



Bio-fertilisation with native plant growth promoting rhizobacteria increases the tolerance of the neotropical legume tree *Caesalpinia spinosa* to water deficit

Irene Cordero^{a,b,c,*}, José J. Pueyo^a, Ana Rincón^a

^a Instituto de Ciencias Agrarias (ICA), Consejo Superior de Investigaciones Científicas (CSIC), Serrano 115-bis, Madrid 28006, Spain

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

^c Department of Community Ecology, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zuercherstrasse 111, Birmensdorf 8903, Switzerland

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ABSTRACT

Bio-fertilisation with plant growth promoting rhizobacteria (PGPR) can be extremely beneficial for plant development and growth under harsh environments. PGPR have been recently successfully applied in restoration programmes, but locally adapted strains are needed for successful outcomes. In this study, we evaluated the effect of bio-fertilisation with selected native PGPR strains on the growth and physiological response to drought of *Caesalpinia spinosa* (Mol.) Kuntze (tara), the main tree species of the Atiquipa forest, a highly valuable and unique ecosystem in the middle of the Atacama Desert in Peru. We compared the growth and physiological status of tara seedlings that were bio-fertilised with three PGPR strains, chemically fertilised and a non-fertilised control, under well-watered and under drought conditions. Seedlings inoculated with *Pseudomonas* sp. strain RC5.5 showed enhanced tolerance to drought, although under favourable, stress-free conditions it did not promote growth and only significantly increased quantum yield of photosystem II (F_v/F_m). On the other hand, chemical fertilisation highly enhanced plant growth, but led to substantial hydric demand under water limitation. Under drought, RC5.5 bio-fertilised plants showed lower reduction of leaf relative water content, net carbon assimilation rate, stomatal conductance, and a balanced shoot-to-root ratio compared with control plants. Therefore, strain RC5.5 is highlighted as a valuable candidate to be used as inoculant of tara in reforestation and restoration programmes in arid zones, in particular the Atiquipa forest, or in local tara plantations.

1. Introduction

The importance of belowground microbial-mediated processes and plant-soil feedbacks in ecosystem conservation, management and restoration is being progressively recognized (Jia et al., 2020; Pugnaire et al., 2019; Ramakrishna et al., 2020). Soil microorganisms are essential for plant survival and ecosystem recovery, particularly under harsh environments limiting seedling recruitment, such as drylands (Cordero et al., 2017; Phillips et al., 2016; Ulrich et al., 2020).

Plant growth promoting rhizobacteria (PGPR) are free living or endophytic bacteria that establish a symbiotic relationship with plants, enhancing their fitness and response to stress (Dimkpa et al., 2009; Oleńska et al., 2020). PGPR act by increasing nutrient availability for plants (e.g., by siderophore production, phosphate solubilisation,

enzyme release), but also through non-nutritional mechanisms related to plant signalling (e.g., production of phytohormones such as auxin or abscisic acid) that stimulate germination, growth, and plant water uptake (Oleńska et al., 2020). It has been reported that PGPR are capable of reinforcing the hydric strategy of plants, enabling them to better cope with drought stress (Kannenberg and Phillips, 2017; Rincón et al., 2008b).

Caesalpinia spinosa (Mol.) Kuntze, also known as *Tara spinosa* (Molina) Britton & Rose (Gagnon et al., 2016), usually known as tara, is the dominant tree species in the Atiquipa fog forest, a highly valuable and unique ecosystem in the middle of the Atacama Desert in Peru. Tara provides multiple ecosystem services such as water catchment from fog, pods and seeds rich in tannins and gums, and timber (de la Cruz Lapa, 2004; Larrea, 2011). Wild populations of tara are frequently managed

* Corresponding author at: Department of Community Ecology, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zuercherstrasse 111, Birmensdorf 8903, Switzerland.

E-mail address: irene.cordero@wsl.ch (I. Cordero).

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for seed collection (Cordero et al., 2016; Larrea, 2011), but additionally, tara is sometimes cultivated in different areas of South America, particularly in Peru, Ecuador, and Bolivia. Its pods and seeds have applications in medicine for their antimicrobial and antioxidant capacities (Aguilar-Galvez et al., 2014; Chambi et al., 2013), its tannins are used as dyes (Goycochea Ricci, 2010) and the gums are commercialised within the food and cosmetics industry (Aronson, 1990; Goycochea Ricci, 2010). Tara shows high plasticity and ability to adapt to different edaphoclimatic conditions, such as variable soil pH, soil nutrient content, temperature, or precipitation, which makes it a very flexible tree crop (Murga-Orrillo et al., 2023; Sangay-Tucto and Duponnois, 2018). The persistent deforestation in the Atiquipa forest has seriously endangered this fragile ecosystem, where restoration projects have been implemented to promote tara recruitment and to ensure the sustainability of the local communities (Balaguer et al., 2011; González Molina and Torres Guevara, 2009). Climatic and edaphic conditions in Atiquipa are rather limiting, with a wet season when the fog coming from the ocean is the sole water source, and a dry season that imposes a high drought stress on plants (Balaguer et al., 2011). Tara has developed different mechanisms to tolerate dehydration, such as leaflet closure and the capacity to reach very low leaf water potentials (Cordero et al., 2021). However, a good physiological quality of tara seedlings is essential to guarantee their ability to survive drought stress, particularly at the first stages of establishment in the field (Mediavilla and Escudero, 2004; Ulrich et al., 2020).

Microbial communities may influence tree traits involved in optimizing water and nutrient uptake and drought resistance (Nunes Tiepo et al., 2018; Xi et al., 2018), which represent key factors for seedling establishment and adaptation to water limiting conditions in the field. However, the vast diversity and genetic variability of soil microorganisms makes it difficult to predict which microorganisms will improve tree physiological responses to drought, and the net effect of diverse communities of microorganisms, which requires more study efforts (Ulrich et al., 2020). Local adaptation of beneficial bacteria to their native environment may represent an important asset for improving plant-microbial relationships (Cordero et al., 2017; Revillini et al., 2016). The use of local-adapted PGPR as bio-fertilisers of seedlings in ecological restoration is not widely extended, despite being a sustainable and eco-friendly management approach and an emerging tool with promising results (de-Bashan et al., 2012; O'Callaghan et al., 2022; Singh Rawat et al., 2023). However, there is an increasing number of studies evaluating the potential of specific PGPR strains for ecological restoration, such as the inoculation of *Azospirillum* and other PGPR on cacti and legume trees in degraded areas of the Sonora Desert (Bacilio et al., 2006; Carrillo-Garcia et al., 2000), inoculation of PGPR and rhizobia for the remediation of mine sites (Benidire et al., 2021; Yahaghi et al., 2018) or the use of autochthonous bacterial inoculums on degraded Mediterranean and sub-Mediterranean ecosystems (Azizi et al., 2022, 2021; Barriuso et al., 2008; Ramos Solano et al., 2006). There are even some examples of successful field trials for restoration and reforestation where PGPR inoculation benefited the restoration process (Abdelkrim et al., 2020; Bashan et al., 2012; Chanway et al., 2000).

Local restoration trials in the Atiquipa forest have proven challenging so far (Balaguer et al., 2011). Therefore, the use of locally adapted PGPR strains to inoculate tara trees emerges as a potential solution. Local putative beneficial bacterial strains were isolated in a previous study (Cordero et al., 2017), from the rhizosphere (soil tightly attached to the roots) of juvenile tara trees in the Atiquipa fog forest of Peru. These strains were able to solubilise phosphate, produce siderophores and indoles, and some of them favoured nutrient accumulation in tomato plants (Cordero et al., 2018), confirming their PGPR potential. Therefore, they were selected among the collection of native Atiquipa bacterial strains, as candidates for being tested on tara in this study. Our main objective was to evaluate the effect of bio-fertilisation with selected local PGPR strains on the growth and physiological response of

tara to drought, in a mesocosm experiment. We hypothesise, firstly, that PGPR bio-fertilisation will increase tara growth under favourable, stress-free conditions. Secondly, given that the PGPR may act by both nutritional and non-nutritional mechanisms, we hypothesise that, compared with chemical fertilisation, seedlings bio-fertilised with PGPR would increase the tolerance of tara to drought.

2. Materials and methods

2.1. Plant material and growth conditions

Tara seeds were collected in the fog forest of Atiquipa, Peru (15°45' S 74°22' W). Seeds were sterilised and surface scarified in 98% H₂SO₄ for 45 min, followed by several changes of sterile distilled water and soaked for 48 h. Seeds were individually sown into 300 mL containers with 2:1 (v:v) autoclaved peat:vermiculite, which were placed in a growth chamber with 16 h photoperiod of 250 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 24 /20 °C of temperature day/night and 70 /100% of relative air humidity. Seeds germinated after ~10 days, and two months later seedlings of similar height were transferred to 2.3 L containers filled with 2:1 (v:v) autoclaved peat:vermiculite. The greenhouse conditions were monitored with light, temperature, and relative air humidity sensors (HOBO, Onset Computers, Pocasset, USA). Mean photosynthetically active radiation (PAR) was ~600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 12 h photoperiod, temperature day/night of 19.5 /17.5 °C and 45/60% relative air humidity. Tara seedlings were weekly rotated to minimize potential position effects.

2.2. Bacterial inocula

Three bacterial strains originally isolated from the rhizosphere of tara in the Atiquipa forest were selected by their PGPR capacities (Cordero et al., 2018, 2017): PD1.5 (*Arthobacter* sp., KP267831 GenBank accession number for 16 S rRNA gene sequence), RD17.8 (*Pseudomonas abietaniphila*, KP267838) and RC5.5 (*Pseudomonas* sp., KP267841). To obtain each bacterial inoculum, a single colony from a LB-agar (Bertani, 1951) plate was transferred to 250 mL LB liquid medium and incubated at 28 °C and 150 rpm in an orbital shaker, until reaching 10⁸ cfu·mL⁻¹ (OD_{600 nm} = 1.0). Each bacterial suspension was diluted 1:5 (v:v) with autoclaved phosphate buffer (PBS: 136.75 mM NaCl, 2.68 mM KCl, 10.14 mM Na₂HPO₄, 1.76 mM KH₂PO₄, pH 7.4) prior to inoculation.

2.3. Experimental design

A full factorial experiment with the factors fertilisation and drought was set up to test their effects on the morphology and physiological performance of tara seedlings. The fertilisation factor consisted of five levels: (1) non-fertilised control, (2–4) three bio-fertilisation (BF) treatments i.e., separate inoculation with each bacterial strain, PD1.5, RD17.8 or RC5.5, and (5) chemical fertilisation (CF). Fertilisation treatments started five days after transplantation of seedlings, which were randomly assigned to each treatment (mean height = 5.8 ± 1.5 cm; mean diameter = 1.8 ± 0.3 mm). CF seedlings received 2 g·L⁻¹ of slow-release fertiliser (Osmocote NPK 11:11:18 + 2 MgO₂ + microelements, Projar, Spain). For BF treatments, seedlings were biweekly inoculated with 15 mL of the respective bacterial inoculum for a total of three inoculations. In each inoculation, unfertilised and CF treatments received the same volume of autoclaved LB medium diluted 1:5 (v:v) in PBS as the inoculated seedlings.

The drought factor consisted of two levels: (1) well-watered (W) and (2) drought (D). Drought was gradually applied to avoid sharp substrate water content decrease and to allow seedlings a progressive osmotic and physiologic adjustment to drought (Cordero et al., 2021). Daily water loss was evaluated gravimetrically and the 100% was replaced in well-watered treatment, while drought treatment received 50% of the lost water for 46 days, 25% in the successive 12 days when watering was arrested until the end of the experiment (100 days) (7-month-old

seedlings). The progression of the substrate water content in each treatment along the experiment is shown in [Figure S1](#). Each treatment had a total number of plants of 10–12 ([Table S1](#)). Total number of plants = 108.

2.4. Physiological status of tara seedlings

Midday water potential (Ψ) (11:00–16:00 solar time) of seedlings (only $n = 8$ per treatment instead of 10–12 plants) was measured with a Scholander-type pressure chamber (SKPM 1400, Skye Instruments Ltd., UK). The last two leaflets of a pinnae with a ~ 4 cm rachis were selected, as previously described for legumes with composed leaves ([Cordero et al., 2021](#)). Water potential was measured once at the end of the drought period.

Relative water content (RWC) of one leaflet per plant at midday was calculated as $RWC = (FW - DW) / (FTW - DW)$, where FW is the fresh weight, DW the dry weight and FTW the fully turgid weight, i.e., weight of the leaflet kept at 100% humidity and 4 °C for 48 h. RWC was measured every two weeks in all seedlings.

Carbon assimilation (A), transpiration (E) and stomatal conductance (g_s) were recorded with an infrared gas analyser (Li-Cor 6400, LiCor Inc., USA) at midmorning (9:30–14:00 solar time), every week. Single, attached, mature leaflets were kept inside the cuvette under 750 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of light intensity and constant CO_2 flux of 400 $\mu\text{mol}\cdot\text{s}^{-1}$ for 2 min. Temperature was kept stable at 20 °C. Three consecutive readings were recorded, the average of which was used for statistical analyses. Leaflet area inside the cuvette was measured with the software ImageJ v1.47, and used to recalculate photosynthetic variables by leaf area. Water use efficiency (WUE), which estimates the amount of water invested per unit of biomass, was calculated as $WUE = A / E$.

Fluorescence of chlorophyll *a* was measured with a portable pulse-modulated chlorophyll fluorimeter (PAM 2000, Walz, Germany) before dawn once per week. Maximum quantum yield of photosystem II (F_v/F_m) was calculated as $F_v/F_m = (F_m - F_0) / F_m$, being F_0 the minimal basal fluorescence of dark-adapted leaves and F_m the maximum fluorescence obtained after applying a saturating light pulse (800 ms and 12000 $\mu\text{mol quanta m}^{-2}\cdot\text{s}^{-1}$) ([Genty et al., 1989](#)). Three separate leaves per plant were measured, and the mean of the three readings was used for statistical analyses.

2.5. Growth and morphology of tara seedlings

Seedling height and diameter were measured every 10 days. At harvest, shoots were separated from roots, and were oven-dried separately at 65 °C for 72 h to determine their respective dry weight. Shoot-to-root ratio (S/R) was calculated based on dry weights. One leaflet per seedling was scanned and weighed to determine the specific leaf area ($SLA = \text{area (m}^2) / \text{dry weight (kg)}$). Seedling relative growth rate (RGR) during the drought period was calculated as $RGR = (X_f - X_0) / X_0$; being X_f the final height or diameter (day 100) and X_0 the initial one (day 0). Additionally, the percentage of seedlings with most of their leaflets closed (leaflet angle higher than 70°, measured with a protractor) was periodically recorded.

2.6. Statistical analyses

To evaluate the effects of fertilisation and drought over time, seedling traits repeatedly measured (RWC, A, g_s , WUE, F_v/F_m , height, and diameter) were analysed with linear mixed models considering fertilisation, drought, and time (continuous variable) as fixed factors, and the seedling as a random factor. Non-significant interactions among factors were eliminated from models, following the hierarchical principle. To do this analysis, we used the *lme* function in the “nlme” package ([Pinheiro et al., 2023](#)), and added the “weights” argument to the function to account for different variance distribution among groups.

Seedling traits measured only once (Ψ , SLA, fresh and dry biomass,

R/S, RGR in height and in diameter), and values on the last day of measurement of variables measured over time (RWC, A, g_s , WUE, F_v/F_m , height, and diameter at day 100) were analysed by two-way ANOVA for the fixed factors fertilisation and drought, with the *lme* function. For heterocedastic variables (F_v/F_m), we added the “weights” argument to the function to account for different variance distribution among groups. For RWC, which is a variable constrained between 0 and 100, we used *glmmTMB* function in “glmmTMB” package ([Brooks et al., 2017](#)) instead, using a beta distribution. To identify the effects of fertilisation under favourable, stress-free conditions, only the contrasts between well-watered conditions were performed with post-hoc Tukey test with *emmeans* function in “emmeans” package ([Lenth, 2024](#)).

To assess the effects of drought, we calculated the relative change ratio (RC) of each variable as: (its value under drought divided by the average value under well-watered conditions for each fertilisation treatment $\times 100$) - 100, to obtain a percentage increase/decrease relative to the respective well-watered treatments. For water potential (Ψ), data were converted to positive prior to RC calculation to better visualise the effect of drought (a decrease in a negative variable). As before, data were analysed with *lme* function, using fertilisation as a fixed factor, followed by a post-hoc Tukey test with *emmeans*. For heterocedastic variables (RC of RGR in diameter), we added the “weights” argument to the function as before. RC of g_s and RC of RGR in height were logarithmically transformed to meet model assumptions. One-sample t-test was performed for all RC variables to detect significant deviations from 0 (i.e., significant effect of drought).

Relationships between morphological and physiological variables were evaluated by bivariate Pearson correlation test (linear relationships) or by quadratic or logarithmic regression analyses. All analyses were carried out with R v4.3 ([R Core Team, 2023](#)).

3. Results

Under no water limitation ([Table 1](#)), chemical fertilisation significantly improved tara development, promoting higher net carbon assimilation rate, F_v/F_m and a large increase of all morphological variables (except SLA) with respect to the non-fertilised control. In contrast, biological fertilisation with PGPR strains barely affected tara. The exception was seedling photochemical efficiency F_v/F_m that was significantly improved by inoculation with RD17.8 and RC5.5 compared with the control.

However, under drought, all factors i.e., fertilisation, drought, and time, and specially their interactions had a significant effect on most of the studied variables ([Table S2](#)). There was a significant strong interaction among the three variables, i.e., over time, the relationship between the other two variables changed, having a stronger drought \times fertilisation interaction at the end of the experiment than at the beginning, as expected in a progressive drought experiment. This drought \times fertilisation interaction highlights the fact that fertilisation treatments had different effects under well-watered conditions than under drought, and it was also significant in variables measured only at the end of the experiment ([Table S3](#)). After 100 days of drought, seedling water potential (Ψ) significantly decreased (i.e., became more negative) in all fertilisation treatments ([Fig. 1A](#)). The sharpest Ψ decrease was observed in CF seedlings compared with the other fertilisation treatments (almost three times stronger reduction of Ψ). In fact, CF seedlings did show the greatest leaf water content loss (45% loss), while the lowest RWC loss was observed in inoculated seedlings, particularly with RC5.5 strain (1% loss) ([Fig. 1B](#), [Figure S2A](#)). A significant decrease of leaf RWC was detected in drought-stressed controls (22% loss) and RD17.8 bio-fertilised plants (12% loss) compared to their respective well-watered treatments, although it was not as acute as for CF ([Fig. 1B](#)).

As drought progressed, A and g_s decreased in all treatments but particularly in CF plants (90% and 82% decrease in A and g_s , respectively), while the lowest decrease was observed for seedlings bio-fertilised with RC5.5 (35% and 39% decrease) ([Fig. 1C](#), D; [Figure S2B](#),

Table 1

Effects of biological fertilisation with PGPR strains PD1.5, RD17.8 or RC5.5, or chemical fertilisation (CF) on physiological and morphological characteristics of tara (*Caesalpinia spinosa*) seedlings under favourable conditions (i.e., only well-watered plants) at the end of the experiment. RWC = leaf relative water content; Ψ = water potential at midday; A = net carbon assimilation rate; g_s = stomatal conductance; WUE = water use efficiency; F_v/F_m = maximum quantum yield of PSII; h RGR = height (cm) relative growth rate; \emptyset RGR = diameter (mm) relative growth rate; SLA = specific leaf area; DW = dry weight; S/R = shoot-to-root ratio. Values = average \pm standard deviation, n = 10–12. Different letters denote significant differences among fertilisation treatments by the Tukey test, (p < 0.05).

	Control	PD1.5	RD17.8	RC5.5	CF
Physiological variables					
RWC (%)	81.1 \pm 6.8	81.4 \pm 6.2	82.9 \pm 10.0	81.2 \pm 3.9	82.4 \pm 7.7
Ψ (MPa)*	-1.3 \pm 0.5	-1.2 \pm 0.4	-1.0 \pm 0.4	-1.1 \pm 0.3	-1.4 \pm 0.7
A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	6.8 \pm 1.6	6.1 \pm 1.8	8.4 \pm 1.8	6.5 \pm 2.0	10.7 \pm 2.9
g_s (mmol H ₂ O $\text{m}^{-2} \text{ s}^{-1}$)*	108 \pm 33	115 \pm 54	151 \pm 74	104 \pm 42	161 \pm 80
WUE ($\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$)	5.4 \pm 1.3	5.0 \pm 1.1	5.2 \pm 1.2	5.8 \pm 1.5	6.1 \pm 1.4
F_v/F_m	0.82 \pm 0.01	0.82 \pm 0.02	0.84 \pm 0.01	0.84 \pm 0.01	0.85 \pm 0.01
Morphological variables					
Height (cm) †	9.5 \pm 1.9	10.2 \pm 2.5	10.4 \pm 1.8	10.0 \pm 1.7	28.6 \pm 8.0
Diameter (mm) †	4.8 \pm 0.6	5.1 \pm 0.7	4.9 \pm 0.5	5.0 \pm 0.7	8.5 \pm 1.3
h RGR (%) †	28.1 \pm 13.1	33.6 \pm 18.4	22.3 \pm 10.4	32.7 \pm 20.3	216.5 \pm 48.3
\emptyset RGR (%) †	74.5 \pm 32.6	73.2 \pm 27.4	68.5 \pm 24.9	66.5 \pm 14.8	164.5 \pm 62.9
SLA ($\text{m}^2 \text{ kg}^{-1}$)	8.2 \pm 1.0	8.4 \pm 0.8	8.1 \pm 0.6	8.8 \pm 1.2	8.9 \pm 1.0
Shoot DW (g) †	1.9 \pm 0.7	2.2 \pm 0.8	2.2 \pm 0.7	2.1 \pm 0.8	14.3 \pm 4.2
Root DW (g) †	1.7 \pm 0.9	2.2 \pm 0.8	2.3 \pm 0.8	2.1 \pm 0.7	6.5 \pm 3.1
S/R †	1.3 \pm 0.5	1.0 \pm 0.2	1.0 \pm 0.2	1.0 \pm 0.2	2.1 \pm 0.4

† Logarithmically (ln) transformed variable.

* Square root transformed variable.

C). At the end of the experiment, seedlings inoculated with PD1.5 and RC5.5 were more efficient in the use of water under drought (i.e., no significant effect of drought compared with well-watered seedlings), while WUE significantly decreased for the rest of the fertilisation treatments (i.e., control, RD17.8 and especially CF) (Fig. 1E, Figure S2D). The initial improvement of seedling F_v/F_m by chemical and bio-fertilisation (RD17.8, RC5.5) (Table 1) was maintained under drought conditions only for CF and RC5.5, which showed the smallest decrease on this variable compared to the control (10% decrease in control plants compared to 2% and 3% decrease in CF and RC5.5 plants respectively), although differences among fertilisation treatments were not statistically significant (Fig. 1F, Figure S2E).

At first, chemical fertilisation accelerated the growth in height and diameter of seedlings, which was severely slowed down by drought (Fig. 2, Figure S3, Table S2). The relative growth rate (RGR) in height was not affected by drought in RD17.8 seedlings, while it decreased in all other treatments (Fig. 2C). The RGR in diameter was significantly reduced by drought in control and CF seedlings but not in those bio-fertilised (Fig. 2D). Shoot and root biomass were strongly reduced in CF seedlings under drought (64% and 60% reduction in shoot and root biomass, respectively) (Fig. 3A, B, Figure S4), while the effect was not so strong in the other treatments, especially for root biomass, which was significantly reduced only in CF and RC5.5 seedlings (Fig. 3B). Additionally, bio-fertilisation with PD1.5 and RD17.8 stimulated the root biomass under drought, and it was not significantly different from the

root biomass of CF plants under drought (Figure S4B). The seedling specific leaf area (SLA) significantly increased with drought except in RC5.5 fertilised plants (Fig. 3C). The shoot to root ratio was reduced by drought in control, PD1.5 and RD17.8 seedlings (Fig. 3D). In fact, the S/R ratio was always higher in CF seedlings (i.e., drought and well-watered conditions) compared with the rest of fertilisation treatments (Fig. 3E). Along the experiment, the highest percentage of seedlings with closed leaflets was observed in CF under drought (leaflet closure started on day 43 in CF, and on day 85 in the rest of treatments (Figure S5)).

Ψ was significantly and positively correlated with RWC and g_s (Fig. 4A,B), and this was particularly significant for control and CF seedlings. A significant positive correlation between F_v/F_m and g_s was observed for control, PD1.5 and RD17.8, but not for CF and RC5.5 fertilised seedlings, which preserved their photosynthetic capacity even when stomata were almost completely closed (Fig. 4C). A negative linear correlation between SLA and WUE was also observed, with a lower WUE at higher SLA values for all treatments except bio-fertilisation with RC5.5 and PD1.5 (Fig. 4D).

4. Discussion

Our findings demonstrate that bio-fertilisation with one of the selected PGPR strains significantly enhanced the tolerance of tara to drought, even though it produced no apparent benefit under favourable conditions. On the contrary, chemical fertilisation rapidly fostered seedling growth, but at the expense of a clear physiological imbalance under stress by water limitation. These results support the idea that the sustainable reinforcement of the seedlings physiological endurance (by bio-fertilisation with native microorganisms), rather than promoting their rapid growth (by chemical fertilisation), can be a winning adaptive strategy to survive long under hostile field conditions. Our study highlights the potential applicability of *Pseudomonas* strain RC5.5 as a bio-inoculant for the propagation of tara seedlings in dry environments.

4.1. Response of tara to fertilisation

Chemical fertilisation had a strong positive effect on tara seedling in the absence of drought stress. As shown in similar studies, chemical fertilisation increased seedling photosynthetic capacity and net carbon assimilation rate (Hikosaka, 2004; Zhang et al., 2018), probably due to the higher nitrogen availability that allowed a greater investment in photosynthetic machinery (i.e., chlorophylls or rubisco enzyme) (Lawlor, 2002). The high impact of chemical fertilisation on the morphology of tara clearly reflects the improvement of the seedling nutritional status: as nutrient assimilation increased, also did the productivity of seedlings and thus the biomass production, as often reported (Berenguer et al., 2009; Erefur et al., 2011; Song et al., 2015).

Under favourable conditions, biological fertilisation with PGPR showed a small benefit on tara's physiology: a significant increase on the photochemical efficiency of tara (F_v/F_m) in two out of three bio-fertilisation treatments. This effect has been previously described for different plants (Gamalero et al., 2008). However, bio-fertilisation did not affect any of the other photosynthetic variables (net carbon assimilation, stomatal conductance and water use efficiency). Many studies have demonstrated an improvement in morphological variables of woody species due to PGPR inoculation (Azizi et al., 2022; Cámara Leite et al., 2013; Liu et al., 2011; Martínez et al., 2018; Ramos Solano et al., 2008). However, in the present study we could not detect any significant effects of bio-fertilisation in seedling growth and biomass, although other undetected effects related with the production of bioactive molecules could be operating. Besides direct nutritional and growth promoting effects, PGPR actions on plant hormonal regulation are important in other key aspects, such as improving plant physiological status and hence resistance to stress (Kudoyarova et al., 2019). Two different meta-analyses (Rubin et al., 2017; Schütz et al., 2018) demonstrated that many PGPR have a stronger beneficial effect under

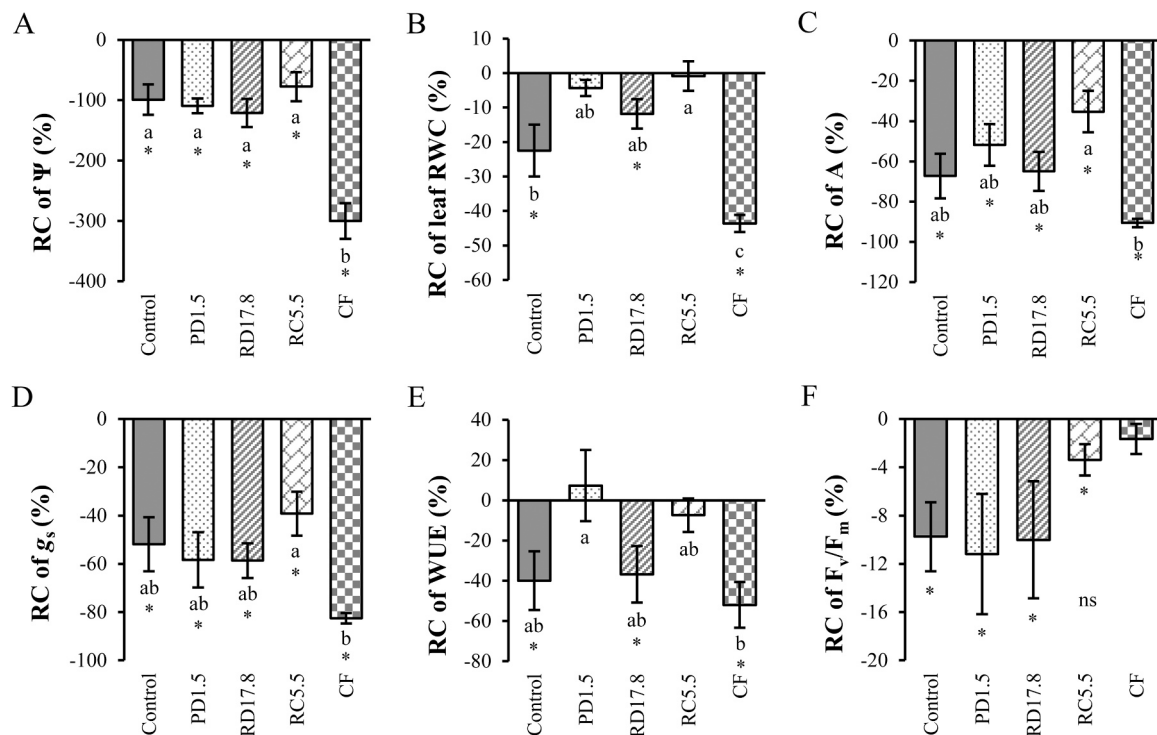


Fig. 1. Physiological status of tara seedlings (*Caesalpinia spinosa*) under drought after 100 days. The relative change (RC) is the percentage of change of a given variable under drought with respect to well-watered conditions calculated as $RC = [(variable\ drought/variable\ well-watered) \times 100] - 100$ for each fertilisation treatment (bio-fertilisation with different PGPR strains: PD1.5, RD17.8 or RC5.5, or chemical fertilisation: CF). A. Seedling water potential at midday (Ψ). B. Leaf relative water content (RWC). C. Net carbon assimilation rate (A). D. Stomatal conductance (g_s). E. Water use efficiency (WUE). F. Maximum quantum yield of PSII (F_v/F_m). Different letters denote significant differences among fertilisation treatments by Tukey test ($p < 0.05$). Asterisks indicate a significant deviation from 0 (a significant effect of drought), by t-test ($p < 0.05$). ns = not significant. Values = mean \pm standard error, $n=8$ (Ψ); $n=10-12$ (all other variables).

stressful conditions than under optimum growth conditions. Under stress, plant growth and physiology are limited by several factors such as physiological disorders, hormonal and nutritional imbalance, susceptibility to diseases, ion toxicity, etc. Because of this, the application of beneficial microorganisms under stress is usually more efficient as they could facilitate not only nutritional but also non-nutritional gains for plants, helping them to recover their equilibrium (Kudoyarova et al., 2019; Nadeem et al., 2014).

4.2. Response of tara to drought and interaction with the fertilisation method

Seedling water status was highly influenced by the fertilisation method: chemically fertilised plants showed a stronger and faster dehydration than the bio-fertilised ones, reaching lower leaf Ψ and RWC values. The rapid initial growth of chemically fertilised plants by direct nutrient supply increased their water demand, depleting rapidly the water available in the pots and subjecting the plants to a severe drought stress. On the other hand, biological fertilisation improved the water status of plants, which showed a smaller decrease in relative water content than controls under drought, particularly with strain RC5.5. Beneficial effects of PGPR on plant water status have been previously described in other plant species (Azizi et al., 2022; Cohen et al., 2015; Kudoyarova et al., 2024; Liu et al., 2023; Naseem and Bano, 2014; Rincón et al., 2008a). These effects could be related to the capacity of some rhizobacteria to produce exopolysaccharides (Naseem and Bano, 2014; Sandhya et al., 2009) and/or by alteration of the plant hormone homeostasis e.g., increase abscisic acid concentration (Bhattacharyya et al., 2021; Poudel et al., 2021).

Photosynthetic parameters (net carbon assimilation, stomatal conductance and water use efficiency) were severely reduced in chemically fertilised seedlings after drought, with the exception of F_v/F_m .

Higher F_v/F_m associated to fertilisation in stressed plants has been previously reported (Shangguan et al., 2000; Yin et al., 2009). Recently, Roy et al. (2021) have showed that at high water and N-P fertilisation rates, chlorophylls and carotenoid contents significantly increased, while at low water availability and high chemical fertilisation, the content of oxidative stress related molecules and enzymes raised (e.g., proline, soluble sugars, catalase, peroxidase).

Biological fertilisation improved the photosynthetic capacity of seedlings, which showed lower reductions in WUE (RC5.5 and PD1.5 strains), and A, g_s , and F_v/F_m (RC5.5) under drought. An enhanced photosynthetic capacity of plants under water deficit mediated by inoculation with selected bacteria has been previously documented (Azizi et al., 2022, 2021; Gururani et al., 2013; Liu et al., 2013). Additionally, it has been demonstrated that inoculation with PGPR reinforces the hydric strategy of woody plants (dehydration tolerance vs avoidance) (Kannenberg and Phillips, 2017; Rincón et al., 2008b), as observed in the present study. Tara has a dehydration tolerance strategy (definition as in Volaire, 2018) under greenhouse conditions (Cordero et al., 2021). This strategy was later strengthened under drought conditions by bio-fertilisation with RC5.5, as seedlings were able to maintain their stomata open for longer time than the controls, striking a cost-benefit balance that allowed them to increase their carbon assimilation despite the water shortage.

Drought had an evident effect on the morphology of tara seedlings, reducing relative growth rate and biomass, as observed in numerous drought studies (Álvarez and Sánchez-Blanco, 2013; Eilmann et al., 2011; Galmés et al., 2005; Zhang et al., 2011). Drought stress prevents carbon assimilation and thus the production of photo-assimilates and ultimately, biomass. Chemical-fertilised seedlings suffered the strongest reductions in biomass, but they still kept higher values of shoot weight and height under drought than the bio-fertilisation treatments. On the other hand, bio-fertilisation with PD1.5 and RD17.8 stimulated root

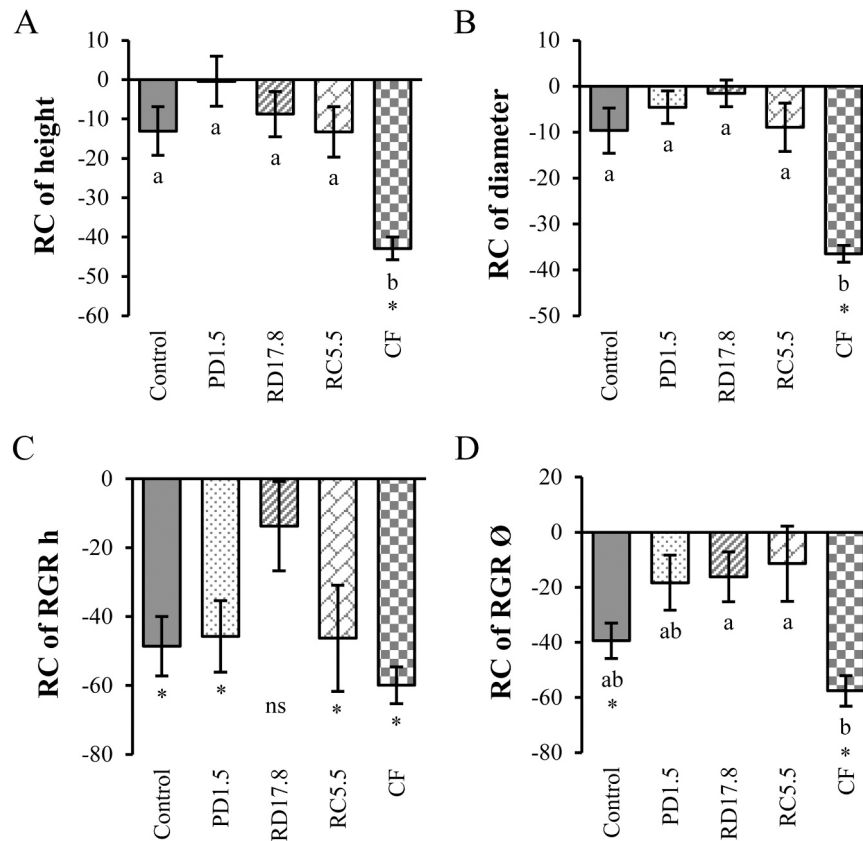


Fig. 2. Growth of tara (*Caesalpinia spinosa*) seedlings. The relative change (RC) is the percentage of change of a given variable under drought with respect to well-watered conditions calculated as $RC = [(variable\ drought/variable\ well-watered) \times 100] - 100$ for each fertilisation treatment (bio-fertilisation with different PGPR strains: PD1.5, RD17.8 or RC5.5, or chemical: CF). A. Height at the end of drought (day 100). B. Diameter (at day 100). C. Relative growth rate in height during the drought period (RGR h). D. Relative growth rate in diameter during the drought period (RGR Ø). Different letters denote significant differences among fertilisation treatments as described in Fig. 1. Values = mean \pm standard error, n=10–12. ns = not significant.

biomass accumulation under drought in a similar manner to chemical fertilisation, and therefore, these two bacterial strains did show a plant growth promoting effect. Similar rhizobacterial-induced PGP effects have been previously observed in plants of agronomical interest (Belimov et al., 2009; Cordero et al., 2018; Sarma and Saikia, 2013) and in other woody species (Azizi et al., 2022; Liu et al., 2013; Marulanda et al., 2006).

Similar to other species (Anyia and Herzog, 2004), SLA increased with drought in all fertilisation treatments, except in the case of bio-fertilisation with RC5.5. Lower SLA (i.e., higher investment in leaf mass than in leaf area) could be related to the investment in structural components such as starch or protective secondary metabolites (Franco et al., 2005; Roche et al., 2004). Under water deficit, plants reduce carbon assimilation and the scarce photo-assimilates have to be rationed and efficiently invested in suited molecules to better cope with the new situation. Under these conditions, higher SLA (loss of leaf biomass) could imply a decrease in the investment in structural carbohydrates, prioritizing the maintenance of the photosynthetic machinery (Jiménez et al., 2009) and/or the biosynthesis of oxidative stress-reducing molecules (Roy et al., 2021). The fact that biological fertilisation with RC5.5 did not produce this increase in SLA can be interpreted as a protective effect of this PGPR, reducing the negative effects of drought, probably by means of non-nutritional mechanisms. Additionally, there was a negative correlation between SLA and WUE (i.e., at higher SLA, lower WUE) and this effect was particularly pronounced in chemically fertilised and non-fertilised control plants. The correlation between these variables has been previously described (Chaturvedi et al., 2014; Hoffmann et al., 2005) and it denotes that thinner leaves are less efficient in their use of water, losing more water through evapotranspiration and showing less

assimilation, effects that were not so strongly evident in the bio-fertilised plants in this study.

Shoot-to-root ratio was significantly more disproportioned in chemically-fertilised plants (~ 2) than in any other treatment, both under well-watered conditions and under drought. However, this ratio was close to 1 in all biologically fertilised plants. Moreover, RC5.5 fertilised plants did not show a significant reduction of S/R ratio under drought, as opposed to the other bio-fertilisation treatments and the controls. Root development and a balanced shoot-to-root proportion is vital in woody species to increase their survival opportunities in the field, particularly in areas prone to drought stress. Higher root biomass increases the probability of water capture and also reduces water loss through evapotranspiration due to the reduction in leaf area (Padilla et al., 2009; Schwinning and Ehleringer, 2001). Therefore, the balanced ratio observed in RC5.5 plants under drought represents a clear benefit of the bio-fertilisation with this strain and a preferable choice respect to chemical fertilisation.

Overall, these results indicate that inoculation with native selected rhizobacteria (RC5.5) improves the physiological response of tara to drought, confirming our second hypothesis. The mechanisms behind these effects are multiple and complex (Kudoyarova et al., 2024; Oleńska et al., 2020). These mechanisms could be nutritional, as PGPR might facilitate plant nutrient acquisition (Richardson et al., 2009; Vessey, 2003). Two of the selected PGPR in this study (RD17.8 and RC5.5) were able to solubilise phosphate and to produce siderophores (Cordero et al., 2017) and PD1.5 and RC5.5 favoured nutrient accumulation (N, P and K) in tomato plants (Cordero et al., 2018), so a nutritional improvement in tara cannot be ruled out. On the other hand, non-nutritional mechanisms can be also involved, such as changes in plant hormonal balance

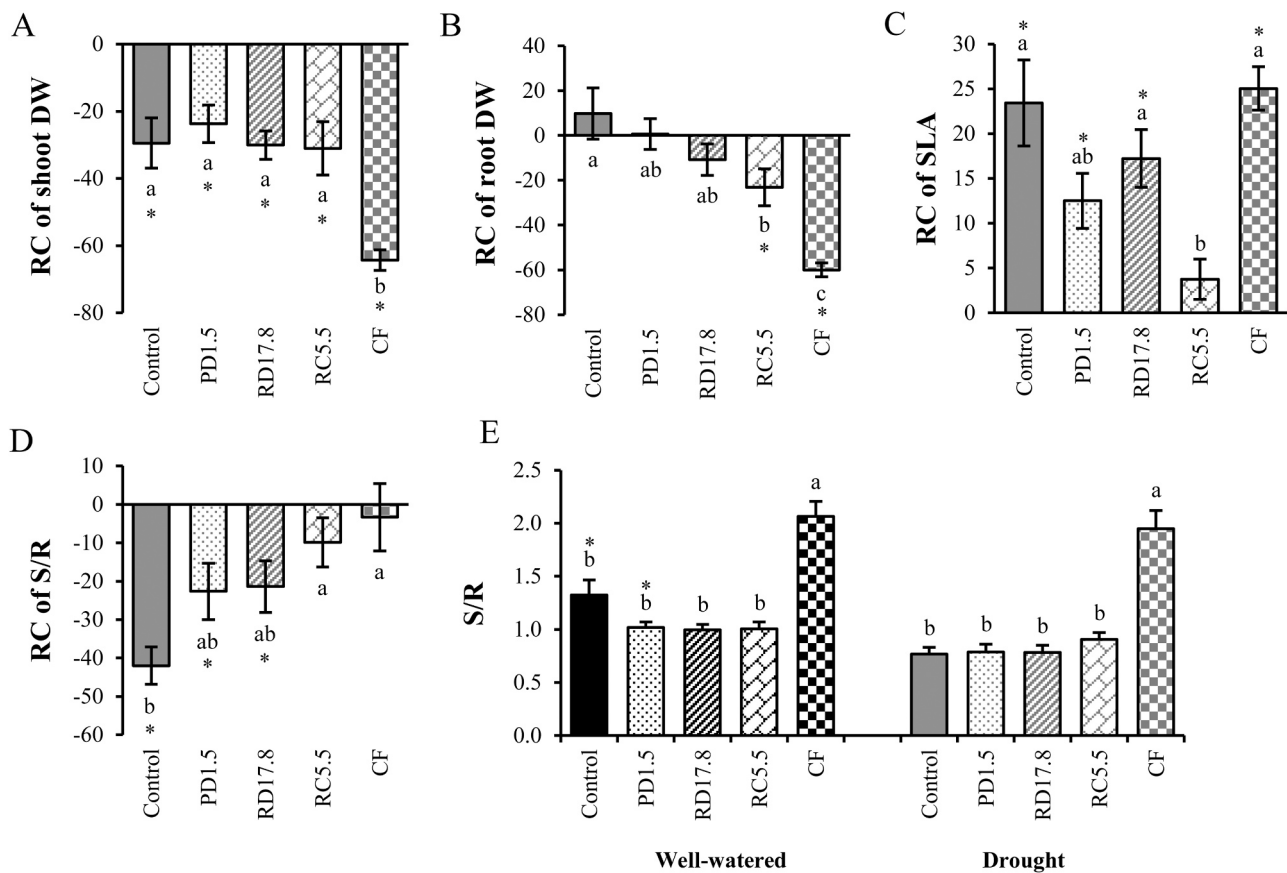


Fig. 3. Morphological characteristics of tara (*Caesalpinia spinosa*) seedlings subjected to drought for 100 days. The relative change (RC) is the percentage of change of a given variable under drought with respect to well-watered conditions calculated as $RC = [(variable\ drought/variable\ well\ watered) \times 100] - 100$ for each fertilisation treatment (bio-fertilisation with different PGPR strains: PD1.5, RD17.8 or RC5.5, or chemical: CF). A. Shoot dry weight (DW). B. Root dry weight (DW). C. Specific leaf area (SLA). D. Shoot-to-root ratio (S/R). E. Absolute value of Shoot-to-root ratio under well-watered and drought conditions. Different letters denote significant differences among fertilisation treatments as described in Fig. 1. In E, for each drought treatment, different letters denote significant differences among fertilisation treatments, and for each fertilisation treatment, asterisks indicate significant differences of factor drought. Values = mean \pm standard error, n=10–12.

such as auxins, cytokinins, gibberellins, abscisic acid, or ethylene (Dodd et al., 2010; Oleńska et al., 2020). There are also some biochemical mechanisms that could explain the effects of PGPR in plant water status, such as the increase in the expression of membrane aquaporins in root cells to increase water absorption (Marulanda et al., 2010). Any of these nutritional, hormonal, or biochemical mechanisms could be underpinning the beneficial effect of the bacterial strain RC5.5 observed in tara under water deficit, which merits further investigation.

5. Conclusions

Chemical fertilisation had a strong positive effect on tara's morphology, but under water deficit, it imposed a greater drought stress deteriorating the physiological status and reducing carbon assimilation. On the other hand, biological fertilisation with PGPR did not provide a great benefit on plant growth, but it increased plant tolerance to dehydration, depending on the inoculated strain. Seedlings inoculated with *Pseudomonas* sp. (RC5.5) showed higher leaf RWC, A, g_s , WUE, F_v/F_m under drought than other fertilisation treatments. They also showed a balanced shoot-to-root ratio that did not significantly change under drought. Therefore, RC5.5 is highlighted as a good candidate to be used as an inoculant of plants to be used for reforestation of arid zones, in particular the Atiquipa forest and other tara forests, or in tara crop production in local agricultural fields. The results of this study under controlled conditions represent a considerable progress in the selection of tara inoculants to produce quality seedlings reinforced to withstand transplantation to the field. However, subsequent applications of PGPR

in the field can prove challenging, as they will interact with the local microbiota, and strain survival is often compromised (Timmusk et al., 2023). Therefore, it will be indispensable to test the efficiency of this strain under field conditions, and to consider the whole microbiota effects of plants, before it could be introduced in restoration programs of the Atiquipa fog forest.

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CRediT authorship contribution statement

José J. Pueyo: Conceptualization, Methodology, Supervision, Writing – review & editing. **Ana Rincón:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Irene Cordero:** Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft.

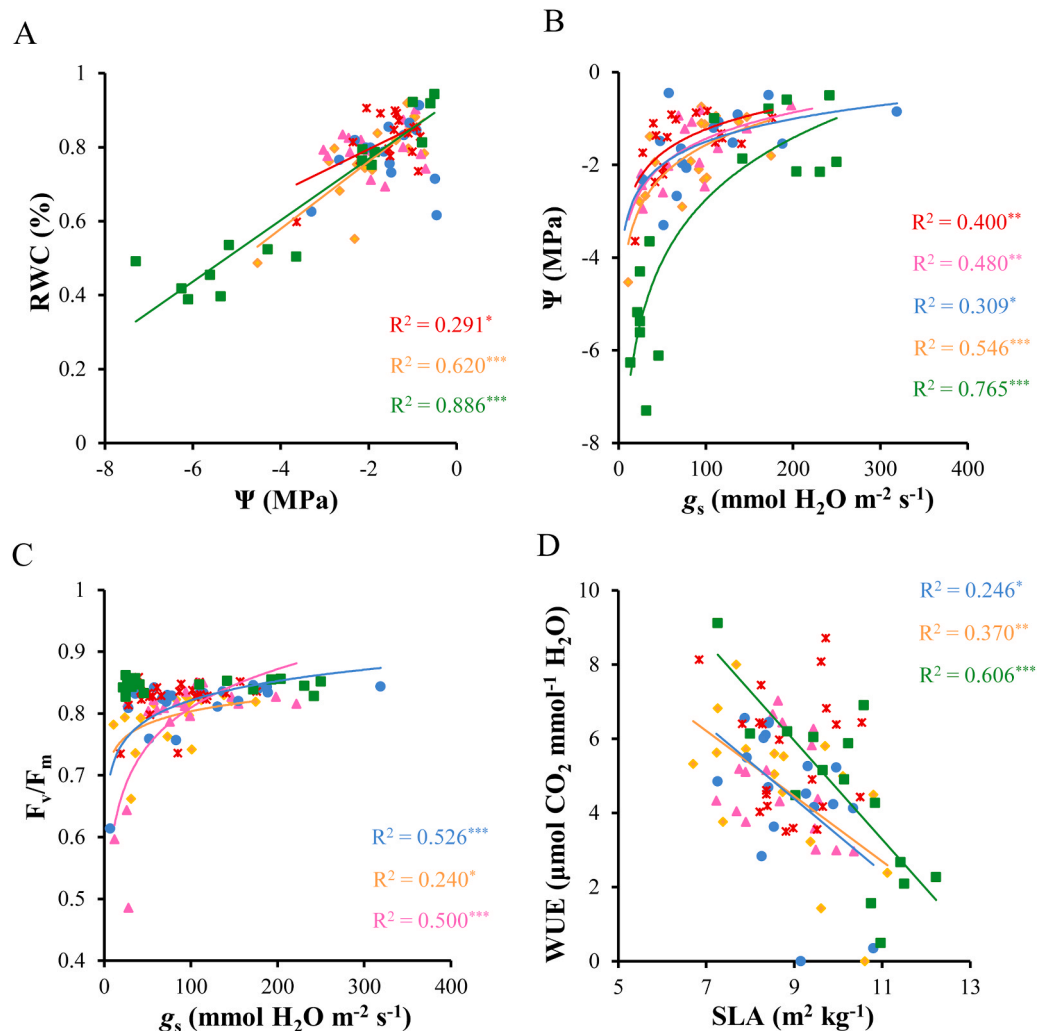


Fig. 4. Relationship between physiological variables of tara (*Caesalpinia spinosa*) seedlings at the end of the drought treatment (day 100). A. RWC vs. Ψ , B. Ψ vs. g_s , C. F_v/F_m vs. g_s , and D. WUE vs. SLA. Different colours and shapes indicate different fertilisation treatments: control (orange diamond), bio-fertilisation with PD1.5 (pink triangle), RD17.8 (blue circle) and RC5.5 (red star), or chemical fertilisation (green square). For each fertilisation treatment, when significant, R^2 and signification value (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) of the linear (A and D) or logarithmic regressions (B and C) are shown. Non-significant regression lines/curves are not drawn. Ψ : water potential; RWC: leaf relative water content; g_s : stomatal conductance; F_v/F_m : maximum quantum yield of PSII; WUE: water use efficiency; SLA: specific leaf area.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the

online version at [doi:10.1016/j.foreco.2024.121786](https://doi.org/10.1016/j.foreco.2024.121786).

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