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Response of Date Palm cv.Barhi Microshoots to Salinity Stress Elevation

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Abstract---Salinity is one of the serious and crucial problem facias the plant in this planet. Fortunately, plants have different lines of defending system that help to overcome such environmental stress. This study uses a different NaCl level to investigate the behavior of date palm microshoots at two stages (multiplication and elongation) of micropropagation protocol. Study found the salinity stress effect on multiplied and elongated shoots at high levels of salt 150 and 200mM. At these levels the morphological and physiological aspects are changed in compression with control and other salt treatments (25-100mM). The physiological aspects recorded elevation in the rates of carbohydrate, proline and abscisic acid when NaCl concentration increasing. However, study concluded the salinity may help to improve some morphological aspect like shoot diameter that help to increase the plant survival during acclimation stage. stage. Concentrations.

Keywords---crucial problem, elevation, environmental, plant, salinity stress.

Introduction

One of the biggest challenges facing the productivity of food crops worldwide is the salinization of arable land and stresses from various sources of biological stress. Exacerbation of these challenges has been observed in arid and semi-arid regions, where continuous changes in climate and shortage of water have reduced. The area of arable land and a decrease in the productivity of many vegetable crops, including salt-tolerant fruit trees such as the date palm, *Phoenix dactylifera*, which is one of the leading fruit crops in Iraq and the Arab world

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(Shareef & Al-Khayri, 2021). Salinity leads to harmful changes in the internal structure of cells and affects the functions of organs, which negatively affects the growth of plants. Therefore, the plant resorts to adopting mechanisms to reduce these damages or those changes. Sodium chloride ions prevent water absorption and threaten the integrity of membranes. Toxic ions such as Na^+ lead to the accumulation of types of Reactive oxygen (ROS). These effects negatively affect plant physiology, including decreased ability to photosynthesize, impaired signaling of protein receptors, and metabolic processes. These effects jointly contribute to reduced growth rates and increased ageing rates (Chiconato et al., 2021).

In the past decade, tissue culture techniques have been recognized as a powerful tool for propagation. In recent years, they have been extensively used in physiological studies of salt and water stress tolerance mechanisms and plant resistance. This technique includes some advantages in using tissue or cell culture for physiological studies, including the homogeneity of cell groups (Gaspar et al., 1996). In the past few years, many studies have shown the contrast of the complex response to biological stresses, especially salinity stresses, between seed cultivars of date palms (Abul-soad & Al-khayri, 2017) (Alhammedi & Edward, 2009).

In view of the importance of the date palm of the Barhee cultivar, the study of the differential responses to salinity is significant. Hence, the main objective of this study is to determine the effect of different concentrations of sodium chloride on the growth and development of vegetative buds of the date palm cultivar 'Barhee' cultured *in vitro*.

Materials and Methods

Experimental materials

Adventitious buds of date palm (*Phoenix dactylifera* L.) cultivar 'Barhee' was obtained from Fadak for Agricultural Plant and Animal Production Private Company, Basrah Governorate, Basrah, Iraq during the period 2019-2021. Buds were multiply continuously on Bud growth and proliferation medium (BGPM) containing Murashige and Skoog inorganic salts (Murashige and Skoog, 1962), 50.000 mg.L⁻¹ sucrose, 100 mg.L⁻¹ myo-inositol, 187.5 mg. L⁻¹ Sodium dihydrogen phosphate dehydrate ($\text{NaH}_2\text{Po}_4 \cdot 2\text{H}_2\text{O}$), 200 mg.L⁻¹ glutamine, 1500 mg.L⁻¹ Calcium nitrate tetrahydrate $\text{Ca}(\text{No}_3)_2 \cdot 4\text{H}_2\text{O}$, 80 mg.L⁻¹ Adenine sulfate, 500 mg.L⁻¹ Polyvinyl pyrrolidone, 0.1 mg.L⁻¹ kinetin(KN), 0.1mg.L⁻¹ benzylaminopurine (BAP) and 0.1 mg.L⁻¹ 6-(γ,γ -Dimethylallylamino)purine (2iP) (Almusawi, et al., 2017).

Impact of salinity on bud growth and proliferation

Adventitious buds obtained from the last step of multiplication were subjected to various concentrations of NaCl (50, 100, 150 and 200 mM) to study the salinity stress on bud growth, proliferation rate and development *in vitro*. To achieve this step a clump of vegetative buds (8- 10 buds) were inoculated to a (BUGM) supplemented with different concentrations of NaCl.

Incubation conditions

The cultures were incubated in growth chamber at 27±2°C. A16/8 h (light/dark) photoperiod provided by 100 w. LED light fixed 40 cm above the rack.

Estimation of morphological parameters

Bud numbers, length and diameter

Bud numbers, length and diameter were calculated after 8 weeks of bud inoculation on (BUGM) supplemented with different concentrations of NaCl.

Fresh weight

The fresh weight (average value) of vegetative buds was assessed directly for three replicates of each treatment.

Estimated Biochemical parameters

Total soluble carbohydrate content

The total Carbohydrate content of dry shoot samples was Spectrophotometrically at 490 nm following the phenol sulfuric acid reagent procedure described by Dubois et al. (1951). The leave chunks (50 mg) were homogenized with phosphate buffer, then centrifuged at 2000 rpm for 25 minutes. 1ml of supernatant was mixed with 0.5 ml of 5% phenol solution and 2.5 ml of 96% sulfuric acid. The mixture was heated for 20 minutes at 30°C in water bath. Total carbohydrate content of samples was computed using a standard curve of glucose and results expressed in mg. 100 g⁻¹ dry weight.

Estimation of Proline

Proline was extracted and determined according to procedure described by Dhawi and Al-Khayri (2008). Results expressed in µg. g⁻¹.

Estimation of Absciscic Acid

Absciscic acid extraction was followed according to method described by Ali *et al.*, (2011). Determination of absciscic acid at 245 nm using UV spectrophotometer (Model UVD-3200). Results expressed in µg.g⁻¹ fresh weight.

Estimation of total Chlorophyll content

The total chlorophyll was estimated according to Howertize (1975). The amount of chlorophyll present in the extract mg.g⁻¹ tissue were calculated using the following equations:

$$\text{mg total chlorophyll} = 20.2 (A_{645}) + 8.02 (A_{663}) \times V / (W \times 1000)$$

where A= absorbance at specific wavelengths

V= final volume of chlorophyll extract at 80% acetone

W= fresh weight of tissue extracted

Browning percentage (%)

The browning percentage was estimation of browning cultures numbers from the total culture numbers for each treatment following the following equation:

$$\text{Browning \%} = (\text{Number of brown affected parts}) / (\text{number of total planted parts}) \times 100. \text{ (Al-Mayahi and Ali, 2021)}$$

Statistical analysis

Data were analyzed using the analysis of variance procedure in the Genstat software. Differences were compared using the least significant difference (L.S.D) at the 1% probability level.

Results

Several variations were observed in most of the morphological and physiological characters of date palm shoots grown on medium supplemented with different concentrations of NaCl.

Effect of salinity stress on morphological parameters

Fresh weight- Multiplication Stage: Initial bud mass was cultured on BGPM medium supplied by varies concentrations of NaCl from 25- 200 mM. Under (100 mM) concentration of NaCl shoots growth was improved, where the highest fresh weight was obtained (22.44 and 25.94 g) during the two interval periods 8 and 12 weeks respectively (Table-1). Moreover, growth was completely ceased at 200 mM NaCl, where the fresh weight recorded less value during the two intervals periods (8 and 12 weeks) in contrast with control and other NaCl treatments Fig-1 and Table-1.

Proliferation- Multiplication Stage: Data in Table-1 demonstrated the positive effect of NaCl treatments except the high concentration 200 mM on multiplication rate of shoots. Where, the high rate of bud numbers (32.05, 31.77, 32.40, 26.00) was observed at 25, 50, 100 and 150 mM NaCl, compared to control treatment (33.66). Results also showed a significant increase in bud diameter with an increase in the NaCl levels in multiplication medium (BGM). Depending on the concentration of NaCl added to the multiplication medium, the shoots diameter recorded the values ranging 1.60 mm to 3.92mm (Table-1), in contrast with control that recorded the less shoot diameter 1.46 mm. Current study indicated an inverse relationship with salinity concentrations and shoot length. The addition of NaCl at 150 and 200 mM to multiplication medium gained the less significant value in shoots length. Cluster⁻¹ (7.06 and 6.53cm) during multiplication stage in compared with other treatments and control(Table 1).

Table 1
Effect of different concentrations of sodium chloride on the vegetative characteristics of the vegetative growths of date palm, Barhee cultivar.

Treatments	NaCl Concentrations (mM)	Fresh weight (g) 8 weeks	Fresh weight (g) 12 weeks	Bud numbers	Bud length (cm)	Bud diameter (mm)
Control						
	0	17.64±1.17	23.01±1.34	33.66±0.96	11.80±1.44	1.46±0.05
	25	18.72 ± 2.07	22.92 ± 0.14	32.05±0.31	11.53±2.13	1.60±0.20
treatments	50	19.51±0.53	22.56±1.67	31.77±0.98	10.63±0.66	1.80±0.10
Nacl	100	22.44±0.65	25.94±0.47	32.40±1.96	9.30±0.55	2.76±0.15
	150	15.15±1.55	18.29±1.62	26.00±2.19	7.06±1.10	3.43±0.35
	200	13.19±2.319	16.39±2.47	18.16±1.27	6.53±0.70	3.92±0.82
LSD		2.275	3.646	3.252	1.841	0.713

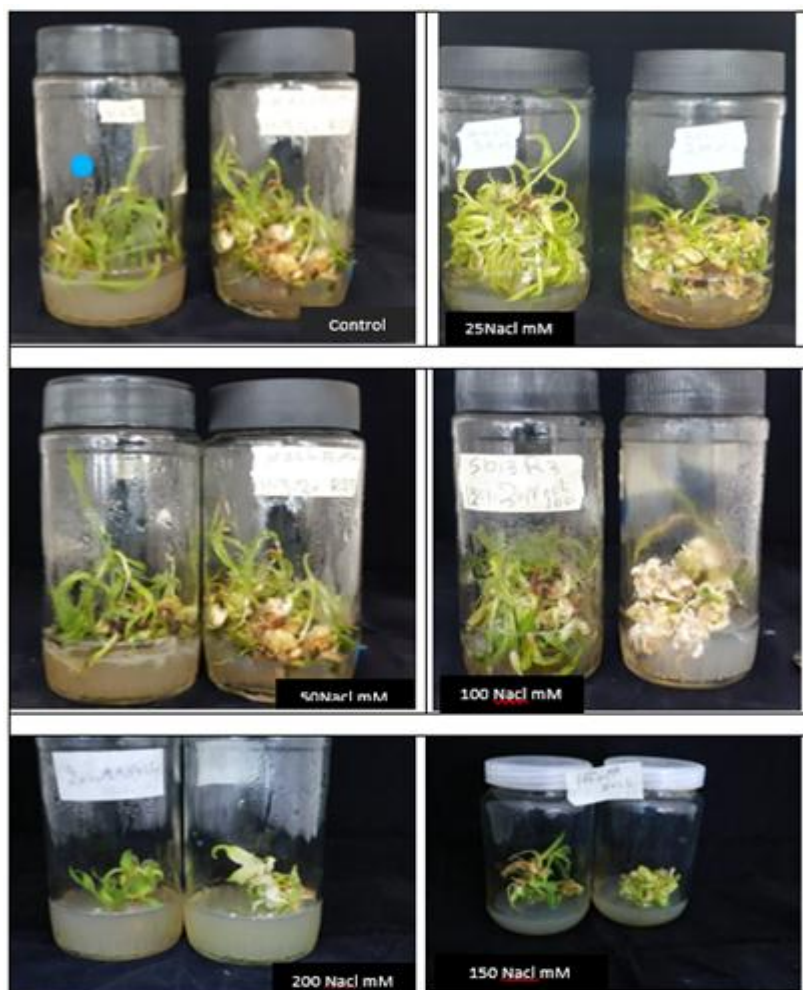


Figure 1. The buds formed in the multiplication medium containing sodium chloride at concentrations (0,25,50,100,150,200) mmol.

Elongation stage- leaf number

Moreover, current study was conducted with various NaCl concentrations in the elongation medium of date palm cultivar 'Barahi' for 12 weeks. Results showed that the leaf numbers were affected by salinity concentrations. In contrast the high leaf numbers per shoot was observed when shoot grown elongation medium supplemented with 25 -200 mM NaCl (Table-2). While less leaf numbers per shoot (2.00) was obtained on elongation medium devoid of NaCl (control).

Shoot elongation and diameter

Results in Table-2 indicated that the addition of NaCl at 100 - 200 mM in this stage decrease the shoot length up to 8.52 cm in compared with other treatments including the control. While the diameter increases with increasing salinity concentrations to 200mM. Where, highest shoot diameter was obtained when

shoots grown on medium enriched with 100 mM NaCl (4.16mm). Following by 150 mM (3.66mm) and 200 mM (3.60mm). where maximum reduction in shoot diameter was observed in control medium (1.73mm) following by 25 mM NaCl (1.85mm) and 50 mM NaCl (2.00mm) Fig-2.

Table 2

Effect of different concentrations of sodium chloride on the vegetative characteristics of the elongation stage of date palm, Barhee cultivar

Treatments	NaCl Concentration (mM)	Leaf numbers. Shoot ⁻¹	Shoot Length(cm)	Shoot Diameter (mm)
Control	0	2.00±0.00	13.73±0.48	1.73±0.08
	25	2.47±0.12	13.77±0.86	1.80±0.26
Treatments Nacl	50	2.50±0.00	13.81±0.09	2.00±0.50
	100	3.57±0.21	11.07±0.01	4.16±0.52
	150	3.00±0.50	8.63±0.32	3.66±0.17
	200	2.92±0.05	8.52±0.02	3.60±0.53
L.S.D		0.338	0.631	0.269

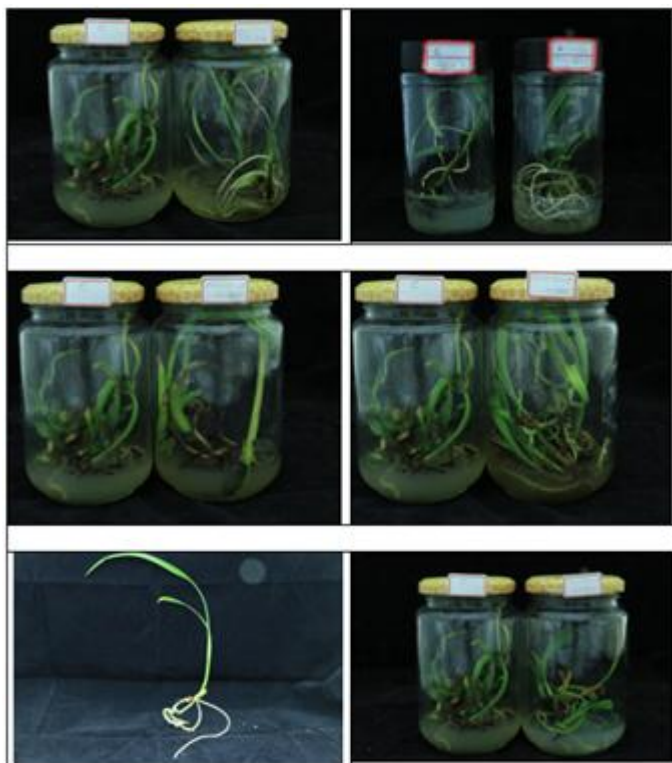


Figure 2. Buds growing on the elongation medium containing sodium chloride and at concentrations (150,200, 0.25,50,100) mmol of sodium chloride.

Biochemical Parameters

Carbohydrate content

The measurement of carbohydrate content was applied in the current study to determine the nature of date palm ability to resist salt stresses *in vitro*. Results presented in Fig-3 indicated a positive relationship between salinity concentrations and total carbohydrate content. Where, the carbohydrate content increase with increasing the NaCl increasing up to the level 200 mM. However, the maximum total carbohydrate content (29.68 mg.100 gm⁻¹) was recorded on (BGM) medium supplemented with 200mM NaCl. While, minimum level of carbohydrate content was obtained on medium containing 25 and 50 mM in as well as the control.

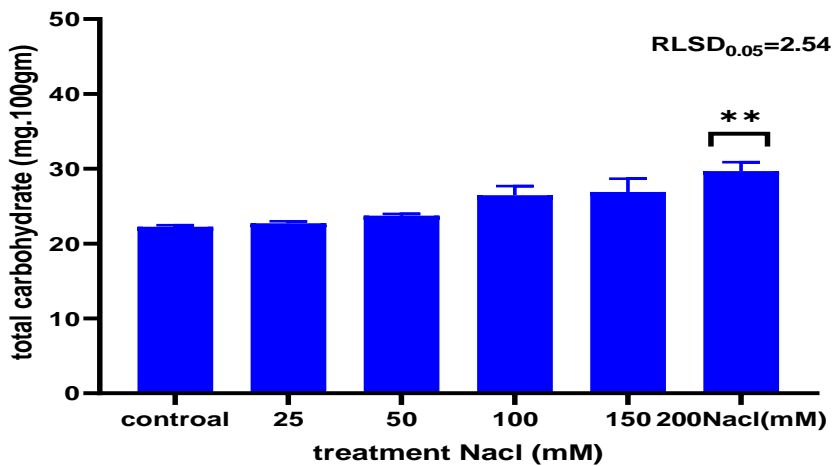


Figure 3. The total carbohydrates content of date palm buds of the Barhee cultivar

Total Chlorophyll content

Results in Fig-4 showed the negatively effect of total chlorophyll content with salinity elevation in the medium. Compared to control, date palm shoots treated with high level of salinity (200 mM) recorded the less value amount to 6.27 mg. 100g⁻¹. Whereas, shoots treated with low level of NaCl (25 and 50 mM) registered (10.28 and 10.39 mg.100g⁻¹) respectively.

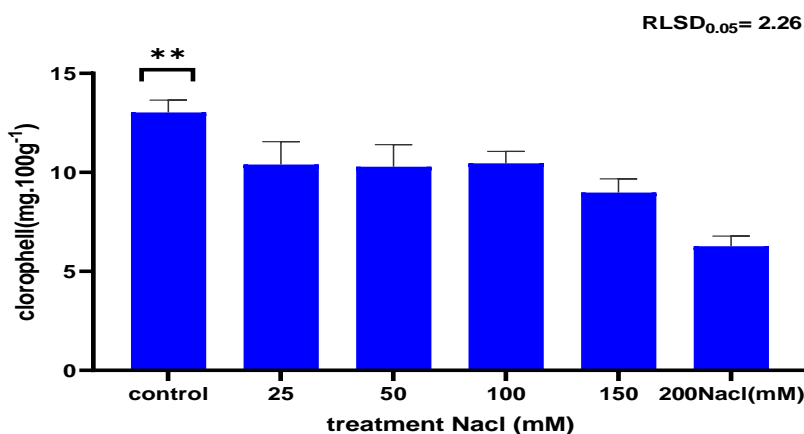


Figure 4. Total chlorophyll content of date palm buds of Barhee cultivar

Browning percentage

Browning is a serious problem in date palm tissue culture. Therefore, vigor strong shoots appeared without browning marks, whereas browning or wilting shoots is a sign of weak and not active culture. However, oxidative browning percentage depending on the NaCl concentrations. Low browning percentage (15%) monitored in low and moderate treatment of NaCl (0-100 mM). Whereas, multiplied shoots treated with 150 and 200 mM NaCl caused more browning than other treatments Fig-5. Results recorded that sever browning was inhibited the growth of multiplied shoots in medium containing high level of NaCl.

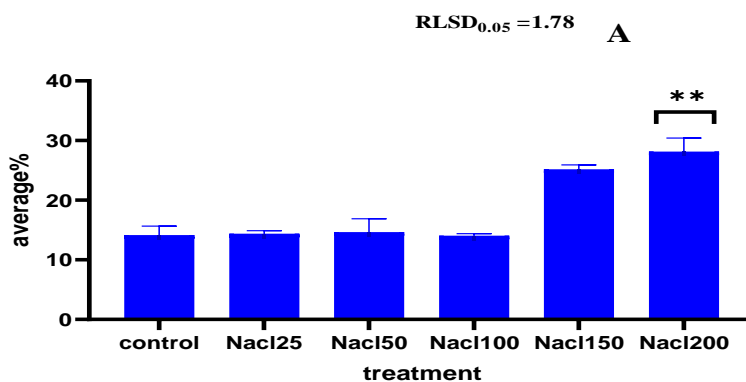


Figure 5. Browning percentage of date palm buds of Barhee cultivar

Proline an Abscisic acid Content

Due to role of proline and abscisic acid in stress tolerance, present study investigated these two factors to evaluate the ability of date palm micro-shoots to survive under salinity stress in vitro. Results revealed the significance increases in proline in multiplied shoots exposed to high levels of NaCl (150 and 200 mM). where, highest proline and abscisic acid were recorded in comparison with the

control treatment. On the contrary, shoots grown on BGM containing low (25, 50 mM) and moderate (100 mM) concentrations of NaCl showed a slightly elevation of proline and abscisic acid Fig- 6. Same results obtained on micro-shoots grown on elongation medium Fig-5.

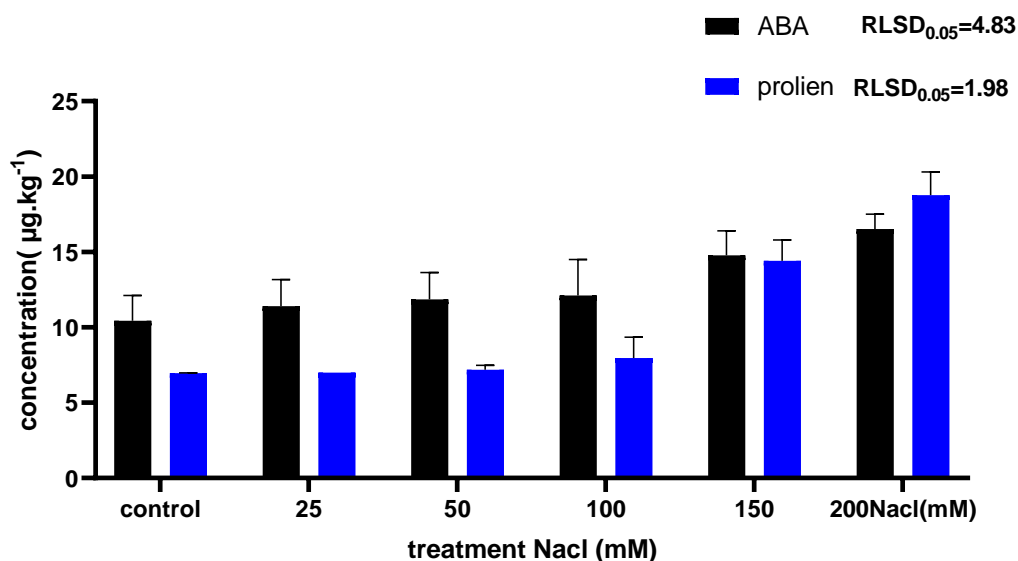


Figure 6. Effect of adding sodium chloride to the elongation medium on the index of buds content of the amino acid proline and abscisic acid ($\mu\text{g.kg}^{-1}$) in the elongation stage of date palm cultivar Barhi

Discussion

Salinity stress is one of crucial environmental factors that determining plants growth and development of in the field (Shareef and Al-Khayri, 2021). In vitro studies of salinity stress exposed the mechanisms used by plants to faces unfavorable conditions. On the other side, salinity stress it might be used to improve plantlet regeneration in tissue culture system. In present study it was found that growth and development decreased with increasing NaCl levels. Same resulted obtained by Alkhateeb et al. (2015). But The reduction in growth and development was combined with capability of survival in this condition, that appeared by increasing the shoots diameter. Increasing in shoot diameter may attributed to ability of microshoots to fight the saline stress by accumulation the carbohydrate to balance the osmotic potential with different NaCl levels. However, Yaish and Kumar, (2015) believed that date palm has a special mechanism to face such condition. But does the date palm exclude or secrete NaCl? or does their tissues possess osmotic tolerance mechanism? In order to answer these questions, it needs to characterizations of date palm verities growing around the world.

Soluble carbohydrate plays important roles to protect membrane structure during extreme environmental conditions like salinity and drought (Helaly et al.,2018). On the other hand, soluble carbohydrate used as antioxidant resulting from

tissue damage (Al Hassan et al., 2015). Also, it contributes to IAA building (Sairanen et al., 2013). In present study, it was found that carbohydrate content increase with increasing salt levels. By this mechanism date palm microshoots able to survived in high levels of NaCl (200 mM).

Chlorophyll a and b as well as total chlorophyll are affected by the salinity stress (Alturki, 2021). In Present study, it was found that the total chlorophyll content decreased with increasing the salt level in the medium. However, inclusion of calcium nitrate to medium with a rate of 1.5 g.L⁻¹ may improve the chlorophyll content at low level of salinity. Where, the calcium nitrate promoted the synthesis of chlorophyll (Shadad et al., 1988). Same result was obtained by Alturki, (2021).

Salinity treatments resulted it in a significant accumulation of proline especially at high level of NaCl. So, increasing proline concentrations promoting growth, antioxidant and protein building to maintaining cell water potential, osmotic balance, and membrane stability. All these things help the cells to prevent leaching and finally plant adaptation to salinity or drought stress (shareef and Al-Khayri, 2021).

ABA content is correlated with signal transduction pathway against environmental stress like high salts and so on (Nagamune et al., 2008). Present study quantified the ABA at multiplication and elongation stages of shoots. It was found that Increasing of ABA correlated with increasing the salt concentrations in the medium. These observations indicate that ABA content linked with degree of how the stress alters cell turgor pressure and relative water content (Verslues and Zho, 2007). Also, Rizwan et al. (2017) mentioned in their review that plant tolerance to different stress can be depend on amendments of endogenous and exogenous ABA. For these reasons date palm shoots able to resist salinity in vitro.

Conclusion

This study enhanced the understanding of salinity effect on morphological and physiological aspects of date palm microshoots cultures at multiplication and elongation stages. This investigation provided a knowledge on morphological growth of shoots grown at different levels of salinity. In addition, study provided an information about carbohydrate, proline and abscisic acid accumulation and used by plant as a defend system to resist the salinity stress. Moreover, this study showed the important of using salinity to improve some morphological aspects of date palm shoots grown in vitro and possibility in tissue culture labs.

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