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DOI:

[10.1093/treephys/tpab034](https://doi.org/10.1093/treephys/tpab034)

Document Version

Accepted author manuscript

[Link to publication record in Manchester Research Explorer](#)

Citation for published version (APA):

Cordero Herrera, I., Dolores Jimenez, M., Delgado, J. A., Balaguer, L., Pueyo, J. J., & Rincon, A. (2021). Local adaptation optimizes photoprotection strategies in a Neotropical legume tree under drought stress. *Tree Physiology*, 41(9), 1641-1657. <https://doi.org/10.1093/treephys/tpab034>

Published in:

Tree Physiology

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Local adaptation optimizes photoprotection strategies in a Neotropical legume tree under drought stress

Irene Cordero^{1,2,3,*}, María Dolores Jiménez⁴, Juan Antonio Delgado⁴, Luis Balaguer^{2,†}, José J. Pueyo¹, Ana Rincón¹

¹ Department of Soil, Plant, and Environmental Quality, Institute of Agricultural Sciences, Spanish National Research Council (ICA-CSIC), Serrano 115-bis, 28006 Madrid, Spain.

² Department of Plant Biology I, Universidad Complutense de Madrid, José Antonio Nováis 10, 28040, Madrid, Spain.

³ Department of Earth and Environmental Sciences, The University of Manchester, Michael Smith Building, Oxford Road, Manchester, M13 9PT, UK

⁴ Department of Biodiversity, Ecology and Evolution, Universidad Complutense de Madrid, José Antonio Nováis 10, 28040, Madrid, Spain

[†]Deceased

* Author for correspondence: Irene Cordero

Tel.: +44 16130 68074; e-mail: irene.cordero@manchester.ac.uk

Keywords

Caesalpinia spinosa (tara), common garden, desiccation, leaflet closure, light stress, local adaptation, phenotypic diversity, photosynthetic response.

Running head: Local adaption and photoprotection under drought

Abstract

Photoprotection is a plant functional mechanisms to prevent photooxidative damage by excess light. This is most important when carbon assimilation is limited by drought, and as such, it entails a trade-off between carbon assimilation vs stress avoidance. The ecological adaptation of plants to local water availability can lead to different photoprotective strategies. To test this, we used different provenances of *Caesalpinia spinosa* (Mol.) Kuntze (commonly known as “tara”) along a precipitation gradient. Tara is a Neotropical legume tree with high ecological and commercial value, found in dry tropical forests, which are increasingly threatened by climate change. Morphological and physiological responses of tara provenances were analysed under three different treatments of drought and leaflet immobilization i.e., light stress, in a common garden greenhouse experiment.

Tara quickly responded to drought by reducing stomatal conductance, evapotranspiration, photochemical efficiency, carbon assimilation and growth, while increasing structural and chemical photoprotection (leaflet angle and pigments for thermal dissipation). Leaflet closure was an efficient photoprotection strategy with overall physiological benefits for seedlings as it diminished the evaporative demand and avoided photodamage, but also entailed costs by reducing net carbon assimilation opportunities. These responses depended on seed origin, with seedlings from the most xeric locations showing the highest dehydration tolerance, suggesting local adaptation and highlighting the value of different strategies under distinct environments. This plasticity on its response to environmental stress allows tara to thrive in locations with contrasting water availability.

Our findings increase the understanding of the factors controlling the functional ecology of tara in response to drought, which can be leveraged to improve forecasts of changes in its distribution range, and for planning restoration projects with this keystone tree species.

Introduction

Global change scenarios predict higher variability and intensification of *El Niño* cycles and fluctuating patterns of fog formation in the Pacific coast of South America, with unforeseeable consequences for dry ecosystems (IPCC 2014, Tognetti 2015). Trees can display different adaptive strategies to deal with adverse environmental conditions, for which the phenotypic and genotypic variability are important features (Wennersten and Forsman 2012). The tree phenotype is defined by the balanced combination of speed on resource acquisition vs tissue longevity, resource overspend vs conservation, hydraulic efficiency vs cavitation resistance, growth vs defence, whose plasticity and/or local adaptation allows trees to thrive in a range of environments (Díaz et al. 2016). Stomatal closure is an effective mechanism of plants to avoid water loss, but it also limits CO₂ diffusion and photosynthesis. In those circumstances, light energy that exceeds the capacity for photosynthesis leads to over-excitation of the photosynthetic apparatus, causing photoinhibition and photodamage (Lawlor and Tezara 2009). This phenomenon is exacerbated in environments close to the equator where the photosynthetically active radiation (PAR) is soaring (Bouvét et al. 2002). Plants display two main mechanisms to escape photoinhibition and photodamage, 1) structural avoidance of the excessive absorption of luminous energy, e.g., reorientation of leaves and chloroplasts, leaflet closure, and 2) chemical adjustments to deal with the excess of absorbed light, e.g., increase of photosynthetic pigments involved in thermal energy dissipation (Raven 2011). Photosynthetic pigments, such as chlorophylls and carotenoids, are involved in light harvesting and photoprotection. They quickly respond to environmental stressors and, therefore, the study of their composition and of the ratios between them provides a diagnosis of the functionality of the photosynthetic apparatus (Esteban et al. 2015).

76 Caesalpinia clade is a group of around 200 species that thrives in an extensive range of
77 environments, including seasonally dry tropical forests and woodlands (Gagnon et al.
78 2019). Species in this clade present abundant adaptations to dry conditions, such as
79 drought deciduousness and rapid post-drought leaf flush (Gagnon et al. 2019).
80 *Caesalpinia spinosa* (Mol.) Kuntze, commonly known as tara, is a legume tree of high
81 ecological (keystone species) and commercial value (high tannins and gums
82 concentration in seeds) widely distributed along the occidental South American coast (de
83 la Cruz Lapa 2004, Larrea 2011). Tara seedlings have been evidenced to present a
84 dehydration-avoiding strategy through a tight stomatal control of photosynthesis and
85 structural photoprotection by leaflet closure triggered by the decrease in air humidity
86 (Balaguer et al. 2011). As a rare exception within the Caesalpinia clade (Gagnon et al.
87 2019), tara is often found as dominant tree of dry tropical forests and in coastal forests
88 maintained by seasonal fogs as the sole water resource (e.g., Lomas biome) (Canziani
89 Amico 2002, Ramírez et al. 2012). The Lomas are ecological valuable formations that
90 trap water and truly biodiversity hotspots (Sotomayor and Drezner 2019). In dry
91 ecosystems, water shortage affects many physiological and morphological parameters,
92 hindering plant survival, growth and/or fitness (McDowell et al. 2008, Lawlor and Tezara
93 2009). However, few studies have evaluated the capacity of *Caesalpinia* species to
94 withstand drought (Dombroski et al. 2011, Pineda-García et al. 2015, Chirino et al. 2017).
95 Previously, we evaluated the response of tara to the environmental conditions of the
96 Atiquipa fog forest (Lomas ecosystem), an invaluable oasis in the middle of the
97 inhospitable Peruvian coastal desert (Balaguer et al. 2011). Strikingly, despite thriving in
98 a very unique environment controlled by fog pulses, tara plants did not show local
99 acclimation or specialisation to these water pulses. Instead, they displayed a rather
100 opportunistic behaviour, which would connect with the proposed human-favoured origin

of this forest (Balaguer et al. 2011). Additional support for this hypothesis is that the Caesalpinia clade presents strong phylogenetic biome conservatism (Gagnon et al. 2019). However, tara is found in remarkably different environments (e.g., fog forests, tropical dry and humid forests), which offers a unique opportunity to study potential morphophysiological and phenotypic variations of this tree species in response to environmental conditions associated with climate change (e.g. light excess, drought).

Our overall objective was to test the response of contrasting tara provenances to water and light stress, evaluated in a common garden greenhouse experiment. We analysed the consequences of the two potential photoprotective strategies of tara by forcing some leaflets to remain open (i.e., structural photoprotection) and by evaluating the effects of the variation of photosynthetic pigments on the maximum photochemical efficiency of plants (i.e., chemical photoprotection). Due to the low genetic variability of tara along its distribution range and its proposed human-favoured dispersal (Balaguer et al. 2011), we initially hypothesized that seedlings from different provenances grown under similar conditions would show similar responses to drought stress. Alternatively, given the ability of tara to thrive in different environments (Canziani Amico 2002, de la Cruz Lapa 2004), possible variations on tara seedling response attributable to local adaptation to the original ecological conditions could be also expected. If the latter scenario is true, we expected that the endurance of seedlings to simulated drought would depend on the availability of water in their origin locations, based on the prediction that tara photoprotective strategy (i.e., leaflet closure and/or pigment variation) would avoid photodamage under water shortage, but with a different carbon assimilation cost depending on the provenance. Specifically, under this alternative hypothesis of local adaptation, we predicted that tara provenances from areas with higher water availability would be more sensitive to combined induced drought and light stress, and accordingly they (i) would completely

close their leaflets and stomata under water stress to preserve their photosynthetic apparatus but with a high assimilation cost; and/or (ii) would imbalance the ratios of photosynthetic pigments, promoting the investment in energy dissipation pigments to avoid photodamage.

Tara-dominated forests are quite abundant in certain areas of its distribution range, particularly in Peru (Larrea 2011). However, in the last century, intensive land use and severe deforestation have threatened many tara-dominated forests and the local human-populations that depend on them (Larrea 2011, Balaguer et al. 2011). Planning successful restoration strategies of these tara-dominated ecosystems is a major goal (Balaguer et al. 2011, Cordero et al. 2017), which requires gaining knowledge about the eco-physiological response of these forests and the ability of the dominant tree, tara, to cope with local environmental constraints.

Materials and Methods

Plant material

Caesalpinia spinosa (Fabaceae) is a long-living non-nodulating drought deciduous tree of 2-5 m height, with hypostomatous bipinnate leaves, terminal yellow inflorescences and long pod-flat fruits with 4-7 seeds (Lewis et al. 2005, Cordero, Ruiz-Díez, et al. 2016). Seeds from different individuals were collected along a latitudinal and water availability gradient in Ecuador and Peru (Fig. 1), in four locations: Andurco, Polán, Jerusalem and Atiquipa. Andurco and Atiquipa locations, respectively, receive the highest and lowest annual water inputs, while Polán and Jerusalem get intermediate amounts of annual water but with different precipitation seasonality (i.e., Jerusalem receives a similar low amount of water throughout the year, while Polán has wetter months, showing a higher value of the precipitation of the wettest quarter of the year).

Seeds were scarified (96 % H_2SO_4 , 45 min), soaked in distilled water for 48 h, and sown in 250 ml containers with 3:1 (v:v) peat:vermiculite. Seedlings were grown for two months in a growth chamber ($250 \mu\text{mol m}^{-2} \text{s}^{-1}$, 24 °C and 60 % humidity) and then transferred to a greenhouse with semi-controlled conditions with 23/20 °C day/night temperature, 45/50 % relative air humidity, and $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR radiation, monitored with four light and temperature sensors placed at plant height, and a sensor for general greenhouse air humidity (HOBO, Onset Computers, Pocasset, MA, USA). After fifteen days of acclimatisation in the greenhouse, 2.5 month old seedlings were transplanted to individual 3.5 L pots (11 x 35 cm) with peat:vermiculite (3:1, v:v), supplemented with 2 g l^{-1} fertilizer (Osmocote NPK 11:11:18 + 2 MgO_2 + microelements), and regularly watered. Plants were grown for approximately one year and rotated weekly to minimize position effects. Mean seedling height and diameter at

the start of the treatments (16 months old seedlings) were of 43.6 ± 7.3 cm and 9.0 ± 0.9 mm, respectively.

Experimental design

A factorial design was used to evaluate the main effects and interactions of 1) seed provenance, 2) leaflet immobilization (i.e., light stress), and 3) drought, on the physiology and growth of *C. spinosa* along a drought and rewetting pulse. The factor immobilization (mobile and immobile leaflets) was tested within the same seedling. Each treatment had eight seedlings ($n = 8$; total of 96 plants). The factor provenance consisted of four treatments: 1) Andurco (AND), 2) Polán (POL), 3) Jerusalem (JER) and 4) Atiquipa (ATQ). The factor leaflet immobilization had two treatments: 1) mobile and 2) immobilized leaflets. In each seedling, two complete pinnae ($\sim 5\%$ of plant's leaflets) were immobilized in fully expanded mature leaves, with transparent Plexiglas pieces installed perpendicularly to the rachis (Fig. 2), in order to avoid the structural photoprotection by leaflet closure and induce light stress. Physiological measurements were carried out on the mobile and its symmetric immobilized pinna. To check for a possible effect of immobilization on the water status of plants, four seedlings per treatment were kept not immobilized throughout the experiment as control.

Drought consisted of three treatments: 1) well-watered control (C), 2) moderate drought (D1), and 3) severe drought (D2). Each pot was daily weighted and the 75 % or the 50 % of the water loss was replaced in D1 and D2 respectively, while control pots received the 100 % of the water lost. Drought lasted 22 days and afterwards all pots were rehydrated during 40 days to initial water content (see Fig. S1 available as Supplementary Data at *Tree Physiology* Online), which is similar to the soil moisture found in Atiquipa forest at the end of the wet season (Balaguer et al. 2011). Substrate water content was monitored with a time domain reflectometer (TDR) (HH2 Moisture Meter with WET-2 sensor,

Delta-T Devices Ltd., UK). The 22 days of drought were enough to achieve very low water potentials and to observe clear effects on the seedling physiology, but not so extreme for them to shed the leaves.

Water status, morphology and seedling growth

Midday water potential (Ψ_{mid}) (12:30-14:00 solar time) was measured with a Schölander chamber (SKPM 1400, Skye Instruments Ltd., UK) in a pinna per plant. The last two leaflets of a pinna with ~4 cm of rachis were selected, as previously described for legumes with composite leaves (Gullo *et al.*, 2003). No interference of immobilization on seedling water potential was found ($p_{\text{immobilization}}=0.676$; $p_{\text{immobilization} \times \text{drought}}=0.737$).

Seedling height and diameter were measured every two weeks for two months since the beginning of the drought period. Relative growth (RG) was calculated as $RG = (X_f - X_o) / X_o$; being X_f the final height or diameter and X_o the initial one.

Leaflet movement and photosynthetic response

To monitor structural photoprotection, leaflet angle was measured with a protractor at midday (11:30-15:00 solar time), from 0° (completely parallel to the horizon; open leaflets) to 90° (vertical position, completely closed leaflets).

To monitor the physiological status of seedlings, net carbon assimilation (A), stomatal conductance (g_s) and transpiration (E) of four seedlings per treatment were measured with a portable photosynthesis system (Li-Cor 6400, LiCor Inc., USA), at midmorning (9:00-11:30 solar time). Mature attached leaflets were kept inside the cuvette under a constant radiation of $750 \mu\text{mol m}^{-2} \text{s}^{-1}$ (previously tested as a non-saturating light that allowed an optimal photosynthetic rate), ~20 °C temperature, and CO_2 flux of $400 \mu\text{mol s}^{-1}$, for 2 min. Three replicated records were logged per pinna and their mean was used for data analyses. Leaflet area inside the cuvette was measured with the software ImageJ v.1.47 and used to recalculate the photosynthetic parameters. Water use efficiency (WUE), i.e.

the amount of water used in the production of biomass instantly, was estimated as the assimilation per transpiration rate (A/E). In order to measure these variables in pinnae with partially closed leaflets, they were forced open to enter the cuvette. This did not damage the leaflets and they recovered their leaflet angle as soon as they were outside the cuvette. However, if the leaflets were completely or almost completely closed, they were inserted as they were, i.e., closed, to avoid damaging them.

The photosynthetic capacity of seedlings was evaluated by measuring the fluorescence of chlorophyll *a* with a Portable Chlorophyll Fluorometer (PAM 2000, Walz, Germany), and maximum quantum yield of photosystem II (F_v/F_m) was determined at predawn in dark adapted leaflets according to Genty *et al.* (1989). Three replicated measurements were made per pinna, and their mean was used as a replicate for analyses. Completely closed leaflets were carefully open to take fluorescence measurements, which did not change leaflet angle afterwards.

To evaluate seedling chemical photoprotection, photosynthetic pigments were measured in four seedlings per treatment previous to the end of the drought period (day 17). One leaflet per plant was collected at midday, immediately frozen in liquid nitrogen and preserved at -80 °C until analysis. After extraction with acetone, chlorophylls (*a* and *b*) and carotenoids, including β -carotene (β -Car), neoxanthin (Neo), lutein (Lut) and the different pigments of the xanthophyll cycle (violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z)), were separated by high-performance liquid chromatography (HPLC, Waters Corp., Milford, MA, USA) as described in Masuda *et al.* (2002). Total chlorophyll content (Chl *a+b*) was expressed by leaflet area. Content of individual carotenoids (β -Car) or the sum of different carotenoids (V+A+Z and total carotenoids) was expressed with respect to the total chlorophyll content (Chl *a+b*). The ratios Chl*a*/Chl*b* and Neo+Lut/ β -Car that indicate the different relative investment on light harvesting

complexes vs reaction centres, and the ratio $A+Z/V+A+Z$ indicative of de-epoxidation of pigments in the xanthophyll cycle were also calculated.

Statistical analyses

The complete dataset was checked for normality, homoscedasticity and outliers. Variables measured repeatedly on the same seedling along time (i.e., Ψ_{mid} , leaflet angle, A , g_s , WUE, F_v/F_m , height, diameter and RG) were analysed by lineal mixed models (LMM) for repeated measures, considering the provenance, leaflet immobilization, drought and time as fixed factors, and the seedling as random factor. Variables measured only once (i.e., pigment contents) were analysed by LMM, considering the same factors except time. Non-significant interactions were eliminated from models, following the hierarchical principle. In all cases, post-hoc comparisons of means among treatments were done by Bonferroni test ($p < 0.05$). Relative change ratio was calculated as the value of the variable under severe drought divided by the value of the mobile well-watered control. Differences of this ratio between immobilization treatments were evaluated by LMM, considering leaflet immobilization as fixed factor, and the seedling as random factor.

The relationship between structural or chemical photoprotection responses and the physiological status of seedlings facing drought was explored by fitting linear or quadratic regressions between leaflet angle and water potential, transpiration, net assimilation and F_v/F_m , and between F_v/F_m and ratios of the different photosynthetic pigments. When the regression analyses included all provenances, a test to confirm parallelism among slopes was performed ($p < 0.05$). Finally, correlation analyses between the response of tara to drought and the climatic variables of the sites of provenance were performed with the non-parametric Spearman correlation test ($p < 0.05$). All analyses were carried out with the software SPSS v.24 (IBM Corporation Software Group, Somers, NY, US).

Results

Photoprotection outcomes under drought: effects of seedling provenance

Drought and leaflet immobilization triggered multiple responses in tara that were dependent on seedling provenance for some traits (Table S1 available as Supplementary Data at *Tree Physiology* Online), such as leaflet water potential, carbon assimilation, stomatal conductance, F_v/F_m , growth rate and pigment contents.

Leaflet water potential (Ψ_{mid}) significantly declined with drought (i.e., 1.5 and 2.3 times lower values than control in moderate and severe drought, respectively), and this effect was dependent on the provenance ($p_{D \times P} < 0.001$) (Fig. 3a; Table S1). Together with the significant negative relations between Ψ_{mid} and leaflet angle ($R^2 = 0.551$; $p < 0.001$), and leaflet angle and transpiration ($R^2 = 0.441$; $p < 0.001$), this likely indicated that leaflet closure was activated to reduce evapotranspiration.

In parallel, drought activated leaflet closure similarly in all provenances (Fig. 3b), which additionally slowed down or even stopped (severe drought) their relative growth (Fig. S2; Table S1; available as Supplementary Data at *Tree Physiology* Online). Coincident with Ψ_{mid} decline, drought and to a lesser extent leaflet immobilization, prompted a quick stomatal closure of seedlings and subsequent drop of net carbon assimilation (reductions of 40 % and 88 % respectively in moderate and severe drought compared with well watered control seedlings, and of 10 % in immobilized compared with mobile leaflets) (Fig. 4a, b; Table S1). Drought negatively affected the WUE of seedlings, i.e., although transpiration decreased, carbon assimilation decreased to a greater extent (Fig. 4c). On average, the provenance from the driest location (ATQ) was the most efficient in the use of water ($p_{provenance} = 0.014$) (Fig. 4c). As drought stress progressed, seedling maximum quantum yield (F_v/F_m) decreased (9 % in severe drought respect to well watered control, no effect of moderate drought), and a significant drought $\times$ immobilization interaction

(i.e., double drought and light stress) was observed (Fig. 4d; Table S1). In fact, a reduction of 15 % in F_v/F_m was observed in immobilized leaflets under severe drought compared to well-watered mobile control leaflets, while the decrease was of 5 % for mobile leaflets, which was indicative of structural photoprotection. The negative effect of severe drought on F_v/F_m on mobile and immobilized leaflets, and on its recovery after drought, was particularly pronounced in AND (wettest) compared with ATQ (driest) provenance (Fig. 4d).

After rewetting, Ψ_{mid} rose, leaflets became nearly horizontal again, and growth restarted, but Ψ_{mid} of POL and JER provenances took longer time to rise (POL did not fully recover) (Fig. 3), and seedlings that had been under severe drought did not completely recuperate the relative growth in diameter (Fig. S2). In the rehydration phase, previous leaflet closure during drought proved to be an effective mechanism for recovering net carbon assimilation in JER and ATQ (i.e., higher A rates in mobile than immobilized leaflets during rehydration) (Fig. 4a).

Drought and leaflet immobilization activated a higher relative investment of seedlings in thermal energy dissipation to protect the photosynthetic apparatus (chemical photoprotection), i.e., Neo+Lut/ β -Car and A+Z/V+A+Z (de-epoxidation state) ratios increased 6/17 % and 47/69 % more than control in moderate/severe drought, respectively, and 2 % and 22 % more in immobilized than mobile leaflets, respectively) (Fig. 5a, b; Table S1). In the case of A+Z/V+A+Z ratio, a drought x immobilization interaction was also observed ($p_{D \times I} < 0.001$), i.e., under severe drought this ratio increased 38 % in mobile leaflets, while it did 118 % in immobilized ones, compared to the mobile well-watered controls. The strength of these effects depended on the provenance; Neo+Lut/ β -Car significantly increased due to drought in AND or to drought and leaflet immobilization in POL (Fig. 5a), and A+Z/V+A+Z increased with severe

drought in both provenances (AND, POL) mainly in immobilized leaflets (double drought and light stress interaction) (Fig. 5b). Total chlorophyll content (Chl *a+b*) remained constant across treatments (Fig. 5c), but Chl*a*/Chl*b* ratio was significantly higher in AND (wettest) than in ATQ (driest) provenance (Fig. 5d), and the opposite happened for β -Car/Chl content (Fig. S3 available as Supplementary Data at *Tree Physiology* Online). Total VAZ pool (V+A+Z/Chl) was also highest in AND provenance (Fig. S3b). No significant effects were detected in the amount of total carotenoids/Chl (Fig. S3c).

Relative change ratio evidenced structural and chemical photoprotection together with a decrease in the physiological activity and growth of seedlings with respect to well-watered control ones, summarizing the main effects of severe drought (Fig. 6). Clear significant benefits of leaflet closure on seedlings were evidenced in the case of depoxidation state (A+Z/V+A+Z) and F_v/F_m (i.e., less photodamage in mobile leaflets), and marginally for Neo+Lut/ β -Car ratio. Net carbon assimilation per unit of leaf area exposed to the sun (A) remained the same in mobile and immobilized leaflets (Fig. 6), suggesting that the main cost of leaflet closure is the reduction of the carbon assimilation opportunities, as mobile leaflets highly reduced the leaf area exposed.

Relationships of seedling traits and climatic variables

The balance between physiological activity and photoprotection depended on seedling provenance (Fig. 7). When considering only mobile leaflets at maximum drought stress (day 22), a clear negative relationship between Ψ_{mid} and leaflet angle was revealed in all provenances, although to a lesser extent in ATQ seedlings (driest area) (Fig. 7a). The higher leaflet closure at maximum drought stress translated into a significant negative relationship of structural photoprotection with transpiration and net carbon assimilation rates for AND and POL (Fig. 7b,c), pointing to higher stomatal closure and assimilation

costs under drought for the provenances with wetter conditions than for the drier ones. In all cases, as leaflets closed, F_v/F_m diminished, particularly in AND seedlings (wettest habitat) (Fig. 7d).

Focusing on the relationship between structural and chemical photoprotection, a negative relationship between F_v/F_m with Neo+Lut/ β -Car was only observed for seedlings from the wettest areas (AND, POL), but not for the driest ones (JER, ATQ) (Fig. 7e). A weak negative regression between F_v/F_m and A+Z/V+A+Z was observed when all data were jointly analysed ($R^2 = 0.041$; $p = 0.048$), but when analysed by provenance, no clear effects were detected (slight trend in AND) (Fig. 7f).

Significant correlations between some drought-responsive tara traits and the climatic conditions of the respective site of seed provenance were found (Fig. 8). A relation between precipitation seasonality and the Ψ_{mid} (positive) (Fig. 8a) and g_s (negative) (Fig. 8b) of seedlings under drought was observed, which was not observed in well-watered seedlings. The number of dry months was also significantly related with the degree of leaflet closure (negative) only in drought seedlings (Fig. 8c), and with the WUE (positive). Finally, there was a significant correlation between the annual water input and F_v/F_m (negative in seedlings under drought) (Fig. 8e) and the reduction of RG in height between well-watered seedlings and drought ones (positive) (i.e., provenances from wetter areas grew more without water limitation, but under drought, their relative height growth was drastically reduced) (Fig. 8f). Most of these correlations were only apparent under drought conditions (induced variation), except WUE, which showed the same trend under well-watered and drought conditions (constitutive variation).

Discussion

Our findings show that tara quickly responded to drought by increasing structural and chemical photoprotection (leaflet angle, pigments related to thermal dissipation), while reducing stomatal conductance and evapotranspiration. Combined drought and light stress negatively affected the photochemical efficiency of seedlings, which further increased the chemical photoprotection response. Main benefits of leaflet closure were the reduction of the evaporative demand and photodamage, while the main cost was the reduction of the carbon assimilation opportunities. But, contrary to that initially expected, this response was dependent on the seed origin, supporting our alternative hypothesis of local adaptation.

Mechanisms to avoid photoinhibition and photodamage under drought and light stress

As water shortage increased, tara closed the leaflets as a mechanism to diminish the exposed leaf area (Fig. 3), subsequently decreasing the evaporative demand and the excess of photonic energy in photosystems. This movement of leaves to reduce light interception and photodamage has previously been observed and experimentally manipulated in other woody species such as *Cistus albidus* L. (Oliveira and Peñuelas 2002) or the legume tree *Robinia pseudoacacia* L. (Arena et al. 2008). Tara displayed a similar photoprotection response in a previous field study (Balaguer et al. 2011), where reduced air humidity and light stress triggered leaflet closure. Under the semi-controlled conditions of the current experiment, tara regulated the orientation of its leaflets closure depending on the stress level (completely closed under severe drought but not under moderate drought) and this movement was reversible, returning to its original position upon rewetting. Leaves less exposed have lower temperatures and therefore less transpiration and water loss (Kao and Forseth 1992). Additionally, plants can decrease water loss by stomatal closure, but in exchange, this limits CO₂ capture and the

availability of C in the carboxylation sites of the chloroplasts, resulting in an excess of energy that may end in photodamage (Lawlor and Tezara 2009, Karabourniotis et al. 2014). In fact, leaf angle is regulated by PSII (Huang et al. 2014), and when F_v/F_m decreases, leaf angle becomes more perpendicular to sunrays to avoid further photoinhibition, as we observed for tara (Fig. 7). In our study, leaflet closure was further evidenced as an effective strategy allowing tara to resist combined stresses (light and drought). A clear decrease in F_v/F_m under severe drought was observed (Fig. 4), especially in immobilized leaflets, which slowly recovered afterwards compared with mobile leaflets, suggesting a certain degree of photodamage (Osmond 1994, Llorens et al. 2003).

Tara also activated chemical photoprotection mechanisms to alleviate negative drought and light stress effects (Fig. 5). Variations in Neo+Lut/ β -Car ratio can be interpreted in terms of differential relative investment in light harvesting complexes (LHC) and reaction centres (RC), as Neo and Lut are carotenoids mainly present in LHC and β -carotene is basically found in RC (Esteban et al. 2015). This ratio has previously been used to estimate differential relative investment in LHC and RC under other environmental stressors such as nitrogen deposition (Arróniz-Crespo et al. 2008, Ochoa-Hueso et al. 2014). In tara, this ratio increased under severe drought, especially in immobilized leaflets, suggesting a greater relative investment in LHC and thus in thermal energy dissipation, while it was lowest in well-watered seedlings, probably implying a greater relative investment in RC, and therefore photosynthesis prioritization. The constant levels of Chl $a+b$ detected for all the factors analysed in our greenhouse experiment could be explained by the similar nitrogen contents observed in leaves across treatments with equal fertilization (see Fig. S4 available as Supplementary Data at *Tree Physiology* Online), since Chl $a+b$ is highly dependent on this variable (Niinemets 1997, Durand and Goldstein 2001). On the other hand, a decrease on the ratio Chl a /Chl b has frequently

been used to inform about plant preferential relative investment on LHC over RC under drought (García-Plazaola and Becerril 2000, Baquedano and Castillo 2006, Junker-Frohn et al. 2019). However, in this experiment, we did not find a significant effect of drought in this ratio (Fig. 5). Finally, the de-epoxidation state of xanthophylls ($A+Z/V+A+Z$) increased under drought, particularly in immobilized leaflets. The xanthophyll cycle plays a key role in leaf defence against the excess of light (Demmig-Adams and Adams 2006). Moreover, not only light but also temperature can induce changes in this ratio and it has been demonstrated that $A+Z/V+A+Z$ can rise upon heating up of photosynthetic tissues even in darkness (Fernández-Marín et al. 2011, Buchner et al. 2015). In this experiment, immobilized leaflets probably suffered an increase on temperature at midday which can partially explain the observed increase of $A+Z/V+A+Z$. These variations in pigment ratios (Neo+Lut/ β -Car and $A+Z/V+A+Z$) concurred with a maximum quantum yield decrease (Fig. 7), highlighting a greater need of tara to invest in thermal energy dissipation in immobilized leaflets under severe drought, i.e., an increase in chemical photoprotection when structural photoprotection (leaflet immobilization) was no longer available. The significant relation found between F_v/F_m and $A+Z/V+A+Z$ could indicate that the decrease in the photochemical efficiency was partially due to an increase in the energy dissipated through the xanthophylls (Martínez-Ferri et al. 2000), although this relationship was weak, clearly suggesting a stronger photodamage effect induced by drought (Fig. 4). However, as F_v/F_m and $A+Z/V+A+Z$ were measured at different times of the day (predawn and midday, respectively), this correlation has to be cautiously observed.

Leaflet closure was thus an efficient photoprotective strategy of tara that generated benefits under drought stress, although at the same time, the reduction of light interception entailed costs in terms of fewer carbon assimilation opportunities. Leaf reorientation to

decrease intercepted light is a balance between photoprotection and carbon assimilation (Valladares and Pugnaire 1999, Pastenes et al. 2005). Additionally, continued severe drought stress led to a sharp decrease of carbon assimilation rate and photochemical efficiency, and consequently to growth arrest.

Photoprotection outcomes under drought relay on seedling provenance

Under semi-controlled conditions, tara displayed a dehydration tolerant strategy i.e. capable of decreasing water potential and stomatal conductance simultaneously to very negative water potential levels (Figs. 3 and 4) (definition as in Voltaire 2018), contrary to that previously observed in the field in tara seedlings, where we reported early stomatal closure and low water potential decrease i.e. dehydration avoidance (Balaguer et al. 2011). Gas exchange under drought and plant's dehydration strategies have been extensively studied, with some species showing a dehydration tolerance strategy while others have a dehydration avoiding one (e.g., Martínez-Ferri et al. 2000, Quero et al. 2011, Katuwal et al. 2020). However, the combined dehydration avoidance/tolerance strategy has been increasingly recognised in the literature (Berger et al. 2016, Voltaire 2018), with a respective prevalence of one or another depending on the plant status (e.g., nutritional conditions, growth stage), and other external factors (e.g., stress intensity or frequency). For example, Berger and Ludwig (2014) demonstrated a range of dehydration avoidance/tolerance strategies in *Lupinus luteus* L. depending on environmental conditions. It is possible that seedlings growing in a controlled environment have a better and consistent nutritional status than under more erratic field conditions, as pointed out when we compared nitrogen leaf contents of the seedlings grown in greenhouse or in the field (see Fig. S4 available as Supplementary Data at *Tree Physiology* Online). Our results could be equally attributable to the plasticity of this species (Reynolds et al. 2018). In fact, we observed a range of dehydration tolerances among tara provenances, with

seedlings from the most xeric origins displaying the greatest abilities to withstand stress (ATQ seedlings showed lower photoinhibition, highest overall water use efficiency and kept their leaflets more open under drought, with lower assimilation costs).

Despite the low genetic variability of this species across its distribution range, evaluated in adult trees (Balaguer et al. 2011), and according to our alternative hypothesis, our results demonstrated clear differences in seedling performance depending on their provenance, therefore pointing to local adaptation. This finding could be related to higher genetic variability in the offspring compared to the adult trees, or to non-genetically based maternal effects (Galloway 2005). Although the genetic diversity of the seedlings used in this experiment was not evaluated, it is possible that the isolation of some of the tara populations have led to certain genetic differences (Rieseberg and Willis 2007), which would deserve further analysis. On the other hand, trees with the same genotype can produce offspring with different phenotypes displaying an enhanced fitness for the particular environment where the mother was growing (Galloway 2005, Marshall and Uller 2007). This maternal effect can be transmitted to the offspring through seed characteristics, transmission of signals from the mother to the maturing zygote, and inheritance of maternal epigenetic states (Roach and Wulff 1987, Penfield and MacGregor 2017). This has been previously demonstrated in other tree species, such as *Quercus ilex*, where common garden experiments showed differences among provenances in the photoprotective mechanisms of seedlings due to local adaptation (Camarero et al. 2012).

As predicted under the hypothesis of local adaptation, tara responses to avoid photodamage under water shortage had different physiological consequences depending on the seed origin. We expected that tara provenances from areas with higher water availability would completely close their leaflets and stomata under water stress to

preserve their photosynthetic apparatus but with a high assimilation cost. This prediction was confirmed and the two provenances with wettest conditions (POL, and especially AND) tightly closed their leaflets under severe drought (Fig. 3), with a higher assimilation cost (stronger negative correlation between the assimilation rate and the leaflet angle, Fig. 7). Moreover, leaflet closure proved to be an effective mechanism for recovering net carbon assimilation in the provenances with driest habitats (JER, ATQ) (i.e., higher assimilation rates in mobile than immobilized leaflets after drought, Fig. 4) but not in POL and AND, emphasizing the higher assimilation cost for the wettest habitats. Additionally, the high precipitation seasonality of AND may have favoured a water saving strategy of seedlings, tightly closing stomata early and with a fast recovery during rewetting, as previously observed in *Eucalyptus microtheca* F.Muell. provenances from highly seasonal areas (Li et al. 2000). Seedlings from the provenance with driest conditions, ATQ, showed in general higher ability to cope with drought. They kept their leaflets more open under severe drought, with lower assimilation costs, showed low photoinhibition and also had the highest overall water use efficiency (Figs. 3 and 4). These results are in accordance to other studies, where tree provenances from more xeric locations showed a higher physiological ability to cope with drought (Nguyen-Queyrens and Bouchet-Lannat 2003, López et al. 2009, Corcuera et al. 2012, Ying et al. 2015, Matías et al. 2019).

We also predicted that combined drought and light stress would imbalance the ratios between different photosynthetic pigments preferentially in provenances from wetter areas, promoting the investment in energy dissipation pigments to avoid photodamage. This hypothesis was also confirmed, as POL and particularly AND (wettest) showed the biggest changes in the pigment balances related to chemical photoprotection (Neo+Lut/ β -Car and A+Z/V+A+Z ratios, Fig. 5). Changes in photosynthetic pigment ratios under

water deficiency depending on seed provenance have been previously reported for other tree species, such as Douglas-fir (Junker et al. 2017) or *Quercus* spp. (Ramírez-Valiente et al. 2015). Additionally, provenances from wetter habitats showed the highest relative growth under not water-limiting conditions and the highest reduction of this rate during water stress (Fig. 8), indicating their high morphological sensibility to drought, as observed in provenances with high precipitations of other tree species such as *Pseudotsuga menziesii* (Mirb.) (Eilmann et al. 2013) or *Arbutus unedo* L. (Vasques et al. 2013). This fact also highlights the trade-off between water-acquisition (i.e., growth) and water-conservation (i.e., drought survival) (Volaire 2018).

The extraordinary capacity of local adaptation to dry conditions of tara could be at the origin of the ecological success of this species (Gagnon et al. 2019), and might favour its persistence under the predicted drier and increasingly more unforeseeable climate (Alberto et al. 2013, Valladares et al. 2014). This can be crucial for the success of restoration actions aiming to keep this keystone species in areas such as the fog forests, and to give economic support to local communities in wide areas of South America (Larrea 2011, Cordero, Jiménez, et al. 2016, Sotomayor and Drezner 2019).

Conclusions

This study evidences the different mechanisms of tara to cope with water and light stress, under semi-controlled conditions. Leaflet closure in tara has a double benefit under water stress, since it diminishes the evaporative demand and avoids photodamage (structural photoprotection), although it also entails costs by reducing the carbon assimilation opportunities. Supporting our alternative hypothesis of a local adaptation, these responses depended on the seed origin, with seedlings from provenances with the most xeric conditions (particularly Atiquipa) displaying the highest capacity to withstand drought. Thus, the observed intraspecific variation is likely the result of local adaptation, which

represents that different photoprotective strategies were revealed useful in different environments. This plasticity on tara response to environmental stress allows it to thrive in localities with contrasting water availability. The results from this study allow us to better understand the ecology and performance of tara under different climatic conditions and future global change scenarios. They can be also greatly helpful to plan efficient ecological restoration or reforestation projects with tara, a keystone legume tree in the dry forests of South America.

Data and Materials availability

All data from this paper will be available to other academic researchers upon reasonable request.

Supplementary data

Additional supporting information may be found in the online version of this article:

Fig. S1 Evolution of soil water content along the experimental time.

Fig. S2 Effects of drought on seedling growth rate.

Fig. S3 Effects of drought and leaflet immobilization on the photosynthetic pigments of tara seedlings.

Fig. S4 Leaf nitrogen content of tara under field and greenhouse conditions.

Table S1 Statistical summary of the main and interactive effects of the factors provenance, drought, leaflet immobilization and time on the physiological and morphological variables of tara seedlings.

Conflict of interest

None declared.

Funding

This work was supported by the SEGARNIEBLA project founded by BBVA Foundation, and grants from Comunidad de Madrid (S2009/AMB-1511) and MINECO (AGL2013-40758-R). IC was supported by the Autonomic Council of Education, Comunidad de Madrid (Spain) (CPI/0243/2008), a Ramon Areces Foundation research grant, and a BBSRC Discovery Fellowship (BB/S010661/1).

Acknowledgments

We gratefully acknowledge Blanca Santamaría-Pérez, Manuela López Valero and Diego Abad Martín for their assistance in the greenhouse. We also acknowledge Luis Villegas Paredes, Percy Jiménez, Luis Albán Contreras and José Manuel Serrano for their assistance collecting tara seeds. We finally thank Arthur Broadbent and David Johnson for their valuable comments on the manuscript. We thank the anonymous reviewers for improving a previous version of the manuscript.

The experiment was carried out in the greenhouse facilities of the Institute of Agricultural Sciences, Spanish National Research Council (ICA-CSIC), Madrid, Spain.

Authors' contributions

IC, MDJ, JAD, LB, JJP and AR contributed to the design of the experiments. IC, MDJ and JAD collected the seeds in Peru. IC performed all experiments and measurements, eventually assisted by JAD and MDJ. IC, MDJ, JAD, LB, JJP and AR analysed and interpreted data. IC, MDJ, JAD, JJP and AR contributed to the writing of the manuscript.

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Figure captions

Fig. 1 Latitudinal gradient and climatic diagrams of the different provenances of tara (*Caesalpinia spinosa*). Climatic and bioclimatic data were obtained from WorldClim database (1950-2000) (Hijmans 2014). Solid bars indicate mean precipitation and the black line represents mean temperature. In Atiquipa, fog collection data are included (slashed bars), monthly means and standard error of five consecutive years (2003-2007) (Cordero, Jiménez, et al. 2016). Bioclimatic variables: annual water input (WI), precipitation seasonality (S) and precipitation of the wettest quarter (PWQ). m asl: meters above sea level. ^{1, 2} Holdridge life zone: ¹Ministry of Agriculture and Livestock (1978), ²INRENA (1995).

Fig. 2 (a) Mature tara pinnae (circles) and leaflets (arrows). (b) Immobilized leaflets (white arrow) in a mature tara pinna from a drought exposed seedling.

Fig. 3 Effects of drought on (a) midday water potential (Ψ_{mid}) and (b) leaflet's angle at midday of different provenances of *Caesalpinia spinosa* (AND = Andurco, POL = Polán, JER = Jerusalem, ATQ = Atiquipa). In each graphic, the dashed vertical line indicates the end of drought and the start of rewetting (day 22). Grey diamonds = control, dark grey squares = moderate drought D1; black triangles = severe drought D2. In (b) filled lines = mobile leaflets; dashed lines = immobilized leaflets. Values = mean $\pm$ standard error; n=8. Data were analysed by linear mixed models with the seedling as random factor. At each day, asterisks indicate significant drought effect, and dots and crosses indicate significant effects of leaflet immobilization and its interaction with drought, respectively (p<0.05). Values in the top of each graph indicate overall marginal means $\pm$ standard error of the factor provenance in the models (Table S1) for that variable, and in each case, different

capital letters denote significant differences across tara provenances according to Bonferroni test ($p < 0.05$).

Fig. 4 Physiological response of *Caesalpinia spinosa* to drought (light grey diamonds = control, dark grey squares = moderate drought D1; black triangles = severe drought D2), leaflet immobilization (filled lines = mobile; dashed lines = immobilized) and time, separately analysed by linear mixed models for different seed provenances (AND = Andurco, POL = Polán, JER = Jerusalem, ATQ = Atiquipa). Dashed vertical line indicates the end of the drought and the start of the rewetting (day 22). (a) Net carbon assimilation (A), (b) stomatal conductance (g_s), (c) water use efficiency (WUE), and (d) maximum quantum yield of photosystem II (F_v/F_m). Values = mean $\pm$ standard error; $n=4$ ($n=8$ for F_v/F_m). At each day, asterisks, dots and crosses indicate significant effects of the factors drought, immobilization and their interaction, respectively ($p < 0.05$). Values in the top of each graph are overall marginal means $\pm$ standard error, when including the factor provenance in the models (Table S1), and different capital letters denote significant differences among treatments. In all cases, differences between treatments were separated by the Bonferroni test ($p < 0.05$).

Fig. 5 Chemical photoprotection of *Caesalpinia spinosa* five days before the end of the drought period, testing the factors drought (D) (white = control, grey = moderate drought D1; black = severe drought D2) and leaflet immobilization (I) (mobile or immobilized) for each seed provenance (AND = Andurco, POL = Polán, JER = Jerusalem, ATQ = Atiquipa). Data were analysed by linear mixed models with the seedling as random factor. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (a) ratio (neoxanthin + lutein) / β -carotene; (b) depoxidation state (A = antheraxanthin, Z = zeaxanthin; V = violaxanthin); (c) Chlorophyll

a and *b* content ($\mu\text{mol m}^{-2}$); (d) Chlorophyll *a/b* ratio. Values = mean $\pm$ standard error; $n=4$. Within each immobilization treatment, different lower-case letters denote significant differences among drought treatments. For a given drought treatment, asterisks indicate significant differences due to leaflet immobilization. Values in the top of each graph are overall marginal means $\pm$ standard error, when including the factor provenance in the models (Table S1), and similar capital letters denote no significant differences among treatments. In all cases, differences between treatments were separated by the Bonferroni test ($p<0.05$).

Fig 6 Benefits of leaflet closure under severe drought at the end of the drought period, expressed as relative change ratio of severe drought (D2) in mobile and immobilized leaflets compared to well-watered control mobile leaflets. Dashed line indicates no difference with the control. Values above and below that line correspond to respective increases or decreases of that variable compared to the control. Significance of the differences between mobilization/immobilized treatments for the same variables are shown: * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Neo+Lut/ β -Car = ratio (neoxanthin + lutein) / β -carotene; A+Z/V+A+Z = de-epoxidation state (A = antheraxanthin, Z = zeaxanthin; V= violaxanthin); A = Net carbon assimilation; *g* = stomatal conductance; WUE = water use efficiency; F_v/F_m = maximum quantum yield of photosystem II.. Values = mean $\pm$ standard error; $n=16$ ($n=32$ for F_v/F_m).

Fig. 7 Relationships between physiological variables of *Caesalpinia spinosa* seedlings at the end of the drought (day 22). Different colours and shapes indicate different provenances: Andurco AND (filled black circle, black line), Polán POL (filled grey square, grey line), Jerusalem JER (open grey triangle, dashed grey line) and Atiquipa

ATQ (open black diamond, dashed black line). Mobile leaflet data were used for regressions including the leaflet angle (a, b, c, d), and all data (mobile and immobilized leaflets) for the rest of analyses. For each provenance, when significant, R^2 and significance value (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) of the linear (a, b, c, e) or quadratic regressions (d, f) are shown. Non-significant regression lines/curves are not drawn. Ψ_{mid} : midday water potential; E: transpiration rate, A: net carbon assimilation, F_v/F_m : maximum quantum yield of PSII, WUE: water use efficiency, Neo+Lut/ β -Car: ratio (neoxanthin + lutein) / β -carotene, A+Z/V+A+Z: de-epoxidation state.

Fig. 8 Correlations between physiological-morphological traits of *Caesalpinia spinosa* seedlings and the climatic variables of the sites of provenance: Andurco AND (filled circle), Polán POL (filled square), Jerusalem JER (open triangle) and Atiquipa ATQ (open diamond). Blue = well-watered control, red = severe drought D2. Data from days 17 and 22. Data are mean $\pm$ standard error. Ψ_{mid} : midday water potential; g_s : stomatal conductance; WUE: water use efficiency; F_v/F_m : Maximum quantum yield of PSII. Reduction of RG h: difference of the relative growth in height in the treatment of severe drought compared with the control. Bioclimatic variables (annual water input and precipitation seasonality) were extracted from the WorldClim database (Hijmans 2014) and the number of dry months was calculated following the Gaussen index, which defines a dry month depending on the mean precipitation (P) and temperature (T) when $P < 2T$. In each graph, the value of the Spearman correlation coefficient and its significance (^{ns} = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) are shown.

Figure 1

Figure 2

(a)

(b)

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8