ORIGINAL ARTICLE

Mechanisms of Enhancing Photosynthetic Gas Exchange in Date Palm Seedlings (*Phoenix dactylifera* L.) under Salinity Stress by a 5-Aminolevulinic Acid-based Fertilizer

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Abstract Photosynthetic gas exchange characteristics, salt uptake, pigment contents, and electrolyte leakage were examined in date palm seedlings (*Phoenix dactylifera* L.) subject to seawater treatments at 1-, 15-, and 30-mS cm^{-1} salinity levels in the presence or absence of 0.08% ALA-based (5-aminolevulinic acid-based) functional fertilizer commercially known as Pentakeep-v. Date palm seedlings accumulated significant amounts of Na$^+$ in the foliage with increasing salinity, about a threefold increase in the accumulated Na$^+$ between the control and 30-mS cm^{-1} salinity treatment. Electrolyte leakage indicated a significant reduction in membrane integrity as salinity increased. A strong linear correlation was observed between the chlorophyll (chl) a/b ratio and assimilation rate throughout salinity treatments. The slope (*b*) and the correlation coefficient between the chl a/b ratio and assimilation suggested that salinity reduced assimilation predominantly via the reduction in chlorophyll a contents ($r^2 = 0.885$ and $b = 1.77$, $P < 0.05$). Plants treated with Pentakeep-v showed a similar response with increasing salinity but at higher levels of both chl a/b ratios and assimilation rates. Mechanistic analysis of *A*:*Ci* response curves showed that photosynthetic gas exchange in seedlings of the date palm

was significantly reduced with increasing salinity due to gas phase limitation (S_L) as evident by stomatal conductance (g_s) values. Salinity did not induce any change in the carboxylation efficiency of the rubisco enzyme (Vc,max), or in the rate of electrons supplied by the electron transport system for ribulose 1,5-bisphosphate (RuBP) regeneration (Jmax). Accelerated carbon loss through respiration has significantly contributed to the described reduction in assimilation and increased CO_2 compensation point (Γ). Only at the 30-mS cm^{-1} salinity level did treatment with Pentakeep-v reduce Na$^+$ accumulation in the leaves, and caused a reduction in K$^+$ selective uptake, leading to a concomitant reduction in K$^+$/Na$^+$ ratios. Pentakeep-v significantly improved chl a contents in all treatments, which was subsequently reflected in total chlorophyll and chl a/b ratios. The non-gas-phase components of the photosynthetic process (biochemical factors limiting gas exchange) were significantly improved by Pentakeep-v applications. Specifically, Pentakeep-v enhanced the biochemical efficiency of carbon fixation (Vc,max) and the rate of electron transport required for RuBP regeneration (Jmax) by 37.4% and 17.8%, respectively, over untreated plants at a salinity level of 15 mS cm^{-1}. In addition, Pentakeep-v reduced S_L to values similar to those of control plants (9.07%) and lowered CO_2 compensation points by reducing respiratory CO_2 loss, with increasing salinity to 30 mS cm^{-1}. We, therefore, conclude that the ALA-based fertilizer Pentakeep-v improves salt tolerance in date palm seedlings by increasing photosynthetic assimilation. The latter is mediated via boosting light-harvesting capabilities of the treated plants by increasing chl a content and by reducing stomatal limitation to photosynthetic gas exchange.

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Introduction

The Arabian Peninsula has an extremely arid environment and scarce precipitation. Agriculture activities in this part of the world are mainly dependent on ground water. The expansion of farmland in the past two decades has led to a gradual increase in ground water salinity (Watanabe and others 2004). In the United Arab Emirates (UAE) about 25% of the farmland is now influenced by groundwater salinity which is as high as 17,000 μg g^{-1} (Dakheel 2005), subsequently many farms are now abandoned (Al Hammadi 2006).

Date palms (*Phoenix dactylifera* L.) are the dominant fruit tree plantations in the Arabian Peninsula, where crop productivity is markedly low relative to potential yield. The UAE is fourth in date fruit production with a total of 760,000 tons/year, representing 11.2% of the world production (FAO 2004).

In addition to its xeromorphic leaf features, the date palm displays true halophytic adaptations to salt stress probably more than many other fruit trees (FAO 1982; Ramoliya and Pandey 2003). Their growth and productivity, however, can be greatly reduced by salinity and aridity (Ayers and Westcot 1985; Erskine and others 2004). Salt tolerance in plants is generally age dependent. Seedlings are more sensitive to salinity than mature trees. Most of the studies related to the effect of salinity on date palm are restricted to fruit yield. Salt tolerance of plants determined by fruit yield may not successfully predict salt tolerance of the vegetative phase of the plant (Ramoliya and Pandey 2003).

In higher plants 5-aminolevulinic acid (ALA) is a key precursor of many tetrapyrrole compounds, including porphyrins for chlorophyll biosynthesis (Stobart and Ameen-Bukhari 1984). ALA was reported to restore chlorophyll content in etiolated plants if treated at concentrations higher than 10 mM (Hotta and others 1997b). In addition, several physiologic effects of exogenous ALA have been found to regulate plant growth and increase productivity (Hotta and others 1997b). ALA improves salt tolerance in cotton seedlings through the reduction in sodium uptake (Watanabe and others 2000). Watanabe and his coworkers (2004) reported successful recovery from salt damage in cotton, tomato, and wheat seedlings grown in Saudi Arabia by foliar spraying of ALA.

Application of ALA as a plant growth regulator at rates of 0.18-0.6 mM through foliar spray increased photosynthetic carbon fixation and reduced dark respiration. This effect was linked to light reactions and the nutrient status of the treated plants (Hotta and others 1997a, b; Tanaka and Kuramochi 2001; Watanabe and others 2000). The exact mechanism by which ALA promotes carbon fixation is not yet known.

In this work we explore the mechanisms involved in the effects of salinity on photosynthetic gas exchange in seedlings of the date palm *Phoenix dactylifera* L. and how the ALA-based functional fertilizer, commercially available as Pentakeep-v, alleviates salt stress on the photosynthetic process.

Materials and Methods

Plant Materials and Experimental Design

Fresh seeds of "Lulu" date palm cultivar were soaked in a water bath at 30°C with air bubbling for 2 weeks to hasten germination. The germinated seeds were individually grown in plastic pots (40 × 24 cm) filled with sand and universal plant compost (Stender, Germany) in a 1:1 ratio. Seedlings were grown under open air (temperature range = 19.5–37°C and relative humidity = 37.5–61%) under 40% shade cloth allowing an average PAR of about 600 μmol$_{photon}$ m^{-2} s^{-1}. Seedlings were irrigated with underground water (EC = 1.0 mS cm^{-1}) twice a week and fertilized with NPK (20:20:20 plus trace elements) at 400 g/100 L water (300 ml/pot) once a month.

In December 2005 a factorial experimental design with three levels of irrigation water salinity and two levels of Pentakeep-v, a functional fertilizer containing 5-aminolevulinic acid (Cosmo Seiwa Agriculture Co., Ltd., Japan), in three replicates (10 seedlings/replicate) per treatment was adopted. Selected uniform seedlings were irrigated once a week with well water of EC = 1.0 mS cm^{-1} (control), diluted sea water (DSW) at EC = 15, or 30 mS cm^{-1} (400 ml/pot) for a period of 10 months. Sea water's major components were 580 mM Na$^+$, 14.2 mM K$^+$, 40.6 mM Mg$^+$, and 725 mM Cl$^-$. The Pentakeep-v solution was applied to the pots at a rate of 0.08% (300 ml/pot) and at 10-day intervals. At the time of Pentakeep-v application, DSW or well water was given (300 ml/pot) to the treatments that received no Pentakeep-v.

At the end of June 2006, leaves (the second new leaf from top) were excised from the seedlings for chlorophyll, sodium, and potassium and electrolyte leakage measurements. At the beginning of October, two seedlings per replicate for each treatment were moved to a greenhouse (28°C and 70–80% relative humidity) for 3 weeks before photosynthetic gas exchange was measured.

Gas-Exchange Measurements

Assimilation (*A*, μmol m^{-2} s^{-1}) versus substomatal CO$_2$ concentration (*Ci*, Pa) response curves (*A:Ci*) of seedling leaves were measured for at least two leaves per replicate using portable photosynthesis open systems (Li-6400; Li-cor Inc., Lincoln, NE, USA). The *A:Ci* response curve was

generated after setting the environment inside the leaf chamber to mimic environmental conditions that the plants may experience in the field. Leaf-to-air vapor pressure deficit (vpd) was maintained at 1.5 kPa and leaf temperature (t_l) was adjusted to 25°C. Light intensity was adjusted above plant light saturation capacity at 1000 μmol_{photon} m^{-2} s^{-1} and was provided by a blue/red light (Li-6400-02 LED light source) fitted to the leaf chamber. A CO_2 regulator (Li-6400-01) provided different CO_2 concentrations (Ca) in the range of 800–50 μmol mol^{-1}.

The A:Ci measurements started at a CO_2 concentration close to ambient (390 μmol mol^{-1}) and continued down to 50 μmol mol^{-1} in a stepwise fashion, then up again to 800 μmol mol^{-1} through the ambient CO_2 concentration. At each step gas exchange variables were recorded after achieving steady-state conditions. Measurements were made for at least two replicates per treatment. Calculations of gas exchange parameters were performed according to von Caemmerer and Farquhar (1981). Data from each A:Ci curve were fitted into a nonrectangular hyperbola equation (Jones 1992) using Photosynthesis Assistant software (v1.1, Dundee Scientific, Dundee, Scotland). Mechanistic analysis of each curve (Farquhar and others 1980; Harley and others 1992) was used to estimate the maximal Rubisco-catalyzed carboxylation velocity (Vc,max), and maximal electron transport rate (Jmax).

Stomatal Limitation to Photosynthesis

The relative contributions of stomatal limitation to photosynthesis, which is the proportional decrease in light-saturated (A) attributed to the stomatal component (S_L, %), was calculated according to Farquhar and Sharkey (1982):

$$S_L = [(A_{390} - A)/A_{390}] \times 100$$

A_{390} is the photosynthetic rate at Ci = 390 μmol mol^{-1} and A the photosynthetic rate at Ca = 390 μmol mol^{-1}. A_{390} was taken from the average [CO_2] curves representing net CO_2 assimilation versus intercellular CO_2 concentration (A:Ci curves) and A from average CO_2 curves representing net CO_2 assimilation versus ambient CO_2 concentration (A:Ca curves).

Chlorophyll Measurement

Chlorophyll a and b and total chlorophyll were measured in leaf samples as described by Hiscox and Israelstam (1997). One hundred milligrams of leaf tissue was used for each replicate/treatment. Chlorophyll was extracted by 50 ml dimethyl sulfoxide (DMSO) without grinding at 65°C through overnight incubation. The OD values at 645 and 663 nm were read by a PU8625 Series UV/Visible spectrophotometer (Philips, UK) against a DMSO blank. Chlorophyll contents (mg/g FW) were calculated according to Arnon (1949).

Electrolyte Leakage Measurement

Two leaves per replicate/treatment were directly washed with distilled water and lightly cleaned with tissue papers. Leaf segments (3×3 cm) were cut from the middle of each leaf, cut into two pieces, and incubated in 40 ml of deionized water at 6°C overnight. Samples were warmed up to room temperature (22 ± 2°C), placed in a shaker for 1 h, and vortexed for a few seconds. Electrolyte leakage before killing was measured with an electrical conductivity digital meter (Orion model 150). Samples were killed by autoclaving (JKA-J.39 Autoclave, Japan) at 121°C for 20 min to release all electrolytes, cooled to 22 ± 2°C, placed in a shaker for 1 h, and vortexed. Total electrolyte leakage was measured and percentage of electrolyte leakage was calculated according to Ingram and Buchanan (1984).

Determination of Potassium and Sodium Concentration

Leaf samples were collected, washed with distilled water, and oven-dried for 48 h at 65°C in paper bags. Samples were ground and wet digested as described by Chapman and Pratt (1961). Potassium and sodium concentrations were determined by an inductively coupled plasma atomic emission analyzer (Varian-Vista-MPX, CCD simultaneous ICP-OES Axial).

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to analyze the main effects of salinity, Pentakeep-v, and their interactions. One-way ANOVA was used to analyze each single treatment using the statistical package MSTATC (Michigan State University, East Lansing, MI). Comparisons between means were made by an F test and the least significant differences (LSD) at P = 5%. Regression analyses in all figures were made using Sigma plot 7.101 (SPSS Inc., Chicago, IL, USA).

Results

Salinity treatments through irrigation with seawater at conductivities of 1, 15, and 30 mS cm^{-1} significantly increased Na$^+$ content in leaves of the treated date palm

Table 1 Effect of salinity and Pentakeep-v applications on K^+ and Na^+ contents and K^+/Na^+ ratios in leaves of the date palm *Phoenix dactylifera* seedlings

Treatments	K^+ (%)	Na^+ (%)	K^+/Na^+ ratio
Salinity (S) mS cm^{-1}			
1	0.88 ± 0.02 b	0.23 ± 0.00 d	3.83 ± 0.17 a
15	1.17 ± 0.02 a	0.42 ± 0.00 c	2.77 ± 0.06 c
30	1.10 ± 0.03 a	0.66 ± 0.02 a	1.70 ± 0.11 e
Pentakeep (P) 0.08%			
1 + P	0.75 ± 0.01 c	0.24 ± 0.00 d	3.20 ± 0.08 b
15 + P	0.92 ± 0.03 b	0.43 ± 0.01c	2.13 ± 0.03 d
30 + P	0.92 ± 0.04 b	0.55 ± 0.04 b	1.70 ± 0.06 e
Salinity (S)	***	***	***
Pentakeep (P)	***	NS	***
36 S × P	NS	NS	NS

Values are mean ±SE, n = 3. Means within each column followed by the same letter are not significantly different at level P = 0.05. ***, significant at level P = 0.001. NS = not significant at level P = 0.05

seedlings (Table 1). Selective uptake of K^+ initially increased at 15 mS cm^{-1}. Further increase in salinity showed no increase in leaf K^+ content. Subsequently, K^+/Na^+ ratios were significantly lower as salinity increased (control, 3.83 > 15 mS cm^{-1}, 2.77 > 30 mS cm^{-1}, 1.7) (P = 0.05).

Except for the 30-mS cm^{-1} treatment, application with Pentakeep-v (ALA-based functional fertilizer) did not affect Na^+ accumulation in the leaves. Potassium accumulation was significantly reduced in the presence of Pentakeep throughout the treatments, leading to a concomitant reduction in K^+/Na^+ ratios with the same exceptions (Table 1).

Chlorophyll a content was gradually reduced with salinity. Only at the highest salinity range applied was reduction in chl a significant (Table 2). Chl b, on the other hand, showed no significant change with salinity. Total chlorophyll followed a similar pattern to chl a (Table 2). Pentakeep treatments significantly improved chl a contents in all treatments, including the control. Despite the insignificant increase of chl b in leaves treated with Pentakeep at all salinities, total chlorophyll was significantly increased by Pentakeep treatments (Table 2). The chlorophyll a/b ratio of the control seedlings was typical of healthy C_3 plants but drastically reduced with increasing salinity. Pentakeep efficiently increased the ratio to values even above the control plants (Table 2).

The extent of membrane damage was indirectly assessed by solute leakage of salinity-treated seedlings in the presence or absence of Pentakeep. An almost twofold increase in electrolyte leakage was observed in plants treated with 30-mS cm^{-1} salinity compared with the control (4.45% and 2.78%, respectively). Pentakeep application did not show any significant reduction in the electrolyte leakage values (Table 2).

Mechanistic analysis of A/Ci response curves (Figure 1) focuses on (1) the initial linear slope below Ci concentrations of approximately 20 Pa, where ribulose bisphosphate (RuBP) is saturated and Rubisco activity limits carboxylation; and (2) the slower rise of the curve beyond its inflection point which represents the phase in which higher Ci levels result in the limiting factor being the supply of RuBP. Subsequently, the effect of salinity and Pentakeep on the biochemical factors controlling the photosynthetic gas exchange in date palm seedlings was measured by the velocity of carboxylation by Rubisco (Vc,max) and the

Table 2 Effect of salinity and Pentakeep-v applications on chlorophyll contents and electrolyte leakage in leaves of the date palm *Phoenix dactylifera* seedlings

Treatments	Chlorophyll concentration (mg/g fw)				Electrolyte leakage (% conductivity)
	Chl a	Chl b	Total	a/b ratio	
Salinity (S) mS cm^{-1}					
1	1.05 ± 0.03c	0.35 ± 0.00b	1.40 ± 0.03d	2.93 ± 0.03bc	2.78 ± 0.02d
15	0.93 ± 0.04c	0.41 ± 0.02b	1.34 ± 0.05d	2.30 ± 0.04cd	3.12 ± 0.11cd
30	0.67 ± 0.04d	0.40 ± 0.04b	1.07 ± 0.04e	1.73 ± 0.03d	4.45 ± 0.31ab
Pentakeep (P) 0.08%					
1 + P	1.95 ± 0.02a	0.55 ± 0.00a	2.50 ± 0.02a	3.57 ± 0.02b	2.91 ± 0.01d
15 + P	1.84 ± 0.02a	0.40 ± 0.03b	2.24 ± 0.05b	4.73 ± 0.03b	3.76 ± 0.20bc
30 + P	1.57 ± 0.01b	0.45 ± 0.01ab	2.02 ± 00c	3.50 ± 0.01a	4.51 ± 0.16a
Salinity (S)	***	NS	**	***	***
Pentakeep (P)	***	*	***	NS	NS
S × P	NS	NS	NS	NS	NS

Values are mean ± SE, n = 3. Means within each column followed by the same letter are not significantly different at level P = 0.05. *** and *, significant at level P = 0.001 and 0.1, respectively. NS = not significant at level P = 0.05

Fig. 1 $A{:}C_i$ response curves of date palm seedlings subject to salinity and Pentakeep-v treatments. Each curve is fitted into a nonrectangular hyperbola (**A**) without Pentakeep-v (●), r^2 = 0.999, 0.998, and 0.998, and (**B**) in the presence of Pentakeep-v (○), r^2= 0.994, 0.999, 0.999 for 1-, 15-, and 30-mS cm^{-1} salinity treatments, respectively. All regression coefficient values (r^2) are significant at $P < 0.05$. Each value is a mean ± SE, n =3
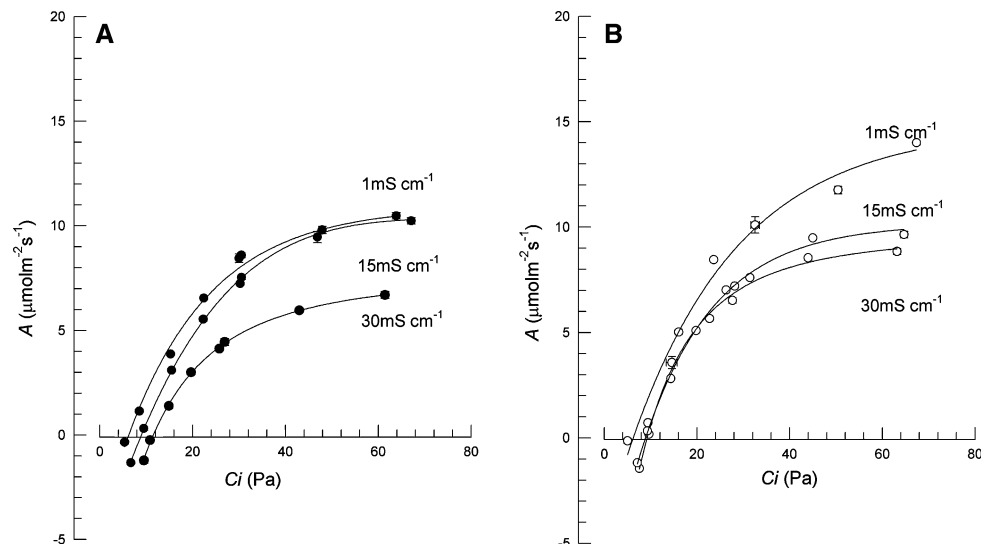
PAR-saturated rate of electron transport (Jmax) for RuBP regeneration represented in Figure 2.

Despite the significant reduction in the photosynthetic gas exchange as salinity increased (control: 8.59 ± 0.12 to 30 mS cm^{-1}, 5.67± 0.20 μmol m^{-2} s^{-1}, Figure 3A), the maximum Rubisco-catalyzed carboxylation rate (Vc,max) and the PAR-saturated rate of electron transport (Jmax) for RuBP regeneration (non-gas-phase limitation) were not significantly affected by the salinity range used in the present study (Figure 2A, B). Pentakeep, however, with the exception of the highest salinity level applied, enhanced the biochemical efficiency of carbon fixation (Vc,max) and the rate of electron transport required for RuBP regeneration (Jmax). For example, at a salinity of 15 mS cm^{-1}, Pentakeep increased Vc,max and Jmax by 37.4% and 17.8%, respectively, over the untreated plants. At 30 mS cm^{-1}, Jmax was improved by more than 20% over values for the untreated seedlings. Increasing salinity gradually increased CO_2 production by leaves through respiration (Figure 2C). Pentakeep inconsistently reduced respiration in the higher-salinity treatments but failed to do so at lower-salinity treatments.

Typically, stomatal conductance (g_s) decreased with increasing salinity leading to a concomitant reduction in gas exchange including photosynthesis (Figure 3A, B). Pentakeep, however, significantly increased stomatal conductance at both ends of treatment, namely, 1 and 30 mS cm^{-1}. The percentage of the contribution of stomatal factor (gas phase limitation) to the apparent reduction in the photosynthetic gas exchange (S_L) with increasing salinity is analyzed in Figure 3C. S_L increased with a maximum (25.02 ± 1.31%) for the 30-mS cm^{-1} treatment compared with the control (1 mS cm–1, 6.74 ± 0.29%). Pentakeep treatments significantly reduced S_L: 9.34 ± 0.35% and 18.79 ± 1.71% for 1- and 30-mS cm^{-1} treatments.

Interestingly, at moderate salinity (15 mS cm^{-1}) Pentakeep reduced stomatal limitations to photosynthesis to values similar to that of control plants (9.07 ± 0.76%, Figure 3B).

The control plants showed CO_2 compensation values typical of C_3 plants (60-80 μmol mol^{-1}) and typically decreased with increasing salinity (Figure 4). The slope (b) of the regression line of Pentakeep-treated seedlings was much lower than that of untreated seedlings (0.77 and 1.177, respectively).

Discussion

Potassium is an indispensable component for photosynthesis in higher plants, particularly in true halophytes. Maathuis and Amtmann (1999) showed that the capacity of plants to tolerate salinity depends strongly on the K$^+$ status in the leaf. In the present study, selective K$^+$ uptake was evident in leaves of the control plants, where the K$^+$/Na$^+$ ratio was very high (3.83). Hassan and El Samnoudi (1998) showed that selective uptake of potassium by date palm seedlings increased with increasing salinity within a moderate range. As salinity increased, K$^+$/Na$^+$ ratios dropped sharply (for example, 1.70 at 30 mS cm^{-1}) (Table 1). This sharp reduction is not attributed to the reduction in K$^+$ uptake but rather to the sharp increase in Na$^+$ uptake. Amtmann and Sanders (1999) showed that the inward K$^+$ channel is about 100-fold selective for K$^+$ over Na$^+$. As salinity increases to full seawater strength, Na$^+$ concentration is about 45 times that of K$^+$; subsequently, Na$^+$ will enter root cells in significant quantities via K$^+$ channels. Date palm seedlings accumulated a significant amount of Na$^+$ in the foliage with increasing salinity (about a threefold increase in the accumulated Na$^+$ between the control and 30 mS cm^{-1}). Many halophytes, however, use Na$^+$ as an
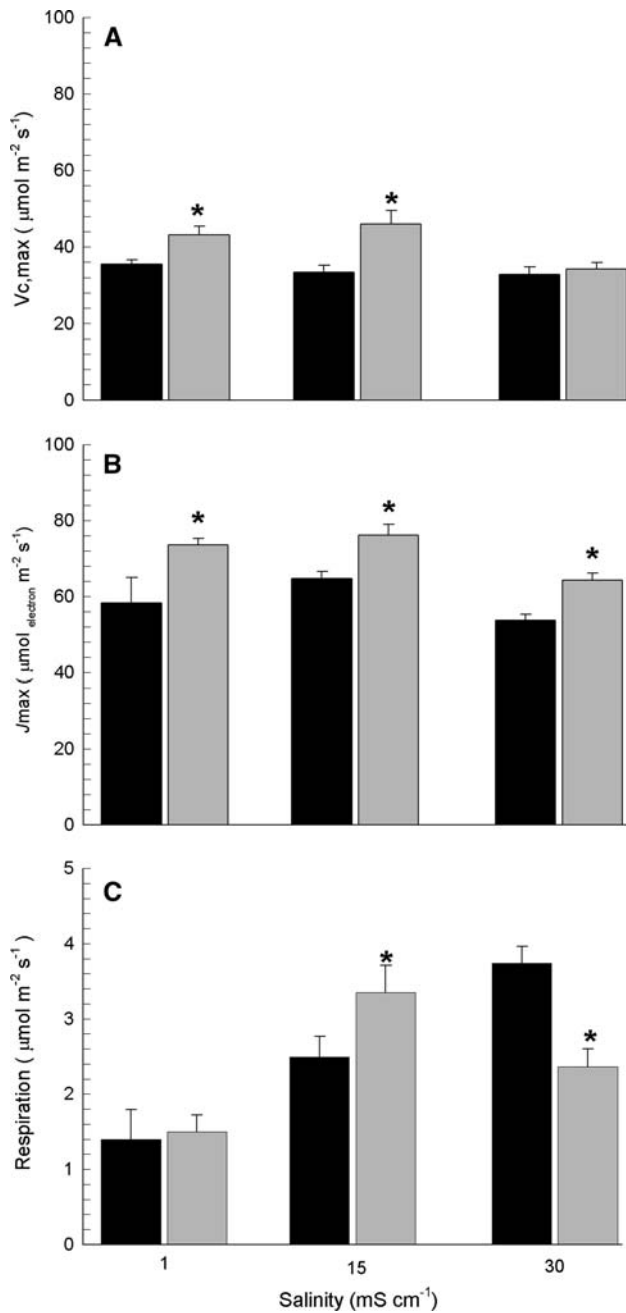
Fig. 2 Results of the mechanistic analysis of the A:Ci response curves of date palm seedlings under salinity and Pentakeep-v treatments. Effect of salinity on (**A**) $V_{c,max}$, (**B**) J_{max}, and (**C**) respiration rate in seedlings treated (gray bars) or untreated (black bars) with Pentakeep-v. Values are mean ± SE, $n = 3$. * indicates significant difference between treatments at $P < 0.05$

Fig. 3 Effect of salinity on (**A**) A, (**B**) g_s, and (**C**) S_L in date palm seedlings treated (○) or untreated (●) with Pentakeep-v. Values are mean ± SE, $n = 3$. Regression lines in **C** are both significant at $P < 0.05$, $r^2 = 0.75$ and 0.95 with slope (b) = 0.329 and 0.643 for Pentakeep-treated and untreated, respectively

energetically cheap osmoticum (Cram and others 2002; Raven 1997). They restrict its accumulation in the vacuole to maintain turgor pressure and to avoid its toxic effects on enzymes. It remains to be answered, however, as to whether date palms accumulate inorganic osmoregulatory moieties rather than compatible organic solutes and if this mechanism is energetically a cost-effect strategy for salt tolerance.
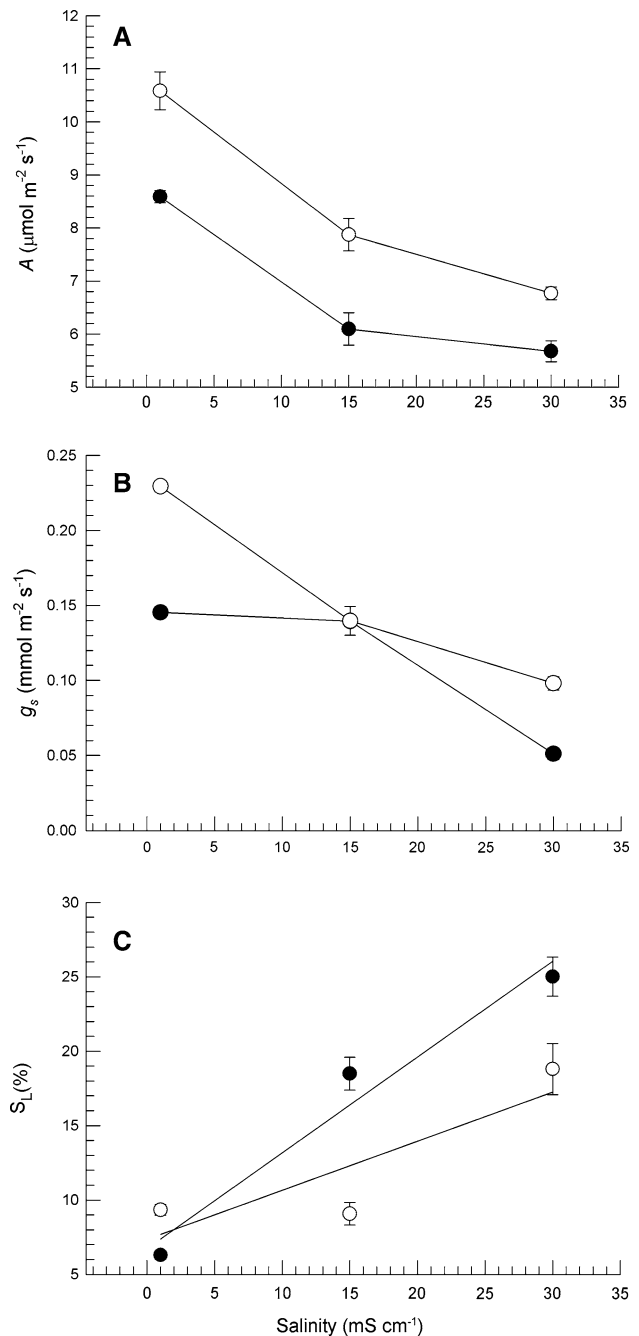
Contrary to the findings of Watanabe and others (2000) on cotton seedlings, date palm seedlings treated with Pentakeep showed a significant reduction in the selective K$^+$ uptake over Na$^+$ leading to a further reduction in the K$^+$/Na$^+$ ratio compared with the untreated plants. Except for the marginal reduction in Na$^+$ accumulation in leaves of plants treated with 30 mS cm^{-1}, there was no significant
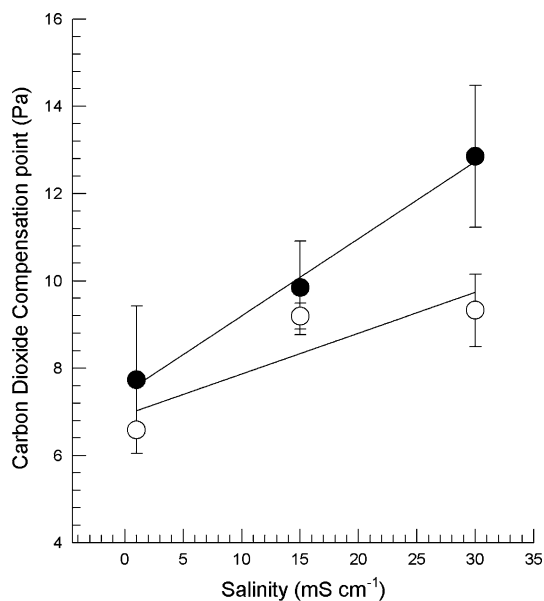
Fig. 4 Correlation between salinity and CO_2 compensation point (Γ) in date palm seedlings treated (○) or untreated (●) with Pentakeep-v. Values are mean ± SE, $n = 3$. Regression lines are both significant at $P < 0.05$, $r^2 = 0.77$ and 0.99 with slope (b) = 0.094 and 0.177 for Pentakeep-treated and untreated, respectively

Fig. 5 Correlation between chl a/b ratio and A in date palm seedlings treated (○) or untreated (●) with Pentakeep-v. Values are mean ± SE, $n = 3$. Regression lines are both significant at $P < 0.05$, $r^2 = 0.875$ and 0.885 with slope (b) = 2.45 and 1.77 for Pentakeep-treated and untreated, respectively

role for Pentakeep in reducing Na^+ accumulation in the other salinity treatments.

High salt concentration impairs cellular electron transport leading to the generation of reactive oxygen species (ROS) which triggers lipid peroxidation, protein degradation, DNA mutations, and membrane damage (Sreenivasulu and others 2000). The electrolyte leakage of date palm seedlings presented in this work is correlated with increasing salinity. An almost twofold increase in electrolyte leakage was observed in plants treated with 30-mS cm^{-1} salinity compared with the control (4.45% and 2.78%, respectively). These values are indications of the degree of impairment to membrane integrity. Contrary to the previous findings of Nishihara and others (2003) on spinach, application of 5-ALA-based fertilizer had no influence on electrolyte leakage in date palm seedlings under salinity. The exact responses to exogenous ALA application, however, vary among different plants, stage of plant development, timing, and applied concentration (Hotta and others 1997b; Nishihara and others 2003).

Assuming that the typical C$_3$ value of the control's chl a/b ratio (2.9) reflects the normal composition of the light-harvesting complexes (Table 2), the ratio's decrease with increasing salinity should reflect a reduction in light harvesting efficiency (Das and others 2002). Salinity was reported to induce a serious decline in chlorophyll content and photosystem II (PSII) activities in many plants (Nishihara and others 2003). The strong linear correlation between chl a/b ratio and assimilation rate supports this
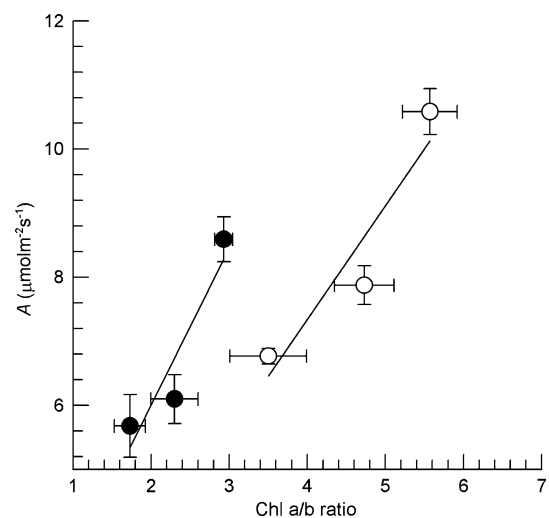
argument (Figure 5). The slope (b) and the correlation coefficient indicate that salinity reduced assimilation mainly via reduction in chlorophyll content (particularly chl a, Table 2) ($r^2 = 0.885$ and $b = 1.77$, $P < 0.05$, Figure 5). Plants treated with Pentakeep showed a similar response with increasing salinity but at higher levels of both Chl a/b and assimilation. The slower decline in assimilation shown in this case (b value twice as low) clearly indicates that Pentakeep may have compensated for chlorophyll damage induced by increasing salinity ($r^2 = 0.875$ and $b = 2.45$, $P < 0.95$). The effectiveness of Pentakeep in enhancing photosynthetic gas exchange can therefore be attributed to the significant improvement of chlorophyll a content and subsequently boosting light-harvesting capabilities of the treated plants.

The assimilation rate in *Phoenix dactylifera* seedlings (A) decreased with increasing salinity. Mechanistic analyses of A:Ci curves (Farquhar and others 1980; Harley and others 1992), as shown in Figures 1 and 2, showed that salinity did not induce any significant change in the catalytic carboxylation efficiency of the Rubisco enzyme or in the rate of electrons supplied by the electron transport system for RuBP regeneration (expressed as Vc,max and Jmax). The 2.5-fold increase in respiration of plants under high salinity compared with the control, however, correlates with the reported reduction in carbon assimilation (Figure 2C). Sobrado and Ball (1999) explained that reduced assimilation in seedlings of a true halophyte subject to saline conditions was due to the high CO_2 loss through photorespiration.

Except for the highest salinity level, seedlings treated with Pentakeep, showed an enhanced biochemical

efficiency of carbon fixation (Vc,max) and an improved rate of electron transport (Jmax) over the untreated seedlings. In Pentakeep-treated seedlings at 30 mS cm^{-1}, Jmax was improved by more than 20% over values for the untreated seedlings (Figure 2A, B).

The typical C$_3$ values for the CO$_2$ compensation point (Γ) recorded herein for the control plants increased with increasing substrate salinity (Figure 4). The slope (b) of the regression line of Pentakeep-treated seedlings was less steep than that of the untreated seedlings (0.77 vs. 1.177, respectively), indicating that in the presence of Pentakeep respiratory loss of CO$_2$, with increasing salinity, was generally much lower than that of the untreated plants. A steeper correlation, on the other hand, reflects the higher amount of CO$_2$ required to compensate for the accelerated respiratory carbon loss at the high-salinity level (30 mS cm^{-1}). Similarly, Hotta and others (1997a, b) reported a reduction in dark respiration and improved carbon fixation in radish seedlings treated with ALA at concentrations of 0.18 and 0.6 mM. Under saline conditions many plants, including halophytes, increase their rate of respiration in an effort to increase their own energy charge required to compensate for the expenditure of metabolic energy in compartmentation of organic and inorganic osmoregulators (Ramoliya and Pandey 2003). This may explain the significant decline in date palm growth rate as affected by salinity (Furr and Ream 1968; Hassan and Abou El-Azayem 1990; Ramoliya and Pandey 2003). A significant portion of the photosynthate that is normally directed toward growth is degraded by accelerated aerobic respiration for the sake of energy.

Using the supply function model of Jones (1992), A:Ci curves were examined to determine whether the decrease in A was mediated through conductance or mainly via the intrinsic photosynthetic capacity of the plant. Stomatal limitation to photosynthesis (S_L) appeared to increase with salinity, reaching a maximum of (22%) the overall limitations to gas exchange at the salinity level of 30 mS cm^{-1} (Figure 3C). Stomatal conductance (g_s) and assimilation rate (A) are in agreement with S_L values. It can be concluded, therefore, that the decrease in the photosynthetic gas exchange (A) reported herein is nonbiochemical and can be attributed mainly to stomatal components. Similar results were reported earlier on true halophytes (Ball and Farquhar 1984; Naidoo and others 1997). It is suggested that reduced stomatal conductance in response to Na$^+$ accumulation may provide a downregulatory mechanism controlling salt loading in plants with limited salt compartmenting capacities (Perera and others 1994; Robinson and others 1997).

Pentakeep, however, significantly enhanced biochemical efficiency of carbon fixation (Vc,max) and, more significantly, improved the rate of electron transport (Jmax) over

the untreated seedlings. This can be explained by the above-discussed role of Pentakeep in boosting light-harvesting capabilities of the treated plants by increasing chlorophyll a content.

Within the salinity range used in the present work, date palm seedlings showed halophytic responses in photosynthetic gas exchange characteristics. Reports by Hussain and others (1993) showed that growth of date palm seedlings was unaffected by salinities at or below 6000 μg g^{-1} (EC = 11.0 mS cm^{-1}), whereas salinities reaching 18,000 μg g^{-1} (EC = 29.5 mS cm^{-1}) showed no toxicity symptoms in the treated seedlings. On the other hand, Furr and Ream (1968) showed that growth of date palm seedlings treated with a salinity of 12,000 μg g^{-1} (EC = 20 mS cm^{-1}) was reduced by 60% of that of the control plants. We report herein that photosynthetic gas exchange in seedlings of the date palm was significantly reduced by salinity due to limitation in gas phase conductance. Salinity within the range applied in the present work did not significantly affect the carboxylation process or the electron supply for RuBP regeneration. Accelerated carbon loss through respiration further contributed to the reduction in assimilation and increased CO$_2$ compensation points at high salinity. The ALA-based functional fertilizer Pentakeep-v improved salt tolerance in seedlings of date palm via increasing photosynthetic assimilation. The latter is mediated by boosting the light-harvesting capacity of the treated plants by increasing chlorophyll a content and by reducing stomatal limitation to photosynthetic gas exchange. Our data are in agreement with the findings of Hotta and others (1997a, b) who suggested that growth and yield of several crops were improved by ALA application due to increased chlorophyll content and increased photosynthetic activity. They successfully linked the ALA mechanism of action to light and nutrient status around the plants.

As in many true halophytic plants, date palms appear to be well adapted to moderate salinity ranges. Xeromorphic leaf characteristics and small stomatal apertures may have contributed to the over all salt tolerance of date palm plants (Ramoliya and Pandey 2003). To the best of our knowledge, this work is the first to look at mechanisms and responses of the photosynthetic processes to salinity stress in date palms and also at the mechanisms involved in alleviation of salt stress in these seedlings through the application of ALA-based products. The present data as well as the available information, however, still fall short in explaining the exact mechanism of osmoregulation, water use strategies, and tolerance boundaries in date palms under an arid environment.

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