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Research paper

Metabolic responses of date palm (*Phoenix dactylifera* L.) leaves to drought differ in summer and winter climate

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Drought negatively impacts growth and productivity of plants, particularly in arid and semi-arid regions. Although drought events can take place in summer and winter, differences in the impact of drought on physiological processes between seasons are largely unknown. The aim of this study was to elucidate metabolic strategies of date palms in response to drought in summer and winter season. To identify such differences, we exposed date palm seedlings to a drought-recovery regime, both in simulated summer and winter climate. Leaf hydration, carbon discrimination ($\Delta^{13}C$), and primary and secondary metabolite composition and contents were analyzed. Depending on season, drought differently affected physiological and biochemical traits of the leaves. In summer, drought induced significantly decreased leaf hydration, concentrations of ascorbate, most sugars, primary and secondary organic acids, as well as phenolic compounds, while thiol, amino acid, raffinose and individual fatty acid contents were increased compared with well-watered plants. In winter, drought had no effect on leaf hydration, ascorbate and fatty acids contents, but resulted in increased foliar thiol and amino acid levels as observed in summer. Compared with winter, foliar traits of plants exposed to drought in summer only partly recovered after re-watering. Memory effects on water relations, and primary and secondary metabolites seem to prepare foliar traits of date palms for repeated drought events in summer. Apparently, a well-orchestrated metabolic network, including the anti-oxidative system, compatible solutes accumulation and osmotic adjustment, and maintenance of cell-membrane stability strongly reduces the susceptibility of date palms to drought. These mechanisms of drought compensation may be more frequently required in summer.

Keywords: anti-oxidative system, compatible solutes, date palm (*Phoenix dactylifera* L.), drought, fatty acids, high temperature, memory effects, nitrogen composition.

Introduction

Global climate change induced by gradually raised atmospheric greenhouse gas concentrations is projected to drive more frequent and prolonged periods of drought and heat waves (IPCC 2014, Grant 2017). These projected changes will not only threaten terrestrial ecosystems globally (Knapp et al. 2017), but also detrimentally impact individual plant species on a regional scale (Rennenberg et al. 2006, Dai 2011, Hasanuzzaman et al. 2013, Kreuzwieser and Rennenberg 2014). Drought is one of the most acute environmental stresses raised by climate change as a major future risk in many areas of the world, particularly in arid and semi-arid environments, where plants are even more prone to water shortage (Sun et al. 2006, Young et al. 2017). Apart from protection by stomatal closure, plants

under drought stress accumulate more carbohydrates, polyols and free amino acids to maintain water uptake and turgor by osmotic adjustment, and increase antioxidant compounds to combat oxidative stress (Rennenberg et al. 2006, Krasensky and Jonak 2012, Rivas-Ubach et al. 2014). High temperature may act as a single stress or interact with drought, impacting water relations, nutrients uptake and photosynthesis, as well as the anti-oxidative system and many other metabolic processes of plants (Rennenberg et al. 2006, Hasanuzzaman et al. 2013). In the context of fluctuated precipitation regimes induced by climate change (Furl et al. 2014, IPCC 2014), drought may occur both in summer and winter, but only aggravated effects of summer drought due to elevated evapotranspirative demand (Rennenberg et al. 2006, Dai 2011) have been documented in evergreen trees (Barber et al. 2000, Arndt et al. 2008, Granda et al. 2014) and other perennial species (Schwinning et al. 2005*a*, 2005*b*). Winter droughts have been characterized not only by decreased precipitation, but also by concurrently increasing winter temperatures (Earles et al. 2018). Some studies have shown strong negative effects on growth of grass and subshrubs (Schwinning et al. 2005*a*, 2005*b*), as well as broad leaved and conifer tree species (Lévesque et al. 2013, Voltas et al. 2013, Costa-e-Silva et al. 2015).

Date palms (*Phoenix dactylifera* L.) are an important cash crop in arid and semi-arid regions (Shabani et al. 2012, Allbed et al. 2017), where more frequent drought periods are projected not only in summer, but also in the winter, e.g., in Africa (Haile et al. 2019) and the Northern Arabian Peninsula region, with a projected maximum winter precipitation decrease of ~30–50% by the end of this century at a rate of −1.36% per decade, concurrent with steadily increasing temperature at a rate of 0.35°C per decade (Almazroui et al. 2016). Although date palms are considered remarkably tolerant to harsh climatic conditions, their distribution and productivity will be significantly affected by more frequent and prolonged drought events (Shabani et al. 2012, Yaish and Kumar 2015, Allbed et al. 2017, Qaddoury 2017). Apart from evolutionarily stress-avoidance strategies, metabolic reconfiguration is often behind stress-adaptation strategies to adverse environmental conditions (Zandalinas et al. 2017*b*). A previous study has shown that heat and drought had little effect on stomatal conductance and photosynthesis (Arab et al. 2016). Heat induced increases of isoprene emission and reactive oxygen species (ROS) contents, while drought strongly impacted fatty acids composition and, hence, membrane structure of date palms (Arab et al. 2016, Du et al. 2019). In both heat and drought conditions, carbohydrate metabolism, cell-wall biogenesis and ROS scavenging processes were likely promoted (Safronov et al. 2017).

Knowledge on the physiological responses of date palms to projected seasonal drought is of particular importance for cultivation and management practices. However, the effects of drought on leaf photosynthetic traits in summer and winter climate were only recently analyzed in date palms. For this purpose, young plants were exposed to simulated summer and winter climate under controlled conditions (Du et al. 2019, Kruse et al. 2019). Optimum temperature of leaf net photosynthesis (T_{opt}) was much lower in plants in the winter climate, i.e., 27.4 ± 0.4C in the winter and 36.0 ± 0.6C in summer, and growth temperature statistically explained about 50% of the variation in T_{opt}, which additionally depended on leaf water status (Kruse et al. 2019). Changes in ROS scavenging, osmotic adjustment, as well as secondary metabolites involved in membrane structure and cell-wall constituents were found to facilitate tolerance to the high temperatures in summer, when foliar hydrogen peroxide (H_2O_2) contents were increased (Du et al. 2019). In addition to declined stomatal conductance, CO_2 assimilation rate and leaf dark respiration, water stress caused significantly decreased (30–80% of control levels) light saturated rates of leaf net photosynthesis at optimum temperature (A_{opt}) and intrinsic water-use efficiency at optimum temperature, but both fully recovered after re-watering. Water-deprived date palm plants operated at greater substomatal CO_2 concentration (C_i) than fully watered plants. Moreover, water deprivation significantly reduced the temperature sensitivity of stomatal conductance. The acclimatory shifts in T_{opt} and A_{opt}, as well as respiratory acclimation reflected a balance between maximization of photosynthesis and minimization of the risk of metabolic perturbations under drought conditions (Kruse et al. 2019). With these results, the date palm was confirmed to constitute an evergreen species well-adapted to hot and semi-arid environments, by slow growth and conservative water-use strategy (Kruse et al. 2019). However, it is still unknown how the altered stomatal aperture as well as carbon (C)gain induced by water deprivation translate into metabolic changes in summer and winter climate.

In the present study, water relations, antioxidant levels, C and N composition, as well as water-soluble metabolites and fatty acids of date palm leaves were analyzed in plant material from the experiment reported by Kruse et al. (2019). With these analyses, we examined the following hypotheses: (i) drought treatment results in elevated foliar antioxidants levels; (ii) compatible solutes including sugars, alcohols and amino acids accumulate in response to drought; (iii) fatty acid content, and hence membrane composition, is modified by drought to achieve greater abundances of unsaturated fatty acids in support of membrane integrity. We further hypothesized that (iv) compared wih winter climate, effects of drought are more prominent in the summer climate, and (v) the altered metabolic profiles largely recover after re-watering under both winter and summer climate.

Materials and methods

Plant materials and experimental design

Two-year-old date palm seedlings purchased from a commercial supplier ('Der Palmenmann,' Bottrop, Germany) were potted into 3.3 l pots filled with a mixture of peat–soil–sand (3:1:7 v/v/v) and about 10 g of Osmocote fertilizer (16% N, 9% P_2O_5, 12% K_2O) was supplied to each pot. Plants were cultured in the greenhouse (day length 12 h, 25/15C, 20/30% relative humidity (day/night)) and irrigated once per week (~150–200 ml per pot). After 2 months, seedlings were transferred to four fully automatized, walk-in phytotron chambers on 10 January 2014 (for more detailed information, refer to Du et al. 2019, Kruse et al. 2019). Conditions in the four phytotron chambers were adjusted to match typical Saudi Arabian summer and winter conditions. Final climatic conditions were achieved in 9 days after the start of the experiment. At this time, average noon temperatures peaked at ~40C in summer and ~25C in the winter climate according to the temperature averages from 2003 to 2012 in Alahsa, Saudi-Arabia (Almazroui et al. 2012, 2016). Minimum temperature and average relative humidity in summer and winter climate chambers were set to 20 and 5C, 20% and 50%, respectively. In the summer treatment, the light period was 4 h longer than for the winter treatment, but with similar maximum irradiance (i.e., photon flux density: 600 µmol m^{-2} s^{-1}). For more detailed climate information, please refer to Kruse et al. (2019). Plants in the winter and summer climate, 24 plants of each, were watered with 40 and 80 ml of de-ionized water every day at 06:00 h, respectively; more water was given to plant in the summer climate to accomplish similar soil water availability (~20% soil volumetric water content). Vapor pressure deficit varied with growth temperature and peaked at ~6.8 kPa in summer and 2.5 kPa in the winter (Kruse et al. 2019). The drought treatment was initiated on 10 February 2014, by withholding 50% water of control levels in the winter and summer climate. Irrigation was further reduced on 20 February 2014 to 25% of control levels. At the end of drought treatment on 11 March 2014, soil water contents were less than 5% in summer and 6–7% in the winter climate compared with 18–22% of controls, and control plants in the summer and winter climates had similar stomatal conductance (see Kruse et al. 2019). At this time, half of both, control and drought exposed plants from summer and winter climate conditions were harvested. Subsequently, the rest of the drought-exposed plants obtained the same amounts of water as control plants for 1 week (re-watering started on 17 March 2014). At the end of this treatment (25 March 2014), plants were harvested representing the recovery from drought. Fully developed mature leaves from six individual plants (per irrigation, per climate) were harvested at midday (12:00–13:00h), and then were analyzed in the present study.

Plant material was immediately frozen and homogenized in liquid N, and kept at −80C until analyses (for more detailed information, refer to Kruse et al. 2019).

Leaf hydration measurements and biochemical analysis

Leaf hydration measurements

Leaf hydration (g H_2O g $^{-1}$ DW) was determined as (FW – DW)/DW, where FW is the fresh mass and DW is the dry mass after drying the samples in an oven at 60C for 2 days (Du et al. 2018*b*).

Total carbon and, nitrogen contents, and carbon isotope

About 1.5–2.0 mg homogenized dry leaf material was weighted into tin capsules (IVA Analysentechnik, Meerbusch, Germany) and combusted in an elemental analyzer (NA 2500; CE Instruments, Milan, Italy) coupled to an Isotope Ratio Mass Spectrometer (Delta Plus/Delta Plus XL; Finnigan MAT GmbH, Bremen, Germany) by a Conflo II/III interface (Thermo-Finnigan GmbH, Bremen, Germany) for leaf C and N contents, and C isotope ($\Delta^{13}C$) analysis as previously described (Peuke et al. 2006).

Thiol and ascorbate measurement

The thiols glutathione (GSH), cysteine and γ-glutamylcysteine were extracted as described by Schupp and Rennenberg (1988). In brief, ~40 mg frozen leaf powder was extracted with 1 ml 0.1 M HCl containing 100 mg pre-washed polyvinylpolypyrrolidone (PVP 6755, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Oxidized glutathione (GSSG) was determined based on the irreversible alkylation of the free thiol groups with N-ethylmaleimide, and the subsequent reduction of oxidized thiols with dithiothreitol (Strohm et al. 1995). Reduced thiols were derivatized with monobromobimane as previously described (Schupp and Rennenberg 1988). Thiol derivatives were separated on an ACQUITY UPLC® HSS (Waters GmbH, Eschborn, Germany) with a C-18 column (2.1 × 50 mm; 1.18 µm mesh size, Waters). Concentrations of thiols were quantified according to a mixed standard solution consisting of GSH, cysteine and γ-glutamylcysteine subjected to the same reduction and derivatization procedure (Samuilov et al. 2016).

Total and reduced ascorbates were determined with a spectrophotometer (UV-DU650, Beckman Coulter Inc., Fullerton, CA, USA) as previously described (Arab et al. 2016). Concentrations of total and reduced ascorbate were calculated according to a standard curve using dilutions of 1.5 mg ml^{-1} L-ascorbic acid (Sigma-Aldrich, Deisenhofen, Germany).

Total amino acids, soluble protein, pigment contents and structural N calculation

Total soluble amino acids (TAA) and soluble protein contents were extracted and determined with photometric methods as

previously described. Glutamine and bovine serum albumin were used as standards for the calculation of TAA and protein contents, respectively (Du et al. 2019).

Foliar chlorophyll a, b and carotene were extracted and its contents were determined using the method of Lichtenthaler and Wellburn (1983). For this purpose, 25 mg of frozen leaf powder was incubated with 8 ml of 80% acetone at 4C overnight with constant shaking. The supernatant containing the pigments was separated from the pellet by centrifugation at 5000*g* for 10 min and measured at 470, 646 and 663 nm using a UV-DU650 spectrophotometer (Beckman Coulter Inc. Fullerton, CA, USA).

Foliar structural N content was calculated by subtracting the N fractions of soluble protein, total amino acids, nitrate and pigments from total N in leaf bulk material as described previously by Du et al. (2019).

Determination of anions

The anions nitrate (NO_3^-), phosphate (PO_4^{3-}) and sulfate (SO_4^{2-}) were determined in aqueous extracts from homogenized frozen material by automated anion chromatography as described previously by Peuke et al. (2006). Separation of anions was achieved on an ion exchange column (AS12A, 4 mm, Dionex, Idstein, Germany) with 2.7 mM Na_2CO_3 and 0.3 mM $NaHCO_3$ as mobile phase. Detection and quantification were performed with a pulsed amperometric detector (Electrochemical detector ED 40 Dionex). Sodium salts of nitrate, phosphate and sulfate were used as standards (Du et al. 2019).

Metabolite analysis by gas chromatography–mass spectrometry

Relative abundances of water soluble low-molecular-weight polar metabolites and fatty acids in leaves were analyzed by a gas chromatography–mass spectrometry (GC–MS) system (Agilent Technologies, Palo Alto, CA, USA) as previously described (Du et al. 2019). The full scan mass covered the range from *m*/*z* 40 to *m*/*z* 550. Briefly, metabolites were extracted from ~50 mg frozen leaf powder, derivatized and separated by a method modified from Lisec et al. (2006). Peak identification and deconvolution of chromatograms were performed using the Quantitative Analysis Module of the Masshunter software (Agilent Technologies). The Golm metabolome database (Hummel et al. 2010) was used for metabolite identification. Peak areas were normalized using the peak area of internal standards, i.e., ribitol and nonadecanoic acid methyl ester (Sigma-Aldrich) for polar metabolites and fatty acids, respectively, and the dry weight of the samples. Abundances of metabolites were indicated by normalized peak areas. Artifact peaks and common contaminants were identified by analysis of 'blanks' prepared in the same manner as biological samples but without leaf powder. Signals corresponding to these artifacts were omitted from interpretation.

Statistical analysis

Raw data were $\log_{10}$ transformed and auto scaled (mean-centered and divided by the standard deviation of each variable) before subjecting to partial least-square discriminant analysis (PLS-DA) using a public web tool (MetaboAnalyst 4.0, http://www.metaboanalyst.ca/) (Chong et al. 2019). Missing values were replaced by half the minimum abundance of respective compounds, assuming that their concentrations were below detection limit. The PLS-DA was performed using all 111 variables including leaf hydration, C and N compositions, antioxidants, anions and metabolites identified from GC–MS. Two-way ANOVA followed by the Tukey's post-hoc test was employed to examine effects of drought, climate, and their interaction on physiological leaf traits. SigmaPlot 12.0 (Systat Software GmbH, Erkrath, Germany) was used for bar plot generation and statistical analysis. Data shown in figures and tables represent means ± standard error (n = 5–6) on a dry weight basis.

Results

Clustering from PLS-DA demonstrated apparent separation of foliar metabolites of control plants and of plants exposed to drought, particularly in the summer climate (Figure 1A and B, R^2 = 0.89, Q^2 = 0.65). Moreover, foliar metabolites of plants grown in the summer climate were separated from those of plants in the winter climate, irrespective of water status (Figure 1A). Compared with less apparent components 2 and 3, the noticeable separation along component 1 explained 21% of the variation, which was mainly determined by catechin, epicatechin, chlorophyll a/b ratio, raffinose, alanine and heptadecanoic acid according to loading values (Table S1 available as Supplementary data at *Tree Physiology* Online). After re-watering, drought-related discriminations appeared only along components 2 and 3 for plants grown in summer and winter climate, respectively (Figure 1C and D, R^2 = 0.91, Q^2 = 0.45). Total N, structural N, C/N ratio, γ-glutamylcysteine, D-cellobiose, threonine, sorbitol and pyrophosphate played a crucial role in component 2, which explained 12.6% of the separation (Table S1 available as Supplementary data at *Tree Physiology* Online). The separation between re-watered and control plants in the winter climate along component 3 axis, which explained 5.3% of the variation, seemed to be mainly attributable to 6-kestose, 5,8,11,14-eicosatetraenoic acid, leaf hydration, phosphoric acid, 9,12-octadecadienoic acid and lyxonic acid as shown in the complementary loading values (Table S1 available as Supplementary data at *Tree Physiology* Online).

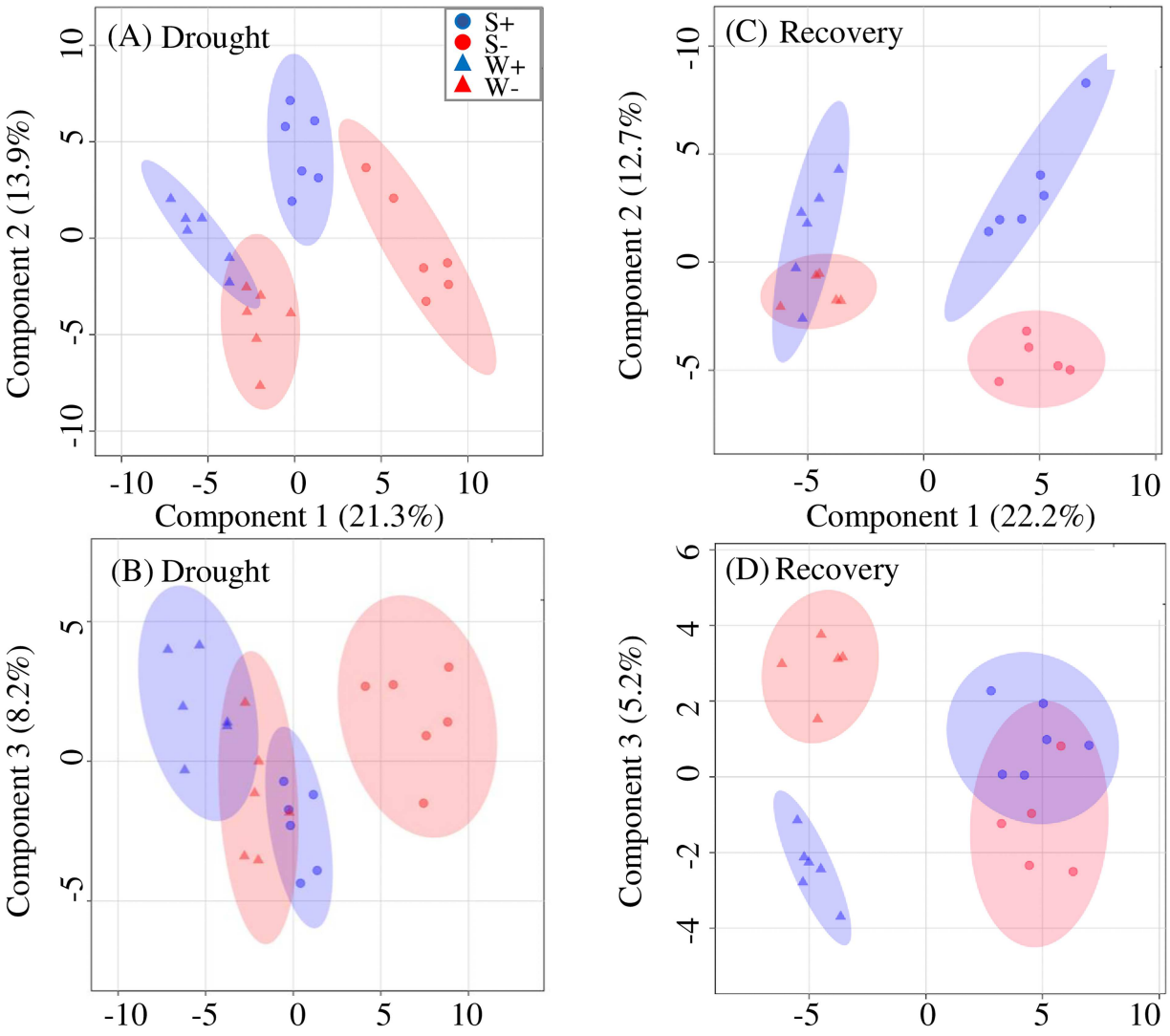

Figure 1. Clustering of metabolites in date palm leaves grown at summer (S, circles) and winter (W, triangles) climate at drought (A and B, component 1/2 and component 1/3, respectively) and recovery (C and D, component 1/2 and component 1/3, respectively) phases. Figure shows scores plot of PLS-DA. Red symbols indicate individual plants of drought treated and re-watered (−), blue symbols indicate individual plants of controls (+) during drought and recovery phases. Semi-transparent shadings indicate 95% confidence regions.

Drought effects in the summer climate

Leaf hydration significantly (P = 0.021) declined to 81% of controls during the drought treatment (Figure 2A, Table S2 available as Supplementary data at *Tree Physiology* Online). Foliar total and reduced ascorbate contents decreased significantly ($P < 0.01$) in drought-treated plants, i.e., 75 and 76% of control levels, respectively (Figure 3), whereas total GSH, GSSG contents and the GSSG/GSH ratio showed significant increases, i.e., 60, 320 and 190% of controls, respectively (Figure 4). Most of the individual amino acids analyzed, particularly β-alanine, glycine, proline, arginine, threonine, tryptophan and phenylalanine, were significantly accumulated in drought-treated leaves (Figures 5 and 6), which resulted in 90% higher TAA contents than in controls (P = 0.038) (Figure 2B). Except for drought-induced accumulation of raffinose (Figure 7A), abundances of most sugars, especially xylose and β-D-galactopyranosyl-1,3-arabinose, were significantly decreased during the drought treatment (Figure 6). Similarly, abundances of the primary organic acids pyruvic acid and glyceric acid, the secondary organic acid 4-hydroxy-benzoic acid, catechin and epicatechin, as well as the amino acids lysine and pyroglutamic acid and an unknown compound (UN-013) largely declined in leaves of drought treated plants compared with controls (Figures 5 and 6). No significant effects of the drought treatment on fatty acids and stearyl alcohol were observed, except for

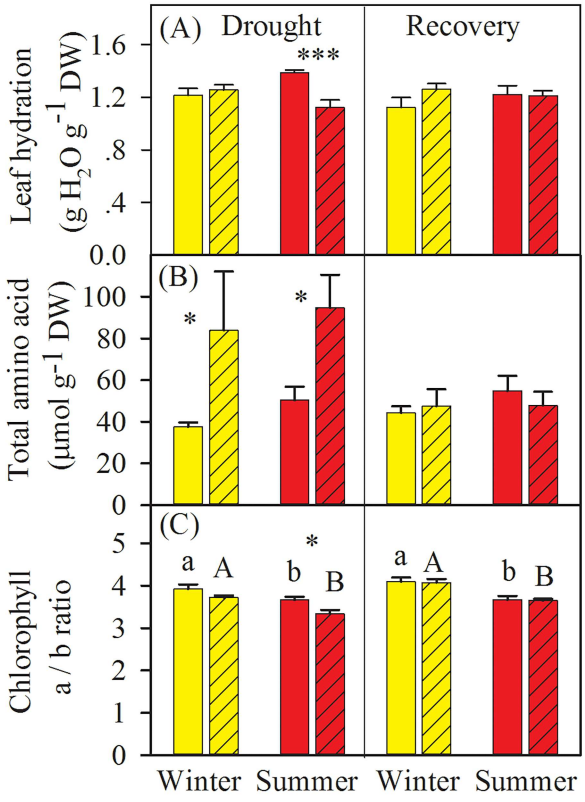

Figure 2. Leaf hydration (a), total amino acid contents (B) and chlorophyll a/b ratio (C) in date palm leaves during drought (left panels) and recovery (right panels) phases in the winter and summer climates. Data shown are means + SE (n = 5–6). Asterisks indicate significant differences ($^*P < 0.05$; $^{***}P < 0.001$) between control (bars without hatching) and drought/re-watered (hatched bars) plants. Significant differences ($P < 0.05$) between summer and winter climate within controls and treatments are indicated by different lowercase and uppercase letters, respectively. Statistical analysis was performed using two-way ANOVA followed by Tukey's post-hoc test, and P-values are given in Table S2 available as Supplementary data at *Tree Physiology* Online.

a significant increase of 5,8,11,14-eicosatetraenoic acid (Figure 6). No significant drought effects were found on total C, total N, soluble protein, pigment, anion, organophosphate and structural N contents, as well as $\Delta^{13}C$ (Table 1, Table S2 available as Supplementary data at *Tree Physiology* Online); only the chlorophyll a/b ratio dropped from 3.7 to 3.3 (Figure 2C).

Drought effects in the winter climate

Unlike in the summer climate, no significant effects of the drought treatment on leaf hydration, total and reduced ascorbate contents were documented in the winter climate (Figures 2 and 3). As in the summer climate, total GSH and GSSG contents were upregulated under drought, 1.4- and 2.1-fold, respectively

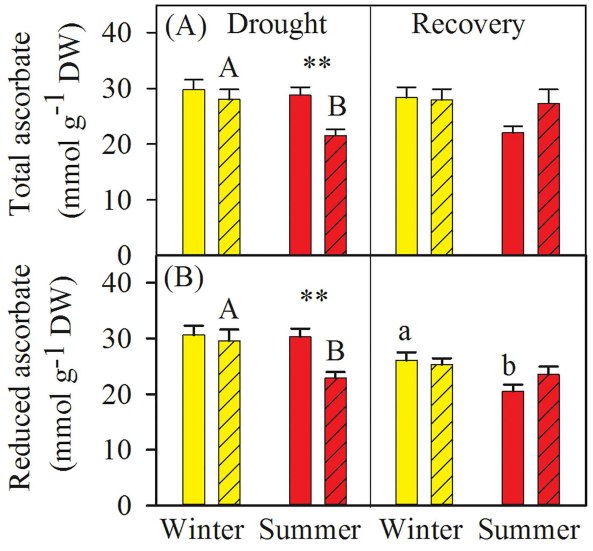

Figure 3. Total (A) and reduced (B) ascorbate contents in date palm leaves during drought (left panels) and recovery (right panels) phases in the winter and summer climates. Data shown are means + SE (n = 5–6). Asterisks indicate significant differences ($^{**}P < 0.01$) between control (bars without hatching) and drought/re-watered (hatched bars) plants. Significant differences ($P < 0.05$) between summer and winter climate within controls and treatments are indicated by different lowercase and uppercase letters, respectively. Statistical analysis was performed using two-way ANOVA followed by Tukey's post-hoc test, and P-values are given in Table S2 available as Supplementary data at *Tree Physiology* Online.

(Figure 4A and B). Similar responses of amino acids to drought treatment were observed like in summer climate, i.e., significantly increased alanine, β-alanine, glycine, proline, arginine, γ-aminobutyric acid, threonine, tryptophan and phenylalanine contents (Figures 5 and 6), which together contributed to a 123% higher TAA content than in controls. Sucrose and β-D-galactopyranosyl-1,3-arabinose were less abundant in drought treated date palm leaves in the winter climate, whereas xylose was more abundant (Figure 6). The galactonic acid content was significantly increased in drought-treated leaves in the winter climate. Most of the phenolics became less abundant during drought treatment, particularly trans-4-hydroxy-cinamic acid (Figure 6). No significant effects of drought treatment on fatty acids and stearyl alcohol were observed, except a significantly increased 5,8,11,14-eicosatetraenoic acid content as also observed in the summer climate (Figure 7C). No significant drought effects were observed on contents of total C, total N, structural N, soluble protein, pigments and anions, as well as $\Delta^{13}C$ in the winter climate (Table 1, Table S2 available as Supplementary data at *Tree Physiology* Online). Abundances of other N containing compounds, alcohols and organophosphates were largely conserved during the drought treatment (Figure 6, Table S2 available as Supplementary data at *Tree Physiology* Online).

Table 1. Total carbon (C), nitrogen (N), soluble protein, pigments, anions and structural N contents (g^{-1} dry weight), and C isotopes in date palm leaves. Data shown are mean ± standard error, n = 5–6. Significant differences ($P < 0.05$) between drought/re-watered (treatment) and control within the same climate are indicated in bold. No significant differences ($P < 0.05$) between the summer and winter climates within controls and treatments, respectively, were found. Statistical analysis was performed using two-way ANOVA followed by Tukey's post-hoc test, P-values are given in Table S2 available as Supplementary data at *Tree Physiology* Online

Parameters	Water	Drought		Recovery	
		Winter	Summer	Winter	Summer
$\Delta^{13}C$ (‰)	Control	−27.72 ± 0.44	−26.94 ± 0.42	−26.47 ± 0.74	−26.88 ± 0.38
	Treatment	−27.57 ± 0.47	−27.29 ± 0.29	−26.34 ± 0.44	−26.11 ± 0.72
Total C (mg g^{-1})	Control	490.04 ± 17.67	480.03 ± 7.12	461.49 ± 7.46	455.66 ± 11.20
	Treatment	469.16 ± 5.17	471.71 ± 4.61	466.47 ± 7.21	451.97 ± 4.29
Total N (mg g^{-1})	Control	14.42 ± 1.10	14.05 ± 0.95	14.88 ± 0.56	**15.36 ± 1.38**
	Treatment	14.49 ± 0.90	15.29 ± 0.86	13.34 ± 0.57	**11.91 ± 0.92**
Soluble protein (mg g^{-1})	Control	24.06 ± 2.74	24.59 ± 2.27	22.80 ± 1.10	22.29 ± 1.84
	Treatment	21.62 ± 1.76	22.57 ± 2.51	18.98 ± 0.97	20.11 ± 2.55
Chlorophyll a (mg g^{-1})	Control	5.42 ± 0.79	4.60 ± 0.45	3.82 ± 0.23	3.84 ± 0.33
	Treatment	4.04 ± 0.30	3.79 ± 0.54	3.60 ± 0.35	2.85 ± 0.25
Chlorophyll b (mg g^{-1})	Control	1.39 ± 0.20	1.26 ± 0.12	0.94 ± 0.07	1.05 ± 0.09
	Treatment	1.08 ± 0.08	1.12 ± 0.14	0.88 ± 0.09	0.78 ± 0.08
Carotene (mg g^{-1})	Control	1.63 ± 0.22	1.31 ± 0.12	1.23 ± 0.06	1.21 ± 0.10
	Treatment	1.34 ± 0.06	1.19 ± 0.15	1.20 ± 0.09	0.97 ± 0.08
Nitrate (μM g^{-1})	Control	2.56 ± 0.78	7.26 ± 4.01	0.60 ± 0.08	**0.61 ± 0.11**
	Treatment	4.93 ± 1.02	1.64 ± 0.17	0.65 ± 0.05	**0.41 ± 0.01**
Phosphate (μM g^{-1})	Control	29.14 ± 5.22	32.47 ± 3.17	12.61 ± 1.00	23.00 ± 2.61
	Treatment	20.49 ± 2.61	41.47 ± 4.79	18.32 ± 5.04	18.00 ± 2.01
Sulfate (μM g^{-1})	Control	50.48 ± 10.74	33.25 ± 7.15	22.11 ± 4.31	**33.58 ± 3.92**
	Treatment	45.67 ± 14.33	46.51 ± 5.58	31.60 ± 9.15	**16.29 ± 1.89**
Structural N (mg g^{-1})	Control	9.25 ± 0.95	8.43 ± 0.65	9.73 ± 0.50	**10.00 ± 1.36**
	Treatment	8.44 ± 1.28	8.77 ± 0.64	8.76 ± 0.38	**7.12 ± 0.79**
C/N ratio	Control	34.95 ± 2.72	34.83 ± 2.03	31.19 ± 0.98	**30.87 ± 2.88**
	Treatment	33.00 ± 2.05	31.30 ± 1.61	35.23 ± 1.53	**38.90 ± 3.06**

Recovery in summer and winter climate

Clustering analysis from PLS-DA showed that the metabolite profile of leaves in the winter climate largely recovered after re-watering except for a slight separation along component 3 (5.3%), whereas the drought effects partially remained or were reversed in leaves of plants in the summer climate (Figure 1C and D). The significant drought effects on leaf hydration, ascorbate, thiols, TAA and chlorophyll a/b ratio (Table 1, Figures 2–4), as well as other foliar metabolites (Figures 5 and 6) disappeared after recovery in the summer climate, except for raffinose which remained higher upon recovery of drought-treated plants (Figure 7A). However, strong increases of sugars, fumaric acid, galactinol, heptadecanoic acid, α-tocopherol and trans-sinapic acid, as well as the markedly decreases of most amino acids, other N compounds and 9,12,15-octadecatrienoic acid were documented in leaves of re-watered plants compared with controls in the summer climate (Figures 5–7). After recovery, total N and structural N contents were significantly decreased compared with controls. Consequently, the C/N ratio increased from 30.9 to 38.9 (Table 1, Table S2 available as Supplementary data at *Tree Physiology* Online).

in the winter climate, the significant drought effects on TAA, GSSG, sugars of xylose, sucrose and β-D-Galactopyranosyl-1,3-arabinose, galactonic acid, amino acids, and trans-4-hydroxy-cinnamic acid all recovered during re-watering (Table 1, Figures 2–6). Total GSH contents in leaves of re-watered plants were dramatically decreased, resulting in significantly lower total GSH contents than in controls after 2 weeks of recovery (Figure 4). Abundances of most sugars, i.e., glucose, galactose the sorbose, 6-kestose, as well as glucose-6-phosphate, lysine, 5,8,11,14-eicosatetraenoic acid, dodecanoic acid, tetradecanoic acid and an unknown compound of NA181001 were highly upregulated after recovery, whereas succinic acid, shikimic acid and 9,12-octadecadienoic acid were less abundant in leaves of recovered plants in the winter climate (Figures 6 and 7C, Table S2 available as Supplementary data at *Tree Physiology* Online).

Discussion

Compensation of drought may be more frequently required in summer climate

In arid and semi-arid regions, like the Arabian Peninsula, intense heating during the day and rapid night thermal radiation results in strong diurnal temperature fluctuations (Drennan 2009). On the other hand, apart from drought in summer, which has

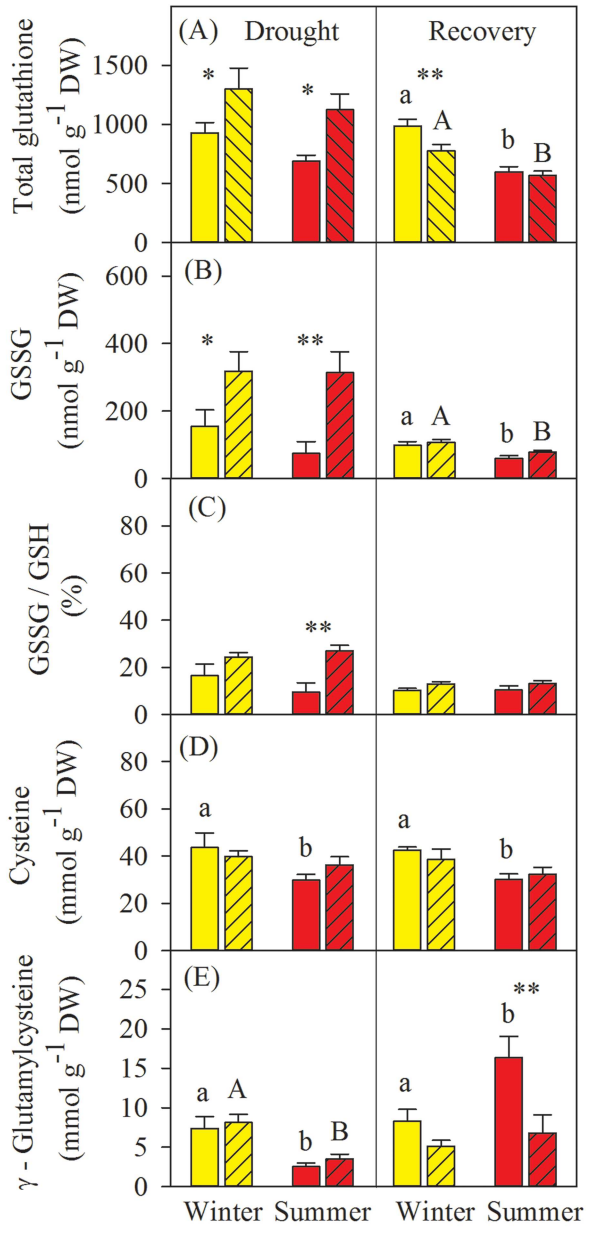

Figure 4. Thiol contents in date palm leaves during drought (left panels) and recovery (right panels) phases in the winter and summer climates. Data shown are means + SE (n = 6). Asterisks indicate significant differences (*P < 0.05; **P < 0.01) between control (bars without hatching) and drought/re-watered (hatched bars) plants. Significant differences (P < 0.05) between summer and winter climate within controls and treatments are indicated by different lowercase and uppercase letters, respectively. Statistical analysis was performed using two-way ANOVA followed by Tukey's post-hoc test, and P values are given in Table S2 available as Supplementary data at *Tree Physiology* Online.

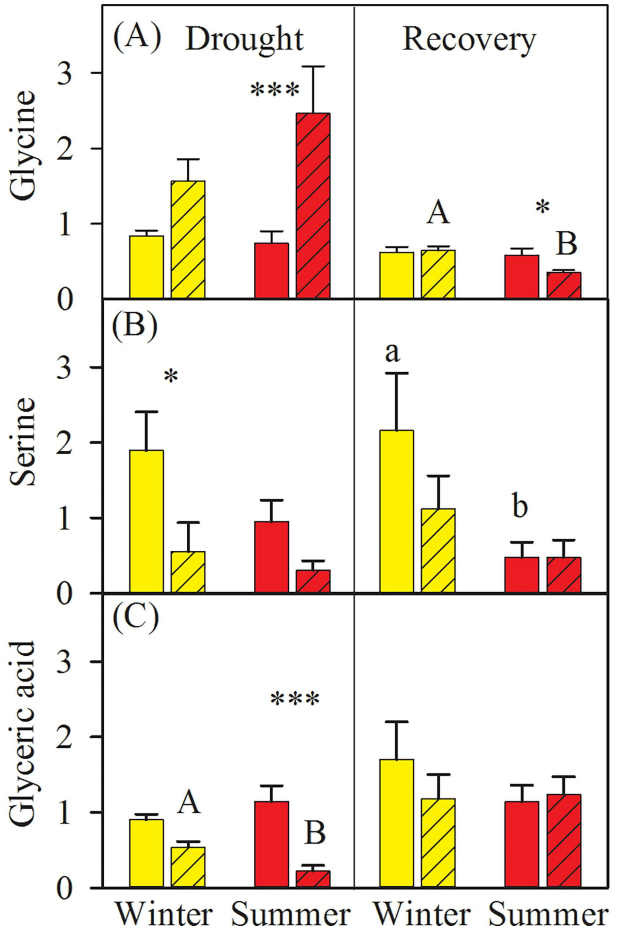

Figure 5. Relative abundances of glycine (A), serine (B) and glyceric acid ((C) in date palm leaves during drought (left panels) and recovery (right panels) phases in the winter and summer climates. Data shown are means + SE (n = 6). Asterisks indicate significant differences (*P < 0.05; ***P < 0.001) between control (bars without hatching) and drought/re-watered (hatched bars) plants. Significant differences (P < 0.05) between summer and winter climate within controls and treatments are indicated by different lowercase and uppercase letters, respectively. Statistical analysis was performed using two-way ANOVA followed by Tukey's post-hoc test, and P-values are given in Table S2 available as Supplementary data at *Tree Physiology* Online.

attracted most attention in the past, drought events in the winter have been documented and/or predicted globally, in the Arabian Peninsula (Almazroui et al. 2016), Africa (Haile et al. 2019), Europe (Vautard et al. 2007, Voltas et al. 2013), the Southeast United States (Hanson and Weltzin 2000) and Australia (Cai et al. 2005), and their frequencies are estimated to increase globally in future due to decreased precipitation and increased temperature (Vicente-Serrano and López-Moreno 2006, Buehler et al. 2011). Schwinning et al. (2005*a*) found that growth for *Oryzopsis hymenoides*, *Gutierrezia sarothrae* and *Ceratoides lanata* was far more sensitive to winter than to summer drought. Similarly, a strong decline of tree diameter growth was also documented in *Quercus suber* during the extremely dry winter in 2012 (Costa-e-Silva et al. 2015). The water loss in the winter climate is lower compared with summer climate due to the lower temperature and therefore, less stomatal closure is required to reduce the water loss upon drought. Compared with less decreased stomatal conductance in the winter, drought may

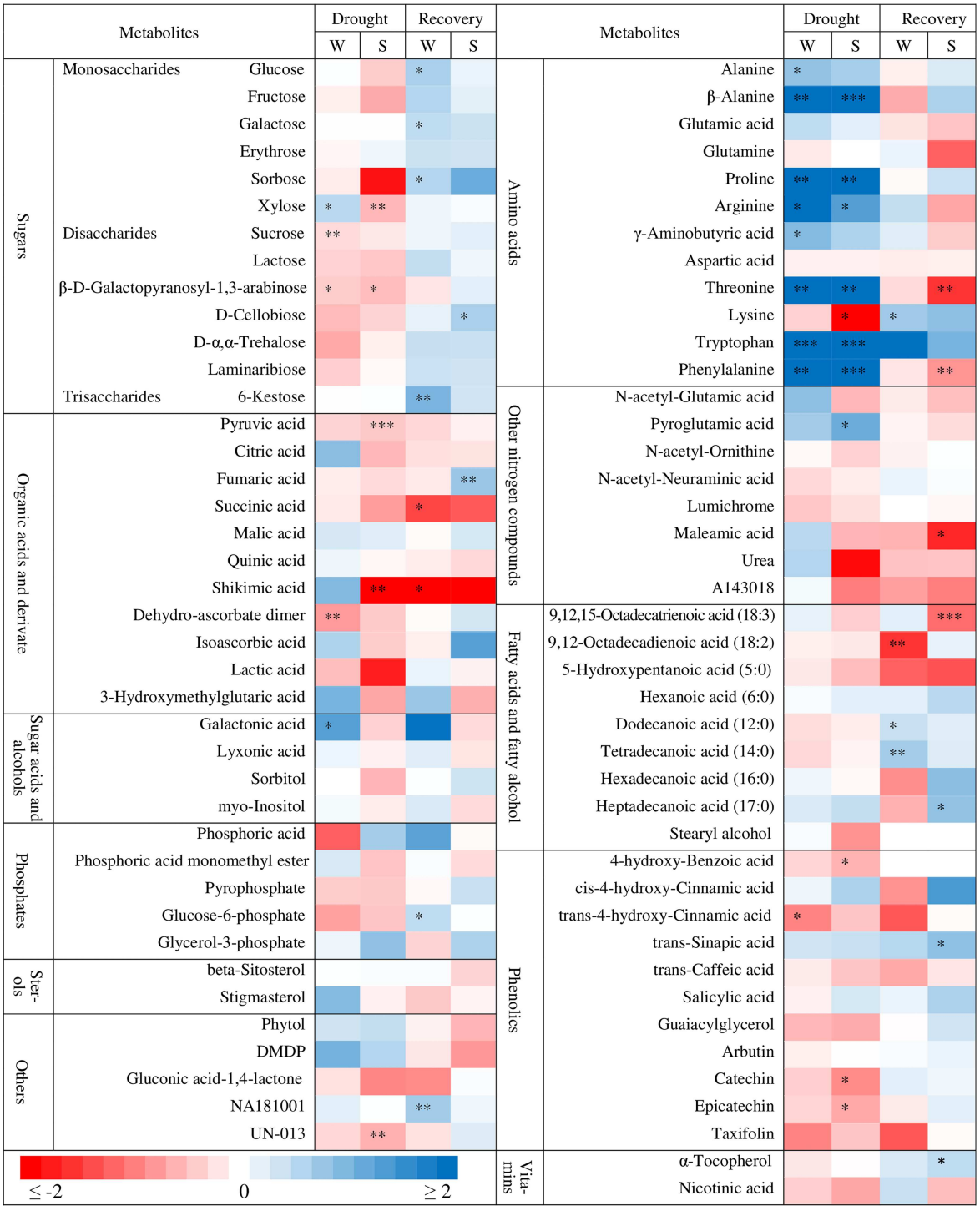

Figure 6. Changes (log_2 treatment/control) of low molecular weight metabolites during drought (left panel) and recovery (right panel) periods in the winter (W) or summer (S) climate, respectively. Statistical analysis was performed using two-way ANOVA followed by Tukey's post-hoc test, and *P*-values are given in Table S2 available as Supplementary data at *Tree Physiology* Online. *, **, ***indicate significant differences between drought and control (drought), or between re-watered and control (recovery), at $P < 0.05$, 0.01 and 0.001, respectively. A143018, N-methyl trans-4-hydroxy-L-proline (2S,4R)-4-hydroxy-1-methyl pyrrolidine-2-carboxylic acid; DMDP, 2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine; A181001, code of an unknown metabolite in Golm library; UN-013, unknown compounds.

be particularly severe in summer (Kruse et al. 2019), because drought-mediated stomatal closure depends on long-distance signaling from the roots to the leaves (Malcheska et al. 2017), which can be impaired under drought conditions (Schachtman and Goodger 2008). Moreover, the frequent co-occurrence of these climate extremes poses enhanced negative influences on

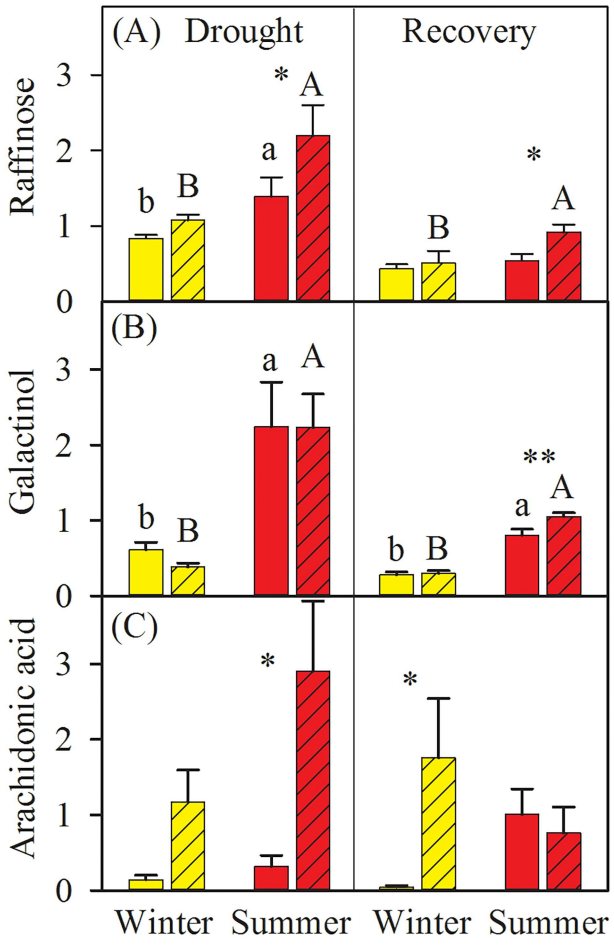

Figure 7. Relative abundances of raffinose (A), galactinol (B) and 5,8,11,14-eicosatetraenoic acid (arachidonic acid) (C) in date palm leaves during drought (left panels) and recovery (right panels) phases in the winter and summer climates. Data shown are means + SE (n = 6). Asterisks indicate significant differences ($^*P < 0.05$; $^{**}P < 0.01$) between control (bars without hatching) and drought/re-watered (hatched bars) plants. Significant differences ($P < 0.05$) between summer and winter climate within controls and treatments are indicated by different lowercase and uppercase letters, respectively. Statistical analysis was performed using two-way ANOVA followed by Tukey's post-hoc test, and P values are given in Table S2 available as Supplementary data at *Tree Physiology* Online.

plant productivity and development (Rennenberg et al. 2006, Dreesen et al. 2012).

To our knowledge, little information is available about on differences in metabolic adaptations of plants between summer and winter climates. In the present study, the effects of drought on foliar traits of young date palms differed in simulated winter and summer climates, as well as during recovery. Consistent with our fourth hypothesis, stronger effects of drought were observed in plants grown under summer climate, i.e., more prominent impacts of drought on leaf hydration, concentrations of total and reduced ascorbates, GSSG, sugars, organic acids, sugar acids, N compounds, fatty acid and phenolic compounds, as well as chlorophyll a/b and GSSG/GSH (Table 1, Figures 2–7). Therefore, subtractive or additive interactive effects of heat and drought together have likely happened in the present study. Similar observations were made by Rennenberg et al. (2006), Arab et al. (2016) and Du et al. (2019), and were attributed to the transcriptional level (Safronov et al. 2017). Similarly, Schwinning et al. (2005*b*) found that summer drought affected the water status *of O. hymenoides*, *G. sarothrae* and *C. lanata* more negatively than winter drought. Additive impacts of stress combinations were observed in other plant species, e.g., *Citrus* leaves (Zandalinas et al. 2017*a*) and *Zea mays* (Obata et al. 2015).

Under stress conditions such as drought, heat, salinity and air pollution, photorespiration is appreciated as an important part of stress responses for preventing ROS accumulation, although it may cause a loss of up to one-third of photosynthetic C fixation (Pick et al. 2013, Dusenge et al. 2019). Previous studies already showed signs of stimulated photorespiratory C flux in date palm leaves under summer climate (Du et al. 2019). In addition, considering the decreased assimilation rate and stomatal conductance in drought-treated plants in comparison with well-watered plants (Kruse et al. 2019), simultaneous occurrence of heat and drought may exacerbate the competition between photorespiration and photosynthesis (Rennenberg et al. 2006), and enhanced photorespiration can be beneficial during drought stress or high light conditions, which both reduce the capacity of the Calvin–Benson cycle to consume the NADPH and ATP generated in photosynthetic electron transport (Dusenge et al. 2019). This view is supported by the extreme accumulation of glycine and a concurrent strong decline of the abundances of its precursors glyceric acid (Kebeish et al. 2007, Pick et al. 2013) and serine, as well as the consequently enhanced glycine/serine ratio observed in drought-treated date palm leaves in the summer climate (Figure 5). Considering that dry and hot climate conditions are generally accompanied by excess light in date palms habitats, a potential protection by photorespiration may be of particular importance for this species (Rennenberg et al. 2006), since it constitutes a less energy-consuming mechanism in comparison to enzymatic and non-enzymatic processes of ROS scavenge for reducing or preventing oxidative damage (Lima Neto et al. 2017).

The foliar metabolic alterations observed in the summer climate only partly recovered after re-watering, particularly of non-structural carbohydrates such as raffinose (Figure 7A). Therefore, our fifth hypothesis has to be rejected. This probably indicates a memory effect of drought, previously observed in *Arabidopsis* plants (Virlouvet and Fromm 2015), as a trait of species such as date palm native to arid and semi-arid areas, which may help plants to better compensate for subsequent drought events (Li and Liu 2016). Plenty of studies have revealed a pervasive legacy of up to 5 years on tree growth and mortality after severe drought (Bigler et al. 2007, Anderegg

et al. 2015, Peltier et al. 2016), and there are also large differences in resistance to and recovery from drought among species (Peltier et al. 2016). The largely upregulated carbohydrate contents after recovery observed in the present study (Figure 6) probably indicate increased post-drought growth (Rivas-Ubach et al. 2014, Peltier et al. 2016, Kruse et al. 2019). The strong decline of amino acid contents upon re-watering after drought further indicates either enhanced consumption for growth and development (Kruse et al. 2019), or increased allocation in the phloem because of elevated sink strength of the roots (Hagedorn et al. 2016, Tegeder and Hammes 2018, Salmon et al. 2019). The decline of N compounds contributed to the reduced total N contents, and consequently increased C/N ratio as also shown in component 2 of the PLS-DA analysis (Figure 1C, Table S1 available as Supplementary data at *Tree Physiology* Online).

Raffinose and galactinol are thought to protect plant cells from oxidative damage induced by heat, drought, salinity or chilling (Nishizawa et al. 2008). In the current study, dramatically increased galactinol abundance ($P < 0.01$) after re-watering concurrent with maintained raffinose accumulation induced by drought (Figure 7A and B) also indicated a drought-mediated memory effect. Apparently, date palms maintain high non-structural carbohydrates concentrations after drought, e.g., D-cellobiose in summer climate, 6-kestose, sorbose, glucose and fructose in the winter climate (Figure 6, Table S1 available as Supplementary data at *Tree Physiology* Online), which might be used to mitigate subsequent periods of drought stress or to compensate its respiratory costs (Chapin et al. 1990). Memory effects might help date palms to compensate environmental constrains to be expected more frequent in future semi-arid and arid climates (Rizhsky et al. 2004, Almazroui et al. 2012, IPCC 2014).

Drought-mediated anti-oxidative capacity

Drought in summer climate may have more severe impact on plants because of concomitant higher temperature that will induce similar damage by osmotic and oxidative stress. Therefore, activating the production of antioxidants and compatible solutes constitutes a useful strategy to counteract these stresses (Wang et al. 2003). Ascorbate and glutathione are two of the most important antioxidants in plant cells involved in basic metabolic responses to abiotic or biotic stress (Noctor and Foyer 1998), and both serve as chemical scavengers of ROS in enzymatic and non-enzymatic reactions (Rennenberg et al. 2006). Drought impacted leaf ascorbate contents differed in summer and winter. Whereas Arab et al. (2016) found a drought-induced increase of foliar total ascorbate at lower temperature, we observed decreased ascorbate contents in drought-treated date palm leaves in the summer climate (Figure 3), indicating an additional temperature effect, as also documented in previous studies on climate/temperature modulated antioxidants in date palm leaves (Arab et al. 2016, Du et al. 2019). In line with our results, García-Plazaola et al. (2008) also reported in a study with 18 tree species that most species had decreased ascorbate contents during the heat and drought event in 2003 in Europe. The declined ascorbate contents were likely due to limited biosynthesis under drought indicated by the decreased precursor abundances, i.e., myo-inositol and gulonic acid-1,4-lactone, glucose and galactose, although not significant (Ishikawa and Shigeoka 2008, Bulley and Laing 2016). Still, enhanced consumption cannot be excluded from the results of these studies, since ascorbate is not only a universal non-enzymatic antioxidant with a high potential for ROS scavenging, but is also involved in a number of other fundamental functions in plants (Akram et al. 2017).

In addition to a decrease in total and reduced ascorbate contents, accumulation of total glutathione, mainly as GSSG, constitutes a characteristic response to enhanced intracellular H_2O_2 (Noctor et al. 2015). Consistent with our first hypothesis, foliar total and oxidized glutathione contents were considerably increased upon drought ($P < 0.05$), particularly in the summer climate (Figure 4). The significantly increased GSSG/GSH ratio observed in the summer climate suggests impaired GSH recycling upon drought treatment, as also observed in *Citrus* trees under combined heat and drought stress (Zandalinas et al. 2017*a*). The declined ascorbate and increased total and oxidized thiols contents in response to drought were fully recovered to control levels after re-watering, particularly total GSH, which was even lower than in controls, indicating an increased turnover (Noctor and Foyer 1998, Arab et al. 2016).

In addition to ascorbate and GSH, also secondary compounds possess high anti-oxidative potential, but only recently received attention as non-enzymatic anti-oxidants involved in the defense against biotic and abiotic stress (Hernández et al. 2004, Ahmed et al. 2015, Varela et al. 2016). Consistent with a positive correlation between ascorbate and flavanol contents observed in drought-induced *Cistus* leaves (Hernández et al. 2004), we found that both ascorbate and flavanol concentrations were decreased during drought treatment (Figures 3 and 6). It is likely that flavanols cooperate with ascorbate in the scavenging of ROS (Rivas-Ubach et al. 2014, Feucht et al. 2016, Davies et al. 2018). In contrast to the elevated concentration of phenolic compounds observed in *Quercus. ilex* in summer and/or drought treatments (Rivas-Ubach et al. 2014), abundances of catechin, epicatechin and taxifolin significantly declined in the present study in response to drought, particularly in summer climate (Figure 6). Similarly, decreased flavanol abundances were documented in date palm leaves under simulated summer climate compared with winter conditions (Du et al. 2019). In contrast, flavanols accumulated in response to acute ozone exposure (Du et al. 2018*b*). Consistent with the present results, Wang et al. (2016) reported that foliar catechin and epicatechin contents significantly decreased in tea plants (*Camellia sinensis*

L) after drought stress. Feucht et al. (2013) found that drought and heat resulted in a complete loss of nuclear flavanols in *Taxus baccata* concomitant with decreased abundances of precursors of secondary metabolites, i.e., shikimic acid, trans-4-hydroxy-cinnamic acid and 4-hydroxy-benzoic acid. It therefore can be concluded that in the present study, drought diminishes the biosynthesis of phenolic compounds (Bettaieb et al. 2011), and/or enhances its consumption due to accelerated oligomerization upon ROS detoxification (Cheynier et al. 2013).

Drought-mediated accumulation of compatible solutes

Osmotic adjustment and stomatal closure are principal mechanisms in plants to cope with water deficit (Warren et al. 2007). In this context, osmotic adjustment may precede stomatal closure, because it allows for continuing photosynthetic CO_2 fixation at low water availability by maintaining cellular turgor pressure and, hence, metabolic activity under stress conditions (Rennenberg et al. 2006). This mechanism is particularly important for young seedlings because osmotic adjustment may provide an ecological advantage for supporting the establishment of roots before they have reached deep soil water (Arndt et al. 2001). Osmo-protectants or compatible solutes are low molecular weight compounds that are electrically neutral, highly soluble and non-toxic at molar concentrations. These features are met by many sugars and sugar alcohols, amino acids and ammonium compounds (Ahn et al. 2011, Rivas-Ubach et al. 2014, Singh et al. 2015). In the present study, abundances of sugar and organic acids involved in tricarboxylic acid cycle largely declined upon drought both in summer and winter climate (Table 1, Figure 6), indicating limited photosynthesis and/or altered C metabolism under drought stress. This assumption is consistent with reduced stomatal conductance and photosynthetic C assimilation upon drought in both summer and winter climates (Kruse et al. 2019).

Instead of decreased concentrations of soluble carbohydrates, amino compounds dramatically accumulated and seemed to serve as osmo-protectants in drought-treated date palm leaves that already start to accumulate under well-watered conditions (Du et al. 2019), but particularly at acute ozone exposure (Du et al. 2018*b*), as well as heat, drought and salinity treatment (Yaish 2015, Safronov et al. 2017). Proline, an important osmolyte and signaling molecule, generally accumulates to protect membranes, proteins and enzymes against various stresses (Singh et al. 2015). In the present study, proline dramatically accumulated in drought-treated date palm leaves in both summer and winter climates (Figure 6). Drought-induced accumulation of proline in *Arabidopsis* plants, was not observed when drought was combined with heat (Rizhsky et al. 2004). Accumulation of proline may facilitate the continued synthesis of N-containing compatible solutes using excess photochemical energy available due to declined stomata opening and, therefore may be of particular significance during drought (Smirnoff et al. 1985, Kruse et al. 2019). Similar responses were reported in other evergreen tree species (Du et al. 2015, 2018*a*, de Miguel et al. 2016), indicating altered N metabolism upon drought. This is not surprising, since the changes of photorespiration discussed above are intimately connected with plant N metabolism, particularly for the formation of serine and glycine (Dusenge et al. 2019). Therefore, our second hypothesis has to be rejected, since sugars and alcohols were not accumulated upon drought.

Drought-modified membrane composition

Cell membranes are the first receptors of stress induced by drought, and decreased membrane lipid content under drought is related to an inhibition of lipid biosynthesis and a stimulation of lipolytic and peroxidative activities (Gigon et al. 2004). Fatty acids are not only essential for membrane function, but are also necessary for growth, development and plant performance (Li et al. 2016), and even a single additional double bond can significantly increase membrane fluidity (Vrablik and Watts 2012). As previously observed, either drought and elevated temperature alone or their combination has eminent effects on fatty acids composition (Arab et al. 2016, Du et al. 2019) and pathways related to fatty acid biosynthesis (Safronov et al. 2017). In line with the report of Toumi et al. (2008) in grapevine (*Vitis vinifera* L.) cultivars in response to water stress, we observed that the unsaturation level of the lipids was increased under drought, particularly in the summer climate, via the accumulation of 5,8,11,14-eicosatetraenoic acid (C20:4), as also previously observed in date palm leaves under higher temperature (Du et al. 2019). The unsaturation level decreased after recovery due to the strong reduction of 9,12-octadecadienoic acid (C18:2) and 9,12,15-octadecatrienoic acid (C18:3) in the winter and summer climate, respectively. The decreased unsaturation level of lipids was further reinforced by the concomitant accumulation of the dodecanoic acid (C12:0), tetradecanoic acid (C14:0) and hexadecanoic acid (C16:0), heptadecanoic acid (C17:0) in the winter and summer climate, respectively (Figure 6, Table S2 available as Supplementary data at *Tree Physiology* Online). The noticeable increase during drought, but decrease after recovery, of the unsaturation level of lipids may constitute a cellular mechanism that contributes to maintaining membrane fluidity under stress (Quartacci et al. 1995). Although the unsaturated fatty acid 5,8,11,14-eicosatetraenoic acid is not commonly found in plant species, it can maintain cell membrane fluidity and react with molecular oxygen owing to its four *cis* double bonds (Brash 2001). It also helps to induce general stress-responsive genes that may play a role in multi-stress responses (Savchenko et al. 2010, Shanab et al. 2018). Together with our previous report

(Du et al. 2019), we argue that increased levels of 5,8,11,14-eicosatetraenoic acid under elevated temperature or drought constitute a mitigation mechanism of date palms to control cell membrane stability and integrity (Sanchez et al. 2018). Therefore, our third hypothesis is fully confirmed.

Conclusions

Date palm leaves have developed a well-orchestrated metabolic network, including the anti-oxidative system, compatible solutes accumulation and osmotic adjustment, as well as maintenance of cell-membrane stability, that strongly reduces their susceptibility to drought. In summer climates, when drought events are particularly frequent, drought tolerance is further supported by memory effects of this metabolic network that prepare the leaves for subsequent drought events. Our results highlight the view that drought events in summer and winter climates have differed impacts on both primary and secondary metabolism of plants, and that drought tolerance requires more elaborate metabolic adjustments in summer climate.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

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

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Authors' contributions

S.A., R.H. and H.R. conceived and managed the project. H.R., J.K., P.A., J.B.W. and J.-P.S. designed the experiment. J.B.W. and J.-P.S. ensured simulation of the Saudi Arabian climate. B.D. performed biochemical measurements, analyzed the data, interpreted the results and wrote the paper. H.R. contributed to writing, reviewing and editing the manuscript. J.B.W., J.-P.S., S.A. and G.A. reviewed the paper.

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