

Salicylic acid-mediated salt tolerance in date palms: comparative analysis of application methods

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Abstract

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In arid environments, tree growth and ecosystem function are limited by salinity stress. Herein, we describe the role of salicylic acid (SA) in ameliorating salt tolerance in date palms (*Phoenix dactylifera* L., cv. Sayer), a woody species characteristic of arid, salinized environments. We examined the role of salicylic acid (SA) delivered via trunk injection and foliar treatment in the tree's physiological acclimatization strategy. Trunk injection of SA at 200 ppm resulted in the highest plant height. SA treatments were associated with increased antioxidant activities (peroxidase and superoxide dismutase), decreased malondialdehyde, and fine-tuned hormone homeostasis (increased auxin and decreased abscisic acid). The trunk injection has much higher efficacy, providing important insights into the adaptation mechanisms of the tree and the efficacy of bioactive compound delivery from the injection site. Our results have implications for tree survival in increasingly salinized arid systems, and together, these findings represent progress toward a mechanistic understanding of ecophysiological pathways of adaptation to environmental stress in trees.

Keywords

antioxidant enzymes, foliar applications, ion homeostasis, *Phoenix dactylifera* L., salicylic acid, salinity stress tolerance, trunk injection

Introduction

Soil salinity limits tree growth by means of many interconnected ecophysiological mechanisms, including 1) osmotic stress resulting from decreased soil water potential, 2) ionic toxicity caused by the accumulation of sodium and chloride, 3) oxidative stress caused by the generation of reactive oxygen species (ROS), and 4) nutrient deficiency due to competitive ion inhibition (FU and YANG, 2023). Beyond these primary effects, chronic salt exposure imposes secondary damage that is particularly detrimental to long-lived woody species. Osmotic stress triggers a rapid reduction in stomatal conductance, leading to photosynthetic downregulation and carbon starvation, which directly impairs woody tissue development, lignin biosynthesis,

and seasonal cambial activity (HAN et al., 2022; LIU et al., 2024). Ionic toxicity, specifically the accumulation of Na⁺ and Cl⁻ in leaf apoplast and mesophyll cells, accelerates chlorophyll degradation, disrupts thylakoid membrane integrity, and induces premature leaf necrosis and abscission in citrus orchards under saline conditions (ALHARBI et al., 2022). Furthermore, ROS overproduction (e.g., H₂O₂, O₂^{•-}, OH[•]) not only causes lipid peroxidation, protein carbonylation, and DNA fragmentation but also triggers programmed cell death in root tips and leaf margins, thereby compromising root hydraulic conductivity and whole-plant water relations (HASANUZZAMAN et al., 2021). Nutrient deficiency under salinity is exacerbated in woody perennials due to their long-term site persistence: Na⁺ competitively inhibits K⁺, Ca²⁺, and Mg²⁺ uptake via ion channels, leading to

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impaired stomatal regulation (K^+ deficiency), weakened cell wall structure (Ca^{2+} deficiency), and reduced photosynthetic enzyme activity (Mg^{2+} deficiency). Collectively, these interconnected stresses result in growth retardation, crown dieback, reduced flowering and seed set, and ultimately increased mortality rates in natural and managed tree populations (LI et al., 2025).

Salicylic acid (SA), a naturally occurring phenolic compound, has been a beneficial growth regulator due to its ability to enhance plant resistance to a range of abiotic stresses, including salinity (KASHIF et al., 2024). Salicylic acid (SA), as an endogenous signaling molecule, is involved in crucial processes that modulate plant stress responses, acting as a signal activator of systemic acquired resistance (SAR) pathways, an effective regulator of antioxidant enzyme activity, and a regulator of cellular homeostasis under stress conditions (SONG et al., 2025). A recent study on salicylic acid demonstrated its ability to mitigate the effects of salt stress in salt-affected (*Zea mays* L., cv. Tall-09 maize) seedlings by adjusting osmotic pressure, alleviating oxidative stress, and restoring ionic balance (BARWAL et al., 2024). Nevertheless, such a mechanistic understanding of SA-mediated adaptation in woody species, with an emphasis on vascular physiology, long-distance signaling, and ecosystem-level consequences, remains scarce (FILGUEIRAS et al., 2019).

The mechanisms for SA delivery are critically dependent on tree vascular physiology for the effectiveness of SA application. The classical foliar application (spray solution onto leaf surfaces) depends on cuticular penetration via stomata and micropores, which is greatly limited by environmental factors (humidity, temperature, wind), has limitations due to cuticular barrier resistance, and is often affected by rapid loss in the external environment (SHAREEF et al., 2021b). Alternatively, trunk injection takes advantage of natural xylem flow driven by transpiration to deliver injected materials directly into the aerial vascular compartment, where they rapidly circulate systemically within the plant (leaves, stems, fruits) and are not subject to uptake barriers (ARCHER et al., 2024). Vascular-guided delivery is especially effective in date palms, which have anatomically unique, scattered vascular bundles along the trunk (unlike the radial vascular arrangement in most dicotyledonous trees). This architectural modification provides mechanical strength in woody tissue but affects compound distribution patterns (AL-MAYAHY et al., 2024).

As the potential of SA to augment stress tolerance is increasingly acknowledged, systematic comparisons of prospective trunk-injection delivery systems with SA application methods are scarce in the literature for woody species under prevailing natural salt-affected sites (ARCHER and ALBRECHT, 2023). Limited research has addressed the interactions between the following aspects, generating significant knowledge gaps in: (1) the relative physiological effectiveness of different SA application strategies in mature palms; (2) the mechanism (or mechanisms) through which salt tolerance is increased; (3) the relationship between application method and efficiency of vascular translocation; and (4) the functional significance

of physiological responses for tree performance and fitness in saline ecosystems.

Woody trees living in saline and drought-prone environments have evolved complex adaptive strategies, including morphological, physiological, and biochemical modifications, to maintain cellular homeostasis, support metabolic processes, and ensure their demographic continuity despite harsh environmental conditions (KUMAR et al., 2023).

Date palms (*Phoenix dactylifera* L.) are among the dominant woody species in the deserts of the Middle East and North Africa and represent vital ecological components for the stability of arid ecosystems and human food security (SWAID and SHAREEF, 2024). Date palms exhibit innate adaptations for salt tolerance compared to most other woody species, and the date palm is used as a model for woody species suitable for arid and saline environments (SHAREEF et al., 2021a).

Here, this study investigates the underlying ecophysiological mechanisms of tree adaptation to soil salinity through the SA module, which regulates ion homeostasis, elicits a tractable protective response, modulates hormonal pathways, and maintains photosynthetic function under environmental stress. This study establishes a mechanistic basis for improved stress tolerance by comparing delivery system efficiency (trunk injection vs. foliar application) and integrating physiological results. It pinpoints effective application pathways to promote *Phoenix dactylifera* L., used here as a model woody species for arid/salinized environments, to elucidate general ecophysiological mechanisms of stress adaptation and ecophysiological functions under salinity limitation.

Materials and methods

The field experiment was conducted in a commercial date palm (*Phoenix dactylifera* L., cv. Sayer) orchard in Basrah, Iraq (30.285504N, 47.694871E). It is a dry climate with an average annual temperature of 26.6 °C and average annual rainfall of 174 mm. The soil conditions were saline, with the mean electrical conductivity (EC) of 12 dS m⁻¹ in the experimental site. To achieve homogeneous growth, 30 Sayer date palm offshoots of the same age (5 years) and size were selected for the experiment. Clonal offshoots were planted at 8 m × 8 m spacing and maintained under normal cultural practices (irrigation, fertilization, and pest control) during the study period. Groundwater was used for irrigation, with an EC of around 4 dS m⁻¹. Salicylic acid treatments (spray and injection) were performed on 15 March (vegetative growth period). Water was used as the carrier for the salicylic acid solution, and injection holes (6 mm in diameter) were drilled into the trunk to sample xylem. As described by ARCHER et al. (2024), the space surrounding the injector was filled with silicon, and salicylic acid was injected at (0, 100, 200 ppm offshoot⁻¹) (volume of solution 100 mL). As part of foliar treatment with salicylic acid, three concentration levels (0, 100, 200 ppm offshoot⁻¹) (volume of solution 100 mL) were pre-

pared for salicylic acid spraying, which occurred in the morning and was mixed with 0.1% Tween 20 as a diffuser.

Experimental design

A completely randomized block design (RCBD) was adopted in this experiment with five replicates per treatment. Six treatments were tested, combining three salicylic acid concentrations (0, 100, and 200 ppm) with two application methods (trunk injection and foliar spraying), along with a control group (no SA application). Each replicate consisted of one tree per treatment, for a total of 30 trees. Samples were taken on 15 November. The control treatment was irrigated with tap water (EC 1.5 dS m⁻¹) to achieve non-saline conditions. The soil EC for the non-saline control was measured at the end of the experiment and was found to be 3.75 dS m⁻¹. The treatment groups were as follows: Control (no SA, no salinity), Control (no SA) under salinity, 100 ppm SA via foliar spraying under salinity, 100 ppm SA via trunk injection under salinity, 200 ppm SA via foliar spraying under salinity, and 200 ppm SA via trunk injection under salinity.

Growth parameters

A tape measure was used to measure plant height to the third completely extended leaf. The new leaf number assayed as [New leaf number = Total of leaves on 1 November 2024 – Total of leaves before treatment (14 March 2024)] was measured for all offshoots. Leaf area (m²) was measured using ImageJ software, following the methodology described by EASLON and BLOOM (2014). Ten leaves were selected for each iteration and scanned for measurement. The leaf area of the plant was measured using the formula: Leaf area = Average leaf area of ten leaves × The number of leaflets per plant.

Chlorophyll concentration

One hundred milligrams of fresh leaves were crushed in 10 ml (CH₃)₂CO acetone (80%) and centrifuged at 2,000 rpm for five minutes, as was done by LICHTENTHALER and WELLBURN (1983). Chlorophyll content was colorimetrically estimated at 663 and 645 nm. This can be expressed in the following equation: Total chlorophyll (mg g⁻¹) C_{a+b} = 1000A₄₇₀ – 3.27C_a – 104C_b / 229.

Relative Water Content (RWC)

Leaf samples were weighed as fresh weight (FW), turgid weight (TW) after immersion in distilled water for 24 hours, and dry weight (DW) after 48 hours at 70 °C. Relative Water Content (RWC) was determined as: RWC (%) = [(Fresh weight – Dry weight) / (Turgid weight – Dry weight)] × 100.

Proline content

Each leaf sample (0.5 g) was mixed with 5 mL of 3% sulfosalicylic acid. The mixture was fractionated, and 3 mL was combined with ninhydrin reagent (3 mL) and glacial

acetic acid (3 mL). The newly prepared mixture was heated in a boiling water bath for 1 h at 90 °C, then cooled rapidly to 25 °C, and the chromophore was formed by adding 4 mL of toluene to the cold solution. The absorbance was measured relative to the blank at 520 nm using a UV-Vis spectrophotometer. A proline solution (0.1 µg mL⁻¹) was used as the standard (BATES et al., 1973).

Malondialdehyde (MDA)

Lipid peroxidation was estimated by measuring malondialdehyde (MDA) content according to the method of HEATH and PACKER (1968). Fresh leaf tissue (0.3 g) was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). After centrifugation, 500 µL of the supernatant was mixed with 500 µL of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA. The mixture was boiled at 95 °C for 30 min, and the absorbance was read at 532 nm, with a correction for non-specific turbidity at 600 nm. The MDA content was calculated using an extinction coefficient of ε = 155 mM⁻¹ cm⁻¹.

Antioxidant enzyme activity

For enzyme extraction, 0.5 g of fresh leaf tissue was homogenized in 5 mL of ice-cold 50 mM phosphate buffer (pH 7.0) containing 1% polyvinylpyrrolidone (PVP) and 0.1 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 12,000 g for 20 min at 4 °C, and the resulting supernatant was stored at –20 °C for subsequent enzyme assays.

Peroxidase (POD) activity was determined by measuring the increase in absorbance at 470 nm over 3 min in a reaction mixture containing 2.9 mL of 50 mM phosphate buffer (pH 6.6), 0.1 mL of 1% guaiacol, 0.1 mL of 0.3% H₂O₂, and 0.1 mL of enzyme extract (ANGEFINI et al., 1990). Superoxide dismutase (SOD) activity was assayed in a reaction mixture comprising 1.5 mL of 50 mM phosphate buffer (pH 7.8), 0.3 mL of 130 mM methionine, 0.3 mL of 750 µM nitro blue tetrazolium (NBT), 0.3 mL of 100 µM EDTA-Na₂, 0.3 mL of 20 µM riboflavin, and 0.3 mL of enzyme extract. The reaction mixture was incubated under fluorescent light (30 µmol m⁻² s⁻¹) for 15 min, and absorbance was measured at 560 nm (BEAUCHAMP and FRIDOVICH, 1971).

Growth regulators' response (IAA and ABA)

The free and conjugated levels of indole-3-acetic acid (IAA) and abscisic acid (ABA) were extracted, purified, and quantified as described by RASTEGAR et al. (2011), with slight modifications. Spectroscopic methods were used to measure IAA and ABA content. For extraction, methanol:chloroform:2 N ammonium hydroxide (12:5:3, v/v/v), with a total volume of 60 mL, was used to extract 1 g of fresh weight per sample. Every 60 mL extract was frozen in a vial for later analysis. Then, 25 mL of distilled water was added to this extract, and subsequently, the chloroform phase was discarded. The water-methanol phase was

evaporated under reduced pressure at 40 °C using a rotary evaporator until approximately 15 mL remained. The pH of the aqueous phase was adjusted to either 2.5, 7, or 11 with 1 N HCl or 1 N NaOH, accordingly. During the three phases, 15 mL of ethyl acetate was added. This step enabled the separation of IAA and ABA, allowing independent measurement of each compound.

Specifically, at pH 2.5, both free IAA and free ABA partitioned into the ethyl acetate phase, while at pH 7, IAA remained in the aqueous phase as a carboxylate anion and ABA was partially extracted. At pH 11, only ABA (in its conjugated or free form) was recovered after re-extraction, effectively separating IAA and ABA into distinct fractions. After extraction and purification, the resulting ethyl acetate fractions were evaporated to dryness under nitrogen gas, and the residues were redissolved in 1 mL of 80% methanol.

For IAA quantification, 0.5 mL of the redissolved extract was mixed with 1 mL of Salkowski reagent (0.5 M Fe Cl₃ in 35% perchloric acid) and incubated at 25 °C in the dark for 30 min. The absorbance was measured at 535 nm using a UV-Vis spectrophotometer. For ABA quantification, an indirect enzyme-linked immunosorbent assay (ELISA) was performed using a monoclonal antibody specific for ABA, with detection at 405 nm after incubation with p-nitrophenyl phosphate. Quantification was based on calibration with appropriate standards (authentic IAA and ABA, 0.1–50 µg mL⁻¹), and results were reported as IAA and ABA concentrations per sample (µg g⁻¹ fresh weight).

Leaves Na, K, and K/Na ratio

Potassium was determined by wet digestion with H₂SO₄/H₂O₂, followed by boiling to dehydrate silica, filtration to yield a clear solution, and flame emission measurement at 766.5 nm (CRESSER and PARSONS, 1979). Sodium was determined by dry ashing at 500 °C for 4–6 h, dissolution of the ash in 2 M HCl, and flame photometry at 589.0 nm (TABATABAI, 1998). Calibration standards for both elements were prepared in the respective digestion matrices and contained 1,000 mg L⁻¹ Cesium (Cs) as an ionization suppressant. Reagent blanks were processed identically, and all concentrations were calculated on a dry weight basis.

Statistical analysis

The collected data were analyzed using ANOVA to determine the effects of salicylic acid concentration, application method, and their interaction on each measured parameter. Duncan's multiple range test ($p \leq 0.05$) was used to identify significant differences among treatments. Data were analyzed using SPSS software (version 26).

Results

Growth parameters and morphological responses under salinity stress

A significant variation in the morphology of Sayer date palm offshoots was observed between salinity treatments and SA application methods (Table 1). Plant height was highest in the control group (no salinity, no SA). Under saline conditions, all growth parameters were significantly reduced compared to the non-saline control, with the highest values for plant height, number of new leaves, and leaf area recorded in the Control (No SA, no salinity) treatment (138.4 cm, 5.4 leaves, 1.10 m²) and the lowest in the Control (No SA) + salinity treatment (108.4 cm, 2.4 leaves, 0.74 m²). Among the salicylic acid treatments applied under salinity, injection SA 200 ppm + salinity yielded the superior coefficients across all traits, achieving the greatest increases over the saline control: plant height (131.4 cm vs. 108.4 cm, a 21.2% increase), number of new leaves (4.4 vs. 2.4, an 83.3% increase), and leaf area (0.93 m² vs. 0.74 m², a 25.7% increase); this treatment was statistically comparable or significantly better than other SA applications. The next most effective treatments were foliar SA 200 ppm + salinity (128.6 cm, 4.0 leaves, 0.91 m²) and injection SA 100 ppm + salinity (126.8 cm, 3.4 leaves, 0.87 m²), while foliar SA 100 ppm + salinity gave the lowest SA-mediated improvements (113.8 cm, 3.4 leaves, 0.82 m²). All SA treatments significantly outperformed the saline control ($p \leq 0.05$), except for foliar 100 ppm in leaf area (which was still significantly higher than the control), confirming that 200 ppm SA applied by injection is the most effective method for mitigating salinity stress.

Table 1. Mean comparisons of date palm Height plant, Number of new leaf, and Leaf area in response to salicylic acid under saline conditions

Treatments	Height plant (cm)	Number of new leaves	Leaf area (m ²)
Control (No SA No salinity)	138.4 ± 1.14a	5.4 ± 0.54a	1.10 ± 0.03a
Control (No SA) + salinity	108.4 ± 3.04e	2.4 ± 0.54d	0.74 ± 0.01e
Foliar SA 100 ppm + salinity	113.8 ± 1.78d	3.4 ± 0.89c	0.82 ± 0.01d
Injection SA 100 ppm + salinity	126.8 ± 2.58c	0.4 ± 0.89c	0.87 ± 0.01c
Foliar SA 200 ppm + salinity	128.6 ± 1.14c	4.0 ± 0.70bc	0.91 ± 0.01b
Injection SA 200 ppm + salinity	131.4 ± 0.89b	4.4 ± 0.54b	0.93 ± 0.02b

Means of 5 replications ± SD. Using Duncan's multiple-range test, means with different letters differ at $p \leq 0.05$.

Physiological stress indicators and photosynthetic parameters

Salinity and SA treatments significantly influenced the physiological responses, such as chlorophyll content, relative water content (RWC), and proline content (Fig. 1). Under saline stress, the chlorophyll content reduced significantly in the control group. Chlorophyll content was also enhanced by SA treatments at 100 ppm and 200 ppm, primarily through trunk injection. Again, the 200 ppm concentration showed the greatest increase, indicating higher salinity tolerance in photosynthesis (Fig. 1a). The high-salinity control had a considerably lower RWC (Fig. 1b). SA treatments (especially 200 ppm) enhanced RWC, indicating a greater ability to avoid water loss under stress. In control conditions, proline was significantly increased under salinity stress. On the other hand, SA treatments, especially at 200 ppm, further increased proline content, suggesting that SA enhances the plant's resistance to osmotic stress in saline environments (Fig. 1c).

Oxidative stress markers and antioxidant enzyme activity

Oxidative damage in the plants was measured as lipid peroxidation and the activities of POD and SOD (Fig. 2).

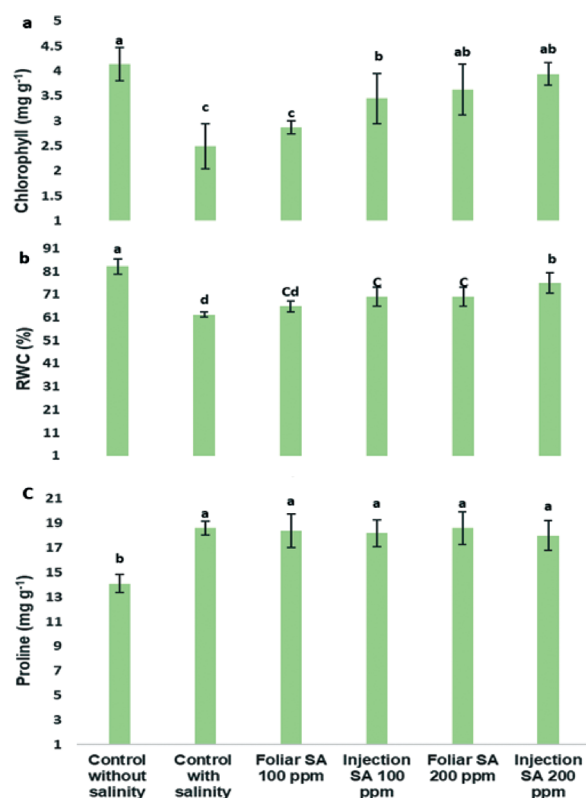


Fig. 1. Change of (a) chlorophyll content, (b) Relative water content (RWC), and (c) Proline in response to salicylic acid in Sayer date palm leaves under saline conditions. The means of five replicates $\pm$ SD. Bars with different letters are different at $p \leq 0.05$ using Duncan's multiple range test.

The saline control group had significantly higher MDA levels. MDA levels decreased compared to the controls, foliar-sprayed (100 ppm and 200 ppm), and trunk-injected (100 ppm and 200 ppm), with the most notable decrease observed with trunk injection at 200 ppm (approximately 37.3%) (Fig. 2a).

The activities of POD and SOD were significantly increased in the SA-treated groups compared to the control under saline conditions (Fig. 2b–c). POD activity in the salinity treatment is lower than in the control without salinity. Trunk injection at 100 and 200 ppm SA resulted

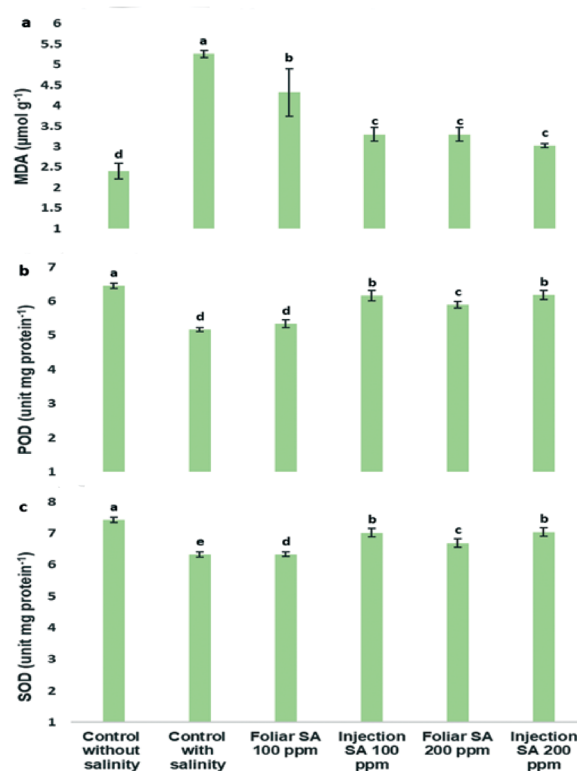


Fig. 2. Change of (a) malondialdehyde (MDA), (b) the activity of peroxidase (POD), and (c) superoxide dismutase (SOD) in response to salicylic acid in Sayer date palm leaves under saline conditions. The means of five replicates $\pm$ SD. Bars with different letters are different at $p \leq 0.05$ using Duncan's multiple range test.

in higher activity than the salinity treatment. The highest POD activity was in the control without salinity. SOD activity showed similar enhancement patterns following trunk injection. SA-treated plants showed 12% higher SOD activity than saline controls, indicating greater enzyme activation than with foliar applications.

Hormonal responses

To analyze how SA affects the levels of different phyto-hormones in plants, we performed hormonal analysis by treating with IAA and ABA, both of which are important stress-response hormones (Fig. 3). The IAA concentration was lower in the salinity treatment compared to the control without salinity. However, treatment with SA, especially trunk injection at 200 ppm, showed the steepest increase

in IAA. Under salinity stress, ABA concentration was significantly elevated in the control group. ABA levels were markedly reduced by SA treatments, especially at the high concentration (200 ppm), particularly in the trunk injection group.

Ion homeostasis and osmotic regulation

Salinity had a pronounced effect on ion content, especially sodium (Na^+) and potassium (K^+) (Table 2). The K^+/Na^+

taining elevated K levels. This led to an increased K^+/Na^+ ratio, close to the ideal ratio found in non-stressed plants. Foliar applications resulted in moderate enhancements, with significant reductions in sodium (moderate shoot salt concentrations at 200 ppm) whilst maintaining acceptable K^+/Na^+ ratios.

Discussion

As an example of how knowledge of such mechanisms will help predict how woody species will sustain physiological function and demographic persistence in environments increasingly exposed to salinity, this work identified core ecophysiological mechanisms underlying tree adaptation to salinity. It demonstrated that salicylic acid coordinates multiple, opposing interactions among linked stress tolerance pathways. Soil salinity represents a combined osmotic and ionic stress that reduces plant-available water and disrupts ion homeostasis, imposing cascading constraints on growth, development, and reproduction (Fu and YANG, 2023).

From an ecophysiological perspective, growth reduction under salinity is adaptive in two main ways: metabolically expensive processes, such as cell expansion and biomass accumulation, are downregulated in favor of survival-related processes, such as ion homeostasis, detoxification, and antioxidant defense (SHAREEF et al., 2021a). This downregulation represents a threshold response: once water and ion gradients move beyond a given physiological range relative to the cytosol, the plant must divert ATP and reducing power from growth to preserve the proton motive force and protect enzyme function. The 21.7 % decline in height under salt stress thus reflects that growth–stress trade-off, whereas the restoration of plant height elongation under salt stress after SA trunk injection indicates that SA rebalances this trade-off. The superior performance of trunk-injected SA suggests efficient systemic translocation through the xylem, consistent with rapid vascular distribution of injected compounds in woody species (VÄISÄNEN et al., 2020; KOPECKÁ and ČERNÝ, 2024). However, the precise mechanism underlying SA-mediated gene regulation and ion transport remains to be fully elucidated. The enhancement of new leaf production under saline stress, coupled with the recovery of IAA levels, demonstrates that meristematic activity and organ initiation are

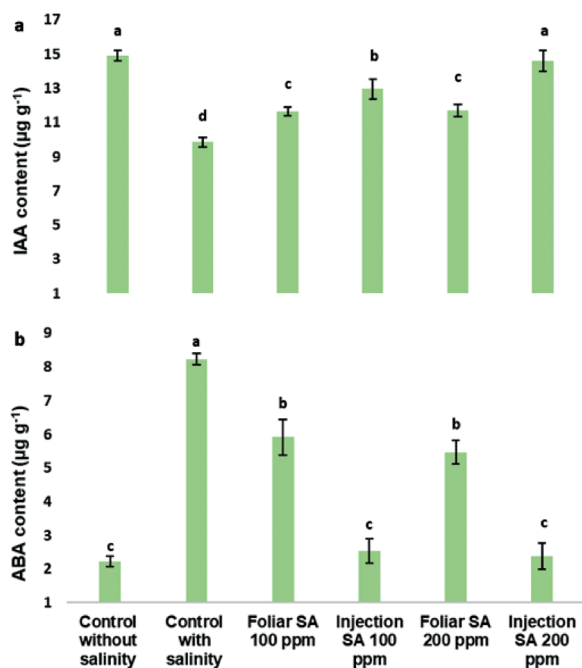


Fig. 3. Changes of (a) indole-3-acetic acid (IAA) content and (b) abscisic acid (ABA) in response to salicylic acid in Sayer date palm leaves under saline conditions. The means of five replicates $\pm$ SD. Bars with different letters are different at $p \leq 0.05$ using Duncan's multiple range test.

ratio increased in SA-treated samples compared with the salinity treatment. The SA-treated plants, particularly at 200 ppm, showed reduced Na^+ accumulation and a subsequent increase in K^+ , resulting in a favorable K^+/Na^+ ratio. Both trunk injection treatments corrected the ion imbalance, with the 200 ppm treatment reducing Na while main-

Table 2. Mean comparisons of date palm leaves in mineral concentrations in response to salicylic acid under saline conditions

Treatments	Na (mg g^{-1})	K (mg g^{-1})	K/Na ratio
Control (No SA, No salinity)	4.38 $\pm$ 0.29d	16.02 $\pm$ 0.37a	3.67 $\pm$ 0.28a
Control (No SA) + salinity	11.01 $\pm$ 0.23a	12.87 $\pm$ 0.10c	1.16 $\pm$ 0.03e
Foliar SA 100 ppm + salinity	7.09 $\pm$ 1.15b	12.18 $\pm$ 0.19d	1.75 $\pm$ 0.29d
Injection SA 100 ppm + salinity	5.96 $\pm$ 0.63c	12.29 $\pm$ 0.07d	2.08 $\pm$ 0.24c
Foliar SA 200 ppm + salinity	5.96 $\pm$ 0.63c	12.31 $\pm$ 0.07d	2.08 $\pm$ 0.24c
Injection SA 200 ppm + salinity	5.46 $\pm$ 0.27c	14.22 $\pm$ 0.71b	2.61 $\pm$ 0.20b

Means of 5 replications $\pm$ SD. Using Duncan's multiple-range test, means with different letters differ at $p \leq 0.05$.

resumed, indicating a restoration of hormonal and ionic status at the shoot apex to the narrow range required to sustain division under chronic stress. The responses were highly dependent on the application method. Trunk injection was 5–10% higher than foliar spray. This distinction emanates from both technical and physiological aspects (SHAREEF, 2025). Foliar sprays must cross the cuticle – a diffusion barrier, along which a large proportion of SA is lost through photodegradation and wash-off – so that only small amounts reach the vascular stream and distal target tissues. Given that xylem flow is driven by water potential gradients, injected SA could be transported passively and efficiently through the tree's hydraulic network, so its distribution should closely reflect the plant's water fluxes. In date palm, due to the distribution of vascular bundles within the trunk, multiple entry points are established when injecting 200 ppm of SA, reducing localized unevenness and promoting system-wide physiological responses with relatively small injected volumes.

In saline conditions, chlorophyll loss is due to Na-induced chlorophyllase activation, disruption of the Mg^{2+} cofactor, ROS-mediated damage to thylakoid membranes, and altered expression of photosynthetic genes (HAMEED et al., 2021). These are all symptoms of divergent ionic and redox homeostasis when Na^+ rises, or ROS is, or becomes, beyond the buffering capacity of chloroplasts to push it into signaling damage. The recovery in chlorophyll content suggests that SA treatment re-establishes a compatible internal milieu, thereby restricting these injury pathways. Concomitantly, proline accumulation under SA treatment is associated with osmotic adjustment and the prevention of ROS (OUYANG et al., 2025). By stabilizing osmotic potential and turgor of mesophyll cells, proline helps maintain stoma opening and water flux, which are required for efficient CO_2 uptake and xylem transport, thereby helping restore photosynthetic functioning and carbon gain. Significant increases in antioxidant enzyme (SOD and POD) activities also attest to SA-induced ROS-scavenging systems that are important for conferring tolerance to environmental stresses. Salinity promotes ROS generation by disrupting photosynthetic and respiratory electron transport, stimulating membrane-bound NADPH oxidases, and recycling polyamines and phenolics (RAO et al., 2025).

At moderate concentrations, these ROS pulses are indispensable signals, but at concentrations above micromolar levels, they damage lipids, proteins, and nucleic acids. SA-treated plants showed 12% higher SOD activity than saline controls, increasing the capacity to convert superoxide ($O_2^{\cdot -}$) into H_2O_2 , while enhanced POD activity removes H_2O_2 by converting it to water (OSEI et al., 2022). This coordinated upregulation keeps ROS within a signaling, non-damaging range. In tobacco seedlings, SA treatment has been shown to upregulate genes involved in the ascorbate–glutathione (AsA–GSH) cycle, including monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), and dehydroascorbate reductase (DHAR) (SONG et al., 2025), suggesting that similar mechanisms may contribute to the enhanced antioxidant capacity observed in our date palm study. These enzymes form a regenerative

antioxidant buffer that can be sustained over long periods of stress without exhausting antioxidant pools, thereby protecting transport proteins, H^+ -ATPases, and other membrane-bound components essential for ion homeostasis and vascular function (ZHENG et al., 2023).

Consequently, MDA content – a marker of lipid peroxidation – declined approximately 37.3% in SA-treated plants relative to saline controls, returning to near-control without salinity values (irrigated with tap water). This is consistent with the hypothesis that SA significantly reduces oxidative membrane damage. ISLAM et al. (2023) similarly reported that treating the plant with salicylic acid reduces lipid oxidation and lowers MDA levels. From an ecophysiological perspective, intact membranes underpin selective nutrient uptake, exclusion of toxic ions, electrochemical gradients that drive transport, and intracellular compartmentalization (LU et al., 2023). For date palms, maintaining root membrane integrity is particularly important for restricting Na^+ influx and sustaining favorable K^+/Na^+ ratios (SABEEM et al., 2022). When membranes remain intact and properly polarized, H^+ -ATPases and ion channels operate near their optimal electrochemical range, allowing roots and leaves to maintain steep ion gradients and functional xylem–phloem exchange even under saline conditions (LI and YANG, 2023).

Hormonal balance is also reshaped by SA. SA treatment restored IAA to near-control levels, while moderating ABA accumulation. IAA is the principal auxin controlling cell growth, meristematic function, and apical dominance, and it is typically reduced under salinity (MUSAZADE et al., 2025). The recovery of IAA levels in SA-treated plants may reflect improved tryptophan availability, suppressing IAA-oxidizing enzymes, and restoring polar auxin transport (SWAID and SHAREEF, 2025). This is consistent with the general role of SA in modulating auxin homeostasis under stress conditions (MUSAZADE et al., 2025). SA-induced moderation of ABA prevents excessive growth inhibition and senescence while preserving essential stress signalling (PANOZZO et al., 2025).

This adjustment of the ABA–IAA balance represents a shift from an acute (emergency) response (high ABA, strong growth arrest) to a chronic tolerance mode where defense and growth run concurrently within a safer physiological range.

In all responses, there is a single central mechanism: ion homeostasis. In the absence of treatment, leaf Na^+ rose from 4.38 to 11.01 $mg\ g^{-1}$ (151%), levels already toxic, inhibiting enzyme activity and cell function. Leaf Na^+ in SA-treated plants decreased to 5.46 $mg\ g^{-1}$, which is approximately a 50% reduction compared with the saline control plants and not significantly different from the non-stressed control plants (irrigated with tap water). Such an enhancement may involve the SA-mediated increase in plasma membrane H^+ -ATPase activity of roots, Na^+/H^+ antiport (NHX) activity in vacuoles, K-selective uptake, and selective xylem loading with K^+ but not Na^+ (ASSAHA et al., 2017; LIU et al., 2019). Similar mechanisms have been reported in other woody species (MÜLLER and SCHEURING, 2025; YUAN et al., 2026), suggesting that they may

modulate conserved ion transport pathways across diverse plant tissues. This functionally restores cytosolic K^+/Na^+ ratios to levels where enzymes and membrane polarization remain largely unaffected, but permits the accumulation of Na^+ in vacuoles as a compatible solute that interacts with water uptake and turgor. This ecophysiological approach offers implications for predictions of tree's persistence in salt-affected habitats, for exploring how community composition and ecosystem processes are likely to change under increasing soil salinization (HUSSAIN et al., 2021), where individuals that can maintain effective vascular transport, controlled ROS signalling, and high K^+/Na^+ ratios will have disproportionately greater contributions to stand-level carbon gain and recruitment.

Conclusions

Salicylic acid, especially when applied by trunk injection at 200 ppm, substantially improved the growth and physiological performance of Sayer date palm offshoots under high salinity. Treated palms showed higher plant height, more new leaves, greater leaf area, and improved chlorophyll content and relative water content compared with untreated saline controls, indicating that SA helps maintain growth under salt stress. These improvements were linked to stronger cellular protection and better stress regulation. Trunk-injected SA reduced lipid peroxidation and enhanced antioxidant enzymes (SOD, POD) while promoting proline accumulation. Together, these changes limited oxidative damage, supported osmotic adjustment, and helped sustain photosynthetic function under saline conditions. SA also rebalanced hormonal status and ion homeostasis, restoring IAA toward control levels, moderating ABA, lowering leaf Na^+ , and improving the K^+/Na^+ ratio. Trunk injection was consistently more effective than foliar spraying, reflecting more direct and efficient delivery through the tissue of the plant.

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