Differences of nitrogen metabolism in date palm (*Phoenix dactylifera*) seedlings subjected to water deprivation and salt exposure

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Abstract

Drought and salt exposure are among the most prevalent and severe abiotic stressors causing serious agricultural yield losses, alone and in combination. Little is known about differences and similarities in the effects of these two stress factors on plant metabolic regulation, particularly on nitrogen metabolism. Here, we studied the effects of water deprivation and salt exposure on water relations and nitrogen metabolites in leaves and roots of date palm seedlings. Both, water deprivation and salt exposure had no significant effects on plant water content or stable carbon (C) and nitrogen (N) isotope signatures. Significant effects of water deprivation on total C and N concentrations were only observed in roots, i.e. decreased total C and increased total N concentrations. Whereas salt exposure initially decreased total C and increased total N concentrations significantly in roots, foliar total C concentration was increased upon prolonged exposure. Initially C/N ratios declined in roots of plants from both treatments and upon prolonged salt exposure also in the leaves. Neither treatment affected soluble protein and structural N concentrations in leaves or roots, but resulted in the accumulation of most amino acids, except for glutamate and tryptophan, which remained stable, and serine, which decreased, in roots. Accumulation of the most abundant amino acids, lysine and proline, was observed in roots under both treatments, but in leaves only upon salt exposure. This finding indicates a similar role of these amino acids as compatible solutes in the roots in response to salt und drought, but not in the leaves. Upon prolonged treatment, amino acid concentrations returned to levels found in unstressed plants in leaves of water deprived, but not salt exposed, plants. The present results show both water deprivation and salt exposure strongly impact N metabolism of date palm seedlings, but in a different manner in leaves and roots.

Keywords

amino acids, carbon accumulation, drought, nitrogen metabolism, roots, salinity

1. Introduction

Drought and salinity are considered the most severe and abundant abiotic stressors with detrimental impacts on plant development, growth and productivity. As a consequence, these environmental constrains cause serious agricultural yield losses, both alone and in combination (Golldack et al. 2014). Drought alone affects 45% of the world's agricultural land (Abdelraheem et al. 2019), and is projected to become more frequent and severe due to climate change (IPCC 2021). Salinity is frequently observed in coastal environments because of direct exposure to saline water, but also inland as a result of inappropriate irrigation and fertilization management of agricultural soils (Machado and Serralheiro 2017, Pulido-Bosch et al. 2018). Particularly, in arid and semi-arid areas, where about 80% of the total cropped land is irrigated, salinization of groundwater and soil is frequently reported (Morris et al. 2003, Pulido-Bosch et al. 2018). Currently, about 20% (45 million ha) of irrigated land is affected by salinity (Shrivastava and Kumar 2015). The continued economic use of these areas makes irrigation and fertilization necessary with an increased risk that salt and drought stress will be further exacerbated.

The general responses of plants to abiotic stress include stomatal closure and inhibition of photosynthesis, accumulation of osmolytes, activation of the antioxidative system, as well as metabolic and molecular adjustments (Bartels and Sunkar 2005, Abdelraheem et al. 2019, Todea et al. 2020). Although plant responses to drought and salt have much in common, particularly during the initial osmotic stress phase (Munns 2002, Munns and Tester 2008), specific effects are also reported in some species (Pérez-Alfocea et al. 1993, Hu and Schmidhalter 2005, Abdelraheem et al. 2019, Todea et al. 2020). Restricted water uptake caused by salinity will quickly result in reductions in stomatal conductance and plant growth, accumulation of compatible solutes, as well as a suite of metabolic changes identical to those caused by drought stress (Munns 2002, Bartels and Sunkar 2005). However, with extended salinity stress, the salt specific ionic phase will start when salt progressively accumulates to

toxic levels through transpiration and results in nutrient imbalances and deficiencies leading to senescence of mature leaves, if ion homeostasis cannot be reached (Munns and Tester 2008, Abdelraheem et al. 2019). In addition to sulfate- and ABA-mediated signalling between roots to shoots under drought and salt stress, Na^+ transported from roots to the leaves also contributes to stomatal closure in the case of salinity (Munns and Tester, 2008, Malcheska et al. 2017, Batool et al. 2018, Du et al. 2021b).

Date palm (*Phoenix dactylifera*) is an economically important perennial plantation crop in several arid and semiarid countries in North Africa, the Middle East and Central America. It is cultivated under a variety of environmental conditions, but encounters both drought and salt stresses naturally or due to irrigation and fertilisation (El Rabey et al. 2015). Nitrogen (N) is an essential factor for growth and development as well as fruit production of date palm (Leghari et al. 2016). Previous studies showed that N metabolic processes were strongly impacted by several abiotic stresses (Yaish 2015, Arab et al. 2016, 2023, Du et al. 2018, 2019, Anli et al. 2020, Du et al. 2021a, 2021b). Therefore, it is important for date palm cultivation to know about N interaction with drought and salt stress (Munns 2002, Bartels and Sunkar 2005, Hu and Schmidhalter 2005, Golldack et al. 2014). However, currently, little is known about the similarity and differences of drought and salt exposure on date palms' N metabolism.

The present study was performed to elucidate how drought and salinity impact the nitrogen metabolism of date palm. For this purpose, seedlings were subjected to either a water deprivation or a salt exposure treatment. Plant water relations, total carbon (C) and N concentrations and stable isotope abundances as well as different N fractions including individual amino acid concentrations in leaves and roots were determined. Specifically, the following hypotheses are addressed: 1) salt expose has greater effects on date palm water relations than water deprivation; 2) both water deprivation and salt exposure result in altered N metabolism, i.e. an accumulation of N compounds as compatible solutes that is greater with increasing time of exposure, particularly for the salt treatment; 3) the partitioning of N

compounds between leaves and roots is altered, and roots react more strongly than leaves to both stresses.

2. Material and methods

2.1 Plant material and growth conditions

About two-year old seedlings of the micro-propagated date palm cultivar Khalas were purchased from Date Palm Developments Ltd. (Somerset, U.K.). Seedlings were planted directly in 5 L pots filled with 70% quartz gravel (3-5 mm diameter, Quarzwerke GmbH, Frechen, Germany) and covered by 4 cm soil substrate (Floragard Vertriebs-GmbH, Oldenburg, Germany). Plants were cultivated in walk-in phytotrons at the Research Unit Environmental Simulation (EUS), Helmholtz Center Munich (Neuherberg, Germany) at photon flux density of 600 μmol m^{-2} s^{-1} (12/12 h, day/night), temperature of 40/20 ^{o}C and relative humidity of 5%/30%, as described previously (Du et al. 2018; Kruse et al. 2019, Ghirardo et al. 2021). Treatments were performed after 2 weeks of acclimation. Salt was applied by irrigating the plants twice a day (at 8:00 and 18:00 h) with 120 mL of a NaCl solution, gradually increasing the concentration every other day, from 100 mM to 200 mM, to 400 mM, until the final concentration of 600 mM NaCl, which was given from the sixth day onwards. For the water deprivation treatment, regular watering was stopped for three days and then only 50 mL de-ionized water per day was supplied to each plant to maintain a low soil water content. During the whole experiment, soil water contents of the control, water deprivation and salt treatments were kept at 21.4±7.5%, 10.7±5.2%, and 31.2±4.7%, respectively (Fig. S1). Soil moisture was measured with a ML2 ThetaProbe connected to a HH2 moisture meter (Delta-T, Cambridge, UK). The higher soil water contents in salt treatment than controls were due to the effect of ionic conductivity of salts on the ML2 ThetaProbe. Since the control of drought stress in the soil was our priority, the generalized calibration of the Theta probe was used.

Leaves and roots of plants from the water deprivation treatment, salt treatment and control groups were harvested on 27 June, 4th July and 25th July 2016, and are referred to as 3d, 10d and 31d samples, respectively. The youngest fully expanded leaf and the whole root of 5 plants from each treatment were harvested between 12:00 to 14:00, immediately cut into small pieces, homogenized in liquid nitrogen to a fine powder and stored at -80^{o}C until further analyses.

2.2 Plant water content measurements and biochemical analyses

Leaf and root water content (g H_2O g $^{-1}$ DW) were determined as (FW-DW)/DW, where FW and DW are the fresh mass and the dry mass after drying the samples in an oven at 60 ^{o}C for 3 d, respectively (Du et al. 2018).

Total C and nitrogen N concentrations as well as carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope abundances were determined as described by Du et al (2018). For this purpose, about 1.5-2.0 mg homogenized dry leaf or root material was weighed 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 (Flash IRMS IsoLink CN, Thermo Scientific GmbH, Bremen Germany) by a Conflo II/III interface (Thermo Scientific GmbH) (Gerschlauer et al. 2019).

Total amino acids (TAA) and soluble proteins were extracted and quantified using photometric methods as described previously (Du et al. 2014). Glutamine and bovine serum albumin were used as standards for the calculation of TAA and protein concentrations, respectively. Structural N concentration was calculated by subtracting the amounts of N in the TAA and soluble protein fractions from the total N in leaf and root material as described previously (Du et al. 2014).

The concentrations of individual amino acids were determined using an Agilent GC/MSD system consisting of an Agilent GC 7890A gas chromatograph (Agilent Technologies) connected to a 5975C Inert XL EI/CI MSD quadrupole MS detector (Agilent Technologies).

Amino acids were extracted, derivatized and separated as reported previously (Du et al. 2018). Amino acid standards (Thermo Scientific Pierce Amino Acid Standard H, Thermo Scientific, Waltham, MA USA) subjected to the same procedure as plant extracts were used for the quantification of individual amino acids.

2.3 Statistical analysis

The software package SigmaPlot 12.0 (Systat Software GmbH, Erkrath, Germany) was used for statistical analyses and the generation of figures. Differences between control and treatments, i.e., drought and salt, were determined by ANOVA and post-hoc tests (Tukey's). Student's t-test was performed to examine differences between leaves and roots within the same treatment and time point. Data were transformed by denary logarithm to match normal distribution when necessary. Data shown in figures and tables represent means ± SD of 5 plants (n=5) on a dry weight basis.

3. Results

Neither water deprivation nor salt exposure affected leaf or root water content, or $\delta^{13}C$ and $\delta^{15}N$ signatures, compared to unstressed plants during the entire experiment (Fig. 1). Compared to water deprivation, salt exposure caused a slightly lower leaf water content at the end of the experiment (Fig. 1A). Water content of roots was higher than that of leaves, irrespective of the treatment ($p < 0.01$, Fig. 1A). Leaves exhibited more negative $\delta^{13}C$ values than roots ($p < 0.001$, Fig. 1B); also $\delta^{15}N$ signatures of roots were generally higher than in leaves (Fig. 1C). Significant effects of on plant growth were only observed on shoot as well as total fresh weight at 31 d, i.e., both drought and salt treatments caused similarly decreased shoot concurrent with total biomass (Fig. S2).

Water deprivation did not affect foliar total C and N concentrations, or foliar C/N ratios, significantly. Whereas, compared to the control and drought stressed plants, salt exposure

resulted in significantly lower total C concentrations after 10d, and increased total N concentrations, resulting in reduced foliar C/N ratios, after 31d (Fig. 2). In roots, both water deprivation and salt exposure lowered total C and increased total N concentrations, but the effects of salt exposure were greater than those of water deprivation at 10d and 31d (Fig. 2 A, B). After 10d, both water deprivation and salt exposure significantly reduced root C/N ratios (Fig. 2C). In general, leaves had higher C and N concentrations, and, therefore, lower C/N ratios (Fig. 2).

Water deprivation and salt exposure did not affect soluble protein and structural N concentrations in leaves or roots significantly compared to controls (Fig. 3A, C). However, both treatments induced higher total amino acid concentrations in leaves and roots (Fig. 3 B). Significant differences between water deprivation and salt exposure were observed in leaf soluble protein after 10d and in total amino acid concentrations after 31d (Fig. 3A, B). In contrast to total amino acid concentrations, soluble protein contents were higher in water derived than salt affected leaves. Leaves contained significantly ($p < 0.01$) higher concentrations of soluble protein and structural N than roots, whereas roots contained significantly ($p < 0.01$) higher concentrations of amino acids than leaves (Fig. 3).

Lysine, glutamate, proline, serine and arginine were the most abundant amino acids in both leaves and roots (Table 1). Both water deprivation and salt exposure had significant effects on the abundances of amino acids. After 3d of treatment, foliar concentrations of leucine, isoleucine, threonine, tyrosine, and phenylalanine as well as root tyrosine concentrations were significantly increased compared to controls, whereas serine concentrations declined during the entire duration of the experiment. After 10d of treatment, valine, isoleucine, methionine, threonine, arginine, tyrosine, and phenylalanine concentrations increased in leaves and roots in response to both water deprivation and salt exposure. Significantly increased proline and lysine concentrations in leaves and roots were only observed after 31d of water deprivation and salt exposure. Compared to water deprivation, salt exposure caused more noticeable accumulation

of valine, leucine, isoleucine, methionine, threonine, lysine, proline, arginine and phenylalanine in leaves after 31d of treatment (Table 1). Significant differences in amino acid concentrations were also observed between leaves and roots, with mostly higher aspartate and alanine concentrations in roots than in leaves, and mostly higher phenylalanine and tryptophan concentrations in leaves than roots. Compared to controls, water deprivation and salt exposure reversed or enhanced the differences in partitioning of serine, leucine, threonine, tyrosine, alanine and arginine between leaves and roots (Table 1).

4. Discussion

4.1 Effects of drought and salinity on water relations

Like drought-induced water deficit, hyperosmotic stress of plants exposed to excess salt also results in water deficit in a comparable way (Golldack et al. 2011). In the present study, neither water deprivation nor salt exposure had significant effects on water relations of date palms as indicated by conserved leaf and root water contents as well as $\delta^{13}C$ abundances between treatments and controls. This was observed after 3 days of treatments and for the subsequent 4 weeks indicating little effects of water deprivation and salinity on stomatal conductance (Fig. 1). Previous observations of drought induced increase in leaf water contents (Arab et al. 2016), decrease of photosynthesis (Kruse et al. 2019, Ghirardo et al. 2021) and sea water induced stomatal closure (Du et al. 2021b) were not found in the present study, possibly due to intraspecific differences, age mediated effects, as well as the differences in the duration and intensity of treatments (Lloret et al. 2009). Moreover, the instantaneous photosynthesis is not always revealed by bulk leaf $\delta^{13}C$ signatures, since the integrative variation in carbon isotope fractionation cannot be directly attributed to current variations in stomatal conductance (Brandes et al. 2007, Werner et al. 2012). The very young seedlings with cotyledons of cultivar Khodry used in the study of Du et al. (2021b) were probably more sensitive than the plants used in the present study. The effects of water deprivation and salinity observed in the present study

are consistent with those in cotton plants (Abdelraheem et al. 2019) and mangroves (*Avicennia germinans*) (Sobrado 1999), but contrast with those in salt treated safflower (*Carthamus tinctorius* L.) (Hussain and Al-Dakheel 2018), that also grow in arid and semi-arid regions and are classified as being drought- and moderately salt-tolerant (Penna et al. 1998, Beyyavas et al. 2011). The present results on date palm water relations indicate strong dehydration avoidance via stomatal closure as previously observed under drought and salt treatments (Kruse et al. 2019, Du et al. 2021) as well as high salt tolerance capacity by strategies of compartmentalization, exclusion and secretion of ions (Munns and Tester 2008, Yaish and Kumar 2015, Arab et al. 2023). Therefore, our first hypothesis that salt exposure has greater effects on date palm water relations than water deprivation has to be rejected. However, it cannot be excluded that water deprivation, and particularly salt exposure, inhibited C assimilation (Du et al. 2021b) and stimulated carbohydrate metabolism (Safronov et al. 2017), as indicated by the significant reduction in total C concentrations, particularly in roots of salt treated plants (Fig. 2). Moreover, in comparison to water deprivation, salt exposure had greater impacts on foliar water and plant C concentrations, probably due to stronger osmotically driven stomatal closure (Sperling et al. 2014, Arab et al. 2016, Patankar et al. 2019, Du et al. 2021a, 2021b, Ait-El-Mokhtar et al. 2022). More significant effects of drought than salt exposure were observed previously in silver fir (*Abies alba* Mill.) seedlings (Todea et al. 2020).

4.2 Effects of water deprivation and salinity on nitrogen metabolism

Metabolic responses to stressful conditions are dynamic and species-specific, and also depend on the type and strength of the stress (Krasensky and Jonak 2012). Nitrogen metabolism is not only a limiting factor for growth and development (Leghari et al. 2016), but is also involved in stress responses of plants (Cui et al. 2019, Ghirardo et al. 2021). Despite being an economically important cultivated tree species, relatively little research has been devoted to understanding the mechanisms of adaptation to salinity in date palm (Yaish and Kumar 2015). In particular,

there is little information on the similarities and differences in the responses of nitrogen metabolism to drought and salt exposure. Previous studies have shown that nitrogen metabolism is strongly impacted by several abiotic stresses (Yaish 2015, Arab et al. 2016, Du et al. 2018, 2019, 2021a, Anli et al. 2020, Ghirardo et al. 2021). Nitrogen containing compounds, particularly amino acids and soluble protein play pivotal roles in coping with stress in date palms (Yaish 2015, El Rabey et al. 2015, Arab et al. 2016, Leghari et al. 2016, Safronov et al. 2017, Yaish et al. 2017, Du et al. 2018, 2019, 2021a, 2021b). Previous studies have documented drought and salinity induced increases in total N and amino acid concentrations in roots and leaves (Arab et al. 2016, Du et al. 2021b, Ghirardo et al. 2021). Similarly, decreased foliar C concentrations together with increased N concentrations were observed in salt treated safflower plants (Hussain and Al-Dakheel 2018). The increased N concentrations are mostly due to the accumulation of amino acids rather than soluble proteins, particularly in leaves of plants under salt exposure (Fig. 2, 3). Compared to water deprivation, which had no significant effect on foliar total N concentrations, salt exposure led to increased foliar total N upon prolonged exposure, which was attributed to the accumulation of amino acids, particularly those derived from pyruvate and oxaloacetate as well as arginine and aromatic amino acids (Table 1). Moreover, in roots the increased total N concentrations induced by water deprivation recovered upon prolonged exposure, but this was not observed in salt exposed plants indicating a long-lasting effect of salinity (Fig. 2). Therefore, our second hypothesis that both water deprivation and salt exposure result in altered N metabolism, i.e. an accumulation of N compounds as compatible solutes that is greater with increasing time of exposure, is partially supported by the altered N metabolism under water deprivation and, particularly, at salt exposure.

In the present study, lysine, glutamate, serine and proline were the most abundant amino acids in both leaves and roots, similar to previous findings in needles of temperate Douglas fir (Du et al. 2014). Accumulation of proline is a general response to abiotic stress in the date

palms (Yaish 2015) as well as in cottons (Liang et al. 2016), and many other plants species (Kaur and Asthir 2015, Cui et al. 2019). An increase in proline concentration is a positive indicator of salinity and drought tolerance because it can serve as a compatible solute (Yaish 2015, Liang et al. 2016). In the present study, proline concentrations were in the range of other studies on date palms (Al-Khayri and Al-Bahrany 2004, Faten and Al-Khayri 2008, Yaish 2015), but significantly increased proline concentrations were only observed after a month of the treatment's onset, in leaves of plants upon salt exposure and in roots of plants from both treatments. Similar effects were also observed for lysine, which also accumulated only upon prolonged stress exposure. As the duration of the treatments increased, foliar concentrations of amino acids returned to the levels in unstressed plants in the water deprived plants, but increased further in the salt exposed plants (Fig. 3, Table 1). Similar accumulation of amino acids was observed in barley (Jones and Storey 1978) and tomato plants (Pérez-Alfocea et al. 1993) treated with PEG and NaCl at the same osmotic pressures. Accumulation of foliar osmolytes was also reported in silver fir (*Abies alba*) seedlings under drought and salt treatments (Todea et al. 2020). The extended effects of salt exposure compared to water deprivation on plant N status observed in the present study are probably due to salt accumulation and concomitant changes in ion homeostasis (Munns 2002). Arginine is considered a marker of tree N status (Funck et al. 2008) and its accumulation indicates both sequestration of N and/or N storage (Schneider et al. 1996, Gessler et al. 1998, Millard and Grelet, 2010). Despite large variation, a large accumulation (>100 fold) of arginine was already observed after 10 days of treatments, indicating not only altered N metabolic adjustment, but also continued N uptake by the plants (Funck et al. 2008, Millard and Grelet 2010). In accordance with insignificant effects on soluble protein concentrations of salt or drought stress in two cotton genotypes with contrasting salt tolerance (Ibrahim et al. 2019), as well as upon drought and other abiotic stresses in date palms (Du et al. 2018, 2019, 2021a), no effects on soluble protein concentrations were observed in either water deprived or salt exposed plants in the present study. Probably the imposed stresses

were not severe enough to cause protein degradation as also previously observed only under combined drought and salinity stress, but not in the sole salt or drought treatment (Ibrahim et al. 2019). This is consistent with the previous results showing that drought had no significant effect on foliar total protein abundance of date palm seedlings, but temperature did (El Rabey et al. 2015, Ghirardo et al. 2021). Whereas, salinity normally resulted in decreased soluble protein contents in date palm leaves (El Rabey et al. 2015, Ait-El-Mokhtar et al. 2021).

4.3 Roots and shoots reacted differently to water deprivation and salt exposure

Little work has been done on roots with regard to either salt or water stress, although roots are directly exposed to salt or drying soil and, therefore, are the most vulnerable part of the plant (Munns 2002). Together with the decreased shoots growth observed at the end of the experiment, the significant decrease in total C concentration in roots of date palms in response to both treatments observed in the present study suggests a restricted C-allocation from the leaves to the roots to maintain the foliar C balance (Gessler et al. 2017). In addition, the increased foliar C concentration upon prolonged salt exposure suggests the accumulation of carbohydrates as compatible solutes in addition to amino acids (Singh et al. 2015). Still, earlier and stronger impacts on N metabolism were observed with salt exposure compared to water deprivation. The partitioning of amino acids between leaves and roots also reacted to both treatments. In general, there were higher concentrations of serine, leucine, threonine and tyrosine in roots than in leaves. However, the partitioning of arginine, which generally has higher concentrations in roots than in leaves, was reversed upon salt exposure, resulting in a large accumulation of arginine in leaves (Table 1). Similarly, accumulation of arginine was documented previously in leaves of date palm under drought and salt treatment (Jana et al. 2019, Du et al. 2021a, 2021b), probably indicating internal translocation from roots to leaves and/or enhanced biosynthesis of arginine (Winter et al. 2015, Arab et al. 2023). Drought and salt also induced the accumulation of phenylalanine in leaves and roots in our experiment, as previously

observed by Du et al. (2021a, 2021b). This result is consistent with previous studies where spraying of phenylalanine improved the salt tolerance of maize (*Zea mays*) and broad bean (*Vicia faba*) and, therefore, can be considered a mechanism to mitigate salt stress by restricting Na^+ but improving K^+ uptake (Abd El-Samad et al. 2011). The accumulation of phenylalanine and tyrosine in leaves and roots also indicates stimulated secondary metabolism under both stresses (Barros and Dixon 2020). In contrast to the general accumulation of other amino acids, serine concentrations decreased significantly in roots after 10 days of treatments (Table 1). Similar decreases of serine concentrations were reported in drought stressed barley (*Hordeum vulgare*) roots (Sicher et al. 2012). Therefore, our third hypothesis that the partitioning of N compounds between leaves and roots is altered, and roots react more strongly than leaves to both stresses has to be rejected, because the partitioning of amino acids of both, leaves and roots were strongly impacted by both stresses.

5. Conclusions

Although date palms are thought to be drought and salt tolerant to some extent, N metabolism of both leaves and roots was altered by drought and salt exposure even after a short exposure time. However, N metabolic adjustment differed significantly between salt and drought exposure in date palms. Apparently, the ability of date palms to regulate their N metabolic and physiological functions differently under different stress conditions plays an important role in the stress tolerance of this species in which growth and development are adapted to arid and semi-arid regions as well as coastal areas.

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Authors' Contributions

S.A., R.H. and H.R. conceived and managed the project. H.R., 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. M.D. performed the IRMS analyses. B.D. performed biochemical measurements, analysed the data, interpreted the results and wrote the paper. H.R. contributed to writing, reviewing and editing the manuscript. All authors reviewed the paper.

Conflict of interest

None declared.

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Table 1. Concentrations of amino acids in leaves and roots of unstressed (control) date palms and date palms subjected to water deprivation or salt exposure. Data are mean ± SD (n = 5). Different letters indicate significant differences between control, water deprivation and salt exposure. Bold values indicate differences between leaves and roots.

Amino acids		Leaf			Root		
		Control	Drought	Salt	Control	Drought	Salt
Serine	3d	1.35±0.44	1.01±0.12	0.92±0.25	1.82±0.12 a	0.61±0.52 b	0.54±0.65 b
(μmol g^{-1})	10d	**0.93±0.46**	**0.85±0.15**	**1.15±0.16**	**1.65±0.16** a	**0.21±0.07** b	**0.22±0.12** b
	31d	1.06±0.42	**1.06±0.26**	1.15±0.24	1.72±0.17 a	**0.20±0.03** b	0.79±0.72 b
Glycine	3d	9.80±4.87	13.21±5.80	43.88±60.66	17.45±4.21	30.25±9.36	18.78±5.08
(nmol g^{-1})	10d	8.67±1.59 a	68.16±35.56 b	43.96±24.62 b	17.44±5.67	24.91±5.18	49.70±47.21
	31d	9.74±6.12 a	69.07±37.37 b	85.64±36.16 b	22.86±15.10	44.28±11.87	49.87±8.52
Valine	3d	27.99±29.90	47.57±24.64	187.82±269.87	42.90±8.81	48.35±17.73	58.03±17.13
(nmol g^{-1})	10d	11.21±1.64 a	268.29±193.11 b	409.27±170.39 b	24.03±14.72 a	102.19±29.11 b	257.79±208.51 b
	31d	16.80±6.94 a	86.67±70.16 b	407.51±110.40 c	42.24±23.69 a	202.40±92.69 b	163.98±87.61 b
Alanine	3d	35.32±13.43	21.10±8.55	**13.29±5.23**	29.88±13.68	44.29±21.68	**68.29±52.20**
(nmol g^{-1})	10d	13.33±8.71	25.54±6.62	**10.44±4.36**	31.94±18.74 a	64.08±17.35 ab	**117.18±29.98** b
	31d	30.04±23.02	**27.49±12.34**	**28.25±18.97**	33.53±22.89 a	**131.47±42.79** b	**159.61±89.85** b
Leucine	3d	4.14±4.82 a	24.02±20.05 b	45.53±42.70 b	6.85±2.29	11.45±1.96	16.08±4.77
(nmol g^{-1})	10d	**1.71±0.37** a	**76.27±44.23** b	**117.12±50.14** b	**4.99±1.87** a	**17.78±3.48** b	**42.63±28.79** b
	31d	2.89±1.40 a	18.40±14.37 b	**66.19±11.42** c	6.18±2.12	17.61±6.40	**13.10±4.70**
Isoleucine	3d	8.39±9.22 a	22.54±14.74 ab	65.25±79.83 b	11.09±2.95	15.46±3.86	21.07±7.78
(nmol g^{-1})	10d	2.85±0.86 a	89.65±52.95 b	184.48±68.16 b	7.00±4.20 a	42.89±22.20 b	109.55±72.59 b
	31d	5.34±1.41 a	29.00±11.67 b	106.16±21.87 c	12.67±7.29 a	51.74±18.65 b	54.06±17.42 b
Aspartate	3d	**25.79±5.10**	**14.56±6.60**	**23.88±21.19**	**172.54±49.74**	**199.05±62.55**	**224.43±84.17**
(nmol g^{-1})	10d	**16.43±7.68**	**34.59±27.98**	**26.08±11.42**	**130.00±14.13**	**179.57±51.76**	**200.90±60.58**
	31d	**18.76±11.06** ab	**15.87±8.16** a	69.60±29.20 b	**127.26±84.83**	**318.21±137.43**	157.53±73.90
Methionine	3d	0.62±0.33	0.88±0.54	2.47±3.01	1.33±0.43	1.22±0.62	2.14±0.97
(nmol g^{-1})	10d	0.41±0.09 a	1.87±0.89 b	6.12±2.81 c	0.65±0.34 a	3.40±2.07 b	10.70±4.67 c
	31d	0.32±0.17 a	**0.70±0.42** a	2.46±0.61 b	0.91±0.62 a	**5.12±2.36** b	6.90±3.07 b
Threonine	3d	1.83±2.32 a	19.51±23.35 b	25.03±21.57 b	4.10±4.03	15.48±11.80	12.21±10.05
(nmol g^{-1})	10d	**0.72±0.19** a	**54.11±20.08** b	76.87±39.60 b	**4.96±3.37** a	**17.94±13.41** b	36.74±8.34 b
	31d	1.22±1.03 a	18.20±16.21 b	70.55±15.63 c	5.58±6.50 a	18.43±7.83 b	25.76±13.21 b
Lysine	3d	43.19±25.64	51.28±6.63	55.94±33.07	58.21±12.77	80.68±24.80	45.63±19.68
(μmol g^{-1})	10d	32.37±10.78	56.52±23.50	**28.24±16.40**	43.00±12.78	58.12±15.25	**65.15±20.00**
	31d	25.86±9.42 a	**38.25±4.26** ab	71.21±23.90 b	47.06±24.39 a	**114.36±31.18** b	84.10±28.91 ab
Glutamate	3d	1.52±0.67	1.95±1.08	2.39±0.68	1.95±0.50	2.52±0.87	2.41±1.37
(μmol g^{-1})	10d	1.27±0.41	2.96±1.28	2.01±0.58	1.33±0.71	2.79±0.32	2.96±1.20
	31d	1.31±0.56	1.70±0.44	2.54±0.46	1.19±0.61	3.34±1.15	2.14±1.08
Proline	3d	0.18±0.06	0.14±0.03	0.28±0.34	0.68±1.14	0.15±0.05	0.48±0.75
(μmol g^{-1})	10d	0.12±0.02	0.14±0.03	0.27±0.04	0.36±0.54	0.15±0.09	0.22±0.04
	31d	0.11±0.02 a	0.16±0.06 a	0.56±0.21 b	0.10±0.05 a	0.27±0.07 b	0.53±0.25 b
Arginine	3d	66.68±118.47	45.30±34.49	879.18±1,716.62	81.14±61.22	175.47±59.14	317.21±332.38
(nmol g^{-1})	10d	**11.13±5.48** a	115.94±100.84 b	387.59±153.25 b	**80.41±46.62** a	189.13±150.17 ab	327.77±99.82 b
	31d	40.03±49.92 a	359.11±540.54 a	**4369.61±1055.75** b	92.78±82.70	297.43±190.94	**352.96±235.49**
Tyrosine	3d	0.94±1.21 a	3.58±2.43 b	14.33±22.41 b	2.68±0.93	3.09±1.22	4.84±1.68
(nmol g^{-1})	10d	**0.37±0.05** a	**26.47±26.47** b	10.06±2.98 b	**1.75±0.84** a	**5.27±1.43** b	15.16±7.30 b
	31d	1.08±0.85 a	5.09±3.31 b	21.74±12.27 b	2.12±1.44 a	10.65±3.91 b	9.76±5.25 b
Tryptophan	3d	**1.23±0.59**	1.10±0.80	1.19±0.47	**0.35±0.14**	0.34±0.19	0.40±0.30
(nmol g^{-1})	10d	0.91±0.43	**4.87±2.44**	1.37±0.74	0.36±0.02	**0.35±0.19**	0.40±0.28
	31d	**1.44±0.47**	1.98±1.20	1.32±0.71	**0.16±0.04**	0.74±0.71	0.40±0.17
Phenylalanine	3d	15.62±16.89 a	24.09±10.89 ab	**148.44±231.94** b	4.54±1.13	5.92±1.81	**7.11±1.81**
(nmol g^{-1})	10d	5.66±0.90 a	**130.52±50.10** b	**210.53±50.20** b	4.23±2.30 a	**10.83±3.97** b	**20.58±10.76** b
	31d	4.33±2.45 a	15.44±17.24 a	**268.41±76.97** b	3.68±1.58	9.51±3.00	**7.88±3.57**

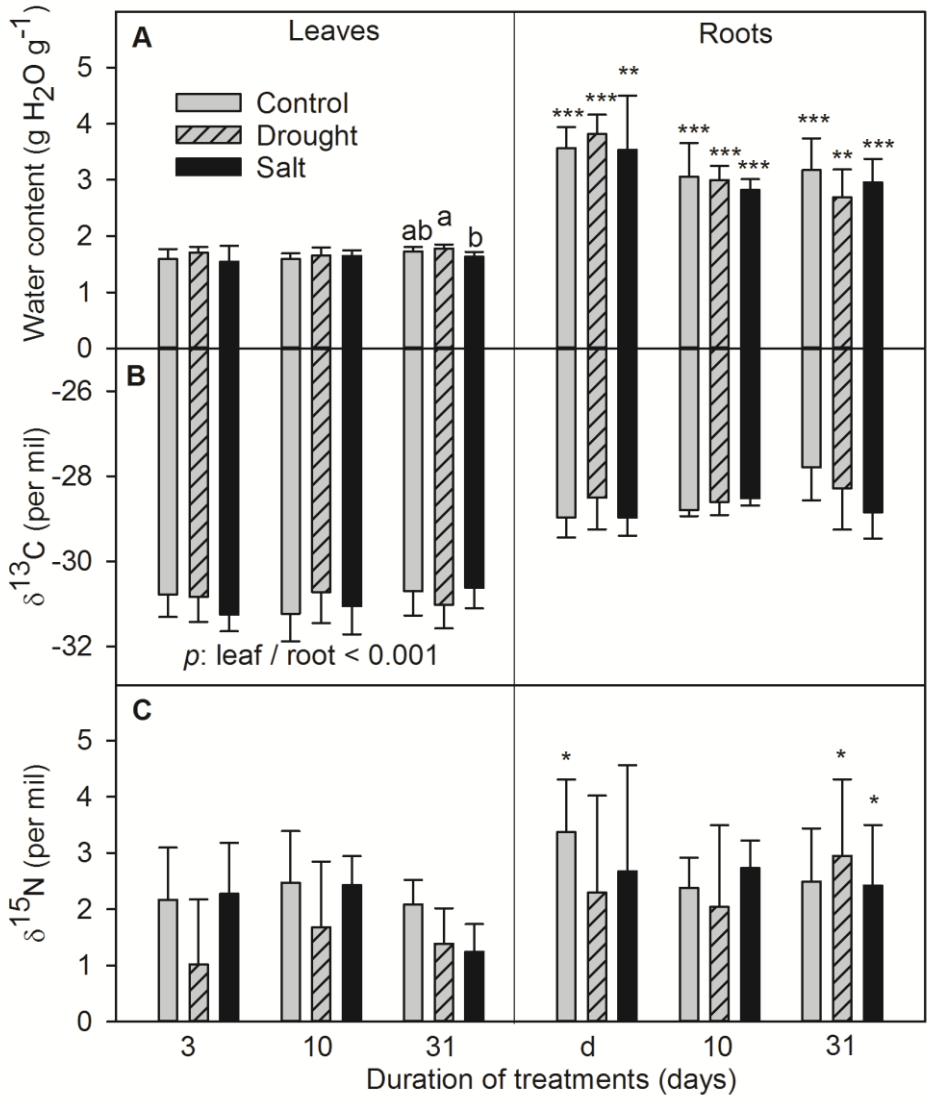

Fig. 1 Water content (A), $\delta^{13}C$ (B) and $\delta^{15}N$ (C) signatures in date palm subjected to water deprivation and salt exposure. Leaves (right panel), roots (left panel); control (grey bars), water deprivation (hatched bars) and salt exposure (black bars) treated plants. Data are mean ± SD (n = 5). Different letters indicate significant differences ($p < 0.05$) between control, water deprivation and salt exposure at the same sampling date. Asterisks indicate significant differences between leaves and roots within the same treatment and sampling date (*, ** and *** indicate $p < 0.05$, 0.01 and 0.001, respectively). For $\delta^{13}C$, all leaves differed significantly from roots ($p < 0.001$).

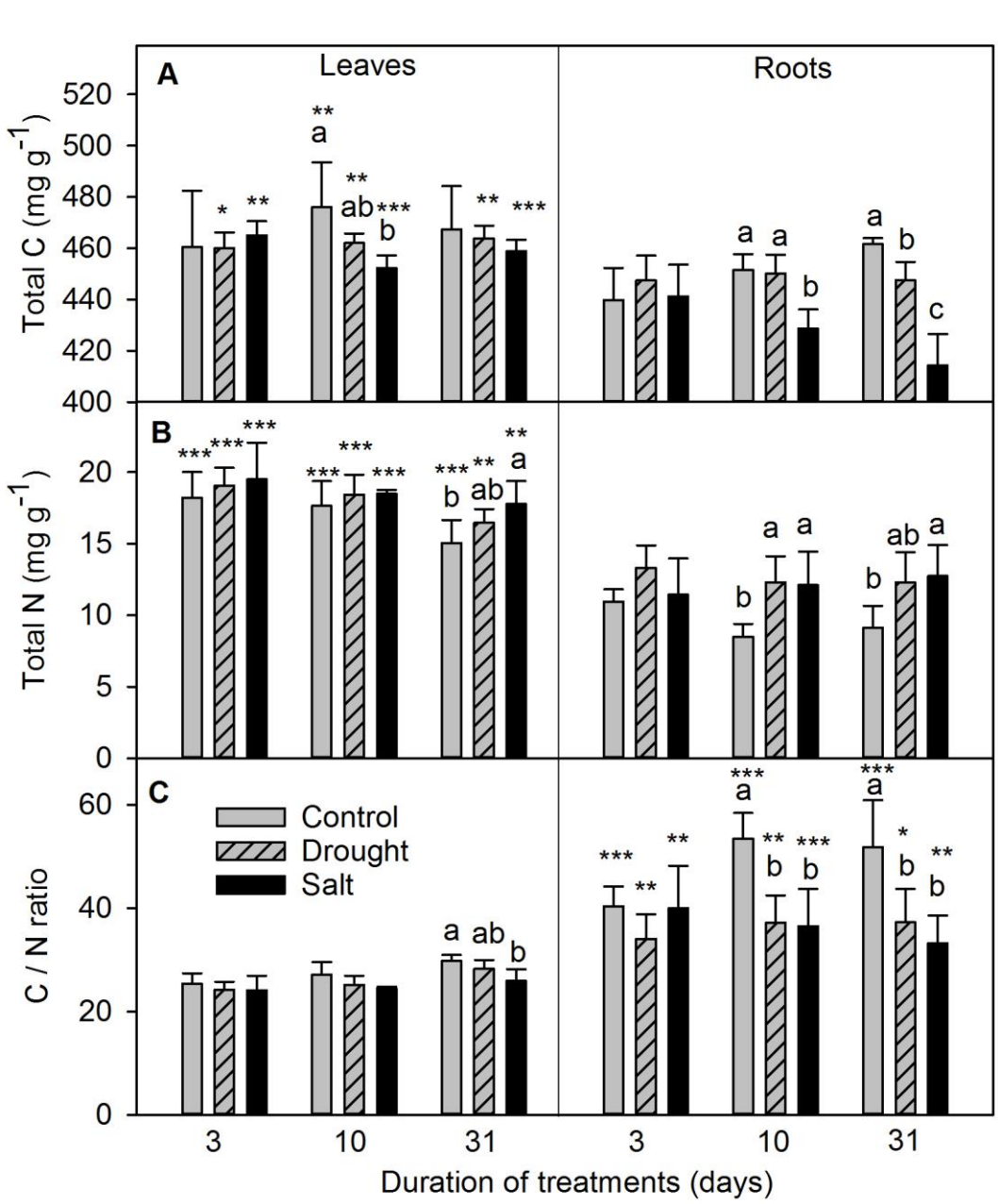

Fig. 2 Total C (A) and total N (B) concentrations, and C/N ratios (C), in date palm subjected to water deprivation and salt exposure. Leaves (right panel), roots (left panel); control (grey bars), water deprivation (hatched bars) and salt exposure (black bars) treated plants. Data are mean ± SD (n = 4-5). Different letters indicate significant differences ($p < 0.05$) between control, water deprivation and salt exposure at the same sampling date. Asterisks indicate significant differences between leaves and roots within the same treatment and sampling date (*, ** and *** indicate $p < 0.05$, 0.01 and 0.001, respectively).

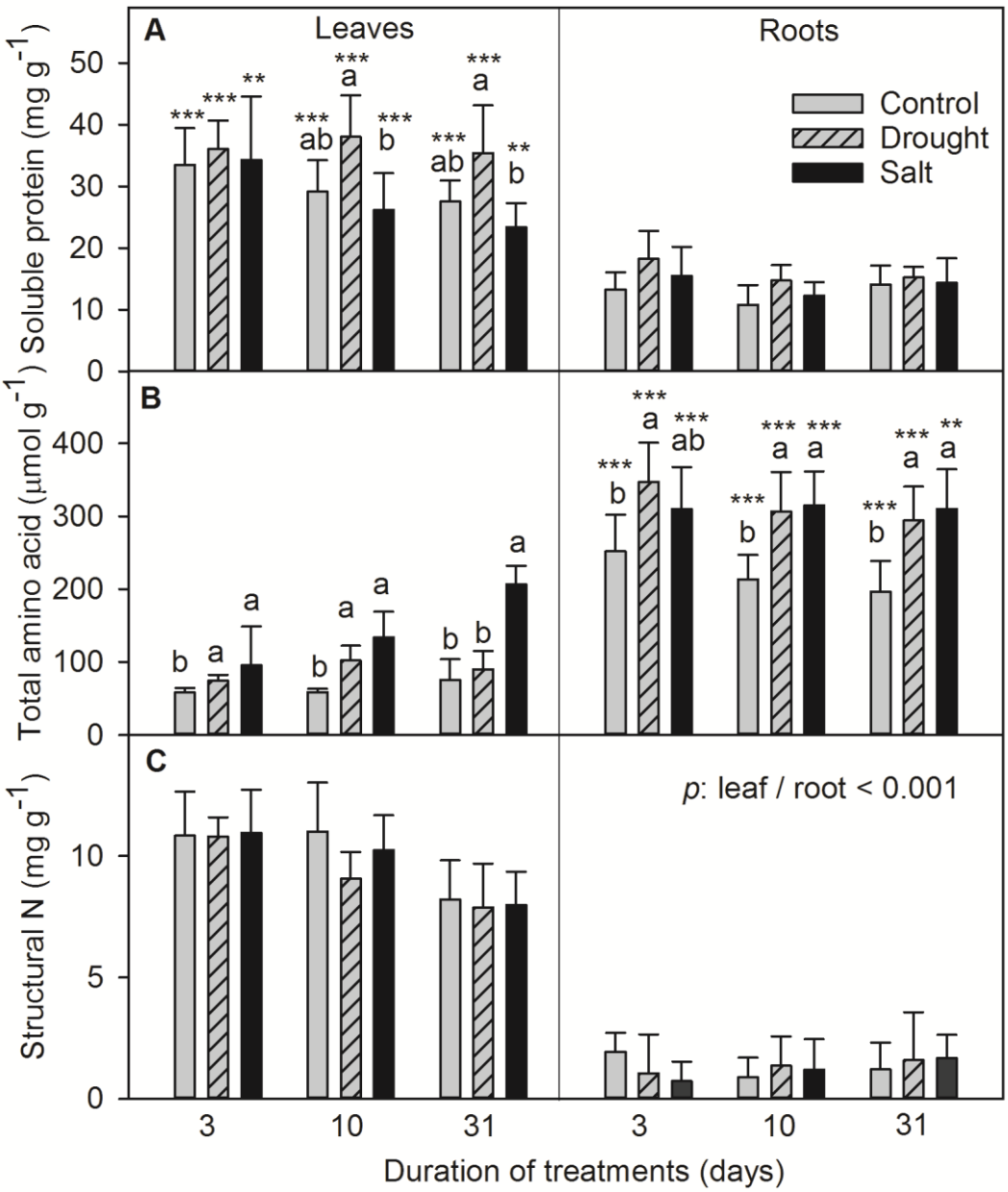

Fig. 3 Soluble protein (A), total amino acid (B) and structural N (C) concentrations in date palm subjected to water deprivation and salt exposure. Leaves (right panel), roots (left panel); control (grey bars), water deprivation (hatched bars) and salt exposure (black bars) treated plants. Data are mean ± SD (n = 5). Different letters indicate significant differences ($p < 0.05$) between control, water deprivation and salt exposure at the same sampling date. Asterisks indicate significant differences between leaves and roots within the same treatment and sampling date (** and *** indicate $p < 0.01$ and 0.001, respectively). For structural N, all leaves were significantly differed from roots ($p < 0.001$).