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Impact of salinity on date palm shoots proliferation and rooting *in vitro*

Rasha, N. Arafa[1]*; Sayed, A. A. Elsayh[2]; Salwa, El-Habashy[2]; Emadeldin, A.H. Ahmed[2]; Eman, H. Afifi[2]; Rabab, W. El Aramany[1]; Esraa, M.E. Hussein[3];Shehata, M.S.H.[1]; Yasser, S.G. Abd Elaziz[2]; Y. A. Abdel Mageed[2]; Ahmed, A.M. Barakat[2] and Dosoky, Hoda A.A.[4]

[1]Central Lab of Date Palm Researches and Development, Agricultural Research Center (ARC), Giza, Egypt.
[2]Horticulture Research Institute, Breeding Department, Agricultural Research Center (ARC), Giza, Egypt.
[3]Horticulture Department Faculty of Agriculture, Sohag University, Egypt.
[4]Horticulture Department, Faculty of Agriculture, Benha University.
Corresponding author E mail: rashanagyarafa2022@gmail.com

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Abstract

The utilization of *in vitro* cultures for the examination of stress responses depends on the idea that cells cultivated *in vitro* exhibit comparable behavior to cells in intact plants that are exposed to situations of water deficiency and salinity. The objective of this study was to investigate the physiological responses of date palm, cv. Bartemoda, shoots proliferation and rooting to salinity stress. A small cluster consisting of 3-5 developed shoots were cultured on MS medium supplemented with BA at 2.0 mg/l + TDZ at 0.5 mg/l with the inclusion of various concentrations of NaCl at (0, 500, 1000, 1500, and 2000 ppm) with five replicates.

The exposure to salt stress resulted in when used 500 ppm NaCl produced significant increases of the shoot number (9.0 shoot/cluster). The number of shoots decreased from 7.0 to 4.0 shoot/cluster with the increase of NaCl concentrations from 1000 to 2000 ppm. The rooting percentage decreased significantly using 1000 ppm NaCl (65%) and was the lowest at 2000 ppm NaCl (30%). The rooting percentage at 500 ppm NaCl was increased significantly (85%) as compared with the control treatment. For healthy *ex vitro* growth of date palm plantlets in a greenhouse, the quality of the shoot and root produced in the laboratory is crucial.

Under salt stress of 1000, 1500, and 2000 ppm, total chlorophyll a, b, and carotenoids levels had decreased significantly. The 500 ppm NaCl treatment resulted in the highest chlorophyll a, b, and carotenoid contents Compared to the control treatment. Proline content in treated shoots was significantly boosted by the salt. The protein content of the shoots was 1.80 and 1.50 (mg/g fw) Under 500 and 1000 ppm salt stress. Increasing the amount of NaCl in the medium caused the protein content to drop. The soluble carbohydrate content of leaves under salt stress was significantly greater with elevated salt concentrations. This study has improved the comprehension of the impact of salt on physiological characteristics of date palm cell cultures. This study proved the genetic stability of the micro-propagation process described using the DNA-based approach RAPD (random amplified polymorphic DNA). The findings suggest that the micro-propagation strategy established in this study was suitable and effective in generating genetically consistent date palm cv. Bartemoda seedlings.

Keywords: *In vitro* date palm development, NaCl concentrations, regeneration shoots, rooting, physiological and biochemical analysis, RAPD-PCR analysis.

1. Introduction

Salinity is the most detrimental environmental factor, especially in the North Africa and Middle East's semiarid and arid regions, where date palms are cultivated **(Al-Kateeb *et al.,* 2002)**. The most significant abiotic stress that adversely impacts development, plant growth and physiological and metabolic activities is salinity stress **(Nikolova and Ivancheva, 2005)**. Whole plant cells react similarly to cells produced *in vitro* under salt conditions. The cell cultures offer homogeneous environmental conditions, genetically and physically matched cells circumstances for researching responses to salt stress by avoiding problems coming from whole plant tissues and varying field settings **(Bartels and Sunkar, 2005)**.

Date palm (*Phoenix dactylifera* L.) is a member of the Arecaceae family and an economically significant tree species that grows in Central America, North Africa and the Middle East **(Yaish *et al.,* 2017)**. The tree has preserved its significance for desert residents as a result of its adaptability to its environment and the breadth of its socioeconomic benefits. Although in their natural environment, date palms are susceptible to a number of challenges, including heat, drought, air pollution and salinity **(Du *et al.,* 2020)**. Date palm tolerates high levels of salinity and is more drought-tolerant than any other fruit crop; nonetheless, drought and saline water inhibit its development and yield production in arid environments. Furthermore, salinity can result in substantial economic losses for date palms **(Al-Muaini *et al.,* 2019)**. This is especially true for salt-susceptible cultivars **(Hazzouri *et al.,* 2020)**, which demonstrated a reduction in photosynthesis, foliar water content, shoot and root growth in response to salt stress **(AlKharusi *et al.,* 2017)**. The effects of salt stress on their growth and productivity are independent of heat and drought **(Alhammadi and Kurup, 2012)**. Enhancing the salt tolerance of commercially farmed date palm cultivars is therefore a pressing need. In this regard, standard breeding techniques for the genetic enhancement of date palm have encountered various difficulties. Because of the perennial nature, high heterozygosity, and extended generation period of the date palm, its traditional breeding process is notably slow and inefficient. Therefore, novel approaches are required to rapidly improve the drought and salt tolerance of date palm cultivars.

Alternative methods for overcoming breeding restrictions and bolstering breeding operations are plant tissue culture techniques. *In vitro* culture is a suitable approach for the investigation of the plant's response to salt conditions and for the isolation and selection of tolerant lines. Other than that, Regardless of the regulatory systems existing at the level of the entire plant, tissue culture allows for the

modulation of stress homogeneity and the characterization of cell behavior under stress. **(Lutts *et al.,* 2004)**.

Regeneration of the date palm *in vitro* occurs through organogenesis and somatic embryogenesis, which are influenced by the genotype and hormones. Somatic embryogenesis from shoot-tip-derived callus is the most effective approach for date palm regeneration used by many growers, as it is amenable to micro-propagation scale-up for commercial purposes. Date palm somatic embryogenesis consists of a number of sequential processes beginning with callus induction, embryogenic callus proliferation, somatic embryo development, and lastly shoot production and roots **(Al-Khateeb, 2008)**. Utilizing plant growth regulators, whose regulate a variety of physiological and biochemical activities, such as synthesis of protein, antioxidant enzymes, and photosynthetic, all of that are directly or indirectly related to the stress tolerance mechanism, is an important strategy for protecting plants from various stresses **(Akram and Ashraf, 2011)**. Under 8-16 dsm^{-1} plant height salt stress, leaf number, fresh weight, and dry weight decreased, whereas proline content increased **(Darwesh, 2014)**.

Rizkallah *et al.* (2007); **Al-Bahrany and Al-Khayri (2012)**; **(Rasha *et al.,* 2020)** have been undertaken to determine the influence of salinity on the behavior of date palms grown *in vitro*. Therefore, in the present study proliferation stage *in vitro* of date palm cv. Bartamoda was evaluated for salinity tolerance represented by NaCl levels in culture medium.

The economic significance of introducing abiotic and biotic stress resistance traits via somaclonal variation in date palm has been widely recognized **(Hadrami and Hadrami, 2009).** Expanding the genetic diversity of date palm cultivars could potentially enhance their ability to tolerate salinity-induced stress. The utilization of several physiological, biochemical and DNA-based molecular markers has great importance in the assessment of somaclonal alterations that occur during *in vitro* culture **(Ahmad *et al*., 2018).** In comparison, the utilization of random amplified polymorphic DNA (RAPD) markers offers a swift, cost-effective, and dependable method for discerning genetic alterations that occur during the process of *in vitro* culturing **(Sudhaa *et al*., 2019)**. With consideration of the methodology's potential in acquiring stress-tolerant variants, the objectives of this research were twofold: (i) to generate salt-tolerant plants by exposing calli from date palm cv. Khalas to varying salt concentrations, and (ii) to assess these regenerated plants for distinct morphological, physiological characteristics, and genetic stability.

2. Material and methods

The work was carried out in The Central Laboratory for Research and Development of Date Palm, Agriculture Research Center, Giza, Egypt, throughout the year of 2023. We studied the effect of salt stress under *in vitro* conditions on the development and growth of shoot and on the rooting of shoots.

2. 1- Shoots regeneration

Salinity stress

A small cluster consisted of 3-5 developed shoots from date palm cv. Bartamoda were used to begin *in vitro* salt tolerance experiments.

The shoots were cultured on **Murashige and Skoog (1962)** (MS) medium contain BA at 2.0 mg/l + TDZ at 0.5 mg/l with the inclusion of various concentrations of NaCl at (0, 500, 1000, 1500, and 2000 ppm) with five replicates, pyridoxine-HCl (0.5 mg/l), nicotinic acid (0.5 mg/l), thiamine (1.0 mg/l), sucrose (35.0 mg/l), agar (6.0 g). Media were dispensed into the culture vessels in aliquots of 35 ml per small jars and capped with polypropylene closures. After that, the media were autoclaved for 20 minutes at 121°C and 1.2 Kg/cm^2. All culture containers were incubated for 8 weeks (two subcultures) in the growth environment at (27°C) and under a 16-hour, 3000 Lux lighting. Each treatment consisted of six replicates (jars), the shoots were transferred to fresh medium every 4 weeks. After the experiment was finished, the following physiological measurements were made (after 8 weeks), average number of shoots/cluster, average number of leaves/cluster and average leaves length (cm).

2. 2- Shoot elongation

To achieve shoot elongation, regeneration shoots were transplanted to the ¾ MS medium contain 3.0 mg/l of gibberellic acid (GA_3) and 0.5 mg/l of NAA **(Hussein and Jawa, 2021)**. The cultured jars were incubated for 8 weeks in the growth chamber at a temperature of 25 2 °C with a light intensity of 3000 lux for a 16-hour photoperiod, and the shoots were moved to the same new media every 4 weeks.

2. 3- Rooting of shoots

To promote root formation, healthy regenerated clusters of unrooted *in vitro* shoots (4-7 mm in length) of date palm cv. Bartamoda were harvested during the elongation phase. Individual typical shoots were cultivated on a medium containing

half-strength MS media with 170 mg/l Na $H_2PO_4.2H_2O$, sucrose 40 g/l, myo-inositol 100 mg/l, glutamine 200 mg/l, 40 mg/l adenine sulfates, 7 mg/l agar, 1.5 g/l activated charcoal and auxins such as 1.5 IBA and 0.5 NAA (mg/l) and different concentrations of NaCl at (0, 500, 1000, 1500 and 2000 ppm)After adjusting the pH to 5.7–5.8, the medium were autoclaved at 121°C for 20 minutes. Each treatment consisted of six replicates (jars), and every four weeks, the shoots were transferred to fresh media. All cultures were incubated for 8 weeks (two subcultures) in the growth room at 27±2°C with a 16 h photoperiod of white fluorescent light. 8 weeks after the inoculation of shoots on the culture media, the percentage of root induction, number of roots per shoot (roots/jar), and root length (cm) were examined.

2. 4- *In vitro* Hardening

Before entering the greenhouse, the plantlets with 2-3 leaves and well-developed roots **(Fig. 1)** were subjected to pre-acclimatization. The plantlets were cultivated in test tubes containing a PGR-free liquid medium with a low sucrose content.

Each test tube's aluminum foil was perforated with a single hole for gaseous exchange between the interior and exterior of the culture tubes. The plantlets were allowed to harden *in vitro* for a few hours in order to change their mode of nutrition from heterotrophic to autotrophic so they could thrive in an *ex vitro* environment.

2. 5- Acclimatization

All plantlets were removed from the tubes and washed with distilled water to remove traces of agar from the roots. The plantlets were additionally submerged in a 3 g/l fungicide solution for up to 5 minutes before being transferred to small plastic bags (19 cm 13 cm) containing soil media in varying proportions (v/v) (peat moss: river sand: yellow sand).Seedlings were kept in the greenhouse for one week with high humidity (85–90%), covered in a white polyethylene sheet that was gradually removed.

After one week, the seedlings were only allowed to breathe for 10–15 minutes before being securely re-covered to prevent further exposure to external dry air. As needed, plantlets were sprayed with copper oxy chloride solution to prevent fungal infestation. After 1–2 months, the transparent plastic sheets were removed and

plantlets were exposed to greenhouse settings. The data was reported as the percentage of plantlets that survived.

2. 6- Biochemical characteristics determination

Chemical analyses were done for leaves of shoots including the following:

2. 6.1- Chlorophyll content determination

The total carotenoids content from fresh leaves were assessed with a UV spectrophotometer (CECL 2021, ENGLAND) at wave max 470 nm **(Lichtenthaler and Wellburn 1983)**, and the total chlorophyll was measured using the method described by **Aron (1949)** at wave max 663 nm.

2. 6.2- Content of proline:

Proline was estimated by a spectrophotometric assay as described by **Bates *et al.* (1973)** method the standard curve (mg/g DW).

2. 6.3- The total soluble protein content

To test the total soluble protein, the **Bradford (1976)** method was employed. Albumin was used to create a standard curve, and the absorbance was measured at 595 nm.

2. 6.4- Measuring the total amount of carbohydrates

A spectrophotometer that measures the absorption spectra at a wavelength of 620 nm was used to assess the total carbohydrates in the embryogenic callus and somatic embryos of the date palm using the **Watanabe *et al.* (2000)** method. A standard glucose curve was created for calculation.

2. 6.5- Activity of peroxidase (POX)

The activity of peroxidase was determined using the **MacAdam *et al.* (1992)** technique for peroxidase (EC: 1.11.1.7) activity.

2. 7- Total genomic DNA isolation

Genomic DNA was extracted from micro-propagated plantlets subjected to four different NaCl treatments (500, 1000, 1500, and 2000 ppm), as well as control plants. The extraction was performed using a modified plant DNA-miniprep methodology based on the method described by (**Arif *et al*., 2010).** The user has provided a numerical reference, indicating the presence of a citation or source. In a concise manner, a quantity of 100-150 mg of recently collected date palm leaves were carefully deposited into a sterile mortar. A combination of 100 mg of sterile

sand and 500 µl of lysis solution was prepared. The lysis buffer consisted of 0.1 µM Tris–HCl (pH 8.0), 0.05 µM EDTA, 0.5 µM NaCl and 0.01 µM β-mercap to ethanol. The plant material was thereafter crushed meticulously using a mortar and pestle. The pulverized leaf material was meticulously put into a sterile Eppendorf tube with a capacity of 1.5 ml. Afterward, an extra 1000 µl of lysis buffer was introduced into the tube, and the contents were fully combined with vigorous vortexing. The leaf extracts were thereafter incubated at a temperature of around 65 °C for 40 minutes, with periodic agitation. After being kept at room temperature for a period of time, the tubes were spun in a centrifuge at a speed of 12,000 revolutions per minute for 5 minutes. The aqueous component, measuring approximately 200 µl in volume, was carefully transferred to a new tube. Subsequently, an equal quantity of a combination comprising chloroform and isoamyl alcohol in a ratio of 24:1 was added. The tubes were well mixed by gentle stirring and then centrifuged at a speed of 12,000 revolutions per minute for 5 minutes, all at room temperature. The aqueous fraction (200 µl) was meticulously transferred to a separate tube, and the DNA was precipitated by adding cold isopropanol (500 µl). The tubes were kept overnight at a temperature of -4 °C, and then underwent centrifugation at a speed of 12,000 revolutions per minute for a period of 10 minutes. The entire liquid portion was removed, and then, the DNA solid was rinsed with 70% cold ethanol at a centrifugal speed of 12,000 revolutions per minute for 10 minutes. The pellet was dried in the air at normal temperature, and then, the DNA was dissolved in 100 µl of MilliQ water. The DNA was quantified, and then the quality was assessed by submitting a volume of 2.0 µl of total genomic DNA to 1% agarose gel electrophoresis in 0.5 TAE buffer. The DNA was stored at a temperature of -20 °C to ease future downstream applications.

2. 7.1- Analysis of genomic DNA using RAPD-PCR

The RAPD amplification method was performed with a total reaction volume of 25 µl. The sample contained template DNA with a concentration of around 50-100 ng. In addition, the reaction mixture contained 2.5 µl of a 10-primer oligo-deoxynucleotide primer from **Table (6)**, 2.5 µl of dNTPs at a concentration of 0.4 µM each, DreamTaq DNA polymerase from The rmo-Scientific, and 2.5 µl of Dream-Taq buffer (10x). A Perkin Elmer thermal cycler was utilized to perform the polymerase chain reaction (PCR). The process began with an initial denaturation stage at a temperature of 95 °C for a period of 5 minutes. Subsequently, a series of 35 cycles were performed, with each cycle comprising denaturation at a temperature of 95 °C for a duration of 35 seconds, annealing at 36 °C for 35 seconds, and extension at 72 °C for 2 minutes. Ultimately, a conclusive extension phase was

carried out at a temperature of 72 °C for a duration of 5 minutes. The PCR findings were analyzed by subjecting them to electrophoresis on a 1% agarose gel using a 0.5× Tris-acetate-EDTA (TAE) buffer. The sizes of all amplicons were confirmed by comparing them to a 1 kilobase (kb) DNA ladder produced by Takara.

Table 1. Sequencing of a Random Amplified Polymorphic DNA (RAPD) marker from date palm Bartamoda cv. mother plant and a sample of shoots treated with NaCl at 500 and 1000 ppm.

Number	Primers	Sequences	Similarity (%)
1	OPA-02	TGCCGAGCTG	92.7
2	OPB-05	TCGCGAGCTG	96.4
3	OPE-15	TGGCCAGCTG	93.2
4	OPO-07	TCGGCAGTCC	91.5
5	OPC-11	ACCGCAGTCG	93.1
6	OPD-08	TGCGCCCTTC	96.5
7	OPG-03	ACGCCTGTCG	92.2

2. 8- Statistical analysis

As explained, a randomized whole block design was used to create this experiment **(Gomez and Gomez, 1984)**. The MSTAT computer program was used to statistically evaluate the gathered data **(MSTAT Development Team, 1988)**. Means of the various therapies were compared using the Duncan's Multiple Range Test, which is defined by **(Duncan, 1955)**.

3. Results and discussion

3. 1- Shoots regeneration

After 8 weeks in the proliferation culture media contained of BA at 2.0 mg/l, TDZ at 0.5 mg/l and various concentrations of NaCl, the growth parameters (number of shoots, number of leaves and leaves length) were significantly affected by the NaCl treatments. As shown in **Table (2)**, the number of shoots decreased from 7.0 to 4.0 shoot/cluster with the increasing of NaCl concentrations from 1000 to 2000 ppm. Among the different NaCl concentrations applied, 500 ppm NaCl did not significantly reduce the number of shoots and produced significant increases of the shoot number (9.0 shoot/cluster) compared with 0.0 ppm NaCl which recorded 5.0 shoot/cluster.

In comparison to all other treatments, the effect of salinity showed that the number of leaves increased significantly (17.0 leaf/cluster) after treatment at 500 ppm NaCl concentration **Table (2)**. Afterwards, a significant gradual decline in the number of leaves took place at 1000, 1500 and 2000 ppm NaCl treatment where the mean number of leaves generally reducing with the increasing in NaCl content in the medium, from 14 leaf/cluster at 1000 ppm NaCl to 5.5 leaf/cluster at 2000 ppm NaCl.

Results revealed that low level of salinity (500 ppm) in culture medium significantly enhanced leaves length to 8.2 cm compared with the control treatment which resulted in leaves length 5.0 cm. Increasing the concentration of NaCl to 1000 ppm gradually decreased the leaves length to 7.0 cm. The length of the leaves reduced from 4.5 to 3.2 cm at high salinity levels of 1500 to 2000 ppm NaCl.

Table 2. Effect of various NaCl concentrations (ppm) on the growth parameters of date palm shoots in the shoots regeneration.

NaCl concentration (ppm)	number of Shoots	number of Leaves	Leaves length (cm)
0.0	5.0 c	10.0 c	5.0 c
500	9.0 a	17.0 a	8.2 a
1000	7.0 b	14.0 b	7.0 b
1500	5.5 c	11.0 c	4.5 cd
2000	4.0 cd	5.5 e	3.2 e

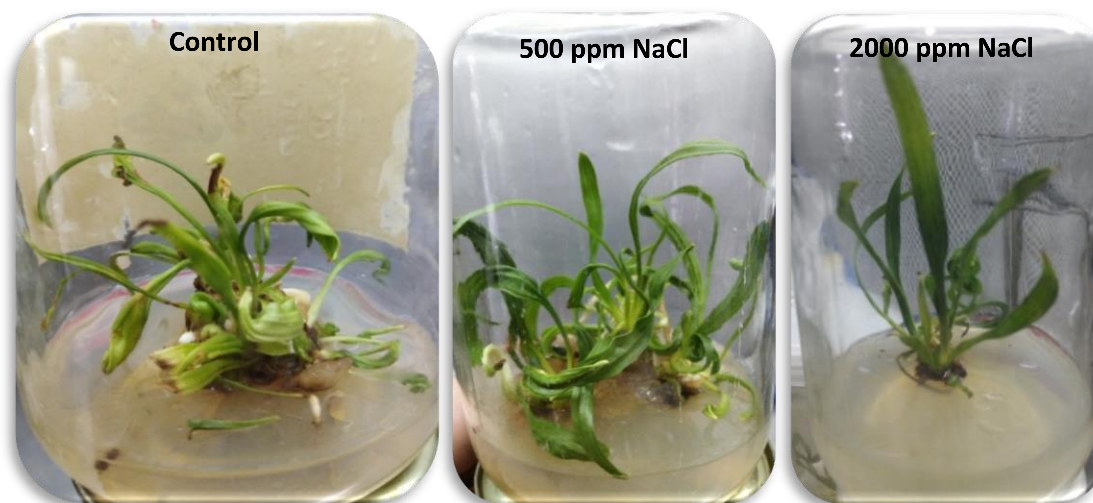

Fig. 2. The proliferation culture media contained of BA at 2.0 mg/l, TDZ at 0.5 mg/l and various concentrations of NaCl after 8 weeks, the number of leaves and leaves length were significantly affected by the NaCl treatments.

According to **Magyar-Tábori*et al.* (2010)** thidiazuron is a highly effective bio-regulator in tissue culture. It is believed that the presence of cytokinins as part of the t-RNA carrier DNA is responsible for the effect of various cytokinins on the events of vegetative multiplication **(Schmülling*et al.*, 1997)** which is mediated by their role in cell division and the elimination of the phenomenon of apical dominance.

One of the most prevalent environmental stressors is salinity. The salinity has a detrimental effect on plant development and growth, reducing seed germination **(Dash and Panda, 2001)**; sea water decreased trunk growth by 20-60% in *Nicotiana tabacum* and *Pistacia terebinthus* **(Germana *et al.*, 2000)**; and length, fresh and dry weights of leaves decreased under high levels of salts (7.22 and 26.5 ds/m) on date palm **(Al- Rokibah *et al.*, 1998)**. The decline in fresh and dry biomass output is a typical unfavorable effect of salt stress on crop plants **(Kusvuran, 2010)**. The growth regulators are involved in modifying plant growth processes, and it is likely that by boosting plant growth, they could lessen the negative consequences of salinity **(Nasser *et al.*, 2001)**.

In early screening at varied NaCl concentrations (160, 240 and 320 µM), we detected no significant differences in growth between cultivars under moderate salinity conditions (160 µM) due to minimal salt stress. Similarly, when the cultivars were exposed to higher levels of salt (320 µM NaCl), no significant differences were seen, perhaps due to the extreme stress reducing their development to comparable

levels. Only the 240 µM stress treatment produced substantial cultivar variations **(Yaish, 2015)**. The findings are consistent with those of **Al-Mansoori and Eldeen (2007)**, they found that at salinities as high as 3.3%, date palm growth significantly decreased. Additionally, when irrigation water salinity rose above 12,000 mg LG1, date palm offshoots of the "Sakoti" and "Bertamouda" cultivars were considerably reduced. Following the addition of a 3.0% (w/v) NaCl solution to the induction medium, irrigation water salinity significantly and completely inhibited date palm immature embryos, according to these researchers. Due to the enhanced osmolality, **Flowers and Lauchli (1983)** discovered that at low concentrations, NaCl exerts a considerable favorable influence on shoot growth *in vitro*. In addition, **El Sharabasy *et al.,* (2008)** found that a low level of salinity (4000 ppm) in the culture medium considerably increased the shoot length of date palm cultivars, but that these values fell dramatically at 8000 and 12000 ppm NaCl. **Prajuabmon *et al.,* (2009)** it was noted that the fresh, dry weight and shoot length relative growth rate of cultivars of rice seedlings grown in high salinity reduced.

The effect of salt stress on number of leaves, plant height and fresh and dry leaf weights may be linked to a drop in turgor pressure, which is important for cell development and division **(Hussain *et al.,* 2008)**.

3.2- Shoot elongation

After a two-month period of cultivation on a ¾ MS medium supplemented with 3.0 mg/l GA_3 and 0.5 mg/l NAA, there was a significant variation in shoot length. However, there was no noticeable change in the total number of roots. The mean length of the shoots was 8.0 cm.

Gibberellins are seen to influence elongation, as it is hypothesized that they promote elongation by stimulating cell division in the apical meristem. Gibberellins, on the other hand, aid in the breakdown of polysaccharides, making them easier for plant tissue to utilize **(Abdul, 1990)**. The results of this study suggest that plant growth regulators promote *in vitro* shoot and root elongation. Among plant growth regulators, auxins and cytokinins are the two most significant subgroups. Auxins and cytokinins have been effectively combined to encourage shoot elongation and root formation in a variety of plant species. Auxins are known to control cell division and elongation as well as to promote the growth of adventitious roots **(Machakova *et al.,* 2008)**. In addition to being essential for plant cell division, cytokinins are also very effective at encouraging the growth and multiplication of shoot buds **(Van Staden *et al.,* 2008)**.GA_3 is a multipurpose PGR that has been effectively used for shoot elongation **(Moshkov *et al.,* 2008)**. In the date palm 'Maktoom' cultivar,

Khierallah and Bader (2007) found that increasing GA_3 concentration increased shoot length.

3. 3- Rooting of shoots

Plantlets that were 5 to 10 cm long, with or without roots, were moved from the multiplication media to the medium for elongation the shoots, and then to the medium for establishing roots. The base of plantlets must be checked for injury during the separation process from the shoot cluster; if it is, the entire plantlet will perish. When cultivated on rooting medium containing 1.5 IBA and 0.5 NAA (mg/l) with various concentrations of NaCl, the plantlet without root and undamaged base had the potential to develop new healthy roots within one month. The cultures were changed to rooting media that only included 1.5 IBA and 0.5 NAA (mg/l) once the shoot length reached 10-15 cm long. Cytokinins were not included in the rooting medium. The NaCl treatments had a considerable impact on the growth metrics (rooting %, number of roots, and roots length) after 8 weeks in the rooting culture media.

Salinity significantly affected the rooting percentage. The rooting percentage decreased significantly at 1000 ppm NaCl from (65%) to the lowest at 2000 ppm NaCl with (30%), as shown in **Fig. (3)**. The rooting percentage at a concentration of 500 ppm NaCl exhibited a considerable increase to 85% compared to the control treatment, which had a rooting percentage of 72%. However, as the salt level went beyond this point, there was a notable decrease in the rooting percentage.

Table 3. Effect of NaCl concentrations on rooting (%) and number of roots of the date palm Bartemoda cv. after 8 weeks of culture.

NaCl concentration (ppm)	Rooting percentage (%)	Number of Roots	Roots length (cm)
0.0	72 b	6.0 b	5.5 b
500	85 a	8.2 a	7.0 a
1000	65 c	5.0 c	4.0 c
1500	50 d	4.3 cd	3.2 d
2000	30 e	2.5 e	2.1 e

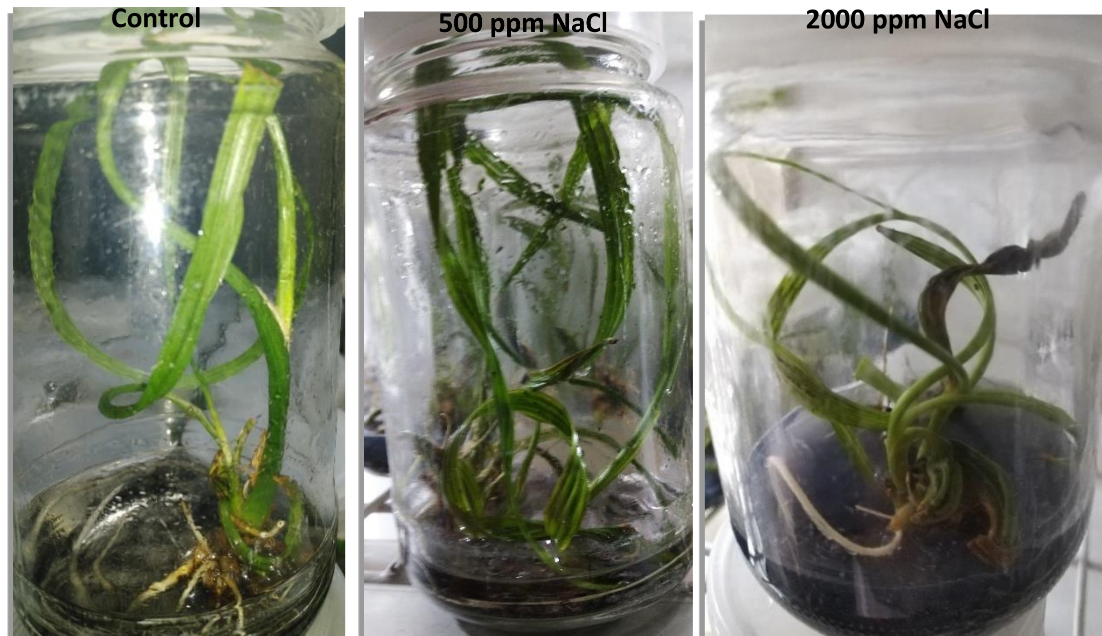

Fig. (3). The well-developed root and shoot system in plantlets are affected by different concentrations of NaCl.

In **Table (3)** illustrates how the number of roots and their length decreased as NaCl concentrations increased from 1000 to 2000 ppm. Of the various NaCl concentrations that were applied, 500 ppm NaCl resulted in a substantial increase in the number of shoot roots (8.2 roots/jar) and a considerable increase in the length of the roots (7.0 cm) as compared to 0.0 ppm NaCl. At 2000 ppm NaCl concentration the number of roots were adversely effected and only 2.0 roots were formed per shoot, whereas the shoot length had sharply decreased to 2.1 cm.

Omar (1988) found that the nutritional medium, which had a concentration of 0.1 mg/l NAA and 0.05 mg/l BA, enhanced roots development and leaf production, respectively. When cultivated plantlets were cultured to a medium containing BA (0.05 mg/l) and NAA (0.1 mg/l), around 80-90% of them established an effective roots structure. Several studies **(Abul-Soad and Al Khayri, 2018; Jatoi *et al*., 2015)** utilized a medium with a concentration of 0.1 mg/l NAA to enhance root development and 0.05 mg/l BA to stimulate leaf growth in the shoot multiplication phase. **Al-Khayri and Naik (2017)** supplemented the MS medium with 0.2 mg/l NAA to promote root development in date palm plantlets obtained from shoot tip cultures. Factors such as light intensity, temperature, and activated charcoal have a significant impact on both root and shoot development, as well as plant growth regulators. Through the utilization of IBA, **Al-memary *et al.* (2020)** found that the mean length of roots was 2.43 cm, while the maximum number of roots per transplant reached 7.00. As to **Jatoi (2013)**, reported that the addition of

40 g/l sucrose to the rooting medium resulted in the growth of adventitious roots, with an average length of 8.5 cm per plantlet. The study found that the addition of 3.0 g/l AC to the rooting soil is necessary for enhancing plantlet growth, as evidenced by the increased length of white roots and leaves. The introduction of alternating current (AC) promoted the development of secondary and tertiary roots.

Also, **Siler *et al.,* 2007** have demonstrated that roots are the most susceptible and among the first plant organs to be affected by salt stress. At 0 µM NaCl, 89.25% of the explants rooted; at 100 µM NaCl concentration, this percentage dropped to 43.25%; at 120 µM NaCl, no rooting occurred. In grapevine and *Citrus macrophylla*, NaCl concentrations over 85 and 100 µM inhibited rooting **(Pe´rez-Tornero *et al.,* 2009)**. Other crops have shown a reduction in rooting percentage in response to the increase in salinity **(Cano *et al.,* 1998)**.

3. 4- *In vitro* Hardening

For the *ex vitro* growth of date palm plantlets in a greenhouse, the quality of the shoot and root produced in the laboratory is crucial, **Fig. (4)**.

In order to accomplish the required development, artificial hormones are also added to the medium during the *in vitro* growth of plantlets, which is based on a heterotrophic mode of nutrition. To finish the *in vitro* propagation method, *in vitro* hardening is a crucial step **(Gabr and Abd-Alla, 2010)**. This was accomplished by gradually exposing plantlets grown in long tubes and prepared for acclimation to autotrophic mode of sustenance. Instead of plastic caps, aluminum foil was used to cover the test tubes, which encourages the transfer of gases between the *in vitro* and *ex vitro* environments. Date palm plantlets were also cultured for a short duration on liquid media containing half the concentration of MS salts and sugar decreased from 30 to 15 g/l.

Fig. (4). *In vitro* hardening for the *ex vitro* growth of date palm plantlets in a greenhouse.

Since the plantlets can't survive when moved into the greenhouse straight after being transferred from regulated *in vitro* conditions, such as heterotrophic mode of nutrition, low light and high humidity, they are frequently unable to be transplanted there. In *ex vitro* settings, humidity of at least 90% was supplied; failure to do so caused plantlets to dry out.

3. 5- Acclimatization

After a period of two months, the date palm plantlets that were cultivated in a greenhouse using a soil medium consisting of peat moss and sand in a 1:1 ratio (v/v) had the best survival rate of 80%. Furthermore, the initial months of acclimation played a vital role in decreasing the mortality rate of plantlets, as the plants developed a stronger vitality on the appropriate soil medium in the greenhouse during this period. Additional factors in greenhouse management, such as tunnel aeration, temperature and humidity, significantly impacted the plants' survival and growth.

The majority of researches have observed a low survival rate (25–35%) during the acclimation stage, which is the most challenging phase of the entire micro-propagation process **(Jatoi*et al.,* 2015)**. **Abul-Soad*et al.* (1999)** produced date palm plantlets by *in vitro* cultivation; yet, the plantlets failed to survive when being moved to *ex vitro* conditions. Enhancing the quality of the roots in plantlets enhances their performance in conditions outside of the laboratory.

Acclimatization is the gradual adaptation of plantlets cultivated *in vitro* to an altered environment **(Mirani *et al.,* 2017)**. Under *in vitro* conditions, plantlets develop physiological and anatomical characteristics that must be adapted when transferred to an *ex vitro* environment. This poor physiological and anatomical development in *in vitro* grown plantlets is a major hindrance to the greenhouse survivability of plantlets. However, well-rooted plantlets with two to three leaves are required for optimum plantlet survival in greenhouses. In addition to these traits, seedlings require high humidity (85–90%) to retain sufficient water in their bodies and low temperatures to perform little transpiration. During acclimatization, **Taha *et al.* (2007)** found that only 25–35% of seedlings survived. **Kurup*et al.* (2014)** determined that cv. Kheneizi had a 60% survival rate when transplanted into pots with a 2:1 mixture of peat moss and vermiculite. **Othmani *et al.* (2009)** reported that date palm cv. Boufeggous had a 60% survival rate, whereas cv. Deglet Nour had an 80% survival rate. 72–84 percent of cv. Khasab plantlets survived following *ex vitro* transplanting **(Al-Khayri, 2010)**.

The most favorable *in vitro* roots were successfully developed within a period of four months. Additionally, about 90% of the *ex vitro* plants succeeded in surviving in a simple combination of sand and peat moss, with a ratio of 1:1. Furthermore, it was shown that the different types or genotypes exhibited independent responses in terms of the growth of shoots and roots. Additionally, it was discovered that trimming the roots before they start growing can be a successful technique for producing thicker adventitious roots and the development of secondary and tertiary roots. This process helps the date palm plantlets derived from tissue culture to adapt successfully and increases their chances of survival **(Jatoi, 2013)**. **Jatoi *et al.* (2015)** achieved a survival rate of more than 95% by successfully cultivating firmly-rooted date palm shoots in a soil mixture consisting of equal parts peat moss and sand.

3. 6- Biochemical characteristics determination

The addition of salt stress of 8–10 dsm^{-1} decreased total soluble carbs, increased proline, and decreased chlorophyll in *Cassia absus* L. **(Hussain *et al.,* 2009)**. According to **Ebrahimian and Bybordi (2012)**, salt stress elevated the protein content in sunflower and the activity of the antioxidant enzymes CAT and POD.

3. 6.1- Chlorophyll content determination

Bhivare and Nimbalkar (1984) stated that plant species differ in their response to salinity with respect to chlorophyll contents.

Under salt stress at 1000, 1500, and 2000 ppm, total chlorophyll a, b, and carotenoid levels decreased significantly to 1.1, 1.0, and 0.4 mg/g fresh weight (FW) for chlorophyll a, respectively, and 0.7, 0.5, and 0.3 mg/g FW for chlorophyll b, respectively. The obtained carotenoid content decreased from 1.7 to 0.7 mg/g FW when the salinity level increased from 1000 to 2000 ppm.

This decrease in pigment content may be attributable to the harmful effects of salinity on pigment production, which increases pigment breakdown and/or damages the chloroplast thylakoid.

On the other hand, The 500 ppm NaCl treatment provided the highest levels of carotenoid, chlorophyll a, and b (1.70, 1.10, and 2.20 mg/g FW, respectively) in comparison to the control treatment.

Table 4. Impact of various concentrations of NaCl on total Chlorophyll content (mg/g fw) in leaves of shoots.

NaCl concentration (ppm)	Chlorophyll a	Chlorophyll b	Carotenoids
0.0	1.2 b	0.8 b	1.6 b
500	1.7 a	1.1 a	2.2 a
1000	1.1 b	0.7 b	1.7 b
1500	1.0bc	0.5 c	1.3 c
2000	0.4 d	0.3 d	0.7 d

Darwesh *et al.* (2006) showed that the chlorophyll content of date palm leaves reduced between 10,000 and 14,000 ppm of salinity. Furthermore, **Abd El-Samed *et al.* (2011)** discovered that the chlorophyll content of *Zea mays* and *Viciafaba* decreased when exposed to 30-90 µM NaCl. Under salt stress, **Al-Mayahi (2015)** reported a decrease in chlorophyll concentration in date palm leaves. Under high stress conditions, the development of photolytic enzymes such as chlorophylls, which is responsible for the breakdown of chlorophyll, led to a decrease in photosynthetic pigment levels. **Schutz and Fangmier (2001)** found that the decrease in chlorophyll due to stress is caused by an increase in reactive oxygen species (ROS) generation within the cell.

Pesserakli and Huber (1987) found that chlorophyll a, b, carotenoids, and total soluble carbohydrates reduced dramatically as salinity increased relative to the control treatment. The decrease in chlorophyll and carotenoid levels was the result of NaCl indirect influence. There is a connection between chlorophyll concentration, photosynthesis, and CO fixation. The decrease in net CO fixation is due to a lack of water, the closure of stomata, the accumulation of apoplast salt, the loss of turgor in mesophyll cells, and the direct toxicity of salt ions. All of these unfavorable circumstances are indications of a decline in general metabolic activity.

The chlorophylls (a and b) are essential for photosynthesis. Reported drop in chlorophyll concentration in salt-stressed date palm **(Al-Mayahi, 2015)**. The high stress conditions led to reductions in photosynthetic pigments due to the instability of protein complexes and the degradation of chlorophyll produced by the heightened activity of chlorophyll-degrading enzymes. According to the research conducted by **Schutz and Fangmir (2001)**, the reduction in chlorophyll levels caused by stress is linked to a rise in the production of reactive oxygen species (ROS) within the cell. During periods of stress, these unstable molecules known as free radicals induce peroxidation, disintegration, and a reduction in chlorophyll levels in plants. The utilization of SA triggers the removal of reactive oxygen species (ROS), which could

potentially increase the concentration of chlorophylls in date palm. An increase in the activity of ascorbate peroxidase and superoxide dismutase may be connected with SA-induced salt tolerance in date palm plants.

Salt stress adversely affects various components of the photosynthetic process, including the chlorophyll concentration, stomatal closure and the reduction of mesophyll conductance to CO_2 diffusion, all of which restrict the amount of CO_2 available for carboxylation **(Sperling *et al.,* 2014)**.

3. 6.2- Proline content:

Data in **Table (5)** showed that the salt stress had a considerable impact on the proline content. Additionally, there was a substantial interaction effect of salt stress on the proline concentration of shoots. Proline content in treated shoots was significantly boosted by the salt.

Under 500 and 1000 ppm salt stress, the shoots' proline levels were 1.5 and 1.8 (mg/g fw), respectively. Proline contents had increased considerably with increasing salinity levels. The date palm shoots grown under 1500 and 2000 ppm NaCl increased significantly in total proline at 2.5 and 2.9 (mg/g fw), respectively in comparison to the control treatment which recorded 1.1 mg/g fw. Proline research has gained importance in the field of plant stress physiology. The leaves of the tolerant plants have higher levels of proline **(Desnigh and Kanagaraj, 2007).**

Table 5. Impact of various concentrations of NaCl on Proline content (mg/g fw) in leaves of shoots.

NaCl concentration (ppm)	Proline content
0.0	1.1 e
500	1.5 d
1000	1.8 c
1500	2.5 b
2000	2.9 a

For the selection and modification of salinity-resistant plants and plant cells, proline acts as a biochemical marker **(El-Hadrami *et al.,* 2011)**.As a stress indicator, it has been discovered that date palm callus accumulates proline in response to an increase in NaCl concentration **(Jasim *et al.,* 2010)**.

Under salinity conditions, plants alter their metabolic processes to adapt to the altered environmental conditions. The buildup of suitable osmolytes, such as proline and soluble carbohydrates, may be one of the ways plants employ to overcome the consequences of salt stress. The production and storage of free amino acids, particularly proline, by plant tissue in response to drought, salt, or water stress is an adaptive reaction **(Yusuf *et al.,* 2007)**. It has been suggested that proline functions as a compatible solute that modifies the osmotic potential of the marker in response to stress.

It has been thought that the rise in proline in response to salt stress indicates damage brought on by salt stress and provides an adequate cytoplasmic osmotic to prevent the cytosol from being dehydrated **(Hasegawa *et al.,* 1986)**. Multiple *in vitro* culture systems subjected to salt stress have confirmed the buildup of endogenous free proline with an increase in medium salinity **(Patnaik and Debata, 1997)**. As the external NaCl concentration raised, the proline content in date palm cultures increased as well.

The greatest concentrations of NaCl inhibited the growth of explants, whereas proline accumulation continued to climb. Similar to this observation, several studies have discovered a link between proline buildup and inhibition of explant growth **(Cano *et al.,* 1996)**. In reaction to increased salinity, **Al-Khayri (2010)** noticed an increase in proline buildup in callus in another article. **Dash and Panda (2001)** indicated that the proline content of *Phaseolus mungo* increased along with rising NaCl concentration and stress duration. Other studies found that NaCl concentrations between 50 and 150 µM boosted proline levels in *Salventianatans* L. **(Jampeetong and Brix, 2009)**. In numerous *in vitro* culture systems subjected to salt stress, proline buildup with increasing medium salinity has been documented **(Htwe*et al.,* 2011). Al-Mansoori and Eldeen (2007)** also demonstrated that proline accumulation significantly increased in calli produced from immature embryos of local date palm cultivars in response to NaCl salt stress. Additionally, **Jasim *et al.* (2010)** demonstrated that the free proline content increased in the medium used to cultivate somatic embryos and calluses on date palms in response to an increase in sodium chloride concentration.

The assays for free proline demonstrated that this amino acid accumulated excessively in the roots and leaves of each plant subjected to stress. It is astonishingly high when leaves are subjected to salt stress. These findings demonstrate that proline synthesis is a frequent response to abiotic stressors **(Yaish, 2015)**.

3. 6.3- Determination of total soluble protein

The protein content and NaCl concentrations in the leaves of shoots varied significantly. Results in **Table (6)** showed that increasing NaCl levels in the medium reduced the protein content, particularly under high levels of salt stress (2000 ppm), where the protein content was (0.38 mg/g fw) as opposed to (2.21 mg/g fw) for the control treatment. the protein content of the shoots Under 500 and 1000 ppm salt stress, was 1.80 and 1.50 (mg/g fw), respectively. When salinity increased, the amount of total proteins rapidly decreased. Date palm shoots grown at 1500 ppm NaCl had a low protein level of 0.95 mg/g.

Table 6. Impact of various concentrations of NaCl on total soluble protein (mg/g fw) in leaves of shoots.

NaCl concentration (ppm)	protein content
0.0	2.21 a
500	1.80 b
1000	1.50 c
1500	0.95 d
2000	0.38 e

When the salinity exceeds the tolerance limit, it can lead to a noticeable reduction in various growth parameters of plants, such as plant height, leaf area, stem diameter, root length, fresh and dry weight and senescence(**Mobayen and Milthorpe, 1980**). The decrease in growth characteristics that occurs when salinity levels rise can be linked to a variety of physiological processes, including a decrease in photosynthesis, protein dehydration, and the harmful effects of ions building up in plant tissue (**Senaratna *et al.,* 2000**).

The complex processes of adaptation to salinity lead to various modifications, including reduced growth, enhanced expression of specific genes, temporary increases in plant regulators, accumulation of osmolytes and protective proteins, increased antioxidative activities, and inhibition of energy-consuming pathways (**Bartels and Sunkar, 2005**). When exposed to high levels of salt, the process of producing proteins may be affected due to changes in how efficiently mRNA is translated or how RNA transcription, transport and stability are regulated. The expression of salt-stress proteins is influenced by both the genetic make-up of the chosen genotype that is salt resistant and the process by which plants adapt to salinity. These findings supported those made by **El-Bassiouny *et al.* (2008)**, who came to the conclusion that the activation of the de novo synthesis of a number of

novel proteins is one of the key mechanisms involved in the cell's defense against salinity stress. By causing the creation of particular, strain-dependent proteins known as the salt-stress proteins, the salinity changed the protein patterns of two Anabaena strains **(Apte and Bhagwat, 1998)**.

The compared to the control treatment, increasing the salt concentration up to 80 µM NaCl considerably enhanced the amount of total soluble proteins in the leaves. When under salt stress, proteins build up and serve as osmotic regulators **(Ahmad *et al.,* 2003)**. Wheat cultivars with salt stress have more amino acids **(El-Bassiouny *et al.,* 2005)**. The treatment with 80 µM NaCl produced the highest levels of total soluble proteins, which raised by 27.9% compared to the control treatment. In response to biotic and abiotic stresses, plants can produce certain proteins, some of which are then depleted by phytohormones such salicylic acid **(Hussein *et al.,* 2007)**. Stress was found to significantly reduce the protein content of date palm shoots *in vitro*. Under stressful circumstances, protein production proceeds at a slower rate and protein breakdown occurs more often **(Ahmad *et al.,* 2013)**. These results in a decrease in the amount of soluble proteins. Similar outcomes in wheat were noted by **(Gong *et al.,* 2005)**. According to some experts, increased activity of catabolic enzymes like protease that were triggered under stress may be the cause of protein degradation.

3. 6.4- Determination of total carbohydrates

The control treatment leaves had a higher soluble carbohydrate content of 19.3 mg/g FW. At higher salt concentrations, leaves under salt stress had a significantly increased amount of soluble carbohydrates.

Results in **Table (7)** showed that the total carbohydrates in the plant leaves, were 25.5 and 29.8 mg/g FW under salinity levels of 500 and 1000 ppm, respectively, indicated that the shoots were significantly more exposed to salt stress. It has been shown that soluble carbohydrates play a role in osmotic adjustment by the fact that the rate of increase in soluble carbohydrate content has been lowered with increasing NaCl concentrations to 17.0 and 12.2 mg/g FW at 1500 and 2000 ppm of NaCl levels, respectively.

Table (7): Impact of various concentrations of NaCl on carbohydrate content (mg/g fw) in leaves of shoots.

NaCl concentration (ppm)	carbohydrates content (mg/g FW)
0.0	19.3 c
500	25.5 b
1000	29.8 a
1500	17.0 cd
2000	12.2 e

Amino acids and sugars are metabolites that frequently have an osmolyte role, reducing the stress caused by salinity **(Acosta-Motos *et al.,* 2017; Al Kharusi *et al.,* 2019a). Pe'rez-Lo'pez *et al.* (2010)** shown that increase of soluble carbohydrates in crops under stressful conditions may be a part of osmotic adjustment. In response to salinity or drought, soluble carbohydrate buildup was frequently observed **(Cheeseman, 1988)**. Salt stress has an impact on the carbohydrates, one of the main organic components of the dry matter. Growing saline concentrations reduced the total carbohydrate content. Under salt stress, reduced photosynthesis and increased photorespiration may be to blame for the decreased carbohydrate content **(Namich *et al.,* 2007)**. When the date palm's shoots were exposed to salt, they contained more total soluble carbohydrates than untreated shoots **(Dawood *et al.,* 2012)**.

Carbohydrates serve a molecular role in the genes that control sugar, which produce a variety of physiological responses include defensive reactions and cellular growth **(Simaei *et al.,* 2011)**. Additionally, the buildup of carbohydrates is essential for reducing the effects of salt stress, either directly or indirectly **(Ackerson, 1985)**. The 80 µM NaCl treatments yielded the highest amount of total soluble carbohydrates (247.33) **(Szepesi, 2006)**. In oat organ plant (root and bud), the soluble sugar was raised with increasing NaCl concentration **(El-Tayeb, 2005)**.

3. 6.5- Peroxidase (POD) activity:

Plant cells have developed complex defensive mechanisms against salt stress, including enzymatic defenses like superoxide dismutase (SOD), POD, and catalase (CAT). Antioxidant resistance mechanisms may offer a method to improve plant stress tolerance. According to the study's data **(Fig. 5)**, POD and CAT activity increased under salt stress and was inversely correlated with 14000 ppm NaCl concentration. The smallest concentrations of POD operations were found in the control treatment. The enzymatic and no enzymatic antioxidant systems are just a

few of the astonishing array of defense mechanisms that plants have against oxidative stress.

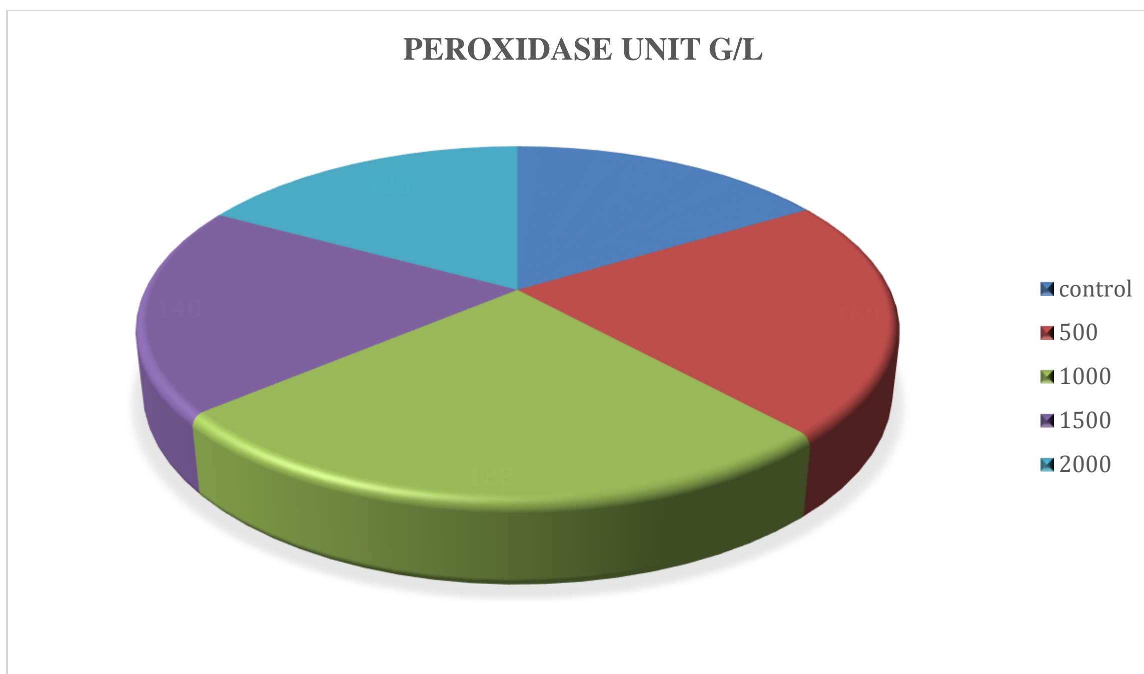

Fig. 5. Effect of salt stress on the peroxidase (unit g/l) activity.

The antioxidant system, which includes the enzymes superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT), and the non-enzymatic antioxidant system, which includes glutathione reductase (GR), superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), is among the impressive array of defense mechanisms that plants have against oxidative stress (Ashraf, 2002). According to **Mittler (2002)** study salinity stimulates and amplifies the activity of these antioxidant enzymes. CAT that involves oxidative stress prevention and hydrogen peroxide decomposition. Therefore, by strengthening the antioxidant defense system, the ability to withstand environmental stress may be dependent, at least in part, on production **(Azevedo *et al.,* 2006)**. Under salt stress of 40–200 µM, POD significantly increased in date palm **(Abdulwahid, 2012)** and *Ctenanthe setosa* **(Mehmet *et al.,* 2013)**.

3. 7- RAPD-PCR analysis

In order to assess the genetic integrity of the chosen regenerate under salt stress conditions, a total of 9 primers were utilized, resulting in the identification of 26 primers that generated observable bands. In each treatment, a total of 140 amplicons were generated by utilizing 26 primers, with sizes ranging from 250 to

6000 base pairs. The primers that were used resulted in the production of 1-10 bands, with an average of 5.4 bands per RAPD primer, as shown in **Fig. 6**. A total of 560 bands were generated through the use of RAPD, which represents the multiplication of the total number of plants studied by the total number of amplicons obtained with all primers. These bands together exhibited a monomorphic pattern throughout all regenerated plants.

Based on the results obtained from physiological, morphological, and biochemical investigations, it was anticipated that a genetic alteration would manifest as somaclonal variation in the regenerate shoots. Hence, the molecular characterization of regenerate shoots was further examined through the utilization of RAPD DNA markers. Nevertheless, as a result of the limited duration of salt stress exposure, the molecular study did not utilize regenerate shoots. The RAPD analysis yielded a consistent pattern in all regenerate shoots, indicating a lack of genetic variation as no polymorphic bands were seen. A total of 560 bands were produced in this investigation using 26 primers, and no polymorphisms were observed. These findings exhibit similarities to the outcomes of other investigations that utilized different plant species and employed PCR-based RAPD analysis (**Lakshmanan *et al*., 2007**), such as the study conducted on date palm (**Moghaieb *et al*., 2011**). The distinct physiological reactions observed with regenerate shoots appeared to be mostly associated with RNA responses rather than DNA responses. It is conceivable that these variations were associated with the extent of the reaction rather than hereditary alterations in regenerate shoots. The potential utility of regenerating and selecting date palm plants acclimated to salt stress in the field lies in their ability to facilitate the large-scale production of salt-adapted plants.

Based on our research findings, it is reasonable to infer that date palm trees produced by this methodology will exhibit enhanced resilience to saline environments compared to untreated counterparts. Abiotic stress tolerance in regenerate shoots can be attributed not only to significant chromosomal alterations or the duplication and deletion of certain areas, but also to single nucleotide variations occurring in the relevant genes. The observed variance in tolerance to salt stress in date palm cv. Bartemoda is primarily attributed to physiological factors rather than genetic factors. Furthermore, the observed alterations in the regenerants can be attributed to either the influence of specific hormones and growth regulators present in the culture medium, or they may be a result of the amelioration of salt stress and the extended duration of culturing in regenerate shoots. In order to identify any alterations occurring at the genetic level, such as point mutations, future investigations may involve conducting whole-genome sequencing studies and/or

utilizing novel breeding tools known as New Breeding Technologies (NBTs) **(Sattar *et al.*, 2017)**. These approaches hold the potential to offer a more comprehensive understanding of the response of *in vitro* regenerated salt-tolerant date palm plants to stress conditions. Furthermore, the insights gained from such studies can contribute to the development of improved strategies for mitigating the effects of salt stress in date palm cultivation. Additional investigations on these regenerants, encompassing molecular analysis by gene sequencing and comprehensive examination at the whole plant level, could potentially yield valuable insights.

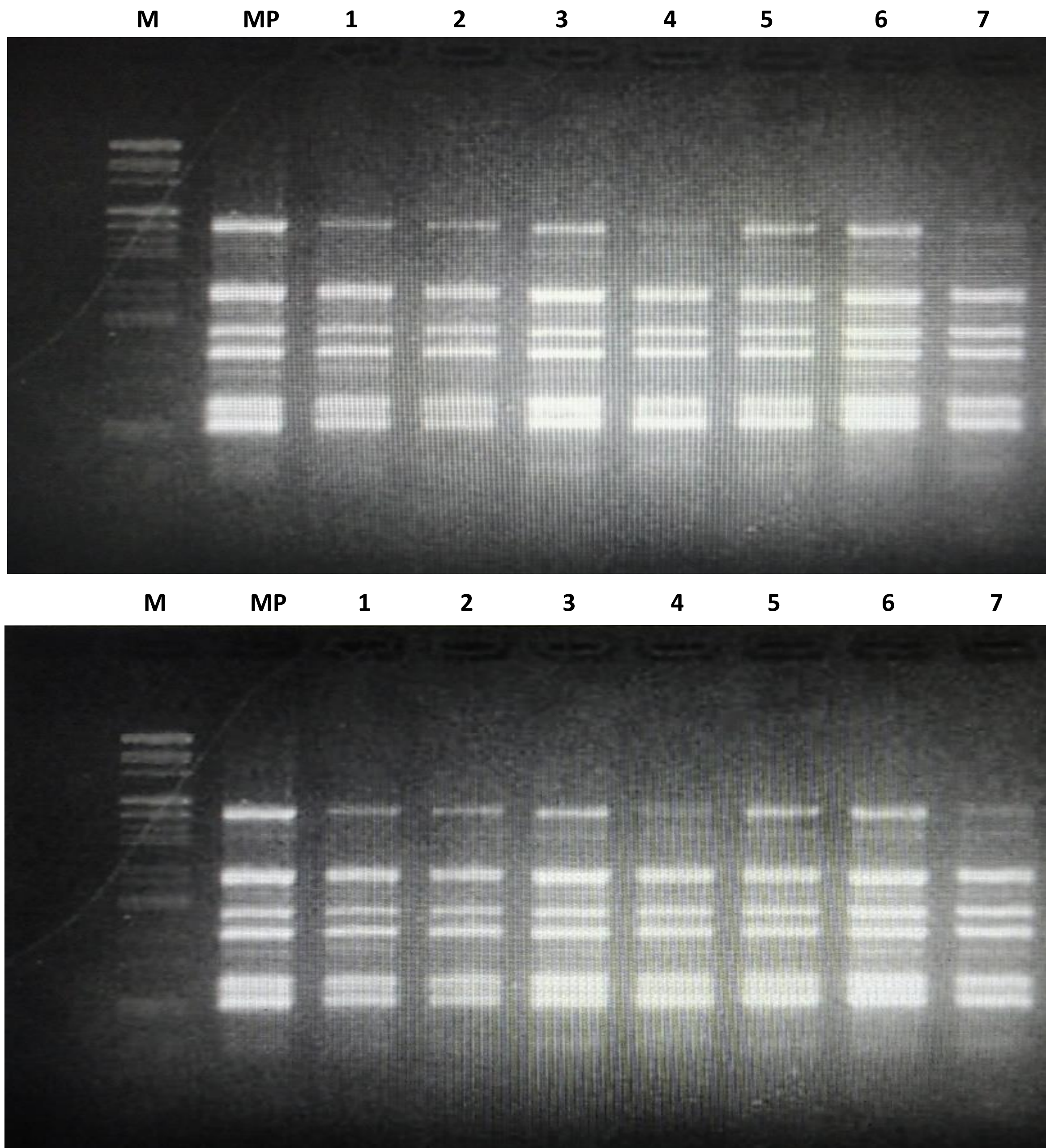

Fig. (6). DNA profiles generated by primers, M: size marker indicated in kb, MP: sample of original plant mother genotype, Lanes 1 to 7: samples of shoots induced in medium containing NaCl at 500 and 1000 ppm. There were no differences in genetic variation (97 and 92%) with two treatments.

4. Conclusion

There were significant increases in both the number of shoots and rooting percentage under salinity stress of 500 ppm NaCl.

The quality of the shoot and root produced in the lab is essential for the ex vitro growth of date palm plantlets in a greenhouse. The highest concentrations of carotenoid, chlorophyll a, and chlorophyll b were obtained at 500 ppm NaCl treatment. The level of proline in the treated shoots was significantly boosted by the salt. Under 500 and 1000 ppm salt stress, the shoots' protein contents were 1.80 and 1.50, respectively. As the concentration of NaCl in the medium increased, the amount of protein dropped. The soluble carbohydrate content of leaves under salt stress was significantly higher with elevated salt concentrations. Despite the fact that the shoot regenerates had a favorable reaction to the elevated salinity levels, it is uncertain whether this response can be attributed to any genetic alterations that occurred during salt stress, as no variations were detected using RAPD analysis. The enhanced efficacy of salt-adapted regenerates in shoot regenerate may be attributed to physiological processes associated with salt adaptation.

Conflicts of interest:

“There are no conflicts to declare”.

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