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In Vitro Response of Shoots Multiplications of Date Palm Phoenix Dactylifera L. Cv Shakar to Jasmonic Acid Application Under Salinity Stress

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Abstract. The study was conducted in the Tissue Culture Laboratory of the Date Palm Research Center - Basra University. shoots adventitious were used resulting from tissue culture of the apical and lateral shoots of date palms cv. Shakar by indirect shoot multiplication technique *in vitro*, where the application of several levels of JA was tested to reduce the effect of stress caused Salt stress using a bilateral interaction between NaCl at levels (0, 50, 100, 200) mM L^{-1} and JA at concentrations (0, 25, 50, 75) µM L^{-1}, these materials were added to the special culture media of multiplication shoots adventitious, the results showed that treatment with NaCl at level (100, 200) mM L^{-1} led to significant inhibition of growth in terms of biomass, water content, and content of chlorophyll and carotene pigments, all of which were greatly improved upon application of JA. Treatment with NaCl salt increased the enzymatic antioxidant activity by enhancing the enzyme activity (POD) and (CAT). The addition of JA to the culture media either alone or under salt stress conditions, significantly improved growth, biomass, water content, chlorophyll, and carotene pigments, increased enzymatic antioxidant metabolism, and regulation of proline synthesis.

Keywords. Date palms , Shoot multiplication , *In vitro* , Jasmonic acid , Salt stress.

INTRODUCTION

Date palms are propagated either sexually through seeds or vegetatively by offshoots, and the technique of micropropagation is considered one of the most promising techniques for propagating palm trees for many reasons, including increasing the cultivated area in the coming years [1], and it is also an excellent tool to clarify the relationship. Between the regulatory level and salt tolerance due to the possibility, it offers to study the physiology of intact plants together with those of individual organs and cells using homogeneous plant material under standardized environmental conditions [2], and this method is currently a major complement to conventional breeding methods [3], as well as the use of tissue culture techniques for date palms in carrying out studies and research on morphological and anatomical characteristics, genetic variants, and resistance to biotic and abiotic stresses [4]. [5], Several physiological studies related to environmental stresses and plant responses to them, including date palms, have been conducted [6].

Salinity is one of the main environmental pressures that challenge plant growth and crop productivity around the world [7]. High levels of salt have a significant effect on the growth and development of plants represented by numbers and lengths of shoots vegetative, a number of leaves and roots, dry weight, fresh weight and water content, Plant hormones in the growth media plays an important role in mitigating the harmful effects of salinity for a number of proliferating date palm varieties *in vitro* [8-10].

Several studies have shown that the leaf content of chlorophyll pigments decreases under salinity stress and its effect is more pronounced in older plant leaves [11]. The salt stress of the plant negatively affects the leaf content of

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the plant pigments responsible for photosynthesis as well as the accumulation of carotenes pigments that act as antioxidants [12,13].

The plant resistance to salinity comes from the standpoint of survival and preservation of the plant type, as the plant can resist salt stress through osmotic and ionic balance and excretion and detoxification by neutralization or equation of free radicals ROS formed as a result of stress such as (superoxide O2-, hydrogen peroxide H2O2 and hydroxyl radical OH). This can cause several damages to membranes or cellular components and as a result, leads to inhibition of growth, and plant tissues are subjected to many enzymatic changes and a change in their content of carbohydrates and proteins when subjected to stress, as studies indicated moderate or severe stress, caused an increase in the activity of anti-enzymes. For oxidation as well as proline while soluble protein decreased under the pressure of salinity [14].

Plants show a wide range of responses to environmental stress conditions by building defensive or warning signaling compounds, as they encourage plants to build different plant hormones, which are basically one of the requirements of the different stages of development in plants and help them in the defensive response to stress [15,16], and it is considered important in regulating plant physiological activities as well as regulating plant resistance to biotic and abiotic stresses [17,18], Jasmonates (JAs) are a newly discovered growth organization in the plant kingdom [19].

Jasmonates (JAs), the most important of which Jasmonic acid (JA) and methyl Jasmonate (MeJA) share various physiological activities in plants and have proven effective in improving plant resistance to different stress conditions, especially salinity, drought, temperature conditions (high and low), and insect infestation, and the extent of their impact depends on the type of plant and The dose used. has important roles that help plants adapt to environmental differences and are derivatives of fatty acid metabolism [20,21], explained that adding methyl jasmonic (MeJA) to the culture media at a concentration of 2 mMol led to the improvement of the growth characteristics of callus tissues and the vegetative growth of citrus plantlets origin Truerstrang growing under saline stress *in vitro,* as indicated [22], that the addition of JA among the components of the nutrient medium for *in vitro* propagation of lavender at a concentration of (0.5) mg L^{-1} led to an increase in the activity of antioxidant compounds, improving growth and development characteristics, While when using higher concentrations (1-2) mg L^{-1} inhibited the growth of explants.

In a study conducted by [23], on soybean plants, it was found that treatment with JA resulted in enhancing the activity of antioxidant enzymes and causing an increase in the concentration of pigments, and confirmed that JA reduced the harmful effects of the high salinity concentration and inhibited the negative effects At low salt concentrations. JA works to regulate plant adaptation to salinity stress and the development of resistance mechanisms in plants [24], Due to the lack of studies on the effect of Jasmonic acid on the growth and development of salt-stressed date palm tissues, the current study aims to investigate the effect of JA and NaCl on the phenotypic and biochemical characteristics of adventitious Shoots of date palm cv. Shakar, *in vitro*.

MATERIAL AND METHODS

Plant Material and Culture Conditions

The study was conducted in the Tissue Culture Laboratory of the Date Palm Research Center - Basra University. Shoot multiplication from adventitious budding was used resulting from tissue culture by indirect multiplication technique of the apical and lateral shoots of date palms seedling cv. Shakar.

MS medium used to experiment of shoot multiplication stage [25], produced by the Indian company HiMedia Laboratories Pvt. Ltd. Sucrose was added to it at a concentration of 4000 mg L^{-1} , 40 mg L-1 Sodium Hydrogen Orthophosphate, 170 mg L^{-1} adenine sulfate, 500 mg L^{-1} Neutral Activated Charcoal. the medium was provided with 5 mg L-1 BA and 1 mg L-1 NAA depending on [26], NaCl was added at concentrations (0 ,50 ,100 , 200) mM L^{-1} and JA at concentrations (0, 25, 50, 75) µM L^{-1}, to the MS shoot multiplication medium. The pH of the medium was adjusted at 5.7 by using HCl and NaOH at one normality concentration. Agar was added at a concentration of 7 g L^{-1} to solidify the medium and then heated to 90°C with continuous stirring using the hotplate magnetic stirrer to facilitate its melting and to obtain a homogeneous mixture of the MS medium. The 40 ml medium was poured into Jars with a capacity of 300 ml. The jars nozzle was closed using aluminum foil. The MS medium was sterilized by autoclave at 121°C and 0.1 MPa for 20 min. The shoot adventitious were cultured on these sterile MS media with (4-5) shoots for each Jar. Subculture was conducted every six weeks [Fig.1]. . The cultures incubated at a temperature of 27± 2, light density at 3000 lux, and photoperiod at 16 h light and 8 h darkness. The morphological

and biochemical characteristics of the adventitious shoots were measured after six months of cultivation in the multiplication medium, with five replicates of the phenotypic characteristics and three replications of the biochemical characteristics.

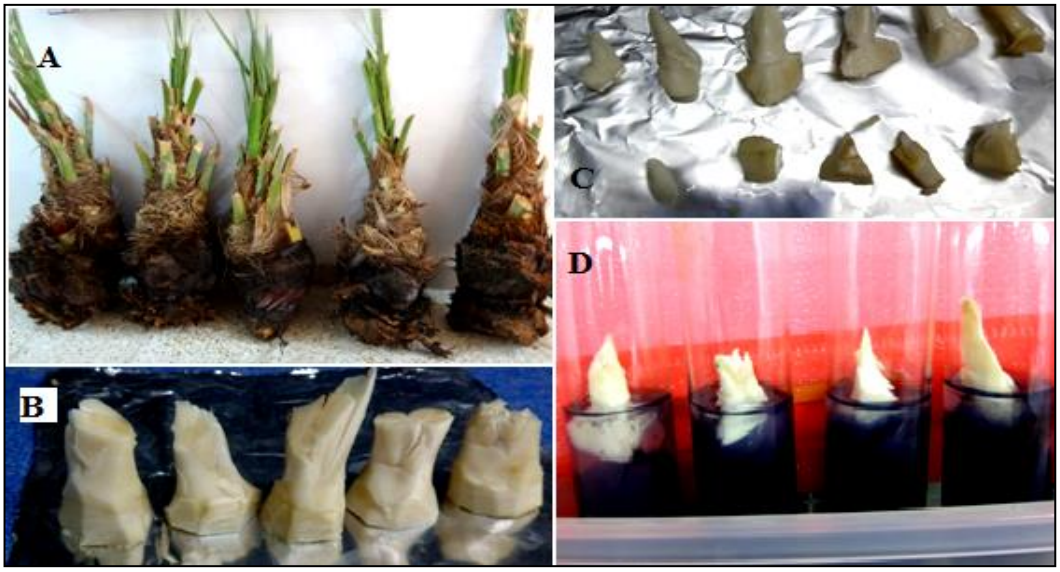

FIGURE 1.. Preparation of tissue culture - A. offshoots of Date palm cv. Shakar was used in the study after removing the large external leaves; B. Excised apical buds; C. Excised Axillary buds; D. Culturing of the explants in a culture media

Estimation of Some Morphological Characteristics

The increase in the number of Shoot multiplication formed for each repeat every six weeks was calculated and its average was extracted for one treatment at the end of the experiment. As for the average length of the adventitious Shoots (cm), the longest of five shoots were selected and their length was measured and the average length of the shoots was calculated for each repeat. Likewise, the fresh weight of the agricultural mass of each Jar was estimated according to [27]. As for the dry weight of the culture mass (mg), it was estimated by drying the samples that were measured for their fresh weight in an electric oven at a temperature of 65 ° C for a period of 72 hours, Then The dry weight was calculated using a sensitive electrical balance, and the water content was estimated using the following equation:

$$WC\% = [FW - DW / FW] \times 100$$

Estimation of Total Chlorophyll, Carotene Content and Chlorophyll Stability Index

Total chlorophyll and carotene pigment were determined by crush of 500 mg of fresh leaves, using 20 cm3 of acetone 80%. Samples were extracted using a centrifuge at 3000g for 10 minutes. The absorbance of pigments was estimated using a Spectrophotometer, where the total chlorophyll was estimated according to the method of [28], at wavelengths (645, 663) nm. As for carotene, it was estimated according to [19] at the wavelengths (474, 663, 647) nm. Acetone was used as a control sample, as for Chlorophyll Stability Index (CSI), it was estimated according to [29], plant pigments and Chlorophyll Stability Index (CSI) were determined according to the following equations:

$$\text{Total chlorophyll} = 20.2 \times DO645 + 8.02 \times DO663 \times V/(W \times 1000)$$

$$\text{Carotene} = 5 \times DO470nm + 2.846 \times DO663nm - 14.876 \times DO647nm$$

$$CSI = [\text{Total Chl. under stress} / \text{Total Chl. under control}] \; X\,100$$

OD= Optical density by wavelength = T. Volume of draw out is (15) ml, w= Fresh weight of sample, (500) mg.

Estimation of The Enzymatic Activity

Extraction of samples: Tissue samples were extracted using the [30] weighed 1 g of tissue after cleaned well and homogenized in 2 ml of Potassium phosphate buffer 0.1M (pH 7) w/v, the extract was filtered with a Miracloth (medical gauze), and centrifuged for 12000 g for 30 minutes at 4°C using refrigerated centrifuge 5804R, produced from the German company Hamburg.

Peroxidase activity (POD): was measured by method of [31], by adding 1 ml of 2 Mm hydrogen peroxide), 1 ml guaiacol (5mM), and 1 ml phosphate buffer (0.1 M, PH 7), the mixture was placed in the spectrophotometer's cell. 0.1 ml of the extract was added to it, and it was reading for 3 minutes with recording the reading every 30 seconds at 30 ° C and a wavelength of 436 nm, the mixture was used without adding the sample as a blank, was calculated the enzymatic activity from the following two equations:

$$\text{Slope} = \Delta \text{absorbance} / \Delta \text{time}$$

$$(\text{POD}) \text{ activity } (\text{U/g}) = \text{Slope} \times (3.1) / 0.1\text{ml} \times 1\text{cm} \times 6.4$$

spectrophotometer's cell size, (0.1ml)Sample size ,(1cm) optical path length, 6.4 molar transmittance constant for guaiacol.

Catalase activity (CAT): was measured by method of [32], using certain proportions of the following materials 1- Substrate (reaction mixture) consisting of equal proportions of H_2O_2 (65 mMol) and Potassium phosphate buffer (60 mMol) pH 7.4, 2- Ammonium molybdates (NH4) 6 Mo7O24.4 H2O), 32.4 mMol. From the plant sample, 0.2 ml of the serum was incubated with 1 ml of substrate for four minutes at 25°C, the enzymatic reaction was stopped with 1.0 ml of 32.4 mmol L-1 ammonium molybdate, the yellow complex of molybdate and hydrogen peroxide(H_2O_2) was measured at 405 nm against blank 3, was calculated CAT enzyme activity from the following equations:

$$\text{CATactivity } (\text{U/g}) = (\text{A(sample)} - \text{A(blank 1)} / \text{A(blank 2)} - \text{A(blank 3)} \text{ X } 271$$

Blank 1 contained 1.0 ml substrate, 1.0 ml molybdate and 0.2 ml serum (without incubation). Blank 2 contained 1.0 ml substrate, 1.0 ml molybdate and 0.2 ml buffer. Blank 3 contained 1.0 ml buffer, 1.0 ml molybdate and 0.2 ml buffer.

Determination of Proline Content

Proline content was estimated following the method of [33]. After extraction with sulphosalicylic acid, a known volume of extract was reacted with ninhydrin reagent, and the absorbance was recorded at 520 nm using a spectrophotometer (CECIL IN Strament,UK), with toluene as a blank. These readings were compared with the standard curve of pure proline, using the following equation:

$$\text{Proline } \mu\text{g/g FW} = [\text{ Proline}(\mu\text{g}) \text{ X Toluene(ml)} / 115.5 *] \text{ X } [5 / \text{w. sample(gm)}]$$

*molecular weight of proline

Estimation of Total Soluble Protein

Total soluble proteins were extracted according to [34], method, by crushing 1 g of fresh tissues in 3 ml of a solution consisting of Tris-HCl buffer (0.1M, pH 7.5) containing Phenylmetmanesulfonyl Fluoride (PMSF) 0.001M, at 4°C, centrifuged at 18000 g for 30 minutes. The filtrate formed was used to estimate the total soluble proteins according to the method of [35], using the Bradford reagent, the process is addition 40 µl of the filtrate to 2 ml of Bradford's reagent, (the prepared by Coomassie Blue100 mg, Ethanol alcohol 50 ml (95%), and phosphoric acid 100 ml (85%) was added to it. The volume was completed to1L of distilled water. The absorbance was estimated using a spectrophotometer at 595 nm. Distilled water was used as a blank sample. (BSA) was used in determining the standard albumin curve based on which total soluble proteins were estimated.

Statistical Analysis

The obtained date were analysis of variance (ANOVA) and the mean difference were compared by least significant difference (L.S.D.) at $p < 0.05$ level with triplicates for treatments, SPSS v.21 was used for statistical analysis.

RESULTS

Growth Parameters

The results of a study showed the effect of different levels of sodium chloride (0, 50, 100, 200) mM and Jasmonic acid (JA) (0,25, 50, 75) μM in the phenotypic growth rate (number and length of shoots, fresh weight, dry weight and water content of the biomass), it indicated a significant decrease in the level of the measured by increasing the concentration of NaCl salt, as the treatment with a high salt concentration (200 mM NaCl) gave the highest significant decrease in the most mentioned characteristics (table. 1). The results also showed in a table (2) the significant effect of adding jasmonic acid to the culture medium devoid of NaCl in improving the studied traits, which appeared clearly in the concentration 50 μM, which significantly outperformed all the treatments under study, while the concentration 75 showed a negative effect on most of the growth parameters.

Table 3 was proved that the addition of JA at a concentration of 25 μm at the level of comparison was very beneficial in increasing the length and number of shoots, with an increase of (15.7, 17.95) % respectively with the control treatment. Also, the results showed in the same table that the addition of Jasmonic acid in several concentrations under low salt stress conditions had a significant effect in improving the phenotypes of the shoot multiplication and the biomass. The treatment achieved (50 mM NaCl + 25 μM JA) was significantly superior to all parameters in the mean of the traits under study.

While jasmonic acid contributed significantly to alleviating the effect of high salt stress and its effect was highly significant, especially in the treatment (100mM NaCl +50μM JA) with an increase of (50, 58.90, 47.68, 22.67, 7.92)% respectively, compared with the treatment (100 mM NaCl + 0 μM JA), as well as for the treatment (200 mM NaCl + 50 μM JA) that did not differ significantly from the treatment (200 mM NaCl+75 μM JA) reduced the effect of salt stress for most of the growth characteristics compared to the treatment (200 mM NaCl + 0 μM JA), which harmed growth rate and biomass (Figure. 2).

TABLE 1. Effect of NaCl concentration on growth parameters and water conten in date palm cultures cv. Shakar *in vitro.*

NaCl mMol L^{-1}	Shoot Number	Shoot Length (cm	Fresh weight of culture biomass (gm)	Dry weight of culture biomass (gm)	Water content of cultures biomass %
0	10.75 ± 2.77	3.39 ± .708	3.81 ± .70	0.67 ± 0.052	83.88 ± 3.60
50	13.15 ± 4.91	5.38 ± 1.76	4.93 ± 1.48	0.69 ± 0.09	85.24 ± 3.00
100	11.80 ± 3.61	4.51 ± 1.36	4.08 ± 1.04	0.66 ± .075	83.19 ± 2.87
200	8.70 ± 2.5	4.23 ± 2.16	3.60 ± 1.64	0.58 ± 0.17	82.18 ± 4.19
LSD	1.05	0.40	0.32	0.04	0.86

TABLE 2. Effect of JA concentrations on growth parameters and water content in date palm cultures cv. Shakar *in vitro*.

JA μMol L^{-1}	Shoot Number	Shoot Length (cm	Fresh weight of culture biomass (gm)	Dry weight of culture biomass (gm)	Water content of cultures biomass %
0	8.90 ± 2.36	2.59± 0.97	3.11± 1.19	0.54± 0.12	82.19± 5.44
25	12.60 ± 5.36	5.15± 1.58	4.48± 1.73	0.65± 0.12	84.39± 3.103
50	13.10 ± 2.55	5.03± 1.23	4.84± 0.69	0.72± 0.06	85.02± 1.43
75	9.80 ± 2.84	4.76± 1.65	3.98± 0.93	0.66± 0.07	82.92± 2.53
LSD	1.05	0.40	0.32	0.04	0.86

TABLE 3. Effect of bilateral overlap NaCl and JA concenetrations on growth parameters and water conten in date palm cultures cv. Shakar in *vitro.*

Treatments		Shoot Number	Shoot Length (cm)	Fresh weight of culture biomass (gm)	Dry weight of culture biomass (gm)	Water content of cultures biomass %
NaCl mMol L^{-1}	JA µMol L^{-1}					
0	0	11.80 ± 1.92^{d}	3.52 ± 0.46^{f}	4.24 ± 0.74^{c}	0.61 ± 0.04^{bc}	89.08 ± 1.83^{a}
	25	14.00 ± 1.22^{c}	4.29 ± 0.28^{de}	4.27 ± 0.42^{c}	0.69 ± 0.02^{ab}	83.76 ± 1.39^{c}
	50	9.80 ± 0.83^{f}	3.22 ± 0.27^{f}	3.79 ± 0.24^{c}	0.65 ± 0.03^{b}	82.84 ± 0.40^{cd}
	75	7.40 ± 0.89^{f}	2.54 ± 0.21^{g}	2.91 ± 0.17^{e}	0.58 ± 0.04^{c}	79.86 ± 0.86^{f}
50	0	9.40 ± 1.14^{e}	3.25 ± 0.33^{f}	3.91 ± 0.32^{c}	0.59 ± 0.05^{c}	84.76 ± 1.27^{bc}
	25	20.20 ± 2.38^{a}	7.56 ± 0.79^{a}	7.17 ± 0.52^{a}	0.77 ± 0.09^{a}	89.22 ± 0.75^{a}
	50	14.20 ± 1.48^{c}	6.15 ± 0.77^{c}	5.04 ± 0.19^{b}	0.72 ± 0.05^{a}	85.66 ± 0.92^{b}
	75	8.80 ± 0.83^{e}	4.59 ± 0.49^{d}	3.56 ± 0.10^{cd}	0.66 ± 0.01^{b}	81.32 ± 0.48^{de}
100	0	8.00 ± 0.70^{ef}	2.32 ± 0.25^{g}	2.82 ± 0.24^{e}	0.58 ± 0.01^{c}	79.24 ± 1.61^{f}
	25	9.00 ± 0.70^{e}	5.07 ± 0.43^{d}	3.76 ± 0.28^{c}	0.65 ± 0.03^{b}	82.60 ± 1.44^{d}
	50	16.00 ± 1.22^{b}	5.65 ± 0.47^{c}	5.39 ± 0.52^{b}	0.75 ± 0.04^{a}	86.06 ± 0.66^{b}
	75	14.20 ± 1.48^{c}	5.01 ± 0.24^{d}	4.35 ± 0.64^{c}	0.65 ± 0.07^{b}	84.88 ± 0.59^{b}
200	0	6.40 ± 1.14^{fg}	1.26 ± 0.39^{h}	1.45 ± 0.27^{f}	0.35 ± 0.05^{e}	75.66 ± 1.03^{g}
	25	7.20 ± 1.30^{f}	3.68 ± 0.26^{f}	2.71 ± 0.26^{e}	0.48 ± 0.04^{d}	81.98 ± 0.45^{d}
	50	12.40 ± 0.54^{d}	5.08 ± 0.37^{d}	5.11 ± 0.14^{b}	0.74 ± 0.03^{a}	85.50 ± 0.39^{b}
	75	8.80 ± 0.83^{e}	6.90 ± 0.80^{b}	5.10 ± 0.46^{b}	0.73 ± 0.04^{a}	85.60 ± 0.60^{b}
$p \leq 0.05$	L.S.D.	1.49	0.56	0.64	0.06	1.21

Total Chlorophyll, Carotene and Chlorophyll Stability Index

The results in Fig. [3. (a,b)] showed clear significant differences between treatments for the effect of NaCl and JA in T. Chl and carotene concentration, also the CSI. The results show that increasing NaCl concentration to 200mM was a negative impact on the studied qualities, while the control treatment significantly outperformed in increasing the rate of these qualities, but it did not differ significantly with the two NaCl (50 and 100) mMol treatments. In same figure, JA at 50 µM concentration treatment were the more significantly than other JA treatments without 25 µM treatment with an increase of (28.29, 27.1, 21.7)% respectively. The results also showed that increasing the concentration of JA when the NaCl concentration was zero led to a nonsignificant decrease in the rate of the T.Chl, and CSI except the treatment (0 mM NaCl + 25 µM JA), which a significant superiority with an increase of (10.11,11.51)% respectively, While in adding JA in several concentrations under salt stress conditions had a significant effect in increasing T. Chl, carotene, and CSI. The treatment (50 Mm.

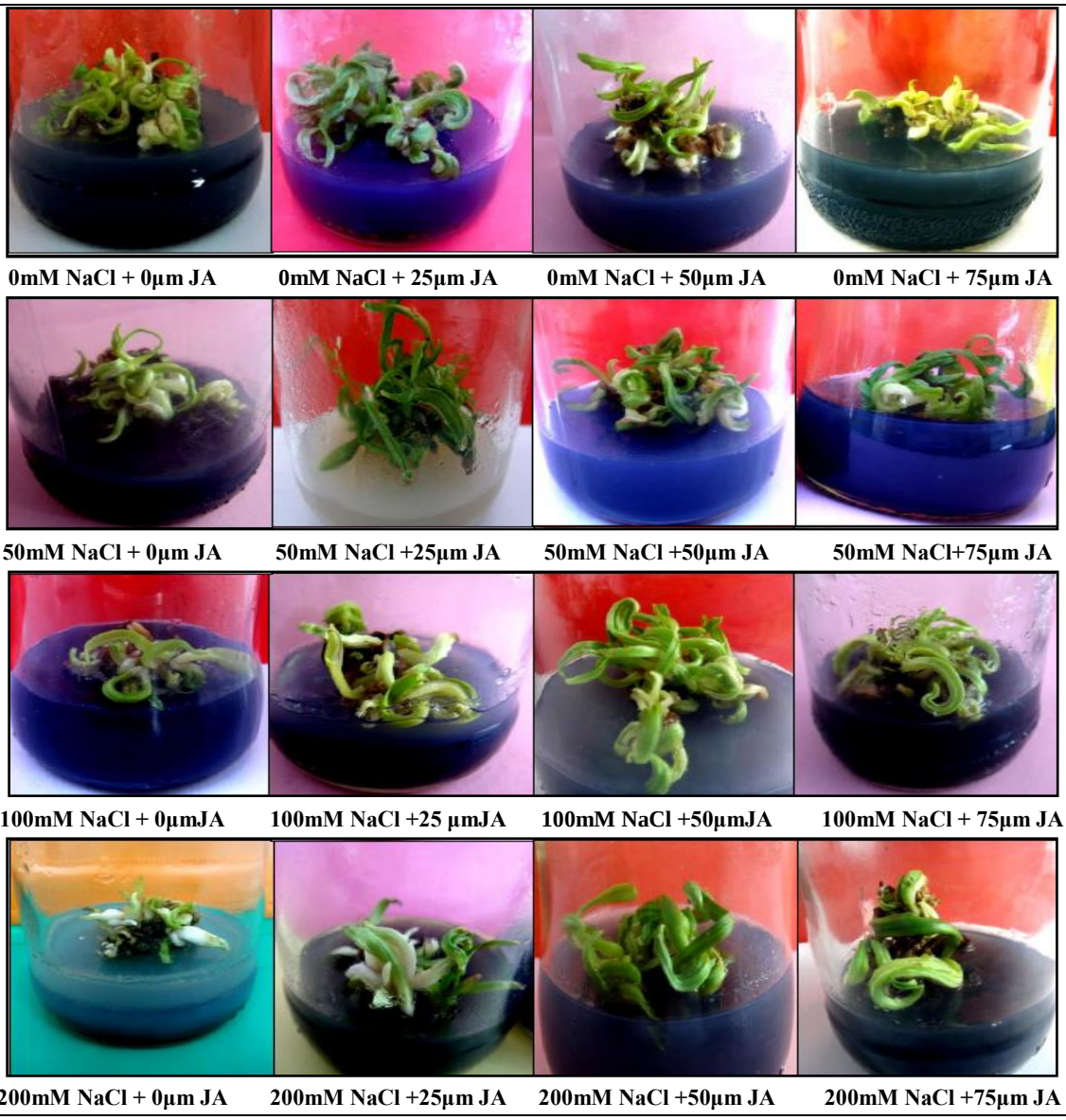

FIGURE 2. Effect of bilateral overlap of NaCl and Jassmonic acid on Growth parameters in date palm cultures cv. Shakar *in vitro*

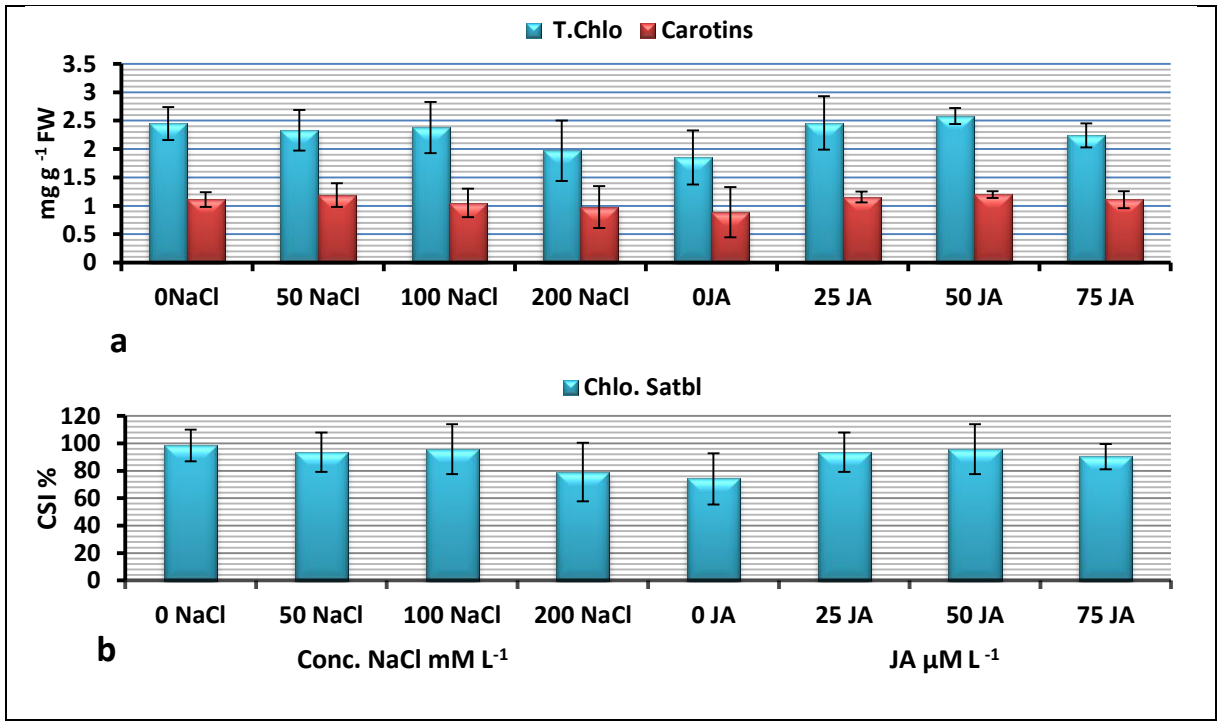

FIGURE 3. Effect of NaCl concentration on and JA concentration in on **a**. Total Chlorophyll and Carotene , **b**. Chlorophyll Stability Index on date palm cultures cv. Shakar *in vitro*. $p < 0.05$. [LSD: T.Ch NaCl = JA= 0.173], [LSD: Carotin NaCl =JA = 0.205],[LSD: CSI NaCl = JA = 3.74**].**

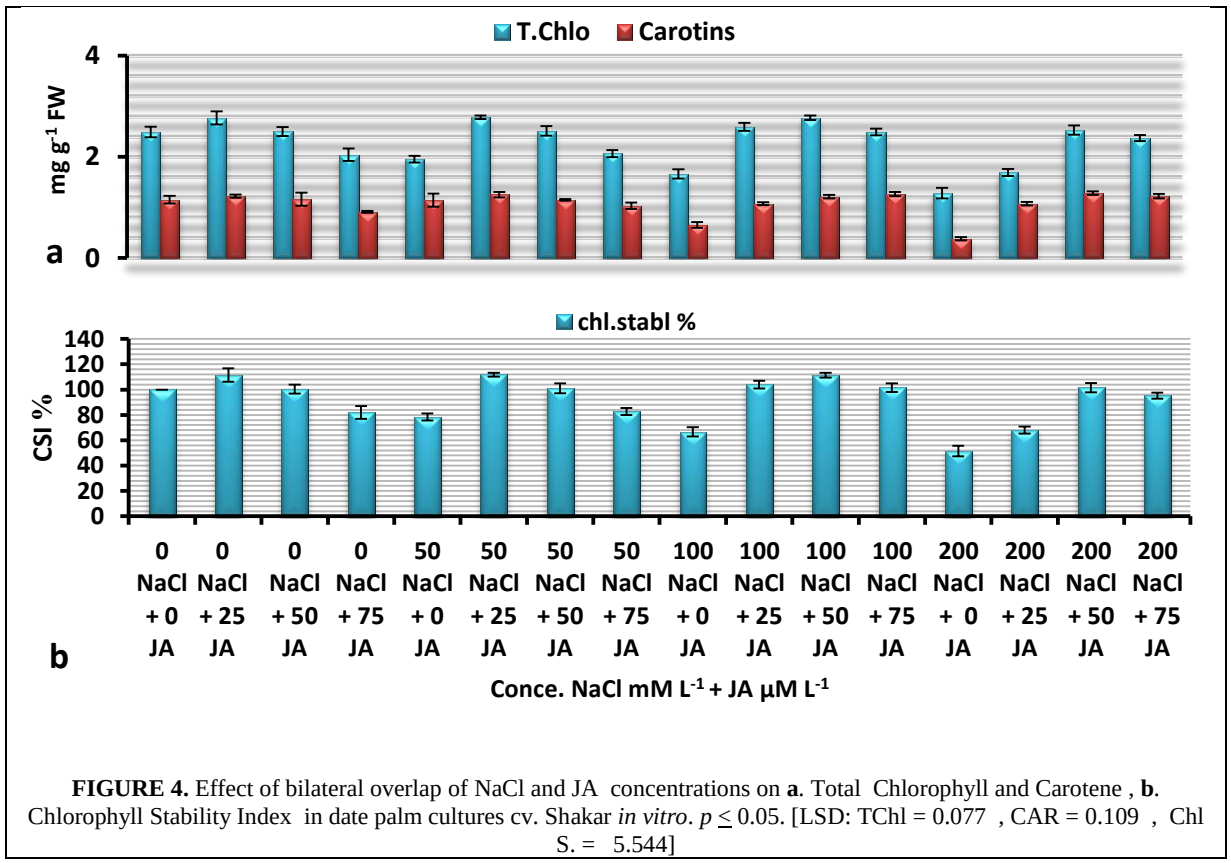

FIGURE 4. Effect of bilateral overlap of NaCl and JA concentrations on **a**. Total Chlorophyll and Carotene , **b**. Chlorophyll Stability Index in date palm cultures cv. Shakar *in vitro*. $p \leq 0.05$. [LSD: TChl = 0.077 , CAR = 0.109 , Chl S. = 5.544]

NaCl + 25 µM JA) achieved a significant superiority in the rate of these traits, which did not differ significantly from the treatment (100 mM NaCl + 50 µM JA) in the mean T.Chl and CSI compared to the treatment (50 mM NaCl + 0µM JA) and the treatment (100 mM NaCl + 0 µM JA) respectively. JA also contributed to mitigating the harmful effect of high salt stress, as the treatment exceeded (200 mM NaCl + 50 µM JA) significantly an average of characteristics with an increase of (49.41, 70.3, 49.2)%, respectively compared to the treatment (200 mM NaCl + 0 µM JA), which had a negative effect on all the studied traits [Figure 4 (a, b)].

Assay of The Enzymatic Activity

When we studied the effect of single factors alone in estimating the effectiveness of peroxidase and catalase enzymes, the results in Fig. (5 showed that there was a significant increase in the activity of POD and CAT enzymes at a concentration of NaCl 200 mMol with an increase of (19.13,26.25)% respectively compared to the concentration NaCl 0 mMol, the results showed in the same figure that there was no significant effect of jasmonic acid in increasing the activity of POD enzyme, but it remained relatively high in all treatments, while the activity of CAT enzyme was increased significantly in treatment 0 JA compared to the other treatments with an increase of 18.15%.

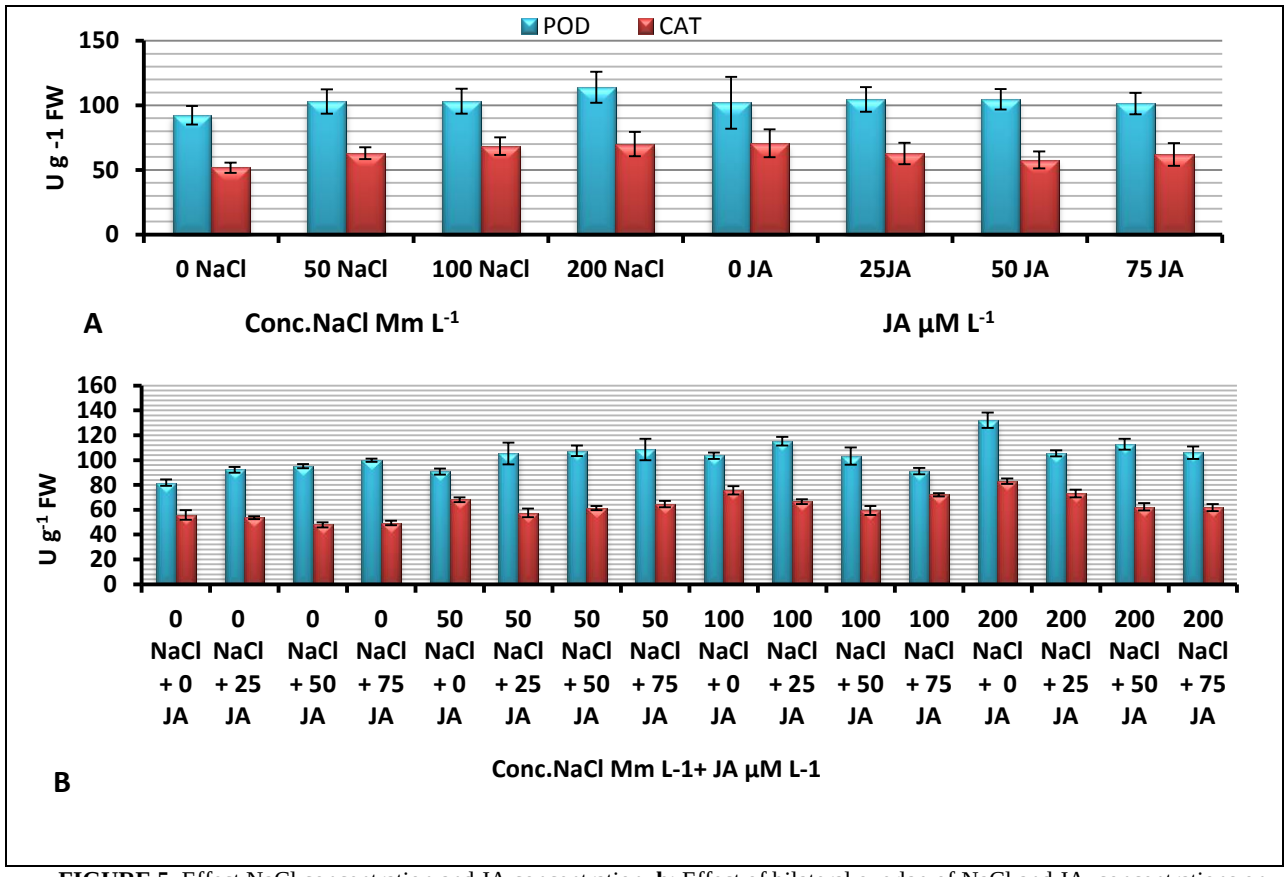

FIGURE 5. Effect NaCl concentration and JA concentration, **b**: Effect of bilateral overlap of NaCl and JA concentrations on antioxidant anzymes activity (POD and CAT) in date palm cultures cv. Shakar *in vitro* at $p \leq 0.05$, **a**[LSD: NaCl or JA POD = 9.475, LSD: NaCl OR JA CAT= 5.253],**b** [LSD: POD = 5.47, CAT =3.714] ,

Figure (6) showed that there was an increase of activity antioxidant enzymes (POD and CAT enzymes) in which were highly significant with an increase in the concentration of NaCl, as it reached its highest activity in the treatment (200mM NaCl+ 0µM JA), with an increase of (38.08, 32.74)% respectively than control treatment. The addition of different concentrations of JA to the NaCl free medium increased the POD activity gradually and CAT enzyme activity decreased in the same treatment, also, adding JA to the treatments subjected to low and medium salt stress led to a significant increase in the activity of POD and CAT compared to the effect of different concentrations of Jasmonic acid alone, except for the treatment (100Mm NaCl + 75µM JA), in which the activity of the POD

enzyme decreased to 91.14 U g^{-1}compared to the treatment (0mM NaCl + 75µM JA) completely free from the effect of salt stress, and the same figure also shows that adding JA to the high salt stress factors (200Mm) led to a significant decrease in the activity of antioxidant enzymes compared to the treatment (200mM NaCl + 0µM JA), but the activity of these The enzymes remained significantly elevated with two concentrations (100,200) mM NaCl compared to the control and non-saline treatments.

Assay of Proline Content

The results showed in (Figure 6) the effect of the single factors (NaCl and JA) on the accumulation of proline in the vegetative shoots of the date palm plantlets, where the accumulation of proline increased significantly in the high concentration of salt (200) mMol compared to the other concentrations, with an increase of 23.31%. For jasmonic acid, the effect was opposite, as the same table showed a significant increase in the accumulation of proline on 0µM JA, were recorded the highest content of 14.77 µg g^{-1} compared with other jasmonic acid treatments.

The results of the analysis indicated the clear significant effect of adding different concentrations of JA at the comparison level, where the treatment (0Mm NaCl+ 75µM JA) recorded the highest proline content of 17.2 µg g^{-1} FW compared to the control treatment (0Mm NaCl+ 0µM JA) . also, The addition of different levels of NaCl increased the content of proline by increasing the level of salt stress, so that it reached its highest value in the treatment (200Mm NaCl + 0µM JA) of 20.81µg g^{-1} FW, which significantly exceeded all the treatments under study. While the addition of different concentrations of jasmonic acid reduced the harmful effect of the salt by reducing the proline content to significantly lower levels in most of the treatments under salt stress conditions, (Figure 5. B).

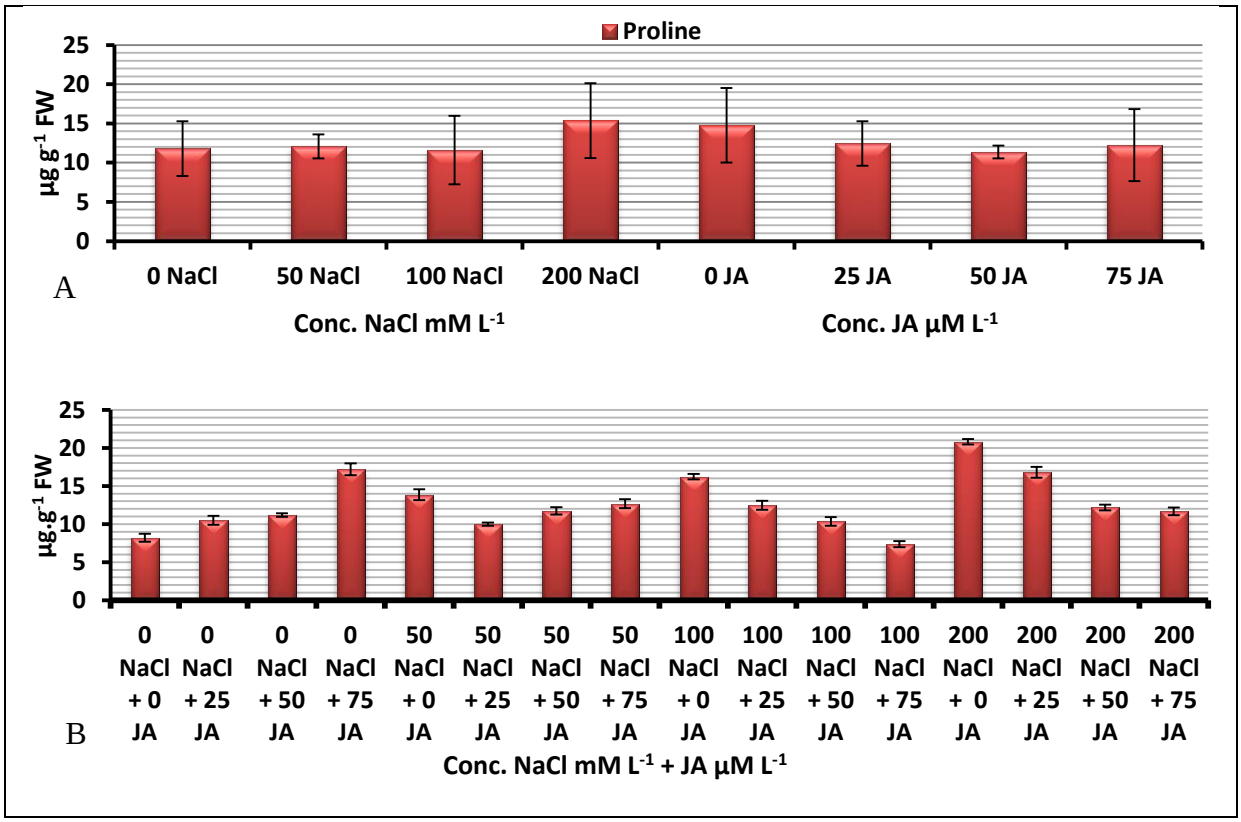

FIGURE 6. Effect NaCl concentration and JA concentration, **b**: Effect of bilateral overlap of NaCl and Jassmonic acid on Proline content in date palm cultures cv. Shakar *in vitro* at $p \leq 0.05$, **a**-[LSD: N OR JA=3.369],**b**-LSD **:** 2.014.

Estimation of Total Soluble Proteins

Figure (7) showed the results of the effect of the individual factors (NaCl and JA) on the concentration of total soluble proteins in the vegetative shoots cells of date palm plantlts, as their concentration decreased at the level of

(200) mmol, but it did not differ significantly with other salt levels. As for jasmine acid, the same table showed a significant increase in protein concentration at 25 µM JA were recorded the highest content of 4.56 compared to the control treatment, with an increase of 29.07%.

The results of the data analysis showed that there was a significant decrease in the total soluble protein concentration in the adventitious shoots with an increase in the concentration of NaCl in the culture medium, where the high salt concentration treatment (200 mM NaCl + 0 µM JA) recorded the lowest content compared to the control treatment. the addition of different concentrations of JA in the comparison level had no significant effect on the protein content, and its content decreased to less than the level of comparison in the treatment of high concentration of Jasminec acid (0 mm NaCl + 75 µM JA).). The results also showed that adding JA to the salinity treatments significantly reduced the effect of salt stress, and adding it had a significant effect in most treatments compared to the treatments that contained different concentrations of salt alone, but it did not differ significantly from the control treatment, except for the treatment (50 ml NaCl + 25 µM JA), which recorded a significant superiority over all studied treatments in protein content of 5.19 mg g^{-1}, (Figure 6. b).

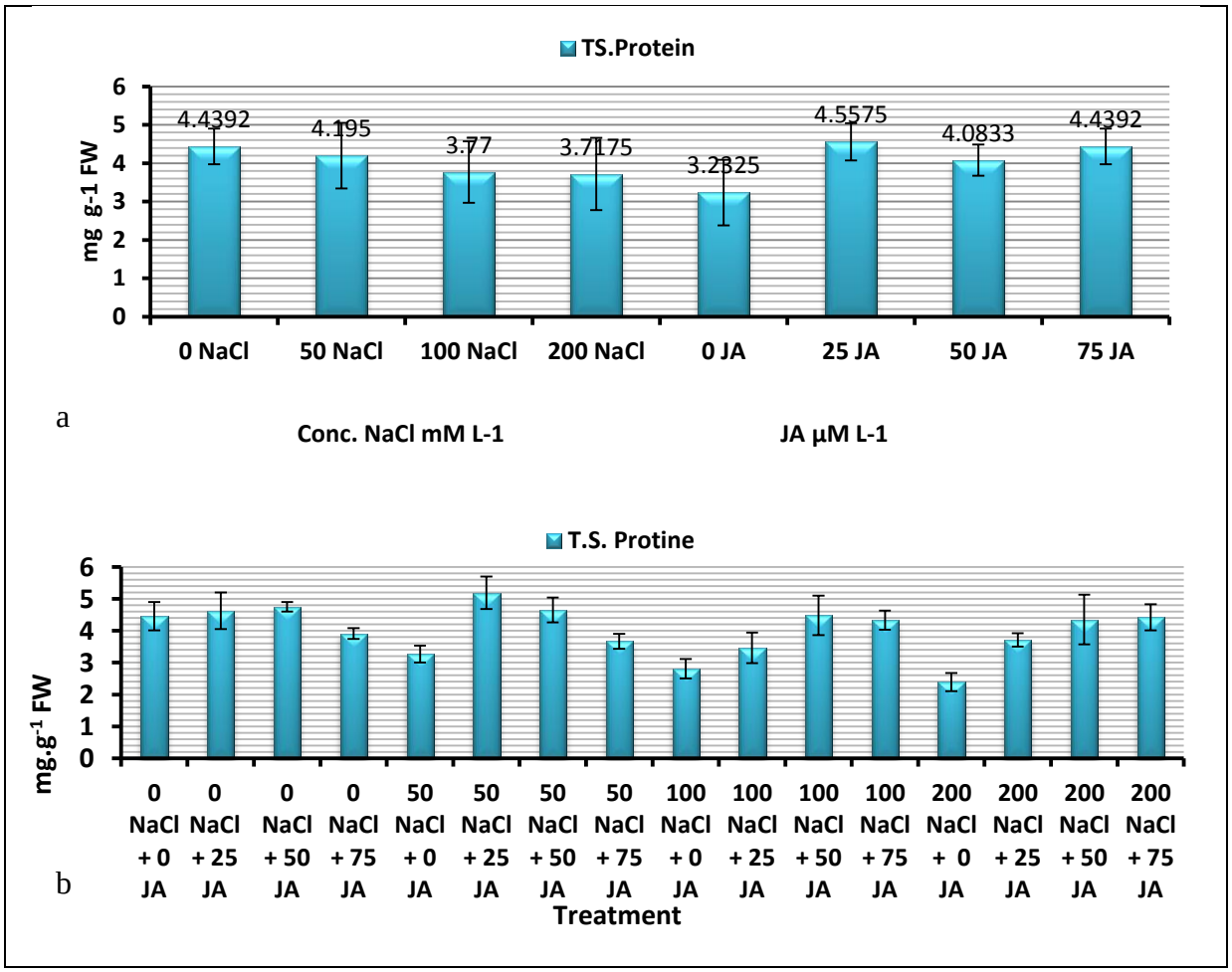

FIGURE 7. a: Effect NaCl concentration and JA concentration, b: Effect of bilateral overlap of NaCl and Jasmonic acid on total soluble proteins content in date palm cultures cv. Shakar in vitro at $p < 0.05$, a-[LSD N OR JA= 0.840], b-LSD = 0.485,

DISCUSSION

Jasmonic acid has been shown to mediate proper plant growth in both normal and stressful conditions, as well as playing a unique role in stress signaling [34,35]. Salinity can reduce plant growth through osmotic effects, toxicity of ions, nutrient uptake imbalance, or a combination of these factors [36,37]. In our present study, shoots multiplication of plants Phoenix dactylifera L. cv Shakar exposed to high concentration of salinity showed a reduction in growth parameters, the growth of date palm reduced substantially with increasing salinity. Our results were similar to [38]. Under salt stress ,decreased water content [39], can potentially retard plant growth [40], this reduction in morphological parameters may be attributed to NaCl-induced inhibition of cell division and cell elongation [42], through direct effects on the efficiency of transport proteins like H+ ATPase and H+ PPase [41]. During prolonged exposure to salt stress, ABA synthesis is stimulated, leading to stomatal closure, resulting in oxidative stress, and a decrease in photosynthesis, aging is accelerated, and plant growth is inhibited [43].

In the present study, the application of JA at a low concentration of 25 mM contributed to enhancing the growth parameters and water content of the adventitious shoots of date palm that were non-stressed by salt while showing a weak response to higher concentrations of JA, as well as, as well as the supplement of JA to the medium enhanced the response to different levels of salt stress. JA has been used for elicitation as well as propagation in several other species, in various studies, with varying results. in lavender, the use of the lower JA concentrations did not influence the growth parameters measured, whereas at the higher concentrations (1 and 1.5 mg dm-3) JA caused growth inhibition and a decrease in plant weight [44]. Simultaneously, medium containing JA (0.5, 1, and 2.0 M) stimulated the growth of potato plants [45]. In Pyrodwarf bear rootstock and Gisela 6 cherry rootstock, high JA concentrations (0.5, 1, and 2 mg/L) inhibited the development and growth of shoots and roots, while a low concentration (0.2 mg/L) promoted these processes [46].

Photosynthesis pigments are a fundamental indicator of the effect of various abiotic stress factors on plants [47]. In our study, chlorophyll pigment synthesis is reduced under salinity stress. In similar studies, Chlorophyll biosynthesis is reduced in NaCl-stressed plants [48,49], as well as chlorophyll stability index [50], treatment with 200 mM NaCl may have damaged pigment molecule ultrastructure by speeding up chlorophyll de gradation or inducing chlorophyllase activity [51]. Salt stress has a negative impact on many aspects of the photosynthetic process, including chlorophyll content and damage to the photosynthetic machinery, with reduced or stopped electron transport for the development of reductants (NADPH) and ATP for the Calvin cycle [52]. On the same level, a clear decrease in the content of carotene has been observed. It is also known that carotene is a non-enzymatic antioxidant, which keeps chlorophyll from being destroyed under conditions of light oxidation by photorespiration or by the activity of free radicals that work to oxidize chlorophylls [53].

The study showed that the chlorophyll and carotene content improved significantly when JA was applied under NaCl stress treatments, as well as, when Jasmonic was applied alone at a low concentration of 25 mM, but its content decreased significantly at higher concentrations of JA. Increased production of carotenoids may terminate radical reactions by binding to the attacking free radicals and degrade to generate non-radical products [54]. Our results were similar to earlier reports. in Soybean, MeJA application enhanced chlorophyll content by neutralizing the inhibitory effect of salt stress on pigments which could be one of the reasons for an increase in the photosynthetic rate [55]. The application of JA on protected NaCl-stressed S. Lycopersicum plants contributed to the preservation maintaining the contents of photosynthetic pigments[56], thereby contributing to the growth by maintaining the production of photoassimilates [57,58].

High salinity leads to the production of reactive oxygen radicals (ROS) in plants that serve to form oxidative forms and ultimately lead to oxidative stress that damages biomolecules (DNA and proteins), inactivates enzymes and causes oxidation of free fats and production of lipid peroxidation [59]. Under salinity stress, the activity of antioxidant enzymes such as CAT and POD. These results show that antioxidant enzymes, which function as a scavenging mechanism, are the first line of protection against oxidative damage when exposed to salt [60,61].

The present study showed that application of JA induced an antioxidant mechanism by regulating the activity of POD, CAT and other enzymes during both standard treatments and NaCl stress treatments. data showed that exogenous application of JA at a concentration of 50 mM increased the activity of peroxidases enzymes (POD) in date palm seedlings root [63]. In soybean plantlets, JA was enhancing antioxidant enzymes activity [62]. In other words, JA protects plants from NaCl damage by enhancing antioxidant metabolism, osmolarity synthesis, and metabolite accumulation when externally application [64]. The mechanism by which JA modifies antioxidant systems is still unknown. Changes in gene transcription, translation, or post-transcriptional modifications may all be

ways for JA to affect enzyme activities. The organ-specific nature of this hormone, on the other hand, indicates that its effects are closely regulated and responsible for directing specific sub-cellular metabolic changes [65].

In vitro culture systems subjected to salt stress have shown proline accumulation in response to increased salinity, providing a biochemical marker useful in the selection and manipulation of plant tolerance to abiotic stress [5]; [61]; [23]. Proline synthesis in date palm plants is a consequence of salt stress that induced changes in osmotic pressure with increased water absorption. This osmoprotectant is produced as a first response mechanism of plants under different types of stress and also is related to the improvement of the salinity tolerance [37]. Furthermore, other proline features are related to antioxidant by reducing the effects of reactive oxygen species (ROS) [20], and plasma membrane [25], Proline production affects protein turnover and stress-protective protein regulation [40]. while soluble protein was mostly decreased [6].Negative effects on the growth of plants exposed to salinity have been reported in several species in a number of studies [46]; [48].

In the present study, the application of JA on culture media contributed to Enhancement in the accumulation of proline and increase of total soluble proteins on salt stress treatments. The free praline content also significantly increased under NaCl stress, while MeJA caused a reduction in free proline content [28], treated plants might have been due to an increased expression of its biosynthesizing genes [48]. Exogenous application of JA increased proline accumulation compared to control in both normal and salt stress treatments [23], providing extra protection to the photosynthetic machinery and energy for rapid stress recovery once the stress was removed [47]. in soybean foliar spray of JA (0.5 mM) was enhancing of total soluble proteins on salt stress treatments. accordingly, Equilibrium in the accumulation of proline may contribute due to the application of JA to salt stress tolerance in Phoenix dactylifera L. through regulation of osmosis, resulting in tissue water content regulation, improved growth, and biomass yield of stressed plants.

CONCLUSION

Shoot multiplication growing in media culture with elevated salt concentrations (200 mM) in response to oxidative salt stress, which in turn reduced growth parameters, biomass, water content, the content of chlorophyll and carotene pigments, chlorophyll stability index, and total soluble proteins, while proline accumulation, POX, and CAT antioxidant enzymes significantly increased in response to salt stress effects. In a similar direction, the application of JA at a concentration of 75 μM reduced most growth parameters at the control level, and a low concentration of 25 and 50 μM of JA was the optimal concentration in pushing plants to physiological and morphological development. This may be since plants growing in industrial agricultural environments need very small amounts of some Plant hormones such as JA to growing and develop in contrast to the high concentrations that harm the plantlet. the application of JA to the culture media under the influence of salinity contributed to the improvement of growth parameters (number and length of shoot multiplication), biomass and water content of cultures, total chlorophyll, carotene, and chlorophyll stability index, as well as enhanced Proline and enzymatic antioxidant activity.

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