

Article

The Regulation of Ion Homeostasis, Growth, and Biomass Allocation in Date Palm Ex Vitro Plants Depends on the Level of Water Salinity

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Abstract: The date palm, a central plant in the fragile oasis ecosystem, is considered one of the fruit species most tolerant to salt stress. However, the tolerance mechanisms involved are yet to be addressed and their evaluation until now was mainly based on heterogenous plant material such as seedlings or limited to *in vitro* experiment conditions. For these reasons, we propose to deepen our knowledge of the morphological and physiological responses to salt stress using acclimated *ex vitro* plants resulting from the propagation of a single genotype. The plants were irrigated with 0, 150, 300, or 450 mM NaCl solutions for four months. Our results showed that the influence of water salinity on growth and ion-homeostasis regulation was very dependent on stress levels. The 150 mM NaCl concentration was found to improve dry biomass by about 35%, but at higher salt concentrations (300 and 450 mM) it decreased by 40–65%. The shoot:root dry mass ratio decreased significantly at the 150 mM NaCl water concentration and then increased with increasing water salt concentration. The leaf:root ratio for Na⁺ and Cl⁻ decreased significantly with increasing water salinity up to a concentration of 300 mM NaCl, and then stabilized with similar values for 300 mM and 450 mM NaCl. In contrast to Na⁺ and Cl⁻, leaf K⁺ content was significantly higher in the leaf than in the root for all salt treatments. Unlike Na⁺ and K⁺, Cl⁻ was expelled to the surface of leaves in response to increased water salinity. Overall, date palm plants appear to be more capable of excluding Cl⁻ than Na⁺ and of changing biomass allocation according to salt-stress level, and their leaves and roots both appear to play an important role in this tolerance strategy.

Keywords: *ex vitro* plants; ions exclusion and uptakes; *Phoenix dactylifera*; salt stress adaptation; salt stress avoidance

1. Introduction

Date palm (*Phoenix dactylifera* L.) has been one of the most common fruit crops in arid and semi-arid regions of the world, primarily in the Middle East, North and South Africa, for at least 5000 years [1]. The high nutritional value of its fruits has made it a desirable crop and it represents the basis of the oasis ecosystem. In addition, the cultivation of the date palm generates significant employment opportunities in rural areas and is often the main source of income for farmers [2]. The ability to tolerate high temperatures and salinity are some of the characteristics behind the success of this crop under the harsh climatic conditions of arid regions. However, the rapid conversion of large proportions of fertile arable lands into saline patches and the increasing soil-salinity levels are seen as a major risk in the near future that threatens the sustainability of the fragile oasis ecosystems, date-palm yield and fruit quality, and consequently food security in several regions [3–6].

Saline stress is one of the greatest environmental threats affecting plant biomass production in agriculture [5]. The effect of salinity on biomass production generally has an impact on its shoot-to-root allocation. Biomass partitioning, due to its direct impact on root-system growth and the plant organ, which is in direct interaction with salts, is considered a very important mechanism in the adaptation strategies to this type of stress [7,8]. At another level, to protect the physiological process responsible for biomass production from ion toxicity, nutritional disorder, and osmotic stress caused by high water and soil salinity, plants generally use two mechanisms [9]: firstly, avoidance, i.e., keeping the salts away from the metabolically active tissues [10]. This is achieved by passive and active exclusion of toxic ions or by their dilution in plant tissues [11]. Secondly, the compartmentalization of accumulated ions in the vacuoles of plant cells [12]. The implication and regulation of each of these mechanisms in the response to saline stress is highly dependent on the species [9,13]. Despite the central role of these mechanisms in the different tolerance strategies adopted by each species to overcome the effects of salinity, they are not sufficiently addressed in the date palm, as recognized by its tolerance to this constraint.

Studies performed on date palm tolerance to salinity have mainly evaluated the concentration of sodium and potassium and the K^+/Na^+ ratio in leaves and/or roots to compare varietal tolerance [14–16]. However, the corresponding regulatory tolerance mechanisms were not sufficiently addressed. Furthermore, the little information available on the physiological response of date palm to salinity, such as enzymatic activity, gas exchange, tissue mineral contents, and nutrient uptake, was obtained from seedlings, a traditional date propagation method, with the inherent uncertainties and potential interfering factors that such material may generate [4,17–21].

Classically, the propagation of date palm was performed through offshoots or seedlings, producing a non-homogeneous plant material. Offshoot plants, in contrast to seedlings, are true-to-type plants, characterized by different growth stages and vigor, a limited number of plants, a short lifespan, and the risk of high disease transmission rates [22,23]. However, the great advance in date palm propagation via *in vitro* culture, and in particular organogenesis, has allowed the maintenance and production of true-to-type plants [24,25] and uniform populations of synchronously developing plant cells [26], thereby permitting the rapid production of several thousand *in vitro* plants with high homogeneous variety [3,27]—a very valuable material for studying date palm response to environmental stress.

Some studies on the physiological response to salinity of homogeneous date palm plant material, generated from *in vitro* tissue culture, have been carried out in recent years, but generally under *in vitro* conditions. The response of date palm plantlets to saline conditions *in vitro* showed that increasing the K^+/Na^+ ratio in the growth media of date palms significantly reduced the absorption of Na^+ and the balance of ion compartmentalization [28]. Despite the importance of this study, which provides important information about the key role of K^+ supply in the response of date palms to salinity in a homogeneous plant material, the role of the translocation and uptake regulation of these ions has not been sufficiently investigated. In addition, the *ex vitro* conditions can severely affect the morpho-physiological behavior of these plants due to environmental factors that can inter-

fer. The *in vitro* culture conditions result in plantlets with altered morphology, anatomy and physiology [29–31]. Acclimatization is essential for correcting *in vitro* anatomical and functional abnormalities while adapting plants to new growth conditions [29,32]. Thus, the investigation of the response of date palms to salinity on vigorous acclimated plants derived from tissue culture of a single genotype is necessary to provide more reliable information on this subject [33,34]. However, the main studies performed with this type of material have evaluated either vegetative growth parameters [33] or proteomic components [34].

The purpose of the present work is to study the morpho-physiological response of date palm plants to different salinity levels of irrigation water and to elucidate the mechanisms of ion exclusion, translocation and uptake involved in salt-stress tolerance in this species using a homogenous, vigorous and acclimated plant material generated from the tissue culture of a single genotype.

2. Materials and Methods

2.1. Plant Material and Stress Treatments

The study used eighteen-month-old date palm (cv. Barhee) commercial plants that originated from tissue culture following *ex vitro* acclimation [27]. At the start of the experiment, all plants had the same number of leaves and a similar height. The plants were grown in black 5 L plastic containers with peat:soil:sand (3:1:7, *v/v/v*). During the experiment, the pots were placed in a greenhouse with a daily average temperature of 25 ± 2 °C, relative humidity (RH) of $65 \pm 2\%$ and photosynthetically active radiation (PAR) of $555 \pm 15.4 \mu\text{mol m}^{-2} \text{s}^{-1}$. Saline treatments consisted of two irrigations per week with 150, 300 and 450 mM NaCl for four months, and control plants were irrigated with 0 mM NaCl water. At each irrigation, a quantity of water equal to the retention capacity of the substrate was added to avoid any water deficit (added water quantity was determined by weighing containers every 3 days). The electrical conductivity (EC) of the substrate was measured according to the saturated paste method with an Accumet meter [35].

The experimental design was a complete randomized block with four treatments, four blocks and three repetitions/block (twelve plants per treatment), giving a total of 48 plants.

2.2. Growth and Morphological Parameters

The morphological parameters, such as the number, thickness and length of leaves; root density; length and area, were determined at the end of the experiment. Length was measured using a ruler and leaf thickness using a high-accuracy digital vernier caliper. The area of leaves and roots, and the root-branching intensity (area occupied by roots per total area of the root system in 2D projection photo, which constitute an indicator of root-system ramification) were estimated by image-analysis methodology, using the “Irregular Area” tool of Motic Image PLUS 2.0 (Motic China Group, Xiamen, China) [36]. Root-hair density is estimated by counting root hairs on the last 1 cm of the roots under a stereo microscope (SZ Olympus, Tokyo, Japan).

At sampling times, plants were rinsed three times with distilled water and then blotted on filter paper. Roots and leaves were separated and their fresh weights (FW) were determined. For dry weight (DW) measurements, organs were dried in an oven at 70 °C for 3 days. Samples for biochemical analysis were frozen in liquid nitrogen and stored at -80 °C. The aerial part (shoot)/root ratio was calculated for DW as a biomass allocation indicator. Water content per leaf area or equivalent water thickness was calculated. This is defined as the ratio between the quantity of water and the area [37].

To calculate relative water content (RWC), fresh leaves were placed in distilled water for 24 h at room temperature for the measurement of turgid weight (TW) [38].

$$\text{RWC}\% = ((\text{FW} - \text{DW}) / (\text{TW} - \text{DW})) \times 100$$

2.3. Leaf and Root Na^+ , Cl^- and K^+ Contents

Oven-dried leaves were ground into a fine powder using a mortar. About 0.3 g of the dried powdered leaves and roots were digested with 0.5% of nitric acid (HNO_3) at room temperature for four days, then the extracts were filtered and used for the determination of nutrient concentration. Chloride was assessed by colorimetry (Büchler); K^+ and Na^+ by flame spectrophotometry (Corning, UK) [39].

2.4. XRD Analysis

The amount of sodium, chloride and potassium on the leaf and root surfaces was determined using an environmental scanning microscope (FEI Quattro S, Thermo Fischer Scientific, Eindhoven, The Netherlands) equipped with an energy-dispersive X-ray diffraction spectroscopy (XRD) device [40].

2.5. Statistical Analysis

The experimental design was a complete randomized block with 4 treatments, 4 blocks and three repetitions/block (twelve plants per treatment), giving a total of 48 plants. The analysis of variance and Tukey's test at $p \leq 0.05$ were used to assess the differences between treatments. All statistical analyses were performed using Statistix 8.0. (Los Angeles, CA, USA).

3. Results

To facilitate and deepen the analysis and interpretation of the results, the differences between the treatments (decrease or increase) were expressed as percentages compared to the values of the control plants.

3.1. Effects of Salinity on Substrate Properties

EC of the substrate decreased slightly from 2.81 dSm^{-1} at the start of the experiment to 2.27 dS m^{-1} at harvest for the salt-free treatment. This may have resulted from the export of ions by plants and/or from the leaching phenomenon. In contrast, after four months of irrigation with 150, 300 and 450 mM NaCl, the EC of the substrate increased greatly from 2.76, 2.72 and 2.85 dS m^{-1} to 16.69, 25.43 and 32.52 dS m^{-1} , respectively.

3.2. Effects of Salinity on Plants' Morpho-Physiological Parameters

For the aerial part, the first obvious symptoms exhibited by salt-stressed plants were detected after two months of treatment with 300 and 450 mM NaCl (Figure 1). These symptoms initially consisted of the appearance of chlorotic lesions on the tips of leaves. The plants irrigated with 150 mM NaCl did not show symptoms of necrosis until the end of the experiment (4 months). In contrast, at final harvest, plants irrigated with 300 mM NaCl showed necrosis in the first and second basal leaves, and some leaflet-tip burn was observed. For the plants irrigated with 450 mM NaCl, the three first basal leaves were severely necrosed and rolled up, and the fourth leaf showed some necrosis (Figure 1).

Concerning the root part, visually, no necrotic symptoms were observed for control plants and those treated with 150 mM NaCl. Increasing salt concentration to 300 mM causes very slight necrosis on the tips of some roots, which becomes more abundant at 450 mM (Figure 1). Plant mortality did not occur during the time course of the experiment.

The morpho-physiological parameters showed different behaviors depending on salt concentrations. The relative water content (RWC), the total area and the number of leaves were not affected by the 150 mM NaCl concentration. Nevertheless, leaf thickness, fresh weight (FW), dry weight (DW) and water content per leaf area increased significantly compared to the salt-free treatment: by 47%, 38%, 35% and 55%, respectively (Table 1).

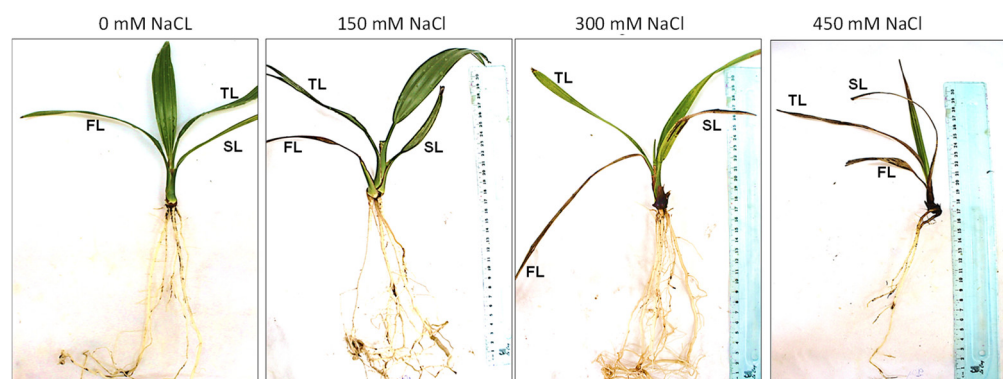


Figure 1. Phenotypes of date palm plants at final harvest after 4 months under different salt concentrations. Treatments were: salt-free conditions (0 mM), plants' exposure to 150, 300 and 450 mM NaCl. FL: first basal leaf, SL: second basal leaf and TL: third basal leaf.

Table 1. Morpho-physiological parameters of date palm leaves grown under greenhouse conditions after 4 months of salt treatments.

Treatments (NaCl)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Leaf Area (mm ²)	Water Content per Leaf Area (g cm ⁻²)	Leaf Number	Relative Water Content	Leaf Thickness (mm)
0 mM	5.60 ± 0.2 b	1.56 ± 0.14 b	104.0 ± 4.7 a	0.038 ± 0.003 c	7.01 ± 0.51 a	83.7 ± 4.2 a	0.37 ± 0.05 b
150 mM	7.72 ± 0.42 a	2.11 ± 0.11 a	97.6 ± 3.5 a	0.058 ± 0.004 a	6.88 ± 0.37 a	79.3 ± 5.1 a	0.54 ± 0.04 a
300 mM	4.54 ± 0.43 c	1.12 ± 0.07 c	71.2 ± 2.1 b	0.048 ± 0.003 ab	5.25 ± 0.21 b	63.7 ± 3.6 b	0.51 ± 0.03 a
450 mM	2.93 ± 0.22 d	0.82 ± 0.08 d	48.4 ± 5.0 c	0.043 ± 0.006 bc	4.25 ± 0.13 c	46.4 ± 3.5 c	0.53 ± 0.03 a

Treatments were: salt-free conditions (0 mM), plants irrigated with 150, 300 and 450 mM NaCl. For each parameter, values (means ± standard error) followed by different letters are significantly different according to Tukey's test at $p \leq 0.05$.

Most morpho-physiological parameters were significantly reduced at higher salt concentrations, 300 and 450 mM NaCl, with a pronounced effect at 450 mM NaCl. When compared to controls (0 mM), the FW, DW, leaf area, leaf number and RWC of plants irrigated with 300 mM NaCl decreased significantly: by 18%, 28%, 31%, 25% and 25%, respectively. These parameters showed a similar reduction, about 45%, when plants were irrigated by 450 mM NaCl (Table 1). In contrast to all other leaf-growth parameters, the different salinity concentrations similarly and significantly improved leaf thickness.

The three water salinity levels differentially affected the root system's growth. Irrigation with 150 mM NaCl water significantly improved the FW, the DW, the branching intensity and the hair density of roots: by 25%, 44%, 23% and 35%, respectively. However, root length and area were not impaired, and the shoot: root ratio exhibited a slight significant decrease at this level of water salinity (Table 2).

The 300 mM NaCl roots exhibited a different trend compared to those irrigated by 150 mM NaCl, and with values significantly below those of stress-free plants. This significant reduction was about 40% for the biomass and root system, and about 30% for the root length and branching intensity. The root-hair density was not significantly affected by this level of salinity. The 450 mM NaCl severely impaired root-growth parameters. In fact, the FW, DW and root-system area and density were all significantly reduced by around 65% compared to controls. However, a lesser but significant reduction, about 30%, was observed for root length and root-hair density compared to the other growth parameters (Table 2). When plants were exposed to very high levels of salinity (i.e., 300 mM and 450 mM), only the shoot:root ratio increased significantly with the increasing water salt concentration.

3.3. Salt's Effects on the Contents of Na⁺, Cl⁻ and K⁺ in Leaf and Root

The irrigation with saline water significantly increased Na⁺ content in leaves by three-, five- and sevenfold, and in roots by four, eight and elevenfold for 150, 300 and

450 mM NaCl-treated plants, respectively, compared to the salt-free treatment (Figure 2). With respect to Cl^- content, control plants and those exposed to 150 mM NaCl showed similar levels in leaves, which subsequently significantly increased in leaves and roots with increasing salt concentrations. These inter-treatment increases in Cl^- content are significantly lower than those observed for Na^+ (e.g., a fivefold increase was reduced to threefold). For all treatments, the Na^+ and Cl^- contents were notably higher in roots than in leaves. Furthermore, Na^+ accumulated at higher levels in leaves than Cl^- , whereas the opposite occurred in roots (Figure 2).

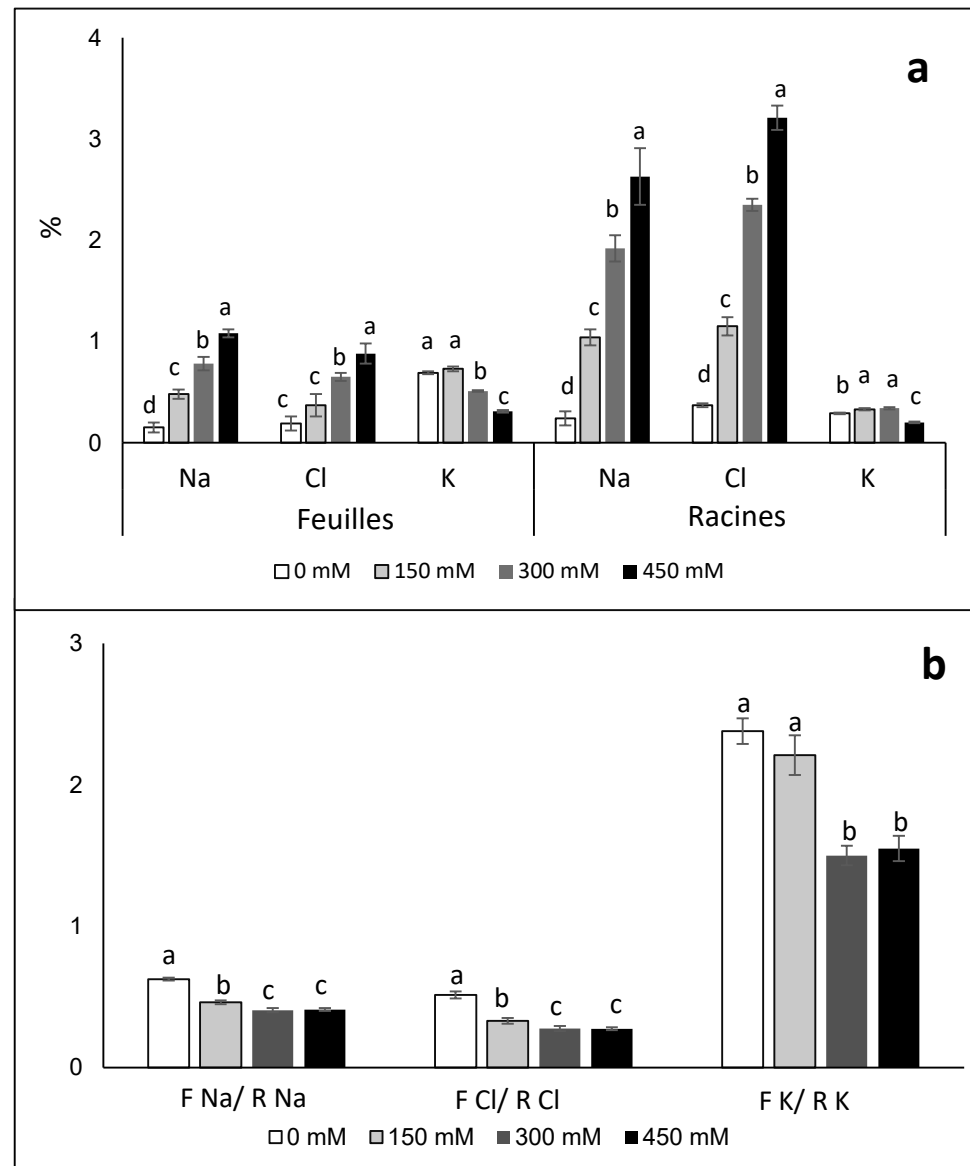


Figure 2. Mineral contents in roots and leaves dry mass (a) and their leaf/root (L/R) ratios (b) in date palm plant under different salt treatments measured after 4 months. For each parameter, values (means \pm standard error) followed by different letters are significantly different (within the same parameter) according to Tukey's test at $p \leq 0.05$. L: leaves; R: roots.

The leaf Na^+ /root Na^+ and leaf Cl^- /root Cl^- ratios decreased significantly with the increase in water salinity of up to 300 mM of NaCl concentration, then stabilized (i.e., similar values for 300 mM and 450 mM NaCl treatments). The significant decrease recorded for plants treated with 150 mM and 300 mM NaCl, compared to the salt-free treatment, was 25% and 35% for Na^+ and 36% and 46% for Cl^- , respectively.

Table 2. Morphological parameters of date palm root system grown under greenhouse conditions after 4 months of salt treatments.

Treatments (NaCl)	Root Fresh Weight (g)	Root Dry Weight (g)	Root Length (cm)	Root System Area (mm ²)	Root-Branching Intensity (%)	Root-Hair Density	Shoot: Root Ratio DW
0 mM	5.48 ± 0.2 b	0.92 ± 0.02 b	30.23 ± 2.6 a	142.08 ± 11 a	51.55 ± 3 b	3.42 ± 0.31 b	1.70 ± 0.04 c
150 mM	6.84 ± 0.42 a	1.33 ± 0.05 a	28.92 ± 1.3 a	139.29 ± 7 a	63.67 ± 5 a	4.62 ± 0.28 a	1.58 ± 0.05 d
300 mM	3.34 ± 0.55 c	0.55 ± 0.07 c	21.33 ± 2.1 b	81.44 ± 3 b	36.49 ± 8 c	3.33 ± 0.24 b	2.04 ± 0.06 b
450 mM	1.91 ± 0.22 d	0.33 ± 0.10 d	20.77 ± 3.5 b	48.37 ± 5 c	17.81 ± 3 d	2.27 ± 0.30 c	2.48 ± 0.04 a

Treatments were: salt-free conditions (0 mM), plants irrigated with 150, 300 and 450 mM NaCl. For each parameter, values (means ± standard error) followed by different letters are significantly different according to Tukey's test at $p \leq 0.05$.

In contrast to Na⁺ and Cl⁻, leaf K⁺ content was significantly higher in leaves than in roots for all salt treatments. The 150 mM salt concentration significantly improved the potassium content of roots and maintained a similar value to the controls for leaves. These two treatments showed a comparable leaf K⁺/root K⁺ ratio. This ratio was significantly reduced by increasingly high NaCl concentrations (i.e., 300 mM and 450 mM of NaCl), resulting in a progressive decrease in leaf potassium content. However, the K⁺ content in the roots was unexpectedly enhanced following irrigation with 300 mM NaCl, whereas it was severely reduced at 450 mM.

The XRD analysis of leaf and root surfaces showed diffraction peaks corresponding to sodium (Na⁺), potassium (K⁺) and chloride (Cl⁻) (Supplementary Materials). The estimated quantification of these elements showed that the leaf surface presented a very low percentage of Na⁺, with similar values between samples. However, the percentage of Na⁺ increased on root surfaces for all salt-treated plants compared to controls, with no similar values among the different salt treatments (Figure 3).

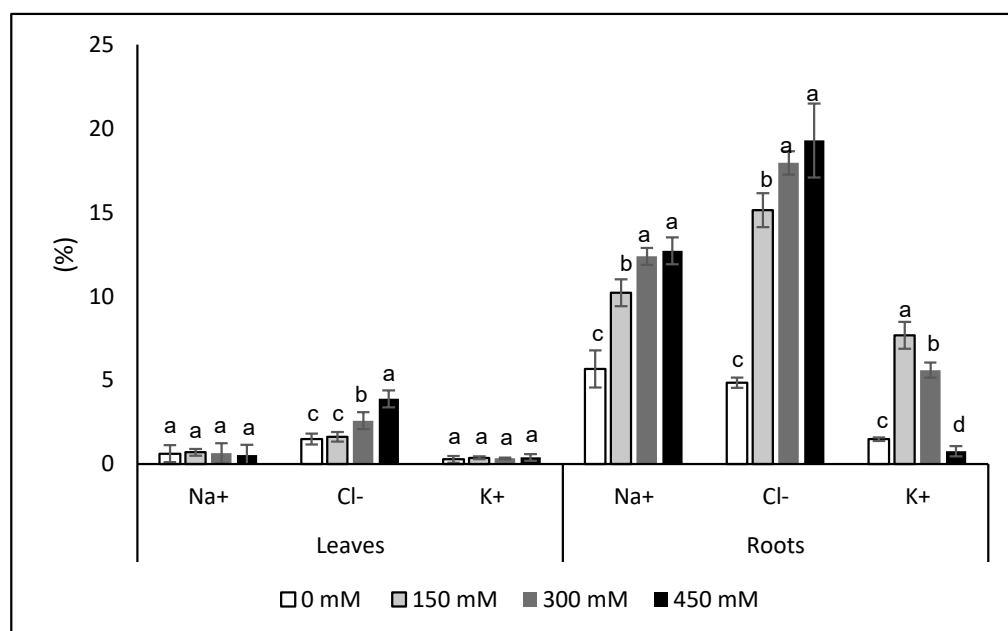


Figure 3. Measurements of sodium, potassium and chloride on the surface of leaves and roots of date palm plants under different salt treatments. For each parameter, values (means ± standard error) followed by different letters are significantly different according to Tukey's test at $p \leq 0.05$.

Like Na⁺, K⁺ was present on the leaf surface of all samples at the same level. Although the level of K⁺ for the root on the surface varied, it did not always follow a successive increase parallel to the increasing salt concentration of the irrigation solution. That is, the

percentage of K^+ in the root surface was highest at 150 mM NaCl, followed by the 300 mM treatment, but both showed significantly higher values than the controls. Plants treated with 450 mM NaCl exhibited the lowest percentage of K^+ on the root surface (Figure 3).

Unlike Na^+ and K^+ , Cl^- accumulated on the surface of both organs (i.e., leaf and root) in response to increased water salinity (Figure 3). However, the concentration of 150 mM NaCl did not affect the accumulation of this ion on the leaf surface. For the root, the accumulation of Cl^- on the surface remains unchanged from the 300 mM NaCl concentration. For all treatments, the amount of Cl^- was higher on the surface of the root than on that of the leaf. In addition, the amount of Cl^- accumulated on the surface of both organs was always greater than that of Na^+ .

4. Discussion

The date palm plants not only experienced no negative effects following irrigation with water containing 150 mM NaCl, but instead showed a significant improvement in root growth and biomass production. Indeed, it is the first time, as far as we know, that an improvement in biomass production and root growth has been observed in young date palm plants irrigated with a relatively high level of water salinity (i.e., 150 mM NaCl). By applying a relatively similar level of salt stress, several previous studies have found similar growth values between stress-free and treated plants [41–44]. This divergence in the results obtained in previous studies may be due to the variability (i.e., intra-variability) of the response of the plants, with a limited homogeneity (seedlings and offshoots), used until now in similar studies, thus making it difficult to detect (i.e., mask) this response. This finding not only emphasizes the importance of using *ex vitro* acclimated plants in date palm research, but it also sheds new light on the date palm's response to salt stress.

The halophytic nature of the date palm has been discussed and suggested by several studies, because of the palm's ability to tolerate high salinity levels [6,18,21,45,46]. The result obtained here shows a significant improvement in the biomass of young date palms, which can constitute a solid argument for this and confirm the salt-loving nature of this species, and even encourages a discussion on the type of halophyte it is, whether facultative or obligatory.

The increase in biomass of the aerial part under the 150 mM NaCl seems to be related to the increase in leaf thickness, since the leaf area and number were not affected. Increases in leaf thickness and water content per leaf area under salinity have been considered as an adaptation of the plants to sequester or dilute toxic ions [47]. In other monocot plants, this behavior has been associated with the development of xeromorphic characteristics and structural modifications, such as increased succulence and/or cuticle and epidermis thickness in leaves [9]. Succulence is an anatomical adaptation that, by increasing the volume of vacuoles, permits the accumulation of larger amounts of water and dissolved ions in leaves, shoots and roots [12]. Changes in these two parameters were generally associated with a decrease in leaf area, but in the case of young date palms, cv. Barhee, this was not observed. This suggests that increasing leaf thickness and water content per leaf area under salt conditions, beyond the adaptation mechanisms, results in an improvement of aerial part biomass in the case of the date palm, or at least in the case of the cv. Barhee.

The biomass of the root system, like the aerial part, increased with an irrigation water salinity of 150 mM. This increase seems to be due to an increase in root-hair density and root-branching intensity. Irrigation water salinity has different effects on root growth. In general, root growth was inhibited by exposure to high salinity [48,49]. Water and soil salinity generate two types of stress: osmotic and ionic. The inhibition of the root system was generally attributed to the ionic effect due to the toxic effects of the sodium and chloride accumulated in the rhizosphere [49–51]. Osmotic stress arises due to the increased sodium ions in the soil, which leads to a reduction in water absorption [52]. The osmotic stress, in the absence of ionic stress like that generated by drought or mannitol (an osmotic agent), seems to improve root branching and hair density [50,53]. In the case of date palm, the ion concentration may not reach a high, toxic level because of its high tolerance to this type of

stress as a halophyte species. The osmotic stress generated, however, could most probably result in increased root branching and hair density, allowing the plant to improve water uptake. This behavior may contribute to preserving the water status (i.e., RWC) of plants subjected to the first level of salt stress.

When the level of saline stress increased, the plants changed their behavior. At 300 mM, plants lose biomass from the aerial part through a decrease in the number and area of leaves and from the root system through the reduction in the root-system area, root-branching intensity and length. However, leaf thickness, water content per leaf area and root-hair density were improved by this level of salinity. This may suggest maintenance of the adaptive response by increasing leaf succulence and thus ion sequestration and dilution mechanisms, which were discussed in the previous paragraph. When the salinity level of the water reached 450 mM, the reductions in the morpho-physiological parameters were aggravated and the root-hair density decreased as well. This result indicates that the ionic and osmotic stresses have reached an important level which inhibits biomass production and growth. The decrease in growth parameters, such as leaf area, root-hair density and branching of the root system, has been described as a common response to salt stress [47,51,54].

Root hair was generally thought to be salt-sensitive [50,55]. However, the results reveal that root-hair density was less sensitive to salinity than the other root-growth parameters, such as root length and branching. A new study has described the lower sensitivity to salinity of very small and young roots compared to larger and older roots, and attributed it to a higher level of NADPH oxidase-activated ROS induction in the first type of root [56]. The root hair plays a key role in the water and nutrient uptake by increasing the absorptive surface area of the root [57], and this is what emphasizes the importance of this result, which may indicate an important role for the root hair in the salt-stress tolerance strategy of the date palm. In fact, the only decrease in root-hair density, at 450 mM NaCl, was accompanied by a severe reduction in plant growth and water status (i.e., RWC) and an intense increase in salt-injury symptoms.

The increase in the symptoms seems to have a specific dynamic related to the leaf age. The symptoms evolve in an ascending direction, from the oldest leaves to the youngest. Salts translocated in the transpiration flow are accumulated and their concentration grows with time. This causes greater salt concentrations in older leaves than those in younger leaves [9]. This is due to an accumulation of toxic ions correlated with leaf age (involving a greater transpiration rate) or probably to a major activity of antioxidative stress mechanisms as observed in the young roots of several other species [56,58,59]. In both cases, one might wonder if the date palm plants sacrifice their old leaves (i.e., get rid of the tissues where the toxic ions are retained) and retain the new leaves and the single meristem that they have, to preserve their growth capacity, which could be a very interesting way to overcome temporary salt stress. A similar response was recorded in rice when facing Fe toxicity, which was suggested as a protection strategy of the newest leaf which may be important for plant survival under an excess of Fe [60].

The shoot:root ratio DW decreased for the 150 mM NaCl water salinity, indicating an increase in biomass allocation to the root part. However, for greater levels of stress (i.e., 300 mM and 450 mM NaCl), the shoot:root ratio increased gradually with NaCl water concentrations. It appears that the allocation of biomass to the roots is progressively reduced under very high salinity levels. It is broadly assumed that plants are able to adjust the relative sizes and allocations of organ systems (e.g., aerial parts, root systems) in response to fluctuations in the external supply of resources [61–65]. Thus, as the species for that shoot:root ratio decrease, they provide more assimilates for root growth in order to overcome the reduction in resource supplies (i.e., water and nutrients) imposed by the decreasing soil water content [65]. This explanation supports the idea discussed above that at 150 mM NaCl, the level of osmotic stress was probably more resented by the plants than that of ionic stress, thus generating a response, like that under water deficit condition. In contrast, species that respond to salinity with a decrease in shoot:root ratio need to reduce

root volume in plants exposed to salinity, which may be a favorable trait limiting their capacity to accumulate toxic ions in the shoot [12,66]. What remains interesting from the observations made is that, in the case of the date palm, it appears that changes in biomass partition are highly dependent on the intensity of the applied salt stress (i.e., the palm adapts with two types of biomass allocation strategies). In any case, further studies would be necessary to evaluate this hypothesis.

The influence of salinity on the ion content, such as that which occurred for the morpho-physiological parameters, was very dependent on the concentration of NaCl in the irrigation water and the organ concerned. The decrease in plant-growth parameters is due to the higher concentration of Na⁺ and Cl⁻ in the growth channel, which affects membrane stability, disturbs photosynthetic activity, and assembles toxic ions at the tissue level, resulting in an imbalance in nutrient uptake, suspending cell division and inducing the production of reactive oxygen species (ROS), which consequently result in a remarkable reduction in the plants [67–69]. The content of Na⁺ and Cl⁻ inside roots increased with the increase in NaCl concentration in the water due to their increase in soil solution, as is well described in date palm and other species [28,49,70–73]. However, a parallel accumulation on the surface of the roots was also observed. The excess of salt in the root zone hinders plant roots from withdrawing water and nutrients from the surrounding soil [54,74]. In addition, the damage symptoms and necrotic spots observed in the roots irrigated with 450 mM NaCl were probably caused by the very high levels of Na⁺ and Cl⁻ accumulated on their surface (Figure 3). Thus, we can suggest that in addition to the negative effects of the accumulation of these ions inside the root, their accumulation on the surface can cause serious damage.

The accumulation of Na⁺ and Cl⁻ on the root surface, beyond the damage that can be attributed to it, can be considered as an indicator of a reduction in ion uptake at the root surface and ion exclusion activity from the inside of the root. This is part of the avoidance mechanism, which allows plants to keep the salts away from the metabolically active tissues through passive exclusion of ions (by a permeable membrane) or active expelling of ions (by ion pumps) [10,11]. Recently, another tolerance strategy for excess ions was described in rice, based on the suppression of genes involved in Fe uptake and translocation [60]. On the other hand, the accumulation of Na⁺ and Cl⁻ inside and outside the roots occurs with different dynamics. Na⁺ accumulation inside the roots was faster than for Cl⁻ when we compared one treatment to another; the opposite occurred on the surface. Considering this result, date palm plants seem not only to exclude toxic ions, but probably exclude Cl⁻ more quickly/efficiently than Na⁺. Na⁺ and Cl⁻ also progressively accumulated in the leaves with increasing water salinity, but the reduction in the leaf:root ratio of their ions indicated a reduction in their translocation from roots to leaves. This reduction in toxic ion translocation was shown to be part of the exclusion mechanism. This regulation process can occur by reducing leaf transpiration, resulting in reduced ion transport and/or operating by retrieving toxic ions from the xylem into the surrounding xylem parenchyma cells of the root through several plasma-membrane-localized ion transporters such as class 1 HKTs [9,71,75–77]. This translocation-reduction capacity seems to depend on the salinity level of the water. The leaf:root ratio of the content of the Na⁺ and Cl⁻ decreased severely when the salinity of the water increased from 0 mM to 150 mM NaCl and continued to decrease, with less intensity, when the salinity reached 300 mM, but did not change if the salinity increased further. The same pattern was also observed for the accumulation of both ions on the root surface. These results lead us to deduce that the exclusion mechanism efficiency has a maximum that, in our case, is reached at a concentration of 300 mM NaCl.

Solute accumulation in leaves is largely dependent on the ability of roots to exclude ions and on the compartmentalization of salts in root and leaf tissues [78]. The irrigation of date palm plants with 150 mM NaCl increased leaf Na⁺ content, but without a negative impact on growth parameters. Hence, there is a probable exclusion of Na⁺ in roots, but its compartmentalization in leaf vacuoles could have occurred.

On the leaf surface, an accumulation of Cl^- is observed when salinity is increased, while surface Na^+ is similar for all treatments. The ability to remove toxic ions from leaves was generally associated with the presence of a salt-secretory structure in the aerial parts of some halophytes [79,80]. However, what is known is that this structure secretes various ions, especially Na^+ and Cl^- , but as far as we know, no structures specialized in the secretion of a specific ion have been described. Furthermore, this possible Cl^- secretion seems to start only when a threshold in Cl^- concentration inside the leaf is reached due to the application of a high level of salt stress (in our case, 300 mM NaCl). At a concentration of 150 mM NaCl, no accumulation of this ion was observed either on the surface or inside the leaf. The lack of an increase in Cl^- content inside the leaf at this water salinity level demonstrates the high efficiency of the mechanism of ion exclusion at the root system in avoiding toxic levels that affect the physiological process and thus plant growth. These observations, despite the new clues that they may suggest about the mechanisms by which the date palm responds to salt stress, show the need to deepen the anatomical, ultrastructural, molecular and physiological analyses to verify them and build a clearer idea of them.

For all salt treatments, Na^+ accumulated at higher levels in the leaves than the Cl^- , while the opposite occurred for the roots. This seems, together with the observations above in the discussion, to indicate a higher efficiency of the date palm in excluding Cl^- than Na^+ . However, this may also suggest that this plant is more sensitive to chloride than to sodium, for which it needs to maintain a lower content. In this sense, it should be remembered that the increase in Na^+ concentration under the 150 mM NaCl treatment did not have any negative impact on plant growth. On the contrary, they accumulated more biomass. In fact, a differential sensitivity to Na^+ and Cl^- has been reported in several species [81].

Potassium is an essential component in protein synthesis, glycolytic enzymes and photosynthesis. Osmotic mediating cell expansion and other turgor-driven processes highlight the crucial role of potassium in plants' salt tolerance [82]. Despite the long-term salt exposure, an increase in the root K^+ was observed for plants irrigated with 150 mM, compared to non-stressed plants. However, as far as we know, this is the first time that an increase in K^+ in roots was observed, which tends generally to decrease with salinity [28,50,70–72,83]. The increase in Na^+ in the nutrient medium is supposed to reduce the K^+ uptake by antagonism [74], but the capacity of K^+ uptake also depends on other factors, such as the root surface [84,85]. Therefore, date palm plants, irrigated with 150 mM NaCl, most likely have improved their K^+ uptake by taking advantage of the increased root surface due to increased root-branching intensity and hair density. The greater amount of K^+ found on the root surface at this salinity level was compared to free salt treatment, which resulted from an increase in K^+ -uptake sites on the root surface, and this is thought to further corroborate this hypothesis. In addition, there was no reduction in K^+ translocation (i.e., leaf K^+ /root K^+ ratio) under 150 mM NaCl, which seems to contribute, in association with the efficient uptake of this ion by the roots, to the maintenance of leaf K^+ content, in a similar amount to that of salt-stress-free plants. This behavior can help to explain why date palms respond positively to the first level of water salinity. Indeed, a strong determinant of salt-stress tolerance in plants is the tissue K^+/Na^+ ratio [76]. The response of date palm plantlets to saline conditions *in vitro* has shown that an increasing K^+ uptake due to an increasing K^+/Na^+ ratio in the growing media of date palm significantly reduced the absorption of Na^+ and also balanced ion compartmentalization [75].

In the case of 300 mM NaCl, the plants maintained a similar root-hair density compared to control, but a higher K^+ root content. This seems to be due to the decrease in K^+ translocation from the root to the aerial part (a decrease in leaf K^+ /root K^+ ratio). Plants treated with 450 mM NaCl showed the lowest content of K^+ inside and on the surface of the root. This result is most probably due to a very serious reduction in the uptake of this element due to the inhibitory effect of Na^+ , accumulated at very high levels inside and outside the roots, in the uptake of K^+ and to the severe reduction in the root surface. The consequences of the reduction in K^+ uptake and translocation were very serious for plant growth. An important decrease in K^+ leaf content resulted in an interruption to a number

of metabolic processes, especially photosynthesis, which is essential to biomass production, since K^+ has an important role in the activation of many enzymatic pathways [86].

5. Conclusions

Date palm plants appear to be more capable of excluding Cl^- than Na^+ in general. The exclusion-mechanism capacity of both ions seems to have a maximum. The exclusion mechanism of these ions allowed plants of all treatments to accumulate a greater amount in roots than in leaves. In contrast, the capacity to uptake K^+ and its translocation allowed a higher content of this ion in the leaf than in the root. Date palm biomass allocation to the root system changes according to the water salinity level. Shoots and roots both appear to play an important role in the tolerance strategy in date palm.

In any case, the results obtained should be followed by studies which focus on the implication of other physiological and molecular mechanisms in palm tree tolerance, not only to NaCl but also to other stressors. Additionally, the potential use of the date palm in phytoremediation in saline soils should be explored because it seems that this application is possibly based on the plants' high capacity to accumulate Na^+ and Cl^- ions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su141912676/s1>, Figure S1: Samples of XRD diffraction pattern of sodium, potassium, and chloride on the surface of leaves and roots of date palm under different salt treatments. Treatments were: salt-free conditions (A, B, for leaves and roots, respectively); plants' exposure to 150 (C, D, for leaves and roots, respectively); 300 (E, F for leaves and roots, respectively); and 450 (G, H for leaves and roots, respectively) mM NaCl.

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