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Research Article

Effect of NaCl on Growth and Development of *in vitro* Plants of Date Palm (*Phoenix dactylifera* L.) 'Khainazi' Cultivar

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Abstract

Background and Objectives: High salinity of soil and water resources is a global issue which is causing substantial decline of productive lands in many arid and semiarid regions of the world due to salinization. Therefore, the main objective of this study was to determine the effect of NaCl on growth and development of *in vitro* plants of date palm (*Phoenix dactylifera* L.) 'Khainazi' cultivar. **Materials and Methods:** The study was carried out in the Tissue Culture Laboratory, Date Palm Research Center, King Faisal University, Kingdom of Saudi Arabia. Ten date palm offshoots of cv. Khainazi, about 3-4 years old and weighing 5-7 kg were used in the experiment. The shoot tip and lateral buds were sterilized in 20% v/v clorex solution for 15 min and then sectioned into 1 cm explants for culture initiation. The modified MS media was supplemented with different concentrations of NaCl ranging from 0, 50, 100, 200, 300 and 400 mmol L^{-1} and incubated at 25 ± 2°C for 16 h in light daily supplied by 65/80 Warm White Weisse 3500 fluorescent tubes. Each treatment was replicated 10 times by following a completely randomized design. Plant observations included dry weight of shoots and roots and length of stem and roots. The concentration of Na, Mg, Ca and K in plant roots and shoots were determined by the Atomic Absorption Spectrophotometer while Cl by a Chloride meter. Experimental data were analyzed by following appropriate statistical procedures. **Results:** Stem and root length and dry weight were progressively reduced in most of the treatments by the addition of 0, 50, 100, 200, 300 and 400 mmol L^{-1} to the growth medium. The reduction in shoot, however, was greater than root. The concentration of Na increased significantly both in the shoots and roots. While the concentration of Ca, Mg and K increased with the addition of NaCl up to 100 mmol L^{-1}, then decreased with increasing the NaCl concentration up to 400 mmol L^{-1} in the growth medium. **Conclusion:** Addition of high NaCl concentration to growth medium for the development of date palm off-shoots using tissue culture technology did not show encouraging results. In conclusion, addition of NaCl solution having concentration above 100 mmol L^{-1} to growth medium proved detrimental to explants to obtain desired date palm off shoots.

Key words: Tissue culture, *in vitro* date palm development, growth medium, 'Khainazi' cultivar, NaCl solution, plant shoots, plant roots, mineral composition (Na, Ca, Mg, K)

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salinity of soil and water resources spread worldwide. This problem poses one of the most serious threats to food production and sustainability of our natural resources Marschner[1]. Agricultural lands have increasingly been reduced in recent years due to salinization, particularly in arid and semi-arid regions of the world.

In the last decade, tissue culture techniques were recognized as a powerful tool for breeding work[2,3]. Not only this, but in recent years tissue culture technology was used intensively in physiological studies of the mechanisms of stress tolerance and/or resistance of plants[4-12]. The tissue culture techniques under these studies may serve as an excellent model system in the investigation of mechanisms operating on the whole plant. Some of the advantages in using tissue or cell culture for physiological studies include: (1) Homogeneity of the cell population, (2) Growth of tissues or cells in defined media under controlled environment, (3) Enable to perform experiments throughout the year, (4) Enable to study the response of tissues or cells isolated from different parts of the plant, (5) Its use to differentiate between mechanisms operating on the cellular level only and on the organization of the cells in the whole plant, (6) The suitability of naked protoplasts for studying aspects of mechanisms related to changes in membrane characteristics and (7) The short time and the limited space needed to execute such type of experiments[2,13-15].

In Saudi Arabia, the cultivated area is constantly declining due to many environmental hazards, particularly the soil and water salinity. The estimated data showed an approximately 12000 ha of cultivated land left in Al-Hassa region which was considered until recently the largest producing date palm area in the Kingdom. Date palm is the major fruit crop in Kingdom of Saudi Arabia. It occupies approximately 72% of the total area under permanent crops in the Kingdom. The estimated number of date palm trees in the Kingdom is 18 million, 12 millions of which are bearing trees with an annual production of 590,000 t. Over 400 varieties of date palm are estimated to exist in Saudi Arabia[16]. Studies on the response of date palm cultivars to salinity hazards in the Kingdom are few and covers a limited number of varieties according to Al-Khateeb[7]. Although date palm is a semi-salt tolerant crop, many cultivars are severely affected with high soil salinity and the yields were greatly reduced[17-19]. Increase of main roots of date palm tree in the previous studies in soil salinity exceeding 1% often prohibits fruiting. However, fruiting is normal if the soil salinity is less than 0.6%. In both cases, callus growth retardation was clearly evident with increasing salinity. Both types of calli also exhibited tissue dehydration symptoms as observed with a significant increase in callus dry weight ratio. Callus Na content increased considerably with increasing NaCl level, whereas K content decreased causing a significant reduction in callus K^+/Na^+ status. El-Sharabasy *et al.*[20] evaluated an *in vitro* technique for salt tolerance of three date palm cultivars Samani, Sewy and Bartamuda cultured on MS medium with 0.0, 4000, 8000 and 12000 mg L^{-1} NaCl after 12 weeks for three subcultures. The effect of salinity on shoot length did not show any significant differences in control 0.0 mg L^{-1}), but it increased significantly for the three cultivars especially Bartamuda cv. to 4000 mg L^{-1} then decreased significantly at 8000 and 12000 mg L^{-1} NaCl. Number of shoots showed the highly significant value at 4000 mg L^{-1} for the Bartamuda cv., however, at 8000 and 12000 mg L^{-1} it decreased and the differences between Sewy and Bartamda cvs. were insignificant followed by Samani cv. The number of burned leaves was the highest at 12000 mg L^{-1} NaCl for the Sewy and Samani cvs. as compared with Bartmuda cv. Significant differences were also found between Bartamuda and the other two cultivars, but the growth vigor of Bermuda was still the highest even at 12000 mg L^{-1} NaCl.

Previously, salinity iwa considered as one of the serious problems in many irrigated agriculture areas. Since date palm is a major cultivated fruit crop in Kingdom Saudi Arabia, therefore, study of differential responses to salinity is inevitable. The main aim of this study was to determine the effect of different concentrations of NaCl on growth and development of *in vitro* Plants of Date Palm (*Phoenix dactylifera* L.).

MATERIALS AND METHODS

This experiment was conducted in the Tissue Culture Laboratory, Date Palm Research Center, King Faisal University, Kingdom of Saudi Arabia from August, 2016 to March, 2017. Ten date palm offshoots of cv. Khainazi about 3-4 years old and weighing 5-7 kg were separated from healthy mother trees from date palm orchard of King Faisal University, Al-Ahsa. Then the offshoots were thoroughly cleaned and the outer leaves were removed to expose the shoot tip and lateral buds region. The exposed region was excised and immediately placed in antioxidant solution containing 15 mg^{-1} ascorbic acid and 100 mg^{-1} citric acid. The shoot tip and lateral buds were sterilized in 20% v/v clorex solution for 15 min, followed by rinsing 3 times with distilled water. The tissues were kept into the previous antioxidant solution until explant excision for culturing. The shoot tip and lateral buds were sectioned into 1 cm explants for culture initiation by following the procedure described by Alkhateeb and Ali-Dinar[21]. One rooted plant resulted from direct organogenesis was transferred to test tube filled with 15 ml of modified MS salts media[22] supplemented with 170 mg L^{-1} $NaH_2PO_4.2H_2O$, 125 mg L^{-1} inositol, 200 mg L^{-1} glutamine, 1 mg L^{-1} thiamine HCl, 1 mg L^{-1} pyridoxine HCl, 1 mg L^{-1} nicotinic acid, 1 mg^{-1} calcium pantothenate; 1 mg L^{-1} biotin, 7 g^{-1} purified agar and 30 g L^{-1} sucrose. The modified MS media was supplemented with different concentrations of NaCl ranging from 0, 50, 100, 200, 300 and 400 mmol L^{-1}. Cultures were incubated at 25±2°C for 16 h daily in light supplied by 65/80 Warm White Weisse 3500 fluorescent tubes. Each treatment was replicated 10 times by following a completely randomized design. Plant growth parameters recorded were dry weights of shoot and root (g) as well as stem and root length (cm).

The samples were washed twice with sorbitol solution. Then the tissues were ground in deionized water followed by digestion for 2-4 h over a 150°C hot surface. Ionic concentration of Na, Ca, Mg and K was determined in roots and shoots by the Atomic Absorption Spectrophotometer and the Cl by a Chloride meter.

Data analysis: Data were subjected to statistical analysis as a randomized complete design according to Gomez and Gomez[23]. All the statistical analysis was performed by analysis of variance (ANOVA) and Duncan multiple range test for significance using the computer facility and SAS software package[24].

RESULTS

Effect of NaCl on plant growth parameters

Plant stem height: Mean length of plant stem ranged between 23 and 15 cm in different NaCl concentrations ranging from 0-400 mmol L^{-1} (Fig. 1). The reduction in plant stem height showed significantly decreasing trend in all the treatments compared to the control treatment when NaCl concentration was increased from 0-400 mmol L^{-1}. The results in Fig. 1 showed that reduction in plant stem height was significant among treatments T-1, T-2 and T-3 treatments when the NaCl concentration increased from 0-100 mmol L^{-1}. Although the plant stem height showed slight increasing trend between treatment T3 and T-4 with increasing the NaCl concentration from 100-200 mmol L^{-1} which is hard to explain, but the difference in stem height was not significant between T-3 and T-4 treatments. However, the reduction in plant height was significant among Treatments T-4, T-5 and T-6 when the NaCl concentration increased from 200-400 mmol L^{-1}. The data showed that plant growth was retarded under high concentration of NaCl which may be attributed to high osmotic potential of the soil solution preventing the uptake of other useful nutrients for plant growth.

Root length: Mean length of plant root ranged between 6 cm and 4.5 cm under different NaCl treatments (Fig. 2). Mean root length of plants showed significant decreasing trend with increasing the NaCl concentration from 0 (control) to 50 mmol L^{-1}. Although the mean plant height showed decreasing trend with corresponding increase in NaCl concentration, but the difference in root length was not significant between T-2 and other high NaCl treatments. The data indicated that high NaCl concentration did not cause any adverse effect on plant root growth and seemed adjusted to high osmotic potential of soil water solution.

Shoot dry weight: It was observed from Fig. 3 that mean shoot dry weight showed continuous decreasing trend with corresponding increase in NaCl concentration. The shoot dry weight ranged between 0.16-0.13 g when NaCl concentration was increased from 0-400 mmol L^{-1}. The results indicated that addition of high NaCl concentration adversely affected the plant growth. The trend of shoot dry weight is similar to the plant stem height.

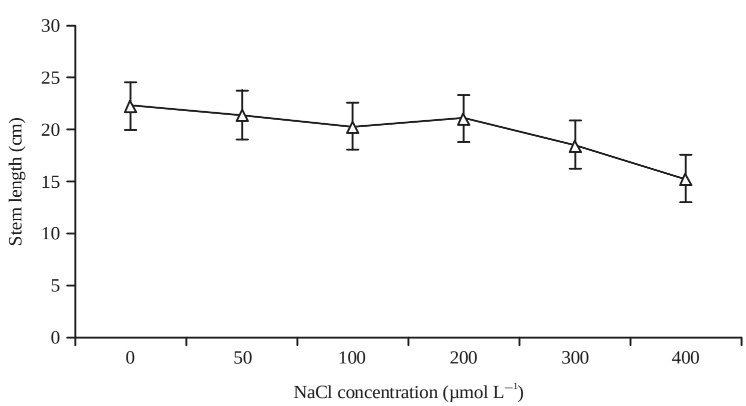

Fig. 1: Stem length (cm) as affected by different NaCl concentrations

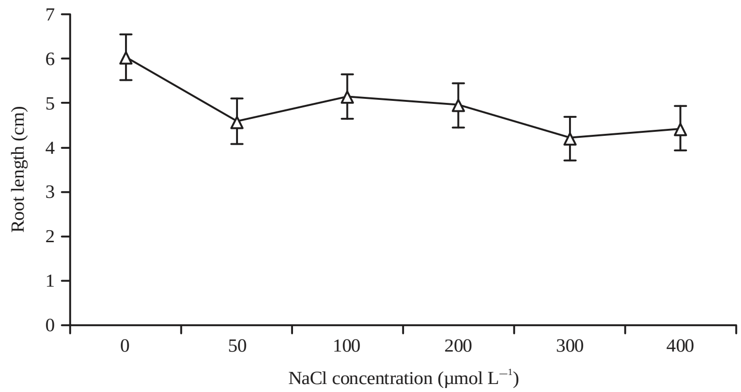

Fig. 2: Root length (cm) as affected by different NaCl concentrations

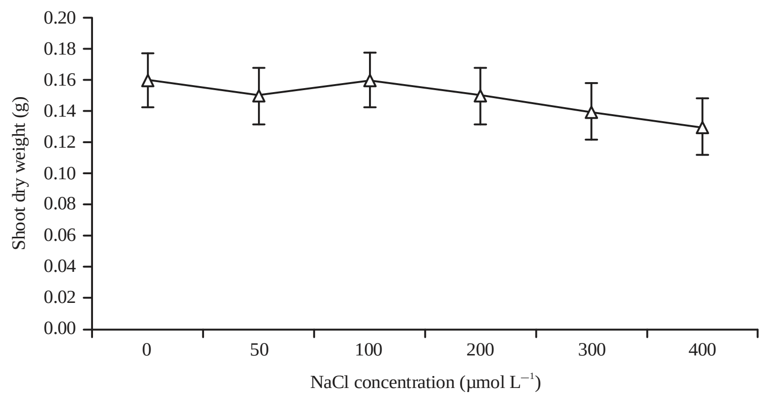

Fig. 3: Shoot dry weight (g) as affected by different NaCl concentrations

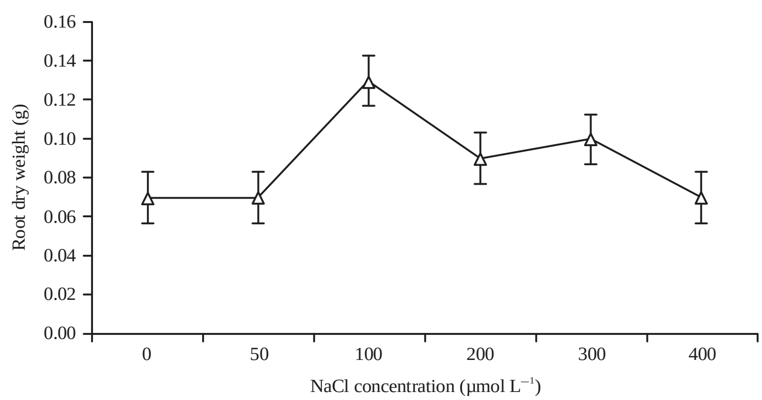

Fig. 4: Root dry weight (g) as affected by different NaCl concentrations

Root dry weight: The mean root dry weight ranged from 0.07-0.13 g under different NaCl concentration treatments (Fig. 4). However, the mean root dry weight increased significantly up to 100 mmol L^{-1} NaCl concentration, but then decreased with increasing the NaCl concentration to 400 mmol L^{-1}. The data in Fig. 4 indicated an increasing trend in root dry weight under high salt concentration. Overall, the plant root growth was affected appreciably in high NaCl concentration and seemed adjusted to high osmotic potential.

Total dry weight: Data in Fig. 5 indicated that overall total dry weight of plant (both the shoot and root dry mass) increased with an increase in high NaCl concentration (Fig. 5). This

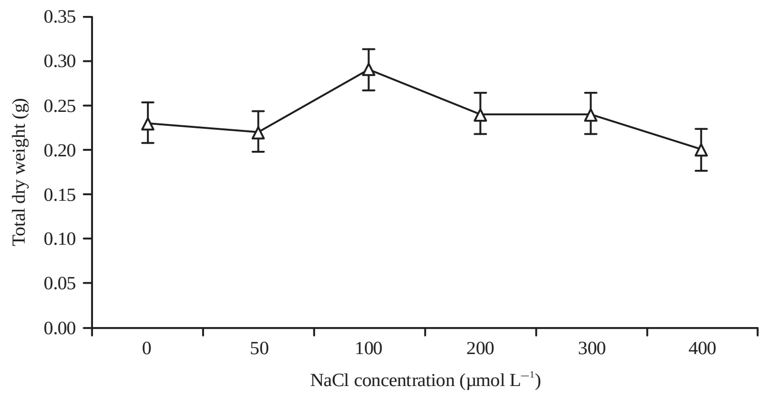

Fig. 5: Total dry weight (g) as affected by different NaCl concentrations

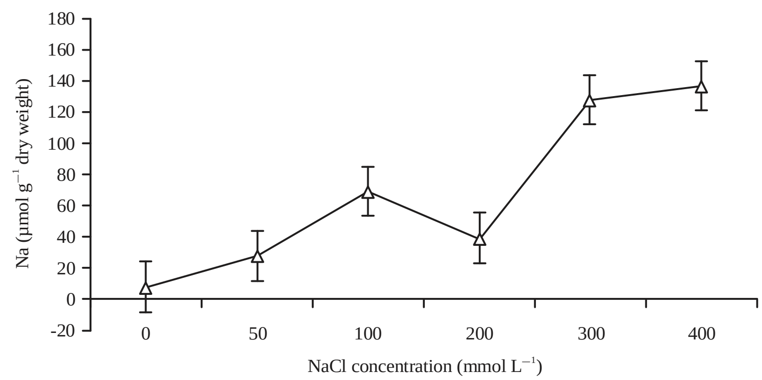

Fig. 6: Na in roots (μmol g^{-1} dry weight) as affected by different NaCl concentrations

suggests that plant needs mineral nutrition for its growth up to certain level after which the high salt concentration adversely affected the plant growth due to high osmotic potential thus causing stunted plant growth. Because at high salt concentration, the plant could not uptake certain mineral elements for its growth due to antagonistic effect among the different plant nutrients present in the soil water solution.

Salt treatments vs concentration of different minerals in plant parts

Sodium (Na) in roots: The analysis of data in Fig. 6 indicated a significant increase in the mean Na concentration in plant roots and ranged between 10 and 138 μmol g^{-1} on dry weight basis. Also, a linear increase was found in Na contents of plant roots with corresponding increase in high NaCl concentration. It is obvious that plant roots absorbed more Na ions due to its high concentration compared to other nutrient ions in the soil water solution.

Potassium (K) in roots: The K contents of plant roots increased from 20-70 μmol g^{-1} with NaCl concentration up to 100 mmol L^{-1}, but later on it was decreased appreciably with an increase in NaCl concentration (Fig. 7). This suggests a competition between nutrient ions from the soil solution. In the present case, Na ion was dominant compared to K ion in the soil solution thus allowing the plant roots to absorb less K ion than the predominant Na ion.

Calcium (Ca) in plant roots: Mean concentration of Ca ion in plant dry mass ranged between 0.60-0.82 μmol g^{-1} in various NaCl treatments (Fig. 8). The pattern of Ca contents in plant roots was similar to that of K contents which showed sharp increase up to 100 mmol L^{-1} with NaCl concentration, but it decreased considerably when the NaCl concentration was increased from 100-400 mmol L^{-1}.

Magnesium (Mg) in plant roots: Mean concentration of Mg in plant roots was from 0.07-0.21 μmol g^{-1} on dry weight basis (Fig. 9). The trend of Mg concentration was comparable with K contents in plant roots which increased with addition of NaCl concentration up to 100 mmol L^{-1}, but later on the Mg contents of plant roots decreased. This variability in the Mg contents of plant roots indicates antagonistic effect among the various mineral elements present in the soil solution due to variation in their atomic weight.

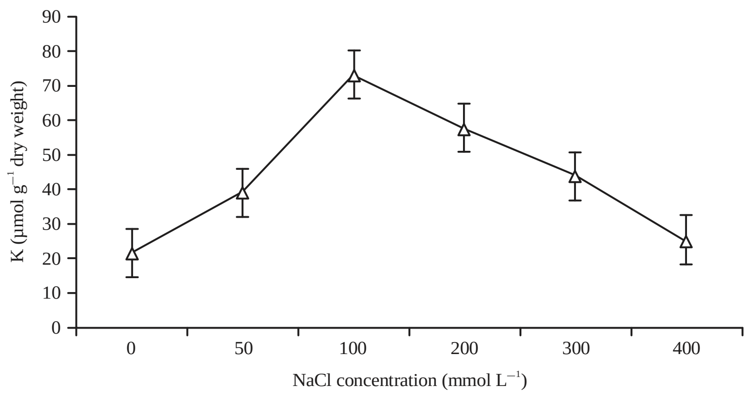

Fig. 7: K in roots (µmol g^{-1} dry weight) as affected by different NaCl concentrations

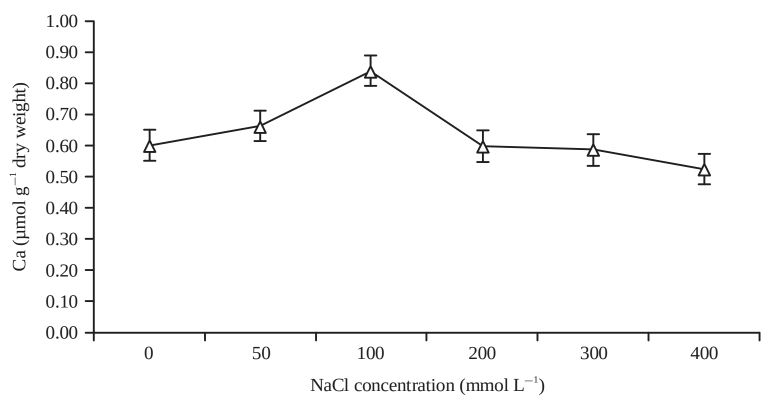

Fig. 8: Ca in roots (µmol g^{-1} dry weight) as affected by different NaCl concentrations

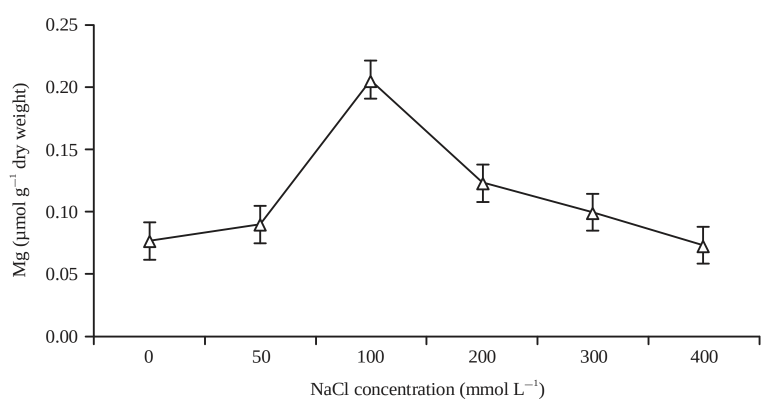

Fig. 9: Mg in roots (µmol g^{-1} dry weight) as affected by different NaCl concentrations

Sodium (Na) contents in plant leaves: The data in Fig. 10 indicated a linear increase in the Na contents of plant leaves and ranged between 15 and 250 µmol g^{-1} on dry weight basis. It is obvious, because addition of high NaCl concentration will significantly increase the Na ion is soil solution compared to other nutrient ions thus resulting in high uptake of Na ion by the plant aerial parts (leaves) during growth period.

Potassium (K) contents in plant leaves: A study of data in Fig. 11 indicate that K contents of plant leaves decreased significantly in high NaCl concentration treatments. Mean K contents ranged between 110-59 µmol g^{-1} when the NaCl concentration was increased from 0-400 mmol L^{-1}. This indicated that K ion uptake by plants was significantly low in the presence of high Na ions

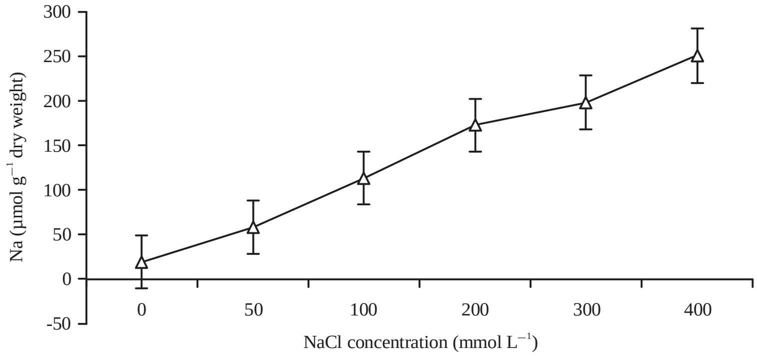

Fig. 10: Na in leaves (µmol g^{-1} dry weight) as affected by different NaCl concentrations

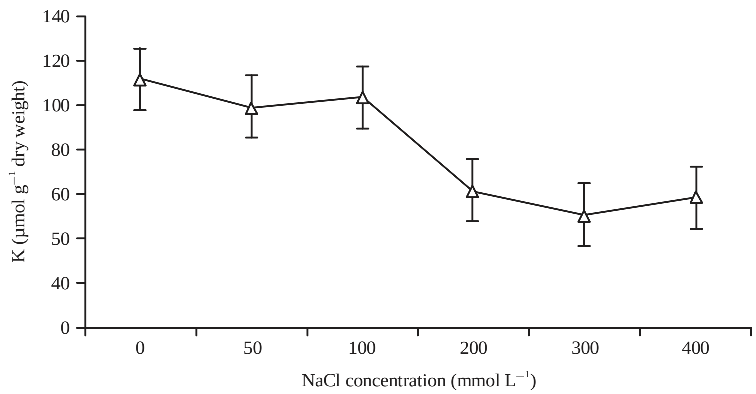

Fig. 11: K in leaves (µmol g^{-1} dry weight) as affected by different NaCl concentrations

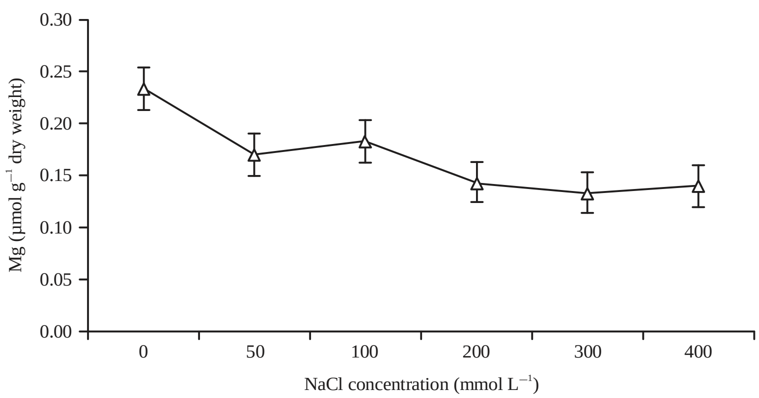

Fig. 12: Mg in leaves (µmol g^{-1} dry weight) as affected by different NaCl concentrations

in soil solution due to antagonistic effect among different plant nutrient ions for their uptake by growing plants.

Magnesium (Mg) contents in plant leaves: The data in Fig. 12 shows a linear decrease in Mg contents of plant leaves with addition of high NaCl concentration. There was a significant decrease in Mg contents ranging from 0.23-0.16 µmol g^{-1} when the NaCl concentration was increased from 0-50 mmol L^{-1}. Although there was a decreasing trend in Mg concentration of plant leaves with increasing the concentration of NaCl, but the difference was not significant at 5% level of significance as indicated from the bars at each salt concentration. This infers that Mg uptake was significantly affected by the growing plants in the presence of high concentration of Na ion in soil water solution due to competition among the different nutrients in the soil solution.

DISCUSSION

Analysis of study data showed that plant growth parameters such as stem length, root length, dry-weight of stem and root as well as the overall dry-weight were significantly affected by the high concentration of NaCl due to high osmotic potential causing stunted plant growth. The study findings are in line with those of Al-Mansoori and Eldeen[25] and Ahmad and Ismail[26], who found significant reduction in growth of date palm with salinity up to 3.3%. Also reduction in date palm offshoots (seedlings) of 'Sakoti' and 'Bertamouda' cultivars were considerable with irrigation water salinity above 12000 mg L^{-1}. These investigators further reported that irrigation water salinity caused significant and total inhibition of date palm immature embryos when 3.0% (w/v) NaCl solution was added to the induction medium. Similarly, El-Sharabasy *et al.*[20] stated that the shoot length of three date palm cultivars namely Samani, Sewy and Bartamuda did not show any significant effect of salinity under control, but it increased in salinity up to 4000 mg L^{-1} and then showed significant reduction with salinity at 8000 and 12000 mg L^{-1} NaCl.

In the present study, the concentration of Na increased with the addition of increasing NaCl concentration both in plant leaves and roots. But the concentration of K, Ca and Mg increased up to 100 µmol L^{-1} NaCl concentration, then a decreasing trend was observed with high NaCl concentration ranging from 200-400 µmol L^{-1}. The study findings agree with those of Taha and Hassan[18] as well as with Yaish and Kumar[19], who found that callus Na^+ content increased appreciably with increasing NaCl level, whereas the concentration of K^+ decreased resulting in significant reduction in callus K^+/Na^+ status. The research finding are in line with the findings of Alkhateeb *et al.*[27], who observed that K/Na ratio of soil salinity is significant to the K status of the callus with high Na contents of soil solution causes considerable reduction in K values of the date palm callus especially under high soil salinity environment.

CONCLUSION

Mean plant stem height and root length decreased significantly with increasing the NaCl concentration in the growth medium from 0-400 mmol L^{-1}. Mean shoot dry weight showed continuous decreasing trend with corresponding increase in NaCl concentration and it ranged between 0.16-0.13 g. However, mean root dry weight increased significantly with 100 mmol L^{-1} NaCl concentration. Overall total biomass of plant (both the shoot and root dry mass) increased with high NaCl concentration. The concentration of Na in plant roots and leaves increased linearly under high concentration of NaCl. While K, Ca and Mg concentration increased sharply with the addition of NaCl up to 100 mmol L^{-1}, but showed a significant reduction with increasing NaCl concentration up to 400 mmol L^{-1}. Mean K contents ranged between 110-59 µmol g^{-1} when NaCl concentration was increased from 0-400 mmol L^{-1}. A linear decrease in Mg contents of plant leaves was found with increasing NaCl concentration.

SIGNIFICANT STATEMENTS

This study tried to test and evaluate date palm off shoots using different concentration of NaCl to determine the level of salinity for healthy date palm off shoot for sustainable plant production. Previously many researchers used tissue culture technology for the development of new strains for high productivity and high salt tolerance under arid environment. The results of this study suggested that production of high salt tolerant date palm cultivar is possible if the NaCl concentration is less than 100 mmol $L^{-1}$for addition to growth medium using tissue culture Technology.

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