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# *In vitro* Responses of Date Palm Cell Suspensions under Osmotic Stress Induced by Sodium, Potassium and Calcium Salts at Different Exposure Durations

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## ABSTRACT

*In vitro* cultures subjected to salt-stress have been shown to exhibit unique characteristics that are useful for identifying stress status. The objective of this study was to investigate the physiological responses of date palm, *Phoenix dactylifera* L. cv. Barhee, callus to salinity stress. Callus were cultured on MS medium supplemented with NaCl, KCl, or $CaCl_2$ at 0.8 MPa (-8 bars) equivalent osmotic potential concentrations. The exposure to salt stress resulted in reduction in callus dry weight as compared to the control. Sodium chloride caused the highest reduction in dry weight followed by KCl then $CaCl_2$. In general, callus water content decreased in response to extending exposure durations regardless of the salt type used. Increasing the exposure duration up to 6 days caused increase in proline content compared to the control. Extending the exposure duration of KCl and $CaCl_2$ to 9 days caused reduction in proline content, due to cell death as indicated by culture browning. Exposure to NaCl initially caused increase in $Na^+$ content but at the ninth day, significant reduction in $Na^+$ content was observed. Increasing salt exposure duration caused significant increase in $K^+$ content as compared to the control, up to 3 days of exposure after which the content decreased but remained higher than the control cultures. The $Na^+/K^+$ ratio was also significantly affected by the salt type and the exposure duration. This study has enhanced the understanding of the influence of salinity on physiological aspects of date palm cell cultures.

**Key words:** Callus, date palm, *in vitro*, salinity, salt stress, tissue culture, proline

## INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous, dioecious tree economically important fruit species (Alshuaibi, 2011). Dates provide numerous health benefits and enrich nutritional value of processed food products (Gad *et al*., 2010). By-products provide raw materials for production of many agricultural products like leaves compost as a peatmoss replacement (Ali, 2008) and nitrogen source (Al-Shaikh *et al*., 2009). Date palm is one of the most resilient plants to adverse environmental conditions which is well adapted to arid regions of the Middle East and North Africa, primarily where saline soils and water scarcity predominate (Zohary and Hopf, 2000; Johnson, 2011). Fruit quality and yield are significantly affected by soil salinity and water status, problems that are gradually mounting as a consequence of agricultural practices and environmental changes (Iqbal *et al*.. 2004; Al-Saikhan, 2008; Marzouk and Kassem, 2011).

Date palm has long juvenile phase which hinders conventional breeding techniques (El-Hadrami and El-Hadrami, 2009). Plant tissue culture techniques provide powerful tools to overcome breeding constraints. Several researchers successfully demonstrated date palm *in vitro* regeneration (Al-Khayri, 2001, 2003; Fki *et al*., 2003; Eshraghi *et al*., 2005; Al-Khayri, 2007; Zouine and El-Hadrami, 2004, 2007; Alkhateeb, 2008; Othmani *et al*., 2009; Al-Khayri, 2010, 2011; Sghaier-Hammami *et al*., 2010). However, regeneration of genotypes tolerant to abiotic stress is yet to be realized. Nonetheless, several studies were conducted to gain an understanding of the behavior of date palm under stress induced by drought (Al-Khayri and Al-Bahrany, 2004; Helaly and EL-Hosieny, 2011) and salinity stress (Al-Khayri, 2002; Al-Mansoori and Eldeen, 2007; El-Sharabasy *et al*., 2008; Jasim *et al*., 2010).

Salinity adversely affects growth, physiological and metabolic processes of whole plants (Ashraf *et al*., 1991; Jat and Sharma, 2006; Lopez *et al*., 2008; Pongprayoon *et al*., 2008; Pak *et al*., 2009; Amirjani, 2010). Cell cultures provide controlled, uniform environment ideal for studying salt-stress response by eliminating complications arising from genetic and morphological variability associated with tissues of whole plants (Stravareck and Rains, 1984).

Salt stress affects numerous physiological and biochemical processes at the cellular level. *In vitro* cultures of different plant species subjected to salt-stress exhibited reduction of callus growth (Kumar and Sharma, 1989; Shah *et al*., 1990; Carceller and D'Ambrogio, 1994; Gangopadhyay *et al*., 1997; El-Yacoubi *et al*., 2010; Htwe *et al*., 2011), increased accumulation of proline (Kumar and Sharma, 1989; Dutta Gupta *et al*., 1995; Patnaik and Debata, 1997; Chaudhary *et al*., 1997; El-Yacoubi *et al*., 2010; Htwe *et al*., 2011) and increased sodium and decreased potassium ion concentrations (Cano *et al*., 1996; Patnaik and Debata, 1997; Chaudhary *et al*., 1997; El-Yacoubi *et al*., 2010).

Previous studies related to date palm response to *in vitro* salt stress focused on sodium chloride (NaCl). These studies have shown that NaCl can exert an influence on callus growth depending on concentration. Al-Khayri (2002) noted a positive effect on the proliferation of shoot tip-derived callus in response at low concentrations of 25 mM NaCl, but higher concentrations were inhibitory. Similarly, Al-Mansoori and Eldeen (2007) reported that NaCl retarded growth of callus derived from zygotic immature embryos and complete inhibition occurred when 3.0% NaCl was incorporated into the induction medium. El-Sharabasy *et al*. (2008) reported that salinity increase shoot length significantly for the three date palm cultivars Bartamuda , Sewy and Samani when 0.4% NaCl was incorporated into the MS medium then decreased significantly at higher concentrations, 0.8 and 1.2% NaCl. Number of shoots showed the high significant value at 0.4% NaCl for the Bartamuda cv., however, at 0.8 and 1.2% NaCl it decreased. Jasim *et al*. (2010) observed that NaCl negatively affected date palm callus and somatic embryos. They found that an increase of NaCl concentrations in the medium led to a decrease of total soluble carbohydrates and proteins content.

In response to salt *in vitro* treatments, accumulation of proline has been observed in plant species (Binzel *et al*., 1987; Chandler and Thorpe, 1987; Hassan and Wilkins, 1988; Zebarjadi *et al*., 2011; Htwe *et al*., 2011). In addition, sodium and potassium ions are involved in the osmotic adjustment (Martinez *et al*., 2004). The $K^+/Na^+$ ratio was positively correlated to proline accumulation and hence callus growth inhibition (Al-Khayri, 2002; Al-Mansoori and Eldeen, 2007).

Studies related to the effect of salts other than sodium chloride on date palm *in vitro* cultures were not encountered; hence, this study was conducted. The objective was to compare the response of date palm callus to osmotic stress induced by three salts, including potassium chloride (KCl), calcium chloride ($CaCl_2$) and sodium chloride (NaCl), in relation to the duration of exposure to the

stress agent. Responses studied were callus dry weight, water content, proline accumulation and $Na^+$ and $K^+$ concentrations and ratio.

## MATERIALS AND METHODS

**Culture establishment and maintenance:** Shoot tips were separated from 3- to 4-year-old offshoots, cv. Barhee, surface sterilized in 70% ethanol for 1 min followed by 15 min in 1.6% w/v sodium hypochlorite (30% v/v Clorox, commercial bleach) containing 3 drops of Tween 20 (Sigma Chem Co, St. Louis, MO) per 100 mL Clorox solution and rinsed with sterile distilled water four times. The tissue was then placed in chilled antioxidant solution consisting of 150 mg $L^{-1}$ each of ascorbic acid and citric acid to prevent browning. Whole leaf primordia and terminal tip longitudinal sections served as explants. Initiation cultures were incubated in darkness at 24±3°C for 12 week after which resultant callus was transferred to callus proliferation medium. After 3 week the callus was transferred to embryogenic callus medium for 9 week. Thereafter, embryogenic callus was maintained on callus maintenance medium. These cultures served as a callus source for the salt-induced stress study. Callus proliferation cultures and subsequent phases were incubated at 24±3°C and a 16 h photoperiod (50 μmol $m^{-2}$ $sec^{-1}$) provided by cool-white fluorescent lamp and transferred to fresh medium at 3 week intervals.

**Callus medium and exposure to various salts:** The basal culture medium consisted of Murashige and Skoog (1962) salts supplemented with (per liter) 170 mg $NaH_2PO_4$, 125 mg myo-inositol, 200 mg glutamine, 1 mg nicotinic acid, 1 mg pyridoxine-HCl, 5 mg thiamine, 30 g sucrose and 7 g agar (purified Agar-agar/Gum agar) (Sigma). Hormonal supplements and activated charcoal were added according to the culture phase as shown in Table 1. Media were adjusted to pH 5.7 with 1 N KOH, dispensed in 125 mL flasks (50 mL per flask) capped with non-absorbent cotton plugs and aluminum foil and autoclaved for 15 min at 121°C 1.1 kg $cm^{-2}$.

To determine the callus responses to salt-induced stress, callus was grown in liquid medium, identical to maintenance medium but without agar, supplemented with 0.8 MPa equivalent osmotic potential of the following salts: : 179.84 mM (12.06 g $L^{-1}$) potassium chloride (KCl), 172.49 mM (11.96 g $L^{-1}$) calcium chloride ($CaCl_2$) and 150.44 mM (9.45 g $L^{-1}$) sodium chloride (NaCl). Each treatment consisted of 18 cultures containing 1 g embryogenic callus per flask. To evaluate the response of callus in relation to time, 3 cultures were analyzed weekly for ion concentrations ($Na^+$ and $K^+$) and proline accumulation. The data were subjected to Analysis of Variance (ANOVA) and the means were separated, where appropriate, with a least significant difference (LSD) at 5% significance level.

Table 1: Hormonal and activated charcoal requirements for date palm *in vitro* culture stages

| | Culture phase | | | |
|---|---|---|---|---|
| Media additives | Culture initiation | Callus proliferation | Embryogenic callus | Callus maintenance |
| 2,4-Dichlorophenoxyacetic acid (2,4-D) | 100 mg $L^{-1}$ (453 μM) | _ | _ | _ |
| 2-Isopentenyladenine (2iP) | 3 mg $L^{-1}$ (15 μM) | 30 mg $L^{-1}$ (147 μM) | 6 mg $L^{-1}$ (30 μM) | 1.5 mg $L^{-1}$ (7 μM) |
| Naphthaleneacetic acid (NAA) | _ | 10 mg $L^{-1}$ (54 μM) | 10 mg $L^{-1}$ (54 μM) | 10 mg $L^{-1}$ (54 μM) |
| Activated charcoal | 1.5 g $L^{-1}$ | 1.5 g $L^{-1}$ | 1.5 g $L^{-1}$ | _ |

**Determination of free proline content:** Extraction and estimation of proline was conducted according to the procedures described by Bates *et al.* (1973). Fresh callus, 500 mg per sample, was homogenized in 10 mL of 3% (w/v) aqueous sulphosalicylic acid and the homogenate was filtered through Whatman No. 2 filter paper. In a test tube 2 mL of the filtrate was mixed with 2 mL acid ninhydrin and 2 mL glacial acetic acid and incubated in 100°C water bath for 1 h. The reaction mixture was terminated by placing in ice bath, extracted with 4 mL toluene and the chromophore phase was aspirated from the aqueous phase. The absorbance was read at 520 nm using LKB Navaspec Model 4049 spectrophotometer.

**Extraction and Estimation of $Na^+$ and $K^+$:** Fresh callus tissue, 500 mg, was placed in a digestion flask to which 8 mL concentrated $HNO_3$ and 2 mL $H_2SO_4$ were added. The flasks were heated gently over a hot plate until the solution became colorless. The digestion was cooled and diluted to 50 mL with distilled water. Sodium and potassium ions were determined using Jenway Model PEP7 flame photometer.

## RESULTS AND DISCUSSION

**Effect of salt stress on callus dry weight:** According to the analysis of variance (Table 2), callus dry weight was affected by salt type and the duration of exposure in a significant two-way interaction. This suggests that the effect of each salt on callus dry weight was dependent upon the exposure duration. The results have shown that callus dry weight increased over time regardless of the salt type used (Fig. 1). However, the exposure to salt stress resulted in reduction in callus dry weight as compared to the control. The extent of reduction in dry weight differed according to salt type. Sodium chloride caused the highest reduction in dry weight followed by KCl then $CaCl_2$.

In the current study, however, inhibitory levels of various salts were used to assess salt stress effect; therefore, reduction in growth as expressed in dry weight was observed as early as 6 days after exposure. Salt exposure for 3 days was not sufficient to decipher the growth inhibitory affect associated with salt stress (Fig. 1). Instead, short durations of salt exposure stimulated callus dry

Table 2: Analysis of variance of the effect of various salt types (NaCl, KCl and $CaCl_2$) on callus dry weight, water content and proline content of date palm callus exposed to 0.8 MPa salt stress for different durations (1, 3, 6, 9 and 12 days)

| Factor | DF | SS | MS | F-value | p-values |
|---|---|---|---|---|---|
| Callus dry weight | | | | | |
| Exposure duration | 4 | 0.0137 | 0.0034 | 21.3049 | 0.0001 |
| Salt type | 3 | 0.0027 | 0.0009 | 5.6510 | 0.0025 |
| Duration x salt | 12 | 0.0070 | 0.0006 | 3.6317 | 0.0010 |
| Error | 40 | 0.0064 | 0.0002 | | |
| Water content | | | | | |
| Salt type | 3 | 26.2547 | 8.7516 | 25.7788 | 0.0001 |
| Duration x salt | 12 | 10.7596 | 0.8966 | 2.6411 | 0.0106 |
| Error | 40 | 13.5795 | 0.3395 | | |
| Proline content | | | | | |
| Exposure duration | 4 | 38.2076 | 9.5519 | 169.7825 | 0.0001 |
| Salt type | 3 | 6.3178 | 2.1059 | 37.4323 | 0.0001 |
| Duration x salt | 12 | 16.6485 | 1.3874 | 24.6602 | 0.0001 |
| Error | 40 | 2.2504 | 0.0563 | | |

p values less than 0.05 are significant

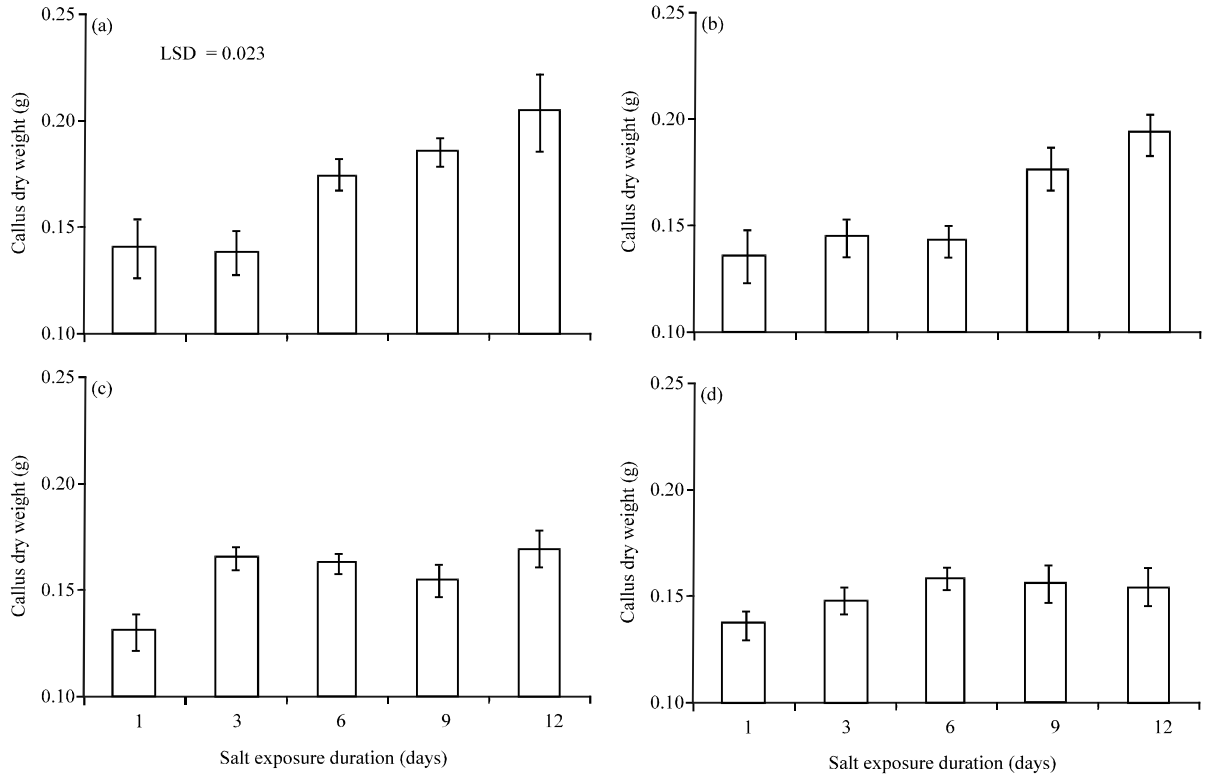


Fig. 1(a-d): Comparison of (a) Control and groups exposed to salt stress (0.8 MPa) induced by (b) $CaCl_2$, (c) KCl and (d) NaCl at different durations (1, 3, 6, 9 and 12 days) on callus dry weight in date palm callus

weight, suggesting that callus growth was stimulated in response to short exposure durations, about 3 days. This stimulation was more obvious with KCl than with $CaCl_2$ and NaCl.

Reduction in callus growth in response to increasing concentrations on NaCl has been observed in *in vitro* cultures of several plant species. For example, *Medicago sativa* L. (Shah *et al*., 1990), sunflower *Helianthus annuus* L. (Carceller and D'Ambrogio, 1994), *Dactylis glomerata* L. (Dutta Gupta *et al*., 1995), mustard *Brassica juncea* L. (Gangopadhyay *et al*., 1997), *Cymbopogon martinii* (Roxb.) (Patnaik and Debata, 1997), *Troyer citrange* (El-Yacoubi *et al*., 2010) and Rice *Oryza sativa* L. (Htwe *et al*., 2011). In addition, low levels of NaCl stimulated somatic embryogenesis in *Sapindus trifoliatus* L. (Unnikrishnan *et al*., 1991). Conversely, stimulatory effect of low levels of NaCl on callus growth was observed for several other plant species such as: *Vigna radiata* (L.) Wilczek (Kumar and Sharma, 1989), *Cymbopogon martinii* (Pandey and Ganapathy, 1984) and *Poncirus trifoliata* (Beloualy and Bouharmont, 1992). Based on such observations, Kumar and Sharma (1989) suggested that the osmotic strength of the culture medium was sub-optimal, hence the enhancement of callus growth in the presence of low levels of NaCl.

In a previous study conducted by Al-Khayri (2002) has shown that low concentrations of salt (25-50 mM NaCl) added to the culture medium stimulated date palm callus growth. Whereas, high concentrations of NaCl, 125 mM, completely inhibited callus growth. This suggests that 125 mM NaCl can be useful for the purpose of *in vitro* selection of date palm tolerant cell lines.

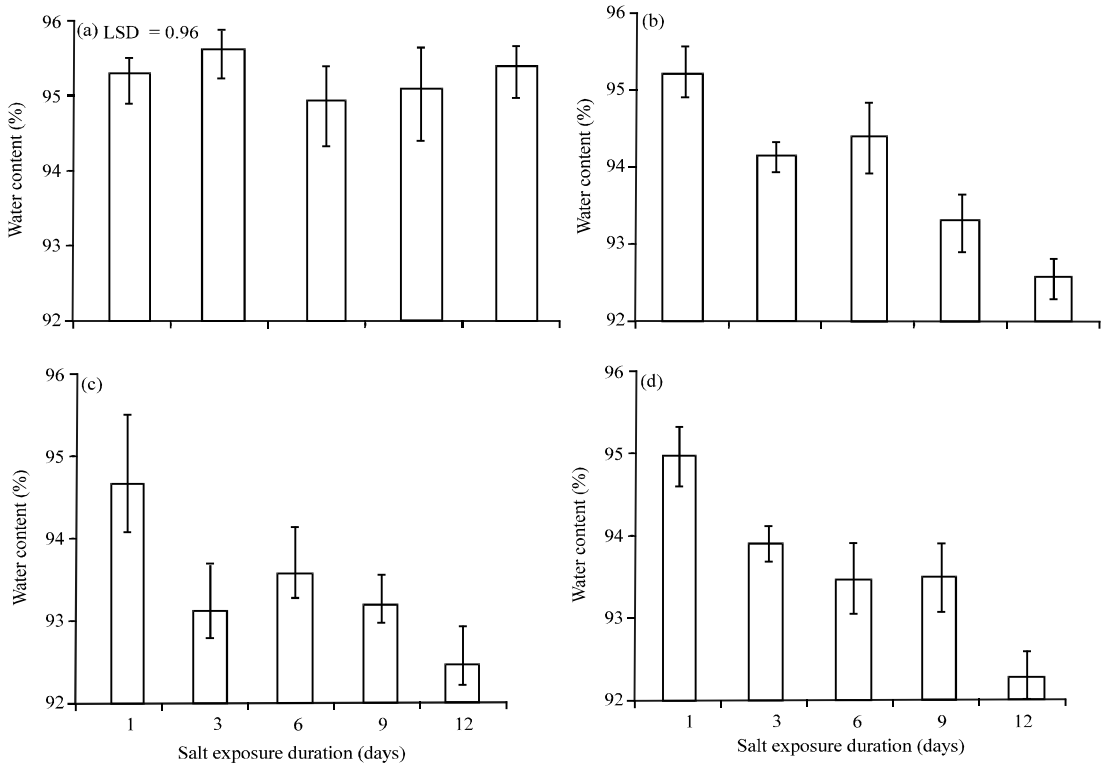


Fig. 2(a-d): Comparison of (a) Control and groups exposed to salt stress (0.8 MPa) induced by (b) $CaCl_2$, (c) KCl and (d) NaCl at different duration (1, 3, 6, 9 and 12 days), on water content in date palm

**Effect of salt stress on callus water content:** Based on the analysis of variance, water content was affected by salt type and the duration of salt exposure in a significant two-way interaction (Table 2). This suggests that the effect of each salt on callus water content was dependent upon the exposure duration. In general, callus water content decreased in response to extending exposure durations regardless of the salt type used (Fig. 2). The exposure to salt stress resulted in reduction in callus water content as compared to the control. The extent of reduction in callus water content differed according to salt type. Sodium chloride caused the highest reduction in callus water content 3.18% followed by KCl 2.94% then $CaCl_2$ 2.84%.

Reduction in water content as a result of increasing salinity has also been reported in *in vitro* cultures of several other plant species such as: *Oryza sativa* japonica type (Lutts *et al.*, 1996), *Triticum durum* (Lutts *et al.*, 2004), *Saccharum officinarum* (Errabii *et al.*, 2006); *Oryza sativa* Thai aromatic rice (Summart *et al.*, 2010) and *Phoenix dactylifera* L. (Al-Mansoori and Eldeen, 2007). Based in such observation Cicek and Cakirlar (2002) suggested that high osmotic pressure resulted from increasing salinity restricted plant cells water uptake.

**Effect of salt stress on free proline accumulation:** Proline provides a biochemical marker useful in selection and manipulation of plants and plant cells resistant to salinity stress (Stravareck and Rains, 1984; Chandler and Thorpe, 1987; El-Hadrami *et al.*, 2011). Accumulation of proline under stress conditions is widely known in callus cultures (Binzel *et al.*, 1987; Chandler and Thorpe, 1987; Hassan and Wilkins, 1988; Zebarjadi *et al.*, 2011; Htwe *et al.*, 2011). Moreover,

as a stress indicator, proline has been observed to accumulate in date palm callus in response to increasing concentration of NaCl (Al-Khayri, 2002; Al-Mansoori and Eldeen, 2007; Jasim *et al.*, 2010).

Proline accumulation with increase in culture medium salinity has been reported in various *in vitro* culture systems subjected to salt stress (Htwe *et al.*, 2011; El-Yacoubi *et al.*, 2010; Summart *et al.*, 2010; Al-Mansoori and Eldeen, 2007; Cano *et al.*, 1996; Kumar and Sharma, 1989; Patnaik and Debata, 1997; Shah *et al.*, 1990). In date palm, a previous study conducted by Al-Khayri (2002) has shown that low concentrations of salt (25 mM NaCl) proline content was unaffected in relation to the NaCl-free control. Whereas, when the level of NaCl was increased to 50 mM and higher, significant accumulation of proline occurred. Similarly, Al-Mansoori and Eldeen (2007) reported that Proline accumulation increased significantly in calli derived from immature embryos of four local date palm cultivars at two distinct stages, which were dedifferentiation and fast growing stages, in response to NaCl salt stress. Also, Jasim *et al.* (2010) reported that an increase in the free proline content was observed in response to an increase of sodium chloride concentration in the date palm culture medium of well developed callus and somatic embryos. In the current study, proline accumulation in response to various salts was also observed. Based on the analysis of variance, proline content was influenced by salt type and the duration of salt exposure as revealed by the significant two-way interaction (Table 2). This suggests that the effect of each salt on callus proline content depends upon the exposure duration.

Increasing the exposure duration up to 6 days caused increase in proline content compared to the control (Fig. 3). Calcium chloride caused the highest increase in proline content 66.6% followed by KCl 62.3% then NaCl 52.2%. Extending the exposure duration of KCl and $CaCl_2$ to 9 days caused reduction in proline content, due to cell death as indicated by culture browning; whereas, NaCl caused an increase in proline content (87.4%) compared to the control. However, 12 days were required to reach death exposure as indicated by the reduction in proline accumulation in the treated callus cultures. Huber (1974) suggested that proline accumulation may be specifically promoted by certain inorganic ions. However, Paleg and Aspinall (1981) mentioned that the numerous responses of the proline accumulating system to inorganic ions do not suggest that differences in internal ion concentration play any crucial role in initiating proline accumulation, but they rather modulate the rate of accumulation, possibly through inhibition or promotion of specific enzyme activities concerned in synthesis or oxidation. Many investigators have suggested that the main role of proline is the ability to act as an enzyme protectant (Solomon *et al.*, 1994), stabilizes membranes and cellular structures (Van Rensburg *et al.*, 1993) during environmental stresses. Proline may also function as an organic nitrogen reservoir ready to be used after stress relief to maintain both amino acid and protein synthesis (Trotel *et al.*, 1996; Sairam and Tyagi, 2004).

**Effect of salt stress on ions content:** Increasing the concentration of salts such as NaCl in the culture medium resulted in a steady increase in the $Na^+$ content in callus cultures (Kumar and Sharma, 1989; Patnaik and Debata, 1997; Unnikrishnan *et al.*, 1991). This response has been also observed in date palm callus cultures (Al-Khayri, 2002; Al-Mansoori and Eldeen, 2007). In the current study, $Na^+$ content was observed to be influence by the salt type and the exposure duration as indicated by the significant two-way interaction obtained from the analysis of variance (Table 3).

Table 3: Analysis of variance of the effect of various salt types (NaCl, KCl and $CaCl_2$) on the content of $Na^+$ and $K^+$ ions and $Na^+/K^+$ ratio of date palm callus exposed to 0.8 MPa salt stress for different durations (1, 3, 6, 9 and 12 days)

| Factor | DF | SS | MS | F-value | p-values |
|---|---|---|---|---|---|
| Sodium content | | | | | |
| Exposure duration | 4 | 533111 | 133277 | 5.0636 | 0.0021 |
| Salt type | 3 | 69014800 | 2300490 | 874.0169 | 0.0001 |
| Duration x salt | 12 | 1337340 | 111445 | 4.2341 | 0.0003 |
| Error | 40 | 1052840 | 26321 | | |
| Potassium content | | | | | |
| Exposure duration | 4 | 1274410 | 318603 | 8.5156 | 0.0001 |
| Salt type | 3 | 2249780 | 749925 | 20.0439 | 0.0001 |
| Duration x salt | 12 | 2700670 | 225056 | 6.0153 | 0.0001 |
| Error | 40 | 1496560 | 37414 | | |
| $Na^+/K^+$ ratio | | | | | |
| Exposure duration | 4 | 0.1450 | 0.0363 | 2.0288 | 0.1087 |
| Salt type | 3 | 29.7772 | 9.9257 | 555.4685 | 0.0001 |
| Duration x salt | 12 | 0.4825 | 0.0402 | 2.2503 | 0.0274 |
| Error | 40 | 0.7148 | 0.0179 | | |

p values less than 0.05 are significant

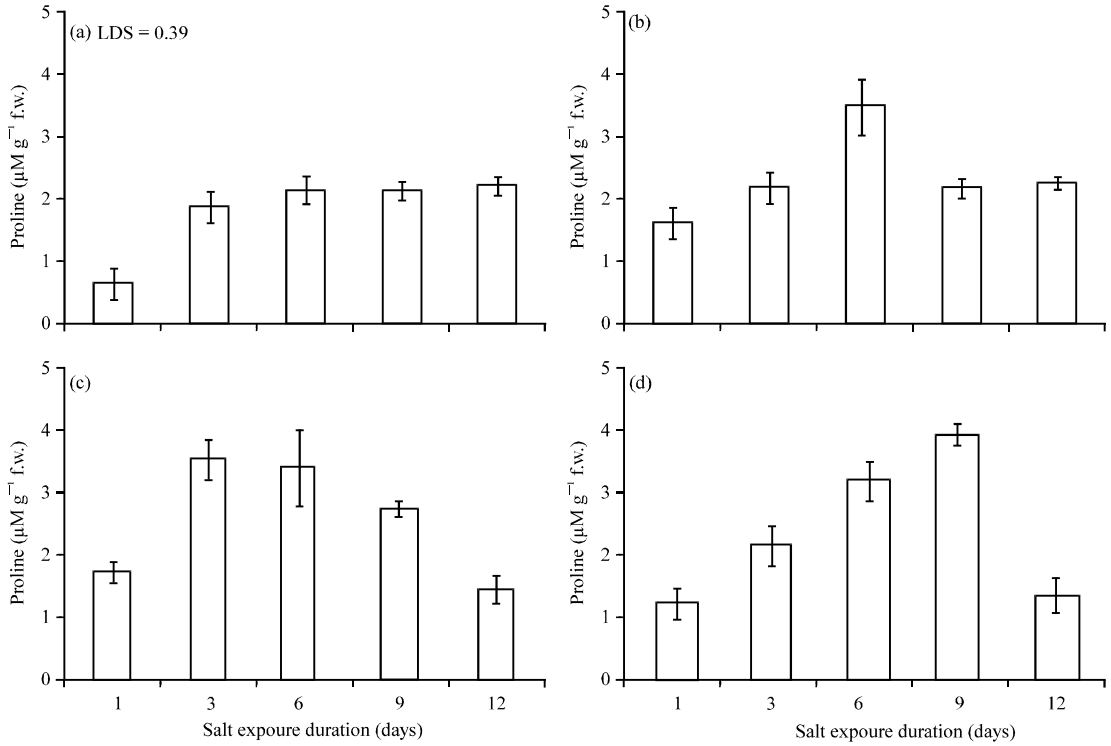


Fig. 3(a-d): Comparison of (a) Control and groups exposed to salt stress (0.8 Mpa) induced by (b) $CaCl_2$, (c) KCl and (d) NaCl at different durations (1, 3, 6, 9 and 12 days), on proline content in date palm callus

Exposure to NaCl initially caused increase in $Na^+$ content more than 5 times (3207.4 µM $g^{-1}$ tissue DW) compared to the control (570.04 µM $g^{-1}$ tissue DW) after third day of NaCl salt

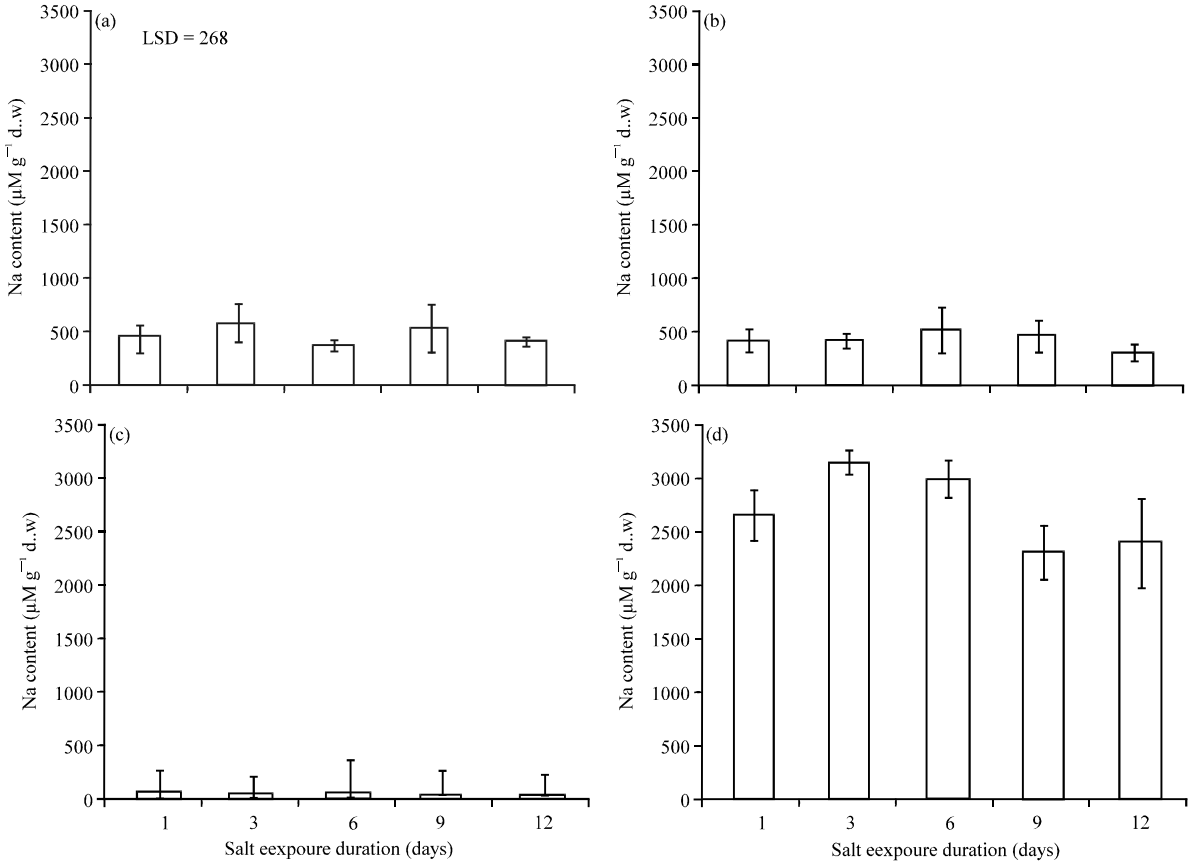


Fig. 4(a-d): Comparison of (a) Control and groups exposed to salt stress (0.8 MPa) induced by (b) $CaCl_2$, (c) KCl and (d) NaCl at different durations (1, 3, 6, 9 and 12 days), on sodium content in date palm callus

exposure then declined starting the sixth day after exposure (Fig. 4). At the ninth day, significant reduction in $Na^+$ content (2355.21 µM $g^{-1}$ tissue DW) was observed; perhaps, this is attributed to reduction in cell growth and death. Sodium ion content was significantly higher than the control as well as other salt type treatments. Comparatively, KCl treatments caused significant reduction in $Na^+$ content (15.06 µM $g^{-1}$ tissue DW) after third day of exposure as compared to the control (570.04 µM $g^{-1}$ tissue DW) as well as $CaCl_2$ (413.53 µM $g^{-1}$ tissue DW) treatments (Fig. 4). In a related study done by Al-Mansoori and Eldeen (2007), callus cultures showed a dramatic increased in $Na^+$ content with increasing NaCl level, whereas $K^+$ content decreased.

The relationship between $K^+$ content and salinity may vary from one species to another. In some cases, the level of $K^+$ has been observed to continuously decline in response to increasing salinity of callus cultures (Kumar and Sharma, 1989; Chaudhary *et al.*, 1997). In the current study, increasing salt exposure duration caused modification in the $K^+$ content. Based on the analysis of variance, $K^+$ content was affected by salt type and the duration of salt exposure in a significant two-way interaction (Table 3). Increasing salt exposure duration caused significant increase in $K^+$ content (approximately 11%) as compared to the control, up to 3 days of exposure after which the content decreased but remained higher than the control cultures (Fig. 5). Potassium ion content

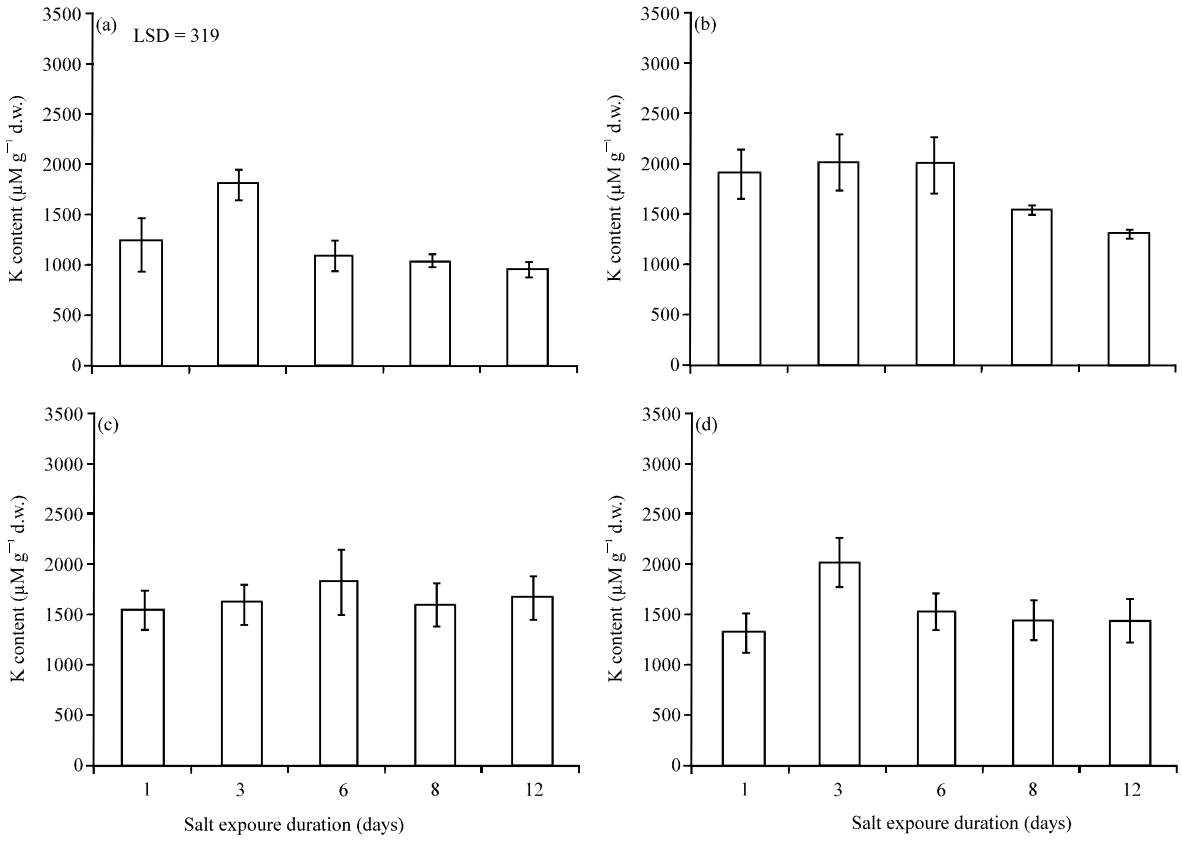


Fig. 5(a-d): Comparison of (a) Control and groups exposed to salt stress (0.8 MPa) induced by (b) $CaCl_2$, (c) KCl and (d) NaCl at different durations (1, 3, 6, 9 and 12 days), on potassium content in date palm callus

was higher in the three salt type treatments compared to the control. Comparatively, KCl treatments was higher in $K^+$ content (1664.43 µM $g^{-1}$ tissue DW) after twelve days of exposure as compared to the control (953.45 µM $g^{-1}$ tissue DW) as well as NaCl (1440.73 µM $g^{-1}$ tissue DW) and $CaCl_2$ (1316.62 µM $g^{-1}$ tissue DW) treatments (Fig. 5). Others have found that at high salt levels resulted in a sharp decline in $K^+$ content (Unnikrishnan *et al*., 1991; Patnaik and Debata, 1997). In a related study (Al-Khayri, 2002), callus cultures showed an initial increase in $K^+$ level in response to 25 mM NaCl but at higher levels a steady decrease in $K^+$ concentration was observed. The salt type and the exposure duration as revealed by the analysis of variance also significantly affected the $Na^+/K^+$ ratio (Table 3). The $Na^+/K^+$ ratio has been observed to increase in response to increasing salinity as previously reported with different plants (Nyman *et al*., 1989; Patnaik and Debata, 1997; Chaudhary *et al*., 1997). In the current study on date palm callus, increasing salt exposure duration to NaCl caused initial decrease in $Na^+/K^+$ ratio at 3 days after exposure (Fig. 6). Subsequently, significant increase in $Na^+/K^+$ ratio was observed at the sixth day while significant reduction was observed thereafter. The $Na^+/K^+$ ratio was significantly higher than the control treatments regardless of the exposure duration. In contrast, $Na^+/K^+$ ratio was significantly lower than the control treatments regardless of the exposure duration when KCl and

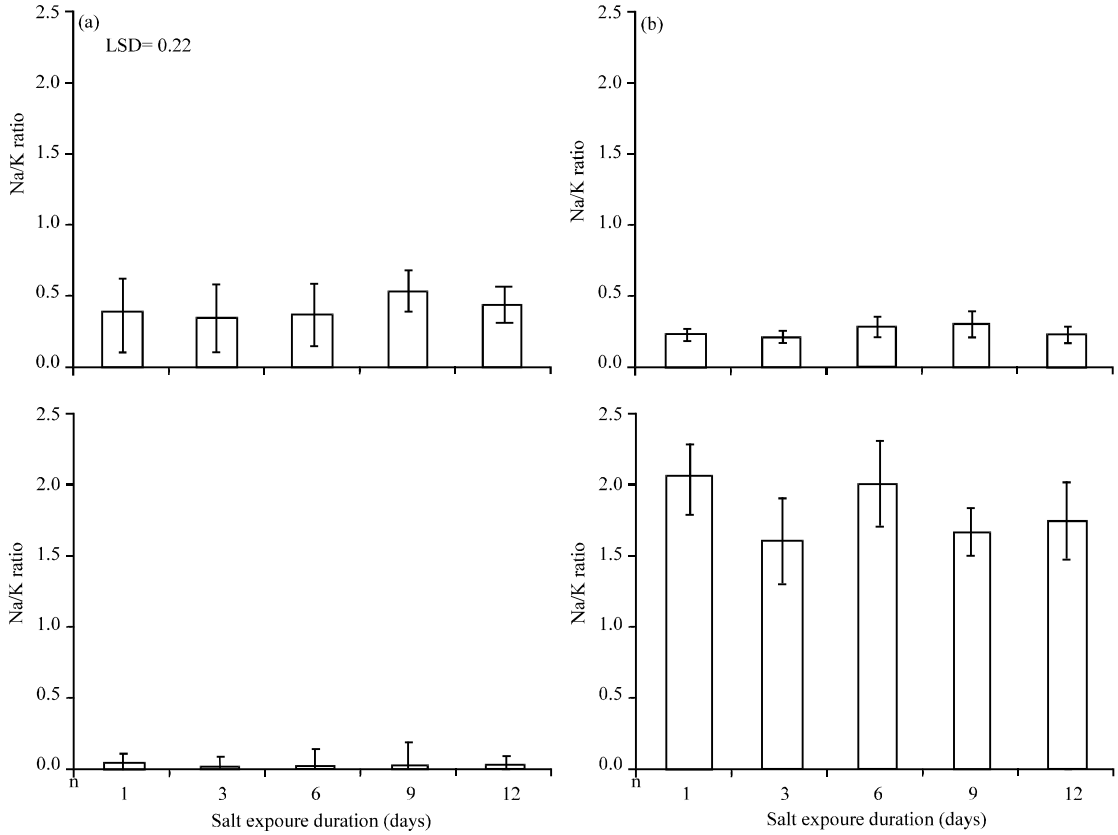


Fig. 6(a-d): Comparison of (a) Control and groups exposed to salt stress (0.8 MPa) induced by (b) $CaCl_2$, (c) KCl and (d) NaCl at different durations (1, 3, 6, 9 and 12 days), on sodium/potassium ratio in date palm callus

$CaCl_2$ were used. Weimberg (1987) mentioned that high concentration of $Na^+$ inside the cells restrain the $K^+$ uptake and as a result it causes the $Na^+/K^+$ ratio to increase and this is might be attributed to the fact that $Na^+$ causes a disturbance in the ion balance in plant by an increase in the $Na^+$ uptake (Cicek and Cakirlar, 2002).

## CONCLUSION

This study has enhanced the understanding of the influence of salinity on physiological aspects of date palm cell cultures. In addition, it provides basic knowledge on growth, proline accumulation and ionic content of date palm *in vitro* cultures in response to salts stress. The behavior of cell cultures to induced stress by various types of salt was clarified. Determination of the inhibitory duration of different salt types is essential for future research involving *in vitro* physiological studies involving somaclonal variation and *in vitro* selection. Further understanding of the response of date palm callus cultures to salt stress can be achieved by studying the effect of salt combinations to simulate natural conditions.

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