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**REVIEW ARTICLE**

# *In vitro* selection of abiotic stress tolerant date palm (*Phoenix dactylifera* L.): A review

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## Abstract

The advent of agricultural biotechnology offers new approaches for the genetic improvement of date palm (*Phoenix dactylifera* L.), an important economic fruit crop in arid regions. In vitro studies were conducted to recover date palm plants exhibiting enhanced tolerance to salinity and drought stress, two major agricultural problems in arid areas. The response of date palm callus cultures to salt stress was investigated by exposing cell suspension and callus cultures to varying concentrations of sodium chloride (NaCl). The reaction of callus to the duration of exposure to NaCl, potassium chloride (KCl) and calcium chloride (CaCl2) was evaluated. Similarly, drought tolerance was assessed in response to varying concentrations of polyethylene glycol (PEG-8000). Significant differences were reported in various parameters measured including callus growth, somatic embryogenesis, proline content and ion distribution. Calli with enhanced tolerance to these selection agents were obtained; however, plant regeneration from these cell lines was not realized. Although the available data provide an insight of the behavior of date palm callus in the presence of salt and drought stress, further research work is required to refine the selection procedures and plant regeneration from the selected tolerant cell lines.

*Key words*: Cell suspension, Date palm, Drought stress, Dry weight, Fresh weight, In vitro selection, PEG, Relative growth rate, Salt stress, Tissue culture

## Introduction

Date palm, *Phoenix dactylifera* L., a monocotyledonous angiosperm diploid species (2n = 36) belongs to the Arecaceae (Palmae) family. It is a dioecious perennial tree species grown mainly for its fruits but the tree is also utilized for ornamental and industrial uses (El Hadrami and Al-Khayri, 2012). Dates are a highly nutritious source of sugar, minerals, and vitamins and have been considered as an antiatherogenic nutrient (Al-Shahib and Marshall, 2003).

Date palm populations are estimated at over 90 million trees worldwide, distributed between latitudes 10° and 30° north of the Equator, mainly in arid, tropical and subtropical regions of Southern Asia and North Africa. Date palm agriculture has expanded to Australia, Southern Africa, South America, Mexico and the United States of America. Dry, hot climate is necessary for pollination and fruit ripening; hence, the limitation in geographical distribution to arid subtropical regions of the world (Zaid and de Wet, 1999; Zohary and Hopf, 2000).

The main date-producing countries exist in regions where water limitation and soil salinity are major agricultural constraints. The problem of salinity existed long before human beings and the start of agricultural practices. From the historical record of the last 6,000 years of civilization, it is evident that people were unable to continue their colonization due to salinity-induced damage of resources (Gelburd, 1985). Salinity inflects restriction on land cultivation and is considered a major factor of poverty and male nutrition for millions of inhabitants (Athar and Ashraf, 2009).

Date palm is tolerant to the adverse environmental conditions, saline soils and water scarcity, predominating in the date palm growing arid regions (Zohary and Hopf, 2000; Johnson, 2011). Generally, the date palm is considered a

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halophytic species which tolerates EC 10 mS $cm^{-1}$ (Ebert, 2000). Glenn et al. (1999) reported that date palm can tolerate about 5 g/l of dissolved salts and considered it as a marginal halophyte; for comparison, sea water typically contains 40 g/l of dissolved salts which mostly consists of sodium chloride (Glenn et al., 1998). In a study conducted by Al-Mulla et al. (2013), tissue culture derived date palm plants were examined for their tolerance to salinity under greenhouse conditions. They found that cvs. Kasab and Barhee tolerated 10 dS/m salinity level; whereas Khalas tolerated up to 20 dS/ m, the highest level tested. The cv. Nabusaif was the least tolerant. Observations were based on the plant height and number of fronds which were inversely related with increasing salinity levels.

Nonetheless, both date quality and yield are affected by soil salinity and water stress. Due to the serious threat of global warming, these problems are expected to be intensified in the date palm growth regions (Jain, 2010, 2012; Jaradat, 2012). For these reasons, breeding efforts geared towards the improvement of tolerance to abiotic stress conditions is of paramount importance for sustainable date palm production. Some of the first reports appeared in the 1950s and 1960s which examined the effects of different salt combinations and concentrations on the date palm plants in situ especially on germination and seedling growth stages (Khudairi, 1958; Furr and Reem, 1968). Khudairi (1958) used the cv. Zahdi to examine the seed germination on sodium chloride solutions. He concluded that seeds germination was not affected by solution concentrations up to 136 mM and germination continued in solutions up to 342 mM. Other recent studies on the effect of water salinity on date palm seedling growth were reported by Al-Rokibah et al. (1998) and Alrasbi et al. (2010). Al-Rokibah et al. (1998) found a difference in response to salt stress among date palm cultivars grown in Saudi Arabia.

In recent ex vitro reports, adding some compounds showed adverse effect on the impact of salinity and helped the date palm plants to tolerate higher salt concentrations by using GA3 at pre-acclimatization stage (Darwish and Mohammad, 2009) or amino acids and yeast for green house grown date palm offshoots (Darwish, 2013). Date palm breeding is restricted by long juvenile phase and high heterozygosity (El Hadrami and El Hadrami, 2009). To augment traditional breeding techniques, biotechnology is an innovative approach for date palm improvement (Jain, 2012). So far, various biotechnological techniques have already been used including induced embryo rescue (Sudhersan and Al-Shayji, 2011) in vitro mutagenesis and selection against bayoud disease (Jain, 2006, 2007; El Hadrami et al., 2005, 2011a), and protoplast culture (Chabane et al., 2007; Rizkalla et al., 2007; Assani et al., 2011). Protoplasts are used for the production of somatic hybrids, and genetic transformation (Saker et al., 2007, 2009; Mousavi et al., 2009; Saker, 2011). Another related approach yet to be studied is the production of haploid date palm. The in vitro regeneration of date palm plantlets with enhanced tolerance to abiotic stress factors is another example of topics requiring research attention. Studies related to the mechanisms of evading salt stress in date palm are also importance. In other halophytes, ion accumulation in the cell in association with ion compartmentalization in the vacuole, is considered the primary mechanism (Hasegawa et al., 1986).

The criterion for salt tolerance is based on yield responses, but field or greenhouse yield evaluations under a range of salinities are labor intensive and can be costly, usually restricted to a defined growth season and prone to variability due to genotype environmental interactions (Velasquez et al., 2005). Cell and tissue culture techniques have been successfully employed for screening and developing stress tolerant varieties of various crops (Patnaik and Debata, 1997). The use of in vitro cultures to study abiotic stress responses is based on the fact that in vitro cultured cells behave similarly to cells of intact plants subjected to water deficit and salinity stress conditions (Attree et al., 1991). As is well evident from the literature on the existence of inter and intra-specific genetic variability for salt tolerance, genetic variability could be exploited for screening and breeding for higher salt tolerance. For example, Moreno et al. (2000) found a great magnitude of genotypic variability in bean (*Phaseolus vulgaris* L.) cultivars for salt tolerance at the seedling stage. Moreover, species differing in drought tolerance at the whole plant level also usually exhibit differences in drought tolerance in cell cultures (Santos-Diaz and Ochoa-Alejo, 1994). Undifferentiated cells and callus cultures eliminate complications associated with genetic and morphological variability inherent to different tissues in whole plants. In vitro selection of somaclones against certain stress factors offers a means for the improvement of date palm tolerance to abiotic stresses including drought and salinity as well as biotic agents such as pathogens and pests (El Hdrami et al., 2011a; Jain 2012). El Hadrami et al. (2011a) reviewed the reasons for such somaclonal variation and their uses in detail.

Cell cultures have served as a very useful tool in trying to clarify mechanisms of salt tolerance operating at the cellular level. Plant cell and tissue culture are also relevant to crop improvement strategies because they offer a method of rapid selection on a mass scale and are useful for the development of breeding techniques to produce salinity tolerant crops (Cherian and Reddy, 2003). Moreover, cell culture systems eliminate all responses associated with stress except those operative at the cellular level (Hasegawa et al., 1984). To study salt stress, the culture medium is supplemented with a critical concentration of salts, commonly sodium chloride (NaCl); whereas, drought stress is normally simulated by augmenting the culture medium with polyethylene glycol (PEG). The critical concentration of NaCl may differ from genotype to another (Ibraheem et al., 2012a). In their study, Ibraheem et al. (2012a) found that the lethal concentration of NaCl on the date palm callus growth of cv. Zaghloul differed from those reported by Al-Khayri (2002) and Al-Khateeb et al. (2002). Abiotic stress affects numerous physiological and biochemical processes at the cellular level and induces osmoregulation mechanisms leading to the accumulation of solutes such as proline (Al-Khateeb et al., 2002; Al-Khayri, 2002; Abbas et al., 2012). Date palm has a wide range of genetic diversity (Elshebly, 2009); however limited information is available concerning variation in salt tolerance among the cultivars currently grown (Al-Juburi, 1992; Al Mansoori et al., 2007). Research on the application of tissue culture techniques for the identification of salt and drought tolerant date palm has been somewhat neglected. This despite the availability of effective in vitro regeneration systems of various date palm cultivars; through both somatic embryogenesis (Fki et al., 2003; Sané et al., 2006; Taha et al., 2007; Zouine and El Hadrami, 2007; Othmani et al., 2009; Al-Khayri, 2010; Fki et al., 2011; Fki et al., 2011; Sané et al., 2012; Al-Khayri, 2013) and organogenesis (Taha et al., 2001; Sudhersan and AboEl-Nil, 2004; Khierallah and Bader, 2007; Abahmane, 2011). Nonetheless, not a single study thus far has reported successful in vitro regeneration of date palm plantlets with enhanced tolerance to abiotic stress factors. The reason for that may be due to the lack of sufficient transformation research on date palm and the non-success in producing embryogenic callus from protoplast cultures. Furthermore, there are no specific studies on date palm genes related to abiotic tolerance. Recently, Gómez-Vidal et al. (2009) identified proteins that might be involved in date palm defense to biotic and abiotic stresses, including a protein related to stress responses by using proteomic studies. In a recent review, Alhammadi and Kurup (2012) reported the use of molecular basis to study the salt stress mechanism for date palm using randomly amplified polymorphic DNA (RAPD) technique. Although, DNA methylation was shown to be involved in regulating gene expression in response to abiotic stresses in different plants (Karan et al., 2012), further molecular research is advisable to elucidate control mechanism in date palm.

Studies so far have focused on the growth behavior and physiological responses of date palm cell culture under stress induced by salinity (Al-Khayri, 2002; Al Mansoori et al., 2007; El-Sharabasy et al., 2008a; Jasim et al., 2010) and drought (Al-Khayri and Al-Bahrany, 2004; El-Sharabasy et al., 2008b; Helaly et al., 2011). Other in vitro stress studies involved date palm seedlings to evaluate response to salinity (Ibraheem et al., 2012b) and PEG-induced drought (Sané et al., 2005). Both studies found differences between date palm cultivars in response to abiotic stress. Ibraheem et al. (2012b) suggested that date palm cvs. Zahdi and Medjool are more tolerant to salinity stress whereas cv. Zaghloul is mostly sensitive (Table 1). In recent studies, the response of somatic embryos to salt stress was investigated (Abbas et al., 2012; Ibraheem et al., 2012a; Al-Zubaidi et al., 2013). The results showed that increasing NaCl concentration significantly decreased the fresh weight of both the embryogenic callus and somatic embryos (Abbas et al., 2012; Ibraheem et al., 2012a). The addition of NaCl to the media caused a significant effect on the germination time and a reduction on the percentages of somatic embryo germination (Ibraheem et al., 2012a; Al-Zubaidi et al., 2013, Ibraheem, 2013). The objective of this paper is to review the research advances on date palm in vitro cultures and highlight future directions to utilize in vitro selection to regenerate date palm plantlets exhibiting tolerance to salinity and drought conditions.

Table 1. The germination of isolated zygotic embryos (%) of 6 date palm cultivars affected by different NaCl concentrations (0, 50, 150, 250, 350 mM) after 8 weeks of culture on PGR-free MS.

| | NaCl concentrations | | | | |
|---|---|---|---|---|---|
| Cultivar | 0 mM | 50 mM | 150 mM | 250 mM | 350 mM |
| Zahdi | 90.91 bc | 100.00 a | 100.00 a | 60.00 e | 28.57 gh |
| Khistawi | 90.00 bc | 90.91 bc | 92.31 bc | 58.33 e | 0.00 j |
| Barban | 89.47 bc | 74.34 d | 50.00 ef | 41.67 fg | 0.00 j |
| Barhee | 91.67 bc | 100.00 a | 53.85 ef | 61.54 e | 8.33 i |
| Zaghloul | 83.33 cd | 75.00 d | 50.00 ef | 25.00 h | 0.00 j |
| Medjool | 100.00 a | 92.86 b | 100.00 a | 54.55 ef | 9.09 i |

Percentages followed by different letters are significantly different using Chi square test at p=0.05.
Source: Ibraheem et al. (2012b)

## 1. Callus Proliferation

Salinity adversely affects numerous physiological and biochemical processes at the cellular level. Research has shown that NaCl can exert positive or negative influence on callus growth depending on concentration. Stimulatory effect of low levels of NaCl on callus growth was observed in some glycophyte plant species (Kumar and Sharma, 1989) whereas others exhibited the opposite response (El Yacoubi et al., 2010; Htwe et al., 2011). In date palm, Al-Khayri (2002) studied the growth and physiological responses of callus, derived from cv. Barhee shoot tip explants, to salinity stress. Callus was cultured on MS medium (Murashige and Skoog, 1962) supplemented with NaCl at concentrations ranging from 0 to 225 mM. At low concentration of NaCl (25 mM), callus growth was enhanced. However, higher concentrations caused declined in callus weight, as compared to the control, and the growth completely ceased at 125 mM NaCl. Similarly, Ibraheem et al. (2012a) found that the callus fresh and dry weights of cv. Zaghloul were enhanced by adding 25 mM NaCl to the proliferation medium. ; whereas, higher levels reduced callus growth which failed to grow at 225 mM (Figure 1a,b). The difference in the lethal doses between cv. Barhee reported by Al-Khayri (2002) and cv. Zaghloul reported by Ibraheem et al. (2012a) suggested that date palm genotypes differ in their response to salt stress in vitro. This variation in response should be taken in consideration in future research dealing with induction of date palm tolerant cell lines and genetic transformation research. In agreement with the two prior citations, Al-Khateeb et al. (2002) investigated the tolerance of calli for five date palm cultivars to different NaCl concentrations ranging from 0 to 300 mM. They reported different responses among the tested cvs. (Khalas, Om Ruhaim, Ruzaiz, Hilali and Barhee). The cvs. Khalas and Om Ruhaim showed higher tolerance than the others.

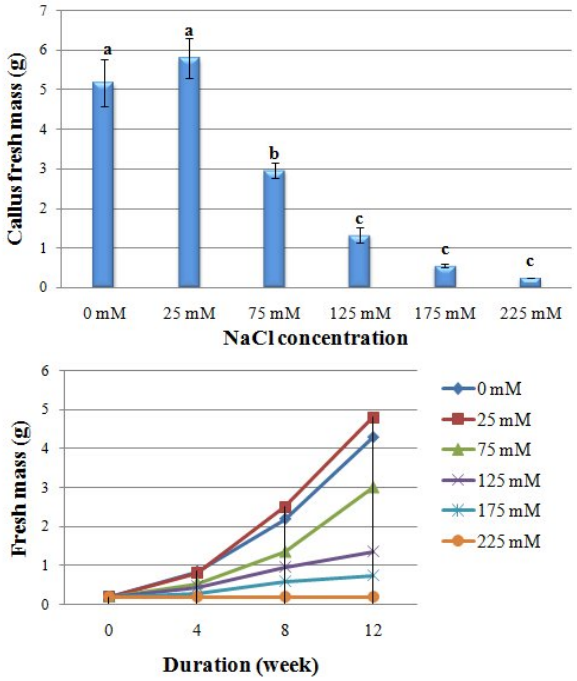


Figure 1. The effect of NaCl concentration on date palm cv. Zaghloul callus growth from 200 mg initial mass: a) Callus fresh mass (g) after 12 weeks [Source: Ibraheem et al. (2012a)]. b) The growth curve of callus growth regarding to the fresh mass evaluated at 4, 8, and 12 weeks from the beginning of the experiment. [Source: Ibraheem (2013)].

In a study reported by Al Mansoori et al. (2007), complete growth inhibition occurred when 3% NaCl was incorporated into the callus induction medium using zygotic immature embryos as explants. Identifying the critical inhibitory concentration for various date palm cultivars would facilitate studies aimed at developing in vitro selection strategies for date palm.

The response of date palm callus to osmotic

stress induced by three different salts was investigated in relation to different duration of exposure to salts (Al-Bahrany and Al-Khayri, 2012). Cell suspensions established from shoot tip-derived callus of cv. Barhee were supplemented with 0.8 MPa equivalent osmotic potential of the following salts: 179.84 mM (12.06 g/L) potassium chloride (KCl), 172.49 mM (11.96 g/L) calcium chloride ($CaCl_2$), and 150.44 mM (9.45 g/L) sodium chloride (NaCl). The results showed increased callus dry weight over time regardless of the salt type tested. 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 $CaCl_2$. Short durations (3 days) of salt exposure enhanced callus dry weight, suggesting that callus growth was stimulated in response to short exposure durations of salt stress. This stimulation was more obvious with KCl than with $CaCl_2$ and NaCl.

On the subject of drought stress, a study by Al-Khayri and Al-Bahrany (2004) revealed that date palm callus fresh weight was significantly higher in cv. Barhee than cv. Hillali when callus was exposed to 10, 15, and 25% PEG-8000 while other treatments resulted in a similar growth response. In both genotypes callus growth was significantly inhibited in response to as low as 5% PEG. The growth of the both genotypes tested (cvs. Barhee and Hillali) almost completely ceased upon exposure to 20% PEG beyond which callus fresh weight decreased. Steady reduction in relative growth rate (RGR) was also observed in response to increasing PEG-concentration in both cultivars. Although, controls of both cultivars exhibited similar RGRs, the RGRs were significantly altered in favor of cv. Barhee when PEG was introduced in the culture medium. This difference diminished when callus was grown on 20 and 30% PEG. Based on the index of tolerance (INTOL) of callus growth, which eliminates inherent differences associated with the relative growth rate of the two cultivars in response to stress, cv. Barhee exhibited higher tolerance to PEG-induced water stress than cv. Hillali. The INTOL values obtained followed a similar pattern as that of the RGR in both cultivars because the RGRs of the controls were almost identical.

The effect of drought stress during the callus induction stage was investigated by Al-Ka'aby and Abdul-Qadir (2011); working with shoot tip explants of the date palm cv. Bream. This study indicated that incorporating PEG-3000 caused significant increase in callus fresh weight as compared to the PEG-free control with no significant difference between the two PEG concentrations tested, 10 and 20%.

In another in vitro drought stress report, El-Sharabasy et al. (2008b) studied isozyme polymorphisms as biochemical markers to distinguish in vitro drought tolerance of embryogenic callus and plantlets of the date palm cvs. Sakkoti, Zaghloul and Sewy. This study revealed cultivar-specific banding patterns in response to different PEG concentrations supplemented to the culture medium. In a study conducted by Al-Khayri and Al-Bahrany (2012), where PEG at 0-15% was incorporated in suspension cultures of cv. Nabout Saif, callus fresh weight was found to reach maximum at 10% PEG.

The widespread development of in vitro techniques to produce massive date palm clones suitable for planting offers new opportunities to apply in vitro selection methods for drought-tolerant cultivars. Sucrose, mannitol or polyethylene glycol (PEG) can be used to achieve selection in vitro for drought tolerant cultivars. These molecules could simulate the mechanical stresses caused by the withdrawal of cellular water due to low water availability (El Hadrami et al., 2011b).

## 2. Morphogenesis

Date palm regeneration through somatic embryogenesis or adventitious organogenesis is affected by the salt concentration of the culture medium. A low concentration of salt, 0.4% NaCl, observed to increase length of in vitro shoots of cvs. Bartamuda, Sewy and Samani ; however, shoot growth reduction was noticed at 0.8% and 1.2% NaCl (El-Sharabasy et al., 2008a).

Adverse effect was reported by Jasim et al. (2010) where 0.5-2% NaCl inhibited callus growth and somatic embryogenesis of cv. Ashkar. However, those effects were alleviated by the addition of proline which significantly improves carbohydrates and proteins content. According to Ibraheem et al. (2012a), the number of somatic embryos of cv. Zaghloul was enhanced in response to 25 mM NaCl to the regeneration medium; however, at 75 mM NaCl the number of resultant somatic embryos was reduced and no embryo formed at 175 mM NaCl (Figure 2a). Similarly, the conversion process of somatic embryos was inhibited at 125 mM NaCl and was absent at 175 mM (Figure 2b).

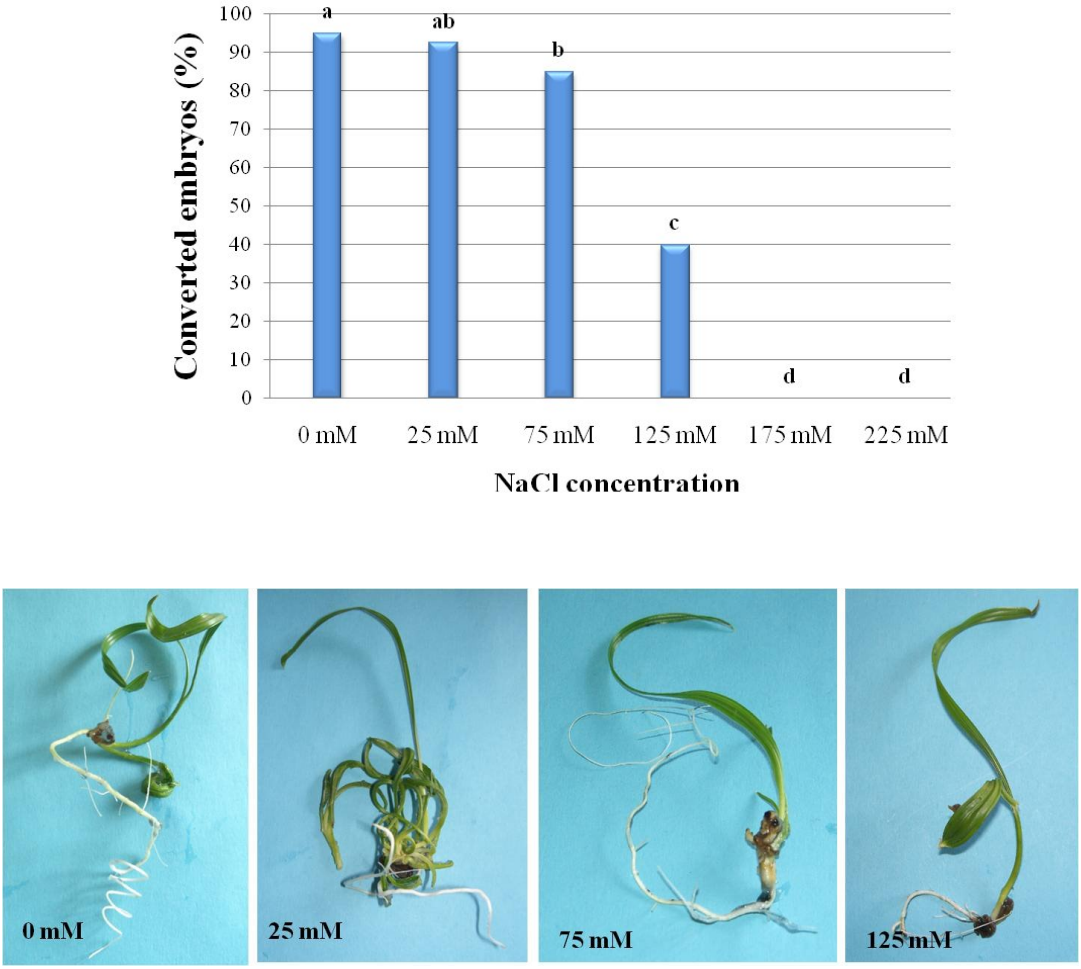


Figure 2. a) The effect of different NaCl concentrations on the number of converted somatic embryos (%), b) Conversion of somatic embryos cv. Zaghloul into plantlets after 12 weeks of culture on MS medium enriched with 1 mg $l^{-1}$ NAA with 0, 25, 75, 125 mM NaCl.
Source: Ibraheem (2013)

Drought stress studies on date palm are rather limited. An investigation by Al-Ka'aby and Abdul-Qadir (2011) observed a significant increase in somatic embryos produced in cv. Bream cultures in response to 10% PEG 3000. This treatment was superior to 20% PEG and the PEG-free control treatments. Working with somatic embryogenesis of cv. Nabout Saif, Al-Khayri and Al-Bahrany (2012) tested PEG at 0-15% and found that increasing PEG concentration resulted in enhancement of the total somatic embryo numbers reaching a maximum number at 10% PEG; however, the highest number of viable embryos was obtained in the presence of 15% PEG. El Dawayati et al. (2012), working with the date palm cv. Gundila, also reported similar positive effect. They obtained viable direct secondary somatic embryos in response to dissertation treatment of abnormal somatic embryos in 20 g $l^{-1}$ PEG for 30 days. They concluded that PEG-induced osmotic stress activates the process of morpho-ontogenetic events leading to the development of new healthy somatic embryos.

## 3. Water Content

In a study by Al-Bahrany and Al-Khayri (2012) where different durations of exposure to various salts were tested, callus water content decreased in response to extending exposure durations regardless of the salt type. The exposure to salt stress resulted in reduction in callus water content as compared to the control in cultivars tested, Barhee and cv. Hillali. 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%. Similarly, Al Mansoori et al. (2007) found reduction of water content in salt-stressed date palm cultures. Reduction in water content as a result of increasing salinity was also observed in other in vitro plant cultures (Errabii et al., 2006).

In relation to drought stress, Al-Khayri and Al-Bahrany (2004) have shown that increasing PEG concentration was associated with a progressive reduction in water content of date palm callus. This general trend was observed in both genotypes tested. Under non-stress conditions, water content of cv. Hillali was higher than that of cv. Barhee. This relationship persisted when callus was treated with 5% PEG; however, at higher PEG concentrations water content of cv. Barhee exceeded that of cv. Hillali. This suggests that the callus ability to retain water under water stress condition is better in cv. Barhee as compared to cv. Hillali. The differences between the two cultivars in relation to water content, however, were significant only at 5 and 15% PEG.

## 4. Proline Accumulation

Proline accumulation in response to increased salinity has been reported in various in vitro culture systems subjected to abiotic stress, thus providing a biochemical marker useful in selection and manipulation of plant tolerance to abiotic stress (Amirjani, 2010; El Yacoubi et al., 2010; El Hadrami et al., 2011b; Htwe et al., 2011). Accumulation of proline in response to salt and drought stress is perceived as a cellular response to provide a compatible cytoplasmic osmotic to protect the cytosol from dehydration (Hasegawa et al., 1986). It has been suggested that the main role of proline is the ability to act as an enzyme protectant (Solomon et al., 1994) and stabilizer of 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 (Sairam and Tygai, 2004).

According to Al-Khayri (2002), date palm cultures showed gradual increases in proline content as the external concentration of NaCl increased. At 25 mM NaCl, proline content was unaffected in relation to the NaCl-free control. This suggests that low NaCl concentration was not sufficient to cause salt stress and consequently proline overproduction was unnecessary. However, when the concentration of NaCl was increased to 50 mM or higher, significant accumulation of proline occurred. Proline accumulation appears to be related to callus growth inhibition. This relationship holds true at low NaCl concentrations (25 to 100 mM), but at higher levels of NaCl callus growth was halted while proline accumulation continued to rise. Similarly, Al Mansoori et al. (2007) reported that proline accumulation increased significantly in calli derived from immature embryos of four local date palm cultivars at two distinct 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 a study by Al-Bahrany and Al-Khayri (2012), proline accumulation of callus cultures of cvs. Barhee and Hillali in response to various salts was determined. Proline content was influenced by salt type and the duration of salt exposure. Increasing the exposure duration up to 6 days caused increase in proline content compared to the control. 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.

In response to in vitro drought stress induced by PEG-8000, endogenous free proline content of date palm callus increased gradually in response to increasing PEG-concentration (Al-Khayri and Al-Bahrany, 2004). The cultivars differed in their sensitivity to various PEG concentrations. For example, at 10% PEG, proline content was unaffected in relation to the PEG-free control in cv. Barhee; whereas, cv. Hillali showed a significant increase in proline accumulation as compared to the control. Nonetheless, cv. Barhee showed more proline accumulation than cv. Hillali at PEG concentrations ranging from 0 to 25%. At 30% proline accumulation in both genotypes started to decline perhaps as an indication of disturbance of metabolic mechanisms and cell death.

## 5. Sodium, Potassium and Chloride Ions Content

In several plant species, 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). In date palm, Al-Khayri (2002) reported a significant increase in $Na^+$ concentration when the callus cultures were augmented with 25 mM NaCl. This concentration coincided with the only increase in $K^+$ content. It is worth noting that the pattern of $K^+$ content in response to increasing NaCl levels more or less parallels callus growth trend. The highest callus growth was obtained on a medium containing 25 mM NaCl, the same concentration that resulted in the highest $K^+$ uptake. At higher concentration of NaCl a steady decrease in $K^+$ concentration was observed. The $K^+$ content remained higher than or equal to the salt-free control up to 100 mM NaCl, beyond which a significant decline in $K^+$ content occurred. The inhibitory concentration of date palm callus growth was identified to be 125 mM NaCl, the same level at which potassium ion concentration was significantly reduced in comparison to the control. This suggests a direct relationship between $K^+$ content and callus growth. Furthermore, the $Na^+/K^+$ ratio increased with increasing salinity of the culture medium. As the concentration of NaCl approached 125 mM, there was a sharp increase in $Na^+/K^+$ ratio which plateaued between 125 to 150 mM and peaked again at 175 mM, beyond which the change in $Na^+/K^+$ ratio became less pronounced. A negative correlation was observed between $Na^+/K^+$ ratio and callus growth in response to increasing external NaCl concentration, particularly with 25 to 125 mM.

In a recent study by Al-Bahrany and Al-Khayri (2012), short-term exposure to NaCl caused a 5-fold increase in $Na^+$ content compared to the control after day 3 of being exposed to NaCl, but starting at day 6, $Na^+$ content began to decline. At day 9, significant reduction in $Na^+$ content was observed. This reduction of ion content may be attributed to damages inflected on cellular membrane transport system and in turn inhibit cell growth and may cause cell death. In response to NaCl stress Sodium ion content was significantly higher than the control as well as other salts tested. Comparatively, KCl treatments caused significant reduction in $Na^+$ content after day 3 of exposure as compared to the control as well as $CaCl_2$ treatments. In a related study carried out by Al Mansoori et al. (2007), callus cultures showed a dramatic increase in $Na^+$ content by increasing NaCl level, whereas $K^+$ content decreased.

Increasing salt exposure duration caused modification in the $K^+$ content in response to different salts Al-Bahrany and Al-Khayri (2012). 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. Potassium ion content was higher in the three salt type treatments compared to the control. Comparatively, KCl treatments induced higher in $K^+$ content after 12 days of exposure as compared to the control as well as NaCl and $CaCl_2$ treatments. Moreover, increasing salt exposure duration to NaCl caused initial decrease in $Na^+/K^+$ ratio at 3 days after exposure. Subsequently, significant increase in $Na^+/K^+$ ratio was observed at day 6 while significant reduction was observed thereafter. The $Na^+/K^+$ ratio was significantly higher than the control treatments regardless of the exposure duration when $CaCl_2$ was incorporated in the culture medium. In contrast, $Na^+/K^+$ ratio was significantly lower than the control regardless of the exposure duration when KCl and $CaCl_2$ were used.

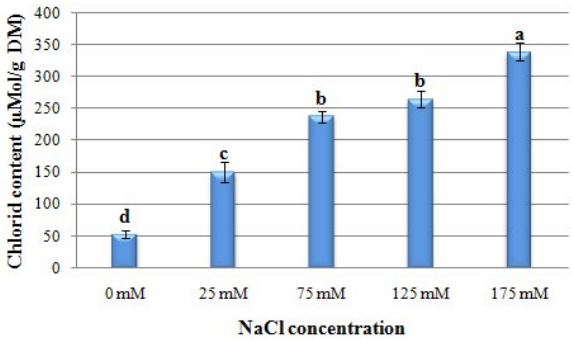


Figure 3. The effect of NaCl concentration on Chloride content (µmol/g DM dry mass) of date palm cv. Zaghloul callus after 12 weeks in vitro; means with different letters are significantly different using Tukey test at p=0.05.
Source: Ibraheem et al. (2012a)

The stress effect of NaCl on date palm calli resulted in significant increase of $Cl^-$ content (Al-Khateeb et al., 2002; Ibraheem et al., 2012a). Ibraheem et al. (2012a) reported that the levels of $Cl^-$ increased linearly with increasing NaCl concentrations (Figure 3). Chloride accumulation was significantly correlated to NaCl concentration ($r2 = 0.96$, $p<0.01$). Osmotic adjustment of salt-adapted cells was due in large part to accumulation of $Na^+$ and $Cl^-$, which occurred against a concentration gradient (Binzel et al., 1983). The chloride content in the callus exposed to salt stress indicated the tolerance of the species cultured. The callus of selected tolerant cells of lemon (*Citrus limon*) accumulated lower $Cl^-$ than the sensitive ones (Piqueras et al., 1996). A comparison of the

$Cl^-$ content between date palm callus and other plant species revealed that the date palm calli accumulated lower chloride than other species like chickpea (*Cicer arietinum)* (Pandey and Ganapathy, 1984). That indicates the high tolerance of date palm to salt stress and ensured the availability to conduct the tissue culture techniques for salt stress studies.

## 6. Conclusions and Prospects

The available in vitro studies related to abiotic stress provide some understanding of the effect of salinity and drought on date palm cell and callus cultures. Parameters investigated include callus growth, differentiation, and biochemical processes like proline accumulation and ion distribution. Although, low levels of abiotic stress agents can induce favorable responses in terms of growth and differentiation, high levels are detrimental resulting in complete growth inhibition and cell death. These studies have contributed to the determination of the inhibitory concentrations, thus meeting the initial requirements for in vitro selection of variants expressing enhanced tolerance to salt or PEG-induced stress. Reports demonstrating successful in vitro regeneration of date palm plantlets with enhanced tolerance to salinity or drought stress were not encountered. Further research is required to optimize in vitro selection and plant regeneration processes to ensure the viability and preserve totipotency of the selected cells for the recovery of date palm plantlets from cell lines selected for tolerance to abiotic stress. Research should also focus on immigrating different abiotic stresses at the same time. This interaction between stresses e.g. salt-drought or salt-heat stresses may lead to new responses and better understanding of the tolerance mechanism. This research should be fulfilled by deep physiological and genetic analysis studies. Transformation experiments are rather neglected in date palm and they are highly required in future to produce abiotic tolerate cultivars. More studies on mutants and somaclonal variations in vitro are also recommended.

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