Wood anatomy and tree-ring stable isotopes indicate a recent decline in water-use efficiency in the desert tree *Moringa peregrina*

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Abstract

The ability of desert plants to adapt to future climate changes and maximize their water-use efficiency will determine their survival. This study uses wood anatomy and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotope analyses to investigate how *Moringa peregrina* trees in the Egyptian desert have responded to the environment over the last 10 years. Our results show that *M. peregrina* tree-ring widths (TRWs) have generally declined over the last decade, although individual series are characterized by high variability and low Rbars. Vessel lumen area percentages (VLA%) are low in wet years but increase significantly in dry years, such as the period 2017–2020. Stable $\delta^{13}\text{C}$ isotope values decrease between 2010 (-23.4‰) and 2020 (-24.9‰), reflecting an unexpected response to an increase in drought conditions. The mean $\delta^{18}\text{O}$ value ($\pm$ standard error, SE) for the first ten rings of each tree from bark to pith (2020–2010) is $33.0\text{‰} \pm 0.85$ with a range of 29.2–36.3‰, which indicates a common drought signal. The intrinsic water-use efficiency (iWUE) declines gradually with time, from $130.0\text{ }\mu\text{mol mol}^{-1}$ in 2010 to $119.4\text{ }\mu\text{mol mol}^{-1}$ in 2020. The intercellular carbon concentration (C_i) and C_i/C_a ratio increase over the same period, likely as a result of decreasing iWUE. The results show that *M. peregrina* trees seem to cool their leaves and the boundary air at the cost of saving water.

Keywords Wood anatomy · Isotopes · Drought stress · *Moringa peregrina* · Water-use efficiency

Introduction

The global extent of arid habitats is expected to increase in the coming decades as a result of rising global air temperatures and shifting precipitation patterns (Huang et al. 2016). The expected increase in drought conditions represents a significant challenge to plant species growing in semiarid and arid environments, as increasing drought will exacerbate their already tenuous existence. Drought stress affects many physiological processes in desert plants like *Moringa peregrina* (Forssk.) Fiori (Moringaceae), a tree species that is well-adapted to the harsh conditions of the Red Sea region, Arabia, and the Horn of Africa (Boulos 1999). The species is a drought-tolerant C_3 plant, multi-stemmed desert tree with high medicinal and ecological value. The

seeds of *M. peregrina* can be used to purify water because they contain a protein that coagulates dispersed particles (Moulin et al. 2019; Nouhi et al. 2019). The seeds are also a source of a high-quality oil, which is used both locally and by the cosmetics industry. There is growing concern about the potential decline of *M. peregrina* in desert regions, both because it is frequently collected for firewood and because increased drought is likely to negatively impact its propagation. The tree grows predominantly on rocky gorge slopes and in valley beds in arid and semi-arid environments (Olson and Carlquist 2001). Due to its frequent occurrence on gorge slopes, this multi-stemmed plant may be negatively impacted by mechanical stresses resulting from increasingly frequent and severe erosion events.

Under drought conditions, plants use stomatal closure to prevent xylem embolism at the cost of not being able to photosynthesize (Sevanto et al. 2014). Extended stomatal closure can result in low overall growth rates, a reduction in xylem formation, and eventual biomass accumulation (Olano et al. 2014; Schuldt et al. 2016) which, for some species, could even shift towards an increased metabolic activity in roots at the expense of the above ground part

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of the plant (Gargallo-Garriga et al. 2014). At the same time, the cambium in the earlywood of desert plants produces more numerous but smaller vessels to facilitate conductivity while minimizing hydraulic failure (Galle et al. 2010; Farahat and Gärtner 2019). Gebauer et al. (2020) recorded a decrease in the cambial activity of drought-stressed European beech, which resulted in lower stem biomass, reduced tree-ring width, and fewer vessels in the tree ring, which were also smaller in size. Thus, wood anatomical traits such as vessel lumen area and wall thickness are useful traits for assessing long-term tree responses and adaptation to drought stress (Fonti et al. 2010).

Although they have rarely been applied in desert ecosystems, tree-ring carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotope ratios can also provide a valuable record of past gas exchange and help in understanding the water-use dynamics of trees in response to environmental conditions (Choury et al. 2017; Santiago et al. 2017; Rahman et al. 2019a, b; Olson et al. 2020). The value of $\delta^{18}\text{O}$ in wood cellulose depends on the $\delta^{18}\text{O}$ value of the source water, the isotope enrichment of leaf water due to transpiration, and cellulose formation (McCarroll and Loader 2004; Treydte et al. 2014). Relatively high $\delta^{18}\text{O}$ values are usually observed in conjunction with drought conditions, but changes in the root activity from shallow to deep soil water can change this relationship (Volkman et al. 2016).

Carbon isotopes are mostly related to leaf physiological properties. Many studies have shown that high intrinsic water-use efficiency (iWUE) is associated with rising CO_2 because the more efficient use of water helps relieve drought stress in plants (Saurer et al. 2014; Rahman et al. 2019a, b). iWUE is an indicator of the balance between photosynthetic carbon assimilation and water loss, both of which are regulated by stomata. Hence, iWUE is related to the intercellular (C_i) and atmospheric (C_a) concentrations of CO_2 . Low stomatal conductance leads to decreased C_i/C_a ratios and an increase in iWUE and $\delta^{13}\text{C}$ values. The trade-off between increasing iWUE and lowering leaf temperatures under hot conditions appears to be highly dependent on site conditions (e.g., soil moisture and heat-induced drought) and the type of photosynthetic pathway (e.g., C_3 , C_4 , or CAM plants) (Olano et al. 2014; Altieri et al. 2015; Chaves et al. 2016).

Moringa peregrina shows morphological plasticity under drought conditions through leaf rolling, reduced leaf surfaces, and increased surface trichomes (Al-Gohary and Hajar 1996). It is also possible that *M. peregrina* may be able to access deep water during drought or to store water during rare flood events, but these possibilities have not yet been studied. Our goal in this study is to shed light on the environmental factors governing tree survival under high temperature and low precipitation conditions. In particular, we aim to elucidate the species' growth response to drought

and its physiological and water uptake strategies using wood anatomy and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotope analyses.

Materials and methods

Study area

Wood samples of *Moringa peregrina* were collected from the arid desert of Hurghada, Red Sea Governorate, Egypt ($27^\circ 4' 13.07'' \text{N}$, $33^\circ 24' 29.30'' \text{E}$, Fig. 1), in mid-January 2020 from trees growing in a rocky gorge along an altitudinal gradient of 200 m (600–800 m a.s.l.). The differences along this gradient related to temperature and/or precipitation can be neglected since they do not differ here. Also, soil conditions along the gradient are not changing. Most of the trees in the area are growing on the eastern slope of the gorge, the shallow soils of which are subject to strong erosion resulting from infrequent but heavy rainfall events (flash flooding). These rainfall events cause local erosion processes that affect the stability of the trees growing along the slope. Although all trees growing on the slope are at risk of mechanical stress, the local nature of slope erosion means that individual trees will experience stress at different times and of different magnitudes.

The trees in Hurghada are young (<20 years) and grow as multi-stemmed trees. The average annual temperature in this area is 22.9°C . January is the coldest month (15.5°C); August is the warmest (29.2°C). The average temperature during the period 2009–2019 was 24.7°C , while the mean total precipitation during the same period was $88.8 \text{ mm year}^{-1}$, with remarkable high precipitation during the years 2018 and 2019 (121.2 - and $102.2 \text{ mm year}^{-1}$, respectively (Fig. 1). No rain occurs between January and September, whereas October is the wettest month, with an

Fig. 1 Mean annual air temperature (T) and annual total precipitation (P) in the studied area during the period 2009–2019. (Source: Climate data organization, <https://en.climate-data.org>)

average of 1 mm (source: Climate Data Organization, <https://en.climate-data.org>, accessed in October 2020). The region is subject to sudden, irregular floods that are local in extent (Youssef et al. 2011). Unfortunately, there are no official records of flash flood events in or around the study area.

Processing and analysis of wood samples

A total of eighteen discs were sampled from the main stems of nine trees (2 discs each) at breast height (1.3 m). Nine tree discs were used to analyze the anatomical structure of the stems, from bark to pith. Transverse microsections (15–20 μm in thickness) were cut using the WSL lab microtome (Gärtner et al. 2015). The microsections were stained with safranin and astra-blue, dehydrated with ethanol, embedded in Eukitt-UV, and stabilized under UV light for about 2 min. High-resolution micro-photos were taken using the Zeiss slide Scanner Axio Z1 at 100 $\times$ magnification.

The wood of *M. peregrina* are known to be diffuse-porous (Farahat and Gärtner 2019), making a purely macroscopic determination of ring boundaries very difficult. Differences in color between latewood and earlywood result from the slightly higher density of earlywood vessels. Color variations also result from differences in the wall thickness of fiber cells caused by frequent but irregular tension wood zones. For this reason, micro-photos were used to define the ring boundaries based on anatomical structures, i.e., changes in the wall thickness of fibers and their tendency to be flattened in the radial direction at the end of the ring. At the end we had to discard one sample because one ring boundary was not clear enough.

The images were also used to measure the vessel lumen area (VLA), vessel wall thickness (VWT), percentage of vessel lumen area related to the analyzed area (VLA%) in each ring, and tree-ring width (TRW) in each of the nine trees using the image analysis program WinCELL (ver. 2019a, Regent Instruments, Canada). A size filter was applied in the program settings to exclude all other cells as either fibers or parenchyma.

Sample preparation and stable isotope analysis

Of the second wood discs taken from the stems, we selected six discs showing the least deviations to analyze the tree-ring (early- and latewood combined) cellulose $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ in the first ten rings of each tree (2020–2010). Depending on the width of the individual ring and its anatomical characteristics, each ring was separated into one, two, or three pieces using a razor blade under a binocular microscope. We used thick woodblocks from the discs to obtain enough wood material for each ring; this was especially important in the case of narrow rings. After cutting, each wood sample

was placed in an Eppendorf vial (2 ml) for milling using an ultracentrifugation mill (Retsch mill ZM200). Then, each wood sample was transferred to a sealed Teflon bag for subsequent α -cellulose extraction. Thereafter, we put all bags in 250-ml Erlenmeyer flasks and used the modified Jayme-Wise method (Green 1963; Wieloch et al. 2011) to extract α -cellulose. Following this method, we added a solution of 7% NaOH to the Teflon bags and heated them in a water bath at 60 $^{\circ}\text{C}$ for 2 h to extract resin, fatty acids, etheric oils, and hemicellulose. This operation was repeated twice. The bags were rinsed three times with distilled water at 60 $^{\circ}\text{C}$ before a solution of 7% NaClO_2 (pH 4–5) was added; the bags were then heated again at 60 $^{\circ}\text{C}$ for 40 h to extract lignin. This process was repeated every 10 h, for a total of three times. The samples were then rinsed again three times with boiling distilled water, squeezed to remove excess water, and placed in an oven at 60 $^{\circ}\text{C}$ for at least 4 h. The yield of all samples was approximately 30–45% cellulose. For each sample, 0.9–1.0 mg of cellulose was packed into silver capsules for isotopic analysis.

Samples were pyrolyzed using a high-temperature elemental analyzer (Pyrocube, Elementar, Hanau, Germany) at 1420 $^{\circ}\text{C}$ to CO gas and then stable carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotope compositions measured using a continuous flow isotopic ratio mass spectrometer (IRMS Delta XP Advantage, Thermo Fisher Scientific, Bremen, Germany) via a variable open split interface (Conflo III, Thermo Fisher Scientific, Bremen, Germany) (Woodley et al. 2012). The analytical errors (standard deviations) of the isotope measurements were less than 0.3‰ and 0.2‰ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively. Based on certified reference values (benzoic acid IAEA-601 and -602, sucrose IAEA-CH-6), the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotopic compositions are presented as the deviation (δ) of the sample from the international standard (VPDB for $\delta^{13}\text{C}$ and Vienna Standard Mean Ocean Water VSMOW for $\delta^{18}\text{O}$) or $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$.

Intrinsic water-use efficiency

To estimate the physiological response of *M. peregrina* trees to the prevailing drought conditions in the study area, we calculated the intrinsic water-use efficiency (iWUE) from the $^{13}\text{C}/^{12}\text{C}$ ratio. According to Farquhar et al. (1982), the isotope discrimination, Δ , observed in plants, results from the preferential use of ^{12}C over ^{13}C during photosynthesis. This relationship is defined as:

$$\Delta = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_{\text{tree}}) / (1 + \delta^{13}\text{C}_{\text{tree}}/1000) \quad (1)$$

where $\delta^{13}\text{C}_a$ and $\delta^{13}\text{C}_{\text{tree}}$ are the isotopic values of atmospheric CO_2 and the wood, respectively. Δ is linearly related to the ratio of intracellular (C_i) to atmospheric (c_a) CO_2 mole fraction as follows (Farquhar and Richards 1984):

$$\Delta = a + (b - a)C_i/C_a \quad (2)$$

where a (4.4‰) is the fractionation associated with the gaseous diffusion of CO_2 through the stomata and b (27‰) is the fractionation associated with Rubisco and other enzymes during carbon fixation. The values of c_a and $\delta^{13}\text{C}_a$ were obtained from Mauna Loa (Hawaii records; <http://www.esrl.noaa.gov/gmd/>). The C_i/C_a ratio reflects the balance between net photosynthesis (A) and stomatal conductance for CO_2 (g_c) according to Fick's law: $A = g_c (C_a - C_i)$. From Eqs. (1) and (2), it follows that C_i can be calculated from the discrimination using literature data for a , b , and $\delta^{13}\text{C}_a$ and measured data for $\delta^{13}\text{C}_{\text{tree}}$:

$$C_i = C_a (\delta^{13}\text{C}_a - \delta^{13}\text{C}_{\text{tree}} - a) / (b - a) \quad (3)$$

Stomatal conductance for CO_2 and water vapor (g_w) are related by a constant factor ($g_w = 1.6g_c$). Accordingly, the last two variables link the leaf-gas exchange of carbon and water. To calculate $i\text{WUE}$ ($\mu\text{mol}/\text{mol}$), we used the relationship $i\text{WUE} = A/g_w$ and the linear relationship between C_i/C_a and Δ (Eq. 1) as follows:

$$i\text{WUE} = (c_a/1.6) [(b - \delta^{13}\text{C}_a + \delta^{13}\text{C}_{\text{tree}}) / (b - a)] \quad (4)$$

Statistical analysis

To calculate the tree-ring index, the first differences of each individual tree series and their mean were calculated. This method was used due to the short time span of the data and our interest in retaining the growth dynamics of the individual trees. The R_{bar} , the mean pairwise correlation coefficient between all individual tree series (Wigley et al. 1984), was calculated on raw, detrended, and first-differenced data of tree-ring widths, vessel lumen area, and wall thickness of vessels. As well, the R_{bar} was calculated for the corrected raw, detrended, and first-differenced data of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values. MATLAB was used to detrend the data by subtracting a linear trend from the raw data. A one-way ANOVA test was used to test the differences in yearly changes in TRW, VLA, VWT, and VLA% using the year (2010–2020) as the independent factor. Pearson's correlation analysis was carried out between the anatomical response variables (vessel lumen area, wall thickness, and tree-ring widths) and the other variables ($\delta^{13}\text{C}_{\text{tree}}$, $\delta^{13}\text{C}_a$, $i\text{WUE}$, C_a , C_i and C_i/C_a). The coefficient of determination (R^2) was used to estimate the strength of the relationship between variables. Statistical analyses were conducted using SPSS.

Results

Characteristics of tree-ring widths

The ages of the sampled trees do not exceed 19 years, and all trees share a common growth period from 2010 to 2020 (11 years; Fig. 2). The tree-ring widths (TRWs) of the sampled trees are relatively wide in all series during the period 2010–2016 and then become narrower and more homogeneous from 2017 onward. The yearly changes in TRW between 2010 and 2020 are significantly different ($F = 4.99$, $p < 0.001$), and the mean TRWs of the non-detrended series show an overall declining pattern. The mean annual growth rate (minimum–maximum) for the 11-year period is $3.73 \text{ mm year}^{-1}$ ($2.72 - 4.80 \text{ mm year}^{-1}$). Due to the variability in growth among the sampled trees, the mean inter-series correlation coefficient (R_{bar}) is low. The R_{bars} for raw, detrended, and first-differenced TRWs are 0.37, 0.09, and 0.04, respectively.

Fig. 2 Tree-ring widths (μm) (a) and indices (first-differenced) (b) for individual series (gray lines). Red lines indicate the mean; red vertical bars indicate the standard error (SE) for the mean values

Vessel traits

From 2010 to 2016, tree rings exhibit relatively high vessel lumen areas (VLA); since 2017, there has been a clear decline in VLA. The year-to-year changes in VLA are not significantly different ($F=0.45$, $p=0.91$). In contrast, VLA% are low in 2010–2016 and then increase significantly from 2017 to 2020 ($F=5.85$, $p<0.001$). The same trend is seen in VWT, but the yearly changes are not significantly different from year to year ($F=1.23$, $p=0.29$; Fig. 3a, b, & c). The mean ($\pm$ SE) of VLA in tree rings (min. – max.) is $13371.1 \pm 3141.2 \mu\text{m}^2$ ($10,809.9$ – $15,783.8 \mu\text{m}^2$). The mean VWT ($\pm$ SE) value (min.–max.) is $9.4 \pm 2.5 \mu\text{m}$ (7.2 – $13.1 \mu\text{m}$). The Rbar values for raw, detrended, and first-differenced VLA of all series are 0.19, 0.22, and 0.15, respectively. Meanwhile, the Rbars for raw, detrended, and first-differenced VWT are 0.04, -0.09 , and -0.02 , respectively.

Stable isotope ratios

Our results indicate that the $\delta^{13}\text{C}$ isotope in tree rings declined gradually between 2010 (-23.4‰) and 2020 (-24.9‰) (Fig. 4a). The population mean $\delta^{13}\text{C}$ value ($\pm$ SE, min. – max.) for all series is $-23.8\text{‰} \pm 0.44$ (-24.6‰ – -23.3‰). It is worth noting that the Rbars for raw, detrended, and first-differenced $\delta^{13}\text{C}$ data are 0.22, 0.05,

and -0.007 , respectively. There are therefore no detectable common signals apart from the overall negative trend in $\delta^{13}\text{C}$.

As with $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ shows a slight decrease between 2010 (33.6‰) and 2020 (32.5‰). The population mean $\delta^{18}\text{O}$ value ($\pm$ SE) is $33.0 \text{‰} \pm 0.85$, with a range of 29.2 – 36.3‰ (Fig. 4b). The Rbars for raw, detrended, and first-differenced $\delta^{18}\text{O}$ data are 0.14, 0.13, and 0.28, respectively.

Intrinsic water-use efficiency and C_i/C_a ratio

The intrinsic water-use efficiency (iWUE) shows a decline with time, from $130.0 \mu\text{mol mol}^{-1}$ in 2010 to $119.4 \mu\text{mol mol}^{-1}$ in 2020. The mean iWUE ($\pm$ SE) is $128.0 \pm 5.3 \mu\text{mol mol}^{-1}$; the minimum and maximum estimated values are 119.4 and $138.1 \mu\text{mol mol}^{-1}$, respectively (Fig. 4c).

The intercellular carbon dioxide concentration (C_i) and C_i/C_a ratio exhibits a gradual increase with time (Fig. 4d). C_i increases from 181.6 ppm in 2010 to 222.2 ppm in 2020, with a mean value ($\pm$ SE) of $196.6 \pm 8.14 \text{ ppm}$ (Fig. 4e). The C_i/C_a ratio increases from 0.44 in 2010 to 0.54 in 2020 and has a mean value ($\pm$ SE) of 0.5 ± 0.01 over the 11-year period. As indicated in Fig. 4f, CO_2 concentrations increased from 389.8 ppm in 2010 to 413.2 ppm in 2020, an increase of 23.4 ppm over 11 years.

Fig. 3 Temporal variation in tree-ring anatomical traits in *Moringa peregrina*: (a) vessel lumen area (VLA, μm^2), (b) vessel wall thickness (WT, μm), and (c) mean vessel lumen % for individual series (gray lines). Red lines indicate the mean; red vertical bars indicate the standard error (SE) for the mean values

Fig. 4 Annual values of the (a) mean stable carbon ($\delta^{13}\text{C}$) and (b) oxygen ($\delta^{18}\text{O}$) isotope ratios; (c) intracellular water-use efficiency (iWUE); (d) intracellular (C_i) CO_2 concentration; (e) the ratio between intracellular (C_i) and atmospheric (C_a) CO_2 concentrations

(C_i/C_a) and (f) atmospheric (C_a , ppm) CO_2 concentrations during the last 10 years. $N=6$ trees. Red lines indicate the mean; red vertical bars indicate the standard error (SE) for the mean values. Gray lines represent individual tree series

Correlations between anatomical and isotopic variables

The relationship between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in the sampled trees is weak ($r=0.3$, $R^2=0.13$) (Table 1, Fig. 5). The relationships between TRW and vessel lumen area and wall thickness is non-significantly correlated ($r=0.51$ and -0.53 at $p \leq 0.05$, respectively, Table 1). TRWs show significant negative relationships with the atmospheric (C_a ; $r=-0.77$, $p \leq 0.01$) and intracellular CO_2 concentrations (C_i ; $r=-0.61$, $p \leq 0.05$). Vessel wall thickness (VWT) also correlates positively and significantly with the atmospheric and intracellular CO_2 concentrations ($r=0.87$ at $p \leq 0.01$ and 0.72 at $p \leq 0.05$, respectively). However, VWT shows significant negative correlations with $\delta^{13}\text{C}$ (at $p \leq 0.05$) and non-significant but also negative correlations with $\delta^{18}\text{O}$.

iWUE has significant negative correlations at $p \leq 0.01$ with C_i ($r=-0.86$, $R^2=0.86$) and C_i/C_a ($r=-0.94$, $R^2=0.94$) and a non-significant negative correlation with C_a (Table 1, Fig. 6). $\delta^{13}\text{C}$ shows a significant positive correlation with iWUE ($r=0.93$ at $p \leq 0.01$) but significant negative correlations (at $p \leq 0.01$) with C_a ($r=-0.84$), C_i ($r=-0.98$), and C_i/C_a ($r=-0.99$). Finally, C_i correlates positively with C_i/C_a ($r=0.97$ at $p \leq 0.01$).

Discussion

Characteristics of tree-rings

The lack of a shared ring-width pattern between trees makes further investigations into potential climate signals very uncertain. This uncertainty is most likely caused by

Table 1 Pearson's correlation analysis of the measured parameters and their significance levels (p) at * $p \leq 0.05$ and ** $p \leq 0.01$. VLA=vessel lumen area, WT=vessel wall thickness, TRW=tree-ring width, iWUE=intrinsic water-use efficiency, C_a =atmospheric

CO_2 concentration, C_i =intracellular CO_2 concentration, $\delta^{13}C$ and $\delta^{18}O$ =carbon and oxygen isotope composition in tree rings, respectively

Variable	VLA	WT	TRW	iWUE	C_a	C_i	C_a/C_i	$\delta^{13}C$
WT	-0.35	1	-	-	-	-	-	-
TRW	0.51	-0.53	1	-	-	-	-	-
iWUE	0.09	-0.33	0.24	1	-	-	-	-
C_a	-0.40	0.87**	-0.77**	-0.59	1	-	-	-
C_i	-0.30	0.72*	-0.61*	-0.86**	0.92**	1	-	-
C_a/C_i	-0.26	0.56	-0.52	-0.94**	0.82**	0.97**	1	-
$\delta^{13}C$	0.24	-0.61*	0.50	0.93**	-0.84**	-0.98**	-0.99**	1
$\delta^{18}O$	-0.30	-0.41	0.31	0.18	-0.40	-0.34	-0.29	0.30

Fig. 5 Relationship between the mean values (‰) of $\delta^{13}C$ and $\delta^{18}O$ in the tree rings of *Moringa peregrina* during the period 2010–2020. $N=6$ trees, R^2 =coefficient of determination, trendline=red dashed line

the infrequent tension wood found in the rings of all trees. This reaction wood indicates that at some point during ring formation, extra stability was needed, possibly in response to very local erosion occurring as a result of short but intense precipitation events (e.g., flash floods). Because these events do not cause homogeneous denudation processes along the slope, not all trees experience the same destabilizing processes at the same time. However, the lack of a common signal in the tree-ring widths does not mean that they are not sensitive to climate. For instance, Panayotov et al. (2011) analyzed the climate signal in the tree-ring chronologies of *Pinus peuce* and *Pinus heldreichii* in Bulgaria and concluded that a climate signal exists but is weak. Levanič et al. (2020) successfully constructed a chronology from the same two

species using tree-ring $\delta^{13}C$, where a better climatic signal was found. Many of our trees exhibit narrow rings during the 2017–2020 period, reflecting some form of common stress (e.g., less rain, high temperatures) during this period. In contrast, most of the trees show relatively wide ring widths in 2012 and 2014, years noted for their catastrophic flash floods.

Different tree-ring parameters often display a wide range of Rbars (Ziaco et al. 2016). Because ring widths are an integrated measure of many growth factors, potential common signals and climatic responses can be distorted (e.g., by mechanical stressors). Low Rbar values can reflect non-climatic influences on tree growth, as well as the presence of reaction wood (Wilson et al. 2017). The low Rbar values for *M. peregrina* tree-ring series (e.g., $rbar_{raw}=0.37$) suggest that a climate signal is present but weak. The regular exposure of trees in the study area to mechanical stress leads to tension wood formation in some rings. This variability in growth depends on the degree to which individual trees experience mechanical stress during their lifetimes. It is also possible that the climate signal is weak because *M. peregrina* has a multi-stemmed (suf-frutescent) growth form, which limits the study of climate responses due to the high variability in growth between the individual stems of the same tree and all individual trees (Driscoll et al. 2020). Worbes (1995) and Brien et al. (2016) hypothesize that the low Rbar values found in tropical trees indicate high growth variability between individuals of the same species. Colombaroli et al. (2016) link the weak common signal in *Pericopsis elata* in the Congo Basin to the microsite conditions rather than to climate. Accordingly, we suggest that the tree-ring widths of *M. peregrina* reflect the microsite conditions in the study area rather than a single common climate signal.

Fig. 6 Relationship between the mean values of intrinsic water-use efficiency (iWUE) and (a) atmospheric CO₂ (C_a, ppm), (b) intracellular CO₂ concentrations (C_i, μmol mol⁻¹), and (c) Ci/Ca ratio of *Moringa peregrina* during the period 2010–2020. $N=6$ trees, R^2 =coefficient of determination, trendline = red dashed line

Anatomical traits

TRWs and VLA are not significantly correlated ($r=0.51$), possibly on account of the irregular zones of tension wood in single rings, which result in a widening of the ring and a reduction in the vessel sizes in the same zone (Heinrich et al. 2007; Heinrich and Gärtner 2008). Nevertheless, an overall trend is observable in both variables. The gradual decline in the VLA of *M. peregrina* rings can be ascribed to the exponential increase in annual air temperature and yearly vapor pressure in the study area over time (Harris et al. 2020). Meanwhile, decreases in the mean VLA and increases in the

VLA% indicate that trees cope with external environmental conditions (e.g., drought or high temperatures) by increasing their vessel densities rather than their lumen area. In doing so, trees improve their conductivity while minimizing the risk of hydraulic failure. Similar findings have been reported for trees subjected to drought conditions (e.g., Galle et al. 2010; Pellizzari et al. 2016; Farahat and Gärtner 2019) or infected by bacterial pathogens (Sabella et al. 2020). A reduction in vessel size and number in the rings of some tree species (e.g., *Quercus ithaburensis* and *Fagus sylvatica*) has been reported for trees experiencing drought stress (Castagneri et al. 2017; Gebauer et al. 2020).

Coping strategy of *M. peregrina* under drought conditions

In light of the anticipated reduction in water availability and increase in arid land area, it is imperative to understand the ecological performance of desert trees and shrubs. It is well known that tree species adapt to increasing C_a by increasing their iWUE, as indicated by increases in tree-ring δ¹³C (Pellizzari et al. 2016; Rahman et al. 2019a, b; Driscoll et al. 2020). This increase in δ¹³C and iWUE is associated with a decrease in C_i/C_a ratios and stomatal conductance (g_s), i.e., partial stomatal closure. The whole process is enhanced by increased water limitation and is associated with low soil moisture or high leaf-atmosphere vapor pressure saturation deficits (Novick et al. 2016). Some authors have warned against using a linear, simplified relationship between δ¹³C and iWUE, since other variables such as mesophyll conductance can influence iWUE (Seibt et al. 2008; Ma et al. 2020). In contrast to the relationship between δ¹³C and iWUE, C_i/C_a shows an increasing trend in the present study, which is associated with decreasing iWUE and may therefore be related to recent growth reductions, i.e., from 2017 to 2020. To explore this possibility, we discuss the possible structural adaptations of *M. peregrina* to the harsh desert environment in the following paragraphs.

The response of tree growth to increased CO₂ is far from simple and appears to depend to a large extent on site conditions. The influence of CO₂ and other climate conditions (e.g., temperature, precipitation) on tree growth further depends on the characteristics of the tree species. Physiological adaptations and water demand of the species determine its interaction with the climatic conditions. Like other species in the genus, *Moringa peregrina* is a C₃ plant (Azza 2014), which means its ability to retain water under drought conditions is weak in comparison to that of C₄ plants, i.e., lower WUE (Chaves et al. 2016). Under drought conditions or periods of high air temperatures, plants that close their stomates and lower their transpiration rates to increase WUE may inadvertently increase their leaf temperatures by up to 7 °C above air temperature (Blum 2015). If this situation

lasts for too long, it can cause severe defoliation and plant mortality. Examples of the cooling effect of leaf transpiration have been reported for Mauritania's hot deserts and the Sonoran Desert of southern California (Lange 1959; Smith 1978).

For the abovementioned reasons, *M. peregrina* is characterized by high structural plasticity under different soil moisture conditions. In an experiment by Al-Gohary and Hajar (1996), *M. peregrina* increased its stomatal density (number of stomates per mm²) from 343.8 under 95–100% soil moisture to 1021.5 under 0.5% soil moisture. *M. peregrina* increases its stomatal density under low soil moisture conditions in order to cool its leaves and continues photosynthesizing. When the stomatal density increases and the stomate size decreases, plants can maintain relatively high transpiration and photosynthetic rates (Bertolino et al. 2019). Accordingly, *M. peregrina* trees seem to cool their leaves and the boundary air at the cost of saving water, i.e., they favor high stomatal conductance at the cost of decreased iWUE. This strategy can explain the gradual decline in iWUE and $\delta^{13}\text{C}$ observed in this study despite the increase in CO₂. Similar findings are reported by Bauters et al. (2020) for the Congo Basin, where a decrease in leaf $\delta^{13}\text{C}$ and iWUE over time was observed in central African trees despite an increase in air temperature. In the present study, the C_i/C_a ratio has a negative relationship with iWUE and $\delta^{13}\text{C}$ but a positive relationship with stomatal conductance. Meanwhile, C_i increases linearly with C_a. These findings are consistent with those of Bauters et al. (2020) and Driscoll et al. (2020) for tropical African trees.

The first-differenced data for $\delta^{18}\text{O}$ shows tendencies toward a common signal, which suggests that the trees are sensitive to a common environmental factor(s), e.g., drought. The range of $\delta^{18}\text{O}$ in the present study (29.2–36.3‰) is consistent with that recorded for trees in semi-arid regions (Xu et al. 2011; Qin et al. 2015) or in the subtropical belt of the Eastern Mediterranean (Lukač et al. 2021). It indicates uptake of enriched water sources or high isotopic enrichment of $\delta^{18}\text{O}$ leaf water caused by high transpiration rates and subsequent assimilation (Sarris et al. 2013; Treydte et al. 2014). Given that *M. peregrina* is perennial and evergreen most of the year despite very limited rainfall, we think that it relies primarily on groundwater. This does not exclude precipitation as a key source of water, since it is the main source of groundwater in the desert. Canadell and Zedler (1995) found that trees with dense root systems can more readily take advantage of the moisture available during the short rainfall episodes that characterize semi-arid areas. Whether precipitation occurs as a light, fleeting event or as a strong flash flood, trees with dense and deep roots systems are better able to take up and store water over long periods.

The relationships between the population means of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ are generally weak, indicating that although both

variables are linked to transpiration, they are largely independent. According to the conceptual model (scenario d, case 1) of Scheidegger et al. (2000), the decrease in $\delta^{13}\text{C}$ over time, in combination with small changes in $\delta^{18}\text{O}$, may be caused by non-significant changes in photosynthesis and strong changes in stomatal conductance. Similar findings by Ferrio and Voltas (2005a, b) have been recorded for *Pinus halepensis* at 23 sites on the eastern Iberian Peninsula.

We can conclude from this work that mechanical stress leads to high growth variability in *M. peregrina* trees and a weak common climate signal. However, *M. peregrina* increases its stomatal conductance under harsh desert conditions (low soil moisture and high temperatures) to cool down its leaves. This results in low iWUE and $\delta^{13}\text{C}$ and high C_i over time. The high average value of $\delta^{18}\text{O}$ (33.0 ‰) is due to the effect of drought conditions on the oxygen isotope discrimination in wood.

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Data availability All data generated or analyzed during this study are included in this published article and no supplementary files attached to the mns.

Declarations

Ethics approval Field studies were carried out according to the Egyptian legislation.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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