Effect of Jasmonic Acid on Stimulating The Growth and Development of Date Palm Callus (Phoenix Dactylifera L.) Cultivar Shukar in Vitro Under Salt Stress Conditions

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Abstract

The study was conducted in the tissue culture laboratory at the Date Palm Research Center, University of Basrah. Using the date palm callus, Shukar cultivar, obtained from culturing the apical and axillary buds in sterile artificial nutrient media, to determine the effect of adding (0, 25, 50, 75) µM concentrations of Jasmonic acid (JA) to the MS media supplemented with several concentrations of NaCl (0, 50, 100, 200) mM were added, on the growth response and morphological development of callus after four months of cultivation in the propagation media. Results showed a significant decrease in growth characteristics in response to an increase in salt level, without the degree of callus browning that's increased with the increase in the level of sodium chloride, especially at the two levels (100, 200) mM. Results showed also that the addition of Jasmonic acid to the saline treatments greatly enhanced the growth parameters of culture callus, as the rate of biomass, water content, relative growth rate, and the number of somatic embryos generated. as well as the degree of browning decreased significantly according to the levels of sodium chloride and the concentrations of added Jasmonic acid. The study demonstrated the effective role of exogenous addition of Jasmonic acid *in vitro* culture systems in enhancing stress tolerance of cells and tissues cultured in saline media.

Keywords: Callus, Date palm, Jasmonic acid, Salt stress, In vitro.

1. Introduction

The date palm trees (*Phoenix dactylifera* L.) were considered one of the most important economic fruit trees in the Middle East [1]. Because of its essential role in the agricultural economy by enhancing food sources and preserving the environment [2]. Salinity is one of the main problems that affect the growth and productivity of palm directly after drought and heat, and the reason is due to the high ionic toxicity that generates acute osmotic pressure that hinders the absorption of water and minerals that nourish cells and plant tissues [3]. The use of modern technologies, tissue culture and studies on plant cells and tissues, it can provide some information about the nature of adaptation mechanisms to salt stress. However, there are some differences between modern and differentiated tissues developed by evolution in functional tasks to determine the mechanisms of tolerance and adaptation to salinity [1, 4]. Plant cell and tissue culture technology can be used to examine plant response to salinity by exposing cells or plant tissues to different levels of salt stress [5], Callus cells are defined as a group of cells derived from specialized source tissues that are cultured under laboratory conditions to form an undifferentiated mass of cells [6, 7]. Plants produce callus cells after exposure to various harsh growing conditions, and this is a regeneration of the plant for a new development program and occurs in most studies by hormonal stimulation [8, 9].

in vitro culture techniques provide ideal environments for studying the response of undifferentiated callus cells to salt stress, where it was found that the performance of the callus expresses the response of the whole plant to salinity stress and through it it is possible to eliminate the genetic and morphological variance of the tissues of the whole plant or the plant type itself [10], some recent studies were conducted using date palm tissue culture technology to determine the response of cells to saline stress using different concentrations of saline solutions within industrial nutrient media. It was noticed that the growth curve is negatively affected with the increase in the concentration of brine solutions. In addition, Na+ accumulated in cellular tissues, and the harmful effect of salts was reduced during simple periods of treatment on callus tissues and somatic embryos [1, 11]. Alturki [12] found that concentrations less than 100 mM have minor effects in determining or inhibiting growth and weight of fresh weight of date palm callus. While it was confirmed that the higher concentrations of 100 mM up to the

concentration of 300 mM significantly affected and inhibited the growth and caused the death of the callus tissue.Hussein and Khierallah [13] explained that treatment with sodium chloride at a concentration of 1500 ppm led to stimulating the growth of date palm callus cultivar Barhi and the rate of fresh weight increased and the formation of vegetative embryos, while high concentrations (3000 and 4000) ppm led to a decrease in the growth rate. It was inhibited and the fresh weight rate decreased to the lowest weight and the vegetative embryos did not form in the individual treatments. At the cellular level, plants display a wide range of responses to environmental stress conditions (biotic and abiotic) by constructing defensive or warning signaling compounds, induces cells to synthesis different plant hormones, which is basically one of the requirements for the different stages of development in the plant and helps it in the defensive response to stress [14, 15], considered important in regulating plant physiological activities as well as regulating plant resistance to biotic and abiotic stresses [16, 17].
Jasmonates (JAs) are a newly discovered growth regulator in the plant kingdom [15, 18]. The most important of them are (Jasmonic acid (JA) and methyl Jasmonate (MeJA), participates in various physiological activities in the plant and has proven its effectiveness in improving the plant's resistance to various stress conditions, especially salinity, drought and temperature conditions (high and low), and insect infestation and the extent of their impact depends on the type of plant and the dose used, as it has important roles that help the plant adapt to environmental differences, and it is considered derivatives of fatty acid metabolism [19, 20]. Esa and Abdul-Hussein [21] showed that the addition of methyl Jasmonic (MeJA) to the nutrient media at 2 mM concentration improved the growth characteristics (fresh weight and dry weight) of callus tissues and vegetative growths of citrus rootstock Troyer Citrang grown under saline stress ex vivo. Where JA works to regulate plant adaptation to salinity stress and develop resistance mechanisms in plants [22, 23]. Andrys *et al*. [24] indicated that the addition of JA within the components of the nutrient media for propagation of lavender plant ex *in vitro* a concentration of (0.5) mg. L^{-1}. increased the activity of antioxidants, bacterial growth, and other pollutants. As well as improving growth and development characteristics, while the use of high concentrations (1-2) mg. L^{-1} led to inhibition of the growth of plant parts. Rawat *et al.* [25] show that the use of MeJA at concentrations ranging from 0.1-20.0 µM within the components of the nutrient media in plant tissue culture reduces the activity of harmful phenolic substances, helps in the production of antioxidants, stimulates the activity of antioxidant enzymes, and reduces the accumulation of reactive oxygen species, he also explained the joint role with other plant growth regulators such as NAA, Kn and BA in improving the response of plant parts to growth and development. In view of the absence of any prior study on the effect of Jasmonic acid on the growth and development of salt-stressed date palm callus, the current research aims to study the effect of Jasmonic acid and sodium chloride and their interactions on some morphological and physiological characteristics of date palm callus, cultivar Shukar, *in vitro*.

2. Materials and Methods

2.1 Preparation of culture media

This study was conducted in the tissue culture laboratory of the Palm and Dates Research Center, University of Basrah. The media for primary callus propagation was prepared using 4.9 g. L^{-1} of media [26] from the Indian company HiMedia Laboratories Pvt., added to it are 40 g. L^{-1} Sucrose, 170 mg. L^{-1} Sodium Hydrogen Ortho Phsphates, 40 mg. L^{-1} adenine sulphates, and 1.5 g. L^{-1} neutralized activated charcoal. The media was supplemented with auxin NAA at a concentration of 10 mg L^{-1} and cytokinin 2ip at a concentration of 1.5 mg L^{-1} [27], the pure sodium chloride salt NaCl produced by the Indian company Central Drug House (P) Ltd. was added. At concentrations (0, 50, 100, 200) mM to the culture media and the acidity of the media was adjusted to pH 5.7 using NaOH and HCl solutions at a concentration of 0.1 N each, Agar was added to the media at a rate of 7 g. L^{-1} and the media was copied to 90 °C for the purpose of dissolving the agar and homogenizing the media. Distribute the media in culture tubes (Pyrex) size of 2.5 x 18 cm at a rate of 20 ml per tube. The culture tubes and all culture tools were sterilized in an autoclave at 121°C and a pressure of 0.1 MPa for 20 minutes. After the sterilization process, the culture tubes were transferred to the Laminar air flow cabinet after being sterilized with UV light, 70% ethanol alcohol and diluted chlorine. Jasmonic acid (JA) produced by the Indian company HiMedia Laboratories Pvt.Ltd has been added at the concentrations are (0, 25,50, 75) µM after sterilization by filtration method, the tubes were closed tightly and kept until the cultivation process.

2.2 Primary callus transplant

The study used the primary callus induced from the cultivation of quarters of the apical buds and axillary buds of date palm offshoots of the cultivar Shukar, which were obtained from one of the orchards of Hilla city in Babylon Governorate at the age of (3-4) years. Where the buds were excised, sterilized, and cultured in the laboratory in the medium of MS for callus induction based on Al Khalifa [28], four months after the formation of the initial callus, it was transferred and cultivated in culture tubes of the propagation medium prepared above and provided with the experimental treatments for the study. Culturing was carried out in Laminar air flow cabinet. After sterilization with UV rays and ethanol alcohol at a rate of 100 mg per tube, the cultured tubes were transferred to the growth chamber, and incubated at 27 ±1 °C and illumination intensity

1000 lux for 16 hours. Day^{-1}. Palette.1. The process of callus propagation took four months, and the culture was re-cultivated every five weeks, after which measurements were made for the morphological and physiological characteristics of the callus, with three replications for each trait (Figure, 1).

2.3 The experimental measurements

2.3.1 Determination of the fresh weight (g.treatment^{-1})

The fresh weight of the callus was calculated directly for one replicate for in each treatment, where the contents of each planting container (refined/treatment) were taken out of the nutrient medium and washed well with sterile distilled water, then placed on filter paper to get rid of water residues, the fresh weight was measured using a sensitive balance and sterilized with 70% ethanol alcohol, this process was carried out inside a stratified air flow cabinet.

2.3.2 Determination of dry weight (g treatment^{-1})

The dry weight of the callus was determined by drying the samples whose fresh weight was measured in an electric oven at a temperature of 65 °C for 48 hours. After the weight was stabilized, the dry weight was calculated using a sensitive electric balance.

2.3.3 Calculation of the average number of somatic embryos for each treatment

2.3.4 Determination of the degree of brown discoloration

The response of the callus to brown discoloration for all treatments was detected by observation, according to the indicator used by [29] depending on the degree scale: -, +, ++, +++ with their interpretation as non-response, weak, medium, high, Respectively.

2.3.5 Determination of water content:

The water content of callus was estimated using the following equation:

$$Callus\ water\ content\ (\%) = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weigh}} \times 100$$

2.3.6 Estimated Relative Growth Rate

The relative growth rate of the callus was estimated using the following equation [30]:

$$Relative\ growth\ rate = \frac{\text{Final fresh weigh} - \text{initial fresh weight}}{\text{Growth periods (120 days)}}$$

2.4 Experimental Design and Statistical Analysis

The experiment was applied as a factorial experiment using CRD (Completely Randomized Design), and the results were analyzed using analysis of variance (ANOVA), the averages were compared according to the LSD (Least Significant) test at a probability level of 0.05, using the SPSS v.21 program to analyze the results. And at a probability level of 0.05.

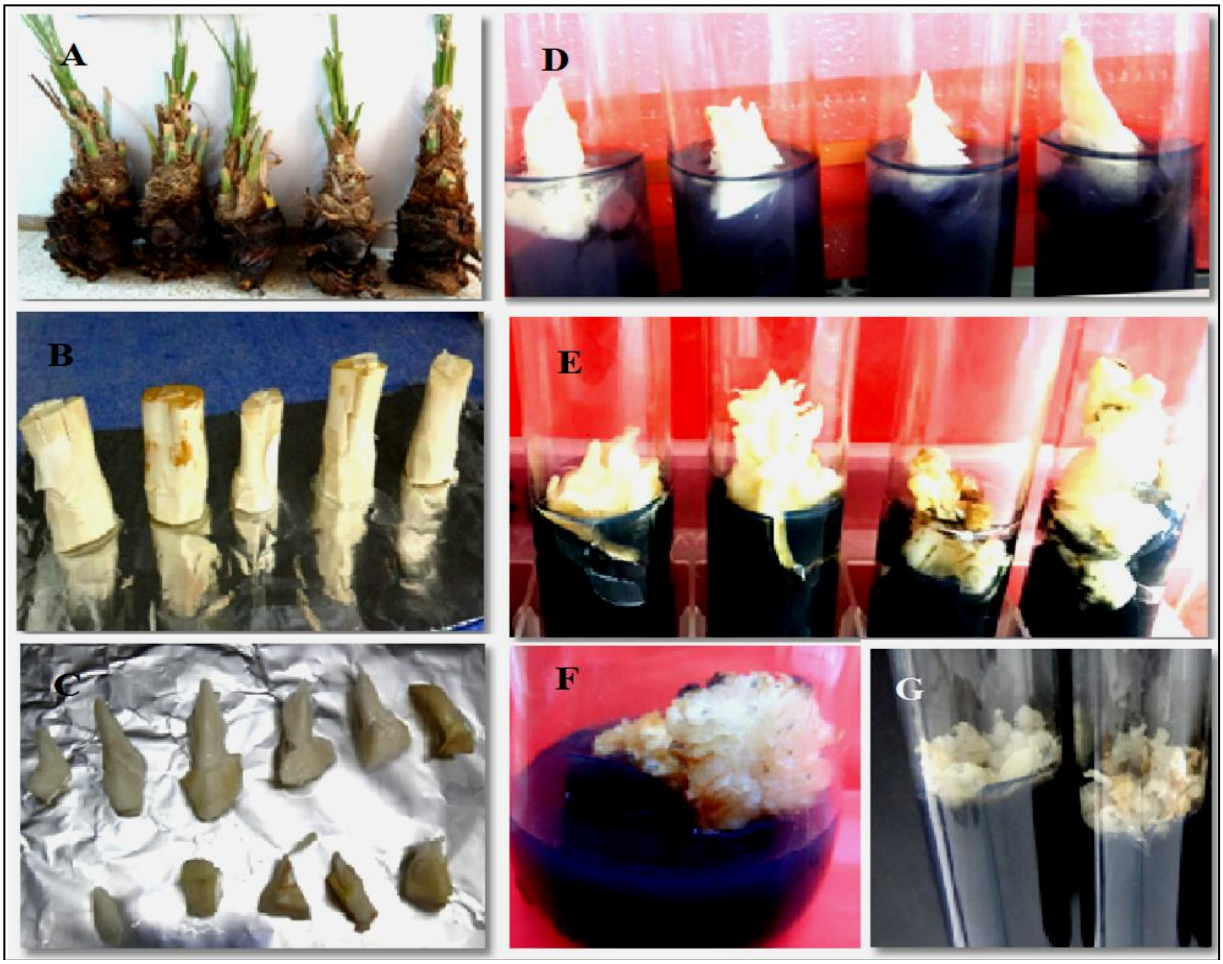

Figure 1. Preparation of the plant part for *in vitro* cultivation: **A** date palm offshoots of Shukar cultivar after removing the outer leaves, **B** the apical bud surrounded by the leaf covers with a part of the calyx tissue, **C** the excised apical and axillary buds, **D** the cultivation of quarters of the excised buds in the center of callus induction **E**, an evolution in the growth of the plant culture and an increase in its size, **F**, the emergence of callus on the surface of the plant culture (buds), **G** transfer of the induced callus to the propagation media.

3. Results

3.1 Fresh weight of the callus

Table 1. show the effect of the rate of single factors (Jasmonic acid, sodium chloride salt) on the fresh weight of callus. Whereas, the addition of NaCl caused a significant decrease in the average fresh weight of callus by increasing the levels of salt stress, except for the 50 mM treatment, which achieved the highest fresh weight rate of callus, which was 2.22 g. treatment^{-1}, with a significant difference from the 200 mM treatment, which achieved the lowest callus fresh weight rate of 1.17 g. treatment^{-1}, while the results showed that the addition of Jassmonic acid to the nutrient medium for callus propagation had contributed to a significant increase in the rate of fresh weight of callus, especially at the concentration 25 µM, which did not differ significantly from the concentration 50 µM, as the two treatments gave the highest rate of fresh weight amounted to (2.00, 1.94) g Treatment^{-1}, respectively.

The same table shows the interference effect of JA and NaCl salt on the fresh weight of callus. Where the results show that the addition of JA acid had a significant effect on increasing the fresh weight of callus under the influence of salt stress, but it did not rise to the level of the NaCl-free control treatment, which significantly outperformed all the treatments under study. Where it gave the highest rate of fresh weight of callus was 2.73 g. treatment^{-1}.The results in the same table showed the significant superiority of the control treatment (0mM NaCl + 0µM JA) overall treatments under study, as it gave the highest rate of the studied characteristic 2.73 g. Treatment 1. The results also showed that JA contributed to enhancing the measured characteristic in the treatments under all levels of saline, but less than the comparison level. At the same time, a sharp

decrease in the fresh weight of the callus was observed in response to the increase in the salt level in the growth medium, so that the treatment (200 mm NaCl + 0 µM JA) recorded the lowest rate of 0.74 g. Treatment^{-1} compared to all treatments under study.

Table 1. Effect of treatment with sodium chloride, Jasmonic acid and bilateral overlap on the fresh weight of date palm callus, Shukar cultivar *in vitro* (g. treatment^{-1}).

Concentration of Jasmonic acid	Concentration of NaCl Mm				Jasmnic
Mµ	0	50	100	200	average
0	2.73 ± 0.04	1.92 ± 0.06	1.44 ± 0.03	0.74 ± 0.06	1.71 ± 0.76
25	2.30 ± 0.05	2.51 ± 0.05	2.03 ± 0.03	1.15 ± 0.07	2.00 ± 0.54
50	1.71 ± 0.04	2.38 ± 0.06	2.29 ± 0.04	1.38 ± 0.03	1.94 ± 0.43
75	1.21 ± 0.01	2.07 ± 0.03	2.32 ± 0.04	1.42 ± 0.04	1.76 ± 0.48
Average NaCl	1.99 ± 0.60	2.22 ± 0.25	2.02 ± 0.37	1.17 ± 0.29	
L S $D_{0.01}$	NaCl * JA = 0.07		JA = 0.1	NaCl= 0.1	

3.2 Dry weight of callus

Table 2. showed that the average dry weight of callus was significantly affected by the experimental factors and according to the added concentrations, and the sodium chloride addition factor did not make a significant difference in the first three treatments except for the high salt stress treatment (200 Mm), which achieved a significant decrease in the mean of the measured characteristic that amounted to 0.176 g. treatment^{-1}, the highest average dry weight of callus in treatment 50mM, which was 0.251 g. treatment^{-1}. The results of the same table showed that the treatment with different concentrations of Jasmonic acid led to a significant difference between the treatments in the measured trait. The treatment 50 µM was superior to the average dry weight of date palm callus, which did not differ significantly from the 25 µM treatment, where the two treatments achieved the highest rate for that trait amounting to 0.240 and 0.236 g respectively.

As for the effect of the interaction between the two experimental factors, the results show in the same table that the control treatment (JA 0Mm NaCl + 0 mM JA) was significantly superior to all the interaction treatments in the dry weight characteristic of callus at a rate of 0.284 g., treatment^{-1}, which did not differ significantly from the treatment of low salt stress (50 mM NaCl + 25µM JA) followed by (JA 50Mm NaCl + 50µM), the results showed a significant effect of adding jassmonic acid to the nutritional medium in improving the studied trait. The two treatments gave a high rate of callus fresh weight of (0.268, 0.263) g. treatment^{-1}, respectively, compared to treatment (JA 50Mm NaCl + 0µM), which achieved a rate of 0.223 g. treatment^{-1}. The results of the study also showed a significant increase in the mean of the measured characteristic within the interaction coefficients under the influence of medium salt stress, especially in the treatment (100 Mm NaCl + 50 µM), which gave a high rate of 0.261 g. treatment^{-1}, with an increase of 12.64% compared to the treatment (JA 100Mm NaCl + 0 µM), which gave a lower rate for the same trait that amounted to 0.228 g. treatment^{-1}, as for the interaction treatments under high salt stress, the treatment (+50µM (200Mm NaCl) that did not differ significantly from the treatment (JA 200Mm NaCl + 75µM) at a rate of (0.210 and 0.205) g. treatment^{-1} respectively, with an increase of (48.10 and 46.83)% respectively compared to the treatment (JA 200Mm NaCl + 0µM), which achieved the lowest mean of the measured characteristic of 0.109 g. treatment^{-1}.

Table 2. Effect of treatment with sodium chloride, Jasmonic acid and bilateral overlap on the dry weight of date palm callus, Shukar cultivar in vitro (g. treatment^{-1}).

Concentration of Jasmonic acid	Concentration of NaCl Mm				Jasmonic
Mµ	0	50	100	200	average
0	0.284 ± 0.014	0.223± 0.004	0.228 ± 0.009	0.109 ± 0.011	0.211 ± 0.067
25	0.258 ± 0.004	0.268 ± 0.005	0.238 ± 0.009	0.179 ± 0.004	0.236 ± 0.037
50	0.226 ± 0.005	0.263 ± 0.012	0.261 ± 0.019	0.210 ± 0.004	0.240 ± 0.027
75	0.219 ± 0.010	0.249 ± 0.003	0.241 ± 0.004	0.205 ± 0.004	0.229 ± 0.019
Average NaCl	0.247 ± 0.028	0.251 ± 0.019	0.242 ± 0.016	0.176 ± 0.042	
L S $D_{0.01}$	NaCl * JA = 0.017		JA= 0.020	NaCl= 0.020	

3.3 Average number of embryos

Table 3. shows that there are significant differences for the effect of adding the two experimental factors on the average number of embryos formed from the callus. it was found that increasing the level of salt stress has contributed to reducing the rate of the number of embryos formed in response to the increase in concentration. While the concentration of 50 mM was significantly superior, and a rate of 6.58 somatic embryos treatment^{-1} was recorded, and it did not differ significantly from the concentration of the comparison 0 mM in the mean of the studied trait. It was noted from the table that there were no significant differences for the factor of Jasmonic acid in the number of vegetative embryos formed, except for the concentration of 75 µM, which recorded the lowest number of vegetative embryos formed, which amounted to 3.58 somatic embryos, with a significant difference from the comparison treatment 0 µM, which recorded an average of 5.67 somatic embryos.

As for the effect of the interaction between the two experimental factors, the comparison treatment (0mM NaCl + 0µM JA) was significantly superior to all treatments under the control level with an average of 11.67 somatic embryos compared to the treatment (0mM NaCl + 75 µM JA) which did not record any rate of the number of somatic embryos. The results in the same table also show that there is no significant effect of Jasmonic acid under the low salt stress level (50µM), and there are non-significant differences between the treatments within this level. While Jasmonic contributed to reducing the effect of medium salt stress, as the treatment (100Mm NaCl + 75Mm JA) recorded an average of 6.00 somatic embryos, with an increase of 38.67% compared to the treatment (100mM NaCl + 0µM JA), which recorded an average of 3.68 somatic embryos. Although there was a significant difference in the average number of embryos under the high salt stress level by increasing the concentration of Jasmonic, it was very slight, as the treatment (200mM NaCl + 0µM JA) recorded the lowest rate for the studied trait which was 0.68 somatic embryos (Figure,2).

Table 3. Effect of treatment with sodium chloride, Jasmonic acid and bilateral overlap on the number of somatic embryos formed for date palm callus of Shukar in vitro cultivar (somatic embryo. treatment^{-1}).

Concentration of Jasmonic acid	Concentration of NaCl Mm				Jasmonic acid
Mµ	0	50	100	200	average
0	11.67 ± 1.53	6.67 ± 0.58	3.68 ± 0.58	0.68 ± 0.58	5.67 ± 4.31
25	6.67 ± 0.58	6.67 ± 0.58	3.00 ± 1.00	1.68 ± 0.58	4.50 ± 2.39
50	4.33 ± 0.58	7.00 ± 1.00	5.33 ± 0.58	2.33 ± 0.58	4.75 ± 1.87
75	0.00	6.00 ± 1.00	6.00 ± 1.00	2.33 ± 0.58	3.58 ± 2.75
Average NaCl	5.67 ± 4.46	6.58 ± 0.79	4.50 ± 1.45	1.75 ± 0.87	
L S $D_{0.01}$	NaCl *JA = 1.21		JA = 1.74	NaCl= 1.74	

3.4 Browning degree of coloration

Table 4. show that the effect of adding specific concentrations of Jasmonic acid JA to the medium of callus propagation under several levels of salt stress of NaCl on the degree of browning, it was noticed from the table that there are significant differences between the treatments in this trait, as it was noticed that there was no brown discoloration of the callus (-) in the comparison treatment (0Mm NaCl + 0µM JA). The degree of brown coloration was medium (++) in the treatment (50mM NaCl + 0µM JA), then it increased more (+++) with the increase in the level of NaCl in the treatment (200mM NaCl + 0µM JA). It was noted from the results that the addition of JA at a concentration of 25 µM at the standard level was weak (+), and the addition of Jasmonic at the same concentration contributed to reducing browning to a large extent at the low level of NaCl so that it was non-existent (-) in the treatment (50mM NaCl + 25µM JA) and it continued Weak (+) in the last two concentrations of Jasmonic acid at the same level of salt stress. Also, it was noted that the degree of brown coloration was medium (++) in the 50 µM Jasmonic acid treatment, and weak (+) in the two treatments (100 mM NaCl + 25 µM JA, +50 µM JA).The same table also shows the effect of adding JA to the callus propagation media under high salt stress, which contributed to reducing the degree of brown discoloration from high (+++) at 75 µM salt-free to weak (+) in the two treatments (100Mm NaCl + 50µM JA, +75µM JA) Figure (2).

Table 4. Effect of the rate of sodium chloride and Jasmonic acid on the degree of brown coloration of date palm callus, cultivar Shukar *in vitro*.

Concentration of Jasmonic acid	Concentration of NaCl mM			
Mµ	0	50	100	200
0	-	++	++	+++
25	+	-	+	+
50	++	+	+	++
75	+++	++	+	+

3.5 Water content of callus %

The results show in Figure (1, A) the effect of single factors (NaCl, JA) on the average percentage of water content of Shukar date palm callus in the propagation stage. The figure shows the significant superiority of NaCl at the level of 50mM, which did not differ significantly from the level of 100Mm, where the two treatments recorded the highest rate of (88.64, 87.66)%, respectively, compared to the 200Mm treatment, which achieved the lowest rate for that trait of 82.07%. The same figure shows the effect of Jasmonic acid on the mean of the measured characteristic, as it shows the significant superiority of the concentration of 25 µM, which did not differ significantly from the concentration of 50 µM, the two treatments achieved averages of (87.71 and 87.26)%, respectively. Figure (2, A) shows a decrease in the percentage of the water content of callus by increasing the concentration of Jasmonic acid, whereby the concentration of 75 µM showed the lowest rate for the studied characteristic.

The results shown in Figure (2, B) also indicate the effect of the interaction between the two experimental factors on the average water content of callus. Where the treatment (0mM NaCl + 0µM JA) achieved a significant superiority in this trait at the control level with an average of 89.64%, and the treatment (0Mm NaCl + 25µM JA) had a similar significant effect at the same level. The same figure shows that there is no significant difference between the treatments under low and medium saline stress except for the treatment (100mM NaCl + 0µM JA), which recorded a lower rate of water content under medium saline stress. While (200mM NaCl + 0µM JA) showed a significant and significant decrease under high salt stress, as this treatment recorded the lowest rate for that characteristic amounted to 73.49% compared to all treatments under study.

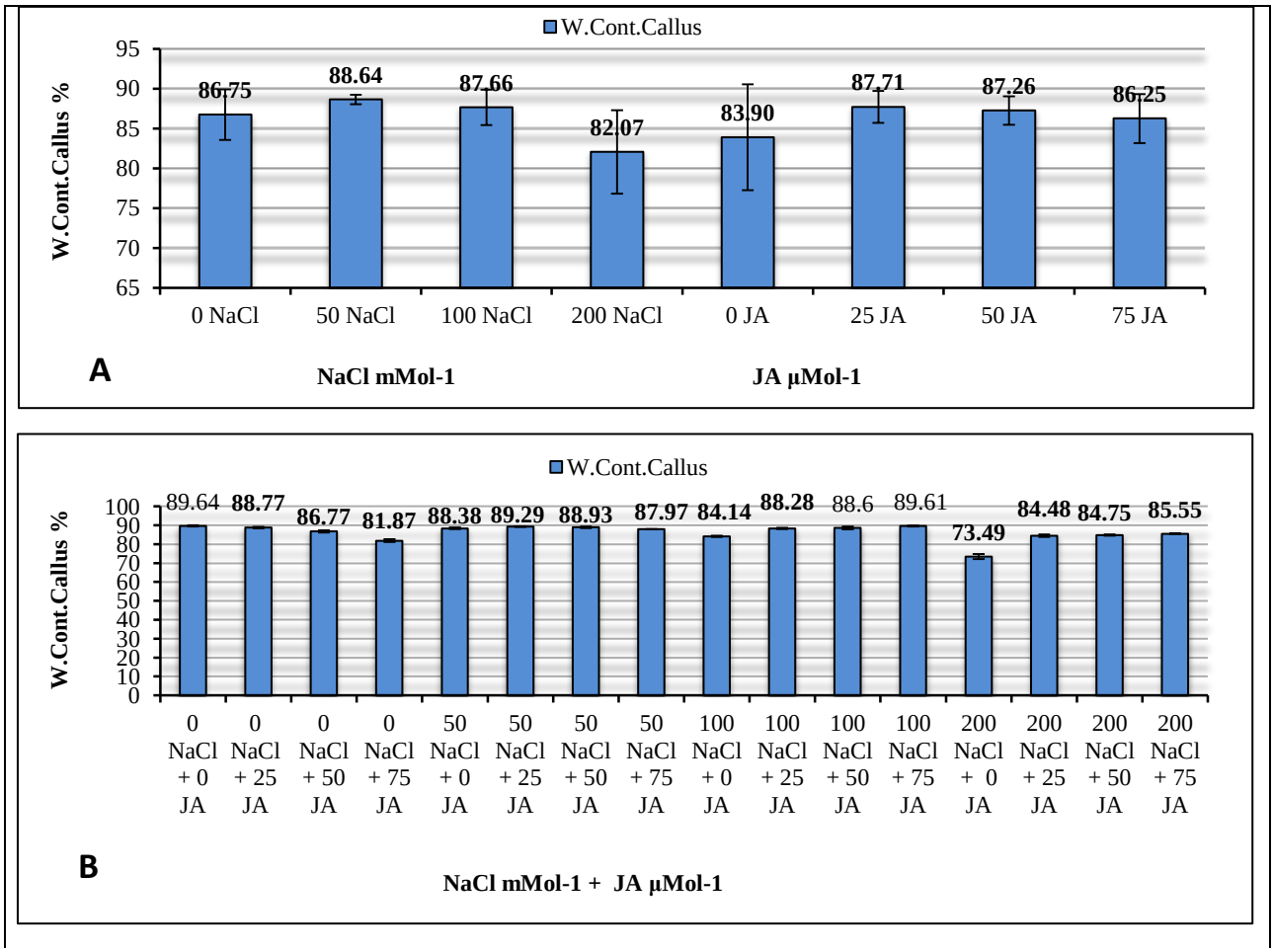

Figure 2. A: Effect of single agents (NaCl, JA), B: Effect of bilateral overlap NaCl and Jasmonic acid concentrations on the water content of Shukar date palm callus *in vitro*, LSD: NACL = JA = 1.23, LSD: NA.JA= 1.74

3.6 Relative growth rate of callus

Figure (3, A) shows the effect of the individual factors of the experiment represented by sodium chloride and Jasmonic acid on the relative growth rate of callus during four months of cultivation in the propagation medium, the results indicate that the

multiplication of callus in the growth medium to which 50 mM of NaCl was added led to a significant increase in callus growth at a rate of 18.08 mg day^{-1} compared to the level of 200 mM, which gave the lowest rate of 9.35 mg day^{-1}. The results in the same figure indicate the effect of adding Jasmonic acid to the growth medium in increasing the rate of the studied trait, where the concentrations of 25 µM and 50 µM were significantly superior at rates of (16.22 and 15.74) mg day^{-1}, respectively, compared to concentration 0 µM, which gave A lower rate of relative callus growth.

The interaction of the experimental workers showed significant differences in the relative growth rate and according to the level of stress to which the callus was exposed during the growth period. Figure (3,B) shows the significant superiority of the control treatment (0mM NaCl + 0µM JA) in the mean of the studied trait in the absence of the effect of NaCl salt, the highest relative growth rate of callus was recorded at 22.33 mg day^{-1}, compared to the treatment (0mM NaCl +75µM JA), which recorded the lowest rate at the same comparison level. The addition of Jasmonic acid with different concentrations and under several levels of salt stress has significantly contributed to improving the relative growth rate of callus. Where the same figure shows the superiority of the treatment (50mM NaCl + 25µM JA) in the mean of the studied trait, which did not differ significantly from the treatment (50mM NaCl + 50µM JA), the two treatments recorded a rate of (20.47, 19.42) mg day^{-1} respectively.

Jasmonic also contributed to alleviating the harmful effect of salt stress at the level of 100 mM in increasing the relative growth rate of callus. Where the treatment (100mM NaCl + 50µM JA) and the treatment (100mM NaCl + 75µM JA) recorded a significant superiority in the mean of the studied trait with an average of (18.64,18.92) mg day^{-1} respectively. With an increase of (37.79, 38.88)% compared to the treatment (100mM NaCl + 0µM JA), which recorded a significant decrease in the growth rate of callus and under the same level of salt stress. Also, the high salt stress had a great and negative impact on the rate of that trait, as the treatment (200mM NaCl + 0µM JA) recorded the highest significant decrease in the relative growth rate of callus, which was 5.72 mg day^{-1}. The addition of Jasmonic acid contributed to improving the harmful effect of salt, specifically in the two treatments (200Mm NaCl + 50µM JA) and (100Mm NaCl +75µM JA), with an increase of (48.37, 49.78)% respectively in the relative growth rate of callus.

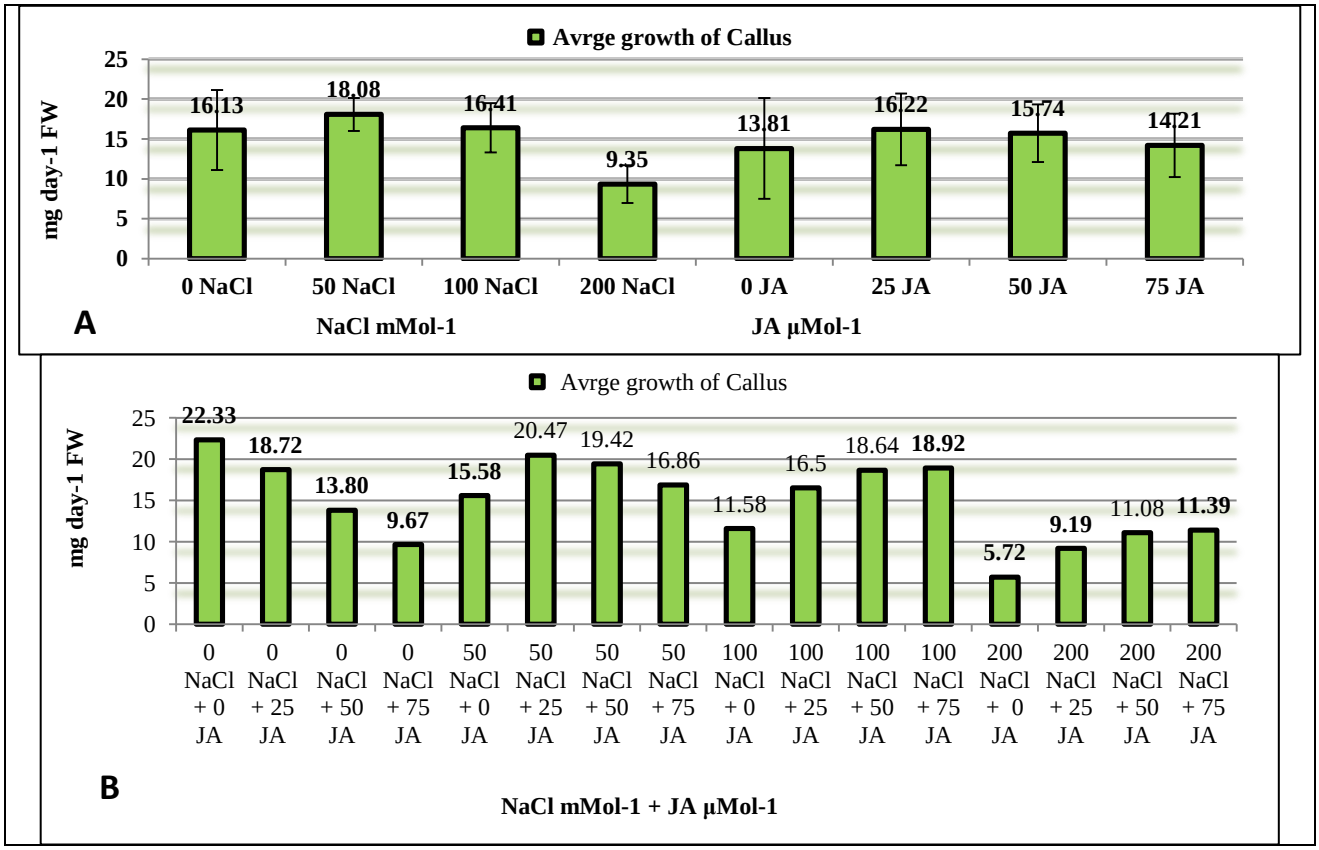

Figure 3. A: Effect of single factors (NaCl, JA), B: Effect of bilateral overlap NaCl and Jasmonic acid concentrations on the relative growth rate of date palm callus cultivar Shukar *in vitro*, LSD: NaCl = JA = 0.81, LSD: Na. JA= 1.14

Figure 4. Effect of different concentrations of Jasmonic acid and sodium chloride salt on morphological growth parameters of date palm callus cultivar Shukar *in vitro*.

4. Discussion

Salinity plays a negative and influencing role in the vital physiological processes at the cell level. Most of the previous studies and research dealt with the study of the effect of NaCl at different concentrations on callus growth. And it has been proven with scientific evidence that it exerts a harmful effect at high levels, while low concentrations may play a stimulating role in the growth of callus in a number of glycophyte plants [31]. Some studies related of other plants showed an opposite response [32,33], although the inhibitory level of NaCl varies from one genotype to another, a tendency to inhibit callus growth in response to increased salt exposure was observed in several related to date palm *in vitro* studies [34, 35, 36, 37], depending on genotypes, salt concentration, and periods of salt exposure. The results of the current study proved that the date palm callus cultivar Shukar followed the same growth inhibition pattern in response to the increase in salt concentration in terms of a decrease in fresh weight, dry weight, water content, number of embryos and relative growth rate, and the decline started at the level of 50 mM until it reached its lowest rate at the level of 200 mM, while the degree of brown coloration of the callus increased with the increase in the salt level due to the oxidation of the phenols secreted by the callus cells, which is one of the non-enzymatic defense systems that the cells exercise during different stress conditions, the decrease in morphological parameters can be attributed to the inhibition of cell division and cell elongation induced by NaCl [38], during direct effects on the efficiency of transport proteins such as H + ATPase and H + PPase during prolonged exposure to salt stress [39]. In similar studies, the response to callus fresh and dry weight and the number of somatic fetuses decreased with an increase in NaCl concentration due to higher concentrations of sodium and chloride ions and osmotic pressure. which severely affected the implanted callus, which causes cell death and brown pigmentation of the medium. These phenomena lead to a decrease in the dry weight of the callus as a result of reduced water and nutrient absorption, loss of osmotic balance of cells and loss of their filling pressure, which leads to the death of most cells and other harmful effects [40]. The decrease in callus growth as a result of treatment with high levels of sodium chloride can be explained by its effect on various physiological and biological processes, such as ionic distribution, water absorption, cellular metabolism, and hormonal balance, as well as its harmful effect on the functions of the protoplast, which in turn led to impeding the growth of date palm callus exposed to salt stress [40]. The ionic disturbance inside the cell caused by the high level of salinity causes the cell to consume a large part of its energy devoted to metabolic activities to adapt to the conditions of salt stress [41]. The salt treatments on the tissues of the date palm callus lead to the accumulation of stress-causing ions, especially the sodium ion Na^+ and the chlorine ion Cl^-. Several studies have shown that sodium chloride salt is more stressful for tissues and plant parts than potassium chloride or calcium chloride [11; 42; 43] As a result of the plant facing abiotic stress, it increases the production of reactive oxygen species (ROS), which leads to the occurrence of oxidative stress, which causes chemical damage to cellular components [5].

In the current study, when applying JA to standard treatments, it resulted in an increase in growth parameters at a concentration of 25 µM, while high concentrations of 75 µM JA caused inhibition of callus growth, decreased relative water content, growth rate, number of embryos, increased media pigmentation, and callus darkening, but they did not cause death of the callus. A similar study showed that high concentration of Jasmonate in culture medium inhibited callus biomass [44]. Jasmonate has also been reported to inhibit growth mainly by disrupting cortical microtubules [45], while the application of JA in NaCl stress treatments that decreased the growth rates, and in particular the salt treatment with 200mM NaCl, JA proved that it improved the growth parameters and mitigated the decrease in the growth caused by NaCl to a large extent, where the addition of JA led to Relative increase in biomass, relative water content, growth rate and number of somatic embryos contributed to a noticeable reduction in the degree of brown discoloration. Growth hormones make plants more resistant to stress and are essential for proper growth and protection in stressful conditions [46]. JA is classified as a growth hormone that has beneficial effects on plant growth and development [47]. It has been shown in other studies and on different plant species that JA mediates proper plant growth in both normal and stressful conditions, in addition to playing a unique role in signaling under stress [48; 19; 49; 50]. Jasmonic acid and its derivatives are known to stimulate a process called induced systemic resistance (ISR) that helps stimulate plant cells to tolerate various types of stress [51].

The improvement of the morphological parameters of the developing callus in the growth medium for saline treatments may be attributed to the role of this hormone in preserving cellular membranes from damage. It is one of the most important negative effects caused by salinity on growth, due to its effect in enhancing the effectiveness of antioxidant enzymes within cells, which work to enhance resistance to salinity damage. In addition to its role in the production of ABA abscisic acid, which is known for its role in mitigating the damage caused by biotic and abiotic stresses to the cells of plants growing in those conditions, and its application led to the synthesis of proline [52; 53; 54]. However, the mechanism by which JA stimulates antioxidant systems is still unknown. Changes in gene transcription, translation, or post-transcriptional gene regulation may all be ways for JA to influence the activities of tensile enzymes, on the other hand, the organ-specific nature of this hormone indicates that its effects are closely regulated and responsible for directing subtle subcellular metabolic changes [52]. In other words, the external application of JA protects plant cells from NaCl damage, it contributes to promoting growth by improving antioxidant metabolism, osmotic balance, and accumulation of secondary metabolites [49].

Conclusions

The callus growing in saline media showed a decrease in the response rate to various morphological growth parameters by increasing the level of NaCl added to the propagation medium. Where the dry weight and the fresh weight of the callus biomass decreased, accompanied by a decrease in the water content, the relative growth rate and the number of embryos formed from the callus. While the degree of brown coloration and pigmentation of the medium increased by increasing the salt concentration. The addition of Jasmonic acid in the standard treatments did not have a significant effect on growth parameters, and the response rate for the measured traits decreased by increasing the concentration of Jasmonic acid. While it was observed that there was an improvement in the response to the addition of Jasmonic acid in the average of the studied traits of the treatments under the influence of salt tension, and the concentration of Jasmonic was directly proportional to the added salt level in increasing the rate of the traits under study. This research provides a supportive study for the role of stress hormones in reducing the harmful effect of sodium chloride on the level of plant cells and tissues in general and for the date palm callus cells in particular.

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