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Differential eco-physiological performance of eight *Eucalyptus* species grown in Tunisian's arid regions

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Abstract: Successful production of Eucalyptus in drylands requires a thorough understanding of the mechanisms involved in their tolerance to abiotic stress as well as drought. The present study aimed to investigate the effects of dry conditions on morpho-physiological characteristics of eight 5-year-old Eucalyptus species trees, Eucalyptus camaldulensis Dehn., Eucalyptus gomphocephala DC., Eucalyptus torquata Luehm., Eucalyptus microtheca F.Muell., Eucalyptus occidentalis Endl., Eucalyptus diversifolia Bonpl., Eucalyptus sargentii Maiden., and Eucalyptus torwood (Spontaneous hybrid), grew in the same arid microhabitat (a plot) characterized by poor soil. The experiments were conducted in summer 2022, a year that was characterized by a low rainfall rate. Thus, at regular intervals, several parameters were assessed. Plant water status, leaf water relations, gas exchanges, and chlorophyll fluorescence in relation to trees growth performance and leaf traits. Results revealed a distinct resistance level to aridity for the studied species, trees were able to grow showing a clear inter-specific variability. Regarding all species, E. camaldulensis, exhibited a particular behavior to preserve their internal functionality. It displayed simultaneously a rapid growth performance with an ability to develop thinner leaves and earlier stomatal closure under water deficit. Although, E. microtheca, E. gomphocephala, and E. diversifolia' growth were low, but trees attempted to maintain a stable inter-dependence functions for survival. For E. occidentalis, E. torquata, E. sargentii, and E. torwood, we suggest that a physiological acclimation took place under severe conditions, they showed convergence responses for many parameters. Their ability to maintain good leaves' succulence and thickness is clearly attributed to a significant RWC (71.5% to 82.4%) related to important LTD, LDMC, and SLW, enabling trees to have important stomata conductance and maintain respectful growth performance. Overall, this comparative study of multiple Eucalyptus species revealed significant variability in their functional and adaptive responses to water deficiency and dry weather conditions, which aids in selecting species that are more appropriate to arid natural environments.

Keywords: Eucalyptus, growth, eco-physiological parameters, plot, aridity

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Introduction

Tunisia, as a southern Mediterranean country, is known for its limited water resources, this has given rise to water scarcity which become noticeable in some areas mainly in arid and semi-arid regions (El Mokh et al., 2021; Boussadia et al., 2023). Erratic rainfall patterns and limited renewable water resources accompanied by an overexploitation of groundwater wealth have worsened the current situation (Melki et al., 2017; Souissi et al., 2022). It is anticipated that by 2050: the average annual temperature will increase by 2.1 °C and a substantial drop in annual rainfall estimated to reach 15% in the southern part of the country (Boughariou et al., 2014; Boughariou et al., 2018). Nevertheless, advances in sylviculture practices have resulted in substantial productivity gains over the past 25 years, but soil water availability coupled with increases heat waves and drought stress still the greatest stressors that affect forests health and trees susceptibility to dieback (Ferraresso Conti Junior et al., 2020).

Eucalyptus (Myrtaceae family) is an important economic and ecological evergreen tree genus in Tunisia that has expanded and developed intensively in the last two decades and is frequently present and cultivated in a diverse spectrum of ecological niches such as in arid regions (Mateus et al., 2021; Chemlali et al., 2022) representing 41397 hectares (ONAGRI, 2019). Its great melliferous value, reforestation interests, uses for dune stabilization, windbreaks, and wayside planting, and ornamental value reinforces the importance of this tree in arid regions (Saadaoui et al., 2022; Chemlali et al., 2022). According to Khouja et al. (2001) and Saadaoui et al. (2022), fifteen species and one hybrid have been cited and identified in those arid zones. It's a fast-growing genus, however in the early stages the plantations are sensitive to abiotic stress and it's common to find high mortality rates and loss of productivity (Garcia et al., 2022). For that, supreme drought could react as an environmental filter for forest species exceptionally sensitive to water deficit, and report a possible ecosystem reorganization (Batllori et al., 2020).

Diversity in Eucalyptus spp. shown an inter and intra-specific variability in responses to stressful conditions specifically drought stress (Amrutha et al., 2019; Saadaoui et al., 2017, 2022; Chemlali et al., 2022). Mechanisms underlying this taxa response to water-deprived conditions have been extensively studied. Correia et al. (2014); Saha et al. (2019) and Amrutha et al. (2019) monitored that exposure to water stress may prompt Eucalyptus clones to develop a combination of responses involving morph-physiological adjustments. However, most of our knowledge comes from short-term studies that ignore longterm acclimatization processes that are especially relevant in this genus (Cano et al., 2014). Combined water deficiency and dry conditions induced perturbation in tree hydraulic status, carbon starvation, and changes in tree above-vs belowground biomass allocation (Maseda et al., 2016). Hence, regulating the stomatal aperture is related to the hydraulic conductance of the leaves, which is a key response to maintain adequate plant water status (Boussadia et al., 2023). These responses restrict the photosynthetic mechanism, particularly the photosystem II (PSII), and contribute to the reduction of photosynthesis of the whole canopy (Edziri et al., 2021). For that, numerous research of Santos et al. (2020) and de Souza Mateus et al. (2022) indicated that those physiological adjustments impair Eucalyptus growth by up to 40%.

The ability of this genus to maintain those physiological processes under drought conditions appears to be an important key to understanding the mechanisms involved in those stressful environments. This raises the question about the value of multispecies studies to shed light on what is adaptive within a given species, even limiting the analysis within a genus. For that, understanding the relationship between eco-physiological traits of local eucalypt plantations appear to be useful tool and easily measurable trait for predicting different species responses to climate change in natural environments.

We therefore concentrated on analyzing the variation in growth and eco-physiological behavior of these eight species grown together in the same micro-habitat. Otherwise, we establish the functional diversity of this collection with a different level of tolerance to combined effects of water deficiency and dry weather aiming at selecting species that are better adapted to those abiotic stresses.

Materials and Methods

Field trials and climatic conditions

The experiment was carried out in a plot in the "Ezzerkine" region belonging to the delegation of Mareth, in the southeast of the governorate of Gabes, Tunisia (33°40'41.39"N, 10°15'32.59"E) at an altitude of 154 m and covers 4030 m². The climate in this region is arid, with low average annual precipitation in 2020 (289 mm), 2021 (212 mm), and in 2022 (50.5 mm; CRDA, 2023). Gabes's geographical position near the Mediterranean coast, the desert, and the Matmata mountain range is behind the unequal and erratic distribution of precipitation patterns. This region is exposed to contrasting climatic influences: the Mediterranean Sea to the east and the Sahara Desert to the south (Abdelkarim et al., 2023). Furthermore, the annual average temperature in this area ranges between 18 and 20 °C respectively, the annual

average evaporation fluctuates between 1500–2000 mm, and the summers are characterized by dry and windy conditions (Mean Winds Velocities exceeding 5 m/s) with high maximum daily temperatures (Jemai et al., 2022).

Experimental design and growth conditions

The experiment setup at our plot represented a complete randomized block design of eight Eucalyptus species (Eucalyptus camaldulensis Dehn., Eucalyptus gomphocephala DC., Eucalyptus torquata Luehm., Eucalyptus microtheca F. Muell., Eucalyptus occidentalis Endl., Eucalyptus diversifolia Bonpl., Eucalyptus sargentii Maiden., and Eucalyptus torwood (Spontaneous hybrid: Eucalyptus torquata × Eucalyptus woodwardii). The plant material (seeds) was initially obtained from the National Institute for Research in Rural Engineering, Water and Forests (INRGREF), sown in April 2017 in polythene bags containing sand (3/4) and manure (1/4) in the nursery of the Agricultural Experimentation Unit, and in December 2017, seedlings were transplanted to an open field. Thus, all eucalypt seedlings (Table 2) were planted in parallel at an inter-row spacing of 7 m and an inter-individual spacing of 6 m. Planting season and spacing were the same for all plants to ensure homogeneity among the sites. All the plants were exposed to arid climatic conditions correlated with a water deficit that has lasted two years since 2020 by full artificial water withdrawal. All measurements were taken during June, July and



Fig. 1. Maps of Tunisia localization, location of the study area (Gabes governorate), and the sampling site around

August of 2022 under a maximum severe water deficit and high temperatures.

Soil sampling and analysis

Soil samples were taken from five soil layers (0–20, 20–40, 40–60, 60–80 and 80–100 cm) using manual helical auger. After that, samples have been enclosed in plastic bags to conserve humidity before taking the appropriate measures of moisture. After that, whole soil samples were air-dried at room temperature, mixed to obtain a homogeneous medium specimen of fine soil, and then sieved through a 2 mm sieve, to eliminate organic fresh residues and gravels. The analyses were carried out according to methods of soil analysis described by Nelson (1982); Nelson & Sommers (1982) and Naanaa & Susini (1988).

The soil moisture was determined using a gravimetric method (or drying method) consisting of determining the moisture content by drying the soil samples in an oven at 65 °C for 24h and measuring the difference between the wet and dry sample weights (Naanaa & Susini, 1988). The soil pH parameter was determined in a 1:5 soil: water suspension (using a pH meter). Furthermore, soil salinity was evaluated by determining the electrical conductivity (EC) at 25 °C on a saturated soil paste using electrical conductivity meter. The content of total calcium carbonate (CaCO₂) was assessed using the Bernard calcimeter method (Nelson, 1982). Total soil organic carbon was determined by the direct colorimetric method after dichromate potassium $(K_2Cr_2O_7)$ and sulfuric acid (H_2SO_4) oxidation (Nelson & Sommers, 1982), and the gypsum content (CaSO₄ $2H_2O$) was evaluated by using a standard method for the determination of sulphate ions (SO₄²⁻), consisting of pretreatment of soil with Barium chloride (BaCl₂) to coat gypsum with BaSO₄ (Naanaa & Susini, 1988). In addition, the soil porosity was evaluated and analyses for mineral content were carried out by ionic chromatography. Those latter parameters were determined according to the methods described by Naanaa and Susini (1988).

Growth assessments

The following in situ growth parameters, including the tree height (cm) was measured from the base to the top of the tree using a graduated ruler with an error of 1 cm. The basal diameter (cm) of each stem were determined at the collar (the base of the trunk) using a vernier caliper. The tree canopy's expansion was assessed by measuring the crown width (cm) using a diameter tape or d-tape, wrapped around the tree, measuring its circumference, the tape is graduated to reflect a conversion from tree circumference to tree diameter. Those measurements were taken during the same summer period. Therefore, to conduct homogenous experiments, we randomly selected three healthy-looking, non-senescent individuals from each species, and the same individuals were used to quantify the following parameters.

Leaf morpho-physiology measurements

Five key morpho-physiological features influencing leaf water economics were determined. At least five fully expanded leaves were used collected from three individuals for each species.

Leaf area (LA; cm²) was determined from scanned and digitized pictures analyzed by Digimizer InK 2014 software (MedCalc Software Ltd, Ostend, Belgium). The other calculated leaf traits were determined according to their respective equations: the specific leaf area (SLA; cm² mg⁻¹) Eq.1 (Datt, 1999); the leaf tissue density (LTD; mg mg⁻¹) Eq.2 (Bacelar et al., 2006); the specific leaf weight (SLW; mg cm⁻²) Eq.3 (Datt, 1999); and the leaf dry matter content (LDMC; mg g⁻¹) Eq.4 (Shipley et al., 2002; Kramp et al., 2022).

$$SLA = LA / DB (Eq. 1)$$
$$LTD = (DB / FB) \times 1000 (Eq. 2)$$
$$SLW = DB / LA (Eq. 3)$$
$$LDMC = DB / FB (Eq. 4)$$

Where LA is the leaf area (cm^2), DB is the dry biomass (g) and FB is the fresh biomass (g).

Plants water relations

Plant water status was characterized by measuring xylem water potential (Ψ_{xylem}) and mid-day leaf water potential (Ψ_{md}). In fact, Ψ_{xylem} has been determined by the method of covered leaves, for which various fully developed leaves have been enclosed in aluminum bags for at least 2 hours before taking the appropriate measures, this prevents leaf water loss and balances the water potential of leaves and xylem (Souden at al., 2020). While Ψ_{md} was assessed from a single mature leaf collected from three randomly selected ramets. Those measurements were taken using the sap pressure chamber method "Scholander" (Model 1000, PMS Instrument Company, Albany, OR, USA) (Scholander et al., 1965).

The leaf water content (WC) provides information about the hydration of vegetal tissues, it was determined according to the method described by Tounekti et al. (2018). Where, FB and DB stand for the fresh biomass and the dry biomass (leaves were oven-dried at 80 °C for 48 h), respectively:

WC (mL
$$H_2O g^{-1}DB$$
) = (FB – DB) / DB (Eq. 5)

The relative water content of the leaf (%RWC) was determined according to the method described by Xu & Leskovar (2015). Leaves were immediately weighed to determine their fresh biomass (FB). Then, brought to saturation overnight at a low temperature (to avoid degradation of the plant material) and placed in distilled water for 4 h and turgid biomass (TB) was recorded. Subsequently, leaves were dried at 60 °C for 72 h and dry biomass was determined (DB). The RWC was deduced as:

RWC (%) =
$$[(FB - DB) / (TB - DB) \times 100]$$

(Eq. 6)

Stomatal conductance

The stomatal conductance (g_s , mol m⁻² s⁻¹) was measured on fully expanded leaves under fully saturating light conditions (2000 μ mol cm⁻² s⁻¹) between 09:30 and 10:30 h, relative humidity of 40–45%, and leaf temperature of 38 ± 2 °C. Measurements were referenced to the abaxial surface of the leaves and were recorded every 30 s using a portable EGM-4 CO₂ Gas Analyzer (Porometer) (PP-SYSTEMS, Amesbury, MA, U.S.A). The measurements were repeated three times for each of the three leaves per tree. A total of three tree per species were used each time.

Fluorescence parameters

The chlorophyll fluorescence was evaluated as previously described (Najar et al., 2019; Aydi et al., 2023), using a fluorometer coupled to an infrared gas exchange analyzer system (IRGA) equipped with a leaf chamber fluorometer (model LI-6400xt, LI-COR Inc., Lincoln, USA) on plants previously adapted to the darkness during 30–40 min using light exclusion clips (FL-DC, Opti-Sciences, Hudson, NH, USA). The evaluation of PSII functioning and its photochemistry variation was estimated by five parameters where F0 (minimum fluorescence), Fm (maximum fluorescence), and Fv (variable fluorescence, equivalent to Fm – F0):

Maximum photochemical quantum yield of PSII (Y):

$$Y = Fv/Fm = (Fm - F0)/Fm$$
 (Eq.7)

- Efficiency of quantum open centers (Just after the transfer of plants in continuous light):

$$\Phi exc = Fv / Fm' = (Fm' - F0') / Fm' (Eq. 8)$$

Proportion of reaction centers open in the PSII:

$$q_p = (Fm' - Fs) / (Fm' - F'0)$$
 (Eq. 9)

NPQ (non-photochemical quenching) (The dissipated energy in heat form):

$$NPQ = (Fm-Fm') / Fm' (Eq. 10)$$

 The quantum yield of electron transport of PSII (estimates the efficiency of all the reaction centers of PSII in the light):

 ϕ PSII = (Fm' – Fs) / Fm' or even ϕ PSII = Φ exc . qP (Eq. 11)

Statistical analysis

Statistical analyses were performed using R software, version R- 4.2.3. The results of the parameters analyzed are presented as mean \pm Standard Deviation (SD) of three independent biological replicates per species. Data were transformed a priori using Z-score normalization, to rescale features to follow standard normal distribution property with mean = 0and SD = 1. A one-factor analysis of variance (ANO-VA) followed by Tukey's post-hoc test for multiple comparisons (where appropriate) was performed to estimate the significance of the results ($p \le 0.05$). The ANOVA was validated graphically a posteriori. For each of the parameters analyzed, correlations were tested using Pearson's correlation coefficient for all parameters. In addition, Linear Discriminant Analysis (LDA) was used to identify the most important variables (group of variables) in discriminating between all species. LDA was performed using appropriate functions from the "MASS" package (Venables & Ripley, 2002). The "dplyr" package (version 1.1.1) was used for data manipulation (Wickham et al., 2023). The "corrplot" package (version 0.84) was used to build a correlogram using the corrplot () function (Wei & Simko, 2017).

Results

Physico-chemical properties of soil

As shown in Table 1, soil moisture increases with depth from 1.95% in the surface layer to 6.77% between 80–100 cm. For all parameters, the soil was considered poor, organic carbon content did not exceed 2.00% (between 0.18 and 0.94%). Additionally, the investigated soils were found to be alkaline. Soil pH ranged between 8.48 and 8.78, there

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Soil depth								
(0-20 cm) $(20-40 cm)$ $(40-60 cm)$ $(60-80 cm)$ $(80-1)$								
Moisture (%)	1.95	3.56	4.88	6.45	6.77			
рН	8.48	8.77	8.77	8.78	8.76			
E C (dS.m ⁻¹)	4.96	9.13	7.82	7.85	8.85			
Soil organic carbon (%)	0.94	0.69	0.49	0.41	0.18			
Porosity (%)	43.62	45.63	43.61	46.67	40.24			
CaCO3 (%)	14.95	12.28	8.24	8.59	8.24			
Gypsum (%)	45.61	56.28	55.27	71.56	49.02			
Mg (ppm)	117.8	229.72	204.96	225.35	196.67			
Ca (ppm)	896.4	829.36	728.01	733.08	663.74			
Sulphate (ppm)	1489.18	1652.66	2154.3	2399.59	2264.85			
Cl (ppm)	932.18	1581.86	1453	1450.1	1316.77			
Na (ppm)	454.93	1017.2	1082.56	1074.27	854.75			

Table 1. Physico-chemical properties of soil in the study site

was no difference across depth. Hence, soils appear formed by highly considerable amounts of gypsum which ranged from 45.61 to 71.56% with moderate contents of calcium carbonate ranged between 8.24 and 14.95%, respectively. This has been shown by the predominance of sulphate ions levels followed by important levels of calcium, chlorine, and sodium ions (Table 1). These features were accompanied by an important soil porosity that exceed 40% and an important level of electrical conductivity (4.96–9.13 dS m⁻¹).

Growth traits and allometry

Unfavorable soil properties, abiotic conditions, and water stress were statistically significant with respect to plant growth variables. As depicted in Table 2, *Eucalyptus* spp. presented a variation in growth rate. Results of ANOVA indicated significant differences between them considering all growth parameters (P < 0.05).

It was stated that *E. camaldulensis* and *E. occidentalis* had the greatest heights of 380 ± 1 cm and 356.67 ± 49.33 cm. However, there were slight differences in comparison to *E. sargentii, E. torwood,* and *E. torquata*

with an average height ranging between 255 to 308 cm, while the lowest ones were intended for *E. microtheca*, *E. diversifolia*, and *E. gomphocephala* (Table 2).

Variance analysis results revealed also that basal diameter showed highly significant differences between studied species ($P = 1.15e^{-07}$), as well as crown width ($P = 6.51e^{-05}$). *E. torquata* was found to have the largest basal diameter of 21 ± 2.65 cm, which was related to its important crown width of 293.33 \pm 30.55 cm along with *E. camaldulensis*. However, *E. microtheca* had the lowest values for both parameters. A convergence trend has also been observed among *E. torwood, E. occidentalis, E. sargentii, E. diversifolia, E. gomphocephala* based on those two parameters, values varied between 10.33 and 26.67 cm for basal diameter and between 366.67 cm and 256.67 cm for crown width (Table 2).

Changes in leaf morph-physiological proprieties

Influences of severe conditions on leaf trait relationships were varied, results showed substantial patterning. Concerning leaf area, fresh biomass,

Table 2. The	performance of	eight Euca	<i>lyptus</i> species'	growth traits u	nder long	-term water o	leficit treatments
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Eucalypt species	Mortality rate (%) —	Plant Morphological Traits					
		Height (cm)	Basal Diameter (cm)	Crown Width (cm)			
E. torquata	24	$255 \pm 30.42b$	21 ± 2.65c	293.33 ± 30.55c			
E. camaldulensis	0	380 ± 1.00c	$25\pm0.05b$	$4.53e+02 \pm 81.85c$			
E. occidentalis	7	356.67 ± 49.33c	22 ± 1.00b	$366.67 \pm 47.26b$			
E. sargentii	0	$308.33 \pm 80.98b$	$18.67 \pm 4.93b$	$330 \pm 17.32b$			
E. microtheca	0	$159.33 \pm 14.01a$	7 ± 1.00a	$116.67 \pm 30.55a$			
E. flocktoniea	100	0	0	0			
E. diversifolia	11	161.67 ± 10.41a	$10.33 \pm 0.58b$	240 ± 10b			
E. torwood	0	$278.33 \pm 46.46b$	$26.67 \pm 3.22b$	$491.67 \pm 65.26b$			
E. gomphocephala	0	178.33 ± 7.64a	$13.33 \pm 0.58b$	$256.67 \pm 30.55b$			
P-value		1.12e-04***	1.15e-07***	6.51e-05***			

Note: Mean ±SD. Different lower-case letters in the same column indicate significantly different values at $p \le 0.05$ levels according to the post-hoc multiple comparisons Tukey test. Level of significance: *** P < 0.01, ** 0.01 ≤ P ≤ 0.02 and * P ≤ 0.05.

	E. torquata	E. camaldulensis	E. occidentalis	E. sargentii	E. microtheca	E. flocktoniea	E. diversifolia	E. torwood	E. gomphocephala	P-value
LA (cm ²)	11.34 ± 1.16b	8.95 ± 1.21b	14.91 ± 0.65b	11.40 ± 1.43b	7.19 ± 2.37b	0	26.56 ± 5.39c	16.38 ± 1.49b	6.15 ± 0.86a	2.34e-07 ***
FB (g)	0.99 ± 0.17b	0.46 ± 0.07b	1.18 ± 0.04b	0.87 ± 0.18b	0.44 ± 0.17b	0	2.64 ± 0.52c	1.71 ± 0.21b	0.22 ± 0.04a	1.14e-08 ***
DB (g)	0.53± 0.06b	0.28 ± 0.02b	0.70 ± 0.02 b	0.50 ± 0.12b	0.27 ± 0.06b	0	1.51 ± 0.27c	1.05 ± 0.09b	0.17 ± 0.07a	2.67e-09 ***
SLA (cm ² mg ⁻¹)	0.02 ± 2.27e-03a	0.03 ± 1.45e-03b	0.02 ± 0.01e-02b	0.02 ± 3.41e-03b	0.03 ± 2.51e-03b	0	0.02 ± 6.66e-04b	0.02 ± 3.22e-04b	0.05 ± 5.21e-03c	8.32e-10 ***
LTD (mg mg ⁻¹)	607 ± 13.21c	591 ± 6.99b	592 ± 16.47b	602 ± 20.71b	562 ± 28.35a	0	592 ± 13.71b	623 ± 14.29b	597 ± 17.79b	0.04 *
SLW (mg cm ²)	52.75 ± 6.21b	29.90 ± 1.37b	48.15 ± 0.57b	45.25 ± 6.39b	34.18 ± 2.80b	0	58.82 ± 2.10b	64.47 ± 1.42b	21.45 ± 2.56a	8.55e-10 ***
LDMC (mg g ⁻¹)	607 ± 13.22c	591 ± 6.10b	592 ± 16.48b	602 ± 20.72b	562 ± 28.36a	0	592 ± 13.71b	623 ± 14.29b	597 ± 17.78b	3.77e-02 *

Table 3. The effects of long-term water deficit treatments on leaf morph-physiological proprieties of the eight *Eucalyptus* species

Note: Mean \pm SD. Different lower-case letters in the same column indicate significantly different values at p \leq 0.05 levels according to the post-hoc multiple comparisons Tukey test. LA – Leaf area; FB – fresh biomass; DB – dry biomass; SLA – Specific leaf area; LTD – Leaf tissue density; SLW – specific leaf weight; LDMC – Leaf dry matter continent. Level of significance: *** P < 0.01, ** 0.01 \leq P \leq 0.02 and * P \leq 0.05.

and dry biomass measurements, statistical analysis highlighted significant differences among the eight studied species with ($P = 2.34e^{-07}$; $P = 1.14e^{-08}$ and $P = 2.67e^{-09}$, respectively). *E. diversifolia* developed bigger leaves and presented the highest values for each parameter (26.56 cm²; 2.64 g; 1.51 g), while, *E. gomphocephala*' leaves were the smallest ones and recorded the lowest values (6.15 cm²; 0.22 g; 0.17 g).

Furthermore, the leaf tissue density parameter showed convergence between *E. camaldulensis, E. torquata, E. occidentalis, E. sargentii, E. diversifolia and E. gomphocephala* species, values varied between 591 to 607 mg mg⁻¹. *E. torwood* exhibited the largest values of 623 ± 14.3 mg mg⁻¹, while, *E. microtheca* expressed the lowest values of 562 ± 28.4 mg mg⁻¹. Additionally, we also noticed convergence in the leaf dry matter content among the studied species, values ranging between 591 to 623 mg g⁻¹. Likewise, the specific leaf weight showed the same trend of convergence between species excepting *E. gomphocephala* that indicated the lowest values of 21.45 \pm 2.56 mg cm² (Table 3).

In depth, we found that specific leaf area (SLA) parameter was the most affected one, showed significant differences between *Eucalyptus* species ($P = 8.32e^{-10}$). We also, noticed that *E. gomphocephala* recorded the highest average of SLA (0.05 ± $5.21e^{-03}$ cm² mg⁻¹), while *E. torquata* showed the lowest one ($0.02 \pm 2.27e^{-03}$ cm² mg⁻¹). *E. torwood, E. occidentalis, E. sargentii, E. diversifolia* have all shown convergence, values vary between 0.02 to 0.03 cm² mg⁻¹ (Table 3).

Chlorophyll Fluorescence (CF) Parameters

Average summer values of Fv/Fm, ϕ PSII, and qP typically known as photochemical quenching parameters, showed insignificant differences between *Eucalyptus* species (*P* = 0.11 for Fv/Fm, *P* = 0.82 for ϕ PSII and *P* = 0.89 for qP). Fv/Fm presented inter-specific variabilities. It was high in *E. microtheca* (0.81 ± 0.02) and weak in *E. torquata* (0.66 ± 0.11). The photosynthetic machinery was less affected by aridity in the former species than in the last one. In studied *Eucalyptus* species, ϕ PSII values ranged between 0.38 and 0.53, while qP values ranged between 0.41 and 0.89. This suggests that electron transport from PSII (qP) through photochemical processes has increased because of stressful conditions.

Significant inter-specific differences were detected for ϕ exc and NPQ parameters (P = 0.03 and $P = 1.2 e^{-03}$). The highest values of ϕ exc (0.65) appeared in *E. gomphocephala* and *E. microtheca*. The non-photochemical quenching (NPQ) describing the loss of quantum yield as heat form showed highly significant difference. Values of NPQ (0.06) showed that *E. camaldulensis* emitted more heat than other species, but *E. microtheca* released the lowest heat (2.23 e^{-02}) (Table 4).

Table 4. The effects of long-ter	m water deficit treatments	on Chlorophyll Fluorescence	(CF) parameters in the eight Eu-
calyptus species			

Eucalypt species	Fv/F m	фехс	qP	φPSII	NPQ
E. torquata	0.66 ± 0.11	$0.57 \pm 0.04a$	0.67 ± 0.12	0.38 ± 0.08	$4.53e-02 \pm 4.93e-03b$
E. camaldulensis	0.71 ± 0.05	$0.56 \pm 0.07a$	0.71 ± 0.51	0.42 ± 0.31	$0.06 \pm 0.02c$
E. occidentalis	0.71 ± 0.01	$0.53\pm0.05b$	0.77 ± 0.17	0.41 ± 0.07	$4.73e-02 \pm 5.51e-03b$
E. sargentii	0.77 ± 0.04	$0.54 \pm 0.05b$	0.81 ± 0.04	0.44 ± 0.03	$0.04 \pm 0.01 b$
E. microtheca	0.81 ± 0.02	$0.65 \pm 0.03a$	0.74 ± 0.05	0.48 ± 0.02	$2.23e-02 \pm 0.02a$
E. flocktoniea	0	0	0	0	0
E. diversifolia	0.78 ± 0.01	$0.6 \pm 0.06a$	0.89 ± 0.06	0.53 ± 0.02	$0.04 \pm 0.01 b$
E. torwood	0.74 ± 0.08	$0.57 \pm 0.01a$	0.71 ± 0.06	0.40 ± 0.04	$3.27e-02 \pm 2.89e-03b$
E. gomphocephala	0.73 ± 0.04	$0.65 \pm 0.02a$	0.71 ± 0.01	0.47 ± 0.02	$2.67e-02 \pm 3.51e-03b$
P-value	0.11	0.03 *	0.89	0.82	1.23 e-03 **

Note: Mean ±SD. Different lower-case letters in the same column indicate significantly different values at P \leq 0.05 levels according to the post-hoc multiple comparisons Tukey test. Fv/Fm, Maximum photochemical quantum yield of PSII; ϕ exc, Efficiency of quantum open centers; qP, Proportion of reaction centers open in the PSII; ϕ PSII, The quantum yield of electron transport of PSII; NPQ, non-photo-chemical quenching. Level of significance: *** P < 0.01, ** 0.01 \leq P \leq 0.02 and * P \leq 0.05.

Plant water relations and stomatal conductance

Withholding irrigation affected plant water status. A highly negative xylem water potential Ψ_{xylem} and Mid-day leaf water potential (Ψ_{md}) were observed in almost all studied *Eucalyptus* spp. Recorded responses were nearly identical for both parameters, but variance analysis results revealed significant differences between the eight species with a P-value < 0.05 ($P = 1.14e^{-05}$ for Ψ_{xylem} ; $P = 7.23e^{-4}$ for Ψ_{md}). Table 5 shows that *E. occidentalis* exhibited the

Table 5 shows that *E. occidentalis* exhibited the most negative values of -5.26 ± 0.22 MPa for $\Psi_{x.}$ and -5.80 ± 0.43 MPa for Ψ_{md} . Whereas, *E. sargentii* showed the lowest negative values for Ψ_{xylem} about -3.87 ± 0.20 MPa and -4.40 ± 0.06 MPa. For the other species, values ranged between -4.83 to -5.11 MPa for Ψ_{xylem} and between -4.77 to -5.53 MPa for Ψ_{md} .

Also, significant differences in RWC results (P = 0.02) indicated that *E. diversifolia* exhibited the lowest values of relative water content around 61.63% compared to the other species that have all shown convergence trend, with values varying between 71.50% to 82.36%.

Furthermore, evaluation of leaves tissue hydration results which was estimated by the water content (WC) demonstrated that: notwithstanding the negative water potential, the current collection of *Eucalyptus* was able to preserve the hydration of their leaves tissues concerning water deficiency. Note that no significant differences have been found between the eight species (P = 0.72), values fluctuated between 0.66 to 0.80 mL H₂O. g⁻¹ DB.

Concerning the stomatal function controlling gas exchanges, the mean values of g_s measured during the three summer months coinciding with the most dry-hot period in the region showed inter-specific variabilities between the studied *Eucalyptus* species ($P = 3.10e^{-05}$). *E. camaldulensis* represented the lowest g_s (17.59 mol m⁻² s⁻¹), whereas *E. sargentii* exhibited the highest g_s values (32.17 mol m⁻² s⁻¹). In the other species, g_s oscillated from 19.43 to 25.97 mol m⁻² s⁻¹. It seems that *E. camaldulensis* accomplished an efficient stomatal regulation reducing water lost by transpiration under hot-dry conditions.

Table 5. Effect of long-term water deficit treatments on physiological parameters of the eight Eucalyptus spp. studied

	-			-	
Eucalypt species	gs (mol m ⁻² s ⁻¹)	Ψxylem (Mpa)	Ψmd (Mpa)	RWC (%)	WC (mL $H_2O. g^{-1} DB$)
E. torquata	$19.43 \pm 2.51b$	$-4.51\pm0.04b$	$-4.9\pm0.03b$	$80.89 \pm 5.45b$	0.79 ± 0.03
E. camaldulensis	$17.59 \pm 1.88a$	$-4.39 \pm 0.29b$	$-4.74 \pm 0.27b$	$78.8\pm1.93b$	0.80 ± 0.09
E. occidentalis	$20.93 \pm 4.44b$	$-5.26 \pm 0.22a$	$-5.8 \pm 0.43a$	$71.53 \pm 2.11b$	0.69 ± 0.15
E. sargentii	32.16 ± 1.01c	$-3.87 \pm 0.20c$	$-4.40 \pm 0.06c$	$82.36 \pm 7.57b$	0.72 ± 0.20
E. microtheca	$19.34 \pm 3.70b$	$-4.46 \pm 0.32b$	$-4.77 \pm 0.44b$	$74.87 \pm 6.15b$	0.90 ± 0.03
E. diversifolia	$25.97 \pm 2.10b$	$-4.18\pm0.08b$	$-4.52 \pm 0.13b$	$61.63 \pm 10.84a$	0.66 ± 0.12
E. torwood	$21.34 \pm 0.45b$	$-5.11 \pm 0.22b$	$-5.53 \pm 0.07b$	$78.11 \pm 7.03b$	0.70 ± 0.16
E. gomphocephala	$18.34 \pm 0.41b$	$-4.83 \pm 0.23b$	$-5.04 \pm 0.58b$	72.11 ± 5.11b	0.81 ± 0.12
P-value	3.1e-05	1.14e-05	7.23e-04	0.02	0.72

Note: Values represent the means \pm SD. Different lower-case letters in the same column indicate significantly different values per $P \le 0.05$ between the eight species for the different treatments.

Relationships between *Eucalyptus* species' structural attributes and functional traits

To illustrate the relationships between variables, a Pearson's correlation matrix was employed to examine the leaf morphophysiological characteristics and tree steam growth parameters of the studied species (Fig. 2).

Thus, there were significant positive correlations between growth parameters: crown width was highly correlated with tree height (r = 0.76; $P = 5.17e^{-06}$) and with basal diameter (r = 0.87; $P = 1.57e^{-12}$). Conversely, SLA had strongly negative correlations with all the leaf traits except for WC (r = 0.67). While the other evaluated leaf parameters exhibited both positive and negative results (Fig. 2).

For, chlorophyll fluorescence parameters, ϕ PSII was significantly positively correlated with all the evaluated parameters except for NPQ (r = -0.34; $P = 9.91e^{-02}$). Fv/Fm ratio was highly positively correlated with qP (r = 0.58; $P = 2.61e^{-03}$), ϕ PSII (r = 0.76; $P = 1.90e^{-05}$) but the NPQ parameter showed strong negative correlations with all the distinct CF parameters.

Relationships between functional and structural traits showed that the plant water status and leaf traits were negatively correlated with chlorophyll fluorescence parameters, except those significant positive relationships that were found between the g_s and qP (r = 0.68; $P = 8.91e^{-05}$), and between Ψ_{md} and ϕ PSII (r = 0.46; $P = 1.04e^{-02}$). Indeed, physiological parameters showed negative correlations with growth traits, only positive correlations were detected between crown width, tree heights, and RWC (r = 0.45; r = 0.47). Furthermore, growth, fluorescence parameters, and leaf traits were found to be negatively correlated. Nevertheless, significant positive correlations were detected between Crown width, NPQ (r = 0.67), RWC (r = 0.48) and LDMC (r = 0.71).

LDA analysis with two axes (LD1 and LD2, where LD is the linear discriminant) explained most of the observed variability (77%) in the analyzed data. The first LD1 (axis 1) accounted for 52% of the total inertia followed by 25% for the second LD2 (axis 2). In general, the results of the LDA analysis of all leaf morph-physiological, steam growth parameters traits, and canopy features assessed in various *Eucalyptus* species were useful tool in establishing a clear distinction between them (Fig. 3).

LD1 axis was the most significant, it showed positive correlations for the variables related to the eight eucalypt species, including leaf fresh biomass, SLA, leaf tissue density, steam height, canopy feature (crown width), chlorophyll fluorescence parameters (Fv/Fm, ϕ exc, qP), and g_s . The LD2 axis showed positive correlations for the variables including leaf area, dry leaf biomass, SLW, LDMC, water species status (Ψ_{xylem} and Ψ_{md}), RWC, WC, ϕ PSII, NPQ, and basal diameter.



Fig. 2. Correlation analysis was performed among the growth, CF, and leaf morph-physiological traits to determine the direction and magnitude of parameters, regardless of the treatment long-term water deficit in *Eucalyptus* species. Blue and red boxes represent positive and negative correlation, respectively. Color intensities are proportional to the correlation coefficients, as shown in the legend to the right



Fig. 3. Linear discriminant analysis (LDA). Biplot between LD1 and LD2 shows the variability and the segregation of the eight *Eucalyptus* species under water stress conditions

EG – Eucalyptus gomphocephala; EM – Eucalyptus microtheca; EC – Eucalyptus camaldulensis; EO – Eucalyptus occidentalis; E.T – Eucalyptus torquata; E.t – Eucalyptus torwood; ES – Eucalyptus sargentii; ED – Eucalyptus diversifolia.

Based on the variability of all the eco-physiological parameters the LD1 and LD2 axes allowed the separation of those species into five groups (Fig. 3). The first one was composed of the E. diversifolia which was situated on the positive side of the LD1 and LD2 axes. The second group was composed of E. camaldulensis, located on the negative side of the LD1 and LD2 axes. The third group included both E. torquata and E. sargentii species and were located on the positive side of the LD1 axis and on the negative side of the LD2 axis. The fourth one regrouped E. torwood and E. occidentalis which was situated on the positive side of the LD1 and LD2 axes. The last group included the *E. microtheca* and *E. gomphocephala*, both species were placed on the negative side of LD1, whereas E. microtheca located on the positive side of LD2 and E. gomphocephala was located on the negative side of LD2.

Discussion

Water is the primary factor affecting tree growth rate and directly impacts the *Eucalyptus* genus's productivity (Reis et al., 2021). Our research was done in 2022, it was a dry year marked by low rainfall rates of around 50.5 mm (mentioned above) and this reinforced the aridity of our plot, which could be attributed to the interplay of various meteorological and environmental factors that have seriously affected precipitation patterns.

The obtained physical and chemical assessments of our soil study area were like those reported in previous soil studies in semi-arid regions of Tunisia (Omar et al., 2017) and particularly in the Gabes region (Bouajila et al., 2021), pointed out a poor soil property. Soil alkalinity can be explained by the high content of the gypsum crust (Boulbaba et al., 2012). According to Bouajila et al. (2021), the presence of high gypsum content and CaCO₃ in the arid soils of Tunisia was linked to the alteration of the geological base material. Furthermore, our results highlighted a significant electrical conductivity, these findings are consistent with those of Hihi et al. (2019) indicated that the southern part of Tunisia has a widespread problem of soil salinity, specifically in the arid and semi-arid areas mainly in the 'Gabes region' where soil salinity poses a serious threat. Studies of Omar et al. (2017) indicated also that Tunisian's arid soils are characterized by lower organic carbon stocks, and this confirm our findings.

So, the evaluation of the combined effects of water deprivation and poor soil certainly provides a variety of responses. Thus, it was previously elucidated that the different *Eucalyptus* species distinctly respond to those abiotic stressors. *E. gomphocephala, E. diversifolia,* and *E. microtheca* trees were significantly smaller and had less potential to grow. In this sense, E. microtheca displays the lowest height, which is related to a small crown width and thin steam. Although, E. gomphocephala, and E. diversifolia were only able to decrease their height and keep the important basal stem diameter related to relative crown width. Hence, reduction in growth and biomass under water stress conditions has been widely reported in other species of this genus, such as with Eucalyptus tereticornis Smith., (Amrutha et al., 2019), E. globulus Labill., (Correia et al., 2014) and E. microtheca (Susiluoto et al., 2007). Conversely, E. camaldulensis and E. occidentalis were the most performant species in our plot in terms of stem growth and canopies features followed by E. torquata, E. torwood, and E. sargentii. These observations are a key factor in estimating the strength of the five previously mentioned species that were able to sustain growth and support a high level of gypsum while experiencing prolonged water deprivation.

According to Singh et al. (2020), variations in growth potential among the *Eucalyptus* genus can be attributed to their differential genetic makeup and the development of a deep root system. It has been revealed by Joshi and Palanisami (2011), Matos et al. (2016), Amrutha et al. (2019) and Chemlali et al. (2022) studies, that in dry areas, Eucalyptus species frequently display an adaptive response, resulting in a gradual increase in their root-to-shoot ratio, this is since plants prioritize minimizing the development of their aerial parts while investing in their root systems. Their ability to withstand dry conditions is enhanced by the development of large root systems; the roots of this genus may extend up to 6–9 meters to optimize their capacity to absorb water and nutrients. Otherwise, minimizing their above-ground biomass results in decreased water loss through transpiration, which in turn contributes to their survival in dry climates.

Despite E. microtheca and E. diversifolia's low growth, extensive other research agreed on their resistance to drought. Results of Susiluoto et al. (2007) and Amrutha et al. (2019) demonstrated that E. microtheca uses an allocation between its foliage and roots as the key mechanism to survive and avoid the destructive effects of drought, whereas changes in photosynthesis and stomatal conductance under water stress were rather modest. These results were following our findings, this species showed moderate physiological responses in favor of its growth performance. As well as, for *E. diversifolia* it has been found that this one develops drought-avoidance features such as a small canopy, multiple smooth stems, simple leaves, extensive lignotuber, and a deep root system (Swaffer et al., 2014).

More broadly, computation of the leaf fresh biomass, leaf area, specific leaf area (SLA) leaf dry matter content (LDMC), specific leaf weight (SLW) and leaf tissue density (LTD) hold for a broad range of leaf thickness and leaf water economics encountered in those species from different growth forms. The SLA was low in all eucalypt species and showed a significant negative correlation with the other leaf trait parameters. The SLA in eucalypt forests is impacted by age, water shortage, and nutrients, as indicated by the results of Nouvellon et al. (2010). We noted that only trees of *E. gomphocephala* yielded small leaves with important values of SLA compared to other species, which was substantiated by small leaf area and lower leaf fresh biomass. On the flip side, leaves of *E. diversifolia* were heavier in terms of fresh biomass and larger, boasting a significant leaf area. Nonetheless, leaf area expansion was lower in E. camaldulensis, E. torquata, E. microtheca, E. occidentalis, E. diversifolia, E. sargentii and E. torwood. Along with, we found that treated species displayed convergence response patterns in terms of LDMC, SLW, and LTD, but the degree of statistical significance varied among them. The lowest SLW values are attributed to E. gomphocephala, however, E. microtheca showed the lowest values related to LDMC and LTD parameters.

As indicated by Xu et al. (2012) study, the leaves of woody plants grown at higher temperatures were thinner: this pattern is caused by a reduction in the thickness of the palisade, spongy, and epidermal layers which is linked to a restriction in leaf mesophyll cells size due to an earlier maturation, this may be beneficial in minimizing the need for transpiration while maintaining of an adequate thermal balance. Xu et al. (2012) also proved that the thickness of Eucalyptus saligna Smith., leaves decreased as a response to dryness due to a reduction in the mesophyll cell size. More than that, according to another study of Gibson et al. (1991) conducted with E. camaldulensis seedlings in dry tropics, it was suggested that the seedlings are more reliant on reducing the leaf area to conserve water, thus, all those outcomes are consistent with our results.

In light of those findings, plant water status greatly decreased and showed a parallel trend for all the treated species. Our results indicated that E. occidentalis exhibited the most negative levels for both xylem (Ψ_{xvlem}) and mid-day leaf water potential (Ψ_{md}) parameters followed by the other studied species, however, E. sargentii showed the lowest negative values. Furthermore, differences in the productivity of leaves, as measured by stomatal conductance (g_{i}) , relative water content (RWC), leaf water content (WC), and chlorophyll fluorescence (CF) highlighted: E. sargentii maintained relatively the highest gs levels, but the lowest values are mainly found in *E*. camaldulensis (Table 5). This may be explained by the fact that E. camaldulensis trees close early stomata to limit transpiration and preserve the internal water

status. While a modest effect of water stress on g_s was detected for the other species, this led us to predict that *Eucalyptus* trees have been slow to close their stomata as well as their ability to maintain meaningful levels of RWC and WC as the water deficit intensified. Hence, research conducted by Ranjbar et al. (2021) emphasized that genotypes that can maintain their hydraulic status without closing their stomata during drought stress are better suited for arid areas. Bearing in mind that mesophyll anatomical differences in leaves may affect g_s (Rahimi-Eichi et al., 2014), and the differences between those eucalypt species could be linked to the compact arrangement of their mesophyll cells (Ranjbar et al., 2021).

As well, our findings revealed that this group of species, mainly *E. camaldulensis, E. occidentalis, E. torwood, E. torquata, E. sargentii, E. gomphocephala,* and *E. microtheca,* were able to sustain theirs leaves succulence and tissues hydration by exhibiting consistent RWC and WC levels. While leaves with moderate levels were observed with *E. diversifolia.* This reflects the water stocking capability of eucalypt leaves, which can be used to maintain cell metabolism during drought conditions (Reis et al., 2021). Tounekti et al. (2018) also highlighted the contrasting abilities of such species to re-saturate their foliar tissue overnight (daily re-saturation ability) and this could explain some of those behaviors in stressful situations.

Accordingly, the outcomes we achieved are in line with those found in a comparative study conducted by Zhou et al. (2016) that investigated the effects of drought stress on two young eucalypt species *E. camaldulensis* and *E. occidentalis*. They discovered that during two months of partial drought, both species' photosynthetic processes were more negatively affected. Even so, after four months, their reactions have not changed, suggesting that photosynthetic acclimation including hydraulic adjustments to minimize water losses was permitted during that period.

Additionally, CF are sensitive physiological markers in plants and may provide a deeper reflection of photosynthetic disturbances, the maximum photochemical quantum yield of PSII (Fv/Fm) is regarded as a specific intrinsic indicator of the clear photosynthesis' response at the level of the PSII and a good biomarker for the selection of drought tolerant species (Reis et al., 2021; León-Burgos et al., 2022). We found that Fv/Fm ratio varied between studied *Eucalyptus* species. Under the dry-hot conditions, the lowest values of Fv/Fm were recorded in E. torquata suggesting the damage of its photosynthetic machinery. However, Fv/Fm was high in E. camaldulensis and E. microtheca suggesting their high resistance to aridity. These findings corroborate with those previously reported by Souden et al. (2020). As well as our results also concur with the findings of Susiluoto et al. (2007) who even found an increase of Fv/Fm

ratio with E. microtheca stressed seedlings, this species increased their uptake of quanta during drought stress but exhibited weaker heat dissipation (NPQ) and photo-inhibition. Electron transport from PSII (qP) through photochemical processes provides also important values with stress (Table 4), strengthened by extensive photo-protection that took place. At the same time, the maximum efficiency of Photosystem II (dexc) was maintained unchanged under longterm water deficit, but a high value was detected for E. gomphocephala and E. microtheca. Results of the photochemical quenching coefficient showed that PSII reaction centers were progressively closed with prolonged water stress. Non-photochemical quenching (NPQ), describing the loss of quantum yield as heat was approximately modest for almost of species and illustrated that E. camaldulensis emits more heat than the other species but E. microtheca exhibited weaker heat dissipation.

Chandra (2004) pointed out that thinner leaves tend to have higher thermal conductivity which leads to improved leaf heat dissipation. As a matter of fact, under drought stress conditions, the leaf mostly transforms solar energy received for electron transfer and phosphorylation into heat (Reis et al., 2021). This study confirmed that chlorophyll fluorescence changed as the water deficiency got worse and the soil dried out. Φ PSII and qP increase with Fv/Fm, indicating chronic photo-protection influencing the photosynthetic productivity instead of reversible photo-inhibition. To sum up, the LDA results validated all these outcomes of convergence/ divergence results across the eight eucalypt species.

Conclusions

The comparative approach between the eight eucalypt species revealed considerable heterogeneity and important distinctions between their functional-adaptive responses. Species reacted differently to severe water deficit conditions with distinct growth patterns manifested by slow or limited growth of aerial parts of some species, compared to noticeable continuous growth for others. Wide modifications in leaf features tend to minimize leaf area expansion and thickness. More deeply, the physiological machinery was protected through extensive photo-protection, limited stomatal conductance, and maintaining leaves succulence and tissue hydration which help trees to preserve their internal water status. Matching the general findings allows us to conclude that E. camaldulensis, E. torquata, E. sargentii, E. occidentalis, and E. torwood were able to maintain high edaphic-climatic suitability with an important growth performance related to a good physiological functionality of the leaves. However, E. microtheca, E. *gomphocephala*, and *E*. *diversifolia* were less efficient in terms of growth, but their leaf trait physiological adaptations highlighted their ability to survive under severe conditions. An ongoing complement research that focuses on the wood growth and anatomy performance of the same collection of eucalypt species, will provide fruitful confirmation of the current results.

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