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Response of date palm offshoots (*Phoenix dactylifera* L.) to the foliar spray of salicylic acid and citric acid under salinity conditions

Hussein J. Shareef[1*], Muayed F. Abbas[2], Abbas M. Jasim[3]

[1]Date Palm Research Centre, University of Basrah, Basrah, Iraq
[2,3]Department of Horticulture and Landscape Design, College of Agriculture, University of Basrah, Basrah, Iraq

Abstract

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Antioxidants enhance the salt tolerance of date palms. A field experiment was carried out on date palm offshoots to improve the salt tolerance of the Sayer cultivar. Salicylic acid and citric acid (500 and 1,000 ppm) were used. The results showed that all growth parameters of plant height, leaf area, and leaf numbers decreased under the salinity conditions. The antioxidant applications increased the plant height, leaf area, carbohydrates, and relative water content compared with the control. Citric acid at 1,000 ppm decreased electrolyte leakage and malondialdehyde. Indoleacetic acid decreased, whereas abscisic acid increased under salinity. The antioxidant application increased indoleacetic, whereas abscisic acid decreased. Proline, protein content, and peroxidase activity increased under antioxidants. Also, the potassium and K/Na ratio increased under antioxidant applications. Citric acid improved the characteristics more than salicylic acid, encouraging farmers to use it for its low cost as an antioxidant to reduce environmental stress damage.

Keywords

antioxidants, citric acid, electrolyte leakage, *Phoenix dactylifera*, salicylic acid

Introduction

Salt stress is exceptionally harmful among all abiotic stresses (drought, salinity, heavy metals, nourishment insufficiency, imbalance of nutrition) that unfavorably influence numerous physiological cycles in plants (Elsheery et al., 2020a). In addition, salt stress incites the amassing of abscisic acid (ABA), which causes enormous decreases in the stomatal opening, stomatal conductance, sub-stomatal CO_2 content RuBisCO and thwarts the exercises of numerous different catalysts (Hala et al., 2020). Salinity prompts metabolic problems, hinders development, and causes negative impacts on plant efficiency (Elsheery et al., 2020b). Further development, advancement, endurance, and the usefulness of date palms are impacted by components that cause oxidative harm created from responsive oxygen species (ROS) (Naser et al., 2016).

The date palm (*Phoenix dactylifera* L.) is a fruit crop capable of withstanding higher temperatures, drought, and salinity than many other crop fruit tree species (Johnson, 2011; Jasim et al., 2016). In date palm plantations areas, such as the Middle East and North Africa, the palms are exposed to soil and water salinity (Khierallah et al., 2015). Enhancing plant salt tolerance is crucial for culturing plants in saline soils, and there are three methods employed: conventional breeding, genetic engineering, and eco-physiological methods. More attention should be paid to eco-physiological ways to enhance plant salt tolerance because it is easy to implement in agricultural practices, ecological restoration, and low cost (Yan et al., 2013). In recent decades exogenous protectants such as plant hormones (salicylic acid) and antioxidants (citric acid) (Gunes et al., 2007; Ibrahim et al., 2013) were used to enhance the plant growth and yield as well as increase stress tolerance under salinity.

Salicylic acid (SA) is an endogenous development controller of phenolic nature, which is involved in physiological cycles, for example, development, photosynthesis,

*Corresponding author:
e-mail: hussein.shareef@uobasrah.edu.iq

nitrate digestion, ethylene creation, and blooming (Hayat et al., 2010; Helaly et al., 2018). SA protects against biotic and abiotic stresses such as salinity and drought stress (Javid et al., 2011; Helaly et al., 2018). The effects of SA on plant tolerance to abiotic stress contradict each other. The same pretreatment with exogenous SA results in opposite responses in different plant species (Yang et al., 2004). SA can exert different effects under various stress situations or with other species is not in contradiction but rather illustrates that different stresses can either be dependent or independent of a SA pathway. The molecule does not have the same effect on different species (Noreen et al., 2009). Thus, protective SA action includes developing anti-stress programs and accelerating growth processes after removing stress factors (Aldesuquy et al., 2013). Many observations suggest that SA being an oxidant, could be linked to oxidative stress (Syeed et al., 2011). Awad et al. (2006) applied foliar salicylic acid (100 and 200 ppm) on tissue culture-derived cv. Khalas date palm plantlets during acclimatization and irrigation with 10,000 ppm seawater for two months. The results showed that the application of salicylic acid increased growth and decreased the effect of salinity.

Citric acid (CA) is a fundamental substrate in the Krebs cycle. The Krebs cycle gives precursors, including explicit amino acids and NADH reducing agents utilized in various biochemical responses (Moradi et al., 2015). CA assumes a fundamental function in invigorating biosynthesis processes and is viewed as a non-enzymatic antioxidant that destroys free radicals created in plants under stress (Faraz et al., 2020). CA produces chemicals when it includes minor amounts to plants, responds quickly with radical intermediates of an auto-oxidation chain, and stops it from progressing (Ghazijahani et al., 2014). Ibrahim et al. (2013) investigated citric acid spraying at 1,000 and 2,000 ppm on cv. Zaghloul date palm growth irrigated with saline water. They found that citric acid significantly increased growth characters in the leaf area relative to the control treatment (untreated palm). Antioxidants could be a potential development controller to improve salt stress tolerance in several plant species (Mohamed et al., 2021). Therefore, this study aimed to determine the response of offshoots of cultivar Sayer to salicylic acid and citric acid under the salinity effect.

Materials and methods

Experiment site

A field experiment was performed at a private date plantation in the Alhartha region, Basrah, Iraq, at Hartha region-Basrah, Iraq (30.654309, 47.753210), on 24 plants, 3–4 years-old cv. Sayer date palm offshoots are affected by salinity stress. The soil is silty clay loam. Offshoots are planted at a spacing of 5 × 5 m. A drip irrigation system was used. Soil electrical conductivity was 15.9 dS m^{-1}, and water soil electrical conductivity was 4.55 dS m^{-1}. The foliar applications were carried out on 1 March. The treatments were replicated four times (one plant for each replicate). The treatments included the: control (spray with distal water), foliar spray salicylic acid at 500 ppm, foliar spray salicylic acid at 1,000 ppm, foliar spray citric acid at 500 ppm, and foliar spray citric acid at 1,000 ppm. After 165 days of treatments, the following data were recorded:

Growth parameters

Plant height was determined by tape measure to the third completely extended leaf. New leaf number of offshoots was measured by applying the equation: New leaf numbers = Total of leaves on 1 November – Total of leaves before treatment on 1 March. Leaf area (m^2) was determined, according to Ahmed and Morsy (1999), at the middle part of each leaf for four leaflets taken according to the following equation: Leaf area (m^2) = (0.37 (length × width) + 10.29 × number of pinnae) / 1,000.

Total chlorophyll content

The chlorophyll extraction was completed according to Lichtenthaler et al. (1983). The new leaf tissue was gathered and frozen, then the leaves (0.25 g) were homogenized with 80% $(CH_3)_2CO$. The removed chlorophyll optical thickness (OD) was estimated at 645 and 663 nm utilizing a spectrophotometer PD-303. The accompanying formulae determined absolute chlorophyll content: total chlorophyll (mg g^{-1}) = 20.2 (OD 645) + 8.02 (OD 663).

The relative water content (RWC)

Fresh leaf was checked (new weight) immediately after a gathering and soaked in purified water at 25 °C for 24 h (Jasim et al., 2016) to determine the excessive weight; the models were dried in an oven at 80 °C for 48 h. RWC = (new weight-dry weight) / (bloated weight-dry weight) × 100.

Electrolyte leakage (EL)

Electrolyte leakage is used to assess membrane permeability, according to Omar et al., 2012. Leaves were washed multiple times with double-distilled water to clear surface contamination. The leaf segments were set in fixed vials containing 10 mL of double-distilled water after hatching a turning shaker for 24 h. The electrical conductivity of the arrangement (EC1) was recorded. The examples were autoclaved at 120 °C for 20 min, and the electrical conductivity was estimated once more (EC2) after the arrangement cooled to room temperature. The electrolyte spillage was portrayed as EC1 / EC2 × 100 and imparted as a rate.

Lipid peroxidation

Lipid peroxidation was determined by estimating the measure of malondialdehyde (MDA) development utilizing the thiobarbituric acid strategy depicted by Stewart and Bewley (1980). The rough concentrate readiness was blended with a similar volume of a 0.5% (w/v) thiobarbituric acid arrangement containing 20% (w/v) trichloroacetic acid. The combination was heated to 95 °C for 30 min, and the response was halted by rapid placement in an ice bath. The cooled combination was centrifuged at 10,000 × g for 10 min, and the absorbance of the supernatant at 532 and 600 nm was perused. After removing the vague absorbance at 600 nm, the MDA focus was determined by its annihilation coefficient of 155 mM^{-1} cm^{-1}.

Soluble protein content

A quantity of 200 mg of fresh leaf tissue was homogenized in 5 mL of potassium phosphate buffer (pH 7). The homogenate was centrifuged at 17,000 × g for 20 min. The supernatant was carefully transferred into test tubes. According to Bradford

et al. (1976), soluble protein extract was estimated, and Bovine serum albumin (BSA) was used as a standard.

Proline concentration

The leaf (0.5 g dry mass) was homogenized with 5 mL of 95% ethanol. The upper layer of the filtrate separated, and dregs were washed by 5 mL of 70% ethanol multiple times, and the upper layer was added to the previous over the compartment. The blend was centrifuged at 6,000 rpm for 10 min at 4 °C, and the supernatant recuperated, and the alcoholic concentrate was kept under refrigeration at 4 °C (Paquin and Lechasseur, 1979). One mL of alcoholic concentrate was added to 10 mL of distilled water and 5 mL of ninhydrin (0.125 g ninhydrin, 2 mL of 6 mM NH_3PO_4, 3 mL of glacial acetic acid); at that point, the blend was set in a water bath for 45 min at 100 °C. The response was halted by putting the test tubes in cold water. The examples were blended in 10 mL benzene. The light retention of the benzene stage was assessed at 520 nm utilizing a PD-303 model spectrophotometer. The proline fixation was resolved to utilize a standard curve. Free proline concentration is communicated as mg g^{-1} D. W. of leaves (Irigoyen et al., 1992).

Antioxidant enzymes

The enzymes were extracted as depicted by Polle et al. (1994) with certain adjustments. New plant tests (0.5 g) were powdered in a fluid nitrogen mortar and homogenized in potassium phosphate buffer (5 mL of 100 mM, pH 7.0) containing 0.5% Triton X-100, 2% (w/v) polyvinylpyrrolidone, 5 mM disodium ethylenediaminetetraacetic acid and 1 mM L-1 ascorbic acid. Homogenates were then centrifuged at 12,000 × g for 20 minutes at 4 °C, and the supernatants utilized in catalyst measures were done at 25 °C. The activity of catalase (CAT) by Góth (1991); Peroxidase activity (POD) was measured by using a guaiacol assay, following Angefini et al. (1990). The activity was expressed on a fresh mass basis (units mg $protein^{-1}$ FW).

Indoleacetic acid and abscisic acid

Indoleacetic corrosive (IAA) and abscisic corrosive (ABA) utilized similar tissue concentrates on guaranteeing information dependability. Tests of assembled date organic products were washed, immediately positioned in fluid nitrogen, and stored at –20 °C. One g of new mass (FM) tests were ground in fluid nitrogen, separated medium-term with 30 ml 80% cold methanol at 4 °C. The accumulate was centrifuged at 2,000 × g and 4 °C for 15 min, and the supernatant was assembled. Then, new chilled methanol was utilized to fill the container multiple times, following the procedures above. The complex and fast methanolic separate was dried in a revolving evaporator and isolated into 10 mL methanol aliquots. IAA and ABA assessed by the imbuement of the mass into a turnaround stage HPLC on a switch stage C18 Section (250 × 4.60 mm, five microns) in an isocratic elution mode using a compact stage involving acetonitrile: water (26:74) with 30 mM phosphoric corrosive as indicated by Tang et al. (2011).

Potassium and sodium concentration

Potassium and sodium concentration was according to Cresser and Parsons (1979). This solution became transparent and was used to determine K and Na concentrations by emission flame photometer.

Chloride concentration

Chloride (Cl) was estimated by potentiometric titration. Dry leaf tissue of 0.2 g was crushed, added to 50 mL of 2% acetic acid, and stirred for 30 minutes — potassium chromate was used as the endpoint indicator (Miller, 1998).

Statistical analysis

A randomized complete block design with six antioxidant treatments replicated four times was used. Duncan, multiple comparison tests performed at $P \leq 0.05$ were conducted using a statistical program, SPSS 21.

Results

Change of plant height, leaf area, and the number of new leaves in response to antioxidants application under salinity conditions

All growth parameters of height plant, leaf area, and new leaves decreased under salinity (15.9 EC). In contrast, antioxidant applications increased all growth parameters (Fig. 1). The citric acid application significantly increased the height of the plant and leaf area related to the control. No significant differences were observed between salicylic acid and citric acid in the new leaf number.

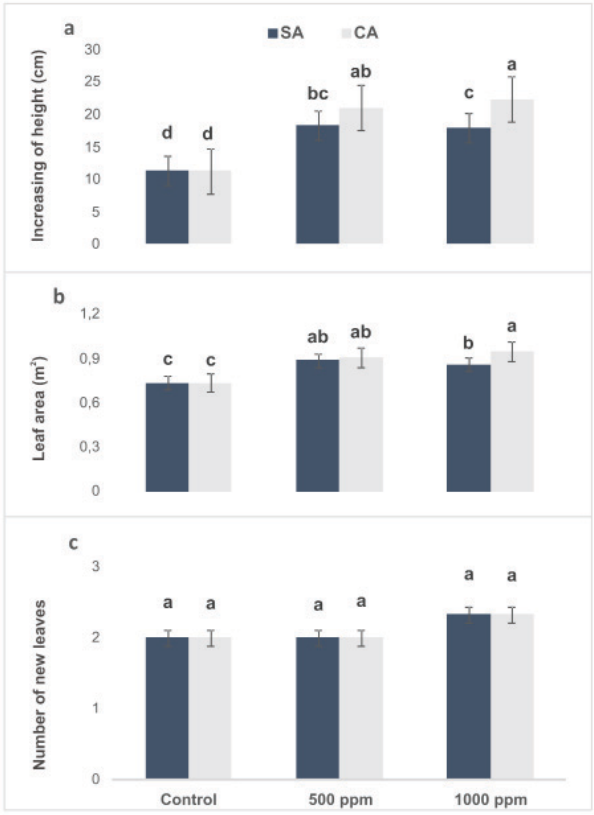

Fig. 1. Change in the height of plant (a), leaf area (b), and the number of new leaves (c) of Sayer cultivar in response to antioxidants application under salinity conditions. The means of four replicates ± SE. Bars with different letters are significantly different at $p \leq 0.05$ after a Duncan correction.

Change of chlorophyll content, carbohydrates content, and relative water content in response to antioxidants application under salinity conditions

Figure 2 shows the change in chlorophyll, carbohydrates, and relative water content under antioxidants application and salinity. Chlorophyll content increased significantly by 1,000 ppm of citric acid. CA significantly increased carbohydrates and relative water content compared to other treatments. Antioxidant applications increased relative water content compared to control. In contrast, the CA application enhanced chlorophyll synthesis in stressed offshoots (Fig. 2). No significant differences were observed between salicylic acid and citric acid in carbohydrates and relative water content.

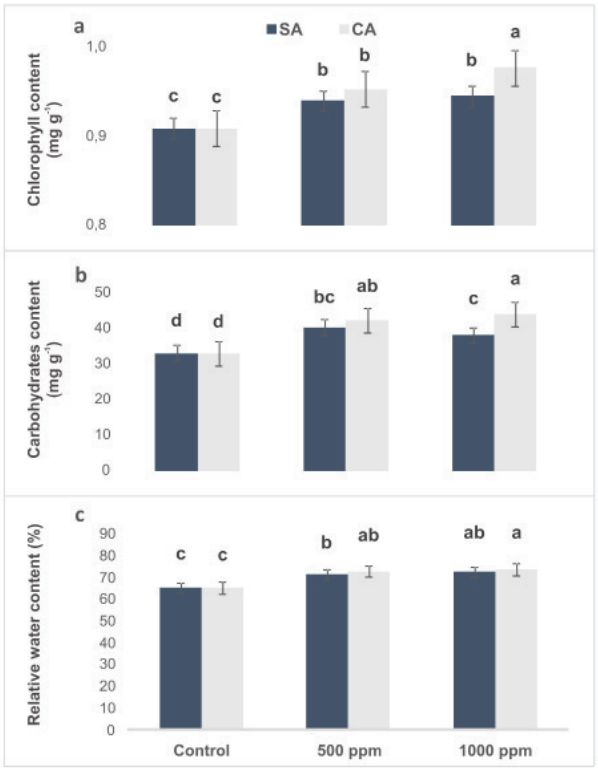

Fig. 2. Change in chlorophyll content (a), carbohydrates content (b), and relative water content (c) of Sayer cultivar in response to antioxidants application under salinity conditions. The means of four replicates ± SE. Bars with different letters are significantly different at $p \leq 0.05$ after a Duncan correction.

Change of proline, protein, peroxidase, and catalase activities in response to antioxidants application under salinity conditions

Antioxidant applications change proline concentration, protein content, and peroxidase (POD) and catalase (CAT) activities to Sayer cv. (Table 1). Proline concentration, protein content, and peroxidase activities increased significantly under antioxidants relative to control. Concentrations of 1,000 ppm of citric acid and salicylic acid increased protein content compared with control. Peroxidase activity increased significantly under citric acid compared with other treatments. At the same time, salicylic acid decreased catalase activity significantly compared with control.

Change of electrolyte leakage and MDA in response to antioxidants application under salinity conditions

The lipid peroxide level, expressed as malondialdehyde content, has been used as evidence of free radical damage to cell membranes. The salinity significantly increased electrolyte

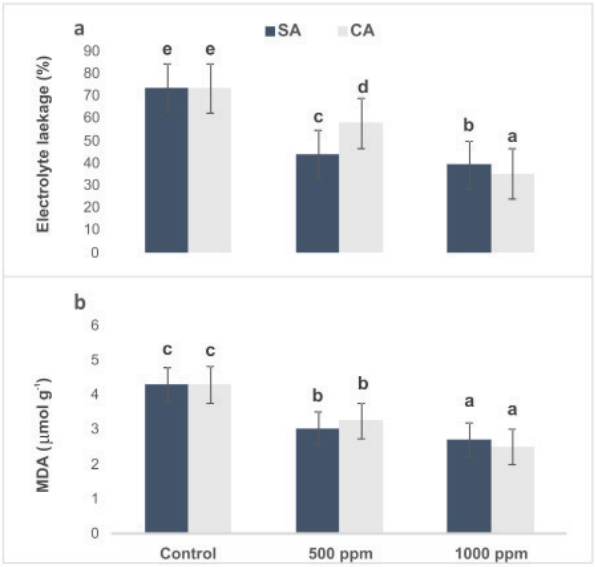

Fig. 3. Change in electrolyte leakage (a) and MDA (b) to Sayer cultivar in response to antioxidants application under salinity conditions. The means of four replicates ± SE. Bars with different letters are significantly different at $p \leq 0.05$ after a Duncan correction.

Table 1. Averages of proline, protein, peroxidase, and catalase activities of Sayer cultivar in response to antioxidants application under salinity conditions

Treatments	Proline (mg g^{-1})	Protein (mg g^{-1})	Peroxidase (Unit mg $protein^{-1}$ FW)	Catalase (Unit mg $protein^{-1}$ FW)
Control	9.13 ± 0.07 b	2.24 ± 0.11 c	4.24 ± 0.18 b	2.30 ± 0.09 c
SA 500 ppm	11.5 ± 0.12 a	3.32 ± 0.12 b	4.33 ± 0.10 b	1.42 ± 0.17 a
SA 1,000 ppm	11.5 ± 0.14 a	3.82 ± 0.15 a	4.21 ± 0.12 b	1.48 ± 0.04 a
Control	9.13 ± 0.77 b	2.24 ± 0.10 c	4.24 ± 0.18 b	2.30 ± 0.09 c
CA 500 ppm	11.5 ± 0.13 a	3.32 ± 0.13 b	4.45 ± 0.08ab	2.20 ± 0.10 bc
CA 1,000 ppm	11.5 ± 0.15 a	3.82 ± 0.14 a	4.63 ± 0.15 a	2.15 ± 0.04 b

Means within each product in the same row with different subscripts have significantly differed at $p < 0.05$.

(Shareef et al., 2020; Shareef et al., 2021; Elsheery et al., 2020b). Antioxidants (such as salicylic acid and citric acid) design chemicals when included in a plant, respond quickly with radical intermediates of an auto-oxidation chain, and prevent it from advancing (Ghazijahani et al., 2014).

Salt stress often triggers drastic changes in cell membrane stability and ultimately influences the membrane sensors (Muchate et al., 2016). Thus, maintaining the cell membrane integrity and stability under stress conditions is critical for vital plant activities. Hanin et al. (2016) showed that cell membrane stability was positively correlated with quality and could indicate plant capacity for salt tolerance. These results indicate that CA or SA improves chlorophyll performance in high salinity environments by reducing oxidative stress.

Salinity generally leads to a change in the balance of endogenous plant hormones and accumulation of ABA with decreased levels of growth stimuli such as IAA (Elhamid et al., 2014). These results apply to research in altering the hormones of date palms under salt stress (Jasim et al., 2016; Fasil et al., 2019; Shareef et al., 2020). The decrease in auxin under the influence of salinity may be attributed to a decrease in the biological structure resulting from an osmotic stress disorder and the conversion to inactive bound forms. The decrease in auxin contents may be due to the conversion of auxins into an inactive compound by some biochemical processes, for example, oxidation and increased IAA-oxidase enzyme activity (Bassiouny and Khalil, 2008). Plant growth is stimulated by maintaining plant tissues under salt stress using several mechanisms to exclude harmful elements from the roots or their collection in cell vacuoles. The sodium and chloride content of the leaves was reduced using antioxidants (Table 2). Antioxidant's role in reducing harmful elements damage may be attributed to preventing absorption or disposal within the tissues.

Conclusion

The salt tolerance mechanism in date palms can be improved by spraying the leaves with antioxidants to bypass the period of oxidative stress resulting from extreme environmental conditions, especially the salinity conditions. Thus, the application of antioxidants may enhance the absorption of essential nutrients. Exogenous application of antioxidants such as citric acid and salicylic acid alleviates the adverse effects of salt stress; this may be a practical approach to improving salt stress tolerance in date palm offshoots. In most of the studied characteristics, citric acid improved the characteristics more than salicylic acid, encouraging farmers to use it for its low cost as an antioxidant that reduces environmental stress damages, especially salt stress.

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