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Research Article

Comparative Water Relations of Two Contrasting Date Palm Genotypes under Salinity

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Salinity is a global agricultural problem, resulting in a significant reduction in the plantation areas and the crop yields, especially in arid and semiarid regions. The date palm is relatively salt-tolerant plant species, although the nature of salt tolerance is poorly understood. In this study, the salt stress responses of a salt-tolerant "Umsila" was compared with salt-susceptible "Zabad" date palm cultivars. Various physiological parameters, plant-water relations, and anatomical characteristics were analyzed. The results revealed that although salinity has negatively affected both cultivars, Umsila exhibited more stable photosynthesis than Zabad as reflected by the quantum yield (Qy) and the stomatal conductance (GS). Similarly, Umsila showed a more dynamic root system and efficient water relations than Zabad as demonstrated by the leaf water potential (LWP) and relative water content (RWC) during salinity. Umsila also accumulated greater abundances of soluble sugars, potassium (K^+), calcium (Ca^{+2}), proline, glycine betaine, and lignin and formed extra layers of Casparian strips in the root tissues when the seedlings were grown under saline conditions. Together, the results obtained from this study have offered some insights into the salt tolerance mechanisms in the date palm.

1. Introduction

Salinity is a worldwide problem resulting in a major reduction in growth and productivity of the crop plants [1]. Important fruit trees such as the date palm (*Phoenix dactylifera* L.) are growing in increasingly saline environments in the Arabian Peninsula, resulting in a significant reduction in the annual yield. For an instant, in Oman, 70% of the cropping areas have been affected by salinity [2]. Salt-tolerant plants use different mechanisms as revealed by several studies [3]. These mechanisms include the reduced Na^+ uptake, accumulation of Na^+ in the vacuoles, the retrieval of Na^+ from the xylem vessels into the xylem parenchyma cells, and the recirculation of Na^+ from the shoot to root and eventually back to the soil through the phloem [1, 4–6].

Determination of salt tolerance mechanism in the date palm requires global omics analysis and rigorous physiological studies [2]. Despite the fact that the association between transcriptomics [7–9], metabolomics [10], and biochemical analyses [11–13] with salinity tolerance was investigated in the date palm, the importance of physiological parameters including water relations has not been thoroughly studied.

Besides the sodium and chloride toxicity, salt stress is known to interfere with the plant-water relations [14–16], affecting osmotic potential and altering cell water turgidity [17, 18], provoking stomatal closure, reducing leaf expansion [19], and impairing photosynthesis [20, 21]. Under salinity, plants adjust their osmotic potential to maintain cell turgor pressure [15, 18, 22]. This procedure involves chiefly osmolytes such as soluble sugars and proline, and other compatible solutes but also certain modifications in the water movement system, which requires lignin and suberin structures [23].

Salinity is enhanced by antioxidant production, which is affected by various factors such as plant hormones and nitric oxide [24, 25]. Water movement through the apoplast and the stomatal aperture, as well as the symplastic movement

via aquaporins across the membranes, is mediated by Ca^{2+} [26]. Ca^{2+} acts as an osmoticum within vacuoles and protects the plasma membrane against Na^+ toxicity by accumulating in the cytoplasm, as well as serves as an essential secondary messenger in the processes associated with cell division, expansion, and photosynthesis [27]. Salinity is known to decrease Ca^{2+} accumulation in plants [28]. Consistently, the exogenous application of Ca^{2+} reduces the negative effects of salt stress by increasing the K^+/Na^+ ratios and improving the antioxidant defense and glyoxalase systems in rice subjected to salt stress [28].

The accumulation of sugars in plants promotes tolerance to salinity by balancing the osmotic potential inside the cell with the external salt concentration, thereby reducing further water loss [29]. Sugars, including starch, occupy a significant percentage of the plant dry weight, and it is also a major source of cellular energy that is required for routine growth and development. Interestingly, the degradation of starch to less complicated sugars under osmotic stress enhances cellular osmoprotection and elevated scavenging activities against reactive oxygen species (ROS) [30].

Lignin is a complex aromatic phenylpropanoid polymer and is a significant component of the secondary cell wall [31, 32]. In general, endodermis tissue is composed of the Casparian strip which consists of a ring of lignin deposited around the endodermal cells and acts as a barrier for water and solute repellants [33, 34]. Salinity induces changes in the cell wall components of root tissues, including an increased deposition of lignin and suberin, which in turn may prevent water loss and alter ion transport pathways [32]. Further, lignification is considered as a defense mechanism in plants [35] because lignin acts as a mechanical support and contributes to the rigidity of cell wall in plants and solute transport across the plants [36].

The purpose of this study was to identify the differences between salt-tolerant "Umsila" and salt-sensitive "Zabad" date palm cultivars concerning water relations, photosynthesis, sugars, proline, glycine betaine, lignin, Na^+, K^+, and Ca^{2+} accumulation during salinity. The results revealed that ion and osmolyte accumulations, photosynthesis, and water system might play an essential role in salinity tolerance in the date palm.

2. Materials and Methods

2.1. Plant Growth Condition and Salt Treatment. Seeds of date palm (*Phoenix dactylifera* L.) fruits, cultivars Umsila and Zabad, were thoroughly washed and then soaked in water overnight. The seeds were sterilized with 75% ethanol for one minute and then rinsed three times with sterile water. Subsequently, the seeds were mixed into a sterilized wet vermiculite bag and incubated at 37°C until germination, which were then transplanted into 2 L pots filled with the sand soil medium. For the establishment, germinated seeds were irrigated using distilled water and covered with clear plastic for two weeks until the first visible sign of shooting was observed. Afterward, seedlings were either irrigated with distilled water (control) or with 240 mM NaCl solution (salt stress) for six weeks. This level of NaCl salinity was known to induce unlethal salt stress for the seedlings [12]. The growing seedlings were fertilized with the all-purpose NPK (14-10-27) fertilizer (0.05 g/L) (Phostrogen, Bayer Garden, UK), twice a month. The fertilizer included all essential macro- and microtrace elements. Pots were incubated in the glasshouse under natural sunlight and at a constant temperature of 30°C. The electrical conductivity (EC), moisture content, and temperature of the soil were monitored by using data loggers (DECAGON, Em50, 2012, WV, USA). Each treatment had three biological replicates, and each replicate included three different plants. At the end of the stress treatment, leaf and root tissues of the control and treated seedlings were separated, thoroughly rinsed with water or a 240 mM salt solution, dried using paper towels, and immediately frozen in liquid nitrogen.

2.2. Photosynthesis and Chlorophyll Fluorescence Measurements. Gas exchange parameters such as the net photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E), and intercellular CO_2 concentration (C_i) were determined using a portable photosynthesis system (LCpro-SD, ADC BioScientific, UK). Photosynthetic flux density was maintained at 869 μmol·m^{-2}·s^{-1}, and reference CO_2 was maintained at a rate of 600 μmol CO_2/mol. Transpiration use efficiency (TUE) was calculated as the following: WUE = A/E. Chlorophyll fluorescence (Fv/Fm) was measured using a rapid screening fluorometer (Pocket Pea, Hansatech Instruments, UK). The values recorded from the fluorometer were analyzed using PEA software.

2.3. Leaf Water Relations. The relative water content was measured as previously described [37, 38]. Leaf water potential (LWP) was determined using a leaf water potential meter (model 600, PMS Instrument Company, Oregon, USA). Osmotic potential (OP) was measured on the sap extracted from leaves and roots of the two cultivars using a vapor pressure osmometer (VAPRO@ Pressure Osmometer, Modele 5600, TX, USA), and the OP at full turgor (OPft) was calculated as previously described [39]. Osmotic adjustment (OA) was then calculated as the difference between OP at full turgor of control and treated plants. The leaf area (LA) of each plant was determined using a portable leaf area meter (CI-202, USA).

2.4. Root Measurements. Root measurements (length, diameter, total surface area, volume, and number of tips) were carried out using root scanner WinRHIZO (RH-R XLR STD) software (version 5.0, Reagent instruments, Inc., Quebec, Canada).

2.5. Total Soluble Sugar Measurements. The total soluble sugars in samples were calorimetrically determined using a previously reported protocol [40]. In brief, 0.5 g of tissue was powdered in 80% ethanol, vortexed vigorously, and boiled at 80°C for 30 minutes. The mixture was then centrifuged at 10,000 g for 15 minutes, and the supernatant was collected. Subsequently, 2 mL of the extract was added to 2 mL of the anthrone reagent on ice, and the mixture was boiled for seven and a half minutes in boiling water bath before the solution could be cooled rapidly. The absorbance readings

were measured at 620 nm. A standard curve was prepared using glucose stock solution (100 mg glucose/100 mL dH_2O), and total sugars were expressed as mg/g.

2.6. Determination of Ca^{2+}, K^+, and Na^+ in Plant Tissues. The concentration of Ca^{2+} was determined in plant tissues using the established protocol [41]. In brief, leaf and root samples were dried at 80°C for 48 h and weighed. The dried samples were digested with 69% HNO_3 acid and H_2O_2 (5 : 1 v/v), and Ca^{2+} concentration was measured by using an atomic absorption spectrophotometer Analyst H 300 (Germany). Subsequently, the Na^+ and K^+ concentrations were quantified using the flame photometer (microprocessor flame photometer, Electronics India, Model 1382, Parwanoo, Himachal Pradesh, India) against Na^+ and K^+ standards of known concentration, following a previously described method [42].

2.7. Determination of Lignin and the Compatible Solutes. Quantification of lignin was carried out using the thioglycolic acid method as previously described [43]. Root tissues (0.2 g) was powdered in liquid nitrogen and incubated in 85% acetone for 48 h. The mixture was then centrifuged at 7,500 × g for 15 min at 7°C, and the supernatant was discarded. The pellet was air-dried and resuspended in 5 mL thioglycolic acid prepared in 2N HCL (1 : 10 v/v) and incubated for 4 h at 25°C. Subsequently, the suspension was centrifuged at 7,500 × g for 15 min, and the resulted supernatant was transferred into a 20 mL tube containing 0.2 mL of 10 M HCl and incubated in an ice bath for 4 h. The supernatant was centrifuged at 7,500 × g for 30 min at 7°C, and the pellet was resuspended in 5 mL of 0.5 N NaOH. Absorbance was determined at 280 nm. The standard curve was prepared using lignin alkali (10–100 μg/mL), and the data were expressed as μg/g DW. Proline and glycine betaine (GB) concentrations were determined by a colorimetric method, according to previously published protocols [44, 45].

2.8. Root Sections and Staining Procedure. To determine the changes in the Casparian band formation, seeds of Umsila and Zabad cultivars were germinated in wet sand and the seeds were irrigated either with distilled water or with a 240 mM NaCl solution and incubated in a growth chamber in the dark at 30°C for six weeks. Freehand cross-sections were taken from the root vascular tissues 1 cm away from the root tip and stained for 1 h in 0.1% (w/v) berberine chloride-hydrate and transferred to 0.5% (w/v) aniline-blue for 30 minutes as previously described [46]. Then, stained sections were examined and viewed under an epifluorescent microscope (NIKON MODEL ECLIPS, DS-Ri2, TOKYO, JAPAN), with UV light using an excitation filter for 4′,6-diamidino-2-phenylindole (DAPI) (EF: 361–389) and a dichroic mirror (DM- 415, NIKON).

2.9. Statistical Analysis. Data were analyzed by using the SPSS statistical package version 21 (IBM Corp, Armonk NY, USA). The test of significance was carried out using pairwise comparison, F-statistics at $p \leq 0.05$.

3. Results

3.1. The Effect of Salinity on the Leaf and Root Tissues. In general, salt stress negatively affected the growth and development of both cultivars. However, both leaf and root systems of Umsila cultivar exhibited better performance than the Zabad (Figure 1(a)). For instance, Umsila exhibited approximately 20% reduction in the leaf area compared with the control, whereas the leaf area in Zabad was more significantly reduced by 27% under salt stress (Figure 1(b)).

Without salt stress (control), the total surface area of the roots was significantly ($p \leq 0.05$) higher in Umsila than in Zabad. Some of the root architecture measurements were negatively affected by salt stress in both cultivars. However, unlike in Zabad, the total root length, surface area, and volume were decreased to a lesser extent ($p \leq 0.05$) in Umsila. By contrast, total diameter and number of tips were significantly ($p \leq 0.05$) increased in response to salinity in Umsila but not in Zabad (Figure 2).

3.2. The Effect of Salinity on Photosynthesis. The net photosynthetic rate (A), stomatal conductance (gs), transpiration (E) and internal CO_2 concentration (C_i), transpiration use efficiency (TUE), and quantum yield of PSII activity (Qy) were determined in leaves of Umsila and Zabad grown under control and salinity. Most of these parameters were significantly ($p \leq 0.05$) reduced in both cultivars when exposed to salinity, although the extent of decrease was greater in salt-sensitive Zabad than salt-tolerant Umsila. The only exception to this general decreasing trend was, TUE which was significantly increased under salinity in Zabad while it was decreased in Umsila (Figure 3(e)). For instance, the percent reduction in A was lower in Umsila (34%) ($p \leq 0.05$) than in Zabad (48%) when compared with their respective controls (Figure 3(a)). Similarly, the reduction in gs was less in Umsila (38%) than in Zabad (67%) ($p \leq 0.05$) compared with their respective control plants (Figure 3(b)). The decrease in C_i was also less in Umsila (30%) than in the control, whereas it was significantly reduced ($p \leq 0.05$) in Zabad (50%) (Figure 3(c)). Additionally, the reduction in E was less in Umsila (21%) compared with the control, whereas it was significantly reduced ($p \leq 0.05$) in Zabad (62%) (Figure 3(d)). A similar pattern was observed for the transpiration use efficiency (TUE) where it was reduced in Umsila by 17% when compared with the control (Figure 3(e)). For the quantum yield of PSII activity, the degree of reduction was less (14%) in Umsila, whereas this parameter was markedly reduced (26%) in Zabad (Figure 3(f)).

3.3. The Effect of Salinity on Plant-Water Relations. Salinity induces osmotic stress both in the soil and in the plant, which disturbs plant-water relations (turgor). However, the degree of disturbance in the plant cell may vary based on the plant's ability to deal with salinity. The leaf water potential (LWP) was significantly ($p \leq 0.05$) decreased both in Umsila and Zabad during salinity (Figure 4(a)). However, the extent of reduction in water potential is much greater in Zabad than in Umsila. The relative water content (RWC) of leaves of Umsila remained unchanged, whereas it

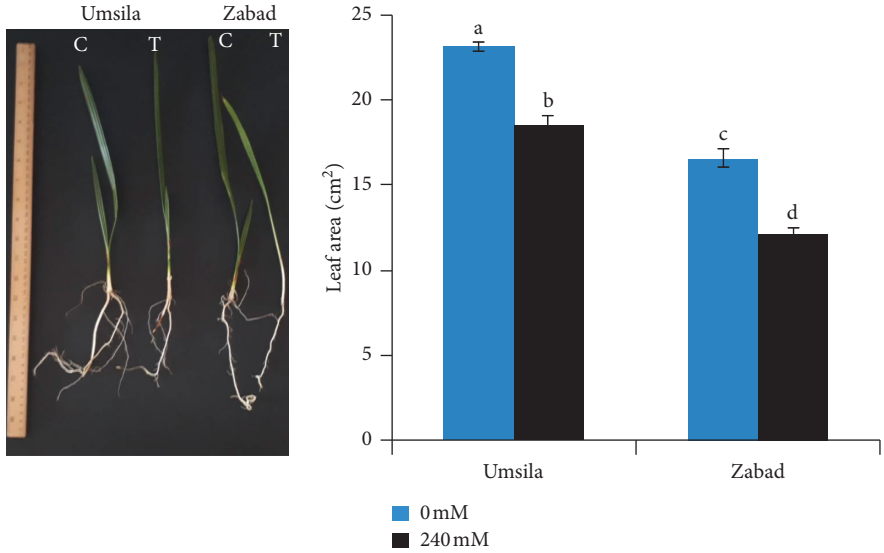

Figure 1: Seedlings phenotype (a) and leaf area (b) of Umsila and Zabad date palm cultivars grown under control and salt stress (240 mM NaCl) conditions. Bars represent mean ± SE ($n = 3$). Statistical differences between the treatments were calculated based on ($p \leq 0.05$).

was significantly ($p \leq 0.05$) reduced in Zabad in response to salinity (Figure 4(b)). Similarly, during salt stress, the leaf osmotic potential (leaf OP) of Umsila was only mildly decreased, whereas it was significantly ($p \leq 0.05$) decreased in Zabad when compared with their respective controls (Figure 4(c)).

Interestingly, the root osmotic potential (root OP) greatly varied between Umsila and Zabad under control conditions, i.e., root OP was much greater in Umsila than in Zabad. During salt stress, OP was reduced in both cultivars but the degree of decrease was significant ($p \leq 0.05$) in Zabad (Figure 4(d)). Without stress treatment or during salt stress, the osmotic potential at full turgor (OPft) of the leaf tissues was almost similar between Umsila and Zabad (Figure 4(e)). However, the osmotic adjustment (OA) was significantly ($p \leq 0.05$) greater in the leaf and root tissues of Umsila but remained low in Zabad tissues when grown under control and salinity conditions (Figure 4(f)).

3.4. The Effect of Salt Stress on Na^+, K^+, and Ca^{2+} Concentrations. The concentration of Na^+ and K^+ was measured in order to determine the effect of salinity on the accumulation of these ions in the plant tissues. The results showed that the concentration of Na^+ was significantly ($p \leq 0.05$) increased in the plant tissues with respect to the control when both cultivars were exposed to salinity. However, that increase was remarkably higher in Zabad tissues (Figures 5(a) and 5(b)). Unlike Umsila, the concentration of K^+ was significantly ($p \leq 0.05$) reduced in the leaf and root tissues of Zabad when exposed to salinity (Figures 5(c) and 5(d)). The reduction in K^+ and the increase in Na^+ led to an increase in the Na^+/K^+ ratio only in the leaf and root tissues of Zabad cultivar (Figures 5(e) and 5(f)). This increase has a negative impact on the ability of the plants to tolerate salinity.

The concentration of Ca^{2+} in the plant tissues was measured in leaf and root tissues in order to determine the effect of salinity on Ca^{2+} accumulation in both date palm cultivars. The Ca^{2+} concentration was significantly ($p \leq 0.05$) increased in 'Umsila' leaf tissues in response to salinity (Figure 6(a)). Surprisingly, Ca^{+2} concentration in the root tissues was almost unaltered under salt stress in both cultivars (Figure 6(b)).

3.5. Differentially Accumulated Sugars in Response to Salinity. Total soluble sugars were measured in the leaf and root tissues of Umsila and Zabad plants grown under control and saline conditions. In general, leaf tissues had more soluble sugars than root tissues of both cultivars. The level of soluble sugars in the leaf and the root tissues of both cultivars was almost similar under control conditions (Figure 7). However, under salt stress, the total sugars were significantly ($p \leq 0.05$) elevated in the leaf and root tissues of Umsila but not in Zabad.

3.6. The Accumulation of Proline and Glycine Betaine in Response to Salinity. Proline and glycine betaine are important compatible solutes that may provide plants with osmoprotection under salinity. Therefore, the concentration of these osmolytes was measured in this experiment in order to determine their role in salinity tolerance in the date palm. The results showed that the concentration of proline was higher than glycine betaine in date palm tissues, and the concentration of proline was higher in the leaves than in the roots (Figures 8(a) and 8(b)). When the seedlings were exposed to salinity, the concentration of proline was significantly ($p \leq 0.05$) increased in the leaf and root tissues of Umsila; however, this concentration was significantly

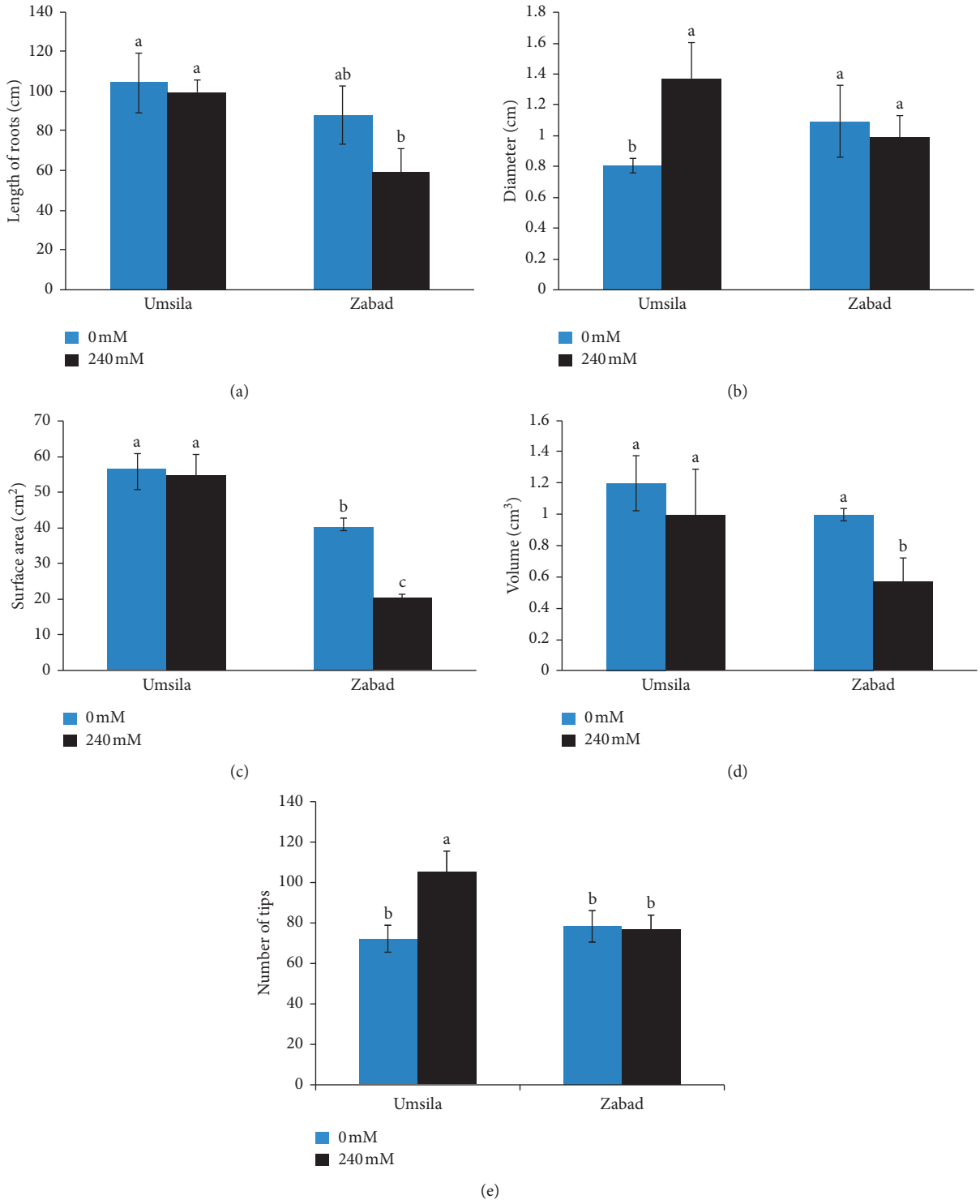

Figure 2: Measurements obtained for the root system including the length (a), diameter (b), total surface area (c), volume (d), and number of tips (e) of Umsila and Zabad seedlings grown under control and saline conditions. Bars represent mean ± SE ($n = 3$). Statistical differences between the treatments were calculated based on ($p \leq 0.05$).

reduced in Zabad tissues (Figures 8(a) and 8(b)). Likewise, the concentration of glycine betaine was significantly ($p \leq 0.05$) increased in the leaf tissues of Umsila, but it was reduced in Zabad in response to salinity (Figures 8(c) and 8(d)).

3.7. The Effect of Salt Stress on Lignin Levels. Total lignin content was quantified in the root tissues of Umsila and Zabad under control and salinity. The lignin levels in the untreated plants of Umsila was slightly higher than Zabad.

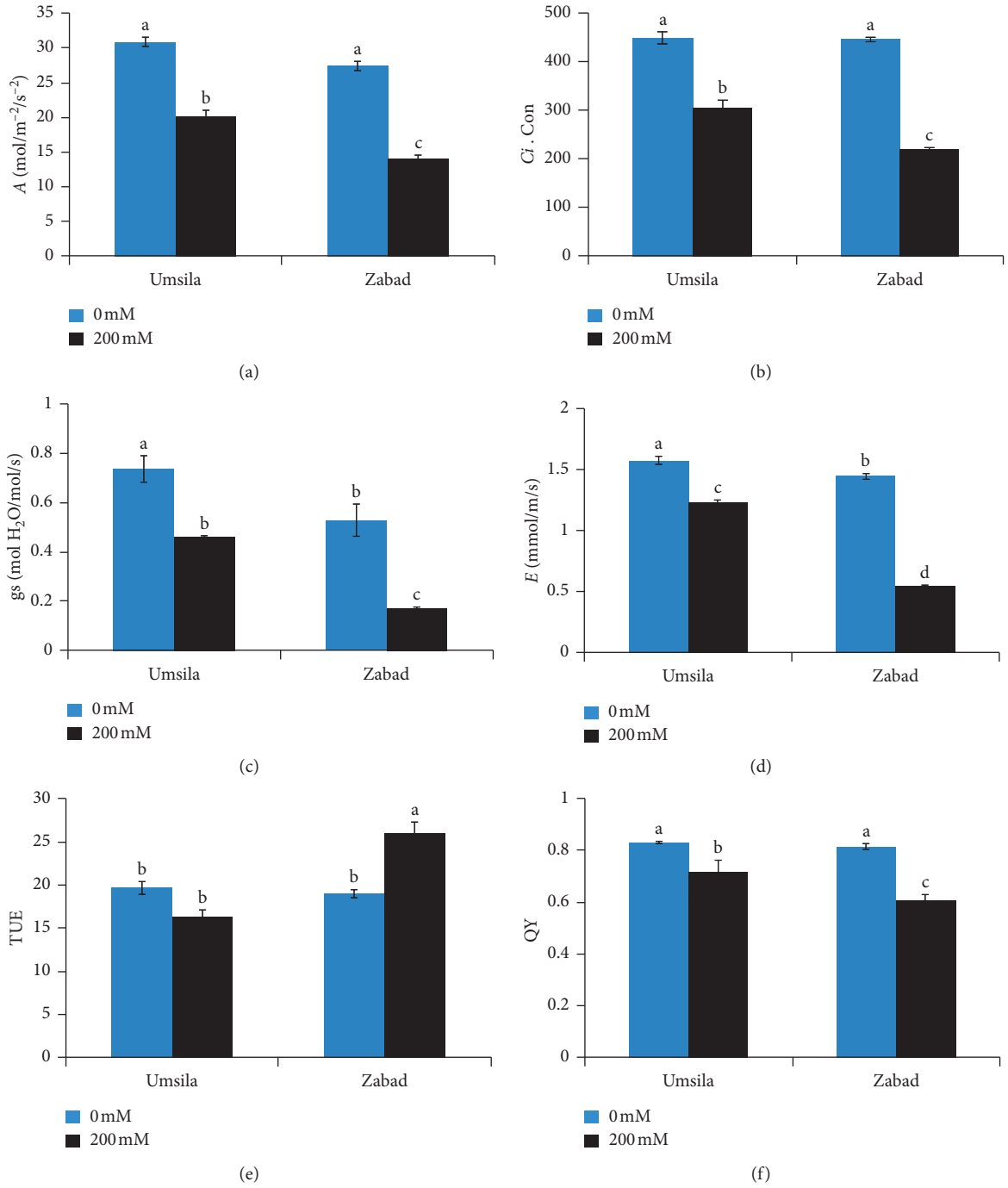

Figure 3: Effect of salinity on photosynthesis rate (A) (a), intercellular CO_2 concentration (Ci) (b), stomatal conductance (gs) (c), transpiration use efficiency (TUE) (d), water use efficiency (WUE) (e), and quantum yield (Qy) (f) in Umsila and Zabad date palm cultivars exposed to control and salinity. Bars represent means ± SE ($n = 3$). Bars with the same letter are not significantly different at the $p \leq 0.05$ level.

However, the lignin in the root of Umsila was slightly higher than those in Zabad under salinity (Figure 9).

3.8. Salinity Promotes Casparian Strip Formation in Umsila. Casparian strips tissue can regulate selective water and mineral transportation and hence contribute to salinity tolerance in plants. A histochemical staining technique was used to investigate the role of Casparian strips in salinity tolerance of date palm cultivars Umsila and Zabad. Staining and measuring of the Casparian strip under the fluorescent microscope revealed a significant ($p \leq 0.05$) increase in the thickness of the Casparian strips of Umsila plants exposed to salinity (Figure 10(a)), whereas there was a significant ($p \leq 0.05$) decrease in this tissue of Zabad cultivar (Figure 10(b)).

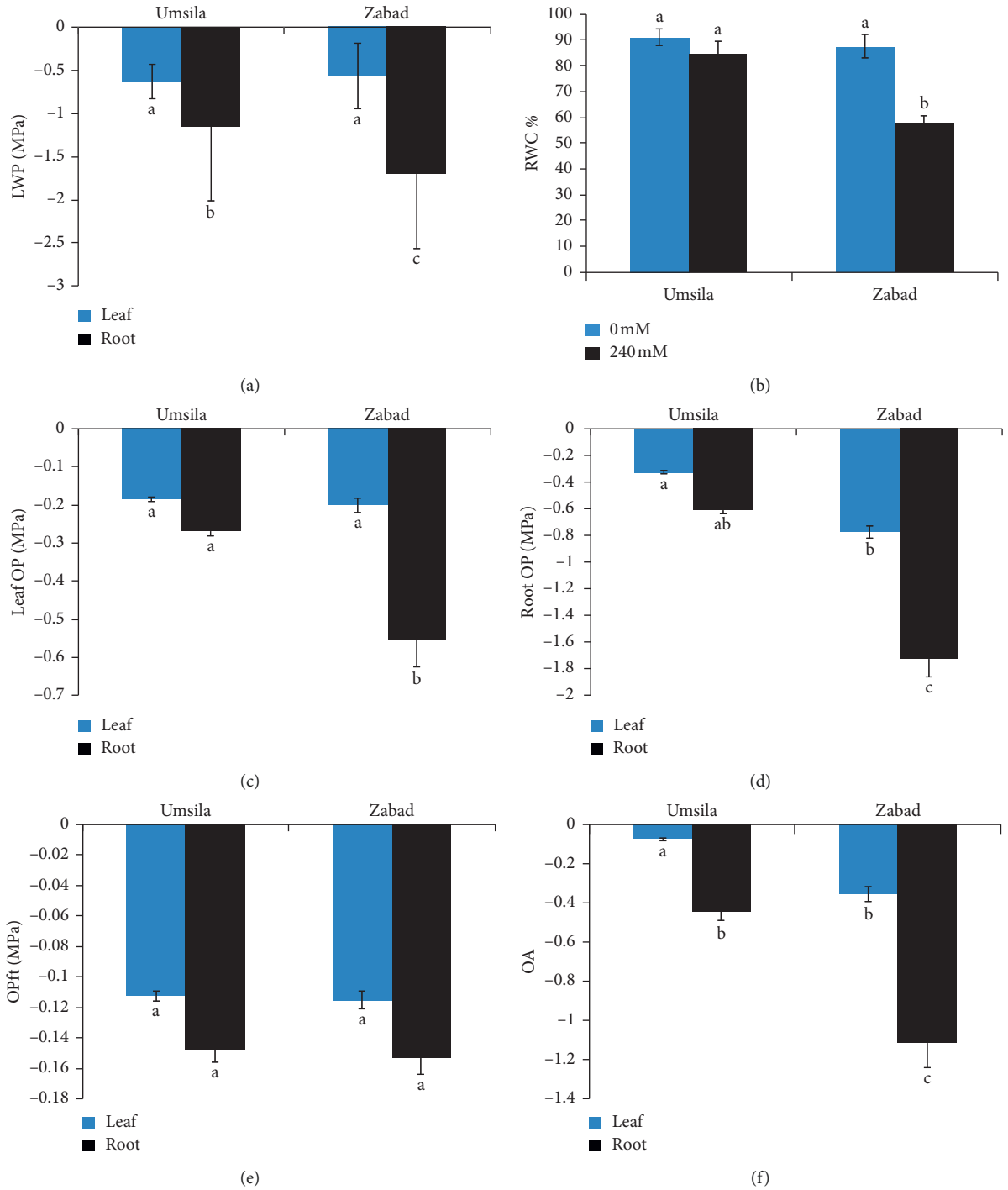

Figure 4: Leaf water potential (LWP) (a), relative water content (RWC) (b), leaf osmotic potential (leaf OP) (c), root osmotic potential (root OP) (d), osmotic potential at full turgor (OPft) (e), and osmotic adjustment (OA) (f) measured in Umsila and Zabad date palm cultivars when grown under control and salinity conditions. Bars represent mean ± SE ($n = 3$). Statistical differences between the treatments were calculated based on $p \leq 0.05$.

4. Discussion

Salinity tolerance is a complex trait involving several pathways and mechanisms that in turn, offer protection to the plant against the harmful effects of salinity. In this study, two date palm cultivars differing in their salinity tolerance capacities were evaluated for water relations, photosynthesis-associated parameters, sugars as well as lignin, and casparian strips to identify the differences between salt-tolerant and salt-sensitive date palm cultivars.

Overall, the adverse effects of salt stress were less severe on the growth parameters of Umsila, a tolerant date palm

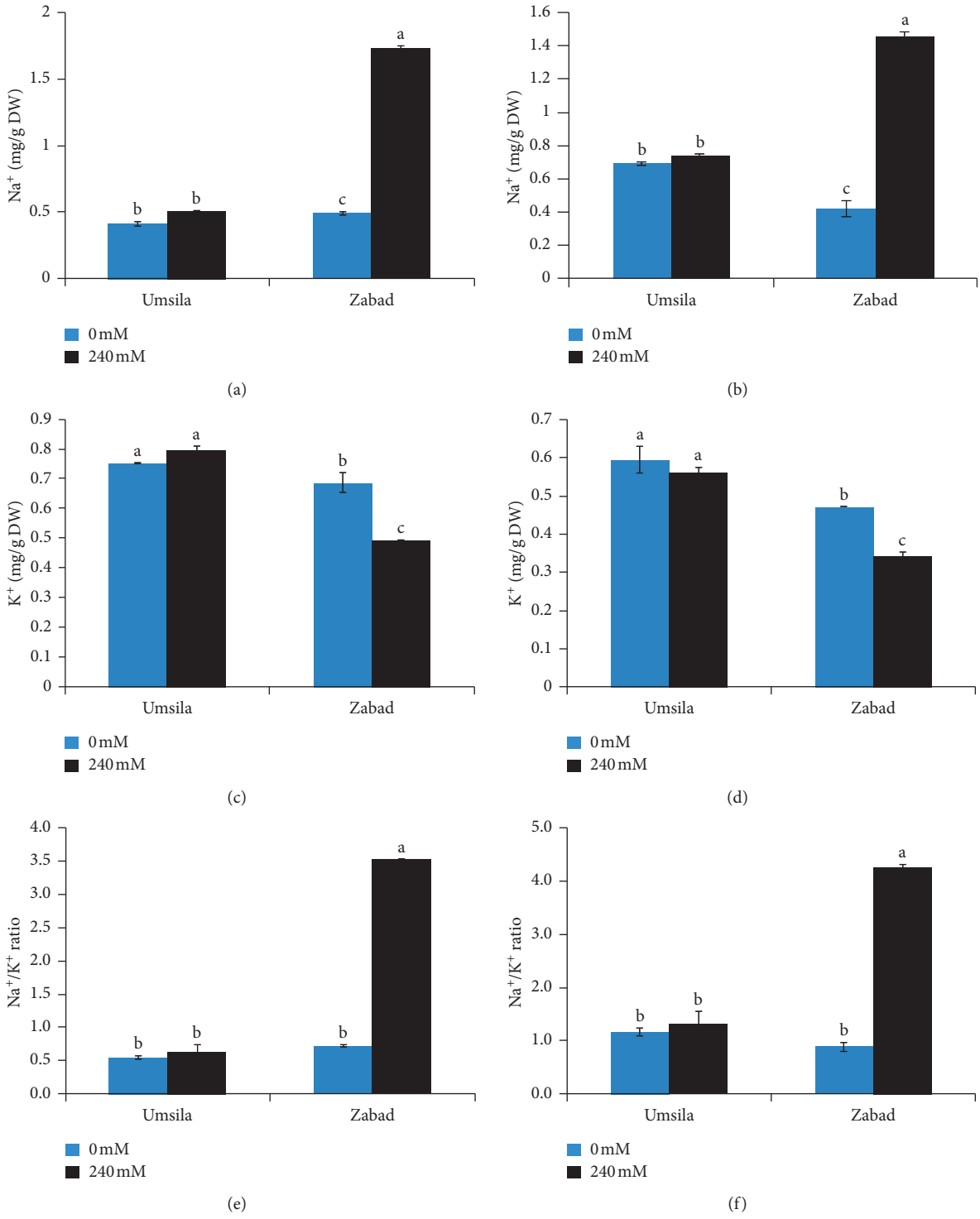

Figure 5: The effect of salinity on the accumulation of sodium (Na^+) in leaves (a) and roots (b), the accumulation of potassium (K^+) in leaves (c) and roots (d), and the Na^+/K^+ ratio in leaves (e) and roots (f) of date palm seedlings when exposed to control (0 mM NaCl) and salinity (240 mM NaCl) conditions. Bars represent the mean ± SE ($n = 3$). Bars with the same letter are not significant at $p \leq 0.05$.

cultivar, than in Zabad. The better performance of salt-tolerant cultivar Umsila could be attributed to better maintenance of various morphological, physiological, and anatomical characteristics. For example, Umsila has a larger root system than Zabad. Moreover, the root system was less affected by salinity in Umsila. In fact, the root diameter and the total root tips have increased in response to salinity. This feature may enhance the water absorption as well as water holding capacity of the root under salinity and, therefore, promote tolerance [47].

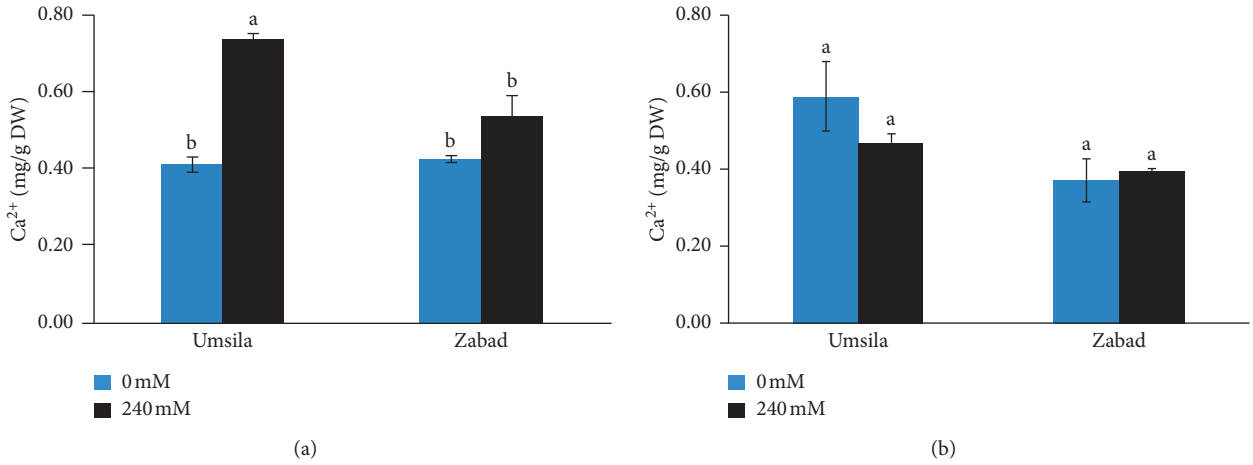

Figure 6: Effect of salinity on the calcium (Ca^{+2}) concentration in leaves (a) and roots (b) of date palm seedlings when grown under control and salinity conditions. Bars represent mean ± SE ($n = 3$). Bars with the same letter are not significant at $p \leq 0.05$.

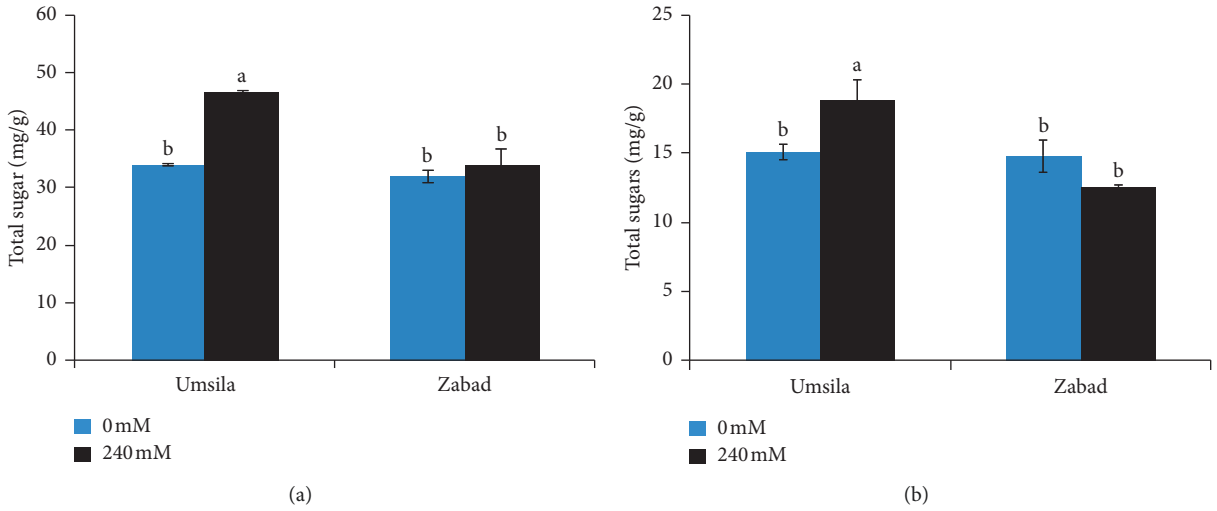

Figure 7: Differential accumulation of soluble sugars in leaves (a) and roots (b) of the salt-tolerant cultivar Umsila and susceptible Zabad cultivars due to salt treatment. Bars represent mean ± SE ($n = 3$). Statistical differences between the treatments were calculated based on $p \leq 0.05$.

Umsila has maintained a better photosynthetic system under salinity than the susceptible cultivar Zabad as reflected by the relevant parameters measured in this study. In fact, maintaining the production of photosynthates such as starch is a natural abiotic response of various salt-tolerant plants [48]. These photosynthates provide an essential source of energy, which is required to combat the harsh conditions and may also transform into smaller molecules while moving toward the sink and, hence, serve as osmolytes that can help plants maintain osmotic potential [48].

Although the photosynthetic rate (A) decreased in Umsila and Zabad, Umsila had a relatively higher value of A than Zabad under salt stress, concomitant with higher levels of gs and E (Figure 3). In the present study, the tolerant cultivar Umsila showed less use of TUE than the susceptible cultivar Zabad (Figure 3). TUE of the photosynthesis procedure is an indicator of efficient photosynthesis machinery [49, 50]. Besides, Qy was less reduced in Umsila than in Zabad. Damage to the photosynthetic machinery is often ascertained by measuring the quantum yield efficiency of PSII (Qy) [51–53]. Generally, higher A is because of higher gs and E is complemented by less use of TUE and, hence, are the major factors contributing in photosynthesis in the date palm as well as in other plant species [50, 54, 55]. Reduction of Ci in the two cultivars under salinity stress could be due to the direct effect of the reduction in stomatal conductance (stomatal limitation) [56, 57]. The decline in photosynthesis could be direct, such as a decrease in CO_2 concentration caused by stomatal closure or mesophyll conductance [58, 59], as it has been reported for other date palm cultivars [50, 60–62]. Umsila maintained a higher leaf area than Zabad under salt stress (Figure 1(b)), an indication of less growth and less photosynthesis reduction in this cultivar [63]. The leaf area is directly correlated with the

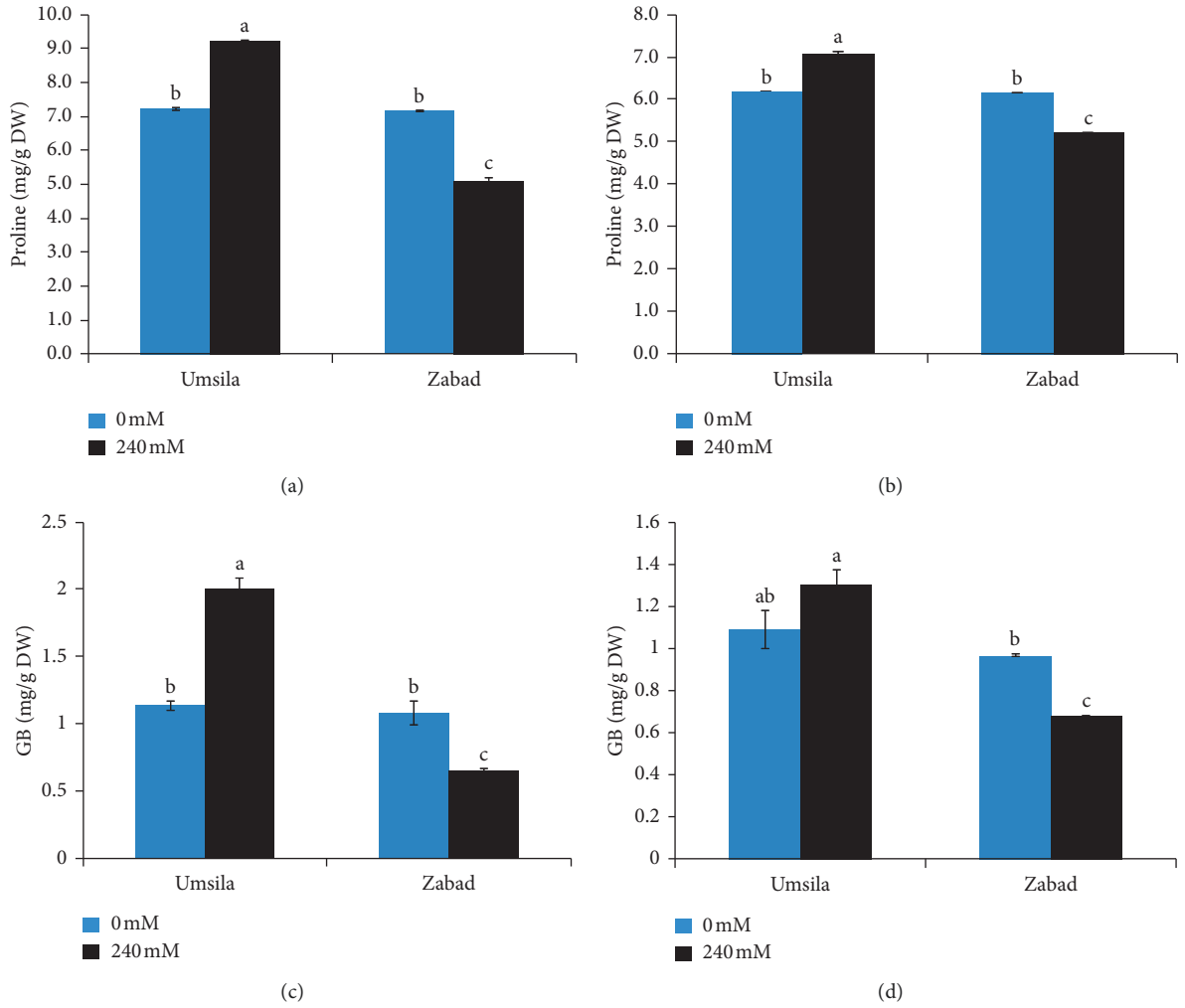

Figure 8: The effect of salinity on the accumulation of proline in leaves (a) and roots (b), and the accumulation of glycine betaine accumulation in leaves (c) and in roots (d) of date palm seedlings when exposed to control (0 mM NaCl) and salinity (240 mM NaCl) conditions. Bars represent the mean ± SE ($n = 3$). Statistical differences between the treatments were calculated based on $p \leq 0.05$.

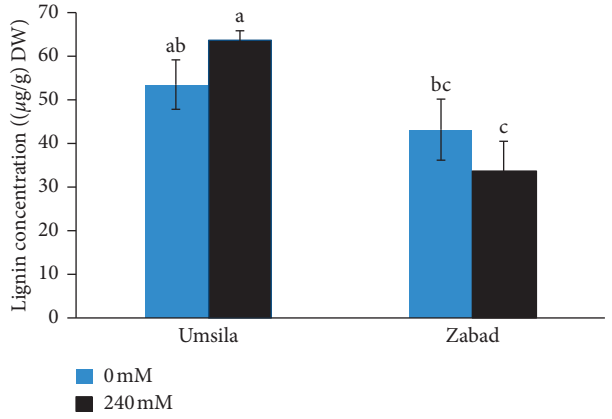

Figure 9: Effect of salinity on the lignin concentration in root tissues of date palm seedlings when grown under control and salinity conditions. Bars represent mean ± SE ($n = 3$). Bars with the same letter are not significant at $p \leq 0.05$.

photosynthetic rate [64]. These results suggest that photosynthesis is a crucial component of the stress-tolerant mechanism in the date palm.

Consistent with the higher photosynthesis efficiency, Umsila seedlings were able to accumulate more soluble sugars in their tissues than Zabad under salinity (Figure 7).

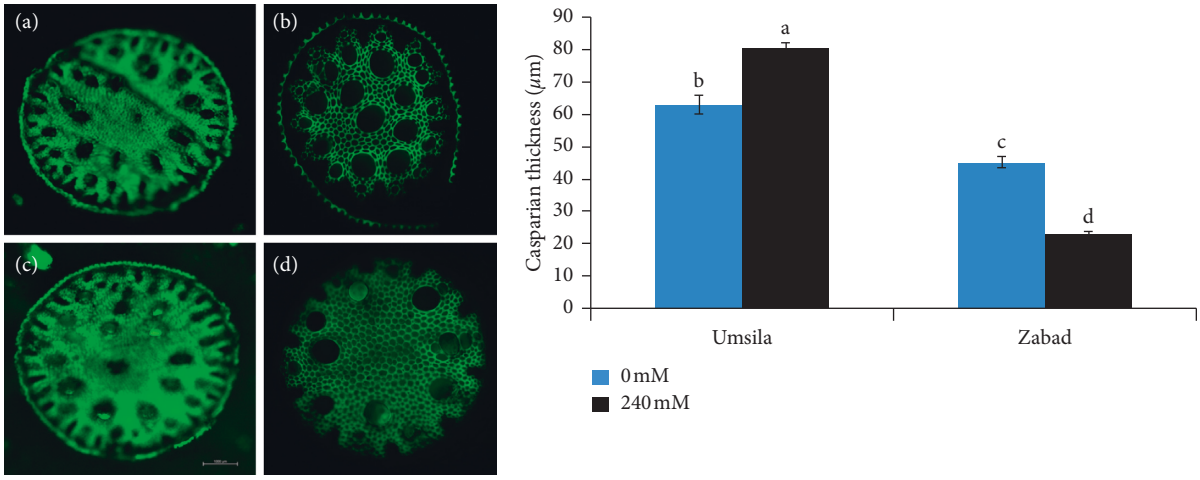

Figure 10: (a) Staining of Casparian strips of the date palm roots. Casparian strips staining of Umsila roots grown under control (a) and salinity conditions (b), and Zabad roots grown under control (c) and salinity conditions (d). (b) Bars represent mean of the Casparian strip thickness measured under the fluorescent microscope ± SE ($n = 3$). Bars with the same letter are not significant at $p \leq 0.05$.

Soluble sugars act as osmolytes to maintain homeostasis in response to adverse environmental stresses [17, 65, 66]. Plants tend to increase the total soluble sugars in their tissues when exposed to salinity, a procedure which enhances salt tolerance [17]. For example, it was previously shown that the tolerant line ICMB 01222 of pearl millet had accumulated higher sugars in the leaves than the sensitive line ICMB 081 line under salt stress [67].

When compared with Zabad, Umsila cultivar maintained better plant-water relations because the seedlings were able to maintain better water potential and osmotic potential in leaves and roots under salinity. This was further reflected from the observation that the degree of reduction in the leaf area was more pronounced in Zabad. Cell expansion is a function of water uptake and cell wall extension [68]. Maintenance of the leaf area is primarily because of cell expansion which in turn is related to water status in the plants under salt stress. Salinity has affected water relations of both Umsila and Zabad by causing pronounced changes in LWP, RWC, OP, and OA. However, the degree of inhibition of these characteristics was much less in Umsila compared with Zabad. The lower OP values in the leaf and root tissues could be attributed to the fact that Zabad accumulates higher amounts of Na^+ than Umsila when grown under salinity. In addition, this reduction in OP might have further contributes toward the reduction LWP which in turn significantly ($p < 0.05$) affected the RWC of the salt-sensitive Zabab as compared with Umsila [11, 12] The overall improved water management system of the salt-tolerant Umsila can be related to the overall genetic makeup of the plant, rendering to its healthy physiology under salinity stress. Also, it was previously shown that transgenic *Arabidopsis* plants overexpressing the date palm gene, had improved water management under salinity and drought conditions.

It was observed that Umsila accumulates significantly ($p < 0.05$) high amounts of the osmolytes and compatible solutes as compared with Zabad under salinity stress. Furthermore, as reported previously, the proline and glycine betaine have increased in response to salinity in the date palm as well as in other plant species [69], where they play a pivotal role in mitigating NaCl-induced K^+ efflux [70], and have a direct protective role for membrane integrity as an osmoprotectant under salt stress and other environmental stresses [71–73], as well as they have an indirect protective role through participating in signal transduction pathways [74]. It was previously reported that the accumulation of glycine betaine in the transgenic lines of wheat under salinity enhanced thylakoid membrane integrity and components resulted in higher photosynthesis [75].

Umsila was able to control Na^+ and retain K^+ content in the leaf and root tissues under salinity. On the other hand, reduction in K^+ ions in Zabad increased the possibility of Na^+ influx induced by the cytosolic K^+ efflux and leakages outside the cell and may cause accumulation of ROS [76]. The fact that Umsila has maintained a lower Na^+/K^+ ratio is an indication that K^+ homeostasis is a critical feature in salt tolerance in the date palm as well as in other plant species [70]. One of the reasons this variation in the Na^+/K^+ ratios could be the high accumulation of lignin in the Umsila than Zabad as observed in this study, these lignins may act as a barrier for the movement of Na^+ and K^+ ions [36]. Additionally, various ion transporters and channels are differentially expressed in different date palm varieties as a result of salinity, and this could further attribute to the variation in the Na^+/K^+ ratios [8, 77].

Despite both the leaf OP and root OP was decreased in Umsila and Zabad under salinity, these parameters including LWP were less affected in Umsila, suggesting that this cultivar displayed a higher ability to extract water under saline conditions than salt-sensitive Zabad. This feature can be contributed to the capacity of this tolerant cultivar to lower the osmotic potential (osmotic adjustment) under salinity, likely through the production of sugars as shown in

this report and also other osmolytes such as proline as previously shown [11]. It was previously shown that despite the low water potentials in *Atriplex* L. under salt stress, it could maintain cell turgor pressure and a favorable water absorption gradient because of the ability of this species to adjust osmotically [78–80].

Na^+ influx depolarizes the plasma membrane [81]. Therefore, the ability of plants to increase Ca^{2+} concentrations is vital for plants to tolerate salinity. In fact, Ca^{2+} can block some of nonselective cationic channels; as a result, it reduces Na^+ permeability into cells, thereby maintaining the Na^+/K^+ ratio balance [82, 83]. Consistently, a previous study showed that water uptake and tolerance of *Capsicum annuum* L. under salinity were improved by exogenous application of Ca^{2+} [84]. In the current study, Ca^{2+} concentrations have increased in Umsila and Zabad leaves, however, remained lower in the root tissues of Zabad than those in Umsila under salinity. Most significantly, the reduction in Ca^{2+} concentrations in Zabad leaves increased the possibility of low relative water content. Reduction in tissue water content under salinity might change cytosolic Ca^{2+} concentrations [68, 85]. A high amount of Ca^{2+} in Umsila leaves can be contributed to less negative LWP, which could maintain cell membrane depolarization and, thus, resulted in opening channels that allow movement of positive ions such as Ca^{+2} and K^+ into cells.

Ca^{2+} concentrations increased significantly in the leaves of Umsila than Zabad. Ca^{2+} substantially reduces the effects of the salt stress in many plants [70], and it is among early signaling events in plants with other signals such as ROS. Ca^+ has a crucial signaling function in plant cells, where it regulates the membrane channel and stomatal movement, which are essential factors responsible for regulating Na^+/K^+ uptake and plant water status as well as photosynthesis regulation [86, 87]. Therefore, it is not unexpected that Ca^+ is actively involved in the salinity tolerance mechanism in date palms.

The Casparian strip thickness has increased in response to salinity in the Umsila cultivar (Figure 7(b)). This may enhance the apoplastic barriers and regulate hydraulic conductivity in roots and, therefore, prevent the nonselective apoplastic bypass of Na^+ into the stele tissues and, hence, increase salinity tolerance [88, 89]. On the same lines, it was proposed that some plant species such as tobacco, beans, and maize tend to accelerate the growth of the exo- and endoderm cells including the Casparian strip tissues in response to salinity [89–91]. Additionally, physical interaction between Na^+ ions and the cation exchange sites on the cell wall might change the chemical composition of the cell wall under salt stress [32], which might help mitigate Na^+ effects to cope with salt stress and decreasing the Na^+/K^+ ratio in Umsila.

Increased deposition of lignin in the stele and cortex tissues in response to salinity was reported in different plant species [92, 93]. Further, a higher lignification level was reported in the root of salt-tolerant but not in the salt-sensitive durum wheat (*Triticum turgidum*) in response to salinity [94, 95]. Moreover, a significant increase in the lignification in the monocotyledonous species reduces salts and other toxic solute movement into the stele [96]. On the other hand, a decreased deposition of lignin increases salt sensitivity to rice [31]. Changes in the lignin content as a response to biotic and abiotic stress was also previously reported in the date palm [97–100]. Consistently, in general, the lignin content was maintained in salt-tolerant Umsila but decreased in Zabad, a slat-sensitive cultivar, under saline conditions.

5. Conclusions

The results obtained from this study revealed that the date palm can tolerate salinity using an efficient photosynthetic system as indicated by the higher Qy and GS found in the tolerant cultivar Umsila and also by better maintenance of plant-water relations, which can maintain relatively better water availability in the plant tissue as observed in the tolerant Umsila. This could be achieved by an efficient OA because of (at least partially) greater accumulation of soluble sugars, Ca^+, and compatible solutes such as proline and glycine betaine compared with salt-sensitive Zabad. Besides, salt tolerance in the date palm is associated with the accumulation of lignin deposition and the formation of Casparian strips, which could perhaps enhance selective ion and water movements across the plant tissues and maintain a balanced Na^+/K^+ ratio as it was shown in the salt-tolerant Umsila cultivar. Taken together, the majority of the measured parameters were less affected in salt-tolerant Umsila compared with salt-sensitive Zabad, suggesting that the impact of salt stress is better counteracted in the tolerant date palm.

Data Availability

All data generated or analyzed during this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

LAK conceived, designed, performed the experiments, analyzed data, wrote the manuscript; RS revised and edited the manuscript; RAY provided resources; and MWY designed the experiment, supervised the experiments, wrote the manuscript, and contributed reagents, materials, and analysis tools.

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