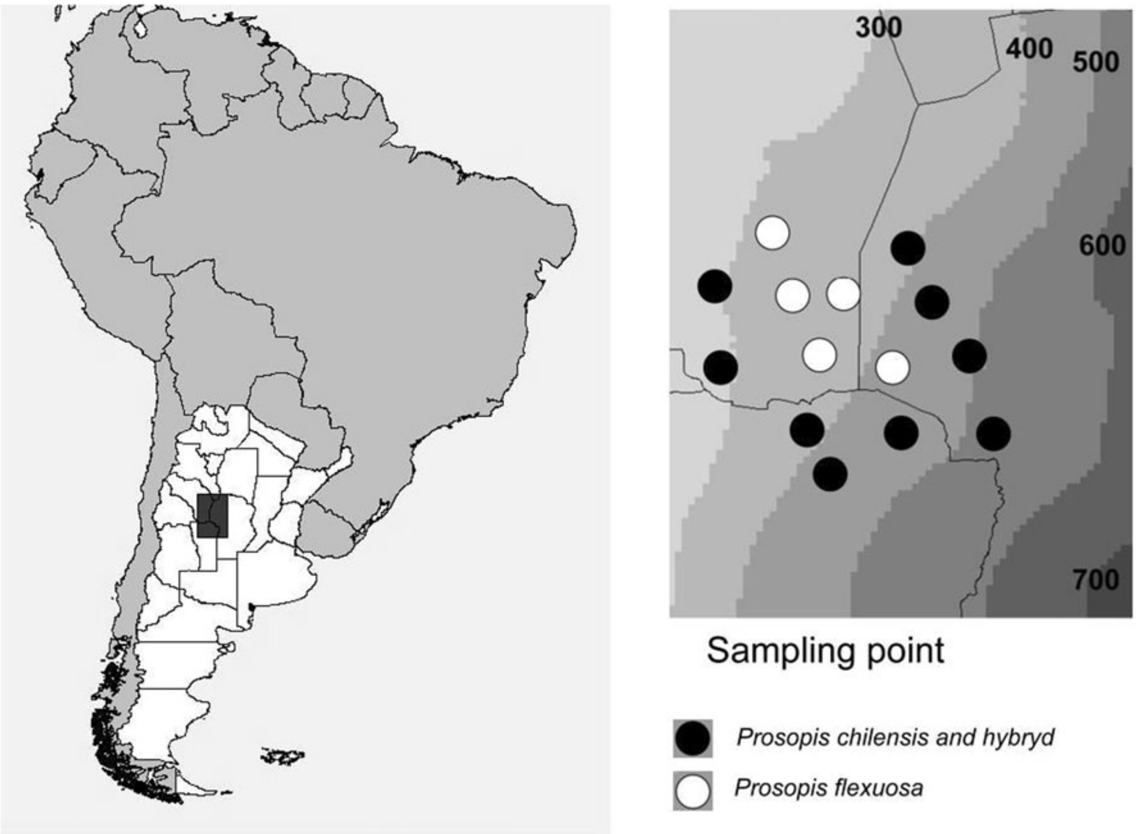


Fig. 1 Map showing the geographical distribution of sampling points of *Prosopis chilensis*, *P. flexuosa* and their hybrids in Arid Chaco region of Argentina, South-America

the two homozygous ADH-A2 and ADH-A3 corroborating the ADH-A23 heterozygous condition of the mother tree.

Treatments and response variables

Plants of the three taxa were produced from seeds and grown in 3 l pots filled with 2:1 parts of mulch and sand, respectively, in a greenhouse with light supplementation, reaching photon flux density of 500 µmoles m^{-2} s^{-1}. Daily temperature and relative humidity ranged between 20 and 35 °C and 35–55 %, respectively. Soil water content was periodically estimated by weighing the pots (every 48 h) and re-watered. A completely randomized design with two factors (taxa and water stress) was applied. Water stress factor had three levels: control-without stress (13–25 % of gravimetric water content, %DW), moderate stress (7–15 %DW) and severe stress (5–10 %DW), which represent plant pre-dawn water potential ranges of −1.6 to −0.5, −3.7 to −1.0, and −5.6 to −1.5 MPa, respectively.

Growth, biomass allocation and survival

Two-month-old plants of each taxon were subjected to drought stress treatments during 75 days (total number of plants = 3 taxa × 3 water stress levels × 10 replicates = 90). Stem basal diameter and plant height were weekly measured during the whole experimental period. At the end of the experiment, plants were harvested and divided into leaves, stem and roots. Biomass samples were dried to constant weight and weighed. Allometric ratios were estimated, such as root weight ratio (root weight: whole plant weight). Survival was estimated in each treatment as the number of live seedlings at the end of experiment in relation to the number of seedling at the beginning of the experiment. The seedling was scored as dead when the stem was completely dry.

Osmotic and elastic adjustment

Pressure–volume (P–V) curves were carried out with 6-month-old plants (six per taxon and treatment) previously subjected to control conditions (no water stress) and moderate water stress (during the previous 3 months), according to the method described by Tyree and Hammel (1972). P–V curves were carried out in apical buds, previously maintained in distilled water during 12 h to reach full turgor. Water potential was measured with a pressure chamber (Bio-control Π10, Buenos Aires, Argentina). Relative water content at turgor loss point (RWC_{tlp}), water potential at the turgor loss point (Ψ_{tlp}), osmotic potential at full turgor (Π_o), and bulk modulus of elasticity (E_{max}) were estimated. The capacity of osmotic and elastic adjustments was determined by comparing parameters (Π_o, E_{max}) between treatments within each taxon.

Maximum hydraulic conductivity

Six-month-old plants (sixteen per taxon) growing under control conditions (no water stress) were used to measure maximum hydraulic conductivity of stem wood and to estimate their vulnerability to cavitation. Xylem water potential (ψ_w) was related to the specific hydraulic conductivity (ks) in stem segments. Each point of the curve corresponds to one individual plant because maximum vessel length was very long, usually of the same length of the whole plant. Maximum vessel length was determined following the compressed air technique (Ewers and Fisher 1989). Therefore, plants were allowed to dehydrate at different levels, each providing a point for the relationship ks–$\psi_{w.}$ The slope of that relationship can provide an estimate of the vulnerability to cavitation. In addition, percent loss of ks (%PLC) versus xylem water potential curves was fitted, and the water potential at which 50 % of PLC (PLC_{50}) occurs was estimated. Xylem water potential was determined by measuring the water potential of a leaf, which previously was covered with an envelope of aluminum foil, about 25 min before measurement, so as to ensure a balance between the water potential of the leaf and that of the stem. After that, the plant was cut at the stem base and a stem piece approx. 45-cm long was submerged in a container of distilled water, and both ends were shaved with a razor blade. The stem was then connected by plastic tubing to a reservoir of distilled water at a pressure of 1 m of water column (0.01 MPa). Flow rate through the stem segment was estimated by weighting the water passing through the stem during 10 min. The water was collected in a plastic vial. The specific hydraulic conductivity was calculated using the formula:

$$\mathrm{ks} = \frac{Q \cdot l}{\Delta_{\psi} \cdot a}$$

where Q is flow in cm^3, $\Delta\Psi$ is potential gradient (in this case 0.01 MPa), l is length of stem segment, a is cross-sectional area of the stem segment (assuming that the whole area corresponds to active sapwood area). The PLC at each water potential value was computed as $\mathrm{ks}/\mathrm{ks}_{max} \times 100$.

Xylem anatomy

Xylem anatomy analyses were carried out in the same set of plants used to make P–V curves (plants under moderate stress and control conditions). Stem transverse sections were cut by hand with a fresh double-edged razor blade, and were mounted on glass slides. Theses samples were examined using an Olympus BX 40 microscope (magnification 100×) and digital pictures were taken with a digital camera (Nikon Cooldpix L 20). Vessel diameter, vessel area and vessel density were measured on 0.25 mm^2 of xylem.

Phenotypic plasticity

Phenotypic plasticity for a given trait was estimated from the relative distance plasticity index (RDPI), proposed by Valladares et al. (2006). This index which ranges from 0 to 1, was calculated as $\mathrm{RDPI} = \Sigma(d_{ij} \rightarrow j'j')/(x_{i'j'} + x_{ij})/n$ where n is the total number of distances and j and j' are two individuals of the same taxon belonging to different treatments (i and i'). One value of RDPI was calculated for each trait and taxon across treatments, and one-way ANOVA was performed to compare RDPI between species.

Statistical analyses

Statistical analyses were carried out with InfoStat software (InfoStat versión 2009. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina). Analyses of variance (ANOVA) were applied for testing differences between treatments within each taxon. Duncan test was used for comparison of means. Auxiliary variables (dummy) were used to compare linear regression models applied to hydraulic conductivity versus xylem water potential between species (InfoStat 2009).

Results

Genetic characterization

The dendrogram (Fig. 2) obtained for the morphological distances of the mother trees separated two main groups at a morphological distance (md) of 0.8. One of those groups included the samples from *P. chilensis*, whereas the other group included samples from the hybrids and *P. flexuosa*. Considering the second group, samples from the hybrids were kept separated from those of *P. flexuosa* at a md of about 0.7. Thereby, hybrids have an intermediate position between *P. chilensis* and *P. flexuosa*.

Important genetic differences at locus ADH-A were observed in the offspring (seeds) between the different taxa (Table 1). *Prosopis chilensis* (C) samples presented a genotypic frequency of 0.98 for homozygous ADH-A33, while *P. flexuosa* (F) had a genotypic frequency of 0.84 for

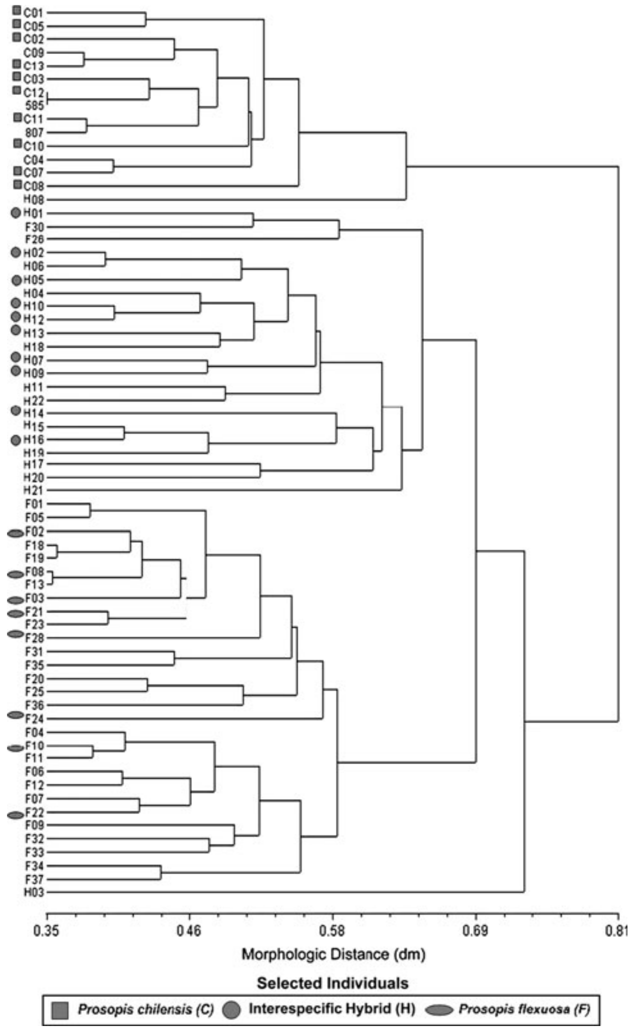


Fig. 2 Dendrogram obtained from morphological distances (md) of mother trees using UPGMA clustering method

ADH-A22. Intermediate genetic characteristics were observed in interspecific hybrid (H) samples, with a frequency of 0.41 for heterozygous ADH-A23. These data resulted in a high differentiation between C and F (d0 CF = 0.92), while H samples were a little more genetically differentiated from F (d0 HF = 0.53) than from C (d0 HC = 0.39) (Table 2).

Table 1 Genotypic and allelic frequency at locus ADH-A from the offspring (seeds) of *P. chilensis* (C), *P. flexuosa* (F) and interspecific hybrids (H)

		C	F	H
Genotypic frequency	ADH-A 22	0.00	0.84	0.19
	ADH-A 23	0.02	0.13	0.41
	ADH-A 33	0.98	0.00	0.39
	ADH-A 12	0.00	0.02	0.01
	ADH-A 13	0.00	0.00	0.00
Allelic frequency	ADH-A 1	0.00	0.01	0.01
	ADH-A 2	0.01	0.92	0.39
	ADH-A 3	0.99	0.07	0.60

Growth, biomass allocation and survival

Under control conditions, *P. chilensis* and hybrid seedlings grew more than *P. flexuosa*, but they were more severely affected (in relative terms) by water stress in both basal diameter and total plant dry weight (total DW) compared to *P. flexuosa* ($P < 0.0001$). Dry weight production was twice in the former species than in the later under control conditions, but the three taxa produced the same dry weight under severe water stress ($P = 0.2223$) (Table 3). Therefore, there was a significant interaction between taxa and drought treatment on total dry weight ($P = 0.0073$).

Leaf dry weight of *P. chilensis*, *P. flexuosa* and the hybrids was reduced under drought stress (Table 3). However, at the end of the experimental period we could not separate the effect of a reduced leaf biomass production than that of leaf shedding due to the drought treatment. In this regard, we observed a strong leaf shedding under severe water stress, where many seedlings completely lost their leaves. Besides, root weight was also reduced in water stress treatments. As a result root weight ratio did not change in response to water stress treatment ($P = 0.0587$). Comparing the different taxa, *P. chilensis* seedlings showed a higher root weight ratio than hybrid and *P. flexuosa* seedlings ($P = 0.0364$) (Table 3).

Survival was very high (100 %) in all three taxa under control conditions and moderate water stress. However, severe water stress severely affected survival in *P. chilensis*, whereas it had a lower deleterious effect on *P. flexuosa* and the hybrids (Table 3). In this regard, mortality was twice in *P. chilensis* than in the other taxa (~70 vs. 30–40 %).

Osmotic and elastic adjustment

Considering control plants, significant differences were observed between the studied taxa for all the studied tissue parameters ($P < 0.001$). *Prosopis chilensis* and the hybrids showed higher water potential values (less negative) at

Table 2 Genetic distance d_0 (Gregorius 1974) for locus ADH-A between the offspring (seeds) of *P. chilensis* (C), *P. flexuosa* (F) and interspecific hybrids

	C	F	H
C	0.00		
F	0.92	0.00	
H	0.39	0.53	0.00

Table 3 Total dry weight (DW), stem basal diameter, root weight ratio (%) and survival in the different treatments: control conditions (without stress), moderate water stress and severe water stress

Taxa	Treatment	Total DW (g)	Basal diameter (mm)	Root weight ratio (%)	Leaf area (cm^2)	Survival (%)
P. chilensis	Without stress	9.07 ± 1.04 a A	4.57 ± 0.26 a A	28.1 ± 1.36 a A	321.0 ± 36.5 a A	100
	Moderate stress	4.75 ± 0.54 b A	3.54 ± 0.20 b A	31.5 ± 3.20 a A	110.2 ± 16.8 b A	100
	Severe stress	2.55 ± 0.32 b A	3.07 ± 0.16 b AB	33.2 ± 1.62 a A	9.800 ± 7.00 c A	30
P. flexuosa	Without stress	4.51 ± 045 a B	3.35 ± 0.07 a B	25.7 ± 1.84 a A	121.9 ± 18.0 a B	100
	Moderate stress	2.84 ± 0.32 b B	2.80 ± 0.09 b B	27.6 ± 1.07 a A	65.30 ± 12.6 b A	100
	Severe stress	1.87 ± 0.29 b A	2.57 ± 0.14 c B	29.3 ± 1.82 a A	4.900 ± 3.10 c A	70
Hybrids	Without stress	8.19 ± 0.77 a A	4.69 ± 0.18 a A	27.5 ± 1.49 a A	235.7 ± 39.8 a A	100
	Moderate stress	3.95 ± 0.44 b AB	3.43 ± 0.14 b A	30.3 ± 1.64 a A	88.10 ± 20.0 b A	100
	Severe stress	2.48 ± 0.30 b A	3.04 ± 0.18 b A	28.3 ± 1.91 a A	9.400 ± 5.90 b A	62.5
	P sp.	<0.0001	<0.0001	0.0364	<0.0001	
	P tr.	<0.0001	<0.0001	0.0587	0.0001	
	P sp × tr	0.008	0.1067	0.7404	0.0006	

Mean and standard error. Different lowercase letters indicate statistical differences between treatments within each taxon. Different capital letters indicate statistical differences between species within each treatment

Significance level: $\alpha = 0.05$

P sp. mean specie effect, *P tr.* mean treatment effect, and *P sp × tr* mean specie x treatment interaction effect

turgor loss point ($\Psi_{\rm tlp}$), osmotic potential at full turgor ($\Pi_{\rm o}$), and higher relative water content at turgor loss point ($\rm RWC_{tlp}$) compared to *P. flexuosa* (Fig. 3). All these parameters indicate an earlier turgor loss (at lower xylem tension) in *P. chilensis* and the hybrids than in *P. flexuosa*. In addition, the hybrids had the lowest bulk modulus of elasticity, indicating a higher cell wall flexibility compared to parental species.

Under moderate water stress, *P. flexuosa* was the only taxon that significantly decreased its $\Pi_{\rm o}$ ($P \leq 0.0001$), nonetheless *P. chilensis* also presented a decrease (but not statistically significant) in this parameter (Fig. 3). *Prosopis flexuosa* also increased the $E_{\max}$ under drought stress conditions, while the other two taxa presented no change in this cell wall parameter. On the other hand, $\Psi {\rm w}_{\rm tlp}$ decreased in *P. chilensis* ($P = 0.036$) and *P. flexuosa* ($P = 0.0039$) under drought stress, whereas no change in this parameter was observed in the hybrids ($P = 0.5584$). Besides, neither taxon changed the $\rm RWC_{tlp}$ under moderate water stress conditions ($P = 0.9493$), but as a whole, it was lower in *P. flexuosa* than in the other two taxa ($P \leq 0.0001$), suggesting that it can tolerate higher cell dehydration without losing turgor compared to the other taxa. There were no statistical interactions between taxa and drought treatment on the P–V curve parameters ($\Pi_{\rm o}$ $P = 0.1889$, $\Psi {\rm w}_{\rm tlp}$ $P = 0.1821$, $\rm RWC_{ppt}$ $P = 0.1422$, and $E_{\max}$ $P = 0.0980$).

Maximum hydraulic conductivity and vulnerability to cavitation

A linear relationship described the response of xylem specific hydraulic conductivity (ks) to xylem water potential (Fig. 4). *Prosopis chilensis* presented the highest maximum specific hydraulic conductivity ($\rm ks_{max}$), and also the largest slope in the fitted linear model, thus suggesting that it was the most vulnerable to cavitation of the three studied taxa (Fig. 4). Moreover, $\rm PLC_{50}$ was −3.7 MPa in *P. flexuosa* and the hybrids, and −2.7 MPa in *P. chilensis*, and the water potential at which no ks was detected ($\rm PLC_{100}$) was −9 MPa in the former taxa and −6 MPa in the latter (Fig. 5). There were no statistical differences in $\rm ks_{max}$, $\rm PLC_{50}$ and the slope of ks–$\psi_{\rm w}$ relationship between *P. flexuosa* and the hybrids, although the linear model fitted to hybrids' data was not significant ($P > 0.05$ and $R^2 = 0.18$). Those variables were different between those taxa and *P. chilensis* ($P < 0.05$).

Xylem anatomy

Under control conditions, *P. chilensis* presented higher mean xylem vessel diameter than *P. flexuosa* and the hybrids ($P < 0.0001$), while under moderate water stress conditions, mean vessel diameter of *P. chilensis* decreased, reaching similar values than those observed in hybrids and *P. flexuosa* (Fig. 6). This last species also decreased the mean vessel diameter, but in a lower degree compared to *P. chilensis*. In contrast, the hybrids had the same vessel size both under high water availability and under water stress conditions ($P = 0.8620$). The same pattern was observed in the vessel area per wood unit area, with significant changes in both *P. chilensis* and *P. flexuosa* ($P = 0.007$) comparing water stress treatments and no change in the hybrids ($P = 0.3933$) (Fig. 6). Considering control conditions, all three taxa differed in the vessel area,

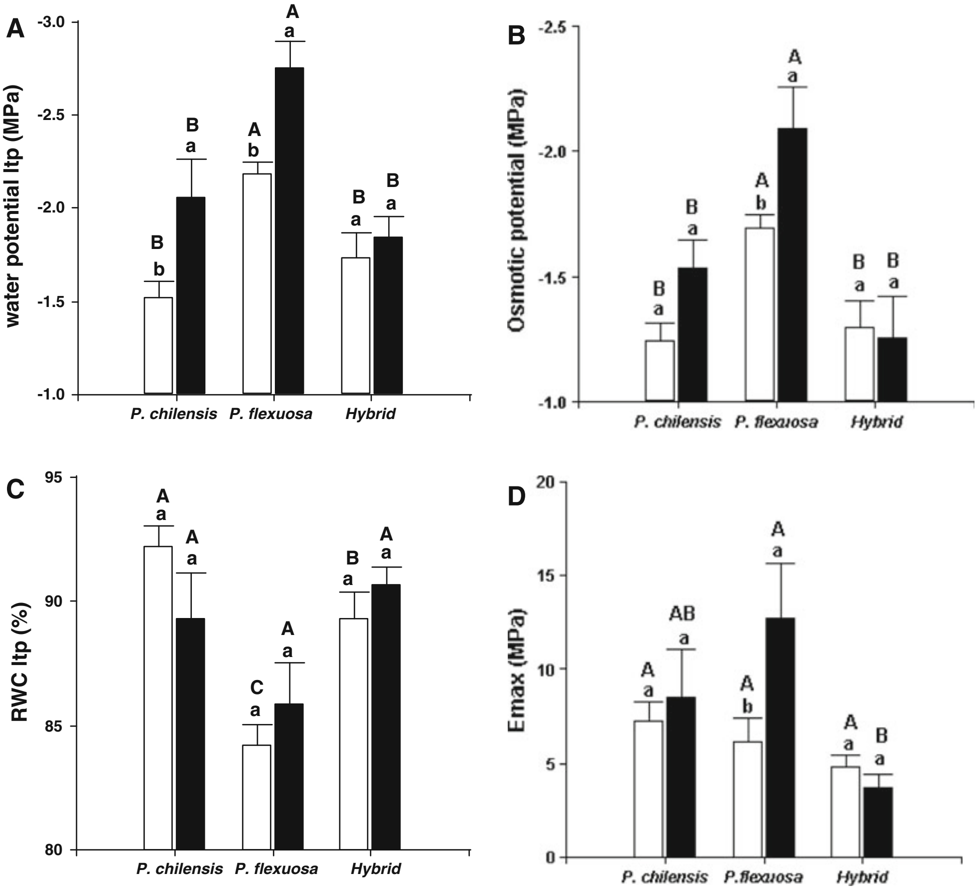


Fig. 3 Water Potential at turgor loss point (**a**), osmotic potential at full turgor (**b**), relative water content at turgor loss point (RWC_{tlp}) (**c**), and Bulk modulus of elasticity (E_{max}) (**d**) estimated in plants growing under moderate drought stress (*white bar*) and without water stress (*black bar*). Mean and standard error. Different *lowercase letter* show statistical differences between treatments per species. Different *capital letter* show statistical differences between species per treatment. Significance level: $\alpha = 0.05$

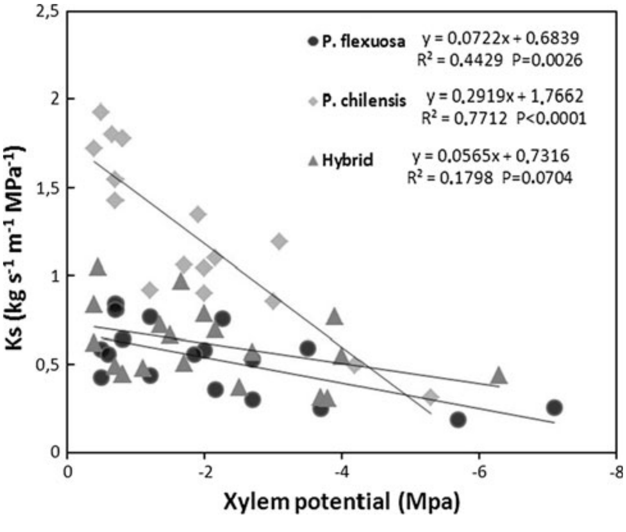


Fig. 4 Relationship between specific hydraulic conductivity of stem wood (ks) and xylem water potential (ψx) measured in control plants of *Prosopis chilensis*, *P. flexuosa* and their interspecific hybrids

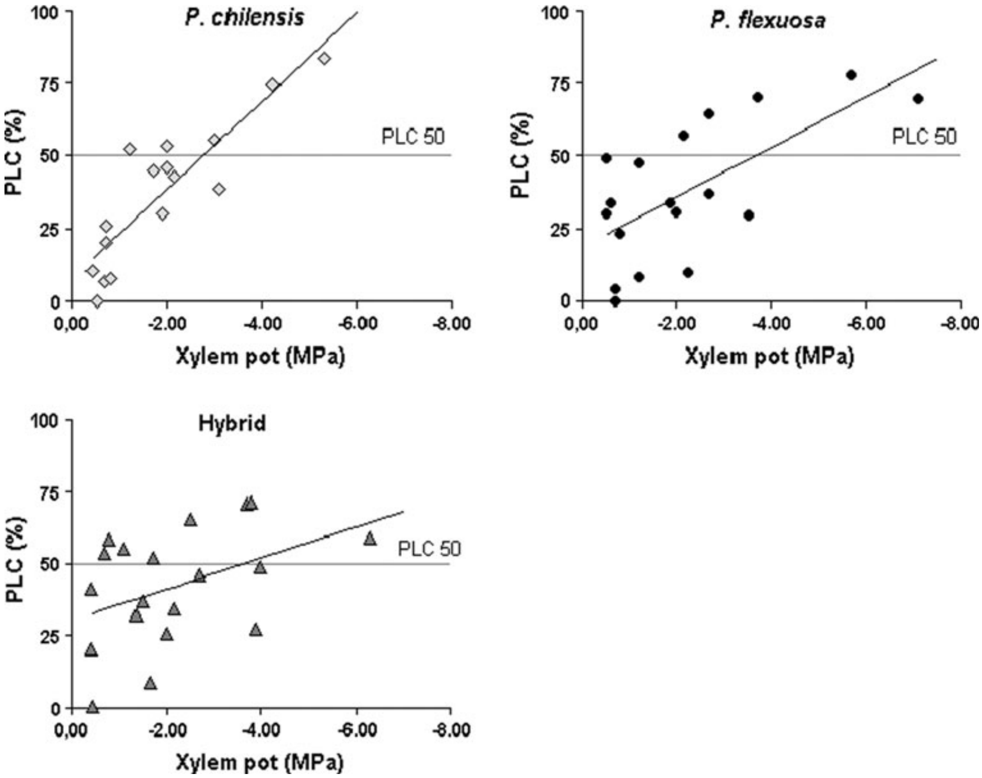
with both parental species having a higher vessel area than the hybrids ($P \leq 0.0001$). It is important to note that it is the mean individual vessel area rather than the whole vessel area, i.e., mainly responsible for maximum hydraulic conductivities (see below). This variable did not differ between *P. flexuosa* and the hybrids (Fig. 6).

The vessel density (number of vessels per unit area) was also affected in both parental species under water stress conditions. However, *P. chilensis* increased the number of vessels ($P = 0.041$) (but they were smaller vessels compared to control conditions), while in *P. flexuosa* the vessel density markedly decreased in response to water shortage ($P = 0.0007$), reaching similar values than those of *P. chilensis* growing under control conditions (note, however, that the size of the vessels was different between species) (Fig. 6). Therefore, there was a significant interaction between taxa and treatment on vessel density ($P = 0.0002$).

Phenotypic plasticity

Hybrids had a lower phenotypic plasticity than at least one parental species for most physiological and xylem anatomy parameters (Fig. 7). Only growth and allometric parameters presented some degree of plasticity in hybrid seedlings. Also, phenotypic plasticity at the physiological level was highest in *P. chilensis* and intermediate in *P. flexuosa*.

Fig. 5 Relationship between percent loss of conductivity (PLC) and xylem potential of *P. chilensis*, *P. flexuosa* and their interspecific hybrids. Regression parameters were: *P. chilensis:* slope = 8.6, $R^2 = 0.77$ and $P < 0.0001$; *P. flexuosa:* slope = 15.3, $R^2 = 0.44$ and $P = 0.0026$; hybrids: slope = 5.4, $R^2 = 0.18$ and $P = 0.0704$



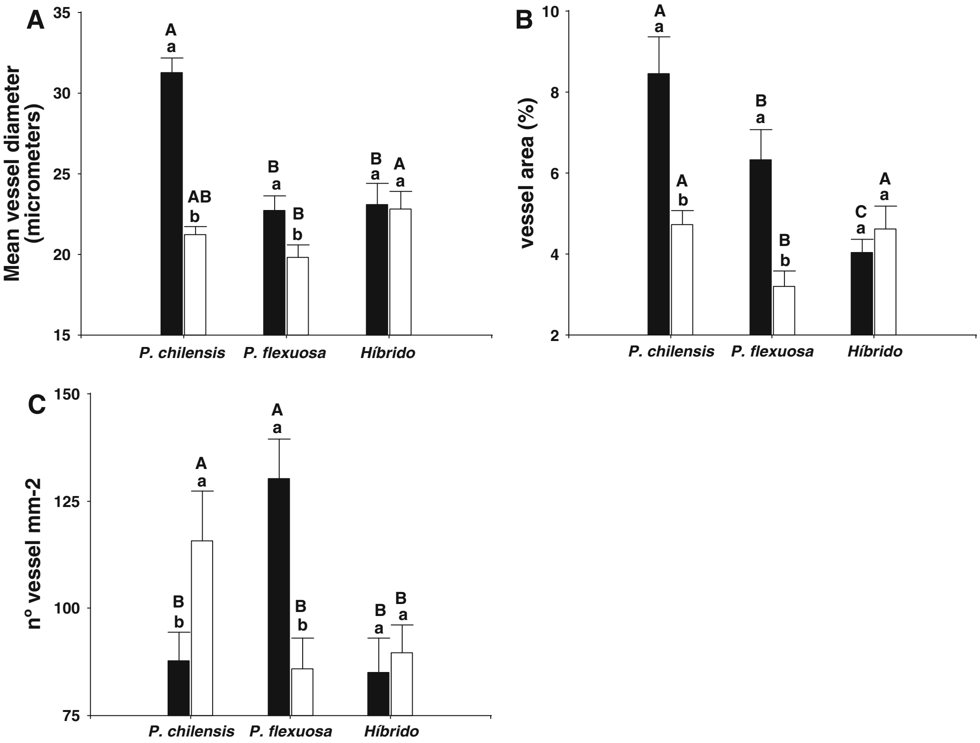


Fig. 6 Mean vessel diameter (**a**), vessel area (**b**) and number of vessels per wood unit area (**c**) of *P. chilensis*, *P. flexuosa* and interspecific hybrids grown under control conditions (*white bars*) and moderate water stress (*black bars*). Mean and standard error. Different *lowercase letters* indicate statistical differences between treatments within each taxon. Different *capital letters* indicate statistical differences between species within each treatment. Significance level: $\alpha = 0.05$

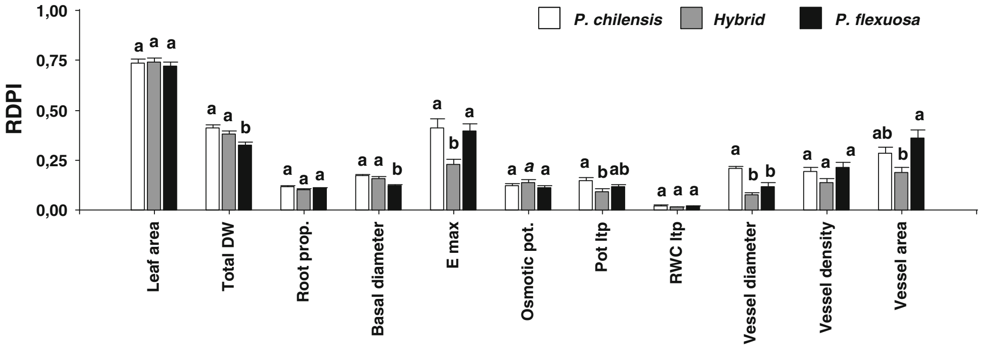


Fig. 7 Relative distance plasticity index (RDPI) for growth and allometric parameters (leaf area, total DW, root prop., basal diameter), physiological parameters (E_{max}, Osmotic pot., Pot_{tlp}, RWC_{tlp}) and xylem anatomy parameters (vessel diameter, vessel density, vessel area). Different *letters* indicate statistical differences between species. Significance level: $\alpha = 0.05$

As expected, because it was the taxon least affected by water stress, *P. flexuosa* presented the lowest plasticity in growth and allometric parameters. Both parental species presented high plasticity in xylem anatomical features. However, they differed in the wood anatomical trait (and the direction of change) that presented a plastic response to water stress conditions. *P. chilensis* showed high plasticity in mean vessel diameter and *P. flexuosa* presented high plasticity in vessel area (Fig. 7).

Discussion

The close correlation between isozyme and morphological analyses (Table 1; Fig. 2) suggests a clear disjunction between *P. chilensis* and *P. flexuosa* and the formation of an intermediate group (hybrid). Moreover, studies about the mating system in this type of hybrid swarm (acording to Verga 1995) have shown that there is a significant degree of genetic isolation of the hybrids in relation to the parental species (Mottura et al. 2005; Córdoba and Verga 2008).

Concerning the drought response of hybrid seedlings compared to that of parental species, the results of this study suggest that the hybrids combine characteristics of both species resulting in a high relative decline in growth similar to that observed in *P. chilensis* but high survival, similar to that observed in the most tolerant species, *P. flexuosa*. It is noteworthy that, even though the relative growth was highly affected in hybrids and *P. chilensis*, the absolute growth of both taxa was similar to *P. flexuosa* growth under drought conditions. This was the result of large differences in growth capacity in the former compared to the latter species. These results are in agreement with the hypothesis of a transgressive behavior of the hybrids, which present high growth capacity when water is highly available (similar to *P. chilensis*) and high survival under drought conditions (similar to *P. flexuosa*). We will discuss the morpho-physiological characteristics of the different taxa that could help understanding the observed patterns.

Considering leaf tissue parameters (i.e., those derived from P–V curves), results of this study allow understanding the different habitat preferences of both parental species and therefore, may contribute to explain their natural spatial distribution. *Prosopis flexuosa* had the lowest osmotic potential at full turgor and it decreased even more under moderate water stress (high osmotic adjustment). Also, in response to water stress, the bulk modulus of elasticity increased, indicating changes in leaf cell wall structure. Both traits contribute to a rapid cell turgor recovery with small water gains (Aranda et al. 2004), and as a whole, tissue parameters of *P. flexuosa* are consistent with a better ability to maintain stable water relations under drought than *P. chilensis*. Hybrids showed similar values of leaf tissue parameters than *P. chilensis*, the least tolerant species (Fig. 3).

On the other hand, *P. chilensis* showed higher maximum ks and vulnerability to cavitation than *P. flexuosa*, which together with the leaf parameters mentioned above, could explain their distribution associated with areas with extra-groundwater contribution (Pockman and Sperry 2000). In contrast to leaf tissue parameters, regarding wood hydraulic characteristics (ks_{max} and PLC_{50}), hybrids presented similar values to *P. flexuosa*, the most tolerant species, at least under control (no water stress) conditions. These traits have a fundamental role in water stress tolerance and survival in many woody species (e.g., Tyree and Sperry 1989; Cochard et al. 2008; McDowell et al. 2008), and therefore, they could be important to understand hybrids' behavior (high survival) under drought stress. Most studies of vulnerability to cavitation have found sigmoid-type relationships between ks and xylem water potential; however, there are some linear-type cases (Jacobsen et al. 2005) similar to those found in this study. However, in contrast to *P. flexuosa*, hybrids showed no change in wood anatomical characteristics responsible for hydraulic conductance when exposed to water stress. Since ks and vulnerabilty to cavitation are usually correlated (e.g., Martínez-Vilalta et al. 2002), the fact that no change occurred in hybrids wood anatomy subjected to drought could be an advantage in the short term, due to ks_{max} maintenance under drought, but it could also imply that new cycles of water stress may probably have a differential effect on hybrids compared to *P. flexuosa*. This hypothesis should be tested with specific experiments to assess the long-term consequences of the lack of wood anatomical plasticity in the hybrids.

The largest vessel diameter found in *P. chilensis* wood can be crucial to explain the observed differences in maximum hydraulic conductivity between species, because this variable increases at the fourth power of vessel radius (Tyree and Zimmermann 2002). According to this, a strong correlation was observed in mean maximum ks and mean vessel diameter of the different taxa. Maximum ks is generally correlated with the maximum rate of carbon fixation (Santiago et al. 2004), as well as to growth rates (Brodribb et al. 2005). Accordingly, *P. chilensis* had higher growth rate than *P. flexuosa* when both species are grown with high water availability. However, interestingly, hybrids presented high growth rates similar to *P. chilensis* under control conditions, although having maximum ks values similar to *P. flexuosa*. In this case, it seems that the hybrids would have greater hydraulic conductance explained by other plant organ (e.g. high root ks), which could support high growth rates with low water conductivity at the stem level. Root and leaf mesophyle conductivities should be measured to understand the contribution of the different organs to whole plant hydraulic conductance, and therefore, to explain differential growth patterns.

Although in different magnitude, *P. chilensis* and *P. flexuosa* showed phenotypic plasticity at morphological, physiological and xylem anatomy parameters under drought conditions (Fig. 6), whereas the hybrids were plastic mostly considering only growth and allometric parameters.

As a whole, the results suggest that the transgressive behavior of hybrids in relation to drought, at least in response to a quite short-term drought period such as that of our experiment, is linked to a particular combination of attributes: leaf tissue parameters were similar to those of *P. chilensis;* wood hydraulic and anatomical characteristics were similar to those of *P. flexuosa*, whereas the phenotypic plasticity in physiological and xylem anatomy traits was lower than that in at least one parental species, highlighting the need of future studies focusing on this interesting phenomenon. This combination of attributes enables hybrids to develop a strategy different from the parental species allowing them to have high growth potential and also high survival under conditions of severe stress. Picotte et al. (2008) found similar results in hybrids of the *Piriqueta caroliniana* complex, hybrids exhibiting intermediate morphological leaf traits (shape and size), and transgressive traits considering other leaf characteristics with adaptive value in relation to drought tolerance, such as hair density. These transgressive characteristics of hybrids could allow them to adapt to new habitats (Schwarzbach et al. 2001; Silim et al. 2001; Rieseberg et al. 2007; Swenson et al. 2008).

On the other hand, the highest drought resistance of *P. flexuosa* would be associated with a moderate plasticity at the physiological and xylem anatomy levels and favorable absolute values of key traits for drought tolerance (low osmotic potential at full turgor, low vulnerability to cavitation, low mean vessel diameter, etc.). *Prosopis chilensis* is the taxon with less water stress tolerance considering the absolute values of all studied traits, but it presented a relatively high phenotypic plasticity in several of those traits.

The construction of a cavitation-resistant xylem has high energy costs for the plant, and therefore it is expected that this investment is coordinated with other traits leading to high drought resistance at the whole plant level. According to this, Vilagrosa et al. (2010) found that several symplastic (measured in leaves) and apoplastic (xylem cavitation) variables were well correlated in Mediterranean species differing in drought resistance and survival under field conditions. The need of such a coordination was also suggested in a study with *Quercus ilex* (Lo Gullo and Salleo 2003), in which the ratio of the minimum diurnal leaf water potential to the leaf water potential at the turgor loss point increased from 0.9 to 1.3, the percent conductivity loss during summer drought increased from 30 to 85 %, and the xylem's capability to recover from embolism decreased dramatically. In the present study, this kind of general coordination was also observed in *P. chilensis* and *P. flexuosa*. The least tolerant species at the leaf level (*P. chilensis*) was also the most vulnerable to cavitation and vice versa. However, it seems that this coordination is broken down in the hybrids, which presented leaf tissue parameters similar to those of the drought-sensitive species, but a xylem with high resistance to cavitation. A similar lack of correlation between leaf and xylem resistance to drought has been observed across eight grapevine cultivars (Alsina et al. 2007). In that study, the authors hypothesized that the uncoupling between leaf and xylem drought resistance could be the result of human selection for grape production oriented to yield and quality, and not to environmental adaptation, combining different vine cultivars and rootstocks. It would be interesting to elucidate the adaptive consequences of such uncoupling in human-selected plants as well as in those formed through natural breeding such as *Prosopis* hybrids.

The present study confirmed that the three taxa taxonomically differentiated by leaf morphology and isozyme markers also functionally differ in relation to their drought stress response. The results suggest that the genetic differences resulting from different evolutionary paths of both studied *Prosopis* species have had a differential effect on the adaptive behavior of individuals. Furthermore, the process of hybridization that naturally occurs between both species, and unlike observations on morphological traits of taxonomic importance, does not result in individuals with intermediate mechanisms related to drought resistance. Instead, it seems that there is genetic recombination leading to the formation of a new taxa, with own characteristics, which may probably have the ability to adapt to new environments, different from those of the parental species. However, the long-term advantages in terms of adaptability to stressful conditions of the observed combination of hybrids' traits deserve future research.

Acknowledgments We gratefully acknowledge INTA (National Institute for Agricultural Technology) of Argentina for funding this study through grant PNFOR 044341 "Domesticación de especies forestales nativas del Parque Chaqueño (Algarrobo)".

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