Effect of different conservation periods with different sucrose concentrations on conserving somatic embryo clusters of date palm (*Phoenix dactylifera* L.) under minimal growth conditions

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ABSTRACT

The present study was conducted to investigate the possibility of using in vitro slow growth storage for date palm (Phoenix dactylifera L.) germplasm conservation to promote germplasm exchange and rapid propagation when necessary. Multiplied somatic embryo clusters of date palm cv. Sukkary were used as the conserved explants. Different conservation periods (4, 8, and 12 months) with different sucrose concentrations (30, 60, 90 or 120 g/l) supplemented to conservation medium which consists of 1/2 strength Murashige and Skoog (MS) basal salts medium, 30 g/l mannitol, 0.05 mg/l (BA) benzyladenin, 0.1 mg/l (NAA) naphthalene acetic acid and 8 g/l agar, under low temperature (15 ℃) and dark incubation conditions, were examined to determine the survival and re-growth capacity of conserved explants after returning to the normal growth conditions. Contents of total soluble sugars (TSS), non reducing sugars (NRS) and reducing sugars (RS) were also determined as physiological changes during conservation periods. Results showed that conservation medium supplemented with 90 or 120 g/l sucrose gave the highest significant value of survival percentage, after 12 month conservation period. Best recovery performance under normal growth conditions for conserved somatic embryo clusters under studied minimal growth conditions was achieved when 90 g/l sucrose was used in conservation medium for 8 months.

***Key words**: Germplasm conservation, In vitro, Sucrose, Low temperature, Phoenix dactylifera L., Slow growth storage.*

INTRODUCTION

Biotechnology approach offered *in vitro* techniques which have been widely used for multiplication and conservation of species whose propagation and storage by classical techniques is problematic. Date palm *(Phoenix dactylifera* L.) is an economical precious tree. It is one of oldest cultivated plants and used as food for 6000 years (Sulieman *et al.*, 2012). Date palm is the most common fruit tree grown in semiarid and arid- regions where it plays an important role in the protection of interplant cropping systems and the stabilization of the ecological system (Hasnaoui *et al.*, 2011). It plays a great socioeconomic important role and is widely used for food and many other commercial purposes. Tissue culture techniques can be used for the propagation and storage of rare or endangered species and crop genetic resources

in agriculture either for the current production of new plants, or preservation of plant genetic resources in order to face the increasing depletion of natural resources (Dodds 1991, Jain 2012). *In vitro* propagation of date palm achieved a major success over conventional methods of propagation, through intensive studies and well applied results using techniques such as somatic embryogenesis and organogenesis (Tisserat 1979, Sharma *et al.* 1984, Eshraghi *et al.* 2005, Othmani *et al.* 2009, Fki *et al.* 2011, Sidky and El-dawyati 2012, Bekheet 2013, Zayed 2014, Mazri *et al.* 2015 and Abd El-Baky and Abdel-Galeil 2016). Germplasm is sum of all the genes present in a given crop and its related species (Rai, 2007). Date palm germplasm is valuable because it contains diversity of genotypes which need to be maintained and improved for endangered, elite and commercial varieties. Advances in biotechnology research have opened new avenues for *in vitro* conservation which has been applied with varying degrees of success to wide range of species and culture systems by cryopreservation or slow growth procedures, depending on the storage duration required (Negri *et al.* 2000, Engelmann 2011 and Sabah *et al.* 2013)

Slow-growth techniques for short and mid-term storage are based primarily on conditions that allow minimal growth of cells, tissues, or organs by reducing temperature or adding osmotic regulators and growth retardants to the medium. These slow-growth techniques are widely used due to their reliability, where the principle of slow growth storage allows a safe use of *in vitro* culture without the disadvantages of frequent sub cultures as genotypes can be effectively conserved without the loss of viability in the form of disease-free stocks in a controlled environment (Thakur *et al.,* 2015). Some studies have been conducted on preserving date palm cultivars by slow growth technique (Bekheet *et al.* 2001, Hassan 2002, Shibli *et al.* 2005, El-Dawayati 2008, El-Ashry *et al.* 2013 and El-Bahr *et al.* 2016). In general, temperature reduction is the most widely applied procedure in slow growth preservation to minimize the growth. However, the temperature requirements appear to vary from species to species and may depend on the agro-climatic conditions in which a particular species is found (Thakur *et al.* 2015). Date palm germplasm conservation has been achieved with the best results in maintaining callus, and shoot tip cultures for short term and mid-term storage at 15°C (El-Dawayati *et al.* 2011 and 2013). Reducing the growth and increasing the storage life by the addition of osmotic agents as sucrose, sorbitol, ribose and mannitol to the culture media proved to be efficient (George *et al.* 2008). Osmotic agents act as a growth retardants by causing osmotic stress to the material under conservation. When added to the culture medium, these carbohydrates reduce the hydric potential and restrict the water availability to the explants (Fortes and Pereira 2001, Shibli *et al.* 2006). In general, sugar solutions can produce an appropriate osmotic potential (Tyagi *et al.* 2006). Osmotic potential is generated differently depending on the plant type; therefore finding the appropriate concentration of the osmoticum is needed in order to identify the optimum conditions for *in vitro* short-term preservation (Levitt 1980, Graham and Patterson 1982). In the present study, the in vitro conservation of Arabian cultivar of date palm Sukkary (from Kingdom of Saudi Arabia) which well established under Egyptian climatic and possesses high fruits quality, was conducted for the first time.

The aim of this investigation was to devise a conservation technique that is easy to establish, cost-effective, and provides the maximum regeneration rate for stored cultures. We tested the effect of sucrose as osmotic agent added to conservation medium at different concentrations (30, 60, 90, or120 g/l)

combined with reduction in incubation temperature at 15 °C and complete darkness, as minimal growth conditions to storage recovered somatic embryos cultures of date palm for three conservation periods (4, 8 and 12 months). Survival and the potential re-growth of conserved somatic embryos after preservation, as browning degree, proliferated shoots number, proliferated shoots length, growth vigor were evaluated. Contents of total soluble sugars (TSS), non reducing sugars (NRS) and reducing sugars (RS) were also determined to understand the physiological changes in sugar metabolism during conservation periods.

MATERIALS AND METHODS

Date palm micropropagation by direct somatic embryogenesis

Offshoots of female date palm adult tree of Arabian cultivar Sukkary, about (3-5 kg weight) was taken from healthy mother plants, all outer leaves and sheath were carefully removed to reach the inner shoot tip (about 6-8 cm length and 3-4 cm width). Shoot apices were washed under running tap water with detergent for 10 min, then kept in antioxidant solution (100 mg/l ascorbic acid + 150 mg/l citric acid) for 1h. Explants were exposed to double surface sterilization with 0.1 mg/l mercuric chloride ($HgCl_2$) for 10 min, then thoroughly rinsed with sterilized distilled water and again with mercuric chloride ($HgCl_2$) for another 50 min, and thoroughly rinsed with sterile distilled water for three times. Sterilized shoot tip explants were divided into four equal longitudinal sections, then cultured on Murashige and Skoog (1962) MS nutrient medium supplemented with 1.0 mg /l 2,4- D, 1.0 mg/l 2iP, NAA 1.0 mg /l, 100 mg/l myo-inositol, 40 mg/l adenine sulfate, 170 mg/l $KH_2PO_4.2H_2O$, 200 mg/l glutamine, 50 g/l sugar, 4 mg/l thiamine HCl and 3 g/l activated charcoal (AC) (MS1). Cultures were transferred every 8 weeks for three subcultures then, transferred to MS nutrient medium supplemented with 0.5 mg/l 2ip, 100 mg/l myo-inositol, 40 mg/l adenine sulfate, 170 mg/l $KH_2PO_4.2H_2O$, 200 mg/l glutamine, 50 g/l sugar and 4 mg/l thiamine HCl (MS2). Cultures were incubated under complete darkness at 27 ± 1°C. Direct adventitious buds and somatic embryos were obtained according to (Abd El-Baky and Abdel-Galeil 2016). Multiplied direct somatic embryo clusters (secondary embryos which serve as explants material) (Fig. 1) were placed on MS nutrient medium (MS3) supplemented with 0.05 mg/l BA ,0.1 mg/l NAA,100 mg/l myo-inositol, 170 mg/l $KH_2PO_4.2H_2O$, 30 g/l sugar and 4 mg/l thiamine HCl. All cultures were incubated at 27 ± 1°C under 16:8-h light/dark. All media were solidified with 6 g/l agar and the pH adjusted to 5.8 prior to autoclaving at 121°C for 20 min.

Conservation of date palm somatic embryos clusters by using different sucrose concentrations under minimal growth conditions

Somatic embryo clusters, about 8-10 embryos (Fig. 2), were obtained as mentioned above (because individually separated somatic embryos were incapable of proliferating further or germinating). Somatic embryo clusters were placed on 40 ml conservation medium consisting of 1/2 strength MS salts, 0.05 mg/l BA, 0.1 mg/l NAA, 30 g/l mannitol and different sucrose concentrations (30, 60, 90 or 120 g/l) were added as different treatments. The pH of all conservation media treatments were adjusted to 5.7 ± 0.1 prior to addition of 8 mg/l agar. The medium was distributed into culture jars (150 ml) where each one contained 40 mL. The culture jars were immediately capped with polypropylin closure and then the medium was sterilized by autoclaving at 121°C for 20 min. The culture jars of each treatment were divided into three

groups according to the three tested conservation periods (4, 8, and 12). All culture jars were conserved at 15 °C under complete darkness.

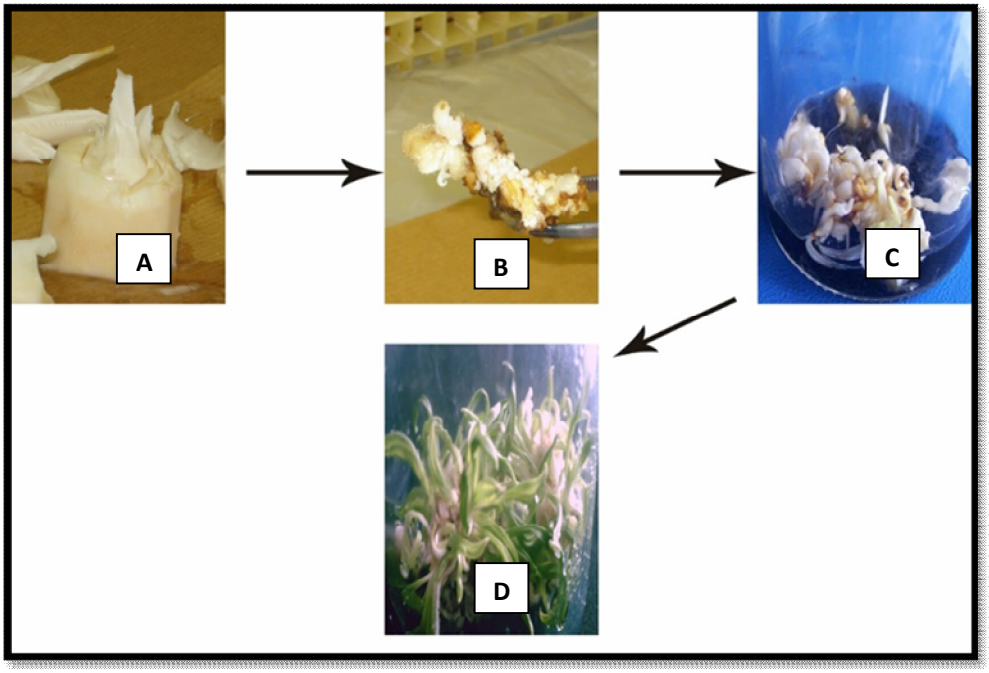

Fig. (1): (A) Sterilized shoot tip explants (B),(C) Induction for direct somatic embryogenesis on (MS1) and (MS2) medium,(D) Received multiplied direct somatic embryos clusters (secondary embryos which serve as explants material) on (MS3) medium.

Fig. (2): Excessed somatic embryo cluster about (8-10 embryos) as Conserved explant.

Recovery growth conditions of conserved somatic embryo clusters

After each conservation period, the conserved somatic embryo clusters were transferred to fresh recovery medium with the same composition (MS3) for further somatic embryos germination and shoot development under normal growth conditions at 27 ± 1 °C and 16:8-h light/dark. Each treatment = 3 replicates and each replicate = 3 culture jars and each jar contained one cluster of somatic embryos. Data were scored as follows:

Survival percentage of the conserved explants were determined after each conservation period on recovery medium under normal growth conditions for 4 weeks. After each conservation period regrowth capacity of conserved explants were scored after three subcultures with (six weeks) intervals on recovery medium under normal growth conditions for browning degree/explant,

Negative result	(-)	1
Below average result	(+)	2
Average result	(++)	3
Good result	(+++)	4
V. good result	(++++)	5

number and shoot length/explant of proliferated shoots from conserved somatic embryo clusters and their growth vigor/explant.

Browning degree and growth vigor data were scored visually according to Pottino (1981) as follows:

Biochemical analysis

The changes in total soluble sugars content (TSS), reducing sugars content (RS) and non reducing sugars content (NRS) of conserved somatic embryos explants were recorded at the end of each conservation period (4, 8 and 12 months) for all treatments.

Determination of the total soluble sugars and reducing sugars

One gram fresh sample was ground in a mortar with 20 ml ethanol 80%, and heated at 70 ^{o}C for 1 h in water bath. The combined extracts were filtered and evaporated to dryness in a water bath at 55 ^{o}C. The dried film was dissolved in 10 ml of 10% aqueous isopropanol. The aqueous isopropanol extract divided to two sections. The first was taken to determine total soluble sugars and the other to determine the reducing sugars. Total soluble sugars were determined in isopropanol extract by using the phenol - sulphuric method according to A.O.A.C. (1980). Reducing sugars were determined in ethanolic extract, using phosphomolybdic method according to Dubois *et al*. (1956).

Experimental Design

The randomized factorial design was used and data were subjected to analysis of variance. Separation of means among treatments was determined using L.S.D test at 5% according to Schroder (1970)**.**

RESULTS AND DISCUSSION

This study was designed to study the effect of different sucrose concentrations (30, 60, 90 or 120 g/l) as osmoticum supplemented in conservation medium in order to determine optimum conditions for minimal growth storage of somatic embryo clusters of date palm cv. Sukkary. It is worth mentioning that mannitol at low concentration 30 g/l was added with each tested concentration of sucrose to increase the effect of storage, because short- and medium-term storage of plant tissues under *in vitro* culture conditions leads to increased oxidative stress and senescence. Mannitol acts as a scavenger of hydroxyl radicals and protects plant tissues against oxidative stress radicals damage (Abebe *et al.* 2003 and Thakur *et al.* 2015). Low concentrations resulted in improved survival of stored cultures (Kovalchuk *et al.* 2009).

The regeneration response of the conserved somatic embryo clusters of date palm Sukarry cv. on recovery medium under normal growth conditions after studied slow

growth conditions can be determined by investigation the following results.

Survival during recovery conditions

All cultures exposed to different levels of sucrose for 4 months of conservation period recorded 100% survival percentage on recovery medium under normal growth conditions, (Table 1)**.** It is clear that survival percentage of conserved somatic embryo clusters decreased significantly with the increase of conservation period. This result agrees with other studies on slow-growth cultures of date palm (EL-Dawayati, 2008 and El-Bahr *et al,* 2016). Manipulation of sucrose concentrations in conservation medium exhibited the least significant value of survival percentage of conserved somatic embryos clusters cultured on conservation medium supplemented with 30 g/l sucrose (62.67) where, the highest significant value of survival percentage of conserved somatic embryos clusters was achieved at 90 g/l sucrose in conservation medium, followed by the 120g/l sucrose (85.62, 79.25, respectively) with no significant differences between both treatments. *In vitro*, slow-growth storage was efficiently used for mid-term conservation of elite clones of *Chlorophytum borivilianum* with sucrose concentrations at 120 g /l which enabled 100% survival from cultures stored for 4 months without any subculture or medium addition **(**Chauhan *et al*., 2016). It could be suggested that high concentrations of sucrose (at 90 g/l or at 120 g/l) in conservation medium, conserved somatic embryo clusters grew very slowly; hence, the medium did not get consumed up to12 months. The role of sucrose as osmoticum at higher concentrations was investigated in view of slow-growth conservation studies. George (1996) reported that more than 100 g/ l sucrose may cause dormancy in Lilium, which explains why sucrose can play an important role in storage of tissue. High concentration of osmoticum in the medium cause a negative water potential and reduce the optimal turgor pressure needed for cell division and growth. Panis *et al*. (1996) reported that sucrose is responsible for lack of moisture in banana, and because of desiccation sensitivity, *in vitro* developed shoots cannot grow well on media supplemented with higher levels of sucrose. Sucrose was efficiently employed for osmotic stability in potato. The addition of osmotic to culture proved to be efficient in reducing growth and increasing the storage life of many in vitro grown tissues of different plant species. According to the high levels of osmotic agents in the medium would inhibit both callus growth and shoot formation (Yasseen, 2012) In the present study, sucrose at 90 g/l was employed as an osmoticum for the maintenance of *in vitro* cultures of somatic embryo clusters of date palm Sukkary cv for 8 months at highest significant result of survival percentage after slow growth conditions (88.29%) (Fig. 3). On the other hand, with sucrose concentration at 30 g/l the cultures stored for 12 months exhibited the poorest survival percentage (22.11%) after slow growth conditions.

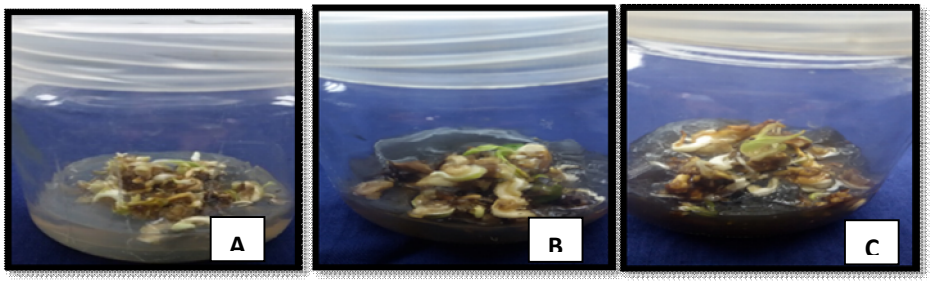

Fig. (3): (A) Conserved explant after 4 months of conservation period on conservation medium supplemented with 90g/l (B) Conserved explant after 8 months of conservation period on conservation medium supplemented with 90g/l. (C) Conserved explant after 12 months of conservation period on conservation medium supplemented with 90g/l.

Table (1): Survival percentage of conserved somatic embryos clusters of date palm Sukkary cv. on recovery medium for 4 weeks after (4, 8 and 12 months) storage with different concentrations of sucrose supplemented in conservation medium, at 15 °C.

Sucrose concentrations g/L(A)	Conservation period (Months) (B			Mean (A)
	4	8	12	
30	100a	65.88 de	22.11g	**62.67 c**
60	100a	76. 95cd	33.96f	**70.30 b**
90	100a	88.29b	68.59d	**85.62a**
120	100a	80.59bc	57.18e	**79.25 a**
Mean (B)	**100a**	**91.66b**	**72.22c**	

Means in the same column followed by the same letters are not significantly different at 0.05 probability level.

Browning appearance during recovery conditions

Browning appearance of regenerated conserved somatic embryo clusters under normal growth conditions for three subcultures were significantly affected by increasing in conservation periods from 4 months (2.39) to 12 months (4.08) as shown in (Table 2). There were no significant differences between the browning degree of regenerated conserved somatic embryo clusters under normal growth conditions for three subcultures after conservation periods for 8 months or 12 months (3.99 and 4.08, respectively). It is noticeable that regardless of the conservation period, sucrose addition into conservation medium at 90 g/l recorded the least significant browning when conserved somatic embryo clusters returned to normal recovery conditions (2.96). According to the effect of different sucrose concentrations supplemented in conservation medium on the browning degree during the recovery of regenerated conserved somatic embryo clusters under normal growth conditions for three subcultures the lowest significant result was recorded when sucrose as osmotic agents was added to conservation medium at 30 g/l for 4 months of conservation period (1.88). Browning as Physiological disorders were induced by increased in concentration of sucrose and extended preservation period during in vitro preservation this was inagreement with (Moges *et al*.2003) in African violet, (Shibli

et al. 2005) in date palm and (Baghdadi *et al*. 2011) in Wild Crocus. From our observation also low temperature at 15°C of minimal growth conditions had an effective role in the browning appearance of regenerated conserved somatic embryo clusters under normal growth conditions for three subcultures after conservation periods. In this concern (Gianní and Sottile 2015) demonstrated that cold storage of plum germplasm by slow growth resulted in necrosis and browning of explants that usually started in the apical region and spread basally over time.

Table (2): Browning degree during recovery conditions of conserved somatic embryos clusters of conserved somatic embryo cluster of date palm Sukkary cv after (4, 8 and 12 months) storage period with different concentrations of sucrose supplemented to conservation medium, at 15 °C.

Sucrose concentrations g/L(A)	Conservation period(Month)(B)			Mean (A)
	4	8	12	
30	1.88 e	4.44 ab	5.00 a	**3.78 a**
60	2.22 de	4.22 b	4.55 ab	**3.66 a**
90	2.66 cd	3.11 c	3.11 c	**2.96 b**
120	2.77 cd	3.33ab	4.55c	**3.55 a**
Mean (B)	**2.39 b**	**3.99a**	**4.08a**	

Means in the same column followed by the same letters are not significantly different at 0.05 probability level

Number of proliferated shoots per explant during recovery conditions

Conserved somatic embryos clusters of date palm cv sukkary responded differently under normal growth conditions according to the length of conservation period and the sucrose concentration as osmotic agent supplemented in conservation medium. Data in (Table 3) showed that the lowest significant value of shoots number converted from conserved somatic embryo under normal growth conditions for three subculture was when the conservation period extended to 12 months (17.83) where conservation period for 4 months recorded the highest value of shoots number converted from conserved somatic embryo under normal growth conditions for three subculture (33.69). It is clear from data increasing in sucrose concentration supplemented in conservation medium from 30 g/l to 90 g/l induced significantly the shoots number which converted from the conserved somatic embryos clusters of date palm when returned to resume their developing under normal growth condition for three subculture. This result is on line with (Tyagi *et al.* 2006) who found that high sucrose (9%) in culture medium was supportive for induction of in vitro rhizomes in *Zingiber officinale*s, and proved to be useful for its in vitro conservation. On the other hand increasing in the sucrose concentration to 120 g/l in conservation medium decreased significantly the shoots number which converted from the conserved somatic embryos clusters of date palm when returned to resume their developing under normal growth condition for three subculture. For the interaction effect between sucrose concentration and conservation period on shoots number which converted from the conserved somatic embryo clusters of date palm cv. Sukkary under normal growth condition for three subculture the results showed that sucrose at 90 g/l

supplemented in conservation medium gave the highest significant value of shoots number which converted from the conserved somatic embryo clusters under normal growth conditions for three subculture after each studied conservation period (4, 8, and 12 months) without significant differences between conservation period for 8 months and 12 months (39.88, 28.37 and 27.81, respectively).

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Table.(3): Shoot number of proliferated shoots per explant during recovery conditions of conserved somatic embryo clusters of date palm Sukkary cv after (4,8 and 12 months) storage period with different concentrations of sucrose supplemented in conservatio medium, at 15 °C.

Sucrose concentrations mg/L(A)	**Conservation period (Month)**			**Mean (A)**
	4	8	12	
30	27.89 b	15.77 de	10.33 f	**17.99 c**
60	28.11 b	18.33 d	11.11 ef	**19.18 c**
90	39.88 a	28.37 b	27.81 bc	**33.04 a**
120	38.89 a	25.70 bc	20.88 c	**28.49 b**
Mean(B)	**33.69 a**	**22.04 b**	**17.83 c**	

Means in the same column followed by the same letters are not significantly different at 0.05 probability level.

Length of proliferated shoots per explant during recovery conditions

Data in (Table 4) illustrated that under normal growth conditions for three subcultures the length of regenerated shoots which proliferated from conserved somatic embryo clusters of date palm Sukkary cv were affected significantly with different sucrose concentrations supplemented in conservation medium during (4, 8 and 12 months) storage period at 15 °C. Clearly from data in Table (4) somatic embryo cluster conserved on conservation medium with the addition of sucrose at 90 mg/l during minimal growth conditions at 15 °C recorded the highest significant length of regenerated shoots under normal growth conditions for three subcultures after conservations periods ,followed significantly by the length of regenerated shoots proliferated from somatic embryos clusters of date palm Sukarry cv preserved on conservation medium supplemented with sucrose at 120 g/l (6.44 and 5.11, respectively), however when sucrose was added to conservation medium at low studied concentrations level at 30 g/l or at 60 g/l the lowest significant length value of regenerated shoots proliferated from conserved somatic embryo clusters under normal growth conditions for three subcultures after conservations periods (3.50 and 3.78, respectively) without significant differences in between. Data demonstrated also that somatic embryo clusters of date palm Sukkary cv. preserved under minimal growth conditions at 15 °C recorded the highest significant length of regenerated shoots under normal growth conditions for three subcultures after conservation period for 4 months (5.42) whereas the increasing in conservation duration to 8 and 12 months decreased the length of regenerated shoots under normal growth conditions for three subcultures after conservation periods (4.46 and 4.25, respectively) without significant differences in between. High sucrose concentration at 90 g/l supplemented in conservation medium gave the highest significant value of shoots length

which converted from the conserved somatic embryos clusters under normal growth conditions for three subculture after each studied conservation period (4, 8, and 12 months) without significant differences among them (6.33 and 6.83, 6.17, respectively).

Table (4): Length of proliferated shoots per explant during recovery conditions of conserved somatic embryo cluster of date palm Sukkary cv after (4,8 and 12 months) storage period with different concentrations of sucrose supplemented in the medium, at 15 °C.

Sucrose concentrations	Conservation period(Months)(B)			Mean (A)
g/L (A)	4	8	12	
30	5.00 d	2.83 e	2.67 e	**3.50 c**
60	5.33 bcd	3.00 e	3.00 e	**3.78 c**
90	6.33 ab	6.83 a	6.17 abc	**6.44 a**
120	5.00 d	5.17 cd	5.17 cd	**5.11 b**
Mean (B)	**5.42 a**	**4.46 b**	**4.25 b**	

Means in the same column followed by the same letters are not significantly different at 0.05 probability leve

Growth vigor of proliferated shoots per explant during recovery conditions

Data in Table (5) determined the visually rating score for growth vigor of conserved somatic embryo clusters of date palm Sukkary cv. when returned to resume their developing under normal growth condition for three subculture after conservation on different sucrose concentrations supplemented in conservation medium under minimal growth conditions at 15 °C for different studied conservation periods. The extracted results showed that the addition of high sucrose concentrations at 90 g/l and 120 g/l to conservation medium had great significant effect on the regeneration potential of conserved somatic embryo clusters under normal growth conditions for three subcultures where healthy full developed green shoots were obtained as the highest significant visually rating score for growth vigor (3.89 and 3.48, respectively without significant differences in between) (Fig. 4) on opposite the visually rating score for growth vigor of conserved somatic embryo clusters of date palm Sukkary cv under normal growth conditions for three subculture was significantly declined when somatic embryo clusters were conserved on conservation medium with the addition of sucrose at low concentrations at 30 g/l and 60 g/l (3.66 and 3.77, respectively without significant differences in between). Weak and bale in color of the developed shoots were observed under normal growth conditions for regeneration (Fig. 4). In addition according to the conservation period effect ,somatic embryos clusters conserved for 4 months showed the best rating score for growth vigor regardless of sucrose concentrations, followed significantly by the results of growth vigor score of regenerated conserved somatic embryos clusters after 8 months then after 12 months (3.05 and 2.77 without significant differences in between) of conservation period. Clearly from results High sucrose concentration at 90 g/l supplemented in conservation medium gave the highest significant growth vigor rating score, that all of converted shoots from the conserved somatic embryo clusters under normal growth conditions for three subculture showed green,

strong and well developed shoots after each studied conservation period (4, 8, and 12 months) (4.11, 3.89 and 3.66, respectively) without significant differences among them.

Table.(5): Growth vigor of proliferated shoots per explant during recovery conditions of conserved somatic embryo cluster of date palm Sukkary cv after (4,8 and 12 months) storage with different concentrations of sucrose supplemented in the medium, at 15 °C.

Sucrose concentrations g/L(A)	**Conservation period(Months) (B)**			**Mean (B)**
	4	8	12	
30	3.66 a	3.33 c	1.99 c	**2.66 b**
60	3.77 a	2.55 bc	1.99 c	**2.77 b**
90	4.11 a	3.89 a	3.66 a	**3.89 a**
120	3.66 a	3.44 ab	3.33 ab	**3.48 a**
Mean (B)	**3.80 a**	**3.05 b**	**2.75 b**	

Means in the same column followed by the same letters are not significantly different at 0.05 probability level

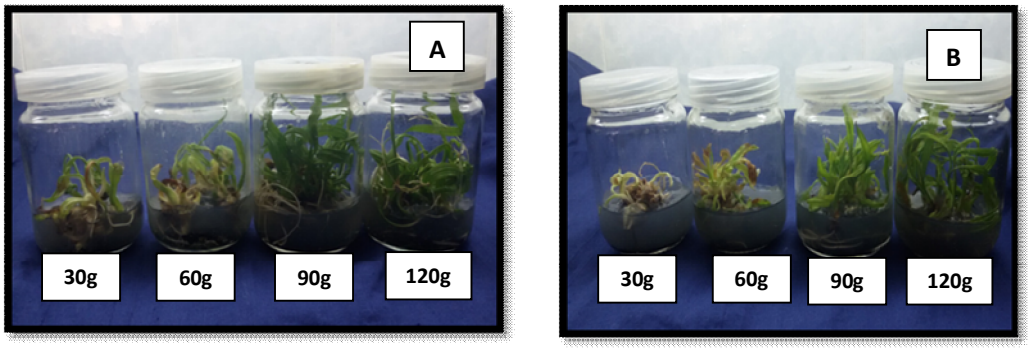

Fig. (4): Conservation medium supplemented with 90 g/l or 120 g/l had great significant effect on the regeneration potential of conserved somatic embryos clusters under normal growth conditions Healthy full developed green shoots were obtained as the highest significant visually rating score for growth vigor after 8 (A) or 12 months(B) of conservation period. Where the addition of sucrose at low concentrations at 30 g/l and 60 g/l resulted in the lowest visually rating score for growth vigor. Weak and bale in color of the developed shoots were observed under normal growth conditions for regeneration after 8 (A) or 12 months(B) of conservation period.

Studies upheld these obtained results Du *et al.* (2012) found that with 90% sucrose was more effective, on conserving two species of lilly which had been conserved on the original medium for more than 15 months. The tube seedlings conserved for 15 °C could turn to normal plantlets after re-growth for one month which showed no obvious difference in morphology.on other hand (Shibli *et al.*, 2005) reported that explant growth in the presence of sucrose depends on its concentration. Survival and regrowth of the date palm callus decreased significantly as the concentration of sucrose increased in the medium. El-bahr *et al.* (2106) found also that different sucrose concentrations at (20, 40 or 60 g/l) supplemented in conservation medium for storage embryonic callus of date palm Bartamoda cv. obviously gave the highest numbers of germinated embryos/culture under recovery conditions without a significant difference among them. It could be suggested that all genotypes retained proliferation capacity under standard conditions and their re-growth capacity seems to be strongly genotype-dependent, closely related to their individual performance in response to the experimental condition of storage as mentioned (Thakur *et al.* 2015).

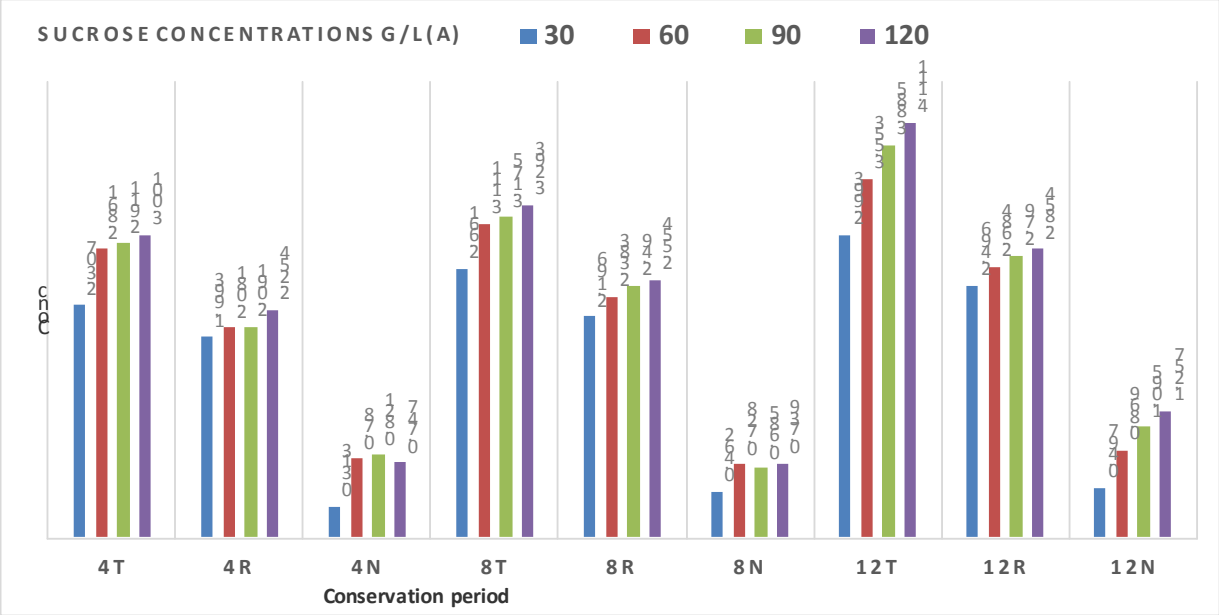

Fig. (5): Detected total soluble sugar, reduced sugar and non reduced sugar contents of conserved somatic embryos of date palm Sukkary cv. on conservation medium supplemented with different sucrose concentrations after different conservation period (4, 8 and 12 months) at 15 °C.

Analysis of total soluble sugars, reduced sugars and non reduced sugars

From data in (Fig. 5) which determined the Changes in total soluble sugars (TSS), reducing sugars (RS) and non-reducing sugars (NRS), in conserved somatic embryos clusters of date palm Sukkary cv. Revealed clearly that the analysis of total soluble sugars (TSS), reduced sugars (RS) contents and non-reducing sugar (NRS) of conserved somatic embryos clusters at the end of each studied conservation period, directly proportional to the increased in conservation period under low temperature of storage at 15 °C. Twelve months of conservation period showed the highest significant values of (TSS) (3.636), (RS) (2.706) and (NRS) (0. 930) contents of conserved somatic embryos clusters, where somatic embryo clusters were conserved for 4 months gave the lowest significant value of (TSS) (2.770), (RS) (2.105). Change in (NRS) content of conserved somatic embryo clusters showed no significant differences between the two duration of storage period for 4 and 8 months. According to sucrose concentration added to conservation medium from presented data in **(**Fig. 5) obviously when sucrose was added at the highest concentrations at 120 g/l the highest significant value of (TSS) (3.468), (RS) (2.554) and (NRS) (0. 914) contents of conserved somatic embryoclusters were achieved. These values are decreased significantly in ascending order with the decrease in the sucrose concentration to record the lowest value of (TSS) (2.654), (RS) (2.228) and (NRS) (0. 930) contents of conserved somatic embryo clusters on conservation medium supplemented with sucrose concentration at 30 g/l. Changes in sugars content (total and reducing and non reducing) due to sucrose-imposed stress were measured in the present study as analysis of these parameters could provide insight into the effect of sucrose concentrations during slow growth conditions on survival and regeneration rate El-dawayati *et al.* (2013**)** showed that the highest significant value of total soluble sugar and reduced sugar contents were recorded when shoot tip explants of date palm Zaghlool conserved on medium supplemented with sucrose at 0.3M at 15˚C under dark for 6and12 months. . In this concern, the exposure of plants to low temperatures induces biochemical and physiological changes, which allow them to withstand this stress (Levitt 1980 and Graham and Patterson 1982). Kaur *et al.* **(**2012) reported that slow growth was associated with changes in sugar metabolism ,they found that changes in starch, total soluble sugars (TSS), non-reducing sugars (NRS), and reducing sugars (RS) in *Dendrocalamus hamiltonii* somatic embryo increased with the increased in storage period when embryos stored for different period storage (30,90 180, 270, 365 day) under liquid paraffin overlay. Kushwaha *et al.* (2007) found that slow growth is generally associated with a slower rate of sugar metabolism in plants. The exogenous sucrose supply may increase the endogenous content of carbohydrate stocks such as starch, sucrose, fructose and glucose in micropropagated plants. It may favor acclimatization and accelerate physiological adaptations (Jo *et al.* 2009). Proline, total sugars, reduced sugars and polyphenols act as main compatible solutes in cotton in order to maintain osmotic balance to protect cellular macromolecules, to detoxify the cells, and to scavenge free radicals under stress condition (Parida *et al*. 2007). It could be suggested that when total and reduced sugars were high enough to be considered the principle solute that may allow plant to overcome low temperature through osmotic adjustment and serve as storage forms of carbon for future under normal growth conditions

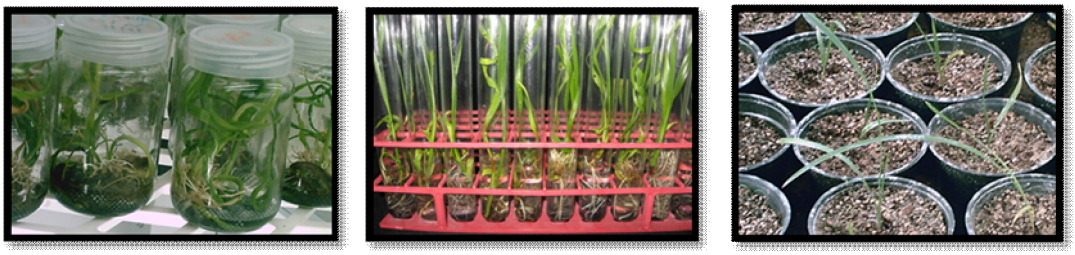

Fig. (6): Elongated shoots on recovery medium which received from all conservation period treatments with sucrose at 90 or 120 g/l were resumed in well manner to rooting and acclimatization stages.

CONCLUSION

The efficacy of each technique used for slow-growth storage was measured by the regeneration percentage and the quality of shoots regenerated after fixed periods of storage. In present study, sucrose at 90 g/L was employed as an osmoticum for the maintenance of in vitro cultures of somatic embryo clusters of date palm Sukkary cv for 8 months at highest significant result of survival percentage after slow growth conditions of regeneration efficiency. In addition, all elongated shoots on recovery medium which received from all conservation period treatments with sucrose at 90 or 120 g/l were succeeded to resume in well manner and to pass to rooting and acclimatization stages (Fig. 6). High sucrose concentration may help to overcome the adverse effects of low temperature during storage period on overall tissue survival and recovery potential. Thus, our study could have a positive economical outcome by promoting germplasm exchange and rapid propagation when necessary. More studies are needed for date palm germplasm conservation for all important varieties need extensive studies to seek the optimal protocols.

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الملخص العربي

تاثير فترات حفظ مختلفة وتركيزات مختلفة من السكروز علي حفظ تجمعات الاجنة الجسدية لنخيل البلح تحت ظروف النمو البطيئة

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ان تقدم مجال التكنولوجيا الحيوية في التقنيات المعملية التي استخدمت بشكل واسع في اكثار وحفظ تلك الانواع التي يكون الاكثارو الحفظ فيها بالتقنيات التقليدية مشكلة. يعتبر نخيل البلح شجرة اقتصادية ذات قيمة. قد اجريت هذه الدراسة لمناقشة امكانية استخدام النمو البطيء في الحفظ المعملي لحفظ المادة الوراثية لنخيل البلح وذلك لتشجيع تبادل المادة الوراثية واكثارها عند الحاجة . تم استخدام تجمع من الاجنة المتضاعفة صنف السكري لنخيل البلح كمنفصل نباتي للتخزين. ولقد درست فترات تخزين مختلفة لمدة ٤,٨,١٢ شهر مع تركيزات مختلفة من السكروز عند ٣٠، ٦٠، ٩٠و١٢٠ جرام /لتر المزودة الي بيئة الحفظ والتي تحتوي علي نصف قوة الاملاح المغذية لبيئة املاح موراشيج وسكوج المغذية بالاضافة الي ٣٠ جرام /لترمانيتول، ٠.٠٥مليجرام /لتر من البنزيل ادنين، ٠.١ مليجرام /لتر نفثالين اسيتيك اسيد و ٨ جرام/ لتر اجار وذلك تحت ظروف التحضين من درجة حرارة منخفضة عند (١٥ درجة سيليزية) واظلام علي قدرة تلك المنفصلات النباتية المخزنة للبقاء حية والمقدرة علي استعادة النمو عند ارجاعها لظروف النموالطبيعية. ولقد تم تقدير المحتوي من السكريات الكلية ،السكريات الغير المختزلة والسكريات المختزلة كتغيرات فسيولوجية خلال فترات الحفظ . اظهرت النتائج ان بيئة الحفظ المزودة بالسكروز عند ٩٠ او١٢٠ جرام /لتر اعطت اعلي قيمة معنوية للبقاء بعد ١٢ شهر من مدة الحفظ . اظهرت المنفصلات النباتية من تجمع الاجنة الجسدية المحفوظة علي بيئة الحفظ المزودة ب ٩٠ جرام /لتر ولمدة ٨ اشهر افضل مقدرة للتضاعف واستعادة النمو تحت ظروف النمو الطبيعية.